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Detoxification of mercury in soil by selenite and related mechanisms

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ABSTRACT

A better understanding of the benefits of selenium (Se) fertilization to alleviate the toxicity of mercury (Hg) on plants and of the underlying mechanisms involved in Hg stress is important for the remediation of soils contaminated by Hg. This study is aimed to explore the effects of the application of selenite to alleviate the toxicity of Hg in soils to plants and related mechanisms involved in this process. The chemical (Hg uptake of pak choi), biological (root and shoot length, root and shoot weight) and physiological effects (antioxidant enzyme activities, non-enzymatic antioxidant contents (proline) and lipid peroxidation products (malondialdehyde)) produced over plants by the application of different doses of Hg and Se to soil has been investigated through a pot experiment, which was conducted with exposure to different dosages of mercuric chloride (0, 1.0, 2.0, and 3.0 mg/kg soil) and sodium selenite (0, 0.5, 1.0, and 2.5 mg/kg soil). Results indicated that single high Hg treatment (3.0 mg/kg Hg) resulted in significantly increase in Hg uptake by plants ($P < 0.01$), thus the growth of pak choi was inhibited. However, the Se application at 1.0 and 2.5 mg/kg led to significantly alleviated Hg uptake by plants ($P < 0.05$). Meanwhile, the low Se (at 0.5 and 1.0 mg/kg) applied to soil induced significantly improvement the growth of pak choi ($P < 0.05$) by elevating the activities of superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase (GSH-Px) enzymes and the content of chlorophyll (SPAD value) as well as suppressed the lipid peroxidation products contents (MDA) and proline. Results collectively indicated that applied Se played an important role in promoting the detoxification of Hg and growth of pak choi under oxidative stress. Notably, this role may only be significant when Se application at the appropriate concentration (≤ 1.0 mg/kg).

1. Introduction

Mercury (Hg) is an extremely toxic pollutant that threatens ecosystem balance and human health by persisting and accumulating in the environment and the food chain (Ullrich et al., 2001). Principal chemical forms of Hg in soil are ionic Hg (Hg^{2+}) and organic Hg (CH_3Hg^+) (Clarkson and Magos, 2006). Hg^{2+} mainly exists under highly oxidant condition in dry land, whereas CH_3Hg^+ only is detected under anoxic conditions in flooded soils through the methylation process, where Hg^{2+} transformed into CH_3Hg^+ by microorganisms (Fernandez-Martinez et al., 2015). Thus, Hg at high concentrations can inhibit growth of plants, and even cause death (Patra and Sharma, 2000). For example, the growth inhibition of tomato (*Lycopersicon esculentum*) ($\geq$ 10 μM Hg) (Cho and Park, 2000), algae (*C. reinhardtii*) ($\geq$ 4 μM Hg) (Elbaz et al., 2010), and *Jatropha curcas* plant ($\geq$ 5 μg/mL Hg) (Marrugo-Negrete et al., 2016) was found in the Hg-treated soil. Because plants exposure to high Hg concentrations result in the excessive accumulation of reactive oxygen species (ROS) such as superoxide radicals (O^{2-}), hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2); which trigger oxidative stress (Sharma et al., 2012). Several studies have reported that tomato (*Lycopersicon esculentum*) (Cho and Park, 2000), cucumber (*cucumerem*) (Cargnelutti et al., 2006) or alfalfa (*Lucerne*) (Sobrino-Plata et al., 2009) exposure to Hg resulted in the imbalance of ROS, which increased lipid peroxidation, inhibited antioxidant system activation and decreased photosynthetic capacity.

The antioxidant system of plants composed enzymes and non-enzymatic components (Miller et al., 2008; Lin et al., 2012). The enzymatic antioxidants include ROS scavengers such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione peroxidase (GSH-Px), which were recognized as typical defense components against heavy metals stress via abolish active oxygen (Sinha et al., 2010; Gao et al., 2010). The non-enzymatic antioxidant like

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proline, an antioxidant amino acid, plays an important role in the detoxification of heavy metal toxic (Alia et al., 2001), because it can protect cells from damage by scavenging ROS (Smirnoff and Cumbes, 1989). Malondialdehyde (MDA), is considered to be a measure of lipid peroxidation, causing membrane impairment and leakage (Cho and Park, 2000). Ultimately, these collective effects of Hg exposure inhibited plants growth, for example, reducing height and biomass of alfalfa (*Lucerne*) plant was also found in the study of Sobrino-Plata et al. (2009).

The above information suggested that plants growth under Hg stress was dependent on antioxidant systems, lipid peroxidation products, and photosynthetic pigments. To developing protective responses of plants and to regulate imbalance of ROS caused by Hg stress, thus the better understanding of Hg detoxification mechanisms is necessary. Although the effects of Hg toxicity on the growth and physiological mechanisms of plants have received considerable attention (Cargnelutti et al., 2006; Gao et al., 2010; Marrugo-Negrete et al., 2016); however, previous studies have mainly focused on adaptive and tolerance mechanisms to Hg toxicity, while no suitable interventions against Hg toxicity was proposed.

Selenium (Se) is a trace element essential to human and animals (Dinh et al., 2017). Whether which is an essential element for plants is still controversial. However, Se often exerts a dual effect on plant growth, in other words, Se stimulate the growth of plants at low levels (Zhao et al., 2013a) and inhibit plants growth at high levels (Han et al., 2013). Se in soils as selenate (SeO_4^{2-}), selenite (SeO_3^{2-}), or organic Se species can readily uptake by plants (Sors et al., 2005; Hawrylak-Nowak, 2013). However, SeO_3^{2-} is the less available species for plants because of its exceptionally high affinity towards strong sorption onto soil particles (Torres et al., 2010; Nakamaru and Altansuvd, 2014). The study of Cartes et al. (2010) reported that treatment with SeO_3^{2-} at 2 μM promoted the growth of ryegrass (*Lolium perenne* L.), while Yao et al. (2009) found that seedlings biomass of wheat (*Triticum aestivum* L.) increased at 0–2.0 mg/kg SeO_3^{2-} treatment. However, the excess of Se ($\geq$ 11.1 mg/kg) has been reported to inhibit the growth of flue-cured tobacco (*Nicotiana tabacum* L.) (Han et al., 2013). Thus, effects of Se concentrations and chemical forms applied in soil both affected the plant growth.

