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Science of the Total Environment 367 (2006) 958 – 967

Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

www.elsevier.com/locate/scitotenv

Effect of scale of Cd heterogeneity and timing of exposure on the Cd uptake and shoot biomass, of plants with a contrasting root morphology

Adriana Manciulea *, Michael H. Ramsey

Centre for Environmental Research, Department of Biology and Environmental Science, School of Life Sciences, University of Sussex, Falmer, Brighton, East Sussex BN1 9QJ, UK

Received 23 June 2005; received in revised form 13 January 2006; accepted 19 January 2006
Available online 4 April 2006

Abstract

A pot experiment was conducted to investigate the influence of spatial heterogeneity of Cd distribution in soil on shoot biomass, shoot metal concentration and total shoot Cd uptake by lettuce (*Lactuca sativa*, variety Tom Thumb) and Indian mustard (*Brassica juncea*). Five different soil treatments had similar overall concentration of Cd per pot, but different scales of heterogeneity and also timing of plant exposure during the growth cycle. The presence and scale of heterogeneity and timing of exposure were found to have significant effects on shoot biomass for both plants (with one exception). The mean values of Cd mass taken up were significantly affected by the presence of heterogeneity and timing only for lettuce. Only the scale of heterogeneity affected the uptake of Cd by Indian mustard, presumably because of its larger root system (~18 cm, compared with ~5 cm for lettuce). These findings have important implications for phytoremediation, and for human health risk assessment where leafy vegetables are grown in situations with highly elevated Cd concentrations.

Keywords: *Lactuca sativa*; *Brassica juncea*; Cadmium; Environmental heterogeneity; Plant metal uptake; Human health risk assessment

1. Introduction

Contamination of the environment with heavy metals due to anthropogenic activities is a major source of potential exposure for humans to toxins. The Cd^{2+} ion is soluble in water, mobile in acid soils and is toxic to plants and animals in very low concentrations (Das et al., 1997; Kabata-Pendias, 2000). In plants, the symptoms of cadmium toxicity are easily identifiable. The most general symptoms are stunted growth and chlorosis. Cadmium is a carcinogenic element that is naturally present in soils and sediments at concentrations which are generally less than 1 mg kg^{-1} (Alloway, 1995).

Soil properties that influence the uptake of Cd by plants include the total and available concentration of Cd, pH and organic matter content (McBride et al., 1997; McBride, 2002; Gray et al., 1999a,b; Peijnenburg

* Corresponding author. Department of Analytical Chemistry, Babes-Bolyai University, Faculty of Chemistry and Chemical Engineering, 11 Arany Janos Street, RO-400028 Cluj-Napoca, Romania. Tel.: +40 264 592889; fax: +40 262 590818.
E-mail address: amanciulea@yahoo.com (A. Manciulea).

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doi:10.1016/j.scitotenv.2006.01.015

et al., 2000; Sauve et al., 2000; DEFRA-EA, 2002), cation exchange capacity (Lehoczky et al., 2000), clay content and the presence of other soluble compounds or ions such as iron (Krishnamurti et al., 2000), zinc (Smilde et al., 1992; McKenna et al., 1993; Narwal et al., 1993; Hart et al., 2002) and chloride (Li et al., 1994; McLaughlin et al., 1994).

