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Low-molecular-weight organic acids exuded by Mangrove (*Kandelia candel* (L.) Druce) roots and their effect on cadmium species change in the rhizosphere

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Abstract

Cadmium (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. In these areas, mangroves have been observed to possess a tolerance to high levels of Cd and it is hypothesized that low-molecular-weight organic acids (LMWOAs) produced at the soil–root interface (rhizosphere) may play an important role in the availability of Cd to these plants. Changes in both LMWOAs and Cd bioavailability, directly or indirectly related to the Cd stress were studied in the laboratory. A rhizobox technique was used for 6 months under growth in air-conditioned greenhouse with natural illumination and the relative humidity of 85%, the temperature ranging from 26 to 32 °C, in increasing Cd concentration stress conditions (0, 5, 10, 20, 30, 40 and 50 ppm). Six-month-old *Kandelia candel* (L.) Druce seedlings which grown in the rhizoboxes were selected to examine their root exudates. The results showed that monocarboxylic acids (formic, acetic, lactic, butyric and propionic acids), and di- and tricarbonxylic acids (maleic, fumaric, citric and L-tartaric acids) were found in root exudates. Citric, lactic and acetic acids being dominant took up 76.85–97.87% of the total LMWOAs in root exudations. Fumaric acid was only found where mangroves were growing on 20 ppm Cd. Root exudates reduced pH by 0.2–0.5 pH units in the rhizosphere compare to the bulk soil. The proportion of exchangeable Cd and Cd bound to carbonate had a positive correlation to total LMWOAs in the rhizosphere soil. Root exudates induced changes in soil Cd species under control conditions, consisting of lower exchangeable Cd compared with increasing stress. Results indicate that the measurement of LMWOAs may be included as early biomarkers in a plant bioassay to assess the phytotoxicity of Cd-contaminated soils on mangrove plants.

Keywords: Low-molecular-weight organic acids; Mangrove; Metal speciation; Rhizosphere

1. Introduction

Heavy metals are common pollutants in urban aquatic ecosystems and in contrast to most pollutants, are not biodegradable and are thus persistent in the environment. Many heavy metals are non-essential to plant and animal metabolism (such as cadmium, chromium and mercury), and are often toxic in low concentrations. Cadmium is of particular concern, because although it is not an essential element (Kabata-Pendias and Pendias, 1992), it is readily absorbed and accumulated in plants, thus increasing the potential for contamination of the food chain (Baker, 1981; McGrath et al., 1997). The severity of impact on coastal habitats has increased dramatically since industrialization. Metal inputs arise from industrial effluents and wastes, urban runoff, sewage treatment plants, boating activities, agricultural fungicide runoff, domestic garbage dumps and mining operations.

Mangrove forests, a complex intertidal ecosystem distributed in the tropics and subtropics, appear to possess a remarkable capacity to retain heavy metals (Harbison, 1986; Lacerda et al., 1993; Tam and Wong, 1996; 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).

Low-molecular-weight organic acids (LMWOAs) released from roots into soil have important functions in mobilizing metal micronutrients and for causing selective enrichment of plant-beneficial soil micro-organisms that colonize the rhizosphere.

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They are known to play important roles in a number of rhizospheric processes, including nutrient (e.g. Fe and Zn) acquisition (Hopkins et al., 1998), plant-microbe associations (e.g. Rhizobium symbiosis with leguminous roots) (Janczarek et al., 1997), regulation of plant growth (Tao et al., 2003), detoxification of potentially harmful elements like Al, Cu, Zn (Jones, 1998) and determination of microbial community structure in the plant rhizosphere (Petra et al., 2004). LMWOAs found in the environment comprise mono-, di-, and tricarboxylic acids including compounds containing unsaturated carbon and hydroxyl groups. The LMWOAs function as ligands increasing the total amount of dissolved cations such as aluminum and iron in soil solutions by complexing of metal cations (Strobel, 2001).

