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Response of soil microbial biomass and enzymes activity to cadmium (Cd) toxicity under different soil textures and incubation times

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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 ecosystems. An incubation experiment was conducted to estimate the response of soil microbial biomass and enzymes activity to Cd toxicity and how this toxicity behaves under different textures. Three different textured soils (sandy loam, clay loam and loam) were collected from three diverse areas across Potohar region of Pakistan and were incubated at five different Cd levels (0.00, 50, 100, 150 and 200 mg kg^{-1}). Microbial biomass carbon (C_{mic}), nitrogen (N_{mic}), phosphorous (P_{mic}), and enzymes activity (dehydrogenase, urease and phosphatase) were quantified at 10, 20 and 30 days interval after Cd-contamination. The application of Cd had strong negative effect on the size of soil microbial biomass and enzyme activities. The highest C_{mic}, N_{mic}, P_{mic}, dehydrogenase, urease and phosphatase (137.30, 16.30, 9.56, 60.93, 5.30 and 33.63 mg kg^{-1} soil) activities were observed at control in loamy texture, whereas the lowest values (21.40, 0.50, 0.20, 6.66, 0.20 and 0.70 mg kg^{-1} soil) were observed at 200 mg Cd kg^{-1} in the sandy loam soil. Moreover, texture and incubation time had profound effect on Cd toxicity to microbial biomass and enzymes activities. The soil texture was positively linked with Cd toxicity, among textural classes, clay loam and loam were more resistive and having maximum quantities of soil microbial biomass and enzyme activities than sandy loam. Cd toxicity increased with the incubation time, therefore the lowest microbial biomass and enzymes activity was observed at thirty days of incubation. Our results revealed that soil microbial biomass and enzyme activities were strongly inhibited by Cd. Furthermore, Cd toxicity changed under different textures deducing that Cd threshold is strongly associated with texture of the soils.

Keywords: Microbial biomass; Enzymes activity; Cadmium; Soil texture.
Abbreviations: Cd_cadmium, C_{mic}_microbial biomass carbon, N_{mic}_microbial biomass nitrogen, P_{mic}_microbial biomass phosphorous, SMB_soil microbial biomass, TOC_Total organic carbon, EA_enzymes activity, CRD_complete randomized design, CEC_cation exchange capacity, EC_electrical conductivity, WHC_ water holding capacity, Ck_control, rpm_revolutions per minute.

Introduction

Environmental pollution is a serious threat to the modern civilization. Many industrial and anthropogenic sources such as waste incinerators, urban traffic, tanning industries, cement factories, excessive use of fertilizers, pesticides, farm yard manure, sewage sludge and rapid urbanization are the major sources of pollution and played cardinal role in the transportation of heavy metals to the cropping fields, pastures and forests (Sanita and Gabbrielli, 1999). Mining, manufacturing, and the use of synthetic products (e.g., pesticides, paints, batteries, industrial waste, and applications of industrial or domestic sludge to the land) can also result in heavy metal contamination of urban and agricultural soils (Kuo et al., 2006; Ramadan and Al-Ashkar, 2007). Heavy metals are not subjected to degradation processes; therefore they remain almost indefinitely in the environment, and accumulate in different parts of the food chain. The food chain contamination is one of the most important pathways for the entry of these toxic pollutants into the human body (Khan et al., 2008). Heavy metals at high concentrations generally affect the growth, morphology and metabolism of microorganisms which leads to decrease in the functional diversity of soil ecosystems (Kandeler et al., 1996; Pennanen et al., 1998). Khan et al. (2010) reported that heavy metals could have long-term hazardous impacts on the health of soil ecosystems and adverse influences on soil biological processes. The inhibition of soil enzymes activities depends on the nature and concentrations of heavy metals (Dick and Tabatabai, 1983; Zheng et al., 1999). Heavy metals usually inhibit enzymes activities by interacting with the enzyme substrate complexes, denaturing the enzyme protein and interacting with their active sites (Megharaj et al., 2003). Among the heavy metals, Cadmium (Cd) is the most ubiquities metal influencing soil biota. At high concentrations, Cd is extremely toxic to soil and aquatic organisms while at low levels, it adversely affects microbial physiology (Stohs and Bagchi, 1995; Sokolova, 2004). The availability of Cd varies with the nature of Cd applied, the soil type and the environmental conditions (Dar and Mishra, 1994; Dar, 1995). Soil contaminated with Cd led to significant decline in the

