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The effect of multiple heavy metals on ascorbate, glutathione and related enzymes in two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*)

Guo-Yong Huang[1], You-Shao Wang[1,2*], Cui-Ci Sun[1,2], Jun-De Dong[1], Zong-Xun Sun[1]

[1]*Key Laboratory of Tropical Marine Environmental Dynamics*
South China Sea Institute of Oceanology, Chinese Academy of Sciences
Guangzhou 510301, China
[2]*Marine Biology Research Station at Daya Bay*
Chinese Academy of Sciences, Shenzhen 518121, China

Key words: Ascorbate-Glutathione cycle, ascorbate peroxidise, glutathione peroxidise, glutathione reductase, heavy metal stress

Abstract

In this study, the effect of multiple heavy metal stress on ascorbate (AsA), glutathione (GSH) and related enzymes was investigated in the leaves, stems and roots of *Kandelia candel* and *Bruguiera gymnorrhiza*. Mangrove seedlings were treated with five different concentrations of a heavy metal mixture (Cd^{2+}, Pb^{2+} and Hg^{2+}). Antioxidants in both the species were analyzed after one month. AsA, GSH, ascorbate peroxidase (APX, EC 1.11.1.11) and glutathione reductase

* Corresponding author: *yswang@scsio.ac.cn*

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(GR, EC 1.6.4.2) exhibited a similar trend with initial increase and subsequent decrease in response to heavy metal stress. At the highest metal concentration, a significant decrease of AsA and GR was observed in *K. candel* and *B. gymnorrhiza*. Glutathione peroxidase (GPX, EC 1.11.1.9) in the leaves, stems and roots of *K. candel* reached their respective maximal values at the highest metal concentration, whereas GPX activity in roots and stems of *B. gymnorrhiza* was similar to the controls at higher metal concentrations. Our results demonstrate that AsA, GSH, APX, GR and GPX in *K. candel* may play more important roles in defending against reactive oxygen species (ROS) than those in *B. gymnorrhiza*.

INTRODUCTION

Heavy metals are common pollutants in urban aquatic ecosystems and are one of the main anthropogenic toxic compounds found in polluted mangrove locales (Caregnato et al. 2008). They are not biodegradable and are thus persistent in the environment (Lu et al. 2007). Many heavy metals (such as cadmium, lead and mercury) are toxic for the plants. They can reduce photosynthesis, cause water imbalance, disturb nutrient accumulation and finally induce visible injuries (Boussama et al. 1999, Patra and Sharma 2000). The toxicity of heavy metals is generally thought to be due to inactivation of enzymes and/or functional proteins by directly binding to them. However, recent studies show that the toxicity may be due to oxidative damage by the generation of reactive oxygen species (ROS) owing to the presence of heavy metals (Tsuji et al. 2002), such as hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH^{\cdot}$) and superoxide radicals ($O_2^{\cdot -}$). These can react very rapidly with DNA, lipids, pigments and proteins and cause lipid peroxidation, membrane damage and inactivation of enzymes (Reddy et al. 2005, Singh et al. 2006).

To minimize the harmful effects of ROS, plants have evolved an effective defense system including both enzymatic antioxidants and non-enzymatic antioxidants that participate in scavenging ROS (Choo et al. 2003). Among the antioxidant defense system, ascorbate (AsA), glutathione (GSH) and related enzymes (such as ascorbate peroxidase, glutathione reductase and glutathione peroxidase) play a pivotal role in direct or indirect scavenging of ROS from plant cells (Noctor and Foyer 1998, Aravind and Prasad 2005). AsA, an essential compound in plant tissues, reacts rapidly with superoxide and singlet oxygen (chemically), and hydrogen peroxide (enzymatically, through ascorbate peroxidase) (Potters et al. 2002). Another important redox metabolite is GSH. It may also directly or indirectly reduce ROS (Noctor and Foyer 1998). What is more, GSH is a precursor of phytochelatins (PCs), which are synthesized by PC synthase. PCs have a particularly high affinity for Cd and are able to form PC–Cd complexes, which are finally transported to vacuole, leading to their detoxification (Cobbett 2000). Ascorbate peroxidase (APX) and glutathione reductase (GR) are two important enzymes participating in scavenging H_2O_2

