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Response of soil microbial biomass and respiration in heavy metal contaminated soil of Multan

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Abstract

The soil fertility depends on the maintenance of microbial processes and activities for the completion of nutrient cycles. The management practices of soil and man-made activities which add contaminants i.e., heavy metals in the soil potentially have long term effects over microbial diversity in different ecosystems. An experiment was conducted to see the effects of heavy metals on different activities (i.e., microbial biomass and respiration) of microorganisms which produce fertility within the agriculture soil. In this case, soil was collected from Agriculture Research Centre at Bahauddin Zakariya University, Multan and samples were treated with different concentrations of Zn, Cu and Cd in single, duplicate and triplicate. The results showed that soil microbial biomass and soil respiration were significantly ($P<0.05$) affected by Zn and Cu individually as well as in the combination of Zn, Cu. The rest Cd did not show any significant affect individually as well as in combination of other heavy metals.

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Introduction

Soil is a complex mixture of various components including different microbes, heavy metals and climate. It provides habitat for all major groups of microorganisms in the form of micro flora (bacteria and fungi) and soil fauna (protozoa and other invertebrates) (Muller *et al.,* 2004). Soil acts as a source and sinks for different nutrients and builds interaction among microorganisms and their food availability through cycling process. Great relationship exists between soil and microbes as they improve soil fertility and play animportant role in the transfer of energy, production of necessary nutrients, decomposition of soil organic matter, degradation of pollutants and maintaining the balance within the soil's' structure (Bauhus and Khanna, 1999, Stevenson, 1982; Verstraete and Top, 1999; Preston *et al.*, 2001).

Soil microbial biomass, respiration, N mineralization and microbial community structure are the key points for the indication of good or bad quality of soil (Hinojosa *et al.*, 2005). These parameters have been put in national and international monitoring programs of soil (Yao *et al.,* 2000). Microbial activities are disturbed due to influence of heavy metals from different sources which indirectly reduce the soil fertility. It has been reported by Obbard, (2001); Kızılkaya *et al.*, (2004); Liao and Xie, (2007); Wang *et al.*, (2007) that soil microbial biomass and respiration are very sensitive to heavy metals and show adverse effects of these heavy metals.

Heavy metals enter into the soil through different ways including natural as well as anthropogenic source which increase the concentration of heavy metals in the soil and in this way the nature of soil is changed (Chen *et al.,* 2011). The heavy metal pollution has become a serious threat to the biological functioning of soil and its living habitants (Lee and Sun, 2014; Perez-de-Mora *et al.,* 2006; Zhao *et al.,* 2012).

Heavy metals are widely spread group of pollutants and persist within organic portion of soils of all types (Friedland *et al.,* 1984; Hernandez *et al.,* 2003). Larger accumulation of these heavy metals with the passage of time can disrupt the soil functioning which indirectly decreases the microbial activity in the form of soil microbial biomass as well as respiration (Baath, 1989; Laskowski *et al.,* 1994; Pennanen *et al.,* 1998).

According to Cambi *et al.,* (2015); Hertmann, (2014) Ellis *et al.,* (2001); Kelly *et al.,* (2003); Lugauskas *et al.,* (2005) heavy metal contamination, especially Cu and Zn can cause shifting of microbial populations from higher regions to lower regions of soil containing heavy metals and in this way the fertility of soil is affected which is a negative sign for the agriculture soil containing microbes.

Pakistan is the country in which population rely on 70% agriculture. Trace metal contamination of soil due to anthropogenic activities especially industrialization (Reimann *et al.,* 2005) is of major concern now-a-days (Allison *et al.,*2008). This blemish of soil depends upon the soil types and the way by which soil is being used (Palumbo *et al.,* 2000). The principal outcome of this soil contamination leads to health related problems to the people whom are exposed (Achakzai *et al.,* 2015). Heavy metals contamination due to growing of industries in Multan as well as in other parts of Pakistan is a major problem for soil fertility, which contaminates the food as well (Zaidi *et al.,* 2005; Khair, 2009). Many studies highlight the importance of contamination of most of agricultural soils of Pakistan including Multan (Shahbaz *et al.,* 2015), Lahore (Jagtap *et al.,* 2010) Peshawar (Sweda *et al.,* 2008), Karachi (Sistla *et al.,* 2012) and some studies in northern region (Sultana *et al.,* 2014). However, no significant work has been conducted in the study area so far to explore the response of soil microbial biomass and respiration in heavy metal contaminated soil.

