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Impact of Lead and Sewage Sludge on Soil Microbial Biomass and Carbon and Nitrogen Mineralization

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Sewage sludge disposal on arable land is viewed as a method to reduce waste accumulation and to enrich soil fertility. However, such disposal can degrade soil ecosystems due to the presence of potentially harmful substances, such as heavy metals. Pb has assumed greater significance because currently its dispersal through anthropogenic activities has exceeded the inputs from natural sources by about 17 fold (Nriagu and Pacyna 1988). Several soil variables such as texture, organic matter content, clay, cation exchange capacity, soil pH, and $CaCO_3$ content influence the toxic effects of heavy metals on soil microbes and their activities (Baath 1989; Elkhatib et al 1991; Dar 1995). Microbes have an essential function in cycling of nutrients through mineralization activities. However, the addition of 375 and 1500 µg Pb g^{-1} soil in sandy loam and clay loam has been reported to cause a 15% decrease in soil microbial respiration (Doelman and Haanstra 1979). Contrarily, in an organic soil microbial respiration and enzyme activities were observed to remain unaltered by the addition of 1000 µg Pb g^{-1} soil (Spadling 1979). While the nitrification process in a sandy loam soil has been reported to be significantly inhibited at 100 µg Pb g^{-1} soil, the addition of similar amount of Pb to alluvial and clay loam had no effect on nitrification and ammonifying and nitrifying bacteria (Wilson 1977; Todorov et al 1987).

Information on microbial biomass vis-a-vis C and N mineralization in relation to available Pb in soils is contradictory and inconclusive. Therefore, the present study was aimed to assess the effects of Pb and sewage sludge on microbial biomass and mineralization processes in soils of varied texture and organic matter content.

MATERIALS AND METHODS

Sewage sludge and soils used in the present study have been described previously (Dar and Mishra 1994). The sewage sludge (organic C, 8.93%; organic N, 0.10%) was collected in polyethylene bags from Sewer Treatment Plant, Okhla, New Delhi. The surface soil (0-15 cm) samples of sandy loam (organic C, 0.47%), loam (organic C, 1.61%), and clay loam (organic C, 0.72%) were collected and root portions removed before the soils were processed and equilibrated to activate native microorganisms (Dar 1996).

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For each soil, half was amended with sewage sludge @ 0.75% (dry-weight basis) and thoroughly mixed. The sludge amended and unamended soils (1 kg each) were supplemented with analytical reagent-grade lead chloride ($PbCl_2$ to yield 0, 100,250, and 500 μg Pb g^{-1} soil. Each treatment was replicated three times in a completely randomized design. The soils were incubated aerobically for 60 d at 30±1°C in 2.5 L capacity plastic containers and soil moisture maintained gravimetrically at 60% water-holding capacity throughout the study period. Soil samples were taken at 0, 1, 10, 20, 30, 45, and 60 d for analytical purposes,

Soil microbial biomass of carbon (μg C g^{-1} oven-dry soil) was estimated by the chloroform - fumigation - K_2SO_4 extraction method (Vance et al 1987), Carbon mineralization, in terms of soil respiration (CO_2-C evolved g^{-1} soil), was determined by the method of Pramer and Schmidt (1964) with slight modification (Dar and Mishra 1994).

For nitrogen mineralization, moist soil (=25 g oven dried) was placed in 150- ml Erlenmeyer flasks with 100 ml of 0.5 M K_2SO_4. Flasks were placed on a rotary shaker for 40 min and contents filtered through Whatman filter paper No. 42. Soil extracts were used for the measurement of NH^+_4-N and NO^-_3-N by steam distillation using MgO and Devarda's alloy (Bremner 1965).

Available Pb content in soils was determined by the DTPA extraction method (Lindsay and Norvell 1978) and analyzed on a double-beam atomic absorption spectrophotometer. The amount of Pb sorbed to soil minerals and organic colloids was the total amount of Pb added minus the amount extracted.

