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Effect of organic amendments on morphological, physiological and root characteristics of red amaranth (*Amaranthus gangeticus***) grown on Cd contaminated saline soils**

M. Z. Hossain^{1*}, M. A. Islam², M. A. Islam³ and K. Q. Kibria¹

1 Soil, Water and Environment Discipline, Khulna University Khulna 9208, Bangladesh 2 Environmental Science Discipline, Khulna University, Khulna 9208, Bangladesh 3 Forestry and Wood Technology Discipline, Khulna University Khulna 9208, Bangladesh

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Abstract

A pot experiment was conducted to investigate the effects of six different types of organic amendments (OAs) applied to Cd contaminated saline soils with varying rates on red amaranth (*Amaranthus gangeticus*) growth. Two levels of salinity (4 dSm⁻¹ and 8 dSm⁻¹) were developed in the experimental soil that was previously contaminated with 5 mg kg⁻¹ Cd. In slightly (4 dSm⁻¹) saline soil, 1% vermicompost (VC) produced 18.77 times higher fresh weight as compared to the control while 32.37 times higher found with 2% cowdung (CD). VC produced the maximum fresh weight in moderately saline soil (8 dSm⁻¹). Other morphological traits were improved with the addition of OAs. VC increased the plant's relative water content, chlorophyll-a and chlorophyll-b. Root characteristics were improved irrespective of the OA rates and salinity. The efficiency of OAs followed the order VC=CD>compost>WT>SD=RH. Therefore, OA could be a promising way of alleviating the saline soil contaminated with Cd.

Keywords: Cd; Salinity; Red amaranth; Morphology; Physiology; Root

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Introduction

Heavy metal contamination of soils has gained global attention due to their hazardous impacts of on soil quality, crop yield and quality (Seleiman and Kheir, 2018), and food safety and human health (Antoniadis *et al*. 2017; Bashir *et al*. 2020). Among the heavy metals, cadmium (Cd) is one of the most toxic and mobile contaminants in surface soils that may arise from anthropogenic sources (Okrikata and Nwosu, 2023; Patil *et al*. 2024). The toxic effects of Cd on plants are well-documented (Azhar *et al*. 2019; Rehman *et al*. 2020). These effects ultimately result in cell death and pose a serious threat to agricultural productivity and food safety (El Rasafi *et al*. 2020; Yildirim *et al*. 2023). The impact of Cd on plants is a matter of great concern due to its potential threat to human health primarily through dietary intake via the food chain (Ma *et al*. 2021; Islam *et al*. 2023; Ghasemi-Soloklui *et al*. 2023). Therefore, it is essential to explore and implement effective remediation strategies to mitigate Cd contamination and its adverse effects on the environment and human health (Ahmad *et al*. 2023; Xing *et al*. 2023).

High levels of salinity in soil is another environmental stress that has rapidly increased worldwide. Climate change, limited rainfall, human induced causes are mainly responsible for increasing salinity in soil. These factors lead to diminish agricultural yields on a global scale and creates a great challenge to global food security (Yupeng *et al*. 2018). Salt stress in soil has been shown to reduce plant growth, biomass, and mineral nutrient uptake (Loudari *et al*. 2022).

Soil salinity has an important impact on elevated levels of bioavailable Cd (Lutts and Lefèvre, 2015). Because salt-based ions can form complexes with Cd and increase Cd mobility (Abbas *et al*. 2018; Parvin *et al*. 2022; Wang *et al*. 2023). Moreover, cations present in salts can compete with Cd for soil adsorption sites. This phenomenon allows the desorption of Cd from the exchange complex which increases Cd mobility in soil. In addition, the ionic strength and pH of the soil solution are altered by soil salinity which affect Cd bioavailability (Raiesi *et al*. 2018).

