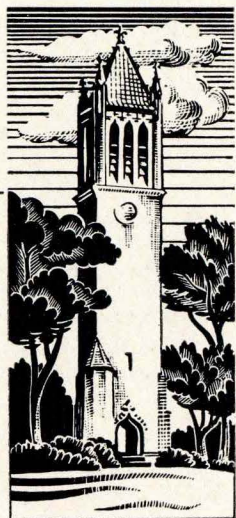


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The NITRATE PROBLEM



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Expressing Nitrate Contents

Nitrate (NO_3^-) contents of feeds and water may be reported as percentages, parts per million, or milligrams per liter of NO_3^- -N, NO_3^- , KNO_3 , or NaNO_3 . See page 13 for a discussion of methods of expressing the nitrate contents.

In this publication nitrate contents are reported on the nitrate (NO_3^-) basis as percentages in feeds and as parts per million in water.

SUMMARY

HAZARDS

Nitrates in Feeds and Water

Nitrates are known to have injurious effects when excessive amounts are ingested by humans or livestock. However, the toxicity of a given amount of nitrate varies tremendously, depending on many factors such as the type of ration and speed of intake.

Death may result from ingestion of variable amounts of nitrates. Smaller amounts may decrease rates of gain and/or milk production, result in abortions and in other ways affect the performance of animals.

Toxic Nitrogen Gases (Silos)

Toxic gases (oxides of nitrogen) are produced in newly filled silos by the reduction of nitrates in the forages. These gases are most dangerous while the silo is being filled and for about 2 to 4 weeks after it has been filled. Gases may persist, however, until sealed silos are emptied or until silo caps are removed from conventional silos. Occasionally, these yellow, red or brown gases may be seen at the top of the silo or at the drain at the bottom. Breathing these gases can result in death or serious illness of humans or livestock.

SOURCES OF NITRATES

Feeds

All plants contain some nitrate, but excessive amounts of toxic significance are most likely to be found in forages (hay, weeds, fodder, silages or pasture) grown on soils that have received heavy applications of manure or nitrogen fertilizers—especially when drouth, shade, disease, herbicide application or other factors interfere with the normal growth and development of the plant. Nitrate contents are usually highest in immature plants. Nitrate concentration is highest in the stems, intermediate in the leaves, and very low in the grain.

Water

Nitrates may build up in water supplies by being leached down through the soil, or by washing in from human, animal and industrial wastes. Toxic nitrate contents are most likely to be found in water from shallow wells (especially wells near accumulations of animal or human wastes); toxic levels (45 ppm) may also be found in ponds and deep wells.

DIAGNOSIS OF NITRATE TOXICITY

An accurate diagnosis of nitrate toxicity is often difficult.

Symptoms and lesions are nonspecific and are often vague and indistinct. There should be definite and reliable information that sufficient nitrates have been ingested or toxic gases inhaled. This usually requires analyses of the feed and water supplies, and results of these analyses may not be available when the information is needed for diagnostic purposes.

For assistance in interpretation and diagnosis of these problems, see your physician or veterinarian.

PREVENTION AND CONTROL

1. Have feed and/or water supplies analyzed for nitrates when conditions favorable for high nitrate content are suspected.
2. Consult your physician, veterinarian or county extension director if you suspect that nitrates may be a problem on your farm.
3. If toxic amounts of nitrates are found in the water supply, (1) obtain water from another source, or (2) reconstruct the well and remove the nitrate source to prevent further contamination. Nitrates can be removed from water by ion exchange, but the process is expensive.
4. Watch for signs of fuming silos, and protect humans and livestock from toxic gases. Always turn on the blower, leaving the distributor pipe close to the silage level, for at least 30 minutes before entering a silo. Even then, do not enter the silo if you have noticed an irritating odor in the area. Fence off the silo to prevent children and animals from entering the area.
5. Test forages that may be suspected of containing excess nitrate before they are fed or put in a silo. If a high nitrate content is detected, wait until the nitrate content decreases to a safe level before grazing or harvesting the forage.
6. *Preserved forages with sublethal nitrate contents* may be mixed with other low-nitrate feeds, fed with high-energy diets and fortified with vitamin A to alleviate some of the unfavorable effects.

LIMITATIONS

A. Nitrates in forages and water

1. Forages may contain toxic amounts of nitrates under certain conditions.
2. Some weeds may contain toxic amounts of nitrates under certain conditions when other plants do not.
3. Herbicides may affect plant growth and thus increase their nitrate content.
4. Fermentation in a silo reduces nitrate but does not destroy all the nitrate present.
5. Water may contain toxic amounts of nitrate, but the amount that is toxic varies under existing conditions.

B. Nitrate toxicity

1. Nitrates are potentially more toxic in all-roughage rations.
2. Forages containing nitrate are not so toxic if the forages are consumed slowly.

3. No single level of nitrate is toxic under all conditions.

4. There is a wide variation in the degree of tolerance to nitrates among the animal species.

5. 40 to 50 grams of nitrate per 100 pounds of animal can be a toxic dose.

6. Tolerances to nitrate are not established by continuous feeding of small amounts.

7. Abortions have occurred in cattle when levels of 0.3 percent nitrate were fed.

8. Low levels of nitrate may affect feed utilization without any signs of toxicity.

9. Carbohydrates hasten the conversion of nitrate (NO_3^-) to nitrite (NO_2^-) and the reduction of nitrite (NO_2^-) to ammonia in ruminant animals.

C. Tests for nitrates

1. Accurate tests for nitrate are not simple or standardized.

The Nitrate Problem

by J. J. Hanway, J. B. Herrick, T. L. Willrich, P. C. Bennett and J. T. McCall

The condition we now know as nitrate poisoning is not new. It is an old problem that has been recognized by livestock producers and the medical profession for at least 100 years. However, the danger of nitrate poisoning has become more important in recent years. Also, an increased awareness of the problem has resulted from information appearing in scientific and popular literature. Greater use of commercial nitrogen fertilizers, increased production and use of nitrate-accumulating feeds, improved feeding practices, improper sewage disposal, water contaminated by animal wastes, and the existence of diseases with similar symptoms have all added to the complexity of the problem.

Although the condition is commonly referred to as nitrate poisoning, toxicity is primarily due to nitrite, a chemically reduced form of nitrate. Nitrites may be formed from nitrates before ingestion by animals or within the digestive tract of the animal after nitrate is consumed. The nitrite, and to some extent the nitrate, is absorbed in the blood of the animal where it is combined with the hemoglobin, making it impossible for the hemoglobin to carry oxygen. If enough hemoglobin is tied up, the animal dies of suffocation.

Small amounts of nitrates are found in most plants and ground water. Under some conditions nitrates accumulate in these materials to toxic levels. Nitrates in well water have resulted in sickness and even death of animals and humans. Most of the early investigations indicated that nitrate toxicity in livestock was due largely to the ingestion of oat hay or cornstalks containing nitrates. However, corn silage, sorgo silage, grass and alfalfa hay and pasture, certain weeds and contaminated water have now been implicated in nitrate poisoning of animals.

