International Journal of Advanced Research in Biological Sciences
www.ijarbs.com

Research Article

Heavy metal Impact on Soil Microbial Biomass, Soil dehydrogenase activity and Soil Respiration rate

***Sonia Sethi, and Saksham Gupta**

Dr. B. Lal Institute of Biotechnology, Malviya Nagar, Jaipur

*Corresponding author: soniakaura198@gmail.com

Abstract

The contamination of agricultural soils by heavy metals is a global problem. Soil texture as a biotic factor represents one of the most important factors that influences the distribution of organic matter and ultimately play decisive role in retention of heavy metals in soil ecosystem. The effects of heavy metals on soil microbial processes were investigated. 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 then analysed for microbial biomass carbon, respiration rate and soil dehydrogenase activity. Soil microbial biomass carbon declined in samples treated with Copper and Zinc. The rate of respiration of the soil microbial populations was equally inhibited by the metals in the Iron and Cadmium treated soils by the end of the experiment. Our results also revealed that soil dehydrogenase activity was strongly inhibited by Copper and Zinc.

Keywords: Microbial biomass carbon, heavy metals, Dehydrogenase activity, Soil Respiration, Microbial Population

Introduction

In the modern civilization, environmental pollution is a serious threat. The major sources of pollution are industrial and anthropogenic sources such as excessive use of fertilizers, pesticides, sewage sludge, rapid urbanization and waste incinerators which play a cardinal role in the transportation of heavy metals [(Sanita and Gabbrielli 1999), (Kuo *et al*., 2006), (Ramadan and Al-Ashkar 2007)]. Heavy metals contamination causes serious problems because they cannot be degraded like organic pollutants and they accumulate in different parts of the food chain. Heavy metal pollution not only cause adverse effects on parameters relating to plant quality and yield but also cause changes in the size, composition and activity of the microbial community [Giller *et al*., (1998)]. Also, the activities of microorganisms that promote plant growth can be altered by high concentration of metals [Wani *et al*., (2007)]. Field studies of metal contamination have demonstrated microbial community size [(Jordan and Le Checalier 1975), (Konopka et al., 1999)] and decreases in activity such as organic matter mineralization [Chander and Brookes (1991)].

Soil microorganisms constitute a large dynamic source and sink of nutrients in all ecosystems and play a major role in plant litter decomposition and nutrient cycling, soil structure, nitrogen fixation and other alterations in soil properties influencing plant growth [Kennedy and Smith (1995)]. Moreover they are very sensitive to environmental change and not only directly influence soil fertility levels [Insam *et al*., 1996)] but also influence the microbial viability, microbial biomass turnover and microbial utilization efficiency of organic carbon, which are important indicators of soil environmental quality [Bardgett *et al*., (1994)]. Microbial biomass, basal respiration and microbial community are possible indicators of soil environmental quality [Sparling (1997)]. Soil microbial biomass has

been found to be sensitive to increased heavy metals concentrations in soil [Giller *et al*., (1998)].

Carbon dioxide evolution, the major product of aerobic catabolic processes in the carbon cycle is also commonly measured and indicates the total carbon turnover. The metabolic quotient, i.e., the ration of basal respiration to microbial biomass, is inversely related to the efficiency with which the microbial biomass uses the indigenous substrates [Anderson and Domsch (1990)] and can be a sensitive indicator for revealing heavy metals toxicity under natural conditions [Wardle and Ghani (1995)].

The microbial biomass in soils comprises a substantial pool of nutrients. Close relationship between microbial biomass and soil fertility indices has been noted. Dehydrogenases (EC.1.1.1.1) are enzymes which catalyse the removal of hydrogen atom from different metabolites [Nelson and Cox (2005)]. Active dehydrogenases are considered to exist in the soil as an integral part of intact cells. They conduct a broad range of oxidative activities that are responsible for degradation of soil organic matter. Soil dehydrogenase activity can reflect changes in the respiratory activity of a given population size in response to changes in the soil environment [Margesin *et al*., (2000)].

In the present work, the effect of metals on microbial processes in the agricultural soil artificially polluted with elevated levels of the metals was studied. The rates of soil respiration, soil biomass carbon and soil dehydrogenase activity were monitored.

