P: ISSN No. 0976-8602 RNI No.UPENG/2012/426228 VOL.-III, ISSUE-IV, October-2014
E: ISSN No. 2349 - 9443
Asian Resonance

Terrestrial Nitrification Studies in the Arid Soils of Purna Basin- A Region Specific Research to Arrest Nitrate

Abstract

Among the chemical fertilizers used for maximizing crop production, nitrogenous fertilizers like amide (most preferred is urea), ammonium (NH_4^+) and nitrate (NO_3^-) containing fertilizers are widely used due to the poor nitrogenous fertilizing capacity of the alluvial to black cotton soils of *Purna* river basin generally encountering indiscriminate nitrogenous fertilization.. The banana bowl in *Khandesh*, *Citrus* zone in *Varhad* and cotton/ cereal crops belt in all north eastern Maharashtra witness nitarte contamination in underground water as these soils are routinely fertilized with heavy dosages of nitrogenous fertilizers.

Thus, the bulky doses of the fertilizers for the past five decades (since 1970s) have been resulted into (i) nitrate contamination in water reservoirs, (ii) erosion through nitrate flush by leaching and floods, (iii) nitrogen enrichment in water aquifers causing threat of eutrophication, (vi) emission of N_2O fluxes to the atmosphere increasing regional warming and (vii) stagnation in crop yield non-commensurate with fertilizer inputs.

The two steps of oxidative nitrification, the formation of nitrite (NO_2^-) from ammonium (NH_4^+) (called nitrosification) and the formation of nitrate (NO_3^-) from nitrite (NO_2^-) (called nitrification), are carried out by different soil chemolithoautotrophic microbial populations, dominated by bacterial genus *Nitrosomonas* involved in nitrosification and another *Nitrobacter* for nitrification. The transfer of nitrate and nitrite ions from surface soil to groundwater, pose a serious human health hazard. The relation of soil microbial nitrification with the nitrate pollution in the environment is under research herewith.

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Keywords: Microbial Nitrification, Nitrate Pollution, Nitrosomonas Sp., Nitrobacter Sp.

Introduction

The Sant Gadge Baba Amravati University's (SGBAU) jurisdiction prevails over the five districts of *Vidarbha* region including Amravati (government's revenue divisional head quarter), Akola, Buldhana, Washim and Yavatmal; that are also the part of North-eastern Maharashtra.

This north-eastern region of the Maharashtra state majorly covers the areas of *Purna* river basin and forest covers on the southern *Satpura* range with the soils of alluvial to black cotton/clay type that have poor to appropriate fertilization capacity.

This region offers the shelter, food, protection and geography to its multi-faceted inhabitants who utilize and manage/ exploit the available natural resources for their livelihood. Among the most important natural resources of this region are soils, water and air whose quality is deteriorating due to ever increasing pollution. Among the various pollutants being added to environment, commercial nitrogenous fertilization seemed to be important from the view of soil erosion, water pollution and climate change.

For alluvial to black clay soils of North eastern Maharashtra, application of nutrients (N:P:K) per ha is recommended by concerned government and non-government agencies through chemical fertilizers, besides fortification with biofertilizers and huge amounts of organic/ green manures etc. to achieve targeted yield to ensure proper fertilization for achieving national food, feed and fiber security. Since, organic manures were not available in adequate quantities due to reduced domestication of livestock, on-farm/ domestic incineration of agricultural residue; farmers mainly used chemical fertilizers only. Among the fertilizers, nitrogenous fertilizers like amide (urea), ammonium and nitrate containing fertilizers are widely used due to the poor nitrogenous fertilizing capacity of these soils. Out of these nitrogenous fertilizers, urea is the cheapest, readily available, easy and safe to apply and hence, most preferred by farmers.

The water quality of this region has shown a steady increase in nitrate contents in the underground as well as surface sources that have mostly approaching the maximal tolerable limits for the safe use of water for domestic and drinking purposes. This increased nitrate and nitrite contents although beneficial for plants for their nitrogen uptake, the mobility of these ions always render its availability to roots for absorption beyond approach.

