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Reduced toxicity of Cu and Zn to mangrove seedlings (*Kandelia candel* (L.) Druce.) in saline environments

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Abstract. We conducted hydroponic and pot experiments to investigate the influence of heavy metals on the growth of *Kandelia candel* (L.) Druce. The growth of *K. candel* decreased with increased concentrations of Cu or Zn in the hydroponic culture solution, but this inhibition was reduced when the culture solution was treated with NaCl. Concentrations of Zn and Cu in root tissue increased significantly with increased concentrations of Cu or Zn in the culture solutions, especially in the absence of NaCl. Accumulations of Cu and Zn in branches and leaves were found only in treatments with high concentrations of heavy metals. Similar results were found in pot experiments, but they were not as prominent as those from the hydroponic culture experiments. The results suggest that toxicity of Cu and Zn to *K. candel* is reduced by the presence of NaCl in the environment.

Keywords: Cu; *Kandelia candel*; Mangrove; Salinity; Toxicity; Zn.

Introduction

Mangroves are generally considered facultative halophytes; salt is not strictly required (Chapman, 1975). There are reports indicating the importance of salinity to some mangroves (Lugo and Snedaker, 1974). Most species of mangrove grow best at salinity levels less than or equal to that of seawater (Snedaker, 1982; Ball, 1988; Burchett et al., 1989). Our colleagues have proven that *Kandelia candel*, a dominant species of mangrove in Taiwan, grows best in media with moderate salinity; the optimum was found to be about 0.5% salinity (Hwang and Chen, 1995).

Various researchers have studied the influence on plants of the interaction between NaCl and metals, but most studied crops in arid regions, which are generally saline (Wallace et al., 1980; Wallace, 1989). In those studies, salinity was considered a stress for glycophytes. Present knowledge of the interaction between NaCl and metals in mangroves is limited mostly to considerations of the competition of NaCl with macro-essential nutrients such as potassium and calcium (Atkinson et al., 1967; Downton, 1982; Naidoo, 1985).

Other than photosynthesis, little is known about the influence of environmental stress on physiological processes in mangroves. This needs to be investigated for a full understanding of the role of the environment in regulating the growth and productivity of mangrove. There is great concern about the influence of mangroves on estuarine ecosystems and their potential as a monitor of pollutants (Montgomery and Price, 1979; Ragsdale and Thorhaug, 1980; de Lacerda et al., 1986).

It has been found that mangroves enhance the accumulation of heavy metals in the mud they are growing in (Harbison, 1986), and our previous studies suggest that this might reduce the effective toxicity of these metals. The concentration which inhibited *K. candel* root development by 50% was estimated to be 3.8 mg liter^{-1} for Cu and 46.3 mg liter^{-1} for Zn (Chiu and Chou, 1991). The interaction of heavy metals and other factors, such as salinity, needs to be clarified. The objective of the our study was to investigate the growth of *K. candel* in environments with high concentrations of heavy metals.

Materials and Methods

Plant Material

Medium size (about 20 cm long), mature viviparous seedlings of *Kandelia candel* were collected from a mother plant in a mangrove forest

Hydroponic Culture Experiments

Viviparous seedlings were planted in a water culture solution containing copper or zinc at various concentrations, and grown under greenhouse conditions. The nutrient culture medium was essentially the same as that described in Chiu and Chou (1991). To investigate the influence of salinity, the basic nutrient was prepared in two forms: salt-treated (8.75 g l^{-1} NaCl) and non-saline solutions. For the experiments with Cu, the Cu concentrations were adjusted to 0.10, 0.50, 1.25, 2.50, 5.00, and 10.0 mg l^{-1} by the addition of $CuSO_4 \cdot 5H_2O$ to the basic medium. For the experiments with Zn, the Zn concentrations were adjusted to 1.00, 5.00, 25.0, and 125 mg l^{-1} by the addition of $ZnSO_4 \cdot 7H_2O$ to the basic medium. The control treatment contained 0.02 mg l^{-1} of Cu and 0.08 mg l^{-1} of Zn in the medium. Each treatment was performed 6 times. The treatments were started on June 2, 1990 and terminated after 12 weeks.