activity of dehydrogenase, urease, alkaline phosphatase and to a smaller extent acid phosphatase (Oliveira and Pampulha 2006; Khan et al., 2007; Tejada et al., 2011). To our knowledge, no study has shown the effect of Cd on microbial biomass and enzymes activity under different soil types across the potohar region of Pakistan. Many regions of Pakistan, especially potohar region have tremendous potential for increasing crop production with proper soil management practices, particularly the improvement of soil quality and health. Therefore, we performed this study with the following objectives: (1) to evaluate the effect of different concentrations of Cd on microbial biomass and enzymes activity; (2) to find out the influence of soil texture on Cd toxicity to soil microbial biomass and enzymes activity and (3) to quantify the effect of incubation time on Cd toxicity to the microbial biomass and enzymes activity.

Results

Effect of cadmium on soil microbial biomass

The higher concentrations of Cd caused sizeable reductions in the values of $C_{mic,}$ N_{mic} and P_{mic} under different textured soils and incubation times (Table 2). Highest values of $C_{mic,}$ N_{mic} and P_{mic} were observed after 10 days of incubation, whereas the lowest were found at 30 days of incubation. In sandy-loam soil, the minimum C_{mic} content (21.40 mg C kg^{-1}) was found at 200 mg Cd kg^{-1} soil, whereas the maximum (66.39 mg C kg^{-1}) was recorded at control (0 mg Cd kg^{-1}). Moreover, significant differences were observed between control and the other treatments (50, 100, 150 and 200 mg Cd kg^{-1}) in this soil at the end of incubation. The lowest amounts of C_{mic} (57.40 and 104.0 mg C kg^{-1}) were quantified due to the addition of 200 mg Cd Kg^{-1} soil in clay-loam and loam soils, respectively. Microbial biomass N values ranged from 8.60 to 0.50 mg kg^{-1} in sandy loam soil at varying concentrations of Cd (from zero to 200 mg Cd kg^{-1}). At the end of the incubation, both clay-loam and loam soils showed higher resistance to the raised Cd toxicity (200 mg Cd kg^{-1}) on the values of N_{mic} (5.60, 11.00 mg kg^{-1} respectively) as compared to the sandy loam soil (0.50 mg kg^{-1}). Incubation time had strong adverse effect on N_{mic} regardless of the soil type, suggesting a progressive decrease in organic substrates availability to microbes with respect to the Cd toxicity. At the control treatment, P_{mic} values were higher than those at the treatments of 50, 100, 150 and 200 mg Cd kg^{-1} under all incubation periods. The highest P_{mic} values (9.56, 8.13 and 6.30 mg kg^{-1}) were observed in loam, clay-loam and sandy-loam at zero mg Cd kg^{-1} after 10 days of incubation, respectively. Conversely, the lowest values of P_{mic} in sandy-loam (0.20 mg kg^{-1}), clay-loam (1.30 mg kg^{-1}) and loam (2.10 mg kg^{-1}) textures were recorded at 200 mg Cd kg^{-1} after 30 days of incubation.

Effect of cadmium on Enzymes activity

The effect of different levels of Cd concentration on soil enzyme activities at different incubation times are presented in Table 3. Higher Cd concentrations exceedingly impede the activities of dehydrogenase, urease and phosphatase enzymes, in all soils (sandy-loam, clay-loam and loam) than the lower concentrations. We observed significant difference in dehydrogenase activity between all the concentrations of Cd at the longest incubation time in sandy-loam soil. On the other hand, non-significant difference in dehydrogenase activity was observed in clay-loam and loam soils at 50, 100 and 150 mg Cd kg^{-1} after 30 days of incubation, which depicted the resistive behavior of these soils. The highest dehydrogenase activity was obtained at zero Cd kg^{-1} (60.90 mg TPF kg^{-1} 24 h^{-1}) in the loam soil after 10 days of incubation, whereas the lowest dehydrogenase activity (6.66 mg TPF kg-1 24 h-1) was detected after 30 days of incubation at 200 mg Cd kg-1 in the sandy loam soil. The maximum urease activity was noted in control (3.30 mg NH_4+-N kg^{-1} 24 h^{-1}) and tends to decrease gradually with the increasing incubation time and Cd toxicity. Conversely, the minimum urease activity (0.20 mg NH_4+N kg^{-1} 24 h^{-1}) was quantified at the utmost Cd level (200 mg kg^{-1}) after 30 days of incubation. The control (Ck) treatment was responsible for the maximum phosphatase activity after 10 days of incubation (14.57, 25.13 and 33.63 mg phenol kg^{-1} h^{-1}) in the sandy loam, clay loam and loam soils, respectively. In contrast, the values of phosphatase activity decreased sharply and reached to their lowest values (0.70, 11.13 and 18.47 mg phenol kg^{-1} h^{-1}) due to the addition of 200 mg Cd kg^{-1} in the sandy loam, clay loam and loam soils, congruently after 30 days of incubation.