Therefore, aim of this research was to explore the problem of soil fertility of Multan because it is city of an agricultural land, contributing largely in the economy of Pakistan. But the quality of land is being deteriorated for some past years due to some industrial activities at certain regions of Multan. For the purpose of improving soil fertility, site study of soil was conducted.

Methods and materials

Sample collection and preparation

Agricultural loamy sand soil samples were collected within the field plot of Agriculture Research Centre (ARC) at Bahauddin Zakariya University Multan. Soil was covered with vegetation and composite soil samples were taken by removing surface vegetation carefully from the depth of 10-20 cm. Samples were dried and sieved to less than 2.5 mm. After sieving, soil samples were sorted to remove the small pebbles, plant material and small fauna and then were mixed thoroughly with trowel. Samples were incubated at 27^{o} C for 8 days to settle disturbance caused due to sampling (Baath *et al.,* 1998).

Prepared soil sample was divided in to eight samples of 1000 g of each and put in to plastic pots of 10 cm in diameters. Solutions of sulphate (sigma) salt of Zn,Cu and Cd dissolved in 100 ml of de-ionized water was applied to each pot (labeled from 1 to 8) in single as well as in combine form (Table 2).Sample after receiving salt solution were thoroughly mixed with spade.

Pot-I was kept as a control and received no metal concentration. Pot-2 received 3600 mg/kg of Zn, Pot-3 was provided with Cu 3600 mg/kg, pot-4 with 3600 mg/kg of Cd, Pot-5 with Zn,Cu at the ratio of 1800:1800 mg/kg, pot-6 with same ratio (1800:1800) of Zn,Cd mg/kg while pot-7 also received the same 1800:1800 mg/kg of Cu,Cd and pot-8 received Zn,Cu,Cd with the ratio of 1200:1200:1200 mg/kg of soil (shown in Table 2). Pots were put for 8 weeks at room temperature of 27-28^{o} C and periodically distilled water was applied to keep the moisture constant in the pots.

Sample analysis

The physico-chemical properties of soil were determined before application of treatments (Table 1).Total metal contents were determined by Atomic Absorption spectrophotometer after digestion and total soil carbons (%) was found by dichromate digestion method following Kalembasa and Jeninson (1973). Soil pH was measured using a pH meter. The Water Holding Capacity (WHC) of the soil was determined by adding 100ml of water to samples of soil placed in weighed funnels containing Whatman No. 42 filter paper. The water drained for 3 hours and the funnels containing the soils were reweighed.

After amendment of heavy metals, soil sample of each pot was analyzed after a few days to 2nd, 4th, 6th, and 8th week. For soil microbial biomass C, the method of Vance *et al.,* (1987) was opted. Fumigation–extraction method was used for the determination of soil microbial biomass. 3 grams of soil sample from each pot were fumigated with chloroform and other 3 g were not fumigated. Microbial biomass C was extracted with K_2SO_4 (0.5 M) solution from both fumigated and non-fumigated samples. The C content was measured in the centrifuged samples by using a soluble-organic C analyzer (Shimadzu TOC-5050A). 2.66 were multiplied with extracted C for microbial biomass calculation.

Soil respiration was determined by IRGA (TORAY PG-100) by placing 50 g of soil with moisture content adjusted to water holding capacity at 50–60% in tightly sealed flasks for incubation at 28 ◦C for 27 days. The CO_2 produced was periodically measured. The data obtained after 27 days of incubation was complied to give a cumulative amount of CO_2 evolved during this period and soil respiration was expressed as ug CO_2/g of soil.

The results were analyzed by two–way Analysis Of Variance (ANOVA).The differences between control and other treatments were measured using least significant level (LSD)

Results and discussion

Soil characteristics determine that P^{H} reading of soil was 6.91, while biomass C and respiration were 167.6

and 2.49 respectively (Table 1).

Table 1. Soil characteristics.

Parameters	Readings
pH	6.91
Carbon (%)	4.66
Biomass Carbon (µgram/gm)	167.6
Respiration	2.49
Water Holding Capacity (%)	50

The pattern of heavy metals amendment in pre-incubated pots was in the sequence of single, double and triple metals with the volume of 3600, 1800:1800 and 1200:1200:1200 mg/kg of soil respectively (Table 2).

Table 2. Concentration of metals in the pots.

