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Effect of bentonite on heavy metal uptake by amaranth (*Amaranthus blitum* cv. Pusa Kirti) grown on metal contaminated soil

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ABSTRACT

A pot culture experiment was conducted to assess the effect of bentonite application on heavy metal uptake by amaranth (*Amaranthus blitum* cv. Pusa Kirti). Amendment of soil with bentonite @ 2.5% significantly improved the plant growth by 76.5 and 41.7% in first and second harvest, respectively. The metal concentration of amaranth reduced by 6.5 & 21.2% for Zn, 30.5 & 29.9% for Cu and 34.4 & 40.2% for Ni at first and second harvest, respectively on application of 2.5% bentonite. Amending the soil with bentonite (2.5%) significantly decreased the bioconcentration factor (BCF) of metals by 74 and 28% for Zn, 38 and 36% for Cu, 44 and 34% for Ni at first and second harvest, respectively. The hazard quotient (HQ) for metal uptake through consumption of amaranth was significantly reduced by application of bentonite @ 2.5% as it immobilises heavy metals in polluted soil.

Key words: Amaranth, bentonite, heavy metal, hazard quotient, remediation.

INTRODUCTION

Peri urban agriculture is the need of the hour due to burgeoning population and ever growing urbanisation. Cultivation of vegetable crops in the marginal lands of suburban area is a common practice followed in developing countries of the world. Peri-urban areas used for vegetable crop cultivation represent a sink for heavy metals because of the excessive accumulation of heavy metals by sewage water irrigation and industrial effluent due to paucity of good quality water and availability of polluted water at free of cost. The metal laden soils affect the food quality and safety by the risk of biomagnifications and bioaccumulation in the food chain because the heavy metals are not biodegradable (Pescod, 1; Sharma *et al.*, 2; Mapanda *et al.*, 3). To reduce the problem of heavy metal transfer from soil to plant a remediation technique, which should immobilise the heavy metals in the soil is warranted. Clay minerals are one of the amendments commonly available, which are integral parts of the soil system, non-toxic, less expensive and posing large specific area (Varrault and Bermond, 4; McBride and Martinez, 5; Malandrino *et al.*, 6). Little research has been conducted on health risk assessment of metal intake through vegetables grown on metal contaminated soil remediated with immobilising agents. In the present study, bentonite clay mineral selected as immobilising agent for the remediation of metal spiked soil. Health risk assessment of metal intake through consumption of amaranth was done by calculation of hazard quotient.

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MATERIALS AND METHODS

Bentonite mineral was procured from Minerals Limited, New Delhi. The soil used for the pot experiment was collected from the top 10 cm of the agricultural area of ICAR-IARI, Research Farm. The soil was spiked with 250 ppm of Zn and 100 ppm each of Cu and Ni by addition of dissolved metal solution and mixing it thoroughly with the soil. Pot culture experiment was conducted during summer 2011 with amaranth (*Amaranthus blitum*) as test crop and to study the residual effect of clay amendment, under greenhouse conditions. Air-dried, grounded, 2 mm sieved 2 kg of treated soil was used in each pot. The bentonite clay mineral was added at 3 levels (T1 = 0.5%, T2 = 1.5% and T3 = 2.5%). A control (T1) without amendment was also set up and the experiment was done in triplicate. Recommended fertilizer dose of 180:90:45 mg pot^{-1} NPK was added for amaranth. Half the dose of nitrogen and full dose of phosphorous and potassium was applied as basal and remaining half of nitrogen was applied 30 days after sowing. Seeds were washed with distilled water and directly sown in the individual pots. The plants were thinned to 5 seedlings per pot after one week of sowing. Deionised water was added throughout the experiment to sustain 60% water holding capacity of the soil.

Two cuttings were taken at 30 day interval and the concentration of Zn, Cu and Ni in the digested samples were determined with Atomic Absorption Spectrophotometer (AAS). Soil samples were extracted with ethylene diamine tetraacetic acid (EDTA), diethylene triamine penta acetic acid (DTPA) and metals in the filtrate were analyzed by flame or graphite AAS. Soil samples were digested with aqua regia (75% concentrated HCl + 25% HNO_3) and analysed with AAS (Jackson, 7).

