Spectrochimica Acta Part B 122 (2016) 40–45

Contents lists available at ScienceDirect

Spectrochimica Acta Part B

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

Investigation of Pb species in soils, celery and duckweed by synchrotron radiation X-ray absorption near-edge structure spectrometry☆

Liqiang Luo *, Yating Shen, Jian Liu, Yuan Zeng

National Research Center of Geoanalysis, Beijing 100037, China

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form 27 May 2016
Accepted 29 May 2016
Available online 31 May 2016

Keywords:
Pb species
Plants
Vegetables
Soils
XANES

ABSTRACT

The Pb species play a key role in its translocation in biogeochemical cycles. Soils, sediments and plants were collected from farmlands around Pb mines, and the Pb species in them was identified by X-ray absorption near-edge structure spectrometry. In soils, $Pb_5(PO_4)_3Cl$ and $Pb_3(PO_4)_2$ were detected, and in sediments, Pb-fulvic acids (FAs) complex was identified. A Pb complex with FA fragments was also detected in celery samples. We found that (1) different Pb species were present in soils and sediments; (2) the Pb species in celery, which was grown in sediments, was different from the species present in duckweed, which grew in water; and (3) a Pb-FA-like compound was present in celery roots. The newly identified Pb species, the Pb-FA-like compound, may play a key role in Pb tolerance and translocation within plants.

1. Introduction

Lead (Pb) pollution in the environment is mainly from the results of anthropogenic activities. Mining and industrial activities have led to heavy pollution in vegetable soils and irrigation system [1–3]. Pb not only affects plant growth and productivity, but also enters into the food chain, thereby poising health hazards to humans and animals [2,4]. Pb is the most toxic trace metal for plants with a toxicity threshold below 1 μM and only mercury is nearly as toxic [5]. Food consumption has been identified as the major pathway of human exposure and is considered to account for more than 90% of exposure, compared to other ways of exposure such as inhalation and dermal contact [6].

Short-term exposure to high levels of Pb can cause brain and kidney damage, and gastrointestinal distress, while long-term exposure may affect blood, liver, and the central nervous and reproductive systems [7]. Chronic low level exposure causes serious damage, in particular to the central nervous system, the vasculature and the kidneys [8]. Pb is a neurotoxicant and is especially harmful to the neurodevelopment of children; postnatal Pb exposure may be associated with a high risk of clinical attention deficit hyperactivity disorder [9]. To minimize the damage caused by Pb exposure, food, which is the primary source of Pb to people, should be kept free of Pb contamination. However, it is estimated that the Pb intake of 2–4 μg kg^{-1} body weight occurs as a results of the intake of edible plant organs in the range of 65–93% of the weekly dietary [8,10].

Although Pb has a low solubility at pH > 5 and low bioavailability, rhizosphere processes can strongly influence its bioavailability and may result in the mobilization of Pb into the food web via plant uptake [8]. The uptake of essential metal ions into roots is hypothesized to be facilitated by membrane transport proteins. However, no transporter has been directly demonstrated to accept Pb(II) ions as the substrate [8], even if such the assumption for Pb was a possibility based on the observation of amelioration of Pb toxicity by the addition of Ca (II) salts [11].

The immobilization of Pb in roots is mainly due to the complexing ability of histidine and the precipitation of Pb as a carbonate, while Pb transport to upper organs is attributed to the formation of Pb-organic acid complexes, and Pb binding in stems and leaves occurs through the formation of carboxylic and amino acid-like complexes [12]. Cell walls may play an important role in the accumulation of metals and extracellular Pb-containing grains in the outermost layer of root cells, which are predominantly composed of pyromorphite [13]. It is thought that Pb is adsorbed first to the root surface outside the roots, and then is bound in the form of phosphate or carbonate inside the plants on various surfaces as a result of the precipitation of Pb compounds [14]. Pb was found to occur in leaves predominantly in the form of cerussite [13] and was present in roots and leaves as Pb acetate. It was also present in leaves as Pb sulfate and sulfide [15]. Typical Pb—S structures were observed through research on bacteria, and the consistency of extended X-ray absorption fine structure (EXAFS) bond-length data and chemically reasonable factors suggests that only three thiolate sulfur ligands coordinate the Pb (II) [16]. Phytochelatins are the principal heavy

☆ Selected Paper from the Colloquium Spectroscopicum Internationale XXXIX (CSI 2015), Figueira da Foz, Portugal, 30 August–3 September 2015.

