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Effects of bisphenol A in soil on growth, photosynthesis activity, and genistein levels in crop plants (*Vigna radiata*)

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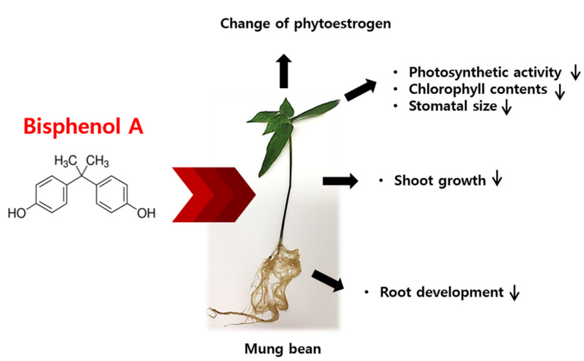
HIGHLIGHTS

- This study investigated the effects of BPA on mung bean (*Vigna radiata*) in soil.
- Changes in growth, photosynthetic activity, and phytoestrogen levels were measured.
- BPA exposure reduced growth, stomatal size, chlorophyll content and photosynthesis.
- The amount of the phytoestrogen genistein increased.
- Genistein is a good specific toxicity endpoint for endocrine-disrupting chemicals.

GRAPHICAL ABSTRACT



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ABSTRACT

The compound bisphenol A (BPA), an endocrine-disrupting compound that can act as an estrogen, is widely used in the industrial manufacture of plastic products and epoxy resins. Because of the widespread use of the compound and its use in soil amendments, there is concern regarding its effects on crop plants, although comparatively little information is available on the ecotoxicity and potential risk of bisphenol. Here, we investigated the toxicity of BPA on mung bean (*Vigna radiata*) by evaluating growth, photosynthesis parameters, and phytoestrogen changes. Adverse effects on shoot growth were observed at a dose of 750 mg BPA/kg dry soil after acute (14 days) and chronic (21 days) exposure, and inhibition of root development was confirmed at a dose of 1000 mg BPA/kg dry soil. Chlorophyll content and stomatal size decreased at doses of 250 and 500 mg BPA/kg dry soil, respectively, and leaf spots due to leaf necrosis were observed in the groups that received 250 mg BPA/kg dry soil. Photosynthetic activity appeared to decrease in the groups that received the highest exposure, although it was not statistically significant. Meanwhile, exposure to bisphenol A increased the level of the phytoestrogen genistein. We propose that changes in genistein levels due to endocrine-disrupting compounds can be considered as a specific toxicity endpoint for endocrine-disrupting chemicals; further studies should explore this effect. This study confirmed the phytotoxicity of BPA at various endpoints and the results provide a basis for future ecological risk assessment for BPA.

1. Introduction

Endocrine-disrupting chemicals (EDCs) are defined as

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"exogenous substances or mixtures that alter the function(s) of the endocrine system and consequently cause adverse health effects in an intact organism, its progeny, or (sub) populations" (World Health Organization et al., 2002). Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl) propane, CAS No. 80-05-7) is a known EDC that is produced in large quantities for use in polycarbonate plastics and epoxy resins (NIH-HHS, 2010; U.S. EPA, 2010). BPA is present in a wide range of consumer products, including plastics and thermal papers (Careghini et al., 2015; Im and Löffler, 2016).

BPA can contaminate the soil environment through sludge and effluent, as well as though biosolids that are used as soil amendments. The half-life of BPA in soil is approximately 30 days, according to EUSES. In Korea, BPA was detected at levels of 0.5–48.68 μg/kg in the soil environment (NIER, 2006). Its concentration in soils was 2340 pg/m^3 in China, 1920 pg/m^3 in Japan, and 17,400 pg/m^3 in India. In the United States, BPA concentrations were 4–14 μg/kg in soil (U.S. EPA, 2010), <32–147 μg/kg in agricultural field soil (Kinney et al., 2008), and 0.55–2 μg/kg at a golf course (Xu et al., 2008). Gibson et al. (2010) reported that BPA concentrations in agricultural field soil irrigated with wastewater in Mexico were 1.6–30.2 μg/kg. Staples et al. (2010) reported a median concentration of BPA in European soils amended with biosolids of 0.24 μg/kg and a 95th percentile concentration of BPA as 140 μg/kg.

A number of studies have confirmed the toxicity of BPA to plants (Ali et al., 2016, 2017; Ferrara et al., 2006; Hu et al., 2014; Jiao et al., 2015, 2017a; 2017b; Qiu et al., 2013; Salazar-Parra et al., 2015; Speranza et al., 2011; Sun et al., 2013; Terouchi et al., 2004; Wang et al., 2015a; b; Zhang et al., 2016b). However, these studies examined hydroponic exposure, not exposure in the field. The effects of chemicals in solution and those in soil are significantly different. Therefore, it is necessary to carry out toxicity evaluations directly in the soil, under realistic conditions.

