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ORIGINAL RESEARCH ARTICLE

Effect of Bentonite amendment on Lead and Cadmium accumulation and plant 2 growth indicators

Fatimah Marzoq Hashim*, Luma Abdalalah Sagban Alabadi

Department of Soil Science and Water, College of Agriculture, University of Al-Qadisiyah, 58001, Iraq

***Corresponding author:** Fatimah Marzoq Hashim, agr23.mas04@qu.edu.iq

ABSTRACT

The objective of this study was to determine the effect of bentonite on lead (Pb) and cadmium (Cd) accumulation in Indian mustard (Brassica juncea), as well as to evaluate the growth parameters of plants grown in soil contaminated with different levels of Pb and Cd. In this study, a pot experiment was conducted using a (3 × 3) factorial arrangement consisting of three levels of lead (10, 100, and 200 mg kg^{-1}), three levels of cadmium (5, 15, and 25 mg kg^{-1}), two bentonite application rates (25% and 50%), and an untreated control. The results showed significant differences ($P \leq 0.05$) in metal concentrations in roots and leaves, as well as in growth parameters such as fresh weight and plant height. A progressive increase in Pb and Cd concentrations was observed in both roots and leaves with increasing contamination levels. In contrast, the application of bentonite at 50% significantly decreased metal concentrations; Pb concentration in roots decreased from 75.03 to 41.20 mg kg^{-1}, while Cd concentration at the highest contamination level decreased from 0.29 to 0.19 mg kg^{-1}, respectively. The results also indicated significant reductions in shoot and root biomass under high Pb and Cd concentrations. In the control treatment, fresh shoot weight was 204.67 g, which decreased by 62.53% under 200 mg Pb kg^{-1} + 25% bentonite and by 61.56% under 25 mg Cd kg^{-1} + 25% bentonite. Fresh root weight followed a similar trend, decreasing to 43.67 g and 46.00 g, respectively, under the same treatments. However, bentonite application at 50% improved all growth attributes compared with the control, particularly at lower contamination levels, where the highest fresh shoot weights (225.33 g and 219.00 g) were recorded under 10 mg Pb kg^{-1} + 50% bentonite and 5 mg Cd kg^{-1} + 50% bentonite, respectively. The results also demonstrated that cadmium was more toxic than lead; however, bentonite reduced the bioavailability of heavy metals and improved soil properties, which consequently enhanced plant growth. At high Pb and Cd contamination levels, Indian mustard tended toward phytostabilization behavior for Pb and phytoextraction behavior for Cd, as the translocation factor (TF ≈ 1) values were higher than the plant accumulation indices. Overall, the findings confirm that bentonite is an effective soil amendment for heavy metal-contaminated soils under varying environmental behavior of each element.

Keywords: bentonite; lead (Pb); cadmium (Cd); heavy metal contamination; Brassica juncea

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1. Introduction

Soil heavy metal contamination, which is among the most severe environmental challenges of modern agricultural systems; Due to industrial development, the increasing usage of fertilizers and pesticides as well as releasing industrial wastes and contaminated irrigation water prompted this problem of accumulating toxic elements (Pb) and cadmium (Cd) in agricultural fields. These elements are either not biodegradable or can be retained in soils for extended periods of time, thus increasing the probability that they will be translocated to plants and subsequently enter the food chain, which threat human health and animal well-being as well as ecosystem stability[1].

According to Liu et al.,[2] and Park et al.,[3] Through a different chemical and physical properties, however, lead and cadmium has two quite opposite behaviors in the soil-plant system. Because of a reaction mechanism in which lead tends to adsorb onto clay minerals and iron and manganese oxides; its stable complex with organic matter makes it less mobile in the soil solution, making it retained probably within the root zone. By comparison, cadmium can be more-soluble and mobile in soils -- that is, it has the ability to move with soil solutions and then bioaccumulate in plant tissues therefore considered as a more hazardous environmental contaminant. Accumulation of these components within plants cause several physiological disorders, such as inhibition of cell division, reduction in photosynthetic activity, disruption of nutrient uptake, stimulation of reactive oxygen species (ROS) formation which generates oxidative stress and ultimately impair plant growth and productivity[4,5]. Additionally, ionic interference among $Pb^{2+}$and Cd^{2} with essential nutrients including iron (Fe) may trigger nutrient antagonism, consequently decreasing the micronutrient concentrations in plant 65 tissue[6].

Accordingly, for the management of heavy metal-contaminated soils, use of soil amendments has been identified as a potential and effective approach. Many of these amendments immobilize toxic elements in soil, reducing their availability and uptake by plants without removing them from the soil[7,8]. Bentonite is one of the most important clay amendments due to its high cation-exchange capacity (CEC), large specific surface area, and plentiful negative charges on its surfaces that impart a high capacity for adsorption and immobilization of divalent cation contaminants such as Pb^{2+} and Cd^{2+}[9,10]. Bentonite application has been shown to efficiently lower heavy metal concentrations in plant tissues[11,12] and is reported to enhance soil physical properties such as porosity, water-holding capacity, and moisture retention[8], which means there would be a better behaviour of the root zone environment for plant growth. However, this may not hold true for all types of metals or their activity and it is said that higher mobility [for e.g. cadmium] compared to lower mobility elements (for e.g. lead 82[13]) will result in more effective bentonite usage.

