

## **IMPACT OF SEWAGE SLUDGE APPLICATION ON SOIL MICROBIAL BIOMASS, MICROBIAL PROCESSES AND PLANT GROWTH- A REVIEW**

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### **ABSTRACT**

**Sewage sludge is a rich source of plant nutrients (C, N, P and K). Beside these sludge often contains heavy metals like Zn, Cu, Fe, Cd, Pd, Ni, Hg and Cr. Some of these metals at low concentration are essential micronutrients. The repeated addition of sludge contaminated with heavy metals may lead to build up of heavy metals in soil and thereby exert adverse effects on soil health. The concentrations of heavy metals close to or less than current permissible limits have no effect on microbial biomass carbon and other activities. Soil microbial biomass carbon and activities increased with the application of uncontaminated sewage sludge. But the application of metal contaminated sewage sludge leads to the decline in microbial biomass C and N. At higher level metal contaminated sewage sludge resulted in the accumulation of organic matter due to less degradation or carbon dioxide evolution. The urease and dehydrogenase activities are adversely affected while other enzyme activities stimulated by metal contaminated sewage sludge. Soil microbial community structure and plant growth are also adversely affected by metal contaminated sewage sludge.**

The generation of sewage is increasing due to rapid urbanization. The worldwide annual production of sewage sludge is estimated to be around 30 millions tons (Nriagu and Pachyna, 1988). The municipalities all over the world are concerned with safe and feasible methods of its disposal. The current methods for disposal include land filling, incineration, dumping in sea and field application for agricultural use. Incineration and land filling are not popular because of the high cost and environmental hazards involved. Therefore, the only viable option left for sludge management is its utilization in agriculture as a source of organic matter and plant nutrients, which is perhaps the most convenient and feasible practice of its disposal. Sewage sludge application provides potential benefits for agricultural soils including increased organic carbon, nitrogen and phosphorus contents and helps in improvement of soil physical properties such as water and nutrient retention. The benefits are of great significance in arid and semi arid regions of the world where, due to high oxidation rate of soil organic matter, soils have poor physical properties and nutrient supplying capacities (Tripathi *et al.* 1990;

Nyamangara and Mzezewa, 2001; Binder *et al.* 2002). The presence of humic substances in sewage sludge will also affect cation exchange capacity (CEC) of the soil and thus affect nutrient retaining capacity of the soil. Total N and P content of sewage sludge varies from 0.18 - 0.75 and 0.15 - 0.90 percent, respectively. The availability of N and P ranges from 50 - 100 percent depending upon the type of sludge (Ministry of Agriculture Fisheries and Food, 1987). In addition to the presence of plant nutrients, sludges often contain heavy metals like Zn, Cu, Ni, Cd, Pb, Hg, Mo, Ar, Se and Cr which could be phytotoxic to plants.

Phytotoxic metal which is usually present in greatest quantity in sewage sludge is Zn followed by Cu and Ni. Among these metals, Cu and Ni are two and eight times more toxic to crops than Zn, respectively (Ministry of Agriculture, Fisheries and Food, 1987). Some of these metals, at low concentrations, are essential micronutrients and their limit in sludge, for use in agriculture, is Cd (20), Cu (1200), Ni (200), Pb (1200), Zn (3000), Hg (25) and Cr (1200) ppm. After the repeated addition of sewage sludge in soil, the level of heavy metals in soil

should not exceed Cd (1-3), Cu (50-140), Ni (30-5), Pb (50-300), Zn (150-300), Hg (1-1.5) ppm, respectively, as described by European Commission (1986). The repeated additions of sewage sludge contaminated with heavy metals may lead to build up of heavy metals in soil and thereby exert adverse effects on soil health (Alloway, 1990; Ortiz and Alcaniz 1993; Sigua *et al.*, 2005). Soil microbial biomass is actively involved in the cycling of major elements (C, N, P and S) and is affected by the organic matter and heavy metals present in the sewage sludge. The adverse effect of heavy metals on soil processes like organic matter decomposition, nutrient mineralization, nitrification, nitrogen fixation and sulphur oxidation may lead to reduced availability of plant nutrients as well as direct phytotoxicity of metals to crops. The effect of addition of sewage sludge on soil microbial biomass and its activities, activities of soil enzyme and plant growth have been reviewed.

