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# Responses of soil enzymes to different heavy metals

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## ABSTRACT

Presence of heavy metals (HM) in agricultural soil is a major hazard to the soil-plant system. In the present study, soil samples (0-5 cm depth) were taken in order to determine the effects of heavy metal pollution on soil enzymes like dehydrogenase, alkaline phosphatase (APA), urease (UA) protease, cellulose, invertase, beta glucosidase and amylase. Results showed that Cadmium (Cd) significantly inhibited the four enzyme activities and Zinc (Zn) inhibited urease and calatase activities. Lead (Pb) was not significantly inhibitory than the other heavy metals for the four enzyme activities and was shown to have a protective role on calatase activity in the combined presence of Cd, Zn and Pb. Overall, all the heavy metals were found to have an inhibitory effect on the soil enzymatic activities. Hence our results suggested that enzymatic activities may be used as a sensitive indicator for assessing changes in soil environment quality.

**Keywords:** Heavy metals, Dehydrogenase activity, Urease activity, Invertase activity, Cellulase activity

## INTRODUCTION

Heavy metals are inherent components of soils, but a great concern today is related to their accumulation due to anthropogenic activities. Under stress conditions caused by adverse anthropogenic effects such as dissemination of chemical pollutants, the development and biochemical activities of soil micro-organisms undergo several alterations. To prevent negative ecological consequences, microbiologically-related parameters should be involved in the indication of soil quality (Filip, 2002). Biological methods can measure the actual impact of contaminant on soil organisms; they show the growth and activity inhibition under stress conditions. Therefore a set of effective, cheap and easily interpretable biological methods should be found.

Many reports have shown that short-term or long-term exposure to heavy metals results in the reduction of microbial diversity and activities in soil (Sandaa et al., 2001; Akmal et al., 2005). Diversity and activity of microbial communities are important indices of soil quality. Soil Enzymes are synthesised by microorganisms and act as biological catalysts to facilitate different reactions and metabolic processes to decompose organic pollutants and produce essential compounds for both microorganisms and plants (Moreno et al., 2006).

Soil enzymatic activities are recognized as a more sensitive bio-indicator than plants and animals of any natural and anthropogenic disturbance (Hinojosa et al., 2004).

The effect of heavy metals on biological activity of soil depends on the physicochemical properties of soil, particularly on its humic content. On the other hand, it is also dependent on concentrations as well as kinds of pollutants or enzymes involved (Moreno et al., 2001). The negative influence of most of the heavy metals

on the activity of soil enzymes was reported by Wyszkowska and Kucharski, (2003). The extent to which enzyme activities are affected depends on Heavy Metals availability as influenced by soil acidity and base saturation, amounts and properties of soil organic matter and clay minerals, as well as interactions with other inorganic constituents, including other metal ions (Tyler, 1981).

The present endeavor was therefore initiated to evaluate the effect of heavy metals (Copper, Iron, Zinc, Cadmium, and Cobalt) on soil enzymatic activity of dehydrogenase, alkaline phosphatase (APA), urease (UA) protease, cellulose, invertase, beta glucosidase and amylase under controlled laboratory conditions.

## METHODOLOGY

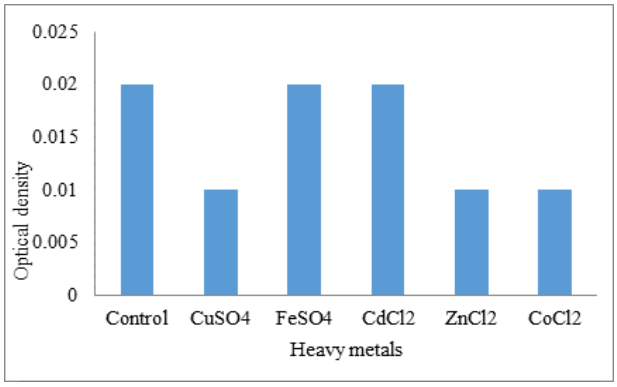
**Soil Sampling:**
Agricultural soil samples were collected from a field plot aseptically from a depth of 5 cm – 15 cm carefully. The samples were sieved (mesh size < 2 mm), sorted to remove stones, plant debris and any visible soil fauna and then thoroughly mixed with hand trowel. The soil was allowed to stabilize for 7days by incubating at 27 °C to permit the disturbance caused by sampling and sieving to subside. The pH of the soil used in the study was 7.23. Analyses were performed in three replications and average values are presented. Analytical grade sulphate and chloride salts of Copper, Iron, Zinc, Cadmium, and Cobalt were added individually to soil samples and incubated in different plastic pots. Samples were taken from the pots and soil enzymatic activity was measured.