Indian mustard (Brassica juncea) is well known for its relative resistance to heavy metals and frequently used in studies examining phytostabilization and phytoextraction pathways, which is supported by the potential uptake of specific elements with differential root accumulation or translocation to aerial tissue[14,15]. In this regard, the evaluation of these plants in different doses of lead and cadmium associated with various rates of bentonite application is a relevant approach, since this complex soil-plant interaction imposes an important constraint on what we understand about heavy metals.

Thus, this work sought to analyze the effects of levels of lead and cadmium associated with two doses of bentonite in metal accumulation for roots and leaves as well as their influence on growth indicators and contamination indices. Its main goal was to evaluate the effectiveness of bentonite in controlling environmental hazards as well as plant performance at heavy metal- contaminated soils.

2. Materials and Methods

A pot experiment was conducted to study the effect of different doses of lead (Pb) and cadmium (Cd), as well as two levels of bentonite application, on heavy metal accumulation and growth parameters of Indian mustard (Brassica juncea). Surface soil (0-30 cm) was sampled from an apparently uncontaminated agricultural location. The soils were air dried, ground and passed through a 2 mm sieve before initial physical and chemical analyses were performed 104 according to standard soil analysis methods[16,17]. High-purity salts (lead nitrate, $Pb(NO_3)_2$ and cadmium chloride or cadmium nitrate $CdCl_2$ or $Cd(NO_3)_2$ were used to jointly fortify the soils for these contamination treatments separately up to levels of 10, 100 and 200 mg Pb kg−1 soil, and 5, 15 and 25 mg Cd kg−1 soil. Soil was well mixed to ensure homogeneity, moistened, and then incubated for a time period until chemical equilibration between the spiked metals and soil components. It is a prerequisite which allows metal stabilization in the soil matrix before planting[18,19].

Due to its high cation exchange capacity and large specific surface area, which enhances the ability of Bentonite to adsorb and immobilize divalent cations such as $Pb^{2+}$and Cd^{2} within soil matrix[10,20], it was used at two application rates: (25%, w/w) and (50%, w/w) in each pot. Bentonite was applied before sowing and well-mixed with the soil. The experiment was arranged factorially in a Completely Randomized Design (CRD) with the appropriate number of replicates per treatment to assess the effects of two levels of bentonite and three levels for each heavy metal alongside a control treatment. Seeds of Indian mustard (Brassica juncea), which has been commonly used for studies on phytostabilization and phytoextraction due to its tolerance to heavy metals[14,21], were sown. Plants were irrigated with magnetic water and cultivated at around the field capacity of soil throughout grow season. Plant height was examined as a sensitive morphophysiological indicator reflecting the plant processes during physiological growth[7,22], measured at the end of the experiment. The measurement of fresh shoot weight was also performed immediately after harvest on a sensitive scale. Roots and leaves were taken from each plot, washed with distilled water, dried at 70°C in a forced ventilation oven until constant dry weight was achieved before being ground prior to the analyses.

The wet digestion method involving nitric acid or a suitable acid mixture[23] was used for digestions of plant samples prior to the determination of trace metals in plant tissues. The Pb, Cd and Fe concentrations were measured using Atomic Absorption Spectrophotometry (AAS) following standard 136 protocols[24].

Indices of plant contamination were computed employing proper equations. Bioconcentration Factor (BCF) was calculated for roots or whole plant over the soil metal concentration. The Bioaccumulation Coefficient (BAC) was 140 calculated as the ratio of metal concentration in the shoot to its soil concentration, and the Translocation Factor (TF) was calculated as the ratio of metal concentrations in shoot to roots [8,14,25].

Statistical analyses were conducted using analysis of variance (ANOVA) and significant differences between treatments were determined at a probability level of ($P \leqslant 0.05$). The Least Significant Difference (LSD_0. Mean comparison was done using the (one way ANOVA) test of Steel and Torrie[26].

