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Nitrate Poisoning

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NITRATE POISONING

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Glossary

ABOMASUM: The fourth or digestive stomach of a ruminant. The three other stomachs are the rumen, reticulum and omasum. The first stomach, or rumen, is often referred to as the paunch.

AEROBIC: In the presence of oxygen.

ANAEROBIC: In the absence of oxygen.

ANOXEMIA: A condition due to deficient aeration of the blood.

ASCORBIC ACID: Vitamin C.

ASSIMILATION: The utilization of absorbed substances by plants or animals in their life processes.

COLIFORM BACTERIA: A group of bacteria which normally inhabit the intestines of animals. The presence of certain species of this group is considered to indicate fecal contamination.

CYANOSIS: A condition in which the surface of the body becomes blue due to insufficient aeration of the blood.

DENITRIFICATION: Conversion of nitrates and nitrites to ammonia or free nitrogen, as by bacteria in soils.

EPICARDIUM: The innermost layer of the membrane covering the heart.

FETUS: The young or embryo of an animal in the womb.

FISTULA: (See RUMEN FISTULA)

GASTROINTESTINAL TRACT: The stomach and intestinal systems of an animal.

GLUCOSE: The sugar occurring in corn syrup.

GRAM: A unit of weight. There are about 453 grams in a pound.

HEMOGLOBIN: The red pigment in the red blood cells of animals. It is found in venous blood, and as it passes through the lungs it combines with oxygen to form a more brilliant red pigment, oxyhemoglobin.

INTRAVENOUSLY: Into a vein.

KILOGRAM: One thousand grams (about 2.2 pounds).

METHEMOGLOBIN: An oxidized form of hemoglobin, brownish-red in color, and differing from oxyhemoglobin in that the oxygen it contains cannot be easily removed.

METHYLENE BLUE: A blue colored dye compound which is relatively easily reduced to its colorless form.

MICROORGANISMS: Microscopic organisms such as bacteria, yeasts and molds.

MILLIGRAM: One-thousandth of a gram.

MUCUS MEMBRANES: The lining membrane of the passages and cavities of the body which communicate directly or indirectly with the exterior, such as the alimentary and respiratory tracts.

NITRIFICATION: Conversion of ammonium salts to nitrites and nitrates as by bacteria in soils.

OXYHEMOGLOBIN: (See HEMOGLOBIN)

PARENCHYMATOUS: Pertaining to the essential and proper tissue of an organ.

PERITONEUM: The lining of the cavity of the abdomen.

PETECHIAL: Pertaining to small crimson or purple spots on the skin or other membranes, resulting from the infiltration of blood from its normal channels into the tissue.

pH: A symbol referring to the acidity or alkalinity of a solution. A solution having a pH of 7 is considered neutral, below 7—acid, and above 7—basic or alkaline.

PHARMACOLOGIC: Pertaining to the study of the properties and action of drugs in the animal organism in relation to their medical use.

RUMEN FISTULA: An opening in the rumen of an animal making possible entrance into the rumen through other than normal passages. In cattle the opening is usually made high on the side of the animal.

SCLERA: The dense, fibrous, opaque, white outer coatings covering the eyeballs except where they are covered by the cornea.

SEROUS COATS OF THE DIGESTIVE TUBE: Watery membranes covering the intestines.

THIONINE: A dye compound similar in some respects to methylene blue.

VASCULAR: Pertaining to the blood or lymph systems of an animal.

VASODILATION: Dilation of a vessel such as a blood or lymph vessel.

Nitrate Poisoning

IN BRIEF

Several cases of heavy cattle losses following the feeding of oat hays have been reported by farmers and ranchers of the Great Plains and Rocky Mountain areas. Oat hay poisoning, or more technically, nitrate poisoning, has been traced to the high nitrate content of the hay.

Though most of the losses reported in South Dakota from nitrate poisoning have involved this feed, oat hay is not the only forage crop found to contain harmful amounts of nitrate. In dry seasons, or when conditions have been unfavorable for normal plant growth, common forage crops, such as barley, wheat, rye hay, corn or sorghum fodder, as well as oat hay, have been found to contain toxic amounts of nitrates.

Most vegetation, including weeds, is frequently high in nitrate content early in the growing season, but the amount usually decreases as the plant matures. This means that plants previously dangerous as a feed become relatively safe when put up for hay after maturing. Native grasses usually contain little nitrate, even during the immature stages, and should not cause nitrate poisoning.

Environmental factors which may affect the nitrate content of plants are:

(1) Drought, particularly if occurring when the plants are immature, may leave the vegetation in a high nitrate state.

(2) High nitrate soils tend to produce crops having a high nitrate content.

(3) Soil deficiency in certain minor elements, such as copper, cobalt, or manganese. It is believed these minor elements favor plant growth and aid in the assimilation of nitrates by plants.

(4) Light intensity, or the degree of shading of plants, is an important factor in nitrate storage and may account for the high nitrate content frequently associated with plants grown in narrow valleys. In laboratory experiments, continuous light resulted in oat plants with lower nitrate content.

(5) Spraying with herbicides, such as 2,4-D, occasionally results in high nitrate content in the plants surviving the treatment. Weeds, such as redroot, ragweed, and Jimson weed, which cattle do not normally graze, are eaten with apparent relish after they have been treated with 2,4-D.

In addition to livestock losses from vegetation, losses are also caused by well water containing nitrate.

Sheep, cattle and horses have been found to be susceptible to nitrate poisoning, but apparently, cattle are most susceptible.

Nitrates are changed to nitrites in the rumen of the animal. These nitrites react so closely with the hemoglobin of the blood that the blood can no longer carry oxygen and the animal dies of asphyxiation. Symptoms resemble closely those of cyanide or prussic acid poisoning. A rapid acceleration of the pulse to 150 per minute, shortened respiration, trembling, weakness, and cyanosis, or "blueness" of the tongue and eyeballs occur.

Intravenous injections of a methylene blue solution have been effective in counteracting the poisoning, and within a few minutes following the treatment, symptoms of poisoning subside.

It would seem advisable to feed any questionable forage to a cull animal, observing the animal closely for several days for any symptoms of distress, before feeding the forage to the herd. Also, a report of nitrate content of forages may be obtained from the Station chemistry department. When the nitrate content is about 1.5 percent or more, the forage should be fed with caution.