#### **Sewage Sludge and Soil Microbial Biomass**

Soil microbial biomass consists of total mass of fungi, bacteria, protozoa and algae per unit weight of soil and is regarded as an undifferentiated single compartment for the purpose of studying energy flows and mineral fluxes in the soil system. It is defined as living part of soil organic matter excluding plant roots and soil animals larger than  $5 \times 10^3 \mu\text{m}^3$  (Jenkinson and Ladd, 1981; Ladd *et al.* 2004). It acts as an agent of transformation of all substances entering in to the soil and represents a relatively labile pool of C, N, P, S and micronutrients. Availability of these nutrients is governed by the rate of turnover through microbial biomass. Brookes *et al.* (1984) found correlation between smaller microbial biomass levels and decreased microbial activity in soils, receiving metal-contaminated sewage sludge from 1942 to 1961 as compared to similar soils with uncontaminated sludge. Heavy metal contamination of soil results in long-term decrease in microbial biomass. Chander and Brookes (1993) reported the effect of addition of uncontaminated and

contaminated sewage sludge on soil chemical and microbiological properties applied twice a year @  $200 \text{ t ha}^{-1}$  on dry weight basis. The presence of neither Zn, Cu nor Ni in soils below current permissible limits was found with decreased amount of microbial biomass carbon. However, Cu 4.9 times and Zn 2.3 times at current permitted EU limits decreased the microbial biomass carbon by 51 and 36 percent, respectively. Soils which contained either Cu or Zn separately at 1.4 times permitted limits contained 12 % less microbial biomass carbon than control. In contrast Cu and Zn in combination at 1.4 and 1.2 times more than permitted limits contained 53 % less microbial biomass carbon than the control soils, suggesting the effect of metals to be additive (Table 1).

Chander and Brookes (1995) studied the short term and long-term effects of addition of sewage sludge with or without Zn, Cu, Ni, or Cd on microbial biomass C and microbial activities under laboratory conditions. Sludges were applied at rates so that total soil concentration of each metal between 1 to 4 times that of EU permitted limits. Sludge addition increased microbial biomass C by 30 % at the lowest rate of application ( $40 \text{ t ha}^{-1}$ ) and 4.5 fold at higher rate ( $160 \text{ t ha}^{-1}$ ) after 4 weeks of incubation. However, after 64 weeks of incubation microbial biomass declined exponentially. Additions of heavy metal contaminated sewage sludge under field condition showed that heavy metals at around or excess of permitted EU limits decreased the proportion of microbial biomass C in total soil organic C. The microbial biomass C as a percentage of soil organic C was about twice as large (1.5 to 2.0 %) in the control soils or soils that received uncontaminated sludge as compared to soil that received sludges with Cu or Zn (0.7-1.0 %) in a field experiment at Lee valley and Ludington (Table 2).

Banerjee *et al.* (1997) showed that sludge application significantly increased the amount of microbial biomass in soil receiving

**TABLE 1:** Soil organic C, total N, pH, total metals, microbial biomass C in soils from Gleadthorpe Experimental Husbandry Farm.

Soil treatments	Sewage sludge (t ha <sup>-1</sup> )	Soil Organic C (%)	Soil total N (%)	pH	Total			MicrobialBiomass C (mg kg <sup>-1</sup> )
					Zn	Cu	Ni	
No sludge (control )	0	1.04	0.10	6.6	42	12	8	169
Uncontaminated sludge	200	1.22	0.12	6.5	66	19	13	183
Zn-sludge (600 kg ha <sup>-1</sup> )	200	1.23	0.13	6.5	220	29	12	185
Zn:Cu-sludge (300:250 kg ha <sup>-1</sup> )	200	1.55	0.11	6.6	127	95	15	186
Cu-sludge (1200 kg ha <sup>-1</sup> )	200	1.41	0.14	6.5	74	197	12	150
Zn-Ni-sludge (150:25 kg ha <sup>-1</sup> )	200	1.21	0.11	6.3	89	23	20	182
Ni-sludge (50 kg ha <sup>-1</sup> )	200	1.20	0.11	6.4	65	23	22	184

