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Effect of Metals Contamination on Soil Microbial Diversity, Enzymatic Activity, Organic Matter Decomposition and Nitrogen Mineralisation (A Review)

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Abstract: Soil is home to remarkable diversity of life from microbes to moles and a well-established ecosystem among these different organisms exists. Microorganisms either independently or in association with other organisms are playing very important role in the plant nutrients recycling. Soil metal concentrations above certain limits disrupt this ecosystem and as a result plant nutrient cycling process.

Metal contamination in soils cause proportions of some groups of microorganisms to increase than the others. Compared with bacteria certain groups of fungi are less affected by soil metal pollution. Phospholipid fatty acid (PLFA) measurements in metal contaminated soils show that within bacterial population, metals are more toxic to gram-positive than gram-negative bacteria. Significant reductions in enzymatic activities occur in soils contaminated with metals and generally metals are more toxic to intra-cellular enzyme activities (e.g. dehydrogenase) than extra-cellular activities (e.g. phosphatase). Similarly, enzymes produced by bacteria are more affected by metal contamination than those produced by fungi. Organic matter decomposition and nitrogen mineralisation, which are carried out by various groups of microorganisms can also be affected by increased metal concentrations in the soils.

Key words: Metal pollution, microbial community structure, enzymatic activity, organic matter decomposition, N mineralisation

Introduction

Metals or heavy metals is the terra applied to a large group of elements with an atomic density greater than 6 g cm^3 (Phipps, 1981). These 'metals are also called trace elements as they occur in concentrations of less than 1% in the rocks of the earth's crust, a primary source of these metals. Metals occur naturally in soils, usually at relatively low concentrations, as a result of the weathering and other-pedogenic processes acting on the rock fragments on which the soil develop. Besides weathering of rocks other major sources of metals to soils are metalliferous mining and smelting, agricultural and horticultural materials (e.g. chemical fertilizers and pesticides), sewage sludges, effluent irrigation, fossil fuel combustion and metallurgical industries etc.

Most of these metals are essential in minute quantities for all living organisms and agricultural land in many parts of the world has been found to be deficient in one or more micro nutrients (an alternative term used for metals). However, numerous studies of metals in ecosystem have indicated that many areas near urban complexes, metalliferous mines or soils subjected to successive sludge applications and effluent irrigations contain high concentrations of metals, for example, As, Cd, Cu, Hg, Ni, Pb and Zn. Almost all of these metals may be toxic to soil microorganisms if found in higher concentration.

Soil organisms including bacteria, fungi, actinomycetes and algae mediate many of the processes that influence soil fertility. For example, microorganisms are dynamically involved in many basic ecologic processes, such as the biogeochemical cycling of elements, and the mineralisation of carbon, nitrogen, sulfur and phosphorus (i.e. organic matter decomposition) (Paul and Clark, 1996). A decrease in the number of species or diversity of microorganisms affects the cycling of plant nutrients in the soil. Similarly, any disturbance in the soil ecosystem can disturb the microbial activity and hence the availability of nutrients. Therefore, the presence and activity of soil microorganisms are of fundamental importance to the productivity of agricultural soils (Smith, 1991).

It has been observed that soils contaminated with metals affect microbial population, community structure (biodiversity), microbial activities and processes (e.g., decomposition and mineralisation). This review paper will

discuss only effects of metals' toxicity on microbial diversity (community structure, biodiversity), enzymatic activity, organic matter decomposition and nitrogen mineralisation in the soil.

Soil microbial diversity: Microbial communities in soil are thought to be highly diverse. A gram of soil may contain as many as 13,000 species of bacteria (Torsvik *et al.*, 1990, 1994) and an unknown diversity of fungi and algae. It is highly unlikely that a test using a single organism or group of organisms from such a complex system could represent all the variety of effects that a pollutant might have on the system on the whole (Bogomolov *et al.*, 1996).