Discussion

Our results indicated that the minimum microbial biomass C, N and P was found at 200 mg Cd kg^{-1} soil. This might be due to the reason that higher Cd concentrations had more toxic effects on soil microbes than the lower concentrations. Low levels of Cd had non-significant impact on soil microbial biomass carbon; whereas high levels of Cd significantly decline the soil microbial biomass carbon (Dar (1996; Liao et al. 2005). Effect of Cd was more prominent in sandy loam than in clay loam and loam. This may be that in clay loam and loam textured soils more cat ion-exchange sites, particularly clay minerals and organic complexes are present, which bind the Cd on exchange complexes. Our results are in agreement with the findings of Dar (1996) who emphasized that maximum toxic effect of Cd was in sandy loam soil than in clay and loamy soils. Matus et al. (2008) observed that soil organic C tends to be associated with the fine fraction of soils and it was significantly greater i.e. three times in clay-rich soils than coarser soils. Soil texture as a biotic factor is important factors that influence distribution of minerals, organic matter retention, microbial biomass and other soil properties (Scott and Robert, 2006). We observed a direct relationship between Cd toxicity and incubation period i.e. the increase in Cd toxicity increase with increased in the incubation period. This confirms the fact that after 30 days of incubation Cd killed down all most all the effective microorganisms. It was estimated by Vig et al. (2003) that Cd toxicity to soil biota varies with time, soil type, speciation, ageing, Cd-source, organisms and the environmental factors. In addition, Sardar et al. (2007) investigated that the extent of inhibition increased significantly with increasing level of heavy metals, and varied with the incubation periods.

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 Cd. Malley et al. (2005) found that an over all reduction on dehydrogenase activity with increasing dosages of Cu, Cd and Zn. 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. Urease known as a very sensitive and imperative hydrolyzing enzyme and it plays crucial role in the hydrolysis of urea (Nannipieri et al., 2002). Urease mainly used as a good and responsive indicator of biological activities and pollution stresses in soils (Hinojosa et al., 2004). Maximum urease activity was achieved in control in loamy

Table 1. Physical and chemical characteristics of experimental soils.

Parameters	Sandy loam	Clay loam	Loam
Sand %	58	30.5	38
Silt %	31	33.5	30
Clay %	11	36	32
Soil pH (1:2.5)	7.85	7.81	7.9
EC (dS m^{-1})	0.29	0.41	0.24
CEC (C mol_c Kg^{-1})	9.61	9.87	10.79
TOC (mg kg^{-1})	75.4	99.4	120.5
Total N (mg kg^{-1})	10.5	12.2	13.3
Available P (mg kg^{-1})	4.5	5.9	6.7
Available Cd (mg kg^{-1})	ND	ND	ND
Total Cd (mg kg^{-1})	0.1	0.3	0.2

ND: not detected.

texture, while minimum at 200 mg Cd kg^{-1} soil after 30 days of incubation in sandy loam. Malley et al. (2005) recorded significant reductions in the urease activity due to increase in the Cu, Cd and Zn levels. The combined effect of polycyclic aromatic hydrocarbons and heavy metals (Cd, Zn and Pb) on soil urease activity largely depends upon the incubation time (Shen et al., 2005). Phosphatase enzyme plays critical roles in P cycles and known as a good indicator of soil fertility and quality (Gil-Sotres et al., 2005). The phosphatase inhibition rate increased with increasing heavy metals, particularly, at the highest levels of Cd. In incubation experiment Khan et al. (2007) mentioned that the alkaline phosphatase activity was significantly decreased by the application of Cd and Pb. Results regarding textures and incubation time indicated that minimum phosphatase activity was recorded in the sandy loam texture, whereas the highest activity was observed in the loamy soil. This might be due to the reason that loam and clay loam soils have greater ability to cope with the Cd toxicity due to the presence of more active sites and mineral complex. Renella et al. (2003) noticed that the phosphatase activity was more sensitive in the sandy than in the finer textured soils. It was found that soil texture is one of the most important factors influencing the enzymes activity as well as, pH, cation exchange capacity and organic matter content (Girvan et al. 2003).