Some aspects of nitrate poisoning pertain to public health. Several cases of "blue babies" have been reported as the result of nitrate poisoning from well water. Water containing more than 10 parts per million of nitrate nitrogen is considered unsafe for infant feeding.

Nitrate Poisoning

E. I. WHITEHEAD and A. L. MOXON[1]

Introduction

Nitrate poisoning has had a long, though spotty, record for afflicting man and livestock. Termed "oat hay poisoning," it has quite recently been brought to the attention of the farmers and ranchers of the Great Plains and Rocky Mountain areas in a report by Newsom et al. (1)[2] of several cases of heavy cattle losses following the feeding of toxic oat hays. In one instance near Franktown, Colorado, on March 31, 1936, 150 head of cattle had been fed oat hay early in the afternoon during a fall of snow and by 7:00 p.m., 67 had died. Experimental feeding of the hay to five steers produced typical symptoms and three died.

At the Wyoming Station, Bradley, Eppson, and Beath (2) studied a number of toxic hay samples and found that they contained abnormally high amounts of nitrate, ranging from 3.2 to 7.2 percent (calculated as potassium nitrate) of the dry matter. Previously, these investigators (3) had shown that the symptoms of toxicity were associated with the appearance of high levels of methemoglobin in the blood of the affected animals, and later they demonstrated that the ingestion of potassium nitrate by steers resulted in the same symptoms as those produced by toxic hays (4).

Methemoglobinemia

The hemoglobin of blood is readily converted to methemoglobin[3] by the action of nitrite,[4] which is formed when nitrate is reduced by microorganisms of the gastrointestinal tract, for example—in the rumen or paunch. Nitrite is absorbed into the blood stream and there reacts with hemoglobin. When nitrite is present in excessive amounts most of the hemoglobin will be changed to methemoglobin. As a result of this transformation, methemoglobin gives the blood a brownish red color instead of the bright red color associated with oxyhemoglobin. Since methemoglobin has little or no oxygen-carrying capacity, the animals die of asphyxiation (lack of oxygen). Frequently, cows surviving nitrate poisoning abort as a result of death of the fetus from lack of sufficient oxygen in the fetal circulation (7, 8). In toxic amounts, that is, a lethal dose of 1 gram of potassium

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[2]Figures in parentheses refer to *Literature Cited,* p. 22.

[3]Average values (grams per 100 milliliters) for the methemoglobin content of normal blood are: man, 0.10; dog, 0.13; cat, 0.13; rat, 0.10; guinea pig, 0.03; rabbit, 0.02. (5).

[4]Average values (micrograms per 100 milliliters) for the nitrite content of blood are: man, 9.45; dog, 9.5; steer, 9.4; monkey (rhesus), 11.0. (6).

nitrate per kilogram of body weight (or about 1 pound of KNO_3 for a thousand pound animal), 70 to 80 percent of the hemoglobin is changed to methemoglobin in a few hours.

The characteristic symptoms of nitrate poisoning have been recorded by Davidson et al. (9):

"The symptoms exhibited by affected animals were usually quite uniform in character, and were markedly increased in severity if the animal were disturbed or forced to move about. A rapid acceleration of the pulse to 150 per minute is soon noted, followed by shortened quickened respirations, trembling of certain groups of muscles, weakness, staggering gait and apparent blindness in some cases. The animal ceases to eat, soon sinks to the ground on its breast, or may roll over and lie quietly on its side with its mouth open. Cyanosis of the tongue and sclera is observed, death usually takes place with little or no struggling.

"On autopsy, petechial hemorrhages are frequently found on the epicardium, respiratory mucous membranes, the peritoneum and the serous coats of the digestive tube. Congestion of the mucous membrane of the abomasum is a constant lesion, the blood is a reddish-brown color, turning lighter on exposure to air."

The similarity of these symptoms to those produced by hydrocyanic acid (prussic acid, cyanide, or cane) poisoning led many invesitgators to seek for the presence of this agent, and tests were usually negative.

Animals Usually Affected By Nitrates

Sheep, cattle, and horses have been found to be susceptible to nitrate poisoning, but apparently cattle are most susceptible (7). This difference can probably be ascribed to differences in ability to reduce nitrate to nitrite, or more specifically to differences in the abilities of the gastrointestinal microorganisms of the respective animals to effect the reduction.

Infrequently, nitrites are found in measurable quantities in hays, but the amount is usually insignificant or the result of special conditions. The formation of nitrite is apparently favored by maintaining the fodder in a slightly moist condition. Olson and Moxon (10) have incubated ground redroot *(Amaranthus retroflexus)* of high nitrate content in the presence of moisture and at varying temperatures and hydrogen-ion concentrations (pH). The most rapid reduction of nitrate occurred when the samples were incubated at 40° C., and pH 7.0 to 8.0. Considering the effect of pH on nitrite formation and the pH of the gastrointestinal tract of cattle (11, 12), it is possible that differences in susceptibilities to nitrate poisoning may be due in part to differences in pH of the rumen contents. Normally, the pH conditions in the rumen of cattle are such that nitrate reduction could take place rapidly.

Bradley, Eppson and Beath (2) concluded that, since nitrite caused methemoglobin formation, the conversion of nitrates to nitrites must occur in the gastrointestinal tract.

In studies of ruminal ingesta (rumen contents) with Merino sheep, Sapiro et al. (13) have shown that the ingesta from well-fed sheep (alfalfa diet) caused nitrate to disappear more rapidly than that from sheep fed a poor quality grass hay. Nitrite increased until nitrate had disappeared, and then nitrite decreased more rapidly with the alfalfa ingesta. The addition of glucose to the ingesta increased the rate of nitrate reduction and subsequently of nitrite disappearance. On giving 20 grams potassium nitrate per 100 pounds of live weight to sheep on the ration of poor quality grass hay, they observed that a mild methemoglobinemia resulted, symptoms of which were alleviated if 30 grams of glucose were fed simultaneously with the nitrate. When these same sheep received 30 grams of potassium nitrate per 100 pounds of live weight, severe symptoms of methemoglobinemia were produced, but with simultaneous glucose feeding the symptoms were less severe. If the ration consisted of about two-thirds of a pound of corn daily and alfalfa free fed, the results were similar except that 50 gram doses of potassium nitrate had about the same effect as the 20 gram dose given the poorly fed sheep.