Fliessbach *et al.* (1994) reported that the toxicity exerted by metals may suppress or even kill sensitive members of the microbial community and lead to a shift in community structure. Some species might be eliminated while others may appear in larger numbers because of reduced competition for substrate. Jordan and Lechevalier (1975) reported that microbial populations in soils close to a zinc-smelter contained greater proportions of bacteria able to grow on media containing high concentration of Zn. Several authors have found that, within the bacterial community, metals are more toxic to Gram-positive than the Gram-negative bacteria (Barkay *et al.*, 1985). Similarly, Duxbury and Bicknell (1983) studied soil, which was contaminated due to industrial complex in New South Wales, and found that Gram-negative bacteria were approximately 15 times more abundant in the metal contaminated soils (Cd, Cu, Ni and Pb) than the Gram-positive bacteria.

Other studies have shown that metal contamination causes a shift in soil microbial community structure from sensitive to resistant microorganisms (Doelman, 1985; Giller *et al.*, 1984). Different physiological groups of bacteria and fungi have shown different response to metal pollution. The soils investigated by Nordgren *et al.* (1986) had metal concentrations from 5 to 75 times higher in the plots closest to the Zn-smelter compared with background levels. The number of bacteria capable of hydrolysing various substrates usually decreased by a factor of 8-11 and chitin hydrolysing bacteria by a factor of 5. In contrast fungal mycelia lengths were not affected by the soil contamination. Their conclusion was that bacteria appeared to be more sensitive to metals than fungi. However, in

earlier work on the same site (Nordgren *et al.*, 1983) they reported that total fungal biomass significantly decreased in metal contaminated soils and this decrease was significantly related to metal concentrations. They also studied fungal species composition and found that abundance of the genus *Penicillium* Link ex Fr decreased from 30 to 40% of the total number of fungal isolates in control sites to about 5% at the sites close to the smelter. In contrast other genus, such as *Paecilomyces* Bainier increased in abundance close to the source of contamination. Another genus *Mortierella* Coemans increased with metal concentration to a peak abundance at about 2,000 μg of Cu g^{-1} and decreased closer to the smelter. In paddy fields polluted with Cu mine drainage, Yamamoto *et al.* (1981) noticed an increase in the fungal population with increased in Cu concentrations. This increase in the fungal population was attributed to an increase in the population of Cu-tolerant fungi. In contrast Pancholy *et al.* (1975) found a significantly larger population of fungi in a control compared with the metal-polluted site. Similarly, Bardgett *et al.* (1994) reported that the eukaryotic (primarily fungi) component of biomass declined linearly with increasing metals contamination, whereas, prokaryotic (bacteria and actinomycetes) contribution changed little.

These conflicting results in population structure probably arise from the metal tolerant microbial populations being developed to different extents (Ohya *et al.*, 1988).

Soil contaminated with Zn (234-571 mg kg^{-1}), Cu (310-751 mg kg^{-1}) and Cd (1,1-2.7 mg kg^{-1}) due to irrigation water containing these metals was investigated by Hiroki (1992) who reported that the number of actinomycetes and bacteria decreased significantly with increasing metals concentrations. In contrast, he did not observe much effect on the number of fungi and suggested that the degree of tolerance to metals was: fungi > bacteria > actinomycetes. Similarly, Maliszewska *et al.* (1985) reported that fungi and actinomycetes are most resistant to metal toxicity than *Azotobacter* sp. and nitrifying bacteria appear to be the most sensitive.

Other studies also showed that bacteria are more sensitive to metal than actinomycetes and fungi (e.g. Huysman *et al.*, 1994). Jordan and Lechevalier (1975) found a decrease of between one to two orders of magnitude in numbers of bacteria along a Zn pollution gradient while fungi and actinomycetes were less affected.