**Soil enzymatic activity:**
The dehydrogenase activity was determined by Casida et al., (1964) method. The protease activity of soil enzyme was estimated by the Ladd and Butler, (1972). The Cellulase activity of soil enzyme was estimated by the Deng and Tabatabai, (1994). The amylase and invertase activity of soil enzyme was estimated by the Ross method, (19660. The alkaline phosphatase activity of soil enzyme was estimated by the Tatabai and Brenner, (1969). The β-glucosidase activity of soil enzyme was estimated by the Eiviza and Tatabai, (1988). Soil Urease activity was determined by Sodium salicilate and Nitroprusside method using different substrate for the reaction.

## RESULTS AND DISCUSSION

**Soil Dehydrogenase activity:**
Soil Dehydrogenase activity was found to be inhibited after the application of Heavy metals. Copper and Zinc showed the maximum inhibitory effect on dehydrogenase activity followed by Cadmium, Cobalt and Iron (Figure-1). Soil contamination also decreased Dehydrogenase content, mining the effect of heavy metals on the physiologically active soil microbial biomass, being reduced by about 50% in relation to the control soil samples. Dehydrogenase enzyme is normally used as an indicator of biological activities in soil and also plays a major role in oxidation of organic matter (Dick et al., 1996). In this study, dehydrogenase activity was significantly inhibited due to the addition of Cu. Malley et al., (2005) found that an overall reduction on dehydrogenase activity. Nweke et al., (2007) concluded that for all the metal ions (Cd2+, Hg2+, Co2+, Zn2+, Fe2+ and Ni2+), there was progressive inhibition in dehydrogenase activity and rhizoplane microbial community with each successive increase in the concentration of metal ions.

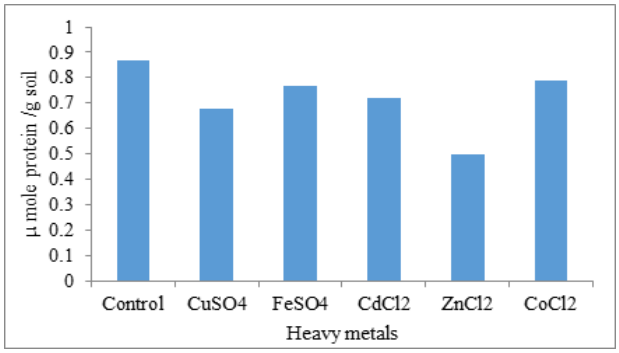
**Figure-1: Effect of heavy metals on soil Dehydrogenase activity**




**Soil Protease activity:**
The addition of Zinc decreased protease activities and protein contents in soil samples

treatments during incubation. Protease activities were negatively correlated with the amounts of exchangeable Zinc. Zinc showed maximum inhibitory effect on Protease activity followed by Iron, Cadmium, Copper and Zinc (Figure-2). Lorenz et al., (2006) found that arsenic contamination showed no effect on protease activity while cadmium contamination had a negative effect on the protease activity of soil. Effron et al., (2004) found that heavy metals inhibited the activities of protease, urease and arylsulfatases of soil. Renella et al., (2005) also found that Cadmium inhibited protease and arylsulphatase but did not affect acid phosphatase and urease.

**Figure-2: Effect of heavy metals on Protease Activity of Soil**

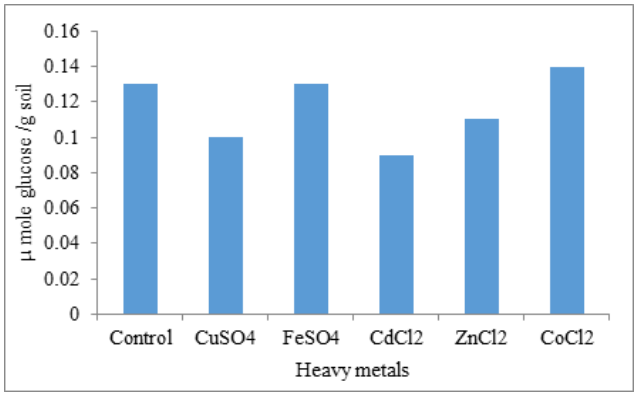



**Soil Cellulase activity:**

Cadmium showed maximum inhibitory effect on cellulase activity followed by Iron, Zinc, Copper and cobalt (Figure-3). Enzyme activities are influenced in different ways by different metals due to the different chemical affinities of the enzymes in the soil system. Khan et al., (2007) found that Cd was more toxic to enzymes than Pb because of its greater mobility and lower affinity for soil colloids. Vig et al., (2003) published a review of the bioavailability and toxicity of Cd towards soil microorganisms and their activities. Geiger et al., (1998) observed that cellulase activities were inhibited at copper concentrations above 200 m M.