Materials and Methods

Physico-chemical analysis

Three different textured soils (loam, sandy loam and clay loam) were collected (0-15 cm), from three distinct sites of Potohar Region of Pakistan, namely Tarnol, khairimurat and Mandra respectively. The samples were transferred into polyethylene bags to the laboratory, air-dried and passed through 2-mm sieve for further use in the incubation study. Soil electrical conductivity (EC) and pH were measured by preparing the 1:2.5 (w/v) soil water suspensions, by using conductivity and pH meter, respectively (Page et al., 1982). For cation exchange capacity (CEC), soil sample of 2.5 g was saturated by shaking with 4 successive 33 ml aliquots of 1.0 M KCl. Excess K^+ in the soil was washed thrice with 20 ml of 95% ethanol, while adsorbed K^+ was extracted with 3 successive additions of 33 ml 1.0 M NH_4OAc. Volume of the extract was made up to 100 ml by adding 1.0 M NH_4OAc and K^+ concentration in the extract was estimated by flame photometry (Anderson and Ingram, 1993). Textural analysis was carried out according to the method of Gee and Bauder, (1986) briefly, 40 g soil sample, 60 ml of 1 % sodium hexametaphosphate and 150 ml of distilled water were added into a beaker for overnight. After stirring for 10 minutes the suspension was shifted to 1000 ml capacity graduated glass cylinder and volume was brought to 1-L. The suspension was stirred with Plunger; first Bouyoucos hydrometer reading was taken after 4 minutes of stirring which gave the percent amount of silt + clay. Whereas second hydrometer reading was taken after 2 hours to determine the percent amount of clay. Soil textural class was determined by using International Soil Science Society (ISSS) triangle. Total organic carbon (TOC) was calculated by the procedure of Page et al., (1982), summarily 2 g soil was taken in an Erlenmeyer flask. Ten ml of I N potassium dichromate ($K_2Cr_2O_7$) was added into flask and the flask was swirled to mix the contents. Then 20 ml of concentrated H_2 SO_4 was added in the suspension and swirling was done for one minute. The flask was allowed to stand for 30 minutes. After 30 minutes, 200 ml of distilled water, 10ml of phosphoric acid and 1 ml of diphenylamine indicator were added in the flask. The contents were titrated against 0.5 N ferrous sulphate solutions until color changes from blue to red. Total nitrogen was determined by adding 30 ml of concentrated H_2SO_4 and 10 g of digestion mixture (K_2SO_4: $FeSO_4$: $CuSO_4$: 10.0:1.0:0.5) in 10 g of soil. Then soil sample was digested on gas heater using Kjeldahl digestion flask. The digested material was cooled and the volume was made up to 250 ml. 10 ml aliquot was taken from this cooled digested mixture for distillation of ammonia in a receiver containing boric acid (4%) and titrated against 0.1 N H_2SO_4 (Buresh et al., 1982). For Available phosphorus, 5 g of soil was taken into 250 ml Erlenmeyer flask along with 100 ml of 0.5 M $NaHCO_3$ solution, flask was shaken for 30 minutes on reciprocating shaker and filtrate was collected. Ten ml of filtrate was added into 50 ml volumetric flask along with 1 ml of 5 N H_2SO_4, volume was made up to 40 ml by adding distilled water. After this, 8 ml of reagent B (ascorbic acid) was added in order to develop the color. Transmittance was recorded after 10 minutes at 880 nm by using Spectrophotometer (Olsen and Sommers, 1982). Available Cd was quantified by extracting 10 g soil with 20 ml of AB-DTPA solution. After 30 minutes the metal content in the extract was measured using the atomic absorption spectrophotometer (Page et al., 1982). For total Cd, 2 gram soil sample and 10 ml of 1:1 HNO_3 were taken and heated at 95°C for 15 minutes. After cooling, 5 ml of concentrated HNO_3 was added and refluxed for an additional 30 minutes at 95°C. Again 5ml of Concentrated HNO_3 was added and refluxed the sample at 95°C until the volume of digest reduced to 5 ml. After cooling, 2 ml of distilled water and 3 ml of 30 % H_2O_2 were added to heat the sample gently to start the effervescence. Heating was continued by adding 30 % H_2O_2 in 1ml increments until the effervescence subsides. Finally, 5ml of concentrated HCl and 10 ml of distilled water were added to reflux the sample for an additional 15 minutes. After cooling, the digest was filtered through what man No. 42 filter paper, diluted to 50 ml and analyzed by atomic absorption spectrophotometer (Soon and Abboud, 1993). Some physical and chemical properties of

Table 2. Effect of Cd on soil microbial biomass carbon, nitrogen and phosphorous at different textured soils and incubation times.