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through the ascorbate–glutathione cycle (Pukacka and Ratajczak 2006). In the cycle, APX reduces H_2O_2 using AsA as an electron donor, resulting in the formation of dehydroascorbate (DHA); DHA is then converted to AsA using GSH as an electron donor leading to the production of oxidized glutathione (GSSG); GSH is regenerated from GSSG by GR at the expense of NADPH (Liu et al. 2007). Glutathione peroxidase (GPX) is another H_2O_2 scavenger which catalyses the reduction of H_2O_2 by reduced glutathione, and defends cells against oxidative damage (Aravind and Prasad 2005). However, there is limited information about GPX in plants under heavy metal stress.

Mangrove forests, a group of inter-tidal plants forming unique communities along tropical and sub-tropical coastal regions, are often subject to effluent discharges, urban and agricultural runoff and solid waste dumping, due to their proximity to urban development (MacFarlane 2002). Many studies suggest mangroves possess a remarkable capacity to retain heavy metals and tolerate relatively high levels of heavy metal pollution (Tam and Wong 1996).

K. candel and *B. gymnorrhiza* are two dominant mangrove species along the south China coast. As for the effects of heavy metals on *Kandelia candel* and *B. gymnorrhiza*, previous studies were mainly concentrated on the distribution and accumulation of heavy metals, and the effects of heavy metals on growth (Tam and Wong 1997, Yim and Tam 1999). Although our laboratory has studied some antioxidative enzymes in *K. candel* and *B. gymnorrhiza* under heavy metal stress (Zhang et al. 2007), the effect of heavy metals on AsA, GSH and related enzymes (APX, GPX and GR) of *K. candel* and *B. gymnorrhiza* has not been studied yet. Therefore, our major aim in the present study was to evaluate what roles AsA, GSH and related enzymes in *K. candel* and *B. gymnorrhiza* play in response to multiple heavy metal (Cd^{2+}, Pb^{2+} and Hg^{2+}) stress.

MATERIALS AND METHODS

Plant material and treatment

Mature propagules of *K. candel* and *B. gymnorrhiza* were collected from Futian Mangrove National Nature Reserve in Guangdong province and Shankou Mangrove National Nature Reserve in Guangxi province, respectively. Undamaged propagules of similar length were planted in plastic pots (five propagules in each pot) filled with sand under greenhouse conditions. Each pot was irrigated with 500 ml of 1/2 Hoagland's solution (containing 10‰ NaCl) every 3 days. After two leaves developed, the seedlings of *K. candel* and *B. gymnorrhiza* were divided into five groups (3 in each group). Four groups were irrigated with 1/2 Hoagland's solution containing multiple heavy metals at

four levels, namely T_1, T_2, T_3 and T_4. The fifth group was irrigated with 1/2 Hoagland's solution without multiple heavy metals and used as the control (C). T_1 represented the irrigation medium containing 1.0 mg l^{-1} Pb^{2+}, and 0.1 mg l^{-1} Cd^{2+} and 0.1 mg l^{-1} Hg^{2+}. The choice of T_1 concentration was based on previous studies on *K. candel* and *B. gymnorrhiza* (Tam and Wong 1997, Yim and Tam 1999, Zhang et al. 2007). T_2, T_3 and T_4 contained heavy metal concentrations that were ×5, ×10 and ×15 higher than T_1, respectively. Each pot was irrigated with 500 ml of corresponding liquid 2 times a week. After one month, the leaves, stems and roots were harvested for analysis of AsA, GSH and related enzymes (APX, GR and GPX).

Extraction and assay of enzyme activities

Extraction of the enzymes was performed as previously described by Iannelli et al. (2002). Fresh tissues were homogenized in a chilled 50 mM potassium phosphate buffer (pH 7.0) containing 0.1% (w/v) ascorbic acid, 0.1% (v/v) Triton X-100 and 1% (w/v) polyvinylpolypyrrolidone. The homogenate was centrifuged at 12,000 rpm for 20 min (4°C). The clear supernatant fraction was used as the source of enzyme.