Cellulase binds to the cellulose in the region of the cellulose-binding domain (Esterbauer et al., 1991). Cellulose binding domains contain plenty of glycine and cysteine which are stabilized by two or three disulfide bonds (Wood and Garcia Campayo, 1990). In other words, the shape of the active site of cellulose is mainly provided by amino acids (glycine and cysteine) and bonds between them (disulfide bonds). The cellulose binding domain also contains tryptophan residues (Teeri et al., 1995). Copper can form complexes with tryptophan residues in the cellulose binding domain, resulting in the inhibition of cellulose.

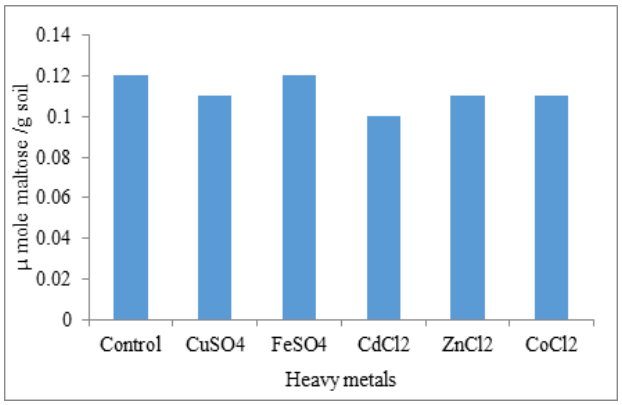
**Figure-3: Effect of heavy metals on Cellulase Activity of Soil**




**Soil Amylase activity:**

The application of heavy metals does not showed significant effect on soil amylase activity. Only Cadmium reduced the activity of soil (Figure-4) and Rogers and Li, (1985) also observed the similar findings with respect to amylase activity.

**Figure-4: Effect of heavy metals on Amylase Activity of Soil**

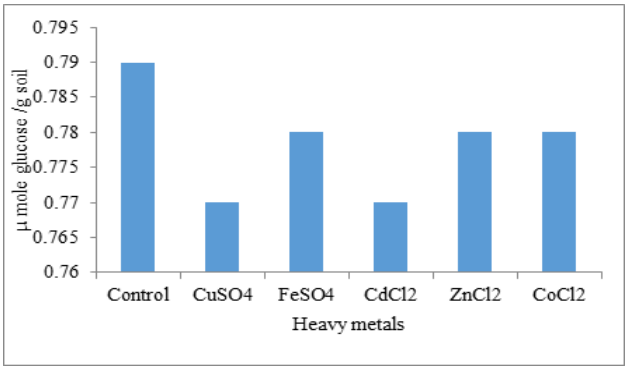



**Soil Invertase activity:**

Cadmium showed maximum inhibitory effect on Invertase activity followed by Zinc, Cobalt, iron

and copper (Figure-5). Balyaeva et al., (2005) found that Pb decreased the activities of urease, catalase, invertase, and acid phosphatase significantly. The difference in the order of effectiveness of inhibition among heavy metals may be related to the different heavy metals ion-soil interactions and their different forms in soil. Usually Cadmium ion has a higher affinity than magnesium ion but a lower affinity than lead ion, copper ion, Zinc ion and nickel ion for these surfaces (Moreno et al., 2001)

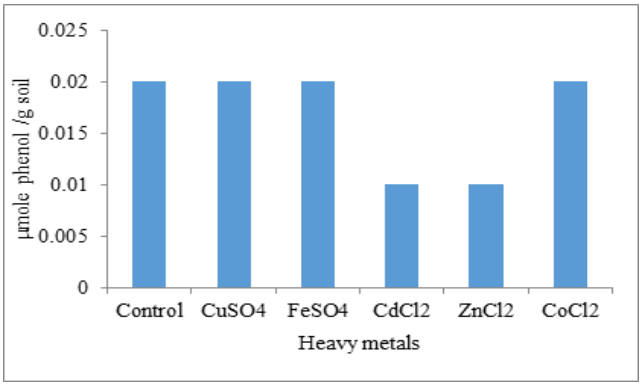
**Figure-5: Effect of heavy metals on Invertase Activity of Soil**




**Soil Alkaline phosphatase activity:**

In this study, Alkaline Phosphatase activities were inhibited by application of Cadmium and Zinc (Figure-6). Such strong influence of cadmium contamination of soil on the activity of acid phosphatase was also reported by Nowak et al., (1999), who determined that it could decrease by 15-25% to 20-30%. Decreased activity of phosphatase in soil was also revealed in studies by Landi et al., (2000).

