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# Effect of di-*n*-butyl phthalate on root physiology and rhizosphere microbial community of cucumber seedlings

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## HIGHLIGHTS

- Cucumbers were grown with DBP supplementation.
- DBP had a significant effect on root physiology of cucumber seedlings.
- The ultrastructural study showed that the organelles were obviously affected by DBP.
- A period of an 'on average' low functional organization was discovered.

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## ABSTRACT

The authors investigated the effects of di-*n*-butyl phthalate (DBP) on root physiology and rhizosphere microbial communities of cucumber seedlings (*sativus* L. cv Jinyan No. 4). Root protein content and root activity were observed to decrease. From the ultrastructural micrographs, visible impact on the mitochondria, endoplasmic reticulum and vacuole were detected. Moreover, the number of starch grains increased, and some were adhered to other cell components which might be the most direct evidence of DBP causing cellular damage. Results of PCR-DGGE (denaturing gradient gel electrophoresis) indicated that DBP significantly changed the abundance, structure and composition of rhizosphere bacteria when the concentration was higher than 50 mg $L^{-1}$. The relative abundances of Firmicutes increased while that of Bacteroidetes decreased. *Bacillus* was detected as the dominant bacteria in DBP contaminated cucumber rhizospheric soil. The amount of Actinobacteridae and *Pseudomonas* decreased until it disappeared in the rhizosphere soil when exposed to DBP concentrations higher than 50 mg $L^{-1}$.



## 1. Introduction

DBP is one of the important members in the phthalate esters family (PAEs) that has been listed as a priority pollutant by the US Environmental Protection Agency [1]. It is a kind of organic compound that is primarily used as plasticizers in the preparation of polyvinyl chloride (PVC) resins, plastic packing films, adhesives, cosmetics, cellulose materials and insect repellents [2]. Like many other PAEs, DBP is not chemically bonded to the polymer products and it migrates readily from the plastics to the environment [3]. Concerns have been raised about D-BP because it is suspected to have mutagenous, carcinogenous, and endocrine-distrupting effects [4,5].

Due to increase in the use of plastic mulching films in agricultural facilities, the content of PAEs in the soil is also on the increase in the agricultural facilities [6]. PAEs play a detrimental role in the physiology of plants by inhibiting plant growth and development [7]. Humans are exposed to PAEs through its entry into the food chain via the contamination of vegetables and food items [8]. It is estimated that the contribution percentages of human exposure to DBP through vegetables, fruits and grains pathways were 42.4%, 2.0% and 7.5% [9]. Yin et al. [10] demonstrated that the decrease of capsaicin content and vitamin C was negatively correlated to the increase the concentration of DBP in capsicum fruit, which suggested that DBP uptake by the plant might be mainly responsible for quality degradation of capsicum fruit. Plant root as medium of absorption and transportation of nutrients, have an important effect on plant growth. Root protein and root activity are used as the general indicators of plant development. Cell ultrastructure could reflect the plants growth whether in good condition.

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Previous researches have proved that microbial community composition show significant differences in the early and late periods of PAEs contamination in the soil [11]. Microbes living in the plant root zone (rhizosphere) exert a profound impact on soil fertility and plant development [12]. Hence, changes in the soil microbial communities may affect functions performed by the microbes, and thus influence overall plant growth and development [13,14]. It means that not only does the contaminant directly affect both the plants and soil microbes, but also plays a detrimental role in the interaction between both components; thus, the further release of DBP into the ecosystem will further increase the level of severity both plants and microorganisms are exposed to.

However, since detailed information about the adverse effects of DBP on plant roots and rhizosphere microorganisms are still not available, and thus requires further study. In this study, cucumber was employed as a target plant because it is one of the most widely consumed vegetables and is usually cultivated in greenhouse conditions. Extensive use of plastic films caused a serious threat to agricultural environment, resulting in significant effect on cucumber [6,15]. Different concentrations of DBP were applied to the cucumber seedlings to investigate their effects on root protein content, root activity and root cell ultrastructure. Changes in bacterial species diversity in the cucumber rhizosphere were also studied using DGGE of the PCR-amplified 16S rRNA gene.

## 2. Materials and methods

### 2.1. Plant materials and stress treatment

Seeds of cucumber *sativus* L. cv Jinyan No. 4 were planted into planting pots at the horticultural garden of Northeast Agricultural University, Harbin, China. The soil was also sampled in the same garden. The soil was a black soil with sandy loam texture. The basic properties of the soil: soil water content, 3.74%; TOC, 3.96%; *N*, 17.1 mg $kg^{-1}$; *P*, 12.9 mg $kg^{-1}$; *K*, 301.17 mg $kg^{-1}$; pH, 7.16; No fertilizer was previously added to the soil. The average temperature in the greenhouse was 27 ± 1 °C during the day and 20 ± 1 °C during the night. The relative humidity was kept at 60–70%. Applications of DBP treatment to cucumber plants were made according to the method described by Zhang et al. [16].