Floods during excessive rains sweep away such a soluble and mobile portion of soil fertility. It is carried towards slopes and deeper areas, partly depositing it to the lands of the river banks, partly adding it to the lakes and constructed damns and major part is taken to the estuaries, finally losing to oceans. All these may seem to be natural process, but is of no use to the terrestrial inhabitants and farmer, who applied and enriched the soil fertility by adopting commercial fertilization programmes. The ongoing addition of nutrients to the lakes and damns turns it to eutrophic, making the water unfit for direct use and recreation. The harm to the established ecosystem is an additional disadvantage.

The past four decades have witnessed the change in the climate of the region by overall unpleasant (a) increase in temperature during summer (upto 48°C) and winter (not dropping below 10°C), (b) change in hydrological cycles increasing unpredictability even in the district of assured rainfall and (c) shift of the annual seasonal cycle abruptly by one month. A study of meteorological records by the Indian government showed that average temperature across the country increased by 0.4°C through the 20th century, whilst sea levels rose by 10-25cm. These trends are predicted with a high degree of confidence to continue and accelerate during the current 21st century. Temperature has a direct impact on the growth and habitats of most of the organisms. It is very clear that a temperature rise even by 1°C reduces the cereal growing season by a week, resulting into immature weak seed and fodder yield.

Thus, maximal reliance on this bulky doses of nitrogenous fertilizers for the past four decades have resulted into (i) an extensive rise in ambient temperatures >45 ^{o}C during summer, due to evolution of green house gases like N_2O through anaerobic denitrification (Smith and Tiedje, 1992; Barnard and Leadley, 2005), (ii) nitrate contamination in underground water reservoirs, (iii) erosion through nitrogenous nutrient loss through leaching of nitrate, (iv) nitrogen enrichment in water aquifers causing threat of eutrophication, (v) increased salinity due to indiscriminate application of merely chemicals and (vi) stagnation/decline in crop yield non-commensurate with inputs.

Review of Research and Development in the Subject

The entry of N to the soils is primarily through biological nitrogen fixation (BNF) and then, application of chemically fixed nitrogen. The other biological process includes organic matter decomposition involving deamination of nitrogenous compounds and denitrification. The nitrogenous entity is NH_3 in aqueous condition, which is subsequently either oxidised to nitrates or reduced back to molecular nitrogen. After the introduction of chemical nitrogenous fertilizers, huge amounts of ammonia yielding chemicals are applied to soils annually for higher yield and productivity.

According to the manner in which the nitrogen is combined with other elements, the nitrogenous fertilizers can be divided into four groups; nitrate, ammonia and ammonium salts, chemical compounds containing nitrogen in the amide form and plant and animal by-products, which further can be divided into two groups depending on their chemical nature either inorganic or organic.

I. Inorganic Nitrogenous Fertilizers: Inorganic substances containing large amount of nitrogen come under this category. It is further divided into following groups according to the form of nitrogen they contain.

a. Nitrate fertilizers (NO_3^-): Nitrogen present in these fertilizers is in nitrate form, NO_3^- which are rapidly dissociated to release NO_3^- ions and readily absorbed by the plants. Nitrate ions are highly reactive, mobile and susceptible to losses due to leaching and under water-logged conditions by denitrification. Those are alkaline in their residual effect in soil. Nitrate fertilizers are sodium nitrate (16% N) and calcium nitrate (15.5% N).

b. Ammonium fertilizers (NH_4^+): Ammonium fertilizers are water soluble and absorbed on the soil colloids. Thus, those are protected from being washed away by run off or by leaching. Some crops like rice, sugarcane, tuber crop, seedlings directly utilise ammonium form of these fertilizers. The absorbed ammonium ions on soil collections are transformed to nitrate slowly and taken up by most of the crops. They are acidic in their residual effect in soil. Ammonium fertilizers are ammonium sulphate ($(NH_4)_2SO_4$, 20.6% N), Ammonium Chloride (NH_4Cl, 25% N), Ammonium phosphate ($NH_4(H_2PO_4)$, 20% N), Anhydrous ammonia (NH_3, 82% N) and Ammonia Solution (NH_3 in water, 20-25% N).

