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Potential of Okra as a hyperaccumulator plant under cadmium exposure

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ABSTRACT

Cadmium (Cd) is a non-essential element and one of the most toxic heavy metals due to its high toxicity for all living organisms. It is easily taken in by plants owing to its high bioavailability, enters the food chain and threatens people's health. Okra (*Abelmoschus esculentus*) was examined for phytoremediation purposes with the focus on accumulation of Cd and physiological mechanisms of Cd tolerance. Pot experiment for 60days was conducted to investigate the effects of different concentrations of Cd (0, 5, 25, 50, 100 and 250 mg kg^{-1} Cd in soil) on okra plant through different physiological and biochemical parameters: plant dry matter content, leaf pigment content, leaf soluble protein, proline activity, antioxidant enzyme of peroxidase (POD) and Cd content. Exposure to Cd enhanced plant growth at 50 mg kg^{-1} without showing symptoms of visible damage and when the Cd concentrations increased to 100 and 250 mg kg^{-1}, the okra biomass were reduced through increasing Cd-stress. The activity of POD was significantly enhanced with increase in Cd concentration. The Cd accumulation in the shoots (stems and leaves) of okra was lower than that in roots and the Cd content in the shoots increased with increasing Cd concentration. The fruit Cd content decreased with increasing Cd treatment which indicated limited ability of transferring Cd from root to fruit. This study suggests that okra has some remediation ability against heavy metal polluted contaminated soil and has potential to decontaminate Cd-polluted soil.

INTRODUCTION

Heavy metal pollution of the environment is caused by human activities such as the discharge of municipal waste, mining activities, smelting, metal manufacturing, and excessive use of pesticides and fertilizers. Contamination of soils with heavy metals impairs ecosystems and human health (Hassan et al., 2018). Among metals, Cadmium is highly toxic to living organisms and humans (Chellaiah, 2018).Cadmium is recognised as a non-essential element and it is ranked fourth in the list of highly toxic elements for plant growth, even at relatively low concentrations (Abdal et al., 2021).Normally, heavy metals present in excess are toxic for plants, which evolved different strategies to avoid the toxic effects of metal including chelation, sequestration, storage and efflux (Karalija et al., 2021). Generally, Cd interferes with the transport and uptake of Ca, P, Mg, K, and Mn (Nazar et al., 2012). World Health Organization (WHO) and Food and Agriculture Organization (FAO) of the United Nations recommend a provisional tolerable weekly intake (PTWI) for Cd of no more than 7 mg/kg of body weight (Munoz et al., 2005). Cadmium causes a wide range of impairments to plant biochemical, physiological and morphological functions (Zhang et al., 2020). Several studies showed that plant metabolism is affected by Cd in different ways; toxicity of Cd caused chlorosis due to reduced plant uptake of Fe and Zn (Xu et al., 2017). Higher toxicity causes stunted growth, necrosis, decreasing chlorophyll content, reduced photosynthetic activity, reduced stomatal conductance (Haider et al., 2021), over-production of Reactive oxygen species (ROS), damage to plant membranes and destruction of cell biomolecules and organelles (Abbas et al.,

2017). Cd toxicity causes overproduction of ROS including hydrogen peroxide (H2O2), superoxide (O^{-}), hydroxyl (•HO) radicles and singlet oxygen, severe damages to carbohydrates, lipids, proteins and nucleic acids in plants (Rehman et al., 2019). The effects of these ROS are mitigated by boosting the activities of antioxidant enzymes (Amjad et al., 2021).The toxic impact of Cd at the high dosages on radish (Varalakshmi & Ganeshamurthy, 2013, 200 mg/kg), wheat (Khan, 2007,100 mg/kg), rice (Huang, 2008,150 mg/kg; Lin, 2016, Cao, 2015, 100 mg/kg), mustard (Gill, 2011, 150 mg/kg),okra (Hameed, 2011, 200mM), and *Abelmoschus manicot* (Wu, 2018, 100 mg/kg) were reported previously. Hence, the present study was aimed to evaluate the tolerance potential of *Abelmoschus esculentus* under high exposure level of Cd for 60days. Okra is one of the most important vegetable members of the Malvaceae family. Okra is grown throughout the tropical and subtropical regions of the world and in warmer parts of the temperate zone. India is the largest producer of okra globally, with a contribution of more than 72% (6 million tonnes) from an area of 0.5 million hectares (NHB, 2020). Tender pods of okra are used as a delicious vegetable. *Abelmoschus esculentus* is a widely used vegetable plant having many industrial and medicinal properties. It is a good source of vitamins A and B, protein and minerals. Dry fruit skin and fibre are used in the manufacture of paper, cardboard and fibre (Ranga et al., 2019).

