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Assessing the Impact of Functionalized Bentonite in Reducing Lead content and uptake in Spinach (*Spinacia oleracea* cv. All Green)

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ABSTRACT: A pot culture experiment was conducted to assess the effect of modified bentonite application on lead (Pb) 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 74.7, 128.8 and 122.6 % in first, second and third harvest, respectively over control. The metal concentration of spinach reduced to 2.17, 1.74 and 1.32 mg kg^{-1} for Pb at first, second and third harvest, respectively on application of 7.5 g kg-1 humic acid bentonite (T10). However, Fe-bentonite, GHA-bentonite and Humic acid-bentonite reduced Pb content in spinach at all the applied doses, but application at 7.5 g kg^{-1} soil was found most significant as compared to control. Between the treatments, highest dry weight of spinach plant was recorded at higher dose of Humic acid-bentonite followed by GHA-bentonite and Fe-bentonite. The hazard quotient (HQ) for metal uptake through consumption of spinach was significantly reduced by application of bentonite @ 7.5 g kg^{-1} as it immobilises heavy metals in polluted soil.

Keywords: Functionalized clays, phytoremediation, metal contamination, risk assessment.

INTRODUCTION

The presence of harmful heavy metals in the soil, air, and water poses a serious threat to both environment and human life. These heavy metals, especially lead (Pb^{2+}), have been found to negatively affect the biological and physiochemical properties of soil, resulting in reduced soil fertility, low organic matter, extreme pH levels, high electrical conductivity, and imbalanced micronutrient availability to plants (Mi *et al.,* 2021; Venkateswarlu and Venkatrayulu 2023). Lead is considered one of the most significant ecological pollutants due to its release into the environment from lead smelters, battery industries, paint, paper, and mining activities (Hannachi *et al.,* 2013). It is particularly toxic, harming the human nervous system, kidneys, and red blood cells (Mutter *et al.,* 2017). Heavy metal pollution also have severe consequences on plant growth and physiology, posing a major global environmental concern (Xie *et al.,* 2018).

Plant growth is adversely affected by heavy metals through limited nutrient availability, inhibited root growth, production of reactive oxygen species (ROS), and reduced photosynthesis, leading to decreased food availability (Xie *et al.,* 2018; Zhang *et al.,* 2021). The toxicity of heavy metals in plants involves complex interactions at the cellular level, affecting signal transduction pathways, genetic processes, and even leading to genetic mutations and programmed cell death (Vrinceanu *et al.,* 2019). According to Alia *et al.* (2015), the shoot and root weights (both fresh and dry) of Spinach crop exhibited significant decrease when exposed to the highest dose of Pb compared to the control treatment. According to Lamhamdi *et al.* (2013), the fresh weight of spinach decreased by approximately 28%, and the dry weight decreased by around 29% when exposed to 15 mM Pb, in comparison to the control treatment. Similarly, several other researchers have also documented the negative repercussions of high Pb concentrations on plant growth (An *et al.*, 2004; Alexander *et al.*, 2006; Lingua *et al.*, 2008). Hasanabadi *et al.* (2015) found that zeolite application increased the alfalfa yields upto 65% compared with the control. Lead from soil gets transferred to plants and is then consumed by animals thus entering the human food chain. Prediction of the potential health risk imparted by consumption of food substances grown on metal contaminated environments and having inconspicuously high metal levels is very crucial. Hazard quotient is a very simple tool often used by researchers to get an understanding about the chronic non-cancer health associated with crops grown on contaminated soils (Saraswat *et al.,* 2023). Restricting the transfer of heavy metals like Pb from transfer into the soil-plant-human continuum has become a challenge for researchers and requires urgent attention.

In recent times, various approaches have been employed to address heavy metal contamination in soil, water, and sediments. These methods encompass physical, chemical, and biological techniques such as thermal treatment, adsorption, chlorination, chemical extraction, ion-exchange, reverse osmosis, membrane separation, electrokinetics, bioleaching, bioremediation, and phytoremediation etc (Wang *et al.*, 2020; Samal *et al.,* 2023; Waghmode *et al.,* 2023). However, adsorption has gained popularity as an efficient and cost-effective approach for extracting heavy metal ions from different sources (Azimi and Eshaghi 2017; Raza *et al.*, 2023). Among the low-cost adsorbents, bentonite clays from the smectite clay group, particularly montmorillonite, have shown promise in adsorbing heavy metal ions, making them suitable for environmental engineering applications like water, sewage, and soil purification (Carraro *et al.*, 2014; Raj *et al.*, 2017; Kumararaja *et al.*, 2017). These mudstones possess unique properties, including a significant specific surface area and a high swelling tendency due to water absorption, making them effective in adsorbing cations and organic substances. Consequently, it was thought proper to synthesise and evaluate the functionalized bentonite clay products to prevent the transfer of Pb from soil to plants cultivated in Pb-contaminated soils. Additionally, the study aimed to evaluate any potential health hazards associated with plants grown in the treated soils.