Poisonous gases produced in a silo by the reduction of nitrates present in the ensiled material can cause sickness or death of animals and people in or near the silo. During the first few weeks after forages are put in a silo, nitrates in the forages are reduced to nitrogen oxides and escape as gases that are very toxic. These gases may be trapped in the silage and released later when the silo is opened.

In view of the complexity of the problem, the following information is presented to create a better understanding of the situation.

NITRATE TOXICITY

LIVESTOCK

Hazards

Nitrate hazards with livestock may be associated with (1) forages being grazed or fed, (2) water or (3) poisonous gases produced in a silo.

Nitrate and nitrite are known to have injurious effects when excess amounts are ingested by animals, but there is considerable confusion and uncertainty about the nature and extent of those effects. Because of the difficulty in getting complete and accurate information, the large number of factors which influence the toxic effect in various species and even within the same species, the confusion and uncertainty are understandable. No single level of nitrate in feeds or water is toxic under all conditions. Types of rations and speed of intake, as well as the level of nitrate in the feed and/or water, all have a definite bearing on toxicity.

Death of animals resulting from ingestion of lethal amounts of nitrates is most often encountered with animals grazing, or fed, forages containing high concentrations of nitrates. However, of equal or greater economic importance is the problem associated with sublethal amounts of nitrate which do not cause death but do affect the performance of the animals. Subclinical

intakes of nitrates are often associated with the water supply rather than with the forages being fed.

Nitrites are much more important toxicologically than are nitrates. However, it is important to realize that nitrates may be converted to nitrites by bacterial action in the digestive tract of an animal. Nitrates, as such, will cause gastroenteritis and diarrhea when ingested in sufficient quantity, but are much more important as material that can be converted to nitrites. The conversion can be carried on by bacteria in the rumen, or paunch, of ruminants, such as cattle, and also occurs to some extent in the digestive tracts of monogastric animals, such as swine. Microorganisms in the rumen also convert nitrites to the ammonium form and subsequently to amino acids and proteins. When this conversion is sufficiently rapid, ingested nitrates may have little apparent effect on the ruminant animal.

A time lapse of at least 5 to 10 hours is required before clinical symptoms can be observed following conversion of nitrate to nitrite in the digestive tract. Nitrites, and to some extent nitrates, are absorbed in the blood of the animal. Nitrite has two properties which are important in causing pathological changes: First, it has an affinity for hemoglobin of the blood. Absorption of nitrite by the hemoglobin results in oxyhemo-

globin being changed to methemoglobin, which does not release oxygen to the cells of the body tissues, and thus results in anoxia. Secondly, nitrite acts as a vasodilator, causing a peripheral circulatory stagnation which adds to the anoxia.

Swine are highly susceptible to nitrite poisoning but are not as often affected as cattle and sheep. The different type of digestive tract, the environmental factors affecting the bacterial inhabitants of their digestive tract, and the smaller amount of roughage consumed by swine apparently make pigs poor converters of nitrate to nitrite and less likely to ingest toxic amounts of nitrates. When nitrites occur in water or plant material consumed by swine, the swine are quite susceptible to the nitrite ion.

Sheep are relatively less affected by nitrite poisoning than cattle, either because of the ability of cattle to serve as better converters or because of the ability of sheep to permit a further breakdown of nitrite to ammonia. Also, in sheep a diet that contains readily fermentable carbohydrates results in decreased nitrite production in the rumen.

Diagnosis

One of the greatest hazards associated with nitrate and nitrite is the problem of arriving at an accurate and basic diagnosis. Many conditions have been popularly associated with nitrate poisoning. Such clinical symptoms as abortion, weakness and poor viability of recently born young, infertility and difficulty of conception, lameness, stiffness, unthriftiness, diarrhea, lower general resistance, anoxia, avitaminosis A, bloat and decreased milk production have been thought to be caused by nitrate and/or nitrite. Many of these conditions are extremely difficult to tie directly to these chemical entities; it is also quite difficult to tie them to any other causative agent.

Research has developed incomplete information on several factors associated with the variable amounts of nitrite which get into the circulatory system and apparently result in a wide range of observable effects. But the incompleteness of the information, the number of factors involved and the natural variations which influence the factors make the task of clinical interpretation and diagnosis very hazardous.

Clinical evidence of methemoglobinemia is revealed in increased heart rate, rapid respiration, dyspnea, salivation, weakness, a normal or subnormal temperature and progressive cyanosis. Fatal cases of nitrite poisoning can be compared to asphyxiation. The critical level of the methemoglobinemia varies with different species, age and various levels of general health. This makes it almost impossible to pinpoint any single degree of methemoglobinemia and say that more will be fatal or less will be nonfatal. The potentially fatal level of methemoglobin in the blood probably ranges between 50 and 90 percent. Symptoms can usually be detected

when about 30 percent of the hemoglobin has been converted to methemoglobin.

Anoxia can result in the development of a number of symptoms and conditions, and their apparent severity will depend upon the degree of anoxia. Symptoms other than those referable to the respiratory system include diarrhea, frequent urination, decreased appetite, listlessness, erythrocytosis and a bluish or slightly muddy discoloration of the mucous membranes. Since any or all of these abnormalities can and do occur in conditions other than nitrite poisoning, the problem of interpretation and diagnosis is quite evident.

In fatal cases of nitrite poisoning the gross lesions that can be found at autopsy are neither characteristic nor impressive. They consist of a generalized congestion, some hemorrhages of the heart, and perhaps a few hemorrhages of the lungs and trachea. Theoretically, the blood should have a brownish color, lacking the bright color of normal blood, but this lesion should be evaluated with considerable caution.

Caution should always be exercised in making a diagnosis of nitrate or nitrite poisoning. Both symptoms and lesions are nonspecific and are often rather vague and indistinct. The most important single factor in arriving at a clinical diagnosis should be rather definite and reliable information that a sufficiently high level of nitrate or nitrite has been ingested during a period of time prior to development of symptoms and lesions. This means analysis of feed and water supplies for nitrate content. Information of this type, applicable to the period of time involved, is seldom available at the time it is needed for diagnostic purposes.

Experimental data indicate that, for forage, a level of 0.3 percent nitrate¹ on a dry weight basis can be considered a maximum safe amount if the ration is otherwise adequate. Economic losses, and eventually mortality, occur above this 0.3 percent level.

Other experimental work with dairy cattle indicated that the M.L.D. 50 (minimum lethal dose in 50 percent of the cases) of nitrate was approximately 45 grams per 100 pounds of body weight. The nitrate content of water is usually reported on the basis of parts per million. If the water analysis report shows 45 parts per million, 1 million grams of water would have to be consumed for an intake of 45 grams of nitrate. One million grams of water equals approximately 280 gallons. The M.L.D. 50 for a 500-pound animal would be 225 grams of nitrate, which means the animal would have to drink 280 gallons of water containing 225 parts per million of nitrate to secure a dangerously toxic dose of nitrate. For purposes of illustration, if we assume that this 500-pound animal drinks 10 gallons per day of the 225 parts per million water, and that there is no nitrate in any of the other food intake, it will take about a month to obtain the dangerously toxic amount of nitrate.

¹See page 13 for different methods of expressing nitrate contents of feeds.