Chemical conditions in the rhizosphere soils are often different from the bulk soil as plant roots exude organic compounds including LMWOAs, It is also expected that roots exudate components to regulate their bioavailability and transport in the soil environment. Moreover, root exudation may be an important mediation in altering the species composition of rhizosphere microflora that function in nutrient transformations, decomposition and mineralization of organic substances, and formation of soil organic matter, all of which are related to soil quality (Petra et al., 2004). This latter aspect is particularly in need of clarification, in view of the ever increasing threat of global environmental change and soil pollution caused by anthropogenic activity. Mangroves have been observed to possess a tolerance to high levels of heavy metals, yet accumulated metals may induce subcellular biochemical changes, which can impact on processes at the organism level. The literature on herbaceous exudation of organic acids is extensive. In contrast, the information that exists for trees is very limited. Equally, the relationship between changes in the composition of exudates in the mangrove rhizosphere is poorly understood. This root-induced chemical change in the soil is of both ecophysiological and silvic significance. Consequently, the analysis of organic acids becomes of importance when understanding the ecophysiology of such plant species in their adaptation mechanisms. With respect to mangrove species, this could aid our understanding of their tolerance in highly polluted areas.

The objective of this experimental study was to determine the effect of Cd stress on root exudation of *Kandelia candel* (L.) Druce, cultivated in a rhizobox. Such experiments were performed in order to consider whether the Cd stress modified the mangrove root exudation of different LMWOAs. The purpose of this was therefore to separate the direct effect of Cd stress on root exudation from the indirect effect involving root soil interaction.

2. Materials and methods

2.1. Plant materials and culture conditions

Mature *K. candel* propagules were collected from Jiulong River Estuary (24°24′N, 117°23′E), Fujian China, in April 2004. The Jiulongjiang Estuary is located in Fujian Province, which is the northern boundary of mangrove forests in China. The region is subtropical where the mean annual temperature and precipitation are 21.1 °C and 1284 mm, respectively. Tides are semi-diurnal with an average range of 4 m. Only complete, undamaged propagules with testa intact and no emergent hypocotyl or radicles were selected for planting. The propagules selected were from 18 to 19.5 g fresh weight. Eighty-one individual 2-month old seedlings were chosen for experimentation. All seedlings were similar in apparent health, height (226 ± 19 mm), and leaf number (4 ± 0.41) (mean ± S.E.). Seedlings were randomly allocated to each treatment ($n = 12$). To each individual rhizobox in each treatment, an appropriate solution of the metal salt was added to arrive at six replicates across the concentration ranges, respectively, of 0, 5, 10, 20, 30, 40 and 50 ppm Cd (as $CdCl_2$) per gram of wet sediment. Propagules were grown for 6 months under glasshouse conditions in sediment (physical and chemical characteristics of soils shown in Table 1) which was supplied from their habitat. The seedlings were planted in a separate rhizobox (Fig. 1, modified from

Table 1
Physical and chemical characteristics of culture soil

Properties	Value
pH (H_2O)	6.32
Salinity (psu)	21‰
Organic matter (C g kg^{-1})	24.4
Water contents	43.68%
TOC (%DW)	1.81
TN (%DW)	0.11
TP (mg g^{-1})	0.623
Percent sand (%DW)	0.57
Percent silt (%DW)	83.62
Percent clay (%DW)	15.81
S (%DW)	0.42
Cd (ppm)	1.07
Fe (%DW)	3.52
Mn (mg g^{-1})	723

Note: Data are three duplicates. DW: dry weight; TOC: total organic carbon; TN: total nitrogen; TP: total phosphorus.

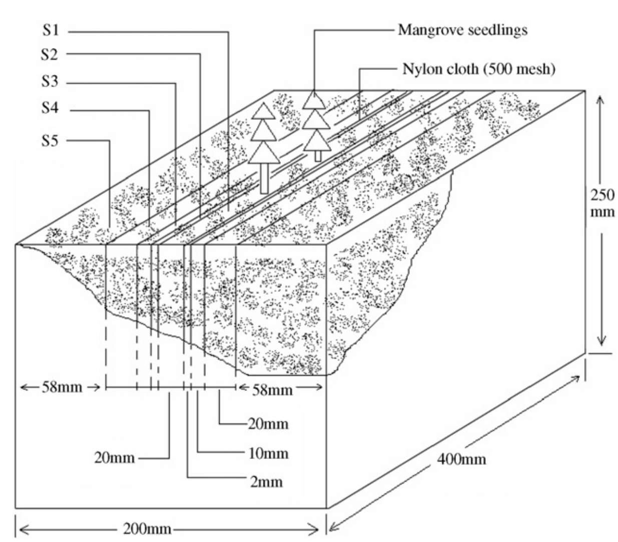

Fig. 1. Sketch diagram of rhizobox (modified from Wang et al., 2001). S1: soil for seedling growth; S2: rhizosphere; S3: near rhizosphere; S4: near bulk soil; S5: bulk soil.

