



नेपाल सरकार
कृषि, वन तथा पर्यावरण मन्त्रालय
कृषि विकास महाशाखा



फोन नं. { ४२११६३९
४२११६०६
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फ्याक्स नं. ४२११९३५

सिंहदरवार, काठमाडौं
नेपाल

पत्र.संख्या.:

च.नं.:

मिति: २०८३/०२/०७

मल नियन्त्रण आदेश, २०८३ को मस्यौदा उपर सुझाव आवाहन ।

कृषि उत्पादन एवं उत्पादकत्व वृद्धि गरी देशको आर्थिक समृद्धिमा टेवा पुऱ्याउन तथा खाद्य सुरक्षा बढाउन आवश्यक पर्ने मलहरुको उत्पादन, आयात, विक्री वितरण एवं गुणस्तर व्यवस्थापनलाई नियमित र नियन्त्रण कार्यलाई सहज, व्यवस्थित, पारदर्शी र प्रभावकारी बनाउन, आवश्यक पदार्थ (नियन्त्रण) ऐन, २०१७ को दफा ३ र ८ ले दिएको अधिकार प्रयोग गरी नेपाल सरकारले रासायनिक मल (नियन्त्रण) आदेश, २०५५ जारी गरेको छ।

रासायनिक मल नियन्त्रण आदेश, २०५५ मा रासायनिक मलको गुणस्तर परीक्षण, अनुगमन, प्रयोगशाला व्यवस्थापन, ट्रेसबिलिटी, लेबलिङ तथा डिजिटल अभिलेख प्रणाली सम्बन्धी व्यवस्था पर्याप्त नरहेको र हाल अन्तराष्ट्रिय बजारमा मलको उपलब्धता, मलको प्रयोगको स्वरूप, किसानको आवश्यकताहरुमा व्यापक परिवर्तन आएकोमा वर्तमान अवस्थामा उक्त आदेशको दायरा समित रहेको छ। उक्त आदेश हाल रासायनिक मलमा मात्र केन्द्रित रहेको हुँदा हाल प्रयोगमा रहेका प्राङ्गारिक मल, जैविक मल, नानो फर्टिलाइजर लगायत मलहरुको समेत व्यवस्था थप गरी समयानुसार विद्यमान व्यवस्था तथा स्पेशिफिकेशनलाई परिमार्जन गरी एउटै मल नियन्त्रण आदेशमा समायोजन गरी उत्पादन, आयात तथा विक्री वितरण हुने मलको गुणस्तर नियमन गर्न आवश्यक रहेकोले मन्त्रालयबाट मल नियन्त्रण आदेश, २०८३ को प्रारम्भिक मस्यौदा तयार गरीएको छ । उक्त प्रारम्भिक मस्यौदामा सम्पूर्ण सरोकारवालाहरुको राय सुझावको लागी मन्त्रालयको वेबसाइटमा अपलोड गरीएको छ । मस्यौदा उपर २०८३।०२।१४ गते भित्र राय सुझाव उपलब्ध गराईदिनुहुन समबद्ध सरोकारवालामा हार्दिक अनुरोध छ ।

सुझाव पठाउने ठेगाना:

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मल (नियन्त्रण) आदेश, २०८३ को प्रारम्भिक मस्यौदा

आवश्यक पदार्थ नियन्त्रण (अधिकार) ऐन, २०१७ को दफा ३ र ८ ले दिएको अधिकार प्रयोग गरी नेपाल सरकारले देहायको आदेश जारी गरेको छ।

परिच्छेद -१

प्रारम्भिक

१. संक्षिप्त नाम र प्रारम्भ: (१) यस आदेशको नाम “मल (नियन्त्रण) आदेश, २०८३” रहेको छ ।

(२) यो आदेश तुरुन्त प्रारम्भ हुनेछ ।

२. परिभाषा: (१) विषय वा प्रसंगले अर्को अर्थ नलागेमा यस आदेशमा,-

(क) “आधिकारिक प्रयोगशाला” भन्नाले मन्त्रालय वा प्रदेश मन्त्रालय अन्तर्गतका मल तथा माटो परीक्षण एवं विश्लेषण गर्न स्थापना र संचालन भएका प्रयोगशाला सम्झनु पर्छ।

(ख) “आधिकारिक विश्लेषक” भन्नाले मलको गुणस्तरको परीक्षण गर्न यस आदेश बमोजिम विभागबाट दफा ३१ बमोजिम नियुक्त मलको विश्लेषण गर्ने व्यक्ति सम्झनु पर्छ।

(ग) “आयातकर्ता” भन्नाले कुनै विदेशी मुलुकबाट नेपालभित्र मल आयात गर्ने व्यक्ति वा संस्था सम्झनु पर्छ।

(घ) “उत्पादक” भन्नाले नेपाल भित्र मल उत्पादन वा सम्मिश्रण गर्न सक्ने पूर्वाधार र न्यूनतम प्रयोगशालाको सुविधा भई दफा ६ बमोजिम मलको सूचीकरण प्रमाणपत्र प्राप्त व्यक्ति वा संस्था सम्झनु पर्छ।

(ङ) “ऐन” भन्नाले आवश्यक पदार्थ (नियन्त्रण) ऐन, २०१७ सम्झनु पर्छ।

(च) “जैविक मल” भन्नाले यस आदेशको अनुसूची -५ अनुसारको मापदण्ड पूरा भएको वाली बिरुवामा पोषक तत्व उपलब्ध गराउने अथवा पोषक तत्व उपलब्ध गराउन सहयोग पुर्याउने शुष्म जिवाणुहरूको निर्दिष्ट संख्यामा जिवित तर सुशुभ्र अवस्थालाई बिभिन्न किसिमका झोल वा ठोस माध्यममा तयार गरीएको मललाई सम्झनु पर्छ।

(छ) “डिलर” भन्नाले नेपालमा उत्पादित वा आयातित मलको बिक्री वितरण गर्ने व्यक्ति वा संस्था वा त्यस्तो व्यक्ति वा संस्थाको एजेन्ट सम्झनु पर्छ र सो शब्दले मल बिक्री वितरण गर्ने उत्पादक वा आयातकर्तालाई समेत जनाउँछ।

(ज) “नानो फर्टीलाइजर” भन्नाले अनुसूची -२ मा उल्लेख भएको स्पेसिफिकेसन बमोजिमका मल सम्झनु पर्छ।

- (झ) “निरीक्षक” भन्नाले दफा २४ बमोजिम नियुक्त भएको मल निरीक्षक सम्झनु पर्छ।
- (ञ) “निर्देशक समिति” भन्नाले दफा ४१ बमोजिमको समितिलाई सम्झनु पर्छ।
- (ट) “प्रदेश मन्त्रालय” भन्नाले प्रदेश स्थित कृषि विषय क्षेत्र हेर्ने मन्त्रालय सम्झनु पर्छ।
- (ठ) “प्राङ्गारिक मल” भन्नाले यस आदेशको अनुसूची-२ अनुसारको स्पेशिफिकेशन पुरा भएको प्राणी अथवा वनस्पतिजन्य सामग्रीहरूलाई पूर्ण रूपमा बिघटन (डिकम्पोज) गरी, धुलो, दानादार अथवा झोलको रूपमा बनाईएको वाली बिरुवाको पोषणका लागि प्रयोग गरीने पदार्थलाई सम्झनु पर्छ। यसमा पोषक तत्व बृद्धि गर्न अथवा अन्य कुनै प्रयोजनका लागि रासायनिक स्रोतका सामग्रीहरू मिसाईएका हुँदैनन्।
- (ड) “प्रादेशिक समिति” दफा ४६ बमोजिमको समितिलाई सम्झनु पर्छ।
- (ढ) “प्राविधिक समिति” भन्नाले दफा ४४ बमोजिमको समितिलाई सम्झनु पर्छ।
- (ण) “बायोस्टिमुलेन्ट” भन्नाले अनुसूची ३ को स्पेशिफिकेशन बमोजिमका पदार्थ सम्झनु पर्छ जसलाई बिरुवामा, बिउमा वा बिरुवाको जरा वरिपरि प्रयोग गर्दा बिरुवाको प्राकृतिक एवं जैविक प्रक्रिया सक्रिय हुन्छ र यसले बालीको प्राकृतिक शक्ति र प्रतिरोधात्मक क्षमता बढाउन सहयोग पुऱ्याउँछ।
- (त) “मन्त्रालय” भन्नाले नेपाल सरकारको कृषि विषय क्षेत्र हेर्ने मन्त्रालय सम्झनु पर्छ।
- (थ) “मल” भन्नाले वाली बिरुवालाई आवश्यक पर्ने खाद्यतत्वहरू उपलब्ध गराउन उपयोग हुने पदार्थहरू सम्झनु पर्छ र सो शब्दले यस आदेशको अनुसूची -३ बमोजिमका स्पेशिफिकेशन भएका बायोस्टिमुलेन्ट समेतलाई जनाउँनेछ।
- (द) “मान्यताप्राप्त प्रयोगशाला” भन्नाले विभाग वा नेपाल नापतौल तथा गुणस्तर विभागबाट मान्यता प्राप्त मलको भौतिक, रासायनिक र जैविक परीक्षण गर्ने प्रयोगशाला सम्झनु पर्छ।
- (ध) “रासायनिक मल” भन्नाले रासायनिक स्रोतको उपयोग मार्फत वाली बिरुवालाई आवश्यक पर्ने खाद्यतत्वहरू उपलब्ध गराउन उपयोग हुने अनुसूची -१ मा उल्लेख भएको कुनै पदार्थलाई सम्झनु पर्छ।
- (न) “विभाग” भन्नाले मन्त्रालय अन्तर्गतको कृषि विभाग सम्झनु पर्छ।
- (प) “सर्भेयर” भन्नाले नेपालमा आयात गरीने मलको परिमाण र गुणस्तरको सर्वेक्षण गर्ने कामको लागि दफा १८ को उपदफा (५) बमोजिम विभागबाट मान्यता प्राप्त संस्था वा सोही दफाको उपदफा (१०) बमोजिम तोकिएको प्रयोगशाला सम्झनु पर्छ।
- (फ) “स्थानीय मल नियमन तथा व्यवस्थापन समिति” भन्नाले दफा ४८ बमोजिमको समिति सम्झनु पर्छ।

(ब) “स्पेशिफिकेशन” भन्नाले मलको भौतिक, रासायनिक र जैविक गुणहरूको मात्रा वा कम्पोजिसन वा पोषक तत्वको मात्रा सम्झनु पर्छ र सो शब्दले विभिन्न मल अनुसारको अनुसूची -१, २, ३, ४ र ५ बमोजिम तोकिएको मापदण्ड समेतलाई जनाउँनेछ ।

परिच्छेद-२

मलको गुणस्तर निर्धारण र नियमन सम्बन्धी व्यवस्था

३. आदेश विपरित हुने गरी मलको उत्पादन, पैठारी वा बिक्री वितरण गर्न नहुने: कसैले पनि यस आदेश विपरित हुने गरी यसै आदेश बमोजिमका मलको उत्पादन, पैठारी वा बिक्री वितरण गर्नु गराउनु हुँदैन ।
४. मलको उत्पादन, आयात वा बिक्री वितरण नियमन र नियन्त्रण गर्ने: (१) नेपालभित्र मलको उत्पादन, आयात, बिक्री वितरण, गुणस्तर व्यवस्थाको नियमन र नियन्त्रण गर्ने दायित्व मन्त्रालयले तोकेको केन्द्रिय निकाय वा पदाधिकारीको हुनेछ ।
 - (२) प्रदेशभित्र उत्पादन र बिक्री वितरण हुने मलको वितरण एवम् गुणस्तर व्यवस्थाको नियमन र नियन्त्रण गर्ने दायित्व प्रदेश मन्त्रालय वा प्रदेश मन्त्रालयले तोकेको निकाय वा पदाधिकारीको हुनेछ ।
 - (३) स्थानीय तहभित्र उत्पादन र बिक्री वितरण हुने प्राङ्गारिक मलको वितरण, गुणस्तर व्यवस्थाको नियमन र नियन्त्रण गर्ने दायित्व सम्बन्धित स्थानीय तह/कार्य पालिकाको हुनेछ ।
५. मलको स्पेशिफिकेशन सम्बन्धी व्यवस्था: (१) नेपालभित्र उत्पादन, आयात, बिक्री वितरण गरिने मलको स्पेशिफिकेशन र सहन सीमा देहाय बमोजिम हुनेछ ।
 - (क) रासायनिक मलको स्पेशिफिकेशन र सहन सीमा अनुसूची-१ को भाग १ र २ मा उल्लेख भए बमोजिम ।
 - (ख) न्यानो फर्टिलाइजरको स्पेशिफिकेशन र सहन सीमा अनुसूची -२ को भाग १ र २ मा उल्लेख भए बमोजिम ।
 - (ग) बायोस्टिमुलेन्टको स्पेशिफिकेशन र सहन सीमा अनुसूची -३ को भाग १ र २ मा उल्लेख भए बमोजिम ।
 - (घ) जैविक मलको स्पेशिफिकेशन र सहन सीमा अनुसूची -४ को भाग १ र २ मा उल्लेख भए बमोजिमको ।
 - (ङ) प्राङ्गारिक मलको स्पेशिफिकेशन र सहन सीमा अनुसूची -५ को भाग १ र २ मा उल्लेख भए बमोजिम ।

६. मलको स्पेशिफिकेशनमा थपघट गर्न सक्ने: (१) दफा ४ मा जुनसुकै कुरा लेखिएको भए तापनि कसैले सो दफामा उल्लेख भए बमोजिमको स्पेशिफिकेशन बाहेकको मल उत्पादन वा आयात गर्न चाहेमा त्यस्तो मल सम्बन्धी विवरण र त्यसरी आयात वा उत्पादन गर्नुपर्ने कारण खुलाई अनुसूचीमा थप गर्नको लागि केन्द्रीय निकायमा अनुरोध गर्नु पर्नेछ ।

(२) उपदफा (१) बमोजिमको निवेदन प्राप्त भएपछि प्राविधिक समितिको सिफारिसमा निर्देशक समितिले त्यस्तो मल नेपालभित्र उत्पादन वा आयात गर्न उपयुक्त ठहराएमा सो मल अनुसूचीमा थप गर्ने व्यवस्थाको लागि मन्त्रालयमा सिफारिस गर्न सक्नेछ ।

(३) उपदफा (२) बमोजिम सिफारिस प्राप्त भएमा मन्त्रालयले नेपाल राजपत्रमा सूचना प्रकाशन गरी सो मल अनुसूचीमा थप गर्न सक्नेछ ।

(४) अनुसूचीमा उल्लेख भएको मलको स्पेशिफिकेशनमा थप गर्ने वा हटाउने कुनै मनासिव कारण भएमा प्राविधिक समितिको सिफारिसमा निर्देशक समितिले मन्त्रालयमा सिफारिस गर्नेछ ।

(५) मन्त्रालयले उपदफा (४) बमोजिम प्राप्त सिफारिस मनासिव ठहर्याएमा नेपाल राजपत्रमा सूचना प्रकाशन गरी स्पेशिफिकेशनमा थप गर्न वा हटाउने सक्नेछ ।

परिच्छेद- ३

मलको सूचीकरण र नवीकरण सम्बन्धी व्यवस्था

७. मल सूचीकरण गराउनु पर्ने: (१) यस आदेशमा उल्लेख भएको स्पेशिफिकेशन बमोजिमका मल उत्पादन गर्न चाहने उत्पादकले उत्पादन एवं विक्री वितरण गर्ने प्रयोजनको लागि मलको सूचीकरण गराउनु पर्नेछ ।

(२) उपदफा (१) बमोजिम सूचीकरण नगरी व्यवसायिक रूपमा मल विक्री वितरण गर्न पाइने छैन ।

(३) अनुसूची -१, २, ३, ४ र ५ बमोजिमका मल उत्पादन गर्न चाहने व्यक्ति वा संस्थाले आफूले उत्पादन गर्न चाहेको मलको सूचीकरण प्रयोजनको लागि प्रत्येक स्पेशिफिकेशनको लागि अनुसूची -६ बमोजिमको राजश्व दाखिला गरी देहायको निकायमा निवेदन दिनु पर्नेछ:-

(क) अनुसूची १, २, ३ बमोजिमका मलको लागि अनुसूची- ७ बमोजिमको ढाँचामा केन्द्रीय निकायमा ।

(ख) अनुसूची -४ र ५ बमोजिमका मलको लागि अनुसूची- ८ बमोजिमको ढाँचामा प्रदेश मन्त्रालय वा प्रदेश मन्त्रालयले तोकेको निकायमा ।

(ग) खण्ड ख मा जुनसुकै कुरा लेखिएको भए तापनि अनुसूची -५ बमोजिमका दैनिक २ मे. टन भन्दा कम परिमाणमा उत्पादन हुने सिटि कम्पोष्ट, धूलो वा दानेदार प्रांगारिक मल, भर्मिकम्पोष्ट र गोठे मल को लागि अनुसूची- ९ बमोजिमको ढाँचामा सम्बन्धित स्थानीय पालिकामा ।

(४) उपदफा (३) बमोजिम प्राप्त निवेदन उपर जाँच गर्दा आवश्यक कागजात पूरा भएको र मलको प्रयोगशालाको परीक्षणको नतिजा अनुसूचीमा तोकिएको स्पेसिफिकेसन बमोजिम पाईएमा सम्बन्धित स्थानीय पालिका र प्रदेश मन्त्रालय वा सो मन्त्रालयले तोकेको निकायले १५ कार्यदिन भित्र र केन्द्रीय निकायले २१ कार्यदिन भित्र सूचीकरण गरी निवेदकलाई अनुसूची- १० बमोजिमको ढाँचामा सर्त सहित सूचीकरण प्रमाणपत्र उपलब्ध गराउनु पर्नेछ ।

(५) उपदफा (४) बमोजिम दिएको सूचीकरणको प्रमाणपत्र तीन वर्षको लागि बहाल रहनेछ ।

७. मल सूचीकरण नहुने: (१) दफा ६ बमोजिम मलको सूचीकरणका लागि प्राप्त निवेदन जाँचबुझ गर्दा देहायका अवस्थामा सम्बन्धित स्थानीय तह, प्रदेश मन्त्रालय वा प्रदेश मन्त्रालयले तोकेको निकाय र केन्द्रीय निकायले मल सूचीकरण गर्न इन्कार गर्न सक्नेछन ।

क. मलको प्रयोगशाला परीक्षण नतिजा यस आदेशमा तोकिएको स्पेसिफिकेसन अनुरूपको नभएमा, वा

ख. सूचीकरण गर्न चाहेको मल पहिले सूचीकरण भएको कुनै मलको ट्रेड मार्क वा व्यापारिक नाम सँग मिल्ने भएमा, वा

ग. भ्रामक वा गलत संदेश दिने गरी मलको व्यापारिक नाम वा ट्रेड मार्क राखेको पाइएमा, वा

घ. आवश्यक विवरण तथा कागजात पेश नगरेमा ।

(२) उपदफा (१) बमोजिम स्थानीय तह, प्रदेश मन्त्रालय वा प्रदेश मन्त्रालयले तोकेको निकाय र केन्द्रीय निकायले कुनै मलको सूचीकरण गर्न इन्कार गरेमा सोको कारण खुलाई निर्णय भएको ५ कार्यदिन भित्र निवेदकलाई जानकारी गराउनु पर्नेछ ।

(३) मल सूचीकरण गराउन निवेदन दिने उत्पादकले उपदफा (३) बमोजिमको जानकारी प्राप्त गरेको मितिले ३५ दिन भित्र सूचीकरण गर्ने निकायको प्रमुख समक्ष उजुर गर्न सक्नेछ र सो सम्बन्धमा सूचीकरण गर्ने निकायले सम्बन्धित स्थानीय समिति वा प्रादेशिक समिति वा प्राविधिक समितिमा पेश गर्नुपर्नेछ । सो सम्बन्धमा सम्बन्धित समितिको निर्णयनै अन्तिम हुनेछ ।

(४) माथि जुनसुकै लेखिएको भएता पनि केन्द्रीय निकायले अनुसूची १ देखि ५ बाहेक देशभित्र उत्पादन हुने विरुवाका वृद्धि वर्धक, विरुवा उत्पादनका लागि माटोको भौतिक वा जैविक गुण सुधारक, डिकम्पोजर्स (Decomposers) र सहयोगी सूक्ष्मजीवहरूको मिश्रण हरूको सूचीकरण प्राविधिक समितिको सिफारिसमा लेबलमा भएबमोजिम गुणस्तर हुने गरि र अन्य सूचीकरण व्यवस्था प्राविधिक समितिले निर्णय गरेबमोजिम सूचीकरण गर्न सक्नेछ ।

८. उत्पादनको अभिलेख तथा विवरण अध्यावधिक गर्नुपर्ने: (१) मल उत्पादकले उत्पादन गरीने मलको हरेक लटको मलको गुणस्तर सम्बन्धमा आधिकारिक वा मान्यताप्राप्त प्रयोगशालाबाट परीक्षण गराइ सूचीकरण गर्ने निकायमा पेश गर्नुपर्नेछ।

(२) उत्पादकले आर्थिक वर्ष समाप्त भएको ३ महिना भित्र आफ्नो वार्षिक उत्पादन र बिक्री समेतको विवरण अध्यावधिक गर्नको लागि अनुसूची-११ बमोजिमको ढाँचामा मल सूचीकरण गर्ने निकायमा पेश गर्नु पर्नेछ।

(३) उपदफा (२) मा जुन सुकै कुरा लेखिएको भए तापनि अनुसूची १ बमोजिमका मल उत्पादन गर्ने उत्पादकले त्रैमासिक रूपमा अनुसूची -१२ मा तोकिएको ढाँचा अनुसारको विवरण केन्द्रीय निकायमा पेश गर्नुपर्नेछ।

(४) उपदफा (२) र (३) बमोजिम प्राप्त विवरणका आधारमा मल सूचीकरण गर्ने निकायले मलको विवरण अध्यावधिक गर्नुपर्नेछ।

९. सूचीकृत मल र उत्पादनको विवरण पेश गर्नुपर्ने: (१) स्थानीय पालिकाले दफा ७ बमोजिम सूचीकरण गरेको मल र दफा ८ बमोजिम अध्यावधिक गरेको विवरण अर्धवार्षिक रूपमा प्रदेश मन्त्रालयमा पेश गर्नुपर्ने छ।

(२) प्रदेश मन्त्रालय वा प्रदेश मन्त्रालयले तोकेको निकायले उपदफा (१) बमोजिम प्राप्त र प्रदेशमा दफा ७ बमोजिम सूचीकरण भएको मल र दफा ८ बमोजिम अध्यावधिक गरेको विवरण अर्धवार्षिक रूपमा केन्द्रीय निकायमा पेश गर्नुपर्नेछ।

(३) केन्द्रीय निकायले उपदफा (२) बमोजिम प्राप्त र केन्द्रीय निकायमा दफा ७ बमोजिम सूचीकरण भएको मल र दफा ८ बमोजिमको विवरण अध्यावधिक गर्नुपर्नेछ।

१०. मल सूचीकरण प्रमाणपत्रको नवीकरण: (१) उत्पादकले आफूले उत्पादन गर्ने मलको सूचीकरण प्रमाणपत्रको अवधि समाप्त हुनु भन्दा कम्तीमा ३५ दिन अगावै अनुसूची -६ बमोजिमको राजश्व दाखिला गरी नवीकरणका लागि सूचीकरण भएको निकायमा निवेदन दिनु पर्नेछ।

(२) सूचीकरण गर्ने निकायले मलको नयाँ लटको प्रयोगशाला विश्लेषण नतिजा र अध्यावधिक कागजात एवं उत्पादन तथा बिक्री वितरणको विवरणका आधारमा तीन वर्षको लागि नवीकरण गर्न सक्नेछ।

(३) उत्पादकले आफूले उत्पादन गर्ने मलको सूचीकरण प्रमाणपत्रको अवधि सकिएको ३ महिना भित्र वा ३ महिना देखि ६ महिना सम्म अनुसूची-६ मा तोकिए बमोजिमको राजश्व दाखिला गरी नवीकरणका लागि सूचीकरण भएको निकायमा निवेदन दिनु पर्नेछ।

(४) उत्पादकले सूचीकरण प्रमाणपत्रको अवधि समाप्त भएको ६ महिनासम्म पनि नवीकरण नगरेमा नवीकरण हुने छैन।

११. सूचीकरण निलम्बन तथा खारेज हुने: (१) यस आदेश कार्यान्वयनका लागि मल निरिक्षकबाट अनुगमनका क्रममा देहायको अवस्था देखि सिफारिस गरेमा सूचीकरण गर्ने निकायले अधिकतम ३ महिनासम्मको लागि मलको सूचीकरण निलम्बन गरी विक्री वितरण रोक्न सक्नेछ।

- (क) सूचीकरण भएको मलको नमुना परीक्षण गर्दा आदेशले तोकेको स्पेशिफिकेशन बमोजिम नभएमा।
- (ख) माग गरेको अभिलेख वा विवरण वा प्रतिवेदन समयमा तोकिए बमोजिम पेश गरेमाको नपाइएमा।
- (ग) मल सूचीकरण गर्दा पेश गरीएका विवरण, कागजात वा आधारहरू पछि झूटो, अपूरो वा भ्रामक पाइएमा।
- (घ) मलको प्याकेजिङमा पोषक तत्वको मात्रा वा प्रयोग विधिबारे गलत वा भ्रामक जानकारी राखेको पाइएमा।
- (ङ) लेबलमा तोकेका आवश्यक जानकारीहरू समावेश नगरेमा वा तोकिएभन्दा फरक लेबलिंग गरेको पाइएमा।

(२) सूचीकरण गर्ने निकायले निलम्बनको जानकारी उत्पादकलाई तुरुन्त गराउनु पर्नेछ।

(३) सूचीकरण निलम्बन भएको उत्पादकले आवश्यक सुधार गरी उपदफा (१) बमोजिम तोकिएको अवधि भित्रै सूचीकरण गर्ने निकायबाट निलम्बन फुकुवा गर्नुपर्नेछ।

(४) सूचीकरण गर्ने निकायले सम्बन्धित समितिको सिफारिसमा देहायको अवस्थामा मलको सूचीकरण खारेजी गर्न सक्नेछ।

- (क) उत्पादकले उपदफा (१) बमोजिम तोकिएको अवधिमा आवश्यक सुधार एवं निलम्बन फुकुवा नगरेमा।
- (ख) मल सूचीकरण प्रमाणपत्रमा उल्लेखित कुनै पनि सर्त वा बन्देजको उल्लंघन गरेमा।
- (ग) यो आदेश वा अन्य प्रचलित कानूनी व्यवस्थाको उल्लङ्घन गरेमा।
- (ज) सूचीकृत मलको उत्पादन बन्द भएमा वा दफा १० मा तोकिएको अवधि भित्र नवीकरण नभएमा।

(५) मल उत्पादकले मलको सूचीकरण खारेजी विरुद्ध सम्बन्धित स्थानीय तहमा प्रमुख समक्ष, प्रदेश मन्त्रालयमा सचिव समक्ष र केन्द्रीय निकायको हकमा विभाग समक्ष निवेदन गर्न सक्नेछ।

(६) उपदफा (५) बमोजिमको निवेदनमा छानबिन गरि सम्बन्धित स्थानीय तह, प्रदेश मन्त्रालय र विभाग बाट भएको निर्णय नै अन्तिम निर्णय हुनेछ।

परिच्छेद-४

मलको आयात निर्यात सम्बन्धी व्यवस्था

१२. रासायनिक मल र नानो फर्टिलाइजर: (१) आदेशको अनुसूची -१ बमोजिमका रासायनिक मल र अनुसूची -२ बमोजिमका नानो फर्टिलाइजर आयात गर्न चाहने आयातकर्ताले आयात अनुमति-पत्रको लागि अनुसूची -६ मा तोकिए बमोजिमको राजश्व दाखिला गरी अनुसूची-१३ बमोजिमको ढाँचामा केन्द्रीय निकायमा निवेदन दिनुपर्नेछ।

(२) उपदफा (१) बमोजिम निवेदन प्राप्त भएमा केन्द्रीय निकायले आवश्यकता अनुसार सर्त तोकी १५ कार्यदिन भित्र अनुसूची-१४ बमोजिमको आयात अनुमति पत्र जारी गर्नेछ।

(३) उपदफा (२) बमोजिमको आयात अनुमतिपत्रको म्याद १ वर्षको हुनेछ।

(४) उपदफा (२) बमोजिम आयात अनुमति प्राप्त आयातकर्ताले रासायनिक मल वा नानो फर्टिलाइजर आयात प्रयोजनको लागि यस आदेशमा तोकिए बमोजिमको रासायनिक मल वा नानो फर्टिलाइजर सम्बन्धी विवरण समेत संलग्न गरी प्रतितपत्र खोल्नको लागि कुनै वाणिज्य बैंकमा निवेदन पेश गर्नुपर्नेछ।

(५) उपदफा ३ बमोजिमको निवेदन परेमा वाणिज्य बैंकले आवश्यक जाँचबुझ गरी केन्द्रीय निकायबाट आयात अनुमति प्राप्त स्पेसिफिकेशन बमोजिमको रासायनिक मल वा नानो फर्टिलाइजर वा दुवै आयात गर्न प्रचलित कानून बमोजिम प्रतितपत्र खोलि दिनु पर्नेछ। यसरी प्रतितपत्र खोल्ने वाणिज्य बैंकले प्रतितपत्र खोलिसकेपछि केन्द्रीय निकायलाई सो कुराको जानकारी तुरुन्त दिनुपर्नेछ।

(६) उपदफा (१) र (२) मा जे सुकै व्यवस्था भए पनि नेपाल सरकारले अनुदानमा उपलब्ध गराउने रासायनिक मलको आयात गर्ने आपूर्तिकर्ताहरूको आयात अनुमति नेपाल सरकारले तोकेबमोजिम हुनेछ।

१३. प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्ट: (१) अनुसूची -३, ४ र ५ बमोजिमका प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्ट नेपालमा आयात गरी बिक्री वितरण गर्न चाहने आयातकर्ताले अनुसूची-१५ को ढाँचामा अनुसूची-६ मा तोकिए बमोजिमको दस्तुर सहित केन्द्रीय निकायमा निवेदन दिनु पर्नेछ।

(२) उपदफा (१) बमोजिमको निवेदन उपर जाँच गर्दा मलको गुणस्तर यस आदेशको स्पेसिफिकेशन बमोजिम रहेमा दफा १४ बमोजिम बाली परीक्षण गर्ने प्रयोजनको लागि आवश्यक पर्ने परिमाण आयात गर्न आयातकर्तालाई केन्द्रीय निकायले पूर्व स्वीकृति प्रदान गर्नेछ।

(३) उपदफा (२) बमोजिम स्वीकृति पाएका आयातकर्ताले आयात गरी ल्याएको प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको दफा १४ को उपदफा (१) बमोजिम बाली परीक्षणको लागि पठाउनु पर्नेछ।

(४) उपदफा (३) बमोजिम बाली परीक्षणको नतिजा बाली उत्पादनमा उल्लेखनीय वृद्धि (Statistically significant) र माटोको गुणस्तर सुधारमा फाइदाजनक देखिएमा प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको प्राविधिक समितिको सिफारिसमा केन्द्रीय निकायले आयातकर्तालाई अनुसूची-१६ को ढाँचामा आवश्यकता अनुसार सर्त तोकी आयात अनुमतिपत्र प्रदान गर्नेछ।

(६) उपदफा (५) बमोजिम प्राप्त आयात अनुमति पत्र १ वर्षको लागि मात्र मान्य हुनेछ।

(७) उपदफा (५) बमोजिम आयात अनुमति प्राप्त आयातकर्ताले प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको आयातको लागि प्रतितपत्र खोल्नको लागि कुनै बाणिज्य बैकमा निवेदन दिंदा यस आदेशमा तोकिए बमोजिमको स्पेशीफिकेशन सम्बन्धी विवरण समेत संलग्न गर्नुपर्नेछ।

(८) उपदफा (७) बमोजिमको निवेदन परेमा बाणिज्य बैकले आवश्यक जाँचबुझ गरी केन्द्रीय निकायबाट आयात अनुमति प्राप्त स्पेशीफिकेशन बमोजिमको प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको आयात गर्न प्रचलित कानून बमोजिम प्रतितपत्र खोल्न दिनु पर्नेछ। यसरी प्रतितपत्र खोल्ने बाणिज्य बैकले प्रतितपत्र खोल्नसकेपछि केन्द्रीय निकायलाई सो कुराको जानकारी तुरुन्त दिनु पर्नेछ।

१४. प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको बाली परीक्षण: आयात गरीने प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको प्रभावकारिता देहाय बमोजिम परीक्षण भएको हुनु पर्नेछ:-

(क) आयात गरीने प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको प्रभावकारिताको बारेमा नेपाल कृषि अनुसन्धान परिषद्ले कम्तीमा दुई सिजनको बाली उत्पादनमा परीक्षण गरी केन्द्रीय निकायलाई परीक्षणको नतिजा त्यस्तो परीक्षण सम्पन्न भएको मितिले तीन महिना भित्र उपलब्ध गराउनु पर्नेछ।

(ख) उपदफा (१) बमोजिमको परीक्षणको लागि लाग्ने नेपाल कृषि अनुसन्धान परिषद्ले तोकेबमोजिमको खर्च परीक्षण गराउने आयातकर्तालेनै व्यहोर्नु पर्नेछ।

(ग) उपदफा (१) बमोजिमको परीक्षणको लागि प्राप्त मल कुनै बाली विशेषका लागि लक्षित रहेमा नेपाल कृषि अनुसन्धान परिषद्ले बाली परीक्षण गर्दा लक्षित बालीलाई समावेश गर्नु पर्नेछ।

१५. आयात अनुमतिपत्रको नवीकरण: (१) आयातकर्ताले आफूले प्राप्त गरेको आयात अनुमति-पत्रको अवधि समाप्त हुनु भन्दा ३५ दिन अगावै अनुसूची-६ मा उल्लेख भएको राजस्व दाखिला गरी अनुमति पत्रको नवीकरण गराउन केन्द्रीय निकाय समक्ष निवेदन दिनुपर्नेछ।

(२) उपदफा (१) बमोजिम नवीकरणको लागि निवेदन परेमा केन्द्रीय निकायले आवश्यक जाँचबुझ गरी नवीकरण गर्न उचित देखिएमा अनुमतिपत्रको अवधि समाप्त नहुँदै १ वर्षको लागि नवीकरण गर्नुपर्नेछ।

तर प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको आयात अनुमति पत्र नवीकरण अधिकतम २ पटक सम्म मात्र हुनेछ। सो पश्चात नवीकरण चाहेमा पुनः दफा १४ बमोजिम बाली परीक्षण गराउनु पर्नेछ।

(३) केन्द्रीय निकायले आयात अनुमति पत्रमा तोकेको सर्तहरू पालना नगर्ने र दफा १७ बमोजिम तोकिएको विवरण पेश नगर्ने आयातकर्ताको आयात अनुमति पत्र नवीकरण गर्न वाध्य हुने छैन।

(४) उपदफा (२) मा जुनसुकै कुरा लेखिएको भए तापनि कुनै आयातकर्ताले आयात गरेको मल अनुगमनका क्रममा गुणस्तरहिन पाइएमा वा सरकारी फार्म केन्द्रहरूमा प्रयोगका क्रममा प्रभावहिन पाइएमा वा आदेश बमोजिम गुणस्तर नभएको ठोस प्रमाण सहित लिखित गुनासो प्राप्त भएमा केन्द्रीय निकायले प्राविधिक समितिको सिफारिसमा दफा १४ बमोजिम बाली परीक्षण गराउन सक्नेछ।

१६. **आयात अनुमतिपत्र खारेज हुने:** (१) केन्द्रीय निकायले मल आयातको लागि प्रदान गरेको आयात अनुमति पत्र देहायको अवस्थामा खारेज गर्न सक्नेछ:-

(क) केन्द्रीय निकायमा पेश गर्नुपर्ने अभिलेख तथा विवरण समयमै पेश नगरेमा

(ख) यस आदेशमा तोकिएको स्पेशिफिकेशन बाहेकका मल आयात तथा बिक्री वितरण गरेमा

(ग) भ्रामक वा गलत संदेश दिने गरी मलको व्यापारिक नाम वा ट्रेड मार्क राखी मल बिक्री वितरण गरेमा

(घ) आयात अनुमति माग गर्दा पेश गरेको मलको लेवल भन्दा फरक लेवल गरी वा स्पेशिफिकेशन भ्रामक तरिकाले उल्लेख गरी मल बिक्री वितरण गरेमा

(ङ) यस आदेश बमोजिम मलको गुणस्तर नभएको प्रमाणित भएमा

(च) कृषि बाहेक अन्य कार्यको लागि मल बिक्री वितरण गरेमा

१७. **आयात अभिलेख तथा विवरण:** (१) आयातकर्ताले आयात गरीएको मलको हरेक लटको लागि अनलौड पोर्टमा मन्त्रालयले तोकेको सर्भेयरले मलको गुणस्तर सम्बन्धमा दिएको प्रमाणपत्र केन्द्रीय निकायमा पेश गर्नुपर्नेछ।

(२) आयातकर्ताले अनुसूची -१७ मा तोकिएको ढाँचा अनुसारको विवरण त्रैमासिक रूपमा केन्द्रीय निकायमा पेश गर्नुपर्नेछ।

१८. **सर्भेयर सम्बन्धी व्यवस्था:** (१) आयातकर्ताले नेपालमा मल आयात गर्दा त्यसको परिमाण, गुणस्तर, प्याकेजिङ र लेबलिङ यस आदेश बमोजिम भए नभएको सम्बन्धमा सर्भेयरद्वारा सर्वेक्षण गराई प्रमाणित गराउनु पर्नेछ।

(२) उपदफा (१) बमोजिमको सर्भेयरको मान्यता प्राप्त गर्नका लागि देहाय बमोजिम योग्यता पुगेको संस्थाले अनुसूची ६ मा तोके बमोजिमको दस्तुर सहित अनुसूची- १८ बमोजिमको ढाँचामा केन्द्रीय निकायमा निवेदन दिनुपर्नेछ।

(क) मल परीक्षण गर्नको लागि नेपाल गुणस्तर तथा नापतौल विभागबाट प्रत्यायन प्राप्त प्रयोगशाला सुविधा भएको

(ख) रासायनिक मल र प्रांगारिक मलहरूको रासायनिक परीक्षणका लागि कृषि विज्ञान वा रसायनशास्त्रमा कम्तीमा स्नातकोत्तर उत्तिर्ण गरी आधिकारीक विश्लेषकको तालिम लिएको जनशक्ति भएको

(ग) जैविक मल परीक्षणका लागि माइक्रोबायोलोजि वा जैविक प्रविधि विषयमा कम्तीमा स्नातकोत्तर उत्तिर्ण गरी आधिकारीक विश्लेषकको तालिम लिएको जनशक्ति भएको

(३) प्राविधिक समितिको सिफारिसमा केन्द्रीय निकायले उपदफा (२) बमोजिमको योग्यता जाँचवुझ गरी सर्भेयरको काम गर्न योग्यता पुगेको संस्थालाई सर्भेयरको काम गर्ने मान्यता प्रदान गर्नको लागि विभागमा सिफारिस गर्नेछ ।

(४) उपदफा (३) बमोजिम सिफारिस गर्दा निवेदकसँग उपलब्ध प्रयोगशाला परीक्षण सुविधा र जनशक्तिका आधारमा यस आदेशको अनुसूची १ देखि ५ सम्मका मल सबै वा केहिका लागिमात्र सर्भेयरको मान्यताको लागि सिफारिस गर्न सक्नेछ ।

(५) विभागले उपदफा (३) बमोजिम सिफारिस भएका संस्थालाई अनुसूची- १९ बमोजिमको ढाँचामा सर्भेयरको प्रमाणपत्र दिनेछ ।

(६) उपदफा (५) बमोजिम प्राप्त प्रमाणपत्रको अवधि १ वर्षको हुनेछ ।

(७) प्रमाणपत्रको अवधि समाप्त हुनु ३५ दिन अगावै प्रमाणपत्र नवीकरणको लागि दफा २१ को उपदफा २ बमोजिमको योग्यता पुगेको प्रमाण सहित केन्द्रीय निकायमा निवेदन दिनुपर्नेछ ।

(८) नवीकरण निवेदन प्राप्त भए पछि उपदफा (३), (४) र (५) को प्रक्रिया पुरा गरी विभागले १ वर्षको लागि सर्भेयरको प्रमाणपत्र नवीकरण गर्न सक्नेछ ।

(९) विभागले सर्भेयरको विवरण अद्यावधिक गरी केन्द्रीय निकायमा पठाउनुका साथै वेभसाइटमा सार्वजनिक गर्नुपर्नेछ ।

(१०) उपदफा (१), (२) र (३) मा जे सुकै लेखिएको भएता पनि विभागको सिफारिसमा मन्त्रालयले आधिकारीक प्रयोगशाला मध्ये कुनै प्रयोगशालालाई यस आदेश बमोजिम सर्भेयरको रूपमा काम गर्न तोक्न सक्नेछ ।

(११) सर्भेयरले आफुले प्रमाणित गरेको मलको सर्भेक्षण प्रतिवेदनको एक प्रति ३० दिन भित्र केन्द्रिय निकायमा पेश गर्नु पर्नेछ ।

१९. सर्भेयरको मान्यता रद्द गर्न सकिने: (१) सर्भेयरले मलको परिमाण, स्पेशिफिकेसन, प्याकेजिङ्ग र लेवेलिङ्ग यस आदेश बमोजिम भए नभएको सम्बन्धमा सर्वेक्षण गरी प्रमाणित गर्दा वदनीयत चिताई वा लापरवाही गरी मलको परिमाण, गुणस्तर, प्याकेजिङ्ग र लेवेलिङ्ग सम्बन्धि बास्तविक कुरा लुकाई

छिपाई नभएको कुरालाई प्रमाणित गरेको पाइएमा केन्द्रीय निकायले त्यस्तो सर्भेयरको मान्यता रद्द गर्न विभागमा सिफारिस गर्न सक्नेछ।

(२) उपदफा (१) बमोजिम सर्भेयरको मान्यता रद्द गर्नु अगाडी विभागले सर्भेयरलाई स्पष्टीकरण पेश गर्ने मौका दिनु पर्नेछ।

(३) उपदफा २ बमोजिम पेश गरेको स्पष्टीकरण चित्तबुझ्दो नभएमा विभागले सर्भेयरको मान्यता रद्द गर्नेछ।

(४) उपदफा (३) बमोजिम मान्यता रद्द भएमा विभागले वेभसाइट मार्फत सो को सूचना सार्वजनिक गर्नु पर्नेछ ।

२०. मल उत्पादनको लागि आवश्यक पर्ने कच्चा पदार्थ आयात सम्बन्धमा: (१) आदेशमा उल्लेख भएको स्पेसिफिकेसन र गुणस्तरको मल उत्पादन गर्न आवश्यक पर्ने कच्चा पदार्थ आयातका लागि तोकिएको आवश्यक कागजात सहित उत्पादकले अनुसूची ६ मा तोके बमोजिमको दस्तुर सहित केन्द्रीय निकायमा निवेदन दिनुपर्ने छ । उत्पादकबाट भएको अनुरोधको जाँचबुझ गरी प्राविधिक समितिको सिफारिसमा केन्द्रीय निकायले उत्पादकलाई परिमाण तोकि कच्चा पदार्थ आयातको आयात अनुमति जारी गर्न सक्नेछ ।

२१. अनुसन्धान प्रयोजनको लागि मल आयात गर्ने सम्बन्धमा: (१) आदेशमा उल्लेख भएको स्पेसिफिकेसन र गुणस्तर बाहेकको मल अनुसन्धान प्रयोजनको लागि आयात गर्न चाहाने व्यक्ति तथा संस्थाले अनुसूची ६ मा तोके बमोजिमको दस्तुर सहित तोकिएको आवश्यक कागजात एवं मल आयात गर्नुपर्ने कारण सहित केन्द्रीय निकायमा निवेदन दिनुपर्नेछ। निवेदकबाट भएको अनुरोधको जाँचबुझ गरी प्राविधिक समितिको सिफारिसमा निकायले परिमाण तोकि आयात अनुमति जारी गर्न सक्नेछ ।

२२. मलको निर्यात सम्बन्धमा: (१) नेपालमा उत्पादित मल निर्यात गर्दा सिफारिस आवश्यक पर्ने भएमा यस आदेश बमोजिम सूचीकरण भएको मलको उत्पादकले केन्द्रीय निकायमा अनुसूची २० बमोजिमको विवरण खुलाई निवेदन दिनु पर्नेछ ।

(२) उपदफा (१) बमोजिम प्राप्त निवेदन उपर जाँचबुझ गरी केन्द्रीय निकायले निवेदकलाई मल निर्यातको सिफारिस गर्नेछ।

(३) उपदफा (१) र (२) मा जुनसुकै व्यवस्था भए तापनि मन्त्रालयले आवश्यक देखेमा मलको निर्यातमा रोक लगाउन सक्नेछ।

परिच्छेद-५

निरीक्षक सम्बन्धी व्यवस्था

२३. मल निरीक्षक नियुक्त गर्ने सम्बन्धमा: (१) नेपालमा उत्पादित एवं आयातित मलको नियमित अनुगमन गरी गुणस्तरयुक्त मल किसानलाई उपलब्ध गराउने कार्यमा सघाउ पुर्याउन देहायका योग्यता पुगेका कर्मचारीलाई विभागको सिफारिसमा मन्त्रालयले मल निरीक्षकको रूपमा नियुक्त गर्न सक्नेछ।

(क) कृषि विषयमा न्यूनतम स्नातक तह उर्तिर्ण

(ख) नेपाल सरकार वा प्रदेश सरकारको निकायमा कम्तिमा रा.प.तृ. श्रेणी वा छैटौँ तहको कृषि अधिकृत नियुक्त भइ कार्यरत रहेको ।

(ग) मल निरीक्षक सम्बन्धी आधारभूत तालिम लिएको ।

(२) मन्त्रालयले उपदफा (१) बमोजिम मल निरीक्षक नियुक्त गरेको जानकारी केन्द्रीय निकाय र सम्बन्धित प्रदेश मन्त्रालयलाई दिनु पर्नेछ ।

२४. मल निरीक्षकको परिचालन सम्बन्धी व्यवस्था: (१) मन्त्रालय अन्तर्गतका निकायमा कार्यरत मल निरीक्षकको परिचालन केन्द्रीय निकायले गर्नेछ।

(२) प्रदेश मन्त्रालय अन्तर्गत कार्यरत मल निरीक्षकको परिचालन कार्यक्षेत्र तोकी प्रदेश मन्त्रालयले गर्नेछ।

(३) उपदफा (२) बमोजिम परिचालन भएका मल निरीक्षकको अद्यावधिक विवरण प्रदेश मन्त्रालयले केन्द्रीय निकायमा पठाउनु पर्नेछ।

(३) उपदफा (१) र (२) बमोजिम परिचालित मल निरीक्षकलाई अनुसूची २१ को ढाँचामा परिचयपत्र उपलब्ध गराउनु पर्नेछ ।

२५. मल निरीक्षकको काम, कर्तव्य र अधिकार: (१) मल निरीक्षकको काम, कर्तव्य र अधिकारहरु देहाय बमोजिम हुनेछन ।

(क) यस आदेश बमोजिम मलको गुणस्तर सुनिश्चित गर्न नियमित अनुगमन गर्ने ।

(ख) मलको उत्पादन, आयात, बिक्री वितरण वा संचिति सम्बन्धी कुनै अभिलेख, विवरण वा कागजात सम्बन्धित उत्पादक, आयातकर्ता तथा डिलरबाट दाखिला गर्न लगाउने वा प्रतिलिपि लिने ।

(ग) मलको अनुगमन गर्दा वा मलको गुणस्तर सम्बन्धी सूचना वा उजुरी प्राप्त भएमा मलको उत्पादन, बिक्री वितरण वा संचय हुने स्थानमा प्रवेश गरी मल वा मल उत्पादन गर्न प्रयोग गरीने बस्तुको विना मूल्य अनुसूची को व्यवस्था अनुसार तीन थान नमुना लिई छुट्टाछुट्टै सिलबन्दि गर्ने र एक थान परीक्षणको लागि आधिकारिक प्रयोगशालामा पठाउने, एक थान केन्द्रीय निकायमा पठाउने र एक थान सम्बन्धित उत्पादक वा व्यवसायीलाई दिने ।

(घ) ऐन वा यस आदेशको विपरीत कुनै काम कारवाही गरेको छ भन्ने उजुरी वा सूचना प्राप्त भएमा त्यसको छानबिन गर्ने ।

(ङ) उपदफा (घ) बमोजिम छानबिन गर्दा ऐन वा यस आदेशको उल्लङ्घन भएको पाइएमा त्यस्तो कार्यमा प्रयोग भएको सर सामान, मल, अभिलेख, कागजपत्र वा अन्य सम्बन्धित सामग्री जफत गर्ने ।

(च) अनुगमनको क्रममा यस आदेश विपरित कार्य गरेको पाइएमा त्यस्तो मलको बिक्री वितरण रोक्का राख्ने वा वितरण कक्षमा सिलबन्दी गर्ने ।

(छ) प्रत्येक निरीक्षणपछि **अनुसूची २२** बमोजिमको लिखित प्रतिवेदन तयार गरी केन्द्रीय निकायमा पठाउने ।

परिच्छेद ६

कसुर एवं सजाय, छानबिन र मुद्दा दायर सम्बन्धी व्यवस्था

२६. कसुर कायम हुने: (१) यस आदेशको व्यवस्था विपरितको कार्य ऐनको दफा ३क बमोजिम कसुर मानिनेछ । ऐनको दफा ४ र दफा ६क मा अनुसार सजाय हुनेछ ।

२७. जफत गर्ने: (१) कसैले यस आदेशको व्यवस्था विपरित मलको उत्पादन, आयात, भण्डार तथा बिक्रीवितरण गरेको पाइएमा त्यस्तो मल मल निरीक्षकले ऐनको **दफा ८ र** यस आदेशको दफा २५ बमोजिमको प्रक्रिया पुरा गरी जफत गर्न सक्नेछ ।

(२) जफत भएको मलको व्यवस्थापन सम्बन्धी व्यवस्था कार्यविधि अनुसार केन्द्रीय निकायले गर्नेछ ।

२८. छानबिन तथा मुद्दा दायर गर्ने: (१) यस आदेश विपरित गरेको कार्यको छानबिन गर्ने कार्य दफा २४ बमोजिम तोकिएको मल निरीक्षकले गर्नेछ ।

(२) उपदफा (१) बमोजिम छानबिनको कार्य समाप्त भए पछि दफा २४ को उपदफा (१) बमोजिम नियुक्त मल निरीक्षकले सरकारी वकिलको राय लिई ऐनको **दफा ७ बमोजिम** मुद्दाको प्रक्रिया गर्नुपर्नेछ ।

२९. स्थानीय प्रशासन र प्रहरीले सहयोग गर्नुपर्ने: (१) ऐन र यस आदेशको पालना गराउने तथा सो सम्बन्धी कानूनी कारवाहीको सिलसिलामा निरीक्षकले सहयोग मागेमा स्थानीय प्रशासन र प्रहरीले निरीक्षकलाई सहयोग पुर्याउनु पर्नेछ ।

३०. असल नियतले गरेको कामको बचाउ: मल निरीक्षकको रूपमा अधिकार प्राप्त कुनै कर्मचारीले यस आदेश बमोजिम आफ्नो कर्तव्यको पालना गर्दा असल नियतले गरेको काम कारवाहीमा निज व्यक्तिगत रूपमा जवाफदेही हुनेछैन।

परिच्छेद-७

प्रयोगशाला, विश्लेषक र मलको परीक्षण सम्बन्धी व्यवस्था

३१. आधिकारिक विश्लेषकको नियुक्ति तथा योग्यता: (१) मलको स्पेशिफिकेशन अनुसारको गुणस्तर सम्बन्धमा परीक्षण तथा विश्लेषण गर्नको लागि विभागले आवश्यकता अनुसार आधिकारीक प्रयोगशाला वा मान्यताप्राप्त प्रयोगशालामा कार्यरत उपदफा (२) बमोजिमको योग्यता पुगेको व्यक्तिलाई केन्द्रीय निकायको सिफारिसका आधारमा आधिकारिक विश्लेषकको रूपमा नियुक्त गर्न सक्नेछ।

(२) आधिकारीक विश्लेषकको पदमा नियुक्त हुनको लागि आवश्यक न्यूनतम योग्यता कृषि वा रसायनशास्त्र वा माइक्रोबायोलोजि वा जैविक प्रविधि विषयमा स्नातकोत्तर तह उत्तिर्ण गरी केन्द्रीय निकायले संचालन गर्ने मल विश्लेषण सम्बन्धि तालिम लिएको हुनुपर्नेछ।

(३) केन्द्रीय निकायले उपदफा १ बमोजिम सिफारिस गर्दा विश्लेषकको शैक्षिक योग्यताको आधारमा यस आदेशको अनुसूचि १ देखि ५ सम्मका मल मध्य कुनै एक वा एक भन्दा धेरै मलका लागि आधिकारिक विश्लेषकको रूपमा नियुक्तिको लागि सिफारिस गर्न सक्नेछ।

३२. आधिकारिक विश्लेषकको काम, कर्तव्य र अधिकार: आधिकारीक विश्लेषकको काम, कर्तव्य र अधिकार देहाय बमोजिम हुनेछ।

(क) आदेशमा व्यवस्था भएको परीक्षण विधि अनुसार प्राप्त मलको नमुनाको परीक्षण तथा विश्लेषण गर्ने।

(ख) मलको नमुनाको परीक्षण र विश्लेषण गरीसकेपछि सो को नतिजा मल निरीक्षकलाई पठाउने।

३३. प्रयोगशालाको काम, कर्तव्य र अधिकार: (१) यस आदेश कार्यान्वयनका लागि आधिकारीक प्रयोगशालाको काम, कर्तव्य र अधिकार देहाय बमोजिम हुनेछ।

(क) मल उत्पादक र मल निरीक्षकले परीक्षणको लागि पठाएको नमूनाको अनुसूची मा भएको परीक्षण विधि र आधिकारीक विश्लेषक प्रयोग गरी परीक्षण र विश्लेषण गराउने।

(ख) मल निरीक्षकले परीक्षणका लागि पठाएको नमूनाको गार्ड नमुना (Guard Sample) लाई ३ महिना सम्म सुरक्षित साथ भण्डारण गर्ने।

(ग) मानक प्रक्रिया (Standard Procedure) अनुसार नलिएको, सिलबन्दी नगरीएको वा लेबलिङ स्पष्ट नभएको नमूनालाई विश्लेषणका लागि अस्वीकार (Reject) गर्ने अधिकार।

(घ) नमूनाको विश्लेषणको नतिजालाई प्रभाव पार्न सक्ने कुनै पनि अतिरिक्त कागजात वा सूचना (जस्तै: उत्पादकको स्पेसिफिकेशन, कच्चा पदार्थको विवरण) माग गर्ने अधिकार।

(ङ) मल परीक्षणका विधिहरूलाई अद्यावधिक (Updating Methods) गर्न र राष्ट्रिय मापदण्डहरू परिमार्जन गर्न सहयोग पुऱ्याउने।

(च) समय-समयमा आफ्ना प्राविधिक कर्मचारीहरूको क्षमता अभिवृद्धि गर्न तालिमहरू सञ्चालन गर्ने।

(छ) परीक्षण सेवाका लागि तोकिएको शुल्क (Analysis Fee) लिने अधिकार।

तर खण्ड (छ) मा जुनसुकै कुरा लेखिए तापनि यस आदेश कार्यान्वयनका लागि मल निरीक्षक बाट प्राप्त मलको नमूना परीक्षणका लागि आफ्नो वार्षिक कार्यक्रम तथा बजेटमा विनियोजन गराई नमूना परीक्षण गर्नुपर्नेछ।

(२) यस आदेश कार्यान्वयनका लागि मान्यताप्राप्त प्रयोगशालाको काम, कर्तव्य र अधिकार देहाय बमोजिम हुनेछ।

(क) मल उत्पादक र मल निरीक्षकले परीक्षणको लागि पठाएको नमूनाको अनुसूची मा भएको परीक्षण विधि र आधिकारीक विश्लेषक प्रयोग गरी परीक्षण र विश्लेषण गराउने।

(ख) मल निरीक्षकले परीक्षणका लागि पठाएको नमूनाको गार्ड नमूना (Guard Sample) लाई ३ महिना सम्म सुरक्षित साथ भण्डारण गर्ने।

(ग) मानक प्रक्रिया (Standard Procedure) अनुसार नलिएको, सिलबन्दी नगरीएको वा लेबलिङ स्पष्ट नभएको नमूनालाई विश्लेषणका लागि अस्वीकार (Reject) गर्ने अधिकार।

(घ) नमूनाको विश्लेषणको नतिजालाई प्रभाव पार्न सक्ने कुनै पनि अतिरिक्त कागजात वा सूचना (जस्तै: उत्पादकको स्पेसिफिकेशन, कच्चा पदार्थको विवरण) माग गर्ने अधिकार।

(ङ) परीक्षण सेवाका लागि तोकिएको शुल्क (Analysis Fee) लिने अधिकार।

३४. मलको नमूनाको स्वतन्त्र विश्लेषण सम्बन्धी व्यवस्था: (१) यस आदेशको कार्यान्वयनका क्रममा मल निरीक्षकले दफा २५ र दफा ३५ बमोजिम लिई परीक्षणका लागि आधिकारिक प्रयोगशालामा पठाइएको मलको नमूनाको र उत्पादक वा व्यवसायीसँग भएको नमूनाको आधिकारिक प्रयोगशालामा वा अन्य कुनै मान्यताप्राप्त प्रयोगशालामा भएको विश्लेषण नतिजामा फरक आई विवाद उत्पन्न भएमा मल निरीक्षकले केन्द्रीय निकायमा पठाएको सम्बन्धित मलको नमूना प्रतिलाई अन्य कुनै आधिकारिक वा

मान्यताप्राप्त प्रयोगशाला वा कुनै अन्तर्राष्ट्रिय रूपमा एक्रिडेटेड प्रयोगशालामा स्वतन्त्र विश्लेषण गर्नुपर्नेछ ।

(२) उपदफा १ बमोजिमको नतिजानै निर्णायक नतिजा हुनेछ ।

३५. मलको नमुना लिने तरिका: (१) सर्भेयर एवं मल निरीक्षककले रासायनिक मलको नमुना लिँदा अनुसूची- १ को भाग ३ मा उल्लेख भए बमोजिमको तरिका अपनाउनु पर्नेछ ।

(२) सर्भेयर एवं मल निरीक्षककले नानो फर्टिलाइजर नमुना लिँदा अनुसूची-२ को भाग ३ मा उल्लेख भए बमोजिमको तरिका अपनाउनु पर्नेछ ।

(३) सर्भेयर एवं मल निरीक्षककले बायोस्टिमुलेन्टको नमुना लिँदा अनुसूची-३ को भाग ३ मा उल्लेख भए बमोजिमको तरिका अपनाउनु पर्नेछ ।

(४) सर्भेयर एवं मल निरीक्षककले जैविक मलको नमुना लिँदा अनुसूची-४ को भाग ३ मा उल्लेख भए बमोजिमको तरिका अपनाउनु पर्नेछ ।

(५) सर्भेयर एवं मल निरीक्षककले प्राङ्गारिक मलको नमुना लिँदा अनुसूची- ५ को भाग ३ मा उल्लेख भए बमोजिमको तरिका अपनाउनु पर्नेछ ।

(६) उपदफा (१), (२), (३), (४) र (५) बमोजिम निरीक्षकले मलको नमुना लिँदा उत्पादक वा आयातकर्ता वा डीलर वा निजको प्रतिनिधि, स्थानीय तहको प्रतिनिधि र सम्बन्धित प्रहरी कार्यालयको प्रतिनिधिको रोहवरमा लिई मुचुल्का गर्नु पर्नेछ ।

(७) उपदफा (६) बमोजिमको प्रतिनिधिहरु मध्ये कुनै प्रतिनिधि उपस्थित नभएमा वा मुचुल्कामा सहीछाप नगरेमा सोही व्यहोरा खुलाइ मल निरीक्षकले प्रमाणित गर्नुपर्नेछ ।

(८) मल निरीक्षकले मलको नमुना लिँदा सम्बन्धित उत्पादक, आयातकर्ता वा डीलरले आवश्यक सहयोग गर्नुपर्नेछ ।

३६. मलको नमुना विश्लेषणको लागि पठाउने तरिका: (१) निरीक्षकले दफा ३६ बमोजिम लिएको सिलबन्दी मलको नमुना एक प्रति विश्लेषणको लागि आधिकारिक प्रयोगशालामा पठाउनु पर्नेछ ।

(२) उपदफा १ बमोजिम विश्लेषणको लागि आधिकारिक प्रयोगशालामा नमुना पठाउँदा मलको नमूनाको साथमा **अनुसूची २३** बमोजिमको औपचारिक पत्र संलग्न गर्नु पर्नेछ ।

(३) मल निरीक्षकले मलको नमूना विश्लेषणका लागि पठाउँदा र प्रयोगशालाले मल प्राप्त गरी विश्लेषण गर्दा देहायबमोजिम गर्नु पर्नेछ ।

क. नमुना पठाउने (निरीक्षक) र प्राप्त गर्ने (प्रयोगशालाको कर्मचारी) ले नमुना दर्ता किताब (Sample Register Book) मा नमुना कोड, मिति र समयसहित हस्ताक्षर गर्ने ।

- ख. नमुनाको प्रतिलाई बन्द र सीलबन्द बक्स वा प्याकेजमा राखेर मात्र पठाउने। यसले ढुवानीका क्रममा हुने क्षतिबाट बचाउँछ।
- ग. मल चिस्यानप्रति संवेदनशील हुने भएकाले, नमुनाको गुणस्तरमा हास आउन नदिन जतिसक्दो चाँडो (४८ घण्टाभित्र) प्रयोगशालामा पुऱ्याउने।
- घ. मलको प्रकार, सङ्कलनको कारण (नियमित अनुगमन वा गुनासो), र कुन मापदण्ड (Specification) अनुसार विश्लेषण गर्नुपर्ने हो, सो स्पष्ट उल्लेख गरी पठाउने।
- ङ. नमुना सङ्कलनको प्रक्रिया, लटको विवरण, रोहबरमा उपस्थित व्यक्तिहरूको विवरण र कुनै असामान्य परिस्थिति भएमा त्यसको उल्लेख गरीएको रिपोर्ट पठाउने।
- च. निरीक्षकले नमुना कानुनी विधिअनुसार सङ्कलन गरी सीलबन्दी गरीएको हो भनी सहिछाप गरीएको।

३७. मलको विश्लेषण विधि: मलको प्रयोगशाला विश्लेषण विधि देहाय बमोजिम हुनेछ ।

- (१) रासायनिक मलको विश्लेषण विधि अनुसूची- १ को भाग ४ मा उल्लेख भए बमोजिम हुनेछ।
- (२) नानोफर्टिलाइजरको विश्लेषण विधि अनुसूची- २ को भाग ४ मा उल्लेख भए बमोजिम हुनेछ।
- (३) बायोस्टिमुलेन्टको विश्लेषण विधि अनुसूची- ३ को भाग ४ मा उल्लेख भए बमोजिम हुनेछ।
- (४) जैविक मलको विश्लेषण विधि अनुसूची ४ को भाग ४ मा उल्लेख भए बमोजिम हुनेछ ।
- (५) प्राङ्गारिक मलको विश्लेषण विधि अनुसूची ५ को भाग ४ मा उल्लेख भए बमोजिम हुनेछ ।

३८. गुणस्तरमा केही कैफियत देखिएको मल सम्बन्धी व्यवस्था: (१) यस आदेशमा अन्यत्र जुनसुकै कुरा लेखिएको भए तापनि सूचीकरण भैसकेको वा नेपाल भित्र आयात भैसकेको मलको यस आदेश बमोजिम निर्धारित स्पेसिफिकेशन बमोजिम भन्दा कम गुणस्तर भई केही कैफियत देखिन आएमा केन्द्रीय निकायले त्यस्तो मल सम्बन्धीत स्थानीय समिति वा प्रदेश समिति वा प्राविधिक समितिको परामर्शमा विक्री वितरण गर्न अनुमति दिन वा नष्ट गर्न आदेश दिन सक्नेछ।

(२) उपदफा (१) बमोजिम मल नष्ट गर्न आदेश दिएकोमा नष्ट गर्दा लाग्ने खर्च सम्बन्धित पैठारीकर्ता, उत्पादक वा डिलरले बेहोर्नुपर्नेछ ।

(३) उपदफा (१) बमोजिम विक्री वितरणको लागि अनुमित दिइएको कम गुणस्तरको मलको व्याग वा बोराको दुवैपट्टी स्पष्टसंग देखिने गरी मलको पोषक तत्वको मात्रा र प्रतिशत समेत उल्लेख गरी रातो अक्षरमा “नन्स्ट्याण्डर्ड” र क्रश चिन्ह (X) अंकित गरेको हुनुपर्नेछ।

(४) कम गुणस्तर रहेका मलको विक्री मूल्य प्राविधिक समितिको सिफारिसमा केन्द्रीय निकायले तोक्नुपर्नेछ ।

(५) उपदफा (४) मा जुनसुकै लेखिए तापनि नेपाल सरकारद्वारा अनुदानित रासायनिक मलको हकमा प्राविधिक समितिको सिफारिसमा निर्देशक समितिले तय गर्नुपर्नेछ ।

परिच्छेद-८

निकाय तथा समिति सम्बन्धी व्यवस्था

- (३९) केन्द्रीय निकाय सम्बन्धी व्यवस्था: (१) मलको उत्पादन, आयात, निर्यात, आपूर्ति, व्यवस्थापन, गुणस्तर नियमन सम्बन्धी कार्य गर्न मन्त्रालयले नेपाल कृषि सेवाको राजपत्रांकित प्रथम श्रेणीको अधिकृतको नेतृत्वमा कृषि विभाग मातहत एक मल गुणस्तर नियन्त्रण केन्द्रको स्थापना गर्नेछ।
- (४०) केन्द्रीय निकायको काम कर्तव्य र अधिकार: केन्द्रीय निकायको काम कर्तव्य र अधिकार देहाय बमोजिम हुनेछ ।

- (क) देशलाई आवश्यक पर्ने मलको वार्षिक परिमाण (Demand Projection) को अनुमान गरी प्रक्षेपण गर्ने ।
- (ख) अनुसूची १, २ र ३ बमोजिमका मल सूचीकरण, नवीकरण र खारेजी गर्ने ।
- (ग) मलको आयात अनुमतिपत्र जारी, नवीकरण र खारेजी गर्ने ।
- (घ) सूचीकृत मल र उत्पादनको विवरण अध्यावधिक गर्ने ।
- (ङ) मलको आयात, निर्यात, आपूर्ति, भण्डार, वितरण व्यवस्थापन, गुणस्तर नियमन सम्बन्धमा आवश्यक मापदण्ड, कार्यविधि तर्जुमाका लागि सिफारिस गर्ने ।
- (च) मलको उत्पादन, आयात, निर्यातमा स्वीकृत मापदण्डको कार्यान्वयन, गुणस्तर परीक्षण र नियमन गर्ने ।
- (छ) मल निरीक्षक नियुक्त गर्न सिफारिस गर्ने, र नियुक्त भएका मल निरीक्षकहरूको परिचालनका लागि प्रदेश मन्त्रालयसंग समन्वय गर्ने ।
- (ज) आदेश कार्यान्वयनका क्रममा मल निरीक्षकले संकलन गरि पठाएका मलको नमूनाको कम्तिमा ३ महिना सम्म सुरक्षित रूपमा भण्डारण र व्यवस्थापन गर्ने ।
- (झ) विवाद उत्पन्न भएको अवस्थामा मलको नमूनाको स्वतन्त्र विश्लेषण गराई परीक्षण प्रतिवेदन उपलब्ध गराउने ।
- (ञ) सर्भेयरको मान्यता र नवीकरणको लागि निवेदन संकलन गरि विभागमा पठाउने ।
- (ट) कच्चा पदार्थ आयातको लागि अनुमति जारी गर्ने ।
- (ठ) अनुसन्धान प्रयोजनको लागि मलको आयात अनुमती जारी गर्ने ।

- (ड) मल निर्यातको सिफारिस जारी गर्ने ।
- (ढ) गुणस्तरमा कैफियत देखिएको मल विक्री गर्न अनुमति वा नष्ट गर्न आदेश दिने ।
- (ण) मल उत्पादन, आयात, निर्यात, आपूर्ति, प्रयोग, वितरण व्यवस्थापन, गुणस्तर नियमन सम्बन्धमा मन्त्रालयलाई बार्षिक प्रतिवेदन तयार गरी पेश गर्ने ।
- (त) मल निरीक्षक परिचालनको लागि कार्यक्रम तथा बजेट व्यवस्थापन गर्ने ।
- (थ) आधिकारिक विश्लेषक र मल निरीक्षकको आधारभूत तालिम तथा पूर्ण: ताजकी तालिम संचालन गर्ने ।
- (द) निर्देशन समितिको सचिवालयको रूपमा कार्य गर्ने ।
- (ध) मन्त्रालयले तोके बमोजिमका अन्य कार्यहरू गर्ने ।

४१. निर्देशक समिति: (१) रासायनिक, प्राङ्गारिक तथा जैविक मल सम्बन्धि नियमन तथा व्यवस्थापन सम्बन्धमा नेपाल सरकारलाई राय सुझाव प्रदान गर्नकालागि राष्ट्रियस्तरको देहाय बमोजिमको एक निर्देशक समिति रहनेछ।

महानिर्देशक, विभाग	संयोजक
प्रमुख, नेपाल कृषि अनुसन्धान परिषद् अन्तर्गतको माटो विज्ञान अनुसन्धान सम्बन्धी कार्य गर्ने केन्द्र सदस्य	
उप महानिर्देशक, नापतौल तथा गुणस्तर विभाग	सदस्य
उप महानिर्देशक, बातावरण विभाग	सदस्य
सहसचिव, मल सम्बन्धी विषय गर्ने महाशाखा, मन्त्रालय	सदस्य
मन्त्रालयबाट मनोनित निजी क्षेत्रका व्यवसायीहरू २ जना (मलको उत्पादक र आयातकर्ता १/१ जना)	सदस्य
प्रमुख, केन्द्रीय निकाय,	सदस्य सचिव

- (२) समितिको बैठक समितिको संयोजकले तोकेको मिति, समय र स्थानमा आवश्यकता अनुसार बस्नेछ।
- (३) समितिको सम्पूर्ण सदस्य संख्याको पचास प्रतिशत भन्दा बढी सदस्य उपस्थित भएमा समितिको बैठकको लागि गणपूरक संख्या पुगेको मानिनेछ।
- (४) समितिको बैठकको अध्यक्षता समितिको संयोजकले गर्नेछ।
- (५) समितिको निर्णय समितिको सदस्य सचिवले प्रमाणित गरी अभिलेख राख्नु पर्नेछ।
- (६) समितिको बैठकमा आवश्यकता अनुसार सम्बन्धित विषयको विज्ञलाई आमन्त्रण गर्न सकिनेछ।
- (७) समितिको सचिवालयको काम केन्द्रीय निकायले गर्नेछ।
- (८) समितिको बैठक सञ्चालन सम्बन्धी अन्य कार्यविधि समिति आफैले निर्धारण गरे बमोजिम हुनेछ।

