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Short communication

Effects of indole-3-acetic, kinetin and spermidine assisted with EDDS on metal accumulation and tolerance mechanisms in ramie (*Boehmeria nivea* (L.) Gaud.)

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ABSTRACT

The effects and mechanisms of indole-3-acetic (IAA), kinetin (KN) and spermidine (Spd) assisted with ethylenediamine disuccinic acid (EDDS) on Cd and Pb accumulation in ramie were investigated by a pot experiment. In the first stage, the optimum concentrations of IAA, KN, and Spd were determined. And the effects of IAA, KN, and Spd at their optimum concentration on the antioxidant enzymes, non-enzymatic antioxidants, and metal accumulation in ramie were evaluated in the secondary stage. The results show that the translocation factor (TF) of Cd and Pb were increased by approximately 47 and 112%, respectively, at the presence of KN in combination with EDDS. In comparison with IAA treatment, Cd and Pb in root symplast were increased, approximately 2.02 and 2.62 times upon the combined application of IAA and EDDS. In addition, the contents of antioxidant enzymes and non-enzymatic antioxidant were increased dramatically through the addition of IAA, KN, and Spd plus EDDS. On the whole, IAA, KN, and Spd assisted with EDDS could significantly alleviate the oxidative stress induced by Cd and Pb in ramie.

1. Introduction

Cadmium (Cd) and lead (Pb) are highly toxic heavy metals, which can be taken up by plants, and further enter into the food chain (Ben Rejeb et al., 2013). Compared to the traditional physical and chemical methods, phytoextraction is proposed as an environmentally friendly in situ remediation technique to maintain soil fertility and structure (Zhang et al., 2013b). However, the efficiency of phytoremediation is low due to limited metal immobilization in soil. Chelate-enhanced phytoremediation has been proposed by using chelant to improve the efficiency of phytoextration. Ethylenediamine disuccinic acid (EDDS) has been proposed as a potential chelant which is biodegradable and thus producing little secondary pollutants (Mühlbachová, 2011).

Excessive Cd and Pb can induce the formation of reactive oxygen species (ROS), such as the superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$), which can cause the breakdown of proteins, lipid peroxidation in membranes, and DNA injury (Liu et al., 2007). Plants have evolved a variety of defense mechanisms to deal with the oxidative stress, including antioxidative enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and ascorbate peroxidase (APX) as well as non-enzymatic compounds such as glutathione (GSH) and ascorbate (AsA) (Groppa et al., 2001).

IAA and KN are phytohormones involved in different developmental processes: IAA can promote cell division and coleoptile elongation (Ouni et al., 2014), and KN can stimulate cell division, leaf expansion, and chlorophyll synthesis (Zhao et al., 2011). Spd involves in regulatory processes such as promotion of growth, DNA replication, cell division and differentiation (Groppa et al., 2001).

Ramie is widely planted in Asian countries such as China, Philippines, India, and Thailand (Liu et al., 2008). Mature ramie has the high potential for the remediation of metal-contaminated soil, due to their large biomass and root system. Despite some

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literatures concerning the response of ramie to Cd toxicity in hydroponic condition (Liu et al., 2007), little information is available on the heavy metals accumulation and tolerance mechanism of ramie in the presence of phytohormones and EDDS. The present study aims (1) to determine the optimum concentration of IAA, KN, and Spd for ramie growth; (2) to evaluate the effects of IAA, KN, and Spd assisted with EDDS on metals accumulation in ramie under Cd and Pb compound stress; (3) to evaluate the roles of IAA, KN, and Spd assisted with EDDS in alleviating Cd and Pb induced oxidative stress by regulating the antioxidant defense system.