Wang et al., 2001), immersed within 27 plastic containers (as holding trays) to mimimize drainage and simulate anoxic, waterlogged conditions. A homemade rhizobox (Fig. 1 modified from Wang et al., 2001) was used to plant *K. candel*. The dimension of the rhizobox (Fig. 1) was 400 mm × 200 mm × 250 mm (length × width × height). The rhizobox was divided into five sections from central to left or right boundary of rhizobox which were surrounded by nylon cloth (300 mesh), viz. a central zone for plant growth (20 mm in width), rhizosphere zones (2 mm in width), near rhizosphere zones (10 mm in width), near bulk soil zones (20 mm in width) and bulk soil zones (58 mm in width). In the rhizobox soil for seedlings growth, rhizosphere, near rhizosphere, near bulk soil and bulk soil zones were designated as S1, S2, S3, S4 and S5, respectively. Seedlings were watered manually with fresh water. Levels of water in the holding trays were maintained at 300 mL and re-percolation was carried out once a week, where the contents of the holding tray were poured through the soil medium and the percolate collected in the same holding tray, creating a closed system to maintain salinity (and metal levels) in the sediment.

2.2. Root exudates collection

Root exudates from the plants were harvested 2 h after the beginning of the photoperiod. The seedlings were removed from the rhizobox and the roots rinsed thoroughly in distilled water, then rinsed again in distilled water containing an antimicrobial agent to stop microbial degradation of the exudates during collection. The roots were then immersed in a collection volume of 250 mL of distilled water for 2 h. After 4-h incubation, root exudates were collected, evaporated to dryness under reduced pressure at 40 °C, dissolved in 5 mL of distilled water and then stored in a refrigerator at −20 °C. Root exudates were standardized with root dry weight, which was measured immediately after the treatment.

2.3. Analyses

2.3.1. Soil analysis

Sediment samples (the top 0–10 cm) which had been collected for seedling culture were analyzed according to Standard Methods (APHA, 1992). Sediment grain size was determined by a Malvern Particle Size Analyser (Malvern Instruments, England). Water content was estimated by drying triplicate samples in an oven at 105 °C for 24 h and establishing the loss of weight. The soil solution was isolated by centrifugation. This was filtered using filter paper and 0.45 μm HVLP membranes (Millipore), and analyzed for dissolved organic carbon and nitrogen by a Shimadzu TOC/TON Analyser. The sediment was dried, ground and processed for total organic carbon and total nitrogen on a Perkin-Elmer 2400 CHNS/O Series II Analyser and a Shimadzu TOC/TON Analyser with a solid sampler. Total phosphorus in the sediment was extracted using Williams procedures and determined by an ascorbic acid method (APHA, 1992). Salinity was measured on filtered water by a hand-held refractometer, and pH using a pH meter. Elements were determined after strong acid digestion on a Varian Liberty inductively coupled atomic emission spectrometer. For the Cd species, soil samples were extracted according the sequential extraction method proposed by Tessier et al. (1979). Physical and chemical characteristics of culture soil are shown in Table 1.

2.3.2. LMWOAs analysis

LMWOAs were analyzed by high performance liquid chromatography (HPLC) as described by Cawthray (2003) and are summarized here: All RPLC analyses were conducted with a Waters (Milford, MA, USA) 600E dual head pump, 717 plus autosampler and a 996 photo-diode array (PDA) detector. Separation was performed on an Alltima C_{18} column (250 mm × 4.6 mm, i.d.) with 5 mm particle size (Alltech Associates, Deerfield, IL, USA). The mobile phase consisted of 25 mM KH_2PO_4 adjusted to pH 2.5 with concentrated *ortho*-phosphoric acid, and methanol at a flow-rate of 1 mL min^{-1}. The system was equilibrated with 30 column volumes at each new mobile phase composition prior to four injections of a mixed organic acid solution. All data were acquired and processed with Millennium32® chromatography software (Waters) with PDA acquisition from 190 to 400 nm. PDA output at 210 nm was used for the quantification of organic acids. Positive identification of organic acids was accomplished by comparing standard retention times. For the analysis of root exudates samples, a gradient elution was employed every 5th sample, using 60% methanol to fully flush the column of hydrophobic compounds from previous injections. The limit of detection (LOD) was defined as a ratio of 3 for signal to noise (S/N), and all values reported for LOD are based on peak area.

