# Nitrates, agriculture and environment

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Nitrogen is a very important nutrient element in agriculture. In soils it occurs in organic and inorganic forms. Inorganic N occurs primarily as nitrate in arable soils. Nitrate is subject to various processes such as plant uptake, leaching from soils among others. Leaching of nitrates from soils is a global phenomenon. Although a lot of attention has been paid world over on this phenomenon, its importance is being felt recently in developing countries like India where the emphasis has been on the problems related to increased food production from limited cultivable land. However, maintaining delicate agro-ecosystems in order to achieve sustainable agricultural productivity while protecting the environment has attracted the attention of scientists and policy makers. This article discusses the phenomenon of nitrate leaching from soils, its impact on man and animals and means to minimize the leaching. Nitrate leaching could be a major threat to environment in different agricultural situations. By proper management of agricultural systems, these leaching losses could be reduced and pollution problems can be minimized.

# Nitrogen conversions in soil-plant systems

Nitrogen occurs in soil in several forms and inter conversion between these forms is the net result of a large number of dynamic processes (Figure 1). Many of these processes are mediated by micro-organisms. While incorporation of ammonium into organic compounds by microbial assimilation is known as immobilization, the reverse process where micro-organisms oxidize organic matter to produce energy and convert organic nitrogen into inorganic nitrogen is known as mineralization and both these processes occur simultaneously. In most soils, ammonium is rapidly converted to nitrate via nitrite by a process called nitrification, where ammonium is oxidized to nitrite and then to nitrate by the action of the aerobic bacteria such as Nitrosomonas, Nitrosospira, Nitrosococcus or Nitrosovibrio and Nitrobacter, Nitrospina or Nitrococcus, respectively. Ammonium is adsorbed on clay minerals and therefore is less mobile but nitrate is highly mobile. Plants take up nitrogen in mineral form (ammonium or nitrate). Nitrate is very soluble and unless intercepted and taken up by plant roots, leach down in the soil along with irrigation or rain water or it is carried away by runoff. Under some conditions, depending on the availability of organic carbon and anaerobic conditions nitrate may undergo bacterial conversion to molecular nitrogen or nitrous oxide, by a process called 'denitrification'. Unlike nitrifying bacteria, denitrifying bacteria include a wide range of bacteria.

Presence of nitrates, excess water and available carbon source are important factors that affect the denitrification process. Denitrification may result in the liberation of nitrogen  $(N_2)$ , nitrous oxide  $(N_2O)$  or nitric oxide (NO). While nitric oxide easily gets converted to nitrate and is brought down again by precipitation, nitrous oxide escapes to the stratosphere and destroys ozone. It also produces a powerful greenhouse effect. Nitrate is also immobilized by microbes but to a lesser extent than ammonium.

#### Role of nitrates

Nitrogen is absorbed by plants in the form of either ammonium ( $NH_4^+$ ) or nitrate ( $NO_3^-$ ), depending on the species, cultivar, age and soil conditions, particularly submergence or otherwise etc. Once nitrate is absorbed by plants, it has to be reduced by the enzyme, nitrate reductase to ammonium and assimilated via glutamate.

# Nitrate leaching and groundwater pollution

Excess nitrates leach down the soil profile with percolating water. Leaching of nitrates is more likely to occur in sandy soils, but it takes place in fine textured soils also<sup>1</sup>.

Nitrate leaching is a global problem. Recently, there have been many studies made in India which point to the danger of nitrate leaching and subsequent pollution of groundwaters. Due to increased agricultural activity which is necessary for enhanced food production and also due to industrial activity, there is an increasing evidence of nitrate pollution of groundwaters. In agriculturally intensive areas in Punjab<sup>2</sup>, Delhi<sup>3</sup>, Maharashtra<sup>4</sup>, Andhra

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Pradesh<sup>5</sup> where fertilizer applications are high, there is ample evidence of pollution of groundwaters by nitrates. Even in semi arid regions in Deccan plateau<sup>6,7</sup> and arid regions of Rajasthan<sup>8</sup>, where the intensity of agriculture is less, nitrate leaching was found prevalent. Another important area is industrial and urban centres where nitrate pollution of groundwater was found rampant<sup>9,10</sup>. This phenomenon was attributed mainly to dumping of animal manures, organic wastes from industries and sewage on to the soil. In most of the above studies, nitrate concentrations in groundwater exceeded the permissible limit of 45 mg l<sup>-1</sup>.

