MICROBIAL POPULATIONS AND ENZYME ACTIVITY IN SOIL TREATED WITH HEAVY METALS

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Abstract. Effects of two concentrations (200 and 2000 $\mu g\ g^{-1}$ soil) of two heavy metals (copper and zinc as sulphates) applied to clay or sandy soil for 12 weeks on the total counts of fungi, bateria and actinomycetes were studied. Activities of three soil enzymes (urease, nitrate reductase and amidase) were also investigated. Application of heavy metals to the clay soil reduced the microbial populations. However, although neither heavy metal showed any significant increasing effect on microorganisms populations in clay soil samples, some stimulatory effects were noted in sandy soil. Activities of urease and nitrate reductase were inhibited by heavy metal application in both soils. Amidase activity was inhibited only with the higher application rate after some experimental periods.

Key words: Cu^{2+}, Zn^{2+}, clay, sandy soils, fungi, bacteria actinomycetes, soil enzymes

1. Introduction

Heavy metal cations in soils may be present in several different physicochemical forms: (i) as simple or complexed ions in soil solution; (ii) as easily exchangeable ions; (iii) organically bound; (iv) occluded by or co-precipitated with metal oxides, carbonates, or phosphates and other secondary minerals; or (v) as ions in crystal lattices of primary minerals (Viets, 1962; McLaren and Crawford, 1973; Soon and Bates, 1982).

Heavy metals usually contaminate the soils by direct application, direct deposition of emissions or by addition of litter. Several trace elements are added to soil as impurities in fertilizers, as components of municipal and industrial wastes or from disposal of sewage sludges.

Some heavy metals are essential for growth of microorganisms (Foster and Waksman, 1939; Perlman, 1949; Bowen, 1966; Florza, 1969) becoming toxic only at high concentrations.

Concern has been expressed over the effect of heavy metal salts on the growth of microorganisms (Ross, 1975; Babich and Stotzky, 1977; 1978; Malliszewska *et al.*, 1985; El-Sharouny, 1989; El-Sharouny *et al.*, 1988, 1990). Also, several investigations were made on the effect of the toxic heavy metals in soils on the mineralization of nitrogen, the nitrification, soil enzymes and respiration (Premi and Cornfield, 1969; Tyler, 1975, 1976, 1981; Vandoni and Zaltieri, 1976; Wilson, 1977; Baldry and Dean, 1980; Rother *et al.*, 1982; Stadelmann *et al.*, 1982; Domsch, 1984; Hattori, 1992).

Amidase activity was detected in soils (Frankenberger and Tabatabai, 1980). The activity of this enzyme in soils deserves special attention because its substrates,

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especially formamide and aximide, are potent N fertilizers (Hunter 1974, Cantarella and Tabatabai, 1980). Also, the importance of urease and nitrate reductase in the nitrogen cycle has been demonstrated by Omar *et al.* (1994).

The present study was therefore designed to investigate the effect of two heavy metals (Cu^{2+} and Zn^{2+}) at two concentrations (200 and 2000 $\mu g\ g^{-1}$ soil) when applied to two types of soil on soil microbial populations and on activities of three soil enzymes (urease, nitrate reductase and amidase).

2. Materials and Methods

2.1. Soil samples

Clay and sandy soil aliquots were collected from different areas cultivated with wheat around Assiut city, Egypt, according to the method described by Johnson *et al.* (1959). These collected soil samples were analyzed to determine the water content (%), the maximum water holding capacity (%), the pH value, the total soluble salts (as % of the dry soil) and the soil texture (Jackson, 1958).

2.2. Application of heavy metals to the soil

Copper (Cu^{2+}) and zinc (Zn^{2+}) sulphates were employed in this experiment. 0.5 kg air dry soil was put in a polyethylene bag and mixed with the metal sulphate solution. Two concentrations of each metal were used; 200 and 2000 $\mu g\ g^{-1}$ soil. The water content of the soil was adjusted to 28% of its maximum water holding capacity to permit good aeration. Treatment were set up in duplicates in addition to the control. Bags were incubated at 28±2 °C. After 1, 4 and 12 weeks soil samples were taken for assaying the counts of their microbial colonies.