Pot Experiments

Samples of Youngho clay loam soil, collected from the upstream region of the Tamshui river, were used in the pot experiment. The soil sample was air-dried, mixed, and subsequently passed through 2 mm mesh sieves. Its physical and chemical properties were as follows: clay 21%, organic C 1.2%, CEC 26 cmol kg^{-1}, and pH 6.4. Mature viviparous seedlings were planted in 1/5000 acre Wagner pots contained with 4 kg of Youngho soil. Different levels of either $ZnSO_4 \cdot 7H_2O$ or $CuSO_4 \cdot 5H_2O$ were added the pots for the corresponding treatments. Half of the pots were further treated with 8 g NaCl to compare the effect of salinity on the toxicity of heavy metal to *K. candel*. Each treatment was performed 3 times. The experiment started on Oct. 4, 1988 under greenhouse conditions; the plants were harvested after 3 months.

Sampling and Chemical Analysis

Samples of *K. candel* were washed thoroughly, rinsed with deionized water, oven dried at 70°C, and then ground into powder for chemical analysis.

The plant samples were digested in a mixture of 25 ml concentrated nitric acid and 15 ml perchloric acid on a hot plate at a maximum temperature of 200°C. Concentrations of heavy metals in soil extracts and plant digests were determined with an atomic absorption spectrophotometer (Perkin-Elmer Model 2380).

Data were analyzed by the Duncan's multiple range test in the SAS statistical package (SAS Institute Inc., 1989).

Results

Response of Plant Growth to Heavy Metals in Hydroponic Culture

The growth rate of *K. candel* decreased significantly in the presence of increasing concentrations of Cu in the growth media (Figure 1). The dry weight of roots, branches, and leaves were significantly inhibited at Cu concentrations above 2.5 mg l^{-1}. The lethal dose was 10 mg l^{-1}. The damage by large doses of Cu was especially severe in the media without NaCl. Because hypocotyls originated from the viviparous seedlings, the increase in dry weight did not vary much throughout the experiment, and consequently caused no significant difference among the treatments. Similar results were obtained in the treatments with Zn (Figure 2). Root development was retarded in culture solutions containing Zn concentrations above 25 mg l^{-1}, and was completely inhibited at 125 mg l^{-1}. The injury was especially severe in the treatments without NaCl—Cu and Zn were more toxic in the absence of NaCl than in the presence of moderate salinity.

The accumulation of Cu was considerably higher in root tissues; the concentration of Cu in the root tissue increased as the concentration of Cu in the culture media increased (Table 1). Significantly higher concentrations of Cu were found in the absence of NaCl, especially in the roots.

The influence of large doses of Zn on the amount of Zn in plant tissue (Table 2) was similar to that of Cu; more Zn was found in root tissue in the absence of NaCl. The concentration of Zn in the root tissue increased with the

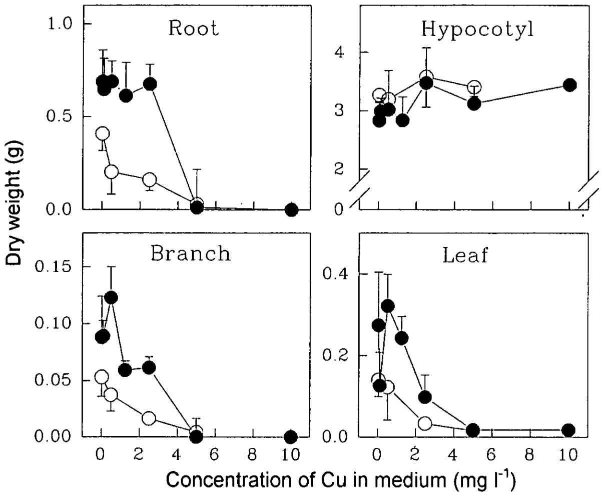

Figure 1. The influence of salinity and Cu on the growth of *K. candel* in hydroponic solution (solid, with NaCl; hollow, without NaCl. Vertical bars indicate the standard deviations of six replicates).

