Nitrogen Transformation in Soil: Effect of Heavy Metals

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A B S T R A C T

Keywords Nitrogen Transformation, Enzyme Activity, Heavy Metals. **Article Info** *Accepted:* 04 April 2017 *Available Online:* 10 May 2017	Nitrogen is the key nutrient factor that influences soil fertility and productivity. It is the mineral nutrient that exists in different forms, but nitrate form is the most preferred form by plants. Irrespective of the form in which N is applied to soil, it undergoes transformation *viz.* mineralization (ammonification, nitrification), denitrification *etc*. by enzymes produced by micro organisms. The rate of these processes are influenced by a number of factors, one such being heavy metals accumulated in soil by various anthropogenic activities like disposal of sewage sludge, domestic and industrial effluents discharge, deposition of air borne particulates from mining on agriculture land *etc*. The heavy metals cause long term hazardous effects on soil eco system and negatively influence the soil biological processes, soil microbial biomass and functions associated with soil N transformation. Hence, there is a need for the study and to monitor heavy metal concentration in soil. The effects of heavy metal contamination on soil are quite alarming and cause huge disturbances in the ecological balance and health of living organisms on earth. Micro organisms and enzymes associated with N transformation in soil are inhibited directly or indirectly by heavy metals. The extent of inhibition depends on the concentration and oxidation state of heavy metals and on soil characteristics.

Introduction

Increased soil pollution with heavy metals, organic and inorganic pollutants due to various human and natural activities has led to a growing need to address environmental contamination. Pollution of the biosphere with toxic metals and other organic and inorganic pollutants has accelerated dramatically since the beginning of the industrial revolution. The primary sources of this pollution are the industrial effluents, mining and smelting of metalliferous ores, metallurgical industries, municipal wastes, pulp and paper mills, distilleries, tanneries and injudicious application of fertilizers, pesticides and sewage.
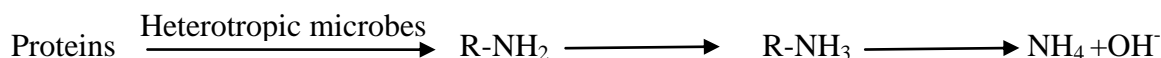
Heavy metals cause hazardous effect on soil microbial biomass and functions, this has negative influence on nitrogen transformation processes, which in turn affects the amount and form of mineral nitrogen present in soil. Hence there is a need to study the impact.

Nitrogen is necessary for all living forms on the earth; it is the basic constituent of proteins, amino acids, nucleic acids, chitin

etc. It is the only element that exists in different forms, but nitrate form is most preferred by crop plants. Irrespective of the form applied to soil, N undergoes transformation in cyclic manner *i.e* nitrogen cycle. A part of this N cycle taking place in soil is the conversion of organic form of nitrogen to inorganic form.

Nitrogen Transformation in Soil- The Important processes in nitrogen transformation in soil are:

Mineralization- ammonification and nitrification
Denitrificaation

$$\text{Proteins} \xrightarrow{\text{Heterotropic microbes}} \text{R-NH}_2 \longrightarrow \text{R-NH}_3 \longrightarrow \text{NH}_4 + \text{OH}^-$$

Micro organisms involved are *Bacillus*, *Clostridium*, *Pseudomonas* and *Streptomyces*

a. Nitrification: The process where NH_4^+ is oxidized to nitrite (NO_2^-) by nitrosomonas and to nitrate (NO_3^-) by nitrobacter bacteria. Others organisms involved are heterotrophic bacteria (*Arthrobacter globiformis, Aerobacter aerogenes, Thiosphaera pantotropha, Streptomyces grisens, and various Pseudomonas spp*), fungi (*Aspergillus flavus)* and Autotrophic *(Nitrosococcus, Nitrosospira, Nitrosolobus, and Nitrosovibrio).*

Denitrification

NO_3^- is mobile because of its high solubility in water, may move via water flow or diffusion into anaerobic soil and is reduced by bacteria to N_2 or N_2O, carried out by *Thiobacillus, Micrococcus* and *Pseudomonas.*

Factors affecting nitrogen transformation in soil

Climate

Mineralization

The process in which nitrogen containing organic complexes are decomposed and converted into inorganic compounds for use by plants.

Mineralisation process consists of two steps:-

Ammonification: The Process of mineralization in which proteins, nucleic acids and other organic components are degraded by micro organism with the eventual liberation of ammonia.

Vegetation
Topography
Soil moisture
 pH

Soil Pollution- mainly includes accumulation of heavy metals by various anthropogenic activities. Now-a-days it is to be considered as an important factor that has major effect on nitrogen transformation in soil.

Heavy Metals

The term heavy metal refers to any metallic element that has a relatively high density and is toxic or poisonous at low concentrations. Metals having specific gravity of more than 5 or having atomic number higher than 20. *Eg*. Al, Si, P, Ni, Cu, Zn, Pb, Ag Cd, Au, Hg, Ti, Sn *etc.*

Sources of Heavy Metals

Sources of heavy metals include geological sources from igneous and sedimentary rocks, atmospheric and hydrosphere sources. Soil pollution is also caused by means other than

the direct addition of xenobiotic (man-made) chemicals such as agricultural runoff waters, industrial waste materials, acidic precipitates, and radioactive fallout. Both organic and inorganic contaminants are important in soil. Among the sources of contaminants, agricultural runoffs, acidic precipitates, industrial waste materials and radioactive fallout. Major contributions of heavy metal contamination in the soil are by irrigation with discharge of industrial effluent and domestic sewage directly on earth surface. Variability of heavy metal contaminants both in their forms and quantity may be due to specific conditions. Some of the major important works made by the researcher on this approach in India can be quoted. Gupta *et al.,* (2007) found that leather industries (Tanneries) located at Jajmau, Kanpur, are the major sources of heavy metal contaminations in the agricultural soil in the surrounding areas where treated effluent has been used for irrigation. Rattan *et al.,* (2005) reported that under Keshopur effluent irrigation scheme, in Delhi, India for 20 years resulted in to significant build up of DTPA extractable Zn (208 %), Cu (170 %), Fe (170 %), Ni (63 %), and Pb (29 %) in sewage irrigated soils. Normally, domestic waste has lower heavy metal content than industrial waste. Soils irrigated by wastewater accumulate heavy metals such as Cd, Zn, Cr, Ni, Pb and Mn in surface soil. In the long term, the use of municipal solid waste (MSW) compost may also cause a significant accumulation of Zn, Cu, Pb, Ni and Cd in the soil and plants (Chopra *et al.,* 2009).

