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Potentially toxic elements in urban soils: size-related bioaccessibility, potentially bioavailability, and risk assessment

Yan Li

Docente guida:	**Coordinatore del Ciclo:**
Prof. Franco Ajmone Marsan	**Prof. Domenico Bosco**
Co-Supervisor	
Dott. Elio Padoan	

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Abstract

Urban soils only take 3% of the global terrestrial surface but support 54% of the world's population, a proportion that is likely to increase 66% by 2050. Urban soils are an essential part of the urban ecological environment, contributing directly and indirectly to the general quality of life.

Recently, rapid urbanization and industrialization have caused a large amount of excessive toxic chemicals emitted to the environment, which pose a threat to human health and urban environment quality. Potentially Toxic Elements (PTE) contamination in the urban environment has become one of the most major environmental issues worldwide in the last few decades, due to their high toxicity, non-degradation, bioaccumulation and bioenrichment.

In most cases, health risk assessment has been conducted considering PTE total concentrations; however, not all the element species are available for absorption and the use of total content may somewhat overestimate the risk. Recently, *in vitro* methods have been developed to evaluate the risk, which is defined as the fraction of an element that is soluble in simulated biological conditions. Meanwhile the choice of a specific particle size fraction is of crucial importance for the evaluation of PTE bioaccessibility, as different soil size fractions may exhibit different properties and composition, affecting the behaviour of pollutants in soil microenviroment.

Turin (Torino) is the third-largest city in Italy, which has a long industrial history and may represent a model for cities with historical contaminations, as the growth of the city interspersed the industrial plants in the residential and commercial area, while the peri-urban area was mostly residential and surrounded by agricultural fields.

Thirty soil sample were collected along a main road across Turin, following the direction of city expansion. *In vitro* oral and lung bioaccessibility and human health risk assessment of PTE were investigated. Lead, Cd, Cu, and Zn were the

most soluble elements in simulated gastric and lung fluids. The average bioaccessibility values suggested that PTE would more bioavailable if ingested than inhaled. A higher bioaccessibility of Pb, Cd, Zn, and Cu may reflect a higher level of anthropogenic pollution. Concerning human health risk, unacceptable non-carcinogenic risk was found through ingestion for children in some urban sited and Pb was the most hazardous elements.

The choice of the optimal size of particles for the PTE bioaccessibility estimation and human health risk assessment is critical. Many studies in recent years started investigating the behaviour of PTE in different soil size fractions but methods, soil fractions investigated and purpose of the studies have been quite dissimilar, making it hard to draw some general conclusions on PTE bioaccessibility and a possible unified method.

Our study provides an overview of the relations between PTE bioaccessibility and soil particle size fractions.

A general trend of higher PTE bioaccessibility in finer fractions was found, which is related to total concentration, element chemical form, and anthropogenic influence. However, it does not mean that the highest PTE bioaccessibility is always found in the finest size fraction, due to the considerable variation in the distribution of pollutants across soils; also, the elements may exhibit different relationships between bioaccessibility and soil particles giving the extreme heterogeneity of the urban soil characteristics.

Lead (Pb) exposure is a significant global concern due to its negative impact on neurological and cognitive development in children. Contaminated soil particles could be eroded and transferred either to the atmosphere (< 10 µm) or to streams; or they may be incidentally ingested (< 200 µm). One of the most cost-effective strategies for the long-term reduction of the risk posed by Pb is chemical immobilization through the addition of phosphorus-containing compounds, the goal of which is the transformation of soil Pb into poorly soluble Pb-phosphates, leading to a decrease of bioavailability and eco-toxicity.

To clarify the effectiveness of a P amendment in reducing the Pb mobility and bioaccessibility in different soil size fractions, an experiment was performed by adding a soluble P compound to a historically contaminated urban soil (RO), a mining soil (MI), and an uncontaminated spiked soil (SP) at different P: Pb molar ratios (2.5:1, 5:1, and 15:1).

In the < 10 μm fraction of soils, P addition reduced the bioaccessible Pb only in the SP soil at the highest dose, with little to no effect on RO and MI soils. Similarly, in the coarse fraction, Pb was immobilized only in the SP soil with all three P doses. These results were probably due to the higher stability of Pb in historically contaminated soils, where Pb dissolution is the limiting factor to the formation of insoluble Pb compounds.

The toxicity and bioaccumulation of PTE are primarily related to their bioavailable fraction, i.e. the portion of a element that actually enters a receptor and cause effects on organism. Meanwhile, the mobility and bioavailability are inextricably linked to PTE geochemical fraction. Therefore, comprehensively understanding the fractionation, mobility and bioavailability of PTE is important to accurately assess the human health risk.

Hence, geochemical fractionation, bioaccessibility, and potential bioavailability of PTE (Cd, Cu, Pb, and Zn) were investigated in selected urban soils by the combined use of sequential extraction (BCR), physiologically based extraction test (PBET, gastrointestinal bioaccessibility), Gamble’s solution (lung bioaccessibility), as well as the diffusive gradient in thin-film (DGT).

A high percentage (more than 50%) of non-residual PTE (the sum of exchangeable, reducible, and oxidisable) indicates a higher bioavailability, pointing out a serious hazard to the urban environment. The bioaccessible concentrations in gastric and lung phases were closely positively correlated with DGT-measured content ($r=0.63-0.99$, $p<0.05$), suggesting the potential use of DGT for the prediction of PTE risk to human health. In addition, the correlation of DGT-measurement with the soluble and reducible fractions of PTE suggests

that DGT could be an easy tool to predict elements quantities released from soil during intense rain episodes or flooding. Our studies provide a more accurate assessment of the urban environmental quality and be helpful for pollution control and urban planning.

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1. Introduction

1.1 PTE in urban soils

Urban soils represent only 3% of the global terrestrial surface, but support 54% of the world's population, and projections show that this proportion will increase to 66% by 2050 (United Nations, 2014). Rapid expansion of urban areas transforms more and more natural and agricultural soils (peri-urban soil) into urban soils, which may increase contamination sources and change the surrounding environment. Urban environments thus become supremely important with regard to human health and wellbeing. Consequently, the management of urban and peri-urban area will be crucially important.

In the last few decades, soil contamination from potentially toxic elements (PTE) has become of great concern worldwide and received increasing attention. Some reviews (Ajmone-Marsan and Biasioli, 2010; Wei and Yang, 2010) have shown that many cities in all over the world are contaminated by PTE, among the most hazardous pollutants in urban environment due to their high toxicity, non-degradation, bioaccumulation and bioenrichment (Ma et al., 2009; Frohne et al., 2014 ; Padoan et al., 2020a).

Urban soils are an essential component of urban ecosystems, contributimg directly or indirectly to the general quality of life of city residents (Ajmone and Biasioli 2010). Due to the complex land use and heterogenous nature of urban soils, an understanding of the the properties, processes, and ecosystem services of intensively disturbed urban soils has decisive implications for urban management.

Most studied PTE include the xenobiotic and highly toxic lead (Pb), arsenic (As), chromium (Cr), cadmium (Cd) and mercury (Hg), as well as some essential elements, such as copper (Cu), zinc (Zn), cobalt (Co), manganese (Mn) and nickel (Ni), that can become toxic when accumulated at high concentrations in soils (Alloway et al., 2013; Li et al., 2018). Studies indicate that the presence of PTE can alter soil physicochemical properties, causing a decrease of biological function and weakened engineering properties (Yang et al., 2021). Meanwhile, anecdotal evidence and empirical research have shown that most PTE are carcinogens and associated with the etiology of some diseases, such as kidney, nervous system, blood and bone diseases (Han et al., 2020b). Thus the study of contamination levels and regional variations of PTE in soils is critical for pollution control and urban planning.

PTE may occur naturally in soils, i.e., atmospheric volcanic emissions, continental dust transport, and element-rick rock weathering (Khan et al., 2021). However, additional contributions in urban areas come from anthropogenic activities such as transportation (fossil fuel combustion, attrition of tyres, petrol and engine oil leaks), industrial activities (mining, smelting and chemical engineering), fertilization, and waste disposal, leading to their build up and gradual release into the environment (Ajmone-Marsan et al., 2019; Mehta et al., 2019). Meanwhile, urban soil is generally regarded as a sink of the pollutants and the center of industry and population agglomeration, which means that, with an intensification of industrial and anthropogenic activities, the severity of PTE accumulation, and the long-time exposure to them, will raise, and so their threat to human health (Yang et al., 2021).

1.2 PTE bioaccessibility and health risk assessment

In recent years, widespread concern has arisen over the implications of human health problems from increasing PTE in soils. In most cases, health risk assessment has been conducted considering PTE total concentration; however, not all the elemental species are available for absorption and the use of total or pseudo-total contents may somewhat overestimate the risk, as already reported from many researchers (Paustenbach, 2000; Han et al., 2020; Mokhtarzadeh et al., 2020). Hence, many different *in vitro* methods have been developed and used to estimate PTE gastrointestinal and lung bioaccessibility.