४२. निर्देशक समितिको काम, कर्तव्य र अधिकार: समितिको काम, कर्तव्य तथा अधिकार देहाय बमोजिम हुनेछ ।

- क. मलको आपूर्ति, वितरण र गुणस्तर व्यवस्थापन नियमित गर्नको लागि मन्त्रालयलाई राय सुझाव सिफारिस गर्ने ।
- ख. नेपालमा उत्पादन, आयात तथा बिक्री वितरण गरीने मलहरूको स्पेसिफिकेसन (Specification) निर्धारण वा थपघट गर्न सिफारिस गर्ने ।
- ग. मल आयात, वितरण र अनुगमन गर्ने विभिन्न सरोकारवाला निकायहरू (कृषि सामग्री कम्पनी, साल्ट ट्रेडिङ कर्पोरेसन, प्रदेश मन्त्रालयहरू निजी क्षेत्र) बीच समन्वय गर्ने र उनीहरूको कार्यप्रगति अनुगमन गर्ने ।
- घ. मलको उत्पादन, आयात, बिक्री वितरण, र मूल्य नियन्त्रण सम्बन्धमा मन्त्रालय लाई परामर्श दिने र थप आवश्यक कानुनी व्यवस्थाका लागि सिफारिस गर्ने ।
- ङ. आवश्यकता अनुसार यस आदेशको उद्देश्य कार्यान्वयन गर्नका लागि उप-समितिहरू गठन गर्ने र तिनीहरूको कार्यक्षेत्र निर्धारण गर्ने ।
- च. गुणस्तरमा कैफियतयुक्त नेपाल सरकारबाट अनुदानित रासायनिक मलको बिक्री मूल्य तय गर्ने ।
- छ. जगेडा (बफर स्टक) मलको व्यवस्थापनको लागि निकायलाई निर्देशन दिने ।

(४३) विभागको काम कर्तव्य र अधिकार: यस आदेश कार्यान्वयनमा विभागको काम कर्तव्य र अधिकार देहाय बमोजिम हुनेछ ।

- क. सभ्यताको मान्यताको प्रमाणपत्र दिने, नविकरण गर्ने र मान्यता रद्द गर्ने ।
- ख. मल निरीक्षक नियुक्तिको लागि मन्त्रालयलाई सिफारिस गर्ने ।
- ग. आधिकारिक मल विश्लेषकको नियुक्ति गर्ने ।
- घ. मल आयात, वितरण र अनुगमन गर्ने विभिन्न सरोकारवाला निकायहरू (कृषि सामग्री कम्पनी, साल्ट ट्रेडिङ कर्पोरेसन, प्रदेश मन्त्रालयहरू निजी क्षेत्र) बीच समन्वय गर्ने र उनीहरूको कार्यप्रगति अनुगमन गर्ने ।
- ङ. मल उत्पादनको अनुगमन नियमन गर्ने ।

४४. प्राविधिक समिति: (१) विभाग र मल निर्देशक समितिको कार्य सम्पादनमा सहयोग पुऱ्याउन देहाय बमोजिमको एक प्राविधिक समिति रहनेछः

प्रमुख , केन्द्रिय निकाय	संयोजक
प्रतिनिधी कृषि अनुसन्धान परिषद् अन्तर्गतको माटो विज्ञान अनुसन्धान सम्बन्धी कार्य गर्ने केन्द्र	सदस्य
विज्ञ प्रतिनिधि, मान्यताप्राप्त प्रयोगशाला	सदस्य
शाखा प्रमुख, मल सम्बन्धी विषय हेर्ने शाखा मन्त्रालय	सदस्य
शाखा प्रमुख, मल सम्बन्धी विषय हेर्ने शाखा विभाग	सदस्य
प्रतिनिधी बातावरण विभाग	सदस्य
प्रतिनिधि, मान्यताप्राप्त प्रयोगशाला (विभागबाट मनोनित १ जना)	सदस्य
शाखा प्रमुख, मल नियमन विषय हेर्ने शाखा, केन्द्रीय निकाय	सदस्य सचिव

- (२) प्राविधिक समितिको बैठक समितिको संयोजकले तोकेको मिति, समय र स्थानमा आवश्यकता अनुसार बस्नेछ ।
- (३) प्राविधिक समितिको सम्पूर्ण सदस्य संख्याको पचास प्रतिशत भन्दा बढी सदस्य उपस्थित भएमा समितिको बैठकको लागि गणपूरक संख्या पुगेको मानिनेछ ।
- (४) प्राविधिक समितिको बैठकको अध्यक्षता समितिको संयोजकले गर्नेछ ।
- (५) प्राविधिक समितिको निर्णय समितिको सदस्य सचिवले प्रमाणित गरी अभिलेख राख्नु पर्नेछ ।
- (६) प्राविधिक समितिको बैठकमा आवश्यकता अनुसार सम्बन्धित विषयको विज्ञलाई आमन्त्रण गर्न सकिनेछ ।
- (७) प्राविधिक समितिको सचिवालयको काम केन्द्रीय निकायको मल नियमन विषय हेर्ने शाखाले गर्नेछ ।
- (८) प्राविधिक समितिको बैठक सञ्चालन सम्बन्धी अन्य कार्यविधि समिति आफैले निर्धारण गरे बमोजिम हुनेछ ।

४५. प्राविधिक समितिको काम, कतवर्ष र अधिकार: (१) प्राविधिक समितिको काम, कतवर्ष र अधिकार देहायबमोजिम हुनेछ ।

- (क) नेपालमा उत्पादन ,आयात तथा बिक्री वितरण गरीने मलहरूको स्पेसिफिकेसन (Specification) र गुणस्तर मापदण्डहरू निर्धारण गर्न वा थपघट गर्न निर्देशक समितिलाई सिफारिस गर्ने ।
- (ख) सूचीकरण इन्कार सम्बन्धि उजुरमा छानविन गरि निर्णय गर्ने ।
- (ग) सूचीकृत मलको खारेजी सम्बन्धमा छानविन गरी केन्द्रीय निकायलाई लाई सिफारिस गर्ने ।
- (घ)सर्भेयरको मान्यता र नविकरणको लागि सिफारिस गर्ने ।
- (ङ)मलको गुणस्तर परीक्षण, भण्डारण, र प्राविधिक पक्षहरूको विश्लेषण गरी सुधारका लागि सिफारिस गर्ने ।
- (च)मलको गुणस्तर तथा वितरण प्रणालीको अनुगमन गरी आवश्यक सुझावहरू निर्देशन समितिलाई पेश गर्ने ।
- (छ) गुणस्तरमा कैफियत देखिएको मलको मूल्य सिफारिस गर्ने ।
- (ज)मल निर्देशक समितिले भने बमोजिमका अन्य कार्य गर्ने ।
- (झ)प्रदेश समिति र स्थानीय समितिसंग समन्वय गर्ने ।

४६. प्रादेशिक समिति: (१) प्रदेशस्तरमा मलको नियमन तथा व्यवस्थापनको लागि एक समिति रहनेछ ।

क) सचिव, प्रदेश सरकारको कृषि विषय हेर्ने मन्त्रालय	-संयोजक
ख) निर्देशक, कृषि विकास निर्देशनालय	-सदस्य
ग) प्रमुख वा प्रतिनिधी, प्रदेश स्थित आधिकारीक प्रयोगशाला	सदस्य
घ) मल उत्पादक प्रतिनिधि १ जना प्रदेश मन्त्रालयबाट मनोनित	सदस्य
ङ) प्रतिनिधि, माटो हेर्ने शाखा, प्रादेशिक कृषि अनुसन्धान निर्देशनालय	सदस्य
च) मल निरीक्षक १ जना प्रदेश मन्त्रालयबाट मनोनित	सदस्य
छ) प्रमुख, मल सम्बन्धी विषय हेर्ने महाशाखा, प्रदेश मन्त्रालय —	सदस्य सचिव

४७.प्रादेशिक समितिको काम, कतव्य र अधिकार:(१) प्रादेशिक समितिको काम, कतव्य र अधिकार देहायबमोजिम हुनेछ ।

- (क)आफ्नो क्षेत्रभित्र प्राङ्गरिक मल तथा जैविक मलको प्रबर्दन, अनुगमन तथा व्यवस्थापन गर्ने ।
- (ख)प्रदेश भित्र बिक्री वितरण गरीने मलको गुणस्तर तथा वितरण व्यवस्थाको अनुगमन, नियमन गर्ने ।
- (ग) प्राङ्गरिक मल तथा जैविक मल सूचीकरणको इन्कार सम्बन्धि उजुरमा छानविन गरि प्रदेश मन्त्रालय वा तोकेको निकायलाई सिफारिस गर्ने ।

- (घ) प्राङ्गारिक मल तथा जैविक मल सूचीकरणको खारेजी सिफारिस गर्ने ।
(ङ) प्रदेश भित्र मलको नियमित आपूर्ति, वितरण व्यवस्था तथा गुणस्तर व्यवस्थापन गर्ने सम्बन्धमा प्रदेश मन्त्रालयालाई राय, सुझाव उपलब्ध गराउने ।

४८. प्रदेश मन्त्रालय वा प्रदेश मन्त्रालयले तोकेको निकायको काम, कतव्य र अधिकार: (१) प्रदेश मन्त्रालय वा प्रदेश मन्त्रालयले तोकेको निकायको काम, कतव्य र अधिकार देहायबमोजिम हुनेछ ।

- (क) प्राङ्गारिक मल तथा जैविक मल सूचीकरण गर्ने ।
(ख) सूचीकरण गरेको मलको उत्पादनको अनुगमन तथा नियमन गर्ने ।
(ग) प्रदेश भित्र विक्री वितरण गरीने मलको गुणस्तर तथा वितरण व्यवस्थाको अनुगमन, नियमन गर्ने ।
(घ) प्राङ्गारिक मल तथा जैविक मल सूचीकरणको खारेजी गर्ने ।
(ङ) मल निरीक्षकलाई यस आदेश बमोजिम मलको गुणस्तर नियमनमा खटाउने र सोको बजेट व्यवस्था गर्ने ।
(च) मल निरीक्षकको परिचालनका लागि केन्द्रीय निकायसँग समन्वय गर्ने ।
(छ) यस आदेश बमोजिम मल तथा मलको गुणस्तर नियमन सम्बन्धि विवरण अध्यावधिक गरि केन्द्रीय निकायमा पठाउने ।

४९. स्थानीय मल नियमन तथा व्यवस्थापन समिति: (१) स्थानीयस्तरमा मलको नियमन तथा व्यवस्थापनको लागि देहायबमोजिमको स्थानीय मल नियमन तथा व्यवस्थापन समिति रहनेछ ।

- | | |
|---|-------------|
| (क) उपप्रमुख वा उपाध्यक्ष, स्थानीय पालिका | -संयोजक |
| (ख) प्रमुख कार्यकारी अधिकृत, स्थानीय पालिका | -सदस्य |
| (ग) प्रमुख, स्थानीय पालिकाको कृषि शाखा | -सदस्य-सचिव |

५०. स्थानीय मल नियमन तथा व्यवस्थापन समिति: (१) स्थानीय मल नियमन तथा व्यवस्थापन समितिको काम, कर्तव्य र अधिकार देहाय बमोजिम हुनेछ ।

- (क) मलको सूचीकरण इन्कार सम्बन्धि उजुरमा छानविन गरि निर्णय गर्ने ।
(ख) मल सूचीकरण खारेजीको लागि स्थानीय तहलाई सिफारिस गर्ने ।
(ग) सूचीकरणको विवरण त्रैमासिक रूपमा प्रदेश समितिलाई उपलब्ध गराउने ।
(घ) स्थानीय स्तरमा उत्पादन भई सूचीकृत मलको नियमित अनुगमन तथा नियमन गर्ने ।

- (ड) स्थानीय क्षेत्र भित्र विक्री वितरण हुने मलको गुणस्तर अनुगमन गर्ने र सो को प्रतिवेदन प्रदेश समितिलाई पठाउने ।
- (च) स्थानीय स्तरमा विक्रीवितरण हुने मलको गुणस्तर विषयमा कृषकहरुको गुनासो बारे छानविन गरि आवश्यकता अनुसार प्रदेश मन्त्रालय वा केन्द्रीय निकायमा पठाउने ।
- (छ) यस आदेश कार्यान्वयनका लागि गरिने अनुगमन निरिक्षण र गुणस्तर कायम गर्ने कार्यमा मल निरिक्षकलाई सहयोग गर्ने ।

५१.स्थानीय पालिकाको काम, कर्तव्य र अधिकार: (१) स्थानीय पालिकाको काम, कर्तव्य र अधिकार देहायबमोजिम हुनेछ ।

- (क) प्रांगारिक मलको सूचीकरण गर्ने ।
- (ख) प्रांगारिक मलको सूचीकरण खारेजी गर्ने ।
- (ग) स्थानीय स्तरमा मल विक्रेता दर्ता गर्ने ।
- (घ) मल सूचीकरण र उत्पादनको विवरण प्रदेश मन्त्रालयमा पठाउने ।
- (ड) स्थानीय स्तरमा उत्पादन भई सूचीकृत मलको नियमित अनुगमन तथा नियमन गर्ने ।
- (च) स्थानीय क्षेत्र भित्र विक्री वितरण हुने मलको गुणस्तर अनुगमन गर्ने र सो को छानविनका लागि मल निरिक्षक परिचालनको लागि प्रदेश मन्त्रालयसँग समन्वय गर्ने ।
- (छ) स्थानीय स्तरमा विक्रीवितरण हुने मलको गुणस्तर विषयमा कृषकहरुको गुनासो बारे छानविन गरि आवश्यकता अनुसार प्रदेश मन्त्रालय वा केन्द्रीय निकायमा पठाउने ।
- (ज) यस आदेश कार्यान्वयनका लागि गरिने अनुगमन निरिक्षण र गुणस्तर कायम गर्ने कार्यमा मल निरिक्षकलाई सहयोग गर्ने ।

परिच्छेद-९

विविध

५२.उत्पादक र आयातकर्ताले पालन गर्नुपर्ने सर्तहरु: (१) मल उत्पादकले निम्न अनुसारका सर्तहरु पालन गर्नुपर्नेछ ।

- (क) उत्पादकले आफ्नै गुणस्तर नियन्त्रण प्रयोगशाला स्थापना गरी आवश्यक परीक्षण उपकरण राख्नुपर्ने र योग्यता प्राप्त प्राविधिक कर्मचारी राख्नुपर्ने ।
- (ख) प्रत्येक लटमा उत्पादन भएको मलको स्पेशिफिकेशन अनुसारको गुणस्तर परीक्षण गर्ने र त्यसको सम्पूर्ण विवरण राख्नुपर्ने ।
- (ग) मल उत्पादन, भण्डारण र ढुवानीका लागि विस्तृत सुरक्षा प्रोटोकल तयार गरी लागू गर्नुपर्ने ।
- (घ) उद्योग परिसरमा आवश्यक सुरक्षा उपकरण र अनुगमन प्रणाली जडान गर्नुपर्ने ।

(ड) प्याकेजिडमा मलको कम्पोजिसन, प्रयोग विधि, मात्रा, उत्पादन मिति, म्याद सकिने मिति र अन्य आवश्यक जानकारी समावेश गरी लेबलिंग सम्बन्धी नियमहरू पालना गर्नुपर्ने।

(च) उद्योग स्थापना गर्नु पूर्व प्रचलित कानून बमोजिम वातावरणीय परीक्षण गराउनु पर्ने।

(२) आयातकर्ताले पालना गर्नुपर्ने सर्तहरू देहाय बमोजिम रहेको छ।

(क) उत्पादकले जारी गरेको स्पेसिफिकेशन बमोजिमको मलको गुणस्तर खुल्ने कागजात/प्रमाणपत्र अनिवार्य रूपमा लिएको हुनुपर्नेछ।

(ख) विदेशी मुलुकबाट मल चलान गर्दा लोड पोर्ट र भन्सार बिन्दुको अनलोड पोर्टमा मलको परिमाण, गुणस्तर, प्याकेजिड र लेबलिङ तोकिएबमोजिम छ वा छैन भनी मन्त्रालयबाट तोकिएको स्वतन्त्र सर्भेयर बाट प्रमाणित गराएको प्रमाणपत्र लिएको हुनुपर्नेछ।

(ग) आयातित मललाई ओस, पानी, मिसावटबाट बचाउनका लागि उपयुक्त भण्डारणको व्यवस्था मिलाएको हुनुपर्नेछ। भण्डारण गर्दा उक्त मलमा भएका पोषक तत्वहरू नष्ट नहुने गरी वा लाभदायक जीवहरू ननासिने गरी वा रासायनिक स्वरूप परिवर्तन नहुने गरी राख्ने व्यवस्था गर्नु पर्नेछ।

(घ) आयातित मलको ब्याच नम्बरका आधारमा बिक्री वितरणको विस्तृत अभिलेख राख्नुपर्नेछ।

(ङ) आयातित मलको परिमाण, गुणस्तर प्रमाणपत्र, बिक्री वितरणको विवरण लगायतका सबै कागजातहरू सुरक्षित रूपमा राख्नुपर्नेछ।

५३. अनुगमन तथा निरीक्षण: (१) यस निर्देशिका बमोजिम मल उत्पादन, आपूर्ति तथा बिक्री वितरण कारोबारमा संलग्न सबै पक्षहरूले आफ्नो जिम्मेवारी अनुरूप कार्य गरे नगरेको सम्बन्धमा निदर्शक समिति, प्राविधिक समिति, र मन्त्रालय तथा कृषि विभागको कृषि उत्पादन समायो नियमन सम्बन्धी विषय हेर्ने शाखाले यस सम्बन्धि सम्पूर्ण क्रियाकलापको अनुगमन, निरीक्षण तथा मूल्याङ्कन गर्न वा गराउन सक्नेछ।

(२) प्रदेशस्तर समितिले आफ्नो क्षेत्र भित्र मलको परिमाण, गुणस्तर र आदेश पालनाको अनुगमन निरीक्षण गर्नेछ।

(३) स्थानीय समितिले आफ्नो क्षेत्र भित्र यस आदेश उत्पादन तथा बिक्री वितरण हुने प्राङ्गारिक मलको सम्बन्धमा अनुगमन तथा निरीक्षण गर्नेछ।

५४. मल विक्रेता दर्ता: (१) यस आदेश बमोजिमका मलहरू बिक्री वितरण गर्न चाहाने विक्रेताहरू सम्बन्धित स्थानीय तहमा दर्ता हुने पर्नेछ।

(२) उपदफा (१) बमोजिम दर्ता भएका विक्रेताहरूको सूचि सम्बन्धित स्थानीय तहले अध्यावधिक गर्नु पर्ने छ र सोको विवरण प्रदेश समितिलाई उपलब्ध गराउनु पर्नेछ ।

(३) उपदफा (१) बमोजिम दर्ता भएका विक्रेताहरूले आफूले कारोबार गर्ने मलको मूल्यसूचि तथा उपदफा (१) बमोजिमको दर्ता प्रमाण पत्र सबैले देखे गरी राख्नु पर्दछ ।

५५. रासायनिक मलको लेवेलिङ्ग र प्याकेजिङ्ग सम्बन्धी व्यवस्था

(१) नेपालमा उत्पादन र आयत गरी विक्री वितरण गरीने मलको प्याकेजिङ्ग र लेवेलिङ्ग सम्बन्धी व्यवस्था अनुसूची- २४ मा उल्लेख भए बमोजिम हुनेछ ।

(२) मलको लेवलमा अनिवार्य रूपमा नेपाली भाषा प्रयोग गर्नु पर्नेछ ।

५६. निर्देशिका बनाउन सक्ने: मन्त्रालयले ऐन र यस आदेशको अधीनमा रही रासायनिक मल सम्बन्धी आवश्यक निर्देशिका बनाई लागु गर्न सक्नेछ र यस्ता निर्देशिकाको पालना गर्नु सम्बन्धित सबैको कर्तव्य हुनेछ ।

५७. अनुसूचीमा हेरफेर वा थपघट गर्न सक्ने: मन्त्रालयले अनुसूचीमा आवश्यकता अनुसार हेरफेर वा थपघट गर्न सक्नेछ ।

५८. खारेजी तथा बचाउ: रासायनिक मल (नियन्त्रण) आदेश, २०५५, प्राङ्गारिक तथा जिवाणु मल निर्देशिका, २०७८ खारेज गरीएको छ । उक्त आदेश एवं निर्देशिका अनुसार यस अघि भएका कार्यवाहीहरू यसै आदेश बमोजिम भएको मानिनेछ ।

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(a) STRAIGHT NITROGENOUS FERTILISERS

1. Ammonium Sulphate (21-0-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Crystallized	
1.2	Particle size: Minimum 90 percent of the material shall pass through 2 mm sieve and be retained on 0.25 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight, minimum	21.0
2.2	Ammoniacal nitrogen percent by weight, minimum	20.5
2.3	Sulphate Sulphur (as S) percent by weight, minimum	23.0
2.4	Free acidity (as H ₂ SO ₄) percent by weight, maximum (0.04 for material obtained from by-product ammonia and by-product gypsum)	0.025
2.5	Arsenic (as As ₂ O ₃) percent by weight, maximum	0.01
2.6	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. Ammonium Sulphate (21-0-0) Granular

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 5.0 mm sieve and be retained on 2 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight, minimum	21.0
2.2	Ammoniacal nitrogen percent by weight, minimum	20.5
2.3	Sulphate Sulphur (as S) percent by weight, minimum	23.0
2.4	Free acidity (as H ₂ SO ₄) percent by weight, maximum (0.04 for material obtained from by-product ammonia and by-product gypsum)	0.025
2.5	Arsenic (as AsP ₃) percent by weight, maximum	0.01
2.6	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

3. Urea (46-0-0) Prilled

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form Free flowing, granules, prilled	
1.2	Particle size: Minimum 80 percent of the material shall pass through 2.8 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight (on dry basis), minimum	46.0
2.2	Biuret percent by weight, maximum	1.5
2.3	Moisture percent by weight, maximum	1.0

3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	
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4. Urea (46-0-0) Granular

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing, granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 2 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight (on dry basis), minimum	46.0
2.2	Biuret percent by weight, maximum	1.5
2.3	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

5. Urea Briquettes (46-0-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: compacted, solid tablets, typically oblong, oblate, or pillow-shaped	
1.2	Particle size: Minimum 90 percent of the material shall pass through 5.7 mm sieve and be retained on 3.8 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight (on dry basis), minimum	46.0
2.2	Biuret percent by weight, maximum	1.5
2.4	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

6. Neem Coated Urea (46-0-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Crystallized or granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 2.8 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight (on dry basis), minimum	46.0
2.2	Biuret percent by weight, maximum	1.5
2.3	Neem oil content soluble in binary mixture of n-hexane-acetone (4:1), per cent by weight, minimum	0.035
2.4	*the total meliacin content in the oily residue, per cent by weight, minimum	1.0
2.5	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

*The total melacin content means consisting of at least three of the major meliacin namely, Azadirachtin 'A' and 'B', nimbin, slantin, 6-desacetyl nimbin and 3-desacetyl salanin.

7. Sulphur Coated Urea (37-0-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.8 mm sieve and be retained on 2 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight (on dry basis), minimum	37.0
2.2	Biuret percent by weight, maximum	1.5
2.3	Elemental Sulphur (as S) per cent by weight, minimum	17.0
2.4	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

8. Calcium Ammonium Nitrate (25-0-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total ammoniacal and nitrate nitrogen percent by weight, minimum	25.0
2.2	Ammoniacal nitrogen percent by weight, minimum	12.5
2.3	Calcium nitrate percent by weight, maximum	0.5
2.4	Moisture percent by weight, maximum	1.0
3		

9. Calcium Ammonium Nitrate (26-0-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total ammoniacal and nitrate nitrogen percent by weight, minimum	26.0
2.2	Ammoniacal nitrogen percent by weight, minimum	13.0
2.3	Calcium nitrate percent by weight, maximum	0.5
2.4	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

10. Urea Ammonium Nitrate (32-0-0) Liquid

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Liquid	

2 Chemical Composition	
2.1	Total nitrogen (Urea, Ammoniacal and Nitrate) per cent by weight, minimum 32.0
2.2	Urea nitrogen per cent weight, maximum 16.5
2.3	Ammoniacal nitrogen percent by weight, minimum 7.5
2.4	Nitrate nitrogen per cent by weight, minimum 7.5
2.4	Specific gravity (at 15 °C) 1.30-1.35
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

11. Calcium Cyanamide (19.5-0-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granulate	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight (on dry basis), minimum	19.5
2.2	Cyanamide nitrogen percent by weight (on dry basis), minimum	15.0
2.3	Nitrate nitrogen percent by weight (on dry basis), minimum	1.5
2.4	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

12. Calcium Nitrate (15.5-0-0) 100% water soluble

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: crystalline solid	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 2 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (Ammoniacal and nitrate form), percent by weight, minimum	15.5
2.2	Nitrate nitrogen as N, percent by weight, minimum	14.5
2.3	Water soluble Calcium (as Ca), percent by weight, minimum	18.5
2.4	Matter insoluble in water, percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

(b) STRAIGHT PHOSPHOROUS FERTILISERS

1. Single Superphosphate (16% P₂O₅ Powdered)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: in a loose, powdery form, the material should be free from excessive lumps and should not form hard cake on storage	
1.2	Particle size: less than 0.5 mm sieve	
2	Chemical Composition	
2.1	Available phosphorous, Neutral Ammonium Citrate soluble phosphates (as P ₂ O ₅) per cent by weight, minimum	16.0

2.2	Water soluble phosphorous (as P ₂ O ₅) per cent by weight, minimum	14.5
2.3	Sulphate Sulphur (as S) per cent by weight, minimum	11.0
2.4	Free phosphoric acid (as P ₂ O ₅) per cent by weight, maximum	4.0
2.5	Moisture percent by weight, maximum	12.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. Single Superphosphate (16% P₂O₅ Granulated)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: the material should be free from excessive lumps and shall not form hard cake on storage	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Available phosphorous, Neutral Ammonium Citrate soluble phosphates (as P ₂ O ₅) per cent by weight, minimum	16.0
2.2	Water soluble phosphorous (as P ₂ O ₅) per cent by weight, minimum	14.5
2.3	Sulphate Sulphur (as S) per cent by weight, minimum	11.0
2.4	Free phosphoric acid (as P ₂ O ₅) per cent by weight, maximum	4.0
2.5	Moisture percent by weight, maximum	5.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

3. Triple Superphosphate (46% P₂O₅)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: the material should be free from excessive lumps and shall not form hard cake on storage	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total phosphates (as P ₂ O ₅) percent by weight, minimum	46.0
2.2	Water soluble phosphates (as P ₂ O ₅) per cent by weight, minimum	42.5
2.3	Free phosphoric acid (as P ₂ O ₅) per cent by weight, maximum	3.0
2.4	Moisture percent by weight, maximum	12.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

4. Rock Phosphate (18% P₂O₅) (Powdered)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: powdered	
1.2	Particle size: Minimum 90 percent of the material shall pass through 0.25 mm sieve and be retained on 0.15 mm sieve.	
2	Chemical Composition	
2.1	Total phosphates (as P ₂ O ₅) percent by weight, minimum	18.0

- 3 Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

(c) STRAIGHT POTASSIUM FERTILISERS

1. Potassium Chloride (Muriate of Potash, 60% K₂O)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: the material should be free from excessive lumps and shall not form hard cake on storage	
1.2	Particle size: Minimum 65 percent of the material shall pass through 1.7 mm sieve and be retained on 0.25 mm sieve.	
2	Chemical Composition	
2.1	Water soluble potassium content (as K ₂ O) percent by weight, minimum	60.0
2.2	Sodium as NaCl percent by weight (on dry basis), maximum	3.5
2.3	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. Potassium Sulphate (50% K₂O)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 5.0 mm sieve and be retained on 1.6 mm sieve.	
2	Chemical Composition	
2.1	Water soluble potassium content (as K ₂ O) percent by weight, minimum	50.0
2.2	Sulphate Sulphur (as S) per cent by weight, minimum	17.5
2.3	Total chlorides (as Cl) per cent by weight, maximum	2.0
2.4	Sodium as NaCl percent by weight (on dry basis), maximum	2.0
2.5	Moisture percent by weight, maximum	2.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

3. Potassium Chloride (Muriate of Potash, 60% K₂O), Granular

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Water soluble potassium content (as K ₂ O) percent by weight, minimum	60.0
2.2	Sodium as NaCl percent by weight (on dry basis), maximum	3.5
2.3	Magnesium (as MgCl ₂) percent by weight, maximum	1.0

2.4 Moisture percent by weight, maximum 0.5

3 Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

4. Potassium Magnesium Sulphate (30% K₂O)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 5.0 mm sieve and be retained on 2 mm sieve.	
2	Chemical Composition	
2.1	Water soluble potassium content (as K ₂ O) percent by weight, minimum	30.0
2.2	Magnesium as MgO, per cent by weight, minimum	10.0
2.3	Sulphate Sulphur as S per cent by weight, minimum	17.0
2.4	Total Chlorides, per cent by weight, maximum	2.5
2.5	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

(d) STRAIGHT SULPHUR FERTILISERS

1. Sulphur (90% S), Powder

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: fine, powder, the material should be free from excessive lumps and shall not form hard cake on storage	
1.2	Particle size: Particle size that can be a few microns in diameter	
2	Chemical Composition	
2.1	Total elemental sulphur (as S) percent by weight, minimum	90.0
2.2	Moisture percent by weight, maximum	1.0
3	Others: The product may contain any inert filler material such as Bentonite etc. up to the maximum extent of 10 percent by weight Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. Sulphur (90% S), Granular

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total elemental sulphur (as S) percent by weight, minimum	90.0
2.2	Moisture percent by weight, maximum	0.5
3	Others: The product may contain any inert filler material such as Bentonite etc. up to the maximum extent of 10 percent by weight Certificate from an independent surveyor or a recognized Government laboratory	

showing there is no contamination with radioactive material

(e) N.P. COMPLEX FERTILISERS

1. Diammonium Phosphate (18-46-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	18.0
2.2	Ammoniacal nitrogen percent by weight, minimum	15.5
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	46.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	39.5
2.5	Moisture percent by weight, maximum	2.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. Ammonium Phosphate Sulphate (16-20-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Ammoniacal nitrogen percent by weight, minimum	16.0
2.2	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	20.0
2.3	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	17.0
2.4	Sulphate Sulphur (as S) per cent by weight, minimum	13.0
2.5	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

3. Ammonium Phosphate Sulphate (20-20-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	20.0
2.2	Ammoniacal nitrogen percent by weight, minimum	18.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	20.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	17.0
2.5	Sulphate Sulphur (as S) per cent by weight, minimum	13.0
2.6	N in the form of urea percent by weight, maximum	2.0
2.7	Moisture percent by weight, maximum	1.0

- 3 Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

4. Ammonium Phosphate Sulphate Nitrate (20-20-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and nitrate) percent by weight, minimum	20.0
2.2	Ammoniacal nitrogen percent by weight, minimum	18.0
2.3	Nitrate nitrogen percent by weight, maximum	2.0
2.4	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	20.0
2.5	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	17.0
2.6	Sulphate Sulphur (as S) per cent by weight, minimum	13.0
2.7	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

5. Nitro Phosphate (20-20-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and nitrate) percent by weight, minimum	20.0
2.2	Ammoniacal nitrogen percent by weight, minimum	10.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	20.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	12.0
2.5	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

6. Urea Ammonium Phosphate (28-28-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	28.0
2.2	Ammoniacal nitrogen percent by weight, minimum	9.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	28.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	24.0
2.5	Moisture percent by weight, maximum	1.5

- 3 Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

7. Mono Ammonium Phosphate (11-52-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal form) percent by weight, minimum	11.0
2.2	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	52.0
2.3	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	44.5
2.4	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

8. Ammonium Phosphate (14-28-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and Urea) percent by weight, minimum	14.0
2.2	Ammoniacal nitrogen percent by weight, minimum	8.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	28.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	24.0
2.5	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

9. NPK 13:33:0:15S

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	28.0
2.2	Ammoniacal nitrogen percent by weight, minimum	13.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	33.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	28.5
2.5	Total elemental plus sulphate sulphur (as S) by weight, minimum	15.0
2.5	Sulphate sulphur (as S) by weight, minimum	7.5
2.6	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

(f) N.P.K COMPLEX FERTILISERS

1. Nitrophosphate with Potash (15-15-15)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and Nitrate) percent by weight, minimum	15.0
2.2	Ammoniacal nitrogen percent by weight, minimum	7.5
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	15.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	4.0
2.5	Water soluble potassium (as K ₂ O) percent by weight, minimum	15.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. N.P.K (10-26-26)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and Nitrate) percent by weight, minimum	10.0
2.2	Ammoniacal nitrogen percent by weight, minimum	7.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	26.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	22.5
2.5	Water soluble potassium (as K ₂ O) percent by weight, minimum	26.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

3. N.P.K (12-32-16)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and Nitrate) percent by weight, minimum	12.0
2.2	Ammoniacal nitrogen percent by weight, minimum	9.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	32.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	27.5

2.5	Water soluble potassium (as K ₂ O) percent by weight, minimum	16.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

4. N.P.K (19-19-19)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) form percent by weight, minimum	19.0
2.2	Ammoniacal nitrogen percent by weight, minimum	5.5
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	19.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	16.5
2.5	Water soluble potassium (as K ₂ O) percent by weight, minimum	19.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

5. N.P.K (20-20-10)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and nitrate) form percent by weight, minimum	20.0
2.2	Neutral ammonium citrate soluble phosphate (as P ₂ O ₅) percent by weight, minimum	20.0
2.3	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	17.0
2.4	Water soluble potassium (as K ₂ O) percent by weight, minimum	10.0
2.5	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

6. N.P.K (15-15-15)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) form percent by weight,	15.0

	minimum	
2.2	Ammoniacal nitrogen percent by weight, minimum	12.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	15.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	13.0
2.5	Water soluble potassium (as K ₂ O) percent by weight, minimum	15.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

7. N.P.K (15-15-15:9S)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) form percent by weight, minimum	15.0
2.2	Ammoniacal nitrogen percent by weight, minimum	12.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	15.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	13.0
2.5	Water soluble potassium (as K ₂ O) percent by weight, minimum	15.0
2.6	Sulphate Sulphur (as S) percent by weight, minimum	9.0
2.7	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

8. Nitrophosphate with Potash (14:14:21)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and nitrate) form percent by weight, minimum	14.0
2.2	Ammoniacal nitrogen percent by weight, minimum	8.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	14.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	9.0
2.5	Water soluble potassium (as K ₂ O) percent by weight, minimum	21.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

9. UREA-Single Super Phosphate Complex Fertilizer

S.N.	Characteristics	Requirements
1	Physical condition	

1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight, minimum	5.0
2.2	Urea Nitrogen, Percent by weight, maximum	4.0
2.3	Available phosphorus (as P ₂ O ₅) percent by weight, minimum	15.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	12.5
2.5	Sulphate Sulphur (as S) percent by weight, minimum	10.0
2.6	Moisture percent by weight, maximum	3.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

(g) MICRONUTRIENTS FERTILISERS

1. Zinc Sulphate Heptahydrate (ZnSO₄·7H₂O)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing crystalline	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Zinc (as Zn) percent by weight, minimum	21.0
2.3	Sulphate Sulphur (as S) per cent by weight, minimum	10.0
2.4	pH (5% solution) not less than	4.0
2.5	Lead (as Pb) percent by weight, maximum	0.003
2.6	Cadmium (as Cd) percent by weight, maximum	0.0025
2.7	Arsenic (as Pb) percent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. Zinc Sulphate Monohydrate (ZnSO₄·H₂O)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Zinc (as Zn) per cent by weight, minimum	33.0
2.3	Sulphate Sulphur (as S) percent by weight, minimum	15.0
2.2	Iron (as Fe) per cent by weight, maximum	1.0
2.4	pH (5% solution) not less than	4.0
2.5	Lead (as Pb) percent by weight, maximum	0.003
2.6	Cadmium (as Cd) percent by weight, maximum	0.0025
2.7	Arsenic (as Pb) percent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

3. Manganese Sulphate

S.N.	Characteristics	Requirements
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1	Physical condition	
1.1	Description and form: Free flowing	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Manganese (as Mn) content percent by weight, minimum	30.5
2.3	Sulphate Sulphur (as S) percent by weight, minimum	17.0
2.4	pH (5% solution) not less than	4.0
2.5	Lead (as Pb) percent by weight, maximum	0.003
2.6	Cadmium (as Cd) percent by weight, maximum	0.0025
2.7	Arsenic (as As) percent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

4. Borax (Sodium Tetraborate) ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) for Soil Application

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Crystalline powder	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Boron (as B) content percent by weight, minimum	10.5
2.3	pH (3.8 % solution)	9.0-9.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) percent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

5. Copper Sulphate Pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing powder/granular	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Copper (as Cu) content percent by weight, minimum	24.0
2.3	pH (5 % solution) not less than	3.0
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) percent by weight, maximum	0.01
2.7	Sulphate Sulphur (as S) percent by weight, minimum	12.0
2.8	Soluble iron and aluminium compound (as Fe) percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

6. Ferrous Sulphate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)

S.N.	Characteristics	Requirements
1	Physical condition	

1.1	Description and form: Free flowing powder	
2	Chemical Composition	
2.1	Matter insoluble in water per cent by weight, maximum	1.0
2.2	Ferrous iron (as Fe) content percent by weight, minimum	19.0
2.3	Ferrous acid (as H ₂ SO ₄) content percent by weight, minimum	1.0
2.3	pH (5 % solution) not less than	3.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) percent by weight, maximum	0.01
2.7	Sulphate sulphur (as S) percent by weight, minimum	10.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

7. Ammonium Molybdate (NH₄)₆Mo₇O₂₄·4H₂O

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing crystalline powder	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Molybdenum (as Mo) content percent by weight, minimum	52.0
2.3	Lead (as Pb) per cent by weight, maximum	0.003
2.4	Cadmium (as Cd) per cent by weight, maximum	0.0025
2.5	Arsenic (as Ar) per cent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

8. Chelated Zinc as Zn-EDTA

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: free flowing crystalline or powder or tablet	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Zinc (as Zn) Content percent by weight, minimum in the form of Zn-EDTA	12.0
2.3	pH (5 % solution) not less than	6.0-6.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) percent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

9. Chelated Iron as Fe-EDTA

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: free flowing crystalline or powder	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0

2.2	Iron content (as Fe) percent by weight, minimum in the form of Fe-EDTA	12.0
2.3	pH (5 % solution) not less than	5.5-6.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) per cent by weight, maximum	0.0025
2.6	Arsenic (as As) per cent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

10. Zinc Sulphate Mono-hydrate (ZnSO₄.H₂O)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: free flowing powder	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Zinc Content (as Zn) percent by weight, minimum	33.0
2.3	Iron (as Fe) percent by weight, maximum	1.0
2.4	pH (5 % solution) not less than	4.0
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) percent by weight, maximum	0.01
2.7	Sulphate Sulphur (as S) percent by weight, minimum	15.0
2.8	Lead (as Pb) percent by weight, maximum	0.003
2.9	Copper (as Cu) percent by weight, maximum	0.1
2.10	Magnesium (as Mg) percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

11. Magnesium Sulphate

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing crystalline form	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Magnesium Content (as Mg) percent by weight, minimum	9.5
2.3	Sulphate Sulphur (as S) percent by weight, minimum	12.0
2.4	Cadmium (as Cd) percent by weight, maximum	0.0025
2.5	Arsenic (as As) percent by weight, maximum	0.01
2.6	Lead (as Pb) percent by weight, maximum	0.003
2.7	pH (5 % solution) not less than	5.0-8.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

12. Boric Acid (H₃BO₃)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Powder/Granular/Crystal	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0

2.2	Boron (as B) Content percent by weight, minimum	17.0
2.3	Lead (as Pb) percent by weight, maximum	0.003
2.4	Cadmium (as Cd) percent by weight, maximum	0.0025
2.5	Arsenic (as As) percent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

13. Zinc Oxide Suspension Concentrate (39.5% Zn)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Dense concentrate of liquid Zn	
2	Chemical Composition	
2.1	Zinc (as Zn) content percent by weight, minimum	39.5
2.2	pH (5 % solution) not less than	9.0+1
2.3	Lead (as Pb) percent by weight, maximum	0.003
2.4	Cadmium (as Cd) percent by weight, maximum	0.0025
2.5	Arsenic (as As) per cent by weight, maximum	0.001
2.6	Specific gravity (at 15 ^o C)	1.71-1.75
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

14. Anhydrous Borax (Na₂B₄O₇)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing	
2	Chemical Composition	
2.1	Matter insoluble in water percent by weight, maximum	1.0
2.2	Boron (as B) content percent by weight, minimum	20.5
2.3	Lead (as Pb) percent by weight, maximum	0.003
2.4	Cadmium (as Cd) percent by weight, maximum	0.0025
2.5	Arsenic (as As) per cent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

15. Chelated Calcium as Ca-EDTA

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing powder	
2	Chemical Composition	
2.1	Ca-EDTA (as Ca) content percent by weight, minimum	9.0
2.2	pH (5% solution)	6.0-7.0
2.3	Matter insoluble in water percent by weight, maximum	0.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) per cent by weight, maximum	0.01

3 Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

16. Chelated Magnesium as Mg-EDTA

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing powder	
2	Chemical Composition	
2.1	Mg-EDTA (as Mg) content percent by weight, minimum	5.0
2.2	pH (5% solution)	6.0-7.0
2.3	Matter insoluble in water percent by weight, maximum	0.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) per cent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

17. Chelated Copper as Cu-EDTA

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing powder	
2	Chemical Composition	
2.1	Cu-EDTA (as Cu) content percent by weight, minimum	12.0
2.2	pH (5% solution)	6.0-7.0
2.3	Matter insoluble in water percent by weight, maximum	0.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) per cent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

18. Chelated Manganese as Mn-EDTA

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing powder	
2	Chemical Composition	
2.1	Mn-EDTA (as Mn) content percent by weight, minimum	10.0
2.2	pH (5% solution)	5.6-6.5
2.3	Matter insoluble in water percent by weight, maximum	0.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) per cent by weight, maximum	0.01
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

(h) FORTIFIED FERTILISERS

1. Boronated Single Superphosphate (16% P₂O₅ Powdered)

S.N.	Characteristics	Requirements
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1	Physical condition	
1.1	Description and form: the material should be free from excessive lumps and should not form hard cake on storage	
2	Chemical Composition	
2.1	Available phosphorous, Neutral Ammonium Citrate soluble phosphates (as P ₂ O ₅) percent by weight, minimum	16.0
2.2	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	14.5
2.3	Sulphate Sulphur (as S) percent by weight, minimum	11.0
2.4	Free phosphoric acid (as P ₂ O ₅) percent by weight, maximum	4.0
2.5	Boron (as B) percent by weight	0.2-0.3
2.6	Moisture percent by weight, maximum	12.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. Boronated Single Super Phosphate (16% P₂O₅ Granular)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: the material should be free from excessive lumps and shall not form hard cake on storage, granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	16.0
2.2	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	14.5
2.3	Sulphate Sulphur (as S) percent by weight, minimum	11.0
2.4	Boron (as B) percent by weight, minimum	0.2-0.3
2.5	Free phosphoric acid (as P ₂ O ₅) per cent by weight, maximum	4.0
2.6	Moisture percent by weight, maximum	5.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

3. Zincated Urea

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granules	
1.2	Particle size: Minimum 80 percent of the material shall pass through 2.8 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (in Urea form) percent by weight, minimum	43.0
2.2	Biuret, percent by weight, maximum	1.5
2.3	Zinc (as Zn) percent by weight, minimum	2.0
2.5	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

4. Boronated NPK Complex (10:26:26)

S.N.	Characteristics	Requirements
1	Physical condition	

1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	10.0
2.2	Ammoniacal nitrogen percent by weight, minimum	7.0
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	26.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	22.5
2.6	Water soluble potassium (as K ₂ O) percent by weight, minimum	26.0
2.7	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

5. Boronated NPK Complex (12:32:16)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	12.0
2.2	Ammoniacal nitrogen percent by weight, minimum	9.0
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	32.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	27.5
2.6	Water soluble potassium (as K ₂ O) percent by weight, minimum	16.0
2.7	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

6. Boronated Diammonium Phosphate (18:46:0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Free flowing granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	18.0
2.2	Ammoniacal nitrogen percent by weight, minimum	15.5
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	46.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	41.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

7. Zincated Diammonium Phosphate (18:46:0)

S.N.	Characteristics	Requirements
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1		Physical condition
1.1	Description and form: Free flowing granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2		Chemical Composition
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	18.0
2.2	Ammoniacal nitrogen percent by weight, minimum	15.5
2.3	Zinc (as Zn) percent by weight, minimum	0.5
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	46.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	39.5
2.6	Moisture percent by weight, maximum	2.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

8. Zincated NPK Complex (10:26:26)

S.N.	Characteristics	Requirements
1		Physical condition
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2		Chemical Composition
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	10.0
2.2	Ammoniacal nitrogen percent by weight, minimum	7.0
2.3	Zinc (as Zn) percent by weight, minimum	0.5
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	26.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	22.5
2.6	Water soluble potassium (as K ₂ O) percent by weight, minimum	26.0
2.7	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

9. Zincated NPK Complex (12:32:16)

S.N.	Characteristics	Requirements
1		Physical condition
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2		Chemical Composition
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	12.0
2.2	Ammoniacal nitrogen percent by weight, minimum	9.0
2.3	Zinc (as Zn) percent by weight, minimum	0.5
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	32.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	27.5
2.6	Water soluble potassium (as K ₂ O) percent by weight, minimum	16.0
2.7	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

10. Boronated Nitrophosphate with Potash (15:15:15)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and nitrate) percent by weight, minimum	15.0
2.2	Ammoniacal nitrogen percent by weight, minimum	7.5
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	15.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	4.0
2.6	Water soluble potassium (as K ₂ O) percent by weight, minimum	15.0
2.7	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

11. Boronated Calcium Nitrate

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and nitrate) percent by weight, minimum	14.5
2.2	Nitrate nitrogen (as N) percent by weight, minimum	13.5
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Water soluble calcium (as Ca) percent by weight, minimum	17.0
2.5	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

12. Zincated SSP (16% P₂O₅)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	16.0
2.2	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	14.5
2.3	Sulphate Sulphur (as S) percent by weight, minimum	11.0
2.4	Zinc (as Zn) percent by weight, minimum	0.5
2.5	Free phosphoric acid (as P ₂ O ₅) per cent by weight, maximum	4.0
2.6	Moisture percent by weight, maximum	12.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

13. Bononated NP Complex (24:24:0)

S.N.	Characteristics	Requirements
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1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	18.0
2.2	Ammoniacal nitrogen percent by weight, minimum	13.5
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	24.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	20.5
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

14. Zincated Bentonite Sulphur

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Elemental Sulphur (as S) percent by weight, minimum	65.0
2.2	Zinc (as Zn) percent by weight, minimum	18.0
2.3	Moisture percent by weight, maximum	1.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

15. Zincated Urea Ammonium Phosphate (28-28-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	28.0
2.2	Ammoniacal nitrogen percent by weight, minimum	9.0
2.3	Zinc (as Zn) percent by weight, minimum	0.5
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	28.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	25.2
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

16. Zincated NPK (14-35-14)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	

2	Chemical Composition	
2.1	Ammoniacal nitrogen percent by weight, minimum	14.0
2.2	Zinc (as Zn) percent by weight, minimum	0.5
2.3	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	35.0
2.4	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	29.0
2.5	Water soluble potassium (as K ₂ O) percent by weight, minimum	14.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

17. Zincated Ammonium Phosphate Sulphate (20-20-0-13)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen percent by weight, minimum	20.0
2.2	Ammoniacal nitrogen percent by weight, minimum	18.0
2.2	Zinc (as Zn) percent by weight, minimum	0.5
2.3	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	20.0
2.4	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	17.0
2.5	Sulphate Sulphur (as S) percent by weight, minimum	13.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

18. NP fortified with Sulphur (24-24-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	24.0
2.2	Ammoniacal nitrogen percent by weight, minimum	7.5
2.3	Nitrogen in the form of Urea percent by weight, maximum	16.5
2.4	Zinc (as Zn) percent by weight, minimum	0.5
2.5	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	24.0
2.6	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	20.0
2.7	Total Sulphur (as S) percent by weight, minimum	8.0
2.8	Elemental Sulphur (as S) percent by weight, minimum	4.0
2.9	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

19. Boronated Ammonium Phosphate Sulphate (20:20:0:13)

S.N.	Characteristics	Requirements
1	Physical condition	

1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	20.0
2.2	Ammoniacal nitrogen percent by weight, minimum	18.0
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	20.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	17.0
2.6	Sulphate Sulphur (as S) percent by weight, minimum	13.0
2.7	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

20. Single Super Phosphate (SSP) fortified with Zinc and Boron (granular)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Boron (as B) percent by weight, minimum	0.2
2.2	Zinc (as Zn) percent by weight, minimum	0.5
2.3	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	16.0
2.4	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	14.5
2.5	Free phosphoric acid (as P ₂ O ₅) percent by weight, maximum	4.0
2.6	Sulphate Sulphur (as S) percent by weight, minimum	11.0
2.7	Moisture percent by weight, maximum	5.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

21. Boronated Sulphur (Granular)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: The product may contain inert filler material as Bentonite etc. up to the extent of 14 percent by weight, maximum	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total elemental Sulphur (as S) per cent by weight, minimum	80.0
2.2	Boron (as B) per cent by weight, minimum	1.2
2.3	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

22. Urea Ammonium Phosphate fortified with 13% Sulphur (20-20-0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	

- 1.2 Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.

2 Chemical Composition	
2.1	Total Nitrogen (ammoniacal and urea) per cent by weight, minimum 20.0
2.2	Elemental Sulphur (as S) per cent by weight, minimum 6.5
2.3	Sulphate Sulphur (as S) per cent, by weight, minimum 6.5
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum 20.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum 17.0
2.6	Moisture percent by weight, maximum 1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

23. Zincated Single Super Phosphate (SSP) (granular)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Zinc (as Zn) percent by weight, minimum	0.5
2.2	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	16.0
2.3	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	14.5
2.4	Free phosphoric acid (as P ₂ O ₅) percent by weight, maximum	4.0
2.5	Sulphate Sulphur (as S) percent by weight, minimum	11.0
2.6	Moisture percent by weight, maximum	5.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

24. Elemental Sulphur fortified with Zinc Oxide in microgranule form

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total elemental Sulphur (as S) per cent by weight, minimum	67.0
2.2	Zinc (as Zn) percent by weight, minimum	14.0
2.3	Moisture percent by weight, maximum	2.0
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

25. NPK fortified with Mg, S, Zn and B (8-21-21)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total Nitrogen (ammoniacal and urea) per cent by weight, minimum	8.0

2.2	Nitrogen in ammoniacal form percent by weight, minimum	5.5
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Zinc (as Zn) percent by weight, minimum	1.0
2.5	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	21.0
2.6	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	18.0
2.7	Water soluble potash (as K ₂ O) percent by weight, minimum	21.0
2.8	Magnesium (as Mg) percent by weight, minimum	1.2
2.9	Total Sulphur (as S) percent by weight, minimum	2.0
2.10	Moisture percent by weight, maximum	1.5

3 Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

26. Diammonium Phosphate fortified with 4% Sulphur (18:46:0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and urea) percent by weight, minimum	18.0
2.2	Ammoniacal nitrogen percent by weight, minimum	15.5
2.3	Total Sulphur (as S) per cent by weight, minimum	4.0
2.4	Elemental Sulphur (as S) percent by weight, minimum	3.0
2.5	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	46.0
2.6	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	41.0
2.7	Moisture percent by weight, maximum	2.5

3 Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

27. NPK fortified with Mg, S, Zn and B (9-24-24)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total Nitrogen (ammoniacal and urea) per cent by weight, minimum	9.0
2.2	Nitrogen in ammoniacal form percent by weight, minimum	6.3
2.3	Boron (as B) percent by weight, minimum	0.2-0.3
2.4	Zinc (as Zn) percent by weight, minimum	0.6
2.5	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	24.0
2.6	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	20.5
2.7	Water soluble potash (as K ₂ O) percent by weight, minimum	24.0
2.8	Magnesium (as Mg) percent by weight, minimum	0.3
2.9	Total Sulphur (as S) percent by weight, minimum	2.1
2.10	Moisture percent by weight, maximum	1.3

3 Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material

28. Boronated NP (28:28:0)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen (ammoniacal and nitrate) percent by weight, minimum	28.0
2.2	Ammoniacal nitrogen percent by weight, minimum	9.0
2.3	Boron (as B) percent by weight, minimum	0.2
2.4	Available phosphorous (as P ₂ O ₅) percent by weight, minimum	28.0
2.5	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	24.0
2.6	Moisture percent by weight, maximum	1.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

(i) 100% Water Soluble Complex Fertilizers

1. Potassium Nitrate (13-0-45)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: crystalline powder or granules.	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total Nitrogen (all in nitrate form) per cent by weight, minimum	13.0
2.2	Matter insoluble in water, percent by weight, maximum	1.5
2.3	Water soluble potash (as K ₂ O) percent by weight, minimum	45.0
2.4	Sodium (as Na) percent by weight, minimum	1.0
2.5	Total chloride (as Cl) percent by weight, minimum	1.5
2.6	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

2. Mono-Potassium Phosphate (0-52-34) (100% water soluble)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Crystalline or granules.	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	52.0
2.2	Water soluble potash (as K ₂ O) percent by weight, minimum	34.0
2.3	Sodium (as NaCl) percent by weight, minimum	0.5
2.4	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

3. Calcium Nitrate (13-0-45) (100% water soluble)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: crystalline or granules.	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total Nitrogen (ammoniacal and nitrate form) per cent by weight, minimum	15.5
2.2	Nitrate nitrogen as N, percent by weight, maximum	14.5
2.3	Matter insoluble in water, percent by weight, maximum	1.5
2.4	Water soluble calcium (as Ca) percent by weight, minimum	18.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

4. Potassium Nitrate Prilled (13-0-45) (100% water soluble)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Prilled	
1.2	Particle size: Minimum 80 percent of the material shall pass through 2.8 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total Nitrogen (all in nitrate form) per cent by weight, minimum	13.0
2.2	Matter insoluble in water, percent by weight, maximum	1.5
2.3	Water soluble potash (as K ₂ O) percent by weight, minimum	45.0
2.4	Sodium (as Na) percent by weight, minimum	1.0
2.5	Total chloride (as Cl) percent by weight, minimum	1.5
2.6	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

5. Potassium Magnesium Sulphate

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Available phosphorous (as K ₂ O) percent by weight, minimum	22.0
2.2	Sulphate Sulphur (as S) per cent by weight, minimum	20.0
2.3	Magnesium as MgO percent by weight, minimum	18.0
2.4	Sodium (as NaCl), per cent by weight, maximum	2.0
2.5	Total chloride (as Cl) percent by weight, maximum	2.5
2.6	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

6. Mono Ammonium Phosphate (12:61:0) (100% water soluble)

S.N.	Characteristics	Requirements
1	Physical condition	

1.1	Description and form: Free flowing granules	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Ammoniacal nitrogen percent by weight, minimum	12.0
2.2	Matter insoluble in water, percent by weight, maximum	0.5
2.3	Sodium as NaCl percent by weight, maximum	0.5
2.4	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	61.0
2.5	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

7. N.P.K (19-19-19) (100% water soluble)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: Granular	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Total nitrogen form percent by weight, minimum	19.0
2.2	Ammoniacal nitrogen percent by weight, minimum	4.5
2.3	Nitrate nitrogen percent by weight, minimum	4.0
2.4	Water soluble phosphorus (as P ₂ O ₅) percent by weight, minimum	19.0
2.5	Urea nitrogen percent by weight, maximum	10.5
2.6	Water soluble potassium (as K ₂ O) percent by weight, minimum	19.0
2.7	Sodium (as NaCl) percent by weight, maximum (on dry basis)	0.5
2.8	Matter insoluble in water percent by weight, maximum	0.5
2.9	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

8. Urea Phosphate 17:44:0 (100% Water Soluble)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: transparent crystals	
2	Chemical Composition	
2.1	Total Nitrogen (all in urea form) per cent by weight, minimum	17.0
2.2	Matter insoluble in water, percent by weight, maximum	0.5
2.3	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	44.0
2.4	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

9. NP (24-24-0) (100% Water Soluble Complex Fertilizer)

S.N.	Characteristics	Requirement
1	Physical condition	
1.1	Description and form: crystalline powder or in a prilled/granular form	
2	Chemical Composition	
2.1	Total nitrogen (Ammoniacal and Nitrate) percent by weight,	24.0

	minimum	
2.2	Nitrate Nitrogen percent by weight, minimum	10.0
2.3	Ammoniacal Nitrogen per cent by weight, minimum	14.0
2.3	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	24.0
2.4	Matter insoluble in water, percent by weight, maximum	0.5
2.5	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

10. Potassium Metaphosphate dimer (0-40-40)

S.N.	Characteristics	Requirements
1	Physical condition	
1.1	Description and form: free-flowing crystalline powder	
1.2	Particle size: Minimum 90 percent of the material shall pass through 4.0 mm sieve and be retained on 1 mm sieve.	
2	Chemical Composition	
2.1	Water soluble phosphorous (as P ₂ O ₅) percent by weight, minimum	40.0
2.2	Water soluble potash (as K ₂ O) percent by weight, minimum	40.0
2.3	Matter insoluble per cent by weight maximum	0.5
2.4	Lead (as Pb) percent by weight, maximum	0.003
2.5	Cadmium (as Cd) percent by weight, maximum	0.0025
2.6	Arsenic (as As) per cent by weight, maximum	0.01
2.7	Moisture percent by weight, maximum	0.5
3	Others: Certificate from an independent surveyor or a recognized Government laboratory showing there is no contamination with radioactive material	

(j) Chemical liquid fertilizer- mixed or sole

Category		Minimum Nutrient Content by weight in %	Other Requirements
A	Primary Nutrient (sole or mixture)	20	Lead < 0.003%, Arsenic < 0.01%, Cadmium <0.0025%
B	Primary Nutrients plus Secondary Nutrients	15+4	
C	Primary Nutrients plus Micronutrient	15+4	
D	Primary Nutrients plus secondary Nutrients plus Micronutrients	15+5+4	
E	Secondary Nutrients (Sole or mixture)	15	
F	Secondary Nutrients plus Micronutrient)	10+2	
G	Micronutrient (sole or mixtures)	10	

(k) Chemical solid (granular/powder/tablet) mixed fertilizer

Category		Minimum Nutrient Content by dry weight in %	Other Requirements
A	Primary Nutrients plus Secondary Nutrients plus Micronutrient	10+6+4	Moisture: % by weight (Maximum) : 12 Lead < 0.003%, Arsenic < 0.01%, Cadmium <0.0025%
B	Secondary Nutrients plus Micronutrients)	8+4	
C	Micronutrient (sole or mixtures)	10	

Note: Primary nutrients = N, P, K; Secondary nutrients = Ca, Mg, S; Micronutrients = Zn, B, Mn, Cu, **Fe**, Mo; N = Total nitrogen, percent by weight; P= Total phosphorus (as P₂O₅) percent by weight; K = Potash content (as K₂O) percent by weight; Ca= Calcium (as Ca) percent by weight; Mg = Magnesium (as Mg) percent by weight; S = Sulphur (as S) percent by weight; Zn = Zinc (as Zn) percent by weight; Mn = Manganese (as Mn) percent by weight; B = Boron (as B) percent by weight; Cu = Copper (as Cu) percent by weight; **Fe Ferrous ion (as Fe) percent by weight**; Mo = Molybdenum (as Mo) percent by weight

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TOLERANCE LIMIT IN PLANT NUTRIENT AND PHYSICAL PARAMETERS FOR VARIOUS FERTILISERS

S.N.	Fertilizers	Tolerance limit
1.	Straight Nitrogenous Fertilizers, Straight Phosphatic Fertilizers, Straight Potassic Fertilizers, Straight Sulphur fertilizers, NP Complex Fertilizers, NPK Complex Fertilizers, Micro Nutrients , Fortified Fertilizers, 100 per cent Water Soluble Mixture of Fertilizers	0.5 units for each nutrient
2.	For mixture of micro nutrients fertilizers	10 per cent of each nutrient or 0.5 unit whichever is less subject to maximum of 7.5 per cent of all the combined nutrients
3.	For Customized fertilizers and fortified fertilizer: Nutrient level % for primary nutrient (NPK) and secondary nutrient (S, Ca, Mg),	0.5 units for each nutrient
4.	For micronutrients	10 per cent of each nutrient or 0.5 unit whichever is less subject to maximum of 7.5 per cent of all the combined nutrients
5.	Particle size	7 units

6.	Moisture	0.3 units
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Note: The tolerance limit besides total nutrient shall also be applicable on the forms of nutrient subject to the condition that it shall not exceed the value of tolerance limit applicable on the total nutrient.

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PROCEDURE FOR DRAWAL OF SAMPLE OF CHEMICAL FERTILIZERS

PROCEDURE FOR DRAWAL OF SAMPLES OF FERTILISERS

1. General requirements of sampling

In drawing samples, the following measures and precautions should be observed: -

- (a) Samples shall not be taken at a place exposed to rain/sun;
- (b) The sampling instruments shall be clean and dry when used;
- (c) The material being sampled, the sampling instrument and the bags of samples should be free from any adventitious contaminations;
- (d) To draw a representative sample, the contents of each bag selected for sampling should be mixed as thoroughly as possible by suitable means;
- (e) (i) The sample shall be kept in suitable, clean dry and air tight glass or screwed hard polythene bottle of about 400 gm capacity or in a thick gauged polythene bag and this shall be put in a cloth bag which shall be sealed with the Inspector's seal. (ii) The sealed cloth bag containing the sample and **Form P** shall be kept in another cloth bag which shall also be sealed with the Inspector's seal. (iii) The identifiable details such as Sample number/Code number or any other details shall be put on the cloth bag containing sample and **Form P** which enables its identification;
- (f) Each sample bag should be sealed air tight after filling and marked with details of sample and type of fertilizer and the name of Inspector who has collected sample.

2. Sampling from bagged material

(i) Scale of sampling

- (a) Lot (for manufacturers/importers)

All bags in a single consignment of the material of the same grade and type drawn from a single batch of the manufacturer/importer shall constitute a lot. If a consignment is declared to consist of different batches of manufacture/import, all the bags of each batch shall constitute a separate lot.

In the case of a consignment drawn from a continuous process, 2000 bags (or 100 tonnes) of the material shall constitute a lot.

(b) Lot (for dealers)

The lot is an identifiable quantity of same grade and type of fertiliser stored at an identifiable place subject to a maximum limit of 100 tonnes.

The lot shall be identified by the inspector based on visible appearance of bags, their packing and storage conditions. The stock of less than 100 tonnes with a dealer may also constitute one or more lots, if the material (fertiliser) of different sources and brand is available in such quantities.

(c) Selection of bags for sampling

The number of bags to be chosen from a lot shall depend upon the size of the lot as given below:

Lot size (No. of bags) (N)	No. of bags to be selected for sampling (n)
Upto 10	1
11-100	2
101-200	3
201-400	4
401-600	5
601-800	6
801-1000	7
1001-1300	8
1301-1600	9
1601-2000	10

All the bags of a lot should be arranged in a systematic manner. Start counting from any bag randomly, go on counting as 1,2,3..... upto r and so on. r being equal to the integral of N/n . Thus every r th bag counted shall be withdrawn and all bags shall constitute the sample bags from where the sample is to be drawn for preparing a composite sample.

(ii) **Sampling from big godowns/high stackings**

If the procedure given in para 2 (i) (c) is not possible to be adopted, the sample should be drawn from the randomly selected fertiliser bags from different layers, from top and from all open sides in a zig zag fashion.

(iii) **Sampling from small godowns**

All the fertiliser bags of the same grade and type of each manufacturer though received on different dates shall be segregated and properly stacked. All bags of same grade and type of fertiliser manufactured by a particular manufacturing unit may be considered as one lot based on their physical conditions and the sample shall be drawn as per procedure laid down in para 2 (i)(c) and 4.

(iv) **Sampling from damaged stock**

(a) In case of torn or lumpy bags, damaged fertiliser bags or sweepings, the stock should be arranged according to identifiable lots. From each lot the number of bags shall be selected as per procedure; 2 (i) (c). If the bags allow the use of sampling probe conveniently, the samples should be drawn by sampling probe.

(b) In case it is not possible to use the sampling probe, the bags may be opened and fertiliser material mixed together uniformly by hammering the big lumps or putting pressure, if required, and then samples drawn by using suitable sample device.

3. Sampling probe

(i) An appropriate sampling instrument to be used by the Inspectors for collection of a representative sample is called sampling probe. The probe may comprise of a slotted single tube with solid cone tip made of stainless steel or brass. The length of the probe may be approximately 60 to 65 cms and the diameter of the tube may be approximately 1.5 cm and the slot width may be 1.2 to 1.3 cms. The probe may be used if the physical condition of the fertilisers and the packing material permits its use.

(ii) In case of High Density Polyethylene packings and also when the fertiliser material is not in free flowing condition, the use of sampling probe may not be possible. In such a case, selected bags for drawing samples may be opened and the fertilisers may be taken out of the bags and spread on a clean surface and samples drawn with the help of a suitable sampling device which may be made of stainless steel or brass cup.

4. Drawal of samples from bags

(i) Drawal of sample and preparation of composite samples.

Draw, with an appropriate sampling instrument, (sampling probe) small portions of the material from the selected bags as per procedure in para 2 (i) (b), 2 (ii), 2 (iii) and 2 (iv) (a). The sampling probe shall be inserted in the bag from one corner to another diagonally and when filled with fertiliser, the probe is withdrawn and fertiliser is emptied in a container/ or on polythene sheet/ or on a clean hard surface and made into one composite sample.

(ii) If the bags do not permit the use of sampling probe, empty the contents of the bags on a level, clean and hard surface and draw a composite sample by the process of quartering as described under para 3 (ii) or 5.

(iii) In case of chelated micro-nutrients and mixtures of micro-nutrients, the three identical containers of the batch, grade, type and manufacturer, shall be selected which shall constitute the composite samples, provided it is not possible to draw a composite sample of the size given under para 4A (iii).

4A. Weight of one sample

One sample of fertiliser shall have the approximate weight, as specified below: -

For straight micro-nutrient	100g
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fertilisers-	
For chelated micro-nutrient fertilisers and mixtures of micro-nutrients	50 g or the maximum packing size of similar quantity.
For other fertilisers and mixtures of fertilisers	400g
For 100% Water Soluble fertiliser and 100% water soluble Mixture of fertiliser	100g

5. Preparation of composite sample

If the composite sample collected from the different selected bags is larger than required weight, its size shall be reduced by method of quartering as detailed below: -

Spread the composite sample on a level, clean, hard surface, flatten it out and divide it into four equal parts. Remove any diagonally opposite parts. Mix the two remaining parts together to form a cone, flatten out the cone and repeat the operation of quartering till a composite sample of required weight is obtained.

6. Preparation of test sample and reference sample

(i) The composite sample obtained above shall be spread out on a clean, hard surface and divided into three approximately equal portions each of the weight as specified in **Para 4 A**. Each of these samples shall constitute the test sample.

(ii) Each test sample shall be immediately transferred to a suitable container as defined under para 1(e). The slip with detailed description may be put inside the sample bag. Each bag shall also be properly labelled as mentioned in para 1(f).

(iii) Each test sample container shall then be sealed with the seals of the inspector, if possible, seal of the manufacturer/ importer/ dealer or purchaser as the case may be, may also be affixed.

7. Sampling from the bulk fertilisers in ships, bulk carriers and bulk containers

(i) Sampling equipments

(a) Sampling cup

The sampling cup can be fabricated from non-corrosive metal. Inside dimensions of cup mouth may be 3/4" X 10" as per the diagram of the sampling cup.

(b) Sampling probe for bulk fertilisers

The sampling probe should be made of non-corrodable material such as stainless steel or brass. It may be slotted double tube with solid cone tip having a length of about 4 1/2' - 5' and diameter of about 1 1/4" to 1 1/2".

(c) Sampling cup or Scoop

The samples from the wharf or silo, as the case may be, can be collected by a sampling cup or a suitable scoop made of stainless steel or brass."

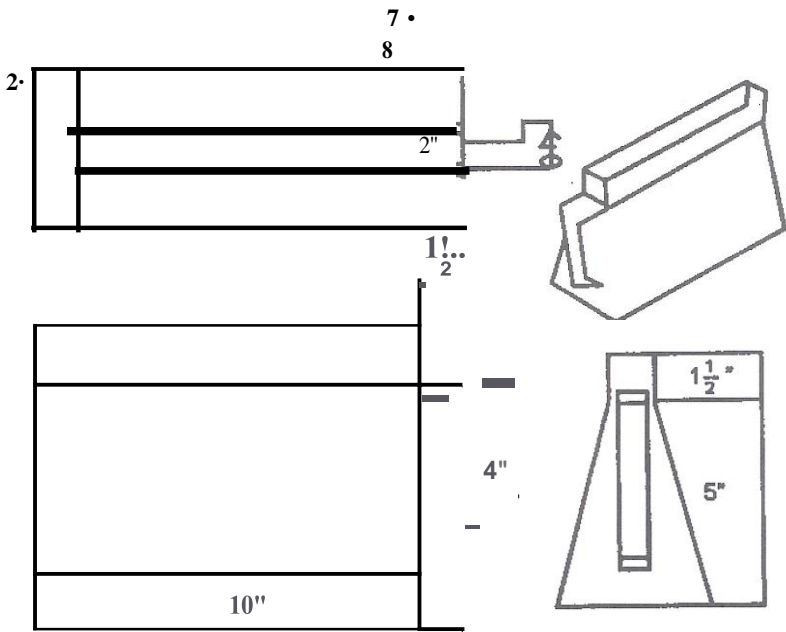


Figure 1 - Sampling cup

(ii) Sampling procedure

(a) Drawal of samples from the ship of bulk fertilisers

A. Sampling from conveyor belt

When the material is unloaded from the ships and transported to temporary/ storage godowns through conveyor belts, the sample can be drawn by passing the sampling cup through the entire stream of material as the material drops from a transfer belt or spout. The long slot in the top of sampling cup should be perpendicular to the falling stream. Pass the cup through the complete stream at a uniform speed, so that the cup will collect approximately equal amounts in each pass but will never overflow.

A minimum of 10 equally timed and speed stream must be taken during the transfer operation. However, the stream samples are not applicable unless uniform continuous flow of fertiliser is maintained for more than 3 minutes while lot is being sampled.

B. Sampling from ship

1. Draw the samples, from the fertilizer being discharged from the ship on the wharf or silo as the case may be, periodically at the regular interval of five hours on first and last day of discharge and three hours on intermediate days. All the samples drawn on a particular day shall be mixed together to make day composite sample. If the day composite sample is larger than required weight, its size shall be reduced by method of quartering as described in **Paragraph 5 of Schedule II of Part A**.

2. Then the ship composite sample shall be prepared by mixing part of each day composite. Further test sample and reference sample shall be prepared as given in **Paragraph 6 of Schedule II of Part A**.

(b) Drawal of samples from bulk carriers-trucks etc.