Effect of heavy metals in soil

The very low general level of their content in soil and plants, as well as the biological role of most of these chemical element, has led them being grouped under the generic name of 'micro elements', when the soil has very high content of such chemical elements, the term 'heavy metal pollution' is used. Hence heavy metals are synonyms to pollution and toxicity (Kebir and Bouhadjera, 2011).

Effect of sewage, sludge: Disposal of municipal solid waste, dumping domestic and industrial sludges load Cd, Cr, Cu, Pb on soil.

Effect of industrial effluents irrigation: Use as irrigation source, discharge, dumping and leaching into aquatic environment cause accumulation of As, Cd, Cr, Pb in soil.

Effect of mining: Strip and underground mining increase the concentration of Cu, Cd, Pb in soil.

Effect of agricultural chemicals and fertilizers: Spraying of metal containing insecticides and fungicides and application of excess fertilizers lead to Cd, Pb, As, Cu contamination in soil.

Effect of heavy metals on soil micro organisms

Although some heavy metals are required for life's physiological processes (e.g., components of metalloenzymes), their excessive accumulation in living organisms is always detrimental (Dmitri and Maria, 2008). Soil micro organisms are the first biota that undergoes direct or indirect impact of heavy metals the number of fungi was relatively higher in heavy-metalpolluted soils than in non-polluted soils (Yamamoto *et al.,* 1981). The populations of bacteria, actinomycetes, and fungi decreased in a forest soil contaminated with Zn at 33,000 mg/kg soil (Jordan and Lechevalier, 1975).

Example 1: Effect of heavy metals on ammonifying bacteria

Bacterial community is more sensitive to heavy metal than fungi according to

Wyszkowska *et al.,* 2008. This affects N transformation in soil as it is mostly carried by bacteria. Number of ammonifying bacteria found to be more in uncontaminated soil. Their population was significantly reduced under Zn, Cd and Cd, Cu, Zn treatments. They found to recover when Cd, Cu and Zn concentration was tripled. Zn was inhibitorier in combination with other metals (Table 5).

Example 2: Effect of Fe on *Nitrosomonas* and *Nitrobacter*

Addiion of 6mg/lt Fe stimulated nitrite production whereas 1.08 mg/lt Mn was poisonous. Inhibitory effect of Mn was counteracted by Cu and Fe (Fig 4).

Nitrite production was stimulated at 6mg/lt Fe followed by 112mg/lt which did not have inhibitory effect. 560 mg/lt inhibited nitrosomonas by forming heavy brown precipitation (Fig 5).

Oxidation of nitrite was completed at 6mg/lt Fe earlier than the absence Fe. The inhibitory effect of different heavy metals at higher concentrations on micro organisms is because heavy metals alter conformational structure of nucleic acids, proteins. This results in disruption of microbial cell membrane integrity or disrupts entire cell (Fig 6).

Example 3: Effect of heavy metals on the growth of Azatobacter in a synthetic medium

The effect of heavy metals on the bacterial growth is shown in Fig 7. In many cases O.D. at 650 nm showed a peak within 2 days and there the value of O.D. became a constant value. Sodium chromate was the most toxic heavy metal and when 125 µM of sodium chromate was added, the growth of Azotobacter was inhibited remarkably, while a concentration of 5 and 25µM also exerted

an inhibitory effect on the growth of Azotobacter. The inhibition of growth of Azotobacter by chromium chloride was less appreciable than that by sodium chromate. However, a concentration of 25 and 125µM of chromium chloride inhibited the growth of Azotobacter. Tungstate and vanadate(meta) did not reduce the O.D. in the case of Azotobacter in this experiment except for a concentration of 125 µM of vanadate which slightly inhibited the growth of Azotobacter**.**

Example 4: Effects of heavy metals on the growth of *Fusarium* in a synthetic medium

The values of O.D. for 27-h fungal cultures in a synthetic medium containing heavy metals are listed in Fig. 2. Tungstate was the most inhibitory on the growth of *Fusarium oxysporum* among heavy metals used in the experiment conducted by Kunio *et al.,* 2012. Even a concentration of 5µM of tungstate was sufficient to inhibit the growth of *Fusarium* and when the tungstate concentration exceeded 25µM, the growth of *Fusarium* was remarkably inhibited. The growth of *Fusarium* was also inhibited considerably by chromate, with a small inhibition at a 5 µM concentration. Chromium chloride induced a slight inhibition at a 25 and 125 µM concentration. Vanadate and molybdate did not inhibit the fungal growth regardless of the concentration but a level of 125 µM of molybdate reduced the growth of *Fusarium* slightly (Fig 8).

Effect of heavy metals on soil enzyme activity

Toxic concentration of heavy metals cause damage to enzymes and inactivate them. Some of the factors responsible for inhibition of N transformation enzymes are-

Heavy metal element: Different heavy metal inhibit at different extent in the order of Cr >

Cd > Zn > Mn > Pb, mostly depends on affinity and mobility.

Heavy metal concentration.