APX was assayed according to Nakano and Asada (1981). The reaction mixture consisted of 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H_2O_2, 0.1 mM EDTA and enzyme extract. The change in absorbance was monitored at 290 nm and the enzyme activity was expressed as μmol of ascorbate oxidized $min^{-1}g^{-1}$ fresh weight (FW).

GR was assayed by estimating the decrease of NADPH at 340 nm as described by Foyer and Halliwell (1976). The reaction mixture consisted of 50 mM phosphate buffer (pH 7.8), 0.15 mM NADPH, 0.5 mM GSSG, 3 mM $MgCl_2$ and enzyme extract. The activity of GR was defined as μmol of NADPH oxidized min^{-1} g^{-1} FW.

GPX was determined by estimating the decrease in the enzymatic reaction of glutathione (except the effect of the non-enzymatic reaction) at 412 nm according to the method of Shi et al. (2001). First, 0.2 ml of enzyme extract was mixed with 0.2 ml of 1.0 mM GSH and 1.0 ml of 1.25 mM H_2O_2 (prewarmed to 37°C) and incubated at 37°C for 5 min. After that, 2.0 ml of 1.67% (w/v) metaphosphoric acid precipitation solution was added, and the mixture was centrifuged at 3000 rpm for 10 min. GSH in the supernatant was determined by mixing 1.0 ml of the supernatant with 1.25 ml of 0.32 M Na_2HPO_4 and 0.25 ml of 0.4 mg ml^{-1} DTNB reagent. The change in absorbance at 412 nm was monitored within 5 min after mixing. Non-enzymatic reaction of GSH (with enzyme inactivation by heat substituted for the enzyme source) was carried through the incubation simultaneously with the sample. A blank was carried

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through 0.2 ml of H_2O and 0.8 ml of 1.67% (w/v) metaphosphoric acid precipitation solution substituted for 1ml of the supernatant; the rest was the same. A standard curve prepared by using GSH was used in the calculation of GPX activity. GPX activity was defined as nmol of GSH oxidized min^{-1} g^{-1} FW.

Extraction and determination of non-enzymatic antioxidants

Extraction of reduced as well as oxidized ascorbate and glutathione was performed as previously described (Knörzer et al. 1996). Fresh tissues were ground with ice-cold 5% (w/v) *m*-phosphoric acid in a cold mortar and pestle. The homogenate was centrifuged at 12,000 rpm for 30 min and the supernatant was collected for analysis of ascorbate and glutathione.

AsA and DHA contents were assayed according to the method of Knörzer et al. (1996). The method is based on the reduction of Fe^{3+} to Fe^{2+} by AsA and the formation of a pink color between Fe^{2+} and bipirydyl. Total ascorbic acid (AsA+DHA) was determined through a reduction of DHA to AsA by dithiothreitol. DHA was estimated by means of the difference between total ascorbic acid and AsA values.

GSH and GSSG pools were measured according to Nagalakshmi and Prasad (2001), which is based on the 5, 5'-dithiobis-nitrobenzoic acid (DTNB)-GSSG reductase recycling procedure. GSSG was determined after GSH had been removed by 2-vinylpyridine derivatization. GSH was determined by subtracting GSSG from the total glutathione content.

Statistical analysis

Statistical analysis was carried out by one-way analysis of variance. The Student-Newman-Keuls analysis was performed to determine the significant difference between treatments. Data are presented as means ± standard deviation of three replicates (three independent replicates of every treatment).

RESULTS

APX, GR and GPX activities

APX activity in the leaves, stems and roots of *K. candel* remained unaltered at T_1 followed by a marked increase at other metal stress levels in comparison with their respective controls (Fig. 1A). Heavy metals significantly enhanced the activity of APX in *B. gymnorrhiza* leaves at all heavy metal stress levels versus the control (Fig. 1B). APX activity in the stems of *B. gymnorrhiza* started to increase significantly at T_2 and reached maximal value at T_3, but decreased to the level of the control at T_4 (Fig. 1B). In the roots of

