Bull. Environ. Contam. Toxicol. (1988) 41:657–663

Environmental Contamination and Toxicology

Effect of Cadmium on Microorganisms and Microbe-mediated Mineralization Process in the Soil*

C. Kumar Naidu and T. K. Ramachandra Reddy

Department of Agricultural Microbiology, University of Agricultural Sciences, Dharwad-580 005, India

Cadmium (Cd) is increasingly used in many industries, culminating in a sharp increase in environmental contamination. Cadmium also reaches the soil through contaminated water from industries, sewage sludge, alloys, plastics, wear of automobiles tires and phosphatic fertilizers (Gupta and Salunkhe 1985). Smelter dust containing cadmium-lead-zinc caused a decrease in microflora and their enzymatic activity in soil (Greszta et al. 1979). Mineralization of nitrogen is essential to the nitrogen nutrition of higher plants. The response of important nitrogen recycling organisms to Cd could be of crucial importance in the balance of nitrogen in the soil. The effect of heavy metals (cadmium, lead, zinc, etc.) on mineralization of nitrogen (Liang and Tabatabai 1978; Rother et al. 1982; Tyler et al 1974) and on pure cultures of microorganisms (Babich and Stotzky 1978; Gadd and Griffith 1978) has been recognized. Although the toxic effect of Cd on fungi in soil, in pure culture (Babich and Stotzky 1977) and at low soil pH levels (Bewly and Stotzky 1983) was tested not much attention was given to study simultaneous effect of Cd on both microorganisms and microbe-mediated mineralization process in the soil at different intervals. This study was performed to determine simultaneously the effect of Cd on both microbial population and microbe-mediated mineralization process in the soil.

MATERIALS AND METHODS

Black cotton soil collected randomly (2" to 6" depth) from 'E' block of the central farm, College of Agriculture, Dharwad, was mixed thoroughly, air dried and passed through a 2 mm sieve. The soil has a pH of 7.5, cation exchange capacity (meq/100g) of 58.2, organic carbon of 0.8%, water holding capacity of 67.0% and clay of 55.2%.

---

* Part of the thesis of senior author approved for the award of the M.Sc.(Ag). degree by the University of Agricultural Sciences, Dharwad 580005, India.

Soil samples equivalent to 50 g of oven dry soil were treated with 0, 10, 50, 100 or 500 ppm of Cd as $CdCl_2H_2O$ (Loba Chemie, Bombay, India). The same soil was thoroughly mixed after adding aqueous urea solution at the rate of 400 ppm and transferred into replicated plastic containers (5 x 4.7 cm). Moisture content was maintained at 60% water holding capacity (W.H.C.) during incubation at 37°C. Three replications from each treatment were drawn at weekly intervals upto 6 weeks for estimation of NH_4-N, NO_2-N and NO_3-N.

Ammoniacal Nitrogen (NH_4-N) was estimated by micro-Kjeldahl distillation (Jackson 1967). Nitrite nitrogen (NO_2-N) and Nitrate nitrogen (NO_3-N) were estimated by the modified Griess Ilosvay method (Bremner 1965) using Elico Spectro Photometer (Model CL-24) at 520 and 420 nm respectively.

Microbial counts from soil were determined from equal amounts of soil sample collected from each replicate for each treatment with Cd and pooled. A composite 10 g sample was used for estimation of bacterial (on Soil extract agar), fungal (on Rose bengal agar) and actinomycetes (on Kuster's agar) by serial dilution method (Allen 1953) at weekly interval up to 8 weeks. The data of three replications were subjected to factorial analysis considering concentration and incubation time as two treatment factors.

