Coupling Pb and Zn bioaccessibility with sequential and HNO_3 extraction in soil from the industrial area of Volos, Greece

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Abstract The urban environment is a complicated system where various anthropogenic sources contribute to the accumulation of metals in soil, leading to potential negative environmental effects. Within this context, we performed single and sequential extractions in contaminated soil from the industrial area of Volos. The objective was to understand how Pb and Zn oral bioaccessibility was related to different pools in soil based on sequential and 0.43 M HNO_3 extractions. Pb was principally found in the reducible fraction (F2; 15-76%), followed by the residual (F4; 9-54%) and the oxidizable (F3; 6-54%), whereas the acid soluble fraction was of minor importance (F1; 2-15%). Zn fractionation was dominated by the residual fraction (20-73%), followed by the oxidizable (10-49%) and the reducible (5-38%). Significant correlations were found between Pb and Zn bioaccessibility and the sum of fractions F1, F2 and F3 (0.50 for Pb and 0.86 for Zn, $p<0.01$), indicating that the applied bioaccessibility solution preferentially targeted the mobile fraction of Pb and Zn in soil. HNO_3-extractable Pb and Zn were highly correlated to bioaccessible Pb and Zn (0.80 and 0.93 respectively, $p<0.01$), suggesting that the HNO_3 can determine the oral bioaccessibility of Pb and Zn in urban soil influenced by industrial activities.

Keywords: soil contamination, toxic elements, geochemical fractionation, bioavailability

1. Introduction

Soil contamination in urban and industrial areas is a significant environmental problem worldwide (Wong et al., 2006). In such environments, potentially toxic elements (PTEs) like Pb and Zn, have been used as tracers of anthropogenic contamination. PTEs enrichment in industrial and urban soil has been well-documented, originating from industrial activities, coal combustion and traffic emissions (Luo et al., 2012; Wei and Yang, 2010).

Risk assessment based on total PTEs content may overestimate the actual risk for the environment. These elements in soil are bound to different geochemical fractions, such as the acid soluble and exchangeable, reducible (Fe and Mn oxides), oxidizable (organically bound) and residual (bound to primary minerals), with different mobility and solubility characteristics (Rao et al., 2008). For human health risk assessment, the oral bioaccessibility of an element is defined as the fraction of the element that is soluble in the gastrointestinal environment, representing the maximum amount of contaminant that is available for absorption. In addition, the 0.43 M HNO_3 extraction has been used to extract the reactive fraction of PTEs, which are sorbed onto amorphous metal oxides (Römkens et al., 2009). Recently, it has been suggested that this specific single extraction may be used as an alternative for PTEs bioaccessibility (Rodrigues et al., 2018). Nonetheless, limited studies have focused on the relationship between PTEs in bioaccessible and reactive pools, and PTEs in different geochemical fractions.

Volos is a medium-sized city, with approximately 150,000 inhabitants. Heavy industrial activities include the operation of one cement production plant about 3 km east of the city center, and a major steel making plant, located some 20 km west of the city. In our previous studies, the soil contamination by PTEs has been addressed (Kelepertzis et al., 2020; Kelepertzis et al., 2021). However, the geochemical partitioning of PTEs has not been studied yet, neither has the evaluation of their reactive fraction. We focus on this study on Pb and Zn because these metals can be considered as indicative of local industrial activities, mostly related to the operation of the steel plant.

The objectives of this study were to (a) determine Pb and Zn fractions contributing to bioaccessible Pb and Zn in soil from the industrial area of Volos, and (b) compare Pb and Zn bioaccessibility to their reactive pools with the aim to evaluate if the 0.43 M HNO_3 extraction is an alternative for Pb and Zn oral bioaccessibility.