3. Results and Discussion

3.1. Bentonite Impact on Lead and Cadmium Accumulation in Roots

The results reported in (**Table 1**) indicate that differences of the accumulation concentrations of lead (Pb) and cadmium (Cd) in roots were significant at the probability level of $P \leqslant 0.05$ between different treatments. Pb: LSD = 2.779; Cd: LSD = 0.02842 and both uptake and accumulation of the elements were influenced by metal contamination levels and bentonite application rates. The Pb contents in root increased with levels of contamination in soils contaminated with Pb. The highest concentration of Pb in roots (75.03 mg kg^{-1}), was recorded from treatment (X5) which represent the maximum level of contamination. This is likely due to the increased metal load in the soil (36 g kg159 1) and the high concentration of Pb^{2+} ions in the soil solution, increasing their mobility towards the root zone and uptake through cell walls and intercellular spaces, especially when adsorption sites and fixation become saturated. These results are in agreement with data from[11,27], who suggested that raising the concentration of Pb within soil enchances root accumulation because of larger concentration gradients formed between the soil solution and root tissues. But, doubling bentonite application rate from 25% to 50%, drastically decreased Pb level in roots of all contamination levels. At the top level of contamination, 168 Pb decreased from 75.03 mg kg^{-1} in treatment (X5) to 41.20 mg kg^{-1} in treatment (X6), indicating the high efficiency of earlier bentonite on the immobilization and reduction of availabilities of Pb. The decrease correlates with the adsorption of the Pb^{2+} ions in bentonite with a net negative surface and increased rate of cation exchange and physical capture phenomena. Similar results were observed by Ahmed[9] and Liu[2] a formula developed by Shaheen[20] and studies conducted by Zulqurnain Haider[28] and Fernández-Calviño[29] reported that the application of amendments based on clay with reduced activity to limit Pb accumulation in roots and transfer Pb into the food chain.

Table 1 showed a similar pattern in root Cd accumulation as well, increasing with increasing soil contamination for the Cd-contaminated soils. The soil 180 containing treatment (C5) with inclusion of 25 mg Cd kg^{-1} soil enriched with 25% bentonite produced the maximum root concentration of Cd at 0.29 mg kg^{-1}. This can be accounted for by the relatively larger amount of Cd^{2+} ions in the soil 183 solution and their higher mobility, which enhances diffusion/adsorption towards root zone and subsequent uptake when fixation sites around roots are filled due to natural saturation compared to Pb. This behavior is consistent with that of Özkutlu et al.[30], who observed a positive correlation between root accumulation and soil Cd contamination.

In contrast, the application of up 50% bentonite reduced Cd concentrations in roots from all contamination levels. The Cd content was reduced from 0.29 mg kg^{-1} (treatment (C5)) to 0.19 mg kg^{-1} (treatment (C6)). The decrease is associated with the high cation exchange capacity of bentonite and its large specific surface area, as well as an abundance of negative charges that increase adsorption and immobilization of Cd 2+ ions on exchange sites to minimize mobility and plant uptake [31]. The interpretation is in accordance with Boostani et al.[12] and Hassan et al.[32].

The relative values of mobility between both elements (Pb and Cd) suggested Pb was more readily translocated to roots at greater concentrations than Cd and had lower mobility in soil as assessed by immobilization process. On the other hand, Cd did not reset as much and was more responsive to soil conditions. Results also supported the efficient role of bentonite in diminishing accumulation of both elements in roots with more efficiency for Pb immobilization over that of Cd.

Table 1. Concentrations of lead (Pb) and cadmium (Cd) in the root system.

Treatments	Lead concentration in roots	Treatments	Cadmium concentration in roots
0Pb + 0 Bentonite = X0	13.97	0Cd + 0 Bentonite = C0	0.12
10Pb + 25 Bentonite = X1	17.40	5Cd + 25 Bentonite = C1	0.18
10Pb + 50 Bentonite = X2	14.20	5Cd + 50 Bentonite = C2	0.13
100Pb + 25 Bentonite =X3	42.60	15Cd+25 Bentonite = C3	0.24
100Pb + 50 Bentonite = X4	32.17	15Cd +50Bentonite = C4	0.18
200Pb + 25 Bentonite = X5	75.03	25Cd+ 25Bentonite = C5	0.29
200Pb + 50 Bentonite = X6	41.20	25Cd+ 50Bentonite = C6	0.19
L.S.D 0.05	2.779	L.S.D 0.05	0.02842

3.2. Effect of Bentonite on Lead and Cadmium Accumulation in Shoots (Leaves)

According to variance analysis, part b of **Table 2**, there was a significant difference at the probability level ($P \leqslant 0.05$) in concentrations of lead (Pb) and cadmium (Cd) accumulated in leaves among various treatments for samples cultivated at both dry and wet conditions. Metal uptake in shoots was influenced by the soil contamination level as well as the bentonite application rate with the least significant difference values of (LSD=4.052) for Pb and (LSD=0.002777) for Cd, respectivelyThe use of 20 t ha−1 bentonite significantly lowered shoot to root translocation factors (TRf), representing effective immobilization in roots, whereas higher rates evoked stress responses leading to metal relocation to aerial tissue with greater TRf values than control plants.The findings indicated that Pb content in the leaves increased with increasing contamination level of soil. The Treatment (X5) with 200 mg Pb kg^{-1} soil containing 25% bentonite has the highest Pb Concentration in leaves (75.40 mg 218 kg^{-1}). This increase is mainly due to the more significant availability of Pb in the rhizosphere zone that can be translocated toward aerial tissues once it exceeds root retention and immobilization capacity. Higher Pb concentration in soil also improves the phytoavailability of this metal which increases its transport from roots to shoots, facilitating toxic effects on plant tissue[33]. Such finding is

in line with Xu et al.[27], who indicated that increasing Pb contamination increases its ability to translocate from roots to shoots.

