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Effect of heavy metals on soil microbial activities during two seasons

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Abstract

Microbes are the important components of soil fertility but their efficiency is reduced due to the entry of heavy metals in the soil through different sources. The present work was conducted to examine the effect of three heavy metals (Zn, Cu, Cd) on the microbial parameters like microbial biomass, soil respiration and metabolic quotient during the season of spring and winter. Experiment was conducted in the pots by the addition of salts of Zn, Cu, Cd with three concentration i.e., conc. 1; 100, 200, 300, conc. 2; 5, 15, 25 and conc. 3; 0.12, 0.24 & 0.36 mg /kg in the soil respectively with respect to the control. Results regarding microbial biomass were significant in all three metals during spring and winter at ($P<0.001$) while in case of soil respiration only Cd showed significance effect in both seasons. Metabolic quotient expressed significance at ($P<0.001$) in case of Cu + Cd during spring and with Zn + Cd at ($P<0.001$) in winter. Overall results show that metals were in sequence of Cd>Cu>Zn in their effects and toxicity and spring was dominant in case of all metals.

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Introduction

Pollution in the soil, ground water and in the atmosphere is mainly caused by the toxic effects of heavy metals due to the deposition of different kinds of heavy metals like Zn, Cu and Cd (Dary *et al.,* 2010; Baki *et al.,* 2017). These heavy metals have been reported to have adverse effect on the activities and biodiversity of soil microbes (Tang *et al.,* 2012; Arifeen *et al.,* 2015). Heavy metals affect all ecosystems and can have longer effect on the biological system of microbial community (Majer *et al.,* 2002) because these heavy metals can decrease microbial activity (Lee *et al.,* 2002). Sometimes exposure of these metals can produce stronger and tolerant microbial populations (Ellis *et al.,* 2003; Bigdeli *et al.,* 2008). According to Preston *et al.,* (2001), soil microbes play a vital role in cycling of organic matter, its decomposition, energy flow and maintenance of terrestrial ecosystem and in this way they can improve the soil fertility on a very low cost (Hernandez *et al.,* 2003) but their high accumulated amount can decrease the microbial biomass, basal respiration and metabolic quotient (Gans *et al.,* 2005; Qahtani, 2015).

Microorganisms since the beginning of their lives are facing the threats of concentrations of different heavy metals (Martinez *et al.,* 2009), and are surviving by the strategy of keeping a balance between the metal concentration and available dose to microbial physiology (Kosolapov *et al.,* 2004; Bong *et al.,* 2010). However, under the elevated concentration of heavy metals (which contaminates the environment) can disrupt the microbial structure and functions (Bong *et al.,* 2010; Aziz *et al.,* 2015). For example, at even milli molar concentrations of Zn can alter the microbial functions by binding with the cell membrane of most of the microbes which can hinder the cell division although being a very important micronutrient element (Silver & Phung, 2005).

It has been investigated that out of other activities, microbial parameters like microbial respiration, biomass and metabolic activities are very important indicator of environmental changes (Wang *et al.,* 2007; Zhang *et al.,* 2008).

Heavy metals show wide range of toxicities under different environmental conditions and toxicity of different heavy metals like Cd, Ni, Zn, Cu & Cr could be changed with different seasons (Hinojosa *et al.,* 2005; Mikanova, 2006). Different studies highlight that soil microbial activities including microbial biomass, soil respiration and metabolic quotient change with the change of soil heavy metals under different environmental conditions during different seasons (Cambi *et al.,* 2015; Preston *et al.,* 2000). The purpose of this research was to measure the toxicity of different heavy metals e.g. Zn, Cu & Cd under different environmental conditions and their relationship with different microbial properties like soil microbial biomass, soil respiration and metabolic quotient under different physicochemical properties of soil.

Materials and Methods

Site selection and soil preparation

Experiment was conducted in Agriculture Research Centre at Bahauddin Zakariya University, Multan. Loamy sandy soil was collected from the area of agriculture land which has not been cultivated since last five years and was lying barren. Collected soil was dried and grinded after removal of small pebbles to less than 2 mm of diameters. Soil Physicochemical properties shown in Table-1 were determined before the use of soil.

Twelve plastic pots of about 20 cm in diameters were filled with 2 kg of soil in each and were labeled from 1 to 12. Three heavy metals (Zn, Cu, and Cd) in the form of salt ($ZnCl_2$, $CuCl_2$ and $CdCl_2$) were supplied to these pots. Out of these pots, 3 were supplied with $ZnCl_2$ with the concentration of 100,200 and 300 mg/kg of soil named as Zn1, Zn2 and Zn3 respectively. Three pots were provided with $CuCl_2$ with the concentration of 5, 15 and 25 mg/kg designated as Cu1, Cu2 and Cu3 respectively while $CdCl_2$ with the concentration of 0.12, 0.24 and 0.36 was mixed with other three pots respectively labeled as Cd1, Cd2 and Cd3.The remaining three pots were kept as control and no metal was added to the soil.

