Applied Clay Science 99 (2014) 171–177

Contents lists available at ScienceDirect

Applied Clay Science

journal homepage: www.elsevier.com/locate/clay

Research paper

Changing elemental uptake of radish seedlings grown in Cd and Pb polluted smectite substrates

Khadija Semhi [a], Norbert Clauer [b,*], Sam Chaudhuri [c]

[a] Department of Earth Sciences, College of Science, Sultan Qaboos University, Al-Khod 123, Muscat, Sultanate of Oman
[b] Laboratoire d'Hydrologie et de Géochimie de Strasbourg, (CNRS/UdS), 1 rue Blessig, 67084 Strasbourg, France
[c] Department of Geology, Thompson Hall, Kansas State University, Manhattan, KS 66506, USA

ARTICLE INFO

Article history:
Received 4 October 2013
Received in revised form 18 June 2014
Accepted 21 June 2014
Available online 9 July 2014

Keywords:
Radish seedlings
Major
Trace and rare-earth elements
Controlled laboratory experiment
Smectite-type substrate

ABSTRACT

Radish seedlings were grown for one month in a smectite substrate under controlled laboratory conditions. The purpose was avoiding as much as possible side effects such as heterogeneity of natural soils, as well as fertilizer and atmospheric supplies. One set of seedlings was watered at the beginning of the survey with plain deionized water, whereas the others were with the same water containing Cd or Pb at 0.5 and 1 mg/L concentrations, to quantify the impact of these metal pollutions on the plants. Significant changes were observed in the uptake of the seedlings watered with the contaminated solutions: Na and K uptakes decreased, whereas those of Al, Fe, Ca and Mn increased. Changes were also detected in the uptake of some rare earths and trace elements after watering with Cd or Pb contaminated solutions. The most concerning uptakes are for the pollutants Cd and Pb that increase by about 795 and 1240 times, respectively, in the contaminated seedlings, suggesting a high tolerance by the radishes for these two metals. The overall increasing elemental uptakes result from replacement of an ion exchange process during growth in the unpolluted substrate by an ion complexation process in the case of growth in the polluted substrate.

Occurrence of a significant Eu anomaly and disappearance of the Gd anomaly in the rare-earth distribution patterns of the contaminated radishes were necessarily induced by organic activity; they also explain the suggested changing uptake process. Rare-earth element distributions represent therefore an efficient tracer to evaluate how much soil pollution by a given metal potentially affects the uptake process of plants, and to postulate that some types of metal pollution, Cd and Pb here, affect specific functions of enzymes and/or of bacterial micro-organisms.

1. Introduction

Heavy metal pollution of soils has continuously increased during the recent decades due to industrial pollution, adversely affecting their micro-organic communities and becoming a serious social issue, especially in agricultural ecosystems (e.g., Atafar et al., 2010; Silver and Misra, 1988; Tyler et al., 1989). Heavy metal pollutants can easily enter the food chain if contaminated soils are used for the production of food crops. Among the most deleterious heavy metals are lead (Pb), cadmium (Cd) and silver (Ag), because of their hazardous impact on the environment (Christine, 1997), the life forms, and even more concerning on human health (e.g., Staessen et al., 1999). For instance, Cd has been shown to cause chlorosis and leaf epinasty, alteration of the chloroplast ultrastructure, inhibition of photosynthesis, and inactivation of enzymes in plants (Gurpreet et al., 2012; Hasan et al., 2008; Khan et al., 2007; Márquez-García et al., 2011; Wahid et al., 2008; Wang et al., 2009). Cadmium and Pb pollutions of soils also inhibit respiration of micro-organic populations (Hattori, 1992; Ohya et al., 1986; Verma et al., 2010), and induce nitrogen mineralization (Chang and Broadbent, 1982; Liang and Tabatabai, 1977) and nitrification (Cela and Sumner, 2002; Liang and Tabatabai, 1978; Perez-Mateos and Gonzalez-Carcedo, 1987). In some instances, they decrease the plant biomass (Kibria et al., 2009; Lux et al., 2010; Rehman et al., 2011; Sengar et al., 2008). Also to be mentioned is the intriguing fact that low and high concentrations of Cd in soils appear to have opposite effects (Chen et al., 2011; Shekar et al., 2011), which could depend on a changing tolerance of some plants to given metals (e.g., Roy and Basu, 2009).