The *in vitro* bioaccessibility is defined as the fraction of an element that is soluble in simulated biological conditions such as, for oral bioaccessibility, the gastric and intestinal juices (Ruby et al., 1999; Juhasz et al., 2009). The determination relies on the extraction of PTE with solutions mimicking the chemical conditions encountered in the human stomach, intestines or lung phase (Turner, 2011). These tests have been mostly developed using one or few elements, but they have been extended to various PTE for which they have not yet been demonstrated to be good analogues of *in vivo* conditions. For these reasons, methods should represent conservative cases and should be suitable for a larger set of PTE, producing repeatable and reproducible results.

Human exposure to PTE in soil can occur via ingestion, inhalation or dermal contact, and soil ingestion has been considered the main pathway, especially for children, since they might ingest soil accidentally through mouthing dirty hands and objects, eating dropped food, or consuming soil directly (Van der Kallen et al., 2020). The most used methods to study the soil ingestion route include the Solubility/Bioavailability Research Consortium method (SBRC), the *in vitro* gastrointestinal method (IVG), the Physiologically Based Extraction Test (PBET), the Deutsches Institutfür Normunge V method (DIN), and the Unified BARGE Method (UBM) which is based partly on the RIVM (the in vitro Digestion Model) method (Ruby et al., 1996; Rodriguez et al., 1999; Oomen et al., 2002; Drexler and Brattin, 2007; Juhasz et al., 2009; Wragg et al., 2011). The differences between these physiologically driven methods are mostly variations in the gastrointestinal fluid composition and extraction parameters as the incubation time in the gastric and small intestinal phases or the solution pH. For example, in the gastric phase, the soil residence time is 1 h for PBET, SBRC, UBM, and IVG, but 2 h for DIN. In the small intestinal phase, the extraction time is 1 h for IVG, 2 h for UBM, 4 h for PBET and, 6 h for DIN (Li et al., 2020a). The different incubation times clearly results in different outcomes of *in vitro* bioaccessibility.

The second most important route for PTE interaction with urban population is inhalation, which involves the soil fine size fractions (e.g. particles <10 μm), as they are easily resuspended by anthropogenic activities and wind erosion. Moreover, PTE in fine particles may easily enter the nasal cavity and lungs through inhalation (Kastury et al., 2018; Li et al., 2020a). Currently, there are three types of extraction techniques for simulated human lung fluids, including artificial lysosomal fluid (ALF) (Midander et al., 2007), simulated lung fluids (SLF) (Taunton et al., 2010), and the more common surrogate fluid named Gamble's solution (GS) (Wragg and Klinck, 2007). The differences between these methods are in compositions of the fluids and in conditions of the *in vitro* methods, including extraction time, pH, sorption sinks, solid to liquid (S/L) ratio, and agitation (Ren et al., 2020). Until now, no unified analytical protocol for the determination of lung bioaccessibility has been adopted due to lack validation via *in vivo*-*in vitro* correlation, and this poses many challenges for methodologies comparison (Ren et al., 2020). Recently, a study (Zhong et al., 2020) obtained a good *in vitro*-*in vivo* correlation using optimized Gamble's solution (Wragg and Klinck, 2007). The method showed good performance for the prediction of lung bioaccessible PTE and has been proposed for human exposure assessment.

Human health risk assessment model, developed by the United States Environmental Protection Agency (USEPA), provides information on hazards posed to humans by a given substance by estimating the potential health risks.

The risk assessment is a multistep that comprises the following: toxicity identification, hazard identification, exposure parameters, exposure assessment, and risk characterization. Exposure of humans to PTE in soils can be categorized into three pathways: inadvertent oral ingestion, dermal contact, and inhalation. The non-carcinogenic risk (HQ) and carinogenic risk (CR) of PTE are assessed based on the guidelines and Exposure Factors Handbook (USEPA, 1989, 1997, 2002).

1.3 Soil size fractions and PTE bioaccessibility

It is widely known that PTE bioaccessibility is strongly dependent from soil characteristics, such as pH, organic matter and Fe, Al and Mn oxides (Petruzzelli et al., 2020). For instance, previous studies have shown that the bioaccessibility of several PTE in soils was significantly correlated with the total element content and soil texture (Cai et al., 2016). An increase in pH will increase the number of negative sites for cation adsorption, decreasing the bioaccessibility of Pb, Cd and Ni (Li et al., 2003). Soil organic matter (SOM), as well as Fe, Al, and Mn oxides, can increase the stability of PTE in soils via inner-sphere complexation reactions, leading to a decrease in bioaccessibility, while SOM could also enhance the dissolution of PTE due to complexation from acid functional groups of low molecular weight SOM components (Palmer et al., 2014; Cai et al., 2017; Yin et al., 2015).

However, the choice of a specific particle size fraction is of crucial importance for the evaluation of PTE bioaccessibility, because smaller sizes have a higher likehood of adhering to children's hands and children are one of the highest risk recepoters. Smaller sizes are also more soluble and more efficiently adsorbed into human tissues that coarse fractions (Yutong et al., 2015). The particle size distribution of soil and its associated PTE content are of primary importance while assessing the potential impact of the contaminant on human health, as different soil particles may exhibit different properties and compositions, influencing PTE behaviour in soil. Various studies have proven in recent years that fine particles (e.g. < 10 um) have higher PTE concentrations than coarser ones due to their large surface area, higher clay minerals and organic matter and to the presence of Fe, Al and Mn odixes, promoting the PTE accumulating owing to adsorption, physical occlusion and complexation processes (Luo et al., 2011; Liu et al., 2018).

In previous studies, different research groups analyzed different soil size fractions to investigate the potential health risk due to PTE exposure. For example, the unified bioaccessibility method, developed by the Bioaccessibility

Research Group in Europe (BARGE), recommends soil sieved at < 250 µm size fraction for the assessment of ingestion bioaccessibility, because of its likely representation of the soil particles that would be expected to adhere to children's hands (Ruby and Lowney, 2012). More recently, the Technical Review Workgroup (TRW) of the US Environmental Protection Agency (USEPA) reviewed recent studies on soil adherence to hands, summarizing the relationship between particle size and adherence, endorsing the use of the < 150 µm size fraction for human health risk assessment from soil exposure (USEPA, 2016). From a former study (Ikegami et al., 2014), emerged that using this fraction would allow to consider, on average, the 98% of particles adhering to children's hands. However, other researchers pointed out that finer particles (e.g., < 63 µm or < 100 µm) are more likely transferred into the human body, thus the use of a coarser fraction may underestimate the risk due to direct soil intake (Yamamoto et al, 2006; Siciliano et al., 2009). Thus, the health risk assessment may be inaccurate, especially when the coarse and fine particles differ significantly in PTE content.

Many studies in recent years started investigating the behaviour of PTE in different soil size fractions but methods, soil investigated fractions and purposes of the studies have been quite dissimilar, making it hard to draw some general conclusions on PTE bioaccessibility and a possible unified method. Up to now, research groups have analysed *in vitro* bioaccessibility in different soil size fractions, however they may not be comparable as no *in vivo* data was obtained in different soil fractions. Therefore, there is a need to provide a review of the relations between PTE bioaccessibility and soil particle size fractions, then to further assess the health risks to human exposure to PTE across different soil particle sizes, and to determine an optimal soil particle size for accurate health risk assessment.

1.4 Bioaccessibility of Pb amended with phosphate

Concern about potential risks from Pb in urban soils have been widely expressed because of the history of soil contamination from numerous anthropogenic activities, including Pb-based paint, gasoline with tetraethyl Pb, coal burning, mining, and smelting (Ajmone-Marsan and Biasioli, 2010). The chemical speciation, structure and reactivity of Pb within these sources are widely variable and exhibits substantial differences in availability and toxicity. However, urban soils can contain all of these sources, and often have several Pb sources within a given location (Haque et al., 2021).

Today, much of modern soil Pb exposure is thought to result from the incidental ingestion and inhalation of resuspended fine soil particles (Kastury et al., 2019; Zahran et al., 2013), as Pb particle size distribution influences its mobility and transfer through ecosystems. Fine Pb-carrying particles (i.e. those < 10 µm in diameter) can be easily detached from soil by erosion and anthropogenic activities and they can then enter the respiratory system (Kastury et al., 2018; Li et al., 2020b) or be transferred to a water body where Pb may become more available. Meanwhile, coarser particles (e.g., < 150 µm) could more likely be ingested, especially by children during their frequent hand-to-mouth activities (Ruby and Lowney, 2012).