2. Materials and methods

2.1. Sampling and samples pre-treatment

A total number of 29 soils (0-10 cm depth) were selected from the sample data set of an earlier survey in the wider area of Volos (Kelepertzis et al., 2020). The criteria for sample selection were the total content of Pb and Zn as determined by a strong acid dissolution. These samples were located around the steel mills (n=7), the cement plant (n=10), as well as within the city core (n=12) (Fig. 1). The land use of the wider area and the rocks outcropping are shown in Fig. 1. Laboratory sample preparation included sieving through 2 mm and subsequently 100 μm nylon sieves to focus on geochemically reactive and bioaccessible particles. Details on sample collection and preparation and analytical methods for the determination of total concentrations of Pb and Zn are described in our previous publication (Kelepertzis et al., 2020).

2.2. Laboratory methods for the evaluation of Pb and Zn bioaccessibility, reactivity and geochemical fractionation

The oral bioaccessibility of Pb and Zn was determined by using a 0.4 M glycine extraction solution (adjusted to pH 1.5 with concentrated HCl), simulating the low pH conditions of the human gastric fluids (USEPA, 2013: Method 1340). Details for the analytical procedure of bioaccessibility assessment following the Simplified Bioaccessibility Extraction Test (SBET) can be found in the study of Kelepertzis et al. (2021).

The reactive forms of Pb and Zn were obtained by mixing 1 g of sample with 40 ml of a 0.43 M HNO_3 solution and shaking for 2 h at room temperature (Rodrigues et al., 2018). The extracts were separated from the solid residue by centrifugation at 3500 rpm for 10 min, and filtration through a 0.45 μm filter.

The soil samples were also subjected to the three-step sequential extraction procedure proposed by the Standards, Measurements and Testing Programme (formerly BCR) of the European Commission (Rauret et al., 1999) (known as modified BCR sequential extraction and named hereafter as BCR). The procedure includes three successive extractions with 0.11 M acetic acid, 0.5 M hydroxylammonium chloride in 0.05 M nitric acid (reducing agent) and 1 M ammonium acetate at pH 2 after digestion with 8.8 M hydrogen peroxide (oxidizing agent). An additional step was included to dissolve elements from the residue remaining after the three extraction steps. The BCR procedure is able to separate elements into geochemical fractions of acid soluble and exchangeable (F1: ion-exchange and carbonate bound), reducible (F2: Fe-Mn bound), oxidizable (F3: organically bound) and residual (F4: primary silicate bound). Details on the BCR sequential extraction method can be found elsewhere (e.g. Rao et al., 2008).

Concentrations of Pb and Zn from the 0.43 M HNO_3 and the sequential extraction procedures were determined by flame atomic spectrometer. Certified reference materials, procedural blanks and analytical duplicates were used for quality control purposes.

2.3. Statistical analysis

All variables were screened for normality of their distribution by the Shapiro-wilk test. Because of violations of normality, the Spearman rho coefficient was used to explore relationships between bioaccessible, reactive and geochemical fractions of Pb and Zn. Plotting of geochemical data was performed with OriginPro 2016 (OriginLab Corp.)

3. Results and Discussion

The percentage bioaccessible and reactive fraction was calculated as follows:

$$\%\ \text{bioaccessibility (or reactivity)} = \frac{C_{bio/react}}{C_{tot}} \times 100$$

where C_{bio} or C_{react} is the bioaccessible or reactive concentration of Pb and Zn (in mg/kg), respectively, and C_{total} is the total content of Pb and Zn (in mg/kg), determined by a strong acid (HNO_3-$HClO_4$-HF) dissolution (Kelepertzis et al., 2020).

The median bioaccessibility was 36% for Pb (range from 14% to 69%) and 37% for Zn (range from 11% to 65%) (Fig. 2a). Considering the reactivity, median Pb and Zn HNO_3-extracted ratios were 58% (range from 28% to 80%) and 37% (range from 15% to 76%), respectively (Fig. 2b). Such Zn bioaccessible and reactive ratios are comparable to those reported for the highly urbanized city of Athens (Kelepertzis and Argyraki, 2015). Nevertheless, Pb reactive and bioaccessible data for Athens soil are higher than those measured in the soil from the industrial area of Volos. Since the pH conditions during both the extraction tests are similar, the observed differences in the metal bioaccessibility and reactivity ratios should be due to the variety of geochemical forms defining the speciation of Pb and Zn in soil.