Cd Treatments	SMB (mg kg^{-1})	Sandy loam			Clay loam			Loam		
		Days after incubation			Days after incubation			Days after incubation		
		10	20	30	10	20	30	10	20	30
0 mg kg^{-1}	C_{mic}	66.9 a	65.3 a	63.3 a	97.0 a	95.0 a	93.9 a	137.3 a	135.4 a	132.7 a
	N_{mic}	8.60 a	8.00 a	7.50 a	11.8 a	11.3 a	10.8 a	16.3 a	15.6 a	15.0 a
	P_{mic}	6.30 a	6.12 a	5.95 a	8.13 a	7.93 a	7.89 a	9.56 a	8.96 a	7.50 a
50 mg kg^{-1}	C_{mic}	64.9 ab	62.3 b	60.4 b	94.2 ab	91.5 ab	90.1 b	134.4 ab	132.3 ab	129.3 b
	N_{mic}	8.10 ab	7.40 ab	6.90 b	11.4 ab	10.8 ab	9.50 ab	16.0 a	15.1 ab	14.7 a
	P_{mic}	5.23 a	3.33 b	2.93 b	7.20 b	5.23 b	4.36 b	8.70 ab	6.83 b	6.10 b
100 mg kg^{-1}	C_{mic}	60.1 b	59.6 b	55.9 c	91.20 bc	87.4 b	86.3 c	131.4 bc	128.7 b	125.3 c
	N_{mic}	7.50 b	6.70 c	6.20 c	10.9 bc	10.2 b	7.00 b	15.3 ab	14.6 b	13.7 b
	P_{mic}	4.13 bc	2.23 c	1.03 c	5.96 bc	4.43 b	3.20 c	7.13 bc	5.86 b	4.93 c
150 mg kg^{-1}	C_{mic}	55.3 cd	31.9 c	31.0 d	87.4 cd	65.2 c	64.4 d	128.2 c	110.3 c	109 d
	N_{mic}	6.70 c	2.70 d	2.10 d	10.3 c	7.20 c	6.70 b	14.8 bc	12.4 c	11.9 c
	P_{mic}	3.16 cd	1.16 cd	0.43 d	4.83 cd	3.40 c	2.26 b	6.20 cd	4.56 c	3.43 d
200 mg kg^{-1}	C_{mic}	49.7 d	22.7 d	21.4 e	83.4 d	58.2 d	57.4 e	124 d	105.4 d	104 e
	N_{mic}	5.80 d	0.90 e	0.50 e	9.60 d	6.00 d	5.60 d	14.2 cd	11.5 d	11.0 d
	P_{mic}	2.30 d	0.50 d	0.20 e	3.90 d	2.30 d	1.30 e	5.10 d	3.30 d	2.10 e

C_{mic} = microbial biomass carbon, N_{mic} = microbial biomass nitrogen and P_{mic} = microbial biomass phosphorus, Values within columns followed by different letters indicate significant difference between the treatments at $P < 0.05$.

Table 3. Effect of Cd on soil enzymes dehydrogenase, urease and phosphatase activity at different textured soils and incubation times.