2.3. Isolation of soil microorganisms

The dilution-plate method was used for the estimation of soil-borne microorganisms (Johnson and Curl, 1972). Modified Czapek's Dox medium in which glucose (10 g L^{-1}) or cellulose (20 g L^{-1}) was used for isolation of glucophilic and cellulose-decomposing fungi at 28±2 °C, respectively. Yeast starch agar (15 g L^{-1}) medium was used for isolation of thermophilic and thermotolerant fungi and actinomycetes (at 45 °C). Rose-bengal (1/15000) was employed to the above media as a bacteriostatic agent (Smith and Dawson, 1944). Nutrient agar medium was applied for soil bacterial isolation (at 28±2 °C). Five Petri-dishes for each treatment were incubated for 7–15 days for fungi and actinomycetes and for 24 hours for bacteria. The developing colonies were counted.

Table I
Some properties determined in samples taken from the field

Soil Property	Clay	Sandy
Water content (%)	10.0	2.0
Max. water holding capacity (%)	81.5	40.9
pH in water (1:2.5 soil/water)	7.5	7.4
Total soluble salts (as percentage of dry soil)	2.3	4.0
Texture: Sandy %	1.1	50.3
Silt %	81.2	47.5
Clay %	17.7	2.2

2.4. Assaying of Soil Enzymes Activity

Soil amidase activity was assayed according to the method of Frankenberger and Tabatabai (1980) using formamide as a substrate. Urease activity of control and treated soils was assayed according to the procedure of Sahrawat (1980). NH_4^+-N released after incubation was estimated by nesslerization. Nitrate reductase activity of control and treated soil was investigated using the procedure of Abdelmagid and Tabatabai (1987). No_2^--N produced was measured using sulfanilic acid reagent (Allen, 1959).

2.5. Statistical Analysis

All data were subjected to one way analysis of variance using computer program (PC-stat). Means were compared by Duncan's multiple range test.

3. Results

The physicochemical characters of the clay and sandy soil samples are presented in Table I. The pH value was nearly similar in both soils. However, the total soluble salts in sandy soil was as much as twice that in clay soil.

Effect of heavy metals on the total count of:

(1) *Glucophilic fungi.* Addition of either heavy metal salt ($CuCO_4$ or $ZnSO_4$) to the clay soil led to a depression in the total count of glucophilic fungi, 4 weeks after treatment (Table II). This effect was significant at both doses of Zn^{2+} (200 and 2000 $\mu g\ g^{-1}$) and at the high dose of Cu^{2+} (200 $\mu g\ g^{-1}$). However, there was a significant increase of the harmful effect of Cu^{2+} by dose. In sandy soil, application of copper sulphate resulted in a decrease in the total count of glucophilic fungi by

the low dose after all periods; but it was significant only 1 and 4 weeks after treatment (Table II). The effect of the high and the low doses of Cu^{2+} and Zn^{2+}, respectively, fluctuated over the experimental periods. Although the high dose of Zn^{2+} induced a significant inhibitory effect until 4 weeks after application, the count of these fungi was significantly higher at the end of 12 weeks.

(2) *Cellulose-decomposing fungi.* Both heavy metals at the applied doses in clay soil drastically and significantly affected the count of cellulose-decomposing fungi (Table II) after 1 and 12 weeks of treatment. Only the high dose of $ZnSO_4$ (2000 μg g^{-1} soil) significantly reduced the count of these fungi, 4 weeks after application. In sandy soil, the fungal count was significantly lowered 1 week after treatment with both heavy metals salt and the effect increased significantly with dose (Table II). All treatments also showed a significant inhibitory effect after 4 weeks but no significant differences were obtained after 12 weeks.

(3) *Thermophilic and thermotolerant fungi.* Despite that the total count of these fungi isolated from heavy metal-amended clay soil on yeast starch agar was depressed after all tested periods, the means did not differ ($P>0.05$) from the controls, except with both doses of $ZnSO_4$ after 1 week of application (Table II). In sandy soil, the low dose of both heavy metals (200 μg g^{-1} soil) induced a significant decrease in fungal count, 1 week after treatment. In contrast, 3 weeks later, the low dose of Zn^{2+} in addition to the high dose of Cu^{2+} significantly enhanced the count of these fungi. However, no significant response was recorded at the end of the experiment.

(4) *Bacteria.* Application of Cu^{2+} or Zn^{2+} to the clay soil for a week resulted in a reduction ($P<0.05$) in the bacterial count only with $CuSO_4$ (200 and 2000 μg g^{-1} soil) (Table II). Enhancement in count of sandy soil bacteria was recorded 1 and 4 weeks after application of both doses of each heavy metal salt.