2. Materials and methods

2.1. Plant materials and treatments

Uncontaminated control soil was collected in Yuelu Mountain, which located in Changsha, China. The basic properties were listed as follows: organic C 18.5 g kg^{-1}; total N 0.870 g kg^{-1}; total P 0.254 g kg^{-1}; total K 15.6 g kg^{-1}; CEC 16.7 cmol kg^{-1}; Cd undetected (<0.025 mg kg^{-1}); Pb undetected (<0.4 mg kg^{-1}). The soil was air dried, ground, and was applied with 30 mg kg^{-1} Cd and 500 mg kg^{-1} Pb (from solutions of $Cd(NO_3)_2{\cdot}4H_2O$ and $Pb(NO_3)_2$, respectively). The mixed soil was incubated for 4 weeks and then put into plastic pots (1.5 kg per pot). One seeding of ramie (from Chinese Academy of Agricultural Sciences, China) in each pot was planted. The ramie seedings were cultivated in Cd and Pb compound-contaminated soil for 40 days.

In the first stage, IAA was used at 0, 1, 10 and 100 μM, while KN or Spd was used at 0, 10, 100 and 1000 μM. The sprays of IAA, KN, and Spd took place at 17:00 each day. After harvest, each plant was measured from the main root apex to the crown and from the crown to the main shoot apex to determine the effects of IAA, KN, and Spd on ramie growth.

In the secondary stage, the leaves were sprayed with 10 μM IAA, 100 μM KN or Spd. These concentrations were based on the first experimental stage. The application of 5 mmol kg^{-1} soil EDDS to soil significantly increased concentrations of Cd and Pb in shoots compared to the treatment of 2.5 mmol EDDS kg^{-1} soil (Luo et al., 2005). Therefore, the soil was supplied with EDDS at 5 mmol kg^{-1} in our study. The sprays of IAA, KN, and Spd also took place at 17:00, while EDDS was applied to the soil one week before the plants were harvested. The treatments were set as T0 (without Cd/Pb, controls), T1 (Cd/Pb), T2 (Cd/Pb/EDDS), T3 (Cd/Pb/IAA), T4 (Cd/Pb/KN), T5 (Cd/Pb/Spd), T6 (Cd/Pb/IAA/EDDS), T7 (Cd/Pb/KN/EDDS), and T8 (Cd/Pb/Spd/EDDS). The ramies were kept in a controlled room with 14 h light period at light intensity of 300 μmol m^{-2} s^{-1}, 25 °C/20 °C day/night temperature and 60–70% relative humidity. After harvested, fresh samples were frozen immediately in liquid nitrogen and stored at − 80 °C for further analysis.

2.2. Metal analysis

Upon harvest the samples were washed with deionized water and the roots were then rinsed with 5 mM $CaCl_2$ for approximately 5 min to displace the metals absorbed (Zhao et al., 2010). The plants were separated into roots, stems, and leaves. The samples were dried, ground, and digested with HNO_3–$HClO_4$ (3:1). The resulting solutions were determined by atomic absorption spectroscopy (Analyst 700, Perkin Elmer, USA).

2.3. Metals distribution in root apoplast and symplast

The metal distribution in root apoplast and symplast were measured using a desorption procedure (Zhao et al., 2010). The entire root system was rinsed using 5 mM $CaCl_2$ for approximately 5 min to displace the metals adsorbed to the root surface. Then the roots were desorbed in 30 mL of 5 mM $CaCl_2$, and the solution was changed every 10 min for 4 times. Then the roots were rapidly frozen in liquid nitrogen to destroy cell membranes, and desorption was continued for 40 min. The metals desorbed in the first 40 min plus the metals remaining in the roots were considered apoplastic fraction. The metals released following the freeze-thaw was considered as symplastic fraction.

2.4. Enzyme and non-enzymatic antioxidant analysis

The activity of antioxidant enzyme (POD, SOD) and GSH content were determined with an assay kit purchased from Nanjing Jian Cheng Bioengineering Institute, Nanjing, China.

APX activity was determined by estimating the rate of ascorbate oxidation (Nakano and Asada, 1987). Leaves (0.2 g fresh weight) were homogenized in ice cold 50 mM phosphate buffer (pH 7.0) containing 0.1% (v/v) Triton X-100, 1% (w/v) polyvinylpyrollidone (PVP) and 2 mM AsA. The leaves were applied at proportions 1:4 (w/v). The homogenate was centrifuged at 4000 rpm for 20 min at 4 °C and the supernatant was used for enzyme assays. APX activity was determined immediately, using a reaction mixture (3 ml) containing 50 mM potassium phosphatebuffer (pH 7.0), 0.1 mM H_2O_2, 0.5 mM AsA and 0.1 mL enzyme liquid. Decrease in absorbance at 290 nm was measured at 25 °C for 3 min ($E = 2.8\ mM^{-1}\ cm^{-1}$).