Source: Chander and Brookes (1993)

single and multiple applications of 0, 50 and 100 tons of sludge ha<sup>-1</sup>. The microbial biomass N was low resulting in mean microbial biomass C:N to 36:1. Bragato *et al.* (1998) studied the relationship between DTPA extractable metals on soil organic C and microbial biomass C in a silty loam soil where dehydrated and composted sewage sludge were applied once a year @ 7.5 and 15 t ha<sup>-1</sup>. After five years, microbial biomass C did not differ at 7.5 t ha<sup>-1</sup> from control. Whereas it was increased by 27 % when the sludge was applied at the rate of 15 t ha<sup>-1</sup>. The total content of Zn, Cu, Ni and Pb in soil treated with sewage sludge did not differ from control. The DTPA extractable Cu and Pb highly correlated only with total soil content of these metals. Garcia *et al.* (2004) reported the effect of addition of 40 t ha<sup>-1</sup> sewage sludge to a degraded soil cropped with barley. Soil properties were affected by sewage sludge amendments up to 9 months but effects disappeared after 36 months. Microbial biomass increased in soil after 9 months but remained similar to unamended soil after 36 months probably due to exhaustion of energy sources. Barajas (2005) reported that the amount of microbial biomass in metal contaminated soils. It was observed about half of that found in soils from the experiment that received uncontaminated organic manure or inorganic fertilizers. The application of metal contaminated sewage sludge leads to the decline in microbial biomass C and N. However, soil

microbial biomass and activities increased with the application of uncontaminated sewage sludge.

#### Effect of Sewage Sludge on Microbial Respiration

Soil respiration measured as CO<sub>2</sub> evolved from soils has been taken as an index of soil microbial activity. Respiration rate is the most widely used index of soil microbial activity. Microbial respiration has been reported to be unaffected by heavy metal at concentrations of current EU permissible limits (Brookes *et al.* 1984). Killham (1985) reported increased diversion of carbon from biosynthesis to maintenance of energy requirements when microorganisms experienced environmental stresses including heavy metals. The microbial metabolic quotient (qCO<sub>2</sub>) was low in control soil whereas sewage sludge treated soil had high qCO<sub>2</sub> indicating high activity per unit microbial biomass. Application of sewage sludge @ of both 7.5 and 15 % gave similar quotients indicating good conditions for sludge degradation (Anderson and Domsch, 1993; Insam *et al.* 1996; Rost *et al.* 2001; Selivanovkaya *et al.* 2002). It was found that there was more total and <sup>14</sup>C-labelled CO<sub>2</sub> evolution from the metal contaminated soil than uncontaminated one during 5 days of addition of <sup>14</sup>C-labelled glucose and maize. As a result, the ratio (biomass <sup>14</sup>C / respired <sup>14</sup>CO<sub>2</sub>) was about 40-50% less in the glucose and maize-amended heavy metal soils during early decomposition (Chander and Brookes, 1991).

**TABLE 2:** Microbial biomass C and organic C in Luddington and Lee Valley Soils

Treatments	Luddington ( Sandy loam soil)			Lee Valley (Silt loam soil)		
	Biomass C (mg kg <sup>-1</sup> soil)	Organic C (mg kg <sup>-1</sup> soil)	BC/OC(%)	Biomass C (mg kg <sup>-1</sup> soil)	Organic C (mg kg <sup>-1</sup> soil)	BC/OC(%)
Control Soil	222	15100	1.47	716	36600	1.96
Uncontaminated sludge	242	15600	1.55	698	40300	1.73
Zn-sludge	244	16500	1.48	702	37700	1.86
Cu-sludge	221	17200	1.28	503	44400	1.13
Ni-sludge	148	22800	0.65	438	461000	0.95

Source: Chander and Brookes (1991)