Materials and Methods

Soil Sampling

Agricultural soil samples were collected from a field plot aseptically from a depth of 5 cm – 15 cm. 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 7 days by incubating at 27 °C to permit the disturbance caused by sampling and sieving to subside [Baath *et al*., (1998)]. The pH of the soil used in the study was 7.23. Analyses were performed in duplicates and average values are presented.

Soil microbial biomass:

Microbial biomass C was determined by the chloroform–fumigation–extraction procedure in which C is extracted by $0.5 molL^{-1}$ K_2SO_4 before and after fumigation [Vance *et al*., (1987)]. Organic C in the extracts was determined colorimetrically. Microbial biomass C was calculated as follows:

$$microbial\ biomass\ C = \frac{E_C}{kEC}$$

where E_c = (organic C extracted from fumigated soils)-(organic C extracted from nonfumigated soils) and k =0.45 [Wu *et al*., (1990)].

Soil Basal respiration:

Basal respiration (CO_2 evolution) was measured by incubating fresh soil equivalent to 20 g dry weight at 28 ± 1^0C in 500mL airtight jars for 28 days, adjusted to 60% of water holding capacity. Respired CO_2 was trapped in 10mL $1 molL^{-1}$ NaOH solution, the CO_3^{2-} was precipitated with $BaCl_2$, and the excess OH ions were titrated with HCl using a phenolphthalein indicator.

Soil Dehydrogenase activity:

Dehydrogenase activity (DHA) was determined by the method of [Tabatabai (1982)] using 1 ml of 3% triphenyl tetrazolium chloride (TTC) solution per 20 g of soil sample (dry weight equivalent). TTC is converted to triphenyl formazan (TPF), a red dye that is detected using a spectrophotometer (485 nm) after incubation for 24 h at 37°C.

Results and discussion

Soil Biomass Carbon

Microbial biomass was found to be inhibited after the application of heavy metals. All the heavy metals Copper, Cadmium, Zinc, Iron, and Cobalt showed the inhibitory effect on the microbial biomass. Copper showed the maximum effect on biomass followed by Zinc, Cadmium, Cobalt and Iron. The results showed that the microbial process of carbon mineralization was inhibited to varied extents by the metals (Figure 1). In other words, carbon was accumulated. Among all the treatments, Cu had the highest rate at followed by Zn by the end of the experiment. The least value was obtained in the Fe treated soil. The other treatments showed subtle interference with the key microbial process but the level was significant.

Microbial biomass carbon is a standard technique used to determine the effect of toxic substances on the soil microbial community [(Chander and Brookes 1993),

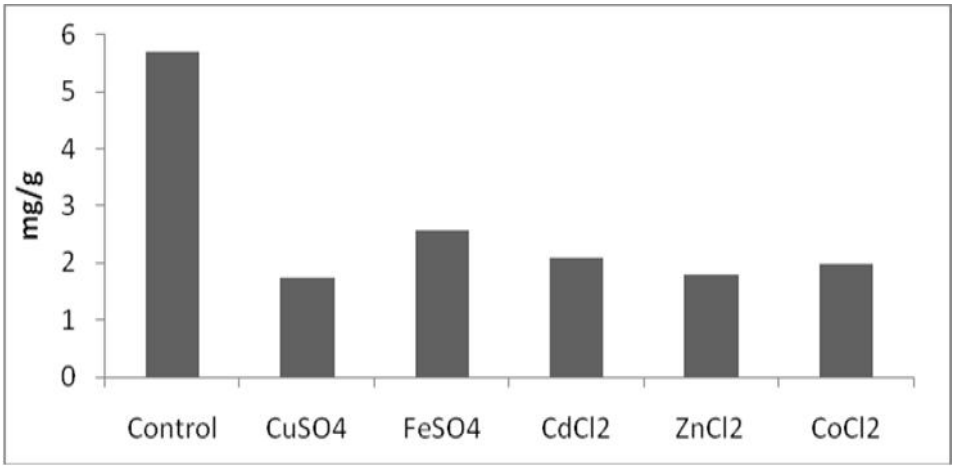

Figure 1. Effect of heavy metals on soil biomass carbon

(Diaz Ravina et al., 1994)]. The results show that the lowest measurements were obtained from the Cu and Zn amendments. Other authors [(Chander and Brookes 1993) and (Kelly and Tate 1998)] have reported similar results, with respect to Zn and Cu. The results indicated that there is a change in the microbial community structure affected by heavy metals, and microbial biomass C/N ratio was a good index of total activity of microflora to express the changes of soil quality affected by heavy metals.