Dar (1996) studied the effect of added Cd and found greater diversity of fungi and higher bacterial populations in control than in the Cd treated soil. Increased Cd concentrations caused a sizeable reduction in the growth of *Penicillium*, *Rhizopus*, *Fusarium* and *Geomyces*, but stimulated the growth of *Aspergillus*, *Trichoderma* and *Paecilomyces*. They concluded that certain species were tolerant to the Cd levels used and, in the absence of competition, proliferated rapidly. Reber (1992) reported that in 3 out of 5 experiments, metal stress decreased the diversity of bacteria. In the other 2 experiments, an unchanged or even higher diversity in the metal contaminated soil was observed which the author attributed to the high pH in those soils.

It is believed that metals from past applications of sewage sludge have decreased the genetic diversity of *Rhizobium leguminosarum biovar trifolii* populations (Hirsch *et al.*, 1993). The population of *Rhizobium leguminosarum biovar trifolii* had been radically altered by long-term exposure to metal contamination, apparently losing those agronomically important strains capable of forming effective symbiotic associates with white and red clover (Hirsch *et al.*, 1993). The acetylene reduction potential of both heterotrophic N₂ fixing soil bacteria and blue-green algae are studied in a metal polluted soil (Brookes *et al.*, 1986; Dahlin *et al.*, 1997). The population size of rhizobia has been found to

decrease as a result of metal contamination at the concentrations around European Community limits (DoE, 1989) for soil receiving sewage sludge (Chaudri *et al.*, 1993). Similarly, in the Woburn experiment, it was found that populations of cyanobacteria (blue-green algae), *Rhizobium leguminosarum biovar trifolii* and mycorrhizal fungi were adversely affected in some soils by metal concentrations which were below the European Community maximum allowable concentration limits for metals in sludge treated soils (McGrath, 1994).

Brookes *et al.* (1986) used the same soil (Woburn soil) to study the effects of metals on nitrogen fixation by blue-green algae. They found that total C₂H₂ reduction decreased by 50% at about 50 μg EDTA-extractable Zn, 20 μg Cu, 2.5 μg Ni and 3 $\mu\text{g g}^{-1}$ total Cd g^{-1} soil. Similarly, Giller *et al.* (1984) found that white clover rhizobia were unable to survive (or at least unable to remain effective) in the presence of metal concentrations close to current limits (DoE, 1989).

It might be possible, that the replacement of sensitive species by resistant species does not change overall microbial indices such as soil respiration or total biomass. Jansen *et al.* (1994) reported that the total number of bacteria in soils estimated by plate counts, with increasing doses of Cd did not change. However, the ratio between resistant and sensitive bacteria increased substantially; resistant and sensitive bacteria were distinguished on the basis of growth in media with added Cd. It has been reported that the usefulness of investigations (Frostegard *et al.*, 1993) involving cultivation of microorganisms on agar plates is limited in that only a minor part of the community is studied, since the majority of soil microorganisms are non-culturable with this technique (Bakken, 1985). One way to examine the entire microbial community structure is to analyze the phospholipid fatty acid (PLFA) composition of the soil, since different subsets of a community have different PLFA patterns (Tunlid and White, 1992). Phospholipids exist only in membranes of living cells (White *et al.*, 1979; Tunlid and White, 1992). Therefore, the content of phospholipids has been used as an indicator of microbial biomass both in aquatic and terrestrial ecosystems (Balkwill *et al.*, 1988; Petersen *et al.*, 1991).

In complex communities like those in soils, it is not normally possible to distinguish specific taxa using PLEA profiles, since most PLFAs exist in different concentrations in a taxonomically wide range of soil microorganisms (Ratledge and Wilkinson, 1988). However, PLFA analyses make it possible to study the dynamics of larger groups of organisms. For example, prokaryotes and eukaryotes can be distinguished and similarly within prokaryotes, the actinomycetes have specific PLFAs (Kroppenstedt, 1985). Gram-negative bacteria can be distinguished by extracting the lipopolysaccharide fatty acids (Parker *et al.*, 1982; Zelles *et al.*, 1994). The ratio of fungal to bacterial biomass can also be measured by measuring fungal and bacterial PLFA (Frostegard and Baath, 1996).