McCarthy and Ross (1994) observed that reduction in CO<sub>2</sub> evolution from bulk soil occurred only at highly metal contaminated sewage sludge. Valsecchi *et al.* (1995) reported that there was a close positive relationship among the metals and the organic C in the soils studied in homogenous area of North Italy. Negative relationship was also observed among heavy metals, soil respiration and the ratio between evolved CO<sub>2</sub>-C and microbial biomass per unit time. Sheppard *et al.* (2005) reported the significant and sustained increase in CH<sub>4</sub> and CO<sub>2</sub> evolution following sludge application to soil cores. The application of metal contaminated sewage sludge at current permissible limits did not affect the microbial respiration and organic matter degradation. However, application at higher levels resulted in the accumulation of organic matter due to less degradation or CO<sub>2</sub> evolution.

#### **Effect of Sewage Sludge on C and N Mineralization**

Mineralization of C and N in the soil is brought about by the activity of a large number of organisms involved in the degradation of organic compounds and is subjected to enormous fluctuations. The conversion of plant and animal residues or soil organic matter to its simple inorganic components in soil is a microbially mediated process, upon which the cycling of the major elements i.e. C, N, P, S take place. It has been reported by many workers that the mineralization rates of soil organic residues are not affected by metal concentrations

near current permissible EU limits (Brookes *et al.*, 1984; McGrath and Ross, 1994). However, no significant effect on decomposition rate of various organic substances was recorded at 100 mg (Cu and Zn) and 10 mg Cd kg<sup>-1</sup> soil (Storjan, 1978). However, presence of metals at high levels decreased the decomposition rate of sewage sludge. Additions of Cu or Zn at concentration more than 400 mg kg<sup>-1</sup> soil inhibited organic matter mineralization by 19-43% in an alluvial soil amended with sewage sludge (Gabteni and Gallali, 1988). Doelman (1986) reported that N mineralization was inhibited at 100 mg kg<sup>-1</sup> (Zn, Cu, and Ni), 200-500 mg kg<sup>-1</sup> (Pb and Cr), and 10-100 mg kg<sup>-1</sup> (Cd). Weritz and Schroeder (1989) found no correlation between heavy metal (Cd, Pb, Zn, Cu) content and carbon mineralization in soils. Boyle and Paul (1989) found that heavy metals introduced in the soil through sewage sludge with metal concentrations more than the current EU limits caused more accumulation of soil organic matter than the uncontaminated sludge. At Luddington (15 % clay) which contained Cu at 3.7 times more than the limit contained 32 % more organic matter than a soil receiving uncontaminated sludge (Chander and Brookes, 1991). Giller *et al.* (1993) reported that soil contaminated with Zn at 3.4 times that of the permitted concentration contained 10% more organic matter than that receiving uncontaminated sludge at Lee valley (21 % clay). These results suggest that heavy metals have pronounced effect on decreasing the turnover rates of organic matter.

### Effect of Heavy Metal from Sewage Sludge on N<sub>2</sub> Fixation

Nitrogen fixation is the process by which nitrogen is taken from its relatively inert molecular form (N<sub>2</sub>) in the atmosphere and converted into nitrogen compounds (such as, notably, ammonia, nitrate and nitrogen dioxide) useful for other chemical processes. Nitrogen fixation is performed naturally by a number of different prokaryotes. Some higher plants, and some animals (termites), have formed associations with diazotrophs and fix nitrogen.

