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# Influence of Heavy Metals on the Carbohydrate and Phenolics in Mangrove, *Aegiceras corniculatum* L., Seedlings

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Mangrove forest, a complex intertidal ecosystem distributed in the tropics and subtropics, appear to possess a remarkable capacity to retain heavy metals (Badarudeen et al. 1996; Machado et al. 2002; Alongi et al. 2004). Recently, mangrove forests have been shown to play an important role in the biogeochemistry of trace metal contaminants in tropical coastal areas, and are considered to have the capacity to act as a sink or buffer and to remove or immobilize heavy metals before they reach nearby aquatic ecosystems (Tam and Wong 1996). However, most of the attention has been paid to the distribution and speciation of heavy metals in mangrove plants and sediments. We have little knowledge about why mangroves could inhabit in the heavily heavy-metal polluted mangrove swamp. Therefore it is important to evaluate the response of mangroves to the heavy metals stress, so as to have an in-depth understand about the high heavy metals tolerance of mangroves.

In this study, we tested the variation of foliar carbohydrate and provided the first comprehensive description of the variation in the contents of phenolic compounds in mangrove (*A. corniculatum* L.) seedlings under heavy metal stress. We also related the variation in the content of phenolic compounds to the antioxidant activities in response to heavy metal in *A. corniculatum* L. seedlings. We aimed to investigate: (1) whether heavy metals induce changes in foliar carbohydrate and phenolic metabolism and, if so (2) could carbohydrate and phenolics be indicators of heavy metal stress of *A. corniculatum* L. seedlings and, (3) do they do any help to the high heavy metals tolerance of mangroves?

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## Materials and Methods

Seeds of *A. corniculatum* L. were collected from Yunxiao mangrove forest (latitude 23°55′180″, longitude 117°25′212″) of Fujian, China. After 2 months of cultivation in greenhouse with natural illumination, the relative humidity 85%, and the temperature ranging from 26 to 32°C, healthy seedlings similar in apparent health, height (106 ± 19 mm), and leaf number (3 ± 0.41) (mean ± SE) were selected for sandy culture in plastic pots (35 cm diameter × 15 cm deep) with Hoagland's nutrient medium (pH ~6.0). Metal salts were supplied with $CuCl_2$, $ZnCl_2$ and $CdCl_2$ in pots to achieve of 0, 5, 10, 20, and 30 mg $L^{-1}$ of Cu, Zn and Cd, respectively. Metals were added as the chloride salt to minimize adverse effects of anion toxicity for mangroves being adapted to high levels of $Cl^-$ in estuarine sediments (Burchett et al. 1984). The experiment had three replicates for each treatment, with a completely randomized block design. Seedlings were watered with nutrient medium and tap water during the growing season when necessary. The experiment was started in January 2006 and ended in June 2006.

Soluble sugars and starch were estimated by anthrone-sulphuric acid method of McCready et al. (1950) using 0.2% anthrone in concentrated $H_2SO_4$ as reagent. Sucrose was used as the standards to prepare standard curves and spectrophotometric readings were taken at 630 nm.

Fresh leaves material was used for the extraction of phenolics. Total phenolics (TP) were extracted with 70% (v/v) acetone and measured with the Prussian blue method (Graham 1992), using tannic acid as the standards to prepare a standard curve. The extractable condensed tannin (Extractable CT), protein-bound condensed tannin (Protein-bound CT), and fibre-bound condensed tannin (Fibre-bound CT) were extracted with 70% (v/v) acetone and determined with butanol–HCl method (Terrill et al. 1992) as modified by Lin et al. (2006). It is important to note that different types of tannins react differently in the assays used to quantify them (Hagerman and Butler 1989; Kraus et al. 2003). Therefore, absorbance data for condensed tannin were converted to a dry fresh weight basis using tannins purified from *A. corniculatum* L. as standards. The tannins were purified and characterized as described by Hagerman (2002). Total condensed tannin (Total CT) was calculated by adding the respective quantities of extractable CT, protein-bound CT and fibre-bound CT.

All the data reported are averaged values of three replicates. Heavy metal was a significant factor therefore analysis of variance (ANOVA) was run using heavy metal as the independent variable. Significance was determined at $p < 0.01$, and Tukey test was used to determine significant differences between treatments. All statistical analyses were performed using SPSS software, Version 11.5, for Windows.