The sample can be drawn as per vertical probing procedure of Association of Official Analytical Chemists. The sampling probe should be about 4 1/2' to 5' length. Draw 10 vertical cuts from the following locations relative to the entire top of the conveyance. The 10 vertical cores are combined into a composite sample.

7	4	8	
3	1	2	5
I	I	I	
10	6	9	

(c) Drawal of samples from bulk material in storage

A. The bulk storage piles (level or flat) upto 100 tonnes could be sampled as per **Figure 2**.

2. Take 10 cores to the maximum possible depth of the probe from the position indicated in the **Figure 2** and all cores are composited.

B. A one sided or sloped pile may be sampled at the points illustrated in **Figure 3**.

3. Withdraw one vertical core of material from location 1 and 6 and two cores at locations 2, 3, 4 and 5. Composite all the probe samples and prepare the composite sample for analysis as per procedure laid down in para, 1, 5 and 6.

(d) Drawal of Samples from Containers

(1) Minimum 5 bags shall be selected randomly from each container.

(2) In case of large number of containers containing similar material, the

containers shall be selected randomly as per following criteria :-

Up to 3 containers	3	From each container	03
- 10 containers		containers	
More than 10 containers		05 containers	

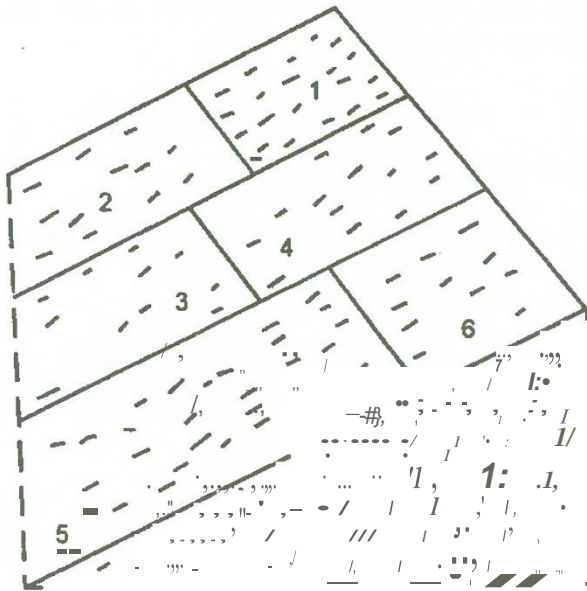
(3) The samples shall be drawn from the bags selected from each container as per the procedure given in Paragraph 4 of Schedule II of Part A. All the samples drawn from each bag of the selected container shall be mixed together to make container composite sample. If the container composite sample is larger than required weight, its size shall be reduced by method of quartering as described in Paragraph 5 of Schedule II of Part A.

(4) All the container composite samples shall be mixed together and test sample and reference sample shall be prepared as given in Paragraph 6 of Schedule II of Part A.

8. Method of sampling of anhydrous ammonia

(i) **Scope**

This method is for use in obtaining samples of anhydrous ammonia. The



method is based on the assumption that the material to be sampled is as claimed and contains only a small amount of impurity primarily water. It is recommended that duplicate samples be taken from each tank or vessel sampled.

(ii) Apparatus

- (a) Tubes for sampling tubes, heat resistant glass, conical centrifuge type 200 ml with lower 100 ml graduated from 0.2 ml in 0.05 ml divisions, 2-4 ml in 0.1 divisions, 4-10 ml in 0.5 ml divisions, and 10-100 ml in 1.0 ml divisions. Tube type is shown in **Figure 5**.
- (b) Stoppers for tubes, rubber, with bent tube vent as in **Figure 5**.
- (c) Sample carrier, constructed of plywood or aluminium as in **Figure 5**.
- (d) Sampling line and connection assembly constructed as in **Figure 4**, with flexible steel sampling nose 48" long, 1/4" NPT coupling at each end and 1/8" insulated steel tubing delivery tip at one end.
- (e) Sample tube adapter, constructed from rubber stopper and 6 mm D.D. glass or steel tubing as in **Figure 5**.
- (f) Protective equipment : Rubber or other non-porous gloves, offering

complete protection to the hands and lower arms, full coverage goggles or approved gas mask.

(iii) Reagents

Charcoal, reagent, 14-20 mesh.

Note: If the sample is expected to contain excessive amount of water (one per cent or more), one piece of the charcoal may be added to each tube before introduction of the sample.

(iv) Procedure

- (a) Place two dry, clean sampling tubes in the sample carrier.
- (b) Connect the sampling line connection assembly to the unloading valve of the tank, vessel, or line to be sampled.

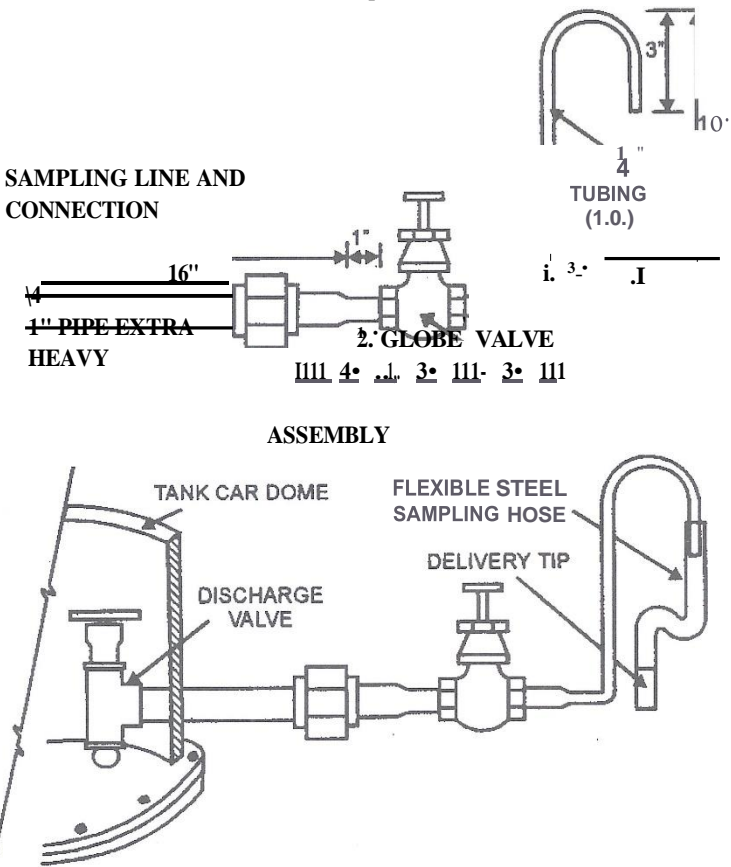


Figure4

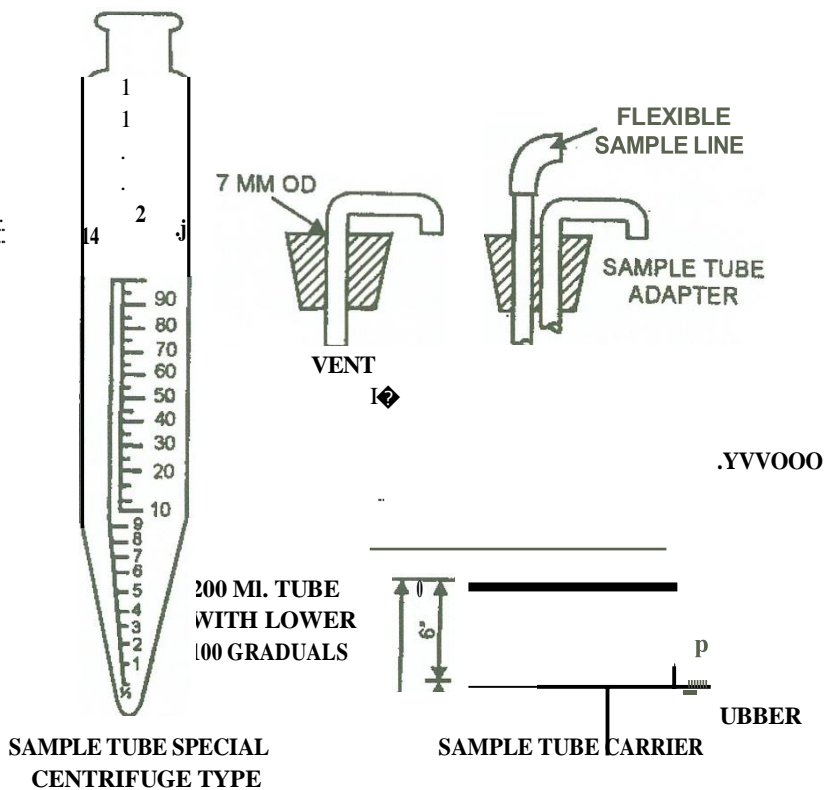


Figure 5

- (c) Open the valves slowly and purge the sampling line connection assembly thoroughly by venting 3 to 4 litres of ammonia. Close the sample line globe valve.
- (d) Remove the vented stoppers from the tubes and insert the adapter end of the sampling line connection assembly.
- (e) Open the sample line valve and slowly fill the sampling tube to the 100 ml mark, close the sample line valve.
- (f) Remove the sampling line adapter and insert the vented stopper in the sampling tube.
- (g) Repeat steps, d, e, and f and fill the second tube.
- (h) Close tank discharge valve and remove the sampling line connection assembly.
- (i) Either know or note the vessel, container, or line pressure of material sampled.

- (j) Tag the collected samples for identification and submit to the laboratory for processing by following methods in this section.

(v) **Precaution**

- (a) Liquid anhydrous ammonia causes severe burns on contact. It evaporates readily releasing the gas which may cause varying degrees of irritation of the skin and mucus membranes and may injure severely the respiratory mucosa with possible fatal outcome.
- (b) Avoid contacting liquid ammonia. In case of contact, immediately flush the affected parts with plenty of water for atleast 15 minutes. Get medical attention at once in case of burns, especially to the eyes, nose and throat, or if the victim is unconscious.
- (c) Ammonia gas in concentrations of 6,000 to 10,000 ppm (by volume) is lethal within a few minutes. Irritation of the eyes, respiratory tract and throat results from concentrations as low as 500 to 1,000 ppm; a concentration of 2,000 ppm produces convulsive coughing and may be fatal after a short exposure i.e. less than half an hour. The maximum concentration tolerated by the skin for more than few seconds is 2 per cent (i.e. when suitable respiratory protection is worn). The maximum allowable concentration for 8 hour working exposure is 50 ppm. This is the least detectable order.
- (d) Obtain medical attention if exposure to the gas produce distress of any type.
- (e) Rubber or other non-porous gloves, offering complete protection to the hands and lower arms must be worn when sampling anhydrous ammonia. Full coverage goggles must also be worn to protect the eyes unless an approved gas mask is used. The gas mask need only to be used if sampling can not be done without possible inhalation of the vapours.

9 Method of sampling of liquid fertilisers (other than anhydrous ammonia)

(i) **Scope**

This method is for use in mixed liquids (fluids), non-pressure nitrogen solutions and suspensions (slurries) (in absence of free-ammonia).

(ii) **Apparatus**

- (a) Glass or polyethylene bottles (capacity 500 ml).
- (b) Nylon cord.
- (c) Missouri type sampling bottles (**Figure SA**).

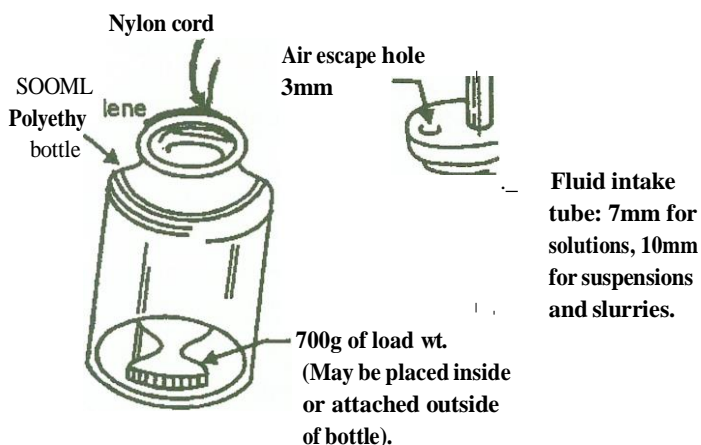


Figure 5A - Sampling bottle

Note : If the bottle is completely full, the sample may not represent the entire depth of the tank and would also be difficult to mix when returned to the laboratory.

(iii) Procedure

- (a) Mix fertiliser solution present in mixing vat, storage tank or delivery tank thoroughly.
- (b) Flush direct tap or delivery line and collect sample in a glass or polyethylene bottle.
- (c) Alternatively, lower the sample bottle by a nylon cord from the top opening of the storage tank to the bottom of the tank.
- (d) Raise slowly while filling at such a rate that the bottle is not quite full when withdrawn.
- (e) Transfer to sample containers and seal tightly.
- (f) Repeat process to obtain three such representative samples.

METHODS OF ANALYSIS OF CHEMICAL FERTILIZERS

METHODS OF ANALYSIS OF CHEMICAL FERTILISERS

1. Preparation of sample for analysis in the laboratory

(i) Procedure

- (a) Reduce gross sample to quantity sufficient for analysis or grind not < 250 g of reduced sample without previous sieving.
- (b) For fertiliser materials and moist fertiliser mixtures, that form a paste on putting pressure, grind in porcelain pestle and mortar to pass sieve with 1 mm sieve.
- (c) For dry mixtures that tend to segregate, grind in a porcelain pestle and mortar to pass through 0.425 mm sieve. In case of Potassium Magnesium Calcium Sulphate, the sample shall pass through 0.150 mm sieve to 0.180 mm sieve.
- (d) Grind as rapidly as possible to avoid loss or gain of moisture during operation.
- (e) Mix thoroughly and store in tightly stoppered bottles.

2. Determination of moisture

oven dry method

(Not applicable to samples that yield volatile substances other than water at drying temperature).

(i) Procedure

- (a) Weigh to the nearest mg about 2 g of the prepared sample in a weighed, clean, dry squat from weighing bottle.
- (b) Heat in an oven for about 5 hours at 99-101 °C to constant weight. Cool in a desiccator and weigh.
- (c) In case of sodium nitrate, ammonium sulphate and potassium salts heat to constant weight at 129-131°C, for urea heat at 70°C + 5°C for 5 hours.
- (d) Report percentage loss in weight as moisture at temperature used.

Calculations

$$\text{Free moisture per cent by weight} = \frac{100(B - C)}{B - A}$$

A = Weight of the bottle.

B = Weight of the bottle plus material before drying.

C = Weight of the bottle plus material after drying.

(Source-*Methods of Analysis*, AOAC. 1965).

(ii) Moisture in ammonium chloride, vacuum desiccator method - (Applicable to ammonium chloride, CAT, DAP, Potassium Schoenite all complexes and NPK mixtures)

- (a) Weigh accurately about 5 g of prepared sample in a weighed shallow porcelain dish and dry for 24 hours in a vacuum desiccator over phosphorus pentoxide and re-weigh.

Calculations

$$\text{Moisture percentage by weight} = 100 \times \frac{W_1}{W_3}$$

W_1 = loss in weight in g on drying, and

W_3 = weight in gm of the prepared sample taken for the test.

(iii) Karl Fischer Method

3. Determination of nitrogen

Methods of determination of total nitrogen, ammoniacal nitrogen, nitrate nitrogen, and urea nitrogen have been described separately in this section. These methods can be adopted both for straight and mixed fertilizers. Scope of each method with various combinations have also been described with each method.

The relevant methods of analysis which have been described are as given below:

- (a) Total nitrogen — for nitrate free samples.
- (b) Total nitrogen — for nitrate containing samples.
- (c) Total nitrogen — for materials with high Cl:NO₃ ratio and to materials containing only water-soluble nitrogen.
- (d) Determination of ammoniacal nitrogen.
- (e) Determination of ammoniacal and nitrate nitrogen.
- (f) Determination of nitrate nitrogen.
- (g) Determination of water insoluble nitrogen.
- (h) Determination of urea nitrogen.
- (i) Determination of cyanamide nitrogen.
- (i) Detection of nitrate

For adopting a specific method as described above, it is necessary to detect the presence of nitrates in the sample before a particular method is adopted. The procedure for detection of nitrates is given below: -

- (a) Mix 5 gm sample with 25 ml hot water and filter.
- (b) To one volume of this solution add 2 volume of sulphuric acid, free from HNO₃ and oxides of N, and let it cool.

- (c) Add a few drops of concentrated FeSO_4 solution in such a manner that fluids do not mix.
 - (d) If the nitrates are present junction shows at first purple, afterward brown, or if only minute quantity is present, reddish colour.
 - (e) To another portion of solution add 1 ml 1 per cent NaNO_3 and test as before to determine whether enough H_2SO_4 was added in the first test. (Reference-Methods of Analysis, AOAC, 1965)
- (ii) Reagents for determination of total nitrogen
- (a) Sulphuric acid — 93-98 per cent H_2SO_4 , N free.
 - (b) Copper sulphate — $\text{Cu SO}_4 \cdot 5\text{H}_2\text{O}$ reagent grade, N free.
 - (c) Potassium sulphate (or anhydrous sodium sulphate) reagent grade.
 - (d) Salicylic acid — reagent grade, N free.
 - (e) Sulphide or thiosulphate solution Dissolve 40 g commercial K_2S in 1 litre distilled water.
(Solution — of 40g Na_2S or 80 g $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$ in 1 litre may be used).
 - (f) Sodium hydroxide — Pellets or solution, nitrate free. For solution dissolve approximately 450 g solid NaOH in distilled water and dilute to 1 litre (Sp. gr. of solution should be 1.36 or higher).
 - (g) Zinc granule reagent grade.
 - (h) Zinc dust Impalpable powder.
 - (i) Methyl red indicator — Dissolve 1 g methyl red in 200 ml alcohol.
- (j) Hydrochloric or sulphuric acid standard solution — 0.5 N or 0.1 N when amount of N is small.
 - (k) Sodium hydroxide standard solution 0.1 N (or other specified concentration).
- (1) Standardize each standard solution with primary standard and check one against another.
 - (2) Test reagents before using, by blank determination with 2 g sugar which insures partial reduction of any nitrates present.

Caution. Use freshly opened sulphuric acid or add dry P_2O_5 to avoid hydrolysis of Nitriles and cyanates. Ratio of salt to acid (Wt: Vol) should be approximately 1:1 at end of digestion for proper temperature control. Digestion may be incomplete at lower ratio, N may be lost at higher ratio.

(iii) Apparatus

- (a) For digestion Use Kjeldahl flask of hard moderately thick, well annealed glass with total capacity approximately 500-800ml. Conduct digestion over heating device, adjusted to bring 250 ml water at 25°C to rolling boil in approximately for 5 minutes. Add 3-4 boiling chips to prevent superheating.
- (b) For distillation Use Kjeldahl or other suitable flask of 500-800ml capacity

fitted with rubber stopper through which passes lower end of efficient scrubber bulb or trap to prevent mechanical carryover of NaOH during distillation. Connect upper end of bulb tube to condenser tube by rubber tubing. Trap outlet of condenser in such a way as to insure complete absorption of ammonia distilled over into acid in receiver.

(iv) Total nitrogen (in nitrate free samples)

(a) *Procedure*

- (1) Weigh 1 g prepared sample and place in the kjeldahl flask (capacity 800 ml).
- (2) Add 0.7 g copper sulphate, 15 g K₂SO₄ or anhydrous Na₂SO₄ and 50 ml conc. ISO.
- (3) Place flask in inclined position and heat gently until frothing ceases (If necessary, add small amount of paraffin to reduce frothing).
- (4) Boil briskly until solution clear and then continue digestion for 30 minutes more.
 - (5) Remove from burner and cool, add 200 ml of water and swirl the flask to dissolve the contents.
 - (6) Transfer to 500 ml volumetric flask giving several washings with water to the digestion flask, cool and make up the volume to the mark.
 - (7) Take 25 ml aliquot in the distillation flask, add 300 ml of water and a pinch of zinc dust.
 - (8) Take 20 ml of standard acid solution in the receiving conical flask. Add 4-5 drops of methyl red indicator and keep the flask at the lower end of condenser in such a way that the lower tip of the condenser is fully immersed in acid solution.
 - (9) Add 30 ml of 45% NaOH to the distilling flask gently so that the contents don't mix.
 - (10) Immediately connect the distillation flask to the distillation assembly and swirl to mix the contents. Heat until all ammonia is distilled (at least 150 ml distillate). Test with red litmus paper, if any ammonia is still coming out.
 - (11) Remove receiving flask. Rinse outlet tube in to receiving flask with a small amount of distilled water.
 - (12) Titrate the contents in the receiver conical flask with N/10 NaOH.
 - (13) Determine blank on reagents using same quality of standard acid in receiver conical flask.

(b) *Calculations*

Where :-

$$1.401(V_1N_1 - V_2N_2) - (V_3N_1 - V_4N_2) \times df$$

Nitrogen per cent (by weight) =

W

- V₁ Volume (in ml) of standard acid taken in receiver flask for sample.
 V₂ Volume (in ml) of standard NaOH used in titrating standard acid in receiver flask after distillation.
 V₃ Volume (in ml) of standard acid taken in receiver flask for blank.
 V₄ Volume (in ml) of standard NaOH used in titrating standard acid in receiver flask after distillation in blank.
 N₁ Normality of standard acid. N₂ Normality of standard NaOH.
 W Weight (in gm) of sample taken.
 df Dilution factor of sample.

(v) *Total nitrogen for nitrate containing samples)*

(Not applicable to samples containing Cl:NO₃ ratio)

(a) *Procedure*

- (1) Place weighed sample (0.7-2.2g) in digestion flask.
- (2) Add 40 ml H₂SO containing 2 grams salicylic acid. Shake until thoroughly mixed and let stand, with occasional shaking, 1 hour or more.
- (3) Then add (i) 5 g Na₂S₂O₅.5H₂O or (ii) 2 g zinc dust (as impalpable powder not granulated zinc or filings).
- (4) Shake the flask and let it stand for five minutes then heat over low flame until frothing ceases.
- (5) Turn off heat, add 0.7 g copper sulphate, 159 powdered ISO, (or anhydrous Na₂SO₄), and boil briskly until solution clears, then at least 30 minutes longer (2 hours for samples containing organic material).

Proceed further as in 6-11 of 3 (iv).

Calculations

Same as in 3(iv)

(Reference *Methods of Analysis*, AOAC, 1965).

(va) Nitrogen (total) (applicable to all fertilisers by modified comprehensive

method)

(a) *Reagents*

- (a) Sulphuric acid — 93-98% H₂SO₄, N Free.
- (b) Potassium sulphate (or anhydrous sodium sulphate) Reagent grade, N Free.
- (c) Sodium hydroxide —45%.

- (d) Methyl red indicator — Dissolve 1 gm methyl red in 200 ml alcohol.
- (e) Standard hydrochloric acid or sulfuric acid solution — N/10. (f1 Sodium hydroxide standard solution — N/10.
- (g) Chromium metal- 100 mesh.
- (h) Alundum — Boiling stones, 8-14 mesh.
- (i) Copper sulphate pentahydrate — Reagent grade.

(b) *Procedure*

- (1) Take accurately 1.0g sample (or quantity containing not more than 60 gm nitrate nitrogen) in Kjeldahl flask. Add 1.2 g chromium powder and 35 ml water.
- (2) Let it stand for 30 minutes with occasional gentle swirling to dissolve all nitrate salts. Add 7 ml hydrochloric acid and let it stand for 30 minutes. Add 15 g K_2SO_4 , 0.7g $CuSO_4 \cdot 5 H_2O$ and 50 ml conc. H_2SO_4 , and proceed as given in method 3 iv a. 3 onward.
(Reference 2.4.05, AOAC 1995).

(vi) *Total nitrogen for materials with high Cl:NO₃ ratio and to materials containing only water soluble nitrogen).*

(a) *Reagents*

Reduced iron powder, electrically reduced, N.F. (National Formulary).

For other reagents see 3(ii).

(b) *Procedure*

Mixed fertilisers

- (1) Place 0.5 — 2.0g sample in Kjeldahl flask and add 2 — 5 g reduced Fe (5 g is enough for 0.185 g NO_3).
- (2) Add approximately 25 ml distilled water, rotating flask at angle to wash down the sample.
- (3) Let it stand for 15 minutes with occasional agitation, to insure complete solution of all soluble salts.
- (4) While rotating flask add 25 ml cold H_2SO_4 (1⁺1) and let it stand until visible reaction ceases, (Use hood or vented digestion unit).
- (5) Add boiling chips and boil 15-20 minutes, but do not take to dryness. (For samples containing organic matter, use 50ml cold H_2SO_4 (1+1), boil for 15-20 minutes, add 0.7g HgO and heat again for 40 minutes).
Cool and proceed further as in 6-11 of 3 (iv).

Calculations

Same as in 3 (iv)

(Reference — *Methods of Analysis*, AOAC 1965).

(vii) Determination of ammoniacal nitrogen

Dissolve 1 gram prepared sample in 250 ml water. Take 25 ml aliquot in 1 litre distillation flask, add approximately 300 ml of water. Add 10 ml of 45% NaOH for fertilisers which do not contain urea.

Add 2 g of freshly ignited carbonate free MgO for fertilisers containing urea.

- (1) Immediately connect the distillation flask to the distillation assembly and swirl to mix the contents. Heat until all ammonia is distilled (at least 150 ml distillate). Test with red litmus paper, if any ammonia is still coming out.
- (2) Remove receiving flask. Rinse outlet tube in to receiving flask with a small amount of distilled water.
- (3) Titrate the contents in the receiver conical flask with N/10 NaOH.
- (4) Determine blank on reagents using same quality of standard acid in receiver conical flask.

Calculations

Nitrogen per cent (by weight) = _____

Where :-

- V₁ Volume (in ml) of standard acid taken in receiver flask for sample.
- V₂ Volume (in ml) of standard NaOH used in titrating standard acid in receiver flask after distillation.
- V₁ Volume (in ml) of standard acid taken in receiver flask for blank.
- V₂ Volume (in ml) of standard NaOH used in titrating standard acid in receiver flask after distillation in blank.
- N₁ Normality of standard acid.
- N₂ Normality of standard NaOH. W
- Weight (in gm) of sample taken.
- df Dilution factor of sample.

(viii) Determination of ammoniacal and nitrate nitrogen (Devarda Method)

(This method is for the determination of total nitrogen when only nitrate or mixture of nitrate and ammoniacal nitrogen is present. This method assumes the absence of urea, calcium cyanamide and organic matter from the sample).

Procedure

- (1) Place 0.35-0.5 g sample in 600-700 ml flask and add 300 ml water, 3 grams Devarda Alloy and 5 ml NaOH solution (42 per cent by weight pouring later down side of flask so that it does not mix at once with contents).
- (2) Allow the flask to stand for 15 minutes.
- (3) By means of Davison [*J. Ind. Eng. Chem.* 11,465. (1919)] or other suitable scrubbing bulb, that will prevent passing over of any spray, connect with condenser, tip of which always extends beneath surface of standard acid in receiving flask.
- (4) Mix contents of distilling flask by rotating.
- (5) Heat slowly at first, and then at a rate to yield 250 ml distillate in 1 hour.
- (6) Collect distillate in measured quantity of standard acid and titrate with standard NaOH solution, using methyl red as an indicator.
- (7) In analysis of nitrate salts dissolve 3.5 or 5.0 g in water, to 250 ml and use 25 ml aliquot.

Calculations

Same as in 3 (iv).

(Reference— *Methods of Analysis*, AOAC, 1965)

(ix) Determination of nitrate nitrogen

Procedure

- (1) Determine total nitrogen as given in serial number 3 at item (v) relating to total nitrogen (for Nitrate containing samples), and item (vi) relating to Total Nitrogen (for material with high C:N O and material containing only water soluble nitrogen).
- (2) Weigh 0.5 g sample into Kjeldahl flask. Add 50 ml distilled water and add 2g Ferrous sulphate heptahydrate and swirl gently, add 20 ml H₂SO₄ and digest over hot flame until all water is evaporated and white fumes appear. Add 0.7 g of Copper Sulphate, 15 g Potassium Sulphate and 50 ml conc. H₂SO₄, and proceed as given in serial number 3 at item (iv) paragraph (a) 3 of this part onwards.

Calculations

Nitrate Nitrogen= Total N(1) — N Found in (2).

(Reference — AOAC 2.4.12—(1995).

(x) **Determination of water insoluble nitrogen**

Procedure

- (1) Place 1 or 1.4 g sample in 50 ml. beaker, wet with alcohol.

- (2) Add 20 ml. water and let it stand for 15 minutes stirring occasionally.
- (3) Transfer supernatant liquid to 11 cm. Whatman No.2 paper in 60°long stem funnel 60mm diameter and wash residue 4 or 5 times by decanting with water at room temperature (20-25° C).
- (4) Finally transfer all residue to filter and complete washing until filtrate measures 250 ml.
- (5) Determine N as in 3 (iv).

(xi) *Determination of urea nitrogen*

(This method is for the determination of urea content of any mixed fertilisers).

(a) *Reagents*

- (1) Neutral urease solution — shake 1 g jack boan meal with 100 ml water for 5 minutes. Transfer 10 ml solution to 250 ml Erlenmeyer flask, dilute with 50 ml water and add 4 drops methyl purple indicator. Titrate with 0.1 N HCl to reddish purple, then back titrate to green with 0.1 N NaOH. From difference in ml calculate amount of 0.1 N HCl required to neutralise remainder of solution (usually approximately 2.5 ml per 100 ml) add this amount of acid and shake well.
- (2) Verify enzyme activity of urease source periodically. Discard any source which does not produce solution capable of hydrolyzing 0.1g urea per 20ml.

(b) *Procedure*

- (1) Weigh **1040.01 g** sample and transfer to 15 cm Whatman No. 12 fluted filter paper.
- (2) Leach with approximately 300 ml water into 500 ml volumetric flask. Add 75
- (3) — 100 ml saturated barium hydroxide solution to precipitate phosphates. Let it settle and test for complete precipitation with few drops of saturated barium hydroxide solution.
- (4) Add 20 ml 10 per cent sodium carbonate solution to precipitate excess barium and any soluble calcium salts.
- (5) Let it settle and test for complete precipitation.
- (6) Dilute to volume, mix and filter through 15 cm Whatman No.12 fluted paper.
- (7) Transfer 50 ml aliquot (equivalent to 1 gm sample) to 200 or 250 ml Erlenmeyer flask and add 1 to 2 drops methyl purple indicator.
- (8)

- (9) Acidify solution with 2N HCl and add 2 to 3 drops excess.
- (10) Neutralise solution with 0.1 N NaOH to first change in colour indicator.
- (11) Add 20 ml neutral urease solution, close flask with rubber stopper and let it stand for 1 hour at 20-25°C.
- (12) Cool the flask in ice water slurry and titrate at once with 0.1 N HCl to full purple colour, then add approximately 5 ml excess.
- (13) Record total volume added, back titrate excess HCl with 0.1 N NaOH to neutral end point.

Calculations

$$\text{Percentage nitrogen} = \frac{(\text{ml } 0.1 \text{ N HCl} - \text{ml } 0.1 \text{ N NaOH}) \times 0.1401}{\text{Weight of sample}}$$

(Xi a) Determination of Cyanamide nitrogen;

Cyanamide nitrogen is precipitated as a silver complex and estimated in the precipitate by Kjeldahl's method.

(a) Reagents

- (i) Ammonical Silver Nitrate Solution- Mix 500 ml of 10% silver nitrate (AgNO₃) solution in water with 500 ml of 10% ammonia solution.
- (ii) Glacial acetic acid
- (iii) Remaining reagents as given in 3(ii)

(b) Procedure :-

- (i) Weigh 2.5 g (W) sample and place it in a small glass mortar.
- (ii) Grind the sample 3 times with water, pouring off the water after each grinding into a 500 ml volumetric flask.
- (iii) Transfer quantitatively the sample into 500 ml volumetric flask, washing the mortar, pestle and funnel with water
- iv) Make up volume to approximately 400 ml. Add 15 ml of glacial acetic acid.
- vii Shake on rotary shaker for 2 hours.
Make up the volume to 500 ml with water, mix and filter.

- (viii) Transfer 25 ml of filtrate into 250 ml beaker.
 - (IX) Add ammonia solution until slightly alkaline and add 20 ml of warm ammoniacal silver nitrate. Yellow precipitate will form.
 - (x) Leave over night. Filter using Whatman No.40 filter paper and wash the precipitate with cold water until it is completely free of ammonia.
 - (xi) Place the filter and precipitate in a Kjeldahl flask.
 - (xii) Add 0.7 g copper sulphate, 15 g Potassium sulphate and 30 ml of H₂SO₄.
 - (xiii) Place flask in inclined position and heat gently.
 - (xiv) Boil briskly until solution becomes clear or pale green.
 - (xv) Continue digestion for 30 minutes more.
 - (xvi) Remove from burner and cool.
 - (xvii) Transfer the contents of Kjeldahl flask to 1 litre capacity, distillation flask, make volume to about 350 ml with water and a pinch of zinc dust. Mix and cool.
 - (xviii) Distill ammonia by adding 10 ml of NaOH (40%) and collect the distillate in receiver conical flask containing 25 ml of 0.1 N HCl or H₂SO₄ containing 5 drops of Methyl red indicator.
 - (xix) Titrate the contents in receiver conical flask with 0.1 N NaOH & calculate the volume of 0.1 N HCl consumed (V).
 - (xx) Determine blank on reagents using same quantity of standard acid in receiver conical flask.
- (c) Calculation :

$$\text{Cyanamide Nitrogen \% (by weight)} = \frac{(\text{Blank}-V) \times 2.8}{w}$$

- (xii) Determination of biuret

(a) *Reagents*

- (1) Alkaline tartarate solution — Dissolve 40 g NaOH in 50 ml water cool add 50 gm of NaKC₂H₃O₆·4H₂O and dilute to 1 litre. Let it stand for one day before use.
- (2) Copper sulphate solution — Dissolve 15 g CuSO₄·5H₂O in CO₂ free water and dilute to 1 litre.
- (3) Biuret standard solution — 1 mg/ml. Dissolve 100 mg reagent grade biuret in CO₂ free water and dilute to 100 ml.

(b) *Preparation of standard curve*

- (1) Transfer series of aliquotes, 2 — 50 ml of standard biuret solution to 100 ml volumetric flask.
- (2) Adjust volume to approximately 50 ml with CO₂ free water. Add one drop of methyl red and neutralize with 0.1 N H₂SO₄ to pink colour.

- (3) Add with swirling 20 ml alkaline tartarate solution and then 20 ml CuSO₄ solution.
- (4) Dilute to volume Shake for 10 seconds and place in water bath for 15 minutes at 30 °C.
- (5) Also prepare reagent blank.
- (6) Determine absorbance of each solution against blank at 555 nm (instrument with 500-570 nm filter is also satisfactory) with 2-4 cm cell, and plot standard curve.

(c) *Procedure*

In urea

- (1) Stir continuously 2-5 g sample in 100 ml approximately 50°C water for 30 minutes.
- (2) Filter and wash into 250 ml volumetric flask and dilute to volume.
- (3) Transfer 25 ml aliquot to 100 ml volumetric flask and proceed as given under preparation of standard curve 3 (xii).

Calculations

From standard curve determine concentration of biuret in final dilution, then per cent of

$$\text{biuret} = \frac{C \times 100}{W}$$

Where, C= Concentration in mg/ml of biuret in final dilution obtained from standard curve.

W = Concentration of original sample in final dilution expressed

(*Reference— Methods of Analysis, AOAC, 1965*)

(xiii) Determination of free acidity in ammonium sulphate

(a) *Reagents*

- (1) Standard sodium hydroxide solution- 0.02 N.
- (2) Methyl red indicator- Dissolve 0.15 g of water soluble methyl red in 500 ml water.
- (3) Methyl red — Methylene blue mixed indicator solution — prepared by mixed equal volumes of 0.2 per cent solution in rectified spirit of methyl red and 0.1 per cent solution in rectified spirit of methylene blue.

(b) *Procedure*

- (1) Dissolve about 20 g of prepared sample, accurately weighed in about 50 ml cold natural water.
- (2) Filter and make up the volume to about 200 ml.

- (3) Titrate with standard sodium hydroxide solution, using one or two drops of methyl red as indicator.
- (4) If satisfactory end point with methyl red is not obtained, methyl red — methylene blue mixed indicator may be used.
- (5) Use preferably a micro biuret for this titration. The filtering medium shall be neutral and shall not contain any alkaline material which would neutralise free acid.

Calculations

$$\text{Free acidity as H}_2\text{SO}_4 \text{ per cent by weight} = \frac{4.904 AN}{W}$$

A = Volume of ml of standard NaOH solution. N

= Normality of standard NaOH solution.

W = Weight in gm of prepared sample taken for the test.
Determination of Arsenic in ammonium sulphate

(c) *Principle*

1. Arsenic V is reduced by KI to Arsenic III and the iodine thus released is reduced in turn by SnCl₂.
2. Hydrogen is liberated by the reduction of zinc with HCl in a nascent state. It also converts Arsenic V to Arsenic III. Acidity of the solution plays a key role in this case.
3. The Arsenic produced is swept out of the solution as Arsine by brisk evolution of hydrogen and it passes through a plug impregnated with lead acetate, which removes any hydrogen sulphide that may be liberated from the solution.
4. H₂S discolours the HgCl₂ paper.

As the concentration of liberated Arsenic as arsine increases, the yellow spot of (HgCl₂) turns to brown spot of (HgCl) and finally to black Hg₃As₂ with further increase in concentration of arsine.

(d) *Reagent*

- (1) Concentrated hydrochloric acid A.R. grade.
- (2) 0.5 M HCl-Mix. 20 ml conc. HCl with 420 ml water.
- (3) Potassium iodide solution — Dissolve 10 g KI in 400 ml water (2.5%).
- (4) 10 per cent stannous chloride solution — Dissolve 25 g SnCl₂.5H₂O in 250 ml conc. HCl.

- (5) 0.75 per cent stannous chloride solution — Mix 75 ml of the 10 per cent stannous chloride solution with 375 ml of concentrated hydrochloric acid and 550 ml of water. Do not keep this solution for a period of more than two days.
- (6) Ethyl alcohol- (Absolute) — 95 per cent.
- (7) Mercuric chloride solution — Dissolve 25 g HgCl_2 in 100 ml of Ethyl alcohol.
- (8) Filter paper — Whatman No.40, 9 cm diameter or comparable grade.
- (9) Mercuric chloride paper Allow 15 Filter paper to soak for 1 hr in alcoholic HgCl_2 solution. Leave it in the dark to dry, then cut into 1 cm square avoiding the edges of each circle. Store in an air tight and light proof container. Prepare freshly each week.
- (10) Lead acetate solution — Dissolve 15 g lead acetate tri hydrate in 100 ml glacial acetic acid.
- (11) Glass wool — Saturate glass wool with above lead acetate solution and allow it to dry. Pack loose plug of this into the tube of each Gutzeit apparatus. Replenish when this is discoloured along half of the length of the tube.

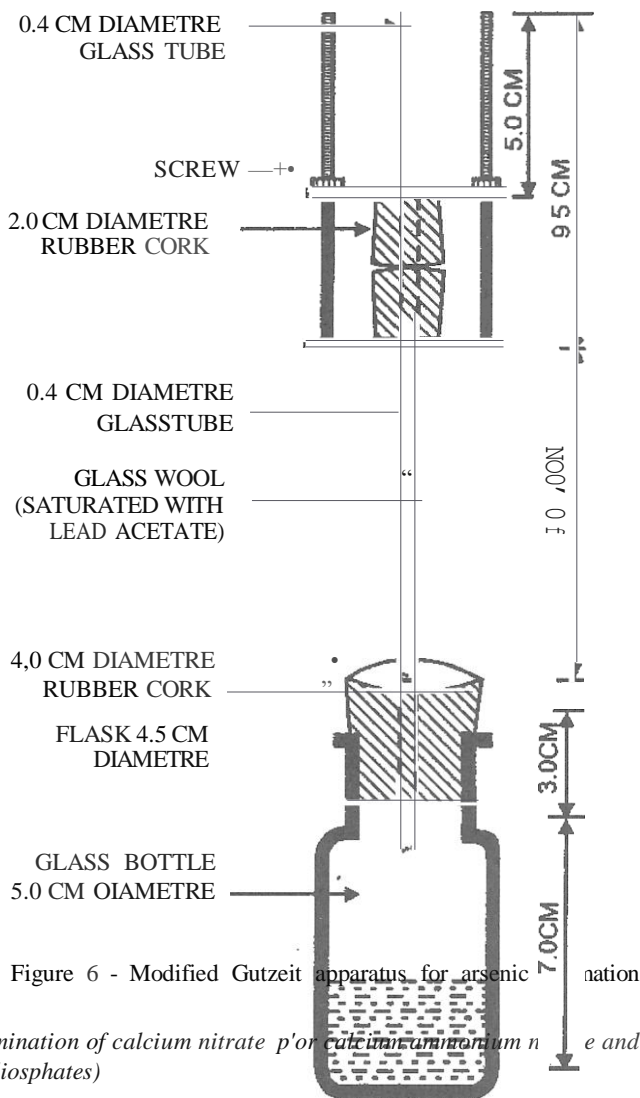
- (12) Zinc pellets — arsenic free zinc pellets AR grade.
- (13) Preparation of arsenic solution — Dissolve 0.416 g of sodium Arsenate ($\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$) in 1000 ml distilled water in volumetric flask after addition of 1 ml of concentrated hydrochloric acid. This is a 100 ppm arsenic solution which may be called standard A. Take 1 ml of standard A and dilute to 100 ml in a volumetric flask. This is a 1 ppm arsenic solution. Which may be called Standard B.
- (14) Preparation of working solution- Take 5 ml, 10 ml, 15 ml, 20 ml, and 25 ml solution of Standard B in respective Gutzeit bottle. The solution taken in Gutzeit bottles contain 5 ppm, 10 ppm, 20 ppm, 25 ppm of arsenic respectively as shown in **Figure 6** and proceed as shown in paragraph C below except as at serial number (i) indicated therein C.

(e) *Procedure*

- (i) Weigh minimum 10 g of fertiliser sample in a Gutzeit bottle.
- (ii) Add 120 ml of water and dissolve the material.
- (iii) **Add** 25 ml of 0.5 M HCl and shake.
- (iv) Add 3 ml potassium iodide solution and wait for 5 minutes.
- (v) Add 10 ml of 0.75% stannous chloride.
- (vi) Add 1-2 g zinc pellets and connect the Gutzeit bottle to the tube without delay, having previously placed a mercuric chloride paper in the head of the Arsenic apparatus.
- (vii) Leave for 30 minutes or till the reaction ceases, remove the mercuric chloride paper immediately, compare the confined spots with standard spot and ascertain the nearest concentration of Arsenic.
- (viii) **Carry** out blank analysis to ensure that reagents are free from Arsenic.

(f) *Calculations*

$$\text{Arsenic as } (\text{As}_2\text{O}_3)\% = \frac{\text{Conc. in ppm} \times 1.32}{10000 \times \text{Wt. of sample}}$$



(xiv) Determination of calcium nitrate or calcium ammonium nitrate and nitrophosphates)

Reagents

- (1) N-Amyl alcohol.
- (2) Dilute hydrochloric acid- approximately 4N.
- (3) Standard calcium solution — Weigh 1.0 g of calcium carbonate dried at $120^{\circ} + 5^{\circ}\text{C}$ and dissolve in the minimum quantity of dilute hydrochloric acid. Dilute the solution to 1 litre in a graduated flask.
- (4) Ammonium chloride — Ammonium hydroxide buffer solution. Dissolve 67.5 g ammonium chloride in a mixture of 570 ml of ammonium hydroxide

(sp. gr. 0.92) and 250 ml water. Also dissolve separately a mixture of 0.931 gm of disodium ethylene diamine tetra-acetate dihydrate and 0.616 g of magnesium sulphate ($Mg SO_4 \cdot 7H_2O$) in about 50 ml of water. Mix the two solutions and dilute to 1 litre.

- (5) Standard disodium ethylene diamine tetra-acetate (EDTA) solution —
- Weigh 3.72 g of disodium ethylene diamine tetraacetate dihydrate in water, and dilute in a graduated flask to 1 litre. The solution shall be standardised frequently against standard calcium solution following the procedure given below.
- (6) Eriochrome black-T indicator solution — Dissolve 0.1 g in 20 ml of rectified spirit. The solution shall be used for not more than a week.

(b) *Procedure*

- (1) Grind quickly about 5 g of the material, accurately weighed, with about 50 ml of amyl alcohol in a pestle and mortar and transfer the contents to a conical flask.
- (2) Wash the pestle and mortar with a few ml of amyl alcohol and add the washings to the flask.
- (3) Shake the contents of the flask manually or in a mechanical shaker for about half an hour and then filter.
- (4) Transfer the filtrate to a separating funnel and extract calcium nitrate completely with water in five to six instalments.
- (5) A few drops of dilute hydrochloric acid may be added during the extraction with water to avoid formation of an emulsion of amyl alcohol with water.
- (6) Concentrate the water extract at low temperature to nearly half its volume.
- (7) Transfer the concentrated solution to a conical flask, add 5 ml of ammonium chloride-ammonium hydroxide buffer solution, 5 drops of eriochrome black-T indicator solution and titrate against standard EDTA solution to a pure blue end point.

Calculations

$$\text{Calcium nitrate per cent by weight} = \frac{8.2 NV}{W}$$

Where N
V

W

Normality of
standard

EDTA

solution

Volume in

ml of

standard

EDTA

solution used

in the

titration,

and

Weight in g

of the

material

taken for

test.

(xv) Determination of chlorides other than ammonium chloride

(a) *Reagents*

- (1) Standard silver nitrate solution — 0.1 N.
- (2) Concentrated nitric acid — conforming to IS: 264-1950.
- (3) Ferric ammonium sulphate solution saturated in water and stabilized by addition of 50 ml nitric acid.
- (4) Standard ammonium thiocyanate solution — 0.1 N.

(b) *Procedure*

- (1) Dissolve about 0.2 g, of the prepared sample, previously dried as in procedure for determination of moisture and accurately weighed, in about 40 ml water.
- (2) Add exactly 50 ml of standard silver nitrate solution and 5 ml of concentrated nitric acid.
- (3) Add 0.5 ml of nitrobenzene and make up the volume of the mixture to exactly 100 ml with water.
- (4) Take exactly 50 ml of the solution and add 2 ml of ferric ammonium sulphate solution.
- (5) Titrate the excess of silver nitrate in this portion with standard ammonium thiocyanate solution.
- (6) Carry out a blank test following the procedure given as above but without using the material.

Calculations

$$\text{Total chlorides (as Cl) per cent by weight (on dry basis)} = \frac{7.094 (V_1 - V_2) N \dots X}{W}$$

Where

V_1 = Volume in ml of standard ammonium thiocyanate used in the blank determination.

V_2 = Volume in ml of standard ammonium thiocyanate solution used in the test with the material.

N = Normality of standard ammonium thiocyanate solution.

W = Weight in g of the dried prepared sample taken for the test.

Express the ammoniacal nitrogen content per cent by weight of the material determined earlier in terms of Cl as follows:

$$\text{Chloride equivalent of the ammoniacal nitrogen content, per cent by weight} = 2.531 \times A \dots \dots \dots Y$$

where A is the ammoniacal nitrogen content determined earlier. Balanced chloride equivalent to sodium chloride (NaCl) per cent by weight = $1.648 \times (X -$

$(X - Y)$ = Balanced chlorides other than ammonium chloride.

(xvi) Determination of oil in Neem Coated Urea

Principle : Oil present in Neem coated urea is extracted with *a binary mixture of n-hexane and acetone and separated from the aqueous phase. The solvent layer is then evaporated and the residue is weighed as oil.

Reagents :

- (i) *a binary mixture of n-hexane and acetone
- (ii) Dilute HCl : **Prepare** 1 : 1 HCl by adding 100 ml. conc. HCl to 100 ml distilled water.

(c) *Procedure*

- (i) Weigh accurately about 200 gm Neem coated urea and transfer it to a 1000 ml separating funnel.
- (ii) Add about 250 to 300 ml warm distilled water and shake gently to dissolve urea.
- (iii) Add 50 ml *a binary mixture of n-hexane and acetone and acidify with 5 ml dil. HCl. Mix well and separate the oily benzene layer.
- (iv) Repeat the extraction of oil from the aqueous layer with fresh 50 ml *a binary mixture of n-hexane and acetone and mix the oily a binary mixture of n-hexane and acetone layer with that obtained in step (iii).
- (v) Give washings to this oily *a binary mixture of n-hexane and acetone with warm distilled water till it is completely free from urea.
- (vi) Transfer the *a binary mixture of n-hexane and acetone layer to an oil free 100 ml beaker. Give 2-3 washings to the separating funnel with about 10 ml benzene each time to transfer completely the oil from the separating funnel to the beaker.
- (vii) Heat the contents in the beaker on a water bath to break any emulsion formed. Filter this oily layer into another previously weighed 100 ml beaker. Give two to three, 10 ml *a binary mixture of n-hexane and acetone washings to the filter paper to transfer the oil completely into the weighed beaker.
- (viii) Evaporate *a binary mixture of n-hexane and acetone by keeping beaker on a water bath at 105°C for about two hours. Cool in a desiccator and weigh the contents to a constant weight. Find out the weight of this oil in the beaker.
- (ix) Carry out a blank test with *a binary mixture of n-hexane and acetone (with equivalent amount consumed in the test) for oily content present, if any, and subtract the same from the test results.

Calculation

*Binary mixture of n-hexane and acetone (oil), ppm = $1000 \times (W_a - W_b) / W$

*Binary mixture of n-hexane and acetone soluble content (oil),

$$\% = (W - W_b) / 10W$$

Where W, is the weight of the oil in the beaker, in mg W, in the weight of oil (in mg) in the blank, and

W is the weight of the sample taken in gm.

*(xviii) Method of Analysis for determination of Meliacin of **Neem Oil in Neem Coated Urea by High Performance Liquid Chromatography**

1. Take 2 kg of urea sample in 5 ltr. capacity borosilicate glass beaker and add

approximately 1 litre. of a binary mixture of n hexane and acetone so as to immerse completely the 2.0 kg urea with n-hexane-acetone and keep it for 4-6 hours, preferably overnight with inermittent stirring the solution with a glass rod.

2. Filter the content of the beaker containing urea dissolve in the n-hexane-acetone through Whatman filter paper no 41 with repeated washings of n hexane and acetone to complete washing of neem oil content from the urea.
3. Evaporate the collected n hexane and acetone soluble Neem extract by vacuum distillation maintaining temperature below 50oC and reduce the quality of n-hexane-acetone to about 15 to 20 ml of Neem Oil extract, the temperature of lask should be maintained so that it should not be dried.
4. Transfer the volume ofn-hexane-acetone soluble extract from the distillation flask to the pre-weighed beaker of 100 ml capacity. Again evaporate the n hexane and acetone from the Neem Oil Extract at a controlled temperature on water bath.
5. Add 5 ml of Methanol : Water (90:10) mixture to the beaker consisting Neem Oil Extract covered with Aluminium foil to check the spillage during the sonicating.
6. Keep the beaker on sonicator and sonicate it for 10 minutes.
7. Transfer the content of beaker into Centrifugal tube and centrifuge it at 2500 rpm for 10 minutes.
8. Collect the upper layer and pass it through C-18 solid phase extraction Cartrige (Supelco or equivalent).
9. Now analyse the Neem Oil Extract derived from the Neem Coated Urea to determine the Azadirachtin, Nimbin and Salannin content by HPLC of following main configuration :

Column	C-18
Detector Temp	UV -Vis
Wave length	Ambient
Mobile Phase	215 rim
	Aceto nitrile: Water(isocratic or gradient flow)

10. Before analysis of sample standardize the **HPLC** using the good purity (>90%) Standard Reference Material of Azadirachtin (A & B) Nimbin, Salannin, 6-deacetyl Nimbin and 3-deacetyl Sallanin.
11. After calibration of the instrument, inject the 20 micro ltr. sample by Syringe or Loop into the HPLC. Wavelength 215 nm acetonitrile water
12. Identify the peak of Azadirachtin(A& B) Nimbin, Salannin, 6-deacetyl Nimbin and 3-deacetyl Sallanin with their retention time.
13. Measure the area of peak and calculate the content of Azadirachtin (A & B) Nimbin, Salannin, 6-deacetyl Nimbin and 3-deacetyl Sallanin with multiplying the dilution factor as per BIS-14299 1995.

Preparation of Reference standard :

Weigh accurately approx 2.0 mg standard reference material (Azadirachtin, Nimbin & Salannin) of known purity into 50 ml volumetric flask and dissolve in Methanol: Water (90:10). Make up to mark and shake well. Take 2 ml of this solution and transfer in solid phase extraction cartridge and elute it into 10 ml volumetric flask with repeated washing with Methnaol : Water (90:10). Make upto mark and use for calibration of HPLC.

Calculation:

- (1) % of Neem oil extracted with a binary mixture of n hexane and acetone :

$$\frac{W2 - W1 \times 100}{\text{Wt of sample (urea) in gm}}$$

Where, W2 = Weight of beaker containing benzene extracted neem oil

W1 = Weight of dry empty beaker

- (2) % Azadirachtin (A and B) in n hexane and acetone extracted Neem Oil:

$$\frac{\text{Test area of Aza} \times \text{Standard Wt} \times 2 \times \frac{5}{10} \times \text{Purity of standard Std. area}}{50 \text{ Wt. of Oil (W2-W1)}}$$

Azadirachtin (ppm) in binary mixture of n-hexane and acetone Neem oil: Azadirachtin % x 10000

- (3) % Nimbin in Benzene extracted Neem Oil:

$$\frac{\text{Test area of Nimbin} \times \text{Standard Wt} \times 2 \times \frac{5}{10} \times \text{Purity of standard Std. area}}{50 \text{ Wt. of Oil (W2-W1)}}$$

Nimbin (ppm) in n hexane and acetone extracted Neem oil: Nimbin % x 10000

- (4) % Salannin in binary mixture of n-hexane and acetone extracted Neem Oil:

$$\frac{\text{Test area of Salanin} \times \text{Standard Wt} \times 2 \times \frac{5}{10} \times \text{Purity of standard Std. area}}{50 \text{ Wt. of Oil (W2-W1)}}$$

Salannin (ppm) in n hexane and acetone extracted Neem oil: Azadirachtin % x 10000

- (5) 6-deacetyl Nimbin

$$\frac{\text{Test area of Aza} \times \text{Standard Wt} \times 2 \times \frac{5}{10}}{\text{Std. area 50 Wt. of Oil (W2-W1)}}$$

(6) 3-deacetyl Salannin

Test area of 3-

$$\frac{\text{deacetyl Salannin}}{\text{Std. area}} \times \text{Standard Wt} \times \frac{2}{50} \times \frac{5}{10} \times \text{Wt. of Oil} \times \text{Purity of standard}$$

(W2-
W1)

x Purity of standard

Azadirachtin (ppm) in binary mixture of n-hexane and acetone extracted Neem oil: Azadirachtin % x
10000

Total percentage of Meliacin = Total %age of Azadirachtin (A and B) + Nimbin+Salannin+6-deacetylNimbin+3-deacetyl Salannin=10000 ppm or 1% (minimum)

4. Determination of phosphates

Methods of determination of total phosphates, water soluble phosphorus, citrate soluble phosphates (available phosphorus), citrate insoluble phosphates and citric acid soluble phosphates have been described separately in this section. These methods are applicable to straight as well as mixed phosphatic fertilisers.

(i) Preparation of solution of the samples

(Separate methods of preparing solutions of the samples have been described according to the nature of the sample).

(a) Reagent

Magnesium nitrate solution - Dissolve 950 g P-free $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ in water and dilute to 1 litre.

(b) Procedure

- (1) Treat 1 gm sample by (A), (B), (C), (D) or (E) method depending on the nature of the sample.
- (2) Cool solution transfer to 200 to 250 ml volumetric flask, dilute to volume, mix and filter through dry filter.
 - (A) (Suitable for materials containing small quantities of organic matter). Dissolve in 30 ml HNO_3 and 3-5 ml HCl and boil until organic matter is destroyed.
 - (B) (Suitable for fertilisers containing much Fe or Al phosphate and basic slag). Dissolve in 15-30 ml HCl and 3-10 ml HNO_3 .
 - (C) (Suitable for organic material like cotton seed meal alone or in mixture). Evaporate with 5 ml of the $\text{Mg}(\text{NO}_3)_2$ solution, ignite and dissolve in HCl.
 - (D) (Generally applicable to materials or mixtures containing large quantities of organic matter). Boil with 20-30ml H_2SO_4 in 200 ml flask adding 2-4 gm of NaNO_3 or KNO_3 at beginning of digestion and small quantity after solution is nearly colourless or adding the nitrate in small portions from time to time. When solution is colourless, cool, add 150 ml water and boil for few minutes. Before adding NaNO_3 or KNO_3 let mixture digest, at gentle heat if necessary, until violence of reaction is over.
 - (E) (Suitable for all fertilisers). Boil gently for 30-45 minutes with 20-30 ml HNO_3 in a suitable flask (preferably a Kjeldahl for samples containing large quantities of organic matter to oxidize all easily oxidisable matter). Cool and add 10-20 ml of 70-72 per cent perchloric acid. Boil very gently until solution is colourless or nearly so and white dense fumes appear in flask. Do not boil to dryness at any time (danger). (With samples containing large quantities of organic matter

temperature should be raised to fuming point approximately 170°C, over a period of 1 hour at least). Cool slightly, add 50 ml water and boil for few minutes. (*Note* Paragraph (A) is applicable to all NPK complexes, NPK mixtures (with small amount of organic fertilisers) and paragraph B is applicable for Rock Phosphate.

(ii) *Gravimetric quinoline, molybdate method for determination of total phosphorus*

(a) *Reagents*

- (1) Citric molybdic acid reagent - Dissolve 54 g, 100 per cent molybdic anhydride (MoO₃) and 12g NaOH with stirring in 400 ml hot water and cool. Dissolve 60 gm citric acid in mixture of 140 ml HCl and 300ml water and cool. Gradually add molybdic solution to citric acid solution with stirring. Cool, filter and dilute to 1 litre. (Solution may be green or blue, colour depends on exposure to light). If necessary add 0.5 per cent KBrO₃ solution drop by drop until green colour becomes pale. Store in dark in polyethylene bottle.
- (2) Quinoline solution - Dissolve 50 ml synthetic quinoline with stirring in mixture of 60 ml HCl and 300 ml water. Cool dilute to 1 litre, and filter. Store in polyethylene bottle.
- (3) Quimociac reagent - Dissolve 70 g of sodium molybdate dihydrate in 150 ml water. Dissolve 60 g citric acid in mixture of 85 ml HNO₃ and 150 ml water and cool. Gradually add molybdate solution to citric acid-nitric acid mixture with stirring. Dissolve 5 ml synthetic quinoline in mixture of 35 ml HNO₃ and 100ml water. Gradually add this solution to molybdate citric-nitric acid solution mix and let it stand for 24 hours. Filter, add 280 ml acetone, dilute to 1 litre with water and mix well. Store in polyethylene bottle.

(b) *Procedure*

- (1) Treat 1 gm sample as prescribed in 4(l) and dilute it to 200 ml.
- (2) Pipette into 500 ml erlenmeyer flask, aliquot containing not more than 25 mg P₂O₅, dilute to approximately 100 ml with water. Proceed with one of the following methods.
 - A. Add 30 ml citric molybdic acid reagent and boil gently for 3 minutes. (Solution must be precipitate free at this stage). Remove from heat and swirl carefully. Immediately add from burette 10 ml quinoline solution with continuous swirling. (Add first 3-4 ml dropwise and remainder in steady stream) or
 - B. Add 50 ml quimociac reagent, cover with watch glass place on hot plate in well ventilated hood, and boil for 1 minute.

After treatment with A or B cool to room temperature, swirl carefully 3-4 time during cooling, filter into gooch with glass fibre filter paper or sintered glass Gooch Crucible Grade 4 (30 ml capacity), previously dried at 250°C and weighed, and wash five times with 25 ml portion of water. Dry crucible and contents for 30 minutes at 250°C. Cool in desicator to constant weight its (C,H N),H₃PO₄. 12 MoO₃, subtract weight reagent blank. Multiply by 0.03207 to obtain weight of P₂O₅.

Report as per cent



(iii) Determination of water soluble phosphorus

Procedure

- (1) Place 1 g sample on 9 cm filter paper and wash with small portion of water until filtrate measure approximately 250 ml.
- (2) Let each portion pass through filter before adding more and use suction if washing would not otherwise be complete within 1 hour.
- (3) If the filtrate is turbid, add 1-2 ml HNO_3 dilute to 250 ml and mix.
- (4) Pipette into 500ml erlenmeyer flask aliquot containing not more than 25mg P_2O_5 .
- (5) Dilute if necessary to 50 ml.
- (6) Add 10 ml HNO_3 (1 plus 1) and boil gently for 10 minutes. Cool and dilute to 100 ml and proceed as 4(ii)(b)(2)(B).

(Reference — Methods of Analysis, AOAC, 1965)

(iv) Citrate insoluble phosphorus

(a) Reagents

(1) Ammonium citrate solution — should have specific gravity of 1.09 at 20°C and pH of 7.0 as determined electrometrically.

Dissolve 370 g crystalline citric acid in 1.5 litre distilled water and nearly neutralize by adding 345 ml NH_4OH (28-29% NH_3). If concentration of ammonia is less than 28 per cent add correspondingly larger volume, and dissolve citric acid in correspondingly smaller volume of water, cool and check pH. Adjust with NH_4OH (1 plus 7) or citric acid solution to pH 7. Dilute solution if necessary to specific gravity of 1.09 at 20°C. (Volume will be approximately 2 litres). Keep in tightly stoppered bottles and check pH from time to time. If pH has changed from 7.0, readjust.

(2) Other reagents and solutions as in 4(i) and 4(ii).

(b) Procedure (acidulated samples and mixed fertilisers)

- (1) After removing water soluble P_2O_5 , in 4(iii) transfer filter and residue within 1 hour to 200 or 250 ml flask containing 100 ml ammonium citrate solution previously heated to 65°C
- (2) Close flask tightly with smooth rubber stopper.
- (3) Shake vigorously until paper is reduced to pulp and relieve pressure by removing stopper momentarily.
- (4) Continuously agitate contents of stopper flask in apparatus equipped to hold contents of flask at exactly 65°C. (Action of apparatus should be such that dispersion of sample in citrate solution is continually maintained and entire inner surface of flask and stopper is continually bathed with solution).

- (5) Exactly 1 hour after adding filter and residue, remove flask from apparatus, and immediately filter contents by suction as rapidly as possible through Whatman No.5 paper or equivalent, using buchner or ordinary funnel with platinum or other cone.
- (6) Wash with distilled water and at 65°C until volume of filtrate is approximately 350 ml, allowing time for thorough draining before adding more water.
- (7) If material is one that will yield cloudy filtrate, wash with 2 per cent NH₄NO₃ solution.
- (8) Determine P₂O₅ in citrate insoluble residue by one of the following methods:

A. Dry paper and contents, transfer to crucible, ignite until all organic matter is destroyed and digest with 10-15 ml HCl until all phosphate dissolve, or

- B. Treat wet filter and contents as in method prescribed in 4 (i) (b) (2) (A) (C), (D) or (E). Dilute solution to 250 ml or other suitable volume, mix well filter through dry paper and determine P₂O₅, as in 4 (ii).

(Reference — *Methods of Analysis*, AOAC, 1965)

- (v) Citrate soluble phosphorus (Available phosphorus)

(Reference S.O. 403 (E) dated 23 May, 1990)

Subtract citrate insoluble P₂O₅ from total P₂O₅ to obtain citrate soluble

'Z_SO₅'

(Reference — *Methods of Analysis*, AOAC, 1965)

- (vi) Citric acid soluble P₀₅ in fertilisers other than basic slag

(a) *Reagents*

- (1) Concentrated hydrochloric acid.
- (2) Concentrated citric acid.
- (3) Calcium carbonate finely ground.
- (4) 5 N sodium hydroxide solution.
- (5) Dilute hydrochloric acid — Dilute 240 ml of concentrated hydrochloric acid with water to 1 litre.
- (6) Citric molybdic acid solution — Stir 54 g of molybdic anhydride (MoO₃) with 200 ml of water, add 11 g of sodium hydroxide and stir the mixture whilst heating to boiling point until the molybdic anhydride dissolves. Dissolve 60 g of citric acid in about 250 to 300 ml of water and add 140 ml of concentrated hydrochloric acid. Pour the molybdate solution into the acid solution, which is stirred throughout the addition. Then cool and if necessary filter the solution through paper pulp pad. Dilute the solution to 1 litre. If the solution is slightly green or blue in colour add dropwise a dilute (1.5 to 1.0 per cent) solution of potassium bromate until the colour is discharged. This reagent should be kept in dark.

- (7) Quinoline solution — Measure 60 ml of concentrated hydrochloric acid and 300 to 400 ml of water into 1 litre beaker and warm to 70° - 80°C. Pour 50 ml of quinoline in a thin stream into the dilute acid while stirring. When quinoline has dissolved, cool the solution dilute to 1 litre, and if necessary filter through a paper pulp filter.
- (8) Sodium hydroxide solution — 0.5 N carbonate free.
- (9) Indicator solution— Mix three volumes of thymol blue solution and two volumes of phenolphthalein solution prepared as follows:
- A. Thymol blue solution - Dissolve 250 mg thymol blue in 5.5 ml of 0.1 N sodium hydroxide solution and 125 ml of industrial methylated spirit. Dilute with water to 250 ml.
 - B. Phenolphthalein solution - Dissolve 250 mg phenolphthalein in 150 ml of industrial methylated spirit and dilute with water to 250ml.
- (10) Hydrochloric acid — 0.5 N.
- (11) Sodium hydroxide solution — 0.1 N.
- (12) Hydrochloric acid — 0.1 N.
- (13) Surface active agent — 0.5 per cent solution of sodium dodecyl benzene sulphonate suitable.

(b) Preparation of solution

- (1) Weigh to the nearest mg about 5 gm of the sample and transfer to a stoppered bottle of about 1 litre capacity.
- (2) Dissolve 10 gm of pure crystallized citric acid (monohydrate) in water, dilute to 500 ml and adjust the temperature to 20°C.
- (3) Add the solution to the sample in the bottle, shaking so as to avoid the possibility of caking.
- (4) Shake the bottle continuously for 30 minutes.
- (5) Pour the whole of the liquid at once on to a large medium-fine filter and collect the filtrate. If the filtrate is not clear, pass it again through the same filter.

(c) Procedure

- (1) Transfer a volume of the solution prepared according to procedure given above containing less than 70 mg of phosphoric acid and preferably about 50 mg to a 500 ml stoppered conical flask marked at 150 ml.
 - (2) Dilute the solution with water to 100 ml.
 - (3) If the sample does not contain calcium add 100 to 200 mg of calcium carbonate.
 - (4) Then add 5 N sodium hydroxide solution dropwise until a faint permanent turbidity or precipitate is formed.
 - (5) Dissolve the precipitate by the dropwise addition of dilute hydrochloric acid, but avoid an excess.
 - (6) Dilute to 150 ml and add 50 ml of the citric-molybdic acid reagent, and heat the solution to incipient ebullition, maintain it at this temperature for 3 minutes and then bring it to the boiling point.
- (7) From burette slowly add 25 ml of the quinoline solution with constant swirling throughout the first few ml, being added dropwise, the rest in slow stream.

- (8) Keep the solution gently boiling during the addition.
- (9) Immerse the flask in boiling water for 5 minutes, then cool it to 15°C in running water.
- (10) Filter with suction the contents of the flask on a paper pulp pad, and wash the flask precipitate and filter with successive small washes of cold water until they are free from acid.
- (11) Transfer the filter pad and precipitate to the original flask, rinse the funnel with water and collect the rinsings in the flask.
- (12) If necessary, wipe the funnel with small piece of damp filter paper to ensure complete removal of the precipitate, and place the paper in the flask.
- (13) Add water to a total of about but not exceeding 100 ml. Stopper the flask and shake it vigorously until the pulp and precipitate are completely dispersed.
- (14) Remove the stopper and wash it with water, returning the washing of the flask.
- (15) Add a measured volume of 0.5 N sodium hydroxide solution sufficient to dissolve the precipitate and leave a few ml in excess.
- (16) Shake the flask vigorously until all the precipitate dissolves. (To facilitate the dispersal of the precipitate, after the addition of 0.5 N sodium hydroxide solution, a few drops of the surface active agent may be added if necessary).
- (17) Add 0.5-1.0 ml of the indicator solution, and titrate the excess of sodium hydroxide with 0.5 N hydrochloric acid, until the indicator changes from violet to green-blue, and then very sharply to yellow at the end point.
- (18) Deduct the number of ml of 0.5 N hydrochloric acid used from the number of ml 0.5 N sodium hydroxide, to ascertain the volume of 0.5 N sodium hydroxide equivalent to the phosphoric acid.
- (19) Carry out a blank determination on all the reagents, omitting only the sample, and using 0.1 N standard alkali and acid instead of 0.5 N for the titration. Calculate the blank in terms of 0.5 N alkali and subtract it from the original result.
- (20) Calculate the amount of phosphoric acid in the portion taken for analysis from the factor 1.0 ml of 0.5 N sodium hydroxide = 1.366 mg P₂O₅.

(Reference — Statutory Instruments 1960, No.1 165, Agriculture, the fertiliser and feeding stuffs Regulations, 1960).

(vii) Method for free phosphoric acid (as P O)

(a) Reagent

1. Acetone
2. Standard sodium hydroxide solution — 0.1 N
3. Bromo cresol green indicator solution — Dissolve 0.1 g bromocresol green in 100 ml of rectified spirit.

(b) Procedure

1. Weigh accurately about 2.5 g of the prepared sample in a 250 ml Erlenmeyer flask.

2. Add 100 ml neutral acetone. Fix to a wrist action shaker. Shake for one hour.
3. Filter rapidly through whatman filter paper No. 1 in to 250 ml erlenmeyer flask, wash with four time, 10 ml portion of acetone.
4. Evaporate acetone as far as possible.
5. Add about 50 ml water and drops of bromocresol green indicator.
6. Titrate with standard NaOH solution, until the colour changes from yellow to blue.

Calculations

$$\begin{array}{l} \text{Free phosphoric acid (as P}_2\text{O}_5\text{)} \\ \text{cent by weight} \end{array} = \frac{7.1 \times N \times V}{W}$$

Where :

N — Normality of standard NaOH solution. V — Volume of standard NaOH used.

W — Weight in g of sample taken for the test.

(Reference IS 6092 (Part -3) 1985)

(viii) Polyphosphate

(a) Outline of the method

The polyphosphate is first hydrolysed to orthophosphate by treating with concentrated nitric acid in hot condition. The polyphosphate content is determined as the difference between water soluble total phosphate and water soluble orthophosphate spectrophotometrically.

(b) Reagents

(1) Reagent 'A'

Dissolve 12.0 g of ammonium molybdate $(\text{NH}_4)_2\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 250 ml distilled water in a beaker. Separately, dissolve 0.2991 g antimony potassium tartarate in 250 ml distilled water in a beaker. Then add both solutions to 1000 ml of 5N sulphuric acid slowly in 2 litre volumetric flask, mix well and make up the volume. Store in pyrex glass bottle in dark and cool place.

(2) Reagent 'B'

Dissolve 1.056 g ascorbic acid in 200 ml of Reagent 'A' and mix well. (NOTE Reagent 'B' should be prepared fresh when required as it deteriorates after 24 hrs.)