To understand how different geochemical phases contribute to Pb and Zn bioaccessibility, the soil samples were separated into 4 fractions via the sequential extraction procedure (Fig. 2c). The most important fraction of Pb based on medians was the reducible (51%), followed by the residual and the oxidizable (21% and 20%, respectively), whereas the acid soluble fraction comprised only 8% of total Pb content. In the case of Zn, the order of its fractionation was: residual (40%) > oxidizable (24%) > reducible (23%) > acid soluble (8%). The sum of fractions F1, F2 and F3 is considered to represent the potential mobile fraction of elements under natural conditions. The mobile fractions of Pb and Zn accounted for 79% (range 46 to 91%) and 60% (range 27-80%), respectively.

To explore how each Pb and Zn fraction contributed to the bioaccessible Pb and Zn, we tested the correlations between Pb and Zn in the different fractions, as well as in the mobile one, with bioaccessible Pb and Zn. For both elements, statistically significant Spearman rho correlations were found between the bioaccessible and the sum of the first three fractions of the BCR (0.50 for Pb and 0.86 for Zn, $p<0.01$) (Fig. 3), indicating that bioaccessibility (%) is governed by the mobile pools of Pb and Zn present in the soil, *i.e.* mostly bound to Fe and Mn oxides and organic matter. Interestingly, no correlations were found between the bioaccessibility of Pb and the each of the F1, F2 and F3 fractions. Regarding Zn, correlations between each Zn fraction and respective bioaccessibility exist, showing that the applied bioaccessibility solution dissolves Zn bound to the first three phases of the BCR.

Moreover, HNO_3-extractable Pb and Zn were correlated with Pb and Zn bioaccessibility (Fig. 3), with correlation coefficients being 0.80 for Pb and 0.93 for Zn ($p<0.05$). This was consistent with results published by Kelepertzis et al. (2015) and Rodrigues et al. (2013) who demonstrated strong correlations between the two methods for a considerable number of soils from Athens and Porto, respectively. One reasonable explanation for the close relationship is the similarity in the extraction pH that is close to 1 in both solutions (HNO_3: pH ~ 1, SBET: pH = 1.5) (Li et al., 2015). These results demonstrate that a single extraction with dilute HNO_3 can be used to determine the bioaccessibility of Pb and Zn in urban soil influenced by industrial emissions.

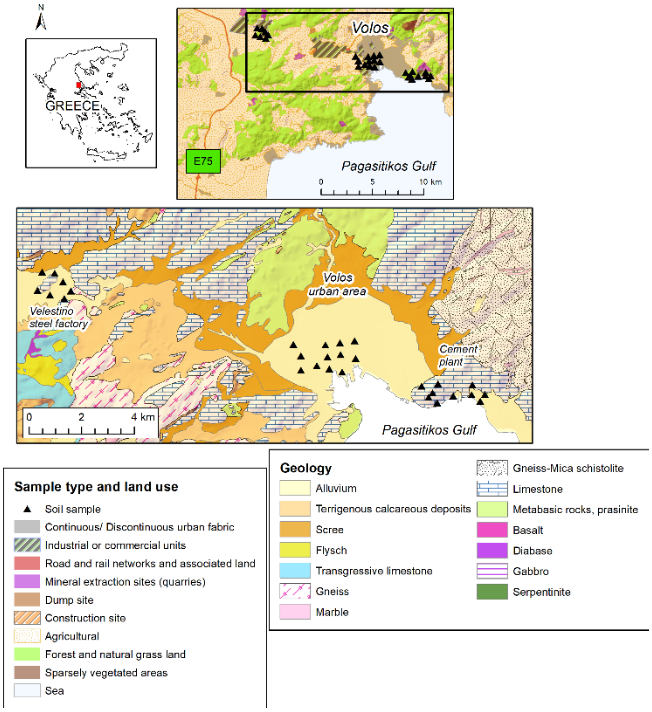

Figure 1. Sampling locations plotted on land use and geological map (Katsikatsos et al., 1978) of the study area.

