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Effect of Cadmium on the Activity of Certain Soil Enzymes

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The effect of different concentrations of Cd (100, 200, 400, 800 and 1600 ppm) on the activity of soil enzymes as like dehydrogenase, invertase and soil alkaline phosphatase were studied after 15 days and 30 days of incubation period. The maximum activity of soil dehydrogenase and invertase enzymes was observed at 100 ppm level of Cd after 15 days of incubation period. Beyond this level a gradual reduction in enzymatic activity was observed. Alkaline phosphatase activity decreased at all applied doses of Cd. A marked reduction in the activities of all these enzymes, over the control, was observed after 30 days of incubation period. A negative correlation was observed between soil pH and soil applied doses of Cd. The above parameters can be used as indicators for cadmium toxicity in soils.

Key words: Cadmium, Dehydrogenase, Invertase, Alkaline phosphatase, pH.

Cadmium (Cd) is a potentially hazardous pollutant and ubiquitous in the environment and has been recognized as one of the most deleterious heavy metal pollutant (Christine, 1997). Cd has most adverse effects on microbial biomass. Its activity play an important role in the biological cycling, soil nutrient cycling and in maintaining soil fertility (Jose *et al*., 2002; Yao *et al*., 2003). Soil enzymes play key biochemical functions in the overall process of organic matter decomposition in the soil system (Sinsabaugh *et al*., 1991). The study of soil enzyme activities is significant because they are intimately involved in catalyzing several important reactions necessary for the life processes of micro-organisms in soils and the stabilization of soil structure, the decomposition of organic wastes, organic matter formation, nutrient cycling, energy transfer and environmental quality (Dick *et al*., 1994). Studies of enzyme activities in soil are also important as they indicate that the activity of soil enzymes support biochemical processes which are essential for the maintenance of soil fertility. Enzymatic activity of soil is a reliable measure of current biological status.

Dehydrogenase activity in soils provides correlative information on the biological activity and microbial populations in the soil. The activity of invertase can serve as an important diagnostic criterion of the suitability of habitation by higher plants. In soil, phosphatases, extracellularly secreted by plants and microorganisms play a key role in the phosphorus cycle.

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Therefore, a pot trial was undertaken to study the effects of different concentrations of Cd on soil dehydrogenase, invertase and alkaline phosphatase activities.

MATERIAL AND METHODS

Experimental soil was a sandy soil with a pH 8.2, moisture 1.66%, and water holding capacity (WHC) 34.95 %, Bulk desity 1.43 g / cc, Specific gravity 4.06, porosity 64.78 % and organic carbon 0.315 % (Table 1).

Different concentrations of cadmium as cadmium chloride were prepared separately by taking corresponding amounts (calculated on the basis of molecular weight) of the chemical per kilogram of soil sample. Cadmium chloride was applied at doses of 100, 200, 400, 800 and 1600 ppm Cd of soil. Pots without the added Cd constituted the control. Experiment was set during the month of May. Three replicates were prepared of each concentration and its moisture content was brought up to 60% water holding capacity by pouring distilled water. This moisture content was maintained throughout the whole experiment.

The activities of the various soil enzymes were based on the release and quantitative determination of the product in the reaction mixture when soil samples were incubated with substrate and buffer solution.

Activities of soil enzymes were measured after 15 days and 30 days of incubation period. The soil dehydrogenase enzyme activity was determined by Lenhard's method modified by Casida *et al.* (1964). The substrate of dehydrogenase enzyme was 3% aqueous TTC (2, 3, 5 Tri phenyl Tetrzolium Chloride) solution. Soil incubation was carried out for 24 hrs at 30°C and activity was measured at a wavelength of 485nm with the help of a spectrophotometer. The activity was expressed as µg TPF g^{-1} soil 24 h^{-1}.

The soil invertase activity was determined according to Scherbakova (1968) using glucose as substrate solution and this absorbance was measured at 540 nm.

The soil alkaline phosphatase was determined according to Tabatabai and Bremner (1969) at a wavelength of 420 nm using p-nitrophenol phosphatase (PNP) solution as substrate.

RESULTS AND DISCUSSION

Heavy metal pollution affects the growth, morphology and metabolism of microorganisms in soil, through functional disturbance, protein denaturation or the destruction of the integrity of cell membranes.

Dehydrogenase enzyme is extracellular enzyme which is involved in microbial oxidoreductase metabolism (Marzadori *et al.* 1996). Wilke (1991) observed that dehydrogenase activity is very sensitive to heavy metals and can be used as a simple toxicity test. Dehydrogenase activity negatively correlated with soil applied concentrations of cadmium (Table 3). Soil contamination with cadmium had a strong antagonistic effect on the activity of soil enzymes. Nanniepieri *et al.* (1997) used enzymatic activity as an index of overall microbial activity in toxicity assays. Olszowska (1998) reported that soil contamination with cadmium can depress the activity of dehydrogenases enzymes by up to 80-95 % relative to the control. In the present study, addition of higher doses of Cd (200- 1600 ppm) resulted in a decrement in enzymatic activity at both the incubation periods (15 & 30 days of incubation period) (Table 3).