2.5. Statistical analysis

Data were analyzed with Origin 8.0 or SPSS 19.0 for windows. The significant differences were detected by the LSD test, taking $P < 0.05$ as the significant level. The results are presented as mean values ± S.E. of three replications.

3. Results and discussion

3.1. Effects of IAA, KN, and Spd on ramie growth

As shown in Fig. 1, the length of ramie roots and shoots were increased by the application of IAA at the presence of Cd/Pb. The probable reason was that IAA promoted cell division and coleoptile elongation (Ouni et al., 2014). Besides, both the root length and shoot height (6.4 and 22.0 cm) exposed to 10 μM IAA were longer than those of other treatments. On the other hand, the length of the root was between 3.5 and 4.5 cm in all treatments (Fig. 1b), indicating that KN did not have a significant effect on the elongation of ramie roots, which was in accordance with the previous study (Zhao et al., 2011). Additionally, the shoots were longer (18.2 cm) at 100 μM and shorter (13.8 cm) at 1000 μM KN, compared to the treatment (15.8 cm) with Cd/Pb alone (Fig. 1b). As shown Fig. 1c, the optimum concentration of Spd was 100 μM, with the root length 6.7 cm and shoot height 25.9 cm. On the whole, the optimum concentrations of IAA, KN, and Spd were 10, 100, and 100 μM, respectively.

3.2. Cd and Pb uptake and distribution in ramie

The effects of IAA, KN, and Spd, alone and combined with EDDS, on Cd and Pb uptake and translocation in ramie are listed in Table 1. Cd and Pb contents in different tissues of ramie decreased following the order of roots > stems > leaves. Lower Cd and Pb in the roots of ramie were determined upon the application of EDDS. On the contrary, the concentrations of Cd and Pb in leaves and stems were increased with EDDS. The result demonstrated that EDDS acting as a chelating agent was useful to facilitate Pb and Cd movement

Table 1
Uptake and distribution of Cd and Pb in ramie exposed to Cd and Pb compound stress for 40 days.

Treatments	Cd content (mg kg^{-1} DW)			TF[a] value	Pb content (mg kg^{-1} DW)			TF value
	Leaves	Stems	Roots		Leaves	Stems	Roots	
T0	UD[b]	UD	UD	/	UD	UD	UD	/
T1	53.00 ± 2.43[c]	309.75 ± 16.01	697.00 ± 35.28	0.219	40.13 ± 2.10	291.50 ± 14.83	1038.50 ± 50.01	0.162
T2	72.88 ± 3.56	324.50 ± 15.25	675.50 ± 19.26	0.294	57.00 ± 2.56	465.00 ± 12.28	992.50 ± 46.21	0.263
T3	19.88 ± 1.01	286.50 ± 14.32	722.50 ± 29.28	0.212	2.88 ± 0.12	305.25 ± 14.78	1017.00 ± 42.12	0.151
T4	29.13 ± 1.52	308.00 ± 14.90*	807.50 ± 39.45	0.209	11.25 ± 0.83	190.00 ± 9.02	1199.00 ± 56.78	0.084
T5	10.13 ± 0.61	204.75 ± 9.56	601.00 ± 30.12	0.179	0.50 ± 0.07	275.75 ± 12.94	905.50 ± 42.31	0.153
T6	31.38 ± 1.60	343.50 ± 17.28	719.00 ± 38.04	0.261	10.75 ± 0.60	500.00 ± 24.00	989.50 ± 32.90	0.258
T7	44.75 ± 2.32	434.75 ± 22.02	744.50 ± 42.73	0.322	26.50 ± 1.47	639.25 ± 30.24	969.00 ± 48.25	0.344
T8	19.25 ± 0.95	293.25 ± 13.45	544.00 ± 26.57	0.287	16.75 ± 0.72	315.50 ± 13.46	888.00 ± 42.01	0.187

* Values without (*) are significantly different from Cd/Pb treatment at $P < 0.05$, while values designated by (*) are not significantly ($P > 0.05$) different from Cd/Pb treatment.
[a] TF: translocation factor: the ratio of metal concentration in shoots to that in roots.
[b] UD: undetected.
[c] Mean value ± standard deviation.

from roots to shoots, which was in agreement with previous studies (Meers et al., 2005).

