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Accumulation, distribution and toxicological effects induced by cadmium on the development of mangrove plant *Kandelia candel* (L.)

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ABSTRACT

Accumulation and distribution pattern of cadmium (Cd) and its toxic effect on growth of the mangrove plant of young *Kandelia candel* seedlings have been examined. This study demonstrated that under high concentration of Cd stress, the total biomass of *K. candel* decreased 41.57 % compare to control (CK). At the end of 90 days exposure to 25 mg/l Cd, the average seedlings stem height and leaf number of the *K. candel* decreased by 30.54 and 42.68 %, respectively. The results showed that *K. candel* seedlings, under the experimental condition, accumulated higher concentration of Cd in their roots (411.29 ± 3.60 mg/kg) when compared to hypocotyls, stems and leaves. More than 95 % of Cd was accumulated mainly in roots. The distribution pattern of Cd concentration in *K. candel* seedlings was found in the following order: roots> hypocotyls > stems > leaves. Based on the leaf symptoms and morphological change of *K. candel* seedlings under heavy metal stress, this study showed that Cd is phytotoxic to *K. candel*.

Key words: cadmium, accumulation, morphological change, mangrove seedlings, *Kandelia candel*, Phytotoxic.

Mangrove is one of the major types that play an important role in the ecological balance of estuaries and seashores in tropical and subtropical zones [1]. Mangroves represent a critical ecological habitat in the coastal environment of tropical and subtropical areas. Many mangrove ecosystems located close to urban development areas may be impacted by effluents from industrial sources and urban runoff that often contains toxic concentrations of heavy metals such as cadmium (Cd), chromium (Cr), copper (Cu), zinc (Zn), and nickel (Ni) [2, 3]. The Cd is a toxic trace metal pollutant and is a nonessential element for both higher plants and animals [4]. It is easily taken up by plants and translocated to different plant parts.

A high accumulation of Cd accumulation in plants poses a potential hazard to human health through the food chain. Heavy metals have been well studied in aquatic environments because they are particulary toxic, even trace amounts modify important bio-physiological functions in plants and animals [2]. The Cd accumulation has been found in large areas of estuaries due to emissions from municipal waste incinerators, car exhausts, residues from metalliferous mining and the smelting industry, and the use of sludge or urban composts, pesticides and fertilizers [5]. Studies performed on mangrove seedlings exposed to heavy metals have demonstrated a relatively low transport of metals from roots to leaves [6]. The Cd inhibits physiological processes in plants such as photosynthesis, chlorophyll synthesis, transpiration, and cell elongation. It can also reduce the uptake and translocation of mineral nutrients and can induce deficiencies and imbalances of essential nutrients thereby resulting in poor growth. The Cd can be up taken and concentrated in plant tissues from soil, which may causes significant damaging effect on the plant. Thus, present study was envisioned to address the following objectives:

1) to investigate the effects of Cd on growth of *K. candel* seedlings;

2) to determine the distribution and accumulation pattern of Cd in the various organs of young *K. candel* seedlings;

3) to assess the toxic effect of Cd on young *K. candel* seedlings.

MATERIAL AND METHODS

Experimental setup. The experiment was set up in a one way completely randomized design (CRD) with three replicates to examine Cd distribution and accumulation and their toxic effect on *K. candel* seedling to 7 treatments.

Field collection and germination. The propagules of *K. candel* were collected from plants grown at the Jiulongjiang mangrove forest stand (24°24′ N, 117°23′ E), Xiamen, Fujian, China. After removal of the bracts, only complete, undamaged propagules with testa intact and no emergent hypocotyls or radicles were selected for planting. Propagules chosen for germination were those collected in the most abundant weight class, 18.0–19.55 g fresh weights. Propagules were planted in plastic pots filled with washed sand. Three plastic pots were placed inside a plastic container (30 cm long × 40 cm wide × 30 cm high). Four propagules were randomly planted in a plastic pot for germination and growth (3×4, $n = 12$). All the pots were placed in the green house. The propagules were kept in a greenhouse under natural lighting with a temperature of 28 ± 5 °C. A quantity of 2 liters tap water was irrigated to each pot 2 times each week. The water level of each container was adjusted daily with tap water (NaCl-free) to compensate the amount of water lost by evaporation. Propagules started to germinate within one month and growth continued therefore. After 3 week, young seedlings were adapted to Hoagland's nutrient solution. The solutions were changed every 7 day to prevent depletion of metals, nutrients and oxygen.