2.3.3. Statistical analysis

Data were analyzed statistically using analysis of variance (ANOVA) and the Duncan's multiple range tests was employed to determine the significance of the differences between treatments. The statistical package used was SPSS statistical software package (Version 11.0) and the confidence limit was 95%.

3. Results

3.1. Composition and concentration of LMWOAs in root exudates

Of the several water extractable LMWOAs that were analyzed, formic, acetic, butyric, malic, lactic, fumaric maleic, citric and L-tartaric acids were the LMWOAs identified in the root exudates (Table 2). The total concentration of LMWOAs under different Cd stress shows large variation. In controls the total LMWOAs concentration was 15.336 μmol g^{-1} DW roots, whereas the total LMWOAs concentration under 10 ppm Cd stresses was found reached a maximum 39.0457 μmol g^{-1} DW roots. When compared with the controls, the lowest Cd application of 5 ppm, induced root exudates least concentration of total LMWOAs. Fumaric acid was only detected in the treatments under 20 ppm Cd stress (Table 2). In root exudates of LMWOAs, acetic, lactic, malic and citric acids were found to be the dominant acids for *K. candel* and they accounted for 8.11%, 18.52%, 2.31% and 68.28% of the total amount of LMWOAs

Table 2
LMWOAs excreted from roots under different cadmium stress

LMWOAs	LMWOAs concentrations in treatments (μmol g^{-1} DW roots)						
	CK	5	10	20	30	40	50
Formic	0.2138 ± 0.0128 c	0.2506 ± 0.0175 c	0.2865 ± 0.0172 c	0.3925 ± 0.0157 b	0.5213 ± 0.0209 a	0.401 ± 0.0160 b	0.5947 ± 0.0238 a
Acetic	1.2452 ± 0.0498 e	2.9861 ± 0.1194 c	3.2342 ± 0.1294 c	8.0556 ± 0.5639 a	2.3611 ± 0.1417 d	5.1389 ± 0.4111 b	1.7361 ± 0.1042 f
Butyric	0.2036 ± 0.0061 e	1.4322 ± 0.0716 b	0.5254 ± 0.0315 d	0.3526 ± 0.0176 e	2.0147 ± 0.1007 a	0.4216 ± 0.0253 d	0.8264 ± 0.0496 c
Malic	0.3537 ± 0.0283 e	3.4553 ± 0.2764 c	1.4634 ± 0.0878 d	3.45 ± 0.2760 c	9.0244 ± 0.5415 b	2.9695 ± 0.1188 d	11.748 ± 0.4699 a
Lactic	2.8415 ± 0.1989 c	0.3388 ± 0.0203 f	2.5683 ± 0.2311 c	5.609 ± 0.3366 a	3.0601 ± 0.1530 b	1.6393 ± 0.0984 d	0.5027 ± 0.0201 e
Fumaric	n.d.	n.d.	n.d.	0.0042 ± 0.0003 a	n.d.	n.d.	n.d.
Maleic	0.0056 ± 0.0003c	0.0063 ± 0.0006 c	0.0179 ± 0.0011bc	0.0223 ± 0.0016 b	0.0424 ± 0.0025 a	0.0182 ± 0.0013 bc	0.0044 ± 0.0003 a
Citric	10.4726 ± 0.8378 b	4.6269 ± 0.2776 e	30.95 ± 2.4760 a	7.9353 ± 0.6348 c	2.7363 ± 0.1915 g	5.3483 ± 0.4813 d	3.607 ± 0.2886 f
L-Tartaric	n.d.	n.d.	n.d.	n.d.	2.5981 ± 0.1819 a	0.1121 ± 0.0056 c	0.6729 ± 0.0471 b
Total	15.336	13.0962	39.0457	25.8223	22.3584	16.0489	19.6922

Data are means ± standard errors of three replicates. Values in each line followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Duncan's test. DW: dry weight. n.d.: not detected.

for the control, respectively (Fig. 2). With a Cd stress increase, the proportions of these were changed and under 10 ppm Cd stress accounted for 8.28%, 6.57%, 3.74% and 79.26, respectively, and under 50 ppm Cd stress 8.81%, 2.55%, 59.65% and 18.31%, respectively (Fig. 2).