More interestingly, the roots of ramie had the highest Cd and Pb contents at the presence of 0.1 mM KN, which could be explained by the fact that KN at high concentration induced cell division and the growth of cultured cells (López et al., 2007). In stems, IAA, KN, and Spd combined with EDDS significantly increased the concentrations of Cd and Pb compared to those without EDDS. The Cd/Pb/KN/EDDS treatment had the highest Cd and Pb contents in stems of ramie (434.75 mg kg^{-1}dw for Cd and 639.25 mg kg^{-1} dw for Pb). The Cd and Pb concentrations in leaves of ramie exposed to Cd/Pb/EDDS increased to 72.88 and 57 mg kg^{-1} dw, approximately 1.38 and 1.42 times than the Cd/Pb treatment.

Compared to the Cd/Pb treatment, IAA, KN, and Spd significantly decreased TF by approximately 3.2, 4.6 and 18.3% for Cd, 6.8, 48.1 and 5.6% for Pb, respectively. Plant hormones such as IAA and KN were able to regulate the absorption and distribution of metal ions (Nenova and Stoyanov, 2000). The decrease of TF might be attributed to the fact that IAA, KN, and Spd inhibited Cd and Pb movement from roots to shoots. Additionally, IAA, KN, and Spd plus EDDS increased TF compared to those deprived with EDDS, and the Cd/Pb/KN/EDDS treatment ranked the highest among all treatments (0.322 for Cd and 0.344 for Pb).

3.3. Metals distribution in root apoplast and symplast

The metals distribution in apoplast and symplast are shown in Table 2. Both Cd and Pb in the roots were largely distributed in the apoplast, indicating that the nonselective apoplastic uptake dominated the translocation of Cd and Pb. It had been demonstrated that the symplastic uptake way was highly selective for essential metals (Cu and Zn), and nonessential metals (Cd and Pb) were limited through this pathway (Zhao et al., 2010). Chelated metals might be taken up mainly through the transpiration steam via the apoplast in roots where the Casparian strip was not fully developed (Tandy et al., 2006; Ben Rejeb et al., 2013). EDDS dramatically increased the contents of Cd and Pb in the symplast. For example, when treated with Cd/Pb/IAA/EDDS, Cd and Pb in symplast was increased to 7.21 and 2.36 mg kg^{-1} fw, approximately 2.02 and 2.62 folds higher than the Cd/Pb/IAA treatment. The probable reason was that EDDS facilitated Cd and Pb passing through membrane or the metals-EDDS complexes had a higher activity to transport across membrane.

3.4. SOD and POD activity

SOD activity in leaves of ramie treated with Cd/Pb and Cd/Pb/EDDS were reduced by 25.9 and 31.4%, respectively (Fig. 2a). The decrease of SOD activity was due to the fact that heavy metals disturbed plant metabolism such as the synthesis of enzyme protein (Vögeli-Lange and Wagner, 1990; Zhang et al., 2013a). In the presence of IAA, KN, and Spd, SOD activity was increased by 44.2, 59.2 and 27.6%, respectively, compared to Cd/Pb treatment. This suggested that KN was more effective in minimizing oxidative damage than IAA and Spd. The increase of SOD activity was probably attributed to the synthesis of enzyme protein (Shah et al., 2001). IAA, KN, and Spd combined with EDDS slightly decreased SOD activity compared to those without EDDS. This probable reason was that the metal concentrations in leaves of these treatments were lower than those without EDDS (Table 1).

SOD can catalyze the dismutation of $O_2^{\bullet -}$ into H_2O_2 and O_2, and subsequently H_2O_2 is detoxified by POD (Zhang et al., 2013a).