Ohya *et al.* (1986) reported a change in PLFA composition of bacteria isolated from soil after amendment with Zn. In another study Frostegard *et al.* (1993) extracted PLFAs directly from the arable soil experimentally exposed to different metal (Cd, Cu, Ni, Pb and Zn) concentrations and incubated for 6 months. Metal pollution resulted in a decrease in the iso-branched PLFAs i15:0 and i17:0 and in the unsaturated 16:1w5 and 16:1 w7c fatty acids, while increases were found for i16:0, the branched br17:0 and br18:0, and the cyclopropane cy17:0 fatty acids. Fatty acid 18:2w6, which is considered to be predominantly of fungal origin, increased as metal inputs increased, except in the Cu contaminated samples, in which it decreased. They also reported a shift from Gram-positive to Gram-negative bacteria as an increase in cy17:0, which is considered to be

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typical for Gram-negative bacteria was observed and a decrease in several iso- and anteiso-branched PLFAs all of which are commonly found in the gram-positive bacteria (Wilkinson, 1988).

In their other study (Frostegard *et al.*, 1996) the effect of Zn contamination on the microbial structure of a forest soil arid an arable soil was investigated. Concentrations of Zn used were 0-256 mmol kg⁻¹ for the forest soil and 0-128 mmol kg⁻¹ for the arable soil. Phospholipid fatty acids analysis (PLFAs) after 18 months of incubation showed that 18:2w6, indicating fungi, increased proportionally due to the metal amendment in both soils. The proportions of several individual bacterial PLFAs changed in both soils due to treatments indicated shifts within the bacterial community in the soils. These results were also supported by Baath *et al.* (1998), who studied the effects of metal-containing sewage sludge on soil microbial community in two agricultural soils which had been contaminated with Zn, Cu and Ni more than 20 years ago. Another short-term laboratory's study by Khan and Scullion (2000) across four different soils also reported increased fungal to bacterial PLFAs' ratio in metal contaminated soils compared with the controls. They also reported that treatments effects were more pronounced in loam than clay soils which they attributed to the greater bioavailability of metals in coarser than in heavier textured soil.

Any shift from bacterial to fungal population could also be assessed by studying the C:N ratio of the soil microorganisms. For example, biomass C to N ratio of bacteria ranges from 3:1 to 5:1, whereas that of fungi varies from 4.5:1 to 15:1 (Paul and Clark, 1996). Khan (1999) observed a general increase in biomass C to biomass N ratio in the grassland soil with higher metal inputs (except Ni) and concluded that this trend could be considered indicative of an increase in fungal relative to bacterial biomass in the high metal soils. In the same study (Khan, 1999) he also observed a consistent increase in ergosterol content with increase in metal inputs and reported that increased ergosterol content in response to metal inputs further support the idea that fungal compared with bacterial biomass increase in high metal soils.

Recently a new biologic assay has been applied for evaluating the changes in the soil microbial community structure. Binlog is a redox system that allows characterization of entire microbial communities, based on the pattern of utilization of 95 different C substrates, and produces a metabolic profile. Knight *et al.* (1997) applied this method to a soil that had been amended in the laboratory with solutions of metal salts and found significant changes in microbial community structure compared with the control. Kelly and Tate (1998) used the same procedure to investigate the effects of elevated metal loadings in the vicinity of a Zn-smelter. They reported significant changes in the microbial population structure in the metal contaminated soil.

Overall, soil metal pollution cause a shift in microbial community structure from sensitive to more tolerant microorganisms. Metals seem to be less toxic to fungal than bacterial population in the soil. Similarly, Gram-positive bacteria are more sensitive to metals than Gram-negative bacteria. It can also be concluded from the above discussion that no single biological property would be sufficient to characterize change and evaluating individual properties with soil metal loadings could lead to conflicting conclusions (Knight *et al.*, 1997). The study of holistic biological parameters such as biologic assay may be used in conjunction with traditional measures of soil microbial population size and activity for assessing soil quality (Kelly and Tate, 1998).