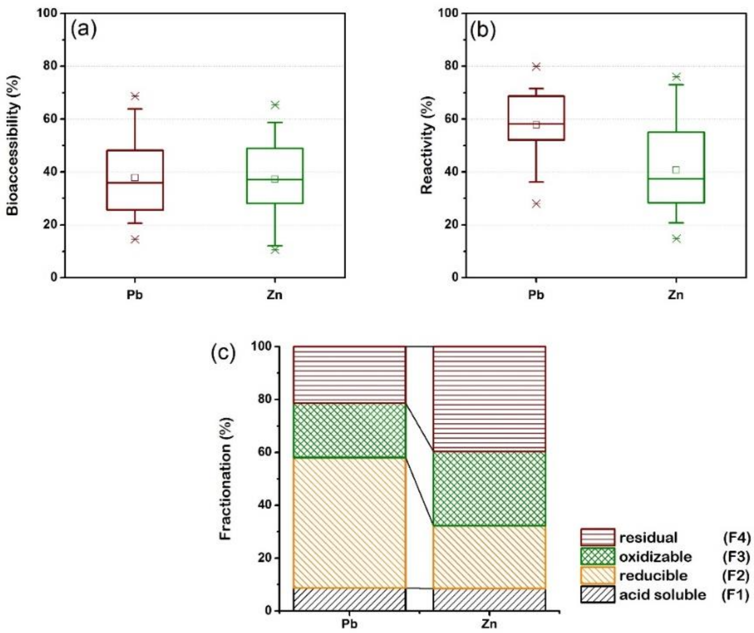

Figure 2. Geochemical results of Pb and Zn based on single and sequential extractions for the soil samples from the wider area of Volos: a) bioaccessibility (%), b) reactivity (%), c) geochemical fractionation (%)

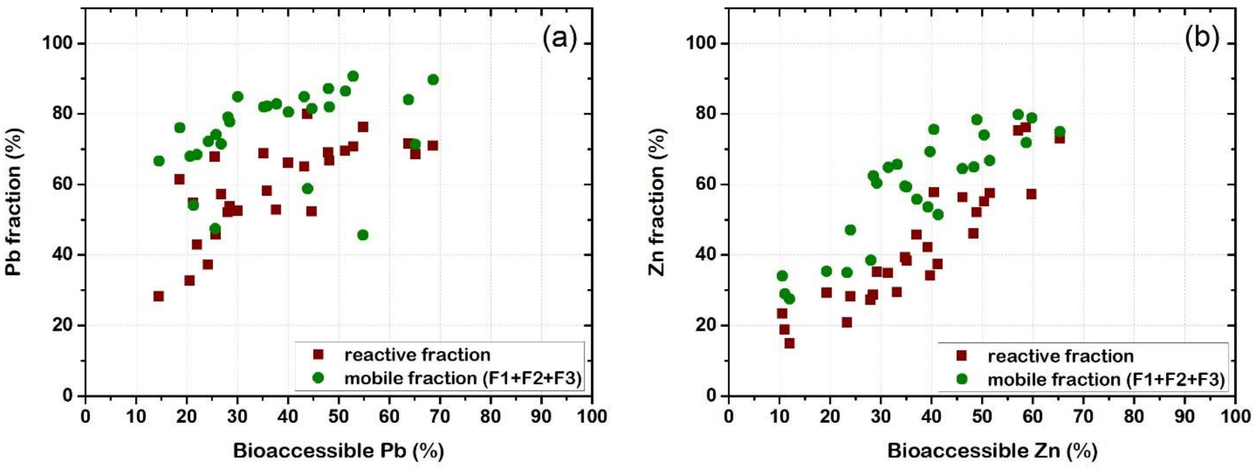

Figure 3. Relationships between bioaccessible Pb and Zn and their reactive fraction, as well as the sum of the first three fractions (F1, F2, F3) from the sequential extraction procedure