Three fourth of world population live in developing countries like India where there is an alarming trend of groundwater pollution by nitrates<sup>2</sup>. Treatment of groundwater for the removal of nitrates or prevention of nitrate from reaching groundwater is possible. Chemical methods such as catalytic removal of nitrate from water<sup>11,12</sup>, abiotic degradation of nitrates using zero valent iron and electrokinetic processes<sup>13</sup>, biotechnological methods such as use of vegetable oil for denitrification 14,15, reduction of agricultural nitrate loading through microbial wetland processes<sup>16</sup>, using sulphur and limestone autotropic denitrification<sup>17</sup> and physical methods such as application of reverse osmosis and nanofilteration<sup>18</sup> have been reported. These processes are at present considered costly even in advanced countries<sup>19</sup>. A recent study conducted in Bulgaria<sup>20</sup> has shown that when groundwater is being tapped in pockets, nitrates from surrounding soil profile can migrate and accumulate in such pockets and this phenomenon was observed in soils which are otherwise low in nitrate concentration. Such studies serve as warning to countries like India of nitrate pollution where groundwater exploitation is growing at a tremendous rate.

# Nitrate content in fruits, vegetables and other food articles

Application of nitrogen fertilizers can cause increase in concentration of nitrates and nitrites in crops. The maximum admissible level of nitrite in vegetables is 1 mg kg<sup>-1</sup>. In India, Usha et al.<sup>21</sup> have studied dietary intake of nitrates. Nitrate (NO<sub>3</sub>) contents of cereals ranged from 20 to 76 mg kg<sup>-1</sup>, pulses 39 to 114 mg kg<sup>-1</sup>, leafy vegetables 30 to 270 mg kg<sup>-1</sup>, roots and tubers 31 to 2043 mg kg<sup>-1</sup> and condiments and spices 145 to 4680 mg kg<sup>-1</sup>. The authors have calculated the percentage contributions of nitrates from diet in Andhra Pradesh: cereals and tubers 41.2; nuts and oil seeds 3.5 and condiments and spices 3.1. Nitrate contents in tea have been studied<sup>22</sup>. 50% of the nitrate in dried tea was found to be released into infusion. In China, different vegetable samples contained 140.6–2762.5 mg kg<sup>-1</sup> nitrate and 0.2–2.85 mg kg<sup>-1</sup> nitrite<sup>23</sup>. In Brazil, the legally permissible limit of nitrate  $(NO_3^-)$  in milk is  $1000 \,\mu g \, l^{-1}$  (ref. 24). However, measurements have shown 50 to 180 µg l<sup>-1</sup> nitrite and 20 to 2100 µg | <sup>-1</sup> nitrate in pasteurized milk<sup>24</sup>. Nitrate contents in some varieties of rice in Pakistan have exceeded the permissible level set by WHO<sup>25</sup>. In Danish market, the

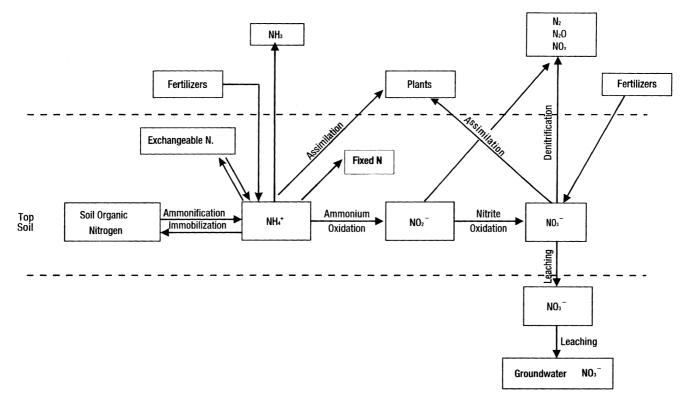


Figure 1. Nitrogen transformations in soil.

