Interactive Impacts of Heavy Metals and Soil Amendments on Enzymatic Activities and Microbial Biomass

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Both organic and inorganic soil additives are frequently used to increase the bioavailability of lead (Pb) and cadmium (Cd) in polluted soils, but these amendments may also affect microbial activity in soils by modifying heavy metal solubility. This research assessed the influence of different soil additives on enzymatic activity and the solubility of Pb and Cd in spiked soils. Soils were spiked with Pb (0, 1000, 1500 mg kg^{-1}) and Cd (0, 100, 150 mg kg^{-1}) artificially. Incubation experiments were carried out with various amendments, such as citric acid (CA; 0, 10 mmol kg^{-1}), ammonium nitrate (AN; 0, 10 mmol kg^{-1}), EDTA (0, 5 mmol kg^{-1}), compost (CO; 0, 10%), and titanium dioxide nanoparticles (TNPs; 0, 100 mg kg^{-1}). The microbial biomass carbon (Cmic) and dehydrogenase activity (DHA) declined by 66% and 47% in Pb_{1500}, and by 54% and 35% in Cd_{150} treatments, respectively. In control soil, compost addition gave the highest value of Cmic and DHA, followed by TNPs, CA, AN, and EDTA. But the mixed application of Pb, Cd, and soil additives caused an overall reduction in microbial activity. Among all the treatments, EDTA alone and in combination with Pb and Cd showed maximum toxicity to soil microorganisms.

Keywords: Lead, Cadmium, Soil Additives, Microbial Biomass, Enzymatic Activity

Introduction:

Cadmium (Cd) and lead (Pb) are highly toxic elements even at trace concentrations, and they play no beneficial role in the growth or developmental processes of living organisms [1][2]. Both metals are non-essential elements; therefore, their presence significantly affects the physiological functioning and growth of living organisms. These metals are naturally present in the environment; however, their concentrations are increasing due to various anthropogenic activities such as ore mining, smelting, the use of lead-based paints, fossil fuel combustion, application of fertilizers and pesticides, irrigation with wastewater, and the disposal of municipal and industrial wastes [3][4][5]. Worldwide, 332 and 7.6 thousand tons per year of Cd and Pb are emitted by anthropogenic sources [6]. Moreover, a study reported that approximately 44% of the total cadmium (Cd) concentration in the environment originates from anthropogenic activities [7]. Globally, approximately 10 million sites have been reported as contaminated, of which more than 50% are polluted with heavy metals [8]. Numerous studies have confirmed that anthropogenic activities are the primary contributors to heavy metal contamination in the environment.

From all the varied natural and anthropogenic sources, these metals eventually find themselves in the soil and impact soil quality, diversity of microorganisms, and microbial community structure [9]. The occurrence of heavy metals interferes with the protein and nucleic acid structure by binding to sulfhydryl groups of proteins and phosphate or hydroxyl groups in nucleic acids, thus disrupting the synthesis and function of proteins and DNA. These molecular interactions result in oxidative stress, lipid peroxidation, and denaturation of cellular components, promoting the inhibition of enzyme function and microbial cell viability [10]. [11] showed that arylsulfatase activity was reduced significantly by 37% and 38%, respectively, in Pb-contaminated (500 mg kg^{-1}) and Cd-contaminated (1 mg kg^{-1}) soil. A 1.2-, 1.7-, and 1.3-fold reduction in microbial biomass carbon (C), dehydrogenase, and alkaline phosphatase activities, respectively, at a lead (Pb) level of 1500 mg kg^{-1} when compared with the control [4]. Cd and Pb along with other essential nutrients are taken up by the plants, accumulate in different compartments [12], affect plants by inhibiting seed germination and reducing plant growth [13], biomass, chlorophyll content, photosynthesis rate, transpiration rate, stomata size and uptake of micro and macro nutrients [14] [15]. The accumulation of these metals in edible crops, vegetables, and fruits affects humans through the food chain.