These studies of the role of rumen microorganisms on nitrite formation have been extended by live animal experiments performed by Lewis (14). Sheep with permanent rumen fistulas were used. Through the fistula, sodium nitrate was introduced into the rumen and samples of the rumen liquor withdrawn for periodic analyses of nitrate, nitrite and ammonium nitrogen. The data secured showed that nitrate is reduced to ammonia by the microorganisms in the rumen and that nitrite, as an intermediate in the reaction, accumulates under certain conditions. Methemoglobin production became gradually greater and the time between administration of the dose and the attainment of maximum methemoglobin values became longer as the amount of sodium nitrate added was increased from 12 grams to 22.5 grams. When the dose was increased to 25 grams, there was a sharp rise in methemoglobin, which the investigators explained could be due to the limiting rate of reduction of nitrite to ammonia and the consequent accumulation of nitrite in the rumen, some of which passes into the blood resulting in a severe methemoglobinemia. Twenty-five grams of sodium nitrate, or 10 grams of sodium nitrite placed in the rumen, or 2 grams of sodium nitrite injected intravenously resulted in a 60 percent conversion of the total hemoglobin (total hemoglobin was 11 to 13 percent on a weight/volume basis) to methemoglobin.

Forages Which Frequently Contain Nitrate

Generally, when hays are the cause of livestock poisoning, oat hay or straw is implicated; most of the cattle losses reported in Colorado (1, 15), Wyoming (2, 3, 7), Saskatchewan (9), and South Dakota (16) from nitrate poisoning have involved this feed. From the latter report, data are taken to illustrate the nitrate content of oat hays which ranchers sent in following cattle losses (Table 1).

Table 1. Potassium Nitrate (KNO_3) Content of Oat Hay Samples (Air-dry Basis)

County in Which Sample Was Grown	Percent KNO_3
Meade	3.48
Meade	3.12
Meade	4.69
Meade	2.06
Meade	0.44
Meade	4.49
Meade	1.93
Meade	5.58
Meade	3.21
Meade	1.10
Meade	1.54
Custer	4.00
Custer	0.41
Custer	3.23
Mellette	5.14
Mellette	2.21
Pennington	5.99
Pennington	5.34
Perkins	1.87
Perkins	3.19

Cereal plants other than oats occasionally contain nitrates in toxic amounts (17), but usually oat plants have the larger nitrate content. There is a general trend of decreasing nitrate content in plants as they mature, so that in many cases plants, previously dangerous as a feed for livestock, become relatively safe when put up as a hay after maturing (the effect of stage of growth on nitrate content of plants is discussed further in another section of this bulletin). Corn plants may accumulate nitrates in extremely high amounts in stalk and sheath tissues of young plants, prior to tasseling, and these stored nitrates may be rapidly assimilated during the development and maturation of the ears (18). Even at maturity, cornstalks frequently contain excessive nitrates and cause cattle losses. Examples of sorghums of low prussic acid content, but containing nitrates in excess have also been noted (16, 19). Considering these general observations, caution should be used when feeding oat, barley, wheat or rye hays, or corn or sorghum fodder grown in years when environmental conditions have been unfavorable to normal plant growth and maturation. It would seem generally advisable to feed any questionable forage to a cull animal, and to observe the animal closely for several days for any symptoms of distress, before feeding the forage to the herd. (A report of nitrate content may be obtained from this department, if samples representative of the forage are sent in for nitrate determination. A fee is charged to cover the cost of the analytical reagents used in supplying this service.)

A few cases of unusual silage fermentations have been reported; two of these have been in Yankton County (in different years) and one in Roberts County. In one case the silo had been filled with drought-damaged corn which had been grown on alfalfa ground. After filling, pungent yellow-brown gases issued from between cracks in the tile of the silo; any vegetation with which these gases came in contact turned yellow. In another instance, samples of silage were analyzed for nitrate and it was found that the yellowed material collected from the top of the silo contained about 6 percent nitric acid. When the fermentation was complete, the yellowed silage was removed from the top of the silo and spread on a field; the remaining silage was fed safely. In a third case, the men filling the silo were sickened by the fumes rising to the top of the silo. The corn which was used in filling this silo came from alfalfa land and, though nearly mature, the stalks contained 2.28 percent potassium nitrate (dry basis), while stalks from an adjacent field contained only a trace of nitrate.

Nitrates have been shown to have a temporary inhibiting effect on the formation of lactic acid (lactic acid is a normal silage fermentation product which, by increasing the acidity of the silage, acts as a preservative) and that the inhibition is due to nitrite formation from part of the nitrate present (20). The extent of nitrate reduction was found to be much greater if the conditions were typical of that in silage fermentations, that is, anaerobic rather than aerobic. A number of microorganisms could bring about the reduction of nitrate to nitrite.

Nitrite in the presence of lactic acid or other fermentation acids exists largely as nitrous acid, which is relatively unstable and decomposes to nitric oxide and nitrogen dioxide. The latter gas is red-brown in color. Nitric oxide will further react with oxygen to yield more nitrogen dioxide. Nitrous acid itself could be an agent in further losses of nitrogen from silage. Principally, this loss could be one of amino nitrogen by a reaction analogous to the Van Slyke reaction for the determination of α-amino nitrogen. Losses of this type have been discussed by Pearsall and Billimoria (21), Vickery et al. (22), and Wilson (23), the latter author reporting nitrogen losses involving nitrite and compounds other than the amino acids. (It may be of interest to note that three patents, two in Germany and one in the United States, have been issued covering silage preservation by addition of small amounts of nitrite (24, 25, 26). The extent of application of this method or its effectiveness is not known by the authors.)

High nitrate contents are not found in the cereal crop plants alone (27). Many weeds, frequently found as gross contaminants in hays, have been shown to contain nitrate. For example, redroot samples collected in 1939 at various locations within the state were analyzed and found to contain nitrates in the amounts indicated in Table 2.