c. Nitrate and Ammonium fertilizers (Nitrate, NO_3^- and ammonium, NH_4^+): These fertilizers contain nitrogen in both nitrate (NO_3^-) and ammonium forms (NH_4^+). The nitrate nitrogen is readily available to plants for immediate need, whereas ammonium nitrogen becomes available to plants at a later stage, when it is transformed by microbiological process to nitrate. They are soluble in water and suitable for most of the crops and soils. They are acidic in its residual effect. Ammonium nitrate (NH_4NO_3, 33-34% N), Calcium Ammonium Nitrate (CAN,

$Ca(NO_3)_2NH_4NO_3$, 25% N), Ammonium Sulphate Nitrate (ASN, $(NH_4)_2SO_4NH_4NO_3$, 26% N) are the examples of Nitrate and Ammonium fertilizers

Organic Nitrogenous Fertilizers

a. These fertilizers contain nitrogen in organic form. These include plant and animal by-products. These are relatively slow-acting, but supply nitrogen for a longer period. Nitrogen rich organic manures fall in this category. Smaller the C/N-ratio, faster will be the ammonification and wider the C/N-ratio, slower will be the ammonification. A wide proportion of C and N in organic matter make it unutilizable as organic manure.
b. Amide Fertilizers (Amine, NH_2 or amide, CN_2): These fertilizers contain nitrogen in organic compounds as amide —NH_2 or —CN_2, not directly available to plants, as such, but quickly converted by soil microbes to ammoniacal and nitrate form and then, utilised. Amide fertilizers are Urea ($CO(NH_2)_2$, 46% N) and Calcium cyanamide ($CaCN_2$, 21% N).
c. Slow release nitrogenous fertilizers: These are newly developed fertilizers which release nitrogen in soil very slowly so that it may be available to the plants for longer period of time. Use of these materials result in better utilization of applied nitrogen by the growing crop plants and reduces losses. The examples are given as follows, Urea-form (Urea+Formaldehyde, 38% N), Oxamide (H_2NCO—$CONH_2$, 31.8 % N), Isobutylidine diurea (IBDU) (Urea+Isobutylaldehyde, $(CH_3)_2$—CH=CH—(NH—CO—$NH_2)_2$, 32.2% N), Crotonilidine diurea (CDU)(Urea+acetaldehyde, 32% N), Guany1 urea (GU, 37% N), N-lignin (Ammonified lignin, 18% N), Sulphur coated urea (SCU, 36-40% N), Metal-ammonium phosphate (Me.NH_4PO_4 x H_2O, 6.1-8.3% N) and Nutricate, Osmocate (Mixed fertilizers coated with various resin containing release controlling agents, additives).

Relatively, few microorganisms are capable of nitrification (Aleem, 1977; Aleem and Alexander, 1958), the process in which ammonium ions (oxidation level of nitrogen = -3) are initially oxidized to nitrite ions (oxidation level of nitrogen = +3) and subsequently to nitrate ions (oxidation level of nitrogen = +5). It is an example of aerobic respiration. These two steps of nitrification are energy-yielding processes from which chemolithotrophic bacteria derive needed energy. The metabolism of the chemolithotrophic nitrifying bacteria changes the oxidation levels of ammonium and nitrite ions when these ions serve as electron donors for chemiosmotic generation of ATP.

Relatively, low amounts of ATPs, however, are generated by the oxidation of inorganic nitrogen compounds. Therefore, large amounts of inorganic nitrogen compounds must be transformed to generate sufficient ATP to support the growth of these chemolithotrophic bacteria. The oxidation of approximately 35 moles of ammonia is required to support the fixation of 1 mole of CO_2. As a consequence of the high amount of nitrogen that must be transformed to support the growth of chemolithotrophic bacterial populations, the magnitude of the nitrification process is typically very high, whereas the growth rates of nitrifiers are generally relatively low compared to those of other bacteria (Fliermans *et al*., 1974).