Various physical, chemical, and biological methods have been adopted for the removal of metal from contaminated soil [2] [16]. Among all remediation techniques, phytoremediation has gained considerable importance. However, the main challenge in remediating metal-contaminated soils through this approach is the limited bioavailability of heavy metals [17]. Therefore, various chelates have been used to increase the bioavailability of Cd and Pb in soil. The application of soil amendments can enhance metal bioavailability; however, they may also influence soil microorganisms either positively or negatively, depending on the type and concentration of the amendments.

Mechanistically, soil additives alter the chemical speciation and bioavailability of metals by chelation, ion exchange, complexation, or redox transformation, which directly affects how metals interact with microbial cell walls and intracellular components [18][19]. Organic chelants like EDTA and citric acid can form stable metal–ligand complexes that mobilize Cd and Pb and, in the process, can increase their uptake by the plant but also short-term increase the metal stress on microorganisms [20]. Compost sources of organic matter, however, immobilize metals by providing binding sites as well as stimulating microbial activity through nutrient enrichment. Inorganic amendments such as ammonium nitrate and titanium dioxide nanoparticles (TiO_2 NPs) can alter soil pH and redox potential, thereby altering microbial enzyme kinetics and impacting processes such as dehydrogenase activity, respiration, and phosphatase activity [21][22].

Soil microorganisms are important in the cycling of nutrients, detoxification of contaminants, and maintenance of soil structure and quality [5]. Soil enzymatic activities such as dehydrogenase, basal respiration, and microbial biomass are highly sensitive to heavy metal pollution [23] and are widely used as indicators of microbial activity and overall soil health [24]. It is crucial to comprehend these mechanistic interactions because the activity of soil amendments has not only additive but also synergistic or antagonistic consequences, depending on how amendments modify metal speciation and microbial metabolic processes. For instance, amendments can decrease metal-induced oxidative stress by stimulating microbial antioxidant activities or increase toxicity by mobilizing soluble fractions of metals [25]. This study is intended to examine the impact of soil additives (organic and inorganic) on the microorganisms of the soil in Cd and Pb-spiked soils. From among the following soil additives, viz., ethylene diamine tetraacetic acid (EDTA; C10H8N2O8), citric acid (CA; C6H8O7), ammonium nitrate (NA; N2H4O3), titanium dioxide nanoparticles (TiO2), and compost, the following were chosen for the present study. Most of the researchers used EDTA and CA for enhancing the phytoextraction of Pb and Cd from soil [5]. Ammonium nitrate and compost are commonly used as nitrogen fertilizers to promote plant growth. Recently, several studies have reported that the application of titanium dioxide nanoparticles (TNPs) enhances the phytoavailable fraction of phosphorus in the soil and increases crop growth, biomass accumulation, and chlorophyll content [26][27]. But limited literature is available showing the effect of CA and EDTA on soil microorganisms and other amendment are not focused. Therefore, the specific objectives were to: a) investigate the comparative effect of soil additives on microbial biomass in spiked soil b) assess the impact of Pb and Cd on soil enzymatic activities in the presence of soil additives.

Material and Methods:

The flow chart diagram of the methodology is presented in Figure 1. Soil samples were collected from the nursery at Malakand, and the samples were air-dried. Afterward, the soil was spiked with a known concentration of heavy metals and left for acclimatization. After the acclimatization period, amendments were added to the samples and incubated in controlled conditions. After the incubation period, the microbial activities were determined through standard procedures. The details methodology is explained in the following sections.

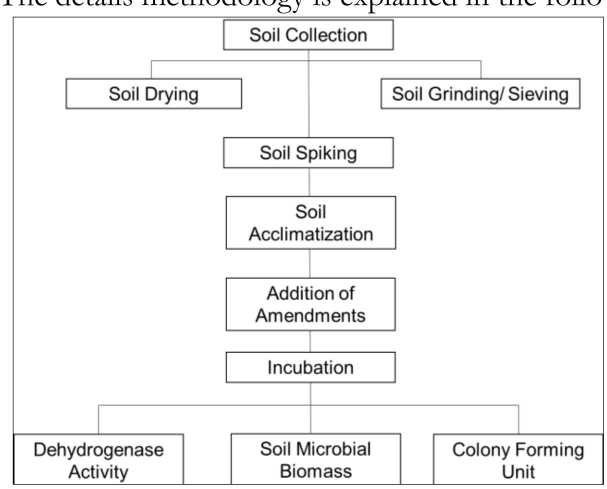

Figure 1. Flow diagram showing the methodology of the study.