Data were analyzed using one way analysis of variance and means compared using Duncan's new multiple range test ($p \leq 0.05$). A simple correlation analysis was performed to estimate the relationship between DTPA extractable Pb and soil microbial biomass (Gomez and Gomez 1984).

RESULTS AND DISCUSSION

Sewage sludge amendment increased soil organic C and N content by 670 and 7.6 μg g^{-1} soil, respectively. The microbial biomass constituted 3.6,2.1, and 3.2% of the total soil organic C in sandy loam, loam, and clay loam soils, respectively. Sewage sludge amendment enhanced soil microbial biomass by 7 to 18%; more in sandy loam and less in loam soil (Table 1). The addition of 100 μg Pb g^{-1} soil caused no significant change in microbial biomass. The 250 μg Pb g^{-1} soil reduced microbial biomass significantly in sandy loam soil, whereas 500 μg Pb g^{-1} soil caused a significant reduction of 16 to 26% in microbial biomass in all the soils; more in sandy loam and less in loam soil. The metal stress on biomass at higher concentration persisted throughout the study period. The soil enrichment with sewage sludge did not reduce the toxic effects of higher concentrations of Pb.

Table 1. Effect of Pb and sewage sludge on soil microbial biomass (µg C g^{-1} soil)

Treatments		Soils					
Sludge (%)	Pb (µg g^{-1} soil)	Sandy loam		Loam		Clay loam	
		30d	60d	30d	60d	30d	60d
0	0	168.3[bc]	142.0[bc]	332.0[abc]	258.2[ab]	231.8[bc]	181.2[bc]
	100	157.7[cde]	140.2[bc]	319.2[bc]	259.4[ab]	226.8[bc]	179.0[bc]
	250	139.8[ef]	124.7[de]	305.3[cd]	241.5[bc]	209.2[cd]	168.5[c]
	500	124.3[f]	108.4[f]	271.8[e]	215.7[d]	185.0[d]	147.8[d]
0.75	0	198.5[a]	157.8[a]	354.3[a]	272.1[a]	265.0[a]	208.1[a]
	100	183.2[ab]	155.1[ab]	347.5[ab]	270.6[a]	257.5[a]	208.9[a]
	250	166.2[bcd]	136.7[cd]	326.3[abc]	254.3[ab]	242.7[bcd]	194.8[ab]
	500	149.7[de]	121.2[ef]	287.5[de]	226.0[cd]	214.8[c]	173.6[c]

Values are means of three replications; means within a column for each treatment followed by the same letter do not differ significantly from each other by Duncan's new multiple range test ($p \leq 0.05$).

The CO_2 evolution in all the three soils decreased with time. The soil organic C mineralization was maximum in sandy loam and minimum in loam soil. About 83 to 88% of the total C mineralized in 60 d period was mineralized in the first 30 d (Table 2). The addition of 100 µg Pb g^{-1} soil had nonsignificant influence on C mineralization,

Table 2. Effect of Pb and sewage sludge on carbon mineralization in soils

Treatments		Carbon mineralization (mg C mineralized g^{-1} soil organic C)					
Sludge (%)	Pb (µg g^{-1} soil)	Sandy loam		Loam		Clay loam	
		0-30 d	30-60 d	0-30 d	30-60 d	0-30 d	30-60 d
0	0	70.6 [bc]	9.4[abc]	46.9[ab]	9.4[abc]	60.4[abc]	10.1[ab]
	100	68.7[cde]	9.0[bc]	46.3[ab]	9.1[ab]	58.6[bcd]	9.8[ab]
	250	60.7[def]	8.5[cd]	44.2[bc]	8.7[ab]	54.7[cd]	9.0[ab]
	500	54.0[f]	7.3[d]	40.0[c]	8.1[b]	49.7[d]	8.4[b]
0.75	0	82.8[a]	10.8[a]	52.0[a]	10.2[a]	68.4[a]	11.1[a]
	100	78.5[ab]	10.4[ab]	51.9[a]	10.0[a]	65.8[ab]	10.9[a]
	250	70.4[bcd]	9.4[abc]	48.7[ab]	9.5[ab]	60.4[abc]	10.0[ab]
	500	60.9[def]	8.3[cd]	45.1[bc]	8.9[ab]	55.1[cd]	9.4[ab]