Actual circumstances in the field are never as simple and uncomplicated as examples. Lesser amounts of water-borne nitrates would have less dangerous effects but would still be of economic significance; other sources of nitrate would be ingested; and the nitrite ion is easily absorbed into the blood stream but not easily discarded. The life span of bovine erythrocytes ranges from about 50 to 60 days. During the month that the 500-pound animal is taking in 45 grams of nitrate, roughly one-half of the total volume of erythrocytes will be destroyed and replaced with new cells. But the conversion of nitrate to nitrite and the absorption of nitrite ions into the blood stream continues during this time, and the oxyhemoglobin of the new cells is quickly changed to methemoglobin. The example assumes that 100 percent of the nitrite is taken into the blood stream. Actually, under field conditions these 100 percent stages are infrequent.

With available information, it is rather impractical to set any single figure of nitrate parts per million in the water supply that can be considered unsafe. The only practical approach seems to be to arbitrarily set some reasonable figure by giving consideration to the actual field circumstances and factors associated with nitrite toxemia. Like the range of critical level of methemoglobinemia, the range of arbitrarily unsafe nitrate in the water supply probably lies between 50 and 100 parts per million.

Treatment and Control

Cases of nitrite poisoning that can be classified as acute will probably be too far advanced for treatment to give satisfactory results. If the less acute cases can be adequately recognized, quite satisfactory results can be expected from intravenous administration of methylene blue. Various dosage rates may be found in the literature, beginning at 1 mg. per pound of body weight as a 1 percent aqueous solution, but with 4 mg. per pound of body weight as a 4 percent aqueous solution as the most frequently recommended rate. Administration of methylene blue is only part of the necessary treatment, however, since the source of the nitrate or nitrite must be located and either eliminated or counteracted.

Elimination of the nitrate and/or nitrite source is largely a matter of substitution. In some instances a change to low-nitrate-nitrite forage and/or water supply is easily accomplished. In other instances when the water supply is the chief source of the nitrate and no other supply is readily available, a decision of economic significance must be made. In such cases the relative costs of the livestock losses, a deionizing system, a different and safe supply of water, or efforts to eliminate the nitrate contamination of the unsafe supply, will determine the course of action to be followed.

Some of the observations developed during the course of investigations of nitrite poisoning in various species of animals can be used to modify, minimize or control

the toxic effects. The type of ration influences the conversion of nitrate to nitrite since the conversion process is favored by poor, inadequate and all-roughage rations. Feeding high-energy feeds increases the animals' tolerance to nitrates. The hydrogen ion concentration of digestive tract contents influences the conversion. A pH of 7 to 8 is optimum for the conversion, and a pH of 6 or below decreases the rate of conversion. Temperature is also a factor, with the conversion increasing with increases of temperature up to 104° and 105°F.

The rate of consumption of food and water of relatively high nitrate content influences the toxic effect, since a rapid rate favors production and absorption of nitrite. Some of the new, easier methods of preserving a crop, such as silage, green chop or hay, have tended to increase the nitrate danger. Green pastures that may be grazed without difficulty may be toxic when fed as hay, green chop or silage, since these practices make it possible for the animal to consume a large quantity of forage in a shorter period of time than would be possible grazing.

Seasonal and weather conditions have considerable influence on the nitrate content of both forage and water supplies. The nitrate content of forages growing in the field may fluctuate between safe and toxic levels within the period of 2 or 3 days as weather conditions vary. Seasonal and weather factors, together with poor management, may also influence the rate of feed and water intake by animals and the subsequent nitrate toxic effects.

HUMANS

Hazards

The nitrate hazard to human health is usually associated with nitrates in the water supply or with poisonous gases produced in silos.

Infants not yet consuming solid foods are particularly subject to nitrate cyanosis or methemoglobinemia (cause of blue babies) resulting from nitrates in the water supply. This disease reduces the ability of the blood to absorb oxygen and may be fatal unless properly treated.

Water containing more than 45 parts per million (ppm) of nitrate is UNSAFE for infant feeding, including formula, regardless of the bacterial quality of the water. Bacterially impure water may be sterilized by boiling. However, boiling and the resulting evaporation concentrates the nitrate in the remaining water and thereby increases the possibility of cyanosis. Water for infants should always be obtained from a source which has tested safe according to both bacterial and nitrate limitations.

Nitrate poisoning appears to be confined primarily to infants. Adults drinking the same water may not be affected, but breast-fed infants of mothers drinking high-nitrate water may be poisoned. Also, cows drink-

ing high-nitrate water may produce milk containing sufficient nitrate to cause infant poisoning.

Humans over 1 year of age may occasionally be affected by nitrate cyanosis. An increasing amount of evidence indicates that consumption of high-nitrate water can produce adverse intestinal pathological conditions resulting in chemical diarrhea similar to that caused by consuming excess sulfates (Epsom salts). Also, consumption of high-nitrate water can have a diuretic effect.

Since the exact tolerance of adults to the consump-

tion of nitrate without undesirable pathological disturbances is not well defined, the conservative course is *not* to use water containing more than 45 ppm of nitrate.

A high nitrate test in the water, particularly when accompanied by a high coliform bacteria test result, also indicates possible water contamination with disease-causing bacteria and viruses.

Diagnosis and Treatment

Consult your doctor.

SOURCES OF NITRATES AND NITRITES

NITRATES IN SOILS

Nitrates found in feeds and water result primarily from nitrates produced in or added to the soil. Most of the nitrate in soils and ground water results from the decomposition of organic nitrogenous materials, such as proteins, in plant and animal materials that are added or returned to the soil. Microorganisms in the soil decompose these organic materials, releasing the nitrogen as ammonium (NH_4^+). In the presence of air, other microorganisms then oxidize this ammonium nitrogen to nitrate (NO_3^-). Nitrates, or other forms of nitrogen which may be readily converted to nitrates, are also added to the soils as commercial fertilizers, and in some locations this may be a primary source of nitrate in the soil and ground water. Other sources, such as rainfall, seldom contribute appreciable amounts of nitrates. Nitrites are rarely found in soils in any significant amounts.

NITRATES IN FERTILIZERS

Commercial nitrogen fertilizers contain nitrogen as nitrate (NO_3^-), ammonia (NH_3) or ammonium (NH_4^+), or urea ($\text{CO}(\text{NH}_2)_2$). Dry fertilizers that contain nitrate, as such, include ammonium nitrate, sodium nitrate, calcium nitrate, potassium nitrate and mixed fertilizers that may contain these compounds in varying amounts. Some fertilizer solutions may contain nitrates as well as the other forms of nitrogen.

The urea form of nitrogen, when applied to soils, is quickly converted to the ammonium form by enzymatic action. The ammonium form of nitrogen is converted to the nitrate form by microorganisms in the soil.

