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Bioaccumulation and Photosynthetic Activity Response of Kenaf (*Hibiscus cannabinus* L.) to Cadmium and Zinc

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ABSTRACT

The response of kenaf (*Hibiscus cannabinus* L.) to Zn/Cd contamination in soil was investigated using pot experiment. Plants were grown on soils containing increasing doses of two metals. Zn and Cd were applied alone or combined. Growth parameters as well as content of photosynthetic pigments, and photosynthetic performance were determined. Soil and plant tissue were analyzed by atomic absorption spectrometry. Metal concentration in plants increased when metal dose in soil increased. The Zn and Cd concentrations were analyzed in order of root >leaf>stem. The Zn-Cd interaction reduced the Cd concentration in plants and alleviates the toxicity of Cd on photosynthetic system, and showed that Cd and Zn acted synergistically to Cd accumulation in plants. The reduction of photosynthetic activity observed did not correlate with the changes in the biomass production. *Hibiscus cannabinus* L. could be an accumulator candidate of Cd and Zn contaminated sites.

Keywords:

Zn-Cd interaction, translocation, phytoremediation, kenaf

1. INTRODUCTION

Metals occur naturally in soils at low concentration, but are considered as soil contaminants at high concentration because of their toxicity which disturbs plant physiology and development (Bertrand and Poirier, 2005; Hasan et al., 2009). Metals are taken up by plant roots and translocated to upper tissues and then poses a potential threat to human health as it enters the food chain (Obata and Umebayashi, 1997). Phytoextraction is a promising technology based on the use of plant tolerating and accumulating a high amount of metal leading to clean up contaminated soil (Raskin et al., 1994; Salt et al., 1995). Species used in phytoextraction would present high biomass production and moderate concentrations of metals in plant tissues (Baum et al., 2006). Such as Industrial crop is not used for food production, example fiber crop would be the best suited candidate for use in phytoremediation (Prasad, 2007). Kenaf (*Hibiscus cannabinus* L.) is annual specie belongs to malvaceae family, fast growing and high biomass producing able

to tolerate different environment conditions (Webber et al., 2002). It is considered as an important bast fiber crop and highly cultivated for its fibrous stem used in many industrial applications such as textile, biocomposites, insulation mat, etc. (Alexopoulou et al., 2013).

The effects of different trace metals on growth and biomass quality of several varieties of kenaf were reported in the studies of Catroga et al. (2005) and Catroga (2009). Tainung 2 is the most efficient variety in the phytoextraction of Cd from soil. Dos Santos et al. (2010) reported the ability of kenaf to accumulate zinc in its tissues. Cadmium and zinc are commonly associated with each other due to their similar geochemical behavior. Cadmium is a potentially toxic metal and zinc plays important role in counteracting Cd toxicity in plants. Several studies are undertaken to understand interaction between Zn and Cd but results are inconsistent. For instance, White and Chaney (1980) found that Cd uptake was reduced in soybean roots and shoots through an application of Zn and Zhou et al. (1994) reported that the Zn-Cd interaction resulted in an increase of Cd

accumulation and a decrease of Zn uptake in a rice plant. Eriksson (1990) concluded that interaction of Cd and Zn is variable with the crop species. Thus, the interaction of Cd and Zn is important in the increasing effect of phytoremediation technology as contaminated soils are concerned.

In this study, pot experiment was designed to investigate the effect of increasing doses of Zn and Cd as well as their interaction on *H. cannabinus*. Because of metal effects on plants are diverse, physiological parameters (photosystem efficiency, chlorophyll content) were used to evaluate metal-induced cellular damage. Growth parameters were also determined on control and polluted soil, in addition to plant metal content of plants in order to evaluate the kenaf potential for contaminated sites remediation.

2. MATERIALS AND METHODS

2.1. Experimental site and soil sampling

A pot experiment was conducted in a greenhouse of National Agronomic Institute of Tunisia. Top soil up to 10 cm depth, was collected from the experimental station of the institute. The soil samples were air-dried crushed to powder and sieved in 2 mm mesh. The soil contamination was performed by adding metals at different levels, ZnSO₄ (1500, 3000, 4500 µM) and CdCl₂ (25, 50 75 µM) either individually or mixed and dissolved in distilled water and then saturated, and air- dried. The Wetting-drying mixing process was repeated several times during 2 months to ensure soil equilibrium.