MATERIALS AND METHODS

1.1. *Plant material and experimental conditions:*

Okra, Arka Anamika (Bhindi) variety was used for this experiment. Seeds of okra (*Abelmoschus esculentus*), hybrid variety Arka Anamika was procured from agro seed stores, Tirunelveli. Seeds were surface sterilized with 0.1% sodium hypochlorite for 5 min. Seeds were thoroughly washed with distilled water and sown in plastic pots with a diameter of 26 cm and a height of 13.5 cm capacity of 2.5 kg. The bottom was covered with plastic tray for water retention. Each pot was then filled with 2 kg of red soil. The experiment consisted of six treatments including one control. Cadmium was used in the form of cadmium chloride which was mixed thoroughly at five levels: 5, 25, 50, 100 and 250 mg kg^{-1}. The soil was additionally supplied with N(NH3NO3), P(KH2PO4) and K(K2SO4) fertilizer to avoid nutrient deficiency as reported by Chi et al.,(2017). The fertilizer was added to the soil in four doses (on days 0, 15, 30 and 45). Each treatment was replicated five times. Four seeds were germinated in each pot. The pots were placed inside the green house below 25˚C for seedling growth. The pots were watered every second day to ensure adequate water content. Plant samples were collected on 15^{th}day, 30^{th}day and 60^{th} day. After 60 days, the plants were harvested from the pots and separated into shoots, roots, and fruits and used for further analysis.

1.2. *Plant biomass*

The roots were separated from the aerial part and then rinsed three times with tape water followed by deionized water. The shoots and roots were oven-dried at 60˚C for overnight. All parts were separately weighed for dry matter content.

1.3. *Determination of photosynthetic pigment*

According to the method of Hiscox and Israelstam, (1979), the content of chlorophyll a and b as well as total carotenoids (Carotene) were determined spectrophotometrically. The samples were collected from the third leaves and pigments were extracted using DMSO (Dimethyl sulfoxide) in a water bath for 30 minutes at 60°C. The absorbance of the resulting extracts was measured at 645, 663 and 470 nm against the DMSO blank.

1.4. *Determination of soluble protein concentration*

The soluble protein concentration was determined according to Bradford (1976) using bovine serum albumin (BSA) as the standard protein.

1.5. *Determination of proline*

Free proline content in leaves was determined according to Bates et al., (1973). Plant materials were homogenized in 10 mL of 3% aqueous sulphosalicylic acid. To 2 mL of filtrate in a test tube, 2 mL of glacial acetic acid and 2 mL ninhydrin were added and heated in the boiling

water bath for 1 h. After that 4 mL toluene was added to the reaction mixture. Toluene layer was separated and the absorbance was recorded at 520nm and proline concentration was calculated using a proline standard calibration curve.

1.6. *Determination of peroxidase (POD)*

Fresh leaf tissue (1g) was homogenized in 3 ml phosphate buffer (0.1M, pH 7.0) using pre-cooled mortar and pestle. The homogenate was centrifuged at 18,000 g at 5˚C for 15 min. The supernatant was used for the assay of the activity of POD. The activity of POD was determined in terms of oxidation of guaiacol by measuring the increase in absorbance at 470 nm (Putter, 1974). The reaction solution was composed of 3 ml buffer (pH7.0), 0.05 mL guaiacol, 0.1 mL enzyme extract and 0.03 mL hydrogen peroxide solution. The reaction was started by addition of H2O2 and the increase in absorbance was recorded at 470 nm.