- (3) Nitric acid
- (4) Sulphuric acid
- (5) Potassium dihydrogen orthophosphate
- (6) P-nitrophenol

(c) Preparation of sample solutions

(1) For determination of total phosphate

- (A) Weigh accurately about 1 g of the sample (W_1) in a beaker.
- (B) Add 30 ml of (1:1) HNO_3 , and boil gently for 15 minutes taking care that sample does not become dry.
- (C) Cool the solution and make up the volume to 1000 ml in a volumetric flask with distilled water.
- (D) Transfer 10 ml of this solution to a 250 ml volumetric flask and make up the volume with distilled water.
- (E) Take 15 ml aliquot from above solution (step-D) into 25 ml volumetric flask and proceed as given in procedure.
- (2) *For determination of total water soluble phosphate*
- (A) Weigh accurately about 1 g of sample (W_2) in a conical flask.
- (B) Add about 100 ml of distilled water and stir for about 30 minutes using a magnetic stirrer.
- (C) Filter the solution in 1000 ml volumetric flask.
- (D) Wash the residue with 50 ml distilled water and collect washing in some volumetric flask.
- (E) Make up the volume with distilled water.
- (F) Take 10 ml of this solution (step-E) to 250 ml beaker.
- (G) Add 50 ml of distilled water and 20 ml of (1:1) HNO_3 .
- (H) Boil for 10 minutes taking care that the sample does not become dry.
- (I) Cool the solution and transfer to 250 ml volumetric flask and make up the volume with distilled water.
- (J) Take 15 ml aliquot from this solution (step-I) into 25 ml volumetric flask and proceed as given in procedure.
- (3) *For determination of total water soluble orthophosphate*
- (A) Weigh accurately about 1 g of sample (W_3) in a conical flask.
- (B) Add about 100 ml of distilled water and stir for about 30 minutes using a magnetic stirrer.
- (C) Filter the solution in 1000 ml volumetric flask.
- (D) Wash the residue with 50 ml distilled water and collect washings in the same volumetric flask.
- (E) Make up the volume with distilled water.
- (F) Take 10 ml of this solution (step-E) to 250 ml of volumetric flask and make up the volume with distilled water.
- (G) Take 1.5 ml of this solution (step-F) in 25 ml volumetric flask and proceed as given in procedure.

Preparation of standard curve

- (A) Weigh accurately 0.1918 g potassium dihydrogen orthophosphate (KH_2PO_4), AR grade salt and transfer to 1000 ml volumetric flask with distilled water and make up the volume.
- (B) Take 50 ml of this solution and dilute to 250 ml in a volumetric flask with distilled water.

- (C) Take following aliquot from above solution (step-B) in separate 25 ml volumetric flask.

Flask No.	Volume of aliquot	PRO, concentration (ppm)
1.	0.5	10
2.	1.0	20
3.	1.5	30
4.	2.0	40
5.	2.5	50
6.	3.0	60
7.	3.5	70
8.	4.0	80
9.	4.5	90
10.	5.0	100

- (D) Develop colour in each flask as per procedure given below.
- (E) Plot the standard curve using absorbance versus P_2O_5 , concentration
- (5) *Procedure*
- (A) Add 1-2 drops of p-nitrophenol indicator to the aliquot in volumetric flask.
- (B) Adjust pH to 5.0 with the help of 2N.NaOH (Yellow colour) and $5NH_2SO_4$, colourless.
- (C) Add 10 ml distilled water. Add 4
- (D) ml of Reagent B.
- (E) Make up the volume to 25 ml with distilled water and mix well.
- (F) After 15 minutes determine the absorbance of blue colour developed at 660 nm wavelength.
- (G) Determine the concentration of P_2O_5 , in the sample solutions, (X) from the standard curve.

Calculations

(1) Total phosphate (as PRO_3), per cent $(A) = \frac{1.667 \times X_1}{W_1}$

(2) Total water soluble phosphate (as P_2O_5), per cent $(B) = \frac{1.667 \times X_2}{W_2}$
 $1.667 \times X_3$

(3) Total water soluble orthophosphate $(C) = \frac{\quad}{W_3}$

- (4) Polyphosphate per cent (as P_2O_5) = B-C, Where X_1 , X_2 , X_3 , are concentration of P_2O_5 , (in ppm) obtained from standard curve and W_1 , W_2 , and W_3 , are respective sample weight.

5. Determination of potassium

Determination of potassium in all kinds of fertilisers is given in this section. Two alternative methods have been described. Any of these two methods may be used depending upon the availability of reagent, and suitability of the the method.

(ii) Sodium tetraphenyl boron method

This method is applicable to both mixed and straight potassium fertilisers.

(a) *Reagents*

- (1) Sodium hydroxide solution — 20 per cent. Dissolve 20 gm NaOH in 100ml distilled water.
- (2) Formaldehyde solution — 37 per cent.
- (3) Sodium tetraphenyl boron (STPB) solution — approximately 1.2 per cent. Dissolve 12 g sodium tetraphenyl boron in approximately 800 ml water. Add 20— 25 gm $\text{Al}(\text{OH})_3$, stir for 5 minutes, and filter (Whatman No.42 paper or equivalent) into 1 litre volumetric flask. Rinse beaker sparingly with water and add to filter. Collect entire filtrate, add 2 ml 20 per cent NaOH solution, dilute to volume with water, and mix. Let it stand for 48 hours and standardize. Adjust to that 1 ml STPB=1 per cent K_2O . Store at room temperature.
- (4) Quaternary ammonium chloride solution — approximately 0.625 per cent. Dilute 50 ml of 12.8 per cent zephiran chloride to 1 litre with water, mix and standardize. Cetyltrimethyl ammonium bromide may be substituted for zephiran chloride. If other concentration is used, adjust volume.
- (5) Clayton yellow - 0.04 per cent — Dissolve 40 gm in 100 ml water.

(b) *Standardisation of solutions*

- (1) Zephiran chloride-To 1.0 ml STPB solution in 125 ml erlenmeyer flask, add 20-25 ml water, 1 ml 20 per cent NaOH 2.5 ml HCHO, 1.5 ml 4 per cent $(\text{NH}_4)_2\text{C}_2\text{O}_4$, and 6-8 drops of indicator (5) above.
- (2) Titrate to pink end point with zephiran chloride solution, using 10 ml semi micro burette. Adjust zephiran chloride solution so that 2.0 ml = 1.0 ml STPB solution.
- (3) Sodium tetraphenyl boron solution - Dissolve 2.5 gm of KH_2PO_4 in water in 250 ml volumetric flask, add 50 ml 4 per cent $(\text{NH}_4)_2\text{C}_2\text{O}_4$ solution, dilute to volume with water and mix. (It is not necessary to bring to boil). Transfer 15 ml aliquot, (51.92 mg (43.10 mg K) to 100 ml volumetric flask, add 2ml 20 per cent NaOH, 5 ml HCHO and 43 ml STPB reagent. Dilute volume with water; mix thoroughly, let stand 5-10 minutes and pass through dry filter. Transfer 50 ml aliquot of filtrate to 125 ml erlenmeyer flask, add 6-8 drops of indicator (5) above and titrate excess reagent with, zephiran solution. Calculate titration values as follows:

F— $34.61/(43\text{ml}-\text{ml Zephiran})=\% \text{K}_2\text{O}/\text{ml STPB reagent}$. Factor applies to all fertilisers if 2.5 g sample is diluted to 250 ml and 15 ml aliquot is taken for analysis. If results are to be expressed as K rather than K_2O , substitute 28.73 for 34.61 in calculating the value of F.

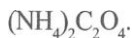
(c) *Preparation of solution*

(1) *Mixed fertilisers*

- A. Place 2.5 g sample in 250 ml volumetric flask.
- B. Add 125 ml water and 50 ml saturated $(\text{NH}_4)_2\text{C}_2\text{O}_4$ solution. Add 1 ml of diglycol stearate solution if needed to prevent foaming.
- C. Boil for 30 minutes, add slight excess of NH_4OH and after cooling dilute to 250 ml. Mix and pass through dry filter.

(2) *Potassium salts (potassium chloride sulphate, potassium magnesium sulphate, and kainite)*

A. Dissolve 2.5 g sample and dilute to X 250 ml without adding NH₄OH and



B. When interfering substances such as NH₄, Ca, Al, etc. are present proceed as in (1) above.

(d) *Procedure*

(1) Transfer 15 ml aliquot of sample solution to 100 ml volumetric flask and add 2 ml 20 per cent NaOH and 5ml HCHO.

(2) Add 1 ml standard STPB solution for each 1 per cent K₂O expected in sample plus additional 8 ml excess to ensure complete precipitation.

(3) Dilute to volume with water, mix thoroughly, let it stand for 5-10 minutes and pass it through dry filter (Whatman 12 or equivalent).

(4) Transfer 50 ml filtrate to 125 ml erlenmeyer flask, add 6-8 drops of indicator

(5) above and titrate excess reagent with standard zephiran solution. Per cent K₂O in the sample = (ml STPB added — ml zephiran) X F where F = per cent - KNO/ml STPB reagent.

(Reference — *Methods of Analysis*, AOAC, 1965).

6. Method of analysis of anhydrous ammonia

(i) *Estimation of water and ammonia*

(a) *Scope*

This method is for the determination of water at 20°C (or room temperature), (and usually ammonia by difference) of any essentially anhydrous ammonia liquid product. Normally, the water will be low (less than 0.5 per cent) or sample of synthetic ammonia.

(b) *Apparatus*

(i) Ammonia sampling tubes.

(2) Fume hood with exhaust dust.

(3) Sample tube holder rack in hood fabricated as convenient to hold number of tubes desired.

(4) Gloves, protective, elbow length.

◊

Reagents

(d) Charcoal, reagent, 14-20 mesh.

Procedure

(1) Remove the sample tubes from the sample carrier and place in the provided sample tube holders in the fume hood.

- (2) Allow the ammonia sample to boil and to evaporate (approximately 30 minutes) spontaneously in the fume hood. Because of the low temperature of the boiling ammonia, atmospheric moisture freezes on the sample container. Exercise care to see that none of this ice contaminates the sample.
- (3) When the boiling of the sample ceases, remove the tube from the holder. Wipe the outer surface of the tube dry and read and record the volume of liquid residue in the tube.
- (4) From the volume recorded, calculate the weight per cent water in the sample. Ignore the small piece of charcoal if used.

Calculations

$$\text{Per cent water} = \frac{A \times F \times 0.890 \times 0.684}{V \times 0.682} \times 100$$

Where

A	Volume of residue in tube (ml)
v	Volume of sample taken (ml)
F	Evaporation factor, taken from Table 1 corresponding to noted sample pressure when sample was obtained.
0.890	density of residue (gm/ml).
0.684	weight fraction of water in residue. 0.682
	density of sample (gm/ml).

(e) *Precaution*

The evaporation in the fume hood should be conducted with the window lowered to protect personnel from possible spray. Whenever it is necessary to handle the sample tubes, protective gloves should be worn.

Note : Determination of residue on evaporation will not usually be necessary for normal ammonia sample. If sample is clear and water residue is clear and colourless, the residue may be considered to be nil. However, should it become necessary to determine the dissolved solids and suspended solids in anhydrous ammonia, proceed, as follows:

(f) *Sampling*

Thoroughly clean and dry the sample tubes, weigh (W_1) each selected tube to the nearest milligram with a similar tube as counterpoise. Obtain the samples as desired earlier.

(g) *Apparatus*

- (i) Analytical balance.
- (2) Ammonia sampling tubes.
- (3) Rubber tubing, 1/4" OD length desired

(h) *Reagents*

Airdry, carbondioxide-free.

(i) *Procedure*

- (1) Start test following elimination of the water content of the sample as given earlier.
- (2) Connect one end of a rubber hose to a source of dry, carbondioxide-free air. Insert the other end of the hose into the sample tube and gently force air through the sample tube until all liquid has been evaporated and no ammonia gas remains in the tube.
- (3) Wipe a counterpoise and the sample tube containing the residue to substantially the same extent to remove moisture which may have condensed on their outer surfaces.
- (4) Weigh the sample tube and residue to nearest milligram.
- (5) Calculate the weight per cent residue of the sample.

$$\text{Calculations}$$
$$(W_2 - W_1) \times F \times 100$$

per cent residue =

$$\frac{\quad}{V \times 0.682}$$

Where

W_1

weight (g) of tube (before sampling).

W_2

weight (g) of tube and residue. volume

V

of sample taken (ml).

F evaporation factor, taken from Table 1 corresponding to noted sample pressure when sample was obtained.

0.682 density of sample (g/ml).

(j) *Precaution*

A check of completeness of evaporation may be made by visual inspection and by carefully smelling the air in the tube making sure that no carbon dioxide or moisture from the breath enters the sample tube.

(k) *Per cent ammonia content (by difference)*

In general, the ammonia content of the sample will be : per cent ammonia= 100 - (per cent water - per cent residue, if determined).

(l) *Precaution*

The evaporation in the fume hood should be conducted with the window lowered to protect personnel from possible spray. Whenever it is necessary to handle the sample tubes, protective gloves should be worn.

(ii) Determination of oil content

(a) *Apparatus*

(1) 500ml conical flask calibrated to hold 300ml (about 200g) of sample and with a bung fitted with glass through which the exit gas can be led away to a safe place. Ensure that the glass tube and exit line are free from constructions.

(2) Platinum dish 75 mm in diameter.

(b) *Reagents*

The reagent used shall be of a recognised analytical reagent quality like petroleum spirit with a boiling range of 40° to 60°C.

(c) *Procedure*

(1) Quickly running the sample (as per the sample procedure) upto the calibration mark, insert the bung.

(2) Immerse the flask in a continuous stream of cold water and allow the ammonia to evaporate slowly.

(3) When the evaporation is complete, remove the bung and gently blow out the last traces of ammonia with a small jet of filtered air free from carbon-dioxide.

(4) Dry the outside of the flask.

(5) Place the flask in an oven at 105 +5°C for 15 minutes to remove the moisture blowing out the last traces with a gentle stream of filtered air at the end of this period; allow to cool.

(6) Add to the flask approximately 100 ml of the petroleum spirit and swirl to dissolve the oil.

(7) Filter the solution through a small filter paper (a Whatman No.31 paper is suitable) direct into the platinum dish previously traced to the nearest 0.1 mg.

- (8) Repeat the operation with two more successive 10 ml portions of petroleum spirit, filtering through the same paper as before into the same dish.
- (9) Evaporate the combined extracts to dryness on a water bath in a fume cup board, taking care to avoid naked lights and finally dry in an oven at 105+5°C for 30 minutes.
- (10) Allow the dish to cool thoroughly in a desiccator and weigh again to the nearest 0.1 mg.
- (11) At the same time, carry out a blank determination on the petroleum spirit and filter paper.
- (12) Make an appropriate correction in the calculation.

Calculations

$$\text{oil content, parts per million by weight} = \frac{(W_1 - W_2) - W_3}{0.68V_2} \times 10$$

Where

W_1 = weight in grams of platinum dish and residue.

W_2 = weight in grams of dish alone.

W_3 — weight in grams of oil in blank determination

V_2 = volume in ml of sample taken.

F — evaporation factor taken from **Table 1** corresponding to noted sample pressure when sample was obtained.

Table 1 - Evaporation factor for different vessel Line Pressures	
Vessel or Line Pressure (Psig) 1	Evaporation factor (F) 2
0	1.000
10	0.963
20	0.940
30	0.920
40	0.900
50	0.885
60	0.870
70	0.860
80	0.850
90	0.840
100	0.830
110	0.821
120	0.813
130	0.805
140	0.797
150	0.789
160	0.782
170	0.776
180	0.770
190	0.764
200	0.758

Note : When a sample of liquid ammonia is transferred to an open flask/tube from a container in which the pressure is higher than the atmospheric that sample quickly reaches the thermodynamics equilibrium with its new environment. Some of the liquid ammonia will evaporate and since the ammonia so lost as vapour contains non-volatile constituents present in the original product, the concentration of these constituents will increase in the liquid sample taken. This may be significant factor in subsequent analysis and fortunately it is possible to calculate from the known thermo-dynamic properties of ammonia an appropriate correction. This correction is usually known in this context as the evaporation factor of flask factor and is simply that fraction by weight of the original liquid ammonia which remains as liquid in the sample. Multiplication of the determined quantity of a non-volatile constituents (oil,

water dissolved or suspended solids, etc.) by the evaporation factor gives a result which will be closer to the true figure. The Table above presents the evaporation factor for different vessel or line pressure.

When a sample is transferred (example by gravity) from a container of a cylinder or the sampler at the same pressure, there should be no loss by evaporation and in this case it is not necessary to use evaporation factor.

Method or analysis of zinc sulphate (both heptahydrate and monohydrate)

(iii) @ iiafiy of reagents

Pure chemicals and distilled water shall be used in tests.

Note : "Pure chemicals' shall mean chemicals that do not contain impurities which affect the results of analysis.

(iv) Determination of matter insoluble in water

(a) Reagents

Dilute sulphuric acid -10 per cent

(b) Procedure

Dissolve 25.0 g of the material in 125 ml of water and add 1 ml of dilute sulphuric acid. Heat the solution to boiling, filter through a weighed and prepared Gooch crucible or sintered glass crucible (G No.4) and wash the residue thoroughly with hot water. Dry the crucible at 110° 45°C to constant **mass**.

Calculations

Matter insoluble in water per cent by mass weight = $4A$ Where A
= weight in g of the residue

(v) Determination of zinc in zinc sulphate by modified EDTA titration method

(a) Reagents

(1) EDTA solution

Dissolve 3.72 g of di-sodium ethylene diamine tetra-acetate dihydrate in distilled water and make up the volume to 1 litre.

(2) Standard zinc metal solution :

Weigh about 1.0 g of zinc metal and record the weight accurately. Express this as W , add HCl (1:1) @ 20ml per g of zinc metal. Keep it for few hours and allow it to dissolve completely. Make up the volume of the solution to exactly 1000 ml.

(3) Ammonium hydroxide (20%) (m/m)

(4) Ammonium chloride - AR grade salt

(5) Sodium cyanide - AR/GR grade salt

(6) Eriochrome black(T) indicator mixture

Mix thoroughly 1 g of eriochrome black (T) indicator with 100 g of AR grade sodium chloride.

(7) Formaldehyde-Acetic acid solution (4%)

Dissolve 100 ml of formaldehyde (37-40%) in about 100 ml of distilled water. Add 40 ml glacial acetic acid and make volume to 1 litre with distilled water.

(8) Hydroxylamine hydrochloride AR grade solid salt

(b) *Procedure*

(1) *Standardisation of EDTA solution*

(A) Take 10 ml of zinc metal solution (standard)

(B) Add about 0.1 g of ammonium chloride and 30 ml of ammonium hydroxide solution (20%). pH of solution should be between 9.5-10.0.

(C) Dilute it by adding about 30 ml distilled water.

(D) Add a pinch of eriochrome black (T) indicator mixture. It will give red colour.

(E) Titrate it with EDTA solution to obtain clear blue end point. Note the volume of EDTA used as V, ml.

(2) *Estimation of zinc in samples*

(A) Weigh accurately 1.0 g of zinc sulphate sample and transfer to 100 ml volumetric flask giving washings with distilled water. Add one drop of concentrated H_2SO_4 and make up the volume with distilled **water**.

(B) Take 10ml of aliquot in beaker. Add 0.1 g of hydroxylamine hydrochloride and 0.1 g of ammonium chloride.

(C) Cautiously add small quantity of sodium cyanide. White precipitate will appear. Continue adding sodium cyanide till white precipitate disappears while swirling the beaker with hand. Add about 0.5 g excess of sodium cyanide.

(D) Dilute it by adding about 30 ml of ammonium hydroxide (20%) and add about 30 ml of distilled water. pH of the solution should be between 9.5-10.0.

(E) Add a pinch of eriochrome black (T) indicator mixture. It will give red colour.

(F) Titrate with EDTA solution till there is a sharp change to violet colour. Note the volume of EDTA used as V (ml).

(G) Add 20 ml of formaldehyde-acetic acid solution into above titrated solution and mix well. Red colour will reappear.

(H) Titrate it with EDTA solution to get blue end point without red tinge 5 ml of formaldehyde-acetic acid solution. If red re-appears, titrate again with EDTA solution to obtain sharp end point from red to blue colour. Note the volume of EDTA used in second titration as V_3 ml.

Calculations

$$\text{Zinc per cent} = \frac{10}{V_1} \times V_3 \times W$$

W = Weight in g of piece of zinc metal taken for preparation of standard zinc solution.

V_1 = Volume of EDTA solution (in ml) used for 10ml of zinc metal solution. V_3 = Volume of EDTA solution (in ml) used for second titration.

(vi) Colorimetric method for determining of copper

(a) *Reagent*

Outline of the method — Sodium diethyldithiocarbamate reacts with slightly acidic or ammoniacal solution of copper in low concentration to produce a brown colloidal suspension of the cupric diethyldithiocarbamate. The suspension can be extracted with an organic solvent and the colour extracted determined spectrophotometrically.

(b) *Apparatus*

Photometer -Any suitable photoelectric colorimeter.

(c) *Reagents*

- (1) Ammonium citrate solution — 40 per cent (m/v)
- (2) Sodium diethyldithiocarbamate solution — 0.1 per cent (m/v).
- (3) Standard copper solution — Clean the surface of a copper wire with sand paper. Dissolve 100 mg of pure copper in dilute nitric acid. Heat the solution nearly to dryness to drive off the acid. Add about 10 ml of water and heat again nearly to dryness. Take up the residue in water, add 25 ml of 1 N sulphuric acid and dilute to 1000 ml. One millilitre of this solution contains
0.1 mg of copper (as Cu).
Alternatively, dissolve 3.928 g of cupric sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in sufficient water containing 1 or 2 ml of concentrated sulphuric acid and dilute to 1000 ml. One millilitre of the solution contains 1.0 mg of copper (as Cu).
- (4) Ammonium hydroxide.

(5) Carbon tetrachloride.

(d) *Preparation of sample*

- (1) Weigh 5 g of sample accurately, dissolve in distilled water and add 1 ml of sulphuric acid. Filter the solution and make up to 250 ml with water in a volumetric flask. Take 50 ml of the above solution in a beaker, heat, pass hydrogen sulphide gas or add sodium sulphide solution and ensure complete precipitation. Filter hot and keep the filtrate for subsequent analysis.
- (2) Boil the residue with dilute nitric acid and filter, if necessary. To the filtered solution add sulphuric acid, evaporate, dilute and filter. Keep the residue for determination of lead.

(e) *Procedure for determination of copper*

Determine copper in the filtrate from (d)(2) above by diethyldithiocarbamate method as detailed below:

Transfer an aliquot containing not more than 0.65 mg of copper to a 100-ml separating funnel. Add 2 ml of ammonium citrate solution and adjust the pH to 8.5 with ammonium hydroxide. The presence of ammonium ions helps to ionize iron, if present, and to prevent rapid fading of developed colour. Add 10 ml of sodium diethyldithiocarbamate solution and develop the colour. Extract the coloured complex with 5-10 ml of carbon tetrachloride.

Separate the carbon tetrachloride layer from aqueous layer and centrifuge for 5 minutes to separate the water droplets. Transfer the carbon tetrachloride solution to a photometric cell and measure its absorbance at the wave length of maximum absorption 440 m μ relative to reagent blank. Calculate the corrected absorbance by subtracting the reading obtained for the solution containing no copper.

Transfer to a series of 100 ml separating funnels aliquot of standard copper solution corresponding to 0, 0.1, 0.2, 0.5, 1.0, 2.0, and 2.5 mg of copper and proceed exactly as

prescribed above. Plot a graph of corrected absorbance of solutions against their copper contents.

Note : If Bismuth is suspected it has to be separated or otherwise accounted for.

Calculations

Calculate the corrected absorbance by subtracting the value obtained for the blank from that obtained for the test solution and read from the calibration curve the corresponding mass of copper.

$$\text{Copper (as Cu), per cent by mass} = \frac{M_1}{M_2} \times 100$$

Where

M_1 = mass in g of copper as determined in the given aliquot of the test solution, and
 M_2 = mass in g of the material present in the aliquot of the test solution.

(vii) Determination of lead by colorometric method using dithizone

(a) Apparatus

Nessler cylinders — 50ml capacity.

(b) Reagents

- (1) Standard lead solution — Dissolve 0.40 g of lead nitrate ($\text{Pb}(\text{NO}_3)_2$) in water containing 2 or 3 ml of concentrated nitric acid and make up the volume to 1000 ml with water. Transfer 10 ml of this solution to a volumetric flask, add 2 or 3 ml of concentrated nitric acid and dilute with water to 1000 ml. One millilitre of this solution contains 2.5 mg of lead (as Pb). The diluted solution shall be freshly prepared.
- (2) Dilute ammonium hydroxide — 1 N, approximately.
- (3) Reagent A—Dissolve 25 g of triammonium citrate or 22 gm of citric acid and 4 g of hydroxylamine hydrochloride in about 200ml of water. Add dilute ammonium hydroxide to bring the pH to 8.5. Dilute the solution to 500ml. Purify this solution by extracting with 15 ml portions of 0.01 per cent dithizone solution until the final colour of the dithizone extract is green. Wash the aqueous remainder portion three times with 25 ml portions of chloroform and finally with 25 ml portion of carbon tetrachloride.
- (4) Dithizone (diphenyl thiocarbazone or phenylazothiomiformic acid) solution — Dissolve 0.001 g of dithizone in 100 ml of carbon tetrachloride, shaking intermittently for 1 hour. Allow to stand overnight and shake once again before using. This shall be kept in a cool and dark place. This gives a 0.01 per cent solution. Filter, if necessary. Dilute 10ml of this solution to 100ml with carbon

tetrachloride in a 100 ml volumetric flask. This shall be prepared fresh before determination. This gives a solution of 0.001 per cent.

Note 1 : Carbon tetrachloride used should be further purified. One litre of carbon tetrachloride is extracted with two portions of 25 ml dilute ammonium hydroxide and then kept over 100 g of activated carbon. Before use, it is decanted and distilled at about 80°C over a little fresh lime.

Note 2 . Sometimes dithizone solid and its 0.01 per cent solution deteriorate on storage. The 0.01 per cent solution should, therefore, be tested before further dilution, by shaking 2 ml of the solution with 5 ml of 1 per cent ammonium hydroxide. If the organic layer is only faintly yellow under these conditions, the solution may be used. If it is deeply coloured it shall be discarded and fresh solution be prepared. The solution as well as the reagent should be stored in a refrigerator and exposure to sunlight should be avoided during analytical work. To increase the stability of 0.01 per cent solution, it should be covered with a thin aqueous layer saturated with sulphur dioxide.

(5) Thymol blue indicator solution — 1 per cent (m/v) solution in rectified spirit.

◊ *Procedure*

(1) Take several aliquots of standard lead solution into a series of separating funnels, add 5 ml of the water and 10 ml of the reagent A. Then add 2 drops thymol blue indicator and bring the pH of the solution to 8.5 by addition of dilute ammonium hydroxide. Add 5 ml of the dithizone solution and shake well for about 10 seconds. Drain the organic layers into stoppered Nessler cylinders.

(2) Dissolve the residue obtained in (iv) (d) (2) above, in dilute nitric acid and dilute with water. Take a suitable aliquot of the solution and transfer to a separating funnel. Develop the colour as prescribed above. Drain the organic layer into a stoppered Nessler cylinder, add 10 ml of dilute ammonium hydroxide, and shake for about 10 seconds. Compare the colour developed with those of the standard solutions. Note the volume of the standard with which the colour of the test solution matches.

Note . If the colour of the test solution is intermediate between two standard solutions, then the experiment is repeated by taking more number of standard solutions in that range and exact colour matching is arrived at.

Calculations

$$\text{Heavy metals (as Pb), per cent by mass} = \frac{100 \times V \times f}{M}$$

where

V = volume in ml of standard lead solution matching with the test solution.

f = mass in g of heavy metals (as Pb) equivalent to 1 ml of standard lead solution, and

M = mass in g of the material in the aliquot taken for the test.

(viii) Determination of magnesium in the sample material

(a) Reagents

- (1) Eriochrome black T indicators — Dissolve 0.1 g of eriochrome black T in 25 ml of methyl alcohol.
- (2) Ammonium hydroxide — ammonium chloride buffer solution — Mix 350 ml of ammonium hydroxide (20 per cent w/w) with 54 g of ammonium chloride. Dilute with water and make up the volume to 1000 ml (The pH of the solution should not be more than 10).
- (3) Standard magnesium solution — 0.01 M weigh 2.4640 g of magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) and dissolve it in water. Make up the volume to one litre.
- (4) Ethylenediamine tetra-acetate (EDTA) solution — Dissolve 3.72 g of disodium ethylenediamine tetra-acetate dihydrate in water and make up the volume to one litre.

(b) Procedure

Standardisation of EDTA solution — Take 10 ml of standard magnesium solution in a conical flask. Add 20 ml of water, one millilitre of eriochrome black T indicator and 25 ml of ammonium hydroxide-ammonium chloride buffer solution. Heat at 40° to 50°C and then titrate with EDTA solution, maintaining the temperature between 40°C and 50°C until the colour changes from wine red to distinct blue.

$$\text{Molarity of EDTA solution} = \frac{10 M_1}{V}$$

Where M_1 = Molarity of standard magnesium solution, and V = Volume in ml of EDTA solution used for titration.

(c) Determination of magnesium in the sample material

Take the filtrate from (iv) (d) (1) (after removal of copper), add a few drops of concentrated nitric acid, boil and cool and then add solid ammonium chloride (about 2 grams), boil and cool, add ammonium hydroxide till the strong smell of ammonia comes and filter the precipitate through sintered crucible, take this filtrate and add dilute sulphuric acid till the solution is acidic (test with methyl red), heat the solution to boil and add excess of di-ammonium-hydrogen phosphate with continuous stirring. Add 10 per cent ammonia solution with continuous stirring till the solution is just alkaline (test with methyl red) while precipitate of zinc ammonium phosphate will be formed (the optimum pH for precipitation is 6-7, allow it to stand for 3-4 hours. Filter through Whatman filter paper no.40. Collect the filtrate in a volumetric flask. Make up the volume (say 100 ml). Take a suitable aliquot (say 10 ml) for the determination of magnesium. Add 20 ml of water, one ml of eriochrome black-T indicator

and 20ml of ammonium hydroxide-ammonia chloride buffer solution. Heat to 40°C to 50°C and titrate with standard EDTA solution, maintaining the temperature between 40°C and 50°C until the colour changes from wine red to distinct blue.

1 ml of 0.01 M-EDTA — 0.2432 mg of Mg'

$$\text{Mg} \quad \frac{X \times 0.2432}{5}$$

Where X = Volume of 0.1 M-EDTA used for titration.

The calculation factor 5 is derived presuming that 5 g of material is taken for test and the filtrate obtained is 100 ml out of which 10 ml is titrate.

(ix) Determination of pH

Dissolve 5 gm of the material in freshly boiled and cooled water. Dilute to 100 ml and mix. Determine the pH value of the solution with a pH meter.

(x) Determination of iron in the sample material
(Spectrophotometric Method)

(This method is applicable for iron contents upto 1 mg in the final aliquot).

(a) *Outlines of the method*— In test solution, iron is determined absorption-metrically as ferrous iron forms red complex with ortho-phenanthroline.

(b) *Apparatus* - Photo—electric-colorimeter.

(c) *Reagents*

(1) Bromophenol blue indicator solution 0.4 per cent solution in 95 per cent ethanol.

(2) Sodium citrate solution — 25 per cent.

(3) Hydroquinone solution — 1 per cent in an acetic acid buffer of pH 4.5 obtained by mixing 65 ml of 0.1 M acetic acid with 35 ml of 0.1 M sodium acetate solution.

(4) Ortho-phenanthroline solution — 0.25 per cent in 25 per cent ethanol.

(5) Dilute hydrochloric acid — 1:3 (V/V)

(6) Standard iron solution — Dissolve 0.7002 g. of ammonium ferrous sulphate (AR) in distilled water containing 2 ml of perchloric acid and dilute to 100 ml with water. One ml of this solution contains 1 mg of iron.

(d) *Preparation of sample solution* — Boil 1 g sample in 20 ml of hydrochloric acid (1:1) for 30 minutes and filter through Whatman No.40 filter paper in 100 ml volumetric flask. Follow with repeated extraction with boiling dilute hydrochloric acid (1:5) decanting the solution each time through the same filter paper. Dilute the filtrate to 100 ml.

(e) *Procedure*

(1) Take 5 ml of the sample solution, add bromophenol blue indicator solution and titrate with sodium citrate solution until yellow colour changes to blue.

(2) Take another 5 ml of sample solution in a 25 ml volumetric flask. Add 1 ml of hydroquinone solution, 3 ml of ortho-phenanthroline solution and an amount of sodium citrate solution equivalent to the above titration in step (1). Dilute with distilled water to 25 ml. Allow the solution to stand for one hour.

(3) Measure the absorbance of test solution at 510 nm using 1 cm cell and also blank with water.

(4) Place 4,8,12,16 and 20 ml of standard iron solution in a series of 100 ml volumetric flasks. To each, add 40 ml of diluted hydrochloric acid and dilute to 100 ml with water.

- (5) Using 5 ml aliquot proceed as for sample solution.
- (6) Measure the absorbance of the solution and plot a graph relating to absorbance to number of mgs of iron.

Calculations

$$\text{Per cent Fe} = \frac{2X}{W}$$

Where

X = Concentration (in mg) of iron in final sample solution aliquot as determined from the graph.

W = Weight in gm of material taken for the preparation of sample solution.

(xi) *Determination of Arsenic (As O)*

The principle, reagent and other matters in this case shall be same as given in paragraph (xiv) relating to determination of Arsenic in Ammonium Sulphate (As_2O_3) under sub-heading "3. Determination of Nitrogen".

(xii) Determination of cadmium

Method of analysis of cadmium in zinc sulphate — fertiliser sample

(l) Quality of Reagents : - Unless specified otherwise, pure chemicals and glass distilled or dematerialized water shall be used in tests.

(a) *Reagents*

(i) Standard cadmium solution:- Weigh out 1 g of pure cadmium metal and transfer it to a 250 ml beaker. Add 50 ml of water and 10 ml of concentrated nitric acid to dissolve the metal completely. Transfer the cadmium solution to a one litre flask with necessary washing. Make up the volume up to the mark. Shake well. This is a 1000 ppm solution of cadmium, (hereinafter called standard A).

Dilute 1 ml of standard A to 100 ml in a volumetric flask. This is a 10 ppm solution of cadmium, (hereinafter called standard B).

(1) Glass distilled water of pH 2.5 ± 0.5:- Dilute 1 ml of 10 per cent Sulphuric acid to one litre with glass distilled water and adjust the pH to 2.5 ± 0.5 with a pH meter using sulphuric acid or sodium hydroxide solution. The water so obtained is called acidified water.

(b) *Preparation of working standards*

Pipette out the following volume of standard B in 100 ml of numbered volumetric flask and make up the volume with acidified water.

Stopper the flask and shake them well. The same acidified water should be used for the preparation of the sample solution. Fresh standards should be prepared and used every time.

Flask No.	Volume of standard B taken (in ml)	Concentration of cadmium after making volume to 100 ml (in ppm)
1	0	0/0
2	2.0	0.2
3	4.0	0.4
4	8.0	0.8
5	12.0	1.2
6	16.0	1.6
7	20.0	2.0

(c) *Procedure*

(1) Preparation of sample solution : Weigh 2 g of zinc sulphate and transfer it to a 100 ml volumetric flask giving repeated washings with **acidified** water. Dissolve the material by shaking well, make up the volume and mix thoroughly. Filter a portion if necessary. For higher concentration of **cadmium** adjust the weight and dilution such that the absorbance of final flaming solution is not more than a 2 ppm solution of cadmium.

(2) Aspirate the standards as well as the sample solution in an atomic absorption Spectrophotometer at a wave length of 228.8 nm using **air acetylene** flame **and** note the corresponding absorbance value for each solution.

Calculations

Draw a graph using concentration (ppm) as the X-axis and absorbance as the Y-axis. Determine the concentration of cadmium in ppm in the sample solution from the graph.

$$\text{Cadmium (as Cd)} \quad \frac{C \times df}{\text{Concentration in ppm}}$$

Where C is the concentration in ppm of final sample solution
 W is the weight of the sample
 df is the dilution factor.

7. Alternate method of analysis of zinc sulphate (both heptahydrate - and monohydrate)

(i) Quality of reagents

1. Unless specified otherwise, pure chemicals and glass distilled or demineralised water shall be used in tests.

Note. 'Pure chemicals' mean chemicals that do not contain impurities which affect the results of analysis.

'Demineralised water' means the water obtained after passing distilled water through a cation and an anion exchange resin or a combined cation-anion exchange resin.

(ii) Determination of zinc

(a) Reagents

- (1) Standard zinc solution — Weigh accurately 1.0 g of zinc metal (AR) in a beaker. Add 20 ml HCl (1:1). Keep it for few hours and allow it to dissolve completely. Transfer the solution to 1 litre volumetric flask. Make up the volume with glass distilled water. This is 1000 ppm zinc solution, (hereinafter called standard A). This solution should be stored in a clean bottle for further use. Dilute 1 ml of standard A to 100 ml to get 10 ppm standard zinc solution designated as standard B.
- (2) Glass distilled or mineralised water of pH 2.5 ± 0.5. Dilute 1 ml of 10 per cent sulphuric acid to one litre with glass distilled or mineralised water and adjust the pH to 2.5 with a pH meter using H₂SO₄ or NaOH. This solution is called acidified water and 5 to 10 litres of this solution should be prepared at a time.
- (3) Preparation of working standards — Pipette the following volume of standard B in 50 ml numbered, volumetric flasks and make the volume with acidified water.

Stopper the flasks and shake them well. Prepare the standard in duplicate. The same acidified water should be used for preparing the solution of unknown fertiliser samples. Fresh standards should be prepared every time when a fresh lot of acidified water is prepared.

Flask No.	Volume of standard B taken (ml)	Concentration of zinc after making volume to 50 ml (ppm)
	0.0	0.0
1		
2	1.0	0.2
3	2.0	0.4
4	3.0	0.6
5	4.0	0.8
6	5.0	1.0
7	7.0	1.4
8	9.0	1.8
9	10.0	2.0

(b) *Procedure*

- (1) Preparation of zinc sulphate fertiliser samples — Weigh 0.25 g of the material on a clean watch glass and transfer it to one litre volumetric flask through the funnel giving repeated washings with glass distilled water and dissolve the material by shaking well, add 1 ml of concentrated H_2SO_4 . Then make the volume upto mark with glass distilled water and shake well.
- (2) Take 5 ml of the prepared solution in 250 ml volumetric flask and make the volume with acidified water. Shake the solution well and filter through Whatman No.42 filter paper in dry clean flasks. The flasks should be rinsed with a 10 to 15 ml of the filtrate and then continue filtration.
- (3) Flaming the solutions — Flame the standards and the filtered samples on atomic absorption spectrophotometer at a wave length of 213.8 nm (Zn line of the instrument).

Calculations

Prepare a standard curve of known concentrations of zinc solution by plotting the absorbance values on Y-axis against their respective zinc concentration on X-axis. Calculate the percentage zinc in zinc fertiliser by multiplying zinc concentration value calculated from standard curve by 20. Example:

Weight of the fertiliser sample	= 0.25g
Volume made.....	1000ml
Further dilution.....	= 50 times
Reading of the samples from atomic absorption.....	= Y Corresponding,
concentration value of zinc from	
standard curve against Y absorbance	= X ppm
Percentage zinc in the fertiliser	= 20(X)

(c) *Precautions*

- (1) Weighing must be done on a electric balance.
- (2) All the glass apparatus to be used should be of coming make and washed with dilute hydrochloric acid (1:4) and washed thoroughly with distilled and then with demineralised water.
- (3) The pipette should be rinsed with the same solution to be measured.
- (4) The outside of the pipette should be wiped with filter paper after taking out from the solution to be measured.
- (5) After using the pipette, place them on a clean dry filter paper in order to prevent contamination.
- (6) To start filtration, only a few drops should be added first in order to wet the filter paper and then continue further filtration.

(iii) Determination of magnesium

(a) Reagents

- (1) Strontium chloride — Dissolve 7.5 g of strontium chloride ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$) in one litre of glass distilled water.
- (2) Standard magnesium solution — Weigh 0.507g of magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) on a clean watch glass and transfer it to one litre flask through the funnel giving several washings to watch glass and the funnel with glass distilled or demineralised water. This is 50ppm Mg solution. Dilute 10ml of 50ppm solution of Mg to 100 ml to get 5 ppm standard Mg solution.
- (3) Preparation of working standards—Pipette the following volume of 5 ppm standard Mg solution in 50 ml numbered volumetric flasks. Add 10 ml of strontium chloride solution to each flask and make up the volume to 50

Stopper the flask and shake them well. Prepare fresh standards every fortnight.

Flask No.	Volume of 5 ppm Mg solution taken (ml)	Volume of strontium chloride added (ml)	Concentration of Magnesium after making the volume to 50 ml (ppm)
1	0.0	10.0	0.0
2	2.0	10.0	0.2
3	4.0	10.0	0.4
4	6.0	10.0	0.6
5	8.0	10.0	0.8
6	10.0	10.0	1.0

(b) Procedure

- (1) Pipette 20 ml of the solution which was prepared for the determination of zinc by dissolving 0.25 g of the fertiliser sample in one litre flask [Step 8(ii) (b)(1)]. Add 10 ml of strontium chloride. Make up the volume to 50 ml.
- (2) Flame the standards and the samples on atomic absorption spectro-photometer at a wave length of 285.5 nm (Mg line of the instrument).

Calculations

Prepare a standard curve of known concentrations of Mg solutions by plotting the absorbance value on Y-axis against their respective concentration values on X-axis. Percentage magnesium in the zinc fertiliser will correspond to the concentration values calculated from the standard curve. Example:

Weight of the fertiliser..... = 0.25 g
Volume made..... = 1000ml
Further dilution..... = 2.5 times

Reading of the sample from atomic absorption spectrophotometer..... = Y
 Corresponding concentration of Mg from standard curve against Y absorbance..... = X ppm
 Percentage magnesium in the fertiliser = X

(iv) Determination of copper

(a) Reagents

- (1) Standard copper solution — Weigh 0.1965 g of copper sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) on a clean watch glass and transfer it to one litre flask through the funnel giving several washings to watch glass and the funnel with glass distilled water. Add one ml of 10 per cent sulphuric acid and make up the volume upto the mark. Stopper the flask and shake the solution well. This is 50 ppm Cu solution and should be stored in a clean bottle for further use. Dilute 10ml of 50 ppm solution of copper to 100 ml to get 5 ppm standard copper solution.
- (2) Glass distilled or mineralised acidified water of pH 2.5 0.5 (same as given in 8(ii)(a)(2)).
- (3) Preparation of working standards — Pipette the following volume of 5 ppm standard copper solution in 50 ml numbered volumetric flasks and make the volume with acidified water.

Flask No.	Volume of 5 ppm standard Cu solution taken (ml)	Concentration of copper after making volume of 50 ml
1	0.0	0.0
2	2.0	0.2
3	4.0	0.4
4	6.0	0.6
5	8.0	0.8
6	10.0	1.0

Stopper the flasks and shake them well. Prepare fresh standards every fortnight.

(b) Procedure

- (1) The solution which was prepared for the determination of zinc by dissolving 0.25 g of the fertiliser sample in one litre flask step 8(ii)(b)(I) should be used for the determination of copper.
- (2) Flame the standards and the samples on an atomic absorption spectro-photometer at a wavelength of 324.8 nm (Cu line of the instrument).

Calculations

Prepare a standard curve of known concentrations of copper solutions by plotting the absorbance values on Y-axis against their respective concentration values on X-axis. Calculate the percentage copper in the zinc fertiliser by multiplying the copper concentration value calculated from the standard curve by 0.4.

Example: -

Weight of the fertiliser samples = 0.25 g
 Volume made..... = 1000 ml
 Reading of the sample from atomic absorption spectrophotometer.
 = Y

Corresponding concentration of copper from standard

curve against Y absorbance..... = X ppm

Percentage copper in the fertiliser..... = 0.4 X

(v) **Determination of lead**

(a) **Reagents**

- (1) Standard lead solutions — Weigh 0.1599g of lead nitrate ($\text{Pb}(\text{NO}_3)_2$) on a clean watch glass and transfer it to one litre flask through the funnel giving several washings to watch glass and funnel with glass distilled or demineralised water. Add 10ml of concentrated distilled nitric acid and make the volume upto the mark. Stopper the flask and shake the solution well. This is 100 ppm lead solution and should be stored in a clean bottle for further use. Dilute 10 ml of 100 ppm solution of lead to 100 ml with 1 per cent nitric acid solution to get 100 ppm standard lead solution.
- (2) 1 per cent nitric acid solution— Dilute 10ml of concentrated distilled nitric acid to one litre with glass distilled water.
- (3) 20 per cent zinc sulphate solution --- Weigh 20g of zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and dilute to 100ml with 1 per cent nitric acid solution.
- (4) Preparation of working standards — Pipette the following volume of 10 ppm standard lead solution in 50ml numbered volumetric flasks. Add 5 ml of 20 per cent zinc sulphate solution to each flask and make the volume with 1 per cent nitric acid solution.

Flask No.	Volume of 10 ppm lead solution taken (ml)	Volume of 20% zinc sulphate solution added (ml)	Concentration of lead after making the volume to 50 ml (ppm)
1	0.0	5.0	0.0
2	2.0	5.0	0.4
3	4.0	5.0	0.8
4	6.0	5.0	1.2
5	8.0	5.0	1.6
6	10.0	5.0	2.0

Stopper the flasks and shake them well.

(b) *Procedure*

- (1) Preparation of zinc sulphate fertiliser samples — Weigh 1 g of the material on a clean watch glass and transfer to 50 ml volumetric flask through the funnel giving washings with 1 per cent nitric acid solution. Dissolve the material and make the volume with 1 per cent nitric acid solution. Samples should be prepared in duplicate.
- (2) Flaming the solutions — Flame the standards and the samples on atomic absorption spectrophotometer at a wavelength of 217nm (Lead line of the instrument).
- (3) Calculations — Prepare a standard curve of known concentrations of lead solution by plotting the absorbance values on Y-axis against their respective lead concentration on X-axis. Calculate the percentage lead in zinc fertiliser by multiplying lead concentration value calculated from standard curve by 0.005.

(vi) *Determination of pH*

(a) *Procedure*

Dissolve 5 g of the material in freshly boiled water. Dilute to 100 ml and mix. Determine the pH value of the solution with pH meter.

(vii) *Determination of matter insoluble in water*

(a) *Procedure*

Dissolve 25.0 g of the material in 125 ml of water and 1 ml dil H₂SO₄. Filter through a weighed and prepared Gooch crucible or sintered glass crucible (G.No.4) and wash the residue thoroughly with water. Dry the crucible at 10^oA5^o C to constant mass.

Calculations

Matter insoluble in water per cent by weight = 4A. Where A= Weight in g of the residue.

(viii) *Determination of iron*

(atomic absorption spectrophotometric method).

(a) *Reagents*

- (1) Standard iron solution (1 000 ppm) — Weigh accurately 1 g pure iron wire and put it in approximately 30 ml of 6 N HCl in a beaker and boil. Transfer it to one litre volumetric flask through the funnel giving several washings to the beaker and funnel with glass distilled water. Make the volume upto the mark. Stopper the flask and shake the solution well. This is 1000 ppm iron solution.
- (2) Glass distilled or demineralised water of pH 2.5 ± 0.5.
- (3) Preparation of working standards — Pipette 10 ml iron stock solution in 100 ml volumetric flask and dilute to volume. This is 100 ppm iron solution. Pipette the following volumes of 100 ppm iron solution in 50 ml volumetric flask and make the volume with acidified water.

Flask No.	Volume of 100 ppm standard iron solution	Concentration of iron after making volume to 50 (PP)

1.	0.0	0.0
2.	1.0	2.0
3.	2.0	4.0
4.	3.0	6.0
5.	4.0	8.0
6.	5.0	10.0
7.	6.0	12.0
8.	7.0	14.0
9.	8.0	16.0
10.	9.0	18.0
11.	10.0	20.0

Stopper the flask and shake the solution well.

(b) *Procedure*

- (1) Preparation of fertiliser sample solution — Weigh exactly 1 g of the material on a clean watch glass and transfer it to a one litre volumetric flask through the funnel giving repeated washings with acidified water and dissolve the material by shaking well. Make the volume upto the mark with acidified water and shake well and filter through Whatman No.42 filter paper in dry clean flask. The flask should be rinsed with a 10-15 ml of filtrate and then continue filtration.
- (2) Flaming the solution — Flame the standards and the filtered sample on atomic absorption spectrophotometer at a wavelength of 248.3 nm using clean air acetylene flame.

Calculations

Prepare a standard curve of known concentrations of iron solution by plotting the absorbance value on Y-axis against the respective iron concentration on X-axis. Determine the concentration of iron in the sample solution from the graph.

$$\text{Total iron (as Fe) per cent} = \frac{X}{10}$$

Where

X — Concentration of iron (in ppm) obtained from the standard curve.

Note . In case a sample has been analysed by both the methods, viz., indicated under the heading '7' and '8' the result obtained by the method indicated under the heading '8' shall prevail.

(ix) Determination of Arsenic (As₂O₃)

The principle, Reagent and other matters in this case shall be same as given in paragraph (xiv) relating to Determination of Arsenic in ammonium sulphate (As₂O₃) under sub-heading "3, Determination of nitrogen".

(x) Determination of cadmium

Same as provided in paragraph (x) under sub-heading 7.

8. Method of analysis of manganese sulphate, agricultural grade

(i) Quality of reagents

Unless specified otherwise, pure chemicals and glass distilled or demineralised water shall be used in tests.

Note : 'Pure chemicals mean chemicals that do not contain impurities which affect the results of analysis. 'Demineralised water' means the water obtained after passing distilled water through a cation and an anion exchange resins or a combined cation-anion exchange resin.

(ii) Determination of manganese

(a) *Reagents*

- (1) Standard manganese solution — Weigh 3.0763 g of manganese sulphate ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$ A.R. grade) on a clean watch glass and transfer it to one litre flask through the funnel giving several washings to watch glass and funnel with acidified water and make the volume up to the mark. This solution will be 1000 ppm Mn. A secondary dilution of 5 ml to 100 ml with acidified water gives a 50 ppm working standard.
- (2) Glass distilled or demineralised water of pH 2.5 \pm 0.2 - Dilute 1 ml of 10 per cent sulphuric acid to one litre with glass distilled or demineralised water and adjust the pH to 2.5 with pH meter using 10 per cent H_2SO_4 or NaOH.
This solution is called acidified water.
- (3) Preparation of working standards — Pipette the following volume of working standard solution in 50 ml numbered volumetric flasks and make the volume with acidified water.

Flask No.	Volume of working standard taken (ml)	Concentration of manganese after making volume to 50 ml (ppm)
1.	0.0	0.0
2.	0.5	0.5
3.	1.0	1.0
4.	1.5	1.5
5.	2.0	2.0
6.	2.5	2.5
7.	3.0	3.0
8.	3.5	3.5
9.	4.0	4.0

Stopper the flasks and shake them well. Prepare the standard in duplicate. The same acidified water should be used for preparing the solution of unknown fertiliser samples. Fresh standards should be prepared every time when a fresh lot of acidified water is prepared.

(b) Procedure

- (i) Preparation of manganese sulphate fertiliser samples - Weigh exactly 0.4g of the material on the clean watch glass and transfer it to a one litre volumetric flask through the funnel giving repeated washings with acidified water and dissolve the material by shaking well. Make the volume up to the mark with acidified water and shake well.
- (2) Take 5 ml of the prepared solution in 250 ml volumetric flask and make the volume with acidified water. Shake the solution well and filter through Whatman No.42 filter paper in dry clean flasks. The flasks should be rinsed with a 10 to 15 ml of the filtrate and then continue filtration.
- (3) Flaming the solutions — Flame the standards and the filtered samples on atomic absorption spectrophotometer at a wavelength of 279.5 nm (Mn line of the instrument).

Calculations

Prepare a standard curve of known concentration of manganese solution by plotting the absorbance value on Y-axis against their respective manganese concentrations on X-axis. Calculate the percentage manganese in manganese fertiliser by multiplying manganese concentration value calculated from the standard curve by 12.5.

(c) Precaution

- (i) Weighing must be done accurately with a precision balance.
- (2) All the glass apparatus to be used should be Corning / Pyrex make and washed with dilute hydrochloric acid (1:4) and washed thoroughly with distilled and then with demineralised water.

- (3) The pipette should be rinsed with the same solution to be measured.
 - (4) The outside of the pipette should be wiped with filter paper after pipetting the solution to be measured.
 - (5) After using the pipette, place them on a clear dry filter paper, in order to prevent contamination.
 - (6) To start filtration, only a few drops should be added first in order to wet the filter paper and then continue further filtration.
9. Alternative method of analysis of manganese sulphate, agricultural grade

(i) Quality of reagents

Unless specified otherwise pure chemicals and glass distilled or demineralised water shall be used in tests.

Note : 'Pure Chemicals' mean chemicals that do not contain impurities which affect the results of analysis. 'Demineralised water' means the water obtained after passing distilled water through a cation and anion exchange resins or a combined cation-anion exchange resin.

(ii) Determination of manganese

(a) Reagents

- (1) Hydrogen peroxide (H_2O_2) — 30 per cent.
- (2) Nitric acid (HNO_3) — concentrated.
- (3) Orthophosphoric acid (H_3PO_4 from steel industry) — 85 per cent.
- (4) Potassium periodate (KIO_4).
- (5) Glass distilled or mineralised water of pH 2.5A0.5 — Dilute 1 ml of 10 per cent H_2SO_4 to one litre with glass distilled or demineralised water and adjust the pH to 2.5 with a pH meter using 10 per cent H_2SO_4 or $NaOH$.
- (6) Standard manganese solution — Weigh 3.0763 g of manganese sulphate ($MnSO_4 \cdot H_2O$ — A.R. grade) on a clear watch glass and transfer it to one litre flask through the funnel giving several washings to the watch glass and the funnel with acidified water and make the volume up to the mark.
This solution will be 1000 ppm Mn. A secondary dilution of 5 ml to 100 ml with acidified water gives a 50 ppm working standard.
- (7) Preparation of working standards — Pipette 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 ml of standard solution of manganese in a series of 11 different 100 ml beakers. Evaporate the solutions to dryness at low temperature on a hot plate or steam bath. Add 5 ml of HNO_3 and 2 ml of 30 per cent H_2O_2 . Cover the beakers with watch glass and digest the contents on a steam bath or hot plate for 30 minutes. Remove the glass cover and evaporate the contents to dryness. Cool the beakers, add 2 ml of HNO_3 and 5 ml of phosphoric acid and heat till boiling. Again cool and mix the contents with 10 ml of demineralised water. Add 0.3 g of KIO_4 , and heat the solutions on water bath until pink colour appears. Add about 20 ml water and heat for about 40 minutes or until there is no more increase in colour intensity. Cool the beakers to room temperature, transfer the solutions to 50 ml volumetric flasks. Make the volume with deionised water and shake thoroughly. The concentration of manganese ion will be as follows.

Flask No.	Volume of working standard taken (ml)	Concentration of manganese after making the volume to 50 ml (ppm)
	0.0	0.0
2	0.5	0.5

3	1.0	1.0
4	1.5	1.5
5	2.0	2.0
6	2.5	2.5
7	3.0	3.0
8	3.5	3.5
9	4.0	4.0
10	4.5	4.5
11	5.0	5.0

Measure the light transmittance at 540 m μ (green filter). Make the standard curve by plotting per cent transmittance against the concentration of manganese.

(b) *Procedure*

- (1) Analysis of manganese sulphate fertiliser sample — Weigh exactly 0.500 g of the material on a clean watch glass and transfer it to a one litre volumetric flask through the funnel giving repeated washings with acidified water and dissolve the material by shaking well. Make the volume up to the mark with acidified water and shake well. Pipette one ml solution in a 100 ml pyrex/corning beaker and proceed further as described above for preparation of standard curve and make the volume to 50 ml. The standard and the test samples should be prepared and processed for estimation at the same time.

Calculations

The percentage of manganese in the manganese sulphate fertiliser sample is calculated by multiplying manganese concentration value calculated from standard curve by 10.

10. Method of analysis of borax (sodium tetraborate decahydrate fertiliser and boric acid)

(i) Quality of reagents

Unless specified otherwise, pure chemicals and glass distilled water shall be used in tests.

Note : Pure chemicals means chemicals that do not contain impurities which affect the results of analysis.

(ii) Determination of boron

(A) *Atomic absorption spectrophotometer method*

(a) *Reagents*

Standard Boron Solution — Weigh 44.095 g of sodium tetraborate decahydrate ($\text{Na}_2\text{B}_4\text{O}_{10} \cdot 10\text{H}_2\text{O}$) - AR grade or 28.5720g of Boric Acid on clean watch glass and transfer it to one litre volumetric flask through the funnel giving several washings to watch glass and funnel with glass distilled water. Make the volume upto the mark. Stopper the flask and shake the solution well. This is 5000 ppm boron solution herein after called standard A. This solution should be stored in clean bottle for further use.

- (1) Preparation of working standards — Pipette the following volume of standard A in 50 ml numbered volumetric flasks and make the volume with water.

Flask No.	Volume of standard A taken (ml)	Concentration of boron after making volume to 50 ml (ppm)
1	0.0	0.0
2	4.0	400
3	6.0	600

4	8.0	800
5	10.0	1000
6	12.0	1200
7	14.0	1400
8	16.0	1600

(b) Procedure

- (1) Preparation of sample solution — Weigh 2.500 g of the material on a clean watch glass and transfer it quantitatively in a 250 ml volumetric flask through the funnel giving repeated washing with water. Make up the volume, stopper the flask and shake well.
- (2) Flaming the solutions — Flame the standards and the sample solution on atomic absorption spectrophotometer at a wavelength of 249.8 nm using nitrous oxide-acetylene flame.

Calculations

Prepare a standard curve of known concentrations of boron solutions by plotting the absorbance value on Y-axis against their respective boron concentration on X-axis. Calculate the percentage boron in the given sample by multiplying boron concentration value obtained from standard curve by 0.01.

Example.

Wt of the fertiliser sample	2.50 g	
Volume made	250ml	
	250	
Dilution factor	<hr/>	= 100
	2.5	

Reading of sample from atomic absorption	— Y
Corresponding concentration value of boron from standard curve against Y absorbance	= X ppm
Percentage of boron in the sample	— 0.01 X

(B) *Titrametric method*

(a) *Outline of the method*

Borax is determined by first converting it to boric acid with hydrochloric acid and then titrating against sodium hydroxide solution after complexing, boric acid with mannitol or sorbitol.

(b) *Reagents*

- (1) Standard hydrochloric acid — 0.1 N
- (2) Standard sodium hydroxide — 0.1 N
- (3) Methyl red indicator — Dissolve 0.1 g of the material in 60 ml of rectified spirit and dilute with water to 100 ml.
- (4) Phenolphthalein indicator — Dissolve 1 g of the material in 100 ml of rectified spirit.
- (5) Mannitol or sorbitol.

(c) *Procedure*

- (1) Take 1 g of sample and transfer to a 250 ml volumetric flask and dissolve it into 100 ml water. Make up the volume to the mark.
- (2) Take 25 ml of aliquot in a conical flask of 250 ml capacity. Add 100 ml of water. Add 2-3 drops of methyl red and titrate with 0.1 (N) hydrochloric acid till the colour changes to red. Add 2-3 ml of the acid in excess. Let the total volume of acid required be (t).
- (3) Take 25 ml of aliquot of the sample in a separate conical flask and add 100 ml of water. Add 2-3 drops of phenolphthalein indicator. Add the same volume (t) of acid into the conical flask as added in step (2). Boil it for 5 minutes and cool it to room temperature. Back titrate the excess acid with 1N Standard sodium hydroxide solution to pink end point.
- (4) Add 10 g of Mannitol and swirl gently. Titrate with standard sodium hydroxide solution to pink end point. Add approximately 2 g of mannitol again. Titrate again to restore the pink end point if colour discharges.

Calculations

Boron (as B) per cent by weight = $10.811 \times V \times N$

Where, V - volume in ml of standard sodium hydroxide used. N - normality of sodium hydroxide, and

W - weight in g of the material taken for the test

{Reference: Based on AOAC 949.03 (2000)}

(iii) Determination of matter insoluble in water

Dissolve 25g material in 500 ml of water and add 1 ml of dilute H_2SO_4 . Filter

through a previously weighed Gooch Crucible or sintered glass Crucible (G 4) and wash residue thoroughly with water. Dry the crucible at $110 \pm 5^\circ\text{C}$ to a constant mass

Calculation : Matter insoluble in water % by weight = $4A$, Where A is the weight in grams of residue";

(iv) Determination of pH

Dissolve 3.8 g of the material in water and make it to 100 ml. Measure the pH value of the solution with the help of suitable pH meter, using glass electrodes.

(v) Determination of lead

Same as method No.8 (v). Substitute zinc sulphate by borax.

11. Method of analysis of copper sulphate fertiliser ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)

(i) Quality of reagents

Unless specified otherwise pure chemicals and glass distilled or demineralised water shall be used in tests.

Note : 'Pure chemicals' mean chemicals which do not contain impurities which affect the results of analysis. Demineralised water, means the water obtained after passing distilled water through a cation and anion exchange resin or a combined cation-anion exchange resin.

(ii) Determination of copper

(A) *Atomic absorption spectrophotometer method*

(a) *Reagents*

As specified in 8 (iv) (a) (1), (2), and (3)

(b) *Procedure*

- (1) Weigh 0.25 g of the material on a clean watch glass and transfer it to one litre volumetric flask through the funnel giving repeated washings with glass distilled water. Add one ml of 10 per cent sulphuric acid and make up the volume.
- (2) Take 5 ml of the prepared solution in 500 ml volumetric flask and make up the volume. Shake the solution well and filter through Whatman No 42 filter paper in dry clean flask. The flask should be rinsed with a 10 to 15 ml of the filtrate and then continue filtration.
- (3) Flaming the solutions - Flame the standards and the filtered samples on an atomic absorption spectrophotometer at a wavelength of 324.8 nm using air-acetylene flame.

Calculations

Prepare a standard curve of known concentrations of copper solution by plotting the absorbance values on Y-axis against their respective concentration values on X-axis

$$\text{Per cent copper in sample} = 10 X \frac{\quad}{W}$$

where W = Weight in g of the material taken for test.

X = Conc. of copper (in ppm) as determined from the graph.

(B) *Iodometric titration method*

(a) *Outline of the method*

Copper is determined with the addition of potassium iodide and titrating the liberated iodine against standard sodium thiosulphate solution.

(b) *Reagents*

- (1) Sodium carbonate - AR grade
- (2) Potassium iodide - AR - crystals
- (3) Acetic acid - AR grade
- (4) Standard sodium thiosulphate solution - 0.1 N
- (5) Starch indicator solution - Titrate 5 g of starch and 0.01 g of mercuric iodide with 30 ml of cold water and slowly pour it with stirring into one litre of boiling water. Boil for three minutes. Allow to cool and decant off the supernatant clear liquid.
- (6) Potassium thiocyanate crystals.

(c) *Procedure*

- (1) Dissolve about 1g of the test sample (accurately weighed), in 50 ml of water.
- (2) Add a pinch of sodium carbonate till a slight turbidity appears. Then add 5 ml of acetic acid, 3 g of potassium iodide and titrate the liberated iodine with sodium thiosulphate solution, using starch as an indicator, until only a faint blue colour remains.
- (3) Add about 2 g of potassium thiocyanate, shake and continue the titration until the blue colour disappears.

Calculations

$$\text{Copper, per cent by mass} = \frac{6.35 \text{ V. N.}}{\quad}$$

Where V — Volume in ml of standard sodium thiosulphate solution.

N = Normality of standard thiosulphate solution, and M = Mass in g of the sample taken for the test.

{Reference : IS — 261 (1982).

(iii) Determination of lead (Pb)

Same as method No.8 (v).

Substitute zinc sulphate by copper sulphate.

(iv) Determination of soluble iron and aluminium compounds (expressed as Fe)

(a) *Outline of the method*

Iron and aluminium are determined gravimetrically by precipitation with ammonium hydroxide.

(b) *Reagents*

1. Concentrated nitric acid - AR grade
2. Ammonium chloride - AR grade
3. Dilute ammonium hydroxide - approximately 15 per cent NH₃ (m/v)
4. Dilute hydrochloric acid - 33 per cent (mlv)

(c) *Procedure*

- (1) Take 10 g of the test sample and add 25 ml of water, 2 ml of nitric acid and 5g of ammonium chloride.
- (2) Make the solution alkaline by adding ammonium hydroxide solution.
- (3) Keep it on a water bath until the precipitate has flocculated, keeping the solution alkaline by the addition of more ammonium hydroxide, if necessary.
- (4) Filter and wash the residue with dilute ammonium hydroxide.
- (5) Dissolve the residue in hot dilute hydrochloric acid.
- (6) Make the solution again alkaline by adding ammonium hydroxide and allow the precipitate to settle.
- (7) Filter and wash the residue with water.
- (8) Dry the residue, ignite and weigh till a constant mass is obtained.

Calculation

Soluble iron and aluminium compounds (as Fe) per cent by mass = $70M_1/M_2$

Where M_1 = Mass in g of the residue obtained and

M_2 = Mass in g of the sample taken for the test

(v) *Determination of matter insoluble in water*

- (a) Dissolve 25.0 g of the material in 125 ml of water. Filter through a weighed and prepared Gooch crucible or sintered glass crucible (G.No. 4) and wash the residue thoroughly with water. Dry the crucible at $110^{\circ}+5^{\circ}$ C to constant mass.

Calculations

Matter insoluble in water per cent by weight = $4A$, where A = weight in g of the residue

(vi) Determination of pH

(a) *Procedure*

Dissolve 5 g of the test sample in water and make up the volume to 100 ml. Determine the pH with glass electrodes using a suitable pH meter.

12. Method of analysis of ferrous sulphate (heptahydrate) ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)

(i) Quality of reagents

Unless specified otherwise, pure chemicals and glass distilled or demineralised water shall be used in tests.

Note . ‘Pure chemicals’ mean chemicals that do not contain impurities which affect the results of analysis. Demineralised water means the water obtained after passing distilled water through a cation and an anion exchange resin or a combined cation-anion exchange resin.

(ii) Determination of ferrous iron

(a) *Outline of the method*

Ferrous iron content is determined by titration with standard potassium permanganate solution.

(b) *Reagents*

1. Dilute sulphuric acid - 4N approximately.
2. Standard potassium permanganate solution - 0.1 N.
3. Orthophosphoric acid.

(c) *Procedure*

1. Weigh accurately about 5 g of the prepared sample and dissolve it in water.
2. Add 1 to 2 ml of dilute sulphuric acid and make up the solution to 250 ml in a volumetric flask.
3. Pipette out exactly 50 ml of the solution in a flask.
4. Add 10 ml of dilute sulphuric acid and 2ml of orthophosphoric acid and titrate with

standard potassium permanganate solution. *Calculations*

Ferrous iron, per cent by weight = $\frac{27.92 \text{ V. N.}}{W}$

W

Where

V = Volume of standard potassium permanganate used.

N = Normality of standard potassium permanganate solution.

W = Weight in g of the material taken for the test.

(Reference : IS: 262-1982)

(iii) **Determination of total iron**

(A) *Titrametric method using potassium dichromate*

(a) *Outline of the method*

Total iron is determined by reducing ferric iron with stannous chloride and then titrating the total ferrous iron with standard potassium dichromate solution.

(b) *Reagents*

- (1) Concentrated hydrochloric acid.
- (2) Stannous chloride solution - Dissolve 6.0 g of stannous chloride crystals ($\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$) in 60 ml of concentrated hydrochloric acid and dilute with water to 100 ml. Keep this solution in stoppered bottle.
- (3) Mercuric chloride solution - saturated.
- (4) Concentrated sulphuric acid.
- (5) Diphenylamine indicator - Dissolve 0.1 g of diphenylamine in 100 ml of concentrated sulphuric acid.
- (6) Standard potassium dichromate solution - 1.0 N.

Procedure

- (1) Weigh accurately about 5 g of the prepared sample and dissolve it in water.
- (2) Add 1 to 2 ml of dilute sulphuric acid and make up the volume to 250 ml in a volumetric flask.
- (3) Take 50 ml aliquot of this solution and add 10 ml of concentrated hydrochloric acid and heat to boiling.
- (4) Reduce the iron by adding stannous chloride solution until the yellow colour of the ferric chloride disappears. Do not add more than 2 to 3 drops of stannous chloride in excess.
- (5) Cool the solution, add rapidly mercuric chloride solution and stir so that a white precipitate of mercurous chloride forms slowly, indicating that a slight excess of stannous chloride is present.
- (6) Allow to stand for 5 to 10 minutes, dilute to 150 to 200 ml and add 5 ml of phosphoric acid and 5 drops of diphenylamine indicator. Titrate with standard potassium dichromate solution until the colour of the solution changes to a deep blue which does not fade on stirring.

Calculations

27.92 V.N.

$$\text{Total iron, per cent by weight} = \frac{\quad}{W}$$

Where V = Volume in ml of potassium dichromate used in titration.

N = Normality of standard potassium dichromate solution and W = Weight in g of the material present in the aliquot.

(Reference IS: 262-1967)

(B) Atomic absorption spectrophotometer method

(a) Regents

(1) Standard iron solution — (1000 ppm) — Weigh accurately 1 g pure iron wire and put it in approximately 30 ml 6 N HCl, in a beaker and boil. Transfer it to one litre volumetric flask through the funnel giving several washings to the beaker and funnel with glass distilled water. Make the volume up to the mark. Stopper the flask and shake the solution well. This is 1000 ppm iron solution.

(2) Glass distilled or mineralised water of pH 2.5 ± 0.5.

(3) Preparation of working standards — Pipette 10 ml iron stock solution in 100 ml volumetric flask and dilute to volume. This is 100 ppm iron solution.

Pipette the following volumes of 100 ppm iron solution in 50 ml numbered volumetric flask and make the volume with acidified water.

Flask No.	Volume of 100 ppm standard iron solution taken (ml)	Concentration of iron after making volume to 50 ml (pPm)
L	0.0	0.0
2	1.0	2.0
3	2.0	4.0
4	3.0	6.0
5	4.0	8.0
6	5.0	10.0
7	6.0	12.0
8	7.0	14.0
9	8.0	16.0
10	9.0	18.0
11	10.0	20.0

Stopper the flask and shake the solution well.

(b) Procedure

(1) Preparation of ferrous sulphate fertiliser sample — Weigh exactly 1.0009 of the material on a clean watch glass and transfer it to a one litre volumetric flask through the funnel giving repeated washings with acidified water and dissolve the material by shaking well. Make the volume up to the mark with acidified water and shake well.

(2) Take 5 ml of the prepared solution in 100 ml volumetric flask and make up the volume with acidified water. Shake the solution well and filter through Whatman No.42 filter paper in dry, clean flasks. The flasks should be rinsed with a 10 to 15 ml of the

filtrate and then continue filtration.

- (3) Flammng the solutions — Flame the standards and the filtered sample on atomic absorption spectrophotometer at a wavelength of 248.3 nm using clean air-acetylene flame.