3.2. Changes in soil pH value and dissolved organic matter

In the rhizobox experiment, the rhizosphere soils were more acidic than the non-rhizosphere soils, with differences in the range of 0.2–0.5 pH units (Table 3). The soil solution pH was higher under control but in a relatively tight range from 6.43 to 6.54. Toward 20 ppm concentration gone a lower pH from 6.08 to 6.42 and 10 ppm Cd treatments produced soil solutions with a pH in a wider range from 5.94 to 6.40 (Table 3). Compared to the control, the rhizosphere soil pH values in each Cd supply treatment were lower. Under Cd stress conditions, *K. candel* acidified the rhizosphere soils to a different degree (Table 3). The concentration of DOC in centrifuged rhizosphere soil solutions under different Cd stress shows a slight variation and ranged from 17.52 to 27.72 mM with the highest concentration found at 50 ppm Cd supply treatments (Table 4). The concentration of DON was in the range of 0.66–1.15 mM without any clear correlation with pH. The concentrations of DON and DOP showed no distinct differences among each treatment. Calculations showed that the C/N ratio ranged from 19 to 29 (Table 4).

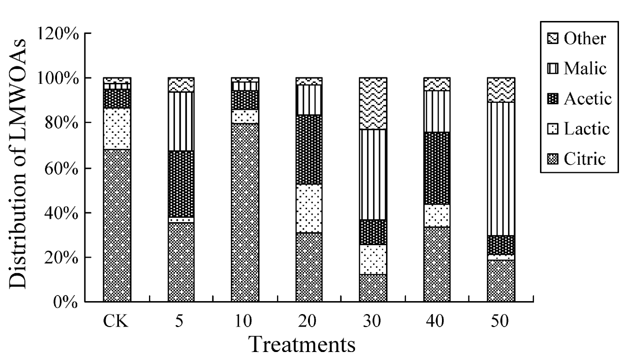

Fig. 2. Distribution of LMWOAs in root exudates under different cadmium stress conditions.

3.3. Change the Cd species in the rhizosphere

The root-induced changes in Cd speciation in the soil can be examined based on the change in the results of the sequential extraction over cultivation time. Results of the sequential extraction process are presented in Fig. 3 as a pie chart illustrating the distribution of exchangeable, carbonate bound, Fe–Mn oxide bound, organic matter and/or sulfide bound and residual Cd found in the rhizosphere soil sample. There was a clear tendency toward a higher proportion of exchangeable Cd at high Cd stress treatments which ranged from 6% to 39% (Fig. 3). Residual Cd showed a marked difference between control and other Cd supply treatments. As shown in Fig. 3, Fe–Mn oxide bound Cd was the dominant species in the sample accounting for more than one third of the total amount (ranged from 33% to 47%), while carbonates bound and residual Cd account for less than 15% (ranged from 14% to 23%), and 1% (ranged from 0% to 6%) of the total, respectively.

4. Discussion

This study showed heavy metal induced root exudates LMWOAs in mangrove *K. candel* where formic, acetic, butyric, malic, lactic, fumaric, maleic, citric and L-tartaric acids were identified in the root exudates (Table 2). The variation among replicates was especially high for formic acid. This acid also occurred in relatively high concentrations in the controls. Lactic acid occurs in the air (Grönberg et al., 1993). This acid is therefore found in high amounts in samples for reasons other than root exudation, especially after air bubbling, which captures the airborne acids. In contrast, differences in LMWOAs exudation patterns were found with the increasing Cd supply (Fig. 2). The exudation of dicarboxylic acids from seedlings was probably interpreted as an adaptation to adverse conditions, especially to toxic Cd concentrations. Previous studies found the exudation of citrate, malate, and related organic acids by maize and wheat in response to high Al^{3+} concentrations as a detoxifying mechanism (Jones and Kochian, 1996). In this study, higher exudation of malic, L-tartaric acids from roots in response to Cd stress were found in root exudates (Table 2), suggesting Cd complexation