1.7 Total Cadmium determination

Samples were dried in an oven at 60˚C. A weight of 0.1 g of dried ground sample was placed in a 75mL Kjeldahl digestion tube and 5mL of concentrated HNO3 was added (Mahfoud et al., 2018). The sample was digested in a KJEL-E-TEK (Kjeldahl) Block digestion for 45 min at 90˚C, and then the temperature was increased to 170˚C until a clear solution was obtained. Concentrated HNO3 was added to the sample (2mL was added at least one times) until the volume was reduced to about 1mL. After cooling, the solution was filtered with whatman No.42 filter paper; made up to 25mL volumetric flask by adding distilled water. It was then transferred to a 50mL polyethylene bottles. The resulting solutions were appropriately diluted with distilled water and analyzed by ICP-OES (Perkin Elmer Optima 5300 DV).

1.8 *TF (Translocation Factor) and BFs (Bioaccumulation Factor)*

The translocation factor (TF) of Cd from shoot to root was calculated as follows:

TF=shoot/root element content

Where, Shoot=Cd in shoots ($\mu g g^{-1}$)

Root=Cd in roots ($\mu g g^{-1}$).

BCF=shoot/soil cadmium content

Where, Shoot=Cd in shoots ($\mu g g^{-1}$)

Soil=Cd concentration in soil ($mg\ kg^{-1}$)

1.9 *Data analysis*

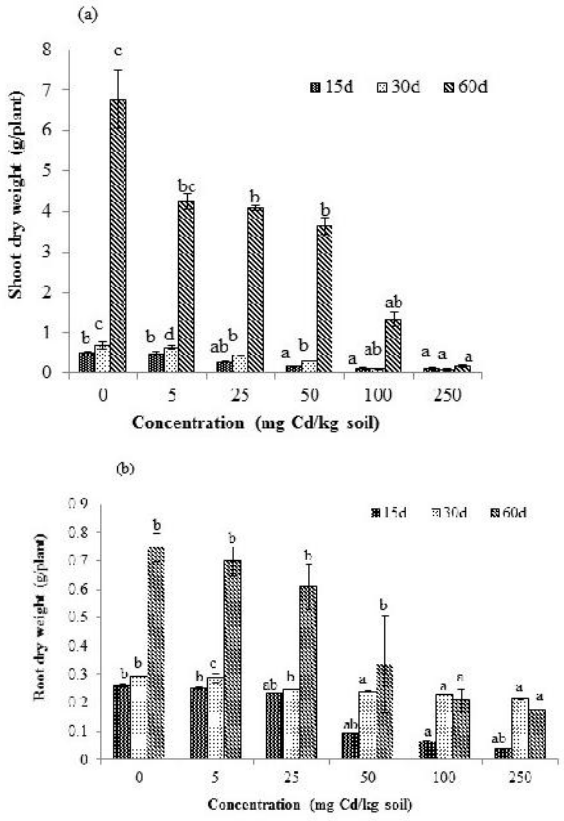
The experiments were established in a completely randomised block design, with six treatments, five replications per each treatment and four plants per pot. The data were processed using IBM SPSS Statistics 22.0 version. The values presented are means (±SD) from three independent repetitions. Data were analysed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests at $P \leq 0.05$ confidence levels.