Brookes *et al.* (1984) reported that concentrations of heavy metals close to or less than current permitted limits decreased heterotrophic N<sub>2</sub> fixation. Several other workers (Smith and Giller, 1992; Chaudhri *et al.*, 1993) also observed decreased N<sub>2</sub> fixation in metal contaminated soils. Laboratory studies confirmed the sensitivity of heterotrophic N<sub>2</sub> fixation at concentrations of 50 mg Cr kg<sup>-1</sup> soil and between 50 to 200 mg Cu kg<sup>-1</sup> (Skujins *et al.*, 1986). McGrath *et al.* (1988) reported that though nodulation of clover occurred but the nodules were ineffective in a sludge amended soil. Time taken to reach maximum nodulation of red clover was delayed by about 3 weeks in the sludge amended soil which led to poor establishment of clover. A drastic decline (>50%) occurred in N<sub>2</sub>-fixation by *R. leguminosarum* bv. *trifolii* in association with white clover in soil containing > 334 mg Zn, 99 mg Cu, 27 mg Ni and 10 mg Cd kg<sup>-1</sup> soil. Chaudhri *et al.* (1992) reported the survival of indigenous population of *R. leguminosarum* bv. *trifolii* in soils spiked with Cd, Zn, Cu, and Ni. No decline in rhizobial population occurred after two months of exposure. After 18 months, however, the number of rhizobia in control soil declined by 90%. No rhizobia could survive at Zn and Cd concentration 385 and 7 mg kg<sup>-1</sup> soil, respectively. Application of Cd, Cu, Zn and Ni rich sewage waters to soil decreased N<sub>2</sub> fixing parameters in pea and Egyptian clover (Chaudhary *et al.*, 2004). McGrath *et al.* (1995)

reported that microbial activity, populations of cyanobacteria, *Rhizobium*, mycorrhizae and total microbial biomass were adversely affected by metal concentration in sewage sludge in field experiments. Nitrogen fixation by free-living bacteria was also inhibited by heavy metals. However, higher pH and increased content of clay and organic matter reduced metal toxicity. Chaudhri *et al.* (2000) enumerated population of *R. leguminosarum* bv. *viciae* and *R. leguminosarum* bv. *trifolii* in soils contaminated for a long period of time with Zn or Cu or Zn plus Cu. Pea and white clover rhizobia were greatly reduced in soils containing > 200 mg Zn kg<sup>-1</sup> soil. Copper also reduced rhizobial population but only at or above the soil concentrations of > 250 mg kg<sup>-1</sup>. Besides potential toxicity of heavy metals to rhizobia and legumes, the root nodulation is also affected considerably.

### Effect of Heavy Metal from Sewage Sludge on Nitrification

Nitrification, the oxidation of ammonia to nitrate, via nitrite, occupies a central position within the global nitrogen cycle. Nitrifying bacteria are the only organisms capable of converting the most reduced form of nitrogen, ammonia, to the most oxidised form, nitrate and also carry out a range of other important processes within the nitrogen cycle. These include denitrification, methane oxidation, degradation of xenobiotics and urea hydrolysis. Nitrification is reduced by high concentration of Zn, Cd, and Pb in the sludge either as a single element or in combination. For this reason, industrial sludge reduced nitrification while domestic sludge had little effect on nitrification. Generally, 17 to 30 percent of the sludge N was nitrified in 16 weeks. At low levels of NH<sub>4</sub><sup>+</sup> (235 ppm N), inorganic N was completely converted to NO<sub>3</sub><sup>-</sup>. But at high levels (> 940 ppm N), a large amount of NH<sub>4</sub><sup>+</sup> was not nitrified even after 16 weeks of incubation.

Liang and Tabatabai (1978) found that addition of 294 mg Ni kg<sup>-1</sup> soil decreased

nitrification. Chang and Broadbent (1982) reported that nitrification is affected in a similar way as N mineralization. Yamoto *et al.* (1986) found no retardation in nitrification up to 50 mg Cd kg<sup>-1</sup> soil. Yadav *et al.* (1986) observed delayed nitrification in sludge-amended soils when Cu concentration goes beyond 100 mg Cu<sup>2+</sup> kg<sup>-1</sup> soil. A potential nitrification rate (PNR) test was used to identify metal toxicity in field contaminated with heavy metals. The test was applied to metal spiked soils, 27 uncontaminated, and 15 contaminated soils by former metal smelting activities. Four different agricultural soils (pH 4.5-6.6) were amended with various metals (0-200 mg Cd kg<sup>-1</sup>) or (0-3,000 mg Cd kg<sup>-1</sup>) and Zn and equilibrated more than nine months prior to testing. The soil Zn EC50s of the potential nitrification rate were between 150 and 350 mg Zn kg<sup>-1</sup>. No continuous decrease in the nitrification with increasing Cd application was observed. The nitrification rate was reduced by 50 to 80% at the highest Cd application in all soils. The PNRs of 27 uncontaminated soils varied widely (0-21 mg N kg<sup>-1</sup>d<sup>-1</sup>), but most of this variability was explained by soil pH ( $R^2 = 0.77$ ). The PNRs of the 15 contaminated soils were 0 to 44% of the values predicted for an uncontaminated soil at corresponding pH. Smolders *et al.* (2003) reported that nitrification rate was significantly reduced by adding Zn in soils and it ranged from 9 to 95% (mean 32%) of the control values at highest doses depending on soil type. Zaman *et al.* (2005) found that the nitrification rate was higher in sewage sludge compost treatment as compared to the chemical fertilizers treatment due to greater availability of minerals and N as a result of higher mineralization and soluble organic C in the former.