In response to the rising Pb contamination levels around the world, substantial studies have been conducted to develop effective remediation techniques in the last decades (Gong et al., 2018). To date, many soil remediation approaches have been tested to reduce the negative impacts of Pb contamination effectively, such as soil washing, immobilization, bioremediation, and phytoremediation (Xu et al., 2021). One of the most cost-effective and convenient remediation strategies for the long-term reduction of the risk posed by Pb is chemical immobilization through the addition of phosphorus-containing compounds, the goal of which is the transformation of labile Pb into poorly soluble Pb-phosphates (i.e., pyromorphite or tertiary Pb-phosphate), leading to a decrease of the element mobility and bioaccessibility (Scheckel et al., 2013). Available examples of field application of P report a variety of P concentrations and addition methods but were, in general, applied to highly contaminated soils.

The amount and type of P best added to moderately contaminated soils is ill-defined (Obrycki et al., 2017). Amendments from 4:1 to 15:1 P: Pb molar ratio have been proposed as effective in reducing bioaccessible Pb (Ryan et al., 2004); however, parameters for P addition vary within sites, as very large P quantities and acidic P amendments, such as phosphoric acid, could pose their own significant environmental concerns including P runoff, possible eutrophication and the possibility of an enhancement leaching of oxyanions such as arsenate (As^{5+}) (Dermatas et al., 2008; Weber et al., 2015). Furthermore, in urban areas the use of acid treatments may not be allowed by municipal governments, due to low public acceptance.

The effectiveness of immobilization of Pb largely depends on the element chemical forms or binding ability, which are governed by soil properties and environmental processes (Palansooriya et al., 2020), such as soil pH, organic matter, soil clay mineral and metal oxide type and concentration.

However, soil presents high spatial heterogeneity in organic matter, iron-bearing minerals, and bacterial composition among different size fraction (Zou et al., 2022), which can affect the mobility and bioaccessibility of Pb, and prevent the formation of insoluble Pb-P compounds. Thus, an important research gap is the assessment of this method’s immobilization efficacy on particles removable through wind or water erosion (< 10 µm) or relevant for incidental ingestion. This necessity is more evident in areas of diffuse, moderate contamination, as in most cases these are close to populations and not managed. Here, soil particles can freely spread to adjacent areas and increase health risk.

1.5 Diffusive gradient in thin films (DGT)

It is well known that the toxicity and bioaccumulation of PTE are primarily related to their bioavailable fraction, the portion of an element that enters a receptor and cause effect on the organism (Naidu et al., 2008), rather than their total concentration (Paustenbach et al., 2000; Padoan et al., 2017; Han et al., 2020). Recent researches have shown that the mobility and bioavailability of PTE in soils are inextricably associated with their geochemical fractionation (Khadhar et al., 2020; Kim et al., 2014). Given the complexity of urban soil properties, the widespread PTE presence in the urban environment and the proximity of people to urban soils, a knowledge of PTE mobility and bioavailability is as important aspect of environment evaluation and is essential for the potential threats for human health.

Until now, *in vivo* experiments (research done with living organism) remain the most reliable methods to assess the bioavailability of PTE in soil; however, these methods tend to be time-consuming and costly in addition to animal ethic issues (Sun et al., 2021). In the last years, a reliable and easy-to-use technique, the diffusive gradients in thin films (DGT), has been developed and widely used globally (Davison and Zhang, 1994).

The DGT technique provides time-integrated measurement of the elemental fractions more relevant for assessing potential bioavailability, such as PTE dissolved in pore water, and labile elements that are readily released from solid phase of the sediment and soil due to weak binding (Amato et al., 2018), and these fractions could be absorbed by organisms, plants, and realistically reflecting PTE risks to human health.

Numerous studies have reported that DGT measurement provides a reliable estimate of PTE uptake by plants (e.g. Tandy et al., 2011; Wen et al., 2020) and, in addition, DGT might also serve in PTE bioaccessibility studies (Camusso and Gasparella, 2006; Zhang and Davison, 2015). For instance, with 18 paddy soils under field condition, Tian et al. (2008) found that DGT-measured element concentrations best predicated the concentrations of Cd ($r^2 = 0.99$, $p < 0.001$), Cu ($r^2 = 0.71$, $p < 0.001$), Pb ($r^2 = 0.56$, $p < 0.001$), and Zn ($r^2 = 0.64$, $p < 0.001$) in rice grain. The results from Bade et al. (2012) showed that DGT measurement can predict Pb, Cu, and Zn bioavailability to earthworms in industrial-contaminated soils. Roulier et al. (2010) also found a significant correlation ($r^2 = 0.89$) between DGT-measured and total concentrations of Cu and Pb in *Chironomus riparius* in freshwater sediments. Further, the application of DGT to assess the bioavailability and potential human health risk in soils has received great attention, and it has been revealed that DGT technique is effective in prediction of the PTE bioavailability in contaminated soils and sediments (Bade et al., 2012; Ren et al., 2015). More recently, the human health risk due to PTE in urban park soils has been assessed by using the DGT method (Sun et al., 2021), while no studies addressed a range of urban soils contaminated from different anthropic activities.

2. Objectives

The main objective of this thesis is to investigate the distribution, size-related bioaccessibility, potentially bioavailability, and health risk of PTE in urban soils.

The first experimental part of this thesis (Chapter 3) focused on the bioaccesibility of PTE in the particle size-associated fractions in the urban and peri-urban area. As previous studies have reported the concentration and sources of PTE, this work highlights the health hazards due to carcinogenic and non-carcinogenic elements via combined ingestion and inhalation pathways exposure based on bioaccessibility data.

As previously mentioned, there was a need to understand the influence of different soil size fractions on PTE bioaccessibility, therefore, a review (Chapter 4) was carried out to compare the most commonly used methods to estimate PTE bioaccessibility in soil particles and to identify the optimal size for soil PTE bioaccessibility and risk assessment.

The second experimental work of this thesis attempted to clarify the effectiveness of a P amendment (KH_2PO_4) in reducing Pb mobility and bioaccessibility in different soil size fractions. In the article addressed in Chapter 5 we used three different soils representative of Pb contamination cases to evaluate the fate of Pb in different soil size fractions, representative of the inhalable (< 10 µm) and ingestible (< 200 µm) portion, when P is added as an amendment.

Finally, the third experiment combined BCR, *in vitro* oral and lung bioaccessibility, and DGT methods to examine the geochemical fractionation, the mobility, bioaccessibility, and the potential bioavailability of PTE. The aim was to explore the correlation for different methods to predict the bioavailability of PTE in urban soils and to provide a more accurate assessment of the urban environmental quality.

3. Health risk assessment via ingestion and inhalation of soil PTE of an urban area

Li Yan[a*], Ajmone-Marsan Franco[a], Padoan Elio[a]

[a] University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco, 10095, Italy

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Abstract

Potentially Toxic Elements (PTE) are common soil contaminants and pose a significant risk to human health. In this study, ingestion (< 150 μm) and inhalation (< 10 μm) bioaccessibility and human health risk due to PTE were investigated in soils of the urban and peri-urban area of Torino. Lead, Cd, Cu, and Zn were observed to be the most soluble elements in simulated gastric and lung fluids. Higher bioaccessible concentrations of Pb, Ni, Co and Sb were observed in the inhalable size fraction (< 10 μm) compared to ingestible one probably because of the higher concentration in fine soil size fraction. Conversely, the relative bioaccessibility of Pb, Cu, Cd, Zn and As was lower, due to the different extracting conditions and to the presumable different elemental speciation. Average values suggested that PTE would be more bioavailable if ingested than inhaled, particularly in urban areas, were the bioaccessible percentages were always higher than in peri-urban sites. Health risk assessment was conducted using bioaccessible concentrations and their corresponding toxicities via ingestion and inhalation exposures. Unacceptable non-carcinogenic risk (HQ > 1) was found through ingestion exposure for children in some urban sites and Pb was the most hazardous element. Carcinogenic risks were under the threshold levels for every soil ($CR < 10^{-4}$), with Cr and As being the dominant contributors to risk. Therefore, necessary soil remediation activities are needed to reduce the risks of human, especially for children, exposure to Pb.

3.1 Introduction

Rapid industrialization and expansion of urban areas lead to the entrance of numerous Potentially Toxic Elements (PTE) to soil (Kabata Pendias, 2010; Ajmone-Marsan and Biasioli, 2010). As PTE tend to accumulate in soils, in cities people exposure to contaminated soils can pose significant human health risk, due mainly to the routes connected to oral ingestion and inhalation (Manjon et al., 2020; Marini et al., 2021). In most cases, health risk assessment has been conducted considering PTE total concentrations; however, not all the elemental species are available for absorption and the use of total or pseudo-total contents may somewhat overestimate the risk, as already reported from many researchers (Paustenbach, 2000; Han et al., 2020a; Mokhtarzadeh et al., 2020). In recent years, different *in vitro* methods have been used for estimating the PTE gastrointestinal bioaccessibility, especially the Simple Bioaccessibility Extraction Test (SBET), which has been widely applied for human health risk assessment (Oomen et al., 2002; Li et al., 2020c).