Values are means of three replications; means within a column for each treatment followed by the same letter do not differ significantly from each other by Duncan's new multiple range test ($p \leq 0.05$).

whereas 250 µg Pb g^{-1} soil caused a significant decrease only in sandy loam soil, irrespective of the sludge amendment during 0-30d incubation. A reduction of 24, 15, and 18% in C mineralization was registered at 500 µg Pb g^{-1} soil in unamended sandy loam, loam, and clay loam soils, respectively, during the first 30 d. The per cent reduction in C mineralization showed no major variation during later periods of incubation. Sewage sludge amendment enhanced soil organic C mineralization by

Table 3. Effect of Pb and sewage sludge on soil nitrogen mineralization (@g g^{-1} soil)

Treatments		Sandy loam*				Loam				Clay loam			
		30 d		60 d		30 d		60 d		30 d		60 d	
Sludge (%)	Pb (@g g^{-1} soil)	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N
0	0	2.7^{cde}	55.6^{b}	4.6^{a}	59.0^{co}	7.7^{ab}	155.5^{ab}	6.3^{bc}	168.2^{bc}	5.3^{ab}	57.8^{cc}	4.9^{b}	66.7^{c}
	100	2.2^{e}	55.1^{b}	3.6^{a}	58.8^{cd}	7.7^{ab}	155.9^{ab}	6.0^{c}	166.9^{bc}	4.8^{b}	56.2^{cd}	5.6^{ab}	65.6^{c}
	250	5.1^{b}	48.6^{bc}	4.8^{a}	55.1^{de}	7.3^{ab}	150.1^{bc}	6.5^{bc}	159.5^{cd}	4.5^{b}	54.6^{de}	5.2^{b}	64.1^{c}
	500	3.2^{cd}	44.8^{c}	4.7^{a}	52.0^{e}	6.1^{b}	142.0^{c}	6.5^{bc}	154.4^{d}	6.1^{ab}	50.5^{e}	5.8^{ab}	61.9^{c}
0.75	0	2.5^{de}	68.9^{a}	4.2^{a}	72.4^{a}	7.8^{a}	167.1^{a}	7.0^{abc}	187.1^{a}	6.7^{a}	67.0^{ab}	7.1^{ab}	79.2^{a}
	100	3.5^{c}	66.7^{a}	4.2^{a}	71.2^{ab}	7.7^{ab}	167.5^{a}	7.3^{abc}	184.8 ^{a}	5.3^{ab}	67.4^{a}	6.8^{ab}	79.3^{a}
	250	3.6^{c}	65.9^{a}	3.9^{a}	68.0^{ab}	7.0^{ab}	162.8^{ab}	7.8^{ab}	178.3^{ab}	5.9^{ab}	61.6b^{c}	6.2^{ab}	79.2^{a}
	500	6.1^{a}	52.5^{bc}	4.5^{a}	64.6^{bc}	5.9^{ab}	158.7^{ab}	8.1^{a}	171.8^{b}	6.2^{ab}	53.9^{de}	7.4^{a}	73.5^{b}

* The initial concentrations of NH_4-N and NO_3-N at 0 d of incubation in sandy loam, loam, and clay loam soils were 8.6 and 40.6, 13.6 and 122.5, and 25.6 and 30.4 @g N g^{-1} soil, respectively.

Values are means of three replications; means within a column for each treatment followed by the same letter do not differ significantly from each other by Duncan's new multiple range test ($p \leq 0.05$).