From the results obtained when a number of common Wyoming

Table 2. Potassium Nitrate (KNO_3) Content of Redroot Pigweed *(Amaranthus retroflexus)*

County in Which Sample Was Grown	Percent KNO_3 (Air-dry Basis)
Lyman	1.88
Lyman	2.40
Lyman	4.27
Lyman	1.77
Lyman	1.17
Brule	5.23
Brule	4.67
Aurora	1.41
Davison	2.08
Davison	2.23
Sanborn	0.82
Kingsbury	1.46
Beadle	0.65
Beadle	1.59
Hand	0.20
Hand	2.15

Table 3. Potassium Nitrate (KNO_3) Content of Some Native Grasses

Sample		Percent KNO_3 (Air-dry Basis)	Stage of Growth
Western wheatgrass	*(Agropyron smithii)*	0.46	early
Needle-and-thread	*(Stipa comata)*	0.77	early
Feather bunchgrass	*(Stipa viridula)*	0.22	early
Stickseed	*(Lappula redowski)*	0.51	early
Blue grama grass	*(Bouteloua gracilis)*	0.12	early
Side-oats grama grass	*(Bouteloua curtipendula)*	0.02	mature

weeds were analyzed for nitrate, Gilbert et al. (17) concluded that thistles, fireweed *(Kochia sp.)*, bindweed, nightshade, white ragweed, wild sunflowers, and redroot are potentially hazardous weeds. In an examination of one hay sample from Gillette, Wyoming, these investigators separated the hay into its constituent parts and determined the nitrate content of the component species. Oat hay contained 5.8 percent potassium nitrate; wheat hay, 3.1 percent; native grasses, 0.7 percent; weeds — prostrate pigweed *(Amaranthus blitoides)*, trembling pigweed *(Amaranthus graecizans)*, redroot pigweed, wild sunflower *(Helianthus annuus)*, oak leaf goosefoot *(Chenopodium glaucum)*, Russian thistle *(Salsola pestifer)*, white ragweed *(Franseria discolor)*, and witchgrass *(Panicum capillare)* — ranged from 4.2 to 7.7 percent, with a weed average of 6.0 percent potassium nitrate.

Native grasses usually contain little nitrate, even during the immature stages, and should not cause nitrate poisoning. Typical of the nitrate values for some South Dakota grasses are those recorded in Table 3 for samples collected in 1939. Eggleton (28) has suggested that nitrite, which occurs naturally in spring grass and increases in amount if the grass is fertilized with ammonium sulfate or sodium nitrate, plays an important part in the so-called "grass tetany" of cattle. Reports of losses of livestock due to this cause have not been received by this laboratory.

The Lower Toxic Limit of Potassium Nitrate in Forages

Bradley, Eppson and Beath (7), on the basis of their toxic hay experiments, concluded that ingestion of 5½ pounds of hay containing 5 percent potassium nitrate (about 125 grams KNO_3) would be necessary to cause the fatal poisoning of a 500 pound animal, and arbitrarily set 1.5 percent potassium nitrate as the lower limit for a toxic hay.

Well Waters, High in Nitrate Content, May Cause Cattle Deaths

Aside from livestock losses caused by high nitrate vegetation, well waters containing nitrate may cause losses. Certain farm wells in the state yield waters containing appreciable amounts of nitrate nitrogen. During the period from 1944 to 1950, eight samples of water were submitted for analysis by farmers who questioned the quality of the water. Table 4 records the nitrate content of these waters. Samples of

Table 4. The Nitrate Nitrogen Content of Some Rural South Dakota Well Waters

Location (Nearest Town)	Nitrate Nitrogen (ppm)*	Year	Remarks
Brookings	510	1944	6 head of dairy cattle lost
Rutland	525	1945	cattle losses
Rutland (new well)	none	1945	
Colman	415	1945	cattle losses
Baltic	485	1948	
Hartford	455	1949	2 head of cattle lost
Colman	235	1949	
Henry	525	1949	
Hazel	500	1949	lost 1 cow; 3 sick

*ppm is an abbreviation for parts per million.

both tank and well waters were supplied from the Brookings and Hartford farms. Water from these tanks contained 750 ppm and 470 ppm of nitrate nitrogen, higher by 240 ppm and 15 ppm nitrate nitrogen, respectively, than the well water pumped into the tanks. Since these samples were received during the winter, it is conceivable that the practice of breaking the ice out of the tank and refilling the tank with well water resulted in concentrating nitrates in the tank water, although evaporation could account for it in part.

Considering the water consumption of a dairy cow to be 12 to 15 gallons per day, a water source containing 415 ppm of nitrate nitrogen would provide a daily dosage of nitrate equivalent to 135 to 170 grams of potassium nitrate.

If cattle are allowed free access to water, high nitrate water would probably not cause fatal poisoning, since the dosage of nitrate received at any one time might be well below the lethal amount. Furthermore, the differences in retention of water and forages in the rumen would have a considerable effect on the amount of nitrate reduction which could take place from either of these nitrate sources. However, the amount of nitrate provided by these waters should be quite sufficient to keep the cattle in a mild to moderately severe state of methemoglobinemia, which would be further aggravated by the presence of small amounts of nitrates in the forage supplied. Several of the water samples were accompanied by hay samples, which ranged from 0.3 to 1.1 percent potassium nitrate. This suggests that the lower toxic level of potassium nitrate in forages should be revised downward when the water supply contains nitrates.

A Wyoming study (7) was made to determine whether a chronic type of poisoning could be produced in an animal, daily drenched with one-half of a minimum lethal dose of potassium nitrate over a two month period. The animal gained weight and in every respect remained normal during the period of treatment. Chronic nitrite poisoning of immature rats over a period of several months was found by Hueper and Landsberg (29) to produce degenerative and parenchymatous lesions in the heart, lungs, brain, kidney and testes. They concluded that these lesions resulted from nutritive disturbances of the vascular walls elicited by anoxemia due to slowing blood flow, nitrite causing the vasodilation.

Treatment of Nitrate Poisoning

In the animal with a mild methemoglobinemia resulting from the ingestion of sub-lethal amounts of nitrate, the methemoglobin formed slowly disappears. In drawn blood, stored at room temperature, 30 to 50 percent of the methemoglobin reverts to hemoglobin in the first two hours, while the remainder changes at a gradually decreasing rate (30). When an animal is acutely poisoned, with methemoglobin values rising to 60 to 80 percent of the total hemoglobin, a convenient and quickly effective treatment is necessary if the animal is to be saved. A treatment to bring about a reversal of methemoglobin formation was devised by Hauschild (31). This treatment, which according to the author led to a reversal of all methemoglobin in 10 minutes in cats and rabbits after the administration of a toxic dose of nitrite, consisted of injecting intravenously a few c.c. of a thionine solution. Hauschild concluded that thionine acts as an oxidation-reduction system (thionine $\rightleftharpoons$ leucothionine) which displaces the system of hemoglobin $\rightleftharpoons$ methemoglobin, in favor of hemoglobin. The lethal dose of thionine, determined with rats, is 10 milligrams per 100 grams of body weight, and the toxicity of this compound must be considered in using it as an antidote.