highest content of nitrate was found in lettuce followed by beetroot, Chinese cabbage, fresh spinach, leek, frozen spinach, white cabbage and potatoes; the total intake of nitrate and nitrite was estimated to be 61 and 0.5 mg day<sup>-1</sup> respectively<sup>26</sup>. In Poland, maximum acceptable limit of nitrates was exceeded in 8.2% of the samples of radish and 65% of lettuce<sup>27</sup>. The nitrate contents in greenhouse vegetables (lettuce, cucumber, radish) were greater than in field-grown vegetables. Reduction of nitrate content in potato<sup>28</sup>, spinach<sup>29,30</sup>, lettuce<sup>29</sup>, kohlarabi<sup>29</sup>, carrot<sup>29</sup>, white mustard<sup>31</sup>, buckwheat<sup>31</sup>, oats<sup>31</sup>, ryegrass<sup>32,33</sup>, green manure crops<sup>34</sup>, cucurbit<sup>35</sup>, fodder crops<sup>35</sup> and radish<sup>36</sup> by the use of nitrification inhibitors has been reported.

#### Nitrates and human health

Nitrates and nitrites in food may cause methemoglobinemia in babies<sup>37</sup>, where due to the oxidation of ferrous iron in haemoglobin to ferric state, the oxygen-carrying capacity of the red blood corpuscles is lost and the affected baby dies. In the US, the upper limit for nitrates in drinking water was set at 45 ppm NO<sub>3</sub> or 10 ppm NO<sub>3</sub>-N (ref. 38). Other health problems associated with nitrate toxicity include oral cancer<sup>39</sup>, cancer of the colon, rectum or other gastrointestinal cancers<sup>40-43</sup>, Alzeimer's disease<sup>44</sup>, vascular dementia of Biswanger type<sup>44</sup> or multiple small infarct type<sup>44</sup>, absorptive and secretive functional disorders of the intestinal mucosa and changes in maturation, differentiation and apoptosis in intestinal crypts<sup>45</sup>, reduced casein digestion 46, multiple sclerosis 47,48, neural tube defects<sup>49</sup>, cytogenetic effect in children<sup>50</sup>, non-Hodkins's lymphoma<sup>41,51,52</sup> and hypertrophy of thyroid<sup>53</sup>. Craig et al. 54 have shown that nitrate consumption leads to a decrease in the ascorbate/nitrite ratio in gastric juice, which regulates the synthesis of potentially carcinogenic N-nitroso compounds and decrease in the ratio leads to increased risk of gastric cancer. Graham et al.55 while reviewing the measurements and association of nitrite and nitrate ions with various clinical conditions such as hypertension, infection, renal and cardiac disease, inflammatory diseases, diseases of the central nervous systems expressed that such associations between disease incidence and drinking water nitrate content are controversial except for methemoglobenemia. However, nitrates are useful for such conditions as cardiovascular diseases, where they reduce platelet aggregation and prevent anginal attacks of both symptomatic and silent types<sup>56</sup>. But continued consumption of nitrates causes tolerance (reduced or no dilation on nitrate administration)<sup>57</sup>. WHO has prescribed a maximum limit of 3.65 mg nitrate kg<sup>-1</sup> body weight for total intake in a day<sup>58</sup>.

#### Toxicity of nitrate to animals

Nitrites and nitrates act as exogenous sources of nitric oxide which is an extreme physiologically active agent in

animals and man<sup>59</sup>. This process manifests itself in the formation of nitrosyl hemoglobin and dinitrosyl iron complexes (DNIC) with thiol groups of proteins. The latter compounds are stable enough to function as a depot of nitric oxide. DNIC degradation is assumed to be associated with the formation of S-nitrothiols, another depot of nitric oxide in the organism. The S-nitrosothiols as well as DNIC can affect various metabolic processes through the release of nitric oxide as well as nitrosonium ion, a powerful nitrosylating agent. Nitrate toxicity varies according to species and, in general, ruminant animals develop methemoglobinemia while monogastric animals exhibit severe gastritis<sup>60</sup>. Unlike nitrate, nitrite is capable of inducing methemoglobinemia in a wide range of species, viz. cattle, sheep, swine, dogs, guinea pigs, rats, chickens and turkeys. The various effects of nitrate on different animals such as intestinal disorders in pigs<sup>61</sup>, pregnancy-related disorders in rats<sup>62</sup>, depression, muscle tremors and incoordination in goats<sup>63</sup>, loss of body weight and reduced water consumption in broiler chicken<sup>64</sup>, sexual disorders in sheep<sup>65</sup>, hyperthyroid in foals<sup>66</sup>, etc. have been reported.