RESULTS AND DISCUSSION

The NH_4-N content in the soil significantly increased upto one week in all the treatments and thereafter it generally decreased at 0, 10 and 50 ppm of Cd application (Table 1a). The initial rise of NH_4-N lead to an increase in NO_3-N (nitrate) accumulation in all the treatments. However, the accumulation of NO_3-N significantly decreased as the concentration of Cd increased (Table 1c). At 100 and 500 ppm of Cd, hydrolysis of urea was significantly poor compared to other treatments, as evidenced by the lower concentration of NH_4-N observed after one week of incubation (Table 1a). At all the four concentrations of Cd, significant accumulation of nitrite (NO_2-N) was observed at every sample interval of time as compared to untreated soil (Table 1b), suggesting that Cd might be toxic to both the steps in soil nitrification. Tyler et al. (1974) observed that more than 100 ppm of Cd decreased NH_4-N production and suggested that general microflora may be more sensitive to Cd. It is seen in this study (Table 2a, b, c) that more than 50 ppm of Cd considerably decreased soil microbial population which apparently affected hydrolysis of urea in the soil. The slow rate of nitrification coupled with the accumulation of NH_4-N and NO_2-N may be attributed to the inhibition of Nitrosomonas and Nitrobacter respectively.

Frago and Fleming (1977) observed in pure culture that 10 ppm of Cd had a slight effect whereas, 100 ppm of Cd significantly decreased the growth of both Nitrosomonas and Nitrobacter. In general nitrification was more sensitive to Cd than ammonification which is in accordance with Rother et al. (1982).

Table 1a. Effect of cadmium on mineralization of urea in the soil : Effect on ammoniacal nitrogen (values in ppm are average of three replicates ± S.D.)

Conc. of Cd in ppm	Incubation time in weeks 0	1	2	3	4	6
0	57 ±1.08	240 ±1.63	64 ±1.08	39 ±1.77	40 ±1.77	10 ±1.08
10	56 ±1.08	241 ±1.47	84 ±1.08	49 ±2.44	48 ±1.77	42 ±1.22
50	48 ±1.41	246 ±2.44	183 ±2.16	156 ±2.16	165 ±2.16	156 ±1.63
100	44 ±1.47	183 ±1.63	185 ±1.63	207 ±2.44	233 ±1.47	215 ±2.44
500	40 ±1.08	166 ±2.16	165 ±2.44	189 ±1.63	208 ±2.16	205 ±2.16

LSD* : Cd, 16.55; Time, 18.13; Cd x Time, 40.55

* Least significant difference at 0.01 level of probability

Table 1b. Effect of cadmium on mineralization of urea in the soil : Effect on nitrite nitrogen (values in ppm are average of three replicates ± S.D.)

Conc. of Cd in ppm	Incubation time in weeks 0	1	2	3	4	6
0	1.1 ±0.14	14.8 ±0.48	2.4 ±0.24	0.9 ±0.08	0.9 ±0.16	0.5 ±0.20
10	1.0 ±0.16	50.9 ±1.45	37.0 ±1.08	26.4 ±1.81	14.2 ±1.20	9.2 ±0.40
50	0.9 ±0.16	19.9 ±0.64	30.6 ±1.24	29.1 ±1.71	24.9 ±0.86	9.4 ±0.16
100	0.8 ±0.14	19.7 ±1.28	31.2 ±0.86	27.1 ±0.78	25.7 ±1.25	7.8 ±0.35
500	0.2 ±0.05	19.9 ±0.21	21.2 ±0.74	13.3 ±1.20	12.5 ±1.08	12.7 ±1.12

LSD* : Cd, 0.96; Time, 1.06; Cd x Time, 2.37

*Least significant difference at 0.01 level of probability

Table 1c. Effect of cadmium on mineralization of urea in the soil : Effect on nitrate nitrogen (values in ppm are average of three replictes ± S.D.)