NITRATES IN PLANTS AND FEEDS

Nitrates in the soil serve as the primary source of the nitrogen required for growth by nonleguminous plants such as corn, cereals, grasses, etc. Bacteria in the nodules on the roots of legumes (alfalfa, clover, beans, etc.) fix nitrogen (N_2) from the air into forms the plants can use, so legumes are not dependent on

nitrates in the soil for their nitrogen supply. However, legumes do use any nitrates present in the soil, and as the amount of nitrate found in the soil increases, the amount of nitrogen fixed by the nodule bacteria associated with the legume decreases. In both legumes and nonlegumes, the nitrates found in the plant have been taken up through the roots from the soil. Nitrites (NO_2^-) are rarely found in plants in appreciable amounts except under very unusual conditions such as severe disease infections.

The nitrates taken up by plants are normally reduced and incorporated into the amino acids, proteins, etc. Since in most plants the primary site of nitrate reduction is in the green leaves, the highest concentrations of nitrates are normally found in the stems and other conductive tissues. Nitrates accumulate in plants only when (1) there is a large amount of nitrate present in the soil or (2) some factor interferes with the normal processes in the plant. The amount of nitrate found in a plant at any given time will depend on the following factors:

1. *The nitrate content of a soil in the rooting zone of the plants.* The nitrate content of a soil depends on several factors and interactions between these factors:
 - a. *Temperature*—The decomposition of organic matter and the conversion of ammonium-nitrogen to nitrate-nitrogen is a microbiological process, and therefore dependent upon temperature. These processes are very slow at temperatures below 50° F., but rates of conversion increase in direct proportion to temperature up to soil temperatures of 80° to 90° F.
 - b. *Moisture*—Microorganisms require moisture, so decomposition of organic matter and production of nitrates will occur only when the soil is moist. In most soils, a large proportion of the organic matter is in the surface layer of soil. This layer may become too dry for microbiological activity even though plants growing on the soil may be obtaining adequate moisture from the subsoil and therefore show no signs of a moisture deficiency.

However, rapid microbial activity and nitrate production commonly occur for a short period after a dry soil has been remoistened, so cycles of drying and rewetting may result in increased nitrate production in the soil.

Nitrates in soils are water soluble and may be leached downward as water moves through the soil. Such leaching (and downward movement of nitrate) must occur when the soil is not frozen and is most likely to occur: (1) during periods of heavy rainfall (or irrigation) or on soils that receive runoff from other areas, (2) on sandy soils or (3) on soils where no vegetation is growing to remove moisture from the soil. Nitrates may also move upward in the soil during dry periods as moisture rises and evaporates at the soil surface.

c. *Aeration.* The soil microorganisms that convert ammonium to nitrate nitrogen require oxygen. Therefore, ammonium-nitrogen resulting from organic matter decomposition may accumulate in waterlogged or heavily compacted soils where no oxygen is present. Furthermore, nitrates present when a soil becomes waterlogged, or oxygen becomes limiting, will be denitrified and lost. Nitrates will therefore not be found in soils under conditions of poor aeration if the temperature and moisture conditions are favorable for microbial activity.

d. *The kind and amount of soil organic matter.* Since much of the nitrate in soil results from decomposition of soil organic matter, the amount of nitrate produced depends upon the kind and amount of soil organic matter present.

e. *Kind and amount of organic residues and manure added to the soil.* Nitrate levels in the soil may be increased or decreased by additions of manure and/or other organic residues. In the decomposition of organic material with a high nitrogen content (low carbon/nitrogen ratio) such as legumes, animal manure, etc., nitrates will be released; but in the decomposition of organic matter with a low nitrogen content (high carbon/nitrogen ratio) such as small grain straw, cornstalks and cobs, etc., nitrates in the soil will be utilized by the microorganisms, and the nitrate level of the soil will decrease. Thus, nitrate production in a soil will be much higher following a legume crop than following a nonlegume and generally will be higher following applications of barnyard manure.

f. *Applications of commercial nitrogen fertilizers.* The effect of added fertilizer nitrogen upon the nitrate level in the soil and the plants will depend on the amount of nitrogen applied and the time and method of application. Applications of large amounts of nitrogen fertilizer to obtain maximum

yields will normally result in an increase in the crude protein content of the plants. As the rate of nitrogen applied increases, there will also be an increase in the nonprotein nitrogen, including nitrates, in the plants. Very high crude protein content of nonleguminous plants generally suggests some accumulation of nitrate.

g. *Plant removal of nitrate from the soil.* Growing plants keep the nitrate levels in most Iowa soils at relatively low levels throughout most of the growing season. Nitrates are most likely to accumulate in the soil during the portion of the season when plants are not growing or in areas such as feedlots, where no plants are allowed to grow.

2. *The plant species being grown and its stage of development*

All plants take up nitrates from the soil, but under any given set of conditions, the amount of nitrate accumulated in the plant will depend on the species being grown and its stage of development. And the amount of nitrate present varies markedly between different parts of the plant.

The nitrates taken up by the plant are normally reduced to other forms by enzymes, such as nitrate reductase, that are produced in green leaves exposed to light. Therefore the nitrate content of green leaves is usually low. However, nitrates may accumulate (1) in the stems and other conductive tissues of the plant through which nitrates are normally moved from the roots to the leaves, and (2) in immature leaves, etc., not yet exposed to sunlight. Grain and fruit seldom contain appreciable amounts of nitrate.

Nitrates will accumulate in the vegetative parts of plants when the plants are shaded, when the nitrate supply to the plants exceeds that which can be used for increased growth, or when some factor other than nitrogen availability limits photosynthesis and growth by the plants.

At normal levels of nitrate in the soil, the nitrate content of plants is usually higher in young plants and decreases as the plants mature. However, at high levels of soil nitrate there may be no decrease, or even an increase, in the nitrate content of the plants as they develop.

An increase in nitrate content of a plant is usually accompanied by (1) a decrease in water-soluble carbohydrate content of the plant and (2) an increase in potassium (or sodium) content of the plant.

Plant species differ in: (1) the season in which they grow, (2) their rooting habits (the kind, extent and activity of their roots), (3) the ratio of stems to leaves at different stages of growth, and (4) many other characteristics. Therefore, at any given time one plant species may contain much larger amounts of nitrate than another. Weeds often contain very high concentrations of nitrate. Oat hay and hay of

some of the other small grains sometimes have very high nitrate contents. Under some conditions grasses, corn, millet and sorghums accumulate excessive amounts of nitrates, but are less likely to do so than the other plants mentioned previously.

3. *Environmental conditions*

Environmental conditions may influence the rate of nitrate production in the soil, the ability of plants to absorb nitrates from the soil, or the ability of plants to reduce the nitrates to other forms in the plant.

High nitrate contents in plants are often associated with drouth conditions, since a lack of moisture interferes with normal plant growth and may result in nitrate accumulation in the plant. However, not all drouth conditions lead to high nitrate contents in plants. Nitrate production from organic matter in the soil is dependent upon having moisture present. Because of this, dry surface soil conditions often result in a nitrogen deficiency, not an excess, in plants. Also, plant roots cannot absorb nitrates from a dry soil. So some moisture in the soil is essential for plant uptake of nitrates. Nitrate accumulation in plants, then, is dependent on having both nitrates and moisture present in the same place in the soil where the plant roots can absorb them.

Nitrate accumulation in plants is most likely to occur during a period of hot, dry weather when transpiration (water loss) from the plant is very rapid and the supply of soil moisture is inadequate to meet this high demand. Under these conditions, the plants wilt and stop growth, and nitrates accumulate in the plants.

