__Original Research Article__

Effect of humic acid functionalized bentonite on heavy metal uptake by Spinach(*Spinacia oleracea* cv. All Green) grown on metal contaminated soil

Abstract

A pot culture experiment was conducted to assess the effect of humic acid functionalized bentonite (HA-B) application on heavy metal uptake by spinach (Spinacia oleracea cv. All Green). Amendment of soil with humic acid functionalized bentonite 7.5 g kg^{-1} significantly improved the plant growth by 180.7%,212%,and 231%in first, second and third harvest, respectively. The metal concentration of spinach reduced by 62.8%, 69.7%, 77.7% for Cd and34.7%, 45.2%, 64.7% for Ni at first, second and third harvest, respectively on application of 7.5 g kg^{-1}humic acid functionalized bentonite. Amending the soil withhumic acid functionalized bentonite (7.5 g kg^{-1}) significantly decreased the bioconcentration factor (BCF) of metals by 36.3%, 40%, 55.2% for Cd and 15.2%, 13%, 34.7% for Niat first, second and third harvest, respectively. The hazard quotient (HQ) for metal uptake through consumption of amaranth was significantly reduced by application ofhumic acid functionalized bentonite @ 7.5 g kg^{-1} as it immobilises heavy metals in polluted soil.

Keywords: Humic acid functionalized clays, phytoremediation, metal contamination, risk assessment.

1. INTRODUCTION

The existence of hazardous heavy metals in soil, air, and water poses a critical hazard to both the environment and human well-being. Among these heavy metals, cadmium and nickel stand out as particularly concerning, as they have been identified to have adverse impacts on the biological and physiochemical attributes of soil. This leads to diminished soil fertility, reduced organic matter content, extreme pH levels, heightened electrical conductivity, and disrupted availability of essential micronutrients for plants [1,2].Cadmium and nickel are regarded as among the foremost ecological pollutants due to their release into the environment from sources like lead smelters, battery industries, paint, paper, and mining operations [3].They exhibit pronounced toxicity, posing risks to the human nervous system, kidneys, and red blood cells [4].The contamination of heavy metals also has grave implications for plant growth and physiological processes, presenting a significant global environmental concern [5].

Heavy metals have a detrimental impact on plant growth by causing limited nutrient availability, inhibited root growth, generation of reactive oxygen species (ROS), and decreased photosynthesis. These effects ultimately lead to reduced food availability [5,6]. The toxicity of heavy metals in plants involves intricate interactions at the cellular level, disrupting signal transduction pathways, genetic processes, and even leading to genetic mutations and programmed cell death [7].[8]found that the shoot and root weights (both fresh and dry) of Spinach crops significantly decreased when exposed to the highest concentrations of cadmium (Cd) and nickel (Ni) compared to the control treatment. Cadmium and nickel from the soil are absorbed by plants and subsequently consumed by animals, thus entering the human food chain. Predicting the potential health risks associated with consuming food grown in environments with elevated metal levels is of utmost importance. The hazard quotient serves as a simple tool often employed by researchers to comprehend the chronic non-cancer health risks associated with crops cultivated in metal-contaminated soils [9].The challenge at hand for researchers is to curtail the transfer of heavy metals like cadmium and nickel from progressing through the soil-plant-human continuum, demanding immediate attention.

In recent times, a range of strategies has been employed to address the issue of heavy metal contamination in soil, water, and sediments. These approaches encompass various techniques falling under the categories of physical, chemical, and biological methods. Examples include thermal treatment, adsorption, chlorination, chemical extraction, ion-exchange, reverse osmosis, membrane separation, electrokinetics, bioleaching, bioremediation, and phytoremediation, among others [10,11].Of these methods, adsorption has gained significant traction due to its efficiency and cost-effectiveness in extracting heavy metal ions from different sources. Among the economical adsorbents, bentonite clays from the smectite clay group, particularly montmorillonite, have demonstrated promise in adsorbing heavy metal ions. This makes them suitable for environmental engineering applications such as water, sewage, and soil purification [12, 13, 14].These mudstones possess distinctive properties, including a substantial specific surface area and a high capacity for swelling due to water absorption. These characteristics make them effective in adsorbing cations and organic substances. Consequently, it was deemed appropriate to synthesize and assess functionalized bentonite clay products with the intention of impeding the transfer of cadmium (Cd) and nickel (Ni) from soil to plants cultivated in metal-contaminated soils.