Soil enzymes: Like other microbial processes any change in the soil micro-environment can disturb soil enzymatic activities. Soil enzymes used as a measure of microbial

activity, give an indication of change, but not an absolute measurement of any specific group of organisms. Some enzymes activities serve as a measure of the microbial intra-cellular activity (e.g. dehydrogenase) while others reflect extra-cellular activity (e.g. phosphatase) in the soil and therefore can be used as a simple metal toxicity test (Rogers and Li, 1985).

Different enzymes are reported to respond differently to metal contamination. For example Brookes *et al.* (1984) observed that phosphatase activity was unaffected, but dehydrogenase activity decreased with increased metals loads. They concluded that as the dehydrogenase enzyme only functions in the living cells, the decrease in activity might be due to Stress on micro-organisms. Reddy *et al.* (1987) supported the Brookes *et al.* (1984) results by observing a reduction in dehydrogenase activity with increasing rates of contaminated sewage sludge addition, with no effect on phosphatase activity.

Dar (1996) compared different soil types and reported that increasing concentrations of Cd decreased dehydrogenase, alkaline phosphatase and arginine-ammonification activities in the sludge amended and unamended soils. However, these effects were less pronounced in the former soil, He also found a more pronounced negative effect of Cd in the sandy-loam than in the loam and clay-loam soils and suggested that the degree of inhibition in enzyme activities by Cd was related to the clay and organic matter contents of the soils. Similar results were also reported by Doelman and Haanstra (1979) who found a significant reduction in dehydrogenase activity in a sandy soil treated with 375 µg Pb and did not observe any toxic effect in clay and peat soils. In their other studies (Daelman and Haanstra, 1989; Haanstra and Doelman, 1991) short- and long-term effects of metals on phosphatase and arylsulphatase activities in 5 different soils were investigated. They reported that at the ED₅₀ value (ecological dose), toxicity did not decrease with time and in sandy soils, was approximately 2.6 mmol kg⁻¹ dry soil for Cd, Cu, and Zn. In the long-term field experiment at Braunschweig (Germany) a detailed study of metal effects on enzyme activities was carried out in the low and high rates of uncontaminated and contaminated sludge amended and inorganic fertilizer soil (Balzer and Ahrens, 1991). Most enzyme activities (phosphatase, protease, glucosidase and catalase) were stimulated by the addition of sludge organic matter, except urease at the highest rate of contaminated sludge and dehydrogenase at both rates of contaminated sludge.

Lower activities of dehydrogenase, urease and acid phosphatase enzymes and no effect on β-glucosidase activity in Cu and Zn polluted soils around a brass smelter in Sweden were recorded by Tyler (1974). Aoyama *et al.* (1993) also reported similar results for Cu added to soil without orchard grass but enhanced activity of this enzyme with added Cu in the orchard grass amended soil. In contrast, Hattori (1989) observed that the activity of β-glucosidase and fungal population was increased at higher Cd inputs. He attributed the increase in the β-glucosidase activity to the increase in the fungal population as fungi are the primary source of β-glucosidase (Hayano and Tubaki, 1985).