Table 2
Metals distributed in root apoplast and symplast.

Treatments	Cd concentration (mg kg^{-1} FW)		Pb concentration (mg kg^{-1} FW)	
	Symplast	Apoplast	Symplast	Apoplast
T0	UD[a]	UD	UD	UD
T1	5.21 ± 0.25[b]	37.26 ± 1.88	1.26 ± 0.05	65.41 ± 3.25
T2	5.56 ± 0.30	35.31 ± 1.96	1.58 ± 0.09	59.85 ± 2.97
T3	3.57 ± 0.19	42.76 ± 2.20	0.90 ± 0.04	69.43 ± 3.50
T4	3.79 ± 0.20	44.41 ± 2.31	1.29 ± 0.06*	63.89 ± 3.18
T5	4.50 ± 0.22	35.86 ± 1.81	1.73 ± 0.08	50.63 ± 2.64
T6	7.21 ± 0.35	37.60 ± 1.90*	2.36 ± 0.12	48.21 ± 2.39
T7	4.39 ± 0.21	39.81 ± 1.94	1.93 ± 0.11	61.30 ± 3.02
T8	5.65 ± 0.28	32.56 ± 1.65	2.17 ± 0.10	47.44 ± 2.34

* Values without (*) are significantly different from Cd/Pb treatment at $P < 0.05$, while values designated by (*) are not significantly ($P > 0.05$) different from Cd/Pb treatment.
[a] UD: undetected.
[b] Mean value ± standard deviation.