2. RESULTS AND DISCUSSION

2.1. Effects of Cd concentration and exposure time periods to Cd stress on okra shoot and root dry weights

Under Cd stress, the dry weights of *Abelmoschus esculentus* (Okra) shoot and root were significantly decreased with increase in Cd concentration. Shoot dry matter of 15th day and 30th day samples decreased from 0.52g/plant to 0.13 g/plant and from 0.7 g/plant - 0.11 g/plant, respectively (Figure 1). Similarly, root dry matter also decreased from 0.26 g/plant to 0.03 g/plant and 0.29 g/plant to 0.21 g/plant, respectively. After 60 days of growth, the dry weight of shoots and roots significantly decreased with increasing Cd concentration from 6.8 g/plant to 0.18 g/plant and 0.75 g/plant to 0.18 g/plant, respectively, when compared with control. Heavy metals may disrupt important bio-processes of plant by irreversible substitution with other essential elements in vital enzymes and therefore, reduce plant growth (Zhixin et al., 2013). Our results are in agreement with those of Yin et al., (2021), Zare et al., (2020), Zhao et al., (2021), and Zhang et al., (2021) who reported that high levels of Cd inhibited the growth of different plant species. Exposing okra plants to different Cd concentrations (5, 25 and 50 $mg\ kg^{-1}$ Cd) for 15 days reduced shoot dry weights by 0.83%, 0.33% and 0.50% compared to control treatment (0 mg Cd), respectively. However, the reduction in root dry weights was not significantly different for 15 days old okra plants when exposed to Cd stress as reported previously (Zare et al., 2020). The Cd stress led to chlorosis and rolling of the basic leaves,

decreased growth and number of leaves. Additionally, 100 and 250 mg kg^{-1} Cd treatment twisted the upper part of leaves and reduced the plant growth. Zhang et al., (2021) reported that high concentrations of Cd twisted the upper leaves and there was a significant reduction in biomass of Canna plant. A few studies reported that lower Cd concentration provoked the plant growth while higher concentration inhibited the growth of plant (Yu et al., 2020) which led to dwarfed growth, black roots, slightly curly leaves, which was the physiological response. With increasing concentrations of Cd, the fruit dry weight decreased significantly when compared with control. After 60days of growth, the fruit dry matter content decreased in the 5, 25, and 50 mg kg^{-1} Cd treatments and the dry weight significantly decreased from 6.14 g/plant to 0.42 g/plant. There was no fruit formation in 100 and 250 mg kg^{-1} Cd treatments indicating that higher dosages affected the plant growth and reproductive capacity severely. These results are in agreement with the reports of Sharma et al., (2010), Raychaudhuri, (2019) and Olivera, (2012) in *Abelmoschus esculentus* and Labidi et al., (2021) in *Cucurbita pepo*.



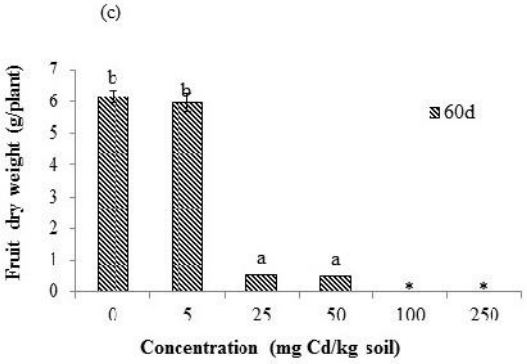


*Figure 1: Shoot dry weight (a), root dry weight (b) and fruit dry weight (c) of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg. Values are means of three independent replicates. For each parameter and sampling day, means followed by different letters are significantly different from each other at P≤0.05. (Note: *-indicate no fruit formation).*

2.2. **Effects of Cd stress on pigments**

The photosynthetic activity is suppressed by heavy metals due to disruptive chlorophyll synthesis. In this study, highest chlorophyll 'a' concentration was observed in control plants when compared with Cd treatments and a similar result was reported by Baweja et al., (2020). With the increase in the cadmium concentration, the pigment content decreased. The chlorophyll 'a', chlorophyll 'b' and carotenoids contents were higher in 30 days old plants then compared with 15 and 60 days old plants (Figure 2). A similar result was reported by Wu et al., (2021) in *Ochromonas*. Zhao et al., (2021) reported that the chlorophyll 'b' content showed a similar change as that of chlorophyll 'a' content, but there was a significant difference between the chlorophyll 'a' and 'b' contents, while our results showed that similar effects appeared in high Cd treatment. The concentration of carotenoids was higher in control plant at 30^{th} day and lower in 60^{th} day plants. There is no impact of Cd on chlorophyll 'b' and carotenoids up to 100 mg/kg Cd in the soil; and similar results were reported in *Cicer* seedlings by Baweja et al.,

(2020).