The non-carcinogenic risk of consumption of amaranth grown on metal spiked soil amended with bentonite was characterized by hazard quotient (HQ), which is the ratio of the average daily dose (ADD; mg kg^{-1} day^{-1}) of metals to their reference dose (RfD; mg kg^{-1} day^{-1}) (Pierzynski *et al.*, 8). RfD is defined as the maximum tolerable daily intake of the specific metal that does not result in any deleterious health effects:

$$HQ = ADD / RfD \quad (1)$$

The values of RfD for Zn and Ni were 0.3 and 0.02 mg kg^{-1} day^{-1}, respectively. For Cu, provisional maximum tolerance daily intake (PMTDI) of 0.5 mg kg^{-1} day^{-1} was used in place of RfD (IRIS, 9). Daily intake of green vegetable was assumed to be 0.2 kg day^{-1} which is the recommended amount from a nutritional point of view. A factor of 0.085 was used to represent on dry weight basis. Average body weight for an adult was assumed to be 70 kg. Thus, the HQ for an adult was calculated as

$$HQ = M_{Plant} * W * F / RfD * 70 \quad (2)$$

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.

Analysis of variance and comparison between means by Duncan's test were carried with SPSS.

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 amaranth 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 @ 1.5% compared to unamended control soil. The biomass yield was found to increase from 4.8 in T_1 (control) to 6.7, 6.9 and 6.8 g pot^{-1} in T_2, T_3 and T_4, respectively at first harvest. At second harvest the biomass yield increased by 76, 88 and 2.9% by application of bentonite @ 2.5,1.5 and 0.5%, respectively over unamended control soil. The above results indicated that application of bentonite improved the plant growth by adsorbing the heavy metals in the soil by the way of increasing adsorption sites, increase in alkalinity and thereby alleviating the heavy metal stress to the plants (Sun *et al.*, 10; Usman *et al.*, 11).

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 amaranth was significantly reduced by the application bentonite (Table 1). Application of bentonite decrased the zinc concentration of amaranth to 339 mg/kg (T_2), 329 mg/kg (T_3) and 326 mg/kg (T_4) from 349 mg/kg (T_1) in control soil at first harvest and the values for second harvest were found to be 289 mg/kg (T_2), 253 mg/kg (T_3), 252 mg/kg (T_4) and 320 mg/ kg (T_1) in control soil. Copper concentration in amaranth was reduced from 48.5 mg/kg in control to 40.7 mg/kg, 34.5 mg/kg and 33.7 mg/kg in on application of bentonite @ 0.5, 1.5 and 2.5%, respectively in first harvest. Similarly, in second harvest the copper content was reduced to 43.1 mg/kg, 40.7 mg/kg and 33.8 mg/kg in 0.5, 1.5 and 2.5% bentonite amended soil, respectively from the control

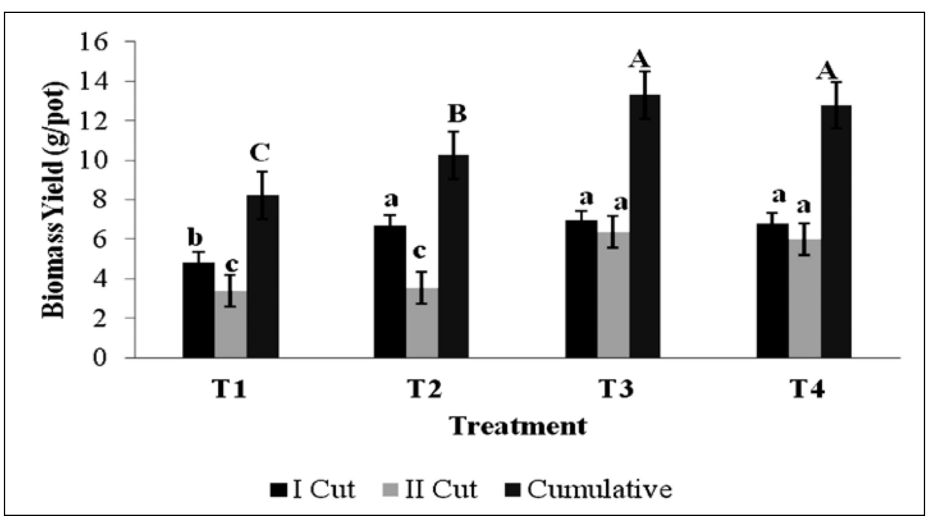

Fig. 1. Effect of bentonite levels on biomass yield (g/pot) of amaranth.