Sample	Metal	Concentration (mg/kg)
1	Control	0
2	Zn	3600
3	Cu	3600
4	Cd	3600
5	ZnCu	1800:1800
6	ZnCd	1800:1800
7	CuCd	1800:1800
8	ZnCuCd	1200:1200:1200

Heavy metal profile of soil prior to amendment was detected only in Zn and Cd (Table 3) while rest of the heavy metals including Pb, Hg, and Cr showed no detection in the soil which was sampled.

The result of heavy metals on the microbial biomass of soil (ug/g) (Table 4) showed that microbial biomass was decreased significantly ($P<0.05$) in individual Zn (i.e., Standard Deviation, S.D = 18.83) and Cu (S.D = 32.2) during respective weeks. In the same way, the combinations e.g., Zn and Cu also exhibited significant ($P\leq0.05$) decrease (S.D =44.5) and Cu, Cd (S.D = 23.9). While the rest including individual Cd (S.D = 4.9), Zn, Cd (S.D = 6.8) and Zn,Cu,Cd (S.D = 6.9) did not significantly affected the soil microbial biomass (Table 4, Fig.1).

The results of heavy metals on the microbial respiration of soil (Table 5, Fig.2) describe the similar story. The individual Zn showed the similar effects of Zn and Cu individually as well as in combination form (e.g., S.D of Zn and Cu, and Zn, Cu = 0.715, 0.713, 0.717 respectively). On an overall basis,It concludes that Zn and Cu individually as well as in combination with Zn, Cu have adversely affect the soil microbial biomass and soil respiration which is similar to the report of Gasper *et al.,* (2005).

Table 3. Metal analysis of soil by A.A.S.

Metal	Concentration (mg/kg)
Zn	13.2
Cu	6.01
Cd	1.12
Ni	N. D
Pb	N. D
Hg	N. D
Cr	N. D

A.A.S= Atomic Absorption Spectrophotometer N.D. = Not Detected.

The results were further checked by inhibitory effects of individual metals (i.e., Zn and Cu) against the microbial biomass and soil respiration during eight weeks (Figs. 3A, 3B, 4A, 4B). The result of microbial biomass showed (Figs. 3A, 3B) that both Zn and Cu produced a sharp decline with the passage of weeks.While on the other hand, results are quite similar in term of inhibitory effects of Zn over soil microbial respiration with the passage of time (Figs. 4A, 4B). The slight differences in the result might be due to high P^H of soil that is alkaline in nature which increases the solubility of Zn ions in the soil and this heavy metal become more mobile (Baath and Arnebrant, 1999).

Besides this, the toxic effects of Zn reduced the microbial biomass on second week by 4.9 mg/kg, on 4^{th} week by 17 mg/kg while on 6^{th} week by 35.3 mg/kg an on 8^{th} week by 42.3 mg/kg. Cu also reduced the microbial biomass by 36.7, 60.8 and 73.3 mg/kg of soil and soil respiration under Zn was reduced by 0.19, 0.72 and 1.21 ug C/g with passage of time. Cu effected the soil respiration by 0.13, 1.21 and 1 ug C/g with passage of weeks. These results shows clear similarity with the report of (Marschner and Kalbitz, 2003; Utgikar *et al.,* 2003) that some heavy metals like Al, Mn and Cr are present in the soil but they have no negative effects but some other heavy metals like Zn, Cu, Cd present in the soil even in low level can strongly affect the microbial biomass and respiration. The similar results were also found by Nwuche and Ugoji (2008).

Table 4. Effect s of heavy metals on the microbial biomass of soil (ug/g).

Sample	1	2	3	4	5	6	7	8
Week & treatment	Control	Zn	Cu	Cd	Zn,Cu	Zn,Cd	Cu,Cd	Zn,Cu,Cd
0	184.8	183.6	182.8	180.9	182.4	182.0	183.1	182.6
2	182.1	177.2	174.5	177.0	173.6	178.5	178.4	181.0
4	182.0	165.0	145.3	176.0	137.8	172.4	163.1	179.5
6	183.4	184.1	122.6	172.0	107.9	167.0	143.5	173.4
8	181.5	139.2	108.2	168.1	76.2	166.5	126.4	165.8
S.D	---	18.83	32.2	4.9	44.5	6.88	23.9	6.9
S.E	---	8.42	14.4	2.19	19.9	3.08	10.7	3.09

S. D = Standard Deviation, S.E = Standard Error.

Table 5. Effect s of heavy metals on the microbial respiration of soil (ug/g).

