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Pathophysiology of Nitrate Toxicity in Humans and its Mitigation Measures

Sunil Gupta[1], R.C. Gupta[2] and A.B. Gupta[3]

[1]Consultant Pediatrician & Neonatologist and Scientist of Environmental Medicine, Krishna Ram Ayurvigyan Shodh Sansthan, Jaipur 302 015, India
[2]Professor and Head, Department of Physiology, SMS Medical College, Jaipur 302 004, India
[3]Professor of Environmental Engineering, MNIT, Jaipur 302 017, India
e-mail: drsunil@gmail.com

ABSTRACT

Naturally occurring nitrate levels in surface and groundwater are generally a few milligrams per liter. However, nitrate concentrations are higher in watersheds that have been intensively used and modified by human activity. Even in many groundwater's, higher levels are found due to sewage percolating through sandy strata and also due to an excessive use of chemical fertilizers. Excessive intake of drinking water with nitrate content beyond permissible limit (100ppm) may cause clinical manifestations of disease known as methemoglobinemia (blue baby syndrome) in infants. In adults intake of high nitrate water may cause stomach disorder or gastrointestinal disease. Recent research by the authors has yielded results to explain the mechanism of nitrate toxicity, the defense system in human body to counteract this toxicity and some other manifestations of nitrates apart from causing methemoglobinemia (recurrent stomatitis, recurrent diarrhea and Recurrent Respiratory Tract Infection) among children, which could be of high importance to the field of environmental health. The current chapter critically discusses the pathophysiology of nitrate toxicity in humans and suggests potential measures for its mitigation in de

Keywords: Nitrate, Toxicity, Human health, Mitigation

INTRODUCTION

Naturally occurring nitrate levels in surface and groundwater are generally a few milligrams per liter. However, nitrate concentrations are higher in watersheds that have been intensively used and modified by human activity. Even, in many groundwaters, higher levels are found due to sewage percolating through sandy strata and also due to an excessive use of chemical fertilizers. World Health Organization (WHO) report, 2004, maintains that extensive epidemiological data support limiting the value of nitrate-nitrogen to 10 mg/L or as nitrate to 50 mg/L (WHO 2004) for human consumption whereas IS-10500 prescribes maximum permissible limits of nitrate in drinking water as 45 mg of NO_3 per liter (IS 10500 1995).

The problem of nitrates is endemic internationally as well as nationally, but so far no compiled data at national level are available. The data available are either of small areas or of scattered zones/areas. Many workers tried to compile the status of nitrate in drinking water in Rajasthan, India (PHED Habitat Survey 1991-93; Kumar et al. 2002).

EPIDEMIOLOGY

The problem of nitrate toxicity is global and has been reported from a number of countries (Prakasa Rao and Puttanna 2006).

International Perspective

Nitrate is a widespread contaminant of ground and surface waters worldwide (Prakasa Rao and Puttanna 2006; Singh et al. 1994). In the United States, the problem appears to be concentrated in the Mid-West and the Far-West, with large areas of Iowa, Illinois, Kansas, Michigan, Wisconsin, Washington and California being heavily affected (Smith et al. 1994; Revenga and Mock 2001). The USGS (Smith et al. 1994) reported that nitrate concentration in the nation's groundwater supply was increasing steadily. In South America, nitrate concentrations in the monitored watersheds are relatively low and follow human land use. The highest nitrate concentrations are found in the Uruguay watershed, where intensive agriculture is practiced. Likewise, nitrate concentrations in water are also greater in the Magdalena watershed of Colombia than in the less densely populated watersheds of the Amazon basin. The nitrate concentrations in South America correspond to lower fertilizer application rates in comparison to Europe.

Nitrate concentrations are higher in watersheds that have been intensively used and modified by human activity such as the Weser, Seine, Rhine, Elbe, and Senegal. High levels are also found in such watersheds in China, South Africa, and the Nile and Mississippi basins. South Africa has some of the highest natural nitrate levels in the world (more than 550 mg/L NO_3^--N) especially in the Kalahari region (Colvin et al. 2008).

National Perspective

Nitrate from sources like mineralized organic matter, fertilizers, organic manures, industrial effluents or urban wastes results in groundwater pollution, which has

received attention in various parts of India. The available data from various studies point to the fact that nitrate pollution from any of the above sources can pose a serious health hazard to humans, animals, birds and aquatic life. The data available on the concentrations of nitrate in groundwater samples from Punjab, Haryana, U.P., Delhi, Orissa, Tamil Nadu, Bihar, A.P., M.P., Maharashtra, Karnataka and other states point to the fact that in many of these samples, the nitrate content in groundwater has been much more than the permissible limits. In many parts of Punjab and Haryana, nitrate level in the groundwater is increasing continuously beyond the critical standard. Nitrate levels in the groundwater over vast agricultural areas can be correlated with intensive irrigated agriculture, use of nitrogenous fertilizers, and groundwater exploitation. Workers tried to evaluate the nitrate pollution and its strategies for reducing it (Prakasa Rao and Puttanna 2006). Nitrate pollution of groundwater by fertilizer use in India has also been reviewed (Singh et al. 2007).

SOURCES

There are two sources of nitrogen nitrate for human beings: exogenous sources and endogenous sources.

Exogenous Sources

Wastes containing organic nitrogen are decomposed in soil or water by microbial action forming ammonia, which is then oxidized to nitrite and nitrate. Since nitrite is easily oxidized to nitrate, it is primarily nitrate that is found in groundwater and surface waters. Nitrate-containing compounds in the soil are generally soluble and readily migrate with the groundwater. Nitrosamines have a short lifespan in ambient air. Vegetables (cauliflower, spinach, collard greens, broccoli, and root vegetables) account for more than 70 percent of the nitrates ingested in the human diet. The remainder of nitrate in a typical diet comes from drinking water (21 percent) and meat and meat products (6 percent). Nitrates are naturally occurring inorganic ions, which are part of the nitrogen cycle, and are commonly found in groundwater and surface waters. The nitrate ion (NO_3^-) is the most stable form of nitrogen in oxygenated environments, thus all nitrogen-containing molecules can act as sources of nitrates.

Water and food

Nitrogen is an essential major element for plant growth (Allan Wild, 1988). Nitrogen nutrition of crops is very often the limiting factor for food production and nutritional quality of food as indicated by its protein content. Thus, nitrogen has a key role to play in feeding the world population. Since a large response to nitrogenous fertilizers is a universal phenomenon, farmers usually apply large doses of fertilizer. But the recovery of applied nitrogen by the crop rarely exceeds 50% and a large fraction of the surplus nitrogen gets transformed into nitrate form

and pollutes the environment including surface and groundwaters. The interaction between the various forms of nitrogen in soil, plants, animals and atmosphere constitutes the nitrogen cycle (Fig. 3.1).

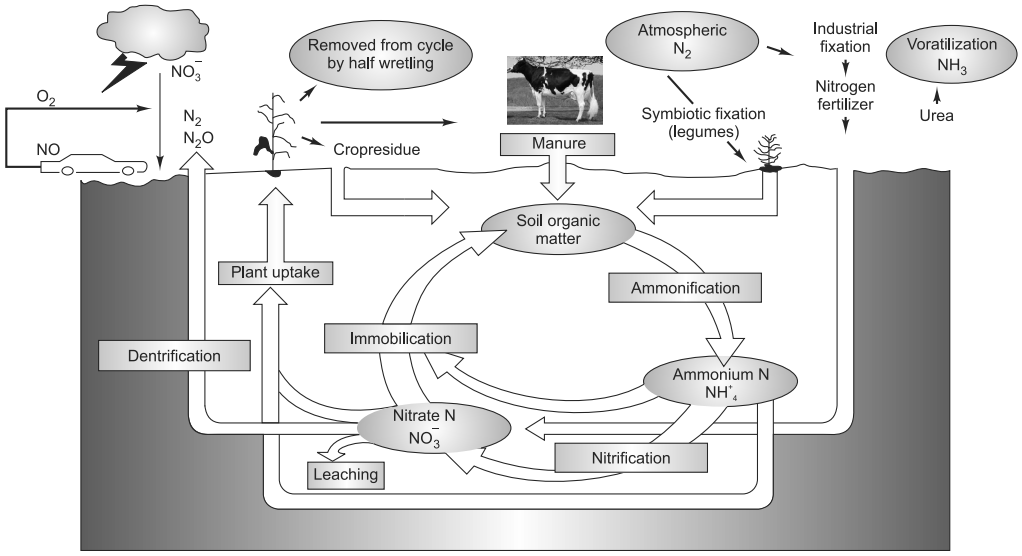


Fig. 3.1. Nitrogen in agricultural system (Wolkoswki et al., Internet publication).

Leafy vegetables

Being a rich source of nutrients and antioxidants, leafy vegetables occupy an important place in the human diet. Some vegetables are known to accumulate high concentration of nitrate under the current practice of heavy fertilization. Vegetables are the major source of daily intake of nitrate by human beings, supplying about 72 to 94% of the total intake (Annexure I). On consumption by humans, part of this nitrate is converted to nitrite and N-nitroso compounds that have detrimental effects on human health.

The nitrate content in the vegetables available in the markets is reaching as high as 4451 mg kg^{-1} fresh weight in chenopodium and 4293 mg kg^{-1} fresh weight in spinach (Anjana et al. 2007a,b).

Other sources of nitrate pollution

Malik (2000) suggested that the presence of nitrate bearing rocks in geological formations as in some parts of Haryana contributed to high nitrate levels in groundwater (Malik 2000) (Table 3.1).