Effects of added metals (Zn, Cu, Ni and Cd) on various enzymes involved in the cycling of C, N, P and S were evaluated by Kandeler *et al.* (1996). A general decrease in the activities of enzymes with increase metal inputs was recorded, however, the degree of effect was different for different enzymes. For example various enzymes related to the cycling of N, P and S showed a considerable decrease in activity. In particular, arylsulfatase and phosphatase activities were more affected. In contrast, enzymes involved in the C-cycling (cellulase and xylanase) were not

affected significantly at the lowest contamination level (300 mg Zn, 100 mg Cu, 50 mg Ni and 3 mg Cd kg⁻¹ soil). Cellulase and xylanase are produced mainly by saprophytic fungi in an aerobic environment (Alexander, 1977). Therefore, Kandeler *et al.* (1996) concluded that since microbial biomass was reduced and cellulase and xylanase were not significantly influenced, low metal contamination probably shifted the balance of the microbial community from bacteria to fungi. It may be concluded from the discussion above that in general the degree of enzyme inhibition varies with concentration and form of added metal, the soil investigated and the enzyme assayed (Ladd, 1985; Nannipieri, 1994). Enzymes produced mainly by fungi seem to be less affected. Similarly, intra-cellular rather than extra-cellular enzymes may be good predictor of soil metal contamination because these enzymes may be an indirect measure of the amount of biomass or at least its state of activity.

Organic matter decomposition: Both natural and man-modified environmental conditions in the soil may markedly affect the rate of decomposer (e.g. microorganisms) activities. Decomposition of organic matter is one of the most important processes in nutrient cycling (Laskowski *et al.*, 1994). Any factor inhibiting this process would tend to result in accumulation of organic matter in undecomposed or partially decomposed form (Bhuiya and Cornfield, 1972). In the 1970s Ruhling and Tyler (1973) studied litter decomposition rate in pine forests under the influence of emissions of Cu, Zn, Cd, Ni and Pb and suggested that litter decomposition rate can be retarded by metals, at least in acidic soils. Babich and Lighthart (1974) have suggested that an accumulation of metals can be toxic to microorganisms and that this phenomenon is responsible for the inhibition of decomposition of organic matter in the heavily polluted forests. Strojan (1978) reported that in heavily polluted regions the organic matter on the forest floor was as high as 213% that of the control.

In agricultural soils the accumulation of organic matter due to long-term metal contamination has rarely been found even at metal concentrations at which other microbial activities are affected (Giller *et al.*, 1998). The large pool of organic matter in the agricultural soils and especially when contamination is due to sewage sludge application makes it difficult to detect a relatively small changes in the organic matter over a period of several years (Bosatta and Agren, 1994). So far enhanced accumulation of soil organic matter in the sewage sludge experiments was only observed in the Lee Valley and Luddington experiments in the U.K. (Chander and Brookes, 1991).

However, under laboratory condition effects of metals could be observed using easily degradable sources of Aoyama and Kuroyanagi (1996) observed decreases in the rates of decomposition and C-mineralisation of cellulose powder, when bordeaux mixture and lead arsenate were applied to the soil in a laboratory experiment. Similar results were reported earlier by Tyler (1975). The rates of cellulose and starch decomposition were lower in metal contaminated soil.

Zwolinski (1994) found that Zn and Cu pollution resulted in a distinct decrease in soil organic matter decomposition under laboratory incubation. A close negative relationship between soil respiration and organic matter decomposition was also noted. Bogomolov *et al.* (1996) investigated the effect of Cu on litter decomposition and reported inhibition of decomposition starting at 100 mg total Cu kg⁻¹ of soil. Cotrufo *et al.* (1995) found soil organic matter decomposition to be influenced by metal contamination shortly after metals were applied and suggested that metal toxicity decreases with the formation of complex substances. Similar results were also reported by

Aoyama *et al.* (1993) who obtained a decrease in the mineralisation rate of added orchard grass in the Cu-enriched soil during the initial period of incubation (up to 1 week). However, they observed that the amount of mineralized C increased with increasing concentration during the latter part of incubation and concluded that the decomposition of orchard grass may be prolonged rather, than depressed when soil is enriched with Cu. In contrast, Strojan (1978) observed that differences in decomposition rate due to metal pollution increased with time. Tyler (1977) also found little effect of pollution level on degradation of different litter fractions during the first few weeks when easily degradable substances were degraded by microorganisms. This could be one reason why Crist *et al.* (1985) did not find any decrease in fresh litter decomposition after addition of 1000 µg Pb g dry weight of litter in a short-terra experiment. These conflicting conclusions may be due to metal affecting microbial degradation of different organic substrates to various extents.