Preparation of Cd solution. The Cd solutions were prepared by dissolving of cadmium chloride salts ($CdCl_2 \cdot 2H_2O$) in 1000 ml of distilled water. From this stock solution, various concentrations (1, 5, 10, 15, 20 and 25 mg/l) of Cd solution were prepared.

Cadmium exposure. Two-month old *K. candel* plants were put in individual plastic containers holding 1000 ml of Hoagland's solution prepared with addition of 0, 1, 5, 10, 15, 20, and 25 mg/l of cadmium chloride ($CdCl_2$). Plants were exposed for 12 weeks under hydroponic conditions. Control plants were cultured with 1000 ml of Hoagland's solution without $CdCl_2$. Twenty four hours after the final exposure of the seedlings to Cd; the plants were harvested for analysis. These exposures were performed in triplicates.

Analyses of samples. At the end of 90 days cultivation of seedlings with heavy metals, the plants were uprooted from the plastic pots and washed thoroughly with tap water and rinsed again in distilled water. Plants were then divided into root, hypocotyls, stem, and leaf portions and taken the fresh weights (FW) of individual parts. The samples were then oven-dried at 70 °C to a constant weight and the dry weight (DW) was determined. Finally, the oven-dried samples were ground with an agate grinder (FW-100, China) to pass through

a 60 mesh sieve. Heavy metals contents were determined after incinerating the samples in a muffle oven at 550 °C for 6 h. Samples (about 0.2 g) were digested for heavy metal analysis with a 90 °C mixture of concentrated nitric acid and hydrogen peroxide, adapting the methods of [7, 8]. Samples were digested in a solution of concentrated nitric acid and hydrogen peroxide, and made to 50 ml volume for root, hypocotyls, stem and leaf tissues. Digested samples were stored in labeled acid-washed glass vials. Samples were analyzed inductively coupled plasma-mass spectroscopy ICP-MS using a PerkinElmer, USA instrument. All concentrations were expressed in mg/ kg on a dry weight basis using weights obtained from oven-dried specimens. Based on the leaf symptoms and morphological changes of *K. candel* seedlings under heavy metal stress, the effects of Cd toxicity were also assessed.

Statistical analysis. Data analysis was accomplished using two statistical programs, namely, Microsoft Excel 2003 package and SPSS 11.5. (Chicago. IL, USA). The descriptive statistics (mean, standard deviation) of the heavy metal concentrations were calculated by using the Microsoft Excel 2003 package. An one way analysis of variance (ANOVA) followed by Duncan's post-hoc test was employed to examine any statistical differences between different treatments in terms of changes in biomass, plant growth parameters, bioaccumulation rate, and distribution of heavy metal concentrations. The differences were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Plant growth. Effects of Cd on *K. candel* growth were assessed by stem height and leaf number. Cd treatments inhibited stem elongation, the difference between CK and Cd treatment was significant (Table 1). High heavy metal concentrations in 25 mg/l caused significant reduction in stem height and leaf number of plants from day 60 onwards (Table 1), indicating metal toxicity on growth of *K. candel*. It was observed that old leaves in the lower position of the plant started to turn yellow and shed off while the young leaves still survived in these metal-treated plants. At the end of the experiment, the stem of plants receiving strong heavy metal stress were found to become deep brown in color from the top to the lower parts. As shown in Table 1, no significant difference in leaf number and plant height was found between 1 mg/l Cd treatment and control. On the other hand, within 30 d treatment, no obvious significant difference was found among the treatments, except for the differences between control and the treatment with 20 and 25 mg/l of Cd. However, increasing Cd concentration (5 ~ 25 mg/l) in the medium induced a significant decline ($P > 0.05$) in average stem height and leaf number. The deleterious effect of Cd became more severe with increasing Cd level and extended time of exposure. For example, at the 90 d of 25 mg/l Cd exposure, the average plant stem height and leaf number of the *K. candel* decreased by 30.54 and 42.87 %, respectively. On the other hand, the leaf area per plant was signifi-