Table 3.1: Other sources of nitrate pollution (Malik RPS 2000)

	Agriculture	Municipal	Industrial
Diffuse sources	Use of synthetic nitrogen fertilizers	Combustion engines in vehicles	Atmospheric emission (oxides of nitrogen) from combustion engines and energy production
	Use of organic manures	Disposal of municipal effluents	Disposal of effluents
Point sources	Accidental spills	Badly designed landfills	
	Improper, shelter less composting pits, heaps	Septic tanks	Disposal of wastes into wells, ponds, injection into tube wells, etc.
		Leaking sewerage systems	Badly designed landfills
Linear sources	Contamination of rivers with major groundwater connections		

Contamination from agro-based industries

The methods of disposal of industrial effluents include diversion to surface water sources (rivers, lakes, canals, etc.), stored in lagoons or ponds for percolation, or spreading or spraying onto the ground surface. The effluents may be treated, partly treated or untreated. The polluted water percolates either directly from the surface water bodies or agricultural fields (when such water is used for irrigation), and pollutes the groundwater. The extent of pollution depends upon the volume of effluents discharged per day and their characteristics (Wakida et al. 2004). Under certain circumstances, forests also contribute to nitrate pollution.

Continuous application of nitrogen fertilizers can cause an increase in the concentration of nitrates in fruits, vegetables and food. Maximum admissible level of nitrites in vegetables is 1 mg/kg (Mondal et al. 2008).

Cooking in aluminum utensils increases the reduction of nitrates to nitrite (WHO 1985a), hence increasing the intake.

Endogenous Sources

Nitrates, known as endogenous nitrate, are also produced in the body. A major pathway for endogenous nitrate production is conversion of arginine by macrophages to nitric oxide and citrulline, followed by the oxidation of nitric oxide to nitrous anhydride and then reaction of nitrous anhydride with water to yield nitrite. Gastrointestinal infections and non-specific diarrhea increase endogenous (non-bacterial) nitrate synthesis, probably induced by the activation of mammalian reticuloendothelial system (WHO 1985b; WHO 1996).

Total Daily Intake of Nitrate

Daily dietary intakes of nitrate and nitrite have been estimated in different countries (Ellen and Schuller 1983). The variation in the quantity of nitrates and nitrites ingested via diet is extremely high. For example, individuals who seldom

eat vegetables and cured meats have a low intake, whereas vegetarians have a relatively high intake. In most European countries, the mean nitrate intake is about 10–30 mg/day. Vegetarians usually have a two-to fourfold higher intake of nitrates than non-vegetarians (WHO 1985c).

KINETICS AND METABOLISMS

About 20% of the ingested nitrate is reduced to nitrite by nitrate-reducing microflora present in the saliva (Eisenbrand et al. 1980) at the base of the tongue (Walker 1995). The factors which influence the oral microflora and hence the reduction of nitrate are nutritional status, infection, environmental temperature and age (more in elderly) (Eisenbrand et al. 1980).

Ingested nitrate is reduced to nitrite by nitrate reducing microflora in the stomach (in favorable conditions, viz. pH ≥ 4) and upper part of intestine and may be at other parts of the human gastrointestinal tract. In normal conditions, the reduction of nitrate to nitrite does not occur in the stomach, but in situations where the stomach pH is high, such as achlorhydria (Ruddell et al. 1978), atrophic gastritis (Walker 1995; Mirvish 1975), artificially fed infants, or patients using antacid or similar drugs, e.g. omeprazole (Farinati et al. 1996; Colbers et al. 1996; Vermeer et al. 2001), the conversion of nitrate to nitrite occurs even in stomach. The high pH of the stomach favors the growth of nitrate reducing organisms.

This nitrite is readily and completely absorbed from both the stomach and the upper small intestine. Approximately 25% of the ingested nitrate is actively secreted into saliva, where it is partly (20%) reduced to nitrite by the oral microflora; nitrate and nitrite are then swallowed and reenter the stomach.

Absorbed nitrite is then rapidly distributed throughout the tissues. Absorbed nitrite is rapidly oxidized to nitrate in the blood, with the formation of methemoglobin.

The following is the flow chart of the fate of ingested nitrate (Li et al. 1997; WHO 1977a) (Fig. 3.2).

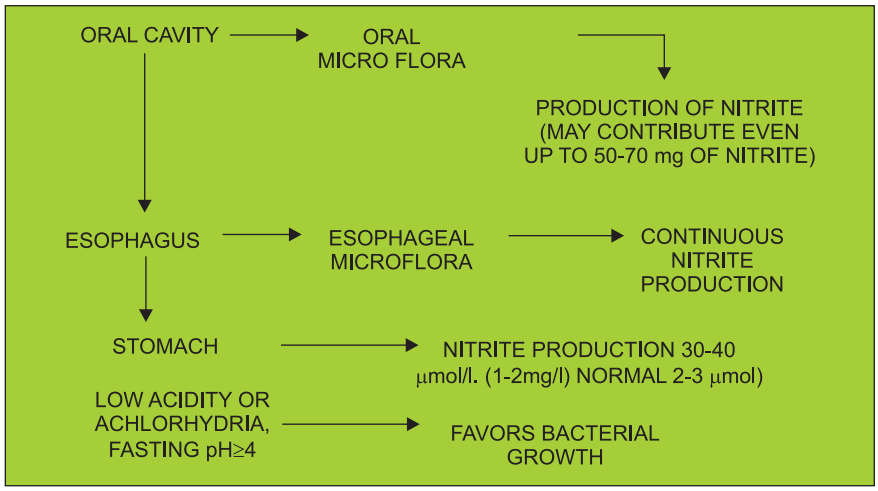


Fig. 3.2. Fate of ingested nitrate (Li et al., 1997; WHO 1977a).

Formation of N-Nitroso Compounds

Formation of N-nitroso compounds is a multiple step process (Choi 1985). First, nitrate is converted into nitrite after consumption. Then the nitrite reacts with natural or synthetic organic compounds (known as secondary amines or amides) in food or water to form new combinations called N-nitroso compounds (either nitrosamines or nitrosamides). Many of these N-nitroso compounds have been found to be carcinogenic in all the animal species tested, although some of the most readily formed compounds, such as N-nitrosoproline, are not carcinogenic in humans. At least 75% of the 120 N-nitroso compounds have been found to be carcinogenic to animals (Gilli et al. 1984; Terblanche 1991). The most common N-nitroso compounds are dimethylnitrosamine (DMN), N-methylmethanamine (DMA), trimethylamine (TMA) and trimethylamine oxide (TMAO). The N-nitroso compounds are carcinogenic in animal species and are probably also carcinogenic to humans. The data from a number of epidemiological studies are at most, only suggestive, relating to carcinogenicity in humans, but it has been reported that a link between cancer risk and endogenous nitrosation as a result of high intake of nitrate and, or nitrite and nitrosatable compounds is possible (RIVM 1989; WHO 1996).

Predisposing Factors

A direct correlation between gastric pH, bacterial colonization and gastric nitrite concentration has been observed in healthy people (Mueller et al. 1986). In individuals with gastrointestinal disorders and achlorhydria, high levels of nitrite have been reported (Ruddell et al. 1978; Dolby et al. 1984). Infections and non-specific diarrhea played a role in the increased endogenous synthesis of nitrate (Lee et al. 1970; Tannenbaum et al. 1978; Green et al. 1981; Hegesh and Shiloah 1982; Bartholomew and Hill 1984; Gangolli et al. 1994).

Trans-Placental Cross

Nitrite has been shown to cross the placenta and cause the formation of fetal methaemoglobinemia in rats (El Nahas et al. 1984).

Half-Life

The half-life of nitrate in the body after ingestion is approximately 5 h (Wagner et al. 1983a). Nitrite was not detected in any of the body fluids studied except saliva where it appeared to increase as the nitrate levels decreased (Cortas and Wakid 1991).

METABOLISM OF INGESTED NITRATE IN HUMAN BODY AT CELLULAR LEVEL

Ingested inorganic or organic nitrates will result in increased oxidation of hemoglobin to methemoglobin and increased production of nitric oxide (Murray et al, 1993;

Waldman et al, 1987; Craven et al. 1978) (Figure 3.1). The conversion of nitrite to nitric oxide is a non-enzymatic process. (Vane et al. 1994; Lowestein et al. 1994; Robertson 1996; Smith et al. 1997). The oxidation of hemoglobin to methemoglobin results in the formation of superoxide radical by the transfer of a single electron. The enzyme, superoxide dismutase, present in the erythrocytes, catalyses the conversion of superoxide radical ($O^{\bullet -}$) to H_2O_2 and O_2. The H_2O_2 is decomposed by glutathion peroxidase or catalase both are present in erythrocytes (Sutton et al. 1976; Winterbourn et al. 1976). Once the rate of oxidation of hemoglobin increases sufficiently in erythrocytes and overwhelms the protective and reductive capacities (e.g. cytochrome b_5 reductase system etc.) of the cells (Bodansky 1951; Jaffe 1981), there is an increased production of reactive free radicals of nitric oxide ($NO^{\bullet}$) and oxygen (Winterbourn et al. 1976).

Fate of Free Radical Nitric Oxide ($NO^{\bullet}$)

Hemoglobin scavenges nitric oxide through the high affinity ferrous sites on heme to form S-nitrosothiol, which has an affinity to nitric oxide 8000 times higher than that for oxygen (Hsia 1998) by binding at β 93 cysteine residue on the globin chain. As hemoglobin binds oxygen in the lungs, its binding affinity to S-nitrosothiol is increased. As hemoglobin releases oxygen at the periphery, its affinity for S-nitrosothiol is reduced and nitric oxide is released in the tissues (Hsia 1998). The thiol group of S-nitrosothiol protects nitric oxide from being scavenged by the binding site on heme. Thus, in addition to carrying oxygen, hemoglobin acts as a carrier of nitric oxide. The enhanced release of nitric oxide from S-nitrosohemoglobin in hypoxic tissue in turn reduces regional vascular resistance.

