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Hormesis effects and implicative application in assessment of lead-contaminated soils in roots of *Vicia faba* seedlings

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ABSTRACT

Chemical analyses and biological methods were combined to investigate oxidative stress, hormesis effect and concerned mechanism in roots of *Vicia faba* seedlings grown in 0–2000 mg kg^{-1} of Pb-treated soils after germination of 20 d. The results showed that U-shaped dose response curves were displayed in superoxide radical (O_2^-) radicals, guaiacol peroxidase (POD) and ascorbate peroxidase (APX) activities, malondialdehyde (MDA) and carbonyl groups as well as activities of endoproteinase (EP) isoenzymes in the roots at low doses of extraneous Pb, indicating reduced oxidative stress and toxic effect. The inverted U-shaped curves were also exhibited in growth height, superoxide dismutase (SOD) and EP activities as well as inducible heat shock protein70 (HSP70) with the increasing extraneous Pb, indicative of enhanced oxidative stress. The enhancement in HSP70, carbonyl groups and EP activities confirmed intracellular proteotoxicity and proteolytic activity in the roots at higher doses of soil Pb. More interestingly, levels of inducible HSP70 were well correlated with those of growth heights ($r = 0.809$, $p < 0.05$), implying that HSP70 induction may be one of the mechanisms underlying the U-shaped growth curve of *V. faba* seedlings in the experiment. The results suggest that traditional threshold models ought to be combined with hormesis effect in assessment of Pb-polluted soils and the threshold dose range of Pb-treated soils is proposed rudimentally as 25–125 mg kg^{-1}.

1. Introduction

Heavy metal contamination has been greatly accelerated since the beginning of industrial revolution. Lead (Pb)-contaminated soil is of widespread occurrence that originates from anthropogenic sources, such as mining and smelting of Pb-ores, burning of coal, effluents from storage battery industries, and automobile exhausts, (Verma and Dubey, 2003), which poses serious hazards to environment and human health. Pb phytotoxicity has been well documented in many species (Schützendübel and Polle, 2002; Verma and Dubey, 2003; Pallavi and Rama, 2005; Liu et al., 2009), generally involving in induction of oxidized cell components, inactivated enzymes, damaged DNA and even dead cells. These adverse effects were generally observed at relatively higher doses of toxicants and possibly show linear or nearly linear dose–response relationship.

The dose–response relationship, however, may not be linear at suitable spacing of doses, which shows a biphasic curve and is usually called hormetic effect or hormesis. When the study design satisfies a priori criteria (i.e., a well-defined no observed adverse effect level (NOAEL), ⩾2 doses below the NOAEL), exposure duration, end-point parameters and statistical analysis protocols, hormesis is frequently encountered and is broadly represented (Calabrese and Baldwin, 2001). The hormesis shows two types of curves: (1) U-shaped curve, which represents a decrease below the control at low doses followed by an increase at higher doses, and (2) inverted U-shaped curve, which represents an increase above the control at low doses followed by a decrease at higher doses (Calabrese and Baldwin, 2003a,b). For hormesis, the U-shaped model denotes dysfunction (e.g. carcinogenesis, mutagenesis, disease incidence, etc.); specifically, low doses produce improvement compared to controls while high-doses above NOAEL lead to excessive dysfunction. The inverted U-shaped model denotes normal function (e.g. growth, fecundity, longevity, etc.), which is called low-dose-stimulation and high-dose-inhibition models (Rodricks, 2003).

Hormetic effects are commonly observed in organisms when studies are designed to assess responses below NOAEL, which demonstrate reduced adverse incident and even lead to seemingly beneficial effect. Their quantitative characteristics are similar for

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species and individuals, which were observed in yeast, bacteria and plants exposed to heavy metals (Oller and Bates, 2004), in terrestrial plants alfalfa, Japanese millet and perennial ryegrass exposed to 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), 2,4-dinitrotoluene (2,4-DNT) or 2,6-dinitrotoluene (2,6-DNT) (Rocheleau et al., 2006), in crop plants and weeds exposed to different antibiotics (Migliore et al., 2003, 2010) as well as human cell lines exposed to sodium arsenite (Yang et al., 2007) and low-dose ionizing radiation (Trosko et al., 2005). Despite its frequent observation, the theory of U-shaped response has been crippled by the lack of a robust mechanism underlying and incomplete in vitro and in vivo correlation (Bae et al., 2008).

The dose–response relationships at low-dose range are more compatible with hormetic effect than threshold models (Calabrese et al., 2006). Accurate description of hormetic dose–response curves (DRC) is a key step for the determination of the efficacy and hazards of the pollutants with the hormetic phenomenon (Qin et al., 2010). However, to our knowledge, how to evaluate pollutant's ecotoxicity by combination of traditional threshold models and hormesis has not been thoroughly documented.

Pb can be absorbed by plants and mainly accumulated in root tissues (Verma and Dubey, 2003). Thus, in this experiment, roots of *Vicia faba* seedlings were employed preferentially as tested materials. The objectives are aimed to (1) investigate hormesis effect and oxidative stress in roots of *V. faba* seedlings grown in concentrations of Pb-treated soils, (2) explore the potential mechanism involved in hormesis effect of Pb on growth curve of the seedlings, and (3) provide implications for evaluation of Pb and other heavy metal-contaminated soils.