Many studies have focused on estimating the impact of contaminants, especially metals, on soil microorganisms, while limited attention has been given to elemental accumulation in plants grown in heavy metal contaminated soils. Kibria et al. (2007) studied the uptake of Cd and Pb by radishes in three types of soils, and they found that the Cd and Pb concentrations correlated expectedly in shoots and roots with the rates of Cd and Pb applications. Also, bioaccumulation of Cd and

* Corresponding author. Tel.: +33 368 85 04 33, +33 680 01 80 49 (cell); fax: +33 368 85 04 02.
E-mail address: nclauer@unistra.fr (N. Clauer).

http://dx.doi.org/10.1016/j.clay.2014.06.029
0169-1317/

Pb was higher in the shoots than in the roots. The present work was set to explore a complementary approach dedicated to quantify the uptake of varied elements by seedlings of a similar radish species grown in a smectite substrate contaminated with Pb or Cd, under strictly controlled growth conditions.

2. Materials and methods

A laboratory experiment was designed to focus on the sole effects of Cd and Pb pollution on the elemental uptake of radish seedlings of the species *Raphanus raphanistrum* by avoiding as much as possible natural effects that are often difficult to identify and quantify. To do so, the plants were grown in a substrate consisting of a single clay mineral, watered periodically with deionized water to maintain wet-soil conditions in a laminar-flow hood at a constant temperature. Expandable smectite was chosen as substrate because it is a common mineral component of soils, able to host elements such as Na, Ca or Mg in interlayer crystallographic sites that are capable of exchanging with external mobile elements and water molecules from substrate solutions (Moore and Reynolds, 1997). Another reason for choosing smectite as a substrate was that its potential in elemental exchange does not alter its crystallographic and constitutive chemical compositions, as only the elements hosted by the mineral interlayers are available for ion exchange.

About 700 g of substrate were stored in pots of 10 cm diameter and 10 cm depth. Approximately 140 radish seeds were sprayed on the substrate of each pot, which was watered with deionized water once a week. No fertilizers were added to the substrate during the experiment. The ambient growth environment remained nearly constant during the experiment at 23 °C with a day/night rhythm of 12/12 h. Five sets of pots were prepared: the non-polluted reference pot and two times two pots with substrate that were polluted by 0.5 and 1 mg/L of Cd in a sulfate form and by the same concentrations of Pb in a nitrate form, at the initial watering of the seeds. The sulfate and nitrate forms of Cd and Pb, respectively, were chosen to test also if the speciation of the two metals had some impact on the extent of the uptake process by the seedlings. After thirty days of growth, the plants in living conditions were collected randomly in separate batches for chemical analysis, each batch consisting of about 20–25 individual seedlings from each pot. Far from “natural” conditions, the experimental conditions were set to avoid uncontrolled factors, but also to reproduce the survey if necessary; the aim being a precise evaluation of the impact of pollutants on plants potentially grown for human consumption.

A previous similar study showed that the mechanism of plant uptake changes after 30 days from particle–surface exchanges into ion-complexation by interaction with organics released by the plant roots (Semhi et al., 2012b). For that reason and to maintain the uptake process as simple as possible, it was decided to run this experiment for 30 days. The plants collected randomly at once from each pot were distributed into five separate batches, each consisting of about 20–25 plants that were processed separately. The reported chemical data represent therefore averages of five independent bulk analyses (Table 1). After harvest, the collected plants were first washed thoroughly with deionized water, cleaned in a gentle ultrasonic bath for about 10 min to remove any solid soil particle that could still adhere at the surface of the roots. After this treatment, the plants were washed again with deionized water, and some roots were selected randomly to check their cleanness of soil particles by optical microscope. Use of the ultrasonic treatment potentially had a contradictory impact: cleaning the plant roots had the advantage of removing soil particles and the disadvantage of removing potentially adsorbed chemical elements. Despite some inconvenience, the choice was for a strict analytical reproducibility that is to say for the gentle ultrasonic treatment.