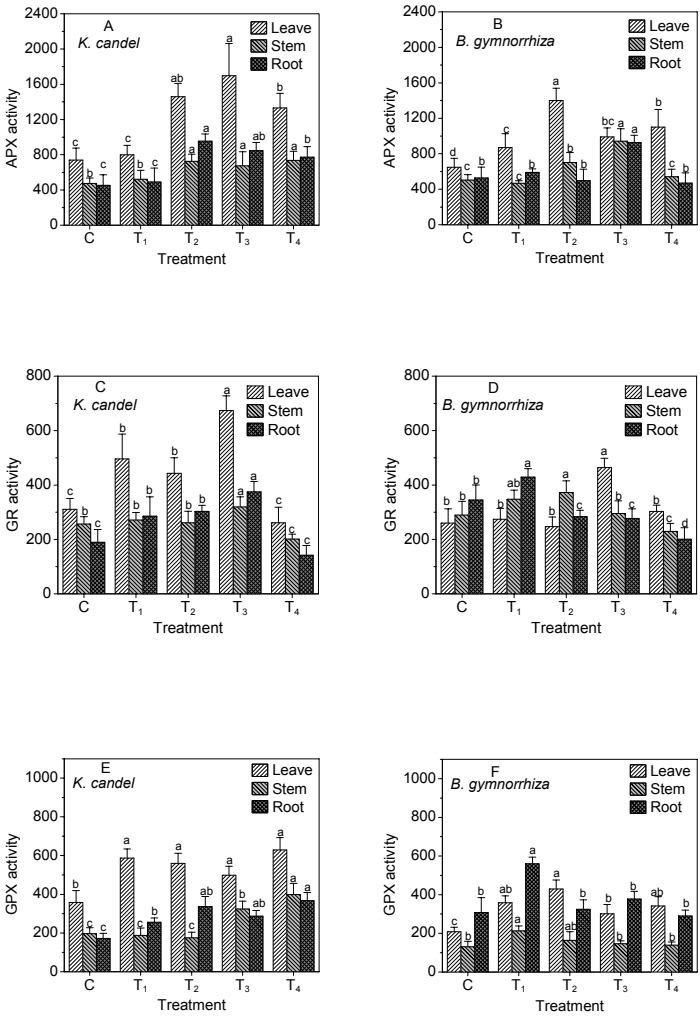


Fig. 1. The activities of APX (μmol min^{-1} g^{-1} FW), GR (μmol min^{-1} g^{-1} FW) and GPX (nmol min^{-1} g^{-1} FW) in the leaves, stems and roots of *K. candel* and *B. gymnorrhiza* exposed to multiple heavy metals for one month. A, C and E for *K. candel*; B, D and F for *B. gymnorrhiza*. Vertical bars indicate the mean of three replications ± SD. Different letters indicate significantly different values at a particular tissue ($P \leq 0.05$).

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B. gymnorrhiza treated with heavy metals, APX had no significant change versus the control except for at T_3 (Fig. 1B).

A significant induction of GR activity was observed in the leaves and roots of *K. candel* at heavy metal stress levels with respect to their respective controls except at T_4 (Fig. 1C). However, in stems of *K. candel*, a significant increase in GR activity was not observed until T_3, but it significantly decreased at T_4 in comparison with the control (Fig. 1C). In leaves of *B. gymnorrhiza* treated with heavy metals, GR activity showed no significant change as compared to the control except at T_3 (Fig. 1D). GR activity of stems in *B. gymnorrhiza* increased up to T_2 and then decreased, which was remarkably lower than the control at T_4 (Fig. 1D). A significant increase in GR activity of roots in *B. gymnorrhiza* was observed at T_1 followed by a significant decrease at all other metal treatments as compared to the control (Fig. 1D).

In *K. candel*, GPX activity in the leaves and roots increased markedly at all heavy metal stress levels when compared to their respective controls, whereas that in stems showed no change at both T_1 and T_2 followed by a significant increase at all other metal treatments when compared to the control (Fig. 1E). A significant enhancement of GPX activity in the leaves of *B. gymnorrhiza* was found at all heavy metal stress levels in comparison to the control with maximum increase at T_2 (Fig. 1F). In the stems and roots of *B. gymnorrhiza* in response to metal treatments, GPX activity significantly increased at T_1 in comparison with their respective controls followed by a decrease to their respective control levels at metal concentrations greater than and equal to T_2 (Fig. 1F).