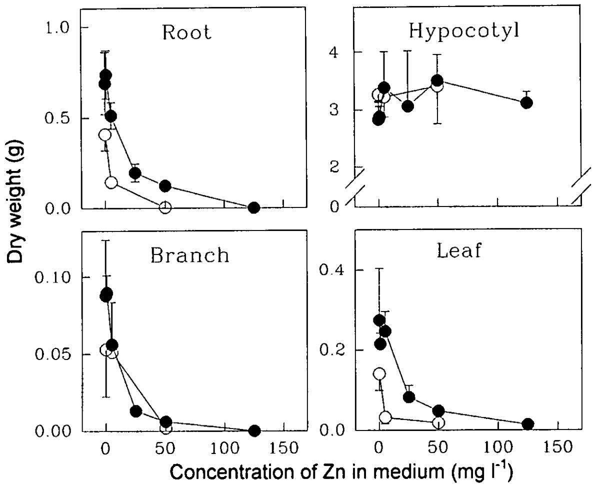

Figure 2. The influence of salinity and Zn on the growth of *K. candel* in hydroponic solution (solid, with NaCl; hollow, without NaCl. Vertical bars indicate the standard deviations of six replicates).

Zn concentration in the culture media, but increased concentrations in hypocotyls, branches, and leaves were only present with high concentrations of Zn.

Plant Growth in Soil Containing Heavy Metals

The interaction of NaCl with Cu and Zn was also found in the pot experiments. Table 3 shows the influence of different concentrations of Cu. The dry weight of root gown in soil containing NaCl was not significantly reduced, even in the presence of 400 mg Cu per kg of soil, but in the absence of NaCl, 400 mg Cu per kg of soil inhibited root development. Inhibition of the growth of leaf and branch was essentially the same as that of root. Similar results were obtained by treatment with Zn (Table 4).

Table 1. The influence of the interaction between NaCl and Cu in hydroponic culture solution on the concentration of Cu in tissues of *K. candel*. Values followed by the same letter show no significant difference in Duncan's multiple range test at the 5% level.

Salinity (%) of medium	Conc. of Cu in medium (mg l^{-1})	Concentration of Cu in plant tissue (μg g^{-1})			
		Root	Hypocotyl	Branch	Leaf
0.875	0.02	19.28d	4.51de	16.01b	17.25b
	0.10	28.95d	2.56e	13.73b	12.94b
	0.50	75.25d	6.32de	10.30b	13.51b
	1.25	128.77d	5.19de	25.42ab	13.85b
	2.50	248.81d	4.71de	38.18ab	12.74b
	5.00	2440.35b	15.45c	–	84.39a
	10.00	–	43.87a	–	18.92b
0	0.02	42.01d	3.52e	11.21b	7.20b
	0.50	258.54d	10.56cd	18.66b	26.98ab
	2.50	1506.62c	10.16cd	62.27a	12.44b
	5.00	4058.93a	27.74b	–	72.07a

Table 2. The influence of the interaction between NaCl and Zn in hydroponic culture solution on the concentration of Zn in tissues of *K. candel*. Values followed by the same letter show no significant difference in Duncan's multiple range test at the 5% level.

Salinity (%) of medium	Conc. of Zn in medium (mg l^{-1})	Concentration of Zn in plant tissue (μg g^{-1})			
		Root	Hypocotyl	Branch	Leaf
0.875	0.08	37.95e	13.62d	35.80b	47.96b
	1.00	123.22e	21.24d	50.47b	69.79b
	5.00	496.97d	29.36cd	84.32b	85.61b
	25.00	1274.41c	25.50d	67.22b	41.66b
	50.00	2430.11b	34.93cd	377.05a	123.53b
	125.00	–	78.61b	–	547.69a
0	0.08	44.38e	11.36d	38.99b	18.61b
	5.00	1061.63c	63.80bc	21.79b	60.11b
	50.00	5430.49a	246.49a	84.21b	235.60ab

Table 3. The influence of NaCl and Cu concentrations in soil on the growth of *K. candel*. Values followed by the same letter show no significant difference in Duncan's multiple range test at the 5% level.