MATERIALS AND METHODS

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. Total and extractable lead in the soil were analysed by digesting the soil with aqua-regia (Quevauviller, 1998) and extracting it with 0.005 *M* DTPA (diethylenetriaminepentaacetic acid) at pH 7.3 (Lindsay and Norvell, 1978), respectively. 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) (Datta *et al.,* 1997). Organic carbon content in the soil was determined using the wet oxidation method (Walkley and Black 1934). The mechanical analysis of soil was done using hydrometer method (Bouyoucos, 1962) 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 (Jackson, 1973). 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 Pb and total Pb concentrations in the soil were 7.2 and 26.5 mg kg^{-1}, respectively.

A. Pot experiment

To assess the effect of modified clay mineral [Iron modified bentonite (Fe-B), Goethite plus humic acid-kaolinite (GHA-K) and Humic acid modified bentonite (HA-B)] on Pb 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 factorial completely randomized design with three replications. Different products were applied to the pots filled with 4 kg soil at a dose of 2.50, 5.00 and 7.5 g kg^{-1} (including control-without clay). The treatments followed in the pot culture study are as follows. T_1: Control pot (without clay), T_2: 2.5 g kg^{-1} Fe-B, T_3: 5 g kg^{-1} Fe-B, T_4: 7.55 g kg^{-1} Fe-B, T_5: 2.5 g kg^{-1} GHA-B, T_6: 5 g kg^{-1} GHA-B, T_7: 7.5 g kg^{-1} GHA-B, T_8: 2.5 g kg^{-1} HA-B, T_9: 5 g kg^{-1} HA-B and T_{10}: 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 samples are harvested at three stages (35 DAS, 65 DAS and 95 DAS) and analysed for lead content. Lead concentration in spinach samples grown in the same soil was determined by using a microwave digester with concentrated (65%) suprapure nitric acid (Guven and Akinci, 2010). The total Pb content in the digest was measured using ICP-MS (Inductively Coupled Plasma Mass Spectrometry).

B. 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/day). 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/day). 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 (Kumararaja *et al.*, 2017). The average body weight for an adult was assumed to be 70 kg. Thus, the HQ for an adult was calculated as (Eq. (1):

$$HQ = \frac{M_{Plant} \times W \times F}{RfD \times 70} \qquad (1)$$

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.

RESULTS AND DISCUSSION

The biomass dry weight of shoots reflects the tolerance ability of plants to an adverse environment. The added amount of functionalized bentonite had a significant effect on the biomass production of spinach during all the three harvest times (Fig. 1). The application of humic acid functionalized bentonite at 7.5 g kg^{-1} improved the growth of spinach by 74.7, 128.8 and 122.6 % in first, second and third harvest, respectively, compared to the unamended soil. Such an improvement in plant growth was achieved by alleviating the heavy metal stress in the plants through humic acid functionalized bentonite amendment (Kumararaja *et al.*, 2016; Raj *et al.,* 2017; Kumararaja *et al.*, 2017). 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 (Datta *et al.*, 2020; Wang *et al.*, 2020; Mi *et al.*, 2021).

Heavy metal accumulation in plants depends on their bioavailability, i.e., the chemical forms which can be taken up by the plants. The concentration of heavy metals in spinach shoots was significantly reduced by the application of humic acid functionalized bentonite (Fig. 2). At first harvest, Pb content of spinach was reduced from 7.2 mg kg^{-1} in the control to 5.82, 4.17 and 2.17 mg kg^{-1} by amending the soil with humic acid functionalized bentonite @ 2.5, 5 and 7.5 g kg^{-1}, respectively. Similarly, in the second and third harvest as well the Pb content was observed to vary in the range of 6.97-1.74 mg kg^{-1} and 6.92-1.32 mg kg^{-1}, respectively.