Soil respiration

Soil Respiration was found to be inhibited after the application of Heavy metals after 7th day of incubation but enhanced after 28 days of incubation upto 40 µg of C/g. In this, Iron showed the maximum inhibitory effect on soil respiration in 7 days followed by Cadmium, Zinc, Copper, and Cobalt (Figure 2). The highest rate of decrease in respiration was recorded in the Fe treatment which was 8 µg of C/g by the 7th day. The rate of respiration in the Zn treated soil declined steadily to 11 µg of C/g during the same period. The relative toxicity of metal ions is known to be influenced by soil conditions. Important factors which influence microbe-metal interactions in soil include pH, the quantity and quality of clay minerals as well as other complex interaction involving the metal ions.

Assay of soil respiration also help to quantify the effects of metals on the total biological activity of soils. Addition of heavy metal salts to soils usually causes an immediate decrease in respiration rates, but responses are determined by the properties of both the metal and the soil. According to Brookes (1995), high levels of artificially added Pb may have no effect in clay soil but may decrease respiration rates in a sandy soil. In this work the respiration rates in the Fe and Zn treated soils decreased significantly. This could be due to the very high level of pollution as well as other interactions involving metals in combined ionic states.

The enhancement of the soil respiration rates may be due to less availability of metals added to the soils because of soil aging processes. Recent studies show that over time, heavy metals in contact with soil particles are less exchangeable thus less available to soil organisms [Wending *et al.*, (2009)]. Oorts *et al*., (2007) confirmed in their studies that Ni solubility declines with aging and the effect is pronounced, compared to Cu and Zn. Similarly, Co availability to organisms in time is reported to be drastically lower in comparison to Ni or Cu. The aforementioned mechsnism may be a result of several processes, such as metal incorporation into mineral structures or chemical complexations with soil solids, diffusion into pore spaces within minerals, precipitation or mineral surface oxidation [Wending *et al.*, (2009)]

Thus, higher sensitivity of microbes from clean soils, after longer exposure to stress, might be caused by higher amount of free ions in soil solution, but it is as well possible that organisms had limited access to an easily degradable carbon source, which declines with time [Baath *et al*., (1998)]. Nikilinska et al., (2005) reported that the slope of the relation between the respiration rate and the metal dose was less steep after 125 day storage than 75 days, what confirms the hypothesis about declined access to easily degradable organic particles.