Sample	1	2	3	4	5	6	7	8
Week & treatment	Control	Zn	Cu	Cd	Zn,Cu	Zn,Cd	Cu,Cd	Zn,Cu,Cd
0	2.44	2.52	2.50	2.55	2.51	2.53	2.45	2.57
2	2.36	2.43	2.28	2.33	1.96	2.49	2.42	2.51
4	2.31	2.12	2.18	2.13	1.74	2.41	2.92	2.28
6	2.27	1.55	1.06	1.72	0.96	1.91	2.16	2.15
8	2.01	0.80	1.01	1.70	0.78	1.84	1.89	2.07
S.D	---	0.715	0.713	0.374	0.717	0.333	0.228	0.219
S.E	---	0.320	0.319	0.167	0.321	0.149	0.102	0.098

S. D = Standard Deviation, S.E = Standard Error.

Lee and Sun (2014) have found the negative effects of Zn, Cu on soil microbial properties while working on the application of these heavy metals to plants and microbes in the form of chelates. According to Landi *et al.,* (2000), heavy metals can reduce soil respiration and biomass C after forming complexes with the substrates or by killing the microorganisms.

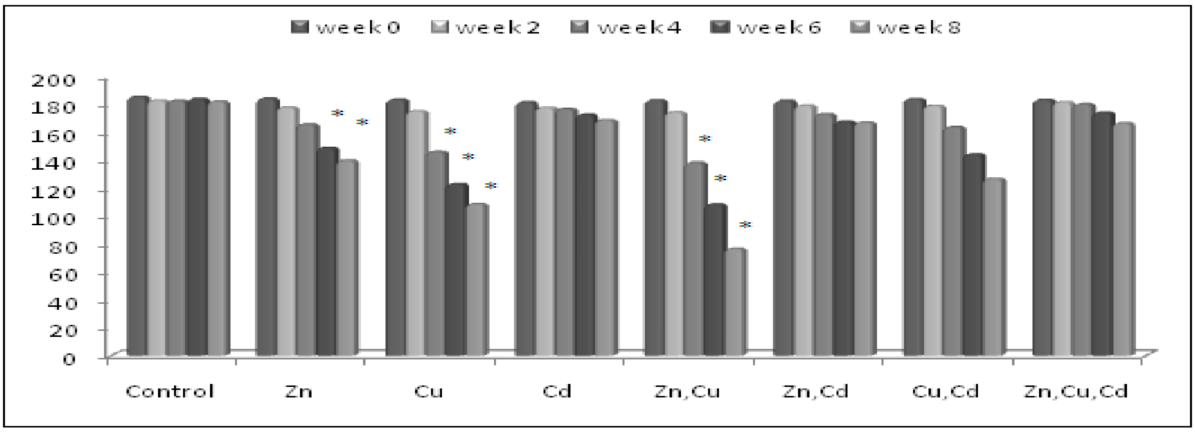

Fig. 1. Effects of heavy metals on the microbial biomass of soil (ug/g). Asterisks indicate significant difference from control treatment (P<0.05) from ANNOVA.

The report of Lourenico, (2010) indicated that plenty of Zn and Cu along with other heavy metals are being produced due to human activities and about 336.75 mg/kg of Zn was observed in soil sample of Sheikhopura which is high level of this heavy metal.