To correctly estimate the risk due to ingestion, in addition to the SBET, or similar extraction methods, studies need to analyse the bioaccessibility only on the potentially ingestible fraction of soil (i.e. the fraction of soil < 150 µm) (Li et al., 2021b).

The second most important route for PTE interaction with urban population is inhalation, which involves the soil fine size fractions (i.e. particles < 10 µm), as they are easily resuspended by anthropogenic activities and wind erosion. Thus, PTE in fine particles may easily enter the nasal cavity and lungs through inhalation (Kastury et al., 2018; Li et al., 2020c). Until now, no unified analytical protocol for the determination of lung bioaccessibility has been adopted, and this poses many challenges for methodologies comparison (Ren et al., 2020). Recently, a new study (Zhong et al., 2020) obtained a good in vitro-in vivo correlation using optimized Gamble solution (Wragg and Klinck, 2007). The method showed good performance for the prediction of lung bioaccessible PTE and has been proposed for human exposure assessment.

Turin is the third-largest city in Italy, which has a long industrial history and may represent a model for cities with historical contaminations, as the industrial activities were concentrated in the city centre while the peri-urban area was mostly residential and surrounded by agricultural fields. Previous studies in this area evidenced this difference between the urban and the peri-urban area (Biasioli et al., 2006; Padoan et al., 2017), however few studies were carried out to the bioaccessibility of PTE in the particle size-associated fractions (Padoan et al., 2017; Pelfrêne and Douay, 2018) and to assess the health risk via the combined ingestion and inhalation pathways, essential to determine the exposure risk. Therefore, the objectives of this study are: (1) to investigate the concentration and distribution of PTE in soils of the urban and peri-urban areas; (2) to assess the gastrointestinal and lung bioaccessibility of PTE; (3) to estimate health hazards due to non-carcinogenic and carcinogenic elements via ingestion and inhalation exposure based on bioaccessibility data.

3.2 Materials and methods

3.2.1 Study area

The metropolitan area of Turin (45°04′ N; 7°41′ E) lies on an alluvial plain in the Piemonte region, in north-west Italy, and has a population of 1.7 million inhabitants. It features a very large amount of vehicular traffic and has a long history of industry, primarily car-manufacturing factories, and metallurgical industries (Padoan et al., 2017).

Soil sampling sites were selected along a main road across the city, on a South-North directory, beginning and ending in the peri-urban area (Figure 3.1). Sites in the peri-urban area (n=10) were surrounded by agricultural fields and sites in the urban area (n=20) were distributed on roadsides and parks.

3.2.2 Soil sampling

Samples collection was conducted in January and May 2020, a total of 30 topsoil samples were collected from the study area. Each sample was taken at a 0-10 cm depth and a composite soil sample at each site was obtained by mixing three sub-samples at a distance of 1 m away from each other. The collected samples were put in plastic bags and homogenized. All samples were air dried in laboratory at room temperature and sieved through a 2 mm plastic sieve to remove stones, plant, and anthropic fragments (plastic, glass, metallic, etc.) before further analyses.

3.2.3 Sample characterization

The pH of soil samples was measured in 1:2.5 soil/water suspensions by using a pH meter with a combined glass electrode, total carbon (TC) and total nitrogen (TN) were measured by an element analyser (CE Instruments, NA2100 Elemental Analyzer, ISO 10694), carbonates were analysed by volumetric method (ISO 10693). Particle size distribution and fraction below 10 μm were measured and collected via the hydrometer method (Padoan et al., 2017). Soil digestion and measurement of pseudo-total PTE were carried out according to

Ajmone-Marsan et al. (2019). A portion of each sample was crushed to pass through 0.15 mm sieves, 1.00 g soil sample was weighed and microwave-digested with *aqua regia* (HCl/HNO_3, 3:1 v/v, Milestone Ethos D, ISO 11466)) and then determined by inductively coupled plasma mass spectrometry (ICP-MS, Perkin Elmer NexION® 350D). All the determinations were carried out in triplicate. Accuracy was checked against a certified reference material for *aqua regia* extractable elements in calcareous soil (CRM 141R).

Along with concentrations in bulk soil (< 2 mm), pseudo-total PTE concentrations were analysed in the < 10 µm and in the < 150 µm fraction using the same procedure.

3.2.4 In vitro gastric and lung bioaccessibility

The gastric bioaccessibility of the elements was determined using the SBET method (Ruby et al., 1999; Oomen et al., 2002). In brief, soil samples sieved at 0.15 mm were used; 0.5 g of sample was weighed and mixed with 50 mL of a 0.4 M glycine solution with pH adjusted to 1.5 by concentrated HCl. The mixture was shaken at 150 rpm, incubated at 37°C for 1 h and then centrifuged at 3000 rpm for 10 minutes, the supernatant was taken and filtered through a 0.45 µm cellulose filter prior to the analysis.

The lung bioaccessibility test was performed using the optimized Gamble Solution (the chemical composition of the solution presented in Appenxid Table S1). Briefly, < 10 µm soil samples were weighed accurately into labelled 50 mL tubes and mixed with solution at a solid: solution ratio of 1:1000; the mixture was then shaken at 37 °C for 24 h. After oscillation, the extracts were centrifuged at 3000 rpm for 10 minutes; the supernatant was taken and filtered through a 0.45 µm cellulose filter. All extraction solutions were freshly prepared, and all the determinations were carried out in triplicate, the extractant was analysed by ICP-MS.

The bioaccessibility was calculated as follows (Du et al., 2020):

$$\text{Bioaccessibility (\%)} = (C_{\textit{in vitro}}/C_{\text{total}}) \times 100$$

Where $C_{in\ vitro}$ is the bioaccessible concentration of PTE as determined using the *in vitro* extraction, and C_{total} is the *aqua regia* concentration in the considered soil fraction.

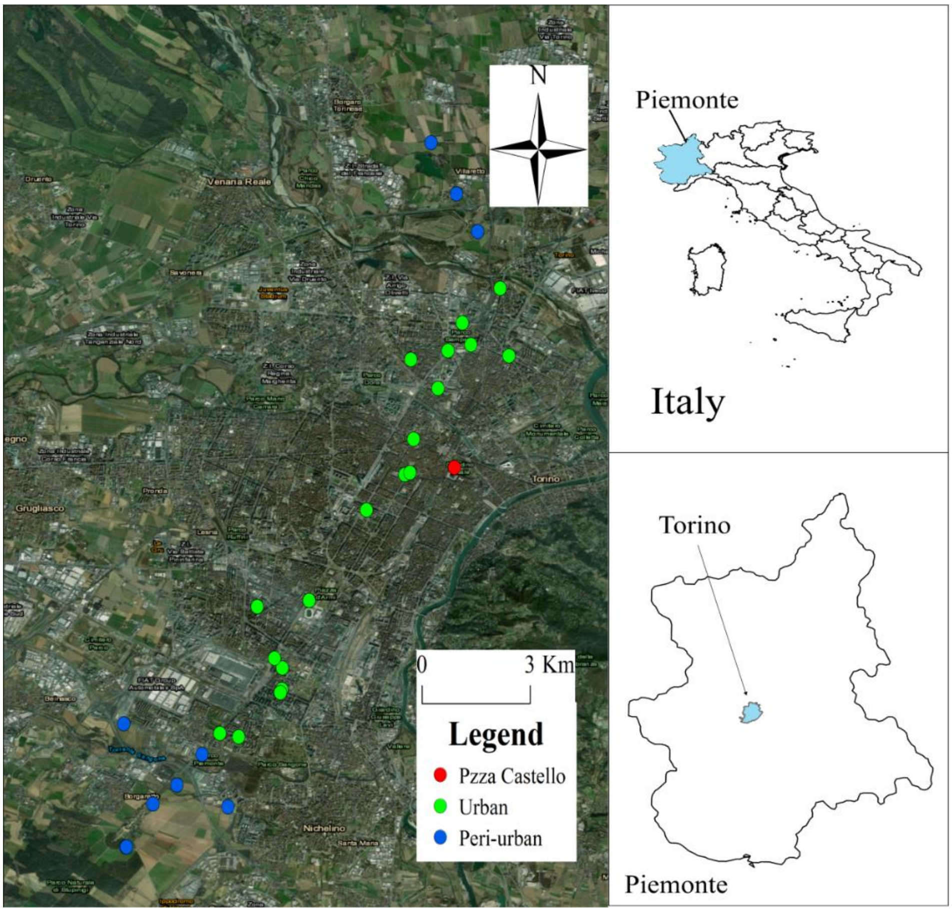


Figure 3.1. Sampling sites and map of the study area in Turin, Italy.

