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Physiological and Antioxidant Responses of Germinating Mung Bean Seedlings to Phthalate Esters in Soil*1

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ABSTRACT

Single phytotoxicity of two representative phthalate esters (PAEs), di-*n*-butyl phthalate (DnBP) and bis(2-ethylhexyl) phthalate (DEHP), was tested in mung bean (*Vigna radiata*) seedlings germinated for 72 h in soils spiked with varying concentrations (0–500 mg kg^{-1} soil) of DnBP or DEHP. PAEs added at up to 500 mg kg^{-1} soil exerted no significant effect on germination but both pollutants significantly inhibited root elongation ($P < 0.01$), DEHP inhibited shoot elongation ($P < 0.01$) and DnBP depressed biomass on a fresh weight basis ($P < 0.05$). Seedling shoot and root malondialdehyde (MDA) contents tended to be stimulated by DnBP but inhibited by DEHP. However, increases in superoxide dismutase, peroxidase, ascorbate peroxidase and polyphenol oxidase activities, as well as glutathione (GSH) content, were induced at higher concentrations (*e.g.*, 20 mg kg^{-1}) of both compounds. Accumulation of proline in both roots and shoots and the storage compounds, such as free amino acids and total soluble sugars, in whole plant was induced under the stress exerted by both PAEs. The general responses of mung bean seedlings indicated higher toxicity of DnBP than DEHP on primary growth, during which root elongation was a more responsive index. MDA and GSH were more sensitive parameters in the roots than in the shoots and they might be recommended as physiologically sensitive parameters to assess the toxicity of PAE compounds in soils in future long-term studies.

Key Words: glutathione, malondialdehyde, phytotoxicity, root elongation, storage compounds

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INTRODUCTION

Phthalate esters (PAEs) are a group of diesters primarily used as plasticizers and are readily released to different environmental media as contaminants because of their weak binding forces with polyolefin molecules (Staples *et al.*, 1997). Reported as endocrine disrupting compounds (EDCs), six PAEs have been nominated by USEPA as priority pollutants. The PAEs compounds di-*n*-butyl phthalate (DnBP) and bis(2-ethylhexyl) phthalate (DEHP) have also been classified by the European Union (EU) as priority pollutants. DnBP and DEHP are the dominant PAEs in the contaminated soil at an electronic waste dismantling area in East China and in the urban soils of Guangzhou in South China (Liu *et al.*, 2009; Zeng *et al.*, 2009), and their concentrations are sometimes 2–3 orders of magnitude higher than those reported in agricultural soils at Roskilde in Denmark (Vikelsøe *et al.*, 2002). DnBP and DEHP have raised wide public concerns because of their potential carcinogenicity and mutagenicity in humans after long-term contact at low concentrations (Hu *et al.*, 2007).

Toxic effects of DnBP and DEHP in contaminated soils are of concern in addition to their toxicity to animals. Their effects on urease, phosphatase, catalase, microorganisms, fauna and the microbial community in soil were significantly greater compared with those of the pristine controls (Chen *et al.*, 2004; Gao and Chen, 2008; Xie *et al.*, 2009; Gao, 2010). DnBP and DEHP in soil may also affect the quality of vegetables such as peppers (*Capsicum* spp.) by decreasing the content of vitamin C in the fruit (An *et al.*, 1999; Yin *et al.*, 2002). Phytotoxicity tests using higher plants are frequently employed for toxicity evaluation of heavy metals (Chen *et al.*, 2010), but there are few for orga-

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nic pollutants such as PAE compounds. Toxic effects of DnBP on the growth of Chinese cabbage (*Brassica rapa* var. *chinensis*) have been reported, including significant accumulation and differences in expression of six protein spots in leaf tissue under high concentrations of the pollutant (Liao *et al.*, 2009).