On the other hand, raising bentonite application rate from 25% to 50% decreased Pb concentration in leaves at all contamination levels drastically. It suggests that efficiency of bentonite in restricting Pb uptake and/or inhibiting internal translocation of Pb in the plant. This effect confirmed that bentonite can immobilize Pb^{2+}ions in soil through adsorption and cation exchange mechanisms, which reduces the concentration of Pb in the soil solution and limits accumulation into roots followed by translocation to leaves. Moreover, the high CEC and cation exchange ability of bentonite increased soil physical properties that could decrease root stress and limit Pb translocation to aerial parts. Such findings are concurrent to that of Zulqurnain Haider[28] and Wang[11]. Pb accumulation in leaves was lowest (10.40 mg kg^{-1}) for treatments at 10 mg Pb kg^{-1} with 50% bentonite, treatment (X2) as compared to the other levels of bentonite which means that bentonite has a great efficiency in reducing the toxicity of Pb on plants especially under low contamination conditions.

The results of cadmium accumulation were consistent with the observations for Pb, showing a trend toward higher leaf concentrations with greater soil contamination. The treatment that gave the highest concentration of a cadmium 243 in leaves (0.29 mg kg^{-1}), and was recorded in treatment C5, which had 25 mg Cd kg^{-1} soil with 25% bentonite. The accumulation of Cd^{2+} in shoots has been ascribed to availability of these ions (through increased solubility) in root zone and their greater mobility (translocation from roots to aerial parts). This behavior indicates that although plants respond to the accumulated metals, Cd is more mobile in plant tissues than Pb due to its higher solubility and lower binding potential with root constituents. This interpretation is in line with Liu et al.[34] and Park et al.[3].

On the other hand, with 50% bentonite it reported a decrease in Cd concentration leaf C5 (0.29 mg kg^{-1}) and C6 (0.21 mg kg^{-1}). This decrease is attributed to the capacity of bentonite particles to immobilize Cd^{2+}ions in soil by surface adsorption processes and cation exchange processes, thus decreasing solid-phase Cd concentration in soil solution and inhibiting root uptake and translocation of Cd from roots to leaves. In addition, the soil physical property improvements may have reduced Cd availability in the plant system. These findings are in line with Bocharnikova et al.[35], El-Naggar et al.[10], Boostani et al.[36], and Hassan et al.[32] all reported that bentonite can 260 reduce the accumulation of Cd in aerial parts of plants.

In general, Pb accumulation in leaves was higher than that of Cd; however, Cd showed greater mobility and easier translocation within the plant which makes it more sensitive to soil conditions. The results also verified that the most effective application rate of bentonite is within the 50% application level, which reduced Pb and Cd translocation to leaves and mitigated their environmental hazards.

Table 2. Concentrations of lead (Pb) and cadmium (Cd) in the leaves.

Treatments	Lead concentrate ion in leaves	Treatments	Lead concentration in leaves
0Pb + 0 Bentonite = X0	10.36	0Cd + 0 Bentonite = C0	0.14
10Pb + 25 Bentonite = X1	14.73	5Cd + 25 Bentonite = C1	0.18
10Pb + 50 Bentonite = X2	10.40	5Cd + 50 Bentonite = C2	0.13
100Pb + 25 Bentonite = X3	36.70	15Cd + 25 Bentonite = C3	0.22
100Pb + 50 Bentonite = X4	30.63	15Cd + 50 Bentonite = C4	0.18
200Pb + 25 Bentonite = X5	75.40	25Cd + 25 Bentonite = C5	0.29
200Pb + 50 Bentonite = X6	62.03	25Cd + 50 Bentonite = C6	0.21
L.S.D 0.05	4.052	L.S.D 0.05	0.002777

3.3. Effect of Lead and Cadmium Levels and Bentonite Rates on Fresh Shoot Weight

As shown in **Table 3**, fresh shoot weight was significantly affected by both contamination levels of lead (Pb) and cadmium (Cd) and bentonite application rates at the probability level, $P \leqslant 0.05$. The Least Significant Difference values (LSD = 2.511) for Pb treatments and (LSD = 2.445) for Cd treatments suggested that vegetative growth was sensitive to metal load and soil environment around root system, respectively.

As Pb and Cd concentrations increased in the soil, a corresponding clear and significant decline of fresh shoot weight was observed. Between the control treatment and 200 mg Pb kg^{-1} + 25% bentonite, together with 25 mg Cd kg^{-1} + 25% bentonite decreased the weight from 204.67 g at the control treatment to 76.67 g, and 79.00 g respectively (**Table 1**). This decrease is likely due to the physiological toxicity of both metals, compared with Pb and Cd hindering cell division, photosynthetic activity and cellular respiration also alters nutrient balance in the plant. They also promote the generation of reactive oxygen species (ROS), which induces oxidative stress that inhibits vegetative tissue growth and biomass accumulation[33,37].

