Research Article

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

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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 Gabbielli 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 ratio 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 0.5molL⁻¹ K₂SO₄ before and after fumigation [Vance et al., (1987)]. Organic C in the extracts was determined colorimetrically. Microbial biomass C was calculated as follows:

$$\text{microbial biomass } C = \frac{E_c}{kEC}$$

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 (CO₂ evolution) was measured by incubating fresh soil equivalent to 20 g dry weight at 28± 1°C in 500mL airtight jars for 28 days, adjusted to 60% of water holding capacity. Respired CO₂ was trapped in 10mL 1molL⁻¹ NaOH solution, the CO₃²⁻ was precipitated with BaCl₂, 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 up to 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 mechanism 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.
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. 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