11 to 17%; more in sandy loam and less in loam soil. However, it did not mitigate the inhibitory effects of Pb.

During 60 d of incubation 14.4, 38.4, and 15.6 µg N g^{-1} soil was mineralized in sandy loam, loam, and clay loam soil, respectively. Of the total N mineralized in 60d, 63, 71, and 46% as recovered in the first 30 d of incubation in sandy loam, loam, and clay loam soils, respectively (Table 3). Sewage sludge amendment increased N mineralization in soils by 11 to 21%. The mean daily mineralization rate during first 30d of incubation in unamended and sludge amended sandy loam soil was 0.30 and 0.49 µg N g^{-1} soil and the respective values for loam and clay loam soils were 0.90 and 1.04, and 0.24 and 0.36 µg N g^{-1} soil. The addition of Pb did not affect the ammonification process; however, the nitrification process was significantly reduced in all the soils treated with 500 µg Pb g^{-1} soil. The decline in N mineralization at 500 µg Pb g^{-1} soil during the 60d period was 48, 35, and 26% in unamended sandy loam, loam, and clay loam soils, respectively. The respective soils when treated with sewage sludge vis-a-vis 500 µg Pb g^{-1} showed corresponding reductions of 38, 28, and 24% in N mineralization over control.

Soils contained traces of DTPA-extractable Pb. In sewage sludge 1.5% of the total Pb content was in available form. About 67-88% Pb added was sorbed in soils; more in clay loam and less in sandy loam (Table 4). Metal sorption decreased with increased concentration of Pb added to soils. Sewage sludge amendment only enhanced Pb sorption in soils by 1.6 to 4.4%. The DTPA-extractable Pb and soil microbial biomass exhibited a significant negative correlation with 'r' values varying from -0.47* to -0.68* ($p \leq 0.05$)

Table 4. Percent Pb sorption in soils

Treatment		Soils		
Sludge (%)	Pb (µg g^{-1} soil)	Sandy loam	Loam	Clay loam
0	100	79.5	87.8	84.0
	250	75.5	85.9	83.1
	500	66.9	78.0	76.0
0.75	100	82.1	89.7	87.1
	250	78.3	87.4	84.6
	500	71.3	81.5	78.4

Carbon and nitrogen turnover in soil occurs through heterotrophic microorganisms and lower concentration of Pb has not influenced their catabolic activities. However, at higher concentrations more soluble forms of Pb seems to have exposed microbes to free metal, therefore, resulting in adverse effects on mineralization processes. The decrease was more prominent in sandy loam than loam or clay loam soil. The presence of clay, organic matter, hydrous oxides and phosphates seem to have influenced the metal mobility through adsorption or chelation on clay minerals, exchangeable organic residues and oxide fractions. The clay loam and loam soils had high phosphate contents (25 and 37 µg g^{-1} soil, respectively) as compared to

sandy loam (4 μg g^{-1} soil). This may also have contributed in reducing Pb availability, as phosphates have previously been reported to be an important sink for Pb (McBride 1989). Therefore, it may be argued that exchangeable sites have an important role in attenuating Pb mobility in soils.

The sewage sludge amendment increased microbial biomass and mineralization activities, but did not reduce the inhibitory effects of Pb, suggesting that Pb was not immobilized by sewage sludge probably due to presence of less available organic substrate and high inorganic contents. The assertions are supported by the previous findings that Pb forms relatively stronger complex with solid organic matter phase (Dowdy and Volk 1986).

Ammonification process remained unaffected by Pb, whereas less nitrate accumulated at higher Pb concentrations. It seems that nitrifying bacteria are more sensitive to Pb contamination than ammonifiers. The prolonged and restrictive effect on C and N mineralization processes suggests that less microbial biomass is produced per unit substrate in the presence of toxic metal. The significant negative correlation of microbial biomass with DTPA - extractable Pb suggested that microbial biomass estimates provide better idea of the changes that might occur due to Pb toxicity.

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