Light is essential for growth of plants and for reduction of nitrates in the plants. Thus plants growing under trees or other vegetation that shade them, or crop plants, such as corn, at high plant populations may contain high concentrations of nitrates. Nitrate contents of plants may be higher in the morning than in the evening and may also be higher on cloudy days than on sunny days.

Any factor that restricts the normal growth and development of a plant, such as drouth, hail, frost or disease, may, if nitrates are available in the soil, result in increased nitrate contents of the plants. Deficiencies of nutrients other than nitrogen will in some, but not all, cases result in increased nitrate contents in the plants.

4. *Herbicidal applications*

Spraying with herbicides such as 2,4-D may result in higher nitrate contents of plants. However, spraying pastures with herbicides to kill weeds that tend to accumulate large amounts of nitrate can reduce the nitrate hazard to livestock since the grasses that are left are less likely to contain toxic amounts of nitrates.

5. *Effect of method of harvesting and storing plant material*

Enzymatic reduction of nitrates in cut forages before they are dry may reduce the nitrate content of the forages. And rain falling on partially or completely dry forage may leach away some of the nitrate. However, since much of the nitrate is usually in the stems of the plants, such leaching is probably seldom of much significance unless the leaching is severe. There will be little or no change with time in the nitrate content of dry forages during storage.

When forages are placed in a silo, anaerobic fermentation results in the reduction of some, but not all, of the nitrates in the plant material to nitrogen oxides. These nitrogen oxides can sometimes be seen as reddish brown or yellow gases rising from the surface of silage within a day or two after forage is placed in the silo. This loss of nitrogen gases may continue for a few days. These gases are extremely toxic and can kill humans or animals. If the silo is covered, these gases accumulate in the top layer of silage and may result in extremely high nitrate contents in this layer. The gases may also be released when the silo is opened. The nitrates in the silage are water soluble and may be present in high concentrations in the liquid that drains from the bottom of the silage.

NITRATES IN WATER SUPPLIES

Sources and Movement of Nitrates in Water

Sources of nitrates include animal excreta, crop residues, human wastes, some industrial wastes or any decomposing animal or plant tissue. Nitrogen fertilizers, naturally occurring deposits of nitrate-containing materials, nitrogen fixed by soil bacteria and silage juices are additional sources of nitrates.

Nitrates and nitrites are water soluble. They move with the water. Any nitrate added to or produced within the soil may be leached or washed away by moving water—either by surface runoff or ground water percolation.

Nitrates in solution move downward through soil, sand layers and porous rock formations toward the water table. The nitrates may then appear in tile drainage water or ground water.

Nitrates are more concentrated below or near the area of waste accumulation or disposal such as manure piles, feedlots, septic tank disposal fields, cesspools, privies, etc.

Excess nitrates are also more apt to be found in ground water under low areas and waterways that collect or convey runoff from higher ground.

Water percolating through decomposing peat soils or through mineral soils which have received a heavy

application of nitrogen fertilizer or manure will move nitrates to the ground water table.

Nitrates leached by percolating water tend to accumulate in the upper portion of the ground water table in the absence of rapid ground water movement. They remain there an indefinite period of time. Nitrate concentration is reduced through dilution by ground water containing little or no nitrate and by ground water movement.

Nitrates move with the ground water. Ground water pumped from a well may contain excess nitrates even though the decomposing organic waste or other nitrogen source may be located a considerable distance away from the well. If the ground water moves rapidly through a porous sand or creviced rock formation with little opportunity for dilution to occur, a well several miles from the nitrogen source may contain high-nitrate water.

Leaching occurs only when the ground is not frozen and when excess rainfall, snow melt or irrigation water causes surface runoff and percolation below the plant rooting zone. Consequently, the nitrate concentration in a water supply will vary according to the season of the year and the amount of excess water.

Occurrence of Nitrates in Pondered Water

Ponds, road ditches and other surface depressions which collect runoff from feedlots and heavily fertilized fields, silo drainage, septic tank discharge, etc., sometimes contain high-nitrate water. Ponds and depressions with few or no green plants growing in them may contain a significant amount of nitrates. However, excess nitrates will not be found in pondered water which contains abundant algae and other plant growth. Apparently the plant growth uses the excess nitrates about as rapidly as they enter the pond with runoff water.

Nitrates may also be lost by denitrification in the anaerobic decomposition of organic matter in the pondered water.

Occurrence of Nitrates in Well Water

Water samples from shallow, dug, bored and driven wells more frequently contain excess nitrates than water from deeper, drilled wells. Shallow wells generally are inadequately constructed to prevent the direct entrance of polluted surface water and subsurface water. Seldom do they extend deeper than the shallowest water-bearing stratum or even the water table, the ground water source most easily polluted by leached nitrates. Shallow wells are also frequently located beside a waterway or other surface depression, which increases the hazard of nitrate pollution.

A drilled well, however, does not assure a nitrate-free water. Many drilled wells are improperly located

and constructed to prevent the entrance of polluted surface and ground water. Even a relatively deep water-bearing stratum may be polluted. Pollutants enter deep strata through old wells which have been abandoned but not filled with concrete, through pumped wells with rusted and perforated casings, through old wells which are wrongly used for sewage and waste disposal, and through drainage wells, natural sinkholes and river valleys.

Very little is known of the variation in nitrate concentration in a well. Test reports indicate that the nitrate level may fluctuate widely. Nitrate levels are generally highest following wet periods and lowest, even down to zero nitrates, during dry periods of the year. Consequently a single sampling and test is of doubtful value. Testing during dry periods may cause a false sense of security. Preferably, a well should be tested immediately following a wet period.

Since babies are particularly susceptible to nitrate poisoning, a well should be tested before bringing a new infant into the home. Additional wet-season tests should be made subsequent to birth during the first 6 to 9 months of the infant's life.

A well supply should also be tested for bacterial purity and nitrate content whenever a change in color, odor or taste causes the continued purity of the water to be questioned. An annual test for water purity following a wet period in the spring is recommended as a routine practice.

Most well waters containing excess nitrates also contain bacterial pollutants. However, some nitrate-containing waters may show no evidence of bacterial pollution by animal or human wastes. Either the nitrates originate from sources other than animal or human wastes, or the nitrate-containing ground water has moved through an adequate distance of soil to filter out the bacteria. Since the nitrates are dissolved in the water, they are nonfilterable.

Occurrence of Nitrites in Water Supplies

Nitrites, a more toxic form of nitrogen oxide than nitrates, are less commonly found in water supplies than nitrates. However, nitrites in toxic concentrations have been found in the following circumstances: (1) Close proximity of a shallow well to a place of waste disposal (the nearer to the source of pollution, the greater the probability of a high nitrite concentration in relation to the nitrate concentration). (2) Small pond collecting silo drainage (nitrites may be concentrated in silage juices). (3) In a shallow well polluted by surface water (under certain conditions, bacteria may convert nitrates to nitrites). (4) In galvanized tanks or waterers used for water storage (during warm weather, zinc may serve as the reducing agent to convert nitrates to nitrites).