Nitric oxide is a biogenic messenger, an endothelial derived relaxing factor (EDRF) (Jaffe 1981; Hsia 1998)) and activates guanylyl cyclase system (Berger et al. 1997) [converts guanosine triphosphate (GTP) to 3′5′ cyclic guanosine monophosphate (cGMP)], raising the cGMP pool and therefore inducing *inter alia* vasodilatation (Berger et al. 1997) by lowering intracellular calcium ion (Smith et al. 1997).

Fate of Free Oxide Radical ($O^{\bullet}$)

In a normal cell, $O^{\bullet -}$ is scavenged by the enzyme superoxide dismutase, and H_2O_2, which is a product of reaction is removed by glutathion peroxidase and catalase (Roediger et al. 1986; Comly 1945). Any $O^{\bullet -}$ that escapes this mechanism leads to the production of much more reactive substances such as hydroxyl radical ($OH^{\bullet}$) and peroxynitrite radical. $O^{\bullet -}$ and H_2O_2 are highly selective in their reaction with biological molecules, whereas $OH^{\bullet}$ attaches everything around it and reacts with other cell constituents, causing irreversible cell damage. This mechanism is likely to become more significant if $O^{\bullet -}$ is produced in abnormally high amount (e.g. excessive nitrate ingestion), or if any of the protective mechanisms are defective (Sutton et al. 1976; Hsia 1998).

Thus, increased consumption of nitrate will lead to (a) increased production of nitrite (Allison et al. 1984; Cole et al. 1980); (b) enhanced absorption of sodium from the intestinal lumen (Roediger et al. 1986); (c) excess $NO^{\bullet}$ (free radical nitric oxide) generation, having vasodilatory effect (Winterbourn et al. 1976; Hsia 1998; Berger et al. 1997; Nitric Oxide 1997), and (d) increased production of $O^{\bullet -}$, which will react with other cell constituents, possibly causing irreversible cell damage (Berger et al. 1997; Gupta et al. 1998) (Fig. 3.3).

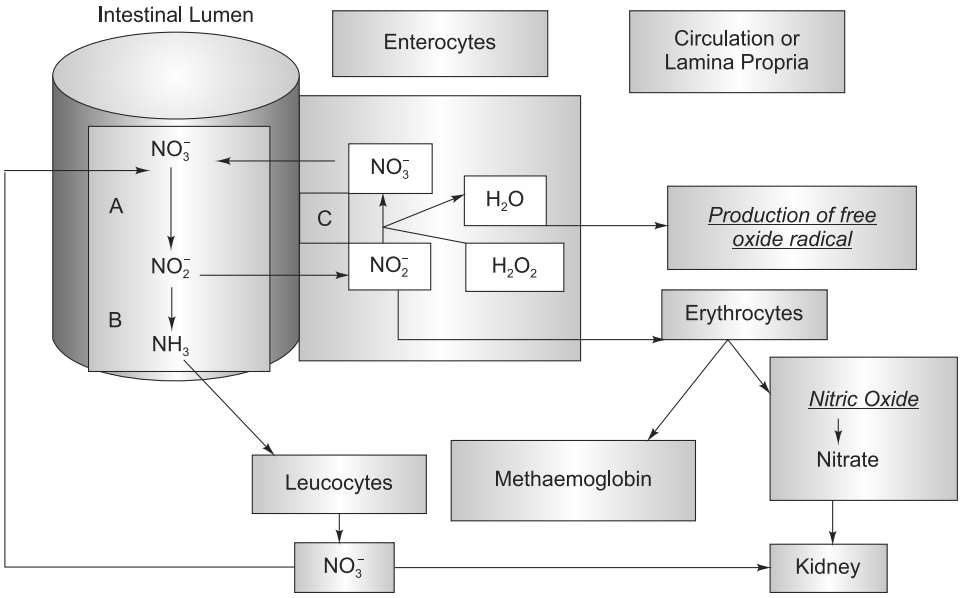


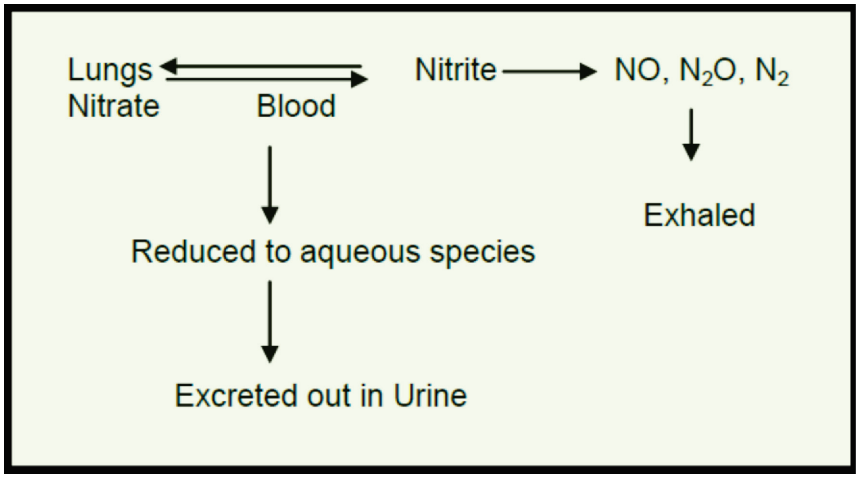
Fig. 3.3. Metabolism of ingested nitrate in human body at cellular level.
A – Bacterial nitrate reductase; B – Bacterial nitrite reductase; C – Catalase

EXCRETION

About 70–75% of ingested nitrate is excreted within 24 hours of ingestion irrespective of the amount of intake. The excretion was noticed high in the first 5 hours. (Bartholomew and Hill, 1984; Wagner et al. 1983b). However, various authors have reported that urinary nitrate excretion may exceed nitrate intake even in infants if the latter is low, as a consequence of endogenous nitrate formation (Hegesh and Shiloas, 1982). Urinary nitrate excretion in infants was reported to be 80–100% of the average intake, but no specific data were given for exposure levels (Turek et al. 1980).

Low levels of nitrate and nitrite were detected in the feces of humans (Saul et al. 1981).

A small amount of ingested nitrate converting to nitrite, changes to oxides of nitrogen (nitric oxide, nitrous oxide, nitrogen, etc.) in blood and gets excreted by exhalation through lungs (as shown in the flow chart given below).



ACUTE TOXIC EFFECTS

Exposure to high nitrate may cause acute toxicity due to acute exposure.

In Humans

Human lethal doses of 4–50 g NO_3^- (equivalent to 67-833 mgNO_3^-/kg BW) have been reported. Toxic doses with methemoglobin formation as a criterion for toxicity ranged from 2 to 5 g (Corré and Breimer, 1979) of NO_3. These values are equivalent to 33–83 and 100–150 mg NO_3^-/kg body weight respectively.

Signs and symptoms of acute nitrate toxicity: Fassett (1973) reported a rapidly occurring severe gastroenteritis with abdominal pain, blood in the urine and feces as symptoms of acute nitrate intoxication. Repeated doses gave rise to dyspepsia, mental depression, headache and weakness. Cyanosis (bluish discoloration) is also present.

In Animal Studies

The studies relating to acute exposure of nitrate were conducted in animals. The acute oral toxicity of nitrate to laboratory animals is low to moderate. LD50 values of 1600–9000 mg of sodium nitrate per kg of body weight have been reported in mice, rats, and rabbits (Til et al. 1988). Ruminants are more sensitive to the effects of nitrate as a result of high nitrate reduction in the rumen; the LD50 for cows was 450 mg of sodium nitrate per kg of body weight. Nitrite is more toxic than nitrate: LD50 values of 85–220 mg of sodium nitrite per kg of body weight have been reported for mice and rats (RIVM, 1989; WHO, 1996).

CHRONIC TOXIC EFFECTS

It is caused due to long-term exposure of non-lethal doses. The following effects have been reported:

Methaemoglobinemia

Nitrates in drinking water have been reported to cause methaemoglobinemia in infants up to 6 months of age.

Mechanism of methemoglobin formation

The essential action in the formation of methemoglobin is an oxidation of ferrous to ferric ion. This oxidation may be brought about in one of the following ways (Bodansky, 1951): by the direct action of the oxidants, or by the action of hydrogen donors in the presence of oxygen, or by auto oxidation. In the presence of nitrites, the ferrous ion of hemoglobin gets directly oxidized to ferric state. Normally, the methemoglobin that is formed is reduced to hemoglobin by cytochrome b_5/ cytochrome b_5 reductase system as follows (Benz, 2001):

$$Hb^{+3} + \text{Red cyt } b_5 \longrightarrow Hb^{+2} + \text{oxy cyt } b_5$$

Oxidised cytochrome b_5 (Red Cyt b_5) is regenerated by the enzyme cytochrome b_5 reductase:

$$\text{oxy cyt } b_5 + \text{NADH} \xrightarrow[\text{Reductase}]{\text{cyt } b_5} \text{Red cyt } b_5 + \text{NAD}$$

Cytochrome b_5 reductase is a NADH dependent enzyme. NADH is supplied from glycolysis (Fig. 3.4).