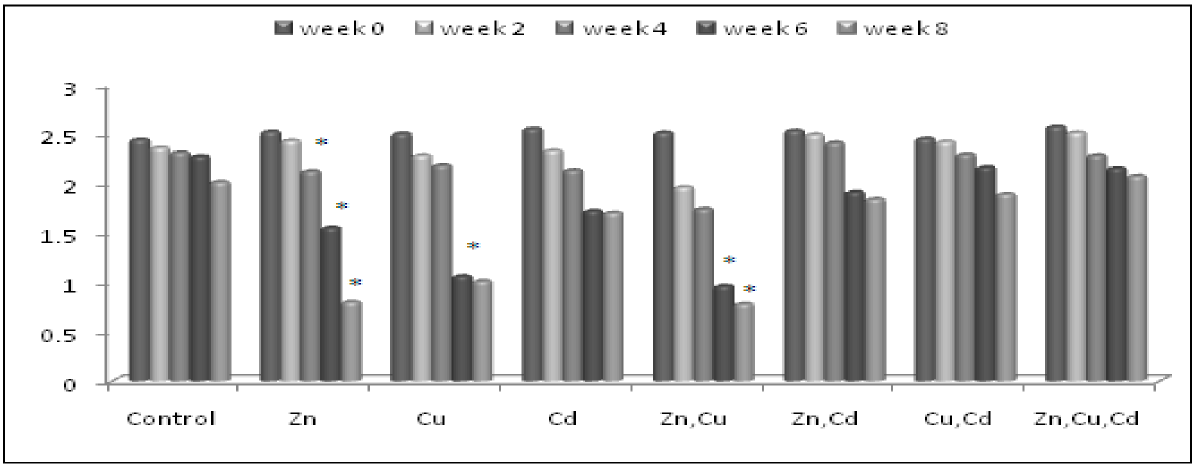

Fig. 2. Effects of heavy metals on the microbial respirationof soil (ug/g). Asterisks indicate significant difference from control treatment (P<0.05) from ANNOVA.

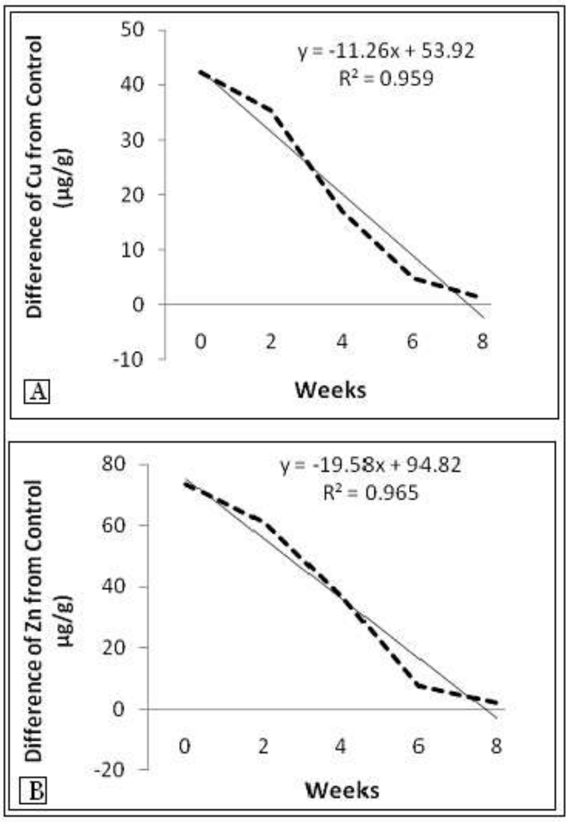

Fig. 3. (A & B). Inhibitory effect of individual Cu and Zn respectively on soil microbial biomass with respect to weeks.

The results distinguish that between both heavy metals (Cu and Zn); Cu has stronger effect as compared to Zn which is also reported by Wang *et al.*, (2015).

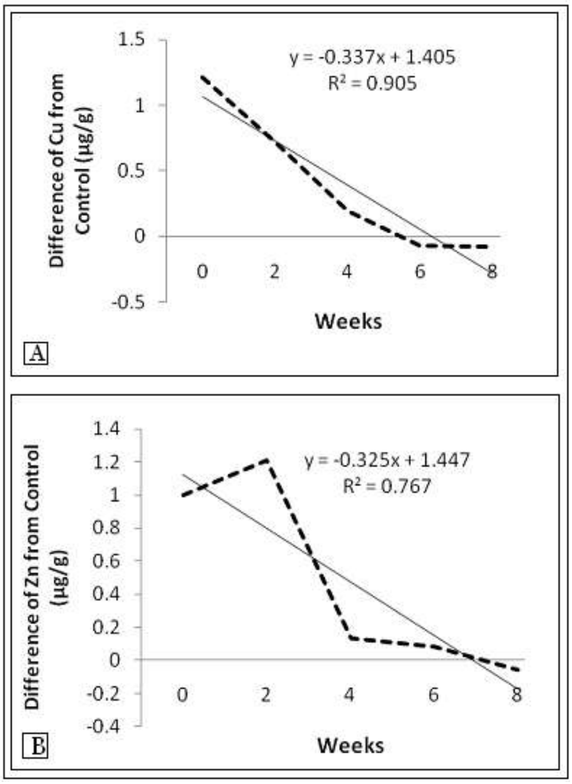

Fig. 4. (A & B). Inhibitory effect of individual Cu and Znrespectively on soil microbial biomass with respect to weeks.