A short-term study was conducted by Yeates *et al.* (1994), who buried cotton fabric strips in the Cu contaminated (up to 700 µg g⁻¹) grassland soil for 28 days and measured the decomposition by both weight loss and reduction in tensile strength. They reported that in plots with medium arid high metal contamination much of the original tensile strength remained compared with the low metal and control arid attributed this effect to the decrease in organic matter decomposition rate in the metal contaminated soil. They did not find any significant loss in weight of cotton strips. However, it was reported that the strips may not have been buried long enough to lose a significant amount of weight. Harrison *et al.* (1988) also reported that change in tensile strength of cotton strips is an earlier indicator of decomposition than the weight.

A close positive relationship between the total metals (Zn, Cu, Cr, Ni, Cd and Pb) and soil organic matter content was observed by Valsecchi *et al.* (1995). The source of metal contamination was irrigation water (urban wastewater). They gave 2 possible hypothesis for this positive relationship: (i) the application of different quantities of organic matter polluted with metals caused, over time, an increase in these elements in the soil, with the highest levels in soils treated with the largest amounts of organic matter, (ii) the metal content of the most polluted soils has interfered with C mineralisation, leading gradually to accumulation of organic C in these soils. Earlier studies have shown that the deposition of Pb from vehicle exhaust and fumes and abraded metals from vehicle components has resulted in metal contamination of roadside soils and vegetation (Lagerwerff and Specht, 1970; Smith, 1976; Harris, 1991). Post and Beeby (1996) studied rates of degradation of cellulose-strips and decomposition of litter bags in roadside soils of low and high metal concentrations. Cellulose-strip degradation was found to be similar in both the low and high metal soils. However, plant litter collected from close to the road side was less readily decomposed than litter collected at a distance from the road. They concluded that the high metal contents in the plant litter close to the roadside compared to the that collected at a distance were the primary factor differentiated the decomposition rates. They further reported that the size and potential activity of the microbial decomposer community appeared not to have been adversely affected by increased soil metal concentrations arising from the traffic pollution. However, metal contamination of roadside herbage did have an inhibitory effect on litter decomposition. More or less similar results were also observed by Hattori (1996), who studied the decomposition rate of organic matter (sludge and rice straw), to which Cd had been added previously, over a 4 weeks of laboratory study. Significant inhibition was found in the decomposition of Cd treated

organic matter compared with the organic matter with no sorption of Cd. In an earlier study by Mathur and Preston (1981), a reduction in organic matter decomposition due to Cu contamination was noticed and they suggested that this decrease may have been happened due to the inactivating of extra-cellular enzymes responsible for organic matter decomposition. Mineralisation of organic matter in soils may also be inhibited directly by metals bound to that organic matter (Hattori, 1996; Post and Beeby, 1996).

N mineralisation: The conversion of organic nitrogen compounds to NH_4^+ and NO_3^- is called nitrogen mineralisation and is performed by different soil microbial species (Van Beelen and Doelman, 1997). N mineralisation in soil is mostly determined in relation to soil fertility. Being a microbial dependent process, all those factors, which affect microbial activities, indirectly affect N mineralisation. Several studies have shown that metals affect N mineralisation, however, results are contradictory (Baath, 1989). Most investigations on the effect of metals on N mineralisation, show a less clear picture than for C mineralisation (Baath, 1989).