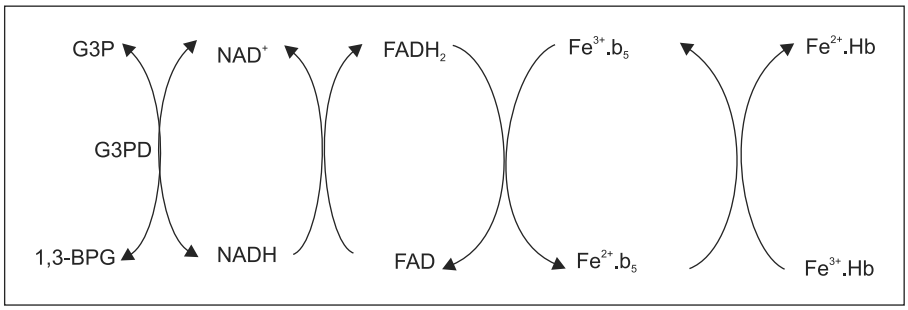


Fig. 3.4. Reduction of methemoglobin (Nagel 2007).

Thus, the enzyme cytochrome b_5 reductase plays a vital role in counteracting the oxidative effects of nitrate ingestion. However, permissible concentration (50 mg/L as nitrate as per (WHO, 2004) normally present in the water or food does not cause any health risk to adults, but infants constitute a vulnerable group for the following reasons (WHO 1977b):

- Relatively higher stomach pH (2.0–5.0), which permits the growth of nitrate reducing organisms such as *Coliforms, E. coli, Pseudomonas fluorescence, B. subtilis, Staph. albus*
- Relatively higher consumption of water per unit weight of body

- The presence of fetal Hb, which readily gets oxidized to methHb.
- Poorly developed cytochrome b_5 reductase system
- Nitrate gets concentrated by repeated boiling of water for feeding
- Bacterial contamination of the water itself or dried milk powder
- Early weaning on to nitrate rich vegetables, e.g. spinach
- Diarrhea causes increase in stomach pH

In India, breastfeeding, which is a common practice especially up to the age of 6 months, protects the infants from nitrate toxicity. The probability of toxicity to fetus in pregnant women cannot be ruled out as transplacental passages of nitrate metabolite (nitrite) have been documented in animal experiments.

While a few cases of methemoglobinemia in infants have been reported to be associated with water nitrate levels of less than 50 mg nitrate ion/L, most cases occur with nitrate level of 90 mg nitrate ion/L or more (Comly 1945; Cornblath et al. 1948; Jaffe 1981; Marshall et al. 1945; Knotek et al. 1964; WHO 1977a). Recently methemoglobinemia has been reported in all age groups (Gupta et al. 2000). It was observed that methemoglobinemia was prevalent among all age groups, but was more severe in infants and older age groups (> 45 years).

Once methemoglobin levels in the blood exceeds 10% of total hemoglobin, it manifests as clinical cyanosis and causes cellular anoxia. The effects of methemoglobin (in relation to %) and clinical presentation are given below (WHO 1977c):

Meth-Hb	Clinical presentation
<10%	No signs and symptoms
10-25%	No symptoms, cyanosis present
25-50%	Cyanosis, dyspnoea, headache
50-60%	Dyspnoea even on lying, cyanosis, disorientation
60%	Lethal levels

Cytochrome b_5 Reductase Adaptation

In several Indian villages, people have been consuming water containing high nitrate concentrations, at times up to 1200 mg nitrate ion/L. High nitrate concentrations are causing methemoglobinemia in all ages (Gupta et al. 2000). It was observed that this methemoglobinemia was more in infants and elderly people (Gupta et al. 2000). Children and adolescents have lower levels of methemoglobin. Adaptation to the reserve of cytochrome b_5 reductase activity with increasing water nitrate concentration to compensate methemoglobinemia was reported to be a possible mechanism. The study (Gupta et al. 1999b) indicated that high nitrate concentrations are hazardous not only to infants but also to groups over 18 years of age. It was reported that adaptation of cytochrome b_5 reductase activity peaks at about 95 mg nitrate ion/L nitrate concentration and gets exhausted by nitrate level 200 mg nitrate ion/L, hence making people more prone to toxicity.

Infant Mortality Rate (IMR)

A study (Super et al. 1981) on African mothers and other studies also (Spalding and Exner 1993; CDC 1996; Dorsch et al. 1984; Fewtrell 2004; Schwiede 2005) reported an increase in infant deaths with increasing exposure of pregnant mothers and infants to nitrate. This may either be due to undetected toxic methemoglobinemia or malformations and weaknesses in the infant caused by fetal nitrate exposure.

It was further suggested that because of high IMR there is a need to revise the nitrate standards for drinking water (Kumar S. et al. 2002).

Nitrate, Nitrite, Nitrosamines and Cancer

Nitrate itself is not carcinogenic, but instead acts as a "procarcinogen", meaning that it reacts with other chemicals (amines and amides) to form carcinogenic compounds (N-nitroso compounds). Under acidic conditions, nitrites (NO^{2-}) are formed naturally from nitrates, and nitrites, in turn, may combine with amines or amides to form N-nitroso compounds (nitrosamines). The endogenous formation of carcinogenic N-nitroso compounds can occur following ingestion of nitrate from drinking water. Nitrate is first reduced in the saliva to nitrite, which can react in the stomach with secondary amines and amides. The nitrosamines most commonly reported in foods are dimethylnitrosamine, diethylnitrosamine, nitrosoproline and nitrosopyrrolidine. Nitrosamines can also be found in some alcoholic beverages and tobacco products.

In animal or human studies, N-nitroso compounds have been associated with 15 different types of cancers, including tumors in the bladder, stomach, brain, esophagus, bone and skin, kidney, liver, lung, oral and nasal cavities, pancreas, peripheral nervous system, thyroid, trachea, acute myelocytic leukemia, and T and B cell lymphoma. These group of N-Nitroso compounds are found to be carcinogenic in a wider range of tumors than any other group of carcinogens (NAS 1977, 1978; IARC 1978; Mirvish, 1991 and 1983). More than one hundred of these N-nitroso compounds have been tested for carcinogenicity in animals, and 75–80% of them have been found to be carcinogens (NAS 1977). IARC (1978) concluded that 11 N-nitroso compounds were carcinogenic in man. In humans, the organs thought to be most at risk from cancer are the stomach, esophagus, nasopharynx, and bladder.

To summarize the carcinogenesis due to high nitrate ingestion is multifactorial. The possible mechanisms may be:

1. Increased formation of nitrosamines (known carcinogen), a metabolic intermediate product of nitrate metabolism.
2. Increased formation of free oxide radicals
3. Interference in NO metabolism

Still there are studies (Hill et al. 1973; Armijo et al. 1975; Cuello, 1976; Zaldivar et al. 1977; RCEP 1979; Fraser et al. 1980) which have drawn no firm conclusion to prove nitrate as a carcinogen.

Common cancers reported due to increased consumption of nitrates are:

1. Gastric cancer (Jaskiewicz et al. 1990; Farinati et al. 1989; Vermeer et al. 2001; Dallinga, 1998)
2. Non-Hodgkins lymphoma (NHL) (Weisenburger, 1990)
3. Colorectal cancer (De Roos et al. 2003 ; Vander et al. 1994)
4. Urinary bladder cancer (Michaud, 2001; Weyer et al. 2001).

Pathophysiology of carcinogenesis due to NO and free oxide radicals

Cancer is the endpoint of a multistep process that includes three fundamental components: initiation, promotion, and progression (Fig. 3.5). Arginine is involved in a number of biosynthetic pathways that significantly influence carcinogenesis and tumor biology. Since the discovery that arginine metabolism generates a ubiquitous signal transduction molecule, nitric oxide (NO), 3 arginine-derived NO has been found to play a significant role in many of the specific events that lead to cancer.

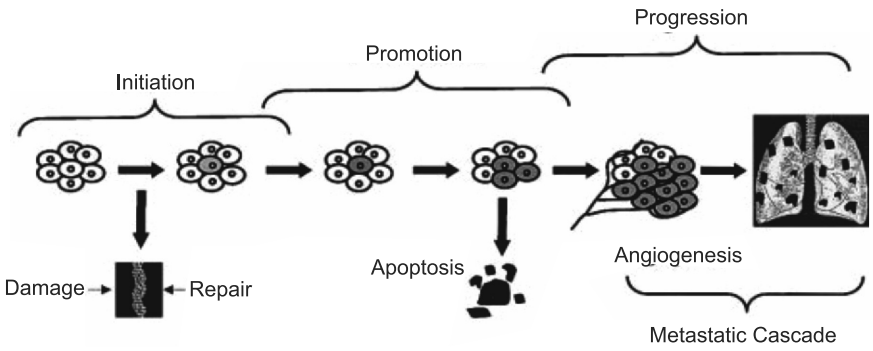


Fig. 3.5. Simplified diagram of the multi-step process resulting in cancer.

Hence, there is a possibility of cancer development in the conditions where NO production is enhanced, e.g.,

1. High nitrate ingestion as has been observed, increases the rate of colorectal cancer
2. Use of silenafil citrate
3. Drugs which have good amount of L arginine
4. Inhibition of cancer growth by NOS inhibitor L-NAME.

Respiratory System

A correlation between drinking water nitrate concentration and pathological changes in bronchi and lung parenchyma has been reported in animal studies (Shuval et al. 1972; Gruener et al. 1970). Changes in the lungs reported were frequent dilation of bronchi with lymphocytic infiltration of mucosa and muscles, frequent purulent bronchial exudates, interstitial round cell infiltration and fibrosis at certain areas.

WHO (1977d) reported an association of increased asthmatic attacks and high airborne nitrate concentrations. A high percentage (40–82%) of cases of acute respiratory tract infection with history of recurrence has been reported in children drinking high nitrate in water (Gupta et al. 2000). These findings were further substantiated (Gupta et al. 1999c) in an animal experiment on rabbits.