The germination stage is crucial for future vegetative and reproductive growth of different higher plant species under the stress of different pollutants. Although germination and root elongation tests have been criticized occasionally as insensitive and representing only a single life stage, they are also supported because the roots are in direct contact with the contaminants in soil during the initial growth stages (Kapustka, 1997). Biomass has been found to be a better indicator for pollutant toxicity than root or shoot elongation (Shi and Cai, 2009). Antioxidant enzyme status is important for understanding EDC effects, including superoxide dismutase (SOD) and peroxidase (POD) in mediating responses to stressors (Zhou *et al.*, 2010; Boojar and Tavakkoli, 2011). Proline, free amino acids (FAA) and total soluble sugars (TSS) can be induced under adverse environmental stresses including drought, salinity and low temperatures (Xu *et al.*, 2001; Liu *et al.*, 2007; Kocsy *et al.*, 2011). Toxicity to individual plants as revealed by the germination tests and effects on antioxidant enzymes and other critical compounds provide useful evidence in assessing the toxicity of organic pollutants to sensitive terrestrial dicotyledonous plant species.

The present study examined the phytotoxicity of the two typical PAE pollutants DnBP and DEHP in soil to mung bean (*Vigna radiata*). Their effects on initial plant growth (germination, root elongation, shoot elongation and biomass) and changes in the activity or content of different enzymes, critical amino acids and some storage compounds such as FAA and TSS were examined. The aim was to compare the potential toxic effects of typical PAE pollutants in soil and to make preparations for further prediction of toxic threshold concentrations. More importantly, it was hoped that the study would provide fundamental data for further health risk assessment and ecosystem effects in China, where soil pollution by PAE compounds is increasing due to the use of plastic films in agriculture.

MATERIALS AND METHODS

Chemicals

DnBP (99.1%) and DEHP (99.6%) were obtained from AccuStandard, Inc., New Haven, USA. The nitro blue tetrazolium (NBT), *L*-methionine (L-Met), ascorbic acid (AsA), trichloroacetic acid (TCA), thiobarbituric acid (TBA), proline, alanine, sulfosalicylic acid, ninhydrin, catechol, anthrone, polyvinylpyrrolidone (PVP), monosodium orthophosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4), hydrogen peroxide (H_2O_2), riboflavin, ethylenediaminetetraacetic acid disodium salt (EDTA-Na_2), sodium hydrate (NaOH), glacial acetic acid and acetone used were all analytical reagents purchased from the National Pharmaceutical Group Chemical Reagent Co., Ltd., Shanghai, China. The glutathione (GSH) assay kit (catalog number A006) was purchased from the Jiancheng Bioengineering Institute, Nanjing, China.

Soil and plant seed preparation

The test soil, collected from a comparatively uncontaminated area at Qixia in Nanjing, Jiangsu Province, East China, is an Alfisol according to the USDA soil classification. The soil had a pH (in water) of 7.4, a clay content of 1.67 g kg^{-1}, an organic matter content of 14.6 g kg^{-1}, and available nitrogen, phosphorus and potassium concentrations of 96.8, 14.4 and 102.8 mg kg^{-1}, respectively. The soil was passed through a 2-mm sieve before use and the background concentrations of the two target pollutants DnBP and DEHP were determined to be 142.6 $\pm$ 4.2 and 194.5 $\pm$ 1.1 μg kg^{-1} soil, respectively.

Batches of soil samples were adjusted to concentrations of DnBP or DEHP of 0, 5, 20, 100 and 500 mg kg^{-1} soil by spraying aliquots of soil with stock solutions of PAEs in acetone. After the acetone had evaporated off, each spiked soil sample was mixed thoroughly before use.

According to the recommendations of the Organization for Economic Co-operation and Development (OECD, 1984) for test species in terrestrial environmental assessment and to promising results from previous studies (Yusuf *et al.*, 2011), seeds of mung bean (*Vigna radiata*) obtained from the Chinese Academy of Agricultural Sciences were selected as our test species for terrestrial environmental assessment. The seeds were surface sterilized by immersion in 10% (v/v) sodium hypochlorite solution for 10 min (USEPA, 1996), rinsed three times with deionized water and soaked in deionized water for 2 h before use. Plastic equipment was avoided throughout the procedure to eliminate background PAE contamination. All the glass Petri dishes were washed and baked in an oven at 400 °C before use.