Heavy metal availability: Availability refers to the fraction of all contaminants of soil that is available to receptor organisms. It depends on soluble and exchange form of heavy metals.

Enzymes: Inhibition depends on nature and type of enzymes, their sensitivity to metal ions.

Example. 1 Inhibition of Urease enzyme by heavy metals

Inhibitory effect on urease enzyme activity at 1000ppm of different heavy metals are in the order of Ag=Hg>Cu>Cd>Co>Ba>Zn>Ni>Fe>Cr>Mn >Pb>Al. Urease is a nickel-containing enzyme that catalyzes the hydrolysis of urea to ammonia. Heavy metal ions react with a sulfhydryl group in the active center of the enzyme and form metal sulfides. Thus inhibit urease enzyme activity.

The enzymes activities were decreased with the increasing concentrations of Cd^{2+} and the incubation periods except for treatments of 0.5 mg/kg Cd^{2+} only and 0.5 mg/kg Pb^{2+} and 0.5mg/kg Cd^{2+} combined. Urease activities were found to be sensitive to the inhibition effect of heavy metals. After 45 days incubation studies done by Jinlong *et al.,* 2013 under the concentrations of 100.0 mg/kg Pb^{2+} and 0.5 mg/kg Cd^{2+} combined, the inhibition rates of soil urease activity was determined at 73.1 % compared to the control (Table 6). The inhibition effect of heavy metals to soil enzyme activities was the results of the changes of chemical conformation mainly due to the coordination reaction. Based hard and soft acids and base theory (HSAB), the active sites in enzyme protein molecular, such as thiol or imidazolyl groups, were preferred coordinated with soft heavy metals.

The influences of combined pollution of Pb^{2+} and Cd^{2+} on soil nitrifying activity after 45 days incubation is listed in Table 7. Disagree with that on soil urease activity, the inhibition effect was appeared in all these amendments including the lower concentration, such as the 0.5 mg/kg Cd^{2+} only treatment and 0.5 mg/kg Pb^{2+} and 0.5 mg/kg Cd^{2+} combined. In comparison with the control, soil nitrifying activity in soil contaminated with 0.5 mg/kg Cd^{2+} was found to be 79.23 ± 4.20 %, lower than the control 83.12% ± 4.16 %. The relative inhibition was increased with the increasing of Pb^{2+} concentration. When the content of Pb^{2+} increased from 0.5 mg/kg to 100.0 mg/kg combined with the constant concentration of 0.5 mg/kg Cd^{2+}, the relative inhibition increased from 4.7 % to 47.6 %.

Soil enzyme activities, soil microbial community structure and biochemical processes usually have complicated relationships among them. It was noted that numerous factors control their relative abundance, *e.g*., original contents of heavy metals, various processes of soil formation, and anthropogenic factors such as the contamination by human activities. In order to evaluate whether there is a synergistic interaction on soil enzyme activities, nutrient cycling and pollutants, the correlation between the relative inhibition of soil urease activity and soil nitrifying activity were depicted in Fig 9, and a significant positive correlation was found between them ($P <$ 0.05). The correlation coefficient was found to be 0.942 (R2), which reflect that heavy metals had similar effect on soil nitrogen cycling and it s relative microbial activity.

Example. 2 Inhibition of Denitrification enzyme activity

Denitrification-related enzymes are generally located within the cell membrane or periplasmic space, expelling heavy metal ions

out of the cell would place them in the immediate contact with denitrification related enzymes, thus limiting utility of such a resistance strategy (Dmitri and Maria, 2008). The fact that denitrification enzymes are located on or near the outer cell surfaces further increases the vulnerability of the entire denitrification pathway to chemical disruption.

Specific inhibition of nitrous oxide reductase by metal has been observed by Hewson and Fuhrman, 2006 resulting in incomplete denitrification leading to emission of nitrous (and possibly nitric) oxides

The relationship in fig 10, shows that although Cr and Cu variability influences DEA variability, a more important role is played by the content of organic carbon and nitrates, which represent the substrates for denitrification activity. It could be expected that only at higher concentrations of metals, their effect on denitrification activity might prevail over other environmental variables. Nitrogen mineralization and nitrification, measured in soils collect in field seemed particularly sensible to Cu contamination, but not to Cr which, being much less mobile then Cu, was probably not enough concentrated to have a relevant impact on those two activities. Denitrification rate was inhibited by both metals, thus appearing suitable as biomarker for soil monitoring for both Cu and Cr. The decrease of mineralization rates as consequence of Cu pollution might reduce the turnover of organic matter and availability of nutrients in the ecosystem. This might be of crucial importance in highly polluted sites.

Example. 3 Inhibition of reductase activity

The extent of inhibition of NO_3^- Reductase, NO_2^- Reductase, NO Reductase and N_2O Reductase depends on different oxidation states and the order of different heavy metals is As(V)>As(III)>Fe(III)>Fe(II)>Se(IV)>Se

(VI). Others metal ions that inhibit the reductase activity are Cd, Hg and W.

The heavy metals inhibit the enzyme activities via various forms like-

by complexing the substrate,
by combining with protein-active groups on the enzyme,
reacting with the enzyme–substrate complex,
masking catalytic active groups,
denaturing protein conformation and
competing with essential metal ions.