2.2. Plant cultivation

Seeds of *H. cannabinus* L. "Tainung" 2 variety were germinated on plastic seed trays. Homogenous seedlings were transplanted into pots. Each pot contained 2 plants. The experiment design was randomized block design. Each pot was considered as a replicate and all the treatments were replicated six times.

Irrigation program has been adapted according to soil temperature and evapotranspiration rate. No fertilizers were applied.

2.3. Zn and Cd analyses

After a five months culture, plants harvested were cut into parts of root, leaf, stem and washed before oven dried at 80°C for 48 hours until constant weight. Then, dried tissues were carefully weighted. The samples were then ground into fine powder. For each plant sample, 0.5 g was digested with 5 ml of 20% HCl then, made up in a 100 ml volumetric flask (Alloway, 1995). Soil samples from each treatment were also oven dried at 80°C grinded and digested using concentrated nitric acid (HNO₃) and perchloric acid (HClO₄). Metal concentrations in the digested samples were determined by the atomic absorption spectrophotometry in a AAS800-PerkinElmer Analyt.

Stem and leaf translocation factors (TF), defined as the ratio of concentration of a metal in stem and leaf to its concentration in root, respectively, were used to estimate the plant's capacity of bioaccumulation.

2.4. Chlorophyll fluorescence analysis

Chlorophyll fluorescence was measured by a chlorophyll portable fluorometer (FIM 1500, Analytical Development Company Limited) on leaves that were mature and fully exposed to the sun. A dark adaptation is needed for the electron transport chains to reach the fully oxidised level (open photosystems) (Maxwell and Johnson, 2000). The fluorometer measures the initial fluorescence (F₀) before excitation of the reaction centres of photosystem II (PSII). The maximal fluorescence (F_m) is measured after the application of a saturation light pulse when all electron acceptors are closed. The ratio $(F_m - F_0) / F_m = F_v / F_m$ can then be calculated which represents the efficiency of the photosystems in the dark-adapted state, where F_v represents the variable fluorescence emission (Maxwell and Johnson, 2000). Fluorescence measurements were

performed on 3 leaves on the 3 replicates per treatment.

2.5. Chlorophyll content

Leaves of *H. cannabinus* from different treatments were collected. Five replicates from each treatment were prepared, 100 mg of leaf tissue was suspended in a test tube containing 10 ml of 80% acetone, mixed and kept at 4°C during 72 hours in dark. Supernatant was withdrawn and absorbance was recorded at 663 and 645 nm in spectrophotometer. The amount of chlorophyll (a) and chlorophyll (b) was calculated according to Arnon method (1949).

2.6. Statistical analysis

Data were analyzed using the program Statistical Analysis System (SAS) by one-way analysis of variance (ANOVA). Differences between means were tested using Least Significance Difference (LSD) test at 0.05.

3. RESULTS

3.1. Plant growth

Exposing kenaf plants to different levels of Zn and Cd resulted in growth reductions as shown in table 1. Increasing Zn concentrations in soil produced a significant growth inhibition of kenaf plant. The greatest adverse effect being on plant's height while the root and shoot dry weight was only significantly affected by 4500 µM Zn ($p < 0.05$). The decrease in plant's height was parallel to a reduction in stem's diameter but no visible symptoms of toxicity, except growth reduction was observed.

A retarded development in Cd-treated plants compared to the controls was observed. In the presence of 50 µM Cd in soil, significant reduction ($p < 0.05$) was found in all growth parameters. Shoot and root dry weight was severely decreased at 75 µM Cd, respectively, 58.17% and 46.17%. The decrease of root growth at 75

μM Cd was characterized by a reduction of lateral roots. The plant height, diameter of stem, shoot and root weight were higher in Cd +Zn treatment (T4) compared to its counterpart in Cd-alone treatment (T6).