#### **Effect of Sewage Sludge on Soil Enzyme Activities**

Soils provide a natural system where extra cellular enzymes released by microbial cells and plant roots become immobilized on clay and

humic substances by physical adsorption onto soil colloids or covalent bonding with soil organic matter. Enzymes in soil are biologically significant as they participate in various transformations processes and influence the availability of various plant nutrients. In soil, microorganisms, active roots and dead cells are the principal sources of enzymes. Soil enzymatic activity is believed to be a sensitive indicator of the effect of environmental factors on microbial functions. Dehydrogenase, phosphatase, cellulase, amidase,  $\beta$ -glucosidase and urease are recognized as important soil enzymes.

Chander and Brookes (1991) found that soil dehydrogenase activity decreased more sharply in soils contaminated with Zn, Cd or Ni alone. On the contrary, Balzer and Ahrens (1991) showed that soil enzyme activities were stimulated by the addition of sewage sludge, except urease at highest rate of contaminated sludge application and dehydrogenase activity at both rates of contaminated sludge. Brendecke *et al.* (1993) reported that four years of sewage sludge application had no significant adverse effect on enzyme activity. However, Reddy and Faza (1989) reported that dehydrogenase activity was 20-60 % less in soils amended with sewage sludge when applied at the rate of 40, 80 and 120 t ha<sup>-1</sup> than the untreated soil after 24 h application. The metallic ions like Cu<sup>+2</sup>, Ni<sup>+2</sup>, Zn<sup>+2</sup> and Cr<sup>+3</sup> are potential inhibitors of various enzymes in soils enriched and unenriched with sewage sludge. The decline in soil enzyme activities may be the effect of decreased enzyme synthesis associated with microbial growth than to direct enzyme inhibition by metals present in sewage sludge. Garcia *et al.* (2004) reported increase in dehydrogenase activity after 9 months in the soils amended with metal contaminated sewage sludge. Kzlkaya and Hepsen (2004) studied the effect of sewage sludge amendment on enzyme activity in soil and earthworm casts. Urease, alkaline phosphatase and arylsulfatase activities increased with increasing sludge application. Sludge

application generally increased arylsulfatase activity at low rate but at higher rates the activity decreased. Zaman *et al.*, (2005) reported the long-term effect of surface application of sewage sludge compost and chemical fertilizer application and found that protease, urease and deaminase activities significantly increased in the sewage sludge compost applied soil than the chemical fertilizer due to the greater availability of organic substances that stimulated microbial activity.