Table 1

Growth parameter under Cd supply after 90 day cultivation

Treatment, mg/l	Stem height, cm	Leaf number, *n*	Total biomass DW, g/pot
CK	20.06 ± 0.66(a)	8.13 ± 0.70(a)	17.63 ± 2.61
1.0	19.13 ± 0.58(ab)	7.93 ± 0.50(ab)	16.53 ± 2.47
5.0	18.00 ± 0.62(abc)	7.50 ± 0.50(ab)	15.40 ± 1.33
10	16.80 ± 0.26(abcd)	7.06 ± 0.60(abc)	14.00 ± 1.07
15	16.20 ± 0.29(bcd)	6.23 ± 0.56(bcd)	12.90 ± 1.75
15	14.90 ± 0.90(cd)	5.56 ± 0.50(cd)	11.30 ± 1.86
25	13.93 ± 0.60(d)	4.66 ± 1.19(d)	10.03 ± 1.02

Note. Mean values in the same column with different letters are significantly different at $P<0.05$ level. The values were the means of 3 replicates.

Table 2

Biomass (DW) of different parts of *K. candel* seedlings under Cd supply after 90 day cultivation

Treatment, mg/l	Root	Hypocotyls	Stem	Leaf
	g/pot			
CK	4.23 ± 0.30	10.13 ± 1.80	1.2 ± 0.10	2.06 ± 0.10
1	3.96 ± 0.55	9.53 ± 1.95	1.13 ± 0.11	1.9 ± 0.20
5	3.53 ± 0.23	9.06 ± 0.66	1.06 ± 0.25	1.73 ± 0.15
10	3.06 ± 0.152	8.5 ± 0.51	0.93 ± 0.11	1.5 ± 0.10
15	2.8 ± 0.20	8.0 ± 0.87	0.8 ± 0.11	1.3 ± 0.10
20	2 ± 0.26	7.53 ± 1.31	0.7 ± 0.10	1.06 ± 0.35
25	1.6 ± 0.52	6.93 ± 0.45	0.6 ± 0.20	0.9 ± 0.40

cantly smaller at higher Cd concentration (data not shown) indicating that irreversible damage to tissue formation was induced under higher Cd level. The development of root system worsened with less root hair. On the 90th day, the Cd treated root system turned to black-brown.

Plant biomass. Plant biomass of root, hypocotyls, stems and leaves decreased with increasing Cd level in the nutrient solution for *K. candel* seedlings after 90 d Cd exposure. For example, at the highest Cd concentration (25 mg/l), the root, hypocotyls, stem, and leaf dry weights were decreased by 62.17, 31.58, 50, and 56.31 %, respectively, when compared to the control (Table 2). The stem biomass decreased with the increase of Cd concentration in the nutrient solutions. The reduction in leaf biomass due to Cd treatment was more obvious than that of the stem biomass (Table 2), suggesting that growth parameters related to the leaves were more sensitive than those associated with the stems. The differences between CK and Cd treated plant stem biomass were significant in this study. The Cd stress resulted in a significant decrease in plant biomass. On the other hand, the final dry matter values of roots ranged from 4.23 to 1.6 g/pot and that of leaves ranged from 2.06 to 0.90 g/pot harvested from Cd treated plants. At higher levels of Cd concentrations, e.g., 5 ~ 25 mg/l as used in this study, a significant decrease in plant stem height, leaf number and corresponding decrease in biomass of *K. candel* plants seedlings was observed. The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis [9]. Some studies reported a marked reduction in photosynthetic rate for different plant species under exposure to Cd stress [9]. Cd may interfere with nutrient uptake by affecting the permeability of plasma membranes. Cd addition decreased the concentration of some nutrients like K, Zn and Mn in wheat root and shoot [10]. Cd inhibits P, K and Mn translocation to shoots, leading to more retention in roots [11]. The water content of leaves decreased by about 10 % compared to control after 90 days cultivation in 25 mg/l of Cd stress. Our results were in line with the findings of Padmaja et al. [12].