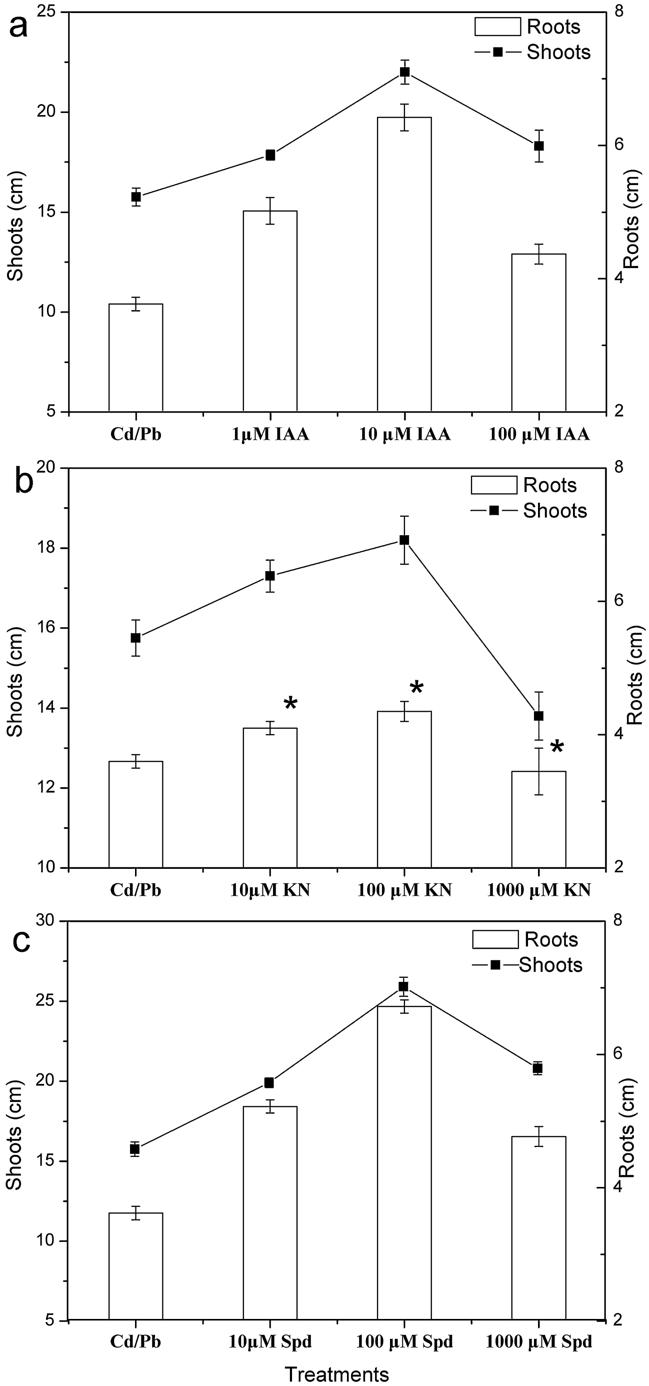

Fig. 1. The root length and shoot height of ramie: (a) Cd/Pb, Cd/Pb/IAA at 1, 10, and 100 μM; (b) Cd/Pb, Cd/Pb/KN at 10, 100, and 1000 μM; (c) Cd/Pb, Cd/Pb/Spd at 10, 100, and 1000 μM. Values without (*) are significantly different from control at $P < 0.05$, while values designated by (*) are not significantly ($P > 0.05$) different from control.

By exposing to Cd/Pb ± EDDS, POD activity were increased by 1.7–2.5-fold (Fig. 2a). The increase of POD activity was probably due to the fact that heavy metals stimulated the synthesis of enzyme (Geebelen et al., 2002). Acting as an inner defense tool, POD inhibited Cd and Pb induced oxidative stress (Wang et al., 2007). The POD activity of Cd/Pb/KN/EDDS treatment was increased to 76.8 U/mg prot, approximately 1.54 times than the Cd/Pb treatment, suggesting that KN induced enzyme synthesis and inhibited lipid peroxidation.

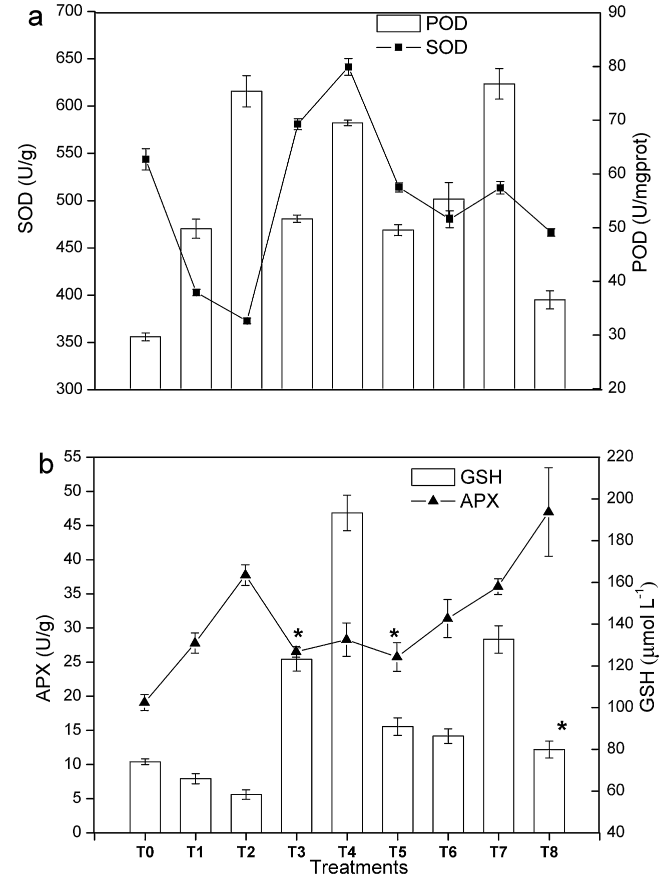

Fig. 2. SOD and POD activities in leaves of ramie (a); APX activity and GSH content in leaves of ramie (b). Values without (*) are significantly different from control at $P < 0.05$, while values designated by (*) are not significantly ($P > 0.05$) different from control.

3.5. APX activity and GSH content

The APX activity increased significantly in all treatments (Fig. 2b). This result suggested that APX had higher activity in continuous detoxification of H_2O_2 under Cd and Pb compound stress. IAA, KN, and Spd combined with EDDS increased APX activity by 18.3, 27.4 and 82.3%, respectively, compared to those deprived of EDDS. A considerable part of Cd and Pb could be deposited in vacuoles as Cd-EDDS and Pb-EDDS complexes producing changes in the enzymatic activity (Vögeli-Lange and Wagner, 1990). These changes were related to heavy metals concentrations, plant characteristics, and the resistance of plant to the heavy metals.