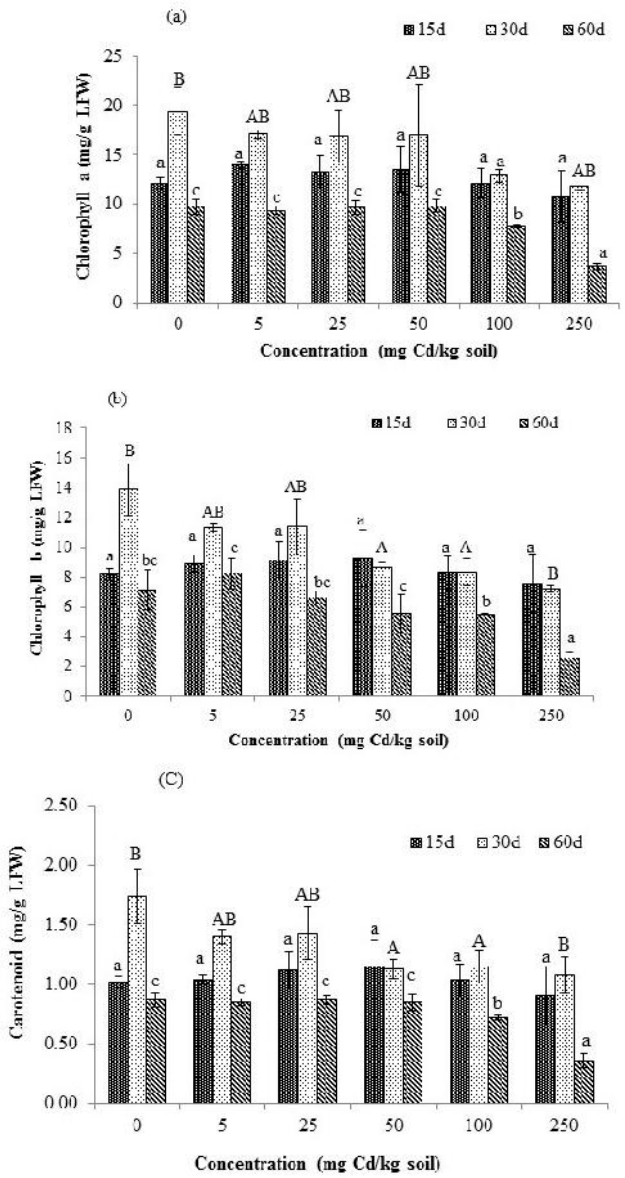


Figure 2: Chlorophyll 'a' (a), chlorophyll 'b'(b) and carotenoids (c) contents of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg. Values are means of three independent replicates and the error bars are standard deviation. For each parameter and sampling day, means followed by different letters are significantly different from each other at P≤0.05.

The decline in chlorophyll and carotenoids content with different cadmium concentration is found to be significant at *P<0.05* when compared with control; and such changes may be accounted by impairment of root transport of Fe reported by Brown et al., (1960).

2.3. Soluble protein content

Total soluble protein content is an important indicator of of reversible and irreversible changes in metabolism and it respond to a wide variety of stressors such as natural and xenobiotic (Aydin, 2013). Data analysis showed that there is a significant difference between the growth periods in terms of protein content (*P≤ 0.05*). Higher total soluble protein contents were observed in 60 days old plant when compared with 15 days and 30 days old plants. Compared with control, there were increases in total soluble protein in Cd treated plants (Figure 3) which is similar to the previous reports with *Sassafras* seedlings (Zhao et al., 2021)and *Vigna radiata* (Rahdarian et al., 2021). Comparing 15, 30 and 60 day old plants, soluble protein contents significantly increased with increasing Cd concentration. The more accumulation indicates that of reversible changes in the metabolism.