* Corresponding author.
E-mail address: luoliqiang@cags.ac.cn (L. Luo).

http://dx.doi.org/10.1016/j.sab.2016.05.017
0584-8547/

metal-detoxifying compounds in plants, and the phytochelating synthase-catalyzed reaction is terminated immediately after the metal ions are chelated by a phytochelatin, apoPCs [17]. In another study, Pb ions in plants were found to have two major coordinations: one with nine oxygen atoms in the first coordination shell and a second coordination with just three oxygen atoms, the authors observed no bonding to biologically important groups (e.g., —S, —N) and no precipitation (—PO_4) [18].

Original information on Pb species, such as Pb-organic ligands, and subcellular location, can become unclear by any chemical treatment, such as chemical digestion or ashing [19]. The distribution and speciation of elements in plants is important for understanding the mechanics of elemental uptake and biotransformation in plants [20]. Thus, a non-destructive and in-situ analytical technique was needed for biogeochemical researches. Synchrotron radiation X-ray absorption near-edge structure (XANES) spectroscopy is an in situ and non-destruction technique that has been successfully used to investigate species of metal(loid)s in environmental samples [21], Pb uptake in divers plant families [18], chemical speciation and cellular deposition of Pb in a fabaceous shrub [15], and accumulated metal speciation in earthworms [22]. Results of XANES analysis have also shown that Pb was bonded to fulvic and humic acids in soils [23], and bioavailable Pb was transformed to organically bound Pb after the addition of tea polyphenols [24].

In general, in plants, Pb forms complexes with PO_4^{3-}, COO^-, NO_3^-, N^- and S^- groups [18]. However, there is no conclusive evidence about the forms of Pb that occur on a cellular or sub cellular level [19]. Indeed, there is conflicting evidence about the chemical environment of Pb in plants [18]. The identification of Pb species in plants is crucial to understanding how plants tolerate Pb. Therefore, it is necessary to conduct further researches into Pb species and clarify the mechanics of Pb uptake, transportation and detoxification in plants.

The aims of this study are to (i) identify Pb species in soils, sediments and plants using XANES, (ii) compare the characteristics of the Pb species present, and (iii) evaluate the roles of Pb-organic groups in tolerance and translocation in plants and crops.

2. Experimental

Plants, soils, sediments and rhizosphere soils were sampled from two vegetable fields near two Pb—Zn mines: one in the Nanjing region (NJ), Jiangsu Province and the other in the Lanping region (LP) of Yunnan Province, China. Plant samples were carefully washed with tap water until no soil and dust were observable, and were again washed at least three times with distilled water. They were then wrapped in medical gauze and hung on ropes to be air-dried. The roots, stems and leaves were separated in the laboratory and freeze-dried. The dried plant parts were ground in a plant grinder to <74 μm, and pressed into pellets for XANES measurements. Parts of soils and sediments were dried and ground to obtain particles <74 μm and pressed into pellets. The other parts of soil samples were kept in plastic bags in the fields. After being brought to the laboratory, they were stored in refrigerators (at 4 °C) for direct XANES measurements. To investigate the original Pb species in the field soils, the loose soil samples from the LP region were randomly measured by adhering it on a 3 M tape.

The following reference materials for fitting Pb species were used: Pb nitrate, Pb acetate ($Pb(Ac)_2$), chloropyromorphite, Pb oxide (II), orthoplumbate (Pb_3O_4), Pb hydroxide ($Pb(OH)_2$), Pb carbonate, Pb sulfate, hexadecyl mercaptide Pb and Pb sulfide. A Pb solution of 62.5 ppm was added into fulvic acid with a standard culture solution (1/2 MS medium) to form a Pb-FA solution. The solution was measured and used as a reference material in the fitting of XANES. The pressed pellets of the powder reference compounds and their solutions were measured in transmission mode using ion chambers filled with argon at ambient pressure.