Soil Spiking for Incubation Experiment:

Uncontaminated soil with a clay loam texture, pH of 7.40, EC (0.333 mS cm^{-1}), and organic matter (0.40%) was air dried, crushed using a ball mill, and then passed through a 2 mm mesh sieve. The prepared soil was stored in separate containers and subsequently spiked with lead nitrate [$Pb(NO_3)_2$] and cadmium sulfate [$CdSO_4$] to achieve the desired concentrations of lead (1000 and 1500 mg kg^{-1}) and cadmium (100 and 150 mg kg^{-1}). After the addition of heavy metals, the soil was mixed manually for 30 days for the acclimatization of heavy metals. A control soil sample with no added lead or cadmium was also made for comparison. The soil was also shaken regularly to maintain evenness, and after the metals had settled, it was made ready for further experimental procedures.

Incubation Experiment:

An incubation experiment was done to study the impacts of lead, cadmium, and different soil amendments on heavy metal solubility and availability, as well as soil enzymes. For this, jars were loaded with various soils—both spiked and not spiked with metals—and different levels of amendments were applied, such as EDTA (0, 5 mmol kg-1), titanium dioxide nanoparticles (TNPs) (0, 100 mg kg-1), citric acid (0, 10 mmol kg-1), compost (0, 10%), and ammonium nitrate (0, 10 mmol kg-1). A total of 120 treatments were established using a completely randomized block design (CRBD), comprising two levels of lead (Pb), two levels of cadmium (Cd), five types of amendments, two concentrations of each amendment, and three replicates per treatment. The jars were kept at 25°C and maintained at a 60% moisture level for 28 days. Soil samples were collected after 28 days for analysis. Three control groups were also established for comparison: (i) a control without any metals or amendments, (ii) a control without metals but with amendments, and (iii) a control with metals but without amendments.

Heavy Metals Extraction:

Following incubation time, samples of soil were divided into two groups. Half of the samples were oven dried for 24 hours at 65ºC, then the soil was shaken with 0.01 M calcium chloride (CaCl2) at a 1:5 ratio (soil: water; w/v) at 200 rpm for 2 hours in an orbital flask shaker. For the analysis of soluble Pb and Cd, the solution was filtered through Whatman filter paper No. 42 and analyzed using an atomic absorption spectrophotometer (AAS, PerkinElmer 900T) following the method.

Soil Enzymatic Activities:

Soil microbial biomass carbon (Cmic) was determined using the fumigation–extraction method and absorbance at 350 nm with a UV–visible spectrophotometer (SPECORD 200 Plus, Germany). Dehydrogenase activity (DHA) in soil was quantified by reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF), where the intensity of the red product was read at 485 nm on the same spectrophotometer. The bacterial colony-forming units (CFU) were enumerated with the help of the serial dilution method on nutrient agar plates [28].

Statistical Analysis:

All values are presented as the mean of three replicates. Least Significant difference LSD at $P < 0.05$ between treatments was tested by analysis of variance (ANOVA) based on Statistix version 10.0 software.

Results:

Soil Dehydrogenase Activity:

Figure 2 illustrates the effects of lead (Pb) and cadmium (Cd) on soil dehydrogenase activity (DHA) following the application of different soil amendments. DHA decreased significantly by 47% and 35% in soils spiked with 1500 mg kg^{-1} of Pb and 150 mg kg^{-1} of Cd, respectively, compared to the control. Application of soil amendments to lead- and cadmium-spiked soils led to an additional reduction in DHA activity when compared to untreated controls. In lead-spiked (1500 mg kg^{-1}) and cadmium-spiked (150 mg kg^{-1}) soils, DHA activity reduced significantly by 43%, 42%, 52%, 45%, and 62% after the use of compost, citric acid, TNPs, ammonium nitrate, and EDTA, respectively, when compared with the control.