In addition, the application of exogenous Se can inhibit Hg uptake by plants through the formation of the Hg–Se insoluble complex in the rhizosphere and/or roots (McNear et al., 2012; Zhao et al., 2013a). Nevertheless, the application of Se result in detoxification of Hg in soils to plants via regulate physiological metabolism, which directly affected the growth of plants, has not yet been studied. Therefore, better understanding the related metabolism of Se application in promoting the detoxification of Hg under Hg stress is necessary for finding an alternative approach for soil Hg remediation.

Earlier studies have investigated Se accumulation in plants by treating plant growth media or soil with SeO_4^{2-} and SeO_3^{2-}. However, given that SeO_3^{2-} plays an important role than SeO_4^{2-} in limiting the absorption and bioaccumulation of Hg in pak choi (Tran et al., 2018). Thus, we used only SeO_3^{2-} in this study.

The main objective of this study was to investigate the effects of the application of Se to ameliorate the toxicity of Hg for pak choi. Our specific objectives were as follows: 1) clarify the role of exogenous Se application on the growth of plants and related mechanism under Hg stress, 2) selection the appropriate concentrations of exogenous Se for detoxify Hg effects.

2. Materials and methods

2.1. Experimental materials

Eum–orthic anthrosol, a typical agricultural soil with an extensive cultivation history in Northern China, was collected at a depth of 0–20 cm at the Northwest A&F University Farm in Shaanxi Province, China. Soil samples were completely air dried at room temperature, homogenized, and passed through a 5 mm sieve. The basic physicochemical properties of the soil were as follows: pH 7.75, cation exchange capacity (CEC) 23.34 cmol/kg, 39.5% clay, calcium carbonate 55.0 g/kg, organic matter 16.33 g/kg, total nitrogen 1.11 g/kg, total Se 0.207 mg/kg and total Hg 0.05 mg/kg. These soil properties were determined in accordance with the procedures described by Bao (2000). Soil pH was determined in water extracts at a soil-to-water ratio of 1:2.5 using a pH meter. Soil organic matter content was measured through hot K_2CrO_7 oxidation and $FeSO_4$ titration. CEC was determined through extraction with NH_4OAC solution and flame spectrophotometry. Clay and carbonate contents were determined through laser particle size analysis and gas volumetric method, respectively. Total nitrogen content was determined through the Kjeldahl method.

Seeds of pak choi (*Brassica chinensis*, Qinbai No. 2) were put into 0.1% mercuric chloride solution 3 min before sowing. Exogenous sodium selenite (Na_2SeO_3) and mercuric chloride ($HgCl_2$) were purchased from a reagent factory in Tianjin, China. Dissolve 0.2189 g of Na_2SeO_3 in 100 mL distilled = deionized water to make 100 mg Se/L stock solution and 0.1357 g of $HgCl_2$ in 100 mL distilled = deionized water to make 100 mg Hg/L stock solution.

2.2. Pot experiments

Se (added as Na_2SeO_3) exposure levels were set at 0, 0.5, 1.0, and 2.5 mg/kg soil, and Hg (added as $HgCl_2$) exposure levels were set at 1.0, 2.0, and 3.0 mg/kg soil (Hu et al., 2014). In addition, one treatment without Se and Hg was prepared as the control. The experiment followed a completely randomized design with three replicates and included 39 pots for 13 treatments (Table 1).

Different concentrations of SeO_3^{2-} or Hg^{2+} solutions were sprayed on dry soil with a plastic nebulizer. The treated soil was homogenized, equilibrated for 30 days, and then transferred to plastic pots (diameter: 18 cm; height: 15 cm). Each pot contained 2.5 kg of the equilibrated soil. The pots were then treated with basal fertilizer comprising 0.15 g/kg N (urea, AR) and 0.033 g/kg P (monopotassium phosphate, AR). Soil moisture content was maintained at approximately 70% water-holding capacity during the equilibration period. Ten pak choi seed were sown in each pot, and seedlings were thinned to five per pot after 10 days of germination. Pots were maintained under greenhouse conditions and were watered periodically to maintain soil moisture at 70% field capacity. Plants were harvested after 38 days.

Table 1
Se and Hg treatment concentrations in pot experiments (including control and single combined treatments).

Treatment	Concentration (mg/kg)		Treatment	Concentration (mg/kg)		Treatment	Concentration (mg/kg)		Treatment	Concentration (mg/kg)	
	Se	Hg		Se	Hg		Se	Hg		Se	Hg
CK	0	0	Hg1.0	0	1.0	Hg2.0	0	2.0	Hg3.0	0	3.0
			Se0.5Hg1.0	0.5	1.0	Se0.5Hg2.0	0.5	2.0	Se0.5Hg3.0	0.5	3.0
			Se1.0Hg1.0	1.0	1.0	Se1.0Hg2.0	1.0	2.0	Se1.0Hg3.0	1.0	3.0
			Se2.5Hg1.0	2.5	1.0	Se2.5Hg2.0	2.5	2.0	Se2.5Hg3.0	2.5	3.0

Note: The experiment followed a completely randomized design with three replicates (n = 13).