Table 3
pH of the rhizosphere to bulk soils after growth of *K. candel*

Distance from the root	Treatments						
	CK	5	10	20	30	40	50
S2	6.43 ± 0.05 a	6.39 ± 0.04 a	5.94 ± 0.08 b	6.08 ± 0.02 b	6.16 ± 0.06 cd	6.27 ± 0.03 e	6.24 ± 0.08 ef
S3	6.45 ± 0.04 a	6.47 ± 0.06 a	6.23 ± 0.06 b	6.42 ± 0.05 a	6.33 ± 0.04 c	6.31 ± 0.07 cd	6.03 ± 0.04 e
S4	6.54 ± 0.07 a	6.19 ± 0.05 b	6.35 ± 0.07 c	6.37 ± 0.06 cd	6.28 ± 0.05 bce	6.34 ± 0.04 cf	6.47 ± 0.06 ac
S5	6.47 ± 0.05 a	6.49 ± 0.07 a	6.40 ± 0.06 a	6.33 ± 0.03 b	6.32 ± 0.09 bc	6.37 ± 0.05 ab	6.33 ± 0.03 b

Data are means ± standard errors of three replicates. Values in each column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Duncan's test.

by these organic acids as a same detoxifying mechanism for Cd.

A wide range of organic acids, which are quantitatively believed to be the most important organic component of tree root exudates (i.e. acetic, succinic and oxalic acids), are also produced by different tree species (Grayston et al., 1996). This study has shown heavy metal induced root exudates LMWOAs in mangrove *K. candel* and a similar range in types of LMWOAs have been identified for other plant species (Chen et al., 2001; Sandnes et al., 2005) where oxalic, formic, fumaric, malic, succinic, citric, acetic and lactic acids were identified. The total amount of LMWOAs in the *K. candel* root exudates studied here ranged from 13.09 to 39.05 μmol g^{-1} DW roots (Table 3) resembling closely the values for other trees root exudates, such as Norway spruce and silver birch (Sandnes et al., 2005) and beech forest (Shen et al., 1996).

In this study, *K. candel* exudates more malic acids than citric acids under higher Cd stress (30 and 50 ppm) (Table 2, Fig. 2). In previous studies, high levels of oxalic and succinic acids have been found in root exudates of temperate rain forests in situ (Chen et al., 2001). Other trees (Sandnes et al., 2005) grown in hydroponic cultures also exhibit this feature. These findings suggest that LMWOAs act as a characteristic of mangrove plants responding to heavy metal stress. Different supplies of Cd in the rhizobox significantly affected on the root exudates. Polle and Schützendübel (2003) pointed out Cd toxic to plants because of its high reactivity with sulphhydryl groups and causes oxidative stress by depletion of antioxidative systems and stimulation of-producing enzymes. This may result in an excess Cd toxicity effect in root functions thus induce different exudations in roots.

The pH value is widely considered to be one of the most important factors affecting metal mobility and bioavailability (Sauvé et al., 2000). In the rhizobox experiment, the rhizosphere soils were more acidic than the non-rhizosphere soils, with differences in the range of 0.2–0.5 pH units, although it has been observed in some cases that roots can make the soil near them more acid (Véronique et al., 2004). The small variation in the pH between the first layer and the other layers (Table 3) is not significant and may relate to the excess uptake of cations over anions in the rhizosphere soil (McGrath et al., 1997). The pH values in the rhizosphere were significantly relate to the total LMWOAs ($P < 0.001$). With Cd concentrations increasing, *K. candel* seemed to be related to an increase in acidification of the rhizosphere. This change in the rhizosphere pH was more likely as a result of exudates producing more acids in the roots. Heavy metals exist in soil solution as soluble ions in the form of inorganic and organic complexes. For ecological considerations, not only the total concentration but also the type of heavy metal species present in the soil solution is of primary importance. This is because metal mobility and availability are closely related to the composition of the solution. In addition, dissolved organic matter (DOM) affects the availability of metals to plant roots (Shoko and Chisato, 2005). The changes in dissolved organic matter in rhizosphere soil (Table 4) can be caused by several processes. As discussed by Qualls and Haines (1991), those possible in the laboratory incubation included: (1) adsorption, precipitation or decomposition; (2) microbial production of additional soluble material; (3) biochemical or physical transformation of one fraction to another.