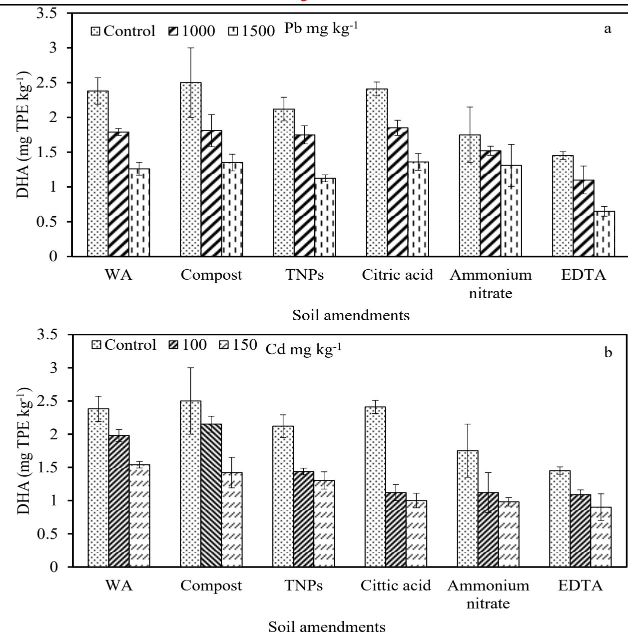

Figure 2. Soil dehydrogenase activity upon amendments application; a = Pb spiked soil; b = Cd spiked soil; WA = without amendments, TNPs = titanium dioxide nanoparticles, EDTA = ethylenediamine tetraacetic acid

Microbial Count:

There was a reduction in the colony-forming units (CFU) of bacteria with the rise in the concentration of lead (0–1500 mg kg^{-1}) and cadmium (0–150 mg kg^{-1}). Addition of compost to the control soil raised CFU 7%, while addition of TNPs, ammonium nitrate, and EDTA decreased CFU 7%, 3.5%, and 25%, respectively (Table 1). When added with heavy metals, CFU levels decreased further. With 1500 mg kg^{-1} Pb and 150 mg kg^{-1} Cd, treatment using TNPs, citric acid, ammonium nitrate, and EDTA inhibited CFU by 32%, 28%, 39%, and 67%, and by 43%, 36%, 50%, and 85%, respectively.

Table 1. Cell enumeration in Pb and Cd-spiked soil upon amendments application

Tereatments	Pb concentration (mg kg^{-1})			Cd concentration (mg kg^{-1})	
	Control	1000	1500	100	150
Without amendment	$2.8x10^5$	$2.0x10^5$	$1.7x10^5$	$1.8x10^5$	$1.6x10^5$
Compost	$2.9x10^5$	$2.4x10^5$	$2.1x10^5$	$2.6x10^5$	$2.3x10^5$
TNPs	$2.6x10^5$	$2.1x10^5$	$1.9x10^5$	$1.8x10^5$	$1.6x10^5$
Citric acid	$3.0x10^5$	$2.5x10^5$	$2.0x10^5$	$2.2x10^5$	$1.8x10^5$
Ammonium nitrate	$2.7x10^5$	$2.1x10^5$	$1.7x10^5$	$1.9x10^5$	$1.4x10^5$
EDTA	$2.1x10^5$	$1.3x10^5$	$0.9x10^5$	$1.0x10^5$	$0.4x10^5$

Pb=lead; Cd=cadmium; TNPs=titanium dioxide nanoparticles; = ethylenediamine tetraacetic acid

Soil Microbial Biomass:

The impact of lead and cadmium on microbial biomass in soil after the application of amendments is shown in Figure 3. Microbial biomass carbon (Cmic) declined with the increasing rates of lead and cadmium when no amendments were applied. With respect to the control (without the addition of lead), Cmic was lowered by 36% and 66% at lead rates of 1000 mg kg^{-1} and 1500 mg kg^{-1}, respectively. In this study, compost, ammonium nitrate, citric acid, and TNPs were added to control soil, and they enhanced Cmic by 58%, 29%, 16%, and 39%, respectively, relative to the un-amended control soil. However, the addition of EDTA reduced Cmic by 27% (Figure 3a). A lower Cmic value was noted in cadmium-spiked soil (Figure 3b). Microbial biomass was reduced considerably by 54% at 150 mg Cd kg^{-1} from the control (no addition of Cd). In this research, the use of compost, TNPs, ammonium nitrate, citric acid, and EDTA at 150 mg Cd kg^{-1} lowered Cmic by 3.1%, 18%, 46%, 38%, and 51%, respectively, relative to soil with 150 mg Cd kg^{-1} alone.

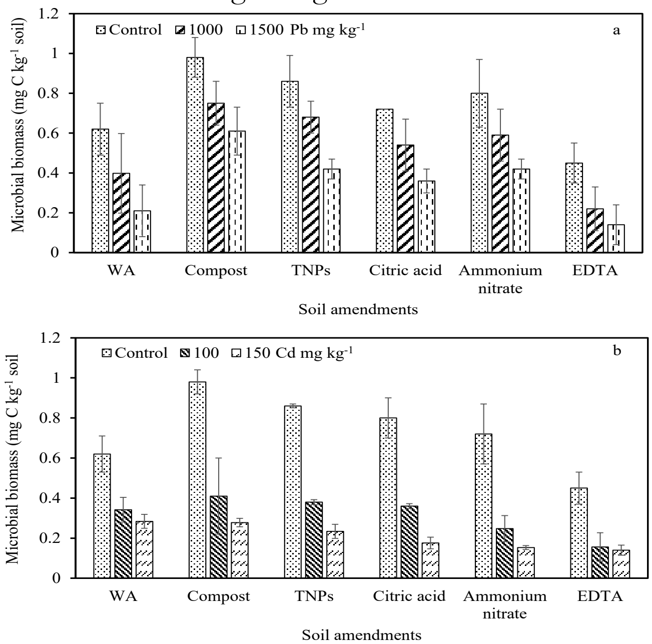

Figure 3. Microbial biomass (C) upon amendments application; a = Pb spiked soil; b = Cd spiked soil; WA = without amendments, TNPs = titanium dioxide nanoparticles, EDTA = ethylenediamine tetraacetic acid

Discussion:

Soil dehydrogenase activity was reduced in the Pb and Cd-spiked soil. With increasing levels of these metals, a reduction in DHA was observed. In a previous study, [9] reported a 2.5-fold decrease in DHA at a lead concentration of 2000 mg kg^{-1} after four weeks of exposure compared to the control. Another report indicated that the maximum DHA occurred in control samples and the minimum in samples with higher cadmium concentrations (200 mg kg-1). The reduction at elevated cadmium concentrations can be attributed to the soil microbial community being affected by the toxic behavior of cadmium, especially in comparison with lower concentrations [23]. The application of soil amendments reduced the soil dehydrogenase activity. The reduction of DHA observed is most probably due to the toxic action of the amendments applied to the microbial populations in the soil.

The CFU was decreased in the heavy metal-spiked soil as compared to the control. However, the application of compost increased the CFU as compared to other amendments. These findings are clear evidence of the sensitivity of microbes in the soil to metal exposure since both Cd and Pb were found to severely inhibit bacterial growth and colony development. The decline in CFU is caused by interference of these metals with microbial nucleic acids and enzymes by binding to phosphate and sulfhydryl groups, resulting in cellular malfunction and death [29][30]. The acute decrease in microbial numbers under EDTA and ammonium nitrate treatments indicates these amendments increased metal mobility and toxicity. EDTA produces soluble metal–chelate complexes that enhance Cd and Pb bioavailability and exacerbate microbial stress [31]. Contrarily, the augmentation of CFU in soils amended with compost indicates the shielding effect of organic matter to maintain energy resources and immobilize toxic metals by complexation [32][33]. Compost improves microbial habitat quality, pH buffers, and provides nutrients so that microbial populations can recover even in the presence of metals. The intermediate response for citric acid and TNPs could be attributed to their dual nature: both can alter metal speciation and microbial metabolism based on concentration and soil chemistry [34][35].