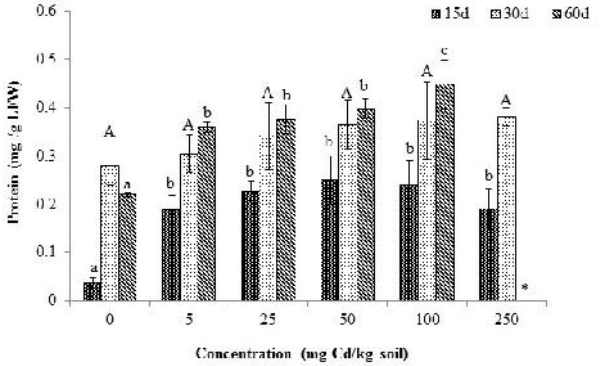


*Figure 3: Soluble protein content of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg. Values are means of three independent replicates. For each parameter and sampling day, means followed by different letters are significantly different from each other at P≤0.05. (Note: *-indicate there was no plant).*

2.4. Proline content

As shown in Figure 4, proline content increased with increasing Cd concentration in the soil. After 60 days of growth, there was a significant increase in the levels of proline content due to Cd stress in Okra plant from 0.13 mg/g FW to 0.17 mg/g FW. Similar results were reported by Sharma et al., (2010). Labidi et al., (2021) reported that proline content in the shoot of *Cucurbita pepo* increased with increasing the Cd treatment. Similar trend was also reported by Zhao et al., (2021). Furthermore, variety of

Arka Anamika exhibited more proline accumulation as observed by Rasheed, (2018) in Okra plant.

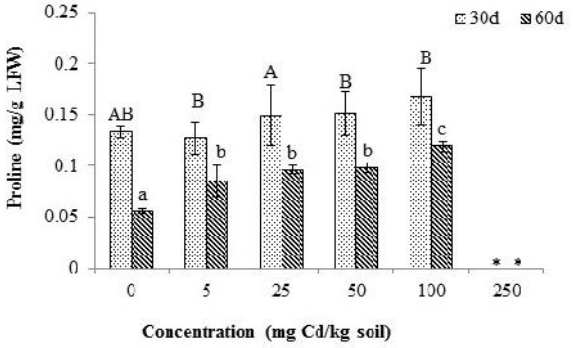


*Figure 4: Proline content of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg. Values are means of three independent replicates. For each parameter and sampling day, means followed by different letters are significantly different from each other at P≤0.05. (Note: *-indicate there was no plant).*

2.5 Peroxidase (POD) enzyme content

As shown in Figure 5, Under Cd stress, the POD enzyme contents in the leaf of okra plants after 15 days of growth increased with increasing Cd treatment from 4 U/mg to 6.4 U/mg. After 30days of growth, the POD activity slowly increased and the highest activity was observed in the highest Cd treatment. This trend is similar to the results reported by Zhao et al., (2021) and Han et al., (2021).

During the experimental period, the antioxidant enzyme POD declined in plants treated with higher Cd treatments after 60 days because of non-protein thiol groups, particularly glutathione which plays an important role in protecting plants from Cd toxicity. Our results are in agreement with Rahdarian et al., (2021) in *Vigna* and Zhang et al., (2021). In the okra plant leaves, POD activity was significantly higher in the Cd treatment than control. The maximum enzyme activity was observed in the 15 days old plants compared with plants after 30 and 60 days of growth.