#### N recoveries

Nitrogen deficiency in agricultural systems is a world wide problem and this is true of India too<sup>67</sup>. The population in India is expected to reach 1.3 billion by 2025 AD and to increase the crop productivity so as to meet the minimum needs of such a large population, large quantities of fertilizers are to be used. In 1998–1999, the estimated NPK fertilizer consumption was 16.8 mt, of which more than 70% was nitrogenous fertilizers<sup>19</sup>. Fertilizer nitrogen recovery in a crop is calculated as follows:

%N recovery in crop = 
$$\frac{-\text{N uptake in fertilized plots}}{-\text{Quantity of N applied}} \times 100.$$

Two crops, viz. wheat and rice consume 70% of the fertilizers but their N recoveries are about 50 and 25–30 per cent, respectively<sup>67</sup>. Such recovery values are commonly found globally (Table 1). Global consumption of N fertilizer was 72.7 mt yr<sup>-1</sup> in 1994 and is estimated to increase to 122.5 mt yr<sup>-1</sup> by the end of the 21st century<sup>68</sup>. Poor nitrogen recovery is of great concern for a number of reasons: (i) nitrogen fertilizers are costly as their manufacture involves consumption of large quantities of nonrenewable sources of energy, namely, naphtha, natural gas, etc.; (ii) it leads to environmental problems like nitrate pollution of groundwater, eutrophication of surface waters, emission of nitrous oxide leading to ozone layer depletion and global warming, emission of ammonia, formation of fog and acid rain.

Nitrate can be washed off from soils by rain or irrigation water. Nitrate in run off water causes eutrophication of surface waters<sup>69</sup>.

### Measurement of nitrate leaching

In order to take rational decisions to control nitrate pollution, data are required regarding the concentration of nitrate leached below 1–2 m depth and the quantity of water flowing down below that depth<sup>70</sup>. The following methods are available for this purpose.

- 1. Extracting the soil solution into a porous ceramic cup<sup>70</sup>.
- 2. Collecting water leached in a lysimeter<sup>70</sup>.
- 3. Collecting water from field drainage systems<sup>70</sup>.
- 4. Computer modelling<sup>70</sup>.
- 5. Collecting nitrate in an ion exchange resin<sup>71</sup>.
- 6. Using bromide as a tracer for nitrate<sup>6</sup>.
- 7. Using <sup>15</sup>N enriched (or depleted) fertilizer<sup>72</sup>.

In India, there are very few references on the movement of nitrate in soil profile. The porous cup method<sup>70</sup>, the bromide tracer method<sup>6</sup> and computer modelling<sup>70</sup> may be more suitable for such studies in India. Data from such studies should help in understanding the process of leaching and in minimizing pollution.

In India, though there are no extensive data, the available information suggests that nitrate contamination of groundwater could be a matter of concern in several areas. It has been claimed that since most of the soils in India are poor in nitrogen and since the quantities of nitrogen fertilizer used are small, nitrate pollution of drinking water is unlikely to be a major problem. However, recently, several reports of highly contaminated drinking water have shown that nitrate pollution can be a problem in certain areas. The number of reports showing nitrate contamination in drinking water in India is small probably because chemical analysis of drinking water is not performed extensively. Quite often the quality of drinking

water is taken for granted if the water is clear and the taste is good. There is an urgent need for performing chemical analysis of drinking water extensively in India. Spatial analysis of such data should help in identifying pockets of contamination. Possible causes of nitrate contamination of drinking water include:

- 1. Cultivation of crops for which high doses of nitrogen fertilizers are applied: e.g. tobacco, vegetables, flowers, etc. It must be emphasized that organic farming does not ensure freedom from nitrate problems.
- 2. Surface disposal of sewage without treatment to remove nitrogen.
- 3. Use of sewage water for irrigation of crops is commonly practiced in India and this may lead to nitrate contamination of groundwater.
- 4. Disposal of industrial effluents containing nitrogen.
- 5. Landfilling: Quarry pits are often filled with municipal wastes which contain large quantities of nitrogen.
- 6. Deforestation: If the foliage is allowed to mineralize *in situ*, nitrate contamination is possible.
- 7. Dairy industry: If large amounts of animal excreta are disposed off on land both ammonia volatilization and nitrification will be rapid. Ammonia may contaminate surface waters and it may get converted to nitrate by the process of nitrification. Nitrate in soil may contaminate surface waters through run off or it may percolate to the groundwater with rain or irrigation water.
- 8. Since the recoveries of nitrogen fertilizers are, in general, below 50%, nitrate contamination of groundwater can take place if water percolating from a large area converges to a point beneath the soil due to hydrological or other reasons.

Analysis of raw and prepared food items also need to be carried out extensively by collecting samples from the market. Food items which are likely to be high in nitrate need to be identified. Vigilance is all the more important in view of the fact that nitrogen fertilizer consumption is expected to increase steeply in the coming years.

<b>Table 1.</b> N-use efficiencies in	different crops and locations
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Crop	Place (country)	N-use efficiency	Ref. and remarks
Corn (Zea mays)	Iowa (USA)	29–45%	80, Anhydrous NH <sub>3</sub>
Corn (Zea mays)	USA	39.6%	81, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , Pot expt.
Wheat	India	50-55%	82
(Triticum aestium L.)	Pakistan	37-53%	83, (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , KNO <sub>3</sub> , Pot expt.
Cotton	Australia	33%	84, Irrigated crop
Spring wheat	Spain	~ 40%	85
Barley	Finland	60-70%	86, Pot expt.
Rice	India	28-34%	87
	Pakistan	49%	88, Pot expt.
Oat	Spain	37.9%	89

# Minimizing nitrate leaching

Although technologies are available for decontaminating nitrate-containing drinking water, these are very costly. Therefore, it is necessary to minimize nitrate leaching from agricultural as well as nonagricultural activities. In developed countries the approach to reducing nitrate pollution is largely based on changing the land use pattern. Any change which may involve conversion from agriculture to nonagricultural use<sup>73</sup>, conversion to grasslands<sup>74</sup>, change in cropping system<sup>75</sup> or reduction in fertilizer dose<sup>76</sup> involving a reduction in food production, would not be appropriate in India and other developing countries where the challenge is to maximize food production with no increase in cultivable land. In developing countries the stress will be on maximizing the fertilizer use efficiency. By adopting the following improved methods of nitrogen fertilizer use, nitrate leaching can be minimized.

- Substituting part of the inorganic fertilizers with organic fertilizers, i.e. adopting integrated nutrient management systems.
- Matching the plant needs and fertilizer applications by using appropriate split applications.
- By using improved crop management practices.
- Use of slow-release fertilizers (urea-aldehyde polymeric compounds, coated fertilizers)<sup>77,78</sup>.
- Use of nitrification inhibitors<sup>77</sup> and urease inhibitors. Chemicals known to be safe should be used.
- Choosing the right cropping systems<sup>79</sup>.
- Intercepting nitrate by means of trees or other deep rooted, nitrate mining crops (e.g. alfalfa) or by digging ditches.
- Establishment of information systems and monitoring networks.
- Education of the population in general and farmers in particular.

Nitrate originating from agriculture could be a major threat to environment in many situations. It is important that proper assessment of nitrate pollution should be made by extensive analysis of soil and groundwater samples. It is possible to minimize this threat by proper management of agricultural systems and by adopting appropriate policy measures.

- 1. Jones, R. D. and Schwab, A. P., Soil Sci., 1993, 155, 272-282.
- 2. Bijay, S., Singh, Y. and Sekhon, G. S., 15th Trans World Congress on Soil Science, 1994, vol. 5a, pp. 174–191.
- Gupta, N. and Chandrasekharan, H., R. Soc. Chem., (spl publ.), 1999, 237, 240–249.
- Deshpande, S. M., Kaplay, R. D. and Kulkarni, S. G., *Pollut. Res.*, 1999, 18, 191–192.
- 5. Srinivasa Rao, N., Hydrol. Sci. J., 1998, 43, 701-714.
- 6. Patra, A. K. and Rego, T. J., Soil Sci., 1997, 162, 656-665.
- Power, N. J. and Shaikh, I. J., Environ. Geol. (Berlin), 1995, 25, 197–204.
- 8. Ozha, D. D., Varshney, C. P., Bohra, T. L. and Sharma, D. C.,