Table 1. Effect of bentonite levels on metal concentration (mg/kg DW) in amaranth.

Treatment	Zinc		Copper		Nickel	
	Harvest I	Harvest II	Harvest I	Harvest II	Harvest I	Harvest II
T_1	349^c	320^c	48.5^c	48.2^c	48.5^c	52.1^c
T_2	339^b	289^b	40.7^b	43.1^b	45.3^c	39.3^b
T_3	329^a	253^a	34.5^a	40.7^b	39.1^b	38.4^b
T_4	326^a	252^a	33.7^a	33.8^a	31.8^a	31.1^a
CD (P = 0.05)	9.5	6.9	3.8	3.9	5.2	4.5

of 48.2 mg/kg. Amendment of soil with bentonite @ 0.5, 1.5 and 2.5% reduced the nickel content by 6.6, 19.4 and 34.4%, respectively in first harvest and 24.4, 26.2 and 40.2%, respectively in second harvest of amaranth. 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. The labile fraction of heavy metals extracted by DTPA and EDTA were significantly reduced by application of bentonite (Fig. 2). Amendment of soil with 2.5% bentonite (T_4) resulted in 15.8, 18.6 and 31.6% reduction of DTPA extractable Zn, Cu and Ni, respectively over the control (T_1). Similarly T_4 reduced the EDTA extractable Zn, Cu and Ni to 139, 44 and 35 mg/kg, respectively from 149, 61 and 41 mg/kg in 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. Application of bentonite raised the soil pH from 8.30 to 8.53 at 2.5% bentonite application (Fig. 3). The raise in pH could be one of the mechanisms of reduced availability of metal ions due to lower solubility of metal ions at higher pH by formation of precipitates (Malandrino *et al.*, 6; Usman *et al.*, 11).

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 Zn varied from 1.78 (T_1) in control to 1.61 (T_2), 1.49 (T_3) and 1.34 (T_4) 0.47 in soil amended with 0.5, 1.5 and 2.5% bentonite, respectively at first harvest. At second harvest BCF of Zn reduced by 8.1, 18.3 and 28% over the control soil by application of 0.5, 1.5 and 2.5% bentonite, respectively. Similarly, BCF of Cu for amaranth varied between 0.37 to 0.23 and 0.33 to 0.21 at first and second harvest, respectively. Amendment of soil with bentonite @ 2.5% reduced the BCF of Ni significantly at first (44.4%) and second (34%) harvest. The BCF decreased in the order of Zn > Cu > Ni for amaranth in both the harvests. Addition of bentonites significantly reduced the translocation of metals to the plants as indicated by the values of BCF.

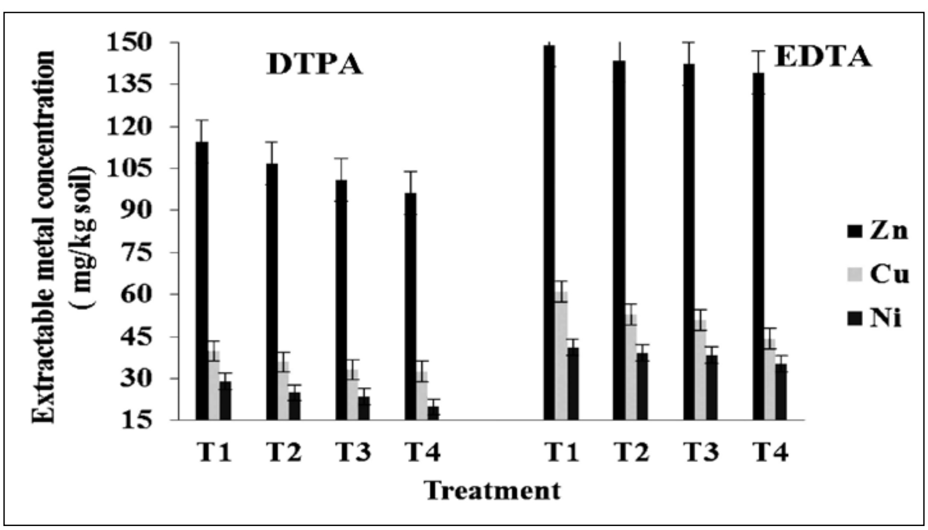

Fig. 2. Effect of bentonite levels on extractable heavy metals in soil.