Effect of heavy metal pollution on soil N transformation processes

Heavy metals can significantly affect soil microbial biomass, thus altering the role of soil microflora, which is mainly involved in organic matter degradation and recycling of soil nutrients. Microbial processes involved in n transformation are particularly important as their rates influence the amount and the form of mineral N present in the soil, which might be immobilized by organisms or lost from the system. Due to their functions and ubiquitous presence, soil micro organisms play a fundamental role in biogeochemical cycles of nutrients; moreover they are actively involved in forming the structure of soil. Rates of this processes influence the amount and the form of mineral N present in the soil, which might be immobilized by organisms or lost from the system. Heavy metal contamination of soil has been demonstrated to affect significantly soil microbial biomass and functions (Bååth, 1989). Among published data, few studies on the impact of heavy metals on N-mineralization and nitrification are available (Babich and Stotzky 1985; Ross and Kaye 1994; Munn *et al.,* 1997; Sauvè *et al.,* 1999; Smolders *et al.,* 2001), and even fewer assessments have been made on denitrification (Sakadevan *et al.,* 1999; Holtan-Hartwig *et al.,* 2002).

Doelman (1986) reported that N mineralization processes will be inhibited at around 1000 mg kg^{-1}Zn, Cu and Ni, 100-500 mg kg^{-1} of Pb and Cr and 10-100 mg kg^{-1} of Cd.

Data in fig 11, showed that the investigated processes had different sensitivity to the two metals. N mineralization rate decreased with increasing total Cu concentration, whereas no clear relationship was observed with Cr (data not shown). The sites that presented lower mineralization rates (NE and E1) were also characterized by higher organic C content as reported by other authors (Wuertz and Mergeay 1997; Castaldi *et al.,* 2004), probably due to a reduced capacity of microflora to decompose organic matter in polluted sites.

The results obtained from the experiment of nitrification in soils containing various heavy metals are presented in Fig. 12. The reduction of nitrification induced by 10 ppm Cr(6) did not persist after 2 days of incubation. However the inhibition by 100 and 1,000 ppm Cr(6) was not alleviated even after 4 days of

incubation. Chromium chloride was less toxic than chromate and only a concentration of 1,000 ppm was able to decrease the amount of nitrate and nitrite. Vanadate was not as toxic as chromate or chromium chloride in terms of the nitrification process but it reduced the amount of nitrate and nitrite at the 1,000 ppm level and the decrease was no longer observed after 2 days of incubation. Addition of molybdate and tungstate did not exert a toxic effect on soil nitrification and even seemed to have a stimulatory effect on nitrogen mineralization. After the addition of 25 mg of ammonium-N to I00 g soil, about 30 mg of nitrate- and nitrite-N was detected after 1 or 2 days of incubation regardless of the concentration levels of the heavy metals. On the contrary about 27 mg of nitrate- and nitrite-N was detected after 1 or 2 days of incubation in a control soil. Thus 3-5 mg of nitrate- and nitrite-N is considered to be mineralized from the organic nitrogen in soils. Data in Fig. 13 indicate the amount of inorganic nitrogen mineralized by ammonification in soils containing 0, 10, 100, 1,000 ppm levels of heavy metals.

Table.1 Total concentration range and limit of heavy metal in soil

Elements	Conc. range (mg/kg)	Limit (mg/kg)
Lead	1-6900	600
Cadmium	0.1-345	100
Arsenic	0.1-102	20
Chromium	0.005-3950	100
Mercury	0.001-1800	270
Copper	0.03-1550	600

Salt *et al.,* 1995 and Riley *et al.,* 1992:

Table.2 Beneficial effects of metal ions

Heavy Metal	Beneficial Effect
Zn	Synthesis of carbohydrates, proteins, phosphate, auxins, RNA and ribosome.
Al	Controlling colloidal properties in cell, activation of dehydrogenases
As	Metabolism of CHO in algae and fungi
Co	Symbiotic and non-nodulating N fixation
Cu	Photosynthesis, respiration, protein and CHO metabolism.
Fe	Photosynthesis, N fixation
Ni	Hydrogenase activity and N fixation.

(Maliwal and Patel, 2011)

Table.3 Biochemical effect of excessive concentrations of heavy metals

Elements	Biochemical processes affected
Ag, Cd, Cu, Hg, Pb,	Permeability of cell membrane
Hg	Inhibition of protein synthesis
Ag, Hg, Pb, Cd, As	Bonding to sulphydryl groups
As, Se, W, F	Competition for sites with essential metabolites
Cs, Rb Sr, Se	Replacement of essential atoms
Ti, Pb, Cd	Inhibition of enzymes, microbial Respiration
Cd, Hg, Pb, Zn	Photosynthesis, Transpiration
Cd	Disturb enzyme activities, inhibition of DNA-mediated transformation in microorganisms, reduced plant-microbes symbiosis
Cu, Ni. Zn, Cd, As	Inhibit the growth, morphology and activities of various groups of microorganisms, symbiotic N_2 fixers

(Maliwal and Patel, 2011)

Table.4 Ranges of the selected microbial groups in heavy metal contaminated and uncontaminated soils of ArcelorMittal steelworks in Cracow, Poland

Sl. No.	Analyzed micro organisms (CFU X g^{-1})	Uncontaminated soil	Heavy metal contaminated soil
	Total nuber of mesophilic bacteria	22.50×10^2 - 10.44×10^6	0-13.15×10^5
	Total number of fungi	84.00×10^1-21.03×10^3	0-57.90×10^3
	Actinomycetes	62-99.50×10^3	0-20.26×10^3
	Azotobacter spp.	0-28.90×10^2	0-57.00×10^1

Anna Lenart-Boron and Piotr Boron, 2015

Table.5 Number of ammonifying bacteria under varied heavy metal contaminated soils