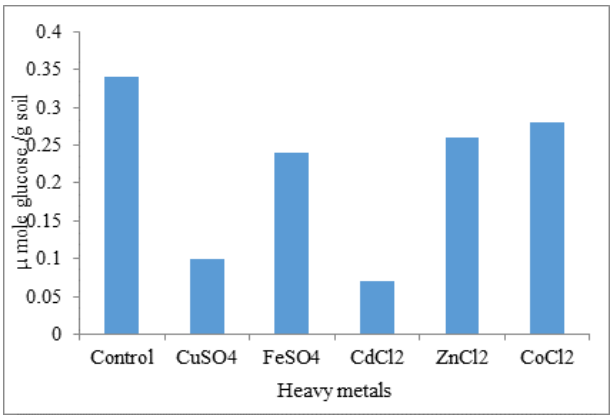
**Figure-6: Effect of heavy metals on alkaline phosphatase Activity of Soil**




**Soil Beta Glucosidase activity:**

Cadmium showed maximum inhibitory effect on Beta-Glucosidase activity followed by Zinc, Iron, Copper and Cobalt (Figure-7). Geiger et al., (1998) found that copper inhibited Beta glucosidase activity more than cellulose activity.

**Figure-7: Effect of heavy metals on Glucosidase Activity of Soil**



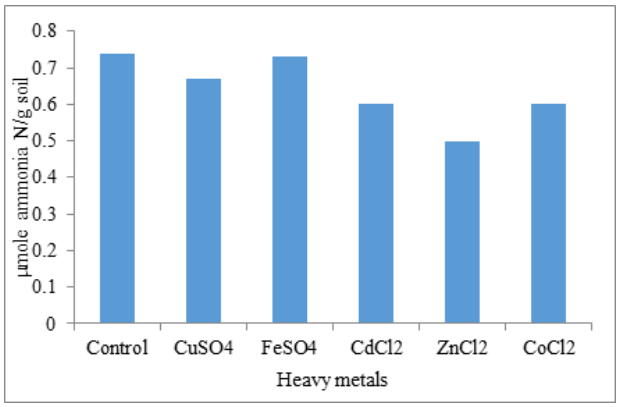

**Soil Urease activity:**

Zinc showed maximum inhibitory effect on Urease activity followed by Copper, Cadmium, Cobalt and iron (Figure-8). Lorenz et al., (2006) found that as contamination significantly affected arylsulfatase activity but not those of xylanase, invertase, protease and alkaline phosphatase; Cd contamination had a negative effect on the activities of protease, urease, alkaline phosphatase and arylsulfatase but no significant effect on that of invertase. Each soil enzyme exhibits a different sensitivity to heavy metals. Shen et al., (2005) and Chanda Mallaiah (2014) reported that the order of inhibition of urease activity generally decreased according to the sequence Cr > Cd > Zn > Mn > Pb (Zheng et al., 1999). Shen et al., (2005) reported that urease and dehydrogenase could be suitable indicators of combined pollution (heavy metals and PAHs), particularly at the early stages of pollution (Nagwasiri Pride Ndasi Yang et al, 2014 and Liu, 2000).

Enzymatic activities can sensitively reflect the biological situation in the soil (Šíša, 1993). There are several reasons why enzymatic analyses could be a good indicator of soil quality: (i) they are strongly connected with

important soil characteristics such as organic matter, physical properties, microbial activity or biomass; (ii) they change earlier than other characteristics; (iii) they involve relatively simple methods compared to other important parameters of soil quality.

**Figure-8: Effect of heavy metals on Urease Activity of Soil**




## CONCLUSION

The present study demonstrated that the addition of Cd and Zn negatively inhibited soil enzyme activities. With regard to the four enzymes, urease is the most sensitive to combined pollution of Cd and Zn with a significant negative correlation between urease activities. Therefore it is feasible to take soil urease activity as the main biochemical index to evaluate multiple metal pollution by soil. Further studies on the possibility of combined toxic effect mechanism for Heavy metal in soil are necessary, since in natural environment, contaminated soil often contain high amounts of multiple metal pollutants, suggesting that more further experiments should be performed to confirm the enzyme kinetics and mechanisms for the effects of heavy metal interaction. Our results show significant inhibition of enzymatic activities by a high level of soil contamination. It is considered that heavy metals mainly inhibit enzymatic reactions through either their complexing with substrate or blocking the functional groups of enzymes or reacting with complex enzyme-substrate. Enzyme activities are influenced in different ways by different metals due to the different chemical affinities of the enzymes in the soil system. The reduction in soil microbes and the inhibition of soil enzyme activities caused by metal contamination negatively affect soil fertility.

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