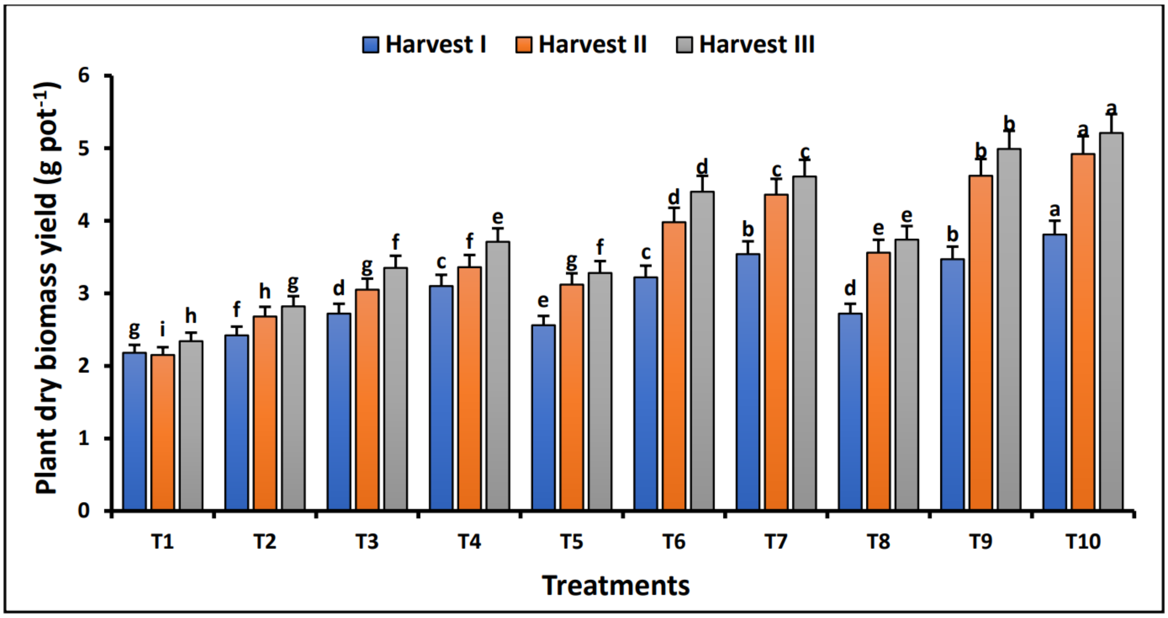

Fig. 1. Effect of modified clay minerals on biomass yield (g pot^{-1}) of amaranth. Bars represent standard deviations at $p < 0.05$. Different letters above the bars represent a significantly different mean at $p < 0.05$. T_1 – Control, T_2 - 2.5 g kg^{-1} Fe-B, T_3 - 5 g kg^{-1} Fe-B, T_4 - 7.5 g kg^{-1} Fe-B, T_5 - 2.5 g kg^{-1} GHA-B, T_6 - 5 g kg^{-1} GHA-B, T_7 - 7.5 g kg^{-1} GHA-B, T_8 - 2.5 g kg^{-1} HA-B, T_9 - 5 g kg^{-1} HA-B and T_{10} - 7.5 g kg^{-1} HA-B (Fe-B; Iron modified bentonite, GHA-B; Goethite and Humic acid modified bentonite; HA-B; Humic acid modified bentonite).