NOx, tobacco and malignancy

Tobacco use causes 20% of cancer deaths worldwide. The International Agency for Research on Cancer predicts 10 million tobacco-related deaths annually by 2020, of which 70% will occur in the developing world (IARC, 2004).

This study conducted by Sleiman et al. (2010) reported that residual nicotine from tobacco smoke sorbed to indoor surfaces reacts with ambient nitrous acid (HONO) to form carcinogenic tobacco-specific nitrosamines (TSNAs). Substantial levels of TSNAs were measured on surfaces inside a smoker's vehicle. Time-course measurements revealed fast TSNA formation, with up to 0.4% conversion of nicotine. Given the rapid sorption and persistence of high levels of nicotine on indoor surfaces—including clothing and human skin—this recently identified process represents an unappreciated health hazard through dermal exposure, dust inhalation, and ingestion. Whereas direct inhalation of SHS is an exposure pathway of concern, nonsmokers, especially infants, are at risk through contact with surfaces and dust contaminated with residual smoke gases and particles (Matt et al. 2004).

N-nitrosamines were found to be unstable in sunlight, rendering the reaction unimportant in outdoor daytime conditions. However, this process can be relevant indoors where N-nitrosamines and HONO are less vulnerable to photochemical decomposition.

Nitrate and animal experiments

Rabbit studies in lungs (Gupta et al. 1999) indicated no damage at 100 ppm nitrate in drinking water. As the nitrate concentration in water increases to 400 ppm (Fig. 3.6), the changes in lungs indicated in the form of congestion, presence of inflammatory cells and breakdown of alveoli.

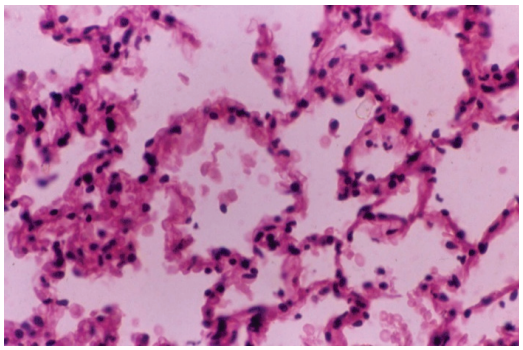


Fig. 3.6. Histopathological changes in lung at 400 ppm (Hematoxylin and eosin, x 400, Gupta et al. 1999).

CARDIOVASCULAR SYSTEM

The cardiac toxicity of nitrate is mediated through its metabolic effects and metabolic products, e.g., methemoglobin formation, increased NO formation, increase in free oxide radicles (ROS) and high levels of compensatory enzymes (Cytochrome b_5 reductase etc.). The following effects have been reported:

1. Early onset of hypertension
2. Inflammation and degeneration of cardiac musculature
3. Causing endothelial dysfunction
4. Making the person prone for ischemia
5. Enhances arteriosclerotic and atherosclerotic process

Early Onset of Hypertension

Earlier onset of hypertension has been reported with high nitrate ingestion (Malberg, 1978). Pomeranz et al. (2000) reported that elevated salt and nitrate levels in drinking water cause an increase of blood pressure in school going children.

Inflammation and Degeneration of Cardiac Musculature

The nitrate toxicity on cardiovascular system has been reported in both animals and human beings. Animal studies in early seventies (Shuval et al. 1972; Gruener et al. 1970) reported correlation among drinking water nitrate concentration, high methemoglobin levels and cardiac muscles (Shuval et al. 1972; Gruener et al. 1970). The changes reported in cardiac muscles were small foci of inflammatory cells and fibrosis, diffuse interstitial cellularity with pronounced degenerative foci, intramural coronary arteries resulting in thinning and dilation in comparison to the control group.

In an animal experiment (Gupta et al. 1999), changes in the cardiac muscles with different concentrations of nitrate in water are given below (Table 3.2).

Table 3.2: The effect of nitrate present in the drinking water on cardiac muscles

Water nitrate (mg/L)	Branching of myosites	Inflammatory cells	Focal degenerative changes
45	NIL	NIL	NIL
100	+	NIL	NIL
200	+	NIL	NIL
400	+	+	+
500	+	+	+

(+) Mild, (++) Moderate, (+++) Severe, NIL: Normal histopathology (No change observed)

Figure 3.7 depicts the changes in the cardiac muscle in the rabbits fed with water containing 400 mg of NO_3 ion per liter (Fig. 3.7). The figure shows branching of this is a medical terminology and is correct. With focal degenerative changes.

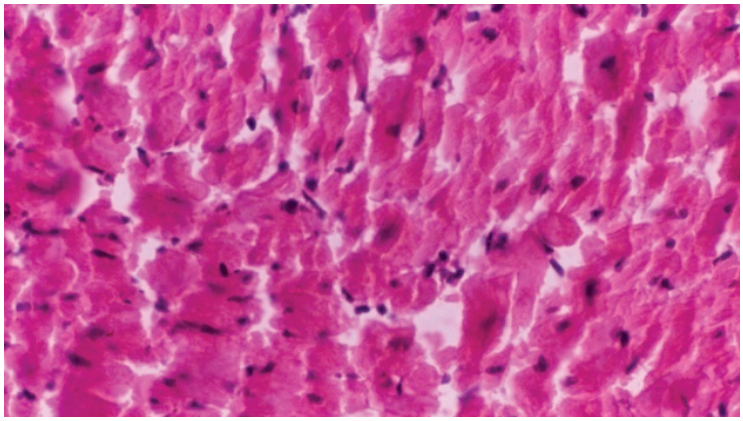


Fig. 3.7. Histopathological changes in the cardiac muscle at 400 ppm (Hematoxylin and eosin, x 400, Gupta et al. 1999).

Endothelial Dysfunction

Endothelial dysfunction, or the loss of proper endothelial function, is a hallmark for vascular diseases, and is often regarded as a key early event in the development of atherosclerosis. An association has been observed between the increase in plasma nitrite/nitrate levels and an excessive production and/or inactivation of NO leading to impaired vascular smooth-muscle reactivity resulting in impairment in endothelium dependent, shear stress-induced vasodilatation.

Making the Person Prone for Ischemia

Nitrate and nitrite have emerged as a viable alternative source of NO under ischemic conditions. NO has been shown to be one of the most important molecules for the prevention of injury from ischemia. Paradoxically, in conditions of inadequate oxygen, the NO is dangerous and may be the cause of ischemia. (Garg and Bryan 2009).

Atherosclerosis

Nitrate is a source of ROS in human body. ROS is known to cause enhanced atherosclerosis. Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized (ox-LDL) by free radicals, particularly reactive oxygen species. When oxidized LDL comes in contact with an artery wall, a series of reactions occurs to repair the damage caused to the artery wall by oxidized LDL. The LDL molecule is globular-shaped with a hollow core to carry cholesterol throughout the body. Eventually, the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover causes narrowing of the artery, reduces the blood flow, and increases blood pressure (Duguid 1960).

GASTROINTESTINAL SYSTEM

In gastrointestinal system, four major findings have been reported:
1. Recurrent diarrhea in children
2. Recurrent stomatitis
3. GI malignancy
4. Histopathological changes in animal study

Recurrent Diarrhea in Children

Gupta et al. (2001) reported a problem of recurrent diarrhea in children upto 8 years of age. They suggested that increased consumption of nitrate leads to (a) increased production of nitrite (Allison et al. 1984; Cole et al. 1980); (b) enhanced absorption of sodium from the intestinal lumen (Roediger et al. 1986); (c) excess $NO^{\bullet}$ (free radical nitric oxide) generation, having vasodilatory effect (Winterbourn et al. 1976; Berger et al. 1997; Nitric Oxide 1997; Smith et al. 1997; Hsia 1998); and (d) increased production of O_2^-, which will react with other cell constituents, possibly causing irreversible cell damage (Berger et al. 1997; Gupta 1998). These changes in enteric mucosa cause hyperemia and edema in the enteric mucosa and later on possibly cause irreversible mucosal damage, and therefore provide high-risk conditions suitable for recurrent diarrhea.

These findings are of interest since infants and children, if given nitrate rich water with ORS (Oral Rehydration Solution) during diarrhea, it will act as aggravating factor for nitrate toxicity (Murray et al. 1993), where the use of WHO oral rehydration solution (ORS) is a normal routine. The use of WHO ORS could be of grave concern, if prepared with water containing high nitrate.

Recurrent Stomatitis

Recurrent stomatitis was another problem reported in people using high nitrate containing drinking water. This finding was observed in all groups and well correlated with increased cytochrome b_5 reductase activity following high nitrate ingestion. The increased activity of enzyme cytochrome b_5 reductase is associated with stomatitis (Gupta et al. 1999a). Another possible cause may be the production of NO, which is a metabolic product of nitrates and a known inflammatory agent.

Histopathological Changes in Animal Study

Animal studies conducted by Gupta et al. (2001) indicated changes in the structure of liver and intestine with high nitrate ingestion. Further, it was observed that the pathological changes were more with increasing nitrate concentrations. The changes observed were as follows:

Liver

The chages in liver with increasing nitrate ingestion are given in Table 3.3 and Fig. 3.8.