Cd Treatments	Enzymes activity	Sandy loam			Clay loam			Loam		
		Days after incubation			Days after incubation			Days after incubation		
		10	20	30	10	20	30	10	20	30
0 mg kg^{-1}	Dehydrogenase	36.3 a	35.3 a	34.2 a	47.6 a	46.6 a	45.5 a	60.9 a	59.9 a	58.6 a
	Urease	3.30 a	2.70 a	2.13 a	4.63 a	3.80 a	3.30 a	5.30 a	4.56 a	3.66 a
	Phosphatase	14.5 a	13.5 a	12.6 a	25.1 a	23.4 a	22.4 a	33.6 a	32.3 a	31.6 a
50 mg kg^{-1}	Dehydrogenase	33.8 a	32.4 ab	31.1 b	46.0 ab	44.8 ab	43.6 a	59.6 a	58.1 a	56.8 ab
	Urease	2.83 b	2.20 ab	1.70 b	4.13 ab	3.06 ab	2.96 ab	4.80 a	3.73 b	3.15 ab
	Phosphatase	13.2 ab	11.9 a	11.0 b	24.4 a	22.0 ab	20.8 ab	31.7 ab	30.9 a	29.6 ab
100 mg kg^{-1}	Dehydrogenase	30.5 b	28.8 b	26.4 c	43.7 bc	42.2 b	40.8 b	57.4 ab	55.9 b	54.0 b
	Urease	2.33 c	1.63 b	1.30 c	3.63 b	2.40 c	1.83 c	4.06 b	2.80 c	2.20 c
	Phosphatase	11.2 b	10.2 b	9.26 c	21.7 b	20.0 b	17.9 c	30.9 bc	29.1 b	28.1 bc
150 mg kg^{-1}	Dehydrogenase	26.2 c	13.9 c	13.1 d	41.0 c	31.0 c	30.3 c	54.7 bc	45.8 c	44.9 c
	Urease	1.80 d	1.13 bc	0.70 d	2.80 c	1.60 d	1.23 d	3.46 b	1.86 d	1.43 d
	Phosphatase	8.86 c	3.26 c	2.06 d	19.5 b	14.9 c	14.3 d	29.2 c	25.0 c	24.3 d
200 mg kg^{-1}	Dehydrogenase	21.1 d	7.90 d	6.66 e	37.5 d	26.0 d	24.7 d	51.3 c	41.7 d	39.6 d
	Urease	1.50 d	0.66 d	0.20 e	2.13 d	1.00 d	0.70 e	2.80 c	1.16 d	0.96 e
	Phosphatase	6.60 d	1.56 d	0.70 e	17.1 c	12.1 d	11.1 e	26.6 d	23.1 d	18.5 e

Units of enzymes: mg TPF kg^{-1} 24 h^{-1}, mg NH_4-N Kg^{-1} 24 h^{-1} and mg phenol kg^{-1} 24 h^{-1} respectively. Values within columns followed by different letters indicate significant difference between the treatments at $P < 0.05$.

tested soils are given in Table 1.

Incubation experiment

The study comprised of laboratory incubation which was carried out in completely randomized design (CRD) with five treatments in triplicate. The sieved soil (250g) was transferred into 500 ml plastic pots, adjusted to 40 % of soil water holding capacity (WHC) by adding distilled water and then pre-incubated at 25°C for seven days (conditioning period). After conditioning, Cd was applied as $Cd(NO_3)_2$ solution to obtain the concentrations of 0 (Ck), 50, 100, 150 and 200 mg Cd kg^{-1} soil. The soil moisture was kept at the predetermined level throughout the incubation period with deionized water by weighing periodically. Soil samples were collected from each pot after 10, 20 and 30 days of incubation.

Analysis of soil microbial biomass

The chloroform fumigation-extraction method was used to measure soil microbial biomass carbon (C_{mic}). Soil sample equivalent to 10 g (fresh soil) was fumigated for 24h at 25°C with alcohol-free chloroform ($CHCl_3$) in a vacuum desiccator containing soda-lime. The fumigated soil was then transferred into a clean empty desiccator and residual $CHCl_3$ was removed from the fumigated soils by repeated evacuations. The fumigated soil was extracted immediately for 30 minutes by using horizontal shaking at 200 rpm with 50 ml O.5 M K_2SO_4 and filtered through a filter paper (Whatman No. 40). The non fumigated control soil (10g fresh soil) was extracted similarly at the time when fumigation commenced. Total organic carbon in the extracts was measured as CO_2 by infrared adsorption after combustion at 760°C using a Shimadzu automatic TOC analyzer (Shimadzu Corp. Japan). Microbial biomass carbon (C_{mic}) was calculated as (Ct_1- Ct_0) x 2.22, where Ct_1 is the extracted carbon (mg kg^{-1}) from fumigated samples ,Ct_0 is the extracted carbon (mg kg^{-1}) from un-fumigated samples and 2.22 is the factor, calculated by 0.45 i.e. 100/45=2.22, here 0.45 is the extractable part of microbial C after fumigation (Wu et al., 1990). For microbial biomass nitrogen (N_{mic}), total N in the K_2SO_4 extract was measured after Kajeldahl digestion. The soil microbial biomass N was calculated as (Nt_1- Nt_0) x 1.85, where Nt_1 is the extracted nitrogen (mg kg^{-1}) in fumigated samples ,Nt_0 is the nitrogen (mg kg^{-1}) in un-fumigated samples and 1.85 is a factor which is obtained via 0.54 (i.e. 100/54=1.85) which is extractable part of microbial N after fumigation (Brookes et al., 1985). In the case of microbial biomass phosphorus (P_{mic}), the fumigated and the non fumigated soil samples were extracted by 0.5 M $NaHCO_3$ (pH 8.5) for 30 min. The concentrations of P were determined using spectrophotometer at 882 nm wave length. The microbial biomass P was calculated as (Pt_1- Pt_0) x 2.5, where Pt_1 is the phosphorus (mg kg^{-1}) in fumigated samples ,Pt_0 is the phosphorus (mg kg^{-1}) from un-fumigated samples and 2.5 is a factor, computed by 0.4 (e.g. 100/40=2.5), while 0.4 is the extractable part of microbial P after fumigation (Brookes et al., 1985).

