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Impact of accumulation of inorganic nitrogen oxides (NO_3 & NO_2) in plants and its implication on human health

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Abstract : Nitrite (NO_2) and nitrate (NO_3) are as a part of nitrogen natural cycle and essential nutrients for plant protein synthesis. Plants take nitrogen in ionic form from soil. Nitrate is a naturally occurring form of nitrogen formed from fertilizers, decaying plants, manure and other organic residues. Due to increased use of synthetic nitrogen fertilizers and livestock manure in agriculture, vegetables and drinking water may contain higher concentrations of nitrate now than in past. Nitrate is also used as a food additive, mainly as preservative and antimicrobial agent. Its level commonly present in food is not toxic to humans but they act as the reservoir for conversion to nitrite by intestinal flora. A high nitrate accumulation in plants results in nitrite production, which then converted into nitric oxide (NO) which together with O_2, could be rapidly catalyzed by nitrate reductase into peroxynitrite ($ONOO^-$). The presence of nitrite and nitrate in food chains is associated with numerous health problems including stomach, gullet and gallbladder cancer and methaemoglobinaemia. This review focuses on the factors for accumulation of nitrate and nitrite in some plants and its potential risks on human health.

Keywords : Nitrate, nitrite, food additive, methaemoglobinaemia, human health

INTRODUCTION

Nitrates are the metals that are present in soils, waters, all plant materials and in metals. They are also found in small concentrations in air as a result of air pollution.[1] Nitrate and nitrite are essential nutrients for plant protein synthesis and play a critical role in nitrogen cycle.[2] Excessive use of nitrogen fertilizers has caused an imbalance of nutrients in the soil which influences the nitrate content of plants.[3,4] Nitrites are formed in nature by the action of nitrifying bacteria as an intermediate stage in the formation of nitrates, but concentrations in plant and water are usually very low.

The nitrate accumulation in plants is a multifactorial process - nutritional, environmental and physiological being the major players. In cereals, light intensity and nitrogen fertilization are major factors.[5] Many nutrients, such as chloride, calcium, potassium, sulphate and phosphorus are also involved in nitrate accumulation, other factors different parts of the plant. This review focuses on the factors affecting accumulation of nitrate, the dietary intake of nitrate and its implications on human health.

Nitrates and nitrites can be categorized into inorganic and organic forms based on their chemical structure. Inorganic nitrate (NO^{3-}) and nitrite (NO^{2-}) are water soluble (as a result of their interaction with the positively charged portions of polar water molecules and commonly exist as salts of nitric acid and nitrous acid, respectively). They are often bound to a metal cation such as Na^+ or K^+ and occur naturally through the fixation of atmospheric nitrogen and oxygen as a part of the environmental nitrogen

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cycle (the cyclic movement of nitrogen in different chemical forms from the environment, to organisms, and then back to the environment. Inorganic nitrites are also produced endogenously through oxidation of nitrous oxide (NO) formed from the enzymatic degradation of L-arginine and through the reduction of nitrate with xanthine oxidoreductase.[6-8]

Vegetables are the major source of the daily intake of nitrate by human beings, supplying about 72 to 94% of the total intake.[9] Under excessive application of nitrogen fertilizers, these vegetables can accumulate high levels of nitrate. Investigations have revealed that high nitrate accumulation in plants results in nitrite production. Nitrite is the compound that is of more health concern.[10]

1. FACTORS RESPONSIBLE FOR NITRATE ACCUMULATION

1.1 Environmental factors

As we know that to fulfill the requirements of nitrogen plants adopts various strategies. Therefore nitrates play an important role in metabolism in higher plants. It is the major nutrient form of nitrogen in most soils and is often the first factor limiting plant growth. In the great majority of cases it is assimilated so rapidly that it's concentration within plant tissues never raises to critical levels.[11] There are several reports where authors have explained the effect of moisture on nitrate concentration. Tisdale and Nelson (1975) and Gomm (1979) reported that waterlogged soils tend to be more anaerobic than moist soils, thus inhibiting microbial conversion of ammonia to nitrate. On the other hand, nitrate is released from the more complex organic forms by microbial activity that requires moisture. The nitrogen thus released, as well as any nitrogen added as fertilizer, must then move, through water, to the absorbing roots.[11-13] Thus a certain amount of moisture is needed in the soil, and either extreme in soil moisture would lead to abnormal conditions. Uptake of nitrate is regarded as very sensitive to temperature because it depends not only on the temperature of the root environment but also upon the temperature of the shoot environment. Santamaria *et al.* (2001) observed an interaction between light intensity, nitrogen availability and temperature on nitrate accumulation in rocket. There is an increase intake of nitrate accumulation at high temperature but low intensity of light. Under high light intensity, an increase in temperature increases the nitrate content but mainly when nitrogen supply is high. The nitrate content was found to be less in the year that had a high rainfall.[14]