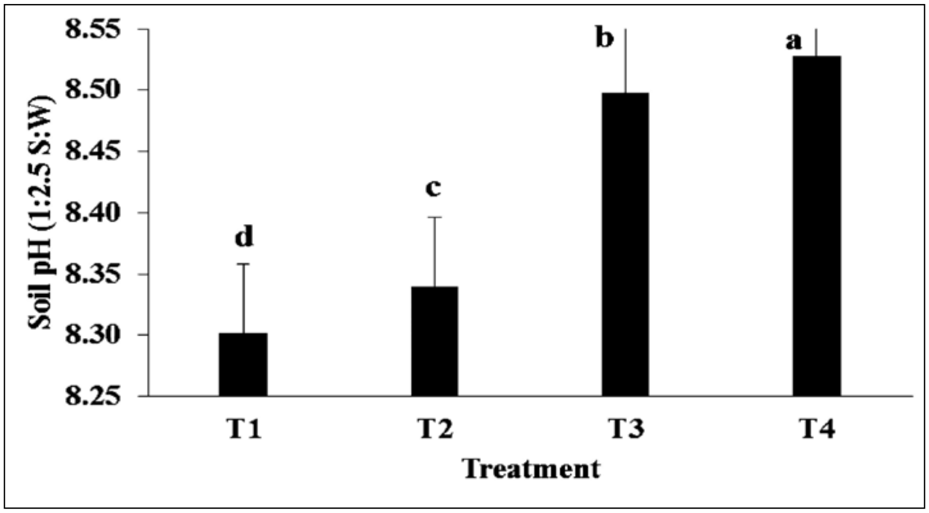

Fig. 3. Effect of bentonite levels on soil pH value.

Table 2. Effect of bentonite levels on bioconcentration factor of metals in amaranth.

Treatment	Zinc		Copper		Nickel	
	Harvest I	Harvest II	Harvest I	Harvest II	Harvest I	Harvest II
T_1	1.78^d	1.40^d	0.37^b	0.33^b	0.53^c	0.44^{bc}
T_2	1.61^c	1.29^c	0.32^b	0.29^{ab}	0.50^c	0.40^b
T_3	1.49^b	1.14^b	0.27^a	0.27^a	0.40^b	0.36^b
T_4	1.34^a	1.01^a	0.23^a	0.21^a	0.29^a	0.29^a
CD (P = 0.05)	0.11	0.12	0.06	0.07	0.08	0.04

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.*, 12). To assess the efficiency of 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 Zn reduced to 0.33, 0.29, 0.26 and 0.41, 0.38, 0.34 for 0.5, 1.5 and 2.5% bentonite amended soil from 0.36 and 0.45 in control at first and second harvest, respectively. Application of bentonite @ 0.5, 1.5 and 2.5% significantly reduced the HQ of Cu by 13.6, 27.3, 40.9% and 10.5, 15.8, 36.8% over the control at first and second harvest, respectively. At first harvest Ni HQ reduced to 0.59, 0.47 and 0.34 by incorporation of bentonite @ 0.5, 1.5 and 2.5% from 0.62 in the control soil. Significant reduction in HQ of Ni was observed at second harvest and the percentage reduction was found to be 8.9, 17.7 and 32.7 on application of 0.5, 1.5 and 2.5% bentonite, respectively. The reduction in hazard quotient on application of 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 indicates that 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 Ni exceeded 0.5 in control soil and amendment with bentonite reduced the values below the safe limlit 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 bentonite @ 2.5% demonstrates the best effectiveness towards the immobilisation of heavy metals in soil. The method

Table 3. Effect of bentonite levels on hazard quotient of metal through consumption of amaranth.

Treatment	Zinc		Copper		Nickel	
	Harvest I	Harvest II	Harvest I	Harvest II	Harvest I	Harvest II
T_1	0.36^d	0.45^d	0.02^d	0.02^{bc}	0.62^d	0.51^d
T_2	0.33^c	0.41^c	0.02^c	0.02^b	0.59^c	0.47^c
T_3	0.29^b	0.38^b	0.02^b	0.02^b	0.47^b	0.42^b
T_4	0.26^a	0.34^a	0.01^a	0.01^a	0.34^a	0.35^a
CD (P = 0.05)	0.01	0.01	0.01	0.01	0.04	0.02

described enables application of 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.

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