Calculations

Prepare a standard curve of known concentrations of iron solutions by plotting the absorbance value on Y-axis against their respective iron concentrations on X-axis. Determine the concentration of iron in the sample solution from the graph.

$$\text{Total iron per cent} = \frac{2.X}{W}$$

Where

X = Concentration of Fe (in ppm) obtained from the standard curve. W = Weight in g of the material taken for the test.

- (iv) Determination of ferric iron

Subtraet the value of Ferrous iron per cent from total iron per cent to obtain the ferric iron per cent in the sample.

(Reference : IS: 262-1967)

- (v) **Determination % EH**

Dissolve 5.0 g of the sample in 100 ml of freshly boiled and cooled water and determine the pH by the means of pH meter using glass electrode .

- (vi) Determination of matter insoluble in water

Same as method No.8 (vii).

- (vii) Determination of lead

Same as method No.8 (v), Substitute zinc sulphate by ferrous sulphate.

- (viii) Determination of free acidity in ferrous sulphate

(a) *Regents*

- (1) Standard sodium hydroxide solution -0.02 N.
- (2) Methyl red indicator - Dissolve 0.15 g of water soluble methyl red in 500 ml water
- (3) Methyl red-Methylene blue mixed indicator solution - prepared by mixed equal volumes of 0.2 per cent solution in rectified spirit of methyl red and 0.1 per cent solution in rectified spirit of methylene blue.

(b) *Procedure*

- (1) Dissolve about 20 g of prepared sample, accurately weighed in about 50 ml cold natural water.
- (2) Filter and make up the volume to about 200 ml.
- (3) Titrate with standard sodium hydroxide solution, using one or two drops of methyl red as indicator.
- (4) If satisfactory end point with methyl red is not obtained, methyl red-methylene blue mixed indicator may be used.

- (5) Use preferably a micro burette *for* this titration. The filtering medium shall be neutral and shall not contain any alkaline material which would neutralise free acid.

Calculations

$$\text{Free acidity as H}_2\text{SO}_4, \text{ per cent by weight} = \frac{4.904 \text{ AN}}{W}$$

A = volume of ml of standard NaOH solution N = Normality of standard NaOH solution

W = Weight in g of prepared sample taken for test

13. Method of analysis of ammonium molybdate ($\text{NH}_4\text{Mo}_2\text{O}_7 \cdot 4\text{H}_2\text{O}$)

- (i) Quality of reagents

Unless specified otherwise, pure chemicals and glass distilled or demineralised water shall be used in tests.

Note. 'Pure chemicals' mean chemicals that do not contain impurities which affect the results of analysis. 'Demineralised Water' means the water obtained after passing distilled water through a cation and anion exchange resins or a combined cation-anion exchange resin.

- (ii) Determination of molybdenum

(A) *Atomic absorption spectrophotometer method*

(a) *Reagents*

- (1) Potassium sulphate — Dissolve 25 g of AR potassium sulphate in 1 litre of distilled water.
- "(2) Standard molybdenum solution — Weigh 1.5 g of molybdenum trioxide (MoO_3) and dissolve in 100 ml of water containing 10 ml of 1:1 HCl and dilute it to 1 litre in volumetric flask. This 1000 ppm standard molybdenum solution"
- (3) Preparation of working standards — Pipette the following volume of 1000 ppm standard Mo solution in 100 ml numbered volumetric flasks. Add 20 ml of potassium sulphate solution to each flask and make up the volume to 100 ml. Stopper the flask and shake them well.

Flask No.	Volume of 1000 ppm Mo solution taken (ml)	Volume of Potassium sulphate added (ml)	Concentration of molybdenum after making the volume to 100 ml (ppm)
i.	0.0	20.0	0
2.	2.0	20.0	20
3.	3.0	20.0	30
4.	4.0	20.0	40
5.	5.0	20.0	50
6.	6.0	20.0	60

(b) *Procedure*

- (1) Preparation of ammonium molybdate fertiliser sample — Weigh exactly 0.1 g of the material on the clean watch glass and transfer it to a 100 ml volumetric flask through funnel giving repeated washings and dissolve the material by shaking well. Make the volume upto the mark.
- (2) Take 10 ml of the prepared solution in a 100 ml volumetric flask, add 20 ml of potassium sulphate solution and make the volume upto the mark.
- (3) Flaming the solution — Flame the standards and the filtered samples on an atomic absorption spectrophotometer at a wavelength of 313.3 nm using nitrous oxide-acetylene flame.

Calculations

Prepare a standard curve of known concentration of molybdenum solution by plotting the absorbance value on Y-axis against their respective molybdenum concentration on X-axis.

$$\text{Where} \quad \text{Per cent of Mo} = \frac{X}{10 W}$$

X = Concentration of molybdenum (ppm) as obtained from the calibration curve.

W = Weight in g of the material taken for the test.

(B) *Colorimetric method for determination of molybdenum*

(a) *Outline of the method*

Molybdenum (vi) in acid solution when treated with stannous chloride (best in the presence of a little ferrous iron) is converted largely into molybdenum (v). This forms a complex with thiocyanate iron, probably largely $\text{Mo}(\text{SCN})_5$, which is red in colour. The latter may be extracted with solvents possessing donor oxygen atoms (3-Methylbutanol is preferred). The colour depends upon the acid concentration (optimum concentration 1M) and the concentration of thiocyanate ion (< 1.0 per cent, but colour intensity is constant in the range 2-10 per cent); it is little influenced by excess of stannous chloride. The molybdenum complex, has maximum absorption at 465 nm.

(b) *Apparatus*

(1) Photometer — Any suitable photoelectric colorimeter .

(c) *Reagents*

(1) Standard molybdenum solution — (0.001 per cent molybdenum) Weigh 0.184 g of A.R. Ammonium molybdate $(\text{NH}_4)_2\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ on a clean watch glass and transfer it to one litre volumetric flask through the funnel giving several washings to watch glass and funnel with glass distilled water. Make the volume upto the mark. Pipette out 10 ml of this solution into 100 ml volumetric flask and make the volume up to the mark. This gives 0.001 per cent Mo solution.

(2) Ferrous ammonium sulphate — Dissolve 10 g of AR ferrous ammonium sulphate salt in 100 ml of very dilute H_2SO_4 .

(3) Stannous chloride solution — Dissolve 10 g of AR stannous chloride dihydrate in 100 ml of 1M — hydrochloride acid.

(4) Potassium thiocyanate solution — Dissolve 10 g of AR potassium thiocyanate salt in 100 ml of distilled water.

(5) Iso-amyl alcohol.

(d) *Preparation of sample*

Weigh 0.1 g of sample on a clean watch glass and transfer it to one litre volumetric flask, through the funnel giving several washings to watch glass and funnel with glass distilled water. Make the volume up to the mark. Pipette out 5 ml of this solution into a 100 ml of volumetric flask and make up the volume up to the mark.

(e) *Procedure*

- (1) Place 1.0, 2.0, 3.0, 4.0 and 5.0 ml of the 0.001 per cent molybdenum solution (containing 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg and 0.05 mg molybdenum) severally in 50 ml capacity separating funnels and diluting each with an equal volume of water.
- (2) Add to each funnel 2.0 ml of conc. HCl, 1.0 ml of ammonium ferrous sulphate and 3.0 ml of the potassium thiocyanate solution.
- (3) Shake gently and then introduce 3.0 ml of the stannous chloride solution.
- (4) Add water to bring the total volume in each separating funnel to 25 ml and
- (5) Pipette 10 ml of redistilled 3-methylbutanol (iso-amyl alcohol) into each funnel and

shake individually for 30 seconds.

- (6) Allow the phases to separate and carefully run out the lower aqueous layer.
- (7) Remove the glass stopper and pour the alcoholic extract through small plug of purified glass wool in a small funnel and collect the organic extract in a 1.0 cm absorption cell.
- (8) Measure the absorbance at 465 nm in a spectrophotometer against a 3-methylbutanol blank.
- (9) Plot absorbance against concentration of standard molybdenum solutions and draw the calibration curve.
- (10) Take 10 ml of the sample solution and determine the absorbance of it by subjecting it to the same treatment as the standard solutions, using calibration curve, determine the corresponding concentration of molybdenum in the sample solution.

Calculations

$$\text{Per cent Mo} = \frac{2000.X}{Y.W}$$

Where

X = Concentration of molybdenum (in mg) of the test solution. Y = Volume of sample solution taken for the test.

W = Wt in g of the material taken for the preparation of the sample solution.

(Reference : Vogel's Text Book of Quantitative Analysis)

(iii) Determination of matter insoluble in water

(a) Procedure

Dissolve 25.0 g of the material in 125 ml of water. Filter through a weighed and prepared Gooch crucible or sintered glass crucible (G.No. 4) and wash the residue thoroughly with water. Dry the crucible at $110^{\circ} + 8^{\circ} \text{C}$ to constant mass.

(b) Calculations

Matter insoluble in water per cent by weight = $4A$ Where

A = Weight in g of the residue.

(iv) Determination of lead

Same as per method No. 8(v). Substitute zinc sulphate by ammonium molybdate.

14. Method of analysis of chelated zinc (as Zn-EDTA)

(i) Reagents

- (a) EDTA solution — (0.05M) Dissolve 18.612 g of disodium ethylene diamine tetra-acetate dihydrate (EDTA) in distilled water and make up the volume to 1 litre.
- (b) Standard zinc solution (1000 ppm) — Weigh accurately 1.0 g of zinc metal in a beaker. Add 20 ml HCl (1: 1). Keep it for few hours and allow it to dissolve completely. Transfer the solution to 1 litre volumetric flask. Make the volume upto the mark.
- (c) Concentrated ammonia solution (sp. gr. 0.88)
- (d) Ammonium nitrate AR grade salt.
- (e) Buffer solution (pH-10) — Dissolve 8.0 g AR grade ammonium nitrate in 65 ml of water and add 35 ml of concentrated ammonia solution (sp. gr. - 0.88)

- (f) Eriochrome black (T) indicator mixture — Mix thoroughly 1 gm of eriochrome black(T) indicator with 100 mg of AR grade potassium nitrate.
- (g) Hydroxylamine hydrochloride — AR grade.
 - (h) Potassium cyanide — AR grade (to be used with extreme care) 15 per cent aq. solution.
 - (i) Manganese sulphate solution — Dissolve 11.15 g of AR grade manganese sulphate in 1 litre of distilled water .
 - (j) Sodium fluoride AR grade.
 - (k) Triethanolamine (1:1)

(ii) *Preparation of sample solution*

Weigh accurately 1.0 g of the sample and transfer it to 100 ml volumetric flask. Make up the volume with distilled water. Keep it overnight.

(iii) *Procedure*

(a) *Standardisation of EDTA solution*

- (1) Take 10 ml of zinc solution (standard).
- (2) Dilute it by adding 30 ml distilled water.
- (3) Add 10 ml of buffer solution and 30-40 mg of indicator mixture.
- (4) Titrate with EDTA solution till clear blue end point is obtained. Note the volume of EDTA used as V_1 ml.

(b) *Standardisation of manganese sulphate solution*

- (1) Take 25 ml of manganese sulphate solution.
- (2) Dilute it by adding 100 ml distilled water .
- (3) Add 0.25 g of hydroxylamine hydrochloride, 2 ml triethanolamine solution and 10 ml of buffer solution.
- (4) Add 30-40 mg of indicator mixture.
- (5) Titrate with EDTA solution till clear blue end point is obtained. Note the volume of EDTA used as V_2 ml.

(c) *Determination of EDTA content of Zn-EDTA fertiliser*

- (1) Take 10 ml of sample solution.
- (2) Dilute it by adding 100 ml of distilled water.
- (3) Add 0.25 g of hydroxylamine hydrochloride, 2ml triethanolamine solution.
- (4) Add 10 ml of buffer solution and 30-40 mg of indicator mixture, red colour will appear.
- (5) Warm to 40°C and titrate with standard EDTA solution (preferably stirring magnetically) to clear blue end point. Note the volume of EDTA used as V_3
- (6) After the end point add 2.5 g of sodium fluoride and stir for one minute.
- (7) Titrate the solution with standard manganese sulphate solution, slowly till a permanent red colour is obtained. Note the volume of manganese sulphate added as V_4 ml.

- (8) Stir for 1 minute.
- (9) Titrate the excess of manganese ions with EDTA solution until the colour changes to pure blue. Note the volume of EDTA used as V, ml.
- (10) After the second end point (step 9) add 1 gm KCN/NaCN.
- (11) Titrate it with manganese sulphate solution till colour changes sharply from blue to red. Note the volume of manganese sulphate solution added as V₆ ml.

Calculations

$$\begin{array}{r}
 \text{Molarity of EDTA solution (M}_1\text{)} = \frac{\text{Molarity of standard zinc solution} \times \text{volume of standard zinc solution taken}}{\text{Volume of EDTA used (V)}} \\
 \\
 \text{Molarity of standard manganese sulphate solution (M}_2\text{)} = \frac{M V_Z}{\text{Volume of standard manganese sulphate solution taken}}
 \end{array}$$

Nos of millimoles of EDTA used in titrating Zn + other metals

(A) = MCV,

Nos of millimoles of EDTA liberated by sodium fluoride (B)

= $M_Z V$, - MCV,

Hence Nos of millimoles of EDTA used for titrating zinc (C) = A - B

But Nos of millimoles of EDTA liberated by KCN (D) = $M_2 \cdot V$, Hence Nos of millimoles of EDTA contained by

Zn- EDTA sample = D — C EDTA per

cent = $372.24(D - C)$

Per cent of manganese in the sample = $24.31 \times B$ Per cent of

free zinc = $65.38 \times C$

Per cent zinc chelated with EDTA = $65.38(D - C)$

(Reference Vogel's Text Book of Quantitative Inorganic Analysis)

Note - In case blue colour appears at step(c)(4), proceed as follows:-

1. Titrate with standard manganese sulphate solution to clear Red end point. Note the volume of manganese sulphate added as V, ml.
2. Add about 1 gram of KCN or NaCN. Swirl the flask cautiously. Blue colour will re-appear.
3. Titrate with standard manganese sulphate solution till blue colour sharply changes to red. Note the volume of manganese sulphate solution add as Vg ml.

Calculations

Per cent chelated Zinc as Zn-EDTA = $65.38(M, Vg)$.

16. Method of analysis of chelated iron (as Fe-EDTA)

(i) Quality of reagents

Unless specified otherwise, pure chemicals and glass distilled or demineralised water shall be used in tests.

Note. 'Pure chemicals' means chemicals that do not contain impurities which affect the result of analysis. 'Demineralised Water' means the water obtained after passing distilled water through a cation and an anion exchange resins or a combined cation-anion exchange resin.

(ii) Determination of chelated iron

(a) Reagents

- (1) Sodium hydroxide solution — 0.5 N, Dissolve 20 g NaOH in water and dilute to 1 litre
- (2) Disodium EDTA solution = 0.66 per cent, Dissolve 0.73 g of $\text{Na}_2\text{H}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$ in water and dilute to 100 ml.
- (3) Iron standard solution
 - (A) Stock solution — (1000 ppm) Dissolve 1.000 g pure Fe wire in approximately 30 ml 6 N HCl with boiling. Dilute to 1 litre in a volumetric flask with distilled water.
 - (B) Intermediate solution — (100 ppm). Pipette 10 ml iron stock solution and 10 ml $\text{Na}_2\text{H}_2\text{EDTA}$ solution in 100 ml volumetric flask and dilute to volume.

(C) Working solution — Pipette the following volumes of 100 ppm intermediate solution in 50 ml numbered volumetric flask and make the volume with 0.5 N HCl.

Flask No.	Volume of 100 ppm standard iron solution taken (ml)	Concentration of Fe after making volume to 50 ml (ppm)
1	1.0	2.0
2	2.0	4.0
3	3.0	6.0
4	4.0	8.0
5	5.0	10.0
6	6.0	12.0
7	7.0	14.0
8	8.0	16.0
9	9.0	18.0
10	10.0	20.0

(b) *Apparatus*

Atomic absorption spectrophotometer with air acetylene flame.

(c) *Procedure*

(1) Preparation of sample solution

- (A) Weigh sample containing approximately 40 mg Fe into 200 ml tall form beaker.
- (B) Wet with 2-3 drops of alcohol and dissolve in 100 ml of water .
- (C) Add 4 drops of 30 per cent H_2O_2 , mix and adjust pH of solution to 8.5 with 0.5 N NaOH. If pH drifts above 8.8, discard solution and repeat analysis.
- (D) Transfer solution to 200 ml volumetric flask, dilute to volume with water and mix.
- (E) Filter solution through quantitative paper.
- (F) Pipette 10 ml filtrate into 200 ml volumetric flask and dilute to volume with 0.5 N HCl.

(2) Flaming the solution

Flame the standards and the sample solution on atomic absorption spectrophotometer at a wavelength of 248.3 nm using air acetylene flame. In same manner determine Fe blank on all reagents used.

Calculations

Prepare a standard curve of known concentration of Fe solution by plotting the absorbance value on Y-axis against their respective Fe concentration on X-axis.

$$\text{Chelated iron per cent} = \frac{(\text{ppm Fe in sample} - \text{ppm Fe in blank}) \times 0.4}{\text{g sample}}$$

g sample

(Reference : Method of Analysis, AOAC, 1984)

(iii) Determination of R

As per method No. 8(vi) (a)

17. Determination of sodium in potassium chloride and potassium sulphate by atomic absorption spectrophotometric method

(i) *Quality of reagents*

Unless specified otherwise, pure chemicals and glass distilled or demineralised water shall be used in tests.

(ii) *Reagents*

- (I) Ammonium oxalate solution Dissolve 40 g of ammonium oxalate in 1 litre of distilled water.
- (2) Standard sodium solution --- (1000 ppm) --- Weigh accurately 2.5421 g of dried NaCl on a clean watch glass and transfer it to one litre volumetric flask. Make up the volume upto the mark. Stopper the flask and shake the solution well. This is 1000 ppm sodium solution.
- (3) Preparation of working standard — Pipette the following volumes of 1000 ppm standard sodium solution in 100 ml volumetric flask and make up the volume with glass distilled water.

Flask No.	Volume of 1000 ppm standard sodium solution taken (ml)	Concentration of sodium after making volume to 100 ml (ppm)
1	0.0	0.0
2	2.0	20.0
3	4.0	40.0
4	6.0	60.0
5	8.0	80.0
6	10.0	100.0
7	12.0	120.0
8	14.0	140.0
9	16.0	160.0
10	18.0	180.0
11	20.0	200.0

Stopper the flask and shake the solution well.

(iii) *Procedure*

- (1) Preparation of sample solution — Weigh exactly 2.5 grams sample on a clean watch glass and transfer it to 250 ml volumetric flask through the funnel giving repeated washings with glass distilled water (about 125 ml of water should be used). Add 50 ml of ammonium oxalate solution. Boil for 30 minutes, cool, dilute to volume, mix and pass through dry filter paper (Whatman No.1 or equivalent).
- (2) Flaming the solution — Flame the standards and the filtered sample on atomic

absorption spectrophotometer at a wavelength of 330.3 nm using clean air acetylene flame.

Calculations

Prepare a standard curve of known concentrations of sodium solution by plotting the absorbance value on Y-axis against their respective sodium concentration on X-axis. Determine the concentration of sodium in the sample solution from the graph.

Sodium (Na) as sodium chloride % = $0.0254 X$ where X is the concentration of sodium in ppm obtained from the standard curve.

(Reference AOAC, 1984).

18. Determination of total chlorides in potassium sulphate by silver nitrate volumetric method

(i) Reagents

- (1) Standard silver nitrate (AgNO_3) solution - 0.1 N
- (2) Potassium chromate (K_2CrO_4) indicator solution -5 per cent

(ii) Procedure

- (1) Weigh accurately about 10 grams of the prepared sample and transfer into 500 ml beaker.
- (2) Add about 250 ml of water and warm gently for about 20 minutes.
- (3) Cool and dilute the solution to 500 ml in a volumetric flask.
- (4) Allow to stand to let any insoluble matter settle.
- (5) Transfer with a pipette 100 ml of the clear solution into a conical flask.
- (6) Titrate with standard AgNO_3 solution (0.1 N) using 1 ml of potassium chromate indicator solution.

Calculations

Total chlorides (as Cl), per cent by

$$\text{Weight (on dry basis)} = \frac{17.73 N, V}{\text{—————}}$$

Where, N = Normality of standard AgNO_3 solution.

19. Methods of analysis of magnesium sulphate

(i) Quality of reagents

Unless specified otherwise, pure chemicals and glass distilled or demineralised water shall be used in tests.

(ii) Determination of magnesium

(A) Atomic absorption spectrophotometric method

(a) Reagents

- (1) Hydrochloric acid 0.5 N.

- (2) Magnesium Standard solution - Dissolve 1.0 g of magnesium metal in 30 ml of 1:1 HCl and dilute to 1000 ml in volumetric flask. This is a 1000 ppm magnesium stock solution.
- (3) Strontium chloride solution — Dissolve 15 g strontium chloride ($\text{Sr Cl}_2 \cdot 6\text{H}_2\text{O}$) in 0.5 N hydrochloric acid and dilute to 100 ml with the same solvent.

(b) *Preparation of working standards*

- (1) Pipette 1.0 ml of 1000 ppm magnesium stock solution to 100 ml capacity volumetric flask and make up the volume with 0.5 N hydrochloric acid. This is 10 ppm Magnesium solution.
- (2) Pipette the following volume of 10 ppm magnesium solution in 100 ml numbered volumetric flask. Add 10 ml of strontium chloride solution to each flask and make up the volume to 100 ml with 0.5 N hydrochloric acid.

Flask No.	Volume of 10 ppm Mg solution taken (ml)	Volume of strontium chloride (ml)	Concentration of Mg (ppm) after making the volume to 100 ml
1	0.0	10	0.0
2	2.0	10	0.2
3	4.0	10	0.4
4	6.0	10	0.6
5	8.0	10	0.8
6	10.0	10	1.0

(c) *Procedure*

(1) Preparation of sample solution

- (A) Weigh 1 g of the sample and place in a 500 ml volumetric flask. Add about 300 ml water and boil for 30 minutes. Allow to cool, dilute to the mark with water, mix and filter.
- (B) Pipette 5 ml of the filtrate (A) into a 100 ml volumetric flask, make up to the mark with water and mix.
- (C) Transfer by pipette 5 ml of the diluted filtrate (B) into 100 ml volumetric flask, add 10 ml of strontium chloride solution; make up to the mark with 0.5 N HCl.
- (2) Blank solution — Prepare a blank solution from which only the sample has been omitted.
- (3) Flaming of solution — Flame the standard and sample solutions on Atomic Absorption Spectrophotometer at wavelength of 285.2 nm using Air Acetylene flame.

Calculations

Plot the calibration curve using the mean absorbances on Y-axis and the corresponding concentrations of magnesium (ppm) on X-axis. Determine the concentration of magnesium in the sample by reference to the calibration curve.

Magnesium per cent = 20X

where X = concentration of magnesium (in ppm) obtained from the standard curve.

(B) *Titrametric method (EDTA titrations)*

(Applicable to the samples which do not contain phosphate as impurity)

(a) *Reagents*

- (1) Buffer solution (pH-10.0) — Dissolve 67.5g ammonium chloride in 200 ml of distilled water, add 570 ml ammonia solution and dilute to 1 litre.
- (2) Potassium hydroxide — Potassium cyanide solution - Dissolve 280 g potassium hydroxide and 66 g potassium cyanide in 1 litre of distilled water.
- (3) Potassium cyanide solution (2%) — Dissolve 2 g potassium cyanide in 100 ml of distilled water.
- (4) Eriochrome black T indicator solution — Dissolve 0.2 g of indicator in 50 ml of methyl alcohol containing 2 g of hydroxylamine hydrochloride.
- (5) Calcium standard solution(1mg/ml) — Dissolve 2.4973 g calcium carbonate, primary standard grade, previously dried for 2 hours at 285° in HCl (1+10). Dilute to 1 litre with distilled water.
- (6) Calcein indicator mixture — Grind together 1 g calcein indicator(2', 7'- bis) bis (Carboxymethyl) amino (methyl fluorescein, sodium derivative sodium salt), 10 g charcoal and 100 g potassium chloride.
- (7) Disodium dihydrogen ethylene diamine tetra acetic acid standard solution (0.4%) -Dissolve 4 g $\text{Na}_2\text{H}_2\text{EDTA}$ in 1 litre of distilled water.
- (8) Triethanolamine (1+1)
- (9) Potassium ferrocyanide solution (4%) Dissolve 4 g potassium ferrocyanide in 100 ml of distilled water.

(b) *Standardisation of calcium solution*

- (1) Pipette 10 ml calcium standard solution into 250 ml erlenmayer flask.
- (2) Add 100 ml of distilled water, 10 ml KOH-KCN solution, 2 drops of triethanolamine solution, 5 drops of potassium ferrocyanide solution and 15+1 mg of calcein indicator.
- (3) Immediately place the flask on a magnetic stirrer in front of daylight fluorescent light with white background.
- (4) While stirring, titrate with EDTA solution to disappearance of all fluorescent green and until solution remains pink. Titrate more than 3 aliquots. From average, calculate calcium titer value.

Calcium Titer
(mg/ml)

$\frac{\text{Volume of calcium standard solution (ml)}}{\text{Volume of EDTA solution used (ml)}}$

From calcium titer, calculate magnesium titer value as follows:

Magnesium titer = Calcium titer \times 0.6064

(c) *Preparation of sample solution*

- (1) Weigh 1g magnesium sulphate fertiliser sample into 250 ml volumetric flask.
- (2) Add 200 ml of distilled water and boil for 30 minutes.
- (3) Cool dilute to volume with water and mix.

(d) *Procedure*

(1) Titration for Ca + Mg

- (A) Pipette 25 ml of aliquot in 250 ml erlenmeyer flask.
- (B) Dilute with 100 ml of distilled water.
- (C) Add 5 ml of buffer solution (pH 10), 2 ml potassium cyanide solution, 2 drops of triethanolamine solution, 5 drops of potassium ferrocyanide solution and 8 drops of eriochrome black T indicator solution.
- (D) Titrate immediately with EDTA solution, stirring and lighting as in standardisation. Colour changes are wine, red, purple, dark blue, to clear blue end point, becoming green if over titrated. Note the volume of EDTA used as V_1 ml

(2) Titration for calcium

- (A) Pipette 25 ml of aliquot in 250 ml erlenmeyer flask.
- (B) Dilute with 100 ml of water.
- (C) Add 10 ml KOH-KCN solution, 2 drops of triethanolamine solution, 5 drops of potassium ferrocyanide solution and 15+1 mg of calcein indicator.
- (D) Titrate immediately with EDTA solution in standardisation. Note the volume of EDTA used as V_2 ml.

Calculation

Magnesium per cent = $(V_1 - V_2) \times \text{Mg Titer EDTA}$

(Reference :AOAC, 14th edition, 1984).

20. Determination of particle size in different fertilisers

(i) *Apparatus*

- (A) Indian standard sieves of 20 cms diameter and 5 cms in height with lid and bottom pan of required sizes.
- (B) Sieve shaker or vibrator with automatic timer and variable cycle control.
- (C) Balance, top loading with suitable capacity and sensitivity of 0.1 gm.
- (D) Weighing pan round suitable for holding sample and approximately 23 cms in diameter.
- (E) Brush either soft, fine wire or stiff.

(ii) *Procedure*

- (A) Take the sieve of required sizes and stack them in progressive order. The biggest sieve size should be on the top and the smallest in the bottom. Place the empty pan at the bottom.
- (B) Put 200 gms of ungrinded sample on the top sieve and place lid on the top of the stack.
- (C) Position and secure sieve stack in shaking apparatus.
- (D) Start shaking action for 5 minutes. If automatic timer is used, set timer for 5

minutes. If vibrator is used, also set vibrator control at 3000 cycle per minute.

Note. If mechanical sieve shaker is not available, use hand sieving. Conduct sieving by appropriate lateral and vertical motions accompanied by jarring action. Continue until no appreciable change is noted in sieve fractions.

- (E) After completion of shaking time, transfer material from each sieve to weighing pan with brush and weight to +/- 0.1 gm.
- (F) Record weight from each sieve (sum of weights from each sieve should agree closely with original sample weight),

Calculations

$$\text{Per cent weight on sieve} = \frac{\text{Weight on sieve (g)} \times 100}{\text{Total weight of sample (g)}}$$

(Reference : Method IV-A TFI -1982)

Note : In case of moist MOP sample, dry the sample at $100^{\circ}+5^{\circ}\text{C}$ for two hours (For urea at $70^{\circ}+5^{\circ}\text{C}$). Cool in a desiccator and then proceed for particle size analysis.

21. Determination of specific gravity of liquid fertilisers

(i) *By pycnometer method*

(a) *Apparatus*

(b) Pycnometer, 25 ml capacity, (Figure 7) (ii) Water bath *Procedure*

- (1) Weigh the empty, dry pycnometer to the nearest mg, after letting it stand for at least 30 minutes at room temperature.
- (2) Fill the dry pycnometer with sample until the neck is about half full.
- (3) Insert the glass pycnometer stopper with a rotary motion to secure a firm seat making sure no air entrapped.
- (4) Place the filled pycnometer in water bath (so that the top of the stopper is slightly above the water level) maintained at a specified temperature (T) for at least 30 minutes.
- (5) Carefully wipe off with soft absorbent paper any liquid from the top flat surface of the stopper while the pycnometer is still in the water bath.
- (6) Then remove the pycnometer, allow to stand at room temperature for 30 minutes, dry weigh to the nearest mg. The true density is calculated as follows:

Calculations

$$\text{True density at temperature T, g/ml} = \frac{S - P}{V}$$

Where

S = Weight of pycnometer full of liquid, g. P =

Weight of empty pycnometer, g.

V = Volume of pycnometer, ml

(ii) *By hydrometer method*

(a) *Apparatus*

- (1) Hydrometer cylinder, of glass or clear plastic 250 ml capacity. The internal diameter of the cylinder shall be atleast 25 mm greater than the outer diameter of the hydrometer used.
- (2) Hydrometer capable of measuring specific gravity of suitable range.
- (3) Constant temperature water bath capable of controlling temperature from 0 to 100°C .

(b) *Procedure*

- (1) Transfer approximately 250 ml of well mixed sample into a clean dry hydrometer cylinder and place in constant temperature water bath maintained at specified

temperature for 30 minutes.

- (2) Depress the hydrometer about 2 scale divisions into the liquid, and then release it.
- (3) When the hydrometer has come to rest floating freely away from the walls of the cylinder, estimate the reading to the nearest 0.001 unit. The correct reading is that point on the scale at which the principal surface of the liquid cuts the scale.
- (4) Record specific gravity reading.

21A. Method of analysis of calcium nitrate

- (i) Determination of total nitrogen - By the method as specified in 3 (viii)
- (ii) Determination of ammoniacal nitrogen - By the method as specified in 3 (vii)
- (iii) Determination of nitrate - By the method as specified in 3 (viii) & (vii)
- (iv) Determination of water insoluble matter - By the method as specified in 8 (vii)
- (v) Determination of water soluble calcium

A. Titrametric Method (EDNA) Titrations

(a) Reagents

- (i) Buffer solution (pH-12.0) - dissolve 225 gm KOH (AR) in 250 ml water. Add 15 ml Triethanolamine and make volume to one litre after cooling the solution.
- (ii) Pattern and Reader's indicator (HHSNNA) - Dissolve 0.2 gm indicator in 100 ml methanol.
- (iii) Calcium standard solution (mg/ml) - Dissolve 2.4973 g calcium carbonates, primary standard grade, previously dried for 2 hours at 285°C, in HCl (1*10). Dilute to 1 litre with distilled water.
- (iv) Disodium dihydrogen ethylene diamine tetra acetic standard solution (0.01 M) - Dissolve 3.7224 gm Na H - EDTA(AR) previously dried at 105 to 110°C for 1 hr in distilled water and make volume to one litre.

(b) Standardisation of calcium solution

- (i) Pipette 10 ml calcium standard solution, into 250 ml erlenmayer flask.
- (ii) Add 10-15 ml distilled water and 20 ml buffer solution. *In case of Potassium Magnesium Calcium Sulphate, add 50 ml distilled water and 20 ml buffer solution.
- (iii) Add few drops of indicator's solution. A red rose colour will appear.
- (iv) Titrate with EDTA till a blue colour is obtained.

Note the volume of EDTA used as V, ml

Volume of calcium standard
solution (ml)

Calcium Titer=-----

(mg/ml) Volume of EDTA solution
used (ml) (v,)

(c) *Preparation of sample solution*

- (i) Weigh 2.5 g calcium nitrate fertiliser sample into 250 volumetric flask.
- (ii) Make up the volume with distilled water. Shake well.
- * (iii) In case of Potassium Magnesium Calcium Sulphate, "weigh 2.5 gm of fresh sample in 250 ml volumetric flask and add 5 ml concentrated HCl, make up the volume and shake well.

(d) *Procedure*

- (i) Pipette 5 ml of aliquot in 250 ml erlenmeyer flask.
 - (ii) Add 10-15 ml distilled water & 20 ml buffer solution.
 - (iii) Add few drops of indicator's solution. A red rose colour will appear.
-

- (iv) Titrate with EDTA till a blue colour is obtained. Note the volume of EDTA used as V_2 ml

Calculations

Calcium per cent = $V_2 \times \text{calcium titer} \times 2$

B. Atomic absorption spectrophotometric method

(I) *Reagent*

Unless specified otherwise pure chemicals and glass distilled or demineralised water shall be used in test.

(a) *Hydrochloric Acid — 3 N*

(b) *Preparation of stock solution*

- (i) Calcium stock solution - Dissolve 2.498 gram CaCO_3 in a minimum amount of 3 N HCl. Dilute to 1 litre (1 000 ppm Ca). Dilute 10 ml to 100 ml. This is 100 ppm stock solution.
- (ii) Lanthanum stock solution - 50 g La/L

Dissolve 58.65 gram La_2O_3 in 250 ml HCl, adding acid slowly. Dilute to 1 litre.

Or Strontium chloride solution

Dissolve 159. strontium chloride ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$) in 0.5 NHCl and dilute to 100 ml

(c) *Preparation of working standard*

Pipette the following volume of 100 ppm calcium stock solution in 100 ml numbered volumetric flasks. Add 10 ml of strontium chloride solution or 20 ml of Lanthanum solution to each flask and make the volume to 100 ml with distilled water.

Flask No.	Volume of 100 ppm Ca solution taken (ml)	Concentration of calcium (ppm) after making the volume 100 ml.
1	0	0
2	1	1
3	2	2
4	3	3
5	4	4
6	5	5

(2) *Procedure*

- (1) Weigh exactly 1.0 g of sample on a clean watch glass and transfer it to 1000 ml volumetric flask through the funnel giving repeated washings with glass distilled water and make up the volume.
- (2) Take 1 ml of prepared solution in 100 ml volumetric flask, add 20 ml of lanthanum solution or 10 ml of strontium chloride solution and make up the volume. Filter, if required.
- (3) *Flaming of the solution*
Flame the standard and sample solution on AAS at wavelength of 422.7 nm using Air - Acetylene flame.

Calculations

Plot the calibration curve using the mean absorbance on Y axis and the corresponding concentration of Ca (ug/ml) at 'X' axis. Determine the concentration of calcium in the sample by reference to the calibration curve :

$$\%Ca = \text{Concentration (kg/ml)} \times 10^2$$

22. Method of analysis of potassium magnesium sulphate

Determination of water soluble K₂O by the method as specified in 5(i) Determination of Magnesium by the method as specified in 19(B) and 19(A) Determination of total chlorides by the method as specified in 18 Determination of sodium by the method as specified in 17

Determination of particle size by the method as specified in 20.

22 A. Analysis of micronutrient fertilisers mixtures by atomic absorption spectrophotometric method

(a) *Reagents*

- (1) Acidified water —Dissolve 10 ml of 10% Sulphuric Acid in 10 litre of double distilled water and adjust the pH to 2.5 (+0.5) with help of a pH meter using H₂SO₄ or NaOH.
- (2) Strontium chloride solution - Dissolve 7.5 g of strontium chloride (SrCl₂.6H₂O) in one litre of distilled water.
- (3) Potassium sulphate solution — Dissolve 25 g of AR potassium sulphate in one litre of distilled water.

(b) *Procedure*

- (i) *Preparation of standard stock solution*

Dissolve the specific quantity of standard material for specific element with the help of suitable solvent as indicated below and make up the volume 1000 ml with distilled water. Further dilute it to suitable flaming range with acidified water in case of Fe, Cu, Mn and Zn. In case of Mg, add 20 ml of strontium chloride and in case of Mo, add 20 ml of potassium sulphate before make up the volume up to the mark, in 100 ml volumetric flask with acidified water.

(ii) Preparation of sample solution

Weigh 10 grams of sample in 500 ml volumetric flask. Add 10 ml of 1:1 HCl and 100 ml distilled water, warm gently. Cool and make up the volume up to the mark with distilled water. Shake well and keep it stand for atleast 1 hour. Dilute it to further suitable working standard range with acidified water in case of Fe, Cu, Mn and Zn. In case of Mg, add 20 ml of strontium chloride and in case of Mo, add 20 ml of potassium sulphate before making up the volume upto mark, in 100 ml flask with acidified water".

Note : In case of micronutrient fertilisers mixtures containing Boron and Molybdenum, separate sample solution should be prepared by taking 10 g of fertiliser material and dissolving it in water cotaining 10 ml of Hydrochloric acid and diluted to 100 ml.

Element	Material	Solvent	Concent-ration in ppm	Flamin g range of standard ppm	Wavelengt h in rim	Flame
Zinc	1.0 g Metal	30 ml 1:1	1000	0.2-2.0	213.9	Air/CMH,
Iron	1.0 g Iron Wire	HCl	1000	2-20	248.3	Air/CMH,
Manga-nese	1.0g Metal	30 ml 1:1 HNO ₃ , 50 ml	1000	0.5-5	279.5	Air/CMH
Copper	1.0g Cooper Turning	Cone. HCl	1000	0.5-1	324.8	Air/CMH,
Boron	28.5720g Boric Acid	30 ml (1:1) HNO ₃ , Warm distille d	5000	400-1600	249.8	NGO/CMH ,
Molyb-denum	1.5g Molyb-Denum Trioxide	water	1000	20-60	313.3	Air/CMH, (Rich) or
Magne-sium	1.09 Metal Powder	10 ml HCl	1000	0.2-t	285.2	Air/CMH,
		30 ml 1:1 HCl				

(iii) Flaming of solutions

Flame the standard solution of specific element at concentration mode. Then flame the sample solution to observe the concentration (in ppm) of specified element.

Calculations

$$\% \text{ Concentration} = X \times \text{dilution factor} \times 10^{*}$$

(X = Concentration in ppm observed by instrument)

23. **Determination of boron in micronutrient fertiliser mixture**
Volumetric method

(a) *Reagents*

- (1) Mannitol
- (2) Bismuth nitrate solution : Dissolve 22 g of bismuth nitrate $[(\text{BiNO}_3)_3 \cdot 5 \text{HNO}]$ in 8 ml of conc. Nitric acid. Warm slightly and dilute to 100 ml with distilled water.
- (3) Nitric acid — Concentrated AR grade.
- (4) Dilute nitric acid — (1:20).
- (5) Sodium hydroxide solution — 10% M/V.
- (6) Sodium hydroxide solution — 0.02 M.
- (7) Bromothymol blue indicator solutions.

(b) *Procedure*

- (1) Weigh accurately 2.5 g of prepared sample in to 250 ml beaker.
- (2) Add 2 ml of conc. nitric acid and 50 ml of water and warm gently and dilute to 100 ml with water.
- (3) Warm the solution (but do not boil).
- (4) Slowly add 5 ml of Bismuth nitrate solution for each 1 per cent of P_2O_5 , per cent in the sample, from a burette with continuous stirring and maintaining the liquid hot during addition.
- (5) Cool and transfer the contents in to 250 ml volumetric flask and dilute up to the mark with distilled water.
- (6) Allow precipitate to settle and dilute up to the mark with distilled water.
- (7) Filter through a dry filter paper (No. 40) rejecting the first few drops in to a beaker.
- (8) Pipette 100 ml of the filtrate in 250 ml beaker, add a few drops of indicator solution.
- (9) Add few drops of 10% NaOH solution with thorough stirring until the indicator turns blue.
- (10) Filter through a dry filter paper (No. 40) into 250 ml beaker and carefully wash the residue several times with cold water. The total volume should be 150 to 200 ml.

- (11) Adjust the pH to about 5 by adding diluted HNO_3 . Warm to about 90°C (do not boil), and stir vigorously to avoid CO_2 . Cool the solution.
- (12) Place in the solution, the electrodes of pH meter and adjust the pH to exactly 6.3 using NaOH solution.
- (13) Add 10 gm of mannitol and again adjust pH to exactly 6.3 with 0.02 N NaOH solution.
- (14) Continue adding mannitol in 10 g portion till pH remains constant at 6.3. Note the total volume of 0.02 N NaOH used for the 1st addition of mannitol (step 13).
- (15) Carry out a blank determination on fertilisers mixture to which borate is not added.

(c) *Calculations*

$$\% \text{ B} = \frac{(\text{A}-\text{B}) \times 0.05405}{\text{W}}$$

Where : A — Volume of 0.02 N NaOH solution used in the sample. B =
Volume of 0.02 N NaOH solution used in the blank.

Note : In case phosphate is not present in the sample, proceed as follows:-

- (i) Take 2.5 g of the sample in 250 ml volumetric flask and make up the volume with distilled water.
- (ii) Proceed as given from step (8) onwards.

24. Determination of sulphur

(The total sulphur includes sum of sulphur present in Sulphate and elemental form).

(A) Determination of Sulphate Sulphur

Method of determination of sulphur present in the sulphate form in various fertilisers for nitrate free and nitrate containing samples is given below :

Classification of procedures

Procedure (a) : For nitrate free samples such as ammonium sulphate, potassium sulphate, zinc sulphate, copper sulphate, ferrous sulphate, manganese sulphate, N.P. and NPK complexes and mixtures.

Procedure (b) : Superphosphate

Procedure (c) : For nitrate containing fertilisers such as ammonium phosphate sulphate nitrate 20:20:0

(i) *Quality of Reagents*

Unless specified otherwise, pure chemicals, glass, distilled or demineralised water shall be used in tests.

Note : (1) "Pure chemicals" mean chemicals which do not contain impurities which affect the results of analysis.

(2) "demineralised water" means water obtained by water passing through a cation and anion exchange resins or a combined cation-anion exchange resins.

(ii) *Reagents*

- (i) Hydrochloric acid-concentrated
- (2) Dilute hydrochloric acid - Dilute 250 ml. of concentrated hydrochloric acid to 1000 ml. of water.
- (3) Barium chloride solution - 2 per cent. Dissolve 20 g of barium chloride in 1000 ml of water.
- (4) Silver nitrate solution - 5 per cent. Dissolve 5 g silver nitrate in 100 ml water.

(iii) *Procedure (a)*

- (1) Weigh about 2.5 g of sample and transfer to 250 ml capacity volumetric flask with the help of dilute hydrochloric acid.
- (2) Make up the volume with dilute hydrochloric acid.
- (3) Apply stopper, shake well and filter through Whatman Filter paper No.40 or equivalent part in a dry beaker if the solution is not clear and transparent.
- (4) Take 25 ml of the filtered aliquot in a beaker of 250 ml capacity. Add 100 ml of water and heat to boil. While stirring add in a slow stream 1 ml of

hot barium Chloride solution for each one per cent sulphur. Sulphur expected in sample plus additional 10 ml in excess to ensure complete precipitation of sulphur as barium sulphate. Boil for a minute.

- (5) Digest the precipitate on a hot plate or water bath for 2 hours at low temperature such that the solution does not boil. Ensure the supernatant liquid to be clear and transparent. Cool to room temperature. Filter into a 30 ml capacity G 4 grade sintered Gooch crucible previously dried at 250° C cooled and weighed. Wash the precipitate 10-12 times with hot water to ensure the precipitate to make it free from barium chloride. The filtrate may be tested with silver nitrate solution to confirm that the precipitate is free from chloride.
- (6) Dry the crucible and its content to 250° C for two hours in a furnace by raising the temperature of the furnace slowly from room temperature. After drying, cool to room temperature in a desiccator to constant weight taken.

Calculations

$$\text{Sulphate Sulphur (as S) per cent by weight} = W \frac{137.4 \times M}{\text{—————}}$$

Where M = weight of precipitate

W = weight of the sample taken

Procedure (b)

Weigh about 2.5 g of sample and transfer to a beaker of 250 ml capacity. Add 25 ml of concentrated hydrochloric acid and 25 ml of water. Heat to boil the solution gently. Boil for 5 minutes and cool. Add 75 ml of water and transfer quantitatively into a volumetric flask of capacity 250 ml with dilute hydrochloric acid solution and make up the volume. Apply stopper, shake well and filter about 50 ml or prepared solution through Whatman filter paper No. 40 or equivalent and proceed as at step (2) of Procedure (a) onward.

Procedure (c)

- (1) Weigh about 2.5 g of sample and transfer to a beaker of 250 ml capacity. Add 10 ml of concentrated hydrochloric acid and grind the sample with the help of a glass rod. Evaporate to dryness on a hot plate. Soak the mass again with few drops of concentrated hydrochloric acid and evaporate to dryness.
- (2) Add 100 ml of dilute hydrochloric acid, heat to boil and cool to room temperature. Transfer the content to a 250 ml volumetric flask. Filter a portion of the solution through a Whatman No. 40 filter paper or equivalent and proceed as at step (2) of Procedure (a) onwards.

Note : Based on AOAC 1995.

(B) Determination of elemental sulphur

(a) Reagents

- (1) Hydrochloric acid - concentrated (4N)
- (2) Sulphur powder (AR Grade)
- (3) Acetone saturated with sulphur - Add 2-3 g of sulphur powder in 250 ml of acetone in stoppered bottle. Shake well and allow it to stand for 1 hour. Filter through whatman filter paper No. 42 and collect filtrate in a stoppered glass bottle.
- (4) Carbon disulphide (AR Grade)

(b) Procedure

Take 1 g of prepared sample in a beaker. Add 50 ml of 4 N HCl boil for 5 minutes. Cool and filter through a sintered glass crucible (G 4). Wash thoroughly with distilled water. Wash the residue 5-6 times with 10 ml portion of acetone saturated with sulphur. Dry at 100°C for 1 hour. Cool in a desiccator and take weight of the crucible. Let the weight be W_1

After taking the weight of the crucible (W_1) wash the crucible 8-10 times with 10 ml portion of carbon disulphide thoroughly under slow suction. Dry the crucible at 100 °C for 1 hour. Cool in a desiccator and weigh. Let this weight be W_2

Calculation :

Elemental sulphur (as S) % by weight = W

$$(W_1 - W_2) \times 100$$

Where "W" is the weight of the sample taken

(C) Total sulphur (as S) per cent by weight = Sulphate sulphur (as per procedure 24A)+elemental sulphur (as per procedure 24 B).

25. Method of analysis of zincated phosphate (suspension)

Determination of total phosphate

By the method as specified in 4(ii) *Determination of citrate soluble phosphates* By the method as specified in 4 (v) *Determination of total zinc*

Procedure

- (i) Dissolve 1 gm of sample in 1:1 HCl for few minutes.
- (ii) Cool, filter and make up the volume suitably and proceed as per the procedure specified at Method No.7(iii) (b) (2) step (b) onwards or Method No.8(ii) (b) step (2) onwards.

26. Method of analysis of Zincated Oxide (suspension)

- (i) Determination of total zinc

Scope: Total extraction of Zinc (Zn), Arsenic (As), Lead (Pb) and Cadmium (Cd) from Zinc Oxide Suspension Concentrate.

Principle: Zinc and heavy metal impurities are extracted from the sample with boiling Aqua Regia.

(a) Sample preparation:

The details of the procedure for drawl of samples of fertilizers have been provided in Schedule II Part A, Serial No. 9 (Method for sampling of liquid fertilizers, other than anhydrous ammonia), however importance of proper shaking and mixing of contents before withdrawal of sample and before drawing aliquot for analysis is particularly emphasized. Since in suspension formulation insoluble materials might settle down over time, thorough mixing of contents would ensure representative composition along the entire depth of the container.

(b) Reagents:

All the reagents should be of analytical grade to ensure negligible concentration of the elements to be determined.

- (1) Glass double distilled water (free from micronutrients)
- (2) Hydrochloric acid 37% HCl (HCl) — 12 mol/l, $p = 1.18$ g/ml
- (3) Nitric acid 65% HNO₃ (HNO₃) — 14.3 mol/l, $p = 1.4$ g/ml

(c) Apparatus:

Apparatus for thermal heating digestion — with reaction vessel and reflux condenser. The vessel should be at least 5 times the volume of the aqua regia used.

In case reaction vessel with reflux condenser is not available, Erlenmeyer flask or high beakers covered with watch glass can be used for the purpose. Ash free filter paper is required if filtration is necessary.

(d) Procedure:

- (1) Weight one gram ($1 + 0.001$ g) of the sample and transfer quantitatively to the reaction vessel
 - (2) Moisten the sample with about 0.5 to 1.0 ml distilled water
 - (3) Mix the contents well and 21 ml of HCl followed by 7 ml of HNO₃ (reagent both drop

wise to reduce foaming).

- (4) Connect the condenser to the reaction vessel and let the mixture stand at laboratory room temperature until effervescence ceases.
- (5) Turn on the heating device and slowly raise the temperature of the reaction mixture to reflux condition. Maintain for 2 hours.
- (6) Ensure that the condensation zone is lower than half of the height of the condenser.
- (7) After 2 hours of reflux, allow to cool and rinse the condenser with 10 ml of distilled water.
- (8) Transfer the contents quantitatively into a 500 ml volumetric flask and dilute to the mark with water. The test solution corresponds to a 500 times dilution of the sample.
- (9) Test solution can be filtered, if necessary (should not be required for Zinc Oxide Suspension concentrate). If filtered, discard the first 20 ml (approx) portion for analysis
- (10) Prepare a blank test solution following the same procedure as the sample. This is to be used for background correction of analysis, in case of any possible contamination through reagents.
- (11) Measurement can be carried out immediately, or can be stored in tightly closed plastic vessels for up to 15 day.

Note:- Addition of one drop of octanol to the reaction vessel can be used as an antifoaming agent.

(e) Determination of Zinc in the extracted material:

Process for analysis of Zinc shall be as per the procedure specified at Method no. 7 (iii) (b) (2) step (B) onwards or method No. 8 (ii) (b) step (2) onward.

Note :- Calculation need to be adjusted accordingly, in view of extent of dilution of original sample.

- (ii) Determination of lead
By the method as specified in 8 (v) after extraction through aqua regia as specified in sub-item (d) of item (i)
- (iii) Determination of specific gravity By
the method as specified in 21
- (iv) For Determination of Arsenic
By the method specified as 8 (ix) after extraction through aqua regia as specified in sub-item (d) of item (i).
- (v) For Determination through Cadmium
By the method specified as 8 (x) after extraction through aqua regia as specified in sub-item (d) of item (i).

27. Determination of Boron in Colemanite

(a) *Reagents*

- (1) Mannitol
 - (2) Bismuth nitrate solution : Dissolve 22 g of bismuth nitrate ($(\text{BiNO}_3) \cdot 5\text{H}_2\text{O}$) in 8 ml of cone. Nitric acid. Warm slightly and dilute to 100 ml with distilled water.
 - (3) Nitric acid - Concentrated AR grade.
 - (4) Dilute nitric acid - (1:20).
 - (5) Sodium hydroxide solution - 10% M/V.
 - (6) Sodium hydroxide solution - 0.5 M.
- (Bromothymol blue indicator solutions.

(b) *Procedure*

- (i) Weigh accurately 2.5 g of sample into 250 ml of beaker.
- (ii) Add 5 ml of concentrated Nitric acid and 50 ml of water, warm gently and dilute to 100

ml cool and transfer the contents into 250 ml volumetric flask and dilute to the mark with distilled water.

- (iii) Filter through a dry filter paper Whatman No.40 rejecting the first few drops into a beaker. Pipette 100 ml of the filtrate into 250 ml beaker, add a few drops of indicator solution.
- (iv) Add few drops of 10% NaOH solution with thorough stirring until the indicator turns blue.
- (v) Filter through a dry filter paper into 250 ml beaker and carefully wash the residue several times with cold water. The total volume should be 150-200 ml. Adjust the pH to about 5 by adding dilute nitric acid. Warm to about 90°C (do not boil) and stir vigorously to avoid carbon dioxide. Cool the solution.

(VI
1)

(V111) Adjust the pH to exactly 6.3 using NaOH solution.

- (ix) Add 10g of mannitol and again adjust pH to exactly 6.3 with 0.5 NaOH solution. Continue adding mannitol in 10 g portion till pH remains constant at 6.3. Note the total volume 0.5 NaOH use for the first addition of mannitol.
- (x)

(xi) Carry out a blank determination to which borate is not added.

(c) *Calculation*

$$\text{Percentage boron} = \frac{(A-B) \times 1.3512}{W}$$

Where A is the volume of 0.5 N NaOH solution used in the sample. B is the volume of 0.5 N NaOH solution used in the blank.

28. Method of Analysis of Customised and Fortified Fertilisers:-

- (i) Determination of nitrogen by method 3 (iv) or 3(v).
- (ii) Determination of ammoniacal nitrogen by method 3 (vii).
- (iii) Determination of neutral ammonium citrate soluble phosphate by method 4 (v).
- (iv) Determination of water soluble phosphorus (available phosphorus) by method 4 (iii).
- (v) Determination of water soluble potash by method 5 (i).
- (vi) Determination of sulphur by method 24.
- (vii) Determination of micro-nutrients in Customised or Fortified Fertilisers.

(a) *Sample Preparation*

1. Weigh accurately 2.5 g sample into a 250 ml beaker. Add 50 ml of 1+1 HCl and cover with a glass.
2. Heat to boiling and continue to boil until volume is reduced to about 25 ml.
3. Dilute to about 100 ml with water and bring to boil.
4. Cool, transfer to a 500 ml volumetric flask and dilute to volume with water.
5. Mix thoroughly and allow to stand until a clear solution is obtained or filter a portion through a dry whatman No.40 filter paper.
6. Transfer 25 ml aliquot to 250 ml volumetric flask, dilute to volume and mix thoroughly.
7. Dilute it further to suitable standard working range with acidified water.
8. Prepare a blank solution in the same manner, omitting the sample.

(b) Preparation of Standard Stock Solution, working standards and determination:

As given in Method 22 A

(c) Determination of Boron-Method 23

29. Determination of Plant Available Silicon (Si(OH)₄)

CaCl₂ extraction of Silicon fertilizer amendment Analysis with UV

(a) Reagent : Do not store any reagent in glass containers, and do not expose to glassware any longer than absolutely necessary while making up reagents. e.g. dissolve reagent/prepare solution in plastic beaker with slightly less than the final volume of solvent, and use volumetric glassware only for a short interval at the very end to make up to volume.

1. Extractant 0.01 M CaCl₂ : Dissolve 7.35 g CaCl₂.2H₂O in de-ionised water, and make up to 5L.
2. Tartaric Acid: Dissolve 100 g of tartaric acid and make up to 500 ml with deionised water. Store in polyethylene bottle. Make up fresh solution when appreciable sediment forms. Store in refrigerator.
3. ANSA reducing agent: Dissolve 25 g of sodium bisulphite (NaHSO₃) in 200 ml water, and

combine with a solution containing 2 g of anhydrous sodium sulfate (Na_2SO_4) and 0.4 g of 1-amino-2-naphthol-4-sulfonic acid in 25 ml of water.

Dilute the combined solution to 250 ml and store in a polyethylene bottle in fridge. Discard if solution darkens in colour.

4. Ammonium paramolybdate tetrahydrate : Dissolve 54 g ammonium molybdate (see note 2) in about 800 ml deionised water in a plastic beaker. Adjust pH to 7 with 5 N NaOH or 0.5 M H_2SO_4 depending on whether the pH needs to be raised or lowered. Make up to 1 L, and store in polyethylene bottle in refrigerator.
5. 0.5M Sulfuric acid : 56 ml of conc. sulfuric acid (from plastic bottle) in 2 L deionised water.
6. 5N NaOH : 20 g of NaOH pellets in 100 ml of deionised water (Make and store in plastic container).

(b) Standards

A minimum of 5 calibration standards must be prepared ; covering the core range from 0 to 5.0 mg/L. Higher standards may be included. Suggested standards are : Si Primary standard - 1000 mg/L Si. Commercial Si standard solution is used.

Si Secondary standard - 50 mg/L Si - Dilute 50 ml primary standard to 1 L with deionised water. Store in tightly stoppered polyethylene bottle.

Working standards: Prepare 0, 0.5, 1, 2, 3, 4, 5, and 6 mg/L Si standards equivalent to 0, 5, 10, 20, 30, 40, 50, 60 mg/kg in the Silicon Fertiliser. Add 0, 0.5, 1, 2, 3, 4, 5 and 6 ml of secondary standard to separate 50 ml volumetric flasks and make up to volume with 0.01 M CaCl_2 .

0.01 M CaCl_2 is the 0 standard.

ml secondary standard in 50 ml flask	mg/L Si in solution	Equivalent Sr fertiliser mg/kg Si
0	0	0
0.5	0.5	5
1	1	10
2	2	20
3	3	30
4	4	40
5	5	50
6	6	60

Method

1. Dry approximately 2-3 grams of Silicon fertiliser in a 105°C oven overnight. Grind the sample if not already in powder form.
2. Weigh 0.4 g of dried Silicon fertiliser into a 70 ml container, and add 40 ml 0.01 M CaCl_2 . Lid, and shake in an end over end shaker overnight (16 hours). Centrifuge sample extract at 2000 rpm for 10 minutes. The extract should be clear.
3. Transfer 1 ml of filtrate into a 20 ml capacity test tube (See note 4)
4. Add 2.5 ml of 0.5 M sulfuric acid
5. Add 2.5 ml ammonium molybdate solution
6. Shake well on a vortex stirrer and wait 5 minutes.
7. Add 1.25 ml tartaric acid solution
8. Add 0.25 ml ANSA reducing solution. Mix well.
9. Prepare a sample blank for each sample in a similar way, but using water instead of the ammonium molybdate solution. Also prepare a reagent blank, using 0.01 M CaCl_2 instead of sample and water instead of the ammonium molybdate solution. The reagent blank is

- used to zero the spectrophotometer prior to calibration.
10. Prepare a standard curve from the working standards by using the same aliquot for the standards as is used for the samples and treat the same way. (See note 5).
 11. The spectrophotometer should be set to 820 nm wavelength. After 30 minutes, transfer reagent blank to spectrophotometer cell, place in machine and zero absorbance. Remove, transfer sample to cell, place in machine and record absorbance reading (i.e. colour intensity due to amount of soluble silica Si in sample). Wash cell before next sample with a small amount of the next sample blank.
 12. Read the concentration of silica Si in the soil sample, mg/kg Si, directly from a regression equation prepared from the standard curve."

Note :

1. Discard ANSA solution if it does not completely dissolve, or yields a dark solution immediately. Try again after changing source of de-ionised water.
2. Molybdate compounds are considered as environmental hazard. Waste containing those elements has to be collected and disposed of according to regional requirements.
3. Avoid use of glass whenever possible. All standards and reagents should be kept in polyethylene or other plastic bottles, particularly NaOH to avoid any possibility of it being stored in glass (NaOH is able to actively dissolve silica in glass).
4. Do not start filling glass test tubes in advances with any of the reagents or sample, at the glass can leach silicon.
5. As the same amount of each reagent is used for both standard solutions and samples, a small amount of Si contamination can be tolerated. The calibration line will allow for minor background contamination. However, if the blue colour in the zero stand becomes too intense, the working range may be pushed beyond the linear range of the calibration line, and all contain solutions must be prepared again. Si contamination may occur in one or more of the following solutions; de-ionised water (used to make up one or all of the solutions), 0.5 sulfuric acid, ammonium para molybdate, tartaric acid or ANSA. Another deionised water supply should be used, if available. If Si is still present, reagents and extractnt may need to be prepared in distilled, no de-onised water.

30. Method of analysis of Sodium Silicate

(i) Determination of Silicon

(a) Chemicals and Glasswares:

- (1) Conc. HCl
- (2) Conc. HNO₃
- (3) Hot Plate
- (4) Platinum Crucible
- (5) Muffle Furnace (Temp. capacity by 1000 °C)
- (6) Desiccators and other routine laboratory glasswares

(b) Procedure:

- (1) Take 1 gm of prepared sample in Teflon or corning beaker, add 2-3 ml HCl solution (1:1) and 2-3 ml HNO₃ solution (1:1).
- (2) Digest the solution on a hot plate till it becomes semi-solid, cool it and again add 3-4 ml HNO₃ solution. Dry it completely on hot plate.
- (3) Add 2-3 ml HCl solution(1:1) and boil it till yellow fumes cease. Filter it with Filter Paper No. 1, wash with 10 ml HCl solution (1:1) one time and 2-3 washing with hot water of 10 ml portion each till yellow colour disappear.
- (4) Transfer the filter paper alongwith residue in pre-weighed platinum crucible, dry for one and halfhour in muffle furnace at 250 °C temperature and finally ignite the residue at 950 °C temperature for 30 minutes.
- (5) Cool the crucible in desiccator and re-weigh and calculate

Calculation:

$$\% \text{ Si as SiO}_2 = \frac{\text{Final weight of crucible} - \text{empty weight of crucible}}{\text{Weight of sample}} \times 100$$

- (i) Determination of sodium
By the method as specified in serial number 17
- (ii) Determination of Specific gravity
By the method as specified in serial number 21

31. Method of analysis of nano Nitrogen

- (1) Determination of Nitrogen — Total Kjeldahl Nitrogen
- (a) Procedure:

- (1) Liquid Nano Fertilizer sample : Take aliquot quantity (10 ml) of sample in the Kjeldahl flask.
- (2) In case of solid Nano Fertilizer Sample : Take one gram of powdered nano-fertilizer sample and transferred to the Kjeldahl flask.
- (3) Add 15 g K₂SO₄, or 12 g anhydrous Na₂SO₄, 0.4 g anhydrous CuSO₄, or 0.6g CuSO₄.5H₂O, and approximately 0.8 g alundum granules.
- (4) Add 37 ml diluted Sulfuric acid with water H₂SO₄, +HNO₃(1+1, v/v) or 20 ml concentrated Sulfuric acid, if adequate ventilation is available.
- (5) Add sufficient test portion mass, precisely 0.1000 to 2.800 g for fertilizers with 30 to 5% nitrogen, respectively. Rinse the inner wall with about 10 ml water.
- (6) Transfer the flask to a preheated (400 °C) Kjeldahl block digester and digest test portions for 75 minutes. (Remove the flask from the heating block and upon cooling (the reaction mixture must be near room temperature). Wash the inner wall with 20-30 ml water and mix.
- (8) Prepare the distillate receiving flask(300 ml Erlenmeyer flask) by adding 30ml of 0.25 N standardized Sulfuric acid to trap the expected total Nitrogen in the test portion.
- (9) Add 2-3 drops of Methyl purple indicator and install the receiver on the outlet tube of the distillation unit, being sure that the distillate outlet tube end is totally immersed in the standardized acid solution.
- (10) Install the digestion tube on the distillation unit. Initiate steam generation and slowly dispense about 80ml (30 -35%) Sodium Hydroxide into the flask.
- (11) Continue steam distillation until about 250 ml or more of steam condensate has been collected in the receiving flask. This usually requires about 6-8 minutes.
- (12) If color changes to green, add more 0.25 N H₂SO₄, to bring the color back to purple and record the amount of acid added.
- (13) Titrate to a grey end point (pH 5.7) with 0.25 N Standard NaOH. The color of the distillate depends upon the amount of total nitrogen in the test portion, which is a function of the amount of ammonia trapped in the receiver flask.
- (14) A green color indicates that the acid in the trap was neutralized by the Ammonia. At this point, add an additional known amount of standardized H₂SO₄, to get to the grey end point.
- (15) The net volume (in ml) of standardized acid would be equal to the total amount of acid initially added to the receiving flask plus the amount of the acid added, after distillation, to reach the grey end point. A blue or Purple colour indicates that there is still acid in the receiving flask, and back titration with NaOH is required.
- (16) The net volume standardized acid would be equal to the amount of acid in the receiving flask minus the amount of base added, after distillation, to reach to the grey end point.

(b) Calculations:

Weight percent total nitrogen is calculated as follows: (net mL

$$\frac{\text{std acid} \times N \text{ of std acid} - (\text{net mL std base} \times N \text{ of std base}) \times 1.4008}{\text{Sample weight, g}}$$

Total N %

Sample weight, g

(ii) **Physical Particle Size** (as per **Transmission Electron Microscope (TEM) Analysis**)

(a) Equipment and Apparatus:

- (1) Transmission Electron Microscope
- (2) Sample grid
- (3) Tweezers, Petri dish, Ethanol and Deionized water

(b) Procedure:

- (1) TEM sample grids carbon coated film that is electron transparent supported by copper mesh or equivalent are suitable.
- (2) Glassware and apparatus used for sample preparation should be cleaned with filtered, demineralized water and stored dry.

- (3) The apparatus used for dispersion and deposition of particles consists of a small glass vial with a screw-on cap, a teflon pillar about 10 mm high that may be inserted into the vial, a petri dish, and a teflon block about 40 mm by 40 mm square.
- (4) Hold the grid with tweezers, dip rinse the grid thoroughly with ethanol. Wick the excess liquid off the grid using filter paper. Place the dried grid onto the clean Teflon block.
- (5) Place a 10 μ L drop of the nanoparticle solution onto the grid.
- (6) Cover the grid with a petri dish lid and let stand at room temperature for getting the grid dry, typically from 5 to 30 minutes, depending on solvent type.
- (7) Transfer the grid on TEM sample holder. Record enough micrographs to image a minimum of 200 nanoparticles per sample per grid square from a minimum of 2 widely separated regions of the grid using a well-aligned and stable TEM, operated at a fixed magnification that allows a large number of nanoparticles to be visible within the micrograph field of view, while ensuring that each individual nanoparticle is recorded with a large number of image pixels.

(c) Precautions:

- (1) Transmission Electron Microscope grids (especially thin film membranes) are very fragile and must be held by their edges with fine tweezers so as not to damage or crack the membrane.
- (2) Exposure of Transmission Electron Microscope grids to the ambient environment should be minimized to reduce the likelihood of dust contamination. Grids should be stored in suitable boxes in dust-free or desiccating cabinets.
- (3) A well-aligned Transmission Electron Microscope is essential to obtain accurate particle size results.
- (4) A minimum of 200 discrete particles should be measured from each of at least two widely separated regions of the sample (that is, different grid squares or membrane regions). Foreign debris in a given image (e.g., dust particles or residues from the rinsing and drying process) should be avoided.
- (5) Particle size results obtained from Transmission Electron Microscope measurements may not coincide with those obtained from other techniques (e.g., dynamic light scattering). This is due in part to differences in the weighted averages determined in each case (e.g., number for Transmission Electron Microscope versus intensity for dynamic light scattering), as well as differences in the physical property that is actually measured (e.g., projected area versus hydrodynamic diffusion area).

(iii) **Hydrodynamic particle size as per Dynamic Light Scattering (DLS) Analysis**

(a) Equipment and Apparatus:

- (1) Dynamic Light Scattering Equipment

- (2) Sample Cuvettes of size
- (3) Deionized water

(b) Procedure:

- (1) For liquid nano-fertilizer sample, 10 ml is sonicated for one minute and used for the analysis. In case for solid nano-fertilizer, fertilizer : distilled water (1:10 ratio) suspension is prepared, sonicated for one minute
- (2) Known standard samples (either one of the nano particles such as Ag, Au or TiO₂) are used to set the machine
- (3) Load sample into the Cuvette
- (4) Pre-rinse filter with solvent (at least 1 ml, depending on filter size and dead volume of filter holder or cartridge).
- (5) After loading syringe with sample and inserting syringe filter, allow the first 4 drops to go to waste. Use the next 4 drops to pre-rinse the cuvette, and discard. The remainder can be used for the sample measurement.
- (6) Load sample into cuvette using minimum amount necessary to ensure liquid level is at least 2 mm above the entrance height of the laser beam for your particular instrument configuration.
- (7) Take care not to touch the cuvette windows with your bare hands while loading. Wipe outside of quartz or glass cuvette with lens paper if needed. Cap the cuvette to prevent dust contamination and solvent evaporation.
- (8) Inspect the cuvette to ensure that air bubbles are not clinging to the optical window area.
- (9) Load the sample into Dynamic Light Scattering machine.
- (10) Perform 3 to 10 independent measurements per sample.

(c) Precautions:

- (1) To measure the size of solid phase nano nitrogen particles, 1 gram of nanoparticles sample should be suspended in 20 ml water followed by 1 minute ultra-sonication before the size measurement carried out by the Dynamic Light Scattering.
- (2) Measurement cuvettes should be cleaned with filtered demineralized water and stored dry.
- (3) The choice of pore size depends on the maximum dimension of the test particles and their tendency to adhere to the filter membrane. p Suspended medium (such as solvent, dispersant, solution) should be filtered prior to sample preparation using a 0.1 or 0.2 μ m filter.
- (4) A typical starting sample concentration is 1 mg/ml.
- (5) Use cuvette with quartz or equivalent optical-quality windows. (Q Pre-rinse cuvette with filtered solvent at least 3 times.

(iv) Zeta Potential Analysis:

- (a) Equipment and Apparatus:

(i) Dynamic Light Scattering (DLS).

(2) Sample Cuvettes of zeta.

(3) Deionized water.

(b) Procedure:

(1) For liquid nano-fertilizer sample, 10 ml is sonicated for one minute and used for the analysis and in case for solid nano-fertilizer, fertilizer: distilled water (1: 10 ratio) suspension is prepared, sonicated for one minute.

(2) Known standard samples (either one of the nano particles such as Ag, Au or TiO₂) are used to set the machine.

(3) Loading Sample into the Cuvette.

(4) Pre-rinse filter with solvent (at least 1 ml, depending on filter size and dead volume of filter holder or cartridge).

(5) After loading syringe with sample and inserting syringe filter, allow the first 4 drops to go waste. Use the next 4 drops to pre-rinse the cuvette, and discard. The remainder can be used for the sample measurement.

(6) Load sample into cuvette using minimum amount necessary to ensure liquid level is at least 2 mm above the entrance height of the laser beam for your particular instrument configuration.

(7) Take care not to touch the cuvette windows with your bare hands while loading. Wipe outside of quartz or glass cuvette with lens paper if needed.

(8) Cap the cuvette to prevent dust contamination and solvent evaporation.

(9) Inspect the cuvette to ensure that air bubbles are not clinging to the optical window area.

(10) Load the sample into Dynamic Light Scattering machine.

(H) Perform 3 to 10 independent measurements per sample.

(c) Precautions:

(1) Measurement cuvettes should be cleaned with filtered demineralized water and stored dry. The choice of pore size depends on the maximum dimension of the test particles and their tendency to adhere to the filter membrane. q suspending medium (such as solvent, dispersant, solution) should be filtered prior to sample preparation using a 0.1 or 0.2

(2) A typical starting sample concentration is 1 mg/ml.

(3) Use cuvette with quartz or equivalent optical-quality windows.

(4) Pre-rinse cuvette with filtered solvent at least 3 times.