Distilled water was showered on each pot to retain the moisture content. Experiment was performed in two seasons (winter and spring) with the same conditions.

Metal analysis

Soil samples at the end of each season were taken. By using method of Ure *et al.,* (1993) oven dried soil samples were prepared for metal analysis by keeping in oven at 70°C for 24 hours and grinding to less than 2mm. A sample of about 2g of soil was digested by the addition of 15ml HCL and 5ml HNO_3 (Aqua Regia digestion) and allowed to cool for 16 hours , this was heated and allowed to reflux for 2 hours. The refluxed sample was cooled and diluted to 50 ml using inductively coupled plasma mass spectrometry (ICP-MS-model 250).

Determination of soil microbial parameters

The method of Vance *et al.,* (1987) was opted for the determination of soil microbial biomass by the chloroform fumigation–extraction method .The concentration of C in the extract was determined by using dichromate digestion. 0.38 was used as efficiency coefficient to eliminate the difference insoluble C between the fumigated and the unfumigated soil to soil microbial biomass.

For the determination of soil respiration method of Smith and Hadly (1990) was followed and soil respiration was measured by using infrared gas analyzer (IRGA-model 7000, LI-COR). Soil sample adjusted at 50% of water holding capacity was incubated for 7 days at 25° C before using the IRGA. Metabolic quotient was measured by combining the soil respiration with the total amount of microbial biomass by using the method Odum (1995)

Results and discussions

Effect of heavy metals on Soil microbial biomass

The results of effects of heavy metals (Zn, Cu, Cd) on the soil microbial biomass have been shown (Table 2) in which all these metal have significant effect at ($P < 0.001$) on the soil microbial biomass in both seasons (Spring and Winter) as shown in Fig. 4. In case of Zn, all three levels were highly significant at ($P < 0.001$) during the spring but there was also remarkable decrease in the winter even at the maximum level of Zn (300 mg/kg soil) and same results have been produced by the rest of the heavy metals. Although, all these heavy metals have highly significant result during the spring but in winter their effects were less significant as compare to that of spring.

Table 1. Soil Physico-chemical parameters of Agricultural Research Centre.

Parameters	Concentrations
pH	6.1-7.8
Organic Matter	8.4%
Zn	3.0 (mg/kg)
Cu	0.31 (mg/kg)
Cd	0.008 (mg/kg)

Over all the results of effects of heavy metals on soil microbial biomass showed that both seasons are different from each other in producing the effects of heavy metals on soil microbial biomass. The winter season showed less significant result ($P<0.05$) as compared to spring time at ($P<0.001$). These results can be supported by the research of Maire *et al.,* (1999) that soil microbial biomass in spring is greater than the winter and it is important phenomenon in assessing the effect of heavy metals during different seasons on the microbial community.

These results can also be verified by (Dormaar *et al.,* 1884) that soil microbial biomass is increased during the spring due to large accumulation of organic matter which is produced as a result of high temperature and mobilization of nutrient over the winter but contradictory results have also been shown by the authors like (Smith, 2002) who suggested that during the spring season soil microbial biomass is decreased due to elimination of substrate during the winter and less production of organic material from the new flora in the soil.

Our results also showed that studied heavy metals have more or less adverse effects on soil microbial community which has been concluded by Wang *et al.,* (2015). According to Sistla *et al.,* (2013) and Blume *et al.,* (2002) soil microbial biomass on the upper and lower layer of soil remains same throughout of year but seasonal changes can effect this due to decrease in the temperature.

Table 2. Effects of different heavy metals on soil microbial biomass (mg/kg) obtained from one way ANOVA.

Microbial Biomass (mg/kg)								
	Spring				Winter			
	To	T1	T2	T3	To	T1	T2	T3
Zn	118.6a ***	92.3c ***	82.6d ***	106.3b ***	74c ns	106a **	84b **	71.3c ns
Cu	118.6a ***	95.3b ***	54.6c ***	95.6b ***	74ab *	49.3c **	71.3b **	77.6a *
Cd	118.6a ***	83b ***	74.6c ***	73c ***	74b **	47d **	66.6c **	137a **

*= $P<0.05$, ** = $P<0.01$, *** = $P<0.001$.

The study of Tripathy *et al.,* (2014) while, working on solid waste showed that other than temperature, organic matter is also helpful in increasing the soil microbial biomass even under the stress of different heavy metals in spring than the winter. So most of the authors have proposed higher microbial biomass in spring than winter due to high temperature and elevated amount of organic matter, even under high concentrations of heavy metals except a few contradictions by some researchers.