Table 3.3: Different nitrate dosage sand histopathological changes in the liver

Nitrate (ppm)	Histopathological changes
100	HP
200	HP, Granular degeneration, mild congestion and inflammation
400	HP, Congestion and inflammation of portal tract
500	HP, Congestion, degeneration and inflammation of portal tract

HP: High power magnification (400X) on microscopic examination

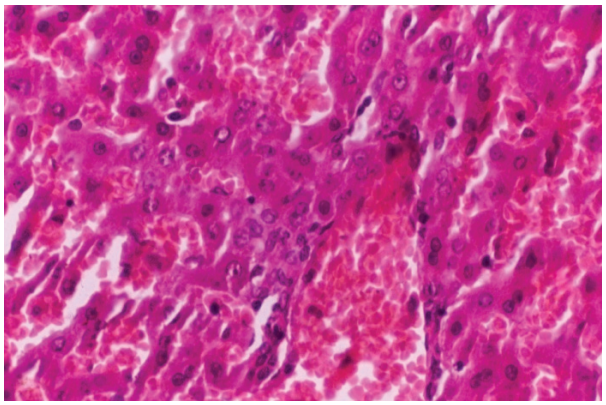


Fig. 3.8. Histopathological changes in the liver at 500 ppm (Hematoxylin and eosin, x 400, Gupta et al. 2001).

Intestine

The histopathological changes in the intestine were not observed at 100 ppm nitrate, but as the nitrate concentration in drinking water increases from 100 ppm, focal collection of inflammatory cells starts at submucosa with slight lymphoid hyperplasia, which was more pronounced as the water nitrate concentration reaches 400 ppm, and at this level, degenerative changes also start (Fig. 3.9).

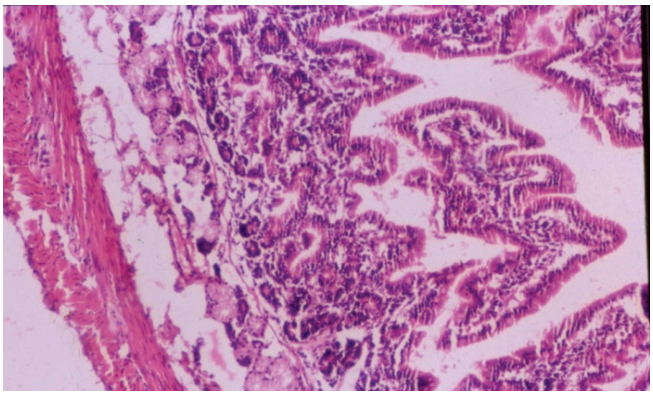


Fig. 3.9. Histopathological changes in the intestine at 400 ppm (LP) (Hematoxylin and eosin, x 100, Gupta et al. 2001).

ABORTIONS

Health effects associated with the ingestion of nitrate-contaminated water include stillbirth, low birth weight, and slow weight gain and even death of the affected animals (Committee on nitrate accumulation, 1972). Spontaneous abortions were also observed in laboratory animals and livestock (Sund et al. 1957; FDA 1972). In 1959, for the first time in humans, spontaneous abortions were reported to have an association with increased methemoglobin levels due to high nitrate ingestion (Muhrer 1959). In subsequent years, some studies (Schmitz 1861; CDC 1996; Fewtrell 2004) found an increased risk of spontaneous abortion or certain birth defects if the mother drank water high in nitrate. Therefore it has been suggested that women who are pregnant or are trying to become pregnant should not consume water containing high nitrate.

BIRTH DEFECTS: MALFORMATIONS

The risk of birth defects due to nitrate exposure is a particular concern because of the fact that it could be due to a single high dose of nitrate early in the pregnancy, which has profound effects on long-term fetal development. Animal studies have indicated that there is a transplacental transfer of N-nitroso compounds to the fetus (Shuval and Gruener 1972) and this fetal exposure can cause cancer later in life (Druckrey 1966).

Extending these findings on human beings, a number of human epidemiology studies were conducted (Knox 1972; Super 1981; Dorsch 1984). A link was found between anencephaly rates and intake of cured meat containing high levels of nitrite (Knox 1972). This study provided the first suggestive evidence in humans that nitrite consumption in food could have adverse impacts on the fetus. A 1984 study (Dorsch et al. 1984) found statistically significant dose response relationships between birth defects of the central nervous system and musculoskeletal system and increasing nitrate concentration of drinking water.

DIABETES

A positive correlation between high nitrate levels in drinking water and increased incidence of type 1 diabetes was observed independently from the length of mother's education, child's or mother's age, place of residence or mother's smoking status (Kostraba 1992; Virtanen et al. 1994; Parslow et al. 1997; Van Maanen et al. 2000). Further studies indicated that consumption of high levels of N-nitroso compounds (NOCs) by human mothers may result in an increased incidence of type 1 diabetes in male offspring (Helgason et al. 1981). In animal studies, NOCs have been reported to be toxic to pancreatic beta cells, providing the rationale for these observations (Wilson et al. 1983). Kostraba (1992) postulated that exposure to nitrate in drinking water causes increased production of free radicals, which may play a role in the etiopathogenesis of insulin-dependent diabetes mellitus (IDDM) (Verge 1994; Mandrup-Poulsen 1996).

THYROID

The thyroid gland contains a iodine trapping transport mechanism, which is accomplished by a membrane protein, the sodium-iodine symporter, which provides sufficient iodine substrate for hormone formation. This trapping mechanism for iodine is shared by other monovalent anions, including pertechnetate, perchlorate, thiocyanate and nitrate, and it is reported that relative potency of perchlorate for inhibiting iodine uptake is 15 and 240 times greater than that of thiocyanate and nitrate, respectively, on a molar concentration basis in serum (Tonacchera M. et al. 2004). Due to inhibition of this trapping mechanism, chronic nitrate exposure causes an inhibition in the accumulation of iodine in the thyroid gland and consequently it may result in thyroid malfunction, causing higher relative risk of goiter (Vladeva et al. 2000) in children, more volume (Van Maanen et al. 1994; Tajtakova M et al. 2006) and weight (Eskiocak 2005; Eskiocak et al. 2005) of thyroid gland in children as well as in adults, and higher frequency of hypoechogenicity (Tajtakova et al. 2006) of thyroid gland. Histomorphological changes reported are retention of lobular architecture, prominent vascular congestion, follicular hyperplasia, a vacuolization and an increase in the colloidal volume (Eskiocak et al. 2005) (Fig. 3.10).

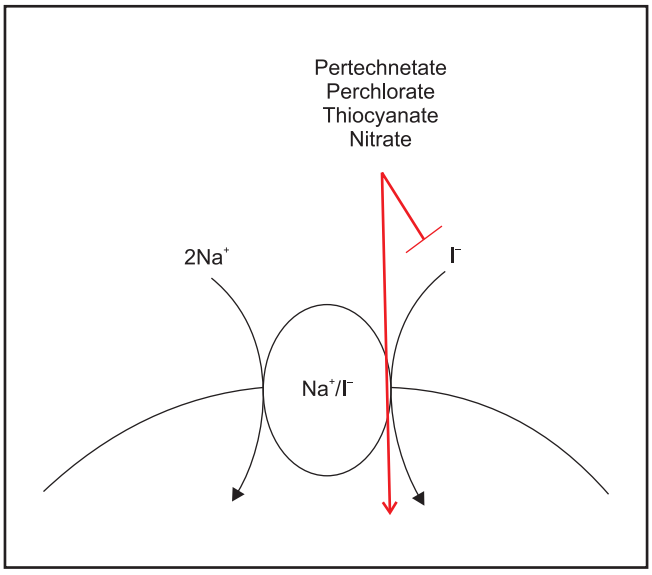


Fig. 3.10. Competition with iodine for sodium-iodine symporter resulting in decreased iodide uptake by thyrocytes.

Several investigators showed that low dose or short-term nitrate intake causes a decrease in thyroid radioiodine uptake (Szokeova et al. 2001), whereas Eskiocak et al. (Eskiocak 2005; Eskiocak et al. 2005) reported that high dose and long-term nitrate exposure results in an increase in the thyroid radioiodine uptake. These findings suggest that the effect of nitrate on thyroid iodine uptake is dose dependent and the inhibition of thyroid iodine uptake may be stronger at higher amounts of nitrate.

In human studies, Van Maanen et al. (1994) reported decreased in TSH levels, whereas Tajtakova et al. (2006) reported an increase in TSH. A decrease in total and free T_3 and T_4 levels at high dose with long-term nitrate exposure (Eskiocak 2005; Eskiocak et al. 2005) has been reported, whereas Szokeova et al (Szokeova et al. 2001) demonstrated that short-term nitrate administration may result in a significantly higher serum level of total T_3.

These findings indicate that short or long-term nitrate exposure may be strongly responsible for the prominent change in thyroid hormone production.

NOx (NITRITE/NITRATE) AND NEPHROTIC SYNDROME

NOx levels in serum obtained from patients with nephritic syndrome showed significantly higher levels than those of healthy controls. Balat et al. (2000) measured plasma and urinary, total NOx in children with minimal change nephritic syndrome (MCNS). In comparison with healthy controls, children with MCNS had increased urinary nitrite excretion. Plasma nitrite levels were high in relapse compared with controls (Balat et al. 2000). Trachtman et al. (1996) also reported that patients with MCNS had increased urinary nitrite excretion regardless of whether the disease was in relapse or remission.

An experiment on rabbit indicated increasing damage to kidney with increasing nitrate concentration in drinking water of the animal (Gupta et al. – unpublished data). Histopathological changes at different nitrate concentration have been depicted in Table 3.4 and Fig. 3.11 given below.