The usual treatment of nitrate poisoning consists of intravenous injection of a methylene blue solution. In 1939 Wendel (32) showed that methylene blue solutions, injected intravenously into dogs previously treated with nitrite, brought about a rapid reduction in methemoglobin levels. Bradley, Eppson, and Beath (4) extended this treatment to cattle and used successfully a 4 percent solution of methylene blue, which was injected with care to prevent the escape of this irritating solution into tissues surrounding the jugular vein. They concluded that 2 grams of methlyene blue would adequately protect a 500 pound animal against the ingestion of hays containing about 300 grams of potassium nitrate (about 14 pounds of plant material with 5 percent KNO_3). Scott (33) has reported using injections of 10 c.c. of a 0.6 percent methylene blue solution (in 0.9 percent saline) to effect a rapid recovery of sheep showing a pronounced anoxemia following injection of 80 milligrams of sodium nitrite per kilogram of body weight.

Public Health Aspects of Nitrate Poisoning

High Nitrate Waters May Cause Methemoglobinemia in Infants

There are several aspects of nitrate poisoning which pertain to public health. Several references to "blue babies," so-called because of the cyanotic symptoms associated with methemoglobinemia, are recorded. In a survey of 243 dug wells tested in 1945 in Iowa, the nitrate content exceeded 20 ppm in 29 percent and 50 ppm in 15 percent of the wells, respectively (34). Two cases of infant methemoglobinemia were recorded in Nebraska by Stafford (35); these involved waters which contained 137 ppm and 144 ppm of nitrate.

The relationship between the nitrate content of rural water supplies and the depth of the wells was examined by Borts (36); of 2,313 wells examined, 1,943 were less than 100 feet in depth and 645 (or 28 percent) contained water with nitrate nitrogen in excess of 20 ppm. Of 69 cases of cyanosis traceable to water, 42 of the well supplies ranged from 51 to 801 ppm of nitrate nitrogen while 53 of the water supplies were also bacteriologically unsafe.

Other cases of cyanosis in infants attributable to water supplies have been recorded for Missouri (37) and for Minnesota (38). In the latter survey 146 cases, including 14 deaths of infants due to methemoglobinemia are discussed; the implicated waters had nitrate nitrogen contents in excess of 20 ppm.

Water containing more than 10 ppm of nitrate nitrogen is considered unsafe for infant feeding (39). In treating cases of methemoglobinemia, both 1 percent methylene blue given intravenously (40) and large doses of ascorbic acid given intramuscularly or sub-cutaneously (37) have been stated to be effective. Naturally, the symptoms will subside when the infant is provided a nitrate-free water. The questions of why all infants are not affected by nitrates in the water supply or why adults seldom show symptoms, remain to be fully answered.

Well waters have been observed to fluctuate in nitrate content. Factors such as percolation of rain or ground waters through heavily fertilized soils or soils in which nitrification is active, and organic pollution from barns and adjacent yards, particularly in view of the frequent association of coliform bacteria with these wells, may be responsible for the observed variability in nitrate content.

Food Poisonings Due to Nitrate or Nitrite

Instances of mass poisonings due to nitrate or nitrite have occurred infrequently. A few of such cases have been reported in Europe (41, 42) in which nitrite broths, used in curing sausages, were determined to be responsible. Also, the inadvertent use of sodium nitrite salt in seasoning instead of table salt (sodium chloride) has resulted in death to several persons served the food (43, 44). Such cases, though rare, serve as a warning to observe caution whenever sodium nitrite, used in preparing certain meat products, is kept with other condiments.

Gilbert et al. (17) found that some vegetables grown in Wyoming soils were high in nitrate. More than 80 percent of the vegetables grown of potassium nitrate and in 1941 a in 1940 contained over 1.5 percent high proportion of the vegetables again contained nitrates. They considered the possible effect of these vegetables on human health. On the basis of 1 gram potassium nitrate as a toxic dosage for humans (15 to 30 grams of KNO_3 representing a lethal dosage) a person would need to eat about one-half pound of beets (85 percent moisture; 0.42 percent KNO_3) or one-third pound of swiss chard (90 percent moisture; 0.67 percent KNO_3) before he would obtain 1 gram of potassium nitrate. They concluded that it was highly improbable that anyone would acquire a lethal dosage from vegetables containing nitrate, because of the amount which must necessarily be consumed.

Some Factors Affecting the Nitrate Content of Plants

Many factors seem to influence the accumulation of nitrates by plants. It would be difficult, considering the data now available, to show that there are definite species differences which result in nitrate accumulation in some plants and not in others, although there are apparently real differences between broad groups of plants, for example —legumes as compared with grasses. A series of studies with controlled environments, growing several species, might furnish evidence of species variability in nitrate accumulation, but present information has been largely obtained with plants growing in a wide range of soil and climate environments and collected at indiscriminate stages of growth.

Variations in Nitrate Content of Plants Taken From the Same Plot

Considerable variation is frequently found in the nitrate contents of plants growing on the same plot of soil, as is shown in Tables 5 and 6 for oat plant samples collected in 1943 and 1945 from bulk fields at the Experiment Farm. These plants were cut from locations within 100 feet of each other and plants representative of an area of 10 feet radius were composited at each location. All samples were collected at 11:00 a.m. on sunny days. The oat plants were rapidly air dried, and in the 1945 study, leaf, stem and sheath, and head fractions of 30 to 45 plants were prepared in addition to the whole plant sample. The plot sampled in 1945 was approximately three-quarters of a mile distant from the 1943 field. Soil samples taken in 1945 revealed relatively low amounts of nitrate nitrogen in the 0 to 6, 6 to 12, and 12 to 24-inch soil profile levels, averaging about 21 ppm (calculated as KNO_3) on June 24, 17 ppm on July 6, and 12 ppm on July 24.