The effects of heavy metals in soil on plants are usually studied under homogeneous conditions. However, contaminated soils in the environment usually have a heterogeneous distribution of heavy metals (Ramsey and Argyraki, 1997; Keller et al., 2003). Recent studies have shown that contaminant heterogeneity of Zn in soils varies between sites with different pollution sources (e.g. 20% RSD at an smelter site and 49% at an ex-firing range), but does not always vary systematically between different scales of measurement (e.g. from 0.01 to 50 m) at one site (Taylor et al., 2005). Previous studies of the effects of metal heterogeneity on plant yield and metal uptake have been carried out on hyperaccumulators such as the Cd/Zn-hyperaccumulator *Thlaspi caerulescens* (Schwartz et al., 1999; Whiting et al., 2000; Lombi et al., 2001; Haines, 2002), the As-hyperaccumulator ferns *Pteris vittata* and *P. cretica* (Caille et al., 2003), and *Brassica juncea* (Podar et al., 2004) used in phytoremediation. The effect of metal heterogeneity on plant uptake and yield has also been found on leafy vegetables such as lettuce (Millis et al., 2004), which has implications for assessment of human exposure to Cd. Some studies have shown that heterogeneity in nutrient supply can also significantly affect the plant biomass and timing of root growth (Hutchings and de Kroon, 1994; Hutchings and John, 2003; Hutchings et al., 2003; Jackson and Caldwell, 1996; Einsmann et al., 1999; Wijesinghe and Hutchings, 1999; Wijesinghe et al., 2001; Day et al., 2003a,b).

The aims of this research were to investigate the effects of the spatial scale of heterogeneity of Cd, and the timing of exposure to Cd within the growth cycle, on shoot biomass, shoot metal concentration, and total shoot Cd uptake (expressed as the mass of Cd) by two plant species with contrasting root morphologies. The selected species, lettuce (*Lactuca sativa*, variety Tom Thumb) and Indian mustard (*Brassica juncea*) are both known to accumulate Cd, but lettuce has a relatively small central tap root with fine lateral roots, whereas Indian mustard has a larger disseminated root structure. This is the first reported study that considers how uptake is affected when the scale of the heterogeneity changes in comparison with the scale of the root ball for two contrasting plants.

2. Materials and methods

2.1. Studies species

Seeds of *L. sativa* L. (variety Tom Thumb) were ordered from Mr. Fothergill's Seed Company, UK, and *B. juncea* (L.) Czernj accession 426308 (provided by USDA/ARS Plant Induction Station of Iowa State University, Ames, IA). This latter species was chosen because of its disseminated root structure and its well established potential for phytoremediation (Salt et al., 1995, 1997; Blaylock et al., 1997). Accession number 426308 was chosen because of its proven efficiency at accumulating metals such as Cd, Zn and Pb (Kumar et al., 1995; Ebbs et al., 1997; Ebbs and Kochian, 1998; Haag-Kerwer et al., 1999).

2.2. Experimental design

This experiment was carried out under controlled conditions in a greenhouse and utilized a randomized experimental design (Fig. 1). There were six treatments: A (control — no added Cd), B (immediate exposure to homogeneous contamination at a intermediate level -15.44 mg Cd kg^{-1}), C (immediate exposure of the plant to the highest metal concentration used -34.5 mg Cd kg^{-1} in each contaminated patch), D (delayed exposure of plant to highest metal concentration), E (optional exposure, 6 cm scale heterogeneity, highest concentration), F (optional exposure, 3 cm fine scale heterogeneity, highest concentration). In the "immediate exposure" treatment the plant grows initially in a Cd contaminated patch and after a certain distance (and therefore time) and delay encounters the clean patch. In the "delayed exposure" treatment — the plant grows initially in a clean patch and only encounters the contaminated patch after a certain distance, and so, after a certain time delay. The alternative is the "optional exposure" treatment, in which the plant seed is at the intersection of contaminated and clean patches. The levels of Cd concentration in the soil were selected to represent heavy contamination, such as those found where home produced vegetable have been grown near sources such as mining or smelting sites (Alloway et al., 1988; Ullrich et al., 1999), but not so high as to be fatally toxic to these plants (Salt et al., 1995, 1997).

Each treatment was replicated eight times. In heterogeneous treatments, the boxes (18×18×25 cm) were divided into either 6×6 cm cells (C, D, E) or 3×3 cm cells (F) using plastic dividers. These dividers were placed in the box and each cell filled separately. The dividers were removed before planting to allow plants to