The two steps of nitrification, the formation of nitrite from ammonium and the formation of nitrate from nitrite, are carried out by different microbial populations. For the most part, the oxidative transformations of inorganic nitrogen compounds in the nitrification process are restricted to several species of autotrophic bacteria. In addition to the chemolithotrophic nitrifying bacteria, some heterotrophic bacteria and fungi are capable of oxidizing inorganic nitrogen compounds but the rates of heterotrophic nitrification are normally four orders of magnitude lower than those of autotrophic nitrification. In soils, *Nitrosomonas* is the dominant bacterial genus involved in the oxidation of ammonia to nitrite, and *Nitrobacter* is the dominant genus involved in the oxidation of nitrite to nitrate. Several other autotrophic bacteria, including ammonia-oxidizing members of the genera *Nitrosospira*, *Nitrosococcus* and *Nitrosolobus* and nitrite-oxidizing members of the genera *Nitrospira* and *Nitrococcus* are also important nitrifiers in different ecosystems. Many of the nitrifying bacteria contain extensive internal membrane networks that are probably the sites of nitrogen oxidation (Eylaro and Schmidte, 1959).

Because relatively few microbial genera make significant contributions to the rates of nitrification, it is not surprising that this process is particularly sensitive to environmental stress. Toxic chemicals can block the nitrification process. Nitrification is an obligate aerobic process, and under anaerobic conditions, such as may exists when high concentrations of organic matter are added to soil or aquatic ecosystems, the nitrification process may cease.

The process of nitrification is very important in soil habitats because the transformation of ammonium ions to nitrite and nitrate ions results in a change from a cation to an anion. Positively charged cations are bound by negatively charged soil clay particles and thus are retained in soils, but negatively charged anions such as nitrate are not absorbed by soil particles and are readily leached from the soil. Nitrification, therefore, in the transfer of inorganic fixed forms of nitrogen from surface soils to subsurface ground water reservoirs. In agriculture, inhibitors of nitrification, such as nitrapyrin, sometimes are intentionally added to soils to prevent the transformation of ammonium to nitrate, ensuring better fertilization of crops.

The transfer of nitrate and nitrite ions from surface soil to groundwater supplies is critical for two reasons: (1) it represents an important loss of nitrogen from the soil, where it is needed to support the growth of higher plants and (2) high concentrations of nitrate and nitrite in drinking water supplies pose a serious human health hazard. Nitrite is toxic to humans because it can combine with blood haemoglobin to block the normal gaseous exchange with oxygen. Additionally, nitrites can react with amino compounds to form highly carcinogenic nitrosamines. Further, nitrate although not highly toxic itself, can be reduced microbially in the gastrointestinal tracts of human infants to form nitrite, causing the 'blue baby syndrome'. This reduction of nitrate does not occur in adults because of low pH of the normal adult gastrointestinal tract. Nitrate and nitrite in the groundwater is a particular problem in agricultural areas, especially banana zone in *Khandesh*, *Citrus* zone in *Varhad* and cotton belt in all north eastern Maharashtra where high concentrations of nitrogen fertilizers are applied to soil. Besides it, cereal, oil-seed, energy crops are abundantly fertilized throughout. The use of nitrification inhibitors in combination with the application of ammonium nitrogen fertilizers are expected to minimize the nitrate leaching problem, and at the same time support better soil fertility and increased plant productivity (Purkhold *et al*., 2001).

Significance of the Proposed Study

The following are accountable outcomes of the project: (i) Nutrient losses would be minimized through environment friendly fertilization of soils, (ii) application of chemical fertilizers and cultivation of crops would be cost-effective, (iii) utilization of nitrogenous fertilizers would be remunerative, (iv) simple, judicious and sustainable integrated plant nutrition management (IPNM) system for increasing the fertilizer use efficiency would be evolved and (v) there should exists the scope for on-site management of available agro-ecological natural resources.

The tangible benefits of adapting technology region-wise at societal level would be as follows: (a) improved crop productivity as a function of sustained efficiency of nitrification: Neither biological nitrogen fixation (BNF) alone, nor application of ammonia releasing nitrogenous fertilization alone, showed appropriate nitrogen use efficiency for robust growth and yield. However, its judicious blend with sustained microbial nitrification would exhibit synergy in more nitrogen uptake in terms of improved biomass and yield, (b) financial savings through recommended agro-practices and minimal usage of applied fertilizers: Using this technology, a farmer could save almost half of the finances spent annually on nitrogenous fertilizers and (c) establishment of cause and effect relationship: The role played by each step of nitrification would be judged and its possible commercial exploitation would be implemented on experimental basis.