The stress that salinity and Cd imposed in soil can be reduced with a focused remediation program. According to research, there are several methods available today for improving the physical, chemical, and biological characteristics of soil (Abbas *et al*. 2018; Loudari *et al*. 2022; Wang *et al*. 2023). The use of organic amendments (OA) in soil is a cost effective strategy for reclaiming saline soils contaminated with Cd and improving crop productivity (Alcívar *et al*. 2018; Gangwar *et al*. 2020; Leogrande and Vitti, 2019). Likewise, OA can function as a biosorbent to facilitate the sorption of Cd from contaminated soils (Anastopoulos *et al*. 2013).

Red amaranth (*Amaranthus gangeticus*) is a popular leafy vegetable in Bangladesh because of its low price, quick-growing character, and higher yield potential (Miah *et al*. 2013). The leaves and stems of red amaranth are rich in protein, fat, Ca, K, P, Na, Fe, ascorbic acid, niacin, and riboflavin (Prakash and Zaidi, 2000). However, red amaranth plant may intake excess amount of heavy metals which can pose threat to the people upon consumption (Adewuyi *et al*. 2010; Guo *et al*. 2020).

The utilization of OA can alleviate the deleterious effects of salinity and Cd stress which leads the improved growth of red

amaranth plants. This could reduce threats to food security and enhance the health of the soil. This study aims to investigate how different OAs affect the morphological, physiological, and root characteristics of red amaranth plants growing in salinity and under cadmium stress.

Materials and methods

Soil and amendments

Before the experiment, surface soil sample (0-30 cm) was collected from the agricultural field of Khulna university campus (22.8022 \degree N, 89.5339 \degree E). The soil sample was prepared by air drying, grinding, sieving through a 2 mm mesh size sieve, mixing and was homogenized before filling the pots. Approximately 0.5 kg of the prepared soil was preserved for the basic physico-chemical characteristics of the studied soil. Cadmium chloride salt $(CdCl₂.2H₂O)$ was added to maintain a contamination level of 5 mg Cd kg-1 soil. In this polluted soil, two salinity levels e.g. slightly saline (4 dSm^{-1}) and moderately saline (8 dSm⁻¹) were developed by adding NaCl, CaCl₂ and $MgCl_2(1:1:1)$. For obtaining the desired Cd and salt levels, soil was spread over a polythene sheet, field capacity water was maintained and left the soil for one month. After one month, cowdung (CD), vermicompost (VC), waste tea leaves (WT), saw dust (SD), rice hull (RH) and ACI commercial organic fertilizer (compost) were added to the Cd laden saline soil with 1% and 2% rates. A control treatment was also kept where no organic amendment was added. The general properties of soil and organic amendments used in this experiment are described in Table I.

Properties	Unit	Soil	CD	VC	WT	SD	RH	Compost	
Sand	$\frac{0}{0}$	14.25			-				
Silt	$\frac{0}{0}$	50.57	-	$\overline{}$	$\overline{}$	$\overline{}$		$\,$	
Clay	$\frac{0}{0}$	35.18	$\qquad \qquad$		$\overline{}$	-		-	
Texture	-	Silty Clay Loam							
pH		7.86	7.31	7.08	6.83	7.37	7.46	8.02	
EC	dSm^{-1}	1.90	3.03	0.49	0.78	0.12	0.09	1.92	
CEC	Cmolc/kg	48	219	68	310	171	226	45	
OC	$\frac{0}{0}$	1.28	24.57	14.82	34.13	27.01	29.32	5.07	
Total N	$\frac{0}{0}$	0.04	1.48	0.90	1.08	0.97	0.91	0.48	
Total P	$\frac{0}{0}$	0.03	0.21	0.30	0.21	0.04	0.05	0.19	
Total K	$\%$	0.05	0.10	0.09	0.26	0.79	0.68	0.10	
Total Cd	$mgkg^{-1}$	2.60	0.32	0.05	0.05	0.05	0.05	0.49	
Bioavailable Cd	$mgkg^{-1}$	0.50	0.001	0.001	0.001	0.001	0.001	0.001	

Table I. General properties of soil and organic amendments

Pot experiment

The experiment was laid out following a completely randomized design with three replications. Ten red amaranth seeds were sown into each pot (16 cm diameter and 18 cm height) containing five kg of organic amended and control soil. After seed germination, 5 seedlings in each pot were maintained. Irrigation was done according to the plant requirement of water and moisture content present in the soil using tap water. After 2 months, the mature plants were harvested and further plant and soil analyses were performed.