Additionally, the study aimed to evaluate any potential health risks associated with plants grown in the treated soils.

2. MATERIALS AND METHODS

Bentonite mineral was procured from Minerals Limited, New Delhi. Humic acid modified bentonite was prepared using standard procedure established by [15].Bulk soil sample (0-15 cm) was collected from the metal-contaminated area of Unnao district, Uttar Pradesh, India (latitude 26˚24’26” N and longitude 80˚26’17” E). The soil sample was air dried, ground and processed before analysing the physico-chemical characteristics using standard procedures. Extractable cadmium and nickel in the soil were analysed by extracting it with 0.005 *M* DTPA (diethylenetriaminepentaacetic acid) at pH 7.3 [16].The soil pH was determined in 1:2 soil-to-water suspension using a digital pH meter equipped with a combined electrode (glass and calomel electrodes) [17].Organic carbon content in the soil was determined using the wet oxidation method [18].The mechanical analysis of soil was done using hydrometer method [19]and the soil texture was determined from the soil textural triangle. The cation exchange capacity (CEC) of the soil was determined using ammonium acetate method [20].The pH, EC, and soil organic carbon (SOC) values of sampled soil were 5.9 g kg^{-1}, 7.2, and 1.30 dS m^{-1}, respectively. The soil was characterized under sandy clay loam textural class (55%-sand, 15 %-silt and 30%-clay) with a cation exchange capacity (CEC) value of 20.46 cmol (p^+) kg^{-1}. The DTPA extractable Cd and Ni concentrations in the soil were 6.45 and 4.46 mg kg^{-1}, respectively.

2.1. Pot experiment

To assess the effect of humic acid functionalized bentonite (HA-B) on Cd and Ni content and uptake by Spinach crop, a pot culture experiment was conducted in *rabi* (Oct-Dec) season (2021-22) using spinach (Variety- All Green) as a test crop at the net house facility of Division of Soil Science and Agricultural Chemistry, ICAR-IARI, New Delhi. Experiment was laid out in a completely randomized design with three replications. Air-dried, grounded, 2 mm sieved 4 kg of treated soil was used in each pot. The treatments followed in the pot culture study are as follows. T_1: Control pot (without clay), T_2: 2.5 g kg^{-1} HA-B, T_3: 5 g kg^{-1} HA-B and T_4: 7.5 g kg^{-1} HA-B. Plant population was maintained at five plants per pot. A uniform basal dose of 108 mg N, 108 mg P_2O_5, and 108 mg K_2O per pot were applied using urea, diammonium phosphate, and muriate of potash, respectively. An additional dose of 108 mg of nitrogen was applied 30 days after sowing. Plants and soil samples are harvested at three stages (35 DAS, 65 DAS and 95 DAS) and analysed for cadmium and nickel content. Cadmium and Nickel concentration in spinach samples grown in the same soil was determined by using a microwave digester with concentrated (65%) suprapure nitric acid [21]and measured using ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

2.2. Bioconcentration factor

The bioconcentration factor is a measure of the potential bioavailability of a specific metal in a particular plant part. It is calculated as the ratio of the metal concentration in plant tissue (measured in mg/kg dry weight, Dw) such as stems, leaves, roots, etc., to the metal concentration in the soil (measured in mg/kg dry weight, Dw) [22].Bioconcentration factor of Cd and Ni was calculated by using the following equation:

$$BCF = \frac{\mathrm{Cplant}}{\mathrm{Csoil}} \qquad (1)$$

Here, Cplant= metal concentration in plant tissue (mg/kg Dw), Csoil= metal concentration in soil (mg/kg Dw).