Object	Number of ammonifying bacteria (cfu 10^8 kg^{-1} of d.m. soil)			
	Contamination level*			
	I		II	
	soil use			
	unsown	sown	unsown	sown
0	137 ± 8	162 ± 9	137 ± 12	162 ± 12
Cd	123 ± 5	155 ± 10	115 ± 9	150 ± 10
Cu	123 ± 8	155 ± 7	106 ± 5	153 ± 9
Pb	106 ± 2	134 ± 5	109 ± 8	138 ± 9
Zn	106 ± 7	134 ± 10	105 ± 9	146 ±14
CdCu	111 ± 5	169 ± 10	114 ± 5	158 ± 7
CdPb	111 ± 7	169 ± 8	103 ± 5	159 ± 5
CdZn	90 ± 7	122 ± 7	65 ± 4	125 ± 5
CdCuPb	90 ± 6	122 ± 6	98 ± 4	158 ± 5
CdCuZn	114 ± 6	180 ± 7	88 ± 6	149 ±6
CdPbZn	114 ± 7	180 ±11	80 ± 4	143 ± 9
CdCuPbZn	102 ± 5	162 ±9	50 ± 2	115 ± 5
Mean	111 ± 1	153 ±2	97 ± 2	146 ± 2

I contamination level in mg per kg d.m. of soil: Cd – 4; Cu – 150; Pb – 100; Zn – 300
II contamination level in mg per kg d.m. of soil: Cd – 12; Cu – 450; Pb – 300; Zn – 900

Wyszkowska *et al.,* 2008

Table.6 Effects of the combined pollution of Pb2+and Cd2+ on soil urease activity

| Treatments	Incubation period		
	10 days	20 days	45 days
CK	4.12 ± 0.16	4.26 ± 0.14	4.20 ± 0.18
0.5 mg/kg Cd^{2+}	4.46 ± 0.23	4.37 ± 0.17	4.53 ± 0.21
0.5 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	4.18 ± 0.19	4.24 ± 0.15	4.27 ± 0.15
1.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	3.88 ± 0.14	3.74 ± 0.24	3.50 ± 0.16
5.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	3.52 ± 0.17	3.32 ± 0.09	3.21 ± 0.13
10.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	3.24 ± 0.13	3.10 ± 0.12	3.01 ± 0.18
50.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	2.12 ± 0.10	1.98 ± 0.14	2.04 ± 0.08
100.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	1.64 ± 0.15	1.28 ± 0.17	1.13 ± 0.12

Jinlong *et al., 2013*

Table.7 Effects of the heavy metals pollution on soil nitrifying activity after 45 days incubation

Treatments	Nitrifying activity	Relative inhibition (%)
CK	83.12 ± 4.16	0
0.5 mg/kg Cd^{2+}	79.23 ± 4.20	4.7
0.5 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	74.20 ± 4.58	10.7
1.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	68.30 ± 3.28	17.8
5.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	62.50 ± 3.41	24.8
10.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	60.12 ± 3.12	28.3
50.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	52.07 ± 3.18	38.4
100.0 mg/kg Pb^{2+} + 0.5 mg/kg Cd^{2+}	43.52 ± 3.16	47.6

Jinlong *et al., 2013*

Fig.1 Schematic representation of nitrogen cycle
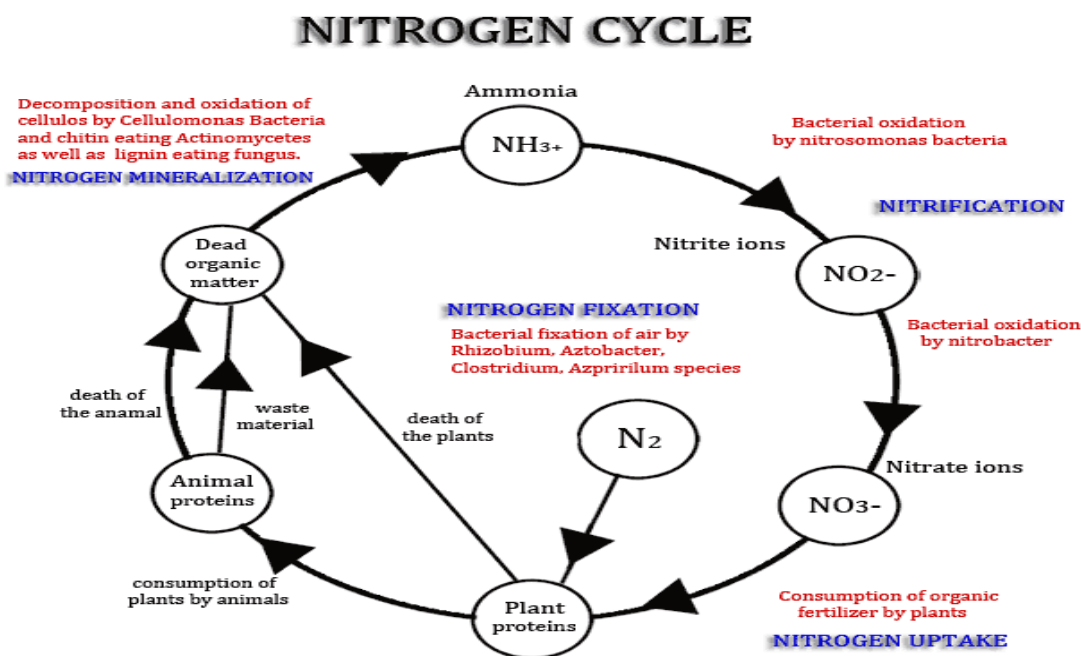
Fig.2 Schematic representation of nitrogen mineralization process