In contrast, million increased the bentonite application rate from 25% to 50%, fresh shoot weight was significantly higher at all levels of contamination for both elements. In the Pb treatment (200 mg Pb kg^{-1} + 50% bentonite) the weight increased to 88.00 g and in the Cd treatment (25 mg Cd kg^{-1} + 50% bentonite) to 89.33 g respectively at day13 post-treatment. The highest values were obtained with lower contamination levels, recorded as 225.33 g in the Pb treatment (10 mg Pb kg^{-1} + 50% bentonite) and 219.00 g in the Cd treatment (5 mg Cd kg^{-1} + 50% bentonite), of which the control treatment was significantly exceeded by these treatments.

Bentonite was found to play an effective role in immobilize the Pb^{2+} and Cd^{2+} ions but with a combined effect of inhibited bioavailability (low dissolution) which has decreased in soil solution as indicated by comparison between treatments through adsorption and cation exchange mechanisms. Compared with untreated control soil, addition of bentonite ameliorated the physical and water properties including increased porosity, improved water-holding capacity and improved moisture retention in soil that provides a better root environment for vegetative growth, as well as reduces metal-induced stress[11,32].

Furthermore, the data showed that at high contamination levels cadmium was more toxic than lead as seen from greater reduction in fresh shoot weight. The overall higher mobility of Cd in soil solution and its moving easily within the plants compared to Pb that is generally more strongly immobilized in the soil, should account for such findings. However, bentonite application displayed an obvious ability to reduce the toxicity of both heavy metals, and thus we can conclude that it is a good soil conditioner which decreased Pb and Cd environmental risks by improving plant growth.

Table 3. Concentrations of lead (Pb) and cadmium (Cd) in fresh shoot biomass.

Treatments	Fresh shoot weight	Treatments	Fresh shoot weight
0Pb + 0 Bentonite = X0	204.67	0Cd + 0 Bentonite = C0	204.67
10Pb + 25 Bentonite = X1	213.67	5Cd +25 Bentonite =C1	208.67
10Pb + 50 Bentonite = X2	225.33	5Cd+50 Bentonite =C2	219.00
100Pb + 25 Bentonite = X3	80.67	15Cd+ 25 Bentonite = C3	85.33
100Pb + 50 Bentonite = X4	93.67	15Cd + 50 Bentonite = C4	97.67
200Pb + 25 Bentonite = X5	76.67	25Cd + 25 Bentonite = C5	79.00
200Pb + 50 Bentonite = X6	88.00	25Cd + 50 Bentonite = C6	89.33
L.S.D 0.05	2.511	L.S.D 0.05	2.445

3.4. Effect of Lead and Cadmium Levels and Bentonite Rates on Dry Shoot Weight

The analysis of variance results for the dry shoot weight are indicated in **Table 4** where significant difference in this trait among treatments at probability level of 0.05 was shown. The Least Significant Difference results obtained were (LSD = 1.537) for Pb treatments and (LSD = 1.027) for Cd treatments which indicated strong influence of both heavy metals and bentonite application rates on plant growth.

A decrease of dry weight with various concentrations of heavy metals was observed. When compared to control treatment, the dry weight was reduced from 43.00 g (control) to 32.00 g at 200 mg Pb kg^{-1} with 25% bentonite and from 38.66 g(control) to 33.33 g at 25 mg Cd kg^{-1} with Weight, followed by bentonite mixture containing (plant materials). That decrease is a reflection of the toxic effects of these two metals. Due to inhibition in photosynthesis, impairment of enzymatic actions and interference with nutrient uptake, these metals cause disruption within the physiological processes in plant. Moreover, they promotes the production of ROS; consequently induce oxidative stress and cell membrane damage which in turn leads to a decline in dry matter accumulation[38,39].

Moreover, the results suggest that cadmium exhibit more toxic effects than lead even at lower doses. It has higher bioavailability and can move more into the plant, while lead is more strongly co-optimized in the soil and accumulates especially in roots[33,40].

On the other hand, for all the levels of contamination the shoot dry weight was enhanced by mixing bentonite specifically at 50% level. At 5 mg Cd kg^{-1} and at the treatment of 10 mg Pb kg^{-1}, the dry weight increased significantly over the control, reaching a weight of 49.00 g (control: 45.23 g) and 48.33 g (control: 41.37), respectively This reduction suggests that bentonite effectively reduces the toxicity of heavy metals.

This effect can be attributed to the colloidal characteristics of bentonite, particularly its high cation exchange capacity and a large surface area that allows it to immobilize heavy metal ions (such as Pb^{2+} and Cd^{2+}) by adsorption mechanisms and via cation exchange. Which decreases their bioavailability and uptake in plants. Bentonite also enhances physical properties of soil, thereby enhancing water-holding capacity as well as soil structure and subsequently increases water and nutrient uptake for dry matter accumulation[32,41]. In addition, clay minerals like bentonite decrease heavy metal bioavailability and enhance plant growth and functionality, which is especially advantageous in polluted soils[8,30].

Table 4. Concentrations of lead (Pb) and cadmium (Cd) in dry shoot biomass.