Plant analysis

After collecting the leaves from the top, the relative water content (RWC) was calculated according to the following formula:

$$
RWC = \frac{FW - D W}{TW - D W} 100
$$

Where, $FW =$ fresh weight of leaves, $TW =$ turgid weight of leaves, DW = dry weight of leaves

To avoid moisture loss from the leaves, the FW was taken as soon as possible after leaf collection. For obtaining the TW, the leaves were kept in a container filled with distilled water for 12 hours until the leaves reached a constant weight which was considered 100% hydration. The TW was determined immediately after removing the leaves from the water. The DW was taken after oven drying fully turgid leaves for 48 hours at 70°C (Turner, 1986). The chlorophyll-a (chl-a) and chlorophyll-b (chl-b) content of the plant was determined by Arnon's (1949) technique. Morphological data for shoot and root were collected using standard methods. Root length was measured by the grid method (Delory *et al*. 2017).

Soil analysis

After the crop harvest, soil samples were taken from each pot, and soil pH and EC were measured. Soil pH was determined by taking 20 g air dried soil where soil to distilled water ratio was 1(w):2.5(v) using a glass membrane electrode pH meter (Hanna, HI110, USA). (Li *et al*. 2005). For the determination of soil EC, 10 g air dried soil was taken with soil to distilled water ratio was $1(w):5(v)$ was determined using a glass membrane electrode EC meter (Hanna, HI2315, USA) (Hardie and Richard, 2012). Bioavailable Cd contents from each potted soil were estimated using the CaCl, extractable technique described by Houben *et al*. (2013).

Statistical analysis

Collected data were analyzed using the statistical package "Statistix 10.0". One-way analysis of variance (ANOVA) technique was used to test the significance of the data. Microsoft Excel was used for the calculation of standard errors of means and graphical presentation. The differences among treatment means were assessed using the Tukey HSD test at 5% probability.

Results and discussion

Morphological characteristics

Fresh weight (g/pot) of red amaranth improved after the addition of organic amendments in saline soil contaminated with Cd (Fig. 1a). In slightly saline soil (4 dSm^{-1}) , application of 1% VC, enhance the fresh weight by more than 18 times as compared to control. This was further enhanced with compost (11 times) followed by CD (8 times) and RH (6 times). However, the increment in fresh weight after the application of WT and SD did not vary significantly. There was a dramatic change in the fresh weight of red amaranth after the application OA with 2%. Here, CD showed the highest increase followed by VC and compost. In moderately saline soil (8 dSm⁻¹), VC increase the red amaranth's fresh weight applied either 1% or 2% rate. But the addition of VC at higher rate was not significant as compared to the low rate of application. CD also showed greater increase in the fresh weight applied to high saline soil. However, other types of OA had relatively less influence on the change in the fresh weight of red amaranth under this condition. Dry weight of red amaranth plant showed variations with varying the types and rates of OA in the experiment (Fig. 1b). In slightly saline soil, lower rate (1%) of VC influenced the dry weight more than that of the other types of OAs. On the other hand, highest dry weight was obtained with CD after rising the rate to 2%. Conversely, VC followed by CD, was found effective for increasing the dry weight applied to moderately saline soil invariably the rate used. Height of the red amaranth plant was also changed as a consequence of the application of OA (Fig. 1c). Application of VC at low rates showed the highest plant height (20.07cm) which did not differ from the height obtained with the application of compost (16.93cm) under low soil salinity. CD produced a height of 13.34 cm under this condition and was insignificantly varied with the height of plant obtained with compost. In this soil, the change in plant height was found only for CD with higher rate (2%) of application. However, in moderately saline soil, CD and VC behave similarly with 1% application rate but the behavior of CD and VC changed when applied with 2% rate. Change in leaf area of red amaranth plant after OA application was also studied under Cd contaminated saline soils (Figure 1d). In slightly saline condition, leaf area was found highest with the application of 1% VC which was 61.43 cm². Compost and CD produced a leaf area of 44.10 cm^2 and 30.85 cm^2 respec-