### 2.2. Root protein content

Root tissues (0.5 g) were ground to fine powder using a chilled pestle and mortar. Five milliliter of 50 mM potassium phosphate buffer (pH 7.0), 1 mM ascorbic acid, 0.2 mM ethylenediamine tetraacetic acid and 2% (w/v) polyvinylpyrrolidone were added to the powder. The homogenate was centrifuged at 4 °C for 20 min at 12,000 g. Supernatant was used for protein content assays. An aliquot of the extract was used to determine its protein content by the method of Redmile-Gordone et al. [17] utilizing bovine serum albumin as the standard.

### 2.3. Root activity

Root activity was measured using the TTC (triphenyl tetrazolium chloride) method [18] and expressed as the deoxidization ability (mg $g^{-1}$ $h^{-1}$). Dehydrogenase was expressed as the deoxidized TTC quantity, which was an index of root activity [19]. Ten milliliter solution of equal quantities of TTC (0.4%) and phosphate buffer was added to root samples (0.5 g) and kept in the dark at 37 °C for 2 h. The reaction was stopped with 1 M $H_2SO_4$. The roots were ground and transferred into a tube with ethyl acetate to a total volume of 10 ml. The solution was measured at the absorbance of 485 nm.

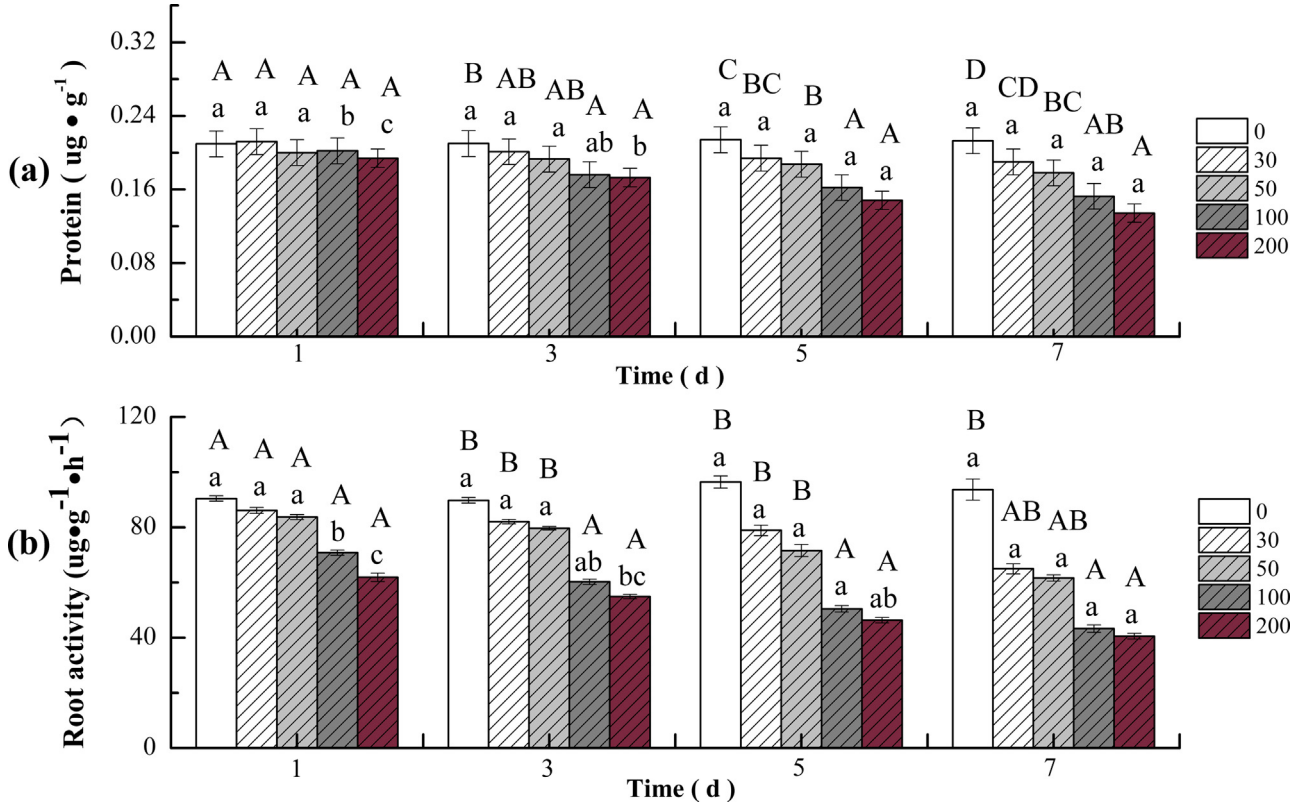


**Fig. 1.** The effect of DBP on root physiology of Cucumber *sativus* L. cv Jinyan No. 4. (a) Protein content. (b) Root activity. Means with different small letters are significantly different from one another under the DBP different concentration treatment, and different capital letters are significantly different from one another under the DBP different treatment times.