(v) **Viscosity Measurement**

(a) Equipment and Apparatus:

(i) Demineralised water.

(2) Viscometer.

(3) Measuring cylinder.

(4) Bottle Adapter.

(b) Procedure:

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)
Calibrate the viscometer with distilled water to set the machine as 1 cps. Viscometer Assembly.

(2) Attach vertical pole to the base using the wrench provided in the case.

(3) Attach the viscometer to the vertical pole.

(4) Connect power cable to the viscometer.

(4) Turn on the viscometer and calibrate.

(5) Starting up — Select and attach required spindle.

(6) Raise the viscometer to the highest level using a screw on the vertical pole. Place beaker (with liquid under the spindle.

(8) Lower the viscometer until the spindle is submerged to the spindle's mark.

(9) Viscometer Operation:- This depend on the software of the instrument make. The analyst (10) should follow the manufacturer's operating instructions for a particular instrument.

(i) Set the viscometer using distilled water to measure 1 cps at room temperature.

(c) Precautions

(i) Wait for 30-60 sec before reading.

(2) Good results are in range 60% -80% of torque.

(3) Results depend on beaker and volume of liquid so use the same beakers for comparison measurements. Viscosity of a Liquid nano-fertilizer N is measured directly.

(4) For solid nano-fertilizer, fertilizer: water suspension should be in 1: 10 ratio with distilled water.

(vi) Measurement of pH

(a) Apparatus:

pH meter, vacuum pump, beaker, pipette, glass rod, china dish, spatula etc.

(b) Reagents:

Buffer solutions of pH 4.0, 7.0 and 9.2: One buffer tablet of the respective pH is dissolved in water and the volume is made to 100 ml.

(c) Procedure:

(1) Take 10 ml of liquid sample, homogenise it and take the pH measurement. For solid/powder samples (1 g dry sample/10 ml water) homogenisation or ultrasonic agitation of the sample for 1 minute followed by pH measurement should be taken after settling of the samples

- (2) pH meter is set at room temperature and calibrated by immersing the electrodes in different buffer solutions of pH 4.0, 7.0 and 9.2.
 - (3) Take the beaker of homogenised samples and dip the electrodes into it and note the pH reading.
 - (4) After each determination, the electrodes must be washed with distilled water and wiped out by ordinary filter paper.
- (d) Precautions:
- (1) Proper homogenisation/sonication must be done.
 - (2) The glass and reference electrode of pH meter should always remain dipped in water.
 - (3) Buffer solutions should be prepared accurately and stored well in glass container.
 - (4) It is desirable to prepare fresh buffer solutions after few days. Connect the pH meter to the stabilizer to avoid the fluctuations in pH readings. Adjust the temperature knob of pH meter at room temperature for correct pH determination.

***32. Method of Analysis of Zinc Polyphosphate**

(i) Quality of reagents

Pure chemicals and distilled water shall be used in tests.

Note: Pure chemicals shall mean chemicals that do not contain impurities which affect the results of analysis.

Demineralised water means the water obtained after passing distilled water through a cation and an anion exchange resins or a combined cationation exchange resin. Glass distilled water means distilled water that is redistilled in a glass distillation apparatus.

(ii) Determination of zinc:

(a) Reagents

- (1) Standard zinc solution- Weigh accurately 1.0 g of zinc metal A.R. grade in a 250 mL beaker.

Add 20 mL HCl (1 : 1) to it and cover with a watch glass. Keep it for a few hours and allow it to dissolve completely. Transfer it to one litre flask through a funnel giving at least 10 washings of beaker and funnel with glass distilled water. Make up the volume using glass distilled water. Stopper the flask and shake the solution well. This is 1000 ppm zinc solution herein after called Standard A. This solution should be stored in a clean bottle for further use. Dilute 10ml of 1000 ppm solution of zinc (Standard A) to 1litre to get 10ppm standard zinc solution designated as Standard B.

- (2) Concentrated hydrochloric acid (HCl).
 - (3) Glass distilled water of pH 2.5. Dilute 1 ml of concentrated hydrochloric acid to one litre with glass distilled water and adjust the pH to 2.5 with a pH meter using HCl or NaOH. This solution is called acidified water and 5 to 10 litres of
-

this solution should be prepared at a time. Do not use sulphuric acid for preparing this acidified water

- (4) Preparation of working standards- Pipette the following volume of Standard B in 50ml numbered volumetric flask and make the volume with acidified water (See Table below). Stopper the flasks and shake them well. Prepare the standard in duplicate. The same acidified water should be used for preparing the solution of unknown fertilizer samples. Fresh standards should be prepared every time when a fresh lot of acidified water is prepared.

Flask No	Volume of standard B taken (ml)	Concentration of zinc after making volume to 50 ml (ppm)
	0	0
2		0.2
	2	0.4
4	3	0.6
5	4	0.8
6	5	1.0
7	7	1.4
8	9	18
9	10	2.0

(b) Procedure

(1) Preparation of zinc polyphosphate fertilizer sample - Weigh 250 mg (0.25 g) fertilizer in a clean watch glass. Transfer into a 250 mL Erlenmeyer flask (conical flask) using a funnel. Wash the watch glass and funnel with 10 mL concentrated hydrochloric acid. Do not use sulphuric acid for dissolving the sample. Do not dilute with water. Place a funnel over the mouth of the Erlenmeyer flask and heat over a hot plate till fumes of acid appear. Allow the solution to cool to room temperature. Carefully add 100 mL water and swirl to mix. Then filter through Whatman I filter paper into a 1L volumetric flask. Wash the Erlenmeyer flask and filter paper repeatedly (at least ten times with 10 mL portions of water) and then dilute to volume. Prepare these solutions in duplicate.

(2) Pipette 10 ml of the prepared solution in 500ml volumetric flask and make the volume with acidified water (hydrochloric acid water).

(3) Flaming the solutions-- Flame the standards and the filtered samples on atomic absorption spectrophotometer at a wavelength of 213.8 nm (Zn line of the instrument).

(c) Calculations

Prepare a standard curve of known concentrations of zinc solution by plotting the absorbance values on Y-axis against their respective zinc concentration on X-axis. Calculate the percentage zinc in zinc fertilizer by multiplying zinc concentration value calculation from standard curve by 20.

Example

Weigh of the fertilizers sample..... = 0.25 g
Volume made..... = 1000 ml
Further dilution..... = 50 times
Reading of the samples from atomic absorption. = Y
Corresponding concentration value of zinc from standard curve against Y absorbance. = X ppm
Percentage zinc in the fertilizer..... = 20X

(d) **Precautions**

- (1) Weighing must be done on an electric or electronic balance.
- (2) All the glass apparatus to be used should be of corning or borosil make and washed with dilute hydrochloric acid (1:4), and subsequently washed thoroughly with distilled water and then with glass distilled water.
- (3) The pipette should be rinsed thrice with the same solution to be measured.
- (4) The outside or the pipette should be wiped with filter paper after taking out from the solution to be measured.
- (5) After using the pipettes, wash with distilled water and place them on a clean dry filter paper in order to prevent contamination.
- (6) To start filtration only a few drops should be added first in order to wet the filter paper and then continue further filtration.
- (7) Do not use sulphuric acid for dissolving the sample or for dilution of solution prior to analysis.

(iii) **Determination of magnesium**

(a) **Reagents**

- (i) Strontium chloride- Dissolve 7.5 g of strontium chloride ($\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$) in one litre of glass distilled water.
- (2) Standard magnesium solution- Weigh 0.507 g of magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) on a clean watch glass and transfer it to one litre flask through the funnel giving several washings to watch glass and the funnel with glass distilled water. This is 50ppm Mg solution Dilute 10ml of 50 ppm solution of Mg to 100ml to get 5 ppm standard Mg solution.
- (3) Preparation of working standards- Pipette the following volume of 5 ppm standard Mg solution in 50ml numbered volumetric flasks. Add 10ml of strontium chloride solution to each flask and make up the volume to 50 ml. Stopper the flask and shake them well prepare fresh standards every for night

Flask No.	Volume of 5 ppm Mg Solution taken (ml)	Volume of strontium chloride added (ml)	Concentration of magnesium after making the flask to 50 ml (ppm)
1	0.0	10.0	0.0
2	2.0	10.0	0.2
3	4.0	10.0	0.4
4	6.0	10.0	0.6
5	8.0	10.0	0.8
6	10.0	10.0	1.0

(b) Procedure

- (1) Pipette 5ml of the solution which was prepared for the determination of zinc by dissolving 0.25g of the fertilizer sample in one litre flask [this section (ii) (b) (1)] Add 50ml of strontium chloride Make up the volume to 250ml.
- (2) Flame the standards and the samples on atomic absorption spectrophotometer at a wave length of 285.5 nm (Mg line of the instrument)
- (c) Calculations
Prepare a standard curve of known concentrations of Mg solutions by plotting the absorbance value on Y-axis against their respective concentration values on X-axis. Percentage magnesium in the fertilizer will correspond to the magnesium concentration value calculated from the standard curve multiplied by 20.

Example:

Weight of the fertilizer = 0.25 g

Volume made.....1000 ml.

Further dilution.....= 50 times

Reading of the sample from atomic absorption spectrophotometer..... = Y

Corresponding concentration of Mg from standard curve against Y absorbance..... = X ppm

Percentage magnesium in the fertilizer..... = 20X

(iv) Determination of lead

(a) Reagents

- (1) Standard lead solution- Weigh 0.1599 g of lead nitrate [$\text{Pb}(\text{NO}_3)_2$] on a clean watch glass and transfer it to one litre flask through a funnel using glass distilled water. Add 10ml of concentrated distilled nitric acid and make the volume upto the mark. Stopper the flask and shake the solution well. This is 100 ppm lead solution and should be stored in a clean bottle for further use. Dilute 10 ml of 100 ppm solution of lead to 100ml with 1 percent nitric acid solution to get 10 ppm standard lead solution.
- (2) 1 percent nitric acid solution- Dilute 10ml of concentrated distilled nitric acid to one litre with glass distilled water.
- (3) 10 percent nitric acid solution- Dilute 10ml of concentrated distilled nitric acid to 100ml with glass distilled water.
- (4) 20 percent zinc sulphate solution- Weigh 20 g of zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$) and dilute to 100ml with 1 percent nitric acid solution.
- (5) Preparation of working standards- Pipette the following volume of 10 ppm standard lead solution in 50 ml numbered volumetric flasks. Add 5ml of 20 percent zinc sulphate solution to each flask and make the volume with 1 percent nitric acid solution

Flask No.	Volume of 10 ppm lead solution taken (ml)	Volume of 20% zinc sulphate solution added (ml)	Concentration of lead after making the volume to 50ml (ppm)
1	0.0	5.0	0.0
2	1.0	5.0	0.2
3	2.0	5.0	0.4
4	4.0	5.0	0.8
5	6.0	5.0	1.2
6	8.0	5.0	1.6

Stopper the flasks and shake them well.

(b) Procedure

- (i) Preparation of zinc polyphosphate fertilizer samples- Weigh 1 g of the material on a clean watch glass and transfer to 100 ml volumetric flask through the funnel using 10 ml of 10 percent nitric acid solution. Let it stand for a few hours till it dissolves. Add 10 mL of 20% zinc sulphate solution to the flask. Make the volume with glass distilled water. Samples should be prepared in duplicate.
- (2) Flaming the solution-- Flame the standards and the samples on atomic absorption spectrophotometer at a wavelength of 217 m (Lead line of the instrument)

(c) Calculations

Prepare a standard curve of known concentrations of lead solution by plotting the absorbance values on Y - axis against their respective lead concentration on X - axis. Calculate the percentage lead in zinc polyphosphate fertilizer by multiplying lead concentration value calculated from standard curve by 0.01.

Example:

Weight of the fertilizer = 1.0 g

Volume made..... = 100 ml.

Further dilution..... = Nil

Reading of the sample from atomic absorption spectrophotometer. = Y

Corresponding concentration of Pb from standard curve against Y absorbance..... = X ppm

Percentage Pb in the fertilizer. = 0.01X

(V) Determination of Arsenic (as As)

The principle and reagents in this case shall substantially be the same as given in Schedule IV, Part D, 12 relating to determination of As in organic fertilizers by Atom Absorption Spectrophotometer (AAS).

(a) Reagents

- (1) Standard sodium hydroxide solution-- approximately 20 percent.
- (2) Standard arsenic trioxide solution - Dissolve 1.320 gm. of resublimed arsenic trioxide (AS₂O₃) in 25 ml. sodium hydroxide solution and neutralize with dilute

sulphuric acid. Dilute with freshly distilled water containing 10ml of concentrated sulphuric acid per liter and make up the volume to 1 liter. This solution contains 1000 ppm As and is solution A.

- (3) Pipette 5 mL of solution A into a 1 L volumetric flask and dilute to volume. This solution contains 5 ppm As and is solution B
- (4) Prepare standards having concentration of 0.05, 0.1 and 0.2 ppm by diluting 1.0, 2.0 and 4.0 ml, respectively of 5 ppm Arsenic solution (solution B) with double distilled water in volumetric flask and make up the volume to 100 ml

(b) Procedure

- (i) Preparation of zinc polyphosphate fertilizer sample - Weigh 1.0 g of fertilizer in a clean watch glass. Transfer into a 250 mL Erlenmeyer flask (conical flask) using a funnel. Wash the watch glass and funnel with 10 mL concentrated hydrochloric acid. Place a funnel over the mouth of the Erlenmeyer flask and heat over a hot plate till fumes of acid appear. Allow the solution to cool to room temperature. Carefully add 100 mL water and so irll to mix. Neutralise the solution with 12 mL 1 N NaOH solution. Transfer to a 1 L volumetric flask and make up volume.
- (2) Estimate Arsenic using vapour generation assembly attached to Atomic Absorption Spectrophotometer as per the procedure given for the instrument. Use the solution of fertilizer directly without further dilution.

(c) Calculations

Prepare a standard curve of known concentrations of As solutions by plotting the absorbance value on Yaxis against their respective concentration values on X-axis. Percentage As in the fertilizer will correspond to the As concentration value calculated from the standard curve multiplied by 0.1.

Example:

Weight of the fertilizer = 1.0 g

Volume made..... = 1000 ml

Furtherdilution..... Nil

Reading of the sample from atomic absorption spectrophotometer..... = Y

Corresponding concentration of AS from standard curve against Y absorbance..... = X ppm

Percentage As in the fertilizer. = 0.1X

(vi) Determination of cadmium

Same as provided in paragraph (x) under subheading 7.

(a) Reagents and procedure same as in subheading 7 (x).

(b) Procedure

- (1) Preparation of zinc polyphosphate fertilizer sample - Weigh 1.0 g of fertilizer in a clean watch glass. Transfer into a 100 mL beaker with 10 mL 20 percent hydrochloric acid. Heat over a hot plate till dissolved. Allow the solution to cool to room temperature. Neutralise with 4 % NaOH solution (1 N NaOH) to pH 2.5. Transfer to a 100 mL volumetric flask and make up volume.
- (2) Aspirate the standards as well as the sample solution for Cd on an atomic absorption spectrophotometer at a wavelength of 228.8 nm using air acetylene flame and note the absorbance for each solution.

(c) Calculations

Prepare a standard curve of known concentrations of Cd solutions by plotting the absorbance value on Yaxis against their respective concentration values on X-axis. Percentage As in the fertilizer will correspond to the Cd concentration value calculated from the standard curve multiplied by 0.01.

Example:

Weight of the fertilizer..... = 1.0 g
Volume made. = 100 ml.
Further dilution. = Nil
Reading of the sample from atomic absorption spectrophotometer..... = Y
Corresponding concentration of Cd from standard curve against Y absorbance. = X ppm
Percentage Cd in the fertilizer. = 0.01X

(vii) Determination of pH

(a) Procedure

Weigh 5 g of fertilizer into a 100 mL beaker. Add 50 mL distilled water, stir and immediately record pH before the suspension sediments.

(viii) Determination of water soluble zinc

(a) Reagents

As described in item (ii) (a) of this serial number relating to "Determination of Zinc".

(b) Procedure

(1) Extraction of water soluble zinc

Weigh 250 mg zinc fertilizer into a 500 mL Erlenmeyer flask (conical flask). Add 100 mL of glass distilled water. Place a stopper at the mouth and shake the solution in a horizontal shaker for 15 min. Filter through a Whatman 1 filter paper into a 500 mL volumetric flask. Wash the Erlenmeyer flask and filter paper repeatedly (at least ten times with 10 mL portions of water). Remove the funnel and filter paper. Add 5 mL concentrated HCl directly to the solution in the volumetric flask and then make up volume. Prepare these solutions in duplicate.

(2) Pipette 5ml of the prepared solution in 50ml volumetric flask and make the volume with acidified water (hydrochloric acid water).

(3) Flaming the solutions-- Flame the standards and the filtered samples on atomic absorption spectrophotometer at a wavelength of 213.8 μ (Zn line of the instrument).

(c) Calculations

Prepare a standard curve of known concentrations of zinc solution by plotting

the absorbance values on Y-axis against their respective zinc concentration on X-axis
Calculate the percentage zinc in zinc fertilizer by multiplying zinc concentration value
calculation from standard curve by 20.

Example.

Weigh of the fertilizers sample.....= 0.25 g
Volume made.....= 500 ml
Further dilution.....= 10 times
Reading of the samples form atomic absorption. = Y
Corresponding concentration value of zinc from standard curve against Y
absorbance.....= X ppm
Percentage water soluble zinc in the fertilizer.....- 2X

(ix) Determination of DTPA soluble zinc

(a) Reagents

(1) Prepare a solution of 0.005 M DTPA (diethylenetriaminepentaacetic acid, $[(\text{HOOCCH}_2)_2\text{NCH}_2\text{CH}_2]_2\text{NCH}_2\text{COOH}$; molecular weight 393.35) by weighing 1.97 g DTPA in a 1000 mL volumetric flask. Add 800 mL water and heat till it is completely dissolved. Cool to room temperature and then make up the volume to 1 litre.

(2) Other reagents as described in item (ii) (a) of this serial number relating to Determination of Zinc?.

(b) Procedure

(1) Extraction of DTPA soluble zinc- Weigh 250 mg zinc fertilizer into a 1 L Erlenmeyer flask (conical flask). Add 250 mL of the DTPA solution. Place a stopper at the mouth and shake the solution in a horizontal shaker for 60 min. Filter through a Whatman 1 filter paper into a 1 L volumetric flask. Wash the Erlenmeyer flask and filter paper repeatedly (at least ten times with 10 mL portions of water). Remove the funnel and filter paper. Add 5 mL concentrated HCl directly to the solution in the volumetric flask and then make up volume. Prepare these solutions in duplicate.

(2) Pipette 10 mL of the DTPA extracted solution into a 500 mL volumetric flask and make up volume with acidified water (hydrochloric acid water).

(3) Flaming the solutions-- Flame the standards and the filtered samples on atomic absorption spectrophotometer at a wavelength of 213.8 nm (Zn line of the instrument).

(c) Calculations

Prepare a standard curve of known concentrations of zinc solution by plotting the absorbance values on Y-axis against their respective zinc concentration on X-axis
Calculate the percentage zinc in zinc fertilizer by multiplying zinc concentration value
calculation from standard curve by 20.

Example.

Weigh of the fertilizers sample= 0.25 g
Volume made.....= 1000 ml
Further dilution.....= 50 times
Reading of the samples form atomic absorption. = Y

Corresponding concentration value of zinc from standard curve against Y absorbance..... = X ppm

Percentage DTPA soluble zinc in the fertilizer..... = 20X

(d) Precautions

- (1) Weighing must be done on a electric or electronic balance
- (2) All the glass apparatus to be used should be of corning or borosil make and washed with dilute hydrochloric acid (1:4) and subsequently washed thoroughly with distilled water and then with glass distilled water.
- (3) The pipette should be rinsed thrice with the same solution to be measured.
- (4) The outside or the pipette should be wiped with filter paper after taking out form the solution to be measured.
- (5) After using the pipettes, place them on a clean dry filter paper in order to prevent contamination.
- (6) To start filtration only a few drops should be added first in order to wet the filter paper and then continue further filtration.
- (7) Do not use sulphuric acid for dilution of solution prior to analysis.

***33 Method of analysis of potassium Thiosulphate and Calcium Thiosulphate (Liquid Fertilizers)**

- (i) Determination of potassium by the method as specified in 5 (ii) ;
- (ii) Determination of Specific Gravity by the method as specified in 21 (ii);
- (iii) Determination of Calcium by the method as specified in 21 A (v);
- (iv) Determination of Arsenic by the method as specified in 8 (ix);
- (v) Determination of Lead by the method as specified in 8(v) ;
- (vi) Determination of Sulphur (as S) in potassium thiosulphate and calcium thiosulphate (Liquid Fertiliser).-

(a) Apparatus

- (i) Electric hot plate, water bath, muffle furnace, Oven;
- (ii) Electronic balance;
- (iii) Desiccators;
- (iv) Gooch crucible;
- (v) Vacuum pump for filtration;
- (vi) Electronic balance (0.001 g capacity);
- (vii) Whatman No 40 and 50 filter papers;
- (viii) Routine laboratory glassware, instruments;

(b) Reagents

- (i) Conc. Hydrochloric acid (37% HCl);
 - (ii) Dilute hydrochloric acid - Take 250 ml of concentrated hydrochloric acid in one litre volumetric flask. Make the volume upto 1000 ml water. Shake and mix it well;
 - (iii) Barium chloride solution: Dissolve 120 gm barium chloride in 1000 ml of water;
 - (iv) silver nitrate (5 percent solution): Dissolve 5 g of silver nitrate in 100 ml of distilled water;
 - (v) Hydrogen per oxide 30 percent (fresh) solution: Dilute liquid hydrogen peroxide to bring final concentration 30 percent;
-

- (vi) Sodium hydroxide solution: Dissolve 40 g sodium hydroxide salt in 100 ml distilled water;
- (vii) Methyl orange indicator: Dissolve 1g methyl orange indicator in 100 ml alcohol. Shake it and store in cool dry Place;
- (viii) Sodium Thiosulphate : AR Grade.

(c) Procedure

1. Oxidation of liquid fertilizer test sample;

(a) Weight 5.0 g sample of potassium thiosulphate (or calcium thiosulphate) liquid fertilisers (up to 0.001 g) accurately in a dry, clean 500 ml graduated flask on an electronic balance. Denote "W" to this as weight of sample. Fill the flask to the mark of 500 ml by using double distilled water'.

(b) Add exactly 50 ml (of the diluted sample from (a) to a 500 ml beaker and add approximately 100 ml of water. Add 2 ml of 40% NaOH solution to it. Add now slowly 2 ml of 30% hydrogen per oxide (H_2O_2) solution. Cover the beaker with watch glass carefully. Heat the solution on low heat on hot plate slowly for about 30 minutes. Cool it and then add slowly 2 ml of hydrogen peroxide 30% solution again. Heat the solution on hot plate slowly. If required add more 1 to 2 ml of hydrogen per oxide solution. Total hydrogen peroxide solution addition should not be more than 6 ml for complete oxidation. Cool the beaker; add 100 ml of water and then mix.

(c) Add a few drops of methyl orange indicator to the solution. Add 2-5 ml of dilute hydrochloric acid and mix it until colour changes to red uniformly. Add a few more drops of acid and mix well with glass rod. Then, keep the beaker on hot plate (below boiling temperature, $<800C$) to bring the solution to colorless within 10 minutes.

Note: If necessary, add a few drops of 30% H_2O_2 to the beakers to bring the solution to colourless.

2. Precipitation of sulphur for gravimetric determination of total sulphur ,-

- (a) Add slowly 15 ml barium chloride to the oxidized sample to ensure complete precipitation of sulphur as barium sulphate;
- (b) Digest the precipitation on a hot plate or water bath for minimum 5 hours at such a temperature that the solution does not boil (around 80^0C). Put a watch glass above the beaker to prevent complete evaporation of the solution. Ensure the supernatant liquid to be clear and transparent. Cool it to room temperature and keep it over night to settle precipitates;
- (c) Take 30 ml capacity G4 grade Sintered Gooch crucible. Dry it in furnace at $2500C$; cool it and record weight of empty crucible(G I);
- (d) Pour the sample with precipitate into 30 ml capacity G4 grade Sintered Gooch Crucible previously weighed. Filter it. Wash the precipitate 10-12 times with hot water to ensure the precipitate to make it free from excess barium chloride. The filtrate may be tested with silver nitrate solution to confirm that the precipitate is free from chloride.

- (e) Dry the crucible having barium sulphate precipitate at 2500 C for two hours in a furnace by raising the temperature of the furnace slowly from room temperature to constant weight taken and note the weight(G2).

3. Calculations :- $(G2-G1) \times 13.74 \times DF$

Total sulphur (as S) percent = _____

Whereas

G1 — weight of Gooch crucible (+ filter paper if used)

G2 = Weight of Gooch crucible + barium sulphate precipitate (+ filter paper if used)

W = Weight of sample

Factor 13.74= Per cent total sulphur (w/w) in barium sulphate = $32 \cdot 100/233.39$

*34. Method of analysis of Phospho Gypsum

1. Determination of calcium as calcium Sulphate Dihydrate in Phospho Gypsum

(i) Standarisation of calcium solution-as per method specified in 21A (b)

(ii) Procedure:-

(a) Take 1 gram of sample in 250 ml dry beaker. Add 50 ml dilute Hydrochloric acid

(i:i)

(b) Boil for 5 minutes on slow heater and cool to room temperature. Add 50 ml distilled water.

(c) Transfer the solution in 250 ml volumetric flask and make the volume to 250 ml with distilled water.

(d) Take 25 ml aliquot in 250 ml washed conical flask and 20 ml distilled water and 20 ml buffer solution. Add few drops of indicator and titrate with EDTA solution till blue color is obtained. Note the volume of EDTA used as V2.

Note:- if the solution is not clear after digestion filter it and then take aliquot.

2. Calculation :-

Calcium (as Calcium Sulphate Dihydrate) per cent.= $V2 \times \text{Calcium titer} \times 3.4 \times 1.$

**35 Method of analysis of potash Fortified Silicon by Spectrophotometer

Determination of Silicon as Si (OH),.

A. Reagents:

(1) Silicon Standard Solution (1000 ppm):— Take 7.5534 gm of $\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$ in 1000 ml of volumetric flask and dissolve in 100 ml distilled water and make the volume to 1000 ml with distilled water. From 1000 ml standard solution take 10 ml

in 100 ml volumetric flask and dilute to the mark with distilled water. Prepare 1 ppm, 2 ppm, 3 ppm and 4 ppm working standard solution in 100 ml volumetric flask accordingly.

(2) Tartaric Acid Solution :- Dissolve 50 gram tartaric acid in water and dilute to 500 ml. Store in plastic bottle.

(3) Ammonium Molybdate Solution:- Dissolve 7.5 gm Ammonium Molybdate

(NH₄)₂MoO₄·4H₂O) in 75 ml water, add 10ml H₂SO₄(1:1) and dilute to 100 ml with distilled water and Store in plastic bottle.

(4) Reducing Solution:- Dissolve 0.7 gm Na₂SO₃ in 10 ml water and add 0.15 gm 1—Amino-2-Naphthol-4- Sulphonic Acid. Stir until dissolved. Dissolve 9 gm NaHSO₃ in 90 ml distilled water, add to first solution and mix properly. Store in plastic bottle.

B. Preparation of Sample Solution.

- (1) Grind requisite quantity of Sample and dry at 105-degree C for two hours.
- (2) Place 0.2-0.5 gm sample in 75 Nickel Crucible.
- (3) Mix 0.3 gm KNO₃ with sample and add 1.5 gm NaOH pellets.
- (4) Cover the crucible with Nickel cover and heat to 5 minutes at dull redness over gas flame (do not fuse in furnace).
- (5) Remove the crucible from flame and swirl melt around sides.
- (6) Cool and add 50 ml distilled water and warm to disintegrate fused cake.
- (7) Transfer the material to 150 ml plastic beaker containing 15 ml 5N HClO₄, (perchloric acid).
- (8) Scrub crucible and lid and wash any residue (with warm distilled water) into beaker.
- (9) Transfer it into 250 ml volumetric flask and dilute to volume.
- (10) Dilute this sample solution for spectrophotometric analysis accordingly.

C. Preparation of Standard Curve

(1) Prepare working standard solution of different strength in 100ml volumetric flask as the like 1 ppm, 2ppm,3 ppm and4 ppm and blank solution. Add 1 ml Ammonium Molybdate solution with swirling. Mix well and let it stand for 10 minutes then add 4 ml tartaric acid solution with swirling and mix well. Add 1 ml reducing solution with swirling, dilute to volume with distilled water. Mix well let it stand for thirty minutes.

(2) Take the absorbance of standard Si solution against blank at 650 nm. Follow same procedure for sample solution and calculate the Si percentage in sample.

Calculation: -

$$(i) \quad \% Si = X \times DF \times 10$$

X = Concentration of sample in ppm

$$(ii) \quad \% \text{ of Silica as Si (OH) } = \%Si \times CF$$

Note :- All the plastic wares are to be used during the analysis.

II Determination of Potash

Determination of water Soluble K₂O by the method as specified in 5 (ii) under The said order.

*36. Method of Analysis Chelated Manganese as Mn-EDTA

Y Reagents

- (i) Glass distilled water or demineralized water of pH 2.5 \pm 0.5:-Dilute 1 ml of 10% H₂SO₄ to one litre with glass distilled water or demineralized water and adjust the pH to 2.5 with a pH meter using 10% H₂SO₄ + NaOH.
- (ii) Di Sodium EDTA solution :- Dissolve 0.73 gm of di sodium EDTA di hydrate in distilled water and make volume to 100 ml.

B. Preparation of standard Manganese metal stock solution [1000 ppm]

- (i) Dissolve 1.0 gm of Manganese metal in 50 ml of conc. Hydrochloric acid and make volume to 1000 ml with distilled water. This is 1000 ppm Manganese stock solution.
- (ii) Intermediate solution (100 ppm):- Pipette 10 ml of Manganese stock solution and 10 ml of Di sodium EDTA solution in 100 ml of volumetric flask and dilute to volume with distilled water.
(Note:- In case the metal stock solution is not exactly 100.0 ppm, the exact titer value of di sodium EDTA required to chelate the metal has to be added.)
- (iii) Working Standard Solution:- Pipette following volumes of 100 ppm intermediate solution in 100 ml Volumetric flask and make volume with acidified water.

Volume of 100 ppm intermediate solution taken	To make volume to 100 ml	Concentration of Mn in ppm after making vol. to 100 ml
1.0 ml	100 ml	1.0 ppm
2.0 ml	100 ml	2.0 ppm
3.0 ml	100 ml	3.0 ppm
4.0 ml	100 ml	4.0 ppm

C. Preparation of sample solution for Chelated Manganese

- (i) Weigh 1.0 gm of sample and transfer into 100 ml volumetric flask and make volume with distilled water. Pipette out 1.0 ml of sample solution and dilute further to make second dilution of 100 ml with distilled water, pipette 5 ml from second dilution and make volume to 50 ml with acidified water to get the above flaming range from 1.0-4.0 ppm.
 - (ii) Flaming the solutions:- Flame the standards and the sample of Chelated Manganese on atomic absorption spectrophotometer at a wavelength of 279.5 nm (Mn line of instrument) using air-acetylene flame.
 - (iii) Prepare a blank solution from which only the sample has been omitted. Flame along with standards and sample.
-

- D. Calculations:- Prepare a standard curve of known concentration of Manganese solutions by plotting the absorbance value on Y-axis against their respective concentrations on X-axis.

Part-I:- **Determination of Total Manganese content in Chelated Manganese sample:-** Total Mn content = Concentration of Mn in ppm obtained after flaming the solution x 10

Part-II :- **Determination of Free Manganese content in Chelated sample:-**

Reagents:- 0.02 M Disodium salt of EDTA— 0.7444 gm of Disodium Salt of EDTA in 100 ml of distilled water. Standardize with 0.02 M standard Zinc or Manganese Metal solution.

- (i) Hydroxylamine Hydrochloride — 0.1-0.2 gm;
- (i) Triethanolamine — 1-2 ml;
- (ii) Buffer solution of pH — 10.0; and
- (iii) Indicator Eriochrome black T (EBT) powder.

Take 10 ml of aliquot from stock dilution of sample (1.0 g in 100 ml). Add to it 0.1 g Hydroxylamine HCl, 2 ml of triethanolamine, 10 ml buffer of pH 10.0, EBT indicator and titrate against 0.02 M disodium EDTA solution.

% Free Manganese = Titration value of EDTA x Atomic weight of Mn metal
Molarity of EDTA x 1

% **Chelated Manganese content** = % Total Manganese - % Free Manganese content

*37. Method of analysis of Chelated Calcium as Ca EDTA

A. Reagents

- (i) Glass distilled water or demineralized water of pH 2.5+/- 0.5:-
Dilute 1 ml of 10% H₂SO₄ to one litre with glass distilled water or demineralized water and adjust the pH to 2.5 with a pH meter using 10% H₂SO₄ or NaOH.
- (ii) Di sodium EDTA solution:- Dissolve 0.73 gm of di sodium EDTA di hydrate in distilled water and make volume to 100 ml.
- (iii) Strontium Chloride (SrCl₂ .6H₂O)- 15.0 gm in 0.5N HCl and dilute to 100 ml.

B. Preparation of Standard stock solution of Calcium 1000 ppm

- (i) Dissolve 2.498 gm CaCO₃ in a minimum amount of 3 N HCl. Dilute to 1000 ml with distilled water. This is 1000 ppm calcium stock solution.
- (ii) Intermediate solution [100 ppm]:- Pipette 10 ml of Calcium stock solution and 50 ml of Disodium EDTA solution in 100 ml volumetric flask and dilute volume with distilled water.

(Note:- In case the metal stock solution is not exactly 100.0 ppm, the exact titer value of disodium EDTA required to chelate the metal has to be added).

- (iii) Working Standard solution.- Pipette following volumes of 100 ppm intermediate solution in 100 ml Volumetric flask and make volume with acidified water.

Volume of 100 ppm intermediate solution taken	Volume of strontium chloride To be added	Concentration of Ca in ppm after making vol. to 100 ml
5.0ml	10ml	5.0ppm
10.0ml	10ml	10.0ppm
15.0ml	10ml	15.0ppm
20.0ml	10ml	20.0ppm

C. Preparation of sample solution for Chelated Calcium

- (i) Weigh 1.0 gm of sample and transfer into 100 ml volumetric flask and make volume with distilled water. Pipette out 5.0 ml of sample solution and dilute further to make second dilution of 100 ml with distilled water, pipette 10.0 ml from second dilution and make volume to 100 ml with acidified water to get the above flaming range from 5.0-20.0 ppm.
- (ii) **Flaming** the solutions
Flame the standards and the sample of Chelated Calcium on Atomic Absorption Spectrophotometer at a wave length of 422.7 nm (Ca line of instrument) using air-acetylene flame.
- (iii) Prepare a blank solution from which only the sample has been omitted. Flame along with standards and sample.

D. **Calculations.-** Prepare a standard curve of known concentration of Calcium solutions by plotting the absorbance value on Y-axis against their respective concentrations on X- axis.

Part-I :- Determination of Total Calcium content in Chelated Calcium sample:-

Total Ca content = Concentration of Ca in ppm obtained after flaming the solution X 2

Part-II :- Determination of Free Calcium in Chelated sample

- (i) Reagents:- 0.02 M Disodium salt of EDTA— 0.7444 gm of Disodium Salt of EDTA in 100 ml of distilled water. Standardize with 0.02 M Standard Zinc and Manganese Metal solution.
- (ii) Buffer solution of pH — 10.0
- (iii) Indicator Eriochrome black T (EBT) powder
- (iv) Take 10 ml of aliquot from stock dilution of sample (1.0 g in 100 ml). Add to it 0.1 g Hydroxylamine HCl, 1 to 2 ml of triethanolamine, 10 ml buffer of pH 10.0, EBT indicator and titrate against 0.02 M disodium EDTA solution.

$$\% \text{ Free Calcium} = \text{Titration value of EDTA} \times \text{Atomic weight of Ca metal} \times \text{Molarity of EDTA} \times 1$$

$$\% \text{ Chelated Calcium content} = \% \text{ Total Calcium} - \% \text{ Free Calcium content}$$

*38. Method of analysis of Chelated Magnesium as Mg-EDTA

A. Reagents

- (i) Glass distilled water or demineralized water of pH 2.5 \pm 0.5. - Dilute 1 ml of 10% H₂SO₄, to one litre with glass distilled water or demineralized water and adjust the pH to 2.5 with a pH meter using 10% H₂SO₄ or NaOH.
- (ii) Di sodium EDTA solution:- Dissolve 0.73 gm of di sodium EDTA di hydrate in distilled water and make volume to 100 ml.

B. Preparation of Standard stock solution of Magnesium 1000 ppm

- (i) Dissolve 1.0 gm of magnesium metal in 30 ml of 1:1 HCl and dilute to 1000 ml in volumetric flask with distilled water. This is 1000 ppm magnesium stock solution.

(ii) Intermediate solution [100 ppm]

Pipette 10 ml of magnesium stock solution and 20 ml of Disodium EDTA solution in 100 ml volumetric flask and dilute volume with distilled water. (Note:- In case the metal stock solution is not exactly 100 ppm, the exact titer value of disodium EDTA required to chelate the metal has to be added).

- (iii) **Working Standard solution** :- Pipette following volumes of 100 ppm intermediate solution in 100 ml Volumetric flask and make volume with acidified water.

Volume of 100 ppm intermediate solution taken	To make volume to 100 ml	Concentration of Mg in ppm after making vol. to 100 ml
0.1 ml	100ml	0.1 ppm
0.2ml	100ml	0.2 ppm
0.4ml	100ml	0.4 ppm
0.6ml	100ml	0.6 ppm

C. Preparation of sample solution for Chelated Magnesium

- (i) Weigh 1.0 gm of sample and transfer into 100 ml volumetric flask and make volume with distilled water. Pipette out 2.0 ml of sample solution and dilute further to make second dilution of 100 ml with distilled water, pipette 1.0 ml from second dilution and make volume to 100 ml with acidified water to get the above flaming range from 0.1-0.25 ppm.
- (ii) Flaming the solutions:- Flame the standards and the sample of Chelated Magnesium on atomic absorption spectrophotometer at a wavelength of 285.2 nm (Mg line of instrument) using air-acetylene.
- (iii) Prepare a blank solution from which only the sample has been omitted. Flame along with standards and sample.

D. Calculations

Prepare a standard curve of known concentration of Magnesium solutions by plotting the absorbance value on Y-axis against their respective concentrations on X-axis.

Part-I :- Determination of Total Magnesium content in Chelated Magnesium sample

Total Mg content = Concentration of Mg in ppm obtained after flaming the solution x 50

Part-II - Determination of Free magnesium in Chelated sample

- (i) Reagents:- 0.02 M Disodium salt of EDTA—0.7444 gm of Disodium Salt of EDTA in 100 ml of distilled water. Standardize with 0.02 M standard Zinc and Manganese Metal solution.
- (ii) Buffer solution of pH — 10.0
- (iii) Indicator Eriochrome black T (EBT) powder
- (iv) Take 10 ml of aliquot from stock dilution of sample (1.0 g in 100 ml). Add to it 0.1 gram Hydroxylamine HCl, 1 to 2 ml of triethanolamine, 10 ml buffer of pH 10.0, EBT indicator and titrate against 0.02 M disodium EDTA solution.

% Free Magnesium = $\frac{\text{Titration value of EDTA} \times \text{Atomic weight of Mg metal}}{\text{Molarity of EDTA} \times 1}$

% Chelated Magnesium content = % Total Magnesium - % Free Magnesium content

*39. Determination of Chelated Copper as Cu-EDTA

A. Reagents

- (i) Glass distilled water or demineralized water of pH 2.5+/- 0.5:- Dilute 1 ml of 10% H₂SO₄ to one litre with glass distilled water or demineralized water and adjust the pH to 2.5 with a pH meter using 10% H₂SO₄ or NaOH.
- (ii) Di sodium EDTA solution:- Dissolve 0.73 gm of di sodium EDTA di hydrate in distilled water and make volume to 100 ml.

B. Preparation of Standard stock solution of Copper 1000 ppm

- (i) Dissolve 1.0 gm of Copper turnings in 30 ml of 1:1HNO₃ and dilute to 1000 ml in volumetric flask with distilled water. This is 1000 ppm copper stock solution.
- (ii) Intermediate solution [100 ppm].-Pipette 10 ml of Copper stock solution and 10 ml of Disodium EDTA solution in 100 ml volumetric flask and dilute volume with distilled water.

(Note:- In case the metal stock solution is not exactly 100.0 ppm, the exact titer value of disodium EDTA required to chelate the metal has to be added).

- (iii) Working Standard solution:- Pipette following volumes of 100 ppm intermediate solution in 100 ml Volumetric flask and make volume with acidified water.
-

Volume of 100 ppm intermediate solution taken	To make volume to 100	Concentration of Cu in ppm after making vol. to 100 ml
0.5 ml	100 ml	0.5 ppm
1.0 ml	100 ml	1.0 ppm
1.5 ml	100 ml	1.5 ppm
2.0 ml	100 ml	2.0 ppm

C. Preparation of sample solution for Chelated Copper

- (i) Weigh 1.0 gm of sample and transfer into 200 ml volumetric flask and make volume with distilled water. Pipette out 2.0 ml of sample solution and dilute further to make second dilution of 100 ml with distilled water, pipette 10.0 ml from second dilution and make volume to 100 ml with acidified water to get between the above flaming range from 0.5-2.0 ppm.
- (ii) Flaming the solutions:- Flame the standards and the sample of Chelated Copper on atomic absorption spectrophotometer at a wavelength of 324.8 nm (Cu line of instrument) using air-acetylene flame.
- (iii) Prepare a blank solution from which only the sample has been omitted. Flame along with standards and sample.

D. Calculations

Prepare a standard curve of known concentration of Copper solutions by plotting the absorbance value on Y-axis against their respective concentrations on X-axis.

Part-I :- Determination of Total Copper content in Chelated Copper sample
 Total Cu content = Concentration of Cu in ppm obtained after flaming the solution x 10

Part-II :- Determination of Free Copper in Chelated sample

- (i) Reagents:- 0.02 M Disodium salt of EDTA— 0.7444 gm of Disodium Salt of EDTA in 100 ml of distilled water. Standardize with 0.02 M standard Zinc/Manganese Metal solution.
- (ii) Acetate buffer (pH= 2.8) — 4.0 gm of Sodium acetate trihydrate + glacial acetic acid 155 ml make volume to 1000 ml.
- (iii) Methanolic PAN Indicator:- 0.2 gm of PAN indicator in 10 ml of methanol.

Take 20 ml of aliquot from stock dilution of sample (1.0 g in 200 ml). Add 10 ml acetate buffer of pH 2.8 and PAN indicator solution and titrate against 0.02 M disodium EDTA solution. End point is Magenta red to yellowish green.

% Free Copper = Titration value of EDTA x Atomic weight of Cu metal x Molarity of EDTA x 1

% Chelated Copper content = Total Copper - % Free Copper content

*40 Estimation of Zinc Content in Chelated Zinc as Zinc Glycine by AAS

D. Principle

The total Zinc in Zinc-Glycine is extracted by wet digestion (as Zinc) and estimated by using Atomic Absorption Spectrophotometer.

1. Instruments / Equipments / Glasswares

- (i) Atomic Absorption Spectrophotometer
- (ii) Hot Plate
- (iii) Analytical Balance weight least count 0.1 mg; and
- (iv) Required Glasswares.

2. Reagents and Chemicals

- i) Zinc Metal (pure);
- ii) Nitric Acid (AR Grade conc.);
- iii) Sulphuric Acid (AR Grade conc.);
- iv) Perchloric Acid (AR Grade — 70%); and
- v) Tri Acid Mixture (10 part of Nitric Acid + 01 part of Sulphuric Acid + 04 parts of Perchloric Acid).

3. Procedure

(a) Preparation of Standard Solutions

- (i) Preparation of 1000 ppm Standard Solution of Zinc - Weigh accurately 500 mg of Zinc metal and dissolve in 10 ml conc. Hydrochloric Acid and make the volume to the volume of 500 ml with distilled water; and
- (ii) Preparation of Working Standard Solution — Take 1 ml, 2 ml, 5 ml, 8 ml, 10 ml aliquot of 100 ppm standard solution in different 100 ml volumetric flasks and add 2 ml nitric acid in each volumetric flask and dilute to volume using distilled water.
- (iii) These solutions will be of 1.0, 2.0, 5.0, 8.0 and 10.0 ppm respectively.

(b) Preparation of Sample Solution

- (i) Take 1.0 gm sample of Zinc Glycine weighed in 250 ml Erlenmeyer (Conical) flask;
- (ii) Add 10 ml of Tri acid mixture (10 part of Nitric acid : 1 part of Sulphuric acid : 4 parts of Perchloric acid);
- (iii) Boil the solution at temperature between 150 to 200 °C on hot plate till the solution is clear and reduce the volume till white fumes appears;
- (iv) Then remove the solution from hot plate, cool it and make up the volume to 250 ml with distilled water;
- (v) Take 5 ml aliquot in 250 ml volumetric flask and make the volume with distilled water; and
- (vi) Run blank solution also to the same level.

(c) Estimation of Zinc in the sample by AAS

Flame the standard solution followed by sample solution at 215.9 nm wavelength.

4. Calculation:- Zinc % by weight :- $(S-B) \times V \times D \times 100 / W \times 10^6$

Where S — Average of concentration in sample reading in ppm, V = Volume of solution

B — Blank reading in ppm, D = Dilution W = Weight of the sample in gm

Note:- Distilled water instead of Milli-Q-Water has been used during analysis of the

sample.

***41. Estimation of Calcium Content in Chelated Calcium as Calcium Glycine by Atomic Absorption Spectrophotometer**

Principle

The total Calcium in Calcium-Glycine is extracted by wet digestion (as Calcium) and estimated by using Atomic Absorption Spectrophotometer.

I. Instruments / Equipments / Glasswares

- (i) Atomic Absorption Spectrophotometer;
- (ii) Hot Plate;
- (iii) Analytical Balance weight least count 0.1 mg; and
- (iv) Required Glasswares.

II. Reagents and Chemicals

- (i) Calcium Carbonate (Assay 99.5%);
- (ii) Nitric Acid (AR Grade conc.);
- (iii) Hydrochloric Acid (Assay 35.8 — 36.5%);
- (iv) Perchloric Acid (AR Grade — 70%);
- (v) Lanthanum Oxide (Assay 99.0%);
- (vi) Acid Mixture (Nitric Acid 2 parts : Perchloric Acid 1 part) ; and
- (vii) Lanthanum Oxide Solution: 5.86% w/v (58.65 gm of lanthanum oxide, 250 ml Hydrochloric acid and make up the volume 1000 ml with Milli-Q water).

III. Procedure

A. Preparation of Standard Solutions

- (i) Preparation of 1000 ppm Standard Solution of Calcium - Weigh accurately 2.50 gm of Calcium Carbonate and dissolve in 1000 ml volumetric flask and make the volume to 1000 ml with distilled water; and
- (ii) Preparation of Working Standard Solution — Take 0.5 ml, 1.0 ml, 2.0 ml, 3.0 ml, 4.0 ml, 5 ml aliquot of 100 ppm standard solution in different 100 ml volumetric flasks and add 10 ml of lanthanum oxide solution in each volumetric flask and dilute to volume using distilled water. These solutions will be of 0.5, 1.0, 2.0, 3.0, 4.0, 5.0 ppm respectively.

B. Preparation of Sample Solution

- (i) Take 1.0 gm sample of Calcium Glycine weighed in 250 ml Conical flask;
 - (ii) Add 10 ml of acid mixture;
-
- (iii) Boil the solution at temperature between 150 to 200 °C on hot plate till the solution is clear & reduce the volume till white fumes appear;
 - (iv) Then remove the solution from hot plate, cool it and add 5 ml Hydrochloric acid and make up the volume to 250 ml with distilled water;
 - (v) Take 2 ml aliquot in 250 ml volumetric flask and add 25 ml lanthanum oxide solution and make the volume with distilled water; and
 - (vi) Run blank solution also to the same level.

C. ESTIMATION of Calcium in the sample by AAS

Flame the standard solution followed by sample solution at 422.67 nm wavelength.

Calculation:- Calcium % by weight :- $(S-B) \times V \times D \times 100 / W \times 10$

Where S = Average of concentration in sample reading in ppm V =
Volume of solution
B = Blank reading in ppm; D — Dilution W =
Weight of the sample in gm

Note:- Distilled water instead of Milli-Q-Water has been used during analysis of the sample.

अनुसूची-2
(दफा ...को उपदफा (8) संग सम्बन्धित)
भाग -१
नानो मलको स्पेसिफिकेशन

1. Nano Nitrogen

S.N.	Characteristics	Requirements
1.1	Particle Size in nanometer (nm) in one dimension a. Physical particle size (as per TEM analysis) b. Hydrodynamic particle size (as per DLS analysis)	<100 <100
	At least 50% of the particles in the samples tested should be in the above range fulfilling the stipulated Department of Biotechnology guideline.	
1.2	Zeta Potential in mV (+/- scale) Liquid nano fertilizer sample (measured directly) Solid nano-fertilizer sample (measured in 1:10 Fertilizer: H ₂ O suspension)	>30

2. Nano Nitrogen and Phosphorus Fertilizer

S.N.	Characteristics	Requirements
1.1	Particle Size in nanometer (nm) in one dimension a. Physical particle size (as per TEM analysis) b. Hydrodynamic particle size (as per DLS analysis)	10-70 <100
	At least 50% of the particles in the tested samples should be in the above range fulfilling the stipulated DBT guidelines	

3. Nano Phosphorus Fertilizer

S.N.	Characteristics	Requirements
1.1	Particle Size in nanometer (nm) in one dimension a. Physical particle size (as per TEM analysis) b. Hydrodynamic particle size (as per DLS analysis)	<100 <100
	At least 50% of the particles in the tested samples should be in the above range fulfilling the stipulated DBT guidelines	
1.2	Zeta Potential in mV (+/- scale)	14-17

4. Nano Zinc

S.N.	Characteristics	Requirements
1.1	Particle Size in nanometer (nm) in one dimension a. Physical particle size (as per TEM analysis) b. Hydrodynamic particle size (as per DLS analysis)	Minimum 50 percent of the material shall be <100
1.2	Zeta Potential or surface charge in mV (+/- scale)	>15

5. Nano Copper

S.N.	Characteristics	Requirements
1.1	Particle Size in nanometer (nm) in one dimension a. Physical particle size (as per TEM analysis) b. Hydrodynamic particle size (as per DLS analysis)	Minimum 50 percent of the material shall be <100
1.2	Zeta Potential or surface charge in mV (+/- scale)	>15

भाग-२
TOLERANCE LIMIT OF NANO FERTILISERS

Not available

भाग-३
PROCEDURE FOR DRAWAL OF SAMPLE OF NANO FERTILISERS

Not available

भाग-४
METHODS OF ANALYSIS NANO FERTILISERS

Not available

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- Humic acid**
- Fulvic acid**
- Amino acid**
- Folic acid**
- Potassium Humate**
- Sea weed extract, Alginate acid**
- Amino proteins**
- Mannitol**
- Fucoidan**
- Alginic**
- Fluvic acid**

-
- Nitrobenzene**
 - Gibberlins**
 - Hydrolized protein**
 - AA**
 - Ethanolamine**
 - Cytokinins**
 - Auxins**
 - Natka**
 - Vitamins**
 - Emulsifiers**
 - Surfactant**
 - Alpha Naphthyl Acetic acid**
 - Stabilizer**
 - Preservatives**
 - Enzymatic stimulator**
 - Triacontanol**
 - Ethepon**

Eg.

This part should be discussed, the ingredients of the product vary.....so its not better to target specific name

a) Humic Acid and Fulvic Acid and their derivatives

1. Humic Acid 5% (Powder)

S.N.	Parameters	Requirements
1.1	Humic Acid percent by weight, minimum	5.0
1.2	pH (1 % aqueous solution)	4.0-5.0
1.3	Specific Gravity	1.0

2. Potassium Humate 49% (Powder)

S.N.	Parameters	Requirements
1.1	Humic Acid percent by weight, minimum	21.0
1.2	pH (1 % aqueous solution)	6.0-8.0

3. Humates and Fulvates-22% (Liquid)

S.N.	Parameters	Requirements
1.1	Total water soluble humates and fulvates per cent weight by volume, minimum	22.0
1.2	pH (1:5 % aqueous solution), minimum	9.0
1.3	Specific Gravity	1.05

4. Humates (12.5%) (Liquid)

S.N.	Parameters	Requirements
1.1	Humic Acid percent by weight, minimum	10.0
1.2	Fulvic Acid percent by weight, minimum	2.5
1.3	pH (10 % aqueous solution)	12-14
1.4	Specific Gravity	1.0-1.2

5. Humic Acid 51% (Granular)

S.N.	Parameters	Requirements
1.1	Humic Acid percent by weight, minimum	51.0
1.2	Fulvic Acid percent by weight, minimum	10.0
1.3	pH (1 % aqueous solution)	10-12
1.4	Bulk Density (25 °C) g/lee	0.5-0.7

b. Seaweed Extract

1. Ascophyllum nodosum 15% (Liquid)

S.N.	Parameters	Requirements
1.1	Alginic Acid percent by weight, minimum	1.5
1.2	Mannitol percent by weight, minimum	1.0
1.3	pH (10% aqueous solution)	4.7 + 1.0
1.4	Specific gravity	1.0-1.1
1.5	Total organic carbon percent by weight, minimum	5

2. Sargassum tenerrimum 2% (Granular)

S.N. Parameters	Requirements
1.1 Alginic Acid per cent by weight, minimum	0.2
1.2 pH (1 :2 aqueous solution)	6.0-8.5
1.3 Bulk density (g/lee)	0.95-1.1

3. Kappaphycus alvarezii 24% (Liquid)

S.N. Parameters	Requirements
1.1 Total carbohydrate per cent by weight, minimum	7.5
1.2 D-Galactose - 4-0- Sulphate per cent by weight, minimum	6.0
1.3 pH (minimum) (1:2 aqueous solution)	3.0-5.0

4. Sargassum tenerrimum 10% (liquid)

S.N. Parameters	Requirements
1.1 Alginic Acid per cent by weight, minimum	2.0
1.2 pH (1 :2 aqueous solution)	8-10
1.3 Organic matter (dry mass basis) per cent by weight, minimum	20.0

c. Mixed formulations of Bio stimulants

1. Mixture of Humic Acid, Amino Acid, Vitamins and Bio-chemicals (powder)

S.N. Characteristics	Requirements
1.1 Humic Acid per cent by weight, minimum	30.0
1.2 Amino Acid (Glycine) per cent by weight, minimum	7.0
1.3 Myoinositol per cent by weight, minimum	2.0
1.4 Vitamin C per cent by weight, minimum	12.0
1.5 Vitamin E per cent by weight, minimum	0.2
1.6 pH (1% aqueous solution)	5.0-6.5
1.7 Total organic carbon per cent by weight, minimum	25.0

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TOLERANCE LIMIT OF BIOSTIMULANTS

S.N.	Bio stimulant	Tolerance limit
(i)	Humic and Fulvic acid and their derivatives	5% of the specified value of active ingredients or the tracer molecule.
(ii)	Seaweed extract namely (Ascophyllum nodosum, Sargassum, Kappaphycus, Graci/aria and Ulva)	

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PROCEDURE FOR DRAWAL OF SAMPLE OF BIOSTIMULANTS

1. General Requirements of Sampling

- 1.0 In drawing, preparing and handling of samples, the following precautions and directions shall be observed -
- 1.1 Sampling shall be carried out by a trained and experienced person as it is essential that the sample should be representative of the lot to be examined.
- 1.2 Samples in their original unopened packets should be drawn and sent to the laboratory to prevent possible contamination of sample during handling and to help in revealing the true condition of the material.
- 1.3 Intact packets shall be drawn from a protected place not exposed to dampness, air, light, dust or soot.

2. Scale of Sampling

2.1 Lot:

All units (containers in a single consignment of type of material belonging to the same batch of manufacture) shall constitute a lot. If a consignment consists of different batches of the manufacture, the containers of the same batch shall be separated and shall constitute a separate lot.

2.2 Batch:- All biostimulants prepared simultaneously in the same process constitute a batch

2.3 For ascertaining conformity of the material to the requirements of the specifications, samples shall be tested from each lot separately.

2.4 The number of packets to be selected from a lot shall depend on the size of the lot and these packets shall be selected at random.

1. Drawal of Samples

1.1 The Inspector shall take three packets as samples from the same batch. Each sample constitutes a test sample.

1.2 Each sample shall be scaled in a cloth bag and be sealed with the Inspector's seal. The sealed cloth bag containing the sample and Form-P shall be kept in another cloth bag which shall also be sealed with the Inspector's seal. Identifiable details such as sample number, code number or any other details shall be put on the cloth bag containing sample and Form-P which enable its identification.

1.3 Out of the three samples collected, one sample so sealed shall be sent to in-charge of the laboratory notified by the **State Government under the sub-clause (1C) of clause 29 or any laboratory notified by the Central Government.** Another sample shall be given to the manufacture or importer or dealer, as the case may be. The third sample shall be sent by the Inspector to his next higher authority for keeping in safe custody. **Any of the latter two samples shall be sent for reference analysis under sub-clause (2) of clause 29B.**

1.4 The number of samples to be drawn from the lot:

Lot/Batch	Number of Samples
Upto 5,000 packets	3
5,001-10,000 packets	4
More than 10,000 packets	5

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METHODS OF ANALYSIS OF BIOSTIMULANTS

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1. Rhizobium

S.N.	Characteristics	Requirements
1.1	Total viable count Colony forming unit (CFU) minimum	5×10^7 cell per gram of powder, granules or carrier material/or per gram capsule content in gelatin base or 1×10^8 cell per ml of liquid.
1.2	Contamination level	No contamination at 10^5 dilutions.
1.3	pH	5.0-7.0
1.4	Efficiency character	Should show effective nodulation on all the species listed on the packet and there should be minimum of 25% increase in dry matter yield in test plant, after 25 Days After Sowing (DAS) when tested as per the method given under controlled conditions.

2. Azotobacter

S.N.	Characteristics	Requirements
1.1	Total viable count Colony forming unit (CFU) minimum	5×10^7 cell per gram of powder, granules or carrier material or per gram gelatin bases capsule content or 1×10^8 cell per milliliter (ml) of liquid.
1.2	Contamination level	No contamination at 10^5 dilutions.
1.3	pH	5.0-7.0
1.4	Efficiency character	The strain should be capable of fixing at least 10 mg of nitrogen per gram of sucrose consumed.

3. Acetobacter

S.N.	Characteristics	Requirements
1.1	Total viable count Colony forming unit (CFU) minimum	5×10^7 cell per gram of powder, granules or carrier material or per gram of capsule content or 1×10^8 cell per milliliter (ml) of liquid.
1.2	Contamination level	No contamination at 10^5 dilutions.
1.3	pH	5.5-6.0 for moist/dry powder, granulated or carrier based and 3.0-6.0 for liquid based.
1.4	Efficiency character	Formulation of yellowish pellicle in semi solid medium N free medium.

4. Azospirillum

S.N.	Characteristics	Requirements
1.1	Total viable count Colony forming unit (CFU) minimum	5×10^7 cell per gram of powder, granules or carrier material or per gram gelatin bases capsule content or 1×10^8 cell per milliliter (ml) of liquid.
1.2	Contamination level	No contamination at 10^5 dilutions.
1.3	pH	5.0-7.0
1.4	Efficiency character	Formation of white pellicle in semisolid Nitrogen free Bromothymol blue media.

5. Mycorrhiza

S.N.	Characteristics	Requirements
1.1	Total viable spores per gram of product, minimum	10 viable spore per gram of finished product.
1.2	Contamination level	No contamination at 10^5 dilutions.
1.3	pH	5.0-7.0
1.4	Efficiency character	1200 Inoculum Potential per gram of finished product by MPN method with 10-fold dilution

6. Phosphate Solubilising Bacteria (PSB)

S.N.	Characteristics	Requirements
1.1	Total viable count Colony forming unit (CFU) minimum	5×10^7 cell per gram of powder, granules or carrier material or 1×10^8 cell per milliliter (ml) of liquid.
1.2	Contamination level	No contamination at 10^5 dilutions.
1.3	pH	5.0-7.0 for moist/dry powder, granulated carrier based and 5.0-7.5 for liquid based.
1.4	Efficiency character	The strain should be capable of solubilizing at least 30 mg per litre of Phosphorus in liquid broth when tested as per the method given using Tricalcium Phosphate or Aluminium Phosphate or Iron Phosphate as Phosphate source.

7. Potassium Mobilising Biofertilizers (KMB)

S.N.	Characteristics	Requirements
1.1	Total viable count Colony forming unit (CFU), minimum	5×10^7 cell per gram of powder, granules or carrier material or per gram of capsule content or 1×10^8 cell per milliliter (ml) of liquid.
1.2	Contamination level	No contamination at 10^5 dilutions.
1.3	pH	5.0-7.0 for powder, granulated carrier based and 5.0-7.5 for liquid based or capsule in gelatin based.
1.4	Efficiency character	The strain should be capable of solubilizing at least 20 mg per litre of Potash in liquid broth when tested as per the method given using Aluminium Potassium Silicate as K source.

8. Zinc Solubilising Bacteria

S.N.	Characteristics	Requirements
1.1	Total viable count Colony forming unit (CFU), minimum	5×10^7 cell per gram of powder, granules or carrier material or per gram of capsule content or 1×10^8 cell per milliliter (ml) of liquid.
1.2	Contamination level	No contamination at 10^5 dilutions.
1.3	pH	5.0-7.0 for powder, granulated carrier based and 5.0-7.5 for liquid based.
1.4	Efficiency character	The strain should be capable of solubilizing at least 20 mg per litre of Zinc in liquid broth when tested as per the method given using Zinc Oxide/ Zinc Carbonate/ Zinc Phosphate as Zinc source.

9. Phosphate Solubilising Fungi

S.N.	Characteristics	Requirements
1.1	Viable spore count, minimum	1×10^6 spores per gram
1.3	pH	1×10^7 viable fungal spores per milliliter of the liquid Liquid: 3.5 to 5.5 Carrier: 6.0 to 7.7
1.4	Contamination level	Nil for liquid inoculums. 1×10^3 cells per gram for carrier-based preparation.
1.5	Efficiency character	The strain should be capable of solubilizing at least 30 mg/ litre of Phosphorus in liquid broth when tested as per the method given using Tricalcium Phosphate or Aluminium Phosphate or Iron Phosphate as Phosphate source.

10. Carrier Based Consortia

S.N.	Characteristics	Requirements
1.1	Individual organism viable count CFU minimum in a mixture of any 2 or maximum three of the micro-organisms	Rhizobium or Azotobacter or Azospirillum 1×10^7 cells per gram (g). PSB 1×10^7 cells per gram (g). KSB 1×10^7 cells per gram (g).
1.2	Total viable count of all the biofertilizers in the product	CFU minimum 3×10^7 cells per gram of carrier/powder.
1.3	Efficiency character	The efficiency character of individual microorganisms to be determined as mentioned in case of individual biofertilizers through quantitative estimation methods.

11. Liquid Consortia

S.N.	Characteristics	Requirements
1.1	Individual organism viable count CFU minimum in a mixture of any 2 or maximum three of the micro-organisms	Rhizobium or Azotobacter or Azospirillum 5×10^7 cells per milliliter (ml). PSB 5×10^7 cells per milliliter (ml). KSB 5×10^7 cells per milliliter (ml).
1.2	Total viable count of all the biofertilizers in the product	CFU minimum 1.5×10^8 cells per milliliter (ml).
1.3	pH	5.0-7.0
1.4	Contamination level	No contamination at any dilution.
1.5	Efficiency character	The efficiency character of individual microorganisms to be determined as mentioned in case of individual biofertilizers through quantitative estimation methods.

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TOLERANCE LIMIT OF BIOFERTILISERS

1. In case of Rhizobium, Azotobacter, Azospirillum, Phosphate Solubilising Bacteria, Potash Mobilising Bacteria, Zinc Solubilising Bacteria, the total viable count shall not be less than 1×10^7 CFU per gm of carrier material in the form of powder or granules or

- 5x10⁷ CFU per ml in case of liquid formulations.
2. In case of Consortia, the total viable count shall not be less than 1x10⁷ in case of carrier based and 1x10⁸ in case of liquid formulations.
 3. In case of Mycorrhizal biofertilizers, total viable spores shall not be less than 8 per gm of finished product.

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PROCEDURE FOR DRAWAL OF SAMPLE OF BIOFERTILISERS

1. General Requirements of Sampling

- 1.0 In drawing, preparing and handling the samples, the following precautions and directions shall be observed.
- 1.1 Sampling shall be carried out by a trained and experienced person as it is essential that the sample should be representative of the lot to be examined.
- 1.2 Samples in their original unopened packets should be drawn and sent to the laboratory to prevent possible contamination of samples during handling and to help in revealing the true condition of the material.
- 1.3 Intact packets shall be drawn from a protected place not exposed to dampness, air, light, dust or soot.

2. Scale of Sampling

2.1 Lot

All units (containers in a single consignment of type of material belonging to the same batch of manufacture) shall constitute a lot. If a consignment consists of different batches of the manufacture, the containers of the same batch shall be separated and shall constitute a separate lot.

2.2 Batch

All inoculants prepared from a batch fermentor or a group of flasks (containers) constitute a batch.

2.3 For ascertaining conformity of the material to the requirements of the specification, samples shall be tested from each lot separately.

2.4 The number of packets to be selected from a lot shall depend on the size of the lot and these packets shall be selected at random.

3. Drawal of Samples

3.1 The Inspector shall take three packets as sample from the same batch. Each sample constitutes a test sample.

3.2 Of the sample of biofertilizers drawn by the Inspector, the label affixed there in shall be defaced and a label containing generic name of biofertilizers or organic fertilizers, expiry date, code number and date of sampling shall be pasted on the upper margin of sample packet/container. The sample shall be kept in cloth bag and be sealed with Inspector seal. The sealed cloth bag containing sample along with 'P' shall be kept in another cloth bag and be sealed with inspector seal. The identifiable details such as code number, date

of sampling, name of biofertilizer shall be put on cloth bag containing biofertilizer with form 'P'.

3.3 Out of three samples collected, one sample so sealed shall be sent to Incharge of the laboratory notified Another sample shall be given to manufacturer or dealer. The third sample shall be sent by the inspector to the next higher authority for safe custody. The appellate authority shall be sent to either of the latter two samples for referee analysis.

3.4 The number of samples to be drawn from the lot

Lot/Batch	Number of samples
Upto 5,000 packets	03
5,001-10,000 packets	04
More than 10,000 packets	05

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METHODS OF ANALYSIS OF BIOFERTILISERS

A. Method of Analysis of Rhizobium Biofertilizers

1. Apparatus

1.1 Pipettes graduated 1 ml and 10 ml

1.2 Dilution bottles or flasks

1.3 Petri dishes clear, uniform, flat- bottomed

1.4 Hot-air oven

Capable of giving uniform and adequate temperature, equipped with a thermometer, calibrated to read up to 250°C and with vents suitably located to assure prompt and uniform heating.

1.5 Autoclave

1.6 Incubator

1.7 Hand tally or mechanical counting device

1.8 pH meter

2. Reagents

2.1 Congo red - one percent aqueous solution

2.2 Medium

Use a plating medium of the following composition:

Medium	Quantity
Agar	20 g
Yeast extract	1 g
Mannitol	10 g
Potassium hydrogen phosphate (K_2HPO_4)	0.5 g
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Sodium chloride (NaCl)	0.1 g
Congo red	2.5 ml

Distilled water	1000 ml
pH	7.0

2.3 Sterilising and preparation procedure for plates:

- 2.3.1 Sterilize the sampling and plating equipment with dry heat in a hot air oven at not less than 160°C for not less than 2 hours.
- 2.3.2 Sterilize the media by autoclaving at 120°C for 20 min. To permit passage of steam into and from closed containers when autoclaved, keep stoppers slightly loosened or plugged with cotton. Air from within the chamber of the sterilizer should be ejected, allowing steam pressure to rise.

Preparation of plating medium and pouring

2.3.3 Prepare growth medium in accordance with the composition of the specific biofertilizer.

2.3.4 Melt the required amount of medium in boiling water or by exposure to flowing steam in partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Melt enough medium which will be used within 3 h. Re-sterilization of the medium may cause partial precipitation of ingredients.

2.3.5 When holding time is less than 30 min, promptly cool the molten medium to about 45°C, and store until used, in a water bath or incubator at 43 to 45°C. Introduce 12 to 15 ml of liquefied medium or appropriate quantity depending on size of the petri dish at 42 to 44°C into each plate. Gently lift the cover of the dish just enough to pour in the medium. Sterilize the lips of the medium containers by exposure to flame.

- a. immediately before pouring.
- b. Periodically during pouring, and
- c. When pouring is complete for each batch of plates, if portions of molten medium remain in containers and are to be used without subsequent sterilization for pouring additional plates. As each plate is poured thoroughly mix the medium with test portions in the petri dish.

2.3.6 By rotating and tilting the dish and without splashing the medium over edge, spread the medium evenly over the bottom of the plate. Provide conditions so that the medium solidifies with reasonable promptness (5-10 min) before removing the plates from level surface.

3. Preparation of Serial Dilutions for Plate Counts

3.1 Dispense 30 g of inoculant to 270 ml of sterile distilled demineralized water and shake for 10 min on a reciprocal shaker or homogeniser. Make serial dilutions up to 10¹⁰. Take 0.1 ml or suitable aliquots of 10⁶ to 10⁹ dilutions using sterile pipettes and deliver to petri dishes containing set medium as given in 2.1 and spread it uniformly with a spreader. Invert the plates and promptly place them in the incubator.

4. Incubation of Plates

4.1 Label the plates and incubate at 28 +/- 2°C for 3 to 5 days for fast growing Rhizobia and 5 to 10 days for slow -growing ones.

4.2 Colony counting aids

Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in centimeter square. Record the total number of colonies with the hand tally. Avoid mistaking particles of undissolved medium or precipitated matter in plates for pin-point colonies. To distinguish colonies from dirt, specks and other foreign matter, examine doubtful objects carefully.

4.3 Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies which absorb congo red and stand out as reddish colonies. Rhizobium stands out as white, translucent, glistening and elevated colonies. Count such colony numbers and calculate figures in terms of per litre, of carrier. Also check for freedom from contamination at 10^5 dilutions.

5. Test for Nodulation

5.1 Pot culture test

Plant nutrient solution

a)	Composition	Concentration	g/l
b)	Potassium chloride	0.001 M	0.0745
c)	Potassium hydrogen phosphate (K_2HPO_4)	0.001 M	0.0175
d)	Calcium sulphate ($CaSO_4 \cdot 2H_2O$)	0.002 M	0.344
e)	Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.001 M	0.246
	Trace elements solution:		
	i) Copper sulphate ($CuSO_4 \cdot 5H_2O$)	0.01 mg/kg	0.78
	ii) Zinc sulphate ($ZnSO_4 \cdot 7H_2O$)	0.25 mg/kg	2.22
	iii) Manganese sulphate ($MnSO_4 \cdot 4H_2O$)	0.25 mg/kg	2.03
	iv) Ammonium molybdate [$(NH_4)_6MO_7O_{24} \cdot 4H_2O$]	0.0025 mg/kg	0.01
	v) Boric acid (H_3BO_4)	0.125 mg/kg	1.43

Prepare the solution No. (e) consisting of trace elements in one litre of stock solution and add final nutrient solution at the rate of 0.5 ml per litre.

f) Iron solution g/100 ml

1) Ferrous sulphate 5

2) Citric acid 5

Prepare the solution No. (f) as 100 ml of stock solution and add final nutrient solution at the rate of 0.5 ml per litre.