Fig.3 Schematic representation of denitrification process

a. NO_3^- Reductase   c. NO Reductase

b. NO_2^- Reductase   d. N_2O Reductase
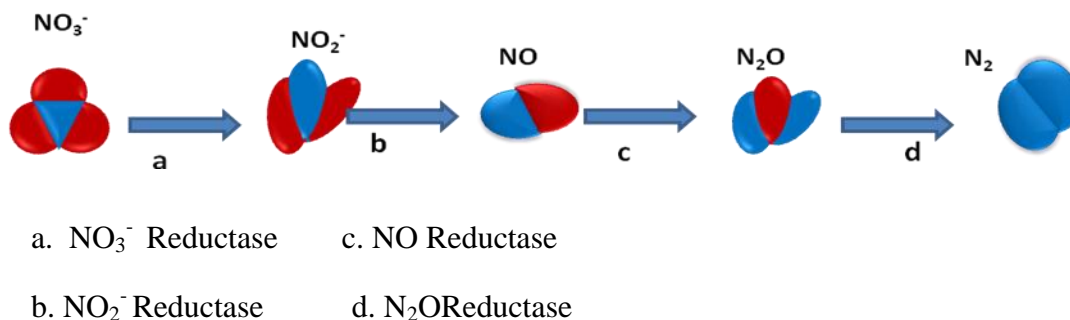
Fig.4 Amount (mg l-1) of nitrate produced from ammonia by enrichment cultures of different metals containing Nitrosomonas spp
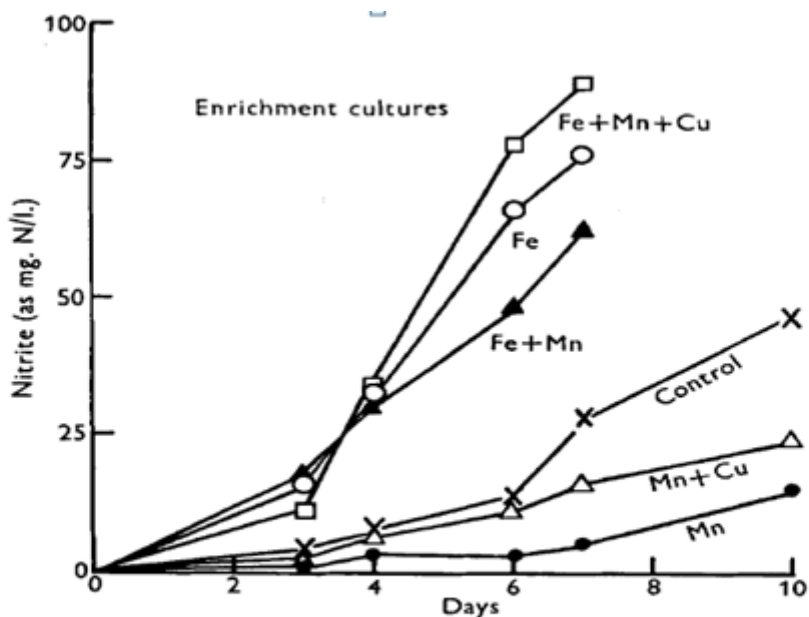
1. Nitrite produced from ammonia by enrichment cultures containing *Nitrosomonas* spp. Metals: ×—×, control (none added); ○—○, iron, 6 mg./l.; ●—●, manganese, 1·08 mg./l.; ▲—▲, iron + manganese; △—△, manganese + copper, 0·14 mg./l.; □—□, iron, manganese, and copper.

Meiklejohn,1953

Fig.5 Effect of large concentration of Fe on oxidation of ammonia to nitrite by Nitrosomonas europea

. Effect of large concentration of Fe on oxidation of ammonia to nitrite by *Nitro-smonas europaea.* ×—×, control (no added Fe); O—O, 6 mg. Fe/l.; △—△, 12 mg. Fe/l.; ●—●, 560 mg. Fe/l.

Meiklejohn,1953

Fig.6 Oxidation of nitrite to nitrate by a strain of Nitrobacter winogradskyi in the presence of metal
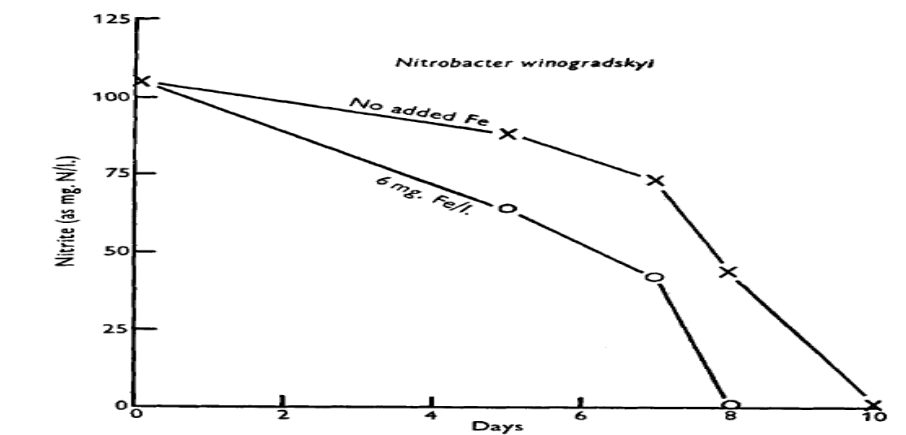
. Oxidation of nitrite to nitrate by a strain of *Nitrobacter winogradskyi.* ×—×, control (no added Fe); O—O, 6 mg. Fe/l. (Average values from duplicate cultures.)