Treatments	Dry root weight	Treatments	Dry root weight
0Pb + 0 Bentonite = X0	43.00	0Cd + 0 Bentonite = C0	43.00
10Pb + 25 Bentonite = X1	47.00	5Cd + 25 Bentonite = C1	45.33
10Pb + 50 Bentonite = X2	49.00	5Cd + 50 Bentonite = C2	48.33
100Pb + 25 Bentonite = X3	35.33	15Cd + 25 Bentonite = C3	36.00
100Pb + 50 Bentonite = X4	40.00	15Cd + 50 Bentonite = C4	42.00
200Pb + 25 Bentonite = X5	32.00	25Cd + 25 Bentonite = C5	33.33
200Pb + 50 Bentonite = X6	38.33	25Cd + 50 Bentonite = C6	39.67
L.S.D 0.05	1.537	L.S.D 0.05	1.027

3.5. Effect of Lead and Cadmium Levels and Bentonite Rates on Fresh Root Weight

The fresh root weight of the different treatments varied significantly at 0.05 probability level (**Table 3**). The values observed were for Pb (LSD = 1.175) and for Cd (LSD =0.6138). Results showed that root fresh weight was the most sensitive indicator of water relations responding to changing conditions relative to other growth parameters.

The increase of fresh root weight obviously occurred in treatments with a low concentration of heavy metals combined with bentonite addition. The weight increased from 69.00 g for control treatments (X0 and C0) to 78.67 g in treatment (X2) with 10 mg Pb kg^{-1} + 50% bentonite, and to 77.00 g in treatment (C2) with 5 mg Cd kg^{-1} + 50% bentonite. It meant that large soil percent of bentonite improve root efficiency in retention water and increase percentage of physiological activity under low level of contamination.

On the other hand, fresh root weight decreased significantly with increasing concentrations of Pb and Cd. Contents of the heavy metal Pb decreased at 100 and 200 mg Pb kg^{-1} with the addition of 25% bentonite in treatments (X3) to reach a weight of only 49.00 g and treatment (X5), which had a lower weight of 43.67 g, respectively. In Cd treatments, moreover, the weight dropped in treatment (C3) and (C5) at 15 and 25 mg Cd kg^{-1} with 25% bentonite to 51.33 g and 46.00 g respectively. This decrease is congruent to the toxicity of both elements.

Heavy Metals Disturb the Root Water Relations, Leading to The Reduced Fresh Weight of Roots Among Plants. Further, these metals also inhibit membrane channel function and cause cell damage in roots tissues; this leads to less water absorption by plant that then results in decreased fresh root mass[5,42]. Among the metals, radium reached effects higher than lead at lower concentration owing to its more rapid diffusion into root cells and consequent severe oxidative stress followed by impairment of water metabolism[39].

Nonetheless, using 50% of the bentonite at the same high contamination levels when compared with 25% has significantly increased fresh root weight. Root weight increased from 49.00 to 61.33 g (treatment X4) at 100 mg Pb kg^{-1} and from 43.67 to 56.00 g (treatment X6) at a higher concentration of Pb, respectively in Pb treatments. The weight was also elevated in Cd treatments with an increase from (51.33 to 67.67g) at treatment (C4) with dose 15 mg Cd kg^{-1} and (46.00 to 59.67 g) at treatment (C6) with dose 25 mg Cd kg^{-1} respectively, as well for data showing the average body dry weight comparison before treatment of families used, significant difference show by different capital letters and p-values <0.05 shown on lower superscripted letter (BCD), indicating statistical significance if differences exist. The results confirm that bentonite alleviates stress in roots caused by metals.

The reduced bioavailability of Pb^{2+} and Cd^{2+} ions is due to the unique layered structure of bentonite, which can adsorb and exchange cations through its layers. Furthermore, bentonite enhances physical characteristics of soil such as raising field capacity (increase in water retention) and redistributing pores leading to greater efficiency of water uptake– increased moisture fractions at roots,[10,37] (Fig. Furthermore,[32,43]. However, under heavy metal stress conditions, the addition of bentonite or expandable clay minerals reduced cellular water loss and improved plant water relations.

C2 recorded 78.67 g and C1 with (77.00 g) response was nevertheless higher than the control treatmentOverall, treatments (X2) and (C2), compared to the control intact further record were verified as best reponse. Indicates that high levels of bentonite not only alleviate toxicity to metals, but also improve the root environment by maintaining an optimal water balance and preventing excessive loss of water due to increased absorption efficiency. These results are in accordance with Rizwan et al. Rizwan et al.[44] and Wang et al.[11], which reported that the immobilization of heavy metals in soil enhances plant water relations and improve fresh biomass, especially when amending soil with high cation exchange capacity clay minerals.

Table 5. Concentrations of lead (Pb) and cadmium (Cd) in fresh root biomass.