Table 3.4: Different nitrate dosages and histopathological changes in the kidney

Nitrate (ppm)	Histopathological changes
100	HP, Tubular changes, Necrosis (+++)
200	HP, Tubular changes, Eosinophilic changes
400	HP, Tubular changes, Eosinophilic changes, granular changes, increased cellularity

HP: High power magnification (400X) on microscopic examination

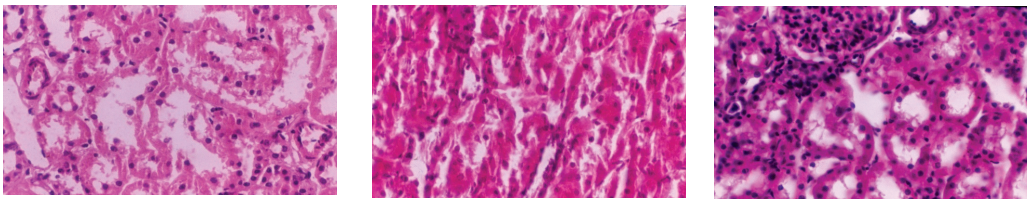


Fig. 3.11. Histopathological changes in kidneys at different nitrate concentrations. 100, 200 and 400 ppm nitrate intake group at A, B and C, respectively. (Hematoxylin and eosin, x 400, Gupta et al. unpublished data) Matter to present in BOX (1.1)

ADRENAL GLAND

High nitrate ingestion affects the adrenal gland. In addition to the effect of nitrite on the adrenal zona glomerulosa in rats, a study in humans indicated that sodium nitrite (0.5 mg of sodium nitrite per kg of body weight per day, during 9 days) causes a decreased production of adrenal steroids, as reflected by the decreased concentration of 17-hydroxysteroid and 17-ketosteroids in urine (Til et al. 1988; Kuper and Til 1995). Similar results were also found in rabbits (Violante et al. 1973). Although the mechanism is not clear, the effects of nitrite seen in rats seem relevant for the hazard assessment in humans.

IMMUNITY

Few studies (CDFA 1989; Kozliuk et al. 1989; Ustyugova 2002) reported the effect of nitrate/nitrite ingestion on immune system. The effect of nitrate ingestion (Ustyugova 2002) on human immune system indicated that nitrate had no effect on lymphocyte growth, but nitrite decreases the proliferation of lymphocytes. Fibroblast growth remains unaffected. A decreased production of Th1 cytokines (interleukin-2, interferon-gamma, and tumor necrosis factor-beta), which is responsible for resistance to a variety of infectious diseases was noted. No effect on the production of Th2 cytokine interleukin-10, which is responsible for disease susceptibility, was noted. Since nitrate/nitrite shifted the balance from a Th1 to a Th2 response in some individuals, exposure to these compounds may decrease these people's responsiveness to infectious diseases. The levels of nitrate used in this study are relevant to human health because they are present in the liquid portion (non-breastfed) of some 2-month-old infant diets in rural Romania. Animal studies also reported an immune suppression due to high nitrate ingestion (Porter et al. 1999).

AIR POLLUTION

Inhalation of NOx causes a wide variety of health and environmental impacts because of various compounds and derivatives in the family of nitrogen oxides, including nitrogen dioxide, nitric acid, nitrous oxide, nitrates, and nitric oxide. It reacts to form nitrate particles, acid aerosols and contributes to formation of acid rain.

Health effects are related to levels of NOx as well as the duration of exposure (CEOHAATS 1996; Bernstein et al. 2004). Low levels of nitrogen oxides in the air irritate eyes, nose, throat, and lungs leading to cough and shortness of breath, tiredness, and nausea. Breathing high levels of nitrogen oxides can cause rapid burning, spasms, and inflammatory swelling of tissues in the throat and upper respiratory tract. High exposures may lead to pulmonary edema leading to hypoxemia and even death. Industrial exposure to nitrogen dioxide may cause genetic mutations, damage a developing fetus, and decrease fertility in women. Industrial exposure to nitric oxide can cause unconsciousness, vomiting, mental

confusion, and damage to the teeth. So far, there is no evidence that nitrogen oxides are potential carcinogens.

A review of studies published in the last decade has shown urban pollution to be environmental cardiovascular risk factors (Maitre et al. 2006). This link was significant for NOx and PM10. A study of short-term effects of nitrogen dioxide on total, cardiovascular and respiratory mortality in 30 European cities found significant association between the two (Samoli et al. 2006). Significant associations of daily changes in particle concentrations, nitrogen dioxide and carbon monoxide were found with hospitalization for respiratory diseases (COPD, pneumonia, asthma) and cardiovascular diseases (Hinwood et al. 2006) . Exposure to indoor NO_2 at levels well below the Environmental Protection Agency outdoor standard (53 ppb) were associated with respiratory symptoms among children with asthma. Each 20-ppb increase in NO_2 increased both the likelihood of any wheeze or chest tightness and days of wheeze or chest tightness (Belanger et al. 2006).

The EPA has established that the average concentration of nitrogen dioxide in ambient air in a calendar year should not exceed 0.053 parts of nitrogen dioxide per million parts of air (0.053 ppm). The Occupational Safety and Health Administration (OSHA) have set a limit of 25 ppm of nitric oxide in workplace air during an 8-hour workday, 40-hour work week. OSHA has also set a 15-minute exposure limit of 5 ppm for nitrogen dioxide in workplace air.

ANIMAL EXPERIMENTS

Clinical manifestations of nitrate experiments on different systems are as follows:

Central Nervous System

The results of experiments on mice indicated a nitrate-induced deviation in behavioral development, and impairment in learning behavior, particularly of the discriminative type (Markel et al. 1989).

Gastrointestinal System

In a 19-month study on mice, the effects of nitrate on gastric epithelium (Ptashekas 1990) indicated atypical changes in the gastric epithelium.

Cardiovascular System

Animal studies (Gruener et al. 1970; Shuval et al. 1972) reported increased cardiac muscle contraction, which correlated directly to methHb levels. At 10–15% methHb, ECG shows shortening of Q-T interval and reduction in T wave.

Embryo Toxicity

Experiments on rats showed anemia, increased meth Hb, and increased mortality in offspring. (RIVM 1989; WHO 1996).

Genotoxicity

In an animal experiment on mice, an increase in chromosome aberrations was found in a group of animals, who were consuming nitrate more than 707 mg/kg BW. (Luca et al. 1985).

Reproductive Behavior

Reproduction in female guinea pig (RIVM 1989; WHO 1996) was grossly impaired by high nitrate ingestion (30,000 mg of potassium nitrate per liter).

Mutagenicity and Related End-Points

Nitrite is mutagenic. It causes morphological transformations in *in vitro* systems. (RIVM 1989; WHO 1996).

Serum Somatomedin Activity

A decrease in serum somatomedin activity due to nitrate administration was also observed (Jahreis et al. 1987).

TREATMENT AND PREVENTION

Treatment

Supplementation of ascorbic acid, methionine, alpha-tocopherol, and methylene blue has been found to be effective in overcoming the problem of nitrate toxicity. These substances are effective in inhibiting nitrosation if present in gastric juices at all times in significant concentrations.

Role of Antioxidants

As ROS are derived from a number of sources including nitrates, it was deemed feasible to readjust the balance of ROS production and detoxification by supplementing antioxidants. How to prevent or treat this oxidative stress? (Armitage et al. 2009).

However, clinical trials testing this hypothesis showed little benefits in reducing respiratory, GIT, cardiovascular etc. events or mortality; some of the treatments even caused harm (Dotan et al. 2009).

Indeed, *in vivo* detoxification of increased ROS by antioxidant supplementation may be impossible due to the poor bioavailability of antioxidants at the correct subcellular localization and at the optimal time point. Antioxidants may also promote new radical chain reactions initiated by their oxidized forms.

Importantly, the global removal of ROS may result in unwanted effects since ROS also regulate important physiological functions. Therefore, the current strategy is to identify and target the major sources of ROS, including NADPH oxidases

(NOx). A major constraint in NADPH oxidase research and translation is the lack of specific inhibitors.

Prevention

At environmental level

Avoid water pollution

- Minimize the contamination of water supplies by nitrates originating from agricultural practice: Avoid inadvertent and excessive use of nitrogenous fertilizers
- Avoid water pollution
- Avoid the habit of open air defecation
- Avoid the stagnation of waste water around the source of water
- Avoid the sewage disposal directly to groundwater table

Removal of nitrate from drinking water—Denitrification

The removal of nitrate from water is costly and difficult to implement and maintain both at domestic as well as community level. The following are the known processes of denitrification:

a. Physiochemical process—The commonly used processes under this category are (a) Reverse osmosis (b) Ion exchange (c) Electrodialysis
b. Biological Treatments—This process is important since it bears similarity with the metabolism of nitrates in living organisms. (Details of these processes have been depicted in annexure II)

At human level

- Breastfeeding only, at least upto the age of 4 months.
- If top feed is necessary, then preferably use cow milk (of course unadulterated) or dry milk preparations reconstituted with water containing low level of nitrate.
- Avoid the use of high nitrate containing food for weaning.
- Avoid the use of nitrate and nitrite as preservative to the minimum extent possible especially to cured and canned meats and fish.
- Use of drinking water with low level of nitrates especially by pregnant mothers.
- Avoiding WHO ORS preparation with locally available high nitrate water during diarrhea.
- Avoid long-term use of anti-acid secretory agents especially in pregnant mothers and children. In case it is necessary to use these drugs, they should be used cautiously and preferably with the use of antioxidants.

Reducing nitrate levels in vegetables

The following hints will reduce nitrates in vegetables. The problem is that some of them will reduce essential nutrients such as vitamin C as well.