1.2 Nutritional factors

During the nitrogen fertilization accumulation of nitrate by the plant tissues are very high. Nazaryuk *et al.* have studied the role of agricultural chemicals in regulating the nitrate accumulation in plants. Nitrate get stored in the vacuole and can be remobilised subsequently when nitrogen supply is insufficient.[15] Accumulation of nitrate in vegetables often depends on the amount and kind of nutrients present in the soil and closely related with the time of application and the amount and composition of the fertilizers applied.[16] Plants accumulate more nitrate as the nitrogen fertilization increases.[17,18] Depending on which plant part is to be consumed, appropriate fertilizer can be selected, eg., the order of nitrate accumulation in cole leaves, upon application of different nitrogen fertilizers is urea, ammonium carbonate, ammonium nitrate, ammonium sulphate; for nitrate accumulation in petioles, it is urea ammonium nitrate, ammonium sulphate, ammonium carbonate.[15]

Accumulation of nitrate is a function of increase supply of nitrate by fertilization and mineralization of soil organic matter. Vegetables supplied with organic fertilizers have a low nitrate content, compared with mineral fertilized or conventionally grown vegetables.[19] Therefore the higher the nitrogen availability (mineral fertilizer >liquid manure = slurry > manure > compost) and the lower the assimilation intensity (e.g. by site conditions and season effects), the greater would be the nitrate accumulation.

Since phosphorous has multiple functions in plant metabolism therefore it has indirect effect on plants. A plant deficient in phosphorus will lack NADPH, on which the nitrogen reducing enzymes depend and any nitrate taken up will thus accumulate.[20] Ahmed *et al.* (2000) have shown a reduction in nitrate content due to increasing phosphorus fertilization. Inorganic phosphorus within the plant is necessary for the metabolism and storage of nitrate, but high concentrations inhibit enzyme reactions, create abnormal pressure in the cell, and accelerate senescence, and the advancing plant age is accompanied by a reduced nitrate uptake and accumulation.[21] Potassium in soils is taken up as an ion by the plant in the K^+ form, and is thus important to the uptake of the nitrate ion (NO3) in maintaining ionic balance. The influence of potassium on nitrate levels is therefore largely catalytic.[22] Potassium is

however, essential in protein synthesis and photosynthesis is decreased with insufficient potassium.

Nitrate accumulation has also been studied in relation to some other chemicals. Foliar application of salicylic acid[21], molybdenum fertilizers[16] and nitrification inhibitors [23,16] and calcium[24] can materially reduce the nitrate content of plants. In lettuce leaves sugar levels and free amino acid concentrations increase under high supply of calcium and replace nitrate in the vacuole of the lettuce cell.[25] There exists a negative correlation between nitrate content and sulfate content in the lettuce plant and, therefore, sulfur deficiency might lead to an increase in nitrate content.

In general, higher nitrate levels are found in plants under any mineral deficiency stress.

1.3 Physiological factors

1.3.1 Genotypic variability

The nitrate content varies markedly with plant species, cultivars of the same species, and even genotypes with different ploidy. The causal factors might include genetic differences among genotypes in enzymes of the nitrogen metabolic pathway (nitrate reductase/nitrite reductase),the rate of nitrate uptake, the rate of uptake of other elements needed for enzyme activity. The differing capacities of nitrate accumulation can also be correlated with differing locations of nitrate reductase activity. The accumulation of nitrate also decreases with increase in carbohydrate concentration in the vacuoles. The sugar concentration and dry matter content of plants are negatively correlated to nitrate accumulation. Thus genotypes with high dry matter content could have high carbohydrate content in their vacuoles and thus need little nitrate to maintain their osmotic value.