Conc. of Cd in ppm	Incubation time in weeks 0	1	2	3	4	6
0	18.0 ±1.08	148.5 ±1.47	304.2 ±0.86	313.8 ±2.13	395.3 ±1.31	341.0 ±1.08
10	18.0 ±1.08	111.5 ±1.87	244.0 ±1.08	276.7 ±5.20	360.7 ±2.14	302.4 ±1.51
50	12.0 ±2.16	53.7 ±0.96	62.4 ±1.39	93.9 ±0.73	168.1 ±1.34	213.5 ±1.47
100	10.0 ±0.70	5.5 ±0.70	14.2 ±0.52	64.2 ±0.80	93.9 ±1.10	152.7 ±1.98
500	1.2 ±0.24	2.5 ±0.70	6.6 ±0.64	24.7 ±1.20	29.6 ±1.04	32.6 ±0.99

LSD* : Cd, 1.73; Time, 1.88; Cd x Time, 3.38

*Least significant difference at 0.01 level of probability

Table 2a. Effect of cadmium on soil microorganisms : Soil bacteria (Values are average of three replicates ± S.D.

Conc. of Cd in ppm	Soil bacteria (10^4/g) Incubation time in weeks 0	1	2	3	4	6	8
0	162.0 ±4.08	159.0 ±8.60	162.6 ±4.92	150.6 ±2.49	156.0 ±6.48	150.3 ±2.86	149.6 ±1.69
10	161.3 ±1.24	151.6 ±8.21	122.0 ±2.94	119.6 ±4.49	129.0 ±1.63	133.6 ±4.49	126.0 ±3.26
50	162.0 ±3.74	129.0 ±1.63	93.6 ±5.31	89.3 ±2.86	89.6 ±3.68	103.0 ±6.16	113.0 ±10.70
100	141.0 ±8.60	111.6 2.05	84.6 ±4.18	74.3 ±2.05	69.0 ±1.63	84.6 ±4.49	94.0 ±2.44
500	128.0 ±6.16	80.3 ±1.69	41.0 ±2.16	34.6 ±4.92	33.0 ±1.63	49.0 ±2.44	71.0 ±3.26

LSD* : Cd, 4.41; Time, 5.22; Cd x Time, 11.68

*Least significant difference at 0.01 level of probability

Table 2b. Effect of cadmium on soil microorganisms : Soil actinomycetes (values are average of three replicates ± S.D.)

Conc. of Cd in ppm	Soil Actinomycetes (10^4/g)						
	Incubation time in weeks						
	0	1	2	3	4	6	8
0	4.1 ±0.12	4.1 ±0.09	3.9 ±0.20	4.1 ±0.24	3.9 ±0.12	4.0 ±0.16	4.3 ±0.35
10	4.0 ±0.16	4.2 ±0.38	4.0 ±0.16	4.2 ±0.18	4.0 ±0.16	3.9 ±0.04	3.9 ±0.09
50	4.1 ±0.16	4.0 ±0.18	3.6 ±0.09	3.8 ±0.12	4.1 ±0.14	4.1 ±0.16	4.1 ±0.16
100	3.7 ±0.12	2.8 ±0.26	3.0 ±0.08	3.0 ±0.16	3.2 ±0.12	3.4 ±0.14	3.6 ±0.12
500	3.3 ±0.12	2.4 ±0.20	1.7 ±0.37	2.0 ±0.12	2.1 ±0.32	2.2 ±0.21	2.5 ±0.29

LSD* : Cd, 0.19; Time, 0.22; Cd x Time, 0.51

Least significant difference at 0.01 level of probability

Table 2c. Effect of cadmium on soil microorganisms : Soil fungi (values are average of three replicates ± S.D)

Conc. of Cd in ppm	Soil fungi (10^4/g)						
	Incubation time in weeks						
	0	1	2	3	4	6	8
0	0.49 ±0.01	0.53 ±0.01	0.51 ±0.01	0.50 ±0.01	0.51 ±0.04	0.47 ±0.01	0.51 ±0.04
10	0.50 ±0.01	0.46 ±0.01	0.44 ±0.01	0.45 ±0.01	0.47 ±0.01	0.48 ±0.01	0.49 ±0.01
50	0.47 ±0.01	0.40 ±0.01	0.32 ±0.01	0.27 ±0.04	0.34 ±0.02	0.40 ±0.03	0.38 ±0.03
100	0.43 ±0.02	0.29 ±0.01	0.27 ±0.01	0.24 ±0.01	0.25 ±0.01	0.26 ±0.01	0.36 ±0.01
500	0.40 ±0.01	0.21 ±0.01	0.16 ±0.02	0.11 ±0.01	0.10 ±0.01	0.11 ±0.02	0.23 ±0.02