Table 3. Effects of different heavy metals on soil microbial respiration (mg CO_2/kg) obtained from one way ANOVA.

Microbial Respiration (mg CO_2-C/kg)								
	Spring				Winter			
	To	T1	T2	T3	To	T1	T2	T3
Zn	0.08b ns	0.2a ns	0.08b ns	0.08b ns	0.31a ns	0.36 ns	0.08a ns	0.09a ns
Cu	0.08a ns	0.07a ns	0.1a ns	0.09a ns	0.07a ns	0.08a ns	0.09a ns	0.05a ns
Cd	0.086b ***	0.13a ***	0.06c ***	0.12a ***	0.07b **	0.073b **	0.16a *	0.09b **

* = $P<0.05$, ** = $P<0.01$, *** = $P<0.001$.

Effect of heavy metals on soil microbial respiration

The results of effects of heavy metals (Zn, Cu, Cd) on the soil microbial respiration have been shown in (Table 3) during the both seasons (spring and winter). Zn and Cu showed no significant effect on the soil microbial respiration while Cd showed highly significant effect at ($P<0.001$) on soil microbial respiration in both seasons (spring and winter). Among the both seasons the effect of Cd in spring was greater than in winter. In case of metabolic quotient, the results of effect of heavy metals shown in (Figure 3; Table 4), Zn showed no significant effect during the spring but in winter results are significant at ($P < 0.05$) and Cu showed the significant result at ($P<0.01$) during the spring and no significant effect have been shown in winter.

The Cd showed the significant results at (P<0.001) in both seasons although somewhat less (P<0.01) in winter than in spring time (P<0.001). Similar results have been produced by the Preston *et al.,* (2000) that toxicity of heavy metals cannot be assumed independent than the toxicity of other heavy metals when present in mixture forms (Degens *et al.,* 2001). Soil microbial respiration is associated with the availability of nutrients in the different seasons under the stress of different heavy metals and during winter due to less availability of nutrients soil microbial respiration is reduced (Smith 2000). Similar results have been suggested by (Lee and Kim, 2002; Rantalainen *et al.,* 2006) that heavy metals are very toxic for the microbial respiration and metabolic quotient but after amendment with organic matter, effects can be minimized.

Table 4. Effects of different heavy metals on soil microbial respiration (mg CO_2-C/kg) obtained from one way ANOVA.

	Metabolic Quotient (mg CO_2-C/g)							
	Spring				Winter			
	To	T1	T2	T3	To	T1	T2	T3
Zn	0.3a ns	0.47a ns	0.44a ns	0.37a ns	0.23a **	0.3a **	0.03b **	0.49 **
Cu	0.3b ***	0.23b ***	0.57a **	0.04c ***	0.23b ns	0.53a ns	0.36ab ns	0.27b ns
Cd	0.3b *	0.54a *	0.33b *	0.56a *	0.23c **	0.42b ***	0.67a ***	0.17c ***

* = P<0.05, ** = P<0.01, *** = P<0.001.

Effect of heavy metals on soil microbial metabolic quotient

The effect of heavy metals on the metabolic quotient has been shown in the (Table 4) in which Zn showed non-significant effect in the spring while during the winter season the effect was significant at (P<0.01) whereas Cu showed no effect on the metabolic quotient in spring as compared to winter with significant effect.

The results showed that Cd affected the microbial parameter more significant in winter as compared to that of spring where less significant effect was observed.

In addition, among all heavy metals, Cd has been found most toxic heavy metals in all seasons which verify the results of (Tang *et al.,* 2012) that Cd present in the soil can negatively effects the microbial communities present in the soil and Dary *et al.,* (2010) already has described that Cd when present even in a minor concentration in the soil around the roots of plants can have adverse effect on them. Macdonald *et al.,* (2007) and Spier *et al.,* (2007) also reported the effects of Cd on the microbial parameters and found that Cd negatively effects soil microbial biomass, basal respiration and metabolic quotient along with effect on enzymatic activities. (Gans *et al.,* 2005) also found the same results that Cd has no biological function and with a small amount can reduce the soil microbial parameters while some contrasting results also have been found by Pennanen *et al.,* (2001) that freshly added heavy metals have no effect on microbial parameters this may be due to their mobility in the soil.

From the above discussion, the authors are of the view that low effect of Zn is due to the fact that Zn is useful nutrient for microbial growth whereas the other two metals have no such contributions towards such growth process.

Conclusion

From the current research work it can be concluded that all the heavy metals showed their more or less effect in both seasons.

The effect of Cd was constant in both seasons as compared to that of rest of the heavy metals. Spring showed higher influence as compared to that of winter and the toxicity was ranked as Cd>Cu>Zn. Among the microbial parameters microbial biomass and metabolic quotient were affected greater as compared to that of microbial respiration.

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