Necker and Kunze (1986) reported a 40% decrease in N mineralisation at $20 \mu\text{g Cd g}^{-1}$ soil compared with the control. However, Walter and Stadelinan (1979) found stimulatory effects at 50 and $500 \mu\text{g Cd g}^{-1}$ soil. Mathur and Preston (1981) did not find any effect of Cu on soil N mineralisation. Similar results were also reported by Preston *et al.* (1979) who reported that mineral N concentrations in field microplots of an organic soil during field study were not affected by experimental increments of Cu contents of 50 to $500 \mu\text{g g}^{-1}$ soil. In contrast, Hattori (1989) observed that the addition of metal salts caused an immediate decrease in soil N mineralisation rate. There are many and various reasons for the diversity of these results. For example, Minnich and McBride (1986) observed no measurable effect of Cu (up to $1445 \mu\text{g g}^{-1}$ soil) on N mineralisation in copper enriched soils and indicated that either the Cu levels were not toxic or that microbial adaptation to Cu inputs had occurred.

Quraishi and Cornfield (1971) studied the effects of 100 and $1000 \mu\text{g Cu g}^{-1}$ on N mineralisation in a calcareous soil treated with $200 \mu\text{g N}$ as dried blood g^{-1} soil and showed that Cu caused 30 and 100% increases, respectively, in the amount of N mineralized during 21 days incubation. They concluded that the Cu deficiency in the untreated soil (control) was inhibiting N mineralisation in soil but did not suggest a mechanism for this effect. Somewhat similar results were obtained by Khan (1999) who studied grassland soil treated with artificially contaminated sewage sludge and found significantly higher mineral N contents in high compared to low metal input soils. He (Khan, 1999) also reported that this increase in mineral N was in line with increased CO_2 evolution in high metal soils over control and concluded that increased mineral N content could be due to higher turnover of organic matter in the high metal soils. In contrast, Liang and Tabatabai (1977) found a decrease of up to 82% in the soil treated with $300 \mu\text{g Cu g}^{-1}$ soil after 20 days of incubation. They suggested that the toxic effect of Cu on N mineralisation could be due to the reaction of Cu ion with sulphhydryl groups in the enzyme systems of the microorganisms involved in the N mineralisation.

Due to different bioavailability of metals in various soils (McGrath, 1994), a study using different soil types was carried out by Dar and Mishra (1994). During 60 days of incubation, Cd at 25 and $50 \mu\text{g g}^{-1}$ soil significantly decreased N mineralisation in the sludge amended sandy loam and loam soils, results which were also in line with CO_2 evolution in those soils. In contrast no effect on N mineralisation was observed in the clay loam soil. As the organic matter contents of the three soils were more or less similar, they suggested that the degree of inhibition of Cd

was more related to clay content than the organic matter. In a recent study by Khan and Scullion (2000) more or less similar results were observed. Metal (Cu, Ni and Zn) treatments caused an initial (over 3 weeks) increase in mineral N content in all (five) high metal soils compared with control. However, after 7 weeks, a significant decrease in sandy loams was observed which was also in line with CO_2 evolution in those treatments.

Some authors have attributed increased N mineralisation to the release of N from microbial cells killed by metal toxicity (Bogomolov *et al.*, 1996). A similar pattern of response was observed by Edwards *et al.* (1994) in soils treated with pesticides. Other authors have reported high concentrations of NH_4^+ in metal contaminated soil and suggested that it might be due to the inhibition of nitrification. For example in a pot experiment, Simon *et al.* (1998) found $3.6 \mu\text{g NH}_4^+ \text{g}^{-1}$ in the control compared to $148.2 \mu\text{g NH}_4^+ \text{g}^{-1}$ in the high Ni fine textured soil and $2.8 \mu\text{g NH}_4^+ \text{g}^{-1}$ in a control compared to $247.9 \mu\text{g NH}_4^+ \text{g}^{-1}$ in the high Ni coarse textured soil.

These conflicting findings with respect to N mineralisation may have arisen because of the differing interpretations of this process. A decrease in N mineralisation or an increase in immobilization by microorganisms may greatly affect the net outcome. In addition, the complex and variable nature of soil may lead to different levels of bioavailability once metals are added to it (McGrath, 1994).

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