Fig. 1. Effects of organic amendments on (a) fresh weight (g/pot) (b) dry weight (g/pot) (c) height (cm) and (d) leaf area (cm2) of red amaranth plant grown on Cd contaminated saline soils. Bars with the same letter are not significantly different at p<0.05 level.

tively. Whereas, the response of other OA was found lower in terms of leaf area. The increased rate of OA's further increased the leaf area of red amaranth but noticeable increase was obtained with CD, VC and compost. In moderately saline soil, OAs behave similarly with the application of either 1% or 2% rate. The order for the increase leaf area followed VC>CD>ACI>WT>SD=RH.

After the treatment of Cd contaminated saline soil with various OAs, the improvement in red amaranth biomass might be attributed to the nutritious significance of OA that improves the soil productivity. Other possible reason might be considered because of their potential to boost the organic

matter mineralization and enhanced crop yield and growth (Shinomol *et al*. 2016; Abbas *et al*. 2020; Ali *et al*. 2020). VC played an important role for increasing plant growth due to its highly porous nature and high nutrients and minerals contents. Such porous structure enhanced physiochemical properties of soil that have a great impact on the movement of nutrients, water and air through soil. It stimulates plant growth and have a significant effect on the absorption efficiency (Blouin *et al*. 2019). Our study revealed that the cation exchange capacity of CD (219 Cmolc/kg) and VC (68 Cmolc/kg) create sufficient voids contributed to adsorption of cadmium from soil. Similar to findings of Adamipour *et al*. (2019) who reported application of vermicompost increased the morpho-physiological indices and mineral nutrient uptake in the plants and could increase the plant yield by alleviating the harmful effects of salinity.

Physiological Characteristics

The effects of various OA's on the relative water content (RWC) in the leaves of red amaranth plant grown under Cd contaminated saline soil is described in Fig. 2a. In slightly saline soil, application of 1% VC, WT, RH and compost produced RWC with insignificant variations. Lower RWC were obtained with the CD and SD. However, with the increased rate of OA, CD increased the RWC in the leaf of the plant. In moderately saline soil, lower RWC were found for WT, SD and RH applied at 1% rate. But when the rate of application increased in this soil, WT regained the RWC in the leaf of the plant as compared to SD and RH. The result indicated that SD and RH were ineffective for the enhancement of RWC in all cases in the study. The results revealed that addition of various kinds of OA can increase chlorophyll-a (chl-a) content in the leaves of red amaranth plant (Fig. 2b). In slightly saline soil (4 dSm^{-1}) , the greatest increase in chl-a in the leaf was recorded by approximately 2.5 times when added the VC either at 1% or 2% over control. Likewise, incorporation of CD, WT, SD, RH and ACI also increased the chl-a content but no significant variations were observed when applied at both rates. The scenario was quite different in moderately saline soil (8 dSm⁻¹) in which the chl-a content doubled with the application of 1% WT. However, VC and ACI acted quite similar in this condition. CD, VC, WT and ACI raised the chl-a content invariably with increased rate but the efficiency was found low. Chlorophyll-b (Chl-b) content in the leaf of red amaranth plant showed variations with the added OA in saline soils contaminated with Cd (Fig. 2c). When 1% OA was added to low saline soil, chl-b content increased over control but the variations were insignificant irrespective of the OA. But the behavior of CD, VC, WT and RH with 2% application rate were similar in soil with low salinity. On the contrary, SD and ACI produced low amount of chl-b in the leaf at this rate of

Fig. 2. Effects of organic amendments on (a) RWC (%) (b) Chl-a (mg/g FW) and (c) Chl-b (mg/g FW) of red amaranth plant grown on Cd contaminated saline soils. Bars with the same letter are not significantly different at p<0.05 level.

application. In moderately saline soil, CD showed the highest chl-b (approximately 3 times) followed by WT (approximately 2.5 times) over control. However, with 2% application rate, OA showed increased chl-b content but the values did not vary among themselves.