- Vignana Parishad Anusandhan Patrika, 1993, **36**, 147–157; CA121: 163383h.
- 9. Pathak, H., R. Soc. Chem., (spl publ.), 1999, 237, 228-239.
- Rangarajan, S., Murugeshan, V., Elango, L. and Elampoornan, T., Indian J. Environ. Prot., 1996, 16, 573–576.
- 11. Vorlop, K. D. and Prusse, V., *Cat. Sci. Ser.* (Environmental Catalysis), 1999, 1, 195–218.
- Daub, K., Emig, G., Choller, M. J., Callant, M. and Dittmeyer, R., Chem. Eng. Sci., 1999, 54, 1577-1582.
- Chew, Chin, F. and Zhang, T. C., Environ. Eng. Sci., 1999, 16, 389-401.
- Hunter, W. J., 5th International in situ On-Site Biorem. Symposium (ed. Allenman, B. C. and Leeson, A.), Battle Press, USA, 1999.
- Hunter, W. J., 5th International in situ On-Site Biorem. Symposium, 1999, vol. 4, pp. 53–58.
- Iverson, Torben, M. and Hoffmann, C. C., Biotechnol. Water Use Conserv. Meas. 96 Workshop, 1996, pp. 609–615.
- Flere, J. M. and Zhang, T. C., Proceedings of the National Conference on Environment and Engineering, pp. 344–349.
- Bohdziewicz, J., Michal, B. and Ewa, W., Desalination, 1999, 121, 139–147.
- Subba Rao, I. V., National Seminar on Developments in Soil Science–1999, Tamil Nadu Agricultural University, Coimbatore, ISSS, IARI, New Delhi, Nov. 1999.
- Pulido-Bosch, A., Lopez-Chicano, M., Mackova, M., Dimitrov, D., Velikov, B., Calaforr, J. M. and Calvache, M. L., IAH, 27th Congress, 1997 (Pub. 1999), vol. 2, pp. 225–231.
- Usha, G., Naidu, A. N. and Kamala, K., Food Compos. Anal., 1993, 6, 242–249.
- Regina, O., Grazyna, P. and Wieslawa, M., Bromatol. Chem. Toksykol., 1998, 31, 343–347, CA130: 266518w.
- 23. Pang, D., Song, Y. and Zhu, Y., Weisheng Yanjiu, 1996, **25**, 251–252, CA126: 211292e.
- Seraphim, K. R., Bastos De Siquera, M. E. P., De Fernicola, N. A. and Galego, G., *Alimenteria (Madrid)* 1998, 296, 49–52, CA130: 51537r.
- 25. Salah-ud-Din, Salariya, A. M., Ashraf, M. and Sheik Abdus Salam, Sci. Int. (Lahore), 1996, 8, 373-374.
- 26. Annette, P. and Soren, S., Food Addit Contem. 1999, 16, 291–299.
- Jozef, S., Anna. P., Rocz. Ponstw. Zakl. Hig., 1999, 50, 17–23, CA13: 227905w.
- 28. Yefimov, V. N., Trusova, L. A. and Diallo, A. P., *Agrokhimiya*, 1992, **8**, 15–25, CA120: 243388h.
- Dressel, J., Jung, J. and Will, H., Gartenbauwissenkschaften, 1984, 49, 106–112, CA101: 190461a.
- 30. Bengtsson, Bengt L., Z. Pflanzenernaehr. Bodenk., 1968, 121, 1-4.
- 31. Kucharski, Jan. Pol. J. Soil Sci., 1993, 26, 127-133.
- 32. Nowakowski, T. Z., Plant Soil, 1968, 28, 165–168.
- Kazimierz, B. and Maria, Z., Rocz. Glebozn., 1979, 30, 45–46, CA91: 192084d.
- Mori, T., Ida, A. and Nakamura, H., Tokai, Kinki Nogyo, Shikensho, 1966, 3, 34–47, CA167: 32046.
- Khashimov, F. Kh., E. K H. and Fatkulina, T. A., Agrokhimiy, 1989, 6, 64–70, CA111: 113999m.
- Jinan, F. and Allen, V. B., Commun. Soil Sci. Plant Anal., 1989, 20, 623–633.
- 37. Vittozi, L., Food Addit. Contam., 1992, 9, 579-585.
- Fan, A. M. and Steinberg, V. E., Regul. Toxicol. Pharmacol., 1996. 23, 35-43.
- Badawi, A. F., Gehen, H., Mohamed, E. H. and Mostafa, H. M., Dis. Markers, 1998, 14, 91–97.
- Paul, K., Ritva, J., Jan, D., Timo, H., Int. J. Cancer, 1999, 80, 852–856.
- 41. Peter, W. J., The Iowa Women's Health, Univ. Iowa, USA, 1998, n. 255