## Results and Discussion

All seedlings survived during the 6-month experiment. One week after treated with heavy metals, numerous brown dots were found on the leaves of *A. corniculatum* L. seedlings, especially in the high treatment concentration groups (more than 20 mg $L^{-1}$), and matured leaves gradually became yellow and defoliated finally. Heavy metal treatments markedly decreased the fresh biomass yields of *A. corniculatum* L. seedlings (Table 1), which showed that heavy metals exposure had been a stress to the seedlings. Soluble sugar content increased with the increase of heavy metals concentration while starch content decreased (Fig. 1), which suggested disturbance of the carbohydrate regulation. Our result was consistent with earlier study in which Cd was reported to increased soluble carbohydrate concentration and decreased starch concentration in the seedlings (Kim et al. 2003).

Foliar TP and CT were markedly enhanced in seedlings exposed to heavy metals than those in control (Table 2). These findings agreed with other studies investigating the effects of heavy metals on *Phyllanthus tenellus* (Santiago et al. 2000), *Capsicum annuum* (Díaz et al., 2001) and *Raphanus sativus* (Sgherri et al. 2003). It was reported that phenolic compounds can act as radical scavengers or radical-chain breakers (Grassmann et al. 2002) and play a beneficial role in protecting plants from the harmful effects of reactive oxygen species generated from exposure to copper (Elzaawely et al. 2006). Thus, stressed plants accumulated increased amount of phenolic compounds, which are considered to be the most important antioxidative plant components (Hu and Skibsted 2002) for protection and recovery from heavy metal injury.

Heavy metal treatments increased foliar concentrations of soluble CT and protein-bound CT, but decreased concentrations of fibre-bound CT, and as a result synthetically increased concentrations of total CT in the present study (Table 2). To our knowledge, there are few comparable results in the literature reporting the direct effect of heavy metals on CT, however, there are some reports showing that phenoilics and certain individual phenolic compounds responded to heavy metal stress, and those responses may contribute to the variations of CT. Some heavy metals, such as Ni and Cu, have been observed to induce leaf accumulation of phenolics (Pandolfini et al. 1992; Baccouch et al. 1998; Santiago et al. 2000). In other cases, levels of phenolic compounds were noted in plants exposed to Ni and Cu (Murch et al. 2003). Loponen et al. (2001) reported both increased and decreased levels of individual phenolic compounds in mountain birch leaves from a Cu and Ni polluted forest site. In their study (+)-catechin and some gallic acid derivatives increased in concentration while some flavonol glycosides decreased slightly towards the CuNi smelters. In the present study, heavy metal stress

**Table 1** Fresh mass yields of *A. corniculatum* L. seedlings grown in sand treated with heavy metals

| | Treatment (mg $L^{-1}$) | Heavy metals | | |
|---|---|---|---|---|
| | | Zn | Cu | Cd |
| Leaves biomass | 0 | 3.17 ± 0.18a | 3.17 ± 0.18a | 3.17 ± 0.18a |
| | 5 | 3.20 ± 0.21a | 2.50 ± 0.23a | 2.60 ± 0.25ab |
| | 10 | 2.50 ± 0.21ab | 2.47 ± 0.27a | 2.04 ± 0.21ab |
| | 20 | 1.92 ± 0.12b | 2.17 ± 0.23a | 1.81 ± 0.14b |
| | 30 | 2.00 ± 0.18b | 2.17 ± 0.09a | 1.73 ± 0.18b |
| Total biomass | 0 | 8.16 ± 0.21a | 8.16 ± 0.21a | 8.16 ± 0.21a |
| | 5 | 8.09 ± 0.36a | 7.54 ± 0.30a | 7.19 ± 0.36b |
| | 10 | 6.90 ± 0.36b | 7.32 ± 0.60a | 5.41 ± 0.44d |
| | 20 | 6.83 ± 0.35b | 6.54 ± 0.31b | 6.35 ± 0.58c |
| | 30 | 6.37 ± 0.38b | 6.59 ± 0.45b | 5.45 ± 0.32d |

Values expressed as mean ± SE (g, fresh weight). Values followed by the same letter in the columns do not differ statistically according to the Tukey test at $p < 0.01$

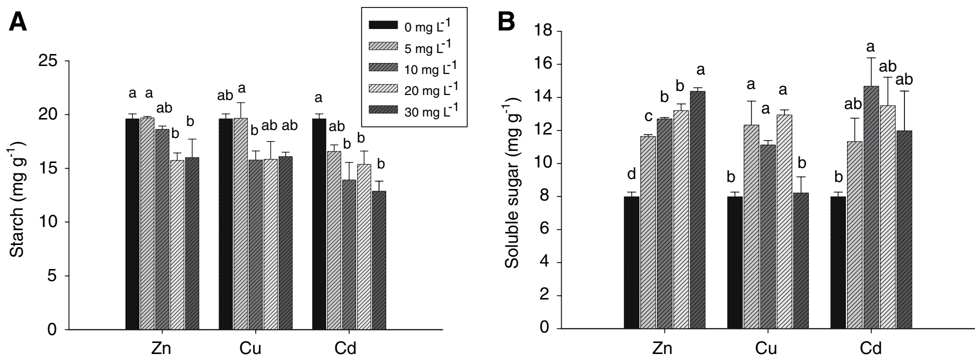


**Fig. 1** Concentrations of foliar starch and soluble sugar in *A. corniculatum* L. seedlings grown in sand treated with heavy metals. Each bar represents mean ± SD (mg $g^{-1}$, dry weight, n = 3)