- Washing and cooking in water (nitrates are soluble in water).
- Peeling, e.g. in potatoes because the nitrates are concentrated in the skin and just below (again, this is part of fail-safe eating).
- Discarding: in leafy vegetables such as lettuce, discarding the stem and mid-rib can decrease up to 40 percent of the nitrates
- A study of French fries showed that peeling reduced the levels by 30 percent; preheating and cutting reduced the nitrate content by a further 20 percent and blanching by 30 percent. After final frying only about five per cent of the original nitrate content remained. (Reheating cold cooked vegetables has been shown not to increase nitrate levels as previously thought).
- For homegrown vegetables, choose low nitrate varieties (iceberg lettuce is one of the lowest and is fail-safe); use slower releasing nitrogen sources such as manure and compost; avoid fertilizing just before harvest; grow vegetables outside if possible (lower light due to plastic or glass covering contributes to higher nitrates); harvest in full sun.
- A number of studies have suggested a protective effect of vitamin C and other nutrients against either nitrates or particular cancers. Some experts think that the naturally occurring nutrients in vegetables are enough to protect against effects of nitrates. The WCRF warns: do not use high-dose supplements to protect against cancer. Research shows that high-dose nutrient supplements can affect our risk of cancer, so it is best to opt for a balanced diet without supplements.

CONCLUSION

Nitrate and nitrite are natural ions that are a part of nitrogen cycle. Naturally occurring nitrate levels in surface and groundwater are generally a few milligrams per liter. In many groundwaters, an increase in nitrate levels has been observed due to water percolating through nitrate rich rocks and owing to the farming practices of using chemical fertilizers.

For human consumption, WHO report, 2004 permits up to 50 mg/L but for short-term exposure only, whereas BIS-10500 permits 45 mg/L (as NO_3) as desirable limit and 100 mg of NO_3 per liter as maximum permissible limit in the absence of alternate source.

Not much literature on human studies is available on nitrate toxicity except reports documenting methemoglobinemia in infants due to high nitrate ingestion. Apart from methemoglobinemia, few studies indicated nitrate as a cause of cancer, but it is still controversial and no firm conclusions have been drawn so far. The other effects observed were increased infant mortality, abortions, birth defects, recurrent diarrhea, recurrent stomatitis, early onset of hypertension, ischemia, CVD, histopathological changes in cardiac muscles, alveoli of lungs and adrenal glands, recurrent respiratory tract infection in children, nephrotic syndrome in children, hypothyroidism and diabetes. Recent studies have indicated that high nitrate ingestion adversely affects the immune system of the body. Recently an

adaptation system to nitrate ingestion has also been reported. This adaptation to an enzyme cytochrome b_5 reductase has been shown to be protective to human beings, but only to a limited extent.

More detailed epidemiological health related studies covering a large sample are required to provide insight to the nitrate toxicity in human beings. It is possible that a detailed study on pathophysiology of nitrate metabolism and its effect on human beings would yield a better understanding of the various diseases caused by nitrates, and their prevention and treatment.

Since it is very difficult and costly to remove nitrate from water because it is chemically non-reactive in dilute aqueous solutions, it is recommended that a simple change in habits and adoption of simple preventive measures may be urgently introduced in government campaign at least in high nitrate belts. Indiscriminate use of nitrogenous fertilizers should be avoided. The most important strategy is to promote breastfeeding up to the age of at least 6 months.

ANNEXURE – 1

Nitrates and Nitrites in Vegetables (mg/kg)

Nitrate					
Food	Low	Average	High	Year	Source*
Beans	6	392	810	2008	EFSA
Beetroot	110	1370	3670	2008	EFSA
Brussels sprouts	1	24	100	2008	EFSA
Cabbage	47	311	833	2008	EFSA
Carrots	21	296	1574	2008	EFSA
Cauliflower	7	148	148	2008	EFSA
Celery	18	1103	3319	2008	EFSA
Garlic	8	69	161	2008	EFSA
Green Beans	9	323	735	2008	EFSA
Leek	5	345	975	2008	EFSA
Lettuce iceberg	210	875	1537	2008	EFSA
Parsnip	2	16	83	2008	EFSA
Peas	1	30	100	2008	EFSA
Potatoes	10	168	340	2008	EFSA
Pumpkin	8	894	4617	2008	EFSA
Rhubarb	28	2943	6550	2008	EFSA
Spinach	64	1066	3048	2008	EFSA

Spinach organic	2138	2005	FSA
Lettuce glasshouse	5700	2004	FSA
Lettuce rucola	4800	2008	EFSA
Lettuce curly	3263	2005	FSA
Lettuce iceberg u/cover	2500	2004	FSA
Lettuce organic	1115	2005	FSA
Lettuce	875	2008	EFSA
Lettuce organic	596	1982	SA

http://www.efsa.europa.eu/cs/BlobServer/Scientific_Opinion/contam_ej_689_nitrate_en.pdf
The EFSA maximum permitted level for nitrates in lettuce is 4,500 mg/kg

ANNEXURE – 2

BIOLOGICAL DENITRIFICATION

The biological reduction of nitrate to nitrite and subsequently to dinitrogen gas requires a suitable electron donor. The electron donor is usually an organic molecule, and methanol is the most commonly used carbon source. Equations 1 and 2 represent the reduction of nitrate to nitrite and nitrite to nitrogen gas respectively, and equation 3 represents the overall reaction using methanol as the electron donor (McCarty et al. 1969).

$$NO_3^- + 0.33\ CH_3OH \longrightarrow NO_2^- + 0.67\ H_2O + 0.33\ CO_2 \quad (1)$$
$$NO_2^- + 0.5\ CH_3OH \longrightarrow 0.5\ N_2 + 0.5\ H_2O + 0.5\ CO_2 + OH^- \quad (2)$$

and overall:

$$NO_3^- + 0.833\ CH_3OH \longrightarrow 0.5\ N_2 + 1.16\ H_2O + 0.833\ CO_2 + OH^- \quad (3)$$

Other organic carbon sources such as ethyl alcohol sucrose, acetone, brewery waste, chemical process waste, corn starch waste, molasses, wharf, sulfide liquor and winery residue, etc. are taken as inexpensive carbon substrates.

Autotrophic denitrification has been studied using hydrogen or various sulfur compounds. Autotrophic denitrifying bacteria use molecular hydrogen or other inorganic compounds as reductants and carbon dioxide as the source of carbon as shown in equation below:

$$5\ H_2 + 2H^+ + 2\ NO_3 \longrightarrow N_2 + 6\ H_2O \quad (4)$$

Some researchers have evaluated the use of reduced sulfur compounds such as sulfide and thiosulfate for the denitrification of water and domestic

and industrial wastewater (Sutton et al. 1979; Amminudin and Nicholas 1973). Sulfate is a by-product of denitrification using sulfur compounds as given in the following equations.

Thiosulfate (Claus and Kutzner 1985)

$$5\ S_2\ O_3^{2-} + 8\ NO_3^- + H_2O \longrightarrow 4\ N_2 + 10\ SO_4^{2-} + 2H^+ \quad (5)$$

Sulfide (Barrenstein et al. 1986)

$$5\ S^{2-} + 8\ NO_3^- + 8\ H^+ \longrightarrow 5\ SO_4^{2-} + 4\ N_2 + 4\ H_2O \quad (6)$$

Though oxygen inhibits denitrification, there have been periodic reports of aerobic denitrification (Marshall et al. 1953; Krul 1976). Recently under controlled conditions in homogeneously suspended bacterial cultures at D.O. concentrations ranging from 10% to twice air saturation, persistent denitrification has been reported by many workers (Meiberg et al. 1980; Gupta and Kumar 1999). Robertson et al. (1988) not only detected the presence of appropriate enzyme, but also demonstrated the production of nitrogen containing gases from nitrate by *Thiosphaera pantotropha* (isolated from desulphurizing, denitrifying wastewater treatment systems) at dissolved oxygen concentrations up to 90% of air saturation.

Biological denitrification has a limited role in the treatment of drinking water because introduction of carbon source and bacteria to water for denitrification would increase the post-treatment cost significantly. Several investigators have evaluated the injection of various substrates and nutrients into aquifers in an effort to simulate *in situ* denitrification. Liquid substrates such as acetic acid, ethanol and treated wastewater have been used and gaseous substances have been evaluated (Kruithof et al. 1988; Soares et al. 1988; Gayle et al. 1989). Application of *in situ* groundwater denitrification depends on the prevention of well clogging and biomass accumulation is thought to be the main cause of *in situ* clogging. In shallow aquifers accumulation of gas may represent a major contributor to the clogging of wells. It is important to take certain precautions while designing an *in situ* denitrification scheme, e.g., complete utilization of carbon sources should be ensured since no organic carbon is allowed to be present in drinking water; clogging of aquifer must be anticipated particularly in soil that provide small pore size; release of N_2 gas must be ensured; movement of bacteria in porous media should be controlled by giving due consideration to factors like ratio of cell size and pore size, shape of microorganisms, flow velocity, injection concentration of bacteria, etc. A proper balance of the above factors should be made to ensure uniform dispersion of bacteria in sub-soil. Biological denitrification has been extensively used for the removal of nitrates from

domestic and industrial wastewaters, which can help prevent nitrate contamination of groundwater, however, only certain advancements in this process have been summarized in the subsequent paragraph.

Gupta (1997) gave a comprehensive review of the literature pertaining to enzyme system of *Thiosphaera pantotropha,* which is responsible for the nitrification and denitrification properties and its potential applications for wastewater treatment. Many laboratory studies were carried out in different suspended growth and fixed film systems to bring out low cost options for a simultaneous removal of carbon and nitrogen from different synthetic samples simulating domestic and industrial waste-waters (Gupta and Gupta 1999, 2001; Gupta et al. 1994).

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