Treatments	wet root weight	Treatments	wet root weight
0Pb + 0 Bentonite = X0	69.00	0Cd + 0 Bentonite = C0	69.00
10Pb + 25 Bentonite = X1	76.33	5Cd + 25 Bentonite = C1	74.67
10Pb + 50 Bentonite = X2	78.67	5Cd +50 Bentonite = C2	77.00

100Pb + 25 Bentonite = X3	49.00	15Cd + 25 Bentonite =C3	51.33
100Pb + 50 Bentonite = X4	61.33	15Cd + 50 Bentonite = C4	67.67
200Pb + 25 Bentonite = X5	43.67	25Cd + 25 Bentonite = C5	46.00
200Pb + 50 Bentonite = X6	56.00	25Cd + 50 Bentonite = C6	59.67
L.S.D 0.05	1.175	L.S.D 0.05	0.6138

Table 5. (*Continued*)

Results in **Table 6** comparing the dry root weight among different treatments at 0.05 probability level, significant differences between each treatment can be observed. Where, the Least Significant Difference values were (LSD = 0.1979) for Pb and (LSD = 0.1743) for Cd. The data show that root responses were more responsive to environmental changes than the shoot system.

At lower contamination levels, application of bentonite resulted in improving dry root weight. The maximum value of dry weight 15.83 g, at 10 mg Pb kg^{-1} and 50% bentonite (X2), was greater than the treatment X0 values of 13.77 g. Treatment (C2) at 5 mg Cd kg^{-1} with 50% bentonite accumulated similarly to that of treatment (C1) recording 15.57 g. Moreover, this results indicate that high amount of bentonite not only decreases toxicity but also improves the growing environment for the roots and supports them grow better since they are already most efficient in absorbing water and nutrients. Which is related to same soil porosity and water holding capacity and is increasing CEC that improves root growth with dry matter gathering[32,45].

However, levels of Pb and Cd contamination showed contrasting trends with dry roots weight decreasing markedly on increasing levels. In treatment (X3), relative to the weight in control, it decreased under 100 and 200 mg Pb kg^{-1} with 25% bentonite, where they were corresponding decreased dropped down to 11.27 g and an approximate of 10.80 g drop more in treatment (X5). Likewise, Cd treated introduced decreases in weight in treatment (C3) to 1146 g and 1097 g(C5) at 15 and 25 mg Cd kg^{-1} with a proportion of bentonite of 25%. These differences were higher than the LSD values and thus, statistically different.

This decrease is due to the direct toxic action of heavy metals on roots, which inhibits the division of mitotic cells in the root meristem zone or also reduces the formation and elongation of root hairs (Oh et al. An indirect effect of salinity occurs when soil solution salinity affects the physical and chemical properties of soils, impairing water and nutrients extraction thereby decreasing root growth and dry matter production[5,39]. Results also illustrated that although cadmium was considerably more toxic than lead, this difference was especially marked at lower concentrations because cadmium is much more bioavailable and enters root cells rapidly through calcium channels[40], causing serious disturbance in metabolism compared to the relatively immobilized form of lead confirmed in soil respiratory rates.

In addition, dry root weight was increased significantly at the high contamination levels with 50% bentonite rate more than the 25% one. Dry root weight had increased from 11.27 to 12.83 g at Pb of 100 mg Pb kg^{-1} and; on the other hand; decreased from 10.80 to 11.73 g at Pb of200mgPb/kg^{-1} after treatment with oxalate-further indicating stabilization effect in case of issued metal ion (Trivalent ions like Fe) use in cleaning treatment or it is better words known as biotreatment (Pb). In a comparable trend, it increased from 11.43 and 10.97 g at 15 and 25 mg Cd kg^{-1} respectively in the treatments of Cd to 13.10 (at 15mg Cd kg-1) and 12.20 (at risking stager of) g respectively These findings indicate that bentonite is successful in reducing metal-induced stress.

Apart from lowering solubility of heavy metals but also transform heavy metals into less bioavailable forms, the immobilization mechanism of bentonite is due to its layered structure allowing an adsorption and cation exchange[13]. This decreases their absorption by roots and enhances the conducive environment for soil growth[30,46].

In summary, the results reveal that root response to heavy metal contamination responds positively and negatively to toxicity levels competing with the ability of soil in attenuation. However, this was not case for bentonite application (50%) which significantly restored root activity by decreasing the bioavailability of heavy metals and improving physical and chemical properties of the soil. These help improve the efficiency of roots to uptake water and nutrients, as well as accumulation of dry matter.

Table 6. Concentrations of lead (Pb) and cadmium (Cd) in dry root biomass.

Treatments	Dry root weight	Treatments	Dry root weight
0Pb + 0 Bentonite = X0	13.77	0Cd + 0 Bentonite = C0	13.77
10Pb + 25 Bentonite = X1	14.87	5Cd + 25 Bentonite = C1	14.13
10Pb + 50 Bentonite = X2	15.83	5Cd + 50 Bentonite = C2	15.57
100Pb + 25 Bentonite = X3	11.27	15Cd +25 Bentonite = C3	11.43
100Pb + 50 Bentonite = X4	12.83	15Cd + 50 Bentonite = C4	13.10
200Pb + 25 Bentonite = X5	10.80	25Cd + 25 Bentonite = C5	10.97
200Pb + 50 Bentonite = X6	11.73	25Cd + 50 Bentonite = C6	12.20
L.S.D 0.05	0.1979	L.S.D 0.05	0.1743

3.6. Plant Contamination Indices for Lead and Cadmium

The relative value of the indices of plant contamination proposed in **Table 7** shows that both lead (Pb) and cadmium (Cd) behave differently, with respect to such elements on how they are managed into the internal biological system by an individual species, i.e.Indian mustard: Brassica juncea Arabidopsis[47].