- 42. Leaf, C. D. and Steven, R. T., *The Role of Nitrate and Nitrite in Human Cancer* (eds Watson, R. E. and Mufti, S. I.), CRC Press, Florida, 1996, pp. 317–324.
- Derache, R. and Derache, P., Cah. Nutri. Diet, 1997, 32, 283–293, CA128: 114315y.
- 44. Tohgi, H., Abe, T., Yamazaki, K., Murata, T., Isobe, C. and Ishizaki, E., *J. Neural Trans.*, 1998, **105**, 1283–1291.
- Ireneusz, G. P., Roez. Panstw. Zakl. Hig., 1998, 49, 321–330, CA130: 263274c.
- Jan, W. and Pokorska-Lis Grazina, Bromatol. Chem. Toksykol., 1998, 31, 229-231.
- Govannoni, G., Heales, S. J. R., Silver, N. C., O'Riorden, J., Miller, R. F., Land, J. M., Clark, J. B. and Thompson, E. J., J. Neurol. Sci., 1997, 145, 77–81.
- 48. Irvine, D. G., Robertson, K., Hader, W. J. and West, R., Appl. Geochem. Supl. 2 (Environ. Geochem.), 1993, 235-240.
- Li, G., Tian, J., Hu, X. and Liu, F., Zonghua Yufang Yixue Zazhi, 1996, 30, 17–19, CA125: 31412q.
- Aspasia, T., Kitsou-Izeli, S., Galla, A., Gourgiotis, D., Papagiorgiou, J., Mitron, S., Molybdas, P. A. and Sinaniotis, C., Arch. Environ. Health, 1996, 51, 458–461.
- 51. Helen, M. W., Non-Hodkin's Lymphoma and Drinking Water Nitrate: A Case Study, Johns Hopkins Univ., USA, 1994, pp. 289.
- Michal, F. D., A population based case control study on the association between nitrate in drinking water and non-Hodkin's lymphoma, Johns Hopkins Univ., USA, 1998, 285 p.
- Van, M., Johannes, M. S., Van D., Mulder, K. A., de Baets, M. H., Paul, C. A. M., Vander, H. D. M., Paul, L. J. M. and Jos, C. K., *Toxicol. Lett.*, 1994, 72, 365–374.
- Craig, M., Andrew, C., Angela, W., Kenneth Mc Coll, *Gastro-enterology*, 1999, 116, 813–822.
- Graham, E., Ian, A., Mehrdad, Y. and Sinnikka, K. M., Clin. Biochem., 1998, 31, 195–200.
- Harald, D., J. Cardiovasc. Pharmacol. (Supl. 2), 1999, 34, 515–520.
- 57. Elkayam, Am. J. Cardiol., 1996, 77, 41c-51c.
- Arnold, R., Kibler, R. and Brunner, B., Z. Enaeprungswiss, 1998, 37, 325–335, CA130: 65478j.
- Vanin, A. F., Free Radicals in Biology and Environment, Kluwer, Dordrecht (NAIO ASI Ser. 3, 27), 1997, pp. 351–363.
- 60. Colleen, S. B., John, B. K., Vet. Hum. Toxicol., 1993, 35, 237-253.
- Egorova, V., Timofeeva, N., Shcherbacov, G., Paskin, V. and Starkenkov, S., EAAP Publ. 88, 1997, pp. 335–338.
- Egorova, V. V., Iezuitova, N. N. and Timofeeva, N. M., Fiziol. Zh. Im. Sechenova, 1993, 79, 93–101, CA120: 25134.
- Mondal, D. B. and Pondey, N. N., Indian J. Anim. Sci., 1999, 69, 157–159.
- Judith, G., Tammy, A., Melissa, B., Arnold, S., J. Appl. Poult. Res., 1996, 5, 330–336.
- Julia, N., Ivan, T., Ivan, N. and Nedka, I., *Zhivotnovud Nauki*, 1997, 34, 149–151, CA128: 304993r.