NaCl (%)	Cu (mg kg^{-1})	Dry weight of plant parts (g)			
		Root	Hypocotyl	Branch	Leaf
0.2	0	1.513a	3.008a	0.692a	0.740ab
	125	1.326ab	2.941a	0.586a	0.460c
	250	1.221ab	3.051a	0.447ab	0.507bc
	400	1.305ab	3.112a	0.477ab	0.635ab
0	0	1.380ab	2.808a	0.618a	0.531bc
	125	1.197ab	2.731a	0.579a	0.355c
	250	1.322ab	2.988a	0.444ab	0.497bc
	400	1.010b	2.880a	0.286b	0.485bc

Table 4. The influence of NaCl and Zn concentrations in soil on the growth of *K. candel*. Values followed by the same letter show no significant difference in Duncan's multiple range test at the 5% level.

NaCl (%)	Zn (mg kg^{-1})	Dry weight of plant parts (g)			
		Root	Hypocotyl	Branch	Leaf
0.2	0	1.513a	3.008a	0.692a	0.740a
	125	1.189ab	3.004a	0.668ab	0.579ab
	250	0.953bc	3.025a	0.489abc	0.360bc
	400	0.595d	2.903a	0.212c	0.344bc
0	0	1.380ab	2.808a	0.618ab	0.531abc
	125	1.410a	3.204a	0.632ab	0.559abc
	250	1.124abc	2.950a	0.438abc	0.272bc
	400	0.774cd	2.750ab	0.404bc	0.224c

Table 5. The influence of the interaction between NaCl and Cu in soil on the Cu content in the tissue of *K. candel*. Values followed by the same letter show no significant difference in Duncan's multiple range test at the 5% level.

NaCl (%)	Cu (mg kg^{-1})	Concentration of Cu in plant (μg g^{-1}plant)			
		Root	Hypocotyl	Branch	Leaf
0.2	0	24.99d	7.34d	31.42ab	25.53a
	125	292.10c	10.67bcd	31.18ab	29.08a
	250	360.30bc	13.50bc	18.02b	10.96b
	400	545.72a	35.52a	33.49a	20.71ab
0	0	24.89d	8.68cd	32.29a	24.89ab
	125	311.78c	10.41bcd	36.03a	29.09a
	250	423.39b	14.72b	31.97ab	25.99a
	400	589.05a	36.33a	30.36ab	18.44ab

Table 6. The influence of the interaction between NaCl and Zn in soil on the Zn content in the tissue of *K. candel*. Values followed by the same letter show no significant difference in Duncan's multiple range test at the 5% level.

NaCl (%)	Zn (mg kg^{-1})	Concentration of Zn in plant (μg g^{-1} plant)			
		Root	Hypocotyl	Branch	Leaf
0.2	0	85.06e	16.08d	74.33ef	89.04a
	125	1387.64d	35.30cd	120.81de	103.07a
	250	3104.97bc	52.53c	153.08cd	115.52a
	400	4004.69ab	310.38a	140.52d	135.06a
0	0	56.12e	31.49cd	59.73f	68.74a
	125	1276.79d	42.73cd	200.32bc	164.90a
	250	2175.27cd	61.52c	227.82b	132.11a
	400	4534.28a	174.29b	346.12a	166.39a

In the presence and absence of NaCl the concentration of Cu in root tissue increased with the concentration of Cu in the soil, while the concentration of Cu in branch and leaf was not significantly different (Table 5). This suggests that Cu accumulates easily in the root and is not translocated to other parts of the plant. These results coincided with those from the hydroponic culture experiments, with the exception that the interaction of NaCl with Cu was not as obvious.