Ascorbate and glutathione contents

AsA content in the leaves, stems and roots of *K. candel* exhibited a significant increase at metal stress levels as compared to their respective controls except at T_4 (Fig. 2A). In *B. gymnorrhiza*, the level of AsA content in the leaves significantly increased at lower heavy metal concentrations and reached its maximum value at T_2, but above T_2, it was slightly lower than the control (Fig. 2B). AsA content in the stems and roots of *B. gymnorrhiza* showed no significant change in comparison with their respective controls except a significant increase in the stems at T_1 and a marked decrease in the roots at T_4 (Fig. 2B). In *K. candel*, a significant increase in DHA content in the leaves, stems and roots was not observed until T_3 versus their respective controls, but DHA content in the stems and roots decreased to their control levels at T_4 (Fig. 2C). In the leaves of *B. gymnorrhiza*, DHA content had no significant change at lower metal concentrations and significant increases at T_3 and T_4 as compared to their respective controls (Fig. 2D). In the stems and roots of

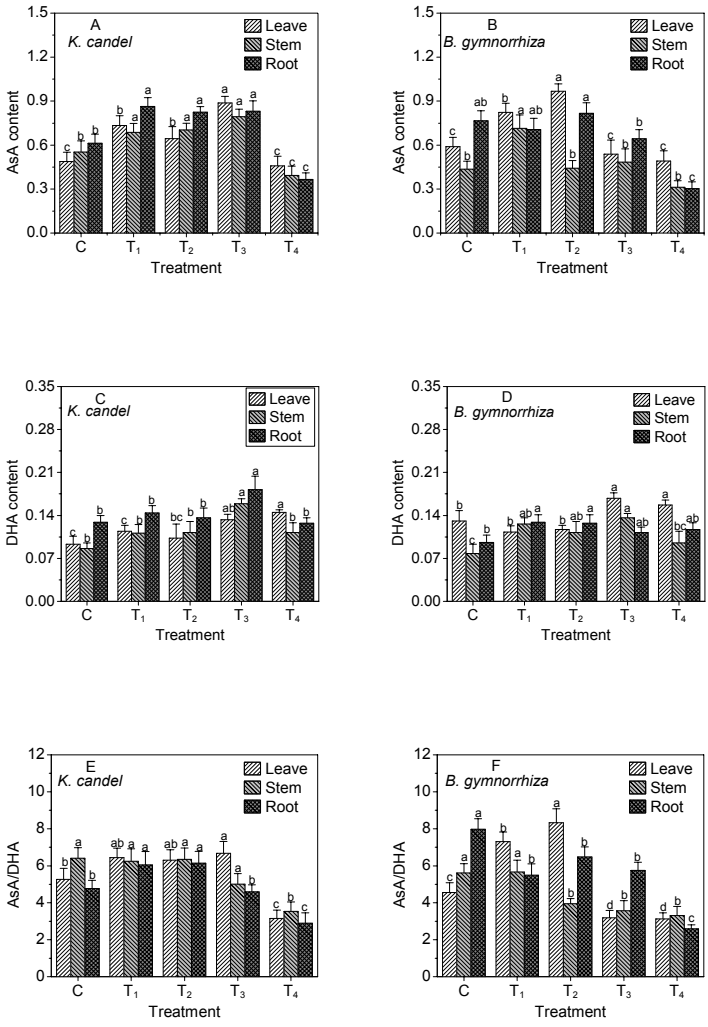


Fig. 2. AsA (μmol g^{-1} FW), DHA (μmol g^{-1} FW), and AsA/DHA in the leaves, stems and roots of *K. candel* and *B. gymnorrhiza* exposed to multiple heavy metals for one month. A, C and E for *K. candel*; B, D and F for *B. gymnorrhiza*. Vertical bars indicate the mean of three replications ± SD. Different letters indicate significantly different values at a particular tissue ($P \leq 0.05$).

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B. gymnorrhiza, DHA content significantly increased at lower metal concentrations and then decreased to control levels at higher metal concentrations (Fig. 2D). As shown in Fig. 2E, the highest metal concentrations resulted in a significant decrease of AsA/DHA ratio in the three parts of *K. candel*. However, a significant reduction of AsA/DHA radio was observed in the stems of *B. gymnorrhiza* at lower metal concentrations (Fig. 2F).