5.2 Preparation

Prepare the nutrient solution by weighing out substances (a), (b) and (d) and dissolving them in a litre of water. To this solution add 0.5 ml of trace elements

solution and 0.5 ml of iron solution. Grind in a mortar 0.344 g of calcium sulphate (c) to a fine consistency and add to the final nutrient solution. Autoclave the nutrient solution thus prepared at 120°C for 20 min.

Notes

1. The nutrient solution may be prepared in the tap water provided the water is soft.
2. The nutrient solution should be shaken well to disperse calcium sulphate before dispensing.
3. If the solution is made up with distilled water, the pH is about 7.2 before autoclaving and falls to 5.5 on autoclaving and rises slowly on standing to about 5.8. However, there is no need to adjust pH. For most tropical legumes, pH of about 6.0 is adequate.

5.3 Procedure

5.3.1 Immerse the seeds in 70 percent alcohol for 1 minute, drain the alcohol and immerse the seeds in freshly prepared 5% Sodium Hypochlorite solution for 3 minutes or in 0.1 percent Mercuric Chloride solution for 3 minutes in a suitable container such as a screwcapped bottle or a test tube with a rubber hung. Drain the Sterilant and wash the seeds several times with sterile water (at least ten times) to get rid of the Sterilant.

5.3.2 Fill earthenware or glazed pot with soil (2 parts soil and 1 part washed coarse sand) (pH 6 to 7) and autoclave for 2 hours at 120 °C. After two days of incubation at room temperature, repeat autoclaving to ensure complete sterility of soil.

5.3.3 Treat surface sterilized seeds with water slurry of inoculant taken from a culture packet @ 1 gm inoculant mixed with 2 ml of sterile water per 50 gm seed and sow the seeds. Keep a set of pots with uninoculated seeds (surface sterilized but not treated with inoculant) as control. Keep minimum of 4-5 pots for each treatment to overcome variations.

5.3.4 Incubate them in a pot-culture house during appropriate seasons for test plants, taking care to separate the inoculated pots from the control pots. Alternatively incubate the inoculated and uninoculated pots in growth chamber/or growth cabinets under controlled environmental conditions having facilities to adjust temperature (28 °C), humidity (65%) and light intensity (10 Kilo Lux) with 16 hours light and 8 hours dark period.

5.3.5 On day 1 of incubation irrigate each pot once to the moisture holding capacity of soil with sterilized growth medium (as specified at 5.1). Subsequently, water the seedling periodically with sterilized water, taking care to prevent splashing of water from inoculated pots to uninoculated ones.

5.3.6 After two weeks of growth, thin down the number of plants in each pot to four uniform plants. At the end of 25 days, separate the plants carefully from the soil under slow running water. Keep inoculated and uninoculated plants separately. Record the number of nodules on each plant, separately from inoculated and un- inoculated pots. Dry the individual plants (shoot+ root) in

an oven at 60 °C for 48 hours, separately and record dry weight. Calculate average dry weight of inoculated and un-inoculated plants.

5.3.7 Calculate the increase in dry matter yield as under

$$\% \text{ increase in dry matter} = \frac{W1 - W2}{W2} \times 100$$

Where, W1 = average dry weight of inoculated plant shoot

W2 = average dry weight of control un-inoculated plant shoot.

5.3.8 If good nodulation is obtained in inoculated plants together with total absence or sometimes presence of stray nodules in controls and if there is a 25% increase in the dry mass of plants over the uninoculated control, it may be concluded that the strain is adequately nodulating and is effective.

B. Method of Analysis of Azotobacter Biofertilizers

1. *Apparatus* - same as Rhizobium

2. *Reagents*:

2.1 Medium

Use a plating medium of the following composition

Medium	Quantity
Agar	20 g
Sucrose (C ₁₂ H ₂₂ O ₁₁)	20 g
Ferric sulphate (Fe ₂ (SO ₄) ₄)	0.1 g
Potassium hydrogen phosphate (K ₂ HPO ₄)	1.0 g
Magnesium sulphate (MgSO ₄ .7H ₂ O)	0.5 g
Sodium chloride (NaCl)	0.5 g
Calcium carbonate (CaCO ₃)	2.0 g
Sodium Molybdate (Na ₂ MoO ₄)	0.005 g
Distilled water	1000 ml
pH	6.8-7.2

2.2 Sterilisation and preparation procedure for plates: same as for Rhizobium

Preparation of Plating Medium and Pouring

3. Preparation of Serial Dilutions for Plate Counts

Dispense 30 g of inoculant to 270 ml of sterile distilled water and shake for 10 min on a reciprocal shaker. Make serials dilutions up to 10¹⁰. Take 0.1 ml or suitable aliquots of 10⁶ to 10⁹ dilutions using sterile pipettes and deliver to petri dishes containing set medium as given in 2.1 and spread it uniformly. Invert the plates and promptly place them in the incubator.

4. Incubation of Plates - Same as Rhizobium

4.1 Label the plates and incubate at 28+/- 3°C for 4 to 6 days.

4.2 Colony counting aids: Same as Rhizobium

Azotobacter chroococcum colonies are gummy, raised with or without striations, viscous and often sticky. The pigmentation varies from very light brown to black. Count the colony number and observe the cyst formation as given below and calculate number per gram of the carrier material.

Grow the vegetative cells at 30°C on Burks agar medium comprising sucrose 20 g, dipotassium hydrogen phosphate 0.64 g, dihydrogen potassium phosphate 0.20 g; sodium chloride 0.20 g; calcium sulphate 0.05 g, sodium molybdate 0.001 g; ferric sulphate 0.003 g, agar 20 g and distilled water 1000 ml. Look for vegetative cells after 18 to 24 h either by simple staining method or through a phase contrast microscope.

Grow the cyst cells on Burks agar medium as given above with 0.3 percent n-butanol in place of the carbon source. Look for cyst formation after 4 to 5 days incubation.

5. Test for Nitrogen Fixation in Pure Culture

5.1 Pure culture medium

5.1.1 Prepare medium as given for *Azotobacter* (2.1 under B), excluding agar.

5.2 Procedure

Select from each *Azotobacter* colony, of the type that has been counted as *Azotobacter chroococcum* in B 4.2. One colony and plate on the medium given in B 2.1. Use this pure culture for inoculating the broth for nitrogen fixation. For this purpose, take 50-ml aliquots of broth in 250-ml conical flasks for inoculation. After 12 days growth at 28°C, test the contents of the flasks for purity by streaking on fresh medium and concentrating over a water-bath (50 to 60°C) to dryness. Wash the dried culture and take it as a sample. The contents of the flasks in inoculated control series should be processed in a similar manner.

5.3 Determination by Kjeldahl Method

- (i) Reagents
- (ii) Sulphuric acid- 93 - 98 percent, N-free
- (iii) Digestion mixture - Mix copper sulphate and potassium sulphate in the ratio 1: 10 and grind them to a fine powder.
- (iv) Sodium hydroxide pellets or solution, N-free - For solution, dissolve about 450 g of sodium hydroxide in water, cool, and dilute to 1 litre (sp gr of the solution should be at least 1.36)

- (v) Zinc granules - reagent grade.
- (vi) Indicators
 - a) Methyl red indicator - Dissolve 1 g of methyl red in 200 ml of ethanol.
 - b) Mixed indicator - Prepare mixed indicator by dissolving 0.8 gm of methyl red and 0.2 g of methyl blue in 500 ml of ethanol.
- (vii) Hydrochloric or sulphuric acid - standard solution 0.5 or 0.1 N when amount of nitrogen is small.
- (viii) Sodium hydroxide standard solution - 0.1 N (or other specified concentration).

Note - Ratio of salt to acid (m/v) should be about 1: 1 at the end of the digestion for proper temperature control. Digestion may be incomplete at a lower ratio, and nitrogen may be lost at higher ratio. Each gram of fat consumes 10 ml of sulphuric acid and each gram of carbohydrate 4.0 ml of sulphuric acid during digestion.

5.3 Apparatus

(i) For digestion - Use Kjeldahl's flasks of hard, moderately thick, well-annealed glass with total capacity approximately 500 to 800 ml. conduct digestion over heating device adjusted to bring 250 ml of water at 25°C to rolling boil in about 5 minutes. To test the heaters, preheat for 10 minutes in the case of gas burners and for 30 minutes in the case of electric heaters. Add 3 to 4 boiling chips to prevent superheating.

(ii) For distillation - Use 500- to 800 ml Kjeldahl's flask fitted with rubber stopper through which passes the lower end of an efficient scrubber bulb or trap to prevent mechanical carry-over of sodium hydroxide during distillation. Connect the upper end of the bulb tube to a condenser by a rubber tubing. Trap the outlet of the condenser in such a way as to ensure absorption of ammonia distilled over with the receiver.

(a) Procedure

Place 0.25 g of the sample in the digestion flask. Add 0.7 Gm mercuric oxide, 15 gm potassium sulphate followed by 25 ml of sulphuric acid. Shake, let stand for about 30 minutes and heat carefully until frothing ceases. Boil briskly until the solution clears and continue boiling further for 90 minutes. Cool, add about 200 ml of water, cool to room temperature and add a few zinc granules.

(b) Tilt the flask and carefully add 50 ml of sodium hydroxide solution without agitation. Immediately connect the flask to the distillation bulb on the condenser whose tip is immersed in 50 ml of standard 0.1 N acid in the

receiving flasks. Rotate the digestion flask carefully to mix the contents. Heat until 150 ml of the distillate collects and titrate excess acid with 0.1 N base using methyl red or mixed indicator. Carry out blank determination on reagents.

Note: Check the ammonia recording periodically, using inorganic nitrogen control, for example, ammonium sulphate.

(c) Calculation: -

(i) Nitrogen content, percent by mass =

(Milliliters of 0.1 N acid for sample - millilitres of 0.1 N acid for blank) x 0.14 mass of sample taken

(ii) Total nitrogen in culture = Total dry mass of sample x percent nitrogen.

(d) Take a 1.0 g of accurately weighed sample each from the inoculated series and from the controls. Put them separately in 250 ml volumetric flask, add 150 ml water, mix the content and make up the volume to 250 ml water. Shake for 5 minutes and centrifuge for 15 minutes at 10000 rev/min. Estimate glucose in the supernatant in triplicate. The difference between the two provides the data of actual amount of glucose consumed. Calculate the amount of nitrogen fixed per gram of sucrose consumed.

5.5 Determination of Glucose - From the supernatant, draw suitable aliquots and estimate reducing sugars (glucose) as follows.

(i) Reagents

(ii) Soxhlet modification of Fehling solution - Prepare by mixing equal volumes of Solution A and Solution B immediately before using.

(iii) Copper sulphate solution (Solution A) - Dissolve 34.639 g of copper sulphate crystals ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) in water, dilute to 500 ml and filter through glass wool or filter paper.

Standardisation of copper sulphate solution: - Using separate pipettes, pipette accurately 5 ml of Solution A and 5 ml of Solution B into a conical flask of 250 ml capacity. Heat this mixture to boiling on an asbestos gauze and add standard invert sugar solution from a burette, about 1 ml less than the expected volume which will reduce the Fehling solution completely (about 48 ml). Add 1 ml of methylene blue indicator while keeping the solution boiling. Complete the titration within 3 minutes, the end point being indicated by change of colour from the blue to red. From the volume of invert sugar solution used, calculate the strength(s) of the copper sulphate solution by multiplying the titre value by 0.001 (mg/ml of the standard invert sugar solution). This would give the quantity of invert sugar required to reduce the copper in 5 ml of copper sulphate solution.

- (iv) **Potassium sodium tartrate (Rochelle salt) Solution (Solution B):-** Dissolve 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in water, and dilute to 500 ml. Let the solution stand for a day, and filter.
- (v) **Hydrochloric acid-sp gr** 1.18 at 20°C (approximately 12 N)
- (vi) **Standard invert sugar solution-**Weigh accurately 0.95 g of sucrose and dissolve it in 500 ml of water. Add 32 ml of concentrated hydrochloric acid, boil gently for 30 minutes and keep aside for 24 hours. Neutralize with sodium carbonate and make the final volume to 1000 ml; 50 ml of this solution contains 0.05 g of invert sugar.
- (vii) **Methylene blue indicator** - 0.2 percent in water.
- (viii) **Procedure:** - Place about 1 g (M), accurately weighed, of the prepared sample of AI into a 250 ml volumetric flask and dilute with about 150 ml of water. Mix thoroughly the contents of the flask and make the volume of 250 ml with water. Using separate pipettes, take accurately 5 ml each of Solution A and Solution B in a porcelain dish. Add about 12 ml of AI solution from a burette and heat to boiling over an asbestos gauze. Add 1 ml of methylene blue indicator and while keeping the solution boiling complete the titration within 3 minutes, the end point being indicated by change of colour from blue to red. Note the volume (H) in ml of AI solution required for the titration.

(ix) Calculation

$$\text{Total reducing sugars, percent by mass} = \frac{250 \times 100 \times S}{H \times M}$$

Where

s = strength of copper sulphate solution,

H = volume in ml of AI solution required for titration, and

M = mass in g of AI taken for the test.

5.6 Determination of Sucrose

i Procedure:- To 100 ml of the stock AI solution, add 1 ml of concentrated hydrochloric acid and heat the solution to near boiling. Keep aside overnight. Neutralize this solution with sodium carbonate and determine the total reducing sugars.

(i) Calculation

Sucrose, percent by mass = (reducing sugars after inversion, percent by mass) - (reducing sugars before inversion, percent by mass) x 0.95.

(a) Nitrogen, mg per gram of sucrose consumed = $2(a - b) - C$

where

a = initial quantity of sucrose taken for the test,

b = mass of sucrose as calculated in (a), and

c = amount of nitrogen fixed per gram of glucose.

C. Method of Analysis of Azospirillum Biofertilisers

Method of determination of total viable count and Pellicle formation

1. Apparatus : same as Rhizobium

2. Reagents

2.1 Medium

Use N-free semisolid medium (Nfb) of the following composition for preparation of MPN tubes

Medium	Quantity
DL-Malic acid	5.0
K ₂ HPO ₄	0.5
MgSO ₄ ·7H ₂ O	0.2
NaCl	0.1
CaCl ₂	0.02
Trace element Soln.	2.0 ml
Fe EDTA (1.64% Soln.)	4.0 ml
Vitamin soln.	1.0 ml
KOH	4.0 ml
Bromothymol blue (0.5% aq.)	2.0 ml
Adjust pH to 6.8-7.0 with KOH	
For semi solid add agar	1.75 g
For solid medium add agar	15.0 g

2.1.1 Trace element solution (g/litre)

Medium	Quantity
Na ₂ MoO ₄ · 2H ₂ O	0.2
MnSO ₄ ·H ₂ O	0.235
H ₃ BO ₃	0.28
CuSO ₄ · 5H ₂ O	0.008
ZnSO ₄ · 7H ₂ O	0.024
Distilled water	1000 ml

Use 2 ml of this solution in one litre of Nfb media

Vitamin solution (g/litre)

Medium	Quantity
Biotin	0.01

Pyridoxin	0.02
Distilled water	1000 ml

Use one ml of this sol. in one litre of Nfb media

2.2 Sterilization and preparation of MPN tubes

2.2.1 Prepare Nitrogen free Bromothymol Blue malate medium as mentioned at paragraph 2.1. Boil to dissolve agar. Quickly dispense 10 ml molten media in 15 x 150 ml test tubes or screw capped culture tubes and close either with cotton plugs or screw caps. Minimum of 25 such tubes shall be needed for each sample.

2.2.2 Sterilize the tubes by autoclaving at 121°C for 20 minutes, as in Rhizobium at paragraph 2.3.2.

3. Preparation of serial dilution for MPN count

Dispense 30 g of Azospirillum biofertilizers in 270 ml of sterile water and shake for 10 minutes on a reciprocal shaker. Make serial dilutions upto 10^{-8} dilution. Pipette out 1 ml aliquots of 10^{-4} to 10^{-8} dilution and deliver it to screw cap tubes or test tubes containing N-free semi solid Nfb media.

4. Incubation of tubes

Label the tubes and incubate at $36 \pm 1^\circ\text{C}$ for 3-4 days in vertical position in a test tubes stand. Do not disturb the medium during the entire period of incubation.

5. Counting

5.1 Count the tubes which have turned blue and have developed typical white sub-surface pellicle.

5.2 Count the tubes as +ve or -ve for the presence of sub-surface pellicle and consider for the purpose of calculation.

5.3 Method for Estimating MPN Count

5.3.1 To calculate the most probable number of organisms in the original sample, select as P_1 the number of positive tubes in the least concentrated dilution in which all tubes are positive or in which the greatest number of tubes is +ve, and let P_2 and P_3 represent the numbers of positive tubes in the next two higher dilutions.

5.3.2 Then find the row of numbers in Table 1 in which P_1 and P_2 correspond to the values observed experimentally. Follow that row of numbers across the table to the column headed by the observed value of P_3 .

5.3.3 The figure at the point of intersection is the most probable number of organisms in the quantity of original sample represented in the inoculum added in the second dilution. Multiply this figure by the appropriate dilution factor to obtain the MPN value.

Table 1 - Most probable numbers for use with 10-fold dilution and 5 tubes per dilution (Cochran, 1950)

		Most probable number for indicated values of P_3					
P_1	P_2	0	1	2	3	4	5
0	0		0.018	0.036	0.054	0.072	0.090
0	1	0.018	0.036	0.055	0.073	0.091	0.11
0	2	0.037	0.055	0.074	0.092	0.11	0.13
0	3	0.056	0.074	0.093	0.11	0.13	0.15
0	4	0.075	0.094	0.11	0.13	0.15	0.17
0	5	0.094	0.11	0.13	0.15	0.17	0.19
1	0	0.040	0.11	0.13	0.15	0.17	0.19
1	1	0.040	0.061	0.081	0.10	0.12	0.14
1	2	0.061	0.082	0.10	0.12	0.16	0.17
1	3	0.089	0.10	0.13	0.16	0.17	0.19
1	4	0.11	0.13	0.15	0.17	0.19	0.22
1	5	0.13	0.15	0.17	0.19	0.22	0.24
2	0	0.046	0.068	0.091	0.12	0.14	0.16
2	1	0.068	0.092	0.12	0.14	0.17	0.19
2	2	0.093	0.12	0.14	0.17	0.19	0.22
2	3	0.12	0.14	0.17	0.20	0.22	0.25
2	4	0.15	0.17	0.20	0.23	0.25	0.28
2	5	0.17	0.20	0.23	0.26	0.29	0.32
3	0	0.078	0.11	0.13	0.16	0.20	0.23
3	1	0.11	0.14	0.17	0.20	0.23	0.27
3	2	0.14	0.17	0.20	0.24	0.27	0.31
3	3	0.17	0.21	0.24	0.28	0.31	0.35
3	4	0.21	0.24	0.28	0.32	0.36	0.40
3	5	0.25	0.29	0.32	0.37	0.41	0.45
4	0	0.13	0.17	0.21	0.25	0.30	0.36
4	1	0.17	0.21	0.26	0.31	0.36	0.42
4	2	0.22	0.26	0.32	0.38	0.44	0.50
4	3	0.27	0.33	0.39	0.45	0.52	0.59
4	4	0.34	0.40	0.47	0.54	0.62	0.69
4	5	0.41	0.48	0.56	0.64	0.72	0.81
5	0	0.23	0.31	0.43	0.58	0.76	0.95
5	1	0.33	0.46	0.64	0.84	1.1	1.3
5	2	0.49	0.70	0.95	1.2	1.5	1.8
5	3	0.79	1.1	1.4	1.8	2.1	2.5
5	4	1.3	1.7	2.2	2.8	3.5	4.3

B. Method of determination of Contamination

Medium	Quantity
KH ₂ PO ₄	0.5 gram
MgSO ₄ . 7H ₂ O	0.2 gram
NaCl	0.1 gram
Yeast Extract	0.5 gram
FeCl ₃ .6H ₂ O	0.015 gram
DL-malic acid	5.0 gram
KOH	4.8 gram
Congo resolution	15.0 gram
Agar	20.0 gram
Water	1000 ml

Adjust to pH 7 with 0.1 N of KOH

Congo red solution weigh 2.5 gin 1000 ml water

Note 1:- Sterilizing and preparation procedure for plates, serial dilution for plate counts, incubation of plates and counting of colonies - Same as of Rhizobium.

Note 2:- On Rojo Congo medium, Azospirillum (*A. lipoferum* and *A. brasilense*) colonies shall appear as scarlet red colonies with dry consistency, diameter of 1.5 to 2 mm, round or irregular form, undulate edge, and rugose surface with ridges radiating from the center.

1.D. Method of Analysis of Phosphate Solubilising Bacterial Biofertilizers

1. **Apparatus:** same as Rhizobium

2. **Reagents**

2.1 **Medium**

Use a plating medium of the following composition:

Medium	Quantity
Glucose	10.0 g
Tri-calcium phosphate	5.0 g
Ammonium sulphate	0.5 g
Magnesium sulphate	0.1 g
Sodium chloride	0.2 g
Yeast extract	0.5 g
Manganese sulphate	Trace
Ferrous sulphate	Trace
Distilled water	1000 ml
Agar	15.0 g

. Sterilizing and preparation procedure for plates - Same as Rhizobium

Preparation of Plating Medium and Pouring - Same as Rhizobium

3.Preparation of Serial Dilutions for Plate Counts - same as Rhizobium

4.Incubation of Plates

4.1 Label the plates and incubate at $28 \pm 1^\circ\text{C}$ for 4 to 6 days.

4.2 Colony counting aids: Same as Rhizobium

Counting

Count the total number of colonies on the plates including colonies with solubilisation zone with the help of a colony counter.

Methods for counting solubilisation zones

- a. Take 10 g of PSBI (BF) in 90 ml in water
- b. Make a ten-fold dilution series up to 10^7
- c. Take 0.2 ml aliquote of 10^5 to 10^7 dilution using sterile pipettes and delivered to petri dishes containing pikowskeyi media.
- d. Spread it uniformly, invert the plates and incubate them up to 2 weeks at $28 \pm 2^\circ\text{C}$.
- e. Count the colonies showing hallow cones and measure their diameter. Minimum acceptable zone is 10 mm in diameter.

5.Determination of Soluble Phosphorus Using Ascorbic Acid

5.1 Apparatus

Spectrophotometer capable of transmission measurements at 840 to 880nm.

Extractant: It is olsen extract.

5.2 Reagents

Ammonium Molybdate $[(\text{NH}_4)_6\text{MO}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$

L-Ascorbic Acid

p-Nitrophenol

$4\text{NH}_2\text{SO}_4$

5.3 Preparation of Reagents

5.3.1 Sulphomolybdic Acid

- Take 20 g of ammonium molybdate and dissolve in 300 ml of distilled water.
- Add slowly 450 ml of 10 N H_2SO_4 .
- Cool the above mixture and add 100 ml of 0.5 percent solution of antimony potassium tartrate.
- Coll and make the volume to one litre. Store in glass bottle away from direct

sunlight.

5.3.2. Preparation of mixed reagent

Add 1.5 g of L-ascorbic acid in 100 ml of the above stock solution and mix. Add 5 ml of this solution to develop colour. Mixed reagent is to be prepared fresh as it does not keep for more than 24 h.

5.3.3 Procedure

- (i) Preparation of Sample
Pure culture medium same as at 2.1 above excluding agar. Prepare broth medium in 100 ml aliquots in 6 nos. 250 ml conical flasks and sterilize in autoclave at 121°C for 20 min.
- (ii) Inoculation of Medium
Select one PSB colony of the type that has been counted as PSB (showing sufficient zone of solubilization) and streak on set medium as described at 2.1 in a Petri dish. Use this pure culture for inoculating the broth. Inoculate 3 flasks and keep 3 flasks as uninoculated control. Incubate the flasks over rotary shaker for 12 days at 28±1°C. After 12 days, filter the contents of each flask separately through Whatman No.42 filter paper or centrifuge at 10,000 rpm for 15 min.
- (iii) Add 10 ml of filtrate/centrifugate to 50 ml of olsen extractant and shake for 30 min over rotary shaker.
- (iv) Filter the suspension through Whatman filter paper No.40. If the filtrate is coloured then add a tea spoon of Dacro-60 (activated phosphorous free carbon), reshake and filter.
- (v) Take a known aliquot (5 to 25 ml) of the extract in a 50 ml volumetric flask.
- (vi) Add 5 drops of p-nitrophenol indicator (1.5 percent solution in water) and adjust the pH of the extract between 2 and 3 with the help of 4NH₂SO₄. The yellow colour will disappear when the pH of the solution becomes 3. Swirl gently to avoid loss of the solution along with the evolution of CO₂.
- (vii) When the CO₂ evolution has subsided, wash down the neck of the flask and dilute the solution to about 40 ml.
- (viii) Add 5 ml of the sulphomolybdic acid mixed reagent containing ascorbic acid, swirl the content and make up the volume.
- (ix) Measure the transmission after 30 min at 880 nm using red filter. The blue colour developed remains stable up to 60 minutes.
- (x) Record the concentration of phosphorus (P) in the extract form from the standard curve and calculate the concentration of soluble phosphorus as follows:

5.3.4. Calculations

- (a) Weight of the substance taken
- (b) Volume of the extractant added
- (c) Volume of the extract taken for P determination
- (d) Volume made after colour developed
- (e) Reading from the standard curve against percent transmission recorded

$$(f) \text{ Soluble phosphorus, percent P} = \frac{Z \times 50 \times 10^{-6} \times 50 \times 100}{Y \cdot X}$$

5.3.4 Preparation of standard curve

Prepare standard curve using 0.1 to 0.6 ppm P in 50 ml volumetric flask. Plot the standard curve by taking concentration of soluble P on x-axis and percent T on y-axis using a semi-log graph paper. It is a straight line relationship between the soluble P and percent T when plotted on a semi-log graph paper.

*5A. Quantification of P solubilization activity

The P solubilization is quantified by inoculating the test culture in Pikovskaya's broth for 5 day at 30°C. Culture suspension is centrifuged at 8000 rpm for 15 min and the supernatant was used (Murphy and Riley, 1962).

(a) Reagents:

- (i) Sulfuric acid (5 N): Dilute 70 ml of concentrated sulfuric acid to 500 ml.
- (ii) Ammonium molybdate: Dissolve 20 gm of ammonium molybdate in water and dilute to 500 ml. Store the solution in a Pyrex glass bottle.
- (iii) Ascorbic acid (0.1 M): Dissolve 1.32 g of ascorbic acid in 75 ml of water. This solution should, if possible, be prepared on the day it is required as the ascorbic acid easily becomes oxidized. If it is necessary to keep the solution it can probably be stabilized by addition of 25 mg of ethylenediaminetetraacetic acid (disodium salt) and 0.5 ml of formic acid per 75 ml of solution.
- (iv) Potassium antimonyl tartrate: Dissolve 0.2743 g of potassium antimonyl tartrate in distilled water and dilute to 100 ml.
- (v) Mixed reagent: Mix thoroughly 125 ml of 5N sulfuric acid and 37.5 ml of ammonium molybdate. Add 75 ml of ascorbic acid solution and 12.5 ml of potassium antimonyl tartrate solution. This reagent should be prepared as required as it does not keep for more than 24 h.

(b) Standard phosphate solution:

Stock phosphate solution: Prepare a solution containing 0.1757 g of potassium dihydrogen phosphate per L. This solution contains 40 mg P (as phosphate)/L. Prepare solutions of different concentrations as given below

Volume from stock (40 mg/L)	Distilled water (mL)	Final Volume (mL)	Final Concentration (mg/L)
5.0	45.0	50.00	4.0
4.0	46.0	50.00	3.2
3.0	47.0	50.00	2.4
2.0	48.0	50.00	1.6
1.0	49.0	50.00	0.8
0.0	50.0	50.00	0.0

(c) Procedure:

1. Pipette 40 ml of the culture supernatant into a 50-ml calibrated flask, add 8 ml of mixed reagent from a tilt measure and make the volume to 50 ml with distilled water, and mix well.
2. Dilute the reaction mixture if required.
3. After not less than 10 min measure the optical density of the solution at 880 nm.
4. Determine the reagent blank in the same manner using uninoculated Pikovskaya medium.
5. The amount of soluble P in the culture supernatant is calculated (z) by plotting the OD values against standard curve prepared using different concentrations of Pas phosphate as detailed in the table. The calibration curve only needs occasional checking as it remains constant and appears to be independent of changes in the batches of reagents.

6. Calculate the solubilized P as:-

$$P (P_2O_5) \text{ mg/L} = z \times X \text{ (Dilution factor used at step 2).}$$

3 Maintenance and Preparation of Culture and Quality Control at Broth Stage

Rhizohium

1. Maintenance of pure cultures

- 1.1 Maintain pure cultures of rhizobia on yeast extract mannitol agar (YEMA) slants of the following composition.

Medium	Quantity
Mannitol	10.0 g
Potassium hydrogen phosphate(K_2HPO_4)	0.5 g
Magnesium sulphate ($MgSO_4 \cdot 7H_2O$)	0.2 g
Sodium chloride($NaCl$)	0.1 g
Calcium carbonate ($CaCO_3$)	1.0 g
Yeast extract	1.0 g
Agar	18.0 g
Distilled water	1 litre
pH	6.8-7.0

1.2 Transfer a loopful of the pure culture to each of the agar slants aseptically in an inoculation room and incubate at 28 \pm 2°C for 3 to 10 days depending upon the species of Rhizobium. Always keep pure cultures at 4°C.

2. Preparation of inoculum cultures

2.1 Prepare yeast mannitol broth of the composition as given in 1.1. minus the agar.

2.2 Transfer a loopfull of the culture.

3. Quality control tests recommended at broth stage

3.1 Qualitative Tests

3.1.1 Check for freedom from visible contaminants

3.1.2 The pH of the bacterial broth shall normally be between 6.5 and 7.5

3.1.3 Smear and gram stain

3.1.3.1 Reagents

a. Ammonium oxalate crystal violet stain weigh 0.2 g of crystal violet and dissolve in 20 ml of 95 percent ethyl alcohol. Dissolve separately 0.8 g of ammonium oxalate in 80 ml of distilled water. Mix the two solutions and filter through a filter paper.

b. Iodine solution

Medium	Quantity
Iodine	1.00g
Potassium Iodide	2.00g
Distilled water	300ml

Weigh the ingredients and dissolve in water. Filter through a filter paper.

c) Erythrosine

Medium	Quantity
Erythrosine	1.00g
Phenol	5.00g
Distilled water	100ml

Weigh the ingredients, dissolve in distilled water and filter through a filter paper.

3.1.3.2 Procedure

Prepare a smear on a clean microscope slide, fix over a flame by gentle and intermittent heating, air cool and flood with ammonium oxalate crystal violet stain for 1 min. After removing the excess of ammonium oxalate crystal violet, wash the slide under a gentle stream of running tap water. Flood the slide with iodine solution for half a minute remove excess stain wash with 95 percent ethyl alcohol and finally wash under a gentle stream of running tap water. Flood the slide with erythrosine stain for about 3 min, wash under a gentle stream of running tap water and dry between the folds of a filter paper. Examine the slide under a compound microscope using an oil immersion objective.

Note:- A smear prepared from undiluted broth should be free from Gram positive

cells . The presence of a few gram positive cells in occasional fields which may be due to dead cells in the medium may be disregarded.

3.1.4 Absence of growth on glucose - Peptone agar

The composition of the glucose - peptone agar is as follows:

Medium	Quantity
Glucose	10.0 g
peptone	20.0 g
Sodium chloride (NaCl)	5.0
Agar (IS 6850)	15.0
Distilled water	1000 ml
Bromocresol purple	10 ml of 1.6 per cent ethyl alcohol solution
pH	7.2

Note:- When a loopful of the broth is streaked into this medium and incubated at 28 +/- 2°C for 24 h, the purple -violet colour of the medium (due to the indicator bromocresol purple) shall not change. If the colour changes to yellow (acidic reaction) or blue (alkaline reaction) the broth is grossly contaminated. Hence, the broth should be rejected.

3.1.5 Streak on yeast extract mannitol agar with Congo red

When a loopful of broth culture is streaked to a plate of this medium and incubated at 28 +/- 2°C for 3 to 10 days, it shall show colonies of bacteria with growth characteristics same as that of the pure culture used in the preparation of the broth, otherwise, the broth should be rejected.

3.2 Quantitative test

3.2.1 Viable or plate counts

Serially dilute one milliliter of the broth to obtain dilutions of the order of 10^6 to 10^9 . Plate 0.2 ml aliquots of the dilutions on YEMA plates and incubate at 28 +/- 2°C for 2 to 6 days, depending on the species of Rhizobium. The counts of viable Rhizobium in the final broth from shake culture or fermentors shall be not less than 10^8 to 10^9 cells / ml. Otherwise, the broth should be rejected.

Azospirillum

1. Maintenance of pure cultures

1.1 Maintain pure culture of Azospirillum on nitrogen free bromothymol blue medium and maintain as semi solid medium

1.2 Transfer a loopful of pure culture to each of the agar culture tube aseptically in an inoculation room and incubate $37\pm 2^\circ\text{C}$ for three days and keep it undisturbed. Always keep pure culture below 5°C.

2. Preparation of inoculum culture and mass culture: Inoculum culture and mass culture of this standard shall be prepared as described for Rhizobium of this

standard.

3. Quality control test recommended at broth stage

3.1 Qualitative test

3.1.1 Check for free from contaminants by preparing slide and observing under microscope.

3.1.2 The pH of bacterial broth shall normally be between 7.0 to 8.0.

3.1.3 Gram staining test shall be carried out as described for Rhizobium of this standard.

3.1.4 See the colour change in the media after 24 hours from inoculation. The colour will change from green to blue.

3.1.5 Watch the pellicle just below the surface of the media. It is checked on the third day after keeping inoculated broth undisturbed.

3.2 Quantitative test

3.2.1 Most probable number (MPN) as given in **Annexure-E**. The counts of Azospirillum in the final broth from shake culture or fermentoes shall be not less than 10^8 to 10^9 cells / ml. Other wise the broth should be rejected.

Azotobacter

1. Maintenance of pure cultures

1.1 Maintain pure cultures of Azotobacter on slants of the following composition

Medium	Quantity
Agar	20g
Sucrose	20g
Ferrous sulphate	1.0g
Dibasic potassium phosphate	10g
Magnesium sulphate	0. g
Calcium carbonate	2.0g
Sodium molybdate	0.005 g

1.2 Transfer a loopful the pure culture to each of agar slants aseptically in an inoculator room and incubate at $28 \pm 2^\circ \text{C}$ for 3 to 10 days depending upon the species of Azotobacter. Always keep culture pure cultures at 5°C .

2. Preparation of inoculum culture

2.1 Prepare Jensen's media broth of the composition as given in 1.1 minus the agar

2.2 Transfer a loop full of the culture into a 100ml/ 250 ml conical flask containing the broth. Incubate the flasks at $28 \pm 2^\circ \text{C}$ on a rotary shaker for 2 to 6 days.

3. Quality control tests recommended at broth stage

3.1 Qualitative test

3.1.1 Check for free from contaminants by preparing slide and observing under microscope.

3.1.2 The pH of bacterial broth shall normally be between 6.5 to 7.0

3.1.3 Gram staining test shall be carried out as described for Rhizobium of this

standard.

3.2. Quantitative test

3.2.1. Viable cell count same as Rhizobium

4. Packing, marking, storage and use

4.1 Packing

Biofertiliser shall be packed in suitable plastic bags/packets, thickness of which shall not be less than 75-100 micron or in suitable plastic bottles.

4.2 Marking

Each polyethylene pack shall be marked legibly and indelibly with the following information:

- (a) Name of the product,
- (b) Name and address of the manufacturer,
- (c) Crop(s) for which intended;
- (d) Type of the carrier used;
- (e) Batch number;
- (f) Date of manufacture;
- (g) Expiry date which shall not be less than 6 months from the date of manufacture in case of carrier based powdered/granulated formulation of Rhizobium biofertiliser, while it shall not be less than twelve months from the date of manufacture in case of liquid based Azotobacter, Azospirillum and PSB biofertilisers.
- (h) Net mass in kg/gram and area meant for;
- (i) Storage instruction worded as under; "STORE IN COOL PLACE AWAY FROM DIRECT SUNLIGHT AND HEAT"
- (j) Any other information required under the standards of Weights and Measures (Packaged Commodities) Rule, 1977.

4.3 Items (c), (f) and (g) shall be printed on a coloured ink background.

4.4 Direction for use of biofertiliser shall be printed briefly on the packets as given below.

The contents of the packet are sufficient enough for seed treatment on to the given area to be broadcasted or given seedlings for root dipping depending on the specified crops as denoted on the packet. Mix the inoculants with seeds gently with the minimum amount of water, taking care to avoid damage to seed coat. Dry the inoculated seed under shade over clean surface gunny bag and sow them immediately.

Use only for the crops mentioned. Use before the expiry date and do not expose to direct sun light or heat.

Biofertiliser is not a chemical fertiliser hence do not mix inoculated seeds or inoculant with agro-chemicals."

4.5 Storage

Inoculant shall be stored by the manufacturer in a cool and dry place away from direct heat preferably at temperature of 20°C. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.

IE Method of Analysis for Mycorrhizal Biofertilisers

1. Estimation of pH
As specified in Schedule IV Part D at serial number 1.
2. Estimation of moisture contents
As specified in Schedule IV Part D at serial number 2 of Fertiliser (Control) Order, 1985.
3. **Estimation of total viable spores**

Harvesting of spores from finished product

A. By wet sieving and decantation method

(a) **Equipment and Reagent:** Stalking sieves with nylon or stainless steel mesh and a large range of pore sizes for isolating spores from the product or sample

- (i) 40-50 micron (0.04 mm) sieve for small sized spores.
- (ii) 100 micron (0.14 mm) sieve for medium sized spores.
- (iii) 250 micron (0.25 mm) sieve for very large sized spores and sporocarps.
- (iv) 450 micron (0.45 mm) sieve for removing root bits and other debris.
- (v) 1 mm sieve for removing root bits and other debris.
- (vi) Wash bottles containing water.
- (vii) Jars for collecting the sieving.
- (viii) Stereomicroscope
- (ix) Petri dishes (11 cm) for observing the sieving under stereomicroscope
- (x) Micropipettes for spore picking.
- (xi) Centrifuge

***(b) Procedure**

- (i) Mix 100 gram Mycorrhizal biofertiliser in a substantial volume of water and decant through a series of sieves arranged in descending order of mesh size;
- (ii) Vigorous washing with water is necessary to free spores from aggregates of clay carrier material or organic materials;
- (iii) Roots and coarse debris are collected on coarse sieves (1 mm and 450 micron), while spores are captured on all the respective finer sieves (250 micron, 100 micron and 40/50 micron);
- (iv) Collect the sieving in jars and view the aliquots for spore count during continuous stirring on magnetic stirrer so that proper separation of spores and carrier could occur (as per suggestions from TERI experts);
- (v) Transfer aliquots from all the finer sieves (250 micron 100 micron and 40/50 micron) are mixed and observed properly on the gridded Petri dishes or plate under stereomicroscope for total viable spore count; and
- (vi) Count the number of spores in plate/dish and express it as spores or gram of the sample.

(B) By Sucrose gradient centrifugation (optional)

- (i) Collect the sieving by the method described above. Transfer the sieving into

centrifuge tubes and centrifuge for 5 minutes at 1,750 rpm in a horizontal rotor.

- (ii) Decant the supernatant liquid carefully and resuspend pellet in 60% sucrose solution. Again centrifuge for 2-5 minutes.
- (iii) Pour the supernatant (with spores) onto a 45 micron sieve and rinse with water to remove the sugar. Transfer the sieving onto the gridded Petri dishes/ plate and observe under stereomicroscope. Count the number of spores in plate/ dish and express it as spores/gram of the sample.

3.2 Spore staining

(a) Equipment and Reagent

- (1) Equipment and reagents for spore extraction as described previously
- (2) 2,5-diphenyl-2N-tetrazolium bromide (MTT)
- (3) Distilled water
- (4) Eppendorf tubes
- (5) Stereomicroscope
- (6) Petri dishes

(b) Procedure

- (i) Prepare 0.25% solution of MTT (2,5-diphenyl-2N-tetrazolium bromide)
- (ii) Avoid exposure of MTT solution to light, as the stain is light sensitive
- (iii) Add freshly collect AMF spores (approximately 50 in number) collected by any of the two methods described above to the staining solution and incubate at 27 °C in sterile Eppendorf tubes in dark
- (iv) Observe the spores for different colour reactions using stereomicroscope under dark field after 24 hours, 48 hours and 72 hours of incubation.
- (v) Spores, which stained red or pink, are treated as viable, as per the following formula:

Number of spore which stained red or pink

$$\% \text{ Spore viability} = \frac{\text{Number of spore which stained red or pink}}{\text{Total number of spore}} * 100$$

4.1 Assessment of Inoculum Potential

(a) Materials needed:

- i. Disposable paper/ plastic cups (250 ml) or PVC tubes (15 cm long; 3.2 cm dia.)
- ii. Plastic bags (30 cm x 20 cm)
- iii. Sterilized diluents (sand: soil mix 1:1)
- iv. Onion or Finger millet (ragi) seeds

(b) Procedure:

- (i) Weigh 30 gram of sample in a plastic bag and add to it 270 gram of sterilized diluents (sand: soil mix 1:1). Shake thoroughly to get 10^{-1} dilution.

- (ii) Remove 30 gram from the 10^{-1} dilution and place it into another bag containing 270 g of sterilized diluents. Shake thoroughly to get 10^{-2} dilution. Make a tenfold series dilution up to 10^{-4} dilution (or higher dilution if needed).
- (iii) Distribute substrate from each dilution into paper/ plastic cups or PVC tubes. Use five replicate cups/ tubes per dilution.
- (iv) Sow seeds of onion/ Finger millet into each cup.
- (v) After emergence, thin down to only one plant per tube/cup and let plants grow in a green house or growth room for 45 days (in paper/ plastic cups) or 25 days (in PVC tubes).
- (vi) At harvest, wash roots free from substrate and stain them with Trypan blue (see the staining procedure given below).
- (vii) Under a dissecting microscope, determine presence or absence of mycorrhizal colonization in each replicate. Counts of positive tube (those containing mycorrhizae) in different dilutions are used to calculate MPN values.
- (viii) Use MPN tables of Cochran (1950) given at IC, 5.3.3 Table 1 under Method of Analysis of Azospirillum Biofertilizers.

Note: Using PVC tubes is advantageous as it gives the result in 25 days with less substrate.

4.2 Methodology and explanation for estimating MPN:

- (i) To exemplify the calculation, let's consider that in the 5 replicates (tubes) in each of the four dilutions (10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}), one obtains a combination of number of positive tubes like: 5,5,3,2;
- (ii) This means that all 5 replicate tubes are positive for mycorrhizal colonization at 10^{-1} and 10^{-2} dilution, 3 replicate tubes are positive at 10^{-3} dilution and 2 replicate tubes are positive at 10^{-4} dilution. For the calculation of MPN, only last three dilutions of a given combination are required;
- (iii) The first number (P1) corresponds to the least dilution in which all (or the highest number of) tubes are positive for mycorrhizal colonization. The two other numbers (P2 and P3) are those corresponding to the next two dilutions. In the example above, the combination would be: P1=5, P2=3 and P3=2;

Now find the row of numbers in MPN Table in which P1 and P2 correspond to the numbers observed experimentally. Follow that row of numbers across the table to the column headed by the observed value of P3. The figure at the point of intersection is the most probable number of organisms in the quantity of original sample represented in the finished product sample. Calculate total IP/gm as follows:-

$$\text{Total IP/gm} = \frac{\text{Value from MPN Table} \times \text{Dilution level of P2}}{\text{Dry mass of product sample taken}}$$

[Example - Using the MPN table, the value given for this combination of positive values is 1.4. To obtain the MPN of infective propagules of Arbuscular Mycorrhizal Fungus (AMF) in the sample, this value has to be multiplied by the middle dilution (in this case 10^{-3}). Therefore, the soil has 1.4×10^3 infective propagules/g sample [$(1.4 \times 10^3$ IP/g or say 1400 IP/g)].

4.3. Staining roots to observe Mycorrhizal colonization

Colonization of root cortex cells by Arbuscular Mycorrhizal Fungus does not alter root morphology. Therefore, to detect and measure mycorrhizal colonization, roots are subjected to a clearing and staining procedure.

(a) Materials needed:

- (i) 10% KOH solution (Potassium Hydroxide)
- (ii) 1% HCl solution (Hydrochloric acid)
- (iii) Lacto Glycerol solution (20 ml Lactic acid, 40 ml Glycerol, 40 ml distilled water)
- (iv) 0.05% Trypan Blue in Lacto Glycerol solution (0.5g/L)

(b) Procedure:

1. Wash roots free from soil debris and rinse in several changes of tap water.
2. Soak roots in 10% KOH at 90 °c for 1 hour or at 120 °c for 15 minutes.
3. Remove KOH and rinse roots with water (2-3 times) to remove KOH
4. Soak roots in 1% HCl solution for 5 minutes.
5. Remove HCl. Do not rinse roots after this step as they must be acidified for proper staining.
6. Stain roots in acidic glycerol solution containing Trypan blue at 90 °C for 1 hour or at 120 °C for 5 minutes.
7. Discard stain and keep roots in lacto glycerol till observation.
8. Cut the root into about 1 cm pieces and mount in microscopic slides with Lactoglycerol, place cover slip and observe under the microscope for mycorrhizal colonization.

F. Method of analysis for Potash *Mobilizing Biofertilizers (KMB)

1. Estimation of total viable count and contamination

1. Apparatus -

- 1.1 Pippettes graduated 1 ml and 10 ml
- 1.2 Dilution bottles or flasks
- 1.3 Petri dishes clear uniform, flat-bottomed
- 1.4 Hot-air oven

Capable of giving uniform and adequate temperature, equipped with a thermometer, calibrated to read upto 250°C and with vent suitably located to assure prompt and uniform heating.

- 1.4 Autoclave
- 1.5 Incubator
- 1.6 Hand tally or mechanical counting device
- 1.7 pH meter
2. Reagents
- 2.1 Medium

Use plating medium of the following composition for total viable count and contamination

Medium for analysis of total viable count and contamination

(Ingredients g/lit)

Medium

Quantity

Manitol	15.0
Yeast extract	3.0
Peptone	2.0
Agar	18.5
Trace element solution	1ml
Distilled water	1000ml

Trace element solution (Ingredients g/lit)

Medium	Quantity
Sodium molybdate	0.20
Boric acid	0.28
Manganese sulphate	0.23
Copper sulphate	0.01
Zinc sulphate	0.03
Distilled water	1000ml

**Medium for studying zone of solubilization in KSB
(Ingredients g/lit)**

Medium	Quantity
Glucose	5.0
Magnesium sulphate	0.005
Ferric chloride	0.1
Calcium carbonate	2.0
Potassium mineral (mica powder)	2.0
Calcium phosphate	2.0
Distilled water	1000ml

2.2 Sterilizing and preparation procedure for plates

2.2.1 Sterilize the sampling and plating equipment with dry heat in a hot air oven at less than 160°C for not less than 2 hours.

2.2.2 Sterilize the media by autoclaving at 120°C for 20 min. To permit passage of steam into and from closed containers when auto claved, keep stoppers slightly loosened or plugged with cotton. Air from within the chamber of the sterilizer should be ejected allowing steam pressure to rise.

2.3 Preparation of plating medium and pouring.

2.3.1 Prepare growth medium in accordance with the composition of the specific biofertiliser.

2.3.2 Melt the required amount of medium in boiling water or by exposure to flowing steam in partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Melt enough medium which will be used within 3 h. Re-sterilisation of the medium may cause partial precipitation of ingredients.

2.3.3 When holding time isles than 30 min. Promptly cool the molten medium to about 45°C, and store until used, in a water bath or incubator at 43 to 45°C.

Introduce 12 to 15 ml of liquefied medium or appropriate quantity depending on size of the petri dish at 42 to 44°C into each plate. Gently lift the cover of the dish just enough to pour in the medium. Sterilize the lips of the medium containers by exposure to flame.

- (a) Immediately before pouring.
- (b) Periodically during pouring, and
- (c) When pouring is complete for each batch of plates, if portions of molten medium remain in containers and are to be used without subsequent sterilization for pouring additional plates. As each plate is poured thoroughly mix the medium with test portions in the petri dish.

2.3.4 By rotating and tilting the dish and without splashing the medium over edge, spread the medium evenly over the bottom of the plate. Provide conditions so that the medium solidifies with reasonable promptness (5-10 min) before removing the plates from level surface.

3. Preparation of Serial Dilution for Plate Counts

3.1 Dispense 10 g of inoculants to 90 ml of sterile distilled demineralized water and shake for 10 min on a reciprocal shaker or homogenizer. Make serial dilutions upto 10^{10} take 1.0 ml or suitable aliquots of 10^6 to 10^9 dilutions using sterile pipettes and deliver to petri dishes containing set medium as given in 2.1 and spread it uniformly with a spreader or use droplet method. Invert the plates and promptly place them in the incubator.

4. Incubation of Plates:

4.1 Label the plates and incubate at $28 \pm 2^\circ\text{C}$ for 4 to 6 days.

4.2 Colony counting aids:

Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in centimeter square. Record the total number of colonies with the hand tally. To distinguish colonies from dirt, specks and other foreign matter, examine doubtful objects carefully.

4.3 Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies which absorb congo red and stand out as reddish colonies. *Fraturia aurentia* (KMB) stand out as white-opaque glistening and domed colonies. Count such colony numbers and calculate figures in terms of per litre, of carrier. Also check for freedom from contamination at 10^5 dilution.

5. Counting

Count the total number of colonies on the plates including colonies with solubilization zone with the help of a colony counter.

6. Method for estimation of K solubilization zones

6.1 Take 10 g of KSB in 90 ml sterile distilled water

6.2 Make a ten-fold dilution series upto 10^7 .

- 6.3 Take 1.0 ml aliquot of 10^5 to 10^7 dilutions using sterile pipettes and deliver to petri dishes containing K-solubilization zone media.
- 6.4 Spread if uniformly, Invert the plates and incubate for up to 2 weeks at $28 \pm 2^\circ\text{C}$.
- 6.5 Count the colonies showing solubilization zones and measure the diameter of solubilization zone. Calculate average zone of solubilization in mm.

7. Method of Analysis for estimation of Potassium solubilisation:

Determination of soluble potash using Flame Photometer

1. Preparation of Sample

Prepare Aleksandrov broth media comprising of 5.0 g glucose; 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$; 0.1 g CaCO_3 ; 0.006 g FeCl_3 ; 2.0 g $\text{Ca}_3(\text{PO}_4)_2$; 3.0 g potassium aluminium silicate in 1:1 of distilled water. The pH of this media is adjusted to 7.2 ± 0.2 by using 1N NaOH. Prepare broth media in 100 ml aliquots in 6 no., 250 ml conical flasks and sterilize in autoclave at 121°C for 20min.

2. Inoculation of Media

Select one Potash Mobilising Biofertilisers colony of the type that has been counted as KSB (showing sufficient zone of solubilization) and streak on Aleksandrov agar medium as described above in a Petri dish. Use this pure culture for inoculating the broth. Inoculate 3 flasks and keep 3 flasks as uninoculated control. Incubate the flasks over rotary shaker for 7 days at $30 \pm 1^\circ\text{C}$.

3. Determination of Soluble K

- (1) After 7 days, centrifuge the broth at 10,000 rpm for 15 min. Collect the supernatant for estimation of potassium by Flame Photometer.
- (2) Add 1ml of filtrate/ supernatant to 50 ml volumetric flask and make up the volume to 50 ml. Estimate K content by feeding the solution to Flame Photometer. The amount of potassium solubilized is calculated from the standard curve by multiplying the observed value with dilution factor.

4. Preparation of Standard curve

- (1) Make a stock solution of 1000 ppm K by dissolving 1.909 g. of AR grade potassium chloride (dried at 60°C for 1h) in distilled water in a 1000 ml volumetric flask; and make up to 1 litre with distilled water.
Prepare 100 ppm standard by diluting 100 ml of 1000 ppm stock solution to 1 litre with distilled water.
- (2) Pipette 0.5, 1.0, 1.5 and 2.0 ml of 100 ppm solution into 100 ml volumetric flasks and make up the volume up to the mark. These solutions contain 0.5, 1.0, 1.5 & 2.0 ppm K (mg/Kg) respectively. Plot standard curve using concentrations against flame photometer values.

Express K solubilized as mg/lit of broth".

G. Method of analysis for Zinc Solubilizing Biofertilizers (ZSB)

2. Estimation of total viable count and contamination

1. Apparatus
 - 1.1 Pipettes graduated 1 ml and 10 ml
 - 1.2 Dilution bottles or flasks
 - 1.3 Petri dishes clear, uniform, flat-bottomed

1.4 Hot - air oven

Capable of giving uniform and adequate temperature, equipped with a thermometer, calibrated to read upto 250°C and with **venus** suitably located to assure prompt and uniform heating.

1.5 Autoclave

1.6 Incubator

1.7 Hand tally or mechanical counting device

1.8 pH meter

2. Reagents

2.1 Medium

Use plating medium of the following composition for total viable count and contamination.

Medium for analysis of Total Viable Count, Contamination and zone of solubilization for Zn solubilizing biofertilizer.

(Ingredients g/lit)

Medium	Quantity
Glucose	10.0
Zinc oxide	1.0
Amm sulphate	0.5
Potassium chloride	0.2
Yeast extract	0.5
Ferrous sulphate	0.01
Manganese sulphate	0.01
Di Pot Hyd. phosphate	0.5
Distilled water	1000ml

2.2 Sterilizing and preparation procedure for plates:

2.2.1 Sterilize the sampling and plating equipment with dry heat in a hot air oven at less than 160°C for not less than 2 hours.

2.2.2 Sterilize the media by autoclaving at 120°C for 20 min. To permit passage of steam into and from closed containers when auto claved, keep stoppers slightly loosened or plugged with cotton. Air From within the chamber of the sterilizer should be ejected allowing steam pressure to rise.

2.3 Preparation of plating medium and pouring.

2.3.1 Prepare growth medium in accordance with the composition of the specific Biofertiliser.

2.3.2 Melt the required amount of medium in boiling water or by exposure to flowing steam in partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Melt enough

medium which will be used within 3 hours. Re-sterilization of the medium may cause partial precipitation of ingredients.

- 2.3.3 When holding time is less than 30 min. promptly cool the molten medium to about 45°C, and store until used, in a water bath or incubator at 43 to 45°C. Introduce 12 to 15 ml of liquefied medium or appropriate quantity depending on size of the petri dish at 42 to 44°C into each plate. Gently lift the cover of the dish just enough to pour in the medium. Sterilize the lips of the medium containers by exposure to flame.
 - a. Immediately before pouring
 - b. Periodically during pouring, and
 - c. When pouring is complete for each batch of plates, if portions of molten medium remain in containers and are to be used without subsequent sterilization for pouring additional plates. As each plate is poured thoroughly mix the medium with test portions in the petri dish.
- 2.3.4 By rotating and tilting the dish and without splashing the medium over edge, spread the medium evenly over the bottom of the plate. Provide conditions so that the medium solidifies with reasonable promptness (5-10 min) before removing the plates from level surface.
3. Preparation of Serial Dilution for Plate Counts :
 - 3.1 Dispense 10 g of inoculants to 90 ml of sterile distilled de-mineralized water and shake for 10 min on a reciprocal shaker or homogenizer. Make serial dilutions upto 10^{10} . Take 1.0 ml or suitable aliquots of 10^6 to 10^9 dilutions using sterile pipettes and deliver to petri dishes containing set medium as given in 2.1 and spread it uniformly with a spreader or used droplet method. Invert the plates and promptly place them in the incubator.
 4. Incubation of Plates:
 - 4.1 Label the plates and incubate at $28 \pm 2^\circ\text{C}$ for 4 to 6 days.
 - 4.2 Colony counting aids :

Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in centimeter square. Record the total number of colonies with the hand tally.
 - 4.3 Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Disregard colonies

which absorb congo red and stand out as reddish colonies. Zinc solubilising biofertilisers stand out as white, translucent, glistening and elevated colonies. Count such colony numbers and calculate figures in terms of per litre, of carrier. Also check for freedom from contamination at 10^5 dilution.

5. Counting

Count the total number of colonies on the plates including colonies with solubilization zone with the help of a colony counter.

6. Method for estimation of Zinc solubilization zones

6.1 Take 10 g of ZSB in 90 ml sterile distilled water.

6.2 Make a ten fold dilution series up to 10^7 .

6.3 1.0 ml aliquot of 10^5 to 10^7 dilutions using sterile pipettes and deliver to petri dishes containing zinc - solubilization zone media.

6.4 Spread it uniformly, invert the plates and incubate for upto 2 weeks at $28 \pm 2^\circ\text{C}$.

6.5 Count the colonies showing solubilization zones and measure the diameter of solubilization zone. Calculate average zone of solubilization in mm.

7. Determination of soluble Zinc using Atomic Absorption Spectrophotometer

(1). Preparation of Sample

Prepare Zinc solubilizing broth media comprising of glucose- 10.0g; ammonium sulphate-1.0g; potassium chloride- 0.2g, dipotassium hydrogen phosphate- 0.1g, magnesium sulphate-0.2g and zinc carbonate/zinc oxide 1 gm in 1:1 of distilled water. The pH of this media is adjusted to 7.0 by using 1 N NaOH. Prepare broth media in 100 ml aliquots in 6 no., 250 ml conical flasks and sterilize in autoclave at 121°C for 20 min.

(2). Inoculation of Media

Select one ZnSB colony of the type that has been counted as ZnSB (showing sufficient zone of solubilization) and streak on ZnSB agar media as described above in a Petri dish. Use this pure culture for inoculating the broth. Inoculate 3 flasks and keep 3 flasks as uninoculated control. Incubate the flasks over rotary shaker for 7 days at $30 \pm 1^\circ\text{C}$.

(3) Determination of Soluble Zinc

(a) After 7 days, centrifuge the broth at 10,000 rpm for 15 min. Collect the supernatant for estimation of solubilized Zinc.

(b) Add 1 ml of filtrate/ supernatant to 50 ml volumetric flask and make up the volume to 50 ml. Estimate Zn content by feeding the solution to Atomic

Absorption Spectrophotometer. The amount of zinc solubilized was estimated by subtracting the soluble zinc of the inoculated sample from the uninoculated control.

- (c) Standard curve should be made by diluting 0.5, 1.0, 1.5, 2.0 and 3.0 ml of 100ppm standard stock solution (commercially available) of Zinc Sulphate with doubled distilled water in volumetric flasks and make up the volume to 100ml to obtain standards having concentrations of 0.5, 1.0, 1.5, 2.0, 3.0 ppm.
- (d) The amount of zinc solubilized is calculated from the standard curve by multiplying the observed value with dilution factor (50).

Express Zn solubilized as mg/lit of broth".

1H Method of analysis for Acetobacter (spp).

1. Appartus -As specified in the Method of Analysis of Rhizobium at serial number A

2. Reagents

2.1 Medium

Use plating medium of the following composition for total viable count and contamination:-

Medium for analysis of total viable count and contamination (ingredients gram/litre)

Medium	Quantity
Sucrose	100g
K ₂ HPO ₄ (Di-Potassium Hydrogen Phosphate)	0.4 g
KH ₂ O ₄ (Potassium di-hydrogen Phosphate)	0.6g
MgSO ₄ (Magnesium Sulphate)	0.2g
Calcium Chloride	0.02g
Sodium Molybdate	0.02g
Ferric Chloride	0.01g
Bromothymol blue solution (0.1% in 0.2 m KOH)	5.0ml
Distilled water	1000ml
pH	5.5
agar agar	18.5g

3. Sterilising and preparation procedure for plates
(Same as specified in the Method of Analysis of Rhizobium)
- 3.1 Preparation of plating medium and pouring
(Same as specified in the Method of Analysis of Rhizobium)
4. Preparation of serial dilutions for plate counts
(Same as specified in the Method of Analysis of Rhizobium)
5. Incubation of plates
(Same as specified in the Method of Analysis of Rhizobium)

5.1 Colony Counting Aids

Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with guide plate and rules in centimeter square. Record the total number of colonies with hand tally. Avoid mistaking particles of undissolved medium or precipitated matter in plates for pin point colonies. To distinguish colonies from dirt, specks and foreign matter, examine doubtful objects carefully.

5.2 Count all plates but consider for the purpose of calculation only those plates showing more than 30 and less than 300 colonies per plate. Acetobacter a nitrogen fixing bacteria stand out as irregular 2-3 mm diameter, smooth flat with bright yellow or yellow with orange centre colour. Count such colony numbers and calculate figures in terms of per litre, of carrier. Also check freedom contamination at 10^5 .

6. Test for Confirmation

1. Apparatus (same as specified in the Method of Analysis of Azospirillum at serial number 1C)

1. Reagent

2.1 Medium (semi solid for pellicle formation) (ingredients gm per liter)

Medium	Quantity
Sucrose	100g
K ₂ HPO ₄ (Di-Potassium Hydrogen Phosphate)	0.4 g
KH ₂ O ₄ (Potassium di-hydrogen Phosphate)	0.6g
MgSO ₄ (Magnesium Sulphate)	0.2g
Calcium Chloride	0.02g
Sodium Molybdate	0.02g
Ferric Chloride	0.01g
Bromothymol blue solution (0.1% in 0.2 m KOH)	5.0ml
Distilled water	1000ml
pH	5.5
agar agar	18.5g

I. Methods of Analysis of Phosphate Solubilizing Fungal Biofertilizer

1. Apparatus required:

- 1.1 Pipettes, graduated, 1 ml and 10 ml
- 1.1 Conical Flasks, 150 ml and 250 ml
- 1.2 Screw-Capped Tubes, 10 ml
- 1.3 Incubator 1.5 Petri Dishes
- 1.4 Hot Air Oven
- 1.5 pH meter
- 1.6 Autoclave
- 1.7 Haemocytometer
- 1.8 Compound Microscope

1.9 Glass Slides and Cover Slips

1.10 Forceps

1.11 Needles

1.12 Glass Roads

2. Preparation of serial dilution for plate count (spore/ cfu)

2.1 Dispense 30 g of PSFI to 270 ml of sterile distilled/demineralised water and shake for 10 min on a reciprocal shaker or homogenizer. Make serial dilutions up to 10^{-7} by suspending to 10 ml aliquot of previous dilution to 90 ml of water. Take 0.1 ml or suitable aliquotes upto 10^{-7} dilutions using sterile pipettes and deliver to Petri dishes containing set medium as given in section 1.2 and spread it uniformly with a spreader. Invert the plates and promptly place them in the incubator.

2.2 Incubation of plates

2.2.1 Label the plates and incubate at $28 \pm 1^\circ\text{C}$ for 2 to 4 days.

2.2.2 Colony counting aids

Count the colonies with the aid of magnifying lens under uniform and properly controlled, artificial illumination. Use a colony counter, equipped with a guide plate and rules in Centimeter Square. Record the total number of colonies with the hand tally. Avoid mistaking particles of undissolved medium or precipitated matter, in plates for pinpoint colonies. To distinguish colonies from dirt, specks and other foreign matter, examine doubtful objects carefully.

2.2.3 Count all plates but consider for the purpose of calculation plates showing more than 30 and less than 300 colonies per plate. Count such colony numbers and calculate figures in terms of per gram/litre of carrier. Also check for freedom from contamination at 10^{-0} dilution.

2.2.4 Counting

2.2.4.1 Count the colonies showing hallow Zones and measure their diameter.

3 Determination of soluble phosphorus using ascorbic acid method:

3.1 Apparatus

Spectrometer capable of transmission measurement at 840 to 880 nm.

Extractant: It is Olsen Extract.

3.2 Reagents

Ammonium Molybdate $[(\text{NH}_4)_6\text{MO}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$

L-Ascorbic Acid

P-Nitro phenol

$4\text{NH}_2\text{SO}_4$

3.3 Preparation of reagents

3.3.1 Sulphomolybdic Acid :-

3.3.1.1 Take 20 g of ammonium molybdate and dissolve in 300 of distilled water.

3.3.1.2 Add slowly 450 ml of 10 N - H_2SO_4 .

- 3.3.1.3. Cool the above mixture and add 100 ml of 0.5 per cent solution of antimony Potassium tartrate.
- 3.3.1.4. Cool and make the volume to one liter. Store in glass bottle away from direct sunlight.
- 3.3.2 Preparation of Mixed Reagent
Add 1.5 g of L-ascorbic acid in 100 ml of the above stock solution and mix. Add 5 ml of this solution to develop colour. Mixed reagent is to be prepared fresh as it does not keep for more than 24 h.

3.3 Procedure

(i) Preparation of Sample

Prepare broth medium in 100 ml aliquots in 6 no., 250 ml conical flasks and sterilise in autoclave at 121°C for 20 min.

(ii) Inoculation of Medium

Select one PSFI colony of the type that has been counted as PSFI (showing sufficient zone of solubilisation) and streak on set medium in a Petri dish. Use this pure culture for inoculating the broth. Inoculate 3 flasks and keep 3 flasks as uninoculated control. Incubate the flasks over rotary shaker for 12 days at 28±1°C.

After 12 days, filter the contents of each flask separately through whatman No.42 filter paper or centrifuge at 10,000 rpm or 15 min.

(iii) Add 10 ml of filtrate/centrifugate to 50 ml of olsen extractant and shake for 30 min over rotary shaker.

(iv) Filter the suspension through Whatman filter paper No.40. If the filtrate is coloured then add a tea spoon of Darco-60 (activated phosphorus free carbon), reshake and filter.

(v) Take a known aliquot (5 to 25 ml) of the extract in a 50 ml volumetric flask.

(vi) Add 5 drops of p-nitrophenol indicator (1.5 per cent solution in water) and adjust the pH of the extract between 2 and 3 with the help of 4N H₂SO₄. The yellow colour will disappear when the pH of the solution becomes 3. Swirl gently to avoid loss of the solution along with the evolution of CO₂.

(vii) When the CO₂ evolution has subsided, wash down the neck of the flask and dilute the solution to about 40 ml.

(viii) Add 5 ml of the sulphomolybdic acid mixed reagent containing ascorbic acid, swirl the content and make up the volume.

(ix) Measure the transmission after 30 min at 880 nm using red filter. The blue colour developed remains stable upto 60 minutes.

(x) Record the concentration of phosphorus (P) in the extract from the standard curve and calculate the concentration of soluble phosphorus as follows:"

3.4 Calculations

- (a) Weight of the substance taken= X g
- (b) Volume of the extract added= 50 ml
- (c) Volume of the extract taken for P determination= Y ml
- (d) Volume made after colour developed= 50 ml
- (e) Reading from the standard curve against per cent transmission recorded = Z ppm
- (f) Soluble Phosphorus percent
$$p = \frac{Z * 50 * 10^{-6} * 50 * 100}{Y * X}$$

3.5 Preparation of standard Curve

Prepare standard curve using 0.1 to 0.6 ppm P in 50 ml volumetric flask. Plot the standard curve by taking concentration of soluble P on x- axis and percent T on y- axis using a semi log graph paper. It is a straight line relationship between the soluble P and percent T when plotted on a semi-log graph paper.

3. Sterilisation and preparation of MPN tubes

(Same as specified in the Method of Analysis of Azospirillum at serial number C)

4. Preparation of serial dilution for MPN count

(Same as specified in the Method of Analysis of Azospirillum at serial number C)

5. Incubation of tubes

(Same as specified in the Method of Analysis of Azospirillum at serial number C)

6. Counting- Yellowish pellicle formation below 1 mm of upper surface of nitrogen free semi solid media. Counting the tubes or plates which have turned yellowish in colour after inoculation and ascertained the presence of pellicle in undistributed medium. To determine usual contamination on the same examine doubtful objects carefully.

7. Method for Estimating MPN count

Count all tubes which have turned yellowish and consider them for the purpose of calculation. Count such type of tubes and tally this count with MPN table (as specified in the Method of Analysis of Azospirillum at serial number C in Table 1) to get the number of cells per gram of carrier or number of cells per ml of liquid.

I Method of analysis of carrier based Consortia of Biofertiliser and liquid consortia of Biofertiliser

- (I) Methods of Analysis of Rhizobium Biofertiliser - Same as specified for Rhizobium at Serial number A.
- (II) Methods of Analysis of Azotobacter - Same as specified for

Azotobacter at serial number B

- (III) Methods of Analysis Azospirillum - Same as specified for Azospirillum at serial number C
- (IV) Methods of Analysis of Phosphate Solubilising Bacteria PSB - Same as specified for Phosphate Solubilising Bacteria at serial number D
- (V) Methods of Analysis of Potash Mobilising Bacteria (KMB) - Same as specified for Potash Solubilising Bacteria at serial number F.