**Table 2** Concentrations of foliar CT and TP in *A. corniculatum* L. seedlings grown in sand treated with heavy metals

| | Treatment (mg $L^{-1}$) | Heavy metals | | |
|---|---|---|---|---|
| | | Zn | Cu | Cd |
| Extractable CT | 0 | 11.62 ± 0.38b | 11.62 ± 0.38c | 11.62 ± 0.38b |
| | 5 | 16.84 ± 0.91ab | 11.80 ± 0.86bc | 15.36 ± 0.17ab |
| | 10 | 17.25 ± 0.05a | 14.26 ± 0.35bc | 17.22 ± 0.96a |
| | 20 | 21.20 ± 1.21a | 18.00 ± 0.45a | 16.72 ± 0.90a |
| | 30 | 20.74 ± 1.15a | 15.29 ± 0.69ab | 14.27 ± 0.42ab |
| Protein-bound CT | 0 | 6.70 ± 0.15bc | 6.70 ± 0.15b | 6.70 ± 0.15ab |
| | 5 | 6.11 ± 0.20c | 6.54 ± 0.40b | 6.03 ± 0.22b |
| | 10 | 7.90 ± 0.18a | 7.99 ± 0.49ab | 7.33 ± 0.49ab |
| | 20 | 7.68 ± 0.12ab | 9.34 ± 0.28a | 8.11 ± 0.27a |
| | 30 | 8.60 ± 0.22a | 8.45 ± 0.09ab | 7.39 ± 0.18ab |
| Fibre-bound CT | 0 | 6.40 ± 0.21a | 6.40 ± 0.21a | 6.40 ± 0.21a |
| | 5 | 4.87 ± 0.07bc | 4.74 ± 0.50ab | 6.03 ± 0.22ab |
| | 10 | 3.74 ± 0.34c | 4.08 ± 0.18b | 5.43 ± 0.05ab |
| | 20 | 5.52 ± 0.31ab | 5.16 ± 0.18ab | 5.05 ± 0.16b |
| | 30 | 5.18 ± 0.12abc | 4.95 ± 0.17ab | 5.35 ± 0.16b |
| Total CT | 0 | 24.72 ± 0.55b | 24.72 ± 0.55b | 24.72 ± 0.55b |
| | 5 | 27.82 ± 1.05b | 23.08 ± 1.68b | 27.42 ± 0.60ab |
| | 10 | 28.89 ± 0.47ab | 26.33 ± 0.66b | 29.98 ± 0.96a |
| | 20 | 34.40 ± 1.59a | 32.50 ± 0.52a | 29.88 ± 1.30ab |
| | 30 | 34.52 ± 0.82a | 28.69 ± 0.92ab | 27.01 ± 0.55ab |
| TP | 0 | 64.53 ± 0.98c | 64.53 ± 0.98c | 64.53 ± 0.98c |
| | 5 | 70.52 ± 0.63bc | 68.10 ± 0.53bc | 68.79 ± 1.53bc |
| | 10 | 73.21 ± 1.42b | 75.89 ± 2.00ab | 80.08 ± 2.18a |
| | 20 | 84.01 ± 1.73a | 83.08 ± 1.37a | 78.18 ± 1.05a |
| | 30 | 74.14 ± 1.12b | 80.26 ± 1.88a | 73.68 ± 0.84ab |

Values expressed as mean ± SE (mg $g^{-1}$, dry weight, n = 3). Values followed by the same letter in the columns do not differ statistically according to the Tukey test at $p < 0.01$

significantly modified foliar CT accumulation in *A. corniculatum* L. seedlings, which suggests demolishment of phenolic metabolism in plants. Some indications of a dose response effect of heavy metals on foliar CT were also observed. However, no statistical difference was found on the same treatment levels of different heavy metal treatment except for the extractable CT, which suggests that Zn, Cu, and Cd might have the same effects on the phenolics metabolism of *A. corniculatum* L. seedlings.