Among the intangible benefits of adapting technology region-wise at environmental level; following are the major. (i) With the reduction in ammonia releasing fertilizers consumption, release of green house gases (CO_2 and N_2O) will be reduced and to that extent pollution will be declined and ambient temperatures will be stabilized. (ii) Efficient usage of applied nitrogenous fertilizers by water retention in root zone would arrest the chances of pollution of underground aquifers. (iii) Appropriate fertilizer management would conserve and recycle the diminishing available resources. (iv) Photosynthetic rate, biomass assimilation and oxygen evolution would be increased. (v) Soil population of beneficial microbes, fertility and sustainability would be improved.

Potential Contribution to knowledge in the Field of Social Relevance or National Importance

1. Strategy to save the finances on the fertilization programme through improved fertilizer use efficiency and reduction in fertilizer losses and doses would be evolved.
2. Understanding the nature of nitrate contamination of water would be improved and thus, less consumption and exposure to nitrates through water could be achieved through reduced nitrate contamination.
3. Overall improvement in the livelihood in the holistic manner might be possible.
4. Requirement and production of the nitrogenous fertilizer would be reduced nationally and globally.
5. Saving of funds in subsidies would be the result for governments.
6. WAPCOS (water and power conservation) would be achieved through minimization of synthetic fertilizer production.
7. Natural resources like soil fertility, water and air quality will be conserved.
8. Possible reduction in global warming through the controlled substrate availability for anaerobic denitrification (Smith and Tiedje, 1992) would be possible.
9. Promising solutions for arresting the pollution and improving global environment would also be possible.

Objectives

Cereal crops viz. sorghum, wheat, rice, maize etc., pulses like grams, oil seeds like soybean, fiber crops e.g. cotton and hemp are of wide preference for cultivation In the north eastern Maharashtra also called as *Vidarbha*/ *Varhad* chiefly and are routinely heavily fertilized by urea for more productivity adding ammonia to soil for its subsequent oxidation. Therefore, it serves imperative to undertake the study of microbe mediated nitrification in the subtropical arid soils of north eastern Maharashtra regions to arrest (i) soil erosion, (ii) nitrate pollution of aquifers and (iii) climate change.

Among the preferred fertilizers, urea is very common, that adds chemically fixed nitrogen to soil in huge quantities. In addition, naturally fixed nitrogen amendment is a regular process occurring in the soil. And therefore, major source of nitrogen for biomass assimilation as well as biological processing after its molecular fixation is ammonia in the soil. This ammonia is available for further treatment like nitrification, where there is shift in the oxidation state of nitrogen from -3 to +5. On this background with the aim to unravel and study this nitrification occurring in the subtropical, dry land, arid soils of these regions, the current project derives the following major Objectives.

1. Identification, locating and study the naturally or artificially occurring conditions in the subtropical, terrestrial agricultural and forest dry land and monsoon-rainfed and irrigated ecosystems of the north-eastern Maharashtra for the share of soil microbial populations towards the contribution to the process of nitrification

2. Reproduction of the microbially mediated nitrifying conditions *in vitro* and *in situ* at laboratory or pilot scale for its comparative study and possibly understanding the mechanisms lying below it.
3. Isolation, characterisation and enumeration of the NH_4-oxidizing microorganisms and its biomass from representative soils from the selected high activity locations and determine their ability of ammonia oxidation per unit of substrate used per unit time to calculate tentatively the annual availability of nitrite per hectare.
4. Isolation, characterisation and enumeration of the NO_2 oxidising microbes and its biomass from soils from the selected high activity locations and determine their ability of nitrite oxidation to NO_3 per unit of substrate used per unit time to calculate the annual NO_3 production per hectare.
5. Co-relating the oxidation of ammonia and nitrite to nitrate through microbial nitrification with nitrogen use efficiency for the soils by the test plants/ crops.
6. Look for the necessary methods for sustainable budgeting of the net available nitrogen to the plant/ crop under study and suggest the mechanisms for the efficient nitrification.