Accumulation of Cd in different parts of K. candel seedlings. The Cd concentration was measured in all the separate four parts of *K. candel* seedlings. The Cd concentration in plant tissue increased markedly with increasing Cd concentration in the Hoagland's solution. There was a significant difference in Cd concentration amongst the various plant parts (Fig. 1). In this experiment, we found that the Cd concentration varies at different plant parts. The Cd concentration in roots ranged from 411.29 to 1.71 mg/kg. The maximum Cd concentration in the dry root matter was 411.29 mg/kg, which was significantly higher than the control. The Cd concentration in hypocotyls ranged 4.05 to 0.68 mg/kg. The Cd concentration in stems ranged from 3.29 to 0.26 mg/kg. The Cd concentration in the leaves ranged from 2.50 to 0.15 mg/kg. The highest Cd concentration was found in roots (411.29 mg/kg, Fig. 1, *A*) and the lowest concentration was found in leaves (2.50 mg/kg, Fig. 1, *D*). The Cd concentration in roots was more than 100 times high-

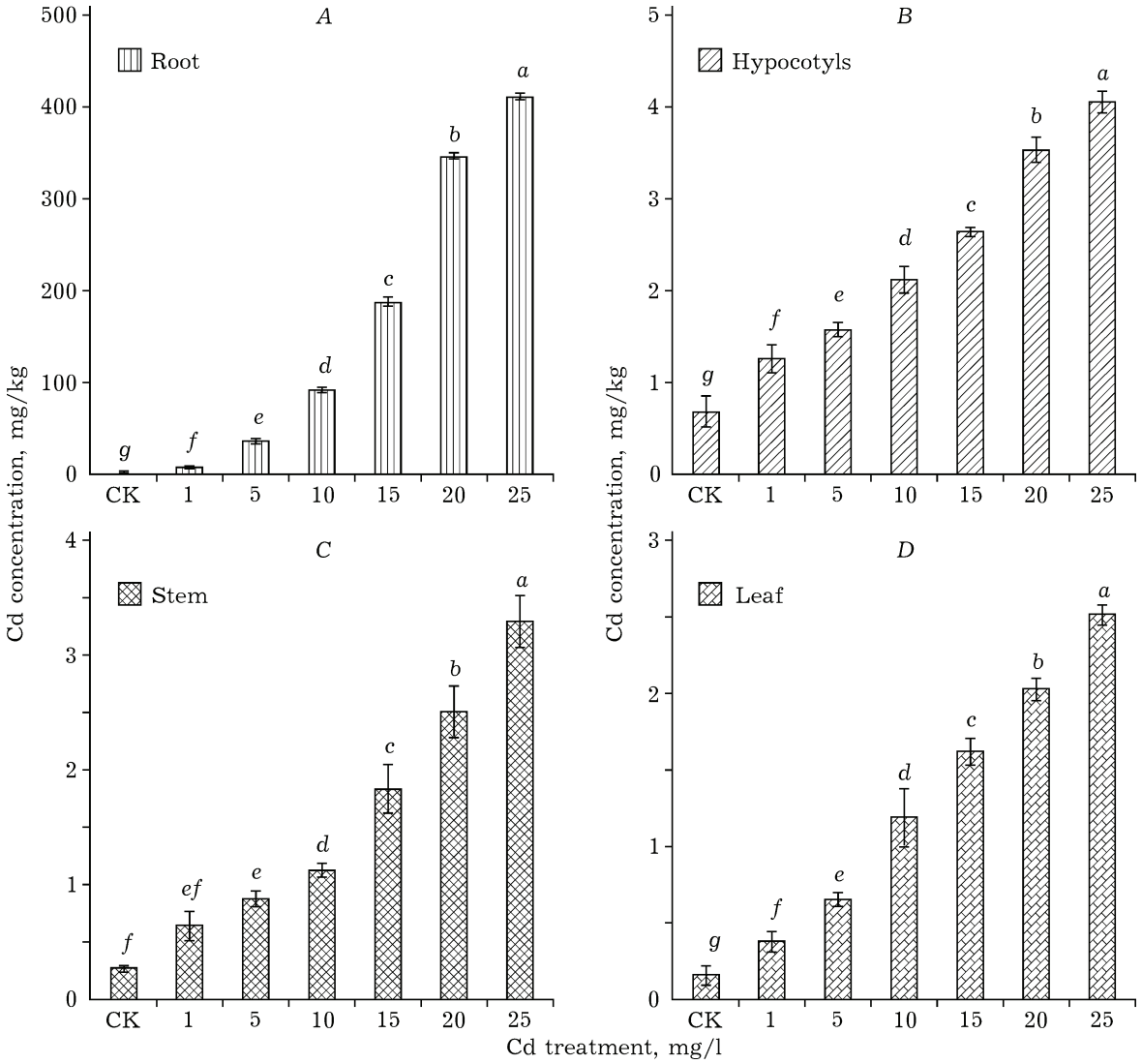

Fig. 1. Cd concentrations in different parts of *K. candel* seedlings under different Cd stresses, *A* – Roots, *B* – Hypocotyls, *C* – Stems, *D* – Leaves (Mean ± S.D). Different letters on the vertical bars indicate statistical significance ($P < 0.05$) of difference between the means according to Duncan's test

er than that of in hypocotyls, 125 times higher than that of in stems, and 165 times higher than that of in leaves, suggesting that roots were the main Cd accumulating organ. Plant root can accumulate high quantities of Cd^{2+} when grown in non-pollution areas as in a medium containing this metal. Cd, as divalent cation, may compete with other cation (including Ca^{2+}, Mg^{2+}, Fe^{2+}) in their transport across membranes. In addition, it was reported that Cd was more easily taken up and accumulated than other heavy metals by plants through the root systems from soil [5]. In the present study, the results showed that *K. candel* seedlings, under the experimental condition, accumulated higher amount of Cd in their roots when compared to hypocotyls, stems and leaves. A number of researchers have found high concentrations of accumulated metals in the tissues of numerous mangrove species including *K. candel*, *Rhizophora* spp. and *Avicennia* spp. [13, 14]. The Cd concentration in plant tissues increases with the increase of its concentration in the nutrient solutions and with the length of exposure period [15], the Cd concentration in the roots being higher than in the aerial parts. The increase in Cd concentration in the roots was not due to the increase in ab-