2.3. Non-cancer health risk assessment

The hazard quotient (HQ) is defined as the ratio of the average daily dose (ADD) of a heavy metal to its corresponding reference dose (RfD). The average daily dose represents the estimated intake of a specific heavy metal in milligrams per kilogram of body weight per day (mg kg^{-1} day^{-1}). The reference dose (RfD) is the maximum tolerable daily intake of that metal, expressed in milligrams per kilogram of body weight per day (mg kg^{-1} day^{-1}). It was assumed that the daily intake of green vegetables is 0.2 kg per day, which is the recommended amount from a nutritional perspective. A factor of 0.082, based on the dry weight basis, was utilized for amaranth [14].The average body weight for an adult was assumed to be 70 kg. Thus, the HQ for an adult was calculated as (Eq. (1)):

$$\mathrm{HQ} = \frac{\mathrm{M\,Plant\ x\ W\ x\ F}}{\mathrm{RfD\ x\ 70}} \qquad (2)$$

where, M_{Plant} is the metal content (mg kg^{-1}) of plant, W is the daily intake of green vegetable (kg kg^{-1} body weight) and F is the factor of conversion of fresh to dry weight. The RfD values allocated for Cd and Ni were 0.001, and 0.02 $mgkg^{-1}day^{-1}$, respectively.

3. RESULTS AND DISCUSSION

The biomass dry weight of shoots usually reflects the tolerance capability of plants to adverse environments. The amount of clay mineral had a significant effect on biomass production of spinach as shown in Fig. 1. Plant shoot biomass increased with increasing level of clay application. The magnitude of increase was highest for the soil amended with bentonite @ 7.5 g kg^{-1} compared to unamended controlsoil. The biomass yield was found to increase from 2.18 in T_1 (control) to 3.84, 4.45 and 6.12 g pot^{-1} in T_2, T_3 and T_4, respectively at first harvest. At second harvest the biomass yield increased by 100, 135 and 212 % by application of bentonite @ 7.5, 5 and 2.5 g kg^{-1}, respectively over unamended control soil. The same trend was followed in third harvest also. The above results indicated that application of humic acid functionalized bentonite improved the plant growth by adsorbing the heavy metals in the soil by the way of increasing adsorption sites and thereby alleviating the heavy metal stress to the plants. Such an improvement in plant growth was achieved by alleviating the heavy metal stress in the plants through humic acid functionalized bentonite amendment [13, 14, 23, 24]. The addition of functionalized clay mineral boosted microbial activity, whereas organic matter mineralization limited the availability of metals and metalloids to microorganisms. Improved soil fertility could potentially be one of the causes for increased plant biomass in bentonite amended treatments [1, 10, 25].

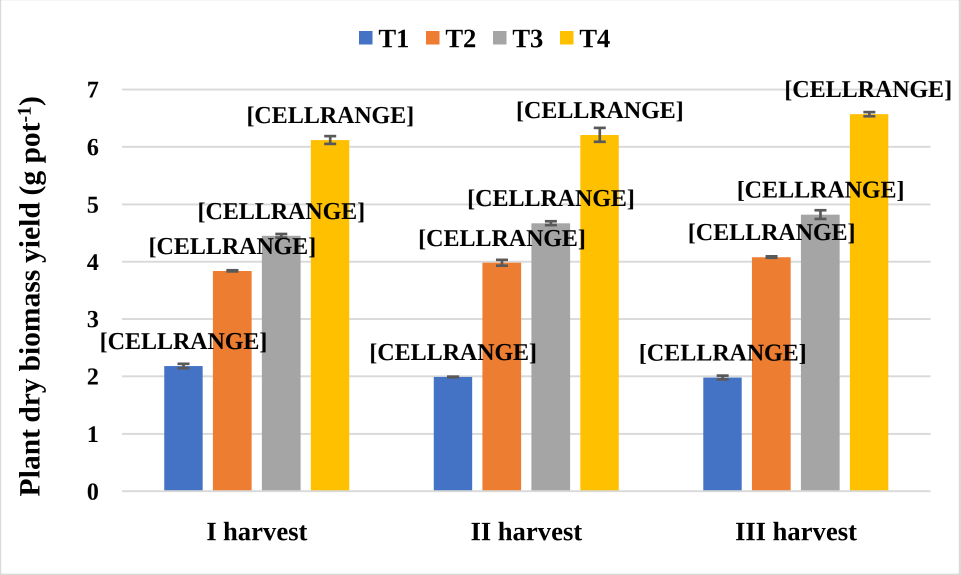

Figure 1. Effect of humic acid functionalized bentonite levels on dry biomass yield (g pot^{-1}) of spinach.