Then, each batch of 20–25 plants was dried at 60 °C for 24 h and weighed carefully. The plant specimens were then ashed in a Pt crucible at about 600 °C for about 45 min. The ash was transferred into a polypropylene beaker and digested in concentrated ultra-pure HNO_3 at about 70 °C for 24 h. The solution was then evaporated slowly to dryness. To ensure complete dissolution of any remaining organic material, a few drops of $HClO_4$ were added to the dried material and the sample evaporated again to dryness by closing the top of the beaker. The final solution was prepared for analysis by dissolving the dried material with a known volume of 1 M HNO_3.

The elemental contents were determined on an Atomic Emission Spectrometer (AES; Jobin Yvon Instruments S.A., JY124) for Si, Al, Mg, Ca, Fe, Mn, Na, K and P, as well as of a few trace elements (Cu, Zn, Ba and Ag), and on an Inductively Coupled Plasma Mass Spectrometer (ICP-MS; Thermo-Electron PQ2) for most of the trace elements (Zr, Rb, Sr, Mo, Cr, Co, Ni, U, Th), and the rare-earth elements (REE). Repeated analysis of two international standards, the basalt B-EN and the glauconite GL-O, on a weekly basis provided an analytical $\pm 2\sigma$ precision of 2.5% for the major elements, 5% for the trace elements and $\pm 10\%$ for the REE. The analytical procedure of Samuel et al. (1985) was followed.

3. Results

After one month of growth, the radish seedlings attained a height of about 20 cm with leaves about 2 cm wide. Optical comparison of the growth density and the size of the seedlings showed that pollution of the substrate with Cd did not modify significantly the growth process of the radishes. Alternatively, those grown in the substrate watered with 1 mg/L Pb had poorer germination, and their leaves were covered by yellow spots, suggesting some chlorophyll depletion, at least. It could be that the leaf disease of the radish seedlings from higher Pb polluted substrate resulted from nitrate form pollution of Pb that could have been more active. Alternatively, some plants develop a tolerance to toxic metals by cell-wall binding, chelation, compartmentation of the metals in vacuoles and enrichment in leaf trichomes (Roy and Basu, 2009), which varies depending on the type of metal and plants (Joshi et al., 2011).

The elemental amounts taken up by the radish seedlings grown in the unpolluted substrate were compared to those taken up by the same radishes grown in the substrate polluted by Cd and Pb at the two concentration levels (Table 1). The ratios above unity ($r > 1$) record increased elementary uptakes under polluted conditions, whereas those below unity ($r < 1$) indicate the opposite, that is to say depleted elemental uptakes under polluted conditions.

3.1. Effect of Cd and Pb substrate pollution on major-element uptake

Comparison of the total amounts of the major elements determined in the radish seedlings grown in the substrates polluted by two concentrations of Cd and Pb shows for both significant changes relative to the contents of the same elements in the unpolluted substrate (Table 1). Surprisingly, the total uptakes for the radishes grown in the Cd polluted substrates increased by a ratio of 1.15 and 1.22, that is to say by about 15 and 22%, respectively for the two Cd concentrations. Those of the plants grown in the Pb-polluted substrates increased less by 1.12 (=12%) in the 0.5 mg/L polluted substrate, and only by 1.04 (=4%) in that polluted by 1 mg/L, which is within analytical uncertainty.

Relative to growth in the unpolluted substrate, the radish seedlings took up very high amounts of Al ($r = 28$) and Fe ($r = 12$), and high amounts of Mn ($r = 4.3$), Ca ($r = 3.0$), P ($r = 2.5$) and Mg ($r = 1.3$) at the 0.5 mg/L Cd pollution level. The amounts of the other taken up elements decreased: Si ($r = 0.5$), Na ($r = 0.9$) and K ($r = 0.8$) (Table 1). The picture remained the same at the 1-mg/L Cd pollution level, again with significant increases in Al ($r = 24$) and Fe ($r = 14$), less in Mn ($r = 4.3$), Ca ($r = 3.6$), P ($r = 2.4$) and Mg ($r = 1.3$). Sodium is the only element that remained essentially stable ($r = 1.0$). The contents of the other elements, Si ($r = 0.4$) and K ($r = 0.9$), decreased about the same as for the 0.5 mg/L Cd pollution (Fig. 1A).

In the case of the 0.5 mg/L Pb pollution, Al and Fe were taken up the most with ratios of respectively 15 and 13 (Table 1). The next elements taken up significantly are Mn ($r = 5.3$), Ca ($r = 2.8$) and P ($r = 2.5$), as