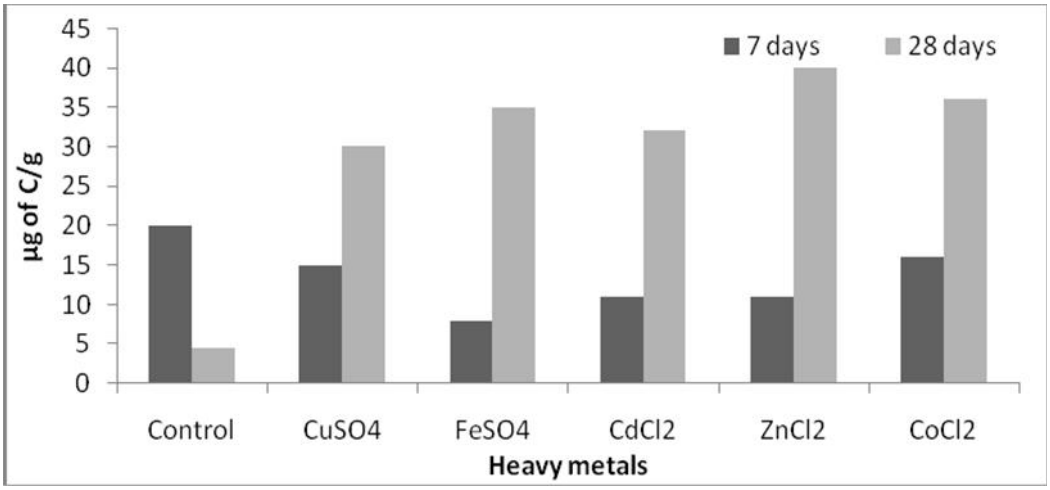

Figure 2: Effect of heavy metals on soil respiration

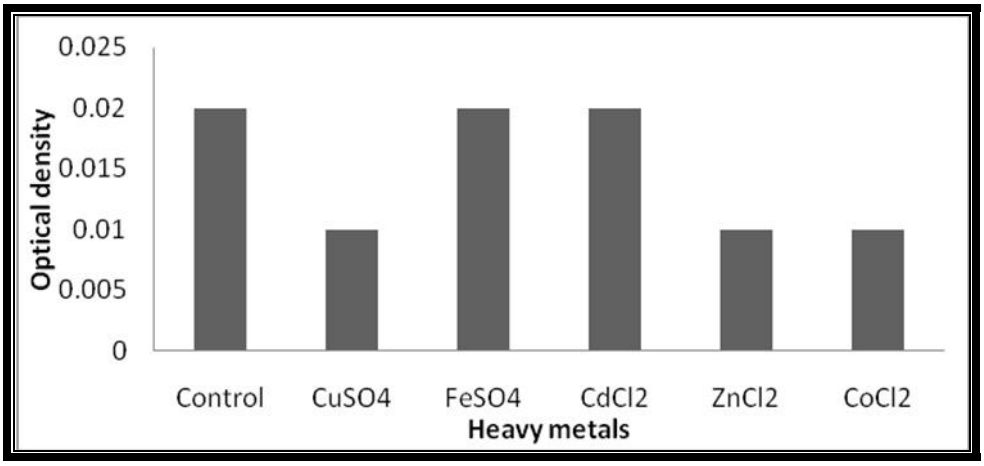

Figure 3: Effect of heavy metals on soil Dehydrogenase activity

Soil Dehydrogenase Activtiy

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. Soil contamination also decreased DHA 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.

Soil enzymes play key biochemical functions in the overall quality and health of the soil. The 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 (Cd^{2+}, Hg^{2+}, Co^{2+}, Zn^{2+}, Fe^{2+} and Ni^{2+}), there was progressive inhibition in dehydrogenase activity and rhizoplane microbial community with each successive increase in the concentration of metal ions.

Conclusion

In the present study, three important biological soil parameters, namely, microbial biomass carbon, soil respiration and soil dehydrogenase activity were found to be negatively affected by the application of copper, iron, cadmium, zinc and cobalt salts. It can be concluded that soils contaminated by heavy metals lead to substantial losses in dry matter and seed yield which

seems to demonstrate interactions between these heavy metals and the growth of plants.

. References

Anderson T.H., Domsch, K.H., (1990): Application of ecophysiological quotients (qCO2 and qD) on microbial biomass from soils of different cropping histories. Soil Biol. Biochem, *22:* 251–255.

Baath E., Diaz Ravina M., Frostegard A., Campell C.D. (1998). Effect of metal rich sludge amendments on the soil microbial community. Appl. Environ. Microbiol, *64* (1): 238 – 245.

Baath E., Frostegard A., Diaz Ravina M., Tunlid A. (1998): Microbial community based measurements to estimate heavy metal effects in soil: The use of phospholipid fatty acid patterns and bacterial community tolerance. Ambio, *27*: 58.

Bardgett R.D., Speir, T.W., Ross, D.J., Yeates, G.W., Kettles, H.A., (1994): Impact of pasture contamination by copper, chromium, and arsenic timber preservative on soil microbial properties and nema-todes. Biol. Fertil. Soils, *18:* 71–79.

Brookes P.C. (1995): The use of microbial parameters in monitoring soil pollution by heavy metals. Biol. Fertile.Soils, *19(4)*: 269-274.

Chander K., Brookes P.C. (1991): Effects of heavy metals from past applications of sewage sludge on microbial biomass and organic matter accumulation in a sandy loam soil and silty loam UK soil. Soil Biol Biochem, *23*:927–932

Diaz Ravina M., Baath E., Frostegard A. (1994): Multiple heavy metal tolerance of soil bacterial communities and its measurement by the thymidine incorporation technique. Applied Environemtal Microbiol, *60(7)*: 2238-2247.

Dick R.P., Breakwell D.P., Turco R.F. (1996): Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. In: Dick R.P., Lal R., Lowery B., Rice Ch.W., Stott D.E. (eds.): Methods of assessing soilquality. SSSA Spec. Publ. No. 49, Madison: 247–271.

Giller, K.E., Witter, E., McGrath, S.P. (1998): Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol. Biochem, *30:* 1389–1414.