The GSH content in leaves of ramie treated with Cd/Pb and Cd/Pb/EDDS decreased by 11.0 and 21.3%, respectively, compared to the controls (Fig. 2b). Previous study reported that diminished GSH content seemed to limit the operation of the ascorbate-glutathione cycle in Cu-treated *Arabidopsis thaliana* (Drążkiewicz et al., 2003). IAA, KN, and Spd increased the GSH content to 1.9, 2.9 and 1.4 times, respectively, compared to Cd/Pb treatment. This result indicated that IAA, KN, and Spd induced the synthesis of GSH, and a remarkable GSH content was essential for phytochelatins synthesis (Srivastava et al., 2007). Phytochelatins were involved in the heavy metal detoxification through the production of metal-chelate, and phytochelatins biosynthesis was dependent on GSH on the presence of heavy metal ions (Srivastava et al., 2007). However, the addition of EDDS with IAA, KN, and Spd reduced GSH content compared to those deprived of EDDS, which was attributed to that Cd and Pb accumulated in leaves at higher concentrations.

4. Conclusions

The high accumulation of Cd and Pb in ramie indicated a great potential of this species in the remediation of Cd and Pb contaminated soils. Optimal growth of ramie was observed when sprayed with 10 μM IAA, 100 μM KN, or 100 μM Spd. In addition, IAA, KN, and Spd combined with EDDS dramaticlly increased TF compared to those deprived of EDDS, and the Cd/Pb/KN/EDDS treatment ranked the highest among all treatments. In roots, Cd and Pb were mostly distributed in the apoplast, and EDDS could increase the amount of Cd and Pb in the symplast. IAA, KN, and Spd assisted with EDDS could enhance the contents of antioxidant enzymes (SOD, POD, and APX) and non-enzymatic antioxidant (GSH). In the future, IAA, KN, and Spd assisted with EDDS could be applied to improve the bioremediation efficiency of Cd and Pb contaminated soils.

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References

Ben Rejeb, K., Ghnaya, T., Zaier, H., Benzarti, M., Baioui, R., Ghabriche, R., Wali, M., Lutts, S., Abdelly, C., 2013. Evaluation of the Cd^{2+} phytoextraction potential in the xerohalophyte *Salsola kali* L. and the impact of EDTA on this process. Ecol. Eng. 60, 309–315.

Drążkiewicz, M., Skórzyńska-Polit, E., Krupa, Z., 2003. Response of the ascorbate–glutathione cycle to excess copper in *Arabidopsis thaliana* (L.). Plant Sci. 164, 195–202.

Geebelen, W., Vangronsveld, J., Adriano, D.C., Van Poucke, L.C., Clijsters, H., 2002. Effects of Pb-EDTA and EDTA on oxidative stress reactions and mineral uptake in *Phaseolus vulgaris*. Physiol. Plant. 115, 377–384.

Groppa, M.D., Tomaro, M.L., Benavides, M.P., 2001. Polyamines as protectors against cadmium or copper-induced oxidative damage in sunflower leaf discs. Plant Sci. 161, 481–488.

López, M.L., Peralta-Videa, J.R., Parsons, J.G., Benitez, T., Gardea-Torresdey, J.L., 2007. Gibberellic acid, kinetin, and the mixture indole-3-acetic acid-kinetin assisted with EDTA-induced lead hyperaccumulation in alfalfa plants. Environ. Sci. Technol. 41, 8165–8170.

Liu, Y.G., Wang, X., Zeng, G.M., Qu, D., Gu, J.J., Zhou, M., Chai, L.Y., 2007. Cadmium-induced oxidative stress and response of the ascorbate-glutathione cycle in *Bechmeria nivea* (L.) Gaud. Chemosphere 69, 99–107.