We carried out the XANES experiments using the 15 U1 and 14 W1 beamlines of the Shanghai Synchrotron Radiation Facility, with an electron beam energy of 3.5 Gev. A double crystal monochromator of Si (111) was applied to get an energy resolution of 2.0×10^{-4}, and a K—B mirror used in a focusing optics system to obtain an ultimate beam size of $2 \times 2\ \mu m^2$. Because the Pb concentrations in the samples were rather low, a defocused beam size of $100 \times 100\ \mu m^2$ was used for the XANES measurements of the collected samples from the two regions. X-ray fluorescence spectra were collected using a Vortex-90EX Si drift detector (SDD) in both fluorescence mode (samples) and combined fluorescence/transmission mode (standards) measurements.

A calibration of Pb L_3 edge at 13.035 keV was carried out with a Pb foil. Energy ranged from 13.005 to 13.155 keV with step size of 0.5 eV. Counting time for each step was 1 s. A Ge filter was used before the SDD to remove interference and to improve signal-to-noise ratio. An Al filter was positioned in the input pathway to attenuate the synchrotron radiation by 31.4% to keep the counting system working normally. X-ray fluorescence of Pb L_3-M_5 was measured to obtain XANES spectra while tuning the excitation energy using the Si (111) monochromator. Under ambient air pressure and room temperature conditions, measurements of XANES spectra of all samples were repeated at least twice to obtain high reproducibility and smooth spectra. After normalization of data, the averages of the XANES spectra were then fitted by least-squares linear combination fitting (LCF).

Conducting XANES analysis at very low concentrations is always a challenge. As such, it is not always possible to get information on Pb species in plants using XANES. For this study, we conducted XANES analysis only on the plants in which the Pb concentrations were high enough to produce reasonable signal-to-noise ratio and XANES spectrum. In this investigation, the Pb concentrations in the fresh vegetables grown in soils and aquatic sediments ranged from 2.61 to 6.86 μg/g, and 1.08 to 6.35 μg/g, respectively, and were therefore not suitable for Pb L_3 XANES measurements. Thus, their dried samples of celery and water-celery were ground into powder, and pressed into pellets, the Pb concentrations of which were 33.73 μg/g and 41.05 μg/g, respectively. In this way, Pb L_3 XANES spectra of celery and water-celery could be recorded by using fluorescence mode.

Principal component analysis (PCA) works by first considering the statistics variance within an experimental data set composed of a group of unknown samples, and then the target transformation (TT) offers the possibility to test which standard species are most likely part of the solution [25]. And then, LCF can be performed to fit XANES spectra of unknown samples. The approaches and successful applications of XANES spectra have been described in detail elsewhere [25–28]. In this study, we used Athena software [29] to perform spectral data mining. All data were baseline-corrected and normalized to 1 at the height of the edge jump. To make a correction of the energy shifts between different measurements, as stated in the Athena software, alignment was conducted by moving the measured data group onto an absolute energy grid defined by standards, here the Pb foil. It is necessary to use the aligned data when the different measurements were made. Using the data matrix that contained XANES spectra of all samples of all field soil and plant samples in which the elemental species were unknown and would be predicted, PCA was first performed to decompose the experimental spectral data matrix into an abstract eigen-spectra matrix and an abstract eigenvector matrix, then to determine the minimum number of significant components [25,29]. By using a few components with the highest eigenvalues, data reconstruction was performed until the required explained variance was reached, which allowed us to decide the minimum number of the principal components that described the experimental spectrum in the unknown sample with the desired level of precision. Then, TT was applied to test the reference compound if it could be described well by the principal components and could be within the dataset of the unknowns. Finally, LCF was performed to fit the experimental spectra of unknown species in the samples.