2. Materials and methods

2.1. Soil treatment and plant materials

For native soil, the pH value was 7.4, total Pb content was 21.05 mg kg^{-1} (dry weight) and organic matter was 1.19%. After spiking, concentrations of Pb-treated soils were 6.25, 12.5, 25.0, 125, 250, 500, 1000 and 2000 mg kg^{-1}, respectively. Control was spiked with deionized water. The soils were allowed to equilibrate naturally for a week. The pH values were measured according to ISO 10390 and ranged from 7.1 to 7.4 before sowing.

Twelve vigorous seeds of *V. faba*. L were sterilized with 0.1% (m/v) sodium hypochlorite solution for 10 min, rinsed with distilled water, and then sown in a pot containing 1.5 kg of Pb-treated soils. After germination, six seedlings with nearly homogeneous properties were reserved. The seedlings were cultured in a growth chamber under controlled conditions (24–25 °C, 70% relative air humidity, a photoperiod of 15/9 h (day/night), photosynthetic active radiation of 200 μmol m^{-2} s^{-1}). They were daily watered and weekly supplemented with equal amount of Hoagland nutrient solution (Lucretti et al., 1999). Roots were harvested after 20 d of germination. Three replicates were performed in two independent experiments.

2.2. Determination of Pb content in roots of V. faba seedlings

Fresh roots were rinsed first with 1 M HCl and then with distilled water. Digestion of dried samples and measurement of Pb contents following dryness at 60 °C were performed according to the previous protocol (Wang et al., 2008a). The Pb content was expressed as μg g^{-1} dry weight. Certified standard samples (GBW07429) and triplicates of all samples were used to ensure accuracy and precision. All results were above the method detection limits (0.02 μg L^{-1}).

2.3. Measurement of shoot heights of the seedlings

Lengths between apical leaf and basal stem were measured to be represented as heights of the seedlings. Six seedlings were measured in each pot, and three replicates were performed in each treatment.

2.4. Determination of O_2^- production in the roots

O_2^- content was determined according to our previous protocol (Wang et al., 2008a), with minor modification. 0.4 g of fresh roots were ground quickly in liquid nitrogen and homogenized at a 1:5 (w/v) ratio in a solution containing 100 mM phosphate buffer (pH 8.0), 10 μM pyridoxal phosphate, 1 mM Na_2EDTA and 5 mM DTT, then centrifuged at 15 000 × *g* for 20 min at 4 °C. The supernatant was collected used as for immediately investigation of O_2^- production. The specific absorption at 530 nm was assayed. O_2^- content was calculated according to the standard curve, which was prepared with sodium nitrite as above.

2.5. Determination of antioxidant enzymes' activities

Crude enzyme extract was prepared according to method described by Romero-Puertas et al. (2004) with minor modification. 1 g of fresh leaves was ground immediately to be fine powder under liquid nitrogen and homogenized in extraction buffer (0.1 M Tris–HCl (pH 8.0), 10% (v/v) glycerol, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.2% (v/v) Triton X-100, 5 mM ascorbate and 1 mM phenylmethylsulphonyl fluoride (PMSF), 1 mM benzamidine, 1 μg mL leupeptin and 2 μg mL apratinin. The extract was centrifuged for 25 min at 15 000 *g* and supernatant was recovered. Soluble protein content was determined by Bradford (1976) with BSA as standard. All operations were performed at 4 °C.

SOD (EC 1.15.1.1) activity was determined according to the previous protocol (Wang et al., 2008a). SOD activity is expressed as units/min. mg protein. One unit of enzyme activity is defined as the amount of enzyme required to inhibit nitroblue tetrazolium chloride (NBT) reduction by 50%. Guiacol POD (EC 1.11.1.7) activity was assayed according to García-Limones et al. (2002), CAT (EC 1.11.1.6) activity according to Bestwick et al. (2001) and APX (EC 1.11.1.11) activity according to Janda et al. (1999).

2.6. Determination of lipid peroxidation and carbonyl contents

Lipid peroxidation was determined by measuring the concentration of 2-thiobarbituric acid reacting substances (TBARS) according to method described by Verma and Dubey (2003). Content of lipid peroxides were quantified and expressed as total TBARS in terms of μmol g^{-1} fresh weight by the extinction coefficient of 155 mM^{-1} cm^{-1}.

Carbonyl contents were assayed using the dinitrophenyl hydrazine according to methods described by Levine et al. (1994), Nemat Alla and Hassan (2006). Crude extraction were prepared with 50 mM potassium phosphate, pH 7.4, containing 1 mM Na_2EDTA, 0.1% Triton X-100 (w/v). After precipitation of the possibly contaminated nucleic acids in the sample with 1% (w/v) streptomycin sulfate, an aliquot of 0.8 mL of the extract was reacted with 0.2 mL of 20 mM dinitrophenyl hydrazine (in 2.5 M HCl) for 1 h with shaking. Proteins were then precipitated with 0.6 M trichloroacetic acid, washed with 1:1 (v/v) ethanol/ethyl acetate, solubilized in 6 M guanidine hydrochloride (pH 2.3) and the absorbance of the hydrazones (derivatized carbonyls) was measured at 370 nm. Carbonyl content was calculated by the extinction coefficient of 22 000 mM^{-1} cm^{-1}. The final content of proteins was quantified by measuring absorbance at 280 nm according to a bovine serum