At the low level contamination (10 mg Pb kg^{-1}), BCF_1 values for Pb treatments reached about 1.74 (with 25% bentonite) and 1.42 (with 50% bentonite). Values greater than one show that uptake capacity at low concentrations is relatively high. These values decreased to 0.38 and 0.21 for the ever-higher contaminated level (200 mg Pb kg^{-1}), accompanied with a decline in the Bioaccumulation Coefficient (BAC) approximately to about values of ~ 0.31–0.38, while the Translocation Factor (TF) varied from 0.73 to 1.51.

Thus, following the criteria for plant classification mentioned above; a phytostabilizer plant must have BCF > 1 and TF < 1, or root retention must prevail over translocation to aerial parts[14,25]. By contrast, Indian mustard phytostabilization behavior in Pb contamination is found under moderate and high levels as most values of BCF < 1 and TF $\leqslant$ 1 are obtained for most treatments. This suggests headroom relative Pb retention in the roots with a limited amount of translocation to leaves. The previous studies Mahar, et al.[21] and Rizwan et al.[44] who emphasized the capacity of Indian mustard to stabilise Pb instead of bioextraction.

Table (7). Plant contamination indices for lead (Pb).

Treatments	BCF1	BCF2	BAC	TF
10Pb + 25 Bentonite = X1	1.74	3.21	1.74	0.85
10Pb + 50 Bentonite = X2	1.42	2.46	1.04	0.73
100Pb + 25 Bentonite = X3	0.43	0.79	0.37	0.86
100Pb + 50 Bentonite = X4	0.32	0.63	0.31	0.95
200Pb + 25 Bentonite = X5	0.38	0.75	0.38	1.01
200Pb + 50 Bentonite = X6	0.21	0.52	0.31	1.51

4. Cadmium Behavior

For cadmium, BCF_1 were low (< 0.1) and from 0.036 at 5 mg Cd kg^{-1} to 0.008 at 25 mg Cd kg^{-1} with the use of bentonite (50%). On the other hand, the Translocation Factor (TF) values were in between 0.92 and 1.11 are nearly one or more than it that indicated ease of transfer Cd from roots to aerial parts.

Based on classification proposed by Baker and Brooks (1989)[31] as well more recent criteria reported by Liu et al.[34] and Park et al.[3]. Even without reaching the height of hyperaccumulator, a high TF in the presence of significant shoot system accumulation implies the possibility for phytoextraction. In this study, the measured values were not above the threshold limits; which are set for hyperaccumulator plants.

Hence, Indian mustard behaved more like partial phytoextraction (except for cadmium as compared to lead). This is possibly because of the better mobility of Cd and its lower affinity with root components, which leads to its relocation to aboveground tissues.

Table 8. Plant contamination indices for cadmium (Cd).

Treatments	BCF1	BCF2	BAC	TF
5Cd + 25 Bentonite = C1	0.036	0.072	0.036	1.00
5Cd + 50 Bentonite = C2	0.026	0.052	0.026	1.00
15Cd + 25 Bentonite = C3	0.016	0.031	0.015	0.92
15Cd + 50 Bentonite = C4	0.012	0.024	0.012	1.00
25Cd + 25 Bentonite = C5	0.12	0.923	0.012	1.00
25Cd + 50 Bentonite = C6	0.008	0.016	0.008	1.11

In general, given the values obtained from **Table 8** and results applying to Indian mustard (Brassica juncea), it can be regarded as a plant species behaving dually. Phytostabilization appears to be favoured at higher contamination levels for both elements and due to its relatively high translocation factor (TF ≈ 1), partial phytoextraction of cadmium, e.g. Further, the reduction in Bioconcentration Factor (BCF) and Bioaccumulation Coefficient (BAC) values with increasing bentonite application indicates its effect on declining bioavailability of both elements. Consequently restricting extraction efficiency and strengthen the stabilization trend. This is in agreement with the observation made by Boostani et al.[12] and Hassan et al.[32], who reported alteration of heavy metals in agricultural systems with clay amendments added.

5. Conclusion

The findings showed that the concentrations of Pb as well as Cd in their tissue grew with rising levels of Pb and Cd contamination. Lead had higher retention in roots while cadmium showed more movement and translocation towards shoot parts. Bentonite (especially at the rate of 50%) was found to effectively mitigate their uptake and bioavailability, improving growth parameters in terms of the fresh and dry weight of both shoots and roots. Further, plant contamination indices also suggested that Indian mustard has a propensity for phytostabilization behavior to lead but commits itself more towards phytoextraction active performance for cadmium weight index based on root accumulation. These results provide general evidence for the role of bentonite as a soil amendment that could be used in management practices to address heavy metal contaminated soils with potential benefits on both mitigating environmental risks and enhancing plant performance.

Conflict of interest

The authors declare no conflict of interest.

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