- Allen, A. L., Townsnd, H. G. G., Doyle, C. E. and Frzet, P. B., Proceedings of the 5th International Symposium on Poisonous Plants, 1997, (Pub. 1998), pp. 334–338.
- 67. Tandon, H. L. S., Fertilizers, Organic Manures, Recyclable Wastes and Bio-Fertilizers, Fertilizer Development and Consultation Organization, New Delhi, 1992, p. 148.
- 68. Prasad, R., Curr. Sci., 1998, 75, 677-683.
- David, M. B., Gentry, L. E., Kovacic, D. A. and Smith, K. M., J. Environ. Qual., 1997, 26, 1038–1048.
- Addiscot, T. M., Whitmore, A. P. and Powlson, D. S., Farming, Fertilizers and the Nitrate Problem., CAB International, Wallingford, UK, 1991, 170 pp.
- Stephano, D., Fco, J., Gholz, H. L., Commun. Soil Sci., Plant Anal., 1986, 17, 989–998.
- Ronalghi, A., Saltanpour, P. N. and Mosier, A. K., Commun. Soil Sci. Plant Anal., 1993, 24, 2629–2639.
- Alkemede, J. R. M., Van Grinven, J. J. M., Wiertz, J. and Kros, J., 1st International Nitrogen Conference, 1998, pp. 101–105.
- Parkinson, R. J., in *Nitrate: Processes Patterns Management* (eds Tim, P. B., Louise, H. A., Stephen, T. T.), Wiley, Chichester, UK, 1993, pp. 321–339.
- Joosten, L. T. A., BuiJze, S. T. and Jansen, D. M., Environ. Pollut. (Supl. 1), 1998, 102, 489–492.
- Moth, I. T. and Ruth, G. and Kurt, N., 1st International Nitrogen Conference (ed. Van der Hoek, K. W.), Elsevier, Oxford, 1998, pp. 771–780.
- 77. Prasad, R. and Power, J. F., Adv. Agron., 1995, 54, 233-281.
- Prakasa Rao, E. V. S. and Rajendra Prasad, *Plant Soil*, 1980, 57, 383–392.
- 79. Yadav, R. L., Exptl. Agric., 1982, 18, 37-42.
- Sanchez, C. A. and Blackmer, A. M., Agron. J., 1988, 80, 102– 108
- Ronaghi, A., Soltanpour, P. N. and Mosier, A. R., Commun. Soil Sci. Plant Anal., 1993, 24, 2629–2639.
- 82. Prasad, R., in *Nitrogen Research and Crop Production* (ed. Tandon, H. L. S.), Fertilizer Development and Consultation Organization, New Delhi, 1996, 173 pp.
- 83. Lodhi, A., Azam, F., Cereal Res. Commun., 1998, 26, 305-312.
- 84. Rocehester, I., Constable, G. and Saffigna, P., Biol. Fert. Soils, 1996, 23, 1-6.
- 85. Videl, I., Longeri, S. and Luis, 1998, Agric. Tech. (Santiago), 48, 137-141, CA110: 74298q.
- 86. Antti, J., Towo, Y., Ann. Agric. Fenn., 1985, 24, 77-87.
- Goswami, N. N., Shinde, J. E. and Sarkar, M. C., Bull. Indian Soc. Soil Sci., 1984, 13, 51–67.
- Lodhi, A., Malik, N. N. and Azam, F., Pak. J. Bot., 1996, 28, 75–83.
- Videl, I., Alfonsa, H., Mitzi, G., Luis, L., An. Eadofol. Agrobiol., 1985, 44, 1433–1438, CA106: 17523c.

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