The raise in bacterial count was not significant statistically. except 4 weeks after addition of either dose of $CuSO_4$. The count was declined insignificantly at the last period.

(5) *Actinomycetes.* The counts of actinomycetes isolated from heavy metal-polluted clay and sandy soils on yeast starch agar at 45 °C were very low or not measurable during the first four weeks of application (Table II). However, $ZnSO_4$ at the two doses significantly increased the count of actinomycetes in sandy soil, 12 weeks after treatment, whereas, the raise in count after the same period in clay soil was not significant with both heavy metals.

Soil enzymes

Urease activity in soils treated with Cu^{2+} and Zn^{2+} at the rate of 200 μg g^{-1} soil was inhibited comparing with that in untreated soil (Table III). In both clay and sandy soils, urease activity was completely diminished at 2000 μg heavy metal g^{-1} soil.

The effects of the metals used on nitrate reductase activity in both clay and sandy soils were to some extent, similar to that of urease activity though much weaker (Table IV).

Table II

Total counts of some soil microorganisms (calculated per mg dry soil) in heavy metal-amended soils, 1, 4 and 12 weeks (W) after application

Microorganism	Heavy metal	Dose $\mu g\ g^{-1}$ soil	Clay soil			Sandy soil		
			1 W	4 W	12 W	1 W	4 W	12 W
Glucophilic fungi		0	14.8	36.8a	25.3	2.7b	11.7a	4.7b
	Cu^{2+}	200	11.3	29.5ab	28.7	0.7c	6.5b	4.4b
		2000	26.0	21.0c	27.7	3.3a	6.2b	6.1b
	Zn^{2+}	200	25.8	23.8bc	22.0	3.0b	6.1b	5.4b
		2000	21.3	26.5bc	26.7	0.8c	3.8c	8.4a
Thermophilic and Thermotolerant fungi		0	9.8a	1.1	7.0	1.9a	2.4b	3.1
	Cu^{2+}	200	9.3ab	1.0	6.3	0.8bc	1.9b	1.8
		2000	8.0abc	0.8	2.3	1.7ab	8.9a	3.3
	Zn^{2+}	200	6.8bc	1.3	5.3	0.7c	9.2a	3.9
		2000	5.3c	1.5	6.7	1.6ab	1.6b	3.7
Cellulose-decomposing fungi		0	31.8a	48.8ab	24.5a	3.9a	11.6a	4.2
	Cu^{2+}	200	18.8b	54.5ab	10.5bc	3.2b	9.0b	3.8
		2000	23.8b	56.0a	8.5c	2.3c	9.3b	4.4
	Zn^{2+}	200	18.0b	47.8b	12.0bc	3.2b	8.8b	4.0
		2000	23.0b	22.5c	14.0b	1.9c	8.3b	4.7
Bacteria		0	261a	292	1008	85.5	405b	224
	Cu^{2+}	200	99b	613	848	103	731a	199
		2000	102.5b	1287	1256	109	672a	187
	Zn^{2+}	200	172ab	472	956	152	629ab	200
		2000	204a	779	876	107	383b	134
Actinomycetes		0	0.0	0.0	40.7	0.0	0.3	1.6b
	Cu^{2+}	200	0.0	0.0	62.7	0.0	0.8	0.5b
		2000	0.0	0.1	76.0	0.0	0.0	0.7b
	Zn^{2+}	200	0.0	0.2	76.0	0.0	0.0	3.7a
	Zn^{2+}	2000	0.0	0.0	61.7	0.0	1.2	3.6a

Values followed by the same letter are not significantly different at 0.05 level.

Amidase activity in soil treated with Cu^{2+} and Zn^{2+} was only marginally inhibited. Only the high application rate (2000 $\mu g\ g^{-1}$ soil) significantly inhibited amidase activity after some experimental periods in both clay and sandy soils.