3.2.5 Human health risk assessment

The non-carcinogenic risk (hazard quotients; HQ) and carcinogenic risk (CR) proposed by US Environmental Protection Agency (USEPA, 2004), have been widely used to quantify the risk of people exposure to PTE contaminated soils. Exposure of humans to PTE in soils can be categorized into three pathways: inadvertent oral ingestion, dermal contact, and inhalation (Paustenbach, 2000). Based on the guidelines and Exposure Factors Handbook (USEPA, 1989, 1997, 2002), chemical daily intake (ADD, mg/kg/day) of PTE through different pathways from soil were calculated using the following equations (1) - (2).

$$\text{ADD}_{\text{ing}}=C_{\text{(Gastric)}}\times \frac{IngR\times EF\times ED}{BW\times AT}\times 10^{-6} \qquad (1)$$

$$\text{ADD}_{\text{inh}}=C_{\text{(Pulmonary)}}\times \frac{InhR\times EF\times ED}{PEF\times BW\times AT} \qquad (2)$$

Where ADD_{ing}, ADD_{inh} are the daily amount of elemental intake via ingestion and inhalation. $C_{\text{(Gastric)}}$ and $C_{\text{(Pulmonary)}}$ are the bioaccessible concentration (mg/kg) in simulated gastric and lung fluids. Other parameters are given in Table 3.1.

The hazard quotients (HQ, Eq. (3)) and the hazard index (HI, Eq. (4)) were used to characterize the non-carcinogenic hazard.

$$HQ_i = \frac{ADD_i}{RfD_i} \qquad (3)$$

$$HI = \sum HQ_i = \sum \frac{ADD_i}{RfD_i} \qquad (4)$$

Where RfDi is the reference dose of the specific element (mg/kg/day). When HQ or HI < 1, it indicates that no potential non-carcinogenic risk for humans, and HQ > 1 or HI >1 indicates adverse health effects (USEPA, 2011).

Carcinogenic risk (CR) was calculated using the dose of PTE multiply the corresponding slope factor (Eq. (5)) and it was assumed that all the element risks were additive (Li et al., 2014b; Luo et al., 2012).

$$CR = ADD_i \times SF_i \qquad (5)$$

$$\text{TCR} = \sum CR \qquad (6)$$

Where SF is the slope factor of carcinogenicity (mg/kg/day). When $10^{-6} < CR < 10^{-4}$ is considered acceptable (USEPA, 2011), while $CR > 10^{-4}$ means a carcinogenic risk to human health (Li et al., 2014b; Guney et al., 2010; USEPA, 1989). The values of RfD and SF for different PTE are shown in Table 3.2.

Table 3.1 Definition and reference value of some parameters for health risk assessment of PTE in soils.

Parameters	Definition	Units	Values		Reference
			Adult	Child	
IngR	Soil ingestion rate	mg/day	100	200	US DOE (2011)
EF	Exposure frequency	day/year	350	350	US EPA (2002)
ED	Exposure duration	year	24	6	US DOE (2011)
BW	Body weight	kg	70	15	US EPA (2002)
AT	Average time	day	365×ED (non-carcinogen)/ /365×70 (carcinogen)		US EPA (2002)
InhR	Soil inhalation rate	m^3/day	20	7.5	US DOE (2011)
PEF	Soil to air particulate emission factor	m^3/kg	1.36×10^9	1.36×10^9	US EPA (2002)

Table 3.2 Summary of reference does (RfD) and slope factor (SF) of different PTE.

Metals	RfD (mg/kg/day)		SF (mg/kg•day)	
	Ingestion	Inhalation	Ingestion	Inhalation
Cd	$1.0\cdot10^{-03}$	$1.0\cdot10^{-02}$		6.3
Cr	$3.0\cdot10^{-03}$[a]	$2.86\cdot10^{-05}$	$5.01\cdot10^{-01}$	$4.2\cdot10^{-01}$
Ni	$2.0\cdot10^{-02}$	$2.0\cdot10^{-02}$		
Zn	$3.0\cdot10^{-01}$	$3.0\cdot10^{-01}$		
Cu	$4.0\cdot10^{-02}$	$4.0\cdot10^{-02}$		
Pb	$3.5\cdot10^{-03}$[b]	$3.5\cdot10^{-02}$	$8.5\cdot10^{-03}$	$4.2\cdot10^{-02}$[c]
As	$3.0\cdot10^{-02}$	$3.0\cdot10^{-03}$	1.5	$4.3\cdot10^{-03}$
References	USDOE, 2011	USDOE, 2011	Adimalla, 2020	Adimalla, 2020

a USEPA (2002)

b WHO (1993)

c Wang et al. (2020)

3.2.6 Statistical analysis

Data processing and statistical analysis were conducted with Microsoft Excel 2010 and Origin 8.0.

3.3 Results and discussion

3.3.1 Physicochemical properties of soils

In Table 3.3, the mean values of the soil physicochemical properties in the urban and peri-urban area are presented. Soils pH in the peri-urban area (agricultural soils) were slightly acidic, however, urban soils were neutral to alkaline, consistently with previous studies highlighting this difference, which may be due to the historical inclusion of extraneous materials (Biasioli et al., 2006). The sand content (50 µm - 2 mm) was almost constant in all samples, with a mean value of 65%. Total carbon (TC) and carbonates content in the urban area were, on average, higher than in peri-urban areas, with carbonate content in line with differences in pH. The higher TC was probably due to the sampling areas, as most of the urban area soils were covered by grass or trees, with a possible variable but low contribution from exogenous organic pollutants such as hydrocarbons or plastics.

The descriptive statistics summary of PTE concentrations in samples is presented in Table 3.4. The mean and median concentrations of all the elements (except As) were higher in the urban area than in peri-urban locations. The concentration of all the elements were higher than the average values of European and world soils (Kabata-Pendias 2010) in both peri-urban and urban areas. Compared to a previous study (Padoan et al., 2017), peri-urban concentrations were lower, while some elements in urban area, such as Cd and Ni, were a little higher.

Little can be said about the spatial trends within the city, as the variability of the distribution of PTE within an urban area is exceedingly high (Ajmone and Biasioli 2010).

Considering only the transect, PTE presented a higher pollution degree in the middle of the city, near the historical centre, and lower concentrations at the edge of the city, in the peri-urban area (Appendix. Figure S1). High concentration of Ni was documented in a roadside park, while Cr presented no obvious polluting sources, confirming that Cr and Ni concentrations in soils were primarily controlled by parent materials (Ajmone-Marsan et al., 2008).

Copper and Zn had similar spatial distributions, indicating that they may originate from the same source. The highest concentrations were found in the central and northern part of the city, coherently with previous studies indicating Cu and Zn as mainly originating from vehicle factories and traffic (Grigoratos and Martini, 2015). Two Pb hotspots were located in the north of the study area, near two gas stations, and in trafficked sites; thus, the high concentrations may derive from fuel leakage or diffuse contamination. Antimony, also, was concentrated in the northern part of the city, which is the oldest industrialized area (Appendix. Figure S2).

These few hints, together with the results of previous studies (Biasioli et al., 2006), suggest the use of distance patterns (e.g. from the city centre) in place of a systematic sampling to evaluate the effects of a city on the transportation and disposal of pollutants within its boundaries giving the heterogeneity of human activities contribution to the content of PTE in urban soils.

Table 3.3 Descriptive statistics of the peri-urban and urban area soil properties.

	pH	TN	TC	Carbonates	Particle size distribution (mass %)				
		%	%	%	< 2 µm	2-20 µm	20-50 µm	50-200 µm	> 200 µm
					<u>Peri-urban area</u>				
Mean	6.5	0.35	3.10	0.76	4.3	19.2	13.2	43.5	19.9
Median	6.3	0.39	3.41	0.65	3.9	19.8	13.5	45.4	21.0
Max	8.0	0.59	5.39	1.77	7.9	26.5	16.8	51.7	31.5
Min	5.7	0.10	0.75	0.33	1.2	11.7	8.9	32.2	7.9
Std.Dev	0.7	0.17	1.54	0.39	2.3	4.7	2.3	5.5	7.3
					<u>Urban Area</u>				
Mean	7.4	0.37	4.02	1.05	4.8	18.3	12.1	34.5	30.3
Median	7.5	0.34	3.56	1.01	4.7	17.8	12.0	31.4	28.6
Max	7.9	0.64	6.36	2.29	8.9	27.8	20.8	58.9	54.9
Min	6.5	0.21	2.16	0.32	0.2	5.7	7.5	21.5	17.5
Std.Dev	0.3	0.12	1.44	0.58	2.0	6.5	3.2	9.9	11.2

Table 3.4 Summary statistics of PTE (mg/kg) in the peri-urban and urban area. (significant differences ($p < 0.05$) between the two areas are represented from different lower-case letters in the same column).