Heavy metals accumulation in plants depends on whether they are in bioavailable form, which can be taken up by plants. The concentration of heavy metals in shoots of spinach was significantly reduced by the application humic acid functionalized bentonite (Table 1). Application of bentonite decreased the cadmium concentration of spinach to 3.78 mgkg^{-1} (T2), 2.98mg kg^{-1}(T3) and 2.05mg kg^{-1}(T4) from 5.52 mg kg^{-1}(T1) in control soil at first harvest and the values for second harvest were found to be 3.15mg kg^{-1}(T2), 3.68mg kg^{-1}(T3), 1.69mg kg^{-1}(T4) and 5.58mg kg^{-1}(T1) in control soil. Similarly in third harvest the cadmium content was reduced to 2.80 mg kg^{-1}, 2.21 mg kg^{-1}, 1.25 mg kg^{-1} in 2.5, 5 and 7.5 g kg^{-1} humic acid functionalized bentonite amended soil, respectively from the control of 5.63mg kg^{-1}. Nickel concentration in spinach was reduced from 3.25mg kg^{-1}in control to 3.05mg kg^{-1}, 2.88mg kg^{-1}and 2.12mg kg^{-1}in on application of humic acid functionalized bentonite @ 2.5, 5 and 7.5 g kg^{-1}, respectively in first harvest. Similarly, in second and third harvest the nickel content was reduced to 2.78 and 2.55mg kg^{-1}, 2.25 and 2.01mg kg^{-1}and 1.67 and 1.12mg kg^{-1}in 2.5, 5 and 7.5 g kg^{-1} humic acid functionalized bentonite amended soil, respectively from the control of 3.15 and 3.18mg kg^{-1}. The bioavailability of heavy metals in soil depends on their concentration in the soil solution and on the release of heavy metal ions from the soil solid phase.

Table 1. Effect of humic acid functionalizedbentonite levels on metal concentration (mg kg^{-1} DW) in spinach.

Treatments	Cd			Ni		
	Harvest I	Harvest II	Harvest III	Harvest I	Harvest II	Harvest III
T$_1$	5.52^{a}	5.58^{a}	5.63^{a}	3.25^{a}	3.15^{a}	3.18^{a}
T$_2$	3.78^{b}	3.15^{b}	2.80^{b}	3.05^{ab}	2.78^{b}	2.55^{b}
T$_3$	2.98^{c}	2.68^{c}	2.21^{c}	2.88^{b}	2.25^{c}	2.01^{c}
T$_4$	2.05^{d}	1.69^{d}	1.25^{d}	2.12^{c}	1.67^{d}	1.12^{d}
CD (P = 0.05)	0.22	0.11	0.10	0.20	0.11	0.12

The labile fraction of heavy metals extracted by DTPA were significantly reduced by application of humic acid functionalized bentonite (Fig. 2). Amendment of soil with 7.5 g kg^{-1} humic acid functionalized bentonite (T$_4$) resulted in 77.7 and 64.7 % reduction of DTPA extractable Cd and Ni, respectively over the control (T$_1$).Application of clay mineral reduced the bioavailability of heavy metals and this might be due to the larger surface area as well as the stronger sorptive capacity of bentonite, which decreases the concentration of cations in the soil solution and thereby reduced the uptake by plants[24].The availability of heavy metals in the soil is influenced by their concentration in the liquid phase and the release of ions from the solid phase. Utilizing clay products may decrease the bioavailability of heavy metals by taking advantage of the larger surface area and enhanced adsorptive capacity of pillared bentonite. This leads to a reduction in the concentration of cations in the soil solution, ultimately resulting in reduced uptake of these metals by plants. This can be attributed to the increased accessibility of a larger surface area with a greater number of ion exchange sites, even when the initial concentration of the ions remains constant [26].The immobilization of heavy metals in soil by bentonite is facilitated by factors such as isomorphic substitution, negative charge, and environmental compatibility. These properties enable bentonite to effectively capture and retain heavy metals, thereby restricting their mobility and mitigating potential adverse effects (Xie *et al.,* 2018). Bentonite's high surface-area value contributes to its strong sorption ability for metal ions, allowing it to trap heavy metal (HM) ions within its structure and enhance isomorphic substitution (Zhang *et al.,* 2021). Studies by Vrinceanu *et al.* (2019) showed that the introduction of bentonite to the soil increased the soil pH, leading to a better retention of HMs in the solid-soil phase and significantly reduced the cadmium and zinc uptake in the above-ground parts. This liming effect resulted in the binding of HMs through long-term diffusion into clay mineral layers.