Table III

Effect of Cu^{2+} and Zn^{2+} on amidase (μg NH_4^+-N/g soil/h.), nitrate reductase (μg NO_2^--N/g soil/h.) and urease (μg NH_4^+-N/g soil/h.) activity in clay and sandy soil

Soil	Treatment	Concentration (μg/g soil)	Weeks after treatment 1	4	12
Amidase					
		0	72.3b	75.6b	69.8a
	Cu^{2+}	200	79.7b	78.2b	65.1a
Clay		2000	65.1c	73.5b	61.5b
	Zn^{2+}	200	88.5a	84.1a	70.0a
		2000	69.1c	76.0b	64.0b
		0	74.0b	69.4b	68.0a
	Cu^{2+}	200	83.0a	76.2a	68.0a
Sand		2000	65.0c	73.0ab	67.0a
	Zn^{2+}	200	82.6a	75.0a	68.0a
		2000	65.5c	63.0b	61.0b
Urease					
		0	41.0a	62.3a	37.1a
	Cu^{2+}	200	8.0c	11.2c	6.2c
Clay		2000	0.0d	0.0d	0.0d
	Zn^{2+}	200	15.1b	22.3b	13.0b
		2000	0.0d	0.0d	0.0d
		0	30.2a	34.1a	32.8a
	Cu^{2+}	200	12.8b	14.0b	11.2b
Sand		2000	0.0c	0.0c	0.0c
	Zn^{2+}	200	12.4b	16.8b	14.0b
		2000	0.0c	0.0c	0.0c
Nitrate reductase					
		0	0.47b	0.78a	0.58a
	Cu^{2+}	200	0.69a	0.52b	0.37b
Clay		2000	0.25c	0.34c	0.18c
	Zn^{2+}	200	0.46b	0.48b	0.33b
		2000	0.26c	0.31c	0.28bc
		0	0.37a	0.39a	0.35a
	Cu^{2+}	200	0.26b	0.28b	0.23b
Sand		2000	0.20bc	0.24bc	0.17c
	Zn^{2+}	200	0.27b	0.35a	0.27d
		2000	0.18c	0.23bc	0.17c

Values followed by the same latter are not significantly different at 0.05 level. Each figure is the mean of three replicates.

Table IV

Inhibition (I) or Stimulation (S) effect of two heavy metals on counts of glucophilic (G), cellulose-decomposing (C) and thermophilic (T) fungi, and bacteria (B) and Actinomycetes (A)

Heavy metal	Dose	Period	Clay soil					Sandy soil				
			G	C	T	B	A	G	C	T	B	A
$CuSO_4$	Low	1 W	I	I[a]	I	I[a]	–	I[a]	I[a]	I[a]	S	–
		4 W	I	S	I	S	–	I[a]	I[a]	I	S[a]	S
		12 W	S	I[a]	I	I	S	I	I	I	I	I
	High	1 W	S	I[a]	I	I[a]	–	S[a]	I[a]	I	S	–
		4 W	I[a]	S	I	S	S	I[a]	I[a]	S[a]	S[a]	I
		12 W	S	I[a]	I	S	S	S	S	S	I	I
$ZnSO_4$	Low	1 W	S	I[a]	I[a]	I	–	S	I[a]	I[a]	S	–
		4 W	I[a]	I	S	S	S	I[a]	I[a]	S[a]	S	I
		12 W	I	I[a]	I	I	S	S	I	S	I	S[a]
	High	1 W	S	I[a]	I[a]	I	–	I[a]	I[a]	I	S	–
		4 W	I[a]	I[a]	S	S	–	I[a]	I[a]	I	I	S
		12 W	S	I[a]	I	I	S	S[a]	S	S	I	S[a]

[a] means significant difference comparable with the control.

4. Discussion

Studies were carried out to evaluate the influence of Cu^{2+} and Zn^{2+} on the total counts of three groups of fungi, bacteria and actinomycetes in clay and sandy soils at two concentrations (200 and 2000 $\mu g\ g^{-1}$ soil). Activities of three soil enzymes (urease, nitrate reductase and amidase) were also studied in response to application of the heavy metals.

In general, both doses of each heavy metal in either soil type caused a noticeable decrease in counts of soil microorganisms. It is worth mentioning also that addition of either heavy metal to the clay soil samples did not show any significant stimulatory effect on the microbial counts (Table IV). A contrasting picture was observed in the sandy soil samples. Results of the present study are in agreement with those of El-Sharouny *et al.* (1988). They showed that treatment of soil with zinc and copper reduced the fungal population counts in some treatments and increased on others. However, Freedman and Hutchinson (1980) found no effect of zinc sulphate on the microfungal community. Evdokimova (1982) showed that addition of $CuSO_4$ to the soil changed the ratios between the functional groups of soil microorganisms. However, on the other hand, CU^{2+} in some types of soil was more toxic to soil fungi than Zn^{2+} (Badura *et al.*, 1979). Moubasher *et al.* (1987) reported that the fungicide, Dithan M-45 which contains zinc, was toxic to fungi when applied to the soil. It is known that many microorganisms can become adapted to heavy metal pollution (Gadd and Griffiths, 1978; Sterritt and Lester,

1980). On the other hand, the toxicity of an environmental contaminant to the biota is affected greatlt by the physiocochemical properties of the recipient environment (Babich and Stotzky, 1982).