It means when Cu is combined with any other heavy metal its effect is significantly increased as observed in the current study. Distance of heavy metal from the source also matters as Boshoff *et al.,* (2014) investigated the source and distance relationship of heavy metal and found that soil nearer to the heavy metal smelter were affected 90% as compared to that of soil away from the smelter. There was gradual decrease of effect of Cu with increase of distance from the source.Keeping in view of the results of Bosholf *et al.*, (2014), soil of this region (Multan) has been found less fertile (Akbar *et al.,* 2006) it means that microbial activities are concerned with the soil fertility and when the activities of microbial communitiesaredisturbed, the efficiency of soil is reduced.

Conclusion

Finally it is concluded that heavy metals including Zn and Cu individually as well as in combined form showed negative effects in decreasing the soil microbial biomass and soil microbial respiration with the passage of weeks. The findings also suggest that fertility of those soils is lost where microbes are disturbed under the effects of heavy metals.

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References

Achakzai M, Shagufta S, Wazir A, Samiullah M, Panezai Z, Anila N. 2015. Comparison of Water Chemistry of Manchar Lake with WHO Drinking Water Quality StandardsAmerican-Eurasian J. Agric. & Environ. Sci. **15,** 1224-1230. http://dx.doi.org/10.5829/idosi.aejaes.2015.15.7.94243

Akbar M, Zaman H, Aashiq L. 2006. Effect of heavy metal on microbial biomass and respiration in Sukkur. Pakistan Journal of Environmental Sciences **11,** 220-223.

Allison SD, Treseder KK. 2008. Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. Global Change Biology. **14,** 2898-2909.

Baath E. 1989. Effects of heavy metals in soil on microbial processes and populations. Water, Air Soil Pollution **47,** 335-379. http://dx.doi.org/10.1007/BF00279331.

Baath E, Diaz-Ravina M, Frostegard A, Campbell CD. 1998. Effect of metal-rich sludge amendments on the soil microbial community. Applied and Environmental Microbiology **64,** 238–245.

Baath E, Arnebrant K. 1999. Growth rate and response of bacterial communities to pH in limed and ash treated forest soils. Soil Biology & Biochemistry **26,** 995-1001. http://dx.doi.org/10.1016/0038-0717(94)90114-7.

Bauhus J, Khanna PK. 1999. The significance of microbial biomass in forest soils. In: Rastin, N., Bauhus, J, Eds. Going Underground – Ecological Studies in Forest Soils. Research Signpost: Trivandrum, India. 77–110 P.

Boshoff M, Jong MD, Dardenne F, Blust R, Bervoets L. 2014. The Impact of metal pollution on soil faunal and microbial activity in two grassland ecosystem. Environment Research **134,** 169-180. http://dx.doi.org/10.1016/j.envres.2014.06.024.

Cambi M, Certini G, Neri F, Marchi E. 2015. The Impact of Heavy Traffic on forest soil: A review. Forest Ecology and Management **338,** 124-138. http://dx.doi.org/10.1016/j.foreco.2014.11.022.

Chen L, Long XH, Zhang ZH, Zheng XT, Rengel Z, Liu ZP. 2011. Cadmium accumulation and transloca- tion in two Jerusalem artichoke (*Helianthus tuberosus* L.) cultivars. Pedosphere **21,** 573-580. http://dx.doi.org/10.1016/S1002-0160(11)60159-8.

Ellis RJ, Neish B, Trett MW, Best JG, Weightman AJ, Morgan P, Fry JC. 2001. Comparison of microbial and meiofaunal community analyses for determining impact of heavy metal contamination. Journal of Microbiological Methods **45,** 171–185. http://dx.doi.org/10.1016/S0167-7012(01)00245-7.

Friedland AJ, Johnson AH, Siccama TG, Mader DL. 1984. Trace metal profiles in the forest floor of New England. Soil Science Society of America Journal **48,** 422-425. http://dx.doi.org/10.2136/sssaj1984.03615995004800020042x.

Gaspar GM, Mathe PL, Szabo B, Uzinger N, Anton A. 2005. After-effect of heavy metal pollution in brown forest soils. Proceedings of the 8th Hungarian Congress on Plant Physiology and the 6th Hungarian Conference on Photosynthesis. ActaBiologicaSzegediensis **49,** 71-72. http://dx.doi.org/10.5402/2011/402647.

Hernandez L, Probst A, Probst JL, Ulrich E. 2003. Heavy metal distribution in some French forest soils: evidence for atmospheric contamination. Science of Total Environment **312,** 195–219. http://dx.doi.org/10.1016/S0048-9697(03)00223-7.