The results from the Zn experiments (Table 6) indicate that the remarkable increase of Zn concentration in root tissue is related to the concentration of Zn in the growth medium. The concentrations in branch and leaf increased likewise, but were never as high as that in the root tissue.

Discussion

Sodium chloride appears to be essential to *K. candel*; its growth was reduced in the absence of NaCl, even in the presence of low concentrations of Cu or Zn (Figures 1 and 2). The absence of NaCl has a synergistic influence on the toxicity of Cu and Zn to *K. candel*. This might be the result of a nutrient imbalance and/or disturbed osmotic regulation.

It has been noted that the primary toxic action of metal ions takes place at the cell surface—alternations in cell membrane properties cause leakage of K^+ and other ions and solutes (Woolhouse and Walker, 1981). This might inhibit the maintenance of positive cell turgor. Our results indicate that the concentrations of Na and K in root tissue were negatively correlated with the concentration of Cu or Zn in the growth media (Chiu et al., unpublished data).

In most soils, metals are present predominantly as complexes. A much larger proportion of Cu than Zn is present as complexes. This causes Zn^{2+} activity to be much higher than that of Cu^{2+} in the absorption sites (Loneragan and Webb, 1993), and supports the results given in Tables 5 and 6—in soil, the uptake of Zn was higher than that of the same concentration of Cu.

The results of metals toxicity in soil were not as obvious as those found in hydroponic treatment. The availability to plants of metals in soil depends on soil pH, organic matter, and phosphate status. Metals are absorbed by organic matter, clay, and hydrous oxides of Fe and Mn. This causes the metal activity to be considerably lower than equilibrium values for known inorganic compounds (Foy et al., 1978), and thus the toxicity of heavy metals in soil is lower. Similarly, sodium is absorbed by soil particles, which reduces the effective salinity of the soil. Thus, the influence of NaCl in the pot experiments was not as significant as it was in the hydroponic experiments.

Inorganic Cl^- can promote the formation of metallic complexes (Hahne and Kroontje, 1973). McLaughlin and Tiller (1994) found that in saline soil solution Cl^- could form a chloro-complex with cadmium that increased the availability of cadmium to the plant. The mechanism by which Cl^- in soil and nutrient solution chelates metals is not clear. It may be that chelators added to the soil increase the solubility of metals and promote metal activity. This would increase the potential of uptake by plants and the consequent toxic influence. Chelator added to nutrient solutions, however, may reduce metal activity and metal uptake dramatically (Foy et al., 1978).

Although metallic complexes formed by Cl^- might increase the bioavailability of metal ions, the interactions between NaCl and heavy metals showed less significance in the pot experiments than in the hydroponic experiments. This might be a result of the strong absorption of the metals by the clay soil. The behavior of chloro-complexes of metals could be one of the reasons that the toxicities of Cu^{2+} and Zn^{2+} were reduced in the saline hydroponic culture solution. Because *K. candel* grows better in moderate salinity, NaCl may not only play a role in the formation of chloro-complexes, but may also act to reduce the toxicity of metals.

We conclude that the toxicity of Cu^{2+} and Zn^{2+} is influenced by the salinity of the growth medium. Interaction of NaCl with Cu^{2+} or Zn^{2+} might be a critical physiological requirement for *K. candel* in heavy-metal polluted environments.

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氯化鈉減輕銅及鋅對水筆仔生長毒害的影響

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水筆仔之生長隨著水耕液中銅或鋅濃度的增加而受到阻礙。尤其當水耕液中無氯化鈉存在時，抑制的現象更爲明顯。根部組織中銅及鋅之濃度隨水耕液中濃度之升高而增加，其中尤以未添加氯化鈉的處理組其增加的程度最爲明顯。至於莖葉部中銅及鋅的濃度只有在高濃度的處理組才略有增加的現象。土耕試驗亦可發現具有上述類似的結果，但不如水耕試驗明顯。此等結果証明氯化鈉具有減輕銅及鋅對紅樹林毒害作用之效果。

關鍵詞：銅；水筆仔；紅樹林；鹽度；毒性；鋅。