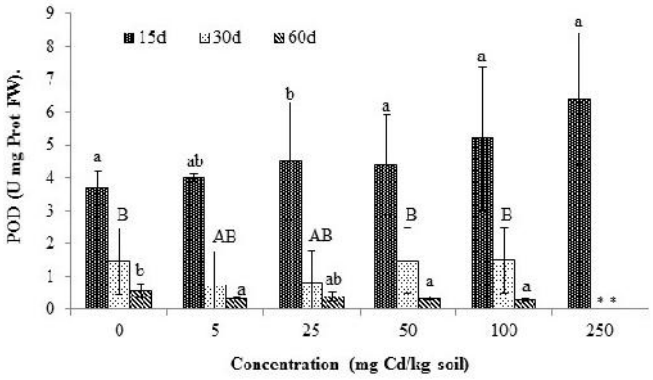
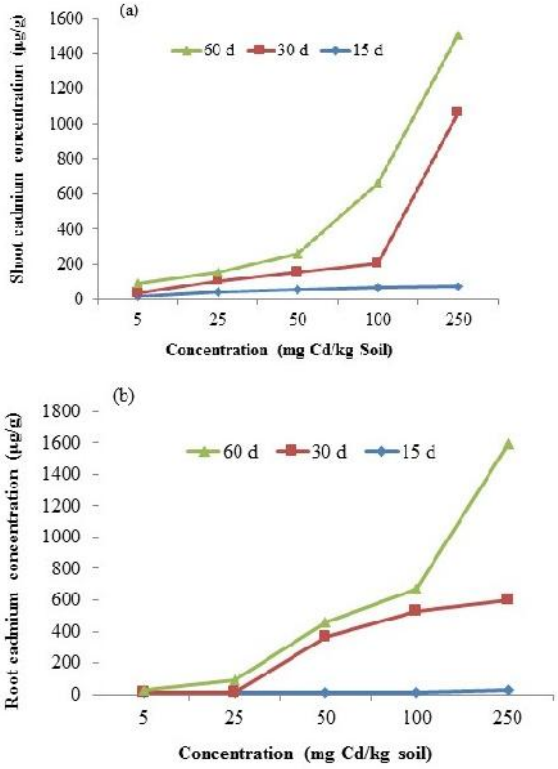


*Figure 5: Peroxidase (POD) enzyme concentration of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg. Values are means of three independent replicates. For each parameter and sampling day, means followed by different letters are significantly different from each other at P≤0.05. (Note: *-indicate there was no plant).*

2.6 Cadmium accumulation and translocation

In most environmental conditions, Cadmium ions enter first the roots and penetrating the root through the cortical tissue and then translocated to the aboveground parts of plants. In general, Cd ions are mainly retained in the roots and only small amounts are transported to the shoots (Hawrylak-Nowak et al., 2014). The results of the present study showed that at increasing Cd concentrations in the soil, significant increase in accumulation of Cd in all plant parts was observed. In 15 days old plants grown in soil amended with Cd at 50 mg kg^{-1} in the soil, the concentration of Cd in *A. esculentus* plants exceeded the accumulation of Cd in plants grown with 100 and 250 mg kg^{-1}. Moreover, the Cd concentration of *A. esculentus* was approximately 10-152% higher in roots and 18-58% higher in shoots compared with *Abelmoschus manihot, Malva sinensis and Abelmoschus rosea* (Wu et al., 2018; Zhang et al., 2021; Liu et al., 2008). After the 60 days of growth, the concentrations of Cd in the root tissues were much greater than in the shoots compared to 30 days old plants. In the fruits, the Cd concentrations were much lower than the Cd concentrations of root and shoot which is in agreement with the results reported by Sharma et al., (2010) in Okra plant. In contrast, in *Brassica* species Cd content was higher in shoots than roots and the accumulation factor slowly increased at low exposure level (Selvam