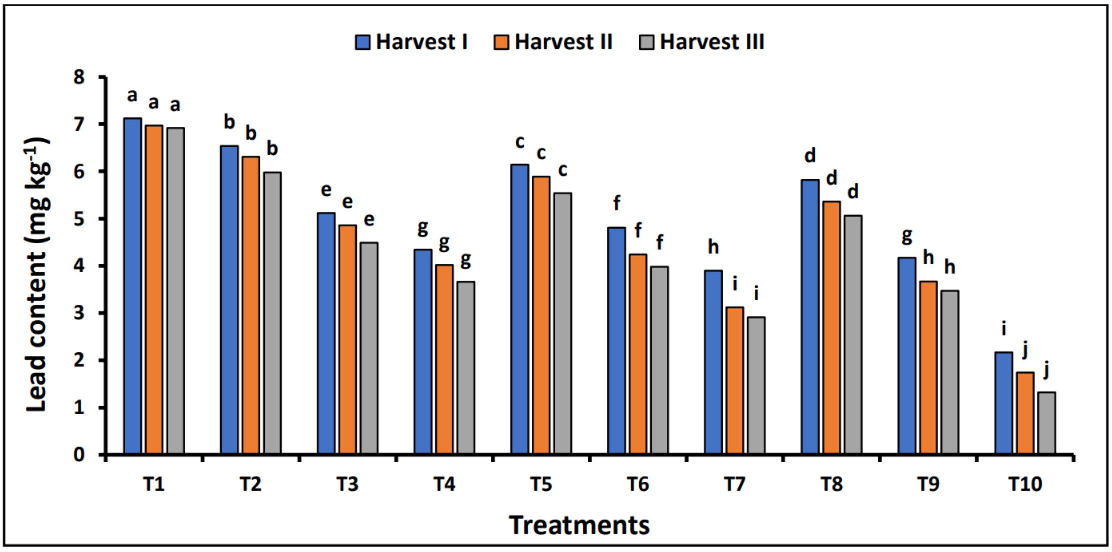

Fig. 2. Effect of functionalized bentonite on Pb content (mg kg^{-1} dry weight) in spinach. Bars represent standard deviations at $p < 0.05$. Different letters above the bars represent a significantly different mean at $p < 0.05$. T_1 – Control, T_2 - 2.5 g kg^{-1} Fe-B, T_3 - 5 g kg^{-1} Fe-B, T_4 - 7.5 g kg^{-1} Fe-B, T_5 - 2.5 g kg^{-1} GHA-B, T_6 - 5 g kg^{-1} GHA-B, T_7 - 7.5 g kg^{-1} GHA-B, T_8 - 2.5 g kg^{-1} HA-B, T_9 - 5 g kg^{-1} HA-B and T_{10} - 7.5 g kg^{-1} HA-B (Fe-B; Iron modified bentonite, GHA-B; Goethite and Humic acid modified bentonite; HA-B; Humic acid modified bentonite).

Overall, the Pb content in each harvest under each treatment decreased relative to the previous harvest. 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 (Hussain and Ali 2021). 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.

In a five-year experiment with *Capsicum annuum* L.-*Brassica pekinensis* L. rotation, Zhang *et al.* (2021) reported that bentonite decreased the leaching of Cd and Pb from the soil. During the first harvest, hazard quotient of Pb (Table 1) was reduced from 0.47 in the control to 0.38, 0.27 and 0.14 by amending the soil with 2.5, 5.0 and 7.5 g kg^{-1} humic acid functionalized bentonite, respectively. Similarly, in the second and third harvest as well the hazard quotient of Pb was observed to vary in the range of 0.46-0.11 and 0.46-0.08, respectively.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).

Table 1: Effect of modified clay mineral on hazard quotient (HQ) of spinach.

Treatments	First harvest	Second harvest	Third harvest
T_1	0.47^a	0.46^a	0.46^a
T_2	0.43^b	0.42^b	0.40^b
T_3	0.34^e	0.32^e	0.30^e
T_4	0.29^g	0.26^f	0.24^g
T_5	0.41^c	0.39^c	0.37^c
T_6	0.32^f	0.28^f	0.26^f
T_7	0.26^h	0.20^h	0.19^h
T_8	0.38^d	0.35^d	0.33^d
T_9	0.27^{gh}	0.24^g	0.23^g
T_{10}	0.14^i	0.11^i	0.08^i
LSD (0.05)	0.02	0.01	0.01

T_1 – Control, T_2 - 2.5 g kg^{-1} Fe-B, T_3 - 5 g kg^{-1} Fe-B, T_4 - 7.5 g kg^{-1} Fe-B, T_5 - 2.5 g kg^{-1} GHA-B, T_6 - 5 g kg^{-1} GHA-B, T_7 - 7.5 g kg^{-1} GHA-B, T_8 - 2.5 g kg^{-1} HA-B, T_9 - 5 g kg^{-1} HA-B and T_{10} - 7.5 g kg^{-1} HA-B (Fe-B; Iron modified bentonite, GHA-B; Goethite and Humic acid modified bentonite; HA-B; Humic acid modified bentonite).

CONCLUSIONS

The present study highlights the positive effects of using humic acid functionalized bentonite clay on the growth and heavy metal tolerance of spinach plants. The addition of functionalized bentonite had a significant and positive impact on the biomass production of spinach during all three harvest times, leading to a substantial increase in plant growth compared to the unamended soil. The study's findings revealed that using humic acid functionalized bentonite at an application rate of 7.5 g kg^{-1} proved most effective in immobilizing heavy metals like Pb. By introducing functionalized bentonite, the chemical adsorption of heavy metals increased, leading to a reduction in their mobility in plants. Bentonite's high surface area and enhanced adsorptive capacity effectively immobilized heavy metals in the soil, reducing their bioavailability and uptake by plants. The reduction in the hazard quotient of heavy metals in spinach was attributed to the decreased metal uptake by plants as a result of their immobilization in the soil due to the application of humic acid functionalized bentonite. The approach outlined in this research allows for the application of modified bentonite in soil to decrease the mobility and availability of heavy metals to plants, consequently reducing the health risks associated with consuming vegetables grown in metal-contaminated soils.

FUTURE SCOPE

The current study provides insights into the short-term effects of humic acid functionalized bentonite on plant growth and heavy metal uptake. However, to better understand the sustainability and persistence of these effects, further research should focus on conducting long-term field experiments. The present study opens promising avenues for the sustainable remediation of heavy metal-contaminated soils and the enhancement of plant growth through bentonite amendments. Combining bentonite amendments with phytoremediation techniques could lead to synergistic effects in remediating contaminated soils.

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Conflict of Interest. None.

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