Liu, Z.T., Yang, Y., Zhang, L., Sun, P., Liu, Z.W., Lu, J., Xiong, H., Peng, Y., Tang, S., 2008. Study on the performance of ramie fiber modified with ethylenediamine. Carbohyd. Polym. 71, 18–25.

Luo, C., Shen, Z., Li, X., 2005. Enhanced phytoextraction of Cu, Pb, Zn and Cd with EDTA and EDDS. Chemosphere 59, 1–11.

Meers, E., Ruttens, A., Hopgood, M., Samson, D., Tack, F., 2005. Comparison of EDTA and EDDS as potential soil amendments for enhanced phytoextraction of heavy metals. Chemosphere 58, 1011–1022.

Mühlbachová, G., 2011. Soil microbial activities and heavy metal mobility in long-term contaminated soils after addition of EDTA and EDDS. Ecol. Eng. 37, 1064–1071.

Nakano, Y., Asada, K., 1987. Purification of ascorbate peroxidase in spinach chloroplasts; its inactivation in ascorbate-depleted medium and reactivation by monodehydroascorbate radical. Plant Cell Physiol. 28, 131–140.

Nenova, V.R., Stoyanov, I.G., 2000. Effects of some growth regulators on young iron deficient maize plants. Biol. Plant. 43, 35–39.

Ouni, Y., Albacete, A., Cantero, E., Lakhdar, A., Abdelly, C., Pérez-Alfocea, F., Barhoumi, Z., 2014. Influence of municipal solid waste (MSW) compost on hormonal status and biomass partitioning in two forage species growing under saline soil conditions. Ecol. Eng. 64, 142–150.

Shah, K., Kumar, R.G., Verma, S., Dubey, R.S., 2001. Effect of cadmium on lipid peroxidation, superoxide anion generation and activity of antioxidant enzymes in growing rice seedlings. Plant Sci. 161, 1135–1144.

Srivastava, S., Mishra, S., Tripathi, R.D., Dwivedi, S., Trivedi, P.K., Tandon, P.K., 2007. Phytochelatins and antioxidant systems respond differentially during arsenite and arsenate stress in *Hydrilla verticillata* (L.f.) Royle. Environ. Sci. Technol. 41, 2930–2936.

Tandy, S., Schulin, R., Nowack, B., 2006. Uptake of metals during chelant-assisted phytoextraction with EDDS related to the solubilized metal concentration. Environ. Sci. Technol. 40, 2753–2758.

Vögeli-Lange, R., Wagner, G.J., 1990. Subcellular localization of cadmium and cadmium-binding peptides in tobacco. Plant Physiol. 92, 1086–1093.

Wang, H.H., Shan, X.Q., Wen, B., Owens, G., Fang, J., Zhang, S.Z., 2007. Effect of indole-3-acetic acid on lead accumulation in maize (*Zea mays* L.) seedlings and the relevant antioxidant response. Environ. Exp. Bot. 61, 246–253.

Zhang, L., Wang, S., Jiao, L., Zhao, H., Zhang, Y., Li, Y., 2013a. Physiological response of a submerged plant (*Myriophyllum spicatum*) to different NH_4Cl concentrations in sediments. Ecol. Eng. 58, 91–98.

Zhang, S., Lin, H., Deng, L., Gong, G., Jia, Y., Xu, X., Li, T., Li, Y., Chen, H., 2013b. Cadmium tolerance and accumulation characteristics of *Siegesbeckia orientalis* L. Ecol. Eng. 51, 133–139.

Zhao, Z.Q., Xi, M.Z., Jiang, G.Y., Liu, X.N., Bai, Z.K., Huang, Y.Z., 2010. Effects of IDSA, EDDS and EDTA on heavy metals accumulation in hydroponically grown maize (*Zea mays.* L.). J. Hazard. Mater. 181, 455–459.

Zhao, Y., Peralta-Videa, J.R., Lopez-Moreno, M.L., Ren, M., Saupe, G., Gardea-Torresdey, J.L., 2011. Kinetin increases chromium absorption, modulates its distribution, and changes the activity of catalase and ascorbate peroxidase in Mexican Palo Verde. Environ. Sci. Technol. 45, 1082–1087.