Analyses of soil enzymes activity

Dehydrogenase activity was determined by the method of Ohlinge (1996). Fleetingly, 20g air dried soil was mixed with 0.2g of $CaCO_3$ and then 6g of this mixture was placed in three different test tubes. Samples were incubated at 37°C for 24 hours after adding 1ml of 3% aqueous solution of Triphenyl Tetrazolium Chloride (TTC) and 2.5ml of distilled water. Then 10 ml of methanol was added and filtered after shaking. The red color intensity was measured by using a spectrophotometer at a wave length of 546 nm. Soil dehydrogenase activity was expressed as mg TPF (Triphenyl formazan) kg^{-1} dry soil 24 h^{-1}. The method of Kandeler and Gerber (1988) was followed to analyze soil urease activity. In summary, 5g soil was taken into 250ml conical flask and 10ml of urea solution was added along with 20ml buffer solution (citric acid, KOH, NaOH) having pH 6.7. The solution was filtered after incubating at 37°C for 24 hours and then 3ml of filtrate was taken into 50 ml flask. Contents were mixed in the flask after adding 20 ml of water and 4 ml of mixed reagent (Phenol + NaOH) in it. Then 4ml of sodium hypochlorite solution was added, mixed and volume was made up to 50ml with distilled water. The absorbance of blue color was checked at 578 nm through spectrophotometer. Soil urease activity was expressed as mg NH_3-N kg^{-1} dry soil $24h^{-1}$. Soil phosphatase activity was determined following the procedure of Alef and Nannipieri (1995). Briefly, 1g soil mixed with 0.2ml of toluene, 4 ml of MUB (modified universal buffer) of pH 11 plus 1ml of p-nitro phenyl phosphatase solution and then the flask was placed in an incubator at 37°C for an hour. Then 1ml of 0.5 M $CaCl_2$ and 4ml of 0.5 M NaOH was added and soil suspension was filtered through filter paper (whatman No.2). Yellow color intensity was measured at 400 nm wavelength using a spectrophotometer. Soil phosphatase activity was expressed as mg phenol produced kg^{-1} dry soil h^{-1}.

Statistical Analyses

All data were statistically tested by one-way analysis of variance (ANOVA) using the M-STATC 98 statistical package for windows. The least significant difference (LSD) was used to indicate the difference between the treatments at $P < 0.05$.

Conclusion

The application of Cd had a significant negative effect on the size of soil microbial biomass (SMB) and enzymes activity (EA). Higher Cd concentrations decreased the SMB and EA more significantly than the lower concentrations. The soil texture and incubation time are important factors in governing the toxicity of Cd on the soil health and quality indicators. The loam and clay loam soils were more resistive than the sandy loam soil regarding Cd toxicity to C_{mic}, N_{mic}, P_{mic}, dehydrogenase, phosphatase and urease activities. It stresses that such Cd-induced changes caused alteration of biological functions in soil ecosystem and consequently affect agricultural sustainability. The date information generated here could be very useful for scientists, researchers and policy makers in evaluating soil quality and ecosystems sustainability under Cd pollution. The present study depicted the dynamics of soil enzymes only up to 30 days after Cd contamination; further long-term studies involving more enzymes and textures are rudimental in improving our understanding about the toxic behavior of heavy metals.

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