Hertmann M, Niklaus P, Zimmermamm S, Schmutz S, Kremer J. 2014. Resistance and resilience of the forest soil micro biome to logging associated compaction. International Society for Microbial Ecology Journal **8,** 226-244. http://dx.doi.org/10.1038/ismej.2013.141.

Hinojosa MB, Carreira JA, Garcia-Ruiz R, Dick RP. 2005. Microbial response to heavy metal polluted soils: community analysis from PLFA and EL-FA extracts. Journal of Environmental Quality **34,** 1789–1800. http://dx.doi.org/10.2134/jeq2004.0470.

Kalembasa SJ, Jenkinson DS. 1973. A comparative study of titrimetric and gravimenetric methods for the determination of organic carbon in soil. Journal of the Science of Food and Agriculture **24,** 1085 – 1090. http://dx.doi.org/10.1002/jsfa.2740240910.

Kelly JJ, Haggblom MM, Tate RL. 2003. Effects of heavy metal contamination and remediation on soil microbial communities in the vicinity of a zinc smelter as indicated by analysis of microbial community phospholipids fatty acid profiles. Biology and Fertility of Soils **38,** 65-71.

Khair MH. 2009. Toxicity and accumulation of copper in Nannochloropsis oculata (*Eustigmatophycea, Heterokonta*). World Applied Sciences Journal**6**, 378–384.

Kizilkaya R, Askin T, Bayraki B, Saglam M. 2004. Microbiological characteristics of soils contaminated with heavy metals. European Journal of Soil Biology **40,** 95-102. http://dx.doi.org/10.1016/j.ejsobi.2004.10.002.

Landi L, Renella G, Moreno JL, Falchini L, Nannipieri P. 2000. Influence of cadmium on the metabolic quotient, L-D-glutamic acid respiration ratio and enzyme activity, microbial biomass ratio under laboratory conditions. Biology and Fertility of Soils **32,** 8–16. http://dx.doi.org/10.1007/s003740000205.

Laskowski R, Maryanski M, Niklinska M. 1994. Effect of heavy metals and mineral nutrients on forest litter respiration rate. Environmental Pollution **84,** 97–102. http://dx.doi.org/10.1016/0269-7491(94)90076-0.

Lee J, Sun K. 2014. Effects of chelates on soil microbial properties, plant growth and heavy metal accumulation. Ecological Engineering **73**, 386–39.

Liao M, Xie XM. 2007. Effect of heavy metals on substrate utilization pattern, biomass, and activity of microbial communities in a reclaimed mining wasteland of red soil area. Ecotoxicology and

Environmental Safety **66,** 17–223.
http://dx.doi.org/10.1016/j.ecoenv.2005.12.013.

Lourenico KL. 2010. Effect of Cu on microbial activities of soil. Eurasian Journal of Ecology **33,** 209-214.

Lugauskas A, Levinskaitė L, Motuzas R, Prosyčevas I. 2005. Effect of copper, zinc and lead acetates on microorganisms in soil. Ekologija **N 1,** 61–69.
http://dx.doi.org/10.5601/jelem.2013.18.4.455.

Marschner B, Kalbitz K. 2003. Control of bioavailability and biodegradation of dissolved organic matter in soils. Geoderma **113,** 211 – 235.
http://dx.doi.org/10.1016/S0016-7061(02)00362-2.

Muller AK, Westergaard K, Christensen S, Sørensen SJ. 2004. The effect of long term mercury pollution on the soil microbial community. FEMS Microbiology Ecology**36,** 11–19.
http://dx.doi.org/10.1111/j.15746941.2001.tb00821.x.

Nwuche CO, Ugoji EO. 2008. Effect of heavy metal pollution on soil microbial activity. International Journal of Environment Science and Technology **5,** 409-414.

Obbard P. 2001. Ecotoxicological assessment of heavy metals in sewage sludge amended soils. Applied Geochemistry **16,** 1405–1411.
http://dx.doi.org/10.1016/S0883-2927(01)00042-7.

Palumbo B, Angelone M, Bellanca A, Dazzo C, HauserS, Neri R, Wilson J. 2000. Influence of inheritance and pedogenesis on heavy metal distribution in soils of Sicily, Italy. Geoderma **95,** 247–266.

Pennanen T, Perkiomaki J, Kiikkila O, Vanhala P, Neuvonen S, Fritze H. 1998. Prolonged, simulated acid rain and heavy metal deposition: separated and combined effects on forest soil microbial community structure. FEMS Microbiology Ecology **27,** 291–300.
http://dx.doi.org/10.1111/j.15746941.1998.tb00545.x.