	Cr	Ni	Cu	Zn	Pb	Co	Cd	Sb	As
Peri-urban area									
Mean	265	**187 b**	**47 b**	**145 b**	**66 b**	20	1.0	**1.2 b**	7.9
Median	206	162	43	130	54	20	0.6	1.0	7.7
Max	461	289	94	322	196	25	4.6	2.5	12.1
Min	158	125	26	72	24	14	0.4	0.7	5.3
Std.Dev	121	56	19	66	48	3.5	1.3	0.5	1.7
Urban area									
Mean	270	**240 a**	**90 a**	**216 a**	**220 a**	23	1.3	**4.1 a**	7.5
Median	236	222	69	167	86	22	0.6	2.8	6.8
Max	665	632	257	551	1174	37	7.9	19.1	11.7
Min	117	104	31	89	27	15	0.3	0.9	0.7
Std.Dev	128	113	56	139	319	5	1.7	4.1	2.8

	Cr	Ni	Cu	Zn	Pb	Co	Cd	Sb	As
Previous study[1]	405	254	128	286	319		0.6	5.4	
European soils[2]	59.5	37	38.9	68.1	32				11.6
Worldwide soils[2]	94.8	29	17.3	70	27	10			6.8
Legislative limit[3]	150	120	120	150	100 ab	11	2	10	

[1] Padoan et al., 2017

[2] Kabata-Pendias, 2010

[3] Metha et al., 2020

3.3.2 In vitro bioaccessibility of PTE in urban and peri-urban areas

Oral bioaccessibility

Bioaccessible percentages and relative concentrations for the studied PTE are presented in Figure 3.2 and Table 3.5 for the urban and peri-urban areas. The data showed that the bioaccessibility of Pb, Zn and Co ($p < 0.05$) in the urban area was significantly higher than in the peri-urban area, although all the elements were more bioaccessible in the urban area. The bioaccessibility trend between elements was similar in both areas; i.e. Pb > Cd, Cu > Zn > Co > As, Ni, Sb > Cr. Moderate to weak correlations between total concentrations and bioaccessibility were observed for Cu ($R^2 = 0.67$), Zn ($R^2 = 0.54$), Pb ($R^2 = 0.43$), and Ni ($R^2 = 0.33$), while there was no clear connection in the case of Cr and Cd (Appendix. $R^2 < 0.10$, Fig. S3). These observations corroborated previous studies where PTE bioaccessibility in soils varied significantly between sampling sites and elements (Wu et al., 2017; Ai et al., 2019). Many factors contribute to the disparity in bioaccessibility values, which one of the most important is the presence of different sources of elements (Kelepertzis, 2014; Liu et al., 2019a) whose possibly include diverse fractions of PTE with different bioaccessibilities (Liu et al., 2019a). High bioaccessibility of Pb, Zn, and Cu has been linked to a higher level of anthropogenic pollution (Liu et al., 2017; Padoan et al., 2017), since elements from anthropogenic sources are generally more soluble in the gastrointestinal environment and thereby more bioaccessible (Luo et al., 2019; Hernandez-Pellon et al., 2018). Huang et al. (2018) also reported that PTE originated in a residential area were more bioaccessible than ones originated in commercial and industrial areas. Furthermore, the PTE speciation need to be considered; the low bioaccessibility of Cr, for example, may be due to the high geogenic contribution of refractory chromium-containing minerals from serpentinites, which cannot be easily solubilized (Sialelli et al., 2011; Biasioli et al., 2006). A very high bioaccessibility of Cd was observed during *in vitro* digestion, as found also in different areas (Luo et al., 2012; Francova et al., 2020) and the results may be associated with the low pH in simulated extraction solutions (Li et al., 2016).

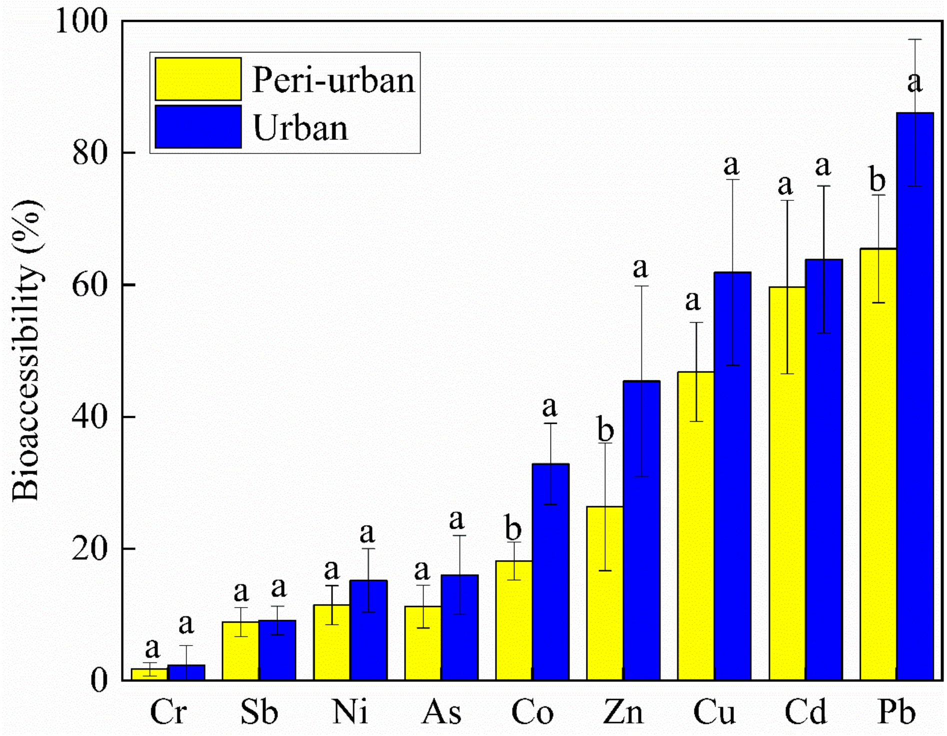


Figure 3.2. Gastric bioaccessibility of PTE in the urban and peri-urban area. Lower-case letters show significant difference ($p < 0.05$) of different element bioaccessibility between areas.

Lung bioaccessibility

Elements associated with fine soil size fraction (< 10 µm) may pose potential health risks because they can directly enter into the lung then to the blood system via inhalation. The results of the total and bioaccessible concentrations, and inhalation bioaccessibility in soils (< 10 µm) were displayed in Table 3.5 and Figure 3.3. Bioaccessible PTE concentrations (Co, Ni, Sb and Pb) through inhalation were higher than ones through ingestion ($p < 0.05$), posing concerns to their possible harm. However, the relative bioaccessibility was lower (Fig 3.4) because of the high total PTE concentrations in the < 10 µm fraction, higher than in coarser fractions. Higher concentrations in the finer fractions have already been reported from many articles and this is due to different phenomenon, such as, in some case, to the increase of sorption due to the higher specific surface of fine particles, according to what was already been reported (Ajmone-Marsan et al., 2008; Ma et al., 2019).

The differences in bioaccessibility may be due also to the different components of the extracting solutions and to pH values of the *in vitro* methods (Hu et al., 2019; Monneron et al., 2020). Many researchers found that pH has a substantial impact on PTE bioaccessibility (Liu et al., 2018). In opposition with these results, PTE bioaccessibility generally decrease with a higher pH (Basta et al., 1993; Li et al., 2020c), however, the complexity of Gamble’s solution could probably have resulted in a different behaviour as, for example, the presence of chlorides in its formula could lead in the formation of metal-chloride complexes which are readily solubilized (Bourliva et al., 2020).

The lung bioaccessibility varied widely among different elements because of the different chemical forms in which the elements could be present in the urban setting. Lead, Cd, Cu, and Co had the highest bioaccessibility, followed by Sb, Zn, As, Ni and Cr. The relatively high bioaccessibility of Cu and Zn may be due to the presence of cysteine in the extraction solution, which provides thiol groups that strongly coordinated with Zn and Cu (Huang et al., 2014). The high bioaccessibility of Cd is also interesting. According to a previous study (Pelfrêne and Douay, 2018), between the major forms of the elements present in the environment, Cd oxide and Cd chloride are easily dissolved in the lung, however, Cd sulfide not.

Lead, Cu, Zn, Ni, and Co had a higher bioaccessibility in the urban than in the peri-urban area, although not statistically significant, while Cr, As, Sb and Cd where more bioaccessible in the peri-urban area. This variability highlighted that PTE release could be influenced by the geological origins and by different anthropogenic processes.