Rhizosphere soil is a dynamic heterogeneous soil zone which is affected by a variety of environmental, biological and chemical factors (McGrath et al., 1997). The effect of organic acids on the Cd species in the rhizosphere soil was most pronounced (Wang et al., 2001). Most of the previous studies of metal pollutions in mangrove sediments have been limited to the total concentration of metals, and it is now widely recognized that the toxicity and the mobility of these pollutants depends strongly on their

Table 4
pH values and dissolved organic matter in rhizosphere soil

Treatments	pH	DOC (mmol L^{-1})	DON (mmol L^{-1})	DOC/DON	DOP (mg PO_4^{3-} L^{-1})
0	6.43 ± 0.05 a	24.12 ± 1.44 a	1.15 ± 0.07 a	21	0.20 ± 0.02 a
5	6.39 ± 0.04 a	20.16 ± 1.23 b	0.84 ± 0.05 b	24	0.19 ± 0.03 ab
10	5.94 ± 0.08 b	19.92 ± 1.18 bc	0.91 ± 0.06 bc	22	0.12 ± 0.02 c
20	6.08 ± 0.02 c	18.24 ± 1.04 bd	0.66 ± 0.05 d	29	0.15 ± 0.02 bc
30	6.16 ± 0.06 cd	17.52 ± 1.02 cd	0.92 ± 0.03 bc	19	0.14 ± 0.01 bc
40	6.27 ± 0.03 e	19.56 ± 1.21 bc	0.92 ± 0.04 bc	21	0.15 ± 0.01 bc
50	6.24 ± 0.08 f	24.72 ± 1.51 a	0.84 ± 0.05 b	29	0.17 ± 0.02 bc

Data are means ± standard errors of three replicates. Values in each column followed by the same letter are not significantly different at $P \leq 0.05$ as determined by the Duncan's test.