Renella (2002) has presented data indicating that combination of heavy metals Cd, Cu and Zn much more inhibits respiration intensity in soil than single heavy metal. Welp (1999) claimed that heavy metals reduced the activity of dehydrogenases by 10 to 90% depending on the rate and type of metals. Motuzas *et al.* (2005) found a significant decrease in activities of dehydrogenases (95- 98%), urease (65- 97%), saccharase (57- 77%) and soil respiration intensity (38- 65%) as compared to unpolluted soils. Murata *et al.* (2005) observed that the determination of soil dehydrogenase activity is a useful tool for assessing the potential impact of heavy metal contamination in soil, even for short term incubation studies

In the present study, the maximum soil dehydrogenase activity was observed at 100 ppm concentration of cadmium after 15 days of incubation period. Beyond this level a gradual

Table 1. Physico-chemical parameters of experimental soil

S.No.	Physico-chemical parameters	Soil
1.	Soil pH	8.2
2.	Soil moisture (%)	1.66
3.	Soil texture	
	Coarse gravel (%)	20
	Gravel (%)	10
	Coarse sand (%)	22
	Fine sand (%)	28
	Slit (%)	18
	Clay (%)	02
4.	WHC (%)	34.95
5.	Bulk density (g/cc)	1.43
6.	Specific gravity	4.06
7.	Porosity (%)	64.78
8.	Organic carbon (%)	0.315

Table 2. Effect of various concentration of cadmium as cadmium chloride on the soil pH after 15 and 30 days of incubation period

Cd Concs. (ppm)	After 15 days	After 30 days
Control	8.30	8.29
100	8.29	8.23
200	8.26	8.18
400	8.22	7.97
800	8.17	7.85
1600	8.12	7.14

reduction in enzymatic activity was observed. At 1600 ppm level of Cd maximum reduction in enzymatic activity was observed. After 30 days of incubation period also a gradual reduction was observed from control to higher doses of cadmium. Dick (1984) observed a positive correlation between percentage organic carbon and soil invertase activity. In the present studies maximum soil invertase activity was observed at 100 ppm dose of soil applied cadmium during 15 days of incubation period. Beyond this level there was a gradual reduction in invertase activity. After 30 days of incubation period a continuous reduction in enzyme activity was observed (Table 4). Similarly, Smejkalova *et al.* (2003) observed that the activity of soil invertase decreased with an increase in heavy metal (Cd, Zn & Pb) concentrations.

The activity of acid and alkaline phosphatase is closely linked to soil pH. Dick and Tabatabai (1984) have shown that acid phosphatase activity is predominant in acid soils and alkaline phosphatase in neutral to alkaline soils. Moreno *et al*. (2003) observed that phosphatase activity was the most sensitive soil enzyme to evaluate soil contamination by heavy metals.

Wyszkowska *et al.* (2006) observed that when the soil pH decreased from 7.1 to 6.4 the activity of alkaline phosphatase decreased 1.8 fold and when soil pH declined from 7.1 to 5.5 the activity of this enzyme decreased by 2.9 fold only. Nowak *et al.* (1999) observed a decrease in the

Table 3. Effect of various concentration of cadmium as cadmium chloride on the soil dehydrogenase enzyme activity in experimental soil after 15 and 30 days of incubation period:

Cd Concs. (ppm)	After 15 days	After 30 days
Control	246.79 ± 12.42	209.55 ± 0.59
100	269.00 ± 12.14	163.413 ± 6.84
200	252.60 ± 6.50	157.946 ± 7.74
400	204.768 ± 6.15	139.811 ± 13.13
800	189.7 ± 4.08	143.607 ± 6.26
1600	179.48 ± 2.95	118.324 ± 15.63

Unit: TPF g^{-1} soil $24h.^{-1}$

Table 4. Effect of various concentrations of cadmium as cadmium chloride on the soil invertase enzyme activity in experimental soil sample after 15 and 30 days of incubation period

Cd Concs. (ppm)	After 15 days	After 30 days
Control	0.591 ± 0.02	0.642 ± 0.09
100	0.604 ± 0.03	0.632 ± 0.02
200	0.569 ± 0.00	0.516 ± 0.05
400	0.552 ± 0.00	0.451 ± 0.07
800	0.553 ± 0.00	0.318 ± 0.03
1600	0.546 ± 0.00	0.149 ± 0.12

Unit: µg glucose g^{-1} soil 24 h^{-1}

Table 5. Effect of various concentrations of cadmium as cadmium chloride on the activity of alkaline phosphatase enzyme after 15 and 30 days of incubation period

Cd Concentrations (ppm)	After 15 days	After 30 days
Control	0.932 ± 0.06	0.920 ± 0.01
100	0.878 ± 0.00	0.650 ± 0.07
200	0.827 ± 0.09	0.680 ± 0.12
400	0.745 ± 0.04	0.52 ± 0.10
800	0.735 ± 0.02	0.44 ± 0.02
1600	0.669 ± 0.02	0.36 ± 0.07

Unit: µg PNP g^{-1} soil h^{-1}

activity of alkaline phosphatase caused by cadmium. Andrade and Silveria (2004) reported reduction in the alkaline phosphatase activity when the soil concentration of heavy metal increased. Wyszkowska and Wyszkowski (2003) observed soil contamination with cadmium depressed the activity of dehydrogenases, urease, alkaline phosphatase and less strongly acid phosphatase. Dar (1996) observed significant negative correlation between the concentration of cadmium in soil and activity of dehydrogenases and phosphatases. A drastic decrease in the activity of soil alkaline phosphatase was observed when sewage sludge containing 329 mg Kg^{-1} Ni was added in soil (Revoredo and Melo, 2007).

A positive correlation was observed between soil pH and soil alkaline phosphatase activities, in the present study. After 15 days and 30 days of incubation period, soil alkaline phosphatase regularly decreased in all applied concentrations of cadmium, however, the decrease in concentration was much more in 30 days incubation period, in comparison to the decrease in concentration in 15 days (Table 5). Soil pH regularly decreased from control to higher doses of treatment in 30 days of incubation period as compared to 15 days of incubation period (Table 2). Dick *et al.* (2000) observed that as soil pH increased, alkaline phosphatase activity also increased.

CONCLUSION

In conclusion, after 30 days of incubation period soil pH, dehydrogenase, invertase and soil alkaline phosphatase activity significantly reduced.

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