Table 5. Potassium Nitrate (KNO_3) Content of Oat Plants at Two Stages of Growth and of Soils on Which the Plants Were Grown (Soil Data in ppm)

Location	Sample	June 14, 1943 (Heading-preflowering Stage) ppm	Percent	July 13, 1943 (Kernels in Milk Stage) ppm	Percent
1	Soils:				
	0—12 inches	48		38	
	12—24 inches	201		126	
	24—30 inches*	211		80	
	Whole plant:		5.15		2.79
2	Soils:				
	0—12 inches	33		26	
	12—24 inches	68		20	
	24—30 inches	82		—	
	Whole plant:		2.51		1.57
3	Soils:				
	0—12 inches	40		32	
	12—24 inches	50		15	
	24—30 inches	56		—	
	Whole plant:		2.19		2.20

*Rocks and gravel below 30 inches prevented sampling to the 36-inch depth.

Table 6. Potassium Nitrate (KNO_3) Content (Percent of Air-dry Material) of Oat Plants at Different Stages of Growth

Location	Fraction	June 24, 1945 (Shooting Stage)	July 6, 1945 (Heads Emerging)	July 24, 1945 (Kernels in Milk Stage)
1	Whole plant	0.66	0.22	0.13
	Leaf blade	0.42	0.21	0.19
	Stem and sheath	1.39	0.35	0.35
	Heads	------	0.10	0.03
2	Whole plant	0.25	0.08	0.15
	Leaf blade	0.11	0.06	0.20
	Stem and sheath	0.39	0.15	0.38
	Heads	------	0.04	0.06
3	Whole plant	0.92	0.30	0.26
	Leaf blade	0.50	0.19	—
	Stem and sheath	1.30	0.34	0.56
	Heads	------	0.08	0.00

Effect of Stage of Growth and Type of Tissue

Table 7 further illustrates the effect of stage of growth on the nitrate content of plants by recording data obtained in 1939 for plant samples collected in Lyman County. Here the downward trend in nitrate content of the plant as it matures is again evidenced.

Not all of the gross tissues of a plant contain the same amounts of nitrate. That nitrates accumulate in the greatest amounts in stalk and sheath tissues of oat plants is shown in Table 6, though these plants were not as high in nitrates as many samples which have been studied. Data for corn plants (collected in 1945) showed larger concentrations of nitrate in the stalks and sheath tissues, the amounts decreasing in

Table 7. Potassium Nitrate (KNO_3) Content of Some Plants at Various Stages of Growth (1939 data)

Kind of Plant	Date Collected	Stage of Growth	Percent KNO_3
Lamb's quarter	June 12	Early bud	3.24
(Chenopodium	June 22	Bud	1.43
album)	July 6	Late bud	1.28
	August 16	Early maturity	0.92
	September 19	Late maturity	1.22
Sunflower	June 12	Bud	4.75
(Helianthus sp.)	June 22	Blossom	1.77
	July 6	Early maturity	2.95
	August 16	Dry	2.81
	September 19	Dry	0.53
Gum plant	June 12	Prebud	1.76
(Grindelia	June 22	Prebud to early bud	1.49
squarrosa)	July 6	Early bud	0.48
	August 16	Bud	0.48
	September 19	Early blossom	0.36
Spiderwort	June 12	Blossom	3.64
(Tradescantia	June 22	Late blossom	1.28
bracteata)	July 6	Mature	1.66

Table 8. Potassium Nitrate Content of Flint (F) and Dent (D) Corn Plants at Different Stages of Growth (Data Expressed as Percent of Air-dry Material)

	July 2		July 13		July 23		August 10		August 29	
1945	F	D	F	D	F	D	F	D	F	D
Entire Plant	4.28	5.05	3.21	3.44	3.68	3.71	1.92	2.05	0.49	0.70
Leaves					1.82	1.48	0.69	0.52	0.74	0.09
Stalks					6.72	6.71	3.39	3.60	1.31	1.48
Sheaths					9.04	10.95	2.69	3.23	0.60	0.99
Shanks							1.78	1.80	2.76	0.74
Ears									0.02	0.03

samples collected progressively later in the season. With respect to nitrate content, these figures would indicate that the flint and dent corn varieties used were quite similar in both concentration and seasonal trend (Table 8). However, flint corn plants left standing in the fields in the fall usually contain more nitrates than adjacent dent corn plants (18). The location of specific plant tissues relative to the root system also influences nitrate content.

Again from data obtained in 1945 (recorded in Table 9), the effect of maturity and of particular corn plant tissues can be determined, and in addition, the decreasing trend in nitrate content of these tissues upward from the base of the plant is discernible. The leaf midrib tissues were found to contain more potassium nitrate than the corresponding leaf blades in the lower sections of the plant; however, above the sixth node these tissues were quite similar in nitrate content. Very high concentrations of nitrates were found in the lower sheath tissues of the July 26 sample; by September 4, the sheath tissue had greatly decreased in nitrate and

Table 9. Potassium Nitrate Distribution in Dent Corn Plants (1945) in Relation to Position of the Tissue (Expressed as Percent of Air-dry Material)

Tissue Analyzed	Nodal Position From Root to Tassel 1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Date of Sampling—July 26													
Leaf blade	2.67	2.07	2.02	1.82	1.29*									
Leaf blade†	0.40													
Leaf mid-rib‡	6.17													
Sheath	13.87	11.41	9.04	7.08										
Internode	9.99	7.96	4.33	2.16										
	Date of Sampling—September 4													
Leaf blade	0.35	0.24	0.17	0.10	0.05	0.05	0.04	0.05	0.04	0.06	0.04	**0.05**	**0.06**	**0.02**
Leaf mid-rib	0.58	0.45	0.37	0.34	0.22	0.22	0.03	0.03						
Sheath	0.90	0.43	0.43	0.31	0.23	0.17	0.15	0.09	0.09	0.07	0.17	0.14	0.04	0.06
Internode	2.09	1.92	1.44	1.00	0.86	0.69	0.66	0.66	0.57	0.58	0.45	0.47	0.68	
Shank		0.61	0.33	0.35	0.19	0.14	0.11							
Ears (fertile)					0.06	0.08	0.07							
Tassel														0.14

*Remainder of plant tissue above node 4.
†Leaf blades, 1 through 4, but minus mid-rib tissue (Ave. value).
‡Leaf mid-ribs, 1 through 4 (Ave. value).

the stalk internodes, having decreased to a lesser degree, then predominated in nitrate content. Since the stalk represented the greatest proportion of the plant weight in all except seedling stages, it was the tissue in which the bulk of the nitrate accumulated.