Heavy metals expose changed the distributions of extractable CT, protein-bound CT and fibre-bound CT significantly (Fig. 2). The pattern of distribution of CT in extractable, protein-bound and fibre-bound fractions is known to be dependent on several factors, such as the total content of condensed tannins, the age of the plant, and certain conditions of climatic or nutritive stress during growth of the plant (Barry and Manley 1986; Iason et al. 1995). In the present study, average across heavy metal treatments ratios of extractable CT to total CT (ECT: TCT) increased significantly (47%–62%) in all three heavy metal species (Fig. 2a), and ratios of fibre-bound CT to total CT (FBCT: TCT) decreased significantly (Fig. 2c), which

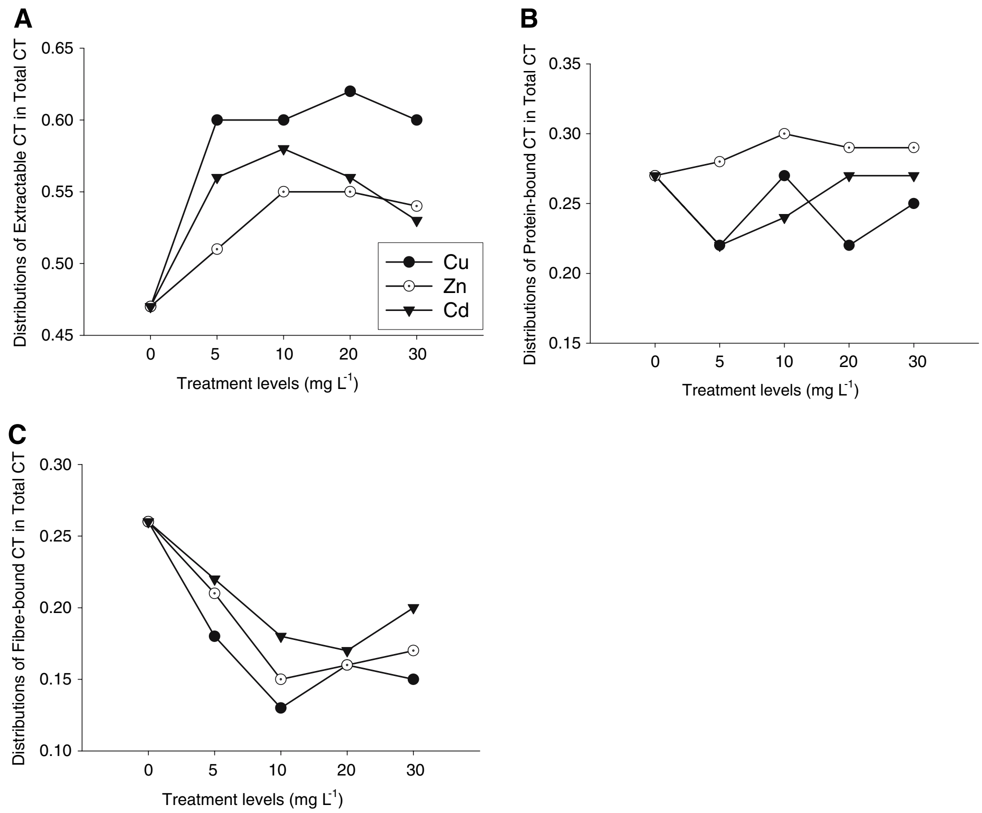


**Fig. 2** Distributions of extractable CT, protein-bound CT, and fibre-bound CT in total CT in the heavy metals exposed *A. corniculatum* L. seedlings

suggests that phenolic metabolism and normal processes in distribution of CT are altered. Our result showed that about half of the foliar total CT (47%–62%) was in the extractable CT fraction. This was consistent with the general pattern showing that species with a high TCT content had a higher proportion of extractable and lower proportion of bound tannin fractions (Frutos et al. 2002). Although Perez-Maldonado and Norton (1996) proved that interchange between free and bound tannin fractions in different parts of the digestive tract was complex, we did find that the distributions of summary CT groups were significantly modified by heavy metal stress, which makes it likely that the heavy metal stress effects of CT could be predicted from the distribution of those fractions in the plant itself.

Heavy metal induced changes in the phenolic compounds may further affect their functions in plant cells. Phenolic compounds, including tannins, are often involved in responses to different kinds of abiotic and biotic stresses (Rivero 2001; Booker et al. 1996; Dixon and Paiva 1995; Yamasaki et al. 1995). It is reported that in plant systems, phenolics can act as antioxidants by donating electrons to guaiacol-type peroxidases (GuPXs) for the detoxification of $H_2O_2$ produced under stress conditions including heavy metal stress (Sakihama et al. 2002). Thus, increased contents of TP and variations of CT found in the present study might play an important role in the seedlings of *A corniculatum* L. responding to heavy metal stress, which is of great important for the plant inhabiting in the heavily heavy metal polluted mangrove swamp.

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