3. Limited grazing and manure application on the watershed area if the pond water is used for livestock watering only—none if the water is used in the home.

Nitrate Removal From Water Supplies

Nitrates in water are not removed by filters, ordinary water softeners or by any added water softening compound. Nitrates are not destroyed by standing, and heating the water merely concentrates the nitrate through the evaporation that occurs.

However, nitrates can be removed by passing the water through two tanks filled with ion exchange resins. The first tank is often a conventional water softener which removes certain positively charged ions (cations) such as calcium and magnesium. The second tank contains an entirely different ion exchange resin which removes negatively charged ions (anions) such as sulfates, nitrates and chlorides.

The cation exchanger always precedes the anion exchanger. Both tanks must be regenerated and backwashed periodically.

The type and cost of regenerant used and the equipment size and cost depend on the quality and quantity of water to be treated. Some regenerants are more corrosive and hazardous to handle, as well as being more expensive, than others. Limited experience with this equipment (only three installations in Iowa, the

first one made in July 1962) indicates that the cost may vary from about \$1 per 1,000 gallons for water of low mineral content to \$5 or more per 1,000 gallons for water of average to high mineral content.

Removal by ion exchange is one alternative for correcting the nitrate problem. The cost and inconvenience of operating nitrate-removal equipment, as well as the hazard resulting from possible equipment failure, must be weighed against the costs, inconveniences and hazards of other alternatives: elimination of the nitrate source, well reconstruction or well abandonment and replacement by a safe supply.

TOXIC GASES PRODUCED IN SILOS

1. Fence off the silo to prevent children from entering the area adjoining the silo.
2. Post a caution sign on the silo when forage is being ensiled and leave it up for about 2 weeks afterward.
3. Do not work in the silo, climb a ladder into the silo or go near it alone if you notice an irritating odor in the area within 3 to 4 weeks after the silo is filled.
4. Leave the distributor pipe in the silo, close to the silage level, to draw off gases.
5. Turn on the blower for at least 30 minutes before entering the silo any time from the time ensiling starts until 10 days after the silo is filled.

METHODS OF EXPRESSING NITRATE CONTENTS OF FEEDS AND WATER

In this publication nitrate contents are expressed on the nitrate (NO₃⁻) basis.

The nitrate contents of feeds and water have been reported in several different ways. They may be reported as nitrate-nitrogen (NO₃⁻-N), nitrate (NO₃⁻), potassium nitrate equivalent (KNO₃) or sodium nitrate equivalent (NaNO₃). This has led to considerable confusion and makes it necessary that one understand which designation is being used for each particular set of data. The most common methods of designating nitrate contents in feeds and water are listed in table 1 together with factors for converting from one method of designation to another. The nitrite forms (NO₂⁻) are also listed in table 1 for purposes of comparison. The *nitrite* form is much more toxic than the *nitrate* form. However, since *nitrate* is the form most commonly found in feeds and water and because it is easier to determine, nitrate serves as the basic reference substance.

In feeds the nitrate content is usually expressed as a percentage (parts per one hundred) of the dry weight of the feed. In water the nitrate content is usually expressed as ppm (parts per million) on a weight basis, i.e. grams of nitrate per million grams of water, or as mg/l (milligrams per liter) or a weight per volume basis. One mg/l is equal to 1 ppm. The conversion

Table 1. Different methods of expressing nitrate and nitrite contents of feeds and water, the molecular or ionic weights of the different substances, and factors for converting from one designation to another.

Method of expressing the form of nitrogen	Chemical formula or designation	Atomic molecular or ionic weight	Factors for converting from one designation to another (relative weights)			
			N=1 ^a	NO ₂ ⁻ =1	NO ₃ ⁻ =1	KNO ₃ =1
Nitrate-nitrogen	NO ₃ ⁻ -N	14	1.0	0.30	0.23	0.14
Nitrite-nitrogen	NO ₂ ⁻ -N	14	1.0	0.30	0.23	0.14
Nitrite	NO ₂ ⁻	46	3.3	1.00	0.74	0.46
Nitrate	NO ₃ ⁻	62	4.4	1.34	1.00	0.61
Sodium nitrate	NaNO ₃	85	6.1	1.85	1.37	0.84
Potassium nitrate	KNO ₃	101	7.2	2.20	1.63	1.00

^a either NO₃⁻-N or NO₂⁻-N.

Examples:

Percent

- 1) 1.0% NO₃⁻-N equals 4.4% NO₃⁻ or 7.2% KNO₃.
- 2) 1.0% NO₃⁻ equals 0.23% NO₃⁻-N or 1.63% KNO₃.
- 3) 1% KNO₃ equals 0.61% NO₃⁻ or 0.14% NO₃⁻-N.

ppm

- 1) 1 ppm NO₃⁻-N equals 4.4 ppm NO₃⁻ or 7.2 ppm KNO₃.
- 2) 1 ppm NO₃⁻ equals 0.23 ppm NO₃⁻-N or 1.63 ppm KNO₃.
- 3) 1 ppm KNO₃ equals 0.61 ppm NO₃⁻ or 0.14 ppm NO₃⁻-N.

factors in table 1 serve for either percentages, ppm, or mg/l in changing from one compound to another as the basis of expressing the results. To change percentages to ppm multiply by 10,000. One percent is equal to 10,000 ppm.

SAMPLING AND TESTING FOR NITRATES AND NITRITES

WATER

Sampling

The water sample to be submitted for analysis should be representative of the water in the well or pond. Therefore, let the water run until all stored water in the pipes and pressure tank has been wasted before collecting a sample of "fresh" water.

Sending Samples for Analysis (Iowa)

A special water sample bottle and mailing container must be used. The bottle and container, plus a report form, may be obtained from: State Hygienic Laboratory, Water Division, Medical Laboratory Building, Iowa City, Iowa.

The completed form must be returned to the laboratory with the water sample.

Note: Detailed sampling instructions are listed in fig. 1 (right). A sample report form is shown in fig. 2.

Fig. 1. Water sampling instructions.

WATER SAMPLING INSTRUCTIONS FOR DRINKING WATER QUALITY

1. Flame sample tap with alcohol torch or other suitable flame.
2. Run water 2 - 3 minutes.
- *3. Uncap bottle without touching inside of cap or lip of bottle. Do not place the cap down while filling the bottle.
4. Fill bottle to within about 1/2 inch of top.
5. Recap and send to laboratory at once. Samples should arrive in Iowa City by Thursday of any week.
6. FILL OUT DATA CARD COMPLETELY AS DIRECTED

White capped bottles are to be used only for sampling supplies containing no chlorine. White capped bottles must be used if nitrate analysis is desired. Black capped bottles are available to sample those supplies subjected to CONTINUOUS CHLORINATION.

Fig. 2. Water sample report form.