Table 10. Potassium Nitrate (KNO_3) Content of Soils and Plants at Several Locations in 1949

Location of Nearest Town	Date	Soils ppm KNO_3	Plants Percent KNO_3
Bruce	June 26	10	0.93
Bruce	June 26	3	0.14
Volga	June 26	9	0.39
Brookings	June 26	86	2.20
Piedmont	July 11	7	0.41
Nemo	July 11	25	2.28
Pringle	July 11	25	1.29
Buelah, Wyoming	July 11	260	5.42

Soil Nitrate Content

Soils having a high concentration of nitrate nitrogen tend to produce crops having a high nitrate content. This is shown in Tables 5 and 10; the latter table presents 1949 data for soils, the samples being representative of the 0 to 24-inch profile, and for oat plants in the "milk to soft dough" stage of kernel development. Similar findings were published in a report by the Soils Research Laboratory at Swift Current, Saskatchewan (45). Nitrates are usually found in highest amounts in soils during the late spring and early summer months, decreasing throughout the summer as the crop growing on them matures. Table 11 illustrates the data obtained for the nitrate content of different soil profile levels under corn plants; these samples were collected in 1945.

The extent of nitrification and the accumulation of nitrates in soils appears to be dependent upon a number of factors as was shown by Finnel (46) and Caster et al. (47), the net effect reflecting the interaction of these factors. Microorganisms which bring about soil nitrification are favored by aeration of the soil,

Table 11. Potassium Nitrate (KNO_3) Content of Soil from Cornfield at Different Dates During the Growing Season

Date of Sampling 1945	PPM of KNO_3 at Different Soil Depths			
	0-6 inches	6-12 inches	12-18 inches	18-24 inches
June 6	61	147	98	—
July 7	121	142	—	80
July 13	133	96	89	58
July 23	103	117	86	50
August 10	39	64	49	39
September 4*	9	17	16	14

*Composite of four locations. All other samples were composites of six locations.

while denitrification is suppressed by aerobic conditions (48). Aeration has been shown to result in increased uptake of nitrate by plants grown in solution cultures (49) and in increased assimilation by roots (wheat seedling) of nitrate previously absorbed (50). It is possible that the nitrate content of crops grown on summer-fallow may exceed that of crops grown on stubble land, since the nitrate content of the fallow soil is generally higher (as a result of continued nitrification without crop depletion of soil nitrate) at the time of seeding (45).

Drought and Its Effect on Nitrate Content of Plants

Drought is a factor which may cause plants to have a high nitrate content. Gilbert et al. (17) and Doughty et al. (45) concluded that continued drought had little effect on the nitrate content of plants. However, the results of greenhouse experiments at this laboratory have indicated that drought could be an important factor. These data are presented in Table 12. Oat plants were grown in 10-inch clay pots filled with weighed amounts of a sandy loam (without fertilizer amendments) and seeded to oats. Two pots were maintained at each of the following soil moisture levels: 15, 20, 25 and 30 percent water (air-dry basis). The data recorded in Table 12 are average values for each of the trials. In another experiment two large tubs, each holding about a cubic foot of the sandy loam soil, were planted with oats. The soil in one container was kept in a dry (near wilting point) state, while the soil of the other was kept moist. After four weeks, samples of plants were taken from each tub and analyzed for nitrate. The soil moisture treatments were then reversed and after a week, the plants were sampled again. The oat plants growing originally on dry soils contained 11.33 percent potassium nitrate (air-dry basis) and their nitrate content decreased to 7.51 percent after seven days of growth on wet soils. The plants grown on wet soils contained 6.62 percent potassium

Table 12. Potassium Nitrate (KNO_3) Content of Oat Plants (Air-dry Basis) as Influenced by Soil Moisture

Treatment (Percent Moisture)	1st Trial (Percent KNO_3)	2nd Trial (Percent KNO_3)
15	9.40	9.97
20	7.10	9.38
25	5.85	3.35
30	5.86	1.63

nitrate, which increased to 9.39 percent after the plants were maintained for a week in a droughty state.

Though it is not always feasible to transpose results obtained in greenhouse experiments to field conditions, these data suggest that drought could have considerable influence on nitrate accumulation by plants, or more specifically, a drought during the later stages of plant growth might result in crops of high nitrate content, as was suggested by Doughty et al. (45). Stahl and Shive (51) have grown oat plants in solution cultures and determined that nitrates were absorbed in minimum quantities during the earliest stages of growth, the quantity absorbed increasing rapidly until it reached a maximum at the blossoming stage, beyond which the absorption rate declined. A drought at a critical stage, such as flowering, could conceivably result in high nitrate oat plants.

Minor Elements and Nitrate Accumulation

Deficiencies of certain minor elements in soils may be a factor in nitrate accumulation by plants and in soil nitrification as well, considering the role of some elements such as cobalt, copper, or manganese as catalysts of certain enzyme systems. The growth of barley plants supplied nitrate in non-aerated solution cultures was observed to be improved in the presence of minute amounts of manganese and copper, with copper having the greater influence on growth and extent of roots of the plants (52). Hewitt et al. (53) have studied nitrate accumulation by cauliflower and determined that small amounts of molybdenum and manganese facilitated the assimilation of nitrates. They conceived possible roles of molybdenum function in the reduction of nitrate nitrogen and of manganese function in protein synthesis from amino acids. More studies bearing on minor element effects in plant metabolism will be needed, if the roles of minor elements in nitrate metabolism are to be fully defined.

Light as a Factor in Nitrate Assimilation by Plants

Light has been shown to be a factor in the assimilation by plants of absorbed nitrate. Lease and Tottingham (54) considered the reduction of nitrate to nitrite to be closely associated with ultra-violet radiations. Plants raised in the greenhouse are usually high in nitrate as compared with similar plants grown outdoors, and since glass panes effectively absorb nearly all of the ultra-violet rays, the accumulation of nitrates in greenhouse plants seems to be explained by the observation of Lease and Tottingham. Data in Table 13 illustrate this effect for oat and redroot plants grown in

Table 13. Potassium Nitrate (KNO_3) Content of Oat and Redroot Plants Grown in the Greenhouse and Outdoors

Sample	Greenhouse (Percent KNO_3)	Outdoors (Percent KNO_3)
Oats:		
1st harvest	7.24	0.45
2nd harvest	6.87	0.15
Redroot	5.70	0.07

pots of soil in the greenhouse and outdoors. Soil moisture content was kept as nearly as possible at 25 percent.