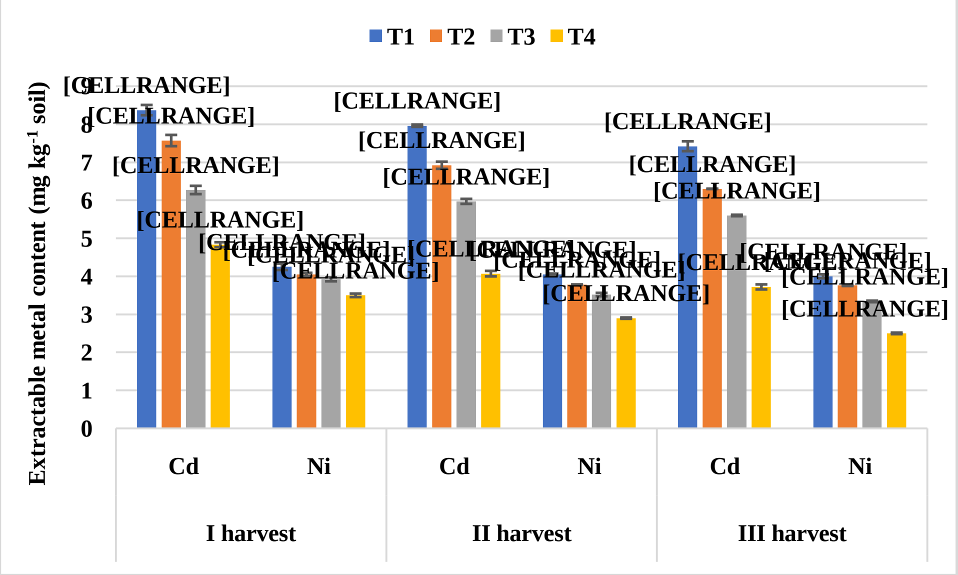

Figure 2. Effect of humic acid functionalized bentonite levels on extractable heavy metals in soil.

Bioconcentration factor (BCF) is the ratio of metal content in edible tissue to that of total metal content in soil. It assessed the efficiency of clay minerals in immobilizing the heavy metals in the soil (Table 2). The bioconcentration factor of Cd varied from 0.66 (T1) in control to 0.50 (T2), 0.48 (T3) and 0.42 (T4) in soil amended with 2.5, 5 and 7.5 g kg^{-1} humic acid functionalized bentonite,respectively at first harvest. At second and third harvest BCFof Cd reduced by 34.2 and 42.1, 35.7 and 48.6, 40 and 55.2 % over the controlsoil by application of 2.5, 5 and 7.5 g kg^{-1} humic acid functionalized bentonite,respectively. Similarly, BCF of Ni for spinach variedbetween 0.72 to 0.61, 0.67 to 0.58 and 0.69 to 0.45at first, second andthird harvest, respectively. Addition of bentonites significantlyreduced the translocation of metals to the plants asindicated by the values of BCF.Food consumption contaminated with heavy metals is a major contributory pathway (more than 90%) to human exposure than any other pathways such as inhalation and dermal contact. Intake of heavy metals at toxic levels by human beings results in several physiological and metabolic disorders (Rattan et al., 2009). To assess the efficiency of humic acid functionalized bentonite on metal immobilization, health risk assessment of vegetable consumption from the clay amended and control soils, hazard quotient was calculated using USPEA protocol (IRIS, 9). The results indicate (Table 3) that the HQ of Cd reduced to 0.89, 0.70, 0.48 and 0.74, 0.63, 0.40 and 0.66, 0.52, 0.29 for 2.5, 5 and 7.5 g kg^{-1} humic acid functionalized bentonite amended soil from 1.29, 1.31 and 1.32 in control at first, second and third harvest, respectively. The reduction in hazard quotient on application of humic acid functionalized bentonites might be due to reduced metal uptake due to immobilization of heavy metal in soil. Values of HQ equal to or more than 1 indicatesthat consumption of food materials may be hazardous to humans due to intake of a particular metal. As the consumption of leafy green vegetables constitute only part of food materials contribute to metal uptake by human beings. If we take into account of other metal sources like drinking water and inhalation of dust, the safe limit of HQ can be considered as 0.5 in risk assessment of contaminated soils. Hence HQ of Cd exceeded 0.5 in control soil and amendment with humic acid functionalized bentonite reduced the values below the safe limit of 0.5. Heavy metals are kept in the soil through exchangeable chemical, physical and biological sorption. Addition of bentonite increases the chemical sorption of heavy metals and reduces the mobility of these metals in environmental conditions as a consequence of complex formation. The results of our studies showed that application of humic acid functionalized bentonite @ 7.5 g kg^{-1} demonstrates the best effectiveness towards the immobilisation o heavy metals in soil. The method described enables application of humic acid functionalized bentonite to soil reduced the mobility and availability of heavy metals to plants and thereby reduce the risk of consumption of vegetables grown on metal contaminated soil. The reduction in hazard quotient was apparently due to the reduced metal uptake by the plants as a result of their immobilisation in the soil (Kumararaja *et al.,* 2016; Raj *et al.,* 2017; Naveenkumar et al., 2023).