STATE HYGIENIC LABORATORY—WATER ANALYSIS

Iowa City, Iowa

Please print with BLACK MEDIUM SOFT lead pencil only

Town County

Collector Date Collected

Sampling Point

Purpose of Sampling

	SENDER
Name	_____
Street	_____
City	_____, Iowa

PLEASE PRINT PLAINLY

Chlorine

Depth of Well

Type of Platform

FEE \$2.00 PER SAMPLE

PLANTS AND FEEDS

Sampling

The nitrate content of plants or feeds from different parts of a field, stack or silo often varies extremely.² Therefore, at any one sampling time it is desirable to obtain separate samples from different places in the field, stack or silo and analyze each sample separately; for plants growing in the field, samples should be collected and tested at different times.

Nitrates may be lost from moist plant or silage samples between the time the sample is collected and the time it is analyzed if the samples are not handled properly. Such losses may be due to enzymatic or microbiological reduction of nitrates in the moist sample or to volatilization of nitrogen gases or acids during drying. Therefore, either (1) the moist plant or feed

²Nitrate content of plants in a field vary between: (1) different plant species, (2) different stages of plant growth, (3) different parts of a plant, (4) different areas in a field, (5) different times of day, and (6) different days as climatic conditions vary. Nitrate contents of feeds in stacks and silos vary because of the above factors and because of differences in: (1) how the hay or silage is handled in the field (including loss of nitrate by leaching from rain) and (2) losses and movement of nitrates during fermentation in the silo.

samples should be frozen as soon as possible after they are collected and kept frozen until they are analyzed, or (2) plant samples (but not silage samples) may be dried immediately in an oven with adequate ventilation at 65°C. (150°F.).

Sending Samples for Analysis

To supply analysts with necessary information, a form similar to the one below should be filled out and sent with plant samples submitted. *Send samples to: Veterinary Diagnostic Laboratory, Iowa State University, Ames, Iowa.*

TESTS FOR NITRATES IN PLANT MATERIAL

1. Quick Field Tests

1.1 Alpha-naphthylamine powder test

1.2 Reagents

1.3 Nitrate test

- (a) 100 gm. BaSO₄ (dried at 110°C)
- (b) 10 gm. MnSO₄·H₂O
- (c) 2 gm. finely powdered zinc

Information for Nitrate Test of Plants and Plant Feeds

1. Date sample was secured _____
2. Name and address of owner _____
3. Veterinarian or extension service personnel forwarding the sample _____
4. Identification of sample (kind of plant, part of plant taken as sample) _____
5. If ensilage, approximate location(s) in stack or silo from which sample was taken _____
6. How long has it been since ensiling? _____
7. If from field, what was the approximate stage of maturity at the time sample was taken? _____
8. Amount, time of application, and kind of any fertilizer used on land prior to or during growth of the sample _____
9. General type of weather condition during the two weeks immediately prior to taking sample, or prior to harvesting for ensiling or curing (wet, dry, hot, cool, humid, etc.) _____
10. Is land on which sample was grown in the path of surface drainage from barnyard or feeding areas? _____
11. What crops were grown on this land during the previous two years, or if not cropped how was the land used? _____
12. Has any of the feed represented by the sample been consumed by any farm animals? _____ What species? _____
13. Has consumption of the feed been associated with any change in health or performance of the animals? _____
14. If answer 13 is "yes", what abnormalities, changes or symptoms have been observed? _____

ENSILAGE AND FRESH PLANT SAMPLES MUST BE FROZEN IMMEDIATELY AFTER THEY ARE TAKEN AND MUST REACH THE LABORATORY WHILE STILL FROZEN.

- (d) 75 gm. citric acid (powdered)
- (e) 4 gm. sulfanilic acid
- (f) 2 gm. alpha-naphthylamine

Grind coarse materials to a fine powder. Mix b, c, e and f separately with portions of the BaSO₄. Then mix all reagents together. (Be sure all equipment is free of nitrate and nitrite.) Store the powder in a thoroughly blackened bottle.

2.3 Nitrite test. If a test for nitrite in the presence of nitrate is desired, omit the powdered zinc and MnSO₄·H₂O.

2.2 Procedure—Split or break open succulent plant tissue. Apply a thin layer of the powder reagent to the freshly exposed surface. Squeeze the plant material until the plant juices wet the powder. Nitrates are indicated by a pink color.

2.1 Diphenylamine test

1.2 Reagents: Dissolve 1 gm. of diphenylamine in 100 ml. of concentrated sulfuric acid. (*This solution is very corrosive and must be handled with care.*)

2.2 Procedure—A drop of the reagent is placed on freshly exposed stem (or nodal) tissue. Development of a blue color indicates the presence of nitrate.

2. Quick Laboratory Tests

1.1 Extraction

1.2 Reagent—20 percent acetic acid. Dilute 2 liters of acetic acid to 10 liters with distilled H₂O.

2.2 Procedure—Shake 0.5 gm. of dry plant material in 100 ml. of hot water for 1 minute and let cool and settle. (5.0 grams of finely chopped, undried plant material may be used in place of the dried material.)³

2.1 Nitrate determination

1.2 Alpha-naphthylamine powder test

1.3 Reagents (as given under quick field tests)

2.3 Procedure—To 1 ml. of the plant extract add 9 ml. of the 20 percent acetic acid solution and 0.8 gm. of the powder reagent. Shake for 1 minute and allow to settle. Compare color with standards prepared in the same way or with permanent standards.

3.3 Permanent Standards—Prepare standards with acid fuchsin in 1 percent acetic acid, saturated with camphor, and calibrate with nitrate standards prepared in the regular way.

2.2 Diphenylamine test

1.3 Reagents: (As given under quick field tests)

2.3 Procedure—Pour 2 drops of the plant extract into a depression on a spot plate. Carefully add 3 to 4 drops of the diphenylamine reagent so it settles below the extract in the cup of the spot plate without mixing. Compare with standards prepared in the same way.

3.2 Interpretation—Color development similar to that with 5 ppm. NO₃⁻-N standard is equivalent to 0.1 percent NO₃⁻-N (0.44 percent NO₃⁻) in the dry plant material.

3. Quantitative Laboratory Tests

1.1 Alpha-naphthylamine method

1.2 Reagents

Reagent 1: (As given under quick field tests)

Reagent 2: Twenty percent acetic acid solution plus 0.2 ppm copper. Dilute 2 liters of glacial acetic acid to 10 liters with distilled water. Add 0.005 gm. of copper sulfate. Mix thoroughly.

2.2 Procedure—Extract 0.1 gm. of dry plant material (1 gm. of fresh plant material) with 20 ml. of hot water. To 1 ml. of plant extract add 9 ml. of reagent 2. Shake for exactly 15 seconds. Shake similarly after 3 minutes. After another 3 minutes shake again and then centrifuge for 3 minutes at 1000 x G. Pour the supernatant solution through a small, loose plug of borosilicate glass wool. Read color in colorimeter at a wave length of 520 mμ and compare with standard curve. Prepare the standard curve by using 5, 10, 20, 30 and 50 ppm standards. These are equivalent to 0.44, 0.88, 1.77, 2.65 and 4.40 percent NO₃⁻ in the dry plant material. (If nitrite is to be determined in the presence of nitrate, omit the zinc, manganese sulfate and cupric sulfate from the reagents.)

2.1 Devarda's alloy—Steam Distillation Method

1.2 Reagents

1N NaCl—Dissolve 58.5 gm. of dry NaCl in NH₃-free distilled water. Dilute to 1000 ml.