GSH content in the two species was significantly affected by heavy metal stress (Fig. 3A and B). In *K. candel*, GSH content in the three parts was significantly increased under heavy metal stress except for a slight but not significant decrease in the leaves at T_1 (Fig. 3A). In *B. gymnorrhiza*, the level of GSH content in the three parts showed a similar pattern with initial increase and subsequent decrease, and the level of GSH content in the stems and roots decreased to their respective control levels at T_4 (Fig. 3B). GSSG content in the leaves of *K. candel* significantly decreased at T_1, then increased and peaked at T_4 (Fig. 3C). In stems and roots of *K. candel*, GSSG content exhibited a significant increase at all metal concentrations except for the stems at T_1 (Fig. 3C). In *B. gymnorrhiza* the GSSG level in the stems and roots at T_2 was greater than at T_3, whereas in leaves GSSG peaked at T_3 (Fig. 3D). The GSH/GSSG ratio in the leaves and roots of *K. candel* peaked at T_2 and was still higher than the controls at T_4 (Fig. 3E). In the leaves of *B. gymnorrhiza*, GSH/GSSG ratio peaked at T_1 and decreased to the control level at T_4 (Fig. 3F). In the roots of *B. gymnorrhiza*, the GSH/GSSG ratio at all heavy metal stress levels was higher than the control, especially at T_1 and T_3 (Fig. 3F). The GSH/GSSG ratio in the stems of the two species under heavy metal stress was similar to their respective controls (Fig. 3E and F).

DISCUSSION

In plants, heavy metals are known to induce oxidative stress, such as over-production of reactive oxygen species (ROS) can cause cellular damage. In order to scavenge ROS, plants have evolved a well-organized enzymatic and non-enzymatic defense system (Reddy et al. 2005, Singh et al. 2006). Therefore, the induction of AsA, GSH and related enzymes is an important protective mechanism to minimize oxidative damage in plants exposed to heavy metals. APX and GR are two main enzymes of the AsA-GSH cycle and act in conjunction to scavenge poisonous H_2O_2 from plant cells through the AsA-GSH cycle. In this study, APX and GR exhibited a similar trend with initial increase and subsequent decrease in response to increasing metal treatment levels (Fig. 1 A-D). This is in agreement with earlier reports about the activities of APX and GR in other plants exposed to heavy metals (Mishap et al. 2006, Liu et al. 2007). The increase of APX and GR activities was observed in different parts of