Perezde-Mora A, Burgos P, Madejon E, Cabrera F, Jaeckel P, Schloter M. 2006. Microbial community structure and function in a soil contaminated by heavy metals: Effects of plant growth and different amendments. Soil Biology & Biochemistry **38,** 327–341.
http://dx.doi.org/10.1016/j.soilbio.2005.05.010.

Preston S, Wirth S, Ritz K, Griffiths BS, Young IM. 2001. The role played by microorganisms in the biogenesis of soil cracks: importance of substrate quantity and quality. Soil Biology & Biochemistry **33,** 1851–1858.
http://dx.doi.org/10.1016/s0038-0717(01)00113-4.

Reimann C, Filzmoser P, Garrett G. 2005. Background and threshold: critical comparison of methods of determination. Science of the Total Environment **346,** 1–16.

Sawada K, Funakawa S, Kosaki T. 2008. Soil microorganisms have a threshold concentration of glucose to increase the ratio of respiration to assimilation. Soil Sci. Plant Nutr **54,** 216–223.
http://dx.doi.org/10.1111/j.1747-0765.2009.00378.

Shabaz M, Hakoomat A, Muhammad S, Saif-ul-Malook, Nawaz S, Qurban A. 2015.Effect of Seed Coating with Boron and Zinc of Zea mays for Various Yield Traits .American-Eurasian J. Agric. & Environ. Sci. **15,** 1304-1311.
http://dx.doi.org/10.5829/idosi.aejaes.2015.15.7.12705

Sistla SA, Asao S, Schimel JP. 2012. Detecting microbial N-limitation in tussock tundra soil: implications for Arctic soil organic carbon cycling. Soil Biology and Biochemistry **55,** 78 84.

Stevenson FJ. 1982. Humus Chemistry: Genesis, Composition, Reactions. John Wiley & Sons, New York **11,** 200-2080.

Sultana N, Alimon AR, Haque KS, Sazili AQ, Yaakub H, Hossain SMJ. 2014. The effect of cutting interval on yield and nutrient composition of different plant fraction of Moringa oleifera tree. J. Food Agri. Environ. **12,** 599-604.

Utgikar VP, Tabak HH, Haines JR, Govind R. 2003. Quantification of toxic inhibitory impact of copper and zinc on mixed cultures of sulfate reducing bacteria. Biotechnology and Bioengineering **82,** 306-312.
http://dx.doi.org/10.1002/bit.10575.

Vance ED, Brookes PC, Jenkinson DS. 1987. Microbial biomass measurements in forest Soils: The use of the chloroform-fumigation method in strongly acid Soils. Soil Biology & Biochemistry **19,** 697-702.

Verstraete W, Top EM. 1999. Soil clean-up: lessons to remember. International Biodeterioration & Biodegradation **43,** 147–153.
http://dx.doi.org/10.1016/S0964-8305(99)00043-8.

Wang Y, Shi J, Wang H, Lin Q, Chen X, Chen Y. 2007. The influence of soil heavy metals pollution on soil microbial biomass, enzyme activity, and community composition near a copper smelter. Ecotoxicology and Environmental Safety **67,** 75-81.
http://dx.doi.org/10.1007/s00253-015-6662-6.

Wang F, Yao J, Liu H, Liu R, Chen H, Yi Z, Yu Q. 2015. Cu and Cr enhanced the effects of various carbon nanotubes on microbial communities in an aquatic environment. Journal of Hazardous Material **292,** 137-145.
http://dx.doi.org/10.1016/j.jhazmat.2015.03.032.

Yao H, Wilson MJ, Campbell CD. 2000. Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microbial Ecology **40,** 223–237.
http://dx.doi.org/10.1007/s002480000053.

Zaidi MI, Asrar A, Mansoor A, Farooqui MA. 2005. The heavy metals concentration along roadside trees of Quetta and its effects on public health. Journal of Applied Sciences **5,** 708–711.
http://dx.doi.org/10.3923/jas.2005.708.711.

Zhao YL, Dolat A, Steinberger Y, Wang X, Osman A, Xie GH. 2012. Biomass yield and changes in chemical composition of sweet sorghum cultivars grown for biofuel. Field Crops Research **111,** 55–64.
http://dx.doi.org/10.1016/j.fcr.2008.10.006.