The possibility that nitrate reduction in plants can be effected in two different ways, one being chemical (as in root tissues) and the other photochemical, has been advanced by Burstrom (55), who also found that increasing light intensities resulted in increased nitrate assimilation in the leaves of wheat plants. The degree of shading of plants is an important factor in nitrate storage and may account for the high nitrate content frequently associated with plants grown on cultivated areas in narrow valleys (17). The effect of length of photoperiod on nitrate accumulation is shown in Table 14. The plants designated as receiving continuous light were supplied with continuous light from a 100 watt bulb in addition to daylight. Plants grown in both soil and solution cultures were studied and in either case continuous light resulted in oat plants with lower nitrate content.

Table 14. Potassium Nitrate (KNO_3) Content of Oat Plants (Air-dry Basis) as Influenced by Daylight and by Continuous Light

Treatment	Pot Grown (Percent KNO_3)	Solution Culture (Percent KNO_3)
Continuous light	4.94	2.04
Daylight only	7.06	3.59

Herbicide Treatment

In a report by Stahler and Whitehead (56) attention was called to the possible effect of herbicides on nitrate accumulation in the treated plants. Beet leaves, inadvertantly sprayed with 2,4 dichlorophenoxyacetic acid (2,4-D), were obtained from seven fields in North Dakota with the assistance of E. A. Helgeson and his associates of the Botany Department, North Dakota Agricultural Experiment Station. Three untreated beet fields adjacent to the treated fields were similarly sampled. The treated fields had been sprayed in mid-August with a Toxaphene mixture to control a late brood of webworm. It was apparent soon after the application that the Toxaphene had been contaminated or mixed with 2,4-D. Analyses of the treated beet leaves showed that they contained a high percentage of potassium nitrate (dry basis) as compared with untreated leaves.[5] These data are presented in Table 15.

It has been observed that weeds such as redroot, ragweed *(Ambrosia sp.)*, and Jimson weed *(Datura stromonium)*, which cattle normally do not graze, are eaten with apparent relish after they have been treated with 2,4-D. This has been attributed in part to the increased succulence of the plants surviving the spray treatment. There have been a number of reports of cattle becoming ill following the ingestion of weeds treated with 2,4-D, but whether these cases of poisoning are attributable to an indirect action

[5]Savage (57) has reported cattle losses due to nitrate poisoning by sugar beet tops. Samples of the beet tops were found to contain 1 to 6 percent potassium nitrate on an "as received" basis (somewhat dry). Of 70 animals turned into the field where the beet tops were piled on the ground, 41 showed symptoms of poisoning, 19 died, and 18 (treated with methylene blue) recovered.

Table 15. Effect of 2,4-D on the Potassium Nitrate (KNO_3) Content of Leaves of Sugar Beets

Sample No.	Treatment	KNO_3 as Percent of Dry Weight of Leaves	
1	None	0.20	
2	None	0.22	
3	None	0.25	
	Average of untreated		0.22
4	2,4-D	1.81	
5	2,4-D	2.26	
6	2,4-D	4.41	
7	2,4-D	4.65	
8	2,4-D	4.68	
9	2,4-D	5.01	
10	2,4-D	8.77	
	Average of treated		4.50

of 2,4-D, such as disturbed plant metabolism, is not known. It has been shown that 2,4-D, in the amounts that might be consumed by cows or sheep grazing pastures sprayed with this material, should not be injurious (58).

Investigations of the nitrate content of cultivated and weedy species sprayed with 2,4-D and other herbicides have been continued. Some of the findings are reported in Table 16. These data substantiate the previous findings, but not all of the data collected showed nitrate accumulation in plants in response to herbicide treatment, this being particularly true of many weed species. This phase of the nitrate problem will be the subject of a future publication, when more data have been collected and studied.

Table 16. Potassium Nitrate Content of Stems of Corn and Oats, and of Foxtail *(Setaria sp.)* **Plants Receiving Post-emergence Sprayings with Herbicides**

Sample	Untreated	TCA	CMU	Niagarthal	EC-3740	EC-3890
Corn stems	3.23	6.98	4.88	3.87	4.42	—
Oat stems	2.08	3.10	4.82	2.36	3.95	3.71
Foxtail	0.41	2.27	1.90	1.34	1.10	2.62

Summary

This bulletin presents a brief survey of the problem of nitrate poisoning, incorporating some of the experimental evidence secured at this laboratory and borrowing liberally of the data obtained by other investigators, so as to make possible a more complete report. It is by no means a comprehensive review of the field, but it is thought that the literature cited (and the references therein) provides an adequate cross-reference on the subject. The pharmacologic aspects of nitrate and nitrite, for example—studies on many of the nitro esters, have been intentionally omitted from the discussion.

Nitrate, as a toxic substance in oat hays and other forages, is reduced to nitrite by microorganisms of the gastrointestinal tract of animals. Nitrite, by its action on the hemoglobin of the blood, converts it to methemoglobin. When a large proportion of the hemoglobin has been changed to methemoglobin, the affected animal shows typical symptoms of poisoning and may die of asphyxiation, since methemoglobin does not readily yield oxygen to the tissues of the body. Many cases of nitrate poisoning of infants by high nitrate well waters have been reported. Some South Dakota well waters have caused cattle losses because of their high nitrate content.

Intravenous injection of a methylene blue solution has been effectively used in cases of nitrate poisoning. Within a few minutes following the treatment, symptoms of poisoning subside.

Oat, barley, wheat or rye hay, or corn or sorghum fodder, grown in years when environmental conditions have been unfavorable for normal plant growth, may contain toxic amounts of nitrates. Vegetation is frequently high in nitrate content early in the growing season, but the amount of nitrate stored usually decreases as the plant matures.

Many environmental factors have been shown to affect the nitrate content of plants. Some of these factors are:

(1) the nitrate content of the soil, high nitrate soils tending to produce crops having a high nitrate content;

(2) drought, particularly if it occurs when the plants are relatively immature, may leave the vegetation in a high nitrate state;

(3) soil deficiency in certain minor elements essential to normal enzyme function;

(4) ultra-violet radiations and increasing light intensities or length of photoperiod favor the assimilation of nitrate by plants; and

(5) spraying with herbicides, which occasionally results in high nitrate contents in the plants surviving the treatment.

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