### **Effect of Sewage Sludge on Microbial Community**

Application of large amount of sewage sludge over a long period of time can lead to the accumulation of pollutants with adverse effect on the soil microflora (Balzer and Ahrens, 1990). The addition of sewage sludge considerably increases the amount of heavy metals in soil, causing changes in soil properties which could be toxic for soil microorganisms (Chaudri *et al.* 1993). It was found that the size and diversity of arbuscular mycorrhizal populations were modified in metal-polluted soils, even in those with metal concentrations that were below the upper limits accepted by the European Union for agricultural soils (Del Val *et al.*, 1999). Khan and Scullion (2000) reported increase in ergosterol content with metal inputs and decreased bacterial-fungal phospholipid fatty acids (PLFA) ratios in most soils. Shi *et al.*, (2002) studied the microbial community composition and activity in soil contaminated with Pb, Cr, and hydrocarbons. Microbial community compositions were estimated from the patterns of PLFA; these were considerably different among the 14 soil samples. The metal sensitivity of the microbial community was determined by extracting bacteria from soil and measuring [<sup>3</sup>H] leucine incorporation as a function of metal concentration. Six soil samples collected in the spring of 1999 had IC(50) values (the heavy metal concentrations giving 50% reduction of microbial activity) of approximately 2.5 mM for CrO<sub>4</sub><sup>-2</sup> and 0.01 mM for Pb<sup>2+</sup>. Much higher levels of Pb were required to inhibit [<sup>14</sup>C]

glucose mineralization directly in soils. Results suggested that the soil microbial community was predominantly affected by hydrocarbons compared to heavy metals. Ghosh *et al.* (2004) studied the effect of arsenic contamination on microbial activities. Linear regression analysis revealed that the bioavailable arsenic exerted greater inhibitory effect on the soil microbial population than the total arsenic content of soils. Rajapaksha *et al.* (2004) studied the influence of heavy metal addition on total, bacterial, and fungal activities for up to 60 days in a laboratory experiment using forest soil contaminated with different concentrations of Zn or Cu. The effects of the metals differed between the different activity measurements. The bacterial activity (thymidine incorporation rate) decreased during the first days with the level of metal contamination, resulting in a 90% decrease at the highest level of contamination. Bacterial activity then slowly recovered to values similar to those of the control soil. Fungal activity (acetate-in-ergosterol incorporation rate) initially increased with the level of metal contamination, being up to 3 and 7 times higher than that in the control samples during the first week at the highest levels of Zn and Cu addition, respectively. The positive effect of metal addition on fungal activity then decreased, but fungal activity was still higher in contaminated than in control soil after 35 days. The different responses of bacteria and fungi to heavy metals were reflected in an increase in the relative fungal/bacterial ratio (estimated using PLFA) with increased metal load. De las Haras *et al.* (2005) observed that fecal coliform numbers decreased significantly after one month of sludge application. However, total coliforms, Clostridia, sulphite-reducers and *Salmonella*, were present in soils even three months after sludge application. Du Plessis *et al.* (2005) reported that bacterial populations responded differently to elevated Cu levels. Protistan numbers in soil from uncultivated land were higher and seemed to be more sensitive to

additional Cu than the numbers of these organisms in soil originating from cultivated land. Muhammad *et al.* (2005) found that higher levels of heavy metal application had significantly affected soil microbial community structure. Pb and Cd addition inhibited the functional activity of soil microbial communities.

### **Effect of Sewage Sludge on Plant Growth**

In spite of containing a number of plant nutrients which will have potential of increasing crop productivity, sewage sludge often contains sufficient quantity of heavy metals which can affect crop plants or human and animal health. Sewage sludges of domestic origin have relatively low contents of metals although it may contain appreciable quantities of Zn but the sewage sludge coming from industrial effluents may carry high levels of heavy metal toxic to plants. Some of the heavy metals are known as essential micronutrient (e.g. Fe, Mn, Zn, Cu) while other like Cd, Ni, Pb and Hg may be toxic to both plants and animals. Sabey and Hart (1975) reported that addition of sewage sludges at the rate of 0, 25, 50, 100 and 125 t ha<sup>-1</sup> to loamy sand affected the germination of sorghum, Sudan grass and pearl millet but sowing of wheat after three months later resulted in increased yield of wheat. Giordana *et al.* (1975) applied sewage sludge at the rate of 0, 50, 100 and 200 t ha<sup>-1</sup> to an acid silt loam soil having pH 5.4. Yields of corn increased by sludge but beans appeared to be more sensitive to high levels of Zn and yields were reduced. Dowdy and Larson (1975) on the studies with vegetable crops receiving up to 450 t ha<sup>-1</sup> sludge dry matter to a coarse sandy soil with pH 5.3. (The sludge contained 1070 mg kg<sup>-1</sup> Zn, 245 mg kg<sup>-1</sup> Cu, 24 mg kg<sup>-1</sup> Ni and 7.4 mg kg<sup>-1</sup> Cd), potato yields were not adversely affected by the sludge treatments.