Table 2. Effect of humic acid functionalized bentonite levels on bioconcentration factor of metals in spinach.

Treatments	Cd			Ni		
	Harvest I	Harvest II	Harvest III	Harvest I	Harvest II	Harvest III

T_1	0.66^{a}	0.70^{a}	0.76^{a}	0.72^{a}	0.67^{b}	0.69^{a}
T_2	0.50^{b}	0.46^{b}	0.44^{b}	0.70^{a}	0.70^{a}	0.68^{a}
T_3	0.48^{c}	0.45^{b}	0.39^{c}	0.73^{a}	0.64^{b}	0.60^{b}
T_4	0.42^{d}	0.42^{c}	0.34^{d}	0.61^{b}	0.58^{c}	0.45^{c}
CD (P = 0.05)	0.02	0.01	0.04	0.03	0.02	0.03

Table 3. Effect of humic acid functionalized bentonite levels on hazard quotient of metal through consumption of spinach.

Treatments	Cd			Ni		
	Harvest I	Harvest II	Harvest III	Harvest I	Harvest II	Harvest III
T_1	1.29^{a}	1.31^{a}	1.32^{a}	0.04^{a}	0.04^{a}	0.04^{a}
T_2	0.89^{b}	0.74^{b}	0.66^{b}	0.04^{ab}	0.03^{b}	0.03^{b}
T_3	0.70^{c}	0.63^{c}	0.52^{c}	0.03^{bc}	0.03^{b}	0.03^{b}
T_4	0.48^{d}	0.40^{d}	0.29^{d}	0.02^{c}	0.02^{c}	0.02^{c}
CD (P = 0.05)	0.06	0.03	0.01	0.01	0.01	0.01

4. CONCLUSIONS

The current study sheds light on the positive outcomes of employing humic acid functionalized bentonite clay in enhancing the growth and heavy metal tolerance of spinach plants. The inclusion of functionalized bentonite exhibited a notable and favourable influence on spinach biomass production across all three harvest intervals, resulting in a significant upsurge in plant growth as compared to untreated soil. The research revealed that the most effective approach for immobilizing heavy metals like cadmium (Cd) and nickel (Ni) was the application of humic acid functionalized bentonite at a rate of 7.5 g kg^{-1}. The introduction of functionalized bentonite enhanced the chemical adsorption of heavy metals, thereby curbing their mobility within plants. The significant surface area and heightened adsorptive capacity of bentonite effectively restrained heavy metals within the soil, diminishing their bioavailability and uptake by plants. The lowered hazard quotient of heavy metals in spinach was attributed to the decreased uptake of metals by plants, brought about by their immobilization in the soil through the application of humic acid functionalized bentonite. The method delineated in this research offers a means to employ modified bentonite within soil to diminish the movement and accessibility of heavy metals to plants. This, in turn, reduces the health hazards associated with the consumption of vegetables grown in soils contaminated with heavy metals.

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