Meiklejohn,1953

**Fig.7** Effect of heavy metals on the growth of Azatobacter in a synthetic medium

1. Effects of heavy metals on the growth of *Azotobacter* in a synthetic medium. ●, 0 μM; ○, 5 μM; □, 25 μM; ■, 125 μM.
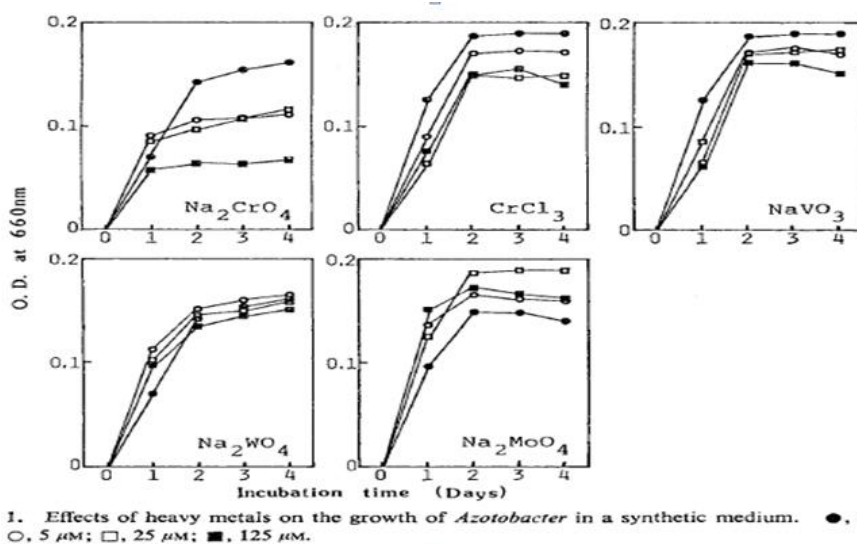
Kunio *et al.*, 2012

Fig.8 Effects of heavy metals on the growth of Fusarium in a synthetic medium

●, control; ○, 5 μM; □, 25 μM; ■, 125 μM.
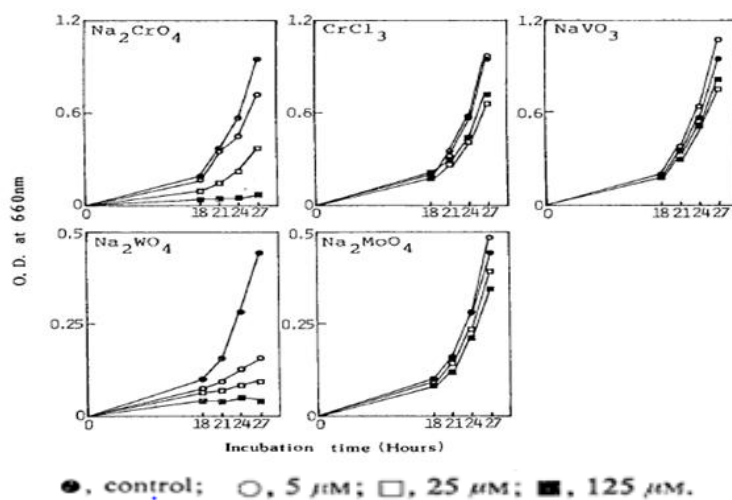
Kunio *et al.*, 2012

Fig.9 Correlative curve of the inhibition of soil urease activity and nitrifying activity
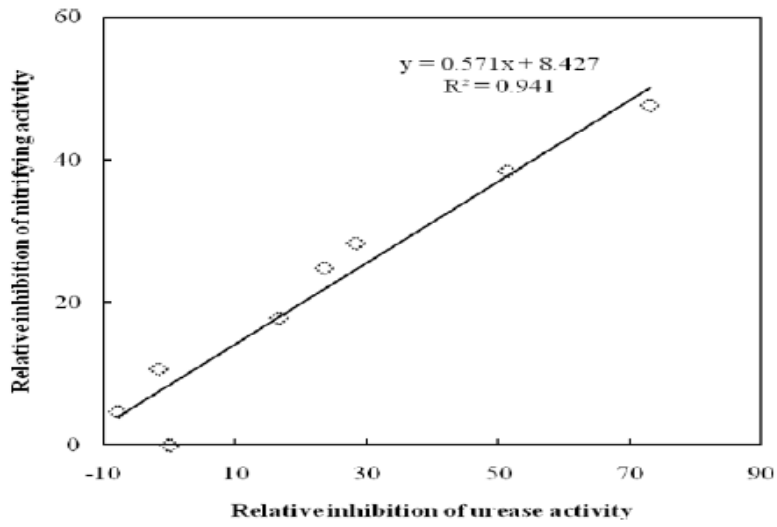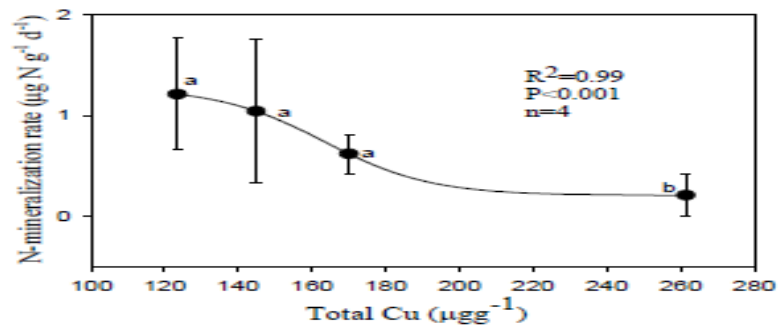