Cellulose decomposition proved to be a process sensitive to soil polluted by heavy metals and it was extremely slow in polluted forest soil (Yevdokimova, 1982). This is in agreement with the present results where counts of cellulose decomposing fungi in sandy soil treated with heavy metals were inhibited (Table IV). Moreover, El-Sharouny *et al.* (1990) also showed that Cu and Zn sulphates induced significant inhibition to cellulose-decomposing fungi in soils. Analysis of soil contaminated with up to 571 μg Zn g^{-1} or 751 μg Cu g^{-1} soil produced a significant decrease in counts of actinomycetes and bacteria (Hiroki, 1992). Nevertheless, counts of these groups of microorganisms in our studies generally tended to increase. Stadelmann *et al.* (1982) showed that low Cd additions to soil stimulated the bacterial counts and growth whereas high additions reduced the spectrum of soil bacteria. Zamani *et al* . (1984) quantitated microbial immobilization of Zn in a sandy soil and found that microbes provided with a nutrient source may accumulate up to 20.5% of the total immobilized zinc. Thus microbes do have an impact on the fate of Zn applied to the soil. This may explain the increasing counts of microorganisms in the present studies. The accumulation of copper by growing microorganisms was also recorded by Baldry and Dean, 1980. They proved that some fungal species such as *Aspergillus niger, Fusarium oxysporum* and *penicillium spinulosum* accumulated 1% copper. In fact, many soil factors may have been responsible for increased tolerance of microorganisms to heavy metals in soil (Babich and Stotzky, 1977).

The deleterious effect of heavy metals on soil enzymes reported herein was observed elsewhere (Hughes *et al.*, 1969; Bremner and Douglas, 1971). Tabatabai (1977) studied the effect of 20 trace elements, including Cu^{2+} and Zn^{2+} on urease activity in some soils. All tested metals inhibited urease activity but the degree of inhibition varied depending to soil type and kind of element used. The inhibitory effect of these metals on the urea-urease system would lead to decrease transformation of urea to NH_4^+ to be utilized by plants and may lead to movement of urea to the ground water. Tabatabai (1977) referred the marked decrease in urease activity by increasing trace-element ions concentration to the reaction of -SH groups of urease, that involved in urease activity, with element ions.

Nitrate reductase activity in clay and sandy soils treated with Cu^{2+} and Zn^{2+} at 200 and 2000 μg g^{-1} soil was decreased compared with control. Metal ions may inhibit enzyme reactions by complexing the substrate, by combining with the protein-active groups of the enzymes or by reacting with the enzyme substrate complex (Frankenberger and Tabatabai 1981). The mode of action is dependent on the type of substrate used. Also, inhibition of nitrate reductase prevent nitrate conversion to NH_4^+-N via NO_2^--N that may cause leaching of NO_3^--N through deeper soil layers. The importance of urease and nitrate reductase in the nitrogen cycle has been demonstrated by Omar *et al.* (1994).

Amidase activity in soil treated with Cu^{2+} and Zn^{2+} was not strongly inhibited comparing with the other two enzymes tested, suggesting that the functional groups of the active sites of amidase may be different. Only the high rate used (2000 μg g^{-1} soil) significantly inhibited amidase activity. In this respect, Frankenberger and Tabatabai (1981) found that, metal ions not strongly inhibited amidase activity. These results supported the findings of Woods and Oris (1974) where thiol groups are not directly involved in the catalytic sites of amidase, even though thiol groups seem necessary for stabilization of the active amidase conformation. Frankenberger and Tabatabai (1980) suggested that, the α-amino groups at the end of polypeptide chain, which do not react with metal ions, seems to be one of the groups involved in the catalytic center of amidase.

In conclusion, the added elements generally caused reduction in populations of microorganisms and soil enzymes activity. Thus most of microbial activities and recycling of nutrients in soil will be affected.

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