Table 1: Effects of soil treatments on growth parameters of kenaf plants

Soil treatment (μM)	Height of plant (cm)	Diameter of stem (mm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)
Control (T0)	96,81 \pm 13,08 ^a	4,93 \pm 0,52 ^a	5,07 \pm 1,09 ^a	0,62 \pm 0,16 ^a
Zn 1500 (T1)	80,30 \pm 9,26 ^b	44,7 \pm 0,49 ^{ab}	4,31 \pm 1,03 ^{ab}	0,66 \pm 0,06 ^a
Zn 3000 (T2)	78,87 \pm 8,52 ^b	4,24 \pm 0,51 ^b	4,10 \pm 0,86 ^{ab}	0,67 \pm 0,13 ^a
Zn 4500 (T3)	72,46 \pm 7,36 ^b	4,16 \pm 0,36 ^b	4,07 \pm 0,72 ^{bc}	0,59 \pm 0,06 ^{ab}
Zn 3000 + Cd 50 (T4)	77,22 \pm 11,44 ^b	4,22 \pm 0,37 ^b	4,09 \pm 1,03 ^{ab}	0,47 \pm 0,08 ^{bc}
Cd 25 (T5)	80,96 \pm 12,40 ^b	4,42 \pm 0,32 ^{ab}	5,16 \pm 0,68a ^{bc}	0,45 \pm 0,12 ^{cd}
Cd 50 (T6)	68,46 \pm 9,47 ^c	4,20 \pm 0,53 ^b	3,59 \pm 0,80 ^c	0,42 \pm 0,07 ^d
Cd 75 (T7)	56,13 \pm 10,30 ^c	3,38 \pm 0,71 ^c	2,90 \pm 0,98 ^c	0,33 \pm 0,12 ^c

Means in same columns followed by the same letter are not significantly different at $p < 0.05$.

3.2. Zinc and cadmium concentrations

The concentration of metals in different plant tissues increases with the increase of metals in soil contamination level as shown in table 2 and table 3. The Zn and Cd concentration in plant tissues were in order of root>leaf>stem regardless soil metal concentration. In Zn-alone treatment, the concentration of Zn in kenaf plants ranged from 41 mg.kg⁻¹ to 285 mg.kg⁻¹, the highest Zn concentration was observed in the root.

The concentration of Cd in tissues increased significantly with increasing Cd in soil, notably in roots.

In the presence of 75 μM Cd, concentration of Cd in roots and leaves was 2 times higher than that in soil. In contract, exposure of kenaf plants to Cd + Zn treatment (T4) resulted in an altered pattern in Cd and Zn concentration in their tissues. The stem and leaf Cd concentration were lower in Cd+Zn treatment (T4) compared to its counterpart in the Cd-alone treatment (T6). In Cd root concentration, 30% of reduction was observed.

Cd and Zn initial levels in soils decreased after cultivation of kenaf plants (Table 4). However, this decrease is not significant according to LSD test at 5%.

Table 2: Zn concentration in leaf, stem and root of kenaf plants

Treatments	Root	Stem	Leaf
Control (T0)	74,19±10,02 ^a	41,085±9,32 ^a	47,475±11,25 ^a
1500µM Zn (T1)	134,76±12,58 ^b	85,53±12,58 ^b	102,285±19,88 ^b
3000µM Zn (T2)	156,6±18,96 ^b	93,3±18,58 ^b	89,985±18,36 ^b
4500µM Zn (T3)	285,15±20,23 ^c	162,9±36,56 ^c	272,7±25,84 ^c
3000µM Zn + 50 µM Cd (T4)	203,7±25,25 ^{bc}	106,39±20,69 ^b	109,68±15,44 ^b

Means in same columns with the different letter are significantly different among treatments at $p<0.05$.

Table 3: Cd concentration in leaf, stem and root of kenaf plants

Treatments	Root	Stem	Leaf
Control (T0)	5,91±1,02 ^a	4,302±0,99 ^a	5,31±0,98 ^a
25 µM Cd (T5)	21,58±2,58 ^b	7,6965±2,36 ^b	26,56±3,33 ^b
50 µM Cd (T6)	40,65±2,56 ^c	14,0985±1,36 ^c	35,34±1,82 ^c
75µM Cd (T7)	38,37±4,01 ^c	15,21±2,83 ^c	27,39±2,33 ^b
3000µM Zn + 50 µM Cd (T4)	29,03±3,98 ^b	16,12±2,12 ^c	30,05±1,58 ^b

Means in same columns with the different letter are significantly different among treatments at $p<0.05$.

Table 4: Metal concentration in soil after plant's harvest

	T0	T1	T2	T3	T4
Zn (mg/kg)	106	184	207	511	362
	T0	T4	T5	T6	T7
Cd (mg/kg)	0,6	6,66	3,24	4,98	7,23

3.3. Zn and Cd translocation from root to leaf and stem

The Zn and Cd translocation from root to upper tissues was significantly influenced by treatments as shown in table 4 and table 5. Results showed also that the stem and leaf translocation factor (TF) decrease with the increase of metal concentration in soil and the highest TF

was found in the control. In Zn treatments, stem TF ranged from 0.65 to 0.50 and leaf TF ranged from 0.86 to 0.54. In Zn+Cd treatment, both TF does not significantly changed ($p<0.05$). In Cd treatments, stem TF ranged from 0,73 to 0,35 and leaf TF ranged from 1,23 to 0,71. In Zn+Cd treatment (T4), the both TF for Cd increased significantly compared to TF in Cd-alone treatment (T6).