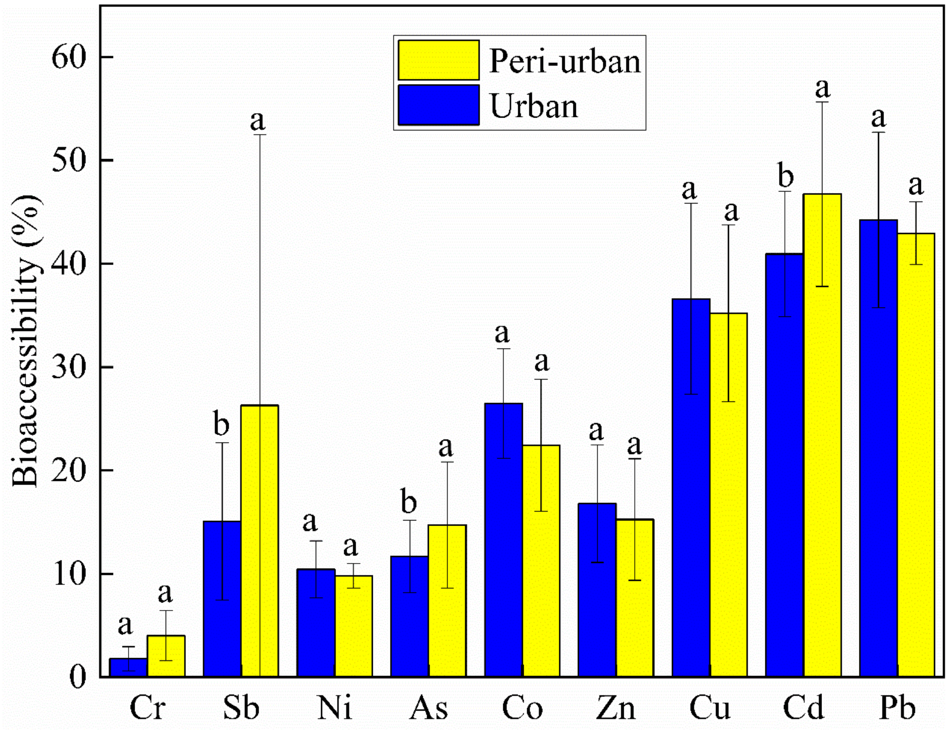


Figure 3.3. Lung bioaccessibility of PTE in the urban and peri-urban area. Lower-case letters show significant difference ($p < 0.05$) of different element bioaccessibility between areas.

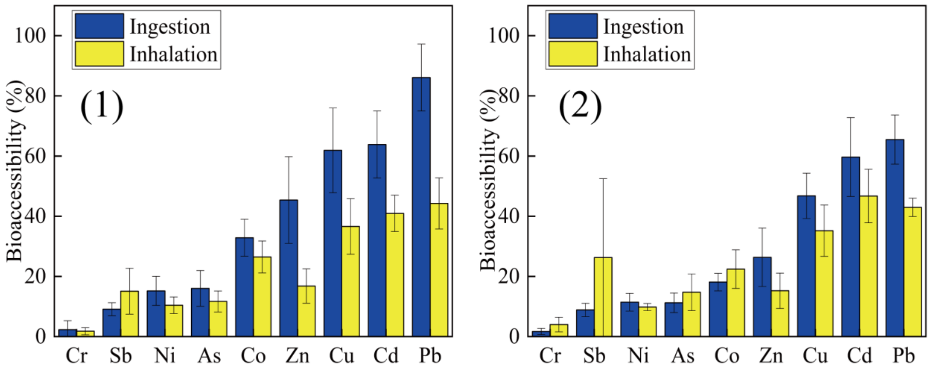


Figure 3.4. Comparison of PTE oral and lung bioaccessibility in urban (1) and peri-urban (2) area.

Table 3.5 Total (< 10µm) and bioaccessible (<150 µm, <10µm) PTE concentrations (mg/kg) in the urban and peri-urban area. Ranges, Median (Med), Averages (Avg) and Standard Deviations (SD). Upper-case letters show significant differences ($p < 0.05$) between ingestion and inhalation in the urban area, while lower-case letters indicate significant differences ($p < 0.05$) in the peri-urban area.

	Urban Area								
	Total concentration (< 10 µm)			Bioaccessible concentration (< 150 µm)			Bioaccessible concentration (< 10 µm)		
	Range	Med	Ave±SD	Range	Med	Ave±SD	Range	Med	Ave±SD
Cr	179-850	358	425±192	1-64	3.4	7±14 A	2-34	5.2	7.6±7.3 A
Co	27-74	42	45±12	4-13	7	7.3±2.1 B	6-19	12.2	12±3 A
Ni	186-932	408	466±207	13-164	31	40±35 B	15-140	38.8	50±31 A
Cu	80-467	147	187±111	15-231	39	63±39 A	28-232	47.9	73±59 A
Zn	192-1720	396	510±382	20-334	66	112±66 A	21-371	65.5	94±90 B
As	13-23	18	18±3	0.6-4.1	1.2	1.3±0.8 A	1.3-3.7	2	2.1±0.7 A
Cd	0.7-11.9	1.3	2.4±2.7	0.2-7	0.5	0.9±1.5 A	0.2-6.1	0.6	1.1±1.4 A
Sb	2-30	5.6	7.9±7.7	0.1-1.8	0.3	0.4±0.4 B	0.2-3.9	0.8	1.1±0.9 A
Pb	88-3426	286	636±938	19-1171	69	209±69 B	35-1669	123.7	301±486 A

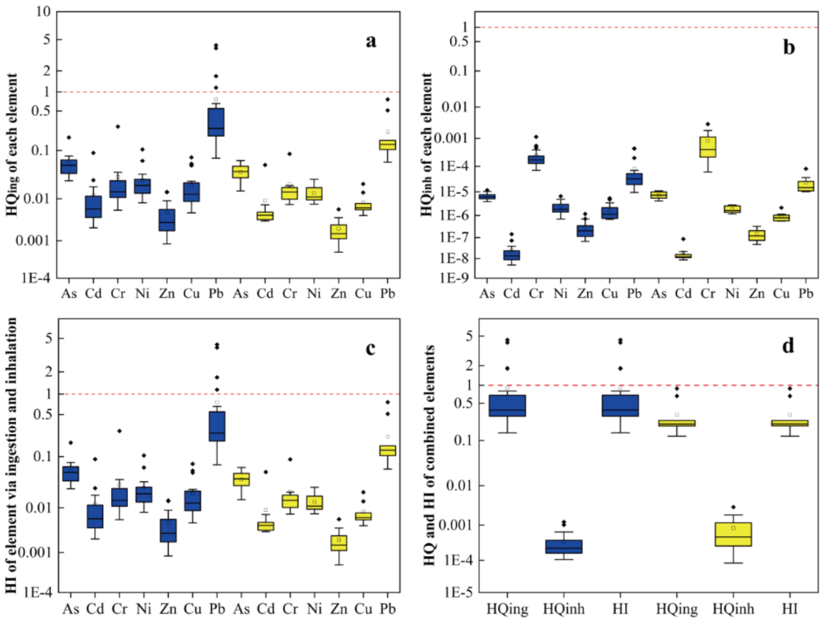
	Peri-urban Area								
	Total concentration (< 10 µm)			Bioaccessible concentration (< 150 µm)			Bioaccessible concentration (< 10 µm)		
	Range	Med	Ave±SD	Range	Med	Ave±SD	Range	Med	Ave±SD
Cr	249-927	368	479±263	1.7-20	3.3	4.8±5.5 b	1.9-90	12.4	25±28 a
Co	22-57	45	40±12	2.1-5	4	3.8±1 b	3.3-15.8	9	8.9±3.6 a
Ni	247-669	357	410±164	12-41	17	21±9 b	25-60	35	40±14 a
Cu	68-222	103	114±43	13-66	19	26±16 b	24-93	47.9	39±21 a
Zn	230-559	254	309±113	12-135	35	47±38 a	15-106	38.3	48±32 a
As	7-25	19.9	18±6	0.3-1.5	0.9	0.9±0.4 b	1.3-3.5	2.3	2.4±0.8 a
Cd	0.8-6.4	1.2	1.7±1.7	0.2-4	0.3	0.7±1.2 a	0.4-3.7	0.51	0.9±1 a
Sb	1.5-5.8	2.9	3.1±1.3	0.1-0.3	0.1	0.1±0.1 b	0.3-1.5	0.6	0.7±0.4 a
Pb	90-653	128	200±174	16-210	36	60±63 b	38-300	55	87±81 a

3.3.3 Human health risk assessment

The non-carcinogenic and carcinogenic risks due to soil PTE via the ingestion and inhalation exposure pathways are presented in Figures 3.5 and 3.6. The mean and median values of HI in this study were lower than 1, suggesting an acceptable average non-carcinogenic risk for the studied soils. However, in some sampling sites in urban area, children exposure to Pb contaminated soil may have adverse health effects (Fig 3.5a).

The health risks through the different exposure routes were in the order of ingestion > inhalation (Fig 3.5 a, b), indicating that exposure to soils by ingestion contributed to the largest to the total calculated health risk (Zhuo et al., 2019; Liu et al., 2020). Comparatively, non-carcinogenic risks for children were higher than for adults (Fig 3. 5 1,2), and higher in the urban area than in peri-urban area (Fig 3.5 d) and the same trend was observed for carcinogenic risk, suggesting that children faced more potential health risks from exposure to elements. The non-carcinogenic risk for each element decreased in the order of Pb > As > Cr > Ni > Cu > Cd > Zn in both areas, which indicated Pb (> 80%) as the main contributor to the estimated human health risk.