Devarda's alloy—granular Devarda's alloy is ground for 4 hours in a ball mill. Longer grinding should be avoided.

Indicator solution—Dissolve 0.013 gm. of bromcresol green and 0.0066 gm. of methyl red in 225 ml. of 90 percent ethanol.

Boric acid solution—Dissolve 20.0 gm. of reagent grade boric acid in 750 ml. of

³When undried plant material is used, the moisture content of the sample should be determined and the results should be expressed on a dry weight basis.

NH₃-free distilled water in a 1000 ml. volumetric flask. Add the 225 ml. of indicator solution to the boric acid solution and dilute to approximately 998 ml. with NH₃-free distilled water. Add 0.1 N NaOH carefully until 1 ml. of the boric acid solution changes from reddish purple to grayish green upon the addition of 1 ml. of distilled water. (This should require about 1 ml. of the NaOH solution.)

MgO—Ignite heavy MgO at 800°C. overnight.

0.00357 N H₂SO₄—Dilute 5 ml. of conc. H₂SO₄ to 100 ml. with distilled H₂O. Add 36 ml. of this diluted H₂SO₄ to approximately 18 l. of NH₃-free distilled water. Distill standard 10 and 20 ppm NO₃-N solutions (equivalent to 0.2 and 0.4 percent NO₃-N in the plant material into boric acid as described under procedure and titrate with the H₂SO₄ solution. Adjust the H₂SO₄ solution to the correct normality by adding NH₃-free distilled water.

2.2 Procedure—Add 100 ml. of hot 1 N NaCl to 0.5 gm. of finely ground, dried plant material (use 5.0 gm. of fresh plant material).⁴ Shake for 10 minutes and allow to settle and cool. Pipet 10 ml. of the plant extract into a 100 ml. micro Kjeldahl distillation flask. Add approximately 0.4 gm. of the ignited heavy MgO. Steam-distill the mixture for 2 minutes at a rate such that 20 ml. of distillate is collected in this time. The distillate may be collected in a 50 ml. Erlenmeyer flask containing 5 ml. of the boric acid solution. This preliminary distillation is to remove any NH₄-N that may be in the sample, and the NH₄-N content can be determined by titrating this distillate with standard H₂SO₄ if desired. Add 0.4 gm. of the finely ground Devarda's alloy to the mixture in the distillation flask and steam-distill for another 2-minute period. Collect the distillate in a 50 ml. Erlenmeyer flask containing 5 ml. of the boric acid solution. The nitrate-nitrogen content is determined by titrating the distillate with 0.00357 N H₂SO₄. (1 ml. is equivalent to 0.1 percent NO₃-N (0.44 percent NO₃⁻) in dry plant material.) (Appropriate corrections must be made for the moisture content of undried samples.) The color change of the indicator is from green to pink. A blank should be run by steam-distilling a mixture containing everything except the sample itself. Five ml. of the boric acid solution can safely hold 200 micrograms of NO₃-N. For greatest accuracy, the aliquot size should be adjusted so 100 to 200 micrograms of NO₃-N is being determined.

⁴See footnote 3 on page 16.

3.1 Phenoldisulfonic acid

1.2 Reagents

Extracting solution—Dissolve 3.5 grams of silver sulfate per liter of water.

Calcium sulfate—Wash with distilled water if necessary to remove nitrates. Dry at 60°C and grind to a fine powder.

Phosphate solution—Dissolve 138 grams of NaH₂PO₄·H₂O in 500 ml. of distilled water. Adjust to pH 6.5 with concentrated NaOH solution and make to 1000 ml.

Calcium carbonate (CaCO₃) suspension—Suspend 1 gram of powdered CaCO₃ in 200 ml. of distilled water.

Hydrogen peroxide (microchemical grade)—Must contain less than 9 ppm nitrate nitrogen, be low in acidity, and assay at least 28 percent H₂O₂.

Phenoldisulfonic acid—Dissolve 25 grams of pure white phenol in 150 ml. of conc. H₂SO₄ and add 75 ml. of fuming H₂SO₄. Mix well and heat at 95° to 100°C for 2 hours or more. Store in tightly stoppered, brown bottle.

Ammonium hydroxide—(1 + 1) Dilute concentrated ammonium hydroxide with an equal volume of distilled water.

Sequestering solution—Suspend 20 grams of Sequestrene AA [(ethylenedinitrilo tetraacetic acid)] in 50 ml. of distilled water. Add 1 + 1 ammonium hydroxide until the Sequestrene dissolves. Make to 1 liter with distilled water. Dilute 5 ml. of this solution to 1 liter with distilled water for use in diluting nitrated phenoldisulfonic acid prior to adding NH₄OH.

2.2 Procedure—Place 0.100 gram of dried, ground plant material in a centrifuge tube or other flask. Add 0.8 to 1.0 grams of calcium sulfate (used as a filter aid). Add 25 ml. of the silver sulfate solution, swirl, and add 1 ml. of the phosphate solution. Shake for 5 to 10 minutes. Centrifuge at 1000 × G for 10 minutes or filter through No. 12 Whatman filter paper.

Transfer an aliquot of the filtrate, containing 5 to 500 micrograms of nitrate nitrogen, to a 100 ml. beaker or evaporating dish. Add 2 ml. of CaCO₃ suspension. Reduce volume to 10 ml. by evaporation if necessary. Add 1.00 ml. of 30 percent H₂O₂. Cover with a watch glass and digest on steam bath for 2 hours. Remove watch glass and evaporate to dryness. Continue heating dry residue for 30 minutes. To the cooled residue add 2.5 ml. of phenoldisulfonic acid from a rapid-delivery pipet to flood the residue rapidly. Mix the residue and reagent with a glass rod. Add 70 ml. of dilute sequestering solution within 5 to 10 minutes after the phenoldisulfonic acid is added. Add an excess of 1 + 1 NH₄OH (usually 15 ml.).

Cool. Make solution up to volume (dilute as needed to give color intensity in proper range for reading). Read the color intensity in a colorimeter using a blue filter in the 400 to 420 $m\mu$ region.

4. Standard Nitrate Solutions

- 1.1 Stock solution — Dissolve 7.216 gm. of dry KNO_3 in 1000 ml. of NH_3 -free distilled water. This solution contains 1000 ppm NO_3-N (4429 ppm NO_3^-) or 0.001 gm. N per ml.

2.1 Standards

Standard (ppm NO_3-N)	Standard (ppm NO_3^-)	Ml. of stock solution	Dilute to:* (ml.)
5	22	5	1000
10	44	10	1000
20	88	20	1000
30	123	30	1000
50	222	50	1000
100	443	100	1000

*For the quick laboratory tests and the alpha-naphthylamine test, dilute with 20 percent acetic acid. For the Devarda's alloy—distillation method, dilute with 1 N NaCl. For the phenoldisulfonic acid method, prepare standards with distilled H_2O .

TESTS FOR NITRATES IN WATER

Nitrates in water may be determined in the same way as described for the laboratory determinations of nitrates in plant extracts. Prepare standards in distilled water.

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