A similar trend was observed for the soil microbial biomass; Cmic was reduced in Pb-spiked soil, and this reduction was accelerated upon application of amendments. This trend reflects a dose-dependent inhibitory influence of heavy metals on soil microbial activity, as [21] have previously reported that Pb exposure decreased microbial biomass and enzyme activities through oxidative stress and denaturation of the enzymes. Cd and Pb can cause changes in membrane permeability and electron transport disruption, resulting in decreased microbial respiration and biomass [36]. In a recent study, Cmic declined progressively with increasing lead concentrations between 500 and 2000 mg kg^{-1} due to the toxic effect of lead on microorganisms in soil. The compost application enhanced the Cmic. The increase in Cmic under the compost, TNP, and ammonium nitrate treatments suggests that a few additives promoted microbial growth without metal stress. Compost possibly supplies available organic carbon and nutrients, enhancing microbial metabolism and biomass production [37]. TNPs possibly enhanced nutrient turnover and root exudation, supporting microbial proliferation indirectly [35]. On the other hand, the reduction in Cmic following EDTA treatment indicates a rise in soluble metal ions through chelation, which harms microbial cells by enhancing the toxic fraction present in the bioavailable form [31].

In lead-spiked soil, the addition of compost enhanced Cmic by 1.9-fold relative to soil treated with lead alone. This elevation can be due to the nutrients and organic matter in the compost that offer a secondary carbon and energy source for microbial development. The addition of TNPs, citric acid, ammonium nitrate, and EDTA also reduced Cmic by 51%, 50%, 46%, and 69% at lead 1500 relative to the control with amendments. These results show that compost alleviates Pb toxicity through immobilization of the metal and modification of soil structure, while EDTA and nitrate treatments exacerbate toxicity through enhanced metal solubility [38][32]. Also reported similar trends, with organic matter additions reversing metal-induced reductions in microbial biomass, while synthetic chelators enhanced them. Soil microbial biomass was reduced in Cd-spiked soil as compared to its respective control. [39] observed a loss of 38% of Cmic at 100 mg Cd kg^{-1}, and this is likely due to the toxic nature of cadmium to soil microorganisms. Microorganisms respond differently to heavy metals depending on their sensitivity levels, and exposure may cause death to microorganisms, thereby decreasing microbial biomass. [39] observed a loss of 38% of Cmic at 100 mg Cd kg^{-1}, and this is likely due to the toxic nature of cadmium to soil microorganisms. Microorganisms respond differently to heavy metals depending on their sensitivity levels, and exposure may cause death to microorganisms, thereby decreasing microbial biomass. The lesser decrease in Cmic in compost-applied Cd soils verifies that organic matter mitigates metal stress through enhanced nutrient cycling and microbial recovery [37]. In contrast, EDTA and citric acid, by creating soluble Cd complexes, enhance the bioavailability and microbial uptake of metals, thus inhibiting microbial metabolism [34].

TNPs and ammonium nitrate effects were intermediate, proposing concentration-responsiveness—moderate concentrations might promote microbial growth, but excessive amounts probably produce reactive oxygen species or induce ionic stress [35][40]. Generally, the interaction between amendments and metals controls whether microbial processes are stimulated or repressed, where balanced amendment application in metal-contaminated soil is necessary.

Conclusions:

For the removal of heavy metals from soil, amendments play a crucial role. But it is important to understand the impacts of amendments on the soil microorganisms. The study found that the toxicity of Pb and Cd harmed soil microbial biomass and dehydrogenase activity. CFU also decreased with an increase in the concentration of both heavy metals. Use of EDTA individually and in combination with Pb and Cd was found to be more toxic than other amendments being tested. Maximum Cmic, DHA, and CFU were seen in compost-amended soil, followed by TNPs, citric acid, ammonium nitrate, and EDTA. From this study, it is concluded that compost is beneficial for the soil microorganisms, and EDTA is more toxic. Before recommending for the field, it is necessary to perform a detailed study on the soil microorganisms and health.

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