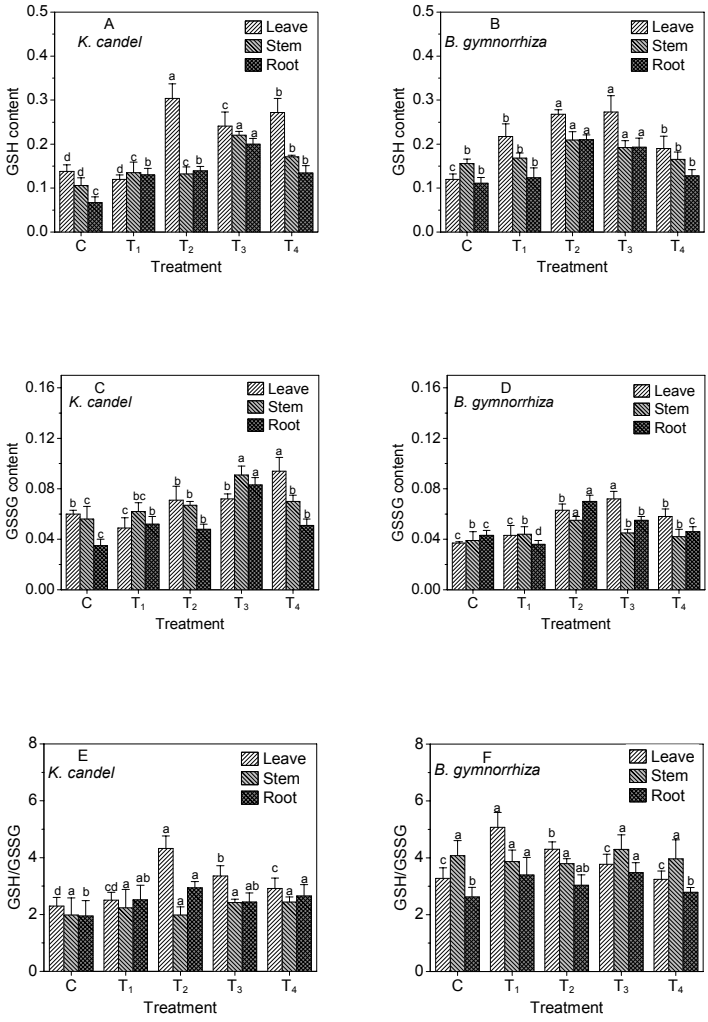


Fig. 3. GSH (μmol g^{-1} FW), GSSG (μmol g^{-1} FW), and GSSG/GSH in the leaves, stems and roots of *K. candel* and *B. gymnorrhiza* exposed to multiple heavy metals for one month. A, C and E for *K. candel*; B, D and F for *B. gymnorrhiza*. Vertical bars indicate the mean of three replications ± SD. Different letters indicate significantly different values at a particular tissue ($P \leq 0.05$).

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both the species exposed to heavy metals (Fig. 1A-D), which suggests an increase in H_2O_2 concentration and their participation in the elimination of H_2O_2 from the two species through the AsA-GSH cycle (Mishap et al. 2006, Liu et al. 2007). However, at higher concentrations of heavy metals, GR activity in both plants showed a significant decrease, especially GR activity in *B. gymnorrhiza* (Fig. 1C and D). GR activity in *K. candel* decreased a lot at T_4 in comparison with the respective controls, whereas that in the roots of *B. gymnorrhiza* began a marked decrease at T_2 (Fig. 1C and D). In addition, at T_4, APX activity in the stems and roots of *B. gymnorrhiza* decreased to their respective control levels, whereas that in the three parts of *K. candel* still significantly increased versus their respective controls (Fig. 1A and B). The discrepancy between *K. candel* and *B. gymnorrhiza* might be due to their different morphological, physiological and growth characteristics.

Glutathione peroxidase (GPX) is another H_2O_2 scavenger (Arvin and Prasad 2005). Our results showed a significant difference in GPX activity between *K. candel* and *B. gymnorrhiza*. GPX activity in three parts of *K. candel* reached the highest levels at T_4 (Fig. 1E). However, GPX activity in three parts of *B. gymnorrhiza* reached their respective maximum values at lower metal concentrations (Fig. 1E and F). The results indicate that GPX activity plays more of a role in scavenging H_2O_2 in *K. candel* than it does in *B. gymnorrhiza*. Previous studies have reported increase, decrease and no change in the activity of GPX in plants in response to heavy metal stress (Nagalakshmi and Prasad 2001, Iannelli et al. 2002, Arvin and Prasad 2005, Caregnato et al. 2008). Caregnato et al. (2008) thought that GPX activity in response to heavy metal exposure is diverse, and depends on the metal in question, the species and plant tissue examined, and on other co-occurring antioxidant defense and metal detoxification mechanisms.

In general, the increase of APX, GR and GPX activities in *K. candel* were more pronounced than in *B. gymnorrhiza* (Fig. 1). The results indicate that *K. candel* is more tolerant of heavy metals than *B. gymnorrhiza,* and these results are in agreement with previous studies conducted in our laboratory (Zhang et al. 2007). In addition, in *K. candel*, the activities of APX, GR and GPX in leaves are markedly higher than those in the stems and roots at all heavy metal stress levels. The results are inconsistent with the findings of Zhang et al. (2007), where SOD, POD and CAT activities in the roots were higher than those in the leaves when *K. candel* was exposed to heavy metals. The data indicate that the response of enzymatic antioxidants in different organs of *K. candel* operate with different mechanisms with respect to ROS elimination (Sinha and Saxena 2006).