LSD* : Cd, 0.020; Time, 0.024; Cd x Time, 0.052

*Least significant difference at 0.01 level of probability

A perusal of the data indicates that all the Cd concentrations, employed in this study, significantly decreased both bacterial (Table 2a) and fungal (Table 2c) population. 10 and 50 ppm of Cd had no effect on actinomycetes whereas, 100 and 500 ppm of Cd significantly reduced their population (Table 2b). Heavy metal polluted soils had a lower microbial population than unpolluted soils (Bisessar 1982; Williams et al. 1977). The reduction in microbial counts in Cd treated soil may be due to uncoupling of respiratory phosphorylation (Bond et al. 1976) and some other aspects of microbial metabolism (Williams and Wollum 1981).

However, after six weeks of incubation of soil with Cd, there appears some adaptation of these organisms to Cd toxicity as evidenced by increase in their population.

Acknowledgments. I(C.K.N) thank University of Agricultural Sciences, Bangalore for providing Graduate Assistantship during research period and Mr. G.G.Nagabushan, Assistant Professor of Agronomy for helping me in statistical analysis.

REFERENCES

Allen ON (1953) Experiments in soil bacteriology, Burgees publ Co., Minneapolis

Babich H, Stotzky G (1977) Effect of cadmium on fungi and on interactions between fungi and bacteria in soil: Influence of clay minerals and pH. Appl Environ Microbiol 33: 1059-1066

Babich H, Stotzky G (1978) Effect of cadimium on the biota: Influence of environmental factors. Adv Appl Microboil 23: 55-110

Bewly RJF, Stotzky G (1983) Effect of combinations of simulated acid rain and cadmium or zinc on microbial activity in soil. Environ Res 31: 332-339

Bisessar S (1982) Effect of heavymetals on microorganisms in soils near a secondarylead smelter. Wat Air Soil Pollut 17: 305-308

Bond H, Lighthast B, Shimabuku R, Russelle L (1976) Some effects of cadmium on coniferous forest soil and litter microcosms. Soil Sci 121: 278-287

Bremner JM (1965), Inorganic forms of nitrogen. In: Black CA (ed) Methods of soil analysis. Agronomy Ser. No.9. Part-2, pp 1179-1237

Frago LL, Fleming RW (1977) Effect of chromate and cadmium on most probable number estimates of nitrifying bacteria in activated sludge. Bull Environ Contam Toxicol 18: 350-354

Gadd GM, Griffith AJ (1978) Microorganisms and heavy metal toxicity. Microb Ecol 4: 303-317

Greszta J, Braniewski S, Marczynska GK, Nosek A (1979) The effect of dust emitted by non-ferrous metal smelters on the soil microflora and selected tree species. Ekol Pol 27: 397-426

Gupta PK, Salunkhe DK (1985) Modern Toxicology: The adverse effects of xenobiotics. 2: 71 Metropolitan Book Co., New Delhi

Jackson MC (1967) Soil chemical analysis. Prentice Hall, New Delhi

Liang CN, Tabatabai MA (1978) Effect of trace elements on nitrification in soils. J Environ Qual 7: 291-293
Rother AJ, Millbank JW, Thornton I (1982) Effects of heavy metal additions on ammonification and nitrification in soils contaminated with cadmium, lead and zinc. Plant soil 69: 239-259
Tyler G, Morjo B, Nilsson B (1974) Effect of cadmium, lead and sodium salts on nitrification in a mull soil. Plant Soil 40: 237-242
Williams SE, Wollum AG (1981) Effect of cadimum on soil bacteria and actinomycetes. J Environ Qual 10: 142-144
Williams ST, McNeilly T Wellington EMH (1977) The decomposition of vegetation growing on metal mine wastes. Soil Biol Biochem 9: 271-275

Received December 3, 1987, accepted March 18, 1988