The addition of OA (CD, VC, WT, SD, RH and compost) effectively minimized the Cd mobility in contaminated soil which might be possible due to the presence of organic substance and functional groups that have great contribution to stabilize Cd through complexation. Furthermore, influence of soil salinity was also reduced with the application of OA. OA increase the porosity of saline soil through which salts may exclude from the soil upon irrigation. The reduced Cd

entry to plant cells make a barrier for lipid peroxidation in the cell. In addition, oxidative stress is reduced in the absence of salinity. This might be the cause of increasing chl-a and chl-b content in the leaves. Shahid *et al*. (2019) found that organic amendments alleviated Cd toxicity in terms of Cd content, lipid peroxidation to bean plants.

Root characteristics

Root fresh weight (RFW) as affected by the addition of various OA on Cd contaminated saline soils depicted in Fig. 3a. The highest RFW was recorded with the application of 1% VC which was 10.11 g/pot in slightly saline soil. This is followed by compost $(4.2 \text{ g}/\text{pot})$, CD $(3.01 \text{ g}/\text{pot})$, RH

Fig. 3. Effects of organic amendments on (a) Root fresh weight (g/pot) (b) Root dry weight (mg/pot) and (c) Root length (cm) of red amaranth plant grown on Cd contaminated saline soils. Bars with the same letter are not significantly different at p<0.05 level

(1.78 g/pot), WT (0.48 g/pot) and SD (0.43 g/pot). After the application of 2% rate, the highest RFW was found with CD which was 15.08 g/pot. RFW was found 11.65 g/pot with VC and 6.78 g/pot with ACI. In moderately saline soil, maximum RFW was found with VC at 1% application rate which was 7.47 g/pot followed by CD which was 4.96 g/pot. With 2% application rate, CD yielded the highest RFW which was 9.60 g/pot and VC produced 7.22 g/pot. Root dry weight (RDW) of red amaranth plant was also affected by the addition of different OA (Fig. 3b). In slightly saline soil, 1% VC showed the highest RDW which was 766.67 mg/pot. Next to VC, compost and CD yielded RDW of 250 mg/pot and 130 mg/pot respectively. However, 2% application rate increase twice the RDW with CD and VC. Root length (RL) was affected by the application of various OA in Cd contaminated saline soils (Fig. 3c). In slightly saline soil, application of 1% ACI exhibited the highest RL which accounted more than 10 times higher than that of control. Increased RL were also found with 2% rate of application of CD, VC, WT and compost but no variations were obtained in this treatment. In moderately saline soil, CD, VC and WT increased the RL in a same way after the application either 1% or 2% rate. The addition of OA caused noticeable improvement in soil properties. This creates a more supportive environment for plant growth and yield. Several researches pointed out that upon decomposition OA provides binding sites for plant nutrients, improve soil structure, and increase microbial activity (Nardi *et al*. 2021; Rashad *et al*. 2022; Tiwari *et al*. 2023). As evidenced from our findings, VC treatment led to the maximum increase in plant growth parameters including root characteristics of plant due to these positive effects of OA on soil fertility and structure.

Conclusion

This study demonstrated the effects of various organic amendments (OA) on the morphological, physiological and root characteristics of red amaranth (*Amaranthus gangeticus*) plants grown in Cd contaminated saline soils. OA application improved the growth parameters depending on the types and rates of OA added to the soil. In slightly saline soil (4dSm⁻¹), morphological, physiological and root characteristics were boosted with VC applied at 1% rate. These parameters were further enhanced with the application of 2% CD and VC. However, WT played an important role in the enhancement of chl-b and RL with higher rate. In moderately saline soil (8dSm⁻¹), growth parameters were accelerated with 1% or 2% of VC and

CD. The findings revealed that VC applied at either 1% or 2% rate plays a dominant role in the enhanced growth parameters of red amaranth plant. Therefore, it is recommended to use VC for the cultivation of red amaranth in Cd contaminated saline soils.

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