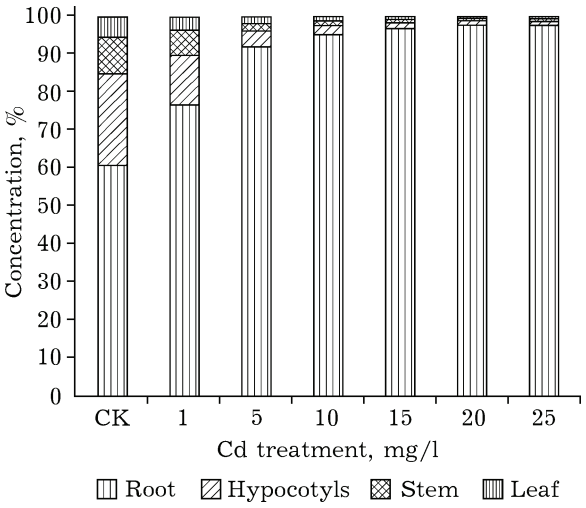

Fig. 2. Distribution pattern of Cd concentration in *K. candel* seedlings

sorption of this element, but due to the concomitant decrease in dry matter accumulation [15]. Although there was a high Cd concentration in roots, it was also found in leaves and stems, demonstrating that this metallic element was not totally immobilized in the root portion, but translocated to the aerial parts as well. A one way ANOVA test showed significant differences in Cd concentrations amongst the four plant parts ($P < 0.05$).

Distribution pattern of Cd concentration in K. candel seedlings. An evaluation of the total Cd concentration in roots, hypocotyls, stems, and leaves of *K. candel* seedlings showed that more than 95 % of Cd was accumulated mainly in roots (Fig. 2). In contrast, the lowest Cd concentration was detected in the leaves. On the other hand, when comparing the heavy metal concentration in different tissues under the exposure of higher concentration (25 mg/l treatment), we observed that Cd concentration in the roots, hypocotyls, stems, and leaves were 411.29 ± 3.60 mg/kg, 4.05 ± 0.12 mg/kg, 3.29 ± 0.22 mg/kg and 2.50 ± 0.06 mg/kg, respectively. An oneway ANOVA followed by Duncan post-hoc test revealed the following order in distribution pattern of Cd concentration in *K. candel* seedlings: roots > hypocotyls > stems > leaves. The Cd content in the leaf was only 0.60 to 8.77 % of those of root (Fig. 2). Our results showed that most of the absorbed Cd by seedlings was accumulated in roots. The variability of distribution of the Cd in the plant may have been caused by compartmentalization and translocation in the vascular tissues. These results also suggest that the translocation of heavy metals to above ground parts of the plants was minimized in order to curtail toxic effects caused by the presence of metal in the media. This observation is consistent with the common behavior of plants in their response towards environmental stress. For example, Study from Australia found that Cu, Zn and Pb accumulated in the tissues of *A. marina* at different concentrations at different plant parts [7]. The Cu and Zn showed some mobility in the plants, being accumulated in leaf tissues at levels of approximately 10 % of the roots. The Pb showed little mobility being accumulated in leaf tissues at levels of only 3% of that of the root levels.

Bioaccumulation of Cd in different parts of K. candel seedlings. Accumulation of Cd in different parts of the plant was calculated using the bioaccumulation factor (*BAF*). The *BAF* provides an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the medium [16], which is calculated as follows:

Table 3

Bioaccumulation factors for Cd in *K. candel*

Treatment, mg/l	Root	Hypocotyls	Stem	Leaf
CK	171.85	68.40	26.71	15.92
1.0	749.38	125.61	63.88	37.78
5.0	723.59	31.49	17.45	13.06
10	927.123	21.18	11.25	11.83
15	1253.81	17.62	12.21	10.76
20	1732.01	17.69	12.52	10.10
25	1645.19	16.22	13.17	10.03

Table 4

Visible symptoms on seedlings of *K. candel* grown in green house with increasing Cd concentrations

Cd in nutrient solution, mg/l	Visible symptoms	Stem growth reduction, %
CK	No symptoms	0
1.0	No obvious symptoms	4.63
5.0	Slight Chlorosis	10.26
10	Chlorosis, reddish-brown discoloration of the leaf blades	16.25
15	Chlorosis (+)	19.22
20	Chlorosis (++), necrosis (+), root worsened with less root hair	25.72
25	Chlorosis (+++), necrosis (++), leaf falling, root becomes shorter and thicker, root hairs sparser and color is black and brown, and stem deep brown in color but plant still survive	30.54

Note. Relative symptom intensity is given in brackets. Percent of growth reduction are given to the 0 mg/l Cd treatment.

$$\text{BAF} = \text{Trace element conc. in plant tissue (mg/kg) at harvest/Conc. of the element is the external nutrient solution added (mg/kg)} \times 100\ \%$$

The Cd content in the tissues generally increased with the increase of Cd concentration in the solution, although the bioaccumulation factors for this metal decreased when Cd solution strengths increased (Table 3).