Table 5: Translocation of Zn from root to stem and leaf of kenaf plants treated with Zn alone and combined Zn and Cd

Treatments	Control (T0)	1500µM Zn (T1)	3000µM Zn (T2)	4500µM Zn (T3)	3000µM Zn + 50 µM Cd (T4)
Stem/root	0,65±0,02 ^a	0,63±0,03 ^a	0,53±0,02 ^b	0,50±0,02 ^b	0,52±0,03 ^b
Leaf/root	0,86±0,05 ^a	0,66±0,02 ^b	0,57±0,07 ^b	0,66±0,05 ^b	0,054±0,08 ^b

Means in same line with the different letter are significantly different among treatment at $p < 0.05$.

Table 6: Translocation of Cd from root to stem and leaf of kenaf plants treated with Cd alone and combined Zn and Cd

Treatments	Control (T0)	25 µM Cd (T5)	50 µM Cd (T6)	75µM Cd (T7)	3000µM Zn + 50 µM Cd (T4)
Stem/root	0,73±0,04 ^a	0,36±0,03 ^b	0,35±0,05 ^b	0,40±0,08 ^b	0,56±0,08 ^a
Leaf/root	1,23±0,1 ^a	0,98±0,05 ^b	0,87±0,09 ^b	0,71±0,07 ^b	1,04±0,09 ^a

Means in same line with the different letter are significantly different among treatment at $p < 0.05$.

3.4. Chlorophyll fluorescence

Chlorophyll fluorescence is measured as a reflection of electron transport in the photosynthetic system of plants. The fluorescence parameters showed significant differences among treatments as shown in table 6. The increase of Cd and Zn in the soil decreases F_v/F_m .

Subjecting kenaf plants to Cd and Zn treatments resulted in significant increase of F_0 that ranged from 329

to 465. Based on the control (T0), the Cd-alone treatment (T6) increases F_0 up to 21%. In the Cd+Zn treatment (T4), an increase in F_0 was also observed but its magnitude was lower than that of (T6) and higher than Zn-alone treatment (T2).

It appeared from the results that the Cd- alone treatment caused the greatest increase in F_0 and reduction in F_v/F_m across the different treatments.

Table 7: Fluorescence parameters of *H. cannabinus* under Zn and Cd treatments

Treatment	F_0	F_v/F_m
Control (T0)	329,21±26,31 ^a	0,837±0,016 ^a
1500µM Zn (T1)	364,33±15,89 ^a	0,834±0,019 ^a
3000µM Zn (T2)	377,00±9,54 ^a	0,796±0,020 ^b
4500µM Zn (T3)	393,67±7,33 ^b	0,770±0,020 ^b
3000µM Zn + 50 µM Cd (T4)	380,33±10,01 ^b	0,769±0,010 ^b
25 µM Cd (T5)	373,00±6,25 ^b	0,793±0,010 ^b
50 µM Cd (T6)	421,33±21,13 ^c	0,768±0,014 ^{bc}
75µM Cd (T7)	465,03±18,42 ^c	0,741±0,012 ^c

Means in same columns followed by the same letter are not significantly different at $p < 0.05$.

3.5. Chlorophyll content

Chlorophyll (a) and (b) varied significantly ($p < 0.05$) among treatments (Table 7). When plants were grown in the presence of Cd and Zn, significant reductions were

observed, especially in the Cd-alone treatment where reduction in chlorophyll (b) reached 75% compared to the control. Chlorophyll (b) content in leaves was significantly higher in Cd +Zn treatment (T4) compared to its counterpart in Cd-alone treatment (T6).

Table 8: Chlorophyll (a) and chlorophyll (b) content ($\mu\text{g}\cdot\text{g}^{-1}$ fresh weight) in leaves of non treated and treated plants

Treatment	Chlorophyll (a)	Chlorophyll (b)
Control (T0)	725,46±21,56 ^a	326,69±34,12 ^a
1500 μM Zn (T1)	727,38±19,51 ^a	151,88±21,89 ^b
3000 μM Zn (T2)	690,22±22,33 ^{ab}	120,89±16,47 ^b
4500 μM Zn (T3)	539,29±34,21 ^b	115,29±9,54 ^b
3000 μM Zn + 50 μM Cd (T4)	606,75±39,54 ^b	110,75±8,58 ^b
25 μM Cd (T5)	417,62±33,16 ^c	97,05±9,78 ^b
50 μM Cd (T6)	383,29±40,01 ^c	84,50±9,46 ^c
75 μM Cd (T7)	295,86±18,01 ^c	81,50±7,11 ^c

Means in same columns followed by the same letter are not significantly different at $p < 0.05$.