1.2.2 Nitrate distribution within the plant

It is well established that nitrate is not uniformly distributed throughout the plant to all the tissues. In terms of grass structure stems usually contain more nitrate than do the roots, roots usually more than the leaves and leaves usually more than floral parts. The upper regions of the stems also tend to have lower nitrate content than the lower regions. The vegetable organs can be listed by decreasing nitrate content as follows: petiole leaf, stem root, inflorescence, tuber, bulb, fruit and seed. The concentration of nitrate in various plant parts could be a measure to evaluate the balance between adequate and excessive available nitrogen for optimum growth of plants during a growth season. Plants that develop fruit or storage organs, such as potato and tomato, usually have low nitrate content in petioles as they approach harvest stage. This decline can be attributed to the translocation of soluble nitrogen to the developing storage organ. Vegetables that do not develop storage organs have a different pattern of nitrate accumulation wherein nitrate often continues to accumulate with the plant's age.

2. DIETARY INTAKE OF NITRATE

Vegetables constitute nearly 72-94 % of the average human dietary intake and are the major source of nitrate. The following plant families are reckoned to belong to the nitrate accumulating group: Amaranthaceae (amaranthus), Chenopodiaceae (beetroot, swiss chard and spinach), Compositeae, Convulvulaceae, Polygonaceae, Solanaceae, Gramineae and Cruciferae.

Human intake of nitrite and nitrate is primarily by dietary ingestion of food crops, vegetables, meat and water. The average adult intake from food has been estimated to be 40-100mg/g for nitrates and 0.03-6 mg/g for nitrites as per WHO's Acceptable Daily Intake (ADI)s. Ysart *et al.* (1999) estimated for the adult human population a total nitrate intake of 93mg /day , normally through potatoes (33%). Green vegetables (21%) , other vegetables (15%), beverages (8.5%), meat products (4.2%) , fresh fruit (3.5%), dairy (3.1%), milk (2.9%), miscellaneous cereals (2.1%), bread (1.6%) and others(5.1%)

3. IMPLICATIONS ON HUMAN HEALTH

3.1 Adverse effects

Naturally nitrate is not toxic for human; however, the conversion of nitrate to nitrite in human body and its accumulation in high concentrations is very dangerous for the body. In digestive tract these substances react with natural amines and produce carcinogens compounds like N-nitrosamine. The N-nitrosamines that are formed in human stomach are known as a risk factor for stomach, gullet and gallbladder cancer.

One of the adverse effects of nitrate is related to combining these compounds with haemoglobin that is present in blood and leads to reducing nitrates to nitrites, which causes a reduction in potential oxygen transport from lungs to body tissues. The principal mechanism involves oxidation of the ferrous iron (Fe^{2+}) in haemoglobin to the ferric state (Fe^{3+}). As a consequence methaemoglobin forms and reduces the oxygen delivery

to tissues. Methaemoglobimea (Blue baby syndromes) may occur in older infants and children due to consuming vegetables. For example in Spain, several infants aged 7-13 months experienced methaemoglobinaemia on consumption of vegetables. Methaemoglobinaemia has been reported to occur in people of different ages with high nitrate ingestion, the infants and above 45 age groups being most susceptible to nitrate toxicity. In pregnant women, the level of methaemoglobin increases from the normal (0.5 to 2.5% of total haemoglobin) to a maximum of 10.5% at the 30th week of gestation and subsequently declines to normal after delivery. Thus, pregnant women might be more sensitive to the induction of clinical methaemoglobinemia by nitrites or nitrates in or around the 30th week of gestation.

Other health problems associated with nitrate toxicity include oral cancer, cancer of colon, rectum or other gastrointestinal regions, Alzheimer's disease, multiple sclerosis, spontaneous abortion or congenital defects.

CONCLUSION

Plants are the major source for nitrogen that consumes it from the soil even under normal circumstances. The accumulation of nitrate means that the rate of assimilation of nitrate to protein has not kept pace with the rate of nitrate uptake from the soil. The use of fertilizers should be however lowered so that nitrate accumulation of the nitrate could be reduced as well as loss of nitrogen to the environment could be minimized.

Since vegetables are considered as the main dietary source of nitrate intake in humans. Therefore breeding of new cultivars that do not accumulate nitrate even under heavy fertilization could be the best way to get rid of.

There are number of evidence regarding the harmful effect of nitrates to human beings. Therefore it is necessary to take preventive measures to reduce the nitrate intake.

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