Methodology

After reviewing literature, completing baseline experimentation and chalking out the strategy of implementation, design of the project methodology was laid as follows (Burlage *et al*., 1998

1. Sample collection: The typically representative types of the soils of the north-eastern Maharashtra like a) dry land, rainfed and arid, b) irrigated, c) forest and d) wetland with various fertilization programmes with typical representative crops of the region in different meteorological seasons will be identified and located for the collection of samples and conducted the *in situ* experiments for the study of the process of microbial nitrification. While doing so, an appropriate mathematical modelling and statistical analysis will be either done or outsourced with the help of software, computing and expertise. Then, the soil samples will be collected, preserved and utilized subsequently for the experimentation.
2. Study of nitrification in controlled conditions: Microbially mediated nitrifying conditions *in vitro* and *in situ* will be reproduced at laboratory or pilot scale for its comparative study and possibly understanding the mechanisms lying below it. This will involve the use of standard microbiological procedures, agrochemical methods and biochemical and analytical tests.
3. Isolation and study of nitrosifying microbes The NH_4-oxidizing microorganisms and its biomass from representative soils from the selected high activity locations will be isolated, characterised and enumerated (Paerl, 1998) and their ability of ammonia oxidation per unit of substrate used per unit time will be determined to calculate tentatively the annual availability of nitrite per hectare. This will involve the application of typical isolation and characterisation techniques used for soil chemo-auto-lithotrophs like nitrosifying bacteria and allied soil biota. The number and biomass of ammonia oxidizing bacteria per unit soil will be enumerated and its ammonia oxidizing capacity per substrate per unit time will be determined. It will be extrapolated to the amount of nitrite produced through nitrosification per annum per unit area. The various possible factors affecting the nitrosification efficiency of the ammonia oxidizing isolates will be studied and taken into consideration while conducting experiments and interpreting the results. The characterised isolates will also be subjected for identification atleast upto genus or species level using Bergey's manual of determinative bacteriology (Holt *et al*., 1994).
4. Isolation and study of nitrifying microbes: The NO_2 oxidising microbes and its biomass from soils from the selected high activity locations will be isolated, characterised and enumerated (Paerl, 1998) and their ability of nitrite oxidation to NO_3 per unit of substrate used per unit time will be determined to calculate the annual NO_3 production per hectare. This will involve the application of typical isolation and characterisation techniques used for soil chemo-auto-lithotrophs like nitrifying bacteria and allied soil biota. The number and biomass of nitrite oxidizing bacteria per unit soil will be enumerated and its nitrite oxidizing capacity per substrate per unit time will be determined. It will be extrapolated to the amount of nitrate produced through nitrification per annum per unit area. The various possible factors affecting the nitrification efficiency of the nitrite oxidizing isolates will be studied and taken into consideration while conducting experiments and interpreting the results. The characterised isolates will also be subjected for identification atleast upto genus or species level using Bergey's manual of determinative bacteriology (Holt *et al*., 1994).
5. Field studies: The oxidation of ammonia and nitrite to nitrate through microbial nitrification will be co-related with nitrogen use efficiency for the selected soils by the test plants/ crops as well as the loss of nitrogen through volatilization and leaching to deeper strata of soils polluting soil aquifers.
6. Enhanced fertilizer use efficiency: Necessary methods for sustainable budgeting of the net available nitrogen to the plant/ crop under study will be looked for and suggested the mechanisms for the improving or decreasing

the efficiency of nitrification, which will be expected to further enhance the nitrogen use efficiency of the soil by the cultivated crop through applied / native nitrogenous fertilization.

Details of Collaboration, if any Intended

The collaboration for extending research work to end users would be done with either a state-run agricultural university or an NGO or Farmers' co-operative organization or a progressive farmer for having field trials and demonstrating the regional technology developed for effective nitrogenous fertilizer use efficiency after successful completion of project.

Acknowledgements

The author gratefully acknowledges the financial assistance received from University Grant's Commission, New Delhi in the form major research project F. No. 41-1134/2012 for implementation of the said research plan, creation of infrastructural facilities and project fellowship.

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