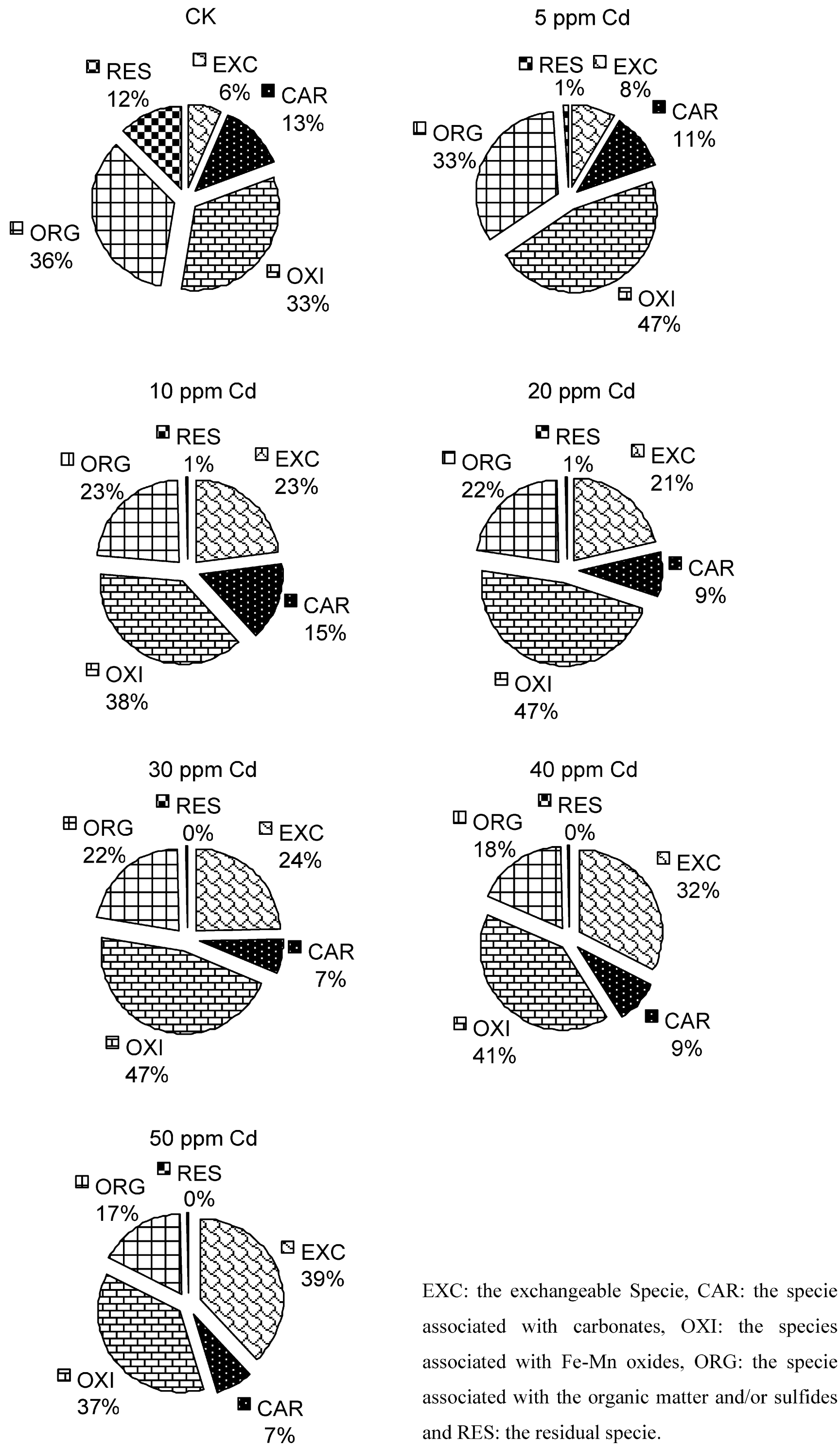

Fig. 3. Distribution of the five cadmium species in the rhizosphere soil.

specific chemical forms and on their binding state (Christine et al., 2002; Welt et al., 2003). Therefore, in the present study, sequential extraction procedures (SEP) were used to investigate some metals' total concentration distribution and chemical form variations. These could then aid the assessment of the potential bioavailability of sediment heavy metals to mangrove fauna and flora in a subtropical mangrove forest. Owing to their relatively high contents compared with the quantities of Cd transformed among the species, the percentage of carbonate associated Cd showed a decreasing tendency (from 13% in CK to 7% in 50 ppm Cd) with increasing Cd supply. However, exchangeable Cd showed an opposite tendency (from 6% in CK to 39% in 50 ppm Cd) (Fig. 3). This indicates the transformation of Cd from less bioavailable pools (most likely the carbonate associated Cd) to the more bioavailable fraction takes place in the rhizosphere. The mobilization of Cd as well as other metals in the rhizosphere has been reported in the literature for various kinds of plants (McGrath et al., 1997; Wang et al., 2001; Christine et al., 2002; Tao et al., 2003; McLaren et al., 2004). The content of exchangeable Cd clearly shows an increasing tendency, reaching a maximum in 50 ppm treatments (Fig. 3). It has been suggested that the increase in exchangeable metal in the rhizosphere was due to root induced differences in the rhizosphere soil (Tao et al., 2003). The metals associated with minerals such as carbonates or oxides can be released under acidic or reducing conditions and root-induced changes in pH and Eh can thereby affect the bioavailability of trace metals in the rhizosphere (Marschner and Römheld, 1996). It appears that it is plant species rather than pH which are responsible to Cd mobilization within the rhizosphere in this study. A similar conclusion was also reached in an experiment conducted by McGrath et al. (1997). So the change in Cd speciation may result from root-induced changes in DOC, redox potential and microbial activity in the rhizosphere.

5. Conclusion

The results of this study show that LMWOAs in the major root exudates change in quality and quantity under Cd stress. This induces a change in the pH value and the Cd spices in the rhizosphere soil of the *K. candel*. In mangrove plants, root exudates LMWOAs could play an important role in the Cd availability to mangrove plants. Cd bioavailability seemed to be affected by factors other than Cd concentrations in soil solution, including the large differences in the solubility of Cd among soils, and possibly, the ability of the plants partly to control Cd uptake. There has been little previous published work on the mechanisms involved in Cd absorption in mangrove plants. Further studies are required in order to fully understand the mechanisms of root exudates under Cd stress. These should include mangrove root uptake processes taking place in the plant root surface and within plant roots under the influences of LMWOAs both in the laboratory conditions and in situ.

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