Insam H., Hutchinson, T.C., Reber, H.H., (1996): Effects of heavy metal stress on the metabolic quotient of the soil microflora. Soil Biol. Biochem, *28:* 691–694.

Jordan M.J., LeChevalier M.P. (1975): Effects of zinc-smelter emissions on forest soil microflora. Can J Microbiol, *21*:1855–1865

Kelly J. J., Tate R.L. (1998): Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter. J. Environ. Qual, *27 (3)*:609-617.

Kennedy A.C., Smith K.L. (1995): Soil microbial diversity and the sustainability of agricultural soils. Plant Soil, *170*:77–86

Konopka A., Zakharova T., Bischoff M., Oliver L., Nakatsu C., Turco R.F. (1999): Microbial biomass and activity in lead-contaminated soil. Appl Environ Microbiol, *65*:2256–2259.

Kuo S., Lai M.S., Lin C.W. (2006): Influence of solution acidity and CaCl2 concentration on the removal of heavy metals from metal-contaminated rice soils. Environ Pollut, *144*: 918-925.

Malley C., Nair J., Ho G. (2005): Impact of heavy metals on enzymatic Activity of substrate and on composting worms Eisenia fetida. Biores Technol, *97*: 1498–1502.

Margesin R.G., Walder A., Schinner F. (2000): The Impact of hydrocarbon remediation on enzyme activity and microbial properties of soil. Acta Biotecnol, *20*:313-333.

Nelson D.L., Cox M.M.(2005): Lehninger Principles of Biochemistry (4th ed).Worths Publisher London. Pp 351-372.

Nikilinska M., Chodak M., Laskowski R. (2005): Characterization of the orest humus microbial community in a heavy metal polluted area. Soil Biol. Biochem, *37 (12)*: 2185.

Nweke C.O., Ntinugwa V., Obah I.F., Ike S.C., Eme G.E., Opara E.C., Okolo J.C., Nwanyanwu V. (2007): In vitro effects of metals and pesticides on dehydrogenase Activity in microbial community of cowpea (Vigna unguiculata) rhizoplane. Afr J Biotechnol, *6*: 290-295.

Oorts K., Ghesquiere U., Smolders E. (2007): Leaching and aging decrease nickel toxicity to soil microbial processes in soils freshly spiked with nickel chloride. Environ. Toxicol. Chem, *26 (6)*: 1130.

Ramadan M.A.E., Al-Ashkar E.A. (2007): The effect of different fertilizers on the heavy metals in soil and tomato plant. Aus J Basic Appl Sci, *1*: 300-306.

Sanita L.T., Gabbrielli R. (1999): Response to cadmium in higher plants. Environ Exp Bot, *41*: 105-130.

Sparling G.P., (1997): Soil microbial biomass, activity and nutrient cycling as indicators of soil health. In: Pankhurst, C.E., Doube, B.M., Gupta, V.V.S.R. (Eds.), Biological Indicators of Soil Health. CAB International, Wallingford, UK, pp. 97–119.

Tabatabai M.A. (1982): Soil Enzymes. In: Methods of soil analysis part 2. Agronomy Monograph, Page

AL.(ed) American Society of Agronomy, Madison, Wincosin. *9*:903-904.

Vance E.D., Brookes P.C., Jenkinson D.S. (1987): Microbial biomass measurements in forest Soils: The use of the chloroform-fumigation method in strongly acid Soils.Soil Biol. Biochem, *19 (6):*697-702.

Wani, P.A., Khan, M.S., Zaidi, A. (2007): Chromium reduction, plant growth-promoting potentials andmetal solubilization by *Bacillus* sp. isolated from alluvial soil. Curr.Microbiol, *54*: 237–243.

Wardle D.A., Ghani, A., (1995): A critique of the microbial metabolic quotient (qCO2) as a bioindicator of disturbance and ecosystem development. Soil Biol. Biochem, *27:* 1601–1610.

Wendling L.A., Kirby J.k., MClaughlin M.J. (2009): Aging effects on cobalt availability in soils. Environ. Toxicol. Chem, *28 (8)*; 1609.

Wu J., Joergensen R.G., Pommerening B., Haussod R., Brookes P.C. (1990): Measurement of soil microbial biomass C by fumigation-extraction-an automated procedure. Soil Biol Biochem, *22*:1167-l169.