Jinlong *et al., 2013*

Fig.10 Denitrification enzyme activity (DEA) plotted versus total Cu and Cr concentrations
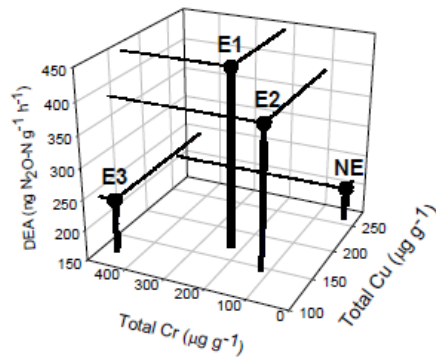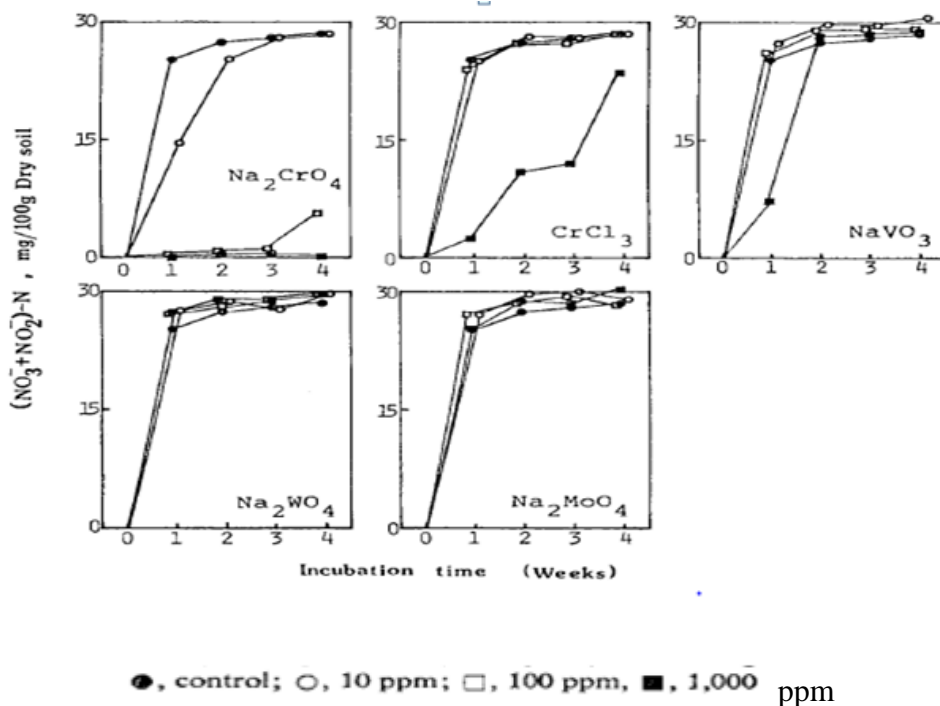

Anna *et al., 2004*

Fig.11 Mineralization rate plotted versus total Cu concentrations

Anna *et al., 2004*

Fig.12 Effect of heavy metals on inorganic N content (nitrate plus nitrite) in a paddy soil during the nitrification of 250 µg NH4+-N-(g soil)-1

●, control; ○, 10 ppm; □, 100 ppm, ■, 1,000 ppm

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Fig.13 Effects of heavy metals on inorganic N values in a paddy soil during ammonification of 250 µg urea-N (g soil)-1
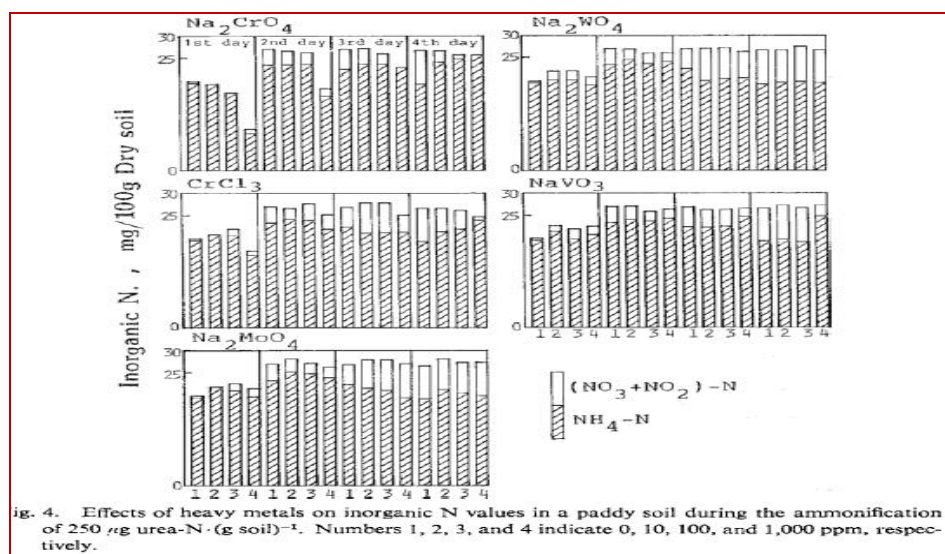

ig. 4. Effects of heavy metals on inorganic N values in a paddy soil during the ammonification of 250 µg urea-N·(g soil)⁻¹. Numbers 1, 2, 3, and 4 indicate 0, 10, 100, and 1,000 ppm, respectively.

Kunio *et al.,* 2012

The results show that the effects of heavy metals on the ammonification of urea were negligible except for a 1,000 ppm level of chromate and chromium chloride. Chromium(6) of a 1,000 ppm level inhibited ammonification during the first 3 days while a

level of 1,000 ppm Cr(3) reduced ammonification only on the first day. Tungstate, molybdate, and vanadate did not exert inhibitory effects on ammonification regardless of the concentration. On the contrary these three metals seemed to promote ammonification or mineralization during the first or two days of incubation, because the amount of mineralized nitrogen in soils polluted by these metals increased somewhat more than in the control soil during these periods.

In conclusion, azotobacter is considered to be more sensitive to heavy metal contamination in soil compared to other soil micro organisms involved in nitrogen transformation. Nitrogen mineralization and nitrification, measured in soils collect in field seemed particularly sensible to Cu contamination, but not to Cr which, being much less mobile then Cu, was probably not enough concentrated to have a relevant impact on those two activities. Denitrification rate was inhibited by both metals, thus appearing suitable as biomarker for soil monitoring for both Cu and Cr.

The effects of heavy metal contamination on soil are quite alarming and cause huge disturbances in the ecological balance and health of living organisms on earth. Micro organisms and enzymes associated with N transformation in soil are inhibited directly or indirectly by heavy metals. The extent of inhibition depends on the concentration and oxidation state of heavy metals and on soil characteristics.

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