अनुसूची-5
(दफा ...को उपदफा (१) संग सम्बन्धित)
भाग -१
प्राङ्गारिक मलको स्पेसिफिकेशन

1. Organic Manure (Powder or Granular) प्राङ्गारिक मल (धुलो वा दानेदार)

S.N.	Characteristics	Requirements
1.1	Particle size	Minimum 90% material should pass through 4.0 mm sieve
1.2	Moisture percent by weight, maximum	25.0
1.3	Total organic carbon percent by weight (dry weight basis), minimum	14.0
1.4	Total nitrogen (as N) percent by weight (dry weight basis), minimum	1.0
1.5	Total phosphates (as P ₂ O ₅) percent by weight (dry weight basis), minimum	0.5
1.6	Total potash (as K ₂ O) percent by weight (dry weight basis), minimum	1.0
1.7	C:N ratio	<20
1.8	pH	6.0-8.5
1.9	Odour	Absence of foul odour
1.10	*Pathogens	
a	<i>E. Coli</i>	Not detected
b	<i>Salmonella</i>	Not detected
c	<i>Shigella</i>	Not detected
1.11	Heavy metal content (mg/kg) (dry weight basis), maximum	
a	Arsenic as (As ₂ O ₃)	10.0
b	Cadmium (Cd)	5.0
c	Chromium (as Cr)	50.0
d	Mercury (as Hg)	0.2
e	Lead (as Pb)	100.0

* मानव वा जीव जन्तु स्वास्थ्य, पर्यावरण तथा जैविक सुरक्षाका दृष्टिकोणले आवश्यक भएमा नियमन गर्ने निकायको निर्णयबाट माथी मापदण्डमा तोकिए बाहेकका जीवाणुको समेत परीक्षण गर्न गराउन सकिने ।

2. Vermicompost (भर्मिकम्पोष्ट)

S.N.	Characteristics	Requirements
1.1	Particle size	Minimum 90% material should pass through 4.0 mm sieve
1.2	Moisture percent by weight, maximum	30
1.3	Total organic carbon percent by weight (dry weight basis), minimum	20.0
1.4	Total nitrogen (as N) percent by weight (dry weight basis), minimum	1.5
1.5	Total phosphates (as P ₂ O ₅) percent by weight (dry weight basis), minimum	1.0
1.6	Total potash (as K ₂ O) percent by weight (dry weight basis), minimum	1.0
1.7	pH	6.0-8.0
1.8	Odour	Absence of foul odour
1.9	*Pathogens	
a	<i>E. Coli</i>	Not detected

b	<i>Salmonella</i>	Not detected
c	<i>Shigella</i>	Not detected
1.10	Heavy metal content (mg/kg) (dry weight basis), maximum	
a	Arsenic as (As_2O_3)	10.0
b	Cadmium (Cd)	5.0
c	Chromium (as Cr)	50.0
d	Mercury (as Hg)	0.2
e	Lead (as Pb)	100.0

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3. Farmyard Manure (गोठे मल)

S.N.	Characteristics	Requirements
1.1	Moisture percent by weight (dry weight basis), maximum	60.0
1.2	Total organic carbon percent by weight (dry weight basis), minimum	20.0
1.3	Total nitrogen (as N) percent by weight (dry weight basis), minimum	1.0
1.4	Total phosphates (as P_2O_5) percent by weight (dry weight basis), minimum	0.5
1.5	Total potash (as K_2O) percent by weight (dry weight basis), minimum	1.0
1.6	C:N ratio	<30
1.7	pH	6.0-8.0
1.8	Odour	Absence of foul odour

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4. City Compost (सिटी कम्पोष्ट)

S.N.	Characteristics	Requirements
1.1	Particle size	Minimum 90% material should pass through 4.0 mm sieve
1.2	Moisture percent by weight, maximum	25.0
1.3	Total organic carbon, percent by weight (dry weight basis), minimum	12.0
1.4	Total of Nitrogen (as N), phosphates (as P_2O_5) and potash (as K_2O) percent by weight (dry weight basis), minimum	1.2
1.5	C:N ratio	<20.0
1.6	pH	6.0-8.0
1.7	Odour	Absence of foul odour
1.8	*Pathogens	
a	<i>E. Coli</i>	Not detected
b	<i>Salmonella</i>	Not detected
c	<i>Shigella</i>	Not detected
1.9	Conductivity (as $ds\ m^{-1}$) not more than	6.0
1.10	Heavy metal content (mg/kg) (dry weight basis), maximum	
a	Arsenic as (As_2O_3)	10.0
b	Cadmium (Cd)	5.0
c	Chromium (as Cr)	50.0
d	Mercury (as Hg)	0.2
e	Lead (as Pb)	100.0

* मानव वा जीव जन्तु स्वास्थ्य, पर्यावरण तथा जैविक सुरक्षाका दृष्टिकोणले आवश्यक भएमा नियमन गर्ने निकायको निर्णयबाट माथी मापदण्डमा तोकिए बाहेकका जीवाणुको समेत परीक्षण गर्न गराउन सकिने ।

5. Phosphate Rich Organic Manure (PROM)(फस्फेटयुक्त प्राङ्गारिक मल)

S.N.	Characteristics	Requirements
1.1	Particle size	Minimum 90% material should pass through 4.0 mm IS sieve
1.2	Moisture percent by weight, maximum	25.0
1.3	Total organic carbon, percent by weight (dry weight basis), minimum	8.0
1.4	Total nitrogen (as N) percent by weight (dry weight basis), minimum	0.4
1.5	Total phosphates (as P ₂ O ₅) percent by weight (dry weight basis), minimum	8.0
1.6	C:N ratio	<20
1.7	pH	6.0-8.0
1.8	Conductivity (as dsm ⁻¹) not more than	10
1.9	*Pathogens	
a	<i>E. Coli</i>	Not detected
b	<i>Salmonella</i>	Not detected
c	<i>Shigella</i>	Not detected
1.10	Heavy metal content (mg/kg) (dry weight basis), maximum	
a	Arsenic as (As ₂ O ₃)	10.0
b	Cadmium (Cd)	5.0
c	Chromium (as Cr)	50.0
d	Mercury (as Hg)	0.2
e	Lead (as Pb)	100.0

* मानव वा जीव जन्तु स्वास्थ्य, पर्यावरण तथा जैविक सुरक्षाका दृष्टिकोणले आवश्यक भएमा नियमन गर्ने निकायको निर्णयबाट माथी मापदण्डमा तोकिए बाहेकका जीवाणुको समेत परीक्षण गर्न गराउन सकिने ।

6. Bio-enriched Organic Manure(बायो एनरीचड प्राङ्गारिक मल)

S.N.	Characteristics	Requirements
1.1	Particle size	Minimum 90% material should pass through 4.0 mm IS sieve
1.2	Moisture percent by weight, maximum	40
1.3	Total organic carbon percent by weight (dry weight basis), minimum	14.0
1.4	Total viable count (within 3 months from the date of manufacture)	5.0x10 ⁶
a	Bacteria-CFU minimum (should contain N or P or K or Zn bacteria or mixture)	5.0 × 10 ⁶ per gram bacteria
b	<i>Mycorrhiza</i>	Minimum 10 viable spore per gram
c	Phosphate solubilizing fungus	1.0 × 10 ⁵ viable spores per gram
1.5	Total nitrogen (as N) percent by weight (dry weight basis), minimum	0.8
1.6	Total phosphates (as P ₂ O ₅) percent by weight (dry weight basis), minimum	0.5
1.7	Total potash (as K ₂ O) percent by weight (dry weight basis),	0.8

	minimum	
1.8	C:N ratio	<18
1.9	pH	6.0-8.5
1.10	Contamination	No contamination at 10 ⁵ dilutions
1.11	Heavy metal content (mg/kg) (dry weight basis), maximum	
a	Arsenic as (As ₂ O ₃)	10.0
b	Cadmium (Cd)	5.0
c	Chromium (as Cr)	50.0
d	Mercury (as Hg)	0.2
e	Lead (as Pb)	100.0

7. Liquid Fermented Organic manure(प्राङ्गारिक झोल मल)

S.N.	Characteristics	Requirements
1.1	Moisture percent by weight	90.0
1.2	Total organic carbon percent by weight (dry weight basis), minimum	14.0
1.3	Total of nitrogen (as N), phosphates (as P ₂ O ₅) and potash (as K ₂ O) percent by weight (dry weight basis), minimum	1.2
1.4	Conductivity (as dsm ⁻¹)	not more than 4.0
1.5	C:N ratio	upto 30
1.6	pH	6.0-8.0
1.7	*Pathogens	
a	<i>E. Coli</i>	Not detected
b	<i>Salmonella</i>	Not detected
C	<i>Shigella</i>	Not detected
1.8	Heavy metal content (mg/kg) (dry weight basis), maximum	
a	Arsenic as (As ₂ O ₃)	10.0
b	Cadmium (Cd)	5.0
c	Chromium (as Cr)	50.0
d	Mercury (as Hg)	0.2
e	Lead (as Pb)	100.0

* मानव वा जीव जन्तु स्वास्थ्य, पर्यावरण तथा जैविक सुरक्षाका दृष्टिकोणले आवश्यक भएमा नियमन गर्ने निकायको निर्णयबाट माथी मापदण्डमा तोकिए बाहेकका जीवाणुको समेत परीक्षण गर्न गराउन सकिने ।

भाग-२

TOLERANCE LIMIT OF ORGANIC FERTILISERS

A sum of nitrogen, phosphorous and potassium nutrients shall not be less than 1% in case of city compost, 2.8% in case of vermicompost, 2.8% in case of organic manure. In case of PROM the phosphate content in terms of P₂O₅ content shall not be less than 7.8%.

भाग-३

PROCEDURE FOR DRAWAL OF SAMPLE OF ORGANIC FERTILISERS

(As per methodology as mentioned under schedule - I,
Part-2)

The Inspector shall draw any sample of Organic Fertiliser in accordance with the procedure of drawal mentioned under Schedule - I, Part -2.

भाग-४

METHODS OF ANALYSIS OF ORGANIC FERTILISERS

1. Estimation of pH

- Make 25 g of compost into a suspension in 50 ml of distilled water and shake on a rotary shaker for 2 hours.
- Filter through Whatman No. 1 or equivalent filter paper under vacuum using a Buchner funnel.
- Determine pH of the filtrate by pH meter.

2. Estimation of Moisture

Method

Weigh to the nearest mg about 5 gm of the prepared sample in a weighed clean, dry petri dish. Heat in an oven for about 5 hours at $65^{\circ} +1^{\circ} \text{C}$ to constant weigh, Cool in a desiccator and weigh. Report percentage loss in weight as moisture content.

Calculation

$$\text{Moisture percent by weight} = \frac{100 (B-C)}{B-A}$$

A	=	Weight of the petri dish
B	=	Weight of the petri dish plus material before drying
C	=	Weight of the petri dish plus material after drying

3. Estimation of Electrical Conductivity

Requirements

-250 ml flask	- Funnel [OD - 75 mm]
-1 00 ml beaker	-Analytical balance
-Potassium chloride [AR grade]	-Filter paper
-Conductivity meter [With temperature compensation system	

Method

- Pass fresh sample of organic fertiliser through a 2-4 mm sieve.
- Take 20gm of the sample and add 100ml of distilled water to it to give a ratio of 1:5.
- Stir for about an hour at regular intervals.
- Calibrate the conductivity meter by using 0.01M potassium chloride solution.
- Measure the conductivity of the unfiltered organic fertiliser suspension.

Calculation

Express the results as millimho's or ds/cm at 25°C specifying the dilution of the organic fertiliser suspension viz., 1:5 organic fertiliser suspension.

4. Estimation of Organic Carbon

Apparatus

- Silica/Platinum crucible 25 g cap.
- Muffle Furnace

Procedure

Accurately weigh 10 g of sample dried in oven at 105° C for 6 hrs, in a pre-weighed crucible and ignite the material in a Muffle furnace at 650- 700°C for 6- 8 hrs. Cool to room temperature and keep in Desiccator for 12 hrs.

Weigh the contents with crucible

Calculation

Calculate the total organic carbon by the following formulae:-

$$\text{Total organic matter\%} = \frac{\text{Initial wt} - \text{final wt.}}{\text{wt. of sample taken}} \times 100$$

$$\text{Total C\%} = \frac{\text{Total organic matter}}{1.724}$$

5. Estimation of Total Nitrogen

As mentioned under Schedule- I, Part-3, 3 (v)

6. Estimation of C: N Ratio

Method

Calculate the C:N ratio by dividing the organic carbon value with the total nitrogen value.

7. Estimation of Phosphate

Preparation of sample - Accurately weigh 10 g oven dried sample in 50 g cap. silica crucible and ignite it to 650°- 700 °C for 6-8 hrs to obtain ash. Cool and keep in a Dessicator.

Transfer the contents to a 100 ml beaker. Add 30 ml 25% HCl. Wash the crucible with 10 ml 25% HCl twice and transfer the contents to Beaker. Heat over hot plate for 10-15 min. Keep for 4 hrs. Filter through Whatman No.1 filter paper. Wash with distilled water 4-5 times (till acid free).

Make up the volume of filtrate to 250 ml in a volumetric flask.

Estimate total P by gravimetric quinoline molybdate method as described under Schedule- I, Part 3, 4(ii).

8. Estimation of Potassium

Flame Photometry Method - Total potassium are usually determined by dry ashing at 650-700°C and dissolving in concentrated hydrochloric acid.

Reagent and Standard Curve

- (1) **Potassium chloride standard solution:** Make a stock solution of 1000 ppm K by dissolving 1.909 g of AR grade potassium chloride (dried at 60°C for 1 hr) in distilled water 1; and diluting up to 1 litre. Prepare 100 ppm standard by diluting 100 ml of 1000 ppm stock solution to 1 litre with extracting solution.
- (2) **Standard curve:** Pipette 0, 5, 10, 15 and 20 ml of 100 ppm solution into 100 ml volumetric flasks and make up the volume upto the mark. The solution contain 0, 5, 10, 15 and 20 ppm K respectively.

Procedure

- * Take 5g sample in a porcelain crucible and ignite the material to ash at 650-700 °C in a muffle furnace.
- * Cool it and dissolve in 5 ml concentrated hydrochloric acid, transfer in a 250 ml beaker with several washing of distilled water and heat it. Again transfer it to a 100 ml volumetric flask and make up the volume.
- * Filter the solution and dilute the filtrate with distilled water so that the concentration of K in the working solution remains in the range of 0 to 20 ppm, if required.

- * Determine K by flame photometer using the K- filter after necessary setting and calibration of the instrument.
- * Read similarly the different concentration of K of the standard solution in flame photometer and prepare the standard curve by plotting the reading against the different concentration of the K.

Calculation: Potash (K) % by weight = $R * 20 * \text{diluting factor}$
 where R= ppm of K in the sample solution (obtained by extra plotting from stand curve).

9. Estimation of Cadmium, Chromium, Lead

Material Required

1. Triacid mixture: Mix 10 parts of HNO₃ (Nitric acid), 1 part of H₂SO₄ (Sulphuric Acid) and 4 parts of HClO₃ (Perchloric Acid)
2. Conical flask, 250ml
3. Hot plate
4. Whatman filter paper No. 42
5. Atomic Absorption Spectrophotometer

Processing of Sample

Take 5.0 g or suitable quantity of oven dried (105°C) sample thoroughly ground and sieved through 0.2 mm sieve in a conical flask.

Add 30 ml triacid mixture, cover it with a small glass funnel for refluxing. Digest the sample at 200°C on a hot plate till the volume is significantly reduced with a whitish residue.

After cooling, filter the sample with Whatman No. 42 filter paper, make up to 100 ml in a volumetric flask.

Preparation of Working Standards

Cadmium -As mentioned under Schedule - I, Part 4, 8(x)

Chromium - Dilute 1, 2, 3 and 4 ml of standard 100 ppm chromium standard solution with doubled distilled water in volumetric flasks and make up the volume to 100 ml to obtain standards having concentrations of 1, 2, 3, 4 ppm

Lead -As mentioned under Schedule - I, Part 4, 8(v)

Measurement of Result

Estimate the metal concentrations of Cd, Cr, Pb by flaming the standard solution and samples using atomic absorption spectrophotometer (AAS) as per the method given for instrument at recommended wavelength for each element. Run a blank following the same procedure.

Expression of Result

Express the metal concentration as mg/g on oven dry weight basis in 3 decimal units.

(Reference: Manual for Analysis of Municipal Solid Waste (compost): Central Pollution Control Board).

10. Estimation of Mercury

Reagents

- (a) Concentrated nitric acid (HNO₃)
- (b) Concentrated sulphuric acid (H₂SO₄)
- (c) Potassium persulphate (5% solution): Dissolve 50g of K₂S₂O₈ in 1 litre of distilled water.
- (d) Potassium permanganate (5% solution): Dissolve 50g of KMnO₄ in 1 litre of distilled water.
- (e) Hydroxylamine sodium chloride solution: Dissolve 120 g of Hydroxyl amine salt and 120 g of sodium chloride (NaCl) in 1 litre distilled water.
- (f) Stannous chloride (20%): Dissolve 20 g of SnCl₂ in 100 ml distilled water.

Materials Required

- (a) Water bath
- (b) Flameless atomic absorption spectrophotometer or cold vapour mercury analyzer.
- (c) BOD bottle, 300 ml

Processing of Sample

- (a) Take 5 g (finely ground but not dried) sample in an oven at a temperature of 105°C for 8 hours for moisture estimation.

- (b) Take another 5 g sample (finally ground but not dried) in a BOD bottle, add to it 2.5 ml of cone. HNO_3 , 5ml of cone. H_2SO_4 and 15 ml of 5% KMnO_4
- (c) After 15 minutes add 8 ml of 5% $\text{K}_2\text{S}_2\text{O}_8$.
- (d) Close the bottle with the lid and digest it on a water bath at 95°C for 2 hours.
- (e) After cooling to room temperature add 5 ml hydroxylamine sodium chloride soln.

Measurement

Reduction of the digested sample is brought out with 5 ml of 20% SnCl_2 immediately before taking the reading, using a cold vapour mercury analyzer.

Expression of Results

Express the mercury concentration as mg/g on oven dry weight basis in 3 decimal units.

(Reference: Manual for Analysis of Municipal Solid Waste (compost). Central Pollution Control Board).

11. Estimation of Arsenic

Processing of sample - Suspend 10 gm finely ground sample in 30 ml aquaregia ($\text{HNO}_3 + \text{HCl}$ in a ratio of 1:3) in a beaker. Keep on hot plate till moist black residue is obtained (do not dry). Add 5 ml aquaregia and allow to dry on hot plate till residue is moist. Dissolve the residue in 30 ml cone. HCl and filter through Whatman No. 1 filter paper in 100 ml volumetric flask. Wash filter paper 3-4 times with double distilled water. Make up the *volume* to 100 ml. Take 1 ml of this solution in 100 ml volumetric flask, add 5ml conc. HCl and 2 gm KI and make up the volume to 100 ml.

Prepare standards having concentration of 0.05, 0.1 and 0.2 ppm by diluting 0.05, 0.1 and 0.2 ml, respectively of standard Arsenic solution with double distilled water in volumetric flask and make up the volume to 100 ml

Measurement - Estimate Arsenic using vapour generation assembly attached to Atomic Absorption Spectrophotometer as per the procedure given for the instrument.

12. Pathogenicity Test

Apparatus

1. Samples of Compost
2. Lactose Broth of Single and Double Strength
3. Culture Tubes
4. Durham Tubes
5. Bunsen Burner
6. Sterile Pipettes
7. Incubator, Autoclaves,
8. Petri-Plates
9. Inoculation Loops

Preparation of Culture Media

A. For Presumptive Test

I. Lactose Broth

Medium	Quantity
Beef Extract	6.0g
Peptone	10.0g
Lactose	10.0g
D.W.	1000ml

B. For Confirmative Test

I. Eosine Methylene Blue Agar Media (EMB Media)

Peptone	10.0 g
Lactose	5.0 g
Sucrose	5.0 g
K ₂ HPO ₄	2.0g
Eosine Y	0.4 g
Methylene Blue	0.06 g
Agar	15.0g
D.W.	1000ml

C. For Completed Test

Beef Extract	3.0g
Peptone	5.0g

Procedures

A. Presumptive Test

1. Prepare 12 tubes of lactose broth for each sample and close the tube with cotton plugs/caps and autoclave at 121°C for 20 min.
2. Fill Durham tubes with sterilized distilled water and keep in beaker and autoclave at 121°C for 20 min.
3. Suspend 30 g of compost sample in 270 ml of sterile distilled water and serially dilute upto 10^{-4} dilution as per Schedule III, Part D, serial number 3 of FCO (1985)
4. Suspend 1 ml suspension from 10^{-1} to 10^{-4} in 3 tubes for each dilution
5. Insert distilled water filled Durham tube in inverted position in each tube and close the tube again
6. Inoculate tubes at 36°C for 24 h in incubator

Result

Production of gas within 24h	Confirms the presence of coliforms in the sample
Production of gas within 48h	Doubtful Test
No Gas Production	Negative Test

B. Confirmative Test

Confirmative test is for differentiating the coliforms from non-coliforms as well as Gram negative and Gram positive bacteria. In this test, the EMB agar plates are inoculated with sample from positive tubes producing gas. Emergence of small colonies with dark centres confirms the presence of Gram negative, lactose fermenting coliform bacteria. Sometimes some of the non-coliforms also produce gas, therefore, this test is necessary.

1. Prepare EMB agar plates with the composition as per the method at Schedule III, Part D, paragraphs 2.3.3 to 2.3.6
2. Inoculate plates with the help of inoculation loop with streaking of samples showing positive/doubtful tests in the presumptive test

3. Incubate plates at $30 \pm 1^\circ\text{C}$ for 12 h in incubator
4. Dark centred or nucleated colonies appear which may differentiate between *E. coli* and *E. aerogenes* based on size of colonies and metallic sheen

Result

E. coli colonies on this medium are small with metallic sheen, whereas *E. aerogenes* colonies are usually large and lack the sheen.

C. Completed Test

This test is required for further confirmation.

Procedure

1. Pick up a single colony from EMB agar plate
2. Inoculate it into lactose broth and streak on a nutrient agar slant
3. Incubate the slants
4. Perform Gram reaction after attaining the growth

Result

Gram-negative nature of bacteria is indicative of a positive completed test.

(i) Total Phosphorus:

(a) Preparation of sample

Accurately weigh 1 g sample and transfer it to 100 ml beaker. Add 30 ml HNO_3 and 10 ml concentrated HCl. Heat over hot plate for 10-15 mins or till the brown fumes disappears. Keep for 4 hrs. Filter through Whatman No. 42 filter paper. Wash with distilled water 4-5 times (till acid free). Make up volume of filtrate to 250 ml in a volumetric flask.

(b) Estimate total P by gravimetric quinoline molybdate method as described under Schedule-11, Part B, 4(ii) of Order, 1985.

(ii) Citric acid soluble phosphorus:

(a) Reagents

Citric Acid solution: To dissolve 20 g of citric acid in water and make up the volume to 1 litre.

(b) Preparation of sample

(a) Accurately weigh 5 g of the sample into a 500-ml dry flask. Make up the volume to 500-ml mark with citric acid solution. Close the flask with a suitable stopper, place it at once in rotary shaking apparatus and shake the flask at the rate of 30 to 40 rev/min for 30 minutes. At the end of this period, remove the flask, filter the solution through a dry filter paper (Whatman No. 42) into a clean dry container.

(b) Estimate P by gravimetric quinoline molybdate method as described under Schedule- II, Part B, 4(ii) of Order, 1985.

(iii) Nitrogen content of water insoluble portion:

Preparation of sample

Accurately weigh 1 g sample into a clean beaker. Stir well with water for a few minutes and filter through a filter paper (Whatman No. 2 or equivalent). Complete the washing until filtrate measures 200-250 ml. Carefully put the filter paper containing the residue into a 500-ml Kjeldahl flask.

Note: Determine N as Schedule-I, Part 4, 3 (iv)

अनुसूची-६

(दफा ६ को उपदफा ३, दफा १० को उपदफा १ र २, दफा १२ को उपदफा १, दफा १३ को उपदफा १, दफा १५ को उपदफा १, दफा १८ को उपदफा २, दफा २० को उपदफा १ र दफा २१ को उपदफा १ सँग सम्बन्धित)

दस्तुर तथा शुल्क सम्बन्धमा

१. वापत	शुल्क ने. रु.
२. मल/उत्पादन सूचीकरण	५०००/- प्रति स्पेशीफिकेशन
३. मल/उत्पादन सूचीकरण नवीकरण	१०००/- प्रति स्पेशीफिकेशन
४. मल/उत्पादन सूचीकरण नवीकरण (म्याद सकिएको तीन महिनासम्म)	२०००/- प्रति स्पेशीफिकेशन
५. मल/उत्पादन सूचीकरण नवीकरण (म्याद सकिएको तीन महिना पछि)	३०००/- प्रति स्पेशीफिकेशन
६. मल आयात अनुमतिपत्र	२०००/- प्रति स्पेशीफिकेशन
७. आयात अनुमतिपत्र नवीकरण	१०००/- प्रति स्पेशीफिकेशन
८. कच्चा पदार्थ आयात अनुमति	१०००/- प्रति पदार्थ
९. अनुसन्धान प्रयोजनको लागि अनुमतिपत्र	१०००/- प्रति स्पेशीफिकेशन
१०.सर्भेयरको मान्यता प्राप्तिको लागि	५०००/- एकमुष्ट
११.सर्भेयरको मान्यता नवीकरणको लागि	१०००/- एकमुष्ट

अनुसूची- ७

(दफा ६ को उपदफा (१) सँग सम्बन्धित)

रासायनिक मल/ न्यानो फर्टिलाईजर/ बायोस्टिमुलेन्टको सूचीकरण गर्न केन्द्रिय निकायमा दिने निवेदनको ढाँचा

मिति:...../...../.....

श्रीमान् प्रमुखज्यू
.....केन्द्रिय निकाय ।
.....

विषय: मल सूचीकरण गरी पाउँ ।

उपरोक्त सम्बन्धमा, रासायनिक मल/ न्यानो फर्टिलाईजर/ बायोस्टिमुलेन्ट उपत्पादन वा सम्मिश्रण गर्न इच्छुक भएकोले मल (नियन्त्रण) आदेश, २०८३ को दफाबमोजिम..... मल सूचीकरण गरी दिनु हुन निम्न अनुसारका आवश्यक कागजात संलग्न गरी देहायको विवरण साथ यो निवेदन पेश गरेको छु ।

(क) मलको विवरण

१. उत्पादक व्यक्ति/संस्था/फर्मको नाम, ठेगाना र सम्पर्क नम्बर :

२. मलको व्यापारिक नाम (ट्रेडनेम) :

३. स्पेसिफिकेशन बमोजिमको नाम र पोषण तत्व लगातका विवरण:

घोषणा:- यस निवेदनमा उल्लिखित मल नेपाल सरकारबाट निर्धारित स्पेसिफिकेशन अनुरूप भएको घोषणा गर्दछु ।

.....
निवेदकको दस्तखत
व्यक्ति/संस्था

(ख) संलग्न कागजातहरू

१. नागरिकता वा राष्ट्रिय परिचयपत्रको प्रतिलिपि ।
२. फर्म/ संस्था दर्ता तथा नवीकरण प्रमाणपत्रको प्रतिलिपि ।
३. प्यान/भ्याट दर्ता प्रमाणपत्रको प्रतिलिपि ।
४. करचुक्ता प्रमाणपत्रको प्रतिलिपि ।
५. मलको ब्राण्ड वा लोगोको प्रतिलिपि ।
६. आधिकारीक वा मान्यताप्राप्त प्रयोगशालाबाट प्राप्त तिन महिना भित्रको मल परीक्षणको नतिजा ।
७. अनुसूची -२४ अनुसार मलको ब्यागमा रहने विवरण ।
८. अनुसूची- ६ मा उल्लेख भएबमोजिम राजश्व दाखिलाको कागजात ।
९. व्यवसायिक योजना (कच्चा प्रदार्थ, वार्षिक उत्पादन र लगानी तथा बजारिकरण योजना खुलेको) ।
१०. प्रचलित कानून बमोजिम तोकिएको स्विकृत संक्षिप्त वातावरणीय अध्ययन वा प्रारम्भिक वातावरणीय परिक्षण वा वातावरणीय प्रभाव मूल्याङ्कन प्रतिवेदन
११. निकायले मागेको अन्य आवश्यक विवरण ।

दृष्टव्य : यस निवेदनपत्रमा कुनै विवरण उल्लेख गर्न ठाउँ नपुग्ने भएमा छुट्टै कागजमा पनि पेश गर्न सकिनेछ ।

अनुसूची-८

(दफा ६ को उपदफा (२) सँग सम्बन्धित)

जैविक मल तथा प्रांगारिक मल सूचीकरणका गर्न प्रदेश मन्त्रालय वा प्रदेशले तोकेको निकायमा दिने निवेदनको ढाँचा

मिति:...../...../.....

श्रीमान् प्रमुखज्यू
प्रदेश मन्त्रालय।

.....

विषय: मल सूचीकरण गरी पाउँ ।

उपरोक्त सम्बन्धमा.....जैविक/ सिटि कम्पोष्ट / प्राङ्गारिक मल / वर्मी कम्पोष्ट / गोठेमल मल उत्पादन गरी बिक्री वितरण गर्न इच्छुक भएकोले मल (नियन्त्रण) आदेश, २०८३ को दफा को उपदफा बमोजिम जैविक मल वा आदेशको दफा ... को उपदफा ... खण्ड ग अनुसार दैनिक २ मे. टन भन्दा बढी परिमाणमा सिटि कम्पोष्ट, प्राङ्गारिक मल, वर्मी कम्पोष्ट र गोठेमल उत्पादन हुनेमलको सूचीकरण गरी दिनु हुन निम्न अनुसारका आवश्यक कागजात संलग्न गरी देहायको विवरण साथ यो निवेदन पेश गरेको छु ।

(क) मलको विवरण

१. उत्पादक व्यक्ति/संस्था/फर्मको नाम, ठेगाना र सम्पर्क नम्बर :
२. मलको व्यापारिक नाम (ट्रेडनेम) :
३. स्पेसिफिकेशन बमोजिमको नाम र पोषण तत्व लगातका विवरण:

घोषणा:- यस निवेदनमा उल्लिखित मल नेपाल सरकारबाट निर्धारित स्पेसिफिकेशन अनुरूप भएको घोषणा गर्दछु ।

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निवेदकको दस्तखत
व्यक्ति/संस्था

(ख) संलग्न कागजातहरू

१. नागरिकता वा राष्ट्रिय परिचयपत्रको प्रतिलिपि ।
२. फर्म/ संस्था दर्ता तथा नवीकरण प्रमाणपत्रको प्रतिलिपि ।
३. प्यान/भ्याट दर्ता प्रमाणपत्रको प्रतिलिपि ।
४. करचुक्ता प्रमाणपत्रको प्रतिलिपि ।
५. मलको ब्राण्ड वा लोगोको प्रतिलिपि ।
६. आधिकारीक वा मान्यताप्राप्त प्रयोगशालाबाट प्राप्त तिन महिना भित्रको मल परीक्षणको नतिजा ।
७. अनुसूची -२४ अनुसार मलको ब्यागमा रहने विवरण ।
८. अनुसूची- ६ मा उल्लेख भएबमोजिम राजश्व दाखिलाको कागजात ।
९. व्यवसायिक योजना (कच्चा प्रदार्थ, वार्षिक उत्पादन र लगानी तथा बजारिकरण योजना खुलेको) ।
१०. प्रचलित कानून बमोजिम तोकिएको स्विकृत संक्षिप्त वातावरणीय अध्ययन वा प्रारम्भिक वातावरणीय परिक्षण वा वातावरणीय प्रभाव मूल्याङ्कन प्रतिवेदन
११. निकायले मागेको अन्य आवश्यक विवरण ।

अनुसूची-९

(दफा ६ को उपदफा (३) ग सँग सम्बन्धित)

सिटि कम्पोष्ट, प्राङ्गारिक मल, गाँड्योला मल वा भर्मिकम्पोष्ट र गोठेमल सूचीकरणका गर्न स्थानीय तहमा दिने निवेदनको ढाँचा

मिति:...../...../.....

श्रीमान् प्रमुख प्रशासकीय अधिकृत ज्यू
..... नगरपालिका/गाउँपालिका
.....

विषय: मल सूचीकरण गरी पाउँ ।

उपरोक्त सम्बन्धमा,..... सिटि कम्पोष्ट, प्राङ्गारिक मल(दाना वा धुलो), वर्मी कम्पोष्ट र गोठेमल मल उत्पादन गरी बिक्री वितरण गर्न इच्छुक भएकोले मल (नियन्त्रण) आदेश, २०८३ को दफा ६ उपदफा (१) बमोजिम दैनिक दुई (२) मे. टन भन्दा कम परिमाणमा उत्पादन हुनेमल सूचीकरण गरी दिनु हुन निम्न अनुसारका आवश्यक कागजात संलग्न गरी देहायको विवरण साथ यो निवेदन पेश गरेको छु ।

(क) मलको विवरण

१. उत्पादक व्यक्ति/संस्था/फर्मको नाम, ठेगाना र सम्पर्क नम्बर :

२. मलको व्यापारिक नाम (ट्रेडनेम) :

३. स्पेसिफिकेशन बमोजिमको नाम र पोषण तत्व लगातका विवरण:

घोषणा:- यस निवेदनमा उल्लिखित मल नेपाल सरकारबाट निर्धारित स्पेसिफिकेशन अनुरूप भएको घोषणा गर्दछु ।

.....
निवेदकको दस्तखत
व्यक्ति/संस्था

(ख) संलग्न कागजातहरू

१. नागरिकता वा राष्ट्रिय परिचयपत्रको प्रतिलिपि ।
२. फर्म/संस्था दर्ता तथा नवीकरण प्रमाणपत्रको प्रतिलिपि ।
३. प्यान/भ्याट दर्ता प्रमाणपत्रको प्रतिलिपि ।
४. करचुक्ता प्रमाणपत्रको प्रतिलिपि ।
५. मलको ब्राण्ड वा लोगोको प्रतिलिपि ।
६. आधिकारीक वा मान्यताप्राप्त प्रयोगशालाबाट प्राप्त तिन महिना भित्रको मल परीक्षणको नतिजा ।
७. अनुसूची -२४ अनुसार मलको ब्यागमा रहने विवरण ।
८. अनुसूची- ६ मा उल्लेख भएबमोजिम राजश्व दाखिलाको कागजात ।
९. व्यवसायिक योजना (कच्चा प्रदार्थ, वार्षिक उत्पादन र लगानी तथा बजारिकरण योजना खुलेको) ।
१०. निकायले मागेको अन्य आवश्यक विवरण ।

अनुसूची-१०
(दफा ६ को उपदफा (४) सँग सम्बन्धित)
मल सूचीकरणको प्रमाणपत्र

{कार्यालयको नाम}
{कार्यालयको ठेगाना}

मिति:.....

श्री
{निवेदकको नाम र ठेगाना}

विषय: मल सूचीकरण गरिएको सम्बन्धमा ।

यस{केन्द्रिय निकाय/प्रदेश मन्त्रालय/स्थानीय तह} मा {मलको नाम} सूचीकरण गर्नको लागि प्राप्त निवेदनका सम्बन्धमा को मिति..... को निर्णय अनुसार बाट उत्पादन गरिएको देहायको निम्न लिखित विवरण अनुसारको मल (नियन्त्रण) आदेश, २०८३ अनुसार सूचीकरण गरिएको व्यहोरा जानकारी गराईन्छ ।

१. उत्पादक/व्यक्ति/संस्था/फर्मको नाम :-
२. ठेगाना :-
फोन नं. :-
मोबाइल नं. :-
३. उत्पादन गर्न अनुमित प्रदान गरिएको मलको व्यापारिक नाम र पोषण तत्वहरू वा जीवाणुहरूको को मात्रा (टेलमा) :-

शर्तहरू:

१. मल (नियन्त्रण) आदेश, २०८३ तथा रासायनिक मल निर्देशिका, २०५७ र राष्ट्रिय मल नीति, २०५८ ले तोकेको प्रकृया पुरा गरी उत्पादन तथा विक्रि वितरण गर्नु पर्नेछ र उत्पादन गरिने मलको स्पेसिफिकेशन आदेशको अनुसूची-१ अनुसार हुनु पर्नेछ ।
२. उत्पादित मल कृषि प्रयोजनका लागि मात्र विक्रि वितरण गर्नु पर्नेछ र गैहकृषि क्षेत्रमा विक्रि वितरण गर्न पाइने छैन ।
३. मल सूचीकरणको प्रमाणपत्र प्रत्येक तीन-तीन वर्षमा अनुसूची- ६ बमोजिम राजस्व दाखिला गरी नविकरण गर्नु पर्नेछ ।
४. मलको गुणस्तर सम्बन्धमा मलको हरेक लटको आधिकारिक वा मान्यताप्राप्त प्रयोगशालाबाट परिक्षण गराइ सूचीकरण गर्ने निकायमा पेश गर्नुपर्नेछ ।
५. आर्थिक वर्ष समाप्त भएको ३ महिना भित्र आफ्नो वार्षिक उत्पादन र विक्रि समेतको विवरण अद्यावधिक गर्नको लागि अनुसूची-११ बमोजिमको ढाँचामा मल सूचीकरण गर्ने निकायमा पेश गर्नु पर्नेछ ।
६. उत्पादकले त्रैमासिक रूपमा अनुसूची -१२ मा तोकिएको ढाँचा अनुसारको विवरण केन्द्रीय निकायमा पेश गर्नुपर्नेछ ।
७. उद्योग परिसरमा आवश्यक सुरक्षा उपकरण र अनुगमन प्रणाली जडान गर्नुपर्ने ।
८. प्याकेजिङमा मलको कम्पोजिसन, प्रयोग विधि, मात्रा, उत्पादन मिति, म्याद सकिने मिति र अन्य आवश्यक जानकारी समावेश गरी लेबलिंग सम्बन्धी नियमहरू पालना गर्नुपर्ने ।
९. उत्पादकले आफ्नै गुणस्तर नियन्त्रण प्रयोगशाला स्थापना गरी आवश्यक परीक्षण उपकरण राख्नुपर्ने र योग्यता प्राप्त प्राविधिक कर्मचारी राख्नुपर्ने ।
१०. मल उत्पादन, भण्डारण र ढुवानीका लागि विस्तृत सुरक्षा प्रोटोकल तयार गरी लागू गर्नुपर्ने ।

अनुसूची-११

(दफा ८ को उपदफा (२) सँग सम्बन्धित)

सूचीकृत मलको सूची अध्यावधिक गर्ने प्रयोजनको लागि पेश गर्नु पर्ने विवरण

उत्पादकको नाम र ठेगाना:

सि.नं.	मलको नाम	मलको प्रकार	मलको परिमाण			उत्पादकको बार्षिक उत्पादन क्षमता
			उत्पादन	बिक्री वितरण	मौज्दात	

विवरण प्रमाणीत गरी पेश गर्ने व्यक्तिको विवरण

संस्था/फर्मको छाप:-

दस्तखत:-

नाम थर:-

पद:-

सम्पर्क नं.:-

अनुसूची-१२

(दफा ८ को उपदफा (३) सँग सम्बन्धित)
त्रैमासिक प्रगति विवरण

मिति

उत्पादकको नाम र ठेगाना:

सि.नं.	मलको नाम..... प्रकार..... महिना.....	मलको नाम..... प्रकार..... महिना.....	मलको नाम..... प्रकार..... महिना.....	जम्मा
उत्पादन				
निकासी				
बिक्री वितरण				
मौज्दात				

विवरण प्रमाणीत गरी पेश गर्ने व्यक्तिको विवरण

संस्था/फर्मको छाप:-

दस्तखत:-

नाम थर:-

पद:-

सम्पर्क नं.:-

अनुसूची-१३

(दफा १२ को उपदफा (१) सँग सम्बन्धित)

रासायनिक मल / नानो फर्टिलाइजरको आयात गर्न केन्द्रिय निकायमा दिने निवेदनको ढाँचा सम्बन्धमा

मिति:...../...../.....

श्रीमान् प्रमुखज्यू
.....केन्द्रिय निकाय ।

.....

विषय: मल आयात अनुमति पत्र पाउँ ।

उपरोक्त सम्बन्धमा, रासायनिक मल/ न्यानो फर्टिलाइजर आयात गर्न इच्छुक भएकोले मल (नियन्त्रण) आदेश, २०८३ को दफाबमोजिम..... मल आयात अनुमति पत्र जारी गरि दिनु हुन निम्न अनुसारका आवश्यक कागजात संलग्न गरी देहायको विवरण साथ यो निवेदन पेश गरेको छु ।

(क) मलको विवरण

१.आयातकर्ताको संस्था/फमको नाम:

२.ठेगाना:

३.मोबाइल Email Address:

४.संस्था/फम प्रमुखको नाम :

५.ठेगाना:

६.मोबाइल नं: Email Address:

७.आयात गर्न चाहेको रासायनिक मल / नानो फर्टिलाइजर:

८.मल आयात गर्न देश:

९.मल आयात शुरु हुने मिति :

१०.मल आपूर्तिकर्ताको नाम ठेगाना

११.हालको कारोबार :

१२.मल कारोबारको लागि आवश्यक पुर्वाधार:

१३.मल आयात गर्ने लगानीको श्रोत:

१४.वार्षिक मलको अनुमानित आयात परिमाणको विवरण :

१५. मल कारोबार गर्न चाहेको जिल्लाहरुको विवरण:

१६. निकायले मागेको अन्य आवश्यक विवरण ।

१७. घोषणा :- यस निवेदनमा उल्लिखित मल नेपाल सरकारबाट निर्धारित स्पेसिफिकेशन वा नेपाल सरकारबाट प्राप्त स्वीकृति अनुरूप भएको घोषणा गर्दछु ।

.....
निवेदकको दस्तखत

व्यक्ति/संस्था

मिति

(ख) संलग्न कागजातहरू

१. निवेदन
२. उद्योग/वाणिज्य दर्ताको प्रमाणपत्र
३. कर चुक्ता प्रमाण पत्र
४. स्थायी लेखा नम्बर (PAN) दर्ता प्रमाण पत्र
५. EXIM code Certification
६. नेपाली नागरिकताको प्रमाणपत्र
७. कम्पनीको प्रबन्धपत्रको उद्देश्यमा मलको आयात तथा कारोबार गर्ने विवरण उल्लेख हुनु पर्ने ।
८. अनुसूची- ६ मा उल्लेख भएबमोजिम राजश्व दाखिला गरेको भौचरको सक्कलै प्रति
९. अनुसूची - बमोजिमको विवरण सहित प्रत्येक मलको लेवलको फोटो
१०. वार्षिक मलको अनुमानित आयात परिमाणको विवरण
११. मल कारोबार गर्न चाहेको जिल्लाहरूको विवरण
१२. उत्पादकले जारी गरेको स्पेसिफिकेशन बमोजिमको मलको गुणस्तर खुल्ने कागजात/ प्रमाणपत्र

दृष्टव्य : यस निवेदनपत्रमा कुनै विवरण उल्लेख गर्न ठाउँ नपुग्ने भएमा छुट्टै कागजमा पनि पेश गर्न सकिनेछ

नविकरणको हकमा

(थप आवश्यक कागजात)

- 1= डिलरको विवरण
- 2= गत वर्षको कारोबारको विवरण
- 3= अनुसूची- ६ मा उल्लेख भएबमोजिम राजश्व दाखिला गरेको भौचरको सक्कलै प्रति

अनुसूची-१४

(दफा १२ को उपदफा (२) सँग सम्बन्धित)
रासायनिक मल / नानो फर्टिलाइजरको आयात अनुमति पत्र

{कार्यालयको नाम}
{कार्यालयको ठेगाना}

मिति:.....

श्री
{निवेदकको नाम र ठेगाना}

विषय: रासायनिक मल / नानो फर्टिलाइजर आयात अनुमतिपत्र ।

यसकेन्द्रिय निकायमा मल आयात अनुमतिपत्र जारी गरिदिनका लागि दिएको निवेदन उपर कार्यवाहि हुँदा मिति.....को निर्णयानुसार निम्न लिखित विवरण अनुसारको त्यस संस्थालाई मल (नियन्त्रण) आदेश, २०८३ तथा राष्ट्रिय मल नीति, २०५८ का प्रावधानहरू पुरा गरी मल आयात गर्न मल आयात अनुमतिपत्र जारी गरिएको व्यहोरा अनुरोध छ।

१. आयातकर्ता संस्था/फर्मको नाम: -
 २. ठेगाना:-
 ३. मोबाइल नं:-
 ४. संस्था प्रमुखको नाम :
 ५. ठेगाना :
 ६. मोबाइल नं.:-
 ७. आयात गर्न अनुमति प्रदान गरिएको मल:
 ८. मल आयात गरिने देश:
 ९. मल आयात शुरू हुने मिति:-
 १०. मल आपूर्तिकर्ताको नाम, ठेगाना:
 ११. हालको कारोबार:
 १२. मल कारोबारको लागि भौतिक पूर्वाधार तथा सुविधाको विवरण:-
 १३. मल आयात गर्ने लगानीको स्रोत:
- शर्तहरू:

(१) आयातकर्ताले पालना गर्नुपर्ने सर्तहरू देहाय बमोजिम रहेको छ।

- क) उत्पादकले जारी गरेको स्पेसिफिकेशन बमोजिमको मलको गुणस्तर खुल्ने कागजात/प्रमाणपत्र अनिवार्य रूपमा लिएको हुनुपर्नेछ।
- ख) विदेशी मुलुकबाट मल चलान गर्दा लोड पोर्ट र भन्सार बिन्दुको अनलोड पोर्टमा मलको परिमाण, गुणस्तर, प्याकेजिङ र लेबलिङ तोकिएको बमोजिम छ वा छैन भनी मन्त्रालयबाट तोकिएको स्वतन्त्र सर्भेयर बाट प्रमाणित गराएको प्रमाणपत्र लिएको हुनुपर्नेछ।
- ग) आयातित मललाई ओस, पानी, मिसावटबाट बचाउनका लागि उपयुक्त भण्डारणको व्यवस्था मिलाएको हुनुपर्नेछ।
- घ) आयातित मलको व्याच नम्बरका आधारमा बिक्री वितरणको विस्तृत अभिलेख राख्नुपर्नेछ।
- ङ) आयातित मलको परिमाण, गुणस्तर प्रमाणपत्र, बिक्री वितरणको विवरण लगायतका सबै कागजातहरू सुरक्षित रूपमा राख्नुपर्नेछ।
- च) आयात गरिएको मल कृषि प्रयोजनका लागि मात्र बिक्री वितरण गर्नु पर्नेछ र गैहकृषि क्षेत्रमा बिक्री वितरण गर्न पाइने छैन।
- छ) आयातकर्ताको प्रमाणपत्र प्रत्येक एक-एक वर्षमा नविकरण गर्नु पर्नेछ।
- ज) मल आयातको विवरण त्रैमासिक रूपमा केन्द्रिय निकायमा उपलब्ध गराउनु पर्नेछ।

अनुसूची-१५

(दफा १३ को उपदफा (१) सँग सम्बन्धित)

प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको आयात गर्न केन्द्रिय निकायमा दिने निवेदनको ढाँचा सम्बन्धमा

मिति:...../...../.....

श्रीमान् प्रमुखज्यू
केन्द्रिय निकाय ।

.....

विषय: मल आयात गरी पाउँ ।

उपरोक्त सम्बन्धमा, प्राङ्गारिक मल/जैविक मल / बायोस्टिमूलेन्टको आयात गर्न इच्छुक भएकोले मल (नियन्त्रण) आदेश, २०८३ को दफाबमोजिम..... मल आयात अनुमति पत्र जारी गरि दिनु हुन निम्न अनुसारका आवश्यक कागजात संलग्न गरी देहायको विवरण साथ यो निवेदन पेश गरेको छु ।

(क) मलको विवरण

१.आयातकर्ताको संस्था/फमको नाम:

२.ठेगाना:

३.मोबाइल Email Address:

४.संस्था/फम प्रमुखको नाम :

५.ठेगाना:

६.मोबाइल नं: Email Address:

७.आयात गर्न चाहेको रासायनिक मल / नानो फर्टिलाइजर:

८.मल आयात गर्न देश:

९.मल आयात शुरु हुने मिति :

१०.मल आपूर्तिकर्ताको नाम ठेगाना

११.हालको कारोबार :

१२.मल कारोबारको लागि आवश्यक पुर्वाधार:

१३.मल आयात गर्ने लगानीको श्रोत:

१४.वार्षिक मलको अनुमानित आयात परिमाणको विवरण :

१५. मल कारोबार गर्न चाहेको जिल्लाहरुको विवरण:

१६. निकायले मागेको अन्य आवश्यक विवरण ।

१७. घोषणा :- यस निवेदनमा उल्लिखित मल नेपाल सरकारबाट निर्धारित स्पेसिफिकेशन वा नेपाल सरकारबाट प्राप्त स्वीकृति अनुरूप भएको घोषणा गर्दछु ।

.....

निवेदकको दस्तखत

व्यक्ति/संस्था

मिति

(ख) संलग्न कागजातहरू

१. निवेदन
२. उद्योग/वाणिज्य दर्ताको प्रमाणपत्र
३. कर चुक्ता प्रमाण पत्र
४. स्थायी लेखा नम्बर (PAN) दर्ता प्रमाण पत्र
५. EXIM code Certification
६. नेपाली नागरिकताको प्रमाणपत्र
७. कम्पनीको प्रबन्धपत्रको उद्देश्यमा मलको आयात तथा कारोबार गर्ने विवरण उल्लेख हुनु पर्ने ।
८. अनुसूची- ६ मा उल्लेख भएबमोजिम राजश्व दाखिला गरेको भौचरको सक्कलै प्रति
९. अनुसूची - बमोजिमको विवरण सहित प्रत्येक मलको लेवलको फोटो
१०. वार्षिक मलको अनुमानित आयात परिमाणको विवरण
११. मल कारोबार गर्न चाहेको जिल्लाहरूको विवरण
१२. उत्पादकले जारी गरेको स्पेसिफिकेशन बमोजिमको मलको गुणस्तर खुल्ने कागजात/ प्रमाणपत्र
१३. मान्यताप्राप्त अन्तर्राष्ट्रिय प्रयोगशालाको पछिल्लो तीन महिना भित्रको परिक्षण प्रतिवेदन
१४. नेपाल कृषि अनुसन्धान परिषद्द्वारा जारी गरिएको बाली परिक्षण प्रतिवेदन/नतिजा ।

दृष्टव्य : यस निवेदनपत्रमा कुनै विवरण उल्लेख गर्न ठाउँ नपुग्ने भएमा छुट्टै कागजमा पनि पेश गर्न सकिनेछ

नवीकरणको हकमा

(थप आवश्यक कागजात)

१. डिलरको विवरण
२. गत वर्षको कारोबारको विवरण
३. अनुसूची- ६ मा उल्लेख भए बमोजिम राजश्व दाखिला गरेको भौचरको सक्कलै प्रति
४. प्रांगारिक मल, जैविक मल र बायोस्टिमुलेन्टको आयात अनुमति पत्र नवीकरण अधिकतम २ पटक सम्म हुनेछ । सो पश्चात नवीकरण चाहेमा पुनः दफा १४ बमोजिम बाली परिक्षण गराउनु पर्नेछ ।

अनुसूची-१६

(दफा १३ को उपदफा (५) सँग सम्बन्धित)
प्राङ्गारिक मल, जैविक मल र बायोस्टिमूलेन्टको आयात अनुमति पत्र

{कार्यालयको नाम}
{कार्यालयको ठेगाना}

मिति:.....

श्री
{निवेदकको नाम र ठेगाना}

विषय: प्राङ्गारिक मल/ जैविक मल / बायोस्टिमूलेन्टको आयात अनुमतिपत्र ।

यसकेन्द्रिय निकायमा मल आयात अनुमतिपत्र जारी गरिदिनका लागि दिएको निवेदन उपर कार्यवाहि हुँदा मिति..... को निर्णयानुसार निम्न लिखित विवरण अनुसारको त्यस संस्थालाई मल (नियन्त्रण) आदेश, २०८३ तथा राष्ट्रिय मल नीति, २०५८ का प्रावधानहरू पुरा गरीमल आयात गर्न मल आयात अनुमतिपत्र जारी गरिएको व्यहोरा अनुरोध छ।

१. आयातकर्ता संस्था/फर्मको नाम: -
 २. ठेगाना:-
 ३. मोबाइल नं:-
 ४. संस्था प्रमुखको नाम :
 ५. ठेगाना :
 ६. मोबाइल नं.:-
 ७. आयात गर्न अनुमति प्रदान गरिएको मल:
 ८. मल आयात गरिने देश:
 ९. मल आयात शुरू हुने मिति:-
 १०. मल आपूर्तिकर्ताको नाम, ठेगाना:
 ११. हालको कारोबार:
 १२. मल कारोबारको लागि भौतिक पूर्वाधार तथा सुविधाको विवरण:-
 १३. मल आयात गर्ने लगानीको स्रोत:
- शर्तहरू:

(१) आयातकर्ताले पालना गर्नुपर्ने सर्तहरू देहाय बमोजिम रहेको छ।

- क) उत्पादकले जारी गरेको स्पेसिफिकेशन बमोजिमको मलको गुणस्तर खुल्ने कागजात/प्रमाणपत्र अनिवार्य रूपमा लिएको हुनुपर्नेछ।
- ख) विदेशी मुलुकबाट मल चलान गर्दा लोड पोर्ट र भन्सार बिन्दुको अनलोड पोर्टमा मलको परिमाण, गुणस्तर, प्याकेजिङ र लेबलिङ तोकिए बमोजिम छ वा छैन भनी मन्त्रालयबाट तोकिएको स्वतन्त्र सर्भेयर बाट प्रमाणित गराएको प्रमाणपत्र लिएको हुनुपर्नेछ।
- ग) आयातित मललाई ओस, पानी, मिसावटबाट बचाउनका लागि उपयुक्त भण्डारणको व्यवस्था मिलाएको हुनुपर्नेछ। भण्डारण गर्दा उक्त मलमा भएका पोषक तत्वहरू नष्ट नहुने गरी वा लाभदायक जीवहरू ननासिने गरी वा रासायनिक स्वरूप परिवर्तन नहुने गरी राखे व्यवस्था गर्नु पर्नेछ।
- घ) आयातित मलको व्याच नम्बरका आधारमा बिक्री वितरणको विस्तृत अभिलेख राख्नुपर्नेछ।
- ङ) आयातित मलको परिमाण, गुणस्तर प्रमाणपत्र, बिक्री वितरणको विवरण लगायतका सबै कागजातहरू सुरक्षित रूपमा राख्नुपर्नेछ।
- च) आयात गरिएको मल कृषि प्रयोजनका लागि मात्र बिक्री वितरण गर्नु पर्नेछ र गैहकृषि क्षेत्रमा बिक्री वितरण गर्न पाइने छैन।
- छ) आयातकर्ताको प्रमाणपत्र प्रत्येक एक-एक वर्षमा नविकरण गर्नु पर्नेछ।
- ज) मल आयातको विवरण त्रैमासिक रूपमा केन्द्रिय निकायमा उपलब्ध गराउनु पर्नेछ।

अनुसूची-१७

(दफा १७ को उपदफा (२) सँग सम्बन्धित)

आयातित मलको त्रैमासिक प्रगति विवरण

मिति.....

१. आयातकर्ता संस्था/फर्मको नाम: -

२. ठेगाना:-

	सि.नं.	प्रकार	आयात	विक्रि	मौज्दात	कैफियत
महिना	मल १.					
	मल.२					
महिना	सि.नं.					
	मल १.					
	मल.२					
महिना	सि.नं.					
	मल १.					
	मल.२					
	जम्मा					

कार्यालय/संस्थाको छाप:-

विवरण पेश गर्नेको,-

दस्तखत:-

नाम थर:-

ठेगाना:-

सम्पर्क नं.:-

अनुसूची-१८

(दफा १८ को उपदफा (२) सँग सम्बन्धित)
सर्भेयरको मान्यताको लागि दिने निवेदनको ढाँचा

श्रीमान् प्रमुखज्यू
केन्द्रिय निकाय ।

.....

विषय: सर्भेयरको मान्यता प्राप्त गर्ने सम्बन्धमा ।

मल (नियन्त्रण) आदेश, २०८३ को दफा १८ को उपदफा ... बमोजिम स्वतन्त्र हैसियतमा मलको गुणस्तर सम्बन्धी कार्य गर्ने मान्यता पाउन निम्न अनुसारका आवश्यक कागजात संलग्न गरी देहायको विवरण साथ यो निवेदन पेश गरेको छु ।

१ संस्थाको नाम:

२ ठेगाना:

३ संस्था स्थापना दर्ता भएको मिति:

४ संस्थाले सम्पादन गर्ने मुख्य कामहरू:

५ मलको सर्भेक्षण गर्न सक्षम जनशक्तिको विवरण:

६ मलको गुणस्तर परीक्षणका लागि आवश्यक पूर्वाधारको विवरण:

७ मलको गुणस्तर परीक्षणका सम्बन्धमा कुनै अन्तर्राष्ट्रिय वा राष्ट्रिय संस्थासंग सम्बन्ध भए सो को विवरण एवं प्रमाण

८ प्रयोगशाला सुविधा विवरण

९ मल सर्भेक्षण सम्बन्धी विगतको अनुभव भए सोको विवरण एवं प्रमाणित प्रतिलिपि

९ संलग्न कागजातहरू:

क) संस्थाको दर्ता/ स्थापनाको प्रमाण वा कुनै लिखतको प्रमाणित प्रतिलिपि

ख) मलको गुणस्तर परीक्षणका सम्बन्धमा कुनै अन्तर्राष्ट्रिय वा राष्ट्रिय संस्थासंग सम्बन्ध भए सो को विवरण एवं प्रमाण

ग) आयकर चुक्ता गरेको प्रमाणपत्र वा निस्साको प्रमाणित प्रतिलिपि:

घ) रासायनिक मल र प्रांगारिक मलहरूको रासायनिक परीक्षणका लागि कृषि विज्ञान वा रसायनशास्त्रमा कम्तीमा स्नातकोत्तर उर्तिण गरी आधिकारीक विश्लेषकको तालिम लिएको जनशक्तिको नियुक्तिपत्र र न्यूनतम योग्यताको प्रमाणपत्र

ङ) जैविक मल परीक्षणका हकमा माइक्रोबायोलोजि वा जैविक प्रविधि विषयमा कम्तीमा स्नातकोत्तर उर्तिण गरी आधिकारीक विश्लेषकको तालिम लिएको जनशक्तिको नियुक्तिपत्र र न्यूनतम योग्यताको प्रमाणपत्र

निवेदकको

नाम:

दर्ता:

ठेगाना:

सही:

मिति:

द्रष्टव्य: यस निवेदन पत्रमा कुनै विवरण उल्लेख गर्न ठाउँ नपुग्ने भएमा छुट्टै कागजमा पेश गर्न सकिनेछ ।

अनुसूची-१९

(दफा १८ को उपदफा (५) सँग सम्बन्धित)
सर्भेयरको प्रमाणपत्र ढाँचा

{कार्यालयको नाम}
{कार्यालयको ठेगाना}
सर्भेयरको प्रमाणपत्र

मिति:.....

श्री

{निवेदकको नाम र ठेगाना}

विषय: स्वतन्त्र सर्भेयरको प्रमाणपत्र ।

मिति..... मा दर्ता/स्थापना भईकार्यालय रहेको श्री.....लाई मल (नियन्त्रण) आदेश, २०८३ को दफा को उपदफा बमोजिमको अख्तियारी प्रयोग गर्ने पाउने गरीको मितिको निर्णयानुसार आ.व..... को लागि अनुसूचीबमोजिमको मलको सर्भेयरको मान्यता दिई यो प्रमाणपत्र दिईएको छ ।

शर्तहरू:

- (१) स्वतन्त्र सर्भेयरले पालना गर्नुपर्ने शर्तहरू देहाय बमोजिम रहेको छ ।
(क) सर्भेयरले आफुले प्रमाणित गरेको मलको सर्भेक्षण प्रतिवेदनको एक प्रति ३० दिन भित्र केन्द्रिय निकायमा पेश गर्नु पर्नेछ ।
(ख) सर्भेयरको प्रमाणपत्रको अवधि १ वर्षको हुनेछ ।

प्रमाणपत्र दिने अधिकारी

नाम:

दर्जा

सही:

मिति:

अनुसूची-२०
(दफा २२ को उपदफा (१) सँग सम्बन्धित)
मलको निर्यातका लागि केन्द्रिय निकायमा दिने निवेदनको ढाँचा

मिति:...../...../.....

श्रीमान् प्रमुखज्यू
केन्द्रिय निकाय ।

.....

विषय: मल निर्यात गरी पाउँ ।

उपरोक्त सम्बन्धमा, मल निर्यात गर्न इच्छुक भएकोले मल (नियन्त्रण) आदेश, २०८३ को दफाबमोजिम..... मल निर्यात अनुमति पत्र जारी गरि दिनु हुन निम्न अनुसारका आवश्यक कागजात संलग्न गरी देहायको विवरण साथ यो निवेदन पेश गरेको छु ।

(क) मलको विवरण

१.निर्यातकर्ताको संस्था/फर्मको नाम:

२.ठेगाना:

३.मोबाइल Email Address:

४.संस्था/फर्म प्रमुखको नाम :

५.ठेगाना:

६.मोबाइल नं: Email Address:

७. निर्यात गर्न चाहेको मल तथा सोको तत्व संरचना :

८.मल निर्यात गर्न देश:

९.मल निर्यात शुरु हुने मिति :

१०.मल निर्यातकर्ताको नाम ठेगाना

११.हालको कारोबार :

१२.मल कारोबारको लागि आवश्यक पूर्वाधार:

१३.मल निर्यात गर्ने लगानीको श्रोत:

१४.वार्षिक मलको अनुमानित निर्यात परिमाणको विवरण :

१५. निकायले मागेको अन्य आवश्यक विवरण ।

घोषणा :- यस निवेदनमा उल्लिखित मल नेपाल सरकारबाट निर्धारित स्पेसिफिकेशन वा नेपाल सरकारबाट प्राप्त स्वीकृति अनुरूप भएको घोषणा गर्दछु ।

.....

निवेदकको दस्तखत

व्यक्ति/संस्था

मिति

(ख) संलग्न कागजातहरू

1. निवेदन
2. उद्योग/वाणिज्य दर्ताको प्रमाणपत्र
3. कर चुक्ता प्रमाण पत्र
4. स्थायी लेखा नम्बर (PAN) दर्ता प्रमाण पत्र
5. EXIM code Certification
6. नेपाली नागरिकताको प्रमाणपत्र
7. कम्पनीको प्रबन्धपत्रको उद्देश्यमा मलको आयात तथा कारोबार गर्ने विवरण उल्लेख हुनु पर्ने ।
8. अनुसूची- ६ मा उल्लेख भएबमोजिम राजश्व दाखिला गरेको भौचरको सक्कलै प्रति
9. अनुसूची - बमोजिमको विवरण सहित प्रत्येक मलको लेवलको फोटो
10. उत्पादकले जारी गरेको स्पेसिफिकेशन बमोजिमको मलको गुणस्तर खुल्ने कागजात/ प्रमाणपत्र

दृष्टव्य : यस निवेदनपत्रमा कुनै विवरण उल्लेख गर्न ठाउँ नपुग्ने भएमा छुट्टै कागजमा पनि पेश गर्न सकिनेछ

अनुसूची-२१

(दफा २३ को उपदफा (३) सँग सम्बन्धित)
मल निरीक्षकको परिचयपत्रको ढाँचा

नेपाल सरकार
कृषि वन तथा पर्यावरण मन्त्रालय

{कार्यालयको नाम}
{कार्यालयको ठेगाना}

मल निरीक्षकको परिचयपत्र

१. नाम:

२. ठेगाना:

३. मोबाइल

४. मल निरीक्षकको परिचयपत्र नम्बर

५. कार्यालय:

Email Address:

निरीक्षकको दस्तखत

प्रमाणित गर्ने दस्तखत

.....

.....

यो परिचयपत्र मल निरीक्षणको लागि मात्र प्रयोग गर्न पाईन्छ ।

अनुसूची-२२

(दफा २६ को उपदफा (१)को खण्ड छु सँग सम्बन्धित)
मल निरीक्षकले निरीक्षणबापत दिने प्रतिवेदनको ढाँचा

प्रतिवेदन

१. उत्पादक/पैठारीकर्ता व्यक्ति/संस्था/फर्मको नाम :-
२. ठेगाना :-
३. फोन नं. :-
४. मोबाइल नं. :- ई-मेल.....
५. उत्पादन/पैठारी गर्न अनुमित प्रदान गरिएको मिति तथा संस्थाको नाम:-
६. मल पैठारी गरिएको देश :-
७. मल उत्पादक/ आपूर्तिकर्ताको नाम, ठेगाना :-
८. मोबाइल नं. :- ई-मेल.....
९. वार्षिक उत्पादन/आयातको विवरण:
१०. पुर्वाधारको अवस्था:
११. प्राविधिकको संख्या तथा दक्षता:
१२. मलको व्यापारिक नाम वा (ट्रेडनेम) :
१३. आदेश बमोजिम अनुमति प्राप्त मलको विसोपना परिमाण :
१४. उत्पादनको हकमा कच्चा प्रदार्थको श्रोत:
१५. सम्मिश्रणयुक्त मल उत्पादन गर्ने भएमा त्यसमा रहने पोषण तत्वको प्रतिशत:
१६. आदेश बमोजिम मलको प्याकेजिङ र लेबेलिङको अवस्था :
१७. मल भण्डारणको अवस्था:
१८. उत्पादकले जारी गरेको स्पेसिफिकेशन बमोजिमको मलको गुणस्तर खुल्ने कागजात:
१९. मलको नाम, पोषण तत्व र आकार

क्र.स	अनुसूचि अनुसारको मलको सिरियल नम्बर	मलको नाम	आकार	परिमाण	पोषक तत्व	कैफियत

२०. मलको गुणस्तर सम्बन्धमा मलको हरेक लटको आधिकारिक वा मान्यताप्राप्त प्रयोगशालाबाट परिक्षण गराइ सूचीकरण गर्ने निकायमा पेश गरेको/नगरेको
२१. आर्थिक वर्ष समाप्त भएको ३ महिना भित्र आफ्नो वार्षिक उत्पादन र विक्रि समेतको विवरण अद्यावधिक गर्नको लागि अनुसूची-११ बमोजिमको ढाँचामा मल सूचीकरण गर्ने निकायमा पेश गरेको/नगरेको
२२. उत्पादक/ आयातकर्ताले त्रैमासिक रूपमा विवरण पेश गरेको /नगरेको
२३. उद्योग परिसरमा आवश्यक सुरक्षा उपकरण र अनुगमन प्रणाली जडानको अवस्था

२४. प्याकेजिङमा मलको कम्पोजिसन, प्रयोग विधि, मात्रा, उत्पादन मिति, म्याद सकिने मिति र अन्य आवश्यक जानकारी समावेश गरी लेबलिंग सम्बन्धी नियमहरू पालना
२५. उत्पादकले आफ्नै गुणस्तर नियन्त्रण प्रयोगशाला स्थापनाको अवस्था
२६. मल उत्पादन, भण्डारण र ढुवानीका लागि विस्तृत सुरक्षा प्रोटोकलको अवस्था
२७. अन्य आवश्यक विवरण
२८. सुभाव तथा आवश्यक कार्यवाही सिफारीस
२९. अनुगमन क्रममा विभिन्न कृयाकलापहरूको फोटो

अनुसूची-२३

(दफा ३७ को उपदफा (२)को सँग सम्बन्धित)
परीक्षणका लागि नमुना सहित पठाउने औपचारिक पत्रको ढाँचा

श्री आधिकारिक विश्लेषक

..... प्रयोगशाला

..... ।

देहायको विवरण भएको र मलको नमुना परीक्षणको लागि त्यस प्रयोगशालामा पठाइएको छ । यो नमुना यथाशीघ्र परीक्षण गरी सोको दुई प्रति प्रतिवेदन पठाउनुहुन अनुरोध गर्दछु ।

- १ पदार्थ, ग्रेड र बाण्डको नाम
- २ नमुना लिइएको मिति
- ३ नमुना लिइएको ठाँउको नाम र ठेगाना
- ४ कारोबारको किसिम
- ५ नमुना लिंदाको पदार्थको भौतिक स्थिति
- ६ नमुनाको कोड नम्बर
- ७ अन्य कुनै कुरा भए सो को विवरण :

निरीक्षकको नाम :

सही :

मिति :

अनुसूची-२४

(दफा ३७ को उपदफा (२)को सँग सम्बन्धित)
मलको प्याकेजिङ र लेबेलिङ सम्बन्धी विवरण

१ प्याकेजिङ

- (क) नेपालमा उत्पादित वा पैठारी भएको मलको विक्री वितरण ५० के.जी .को पोलिप्रोपेलिन वा पोलिईथिलिनको एकसरो एक प्लाई धागोले बुनिएको बोरोमा राखिएको हुनु पर्नेछ। बाहिरी व्यागको बजन २०० ग्राम वा भित्री व्यागको पोलिथिनको मोटाई २०० गेज भन्दा कमको हुनु हुदैन।
- (ख) प्रत्येक व्याग बलियो सिन्थेटिक धागोले सिलाएको हुनु पर्नेछ।
- (ग) खण्ड (क) र (ख) मा जुन सुकै कुरा लेखिएको भएतापनि एक व्याग भन्दा कम खुद्रा मल पनि विक्री गर्न सकिनेछ। तर यसरी विक्री गरिने मल सिल गरिएको प्याकेजमा हुनु पर्नेछ।

२. लेबेलिङ

(क) नेपालमा पैठारी भएको र विक्री गरिने मलको लेबल देहाय बमोजिम हुनु पर्नेछः

(१) बोरोको एकापट्टी (One Side) मा :-

(अ) रासायनिक मलको नाम ।

(आ) उत्पादकको नाम र ट्रेडमार्क वा लोगो।

(इ) उत्पादन भएको (Country Origin) देशको नाम ।

(ई) मलको तत्व प्रतिशतमा एवं सोही क्रममा खुलाइएको र दशमलव पछिको अंकलाई नजिकको पूरा अंकमा मिलाइएको ।

(उ) कुल र खुद बजन किलोग्राममा (Gross and Net Weight in Kg) ।

(ऊ) उत्पादन भएको वर्ष ।

(२) बोरोको अर्कोपट्टी

(अ) पैठारीकर्ताको नाम र ट्रेड मार्क वा लोगो।

(आ) लट नम्बर ।

(इ) पैठारीकर्ताले मलको आन्तरिक प्रयोगका लागि राख्न चाहेको कुनै सूचना वा जानकारी ।

(ई) मल-काँटा नलगाउनुहोस् (Mineral Fertilizer- Use No Hook)

(ख) नेपालमा उत्पादित र विक्री वितरण हुने मलमा देहाय बमोजिमको लेबल हुनु पर्नेछः-

(१) बोरोकी एकापट्टी (One Side) मा

(अ) मलको नाम।

(आ) उत्पादकको नाम र ट्रेड मार्क वा लोगो।

(इ) उत्पादन भएको देशको नाम ।

(ई) मलको पोषण तत्वको प्रतिशत उल्लेख गर्दा दशमलव पछिको अंकलाई नजिकको पूरा अंकमा मिलाउनु पर्ने ।

(उ) खुद र कुल बजन किलो ग्राममा (Net and Gross weight in Kg)

(ऊ) उत्पादन भएको वर्ष ।

(२) बोराको अर्कोपट्टी:-

(अ) उत्पादकको नाम र ट्रेड मार्क वा लोगो ।

(आ) लट नम्बर

(इ) उत्पादकले आन्तरिक प्रयोगका लागि माथि उल्लिखित विवरणका अतिरिक्त अरु कुनै विवरण राख्न चाहे मो राख्न सकिने ।

(ग) खण्ड (क) र (ख) मा जुन सुकै कुरा लेखिएको भएतापनि एक ब्याग भन्दा कम परिमाणको खुद्रा मल पनि बिक्री गर्न सकिनेछ । तर यसरी बिक्री गरिने मल प्याकेजमा उपलब्ध गराउनु पर्ने र भित्रको वस्तु बारे प्याकेजको बाहिरपट्टि स्पष्टसंग लेखिनु पर्नेछ ।

३. रासायनिक सम्मिश्रित युक्त झोल मल, रासायनिक मल तथा सुक्ष्म खाद्यत्वयुक्त मलको लेवलमा देहाय बमोजिमको विवरण उल्लेख हुनु पर्नेछ:-

(क) मलको व्यापारिक नाम (Product name),

(ख) खाद्यतत्वको नाम र मात्रा (Nutrient name and content),

(ग) लक्षित बाली (Target crop),

(घ) प्रयोग विधि तथा समय (Time, method and frequency of application),

(ङ) उत्पादन सामग्री रजिष्ट्रेशन नम्बर (Product registration number),

(च) लट नम्बर (Lot no.),

(छ) उत्पादकको नाम तथा ठेगाना (Name and address of producer),

(ज) उत्पादन मिति (Manufacturing date),

(झ) प्रयोग गर्न सकिने अवधि (Expiry date),

(ञ) मलको परिमाण वा मात्रा (Quantity/amount),

(ट) अधिकतम खुद्रा मूल्य (MRP),

(ठ) हानिकारक तत्व (Toxic elements),

(ड) भण्डारण र प्रयोग गर्दा अपनाउनु पर्ने सावधानी (Precautions to be taken for storage, handling and use),

(ढ) वितरकको विवरण (आयातको हकमा) (Distributors information: in case of imports) ।