References

Katsikatsos, G., Mylonakis, J., Vidakis, M., Hecht, J., Papadheas, G., Dimou, E., Papazeti, E., Skourtsi-Koroneou, V., Hadjicostanti–Tsalachouri, I., Karamicahlou–Kavali, A. et al. 1978. Geological Map of Greece, Volos Sheet; Institute of Geology and Mineral Exploration of Greece: Athens, Greece.

Kelepertzis, E., Argyraki, A. (2015). Geochemical associations for evaluating the availability of potentially toxic harmful elements in urban soils: Lessons learnt from Athens, Greece. *Applied Geochemistry*, **59**, 6-73.

Kelepertzis, E., Argyraki, A., Chrastný, V., Botsou, F., Skordas, K., Komárek, M., Fouskas, A. (2020). Metal(loid) and isotopic tracing of Pb in soils, road and house dusts from the industrial area of Volos (central Greece). *Science of the Total Environment*, **725**, 138300.

Kelepertzis, E., Chrastný, V., Botsou, F., Sigala, E., Kypritidou, Z., Komárek, M., Skordas, K., Argyraki, A. (2021). Tracing the sources of bioaccessible metal(loids) in urban environments: A multidisciplinary approach. *Science of the Total Environment*, **771**, 144827.

Li, S-W., Li, j., Li, H-B., Naidu, R., Ma, L.Q. (2015). Arsenic bioaccessibility in contaminated soils: coupling in vitro assays with sequential and HNO3 extraction. *Journal of Hazardous Materials*, **295**, 145-152.

Luo, X-s., Yu, S., Zhu, Y-g., Li, X-d. (2012). Trace metal contamination in urban soils of China. *Science of the Total Environment*, **421-422**, 17-30.

Rao, C.R.M., Sahuquillo, A., Sanchez, J.F.L. (2008). A review of the different methods applied in environmental geochemistry for single and sequential extraction of trace elements in soils and related materials. *Water Air and Soil Pollution*, **189**, 291-333.

Rauret G, López-Sánchez JF, Sahuquillo A, Rubio R, Davidson C, Ure A, Quevauviller P. (1999). Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *Journal of Environmental Monitoring,* **1,** 57-61.

Rodrigues, S.M., Cruz, N., Coelho, C., Henriques, B., Carvalho, l., Duarte, A.C., Pereira, E., Römkens, P. F.A.M. (2013). Risk assessment of for Cd, Cu, Pb and Zn in urban soils: Chemical availability as the central concept. *Environmental Pollution*, **183**, 234-242.

Rodrigues, S.M., Cruz, N., Carvalho, L., Duarte, A.C., Pereira, E., Boim, A.G.F., Alleoni, L.R.F., Römkens, P.F.A.M. (2018). Evaluation of a single extraction test to estimate the human oral bioaccessibility of potentially toxic elements in soils: Towards more robust risk assessment. *Science of the Total Environment*, **635**, 188-202.

Römkens, P.F., Guo, H-Y., Chu, C-L., Liu, T-S., Chiang, C-F., Koopmans, G.F. (2009). Characterization of soil heavy metal pools in paddy fields in Taiwan: chemical extraction and solid-solution partitioning. *Journal of Soils and Sediments*, **9**, 216-228.

USEPA, 2013: Method 1340. In vitro bioaccessibility assay for lead in soil. SW-846 hazardous waste test methods.

Wei, B., Yang, L. (2010). A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. *Microchemical Journal*, **94**, 99-107.

Wong, C.S.C., Li, X., Thornton, I. (2006). Urban environmental geochemistry of trace metals. *Environmental Pollution*, **142**, 1-16.