Assessment of effects of Cd toxicity on K. candel seedlings. The most obvious symptom of Cd toxicity was the reduction of plant growth. Besides the biomass reduction, the Cd treated seedlings developed even gradients of visible symptoms, which progressively increased with the leaf age. These visible symptoms include uneven chlorosis and necrosis. The chlorosis developed preferentially at the base of the leaf. The necrosis was detected after 4-weeks of exposure to Cd as small dots at the adaxial side of the leaf margins, frequently crossing the veinlets. While chlorosis was observed in all Cd treatments (Table 4), necrosis was restricted to the treatments with Cd concentration of 15, 20 and 25 mg/l. It was observed that old leaves in the lower position of the plant started to turn yellow and tended to dry out at the tip. The plants treated with 25 mg/l Cd concentration showed chlorosis, necrosis, leaf falling, shorter and thicker root, sparser root hairs, black and brown root color, and deep brown stem color. On the other hand, the seedling treated at lower concentrations of Cd had shown no necrosis. The occurrence of chlorosis at high Cd concentrations (such as 20 to 25 mg/l) is probably associated with the decrease in Fe translocation to leaves [17].

Visual symptoms due to Cd toxicity, such as leaf necrosis, chlorosis reddish-brown discoloration of the leaf blades, browning of root system also became more severe with increasing levels of Cd concentrations and length of exposure period indicating that the Cd toxicity caused damage to tissue development and function. This accorded with earlier reports [18]. In contrast, the disturbed water relations to plants comprised one of the main reasons for the heavy metal phytotoxicity [19]. Cd often primarily affects the photosynthetic pigment before photosynthetic function, though Cd is known as an effective inhibitor of photosynthesis [20]. The total chlorophyll content reduced sharply as Cd level exceeds 0.83 mg/kg, which followed similar trends with seedling fresh weight, shoot height and root length [21]. It is well known that Cd can induce essential nutrient deficiency and even to decrease concentrations of many macronutrients in plants [22]. The Cd can influence the absorption of Cu, Fe, Zn and Mn through competition for sites or processes shared by these cations [22]. One possible mechanism, in which elevated concentrations of heavy metals may damage plant tissues, is the stimulation of free radical production by imposing oxidative stress [23, 24]. The Cd is a non-redox metal that is strongly

phytotoxic and causes growth inhibition and plant death. It produces alterations in the functionality of membranes by inducing changes in lipid composition and by affecting the enzymatic activities associated with membranes, such as the H^+- ATPase.

CONCLUSIONS

Present study demonstrates that under high Cd concentration (25 mg/l), the total biomass of *K. candel* decreased 35.81 % compare to control. At the end of 90 days of exposure to 25 mg/l Cd, the average seedlings stem height and leaf number of the *K. candel* decreased by 30.54 and 42.68 %, respectively. The accumulation of Cd was measured in all the separate four parts of *K. candel* seedlings. There was significant difference in Cd accumulation amongst plant parts. In the present study, the results showed that *K. candel* seedlings, under the experimental condition, accumulated higher concentration of Cd in their roots (411.29 ± ± 3.60 mg/kg) when compared to hypocotyls, stems and leaves. More than 95 % of Cd was accumulated mainly in roots. The Cd content in the leaf was only 0.60–8.77 % of that in root. The distribution pattern of Cd concentration in *K. candel* seedlings was in the order: roots > hypocotyls > stems > leaves. Based on the results of this study, it is indicated that Cd is phytotoxic to *K. candel*.

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