AsA is known to play a prominent role in scavenging free oxy-radicals (Singh et al. 2006). In the present study, an increase of AsA content in the two

species was observed at lower heavy metal treatments (Fig. 2A and B). Liu et al. (2007) also reported an increase in AsA content in Cd-treated plants of *Bechmeria nivea*. The enhancement of AsA levels might help the two types of mangrove plants to cope with the heavy metal-induced oxidative damage. AsA content in all three parts of *K. candel* significantly increased under heavy metal stress and peaked at T_3, whereas AsA content in all three parts of *B. gymnorrhiza* decreased to their respective controls at T_3 (Fig. 2A and B). The results suggest that AsA in *K. candel* may play a more important role in coping with the heavy metal-induced oxidative damage than it does in *B. gymnorrhiza*. The AsA/DHA ratio, an important indicator of the redox status of cells, is one of the first signs of oxidative stress (Baccio et al. 2004). In the roots of two plants treated with T_4, there was a significant decrease of AsA and AsA/DHA without a significant variation in DHA (Fig. 2), which may be attributed to high consumption of AsA as an antioxidant to combat oxidative stress (Rai et al. 2004). In addition, from the trends of AsA, DHA and AsA/DHA content (Fig. 2), we can draw the conclusion that *K. candel* may be more tolerant of heavy metals than *B. gymnorrhiza*.

GSH plays several roles in cell metabolism such as redox state regulation, oxidative stress control, and defense against heavy metals (Mendoza-Cózatl and Moreno-Sánchez 2006). A marked increase in GSH content of the two species was observed under heavy metal stress (Fig. 3A and B). The increase in GSH can be correlated with its ability to scavenge single oxygen, peroxides and hydroxyl radicals and is involved in recycling AsA in the AsA-GSH pathway in plant cells under heavy metal stress (Liu et al. 2007). This result is in agreement with other plants exposed to heavy metals (Israr et al. 2006, Mishra et al. 2006). A significant increase in GSH content in both the species can possibly be attributed to heavy metals-induced synthesis of GSH (Mishra et al. 2006). In the GSH biosynthetic pathway, GSH synthesis is catalysed by γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase (GS), which can be induced by heavy metals (Cobbett 2000, Tsuji et al. 2003, Mendoza-Cózatl and Moreno-Sánchez 2006). On the other hand, GSH is supplemented by regeneration from GSSG reduction catalysed by GR (Noctor and Foyer 1998). At T_4, GSH content in the stems and roots of *B. gymnorrhiza* decreased to their respective controls (Fig. 3B), which may result from more consumption for the synthesis of PCs and decreased activity of GR (Fatima and Ahmad 2004, Mendoza-Cózatl and Moreno-Sánchez 2006). The intracellular GSH content depends on the homeostasis between GSH supply and GSH consumption (Lin et al. 2007). The ratio of GSH/GSSG plays an important role in indicating cellular redox status (Smeets et al. 2005). A high GSH/GSSG ratio is necessary to keep glutathione in its reduced form as an antioxidant (Israr et al. 2006). In the present study, when the two mangrove plants were exposed to heavy metals, the GSH/GSSG

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ratio in the leaves and roots had a more obvious change than it did in the stems. (Fig. 3E and F). The result suggests that the GSH-involved defensive mechanism in different parts of the plants possibly play different roles in the detoxification of heavy metals.

Although AsA, GSH, APX and GR exhibited a similar trend with initial increase and subsequent decrease in response to increasing metal treatment levels, the exact correspondence between AsA and GSH contents and APX and GR activities is unusual because the AsA and GSH are involved in enzymatic, as well as in non-enzymatic, antioxidant mechanisms (Baccio et al. 2004).

CONCLUSIONS

All results demonstrate that *K. candel* and *B. gymnorrhiza* can scavenge ROS detoxification through the AsA–GSH cycle or GSH metabolism under relatively low heavy metal stress. However, when *K. candel* and *B. gymnorrhiza* are exposed to higher concentrations of multiple heavy metals, scavenging H_2O_2 through the AsA–GSH cycle weakened in both the species. The changes of non-enzymatic antioxidants (GSH and AsA) and enzymatic antioxidants (APX, GR and GPX) in the leaves, stems and roots of both the species in response to heavy metals were different, especially in *K. candel*; the activities of APX, GR and GPX in leaves were markedly higher than those in the stems and roots under all heavy metal stress levels, suggesting that there may be different defense strategies in the leaves, stems and roots of both the species. Moreover, from the present results, *K. candel* shows a greater tolerance for heavy metal stress than *B. gymnorrhiza*.

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