and Wong, 2009). According to Shivshanker et al., (2004), translocation factors and bioaccumulation factors were greater than one indicate the plant was hyperaccumulator. Wu et al., (2018) reported that the translocation factor was greater in the *Abelmoschus manihot* when exposed to Cd concentrations of 5-60 mg but the values were less with 100 mg Cd in soil which may be due to the fact that the excess Cd in the soil hinders efficient Cd transport. A similar trend was also observed in this study. The accumulation of Cd was lower in the shoots than roots of okra (Sharma et al 2010) and *Canna* (Zhang et al., 2021) plants but when the concentration was increased the accumulation increased after 30 days exposure period. In this study also, the BF decreased from 3.6 to 0.02 with increase in Cd concentration. After 60 days the BF was greater than one which decreased at lower Cd concentrations. Okra plant has the potential to decontaminate Cd-polluted soils. Moreover, the edible portion of treated okra fruits had less Cd accumulation when compared with roots.



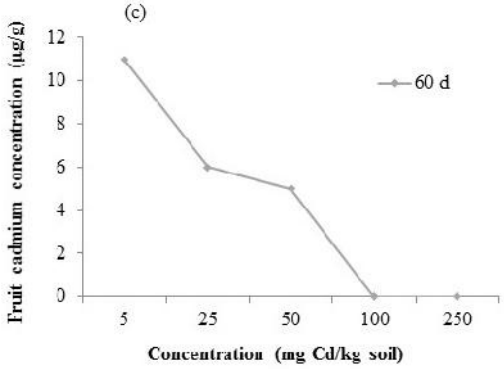


Figure 6: Shoot cadmium concentration (a), root cadmium concentration (b) and fruit cadmium concentration (c) of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg.

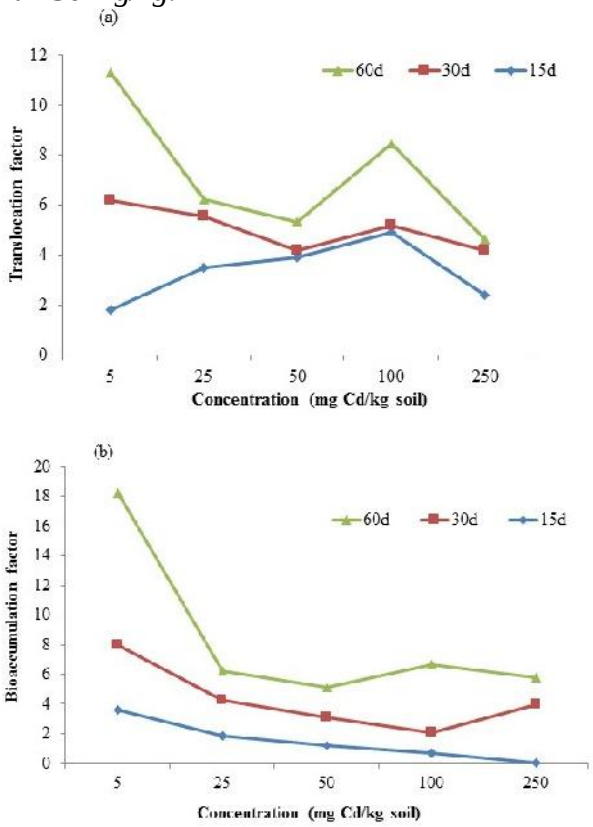


Figure 7: Translocation factor (TFs) (a) Bioaccumulation factor (BFs) of okra plants grown in soil amended with Cd at 0, 5, 25, 50, 100 and 250 mg/kg.

CONCLUSION

Abelmoschus esculentus plants were grown in soil applied with Cd in different dosages. Cd toxicity was pronounced at higher concentration with decreased biomass and reduced photosynthetic

pigment content. Accumulated Cd in edible portion of tested plant was lower than the roots followed by shoots and its hyper- accumulative characteristics under Cd stress indicate that this plant is a potential Cd hyperaccumulator. Some properties of this plant, including biomass, biochemical and physiological characters were enhanced by Cd at lower concentrations (≤ 50 mg/kg) and inhibited at high concentrations. Nonetheless, lowest accumulation of Cd in edible portion suggests it to be a potential vegetable crop for cadmium polluted soils.

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