To date, few studies have examined the phytotoxicity of BPA in soil where plants actually grow. Staples et al. (2010) investigated the toxicity of BPA to six plant species (*Zea mays*, *Avena sativa*, *Triticum aestivum*, *Brassica oleracea*, *Glycine max*, and *Lycopersicon esculentum*) and confirmed a decrease in dry shoot weight among plants grown in BPA-treated artificial soil for 21 days. However, they confirmed only the no-observed-effect concentration (NOEC) for plant growth, not the effect of other factors.

In the present study, the toxicity of BPA on mung bean (*Vigna radiata*) in soil was evaluated after acute or chronic exposure. The aim was to characterize diverse changes in growth and physiological effects (photosynthesis activity, stomatal size, and chlorophyll content), especially changes in phytoestrogen levels in plants exposed to BPA in the soil. Phytoestrogens are plant-specific xenoestrogens (i.e., xenohormones that mimic estrogen activity) (Sirotkin, 2014). Their effects depend on the environment of the plant and on external stressors. The response of phytoestrogens to toxic substances has been little studied; however, when many herbivores are feeding on a plant, the levels of phytoestrogens in plants increase as a defense mechanism, causing endocrine disruption in the herbivores and a decrease in population levels (Hughes, 1988; Kim and Park, 2012; Lee et al., 2009).

We explored whether the phytoestrogen levels in plants changed due to BPA, which is well known as an EDC. Phytoestrogens are mainly categorized as isoflavonoids, flavonoids, coumestrol, and lignans (Salgado and Donado-Pestana, 2013). The isoflavonoid group includes isoflavones such as genistein, which is the most abundant type and the most widely studied phytochemical in soybeans (Müllner and Sontag, 1999; Salgado and Donado-Pestana, 2013). Genistein has both beneficial effects (Makarevich et al., 1997; Messina and Barnes, 1991; Thornton, 2013) and adverse effects (Barrett, 1996; Jin et al., 2007; Kim and Park, 2012) on animal and human health.

In the present study, we observed how exposure to BPA affected genistein levels in plants. As the properties of EDCs differ from those of general toxic substances (for example, they may affect the endocrine systems of organisms), the method of assessing toxicity through effects on genistein levels has not been formalized. We therefore analyzed the toxic endpoints specific to EDCs using a leguminous plant that is known to contain genistein, unlike some other plants. To our knowledge, this is the first report on acute or chronic phytotoxicity of BPA using various parameters and the first to suggest change in genistein level as a specific toxicity endpoint for EDCs.

2. Material and methods

2.1. Test chemicals and species

Bisphenol A (BPA ≥ 99% purity, 2,2-bis(4-hydroxyphenyl) propane) powder was purchased from Sigma Aldrich (St. Louis, Missouri, USA) and dissolved in acetone (99.9%, Duksan, Daejeon, South Korea) for use in the experiments. Seeds of the dicotyledon mung bean (*V. radiata*) were obtained from a commercial seed company (Danong, Namyangju, South Korea), sterilized with 5% hypochlorite solution for 15 min, and rinsed with distilled water. Seeds were incubated in a glass petri dish with wet cotton for 24 h at 25 °C under dark conditions. Germinated seeds were planted in the test soil.

2.2. Test soil preparation

We used LUFA 2.2 soil (Landwirtschaftliche Untersuchungs und Forschungsanstalt). The soil pH was 6.17, the soil contained 4.7% organic matter, and its water-holding capacity (WHC) was 0.473 mL/g. A stock soil of 2000 mg BPA/kg dry soil was prepared by soaking BPA in acetone; the soil was then dried for 24 h to remove the acetone. A solvent control soil was prepared by adding acetone to dry soil and drying for 24 h. The dried soil was mixed using a roller overnight. The concentration of the BPA in soil was altered by mixing the stock soil with solvent control soil to produce test concentrations of 100, 250, 500, 750, and 1000 mg BPA/kg dry soil. We selected these concentrations on the basis of preliminary experiments. These concentrations are much higher than those used in the environment and were set to confirm toxic values of BPA such as the 10% effective-concentration (EC_{10}), the 50% effective-concentration (EC_{50}), and the NOEC in soil. Before use in the plant assays, the test soils were mixed thoroughly on a roller mixer for 24 h at room temperature. Control soil was treated with distilled water. The BPA concentration in soil was analyzed to confirm this concentration and to determine how much BPA decomposed during the experiment.

There were three treatments: (1) untreated dry soil with a BPA concentration of 500 mg/kg soil, diluted with stock soil, (2) soil sampled after a toxicity test with five mung beans for 21 days, and (3) soil sampled after 21 days from a control version of treatment (2) that was watered but not planted with seeds. There were two replicates for each of the three conditions. The soil was frozen immediately after sampling and then thawed and lyophilized to conduct BPA analysis. In the case of treatment (2), BPA analysis of mung bean was also conducted to confirm that BPA uptake by mung bean occurred. Plant samples were also analyzed in duplicate as soil samples. BPA levels were subsequently analyzed by Nanospace SI-2 high-performance liquid chromatography (Shiseido, Tokyo, Japan) with an API4000 triple quadrupole tandem mass spectrometer (Applied Biosystems, Foster City, CA, USA).