MacLean *et al.* (1987) studied the effect of sewage sludge applied @ 44.9 kg ha<sup>-1</sup> in mixture with lime and sawdust. The metal level of Cd, Cr, Cu, Pb, Hg, Ni and Zn increased in soil. The uptake of these metals by grass and

legume plants was variable with Cd, Cu and Zn and was higher in plants growing in sewage sludge. Miller *et al.* (1995) reported the effect of sewage sludge at three application rate in field lysimeters. Sludges were applied at 20, 40, and 100 t ha<sup>-1</sup> along with control. Metal contents were low in barley grain, higher in barley straw and highest in Swiss chard. Metal contents in plants increased with increasing rate of sludge application. Tsadilas *et al.* (1995) studied the influence of sewage sludge application on soil properties and growth of wheat and maize under pot house conditions. Wheat and maize responded well to sludge application. All metals except Fe extracted by DTPA correlated with metal concentration in wheat dry matter. Otabbong *et al.* (1997) studied the effect of sewage sludge application on NH<sub>4</sub>NO<sub>3</sub> extractable Cu, Zn, Pb and Cd as well as metal uptake by barley in sandy loam soil (pH 6.7). Bhogal *et al.* (2003) studied the effect of heavy metal additions in past sewage sludge applications on soil metal availability and the growth and yield of crops at two sites in the UK. From 1994 to 1997, the yields of both cereals and legumes at Gleadthorpe were up to 3 t ha<sup>-1</sup> lower than the control. At Rosemaund, yields were only decreased where total top soil Cu concentrations exceeded 220 mg kg<sup>-1</sup> or 0.7 mg kg<sup>-1</sup> ammonium nitrate extractable Cu. Wang *et al.* (2003) found that *Polygonum hydropiper* growing on contaminated soils in a sewage pond had accumulated 1061 mg kg<sup>-1</sup> of Zn in its shoots. *Rumex acetosa* L. growing near a smelter had accumulated more than 900 mg kg<sup>-1</sup> of Zn both in its shoots and roots. Therefore these species have potential for phytoremediation of metal-contaminated sites. De las Heras *et al.* (2005) studied the effects of sewage sludge applied over a three year period (2001-2003) in a soil on a leafy crop (*Lactuca sativa* L.). The highest yield value was obtained in the second-year harvest, since the last sludge application did not increase yield. Gavalda *et al.* (2005) reported that the quality and quantity of maize were equally good

with both types of fertilization (sewage sludge and inorganic fertilization). Ryser and Sauder (2005) found that the more metal-contaminated soil have lower leaf production rate, plant biomass and delayed the phenological development. Flowering phenology was very sensitive to metals. Leaf life span was reduced at the highest and the lowest metal levels, the latter being a result of advanced seed ripening. Even if the effect of low metal levels on plant growth may be small, the delayed and reduced reproduction may have large effects at population, community and ecosystem level, and contribute to rapid evolution of metal tolerance. Sigua *et al.* (2005) reported that excessive build up of plant nutrients may not occur in beef cattle pastures that repeatedly received sewage sludge while favoring long-term increased forage yield of bahia grass.

## CONCLUSIONS

The use of sewage sludge to fertilize agricultural soils is one of the most practical options for its disposal, but the repeated application of the sewage sludge to soil can lead to the build up heavy metals which can impair the important soil biological properties. The heavy metal contaminated sewage sludge may also adversely affect soil microbial community structure and plant growth. So the continuous monitoring of the sewage sludge and the soils for heavy metals is needed to avoid excessive accumulation of toxic metals in the soils and further transfer to plants, animals and human beings. The sewer waters, sludge and the soils must be monitored continuously for heavy metals so as to avoid excessive accumulation of toxic metals in soils.

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