4. DISCUSSION

Hibiscus cannabinus L. is considered tolerant to excess metal concentrations in soil (Bañuelos et al., 2002; Cartoga et al., 2005; Ho et al., 2008; Bada and Raji, 2010; Nabulo et al., 2011) and is suitable for Cd and Zn in practical phytoextraction (Arbaoui et al., 2013). As expected, results showed a significant increase in the concentration of Cd and Zn in plant tissues of kenaf upon exposure to high concentration in the soil. Several studies have reported that, for successful phytoremediation, it is generally beneficial to select species that show high biomass production combined with a moderate accumulation of metals. In the present study, results showed that kenaf can be suitable regarding

to these conditions.

It has been reported that trace metals, such as Cd, compete for the same transmembrane carriers with other essential nutrients (Ca, Mg, K, Zn) due to relative lack of selectivity of transport systems (Clarkson and Luttge, 1989; Ghosh and Singh, 2005). Interaction of Cd and Zn varied with plants species (Eriksson, 1990). In the case of *H. cannabinus*, the interaction effects showed antagonistic effect of Zn on Cd uptake as evident by the decreased concentration of Cd in plant tissues. As a consequence, stem and leaf translocation factor for Cd in Zn+Cd treatment increased significantly compared to TF in Cd-alone treatment.

Abiotic stress is known to have an influence on different plant fluorescence parameters and pigment

concentrations. Thus, quantification of these parameters is often used as a relative indicator for stress experienced by a plant (Osorio *et al.*, 2012). In plants grown on contaminated soil by trace metals, the cellular mineral balance is disrupted and excess metals are able to replace essential metals in pigments and enzymes, which can alter their function. The chlorophyll (a) and (b) which are the most abundant photosynthesis pigments can be disturbed and their concentrations reduced in the presence of metals, leading to a lower photosynthetic efficiency (Maleva *et al.*, 2012; Osorio *et al.*, 2012). The chlorophyll fluorescence ratio (Fv/Fm) and the initial fluorescence (F₀) estimate the photochemical efficiency of the PSII. A decrease in Fv/Fm and an increase in F₀ indicate that the photosynthetic biosystem functioning is impaired (Maxwell and Johnson, 2000). Our results showed that fluorescence parameters and chlorophyll a and b decreased with the increase of Cd and Zinc in soil. However, this reduction was lower in Zn+Cd than in Cd-alone treatment. In *C. demersum*, Aravind and Prasad (2003; 2004) reported that Zn reduced Cd-induced oxidative stress by its antioxidative capacity and protects macromolecules like proteins and enzymes from

Cd toxicity. Wu and Zhang (2002) showed that the physiological damage caused by Cd toxicity could be alleviated by application of Zn. Köleli *et al.* (2004) reported that Zn protects plants from Cd potential toxicity by improving plant defense against Cd-induced oxidative stress and by competing with Cd for binding to critical cell constituents such as enzymes and membrane protein.

Generally, photosynthesis reduction has a negative effect on biomass. In our study, however, the reduction of photosynthetic activity observed for kenaf plants grown in soils contaminated by Cd and Zn did not correlate with the changes in the biomass production. In fact, with fast-growing plant species, stress-inducing treatments like increased trace metal concentrations in soil led to inhibition in physiological response, however, that was not correlated to reduction of biomass. This phenomenon can be explained by the dilution effect, in which a metal was diluted in tissues of the fast-growing plant (Han *et al.*, 2006).

5. CONCLUSION

The current study showed that the uptake of one metal can be affected by the presence of other metals. kenaf plants have the capacity to uptake and accumulate Zn and Cd in their tissues, especially in roots. The co-presence of both metals reduces the Cd concentration in plants and alleviates the toxicity of Cd on photosynthetic system. It is concluded that there is an antagonistic interaction of Cd and Zn in *H. cannabinus* uptake. Based on this experiment, it is highly suggested that kenaf can be used for phytoremediation of contaminated soils. However, further research is required to evaluate the response of kenaf under natural conditions.

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