In terms of carcinogenic risk, the TCR probabilities for As, Cd, Cr and Pb to children and adults were under the acceptable level ($< 1\times10^{-4}$), indicating no significant risks to adults and children exposed to soils. Soil ingestion was calculated as the most important pathway of exposure (Fig 3.6 a, b), but inhalation has a higher contribution to the carcinogenic risk than to the non-carcinogenic. Chromium (42%) and As (37%) were the dominant contributor to cumulative carcinogenic risk. This was consistent with previous studies revealing As and Cr being the major carcinogen and Pb the major non-carcinogen factors (Eziz et al., 2018; Fan et al., 2019; Bourliva et al., 2020).



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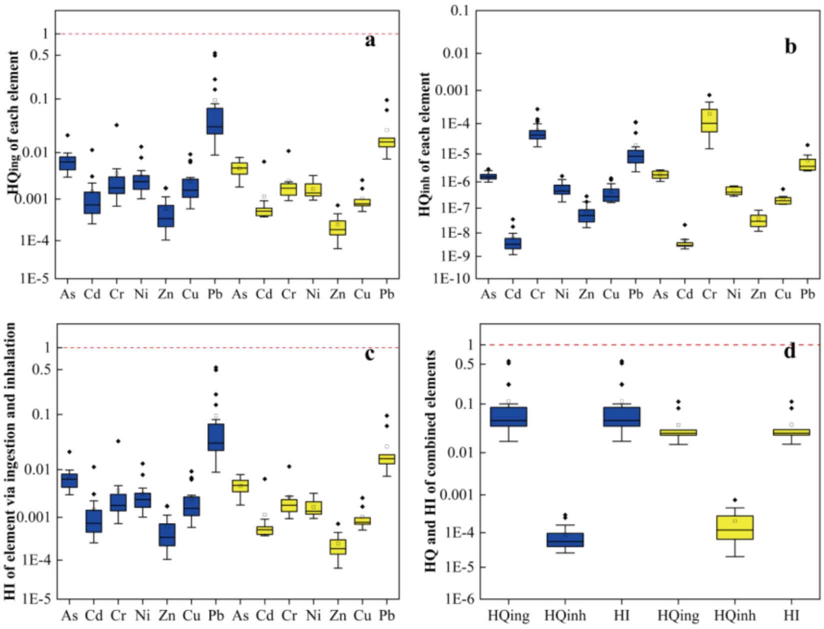

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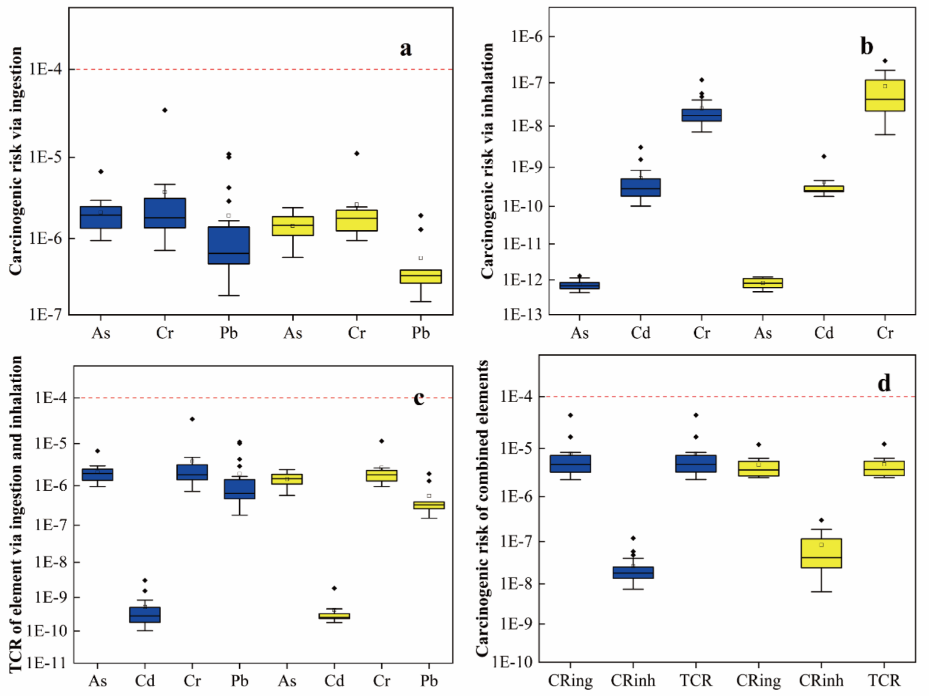
Figure 3.5. Non-cancer Hazard Quotients (HQ) and Hazard Indexes (HI) of PTE in urban (blue) and peri-urban (yellow) areas via ingestion and inhalation

exposure pathways calculated for children (1) and for adults (2). In detail: (a) HQ of each element through ingestion; (b) HQ of each element through inhalation; (c) HI of each element through ingestion and inhalation; (d) HQ and HI of combined elements through ingestion and inhalation.



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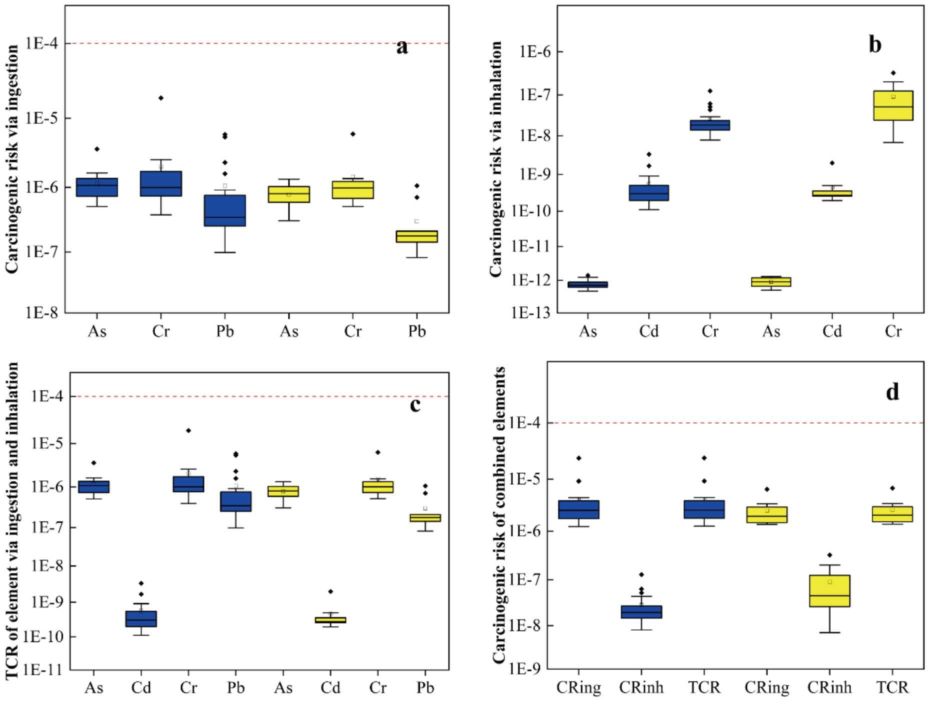

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Figure 3.6. Cancer risk due to PTE in urban (blue) and peri-urban (yellow) areas via ingestion and inhalation exposure pathways for children (1) and adults (2). In detail: (a) CR of each element through ingestion; (b) CR of each element through inhalation; (c) TCR of each element through ingestion and inhalation; (d) CR and TCR of combined elements through ingestion and inhalation.

3.4 Conclusions

In vitro oral and lung bioaccessibility and human health risk assessment of PTE in soil samples from an urban and peri-urban area in Turin were investigated. The average PTE contents and chemico-physical parameters of soils were in line with previous works in the same area. Concerning bioaccessible percentages, they exhibited a decreasing order of Pb > Cd, Cu > Zn > Co > As, Ni, Sb > Cr in the gastric environment and Pb > Cd, Cu > Zn > Co > As, Ni, Sb >Cr regarding lung bioaccessibility. Comparing ingestion and inhalation results, a relative enrichment of bioaccessible concentrations of Pb, Ni, Co and Sb was observed in the inhalable size fraction (< 10 µm) compared to ingestible one. Conversely, the relative bioaccessibility of Pb, Cu, Cd, Zn and As was lower, due to the different components of the extraction solution and extracting pH. The average bioaccessibility values suggested that PTE would more bioavailable if ingested than inhaled. In addition, a higher solubility of Pb, Cd, Zn, and Cu was found using both methods, which may reflect a higher level of anthropogenic pollution.

Human health risk was assessed for the ingestion and inhalation pathways, using the bioaccessible fractions in simulated fluids. Unacceptable non-carcinogenic risk (HQ > 1) was found through ingestion exposure for children in some urban sites and Pb was the most hazardous elements for non-carcinogenic risk. Carcinogenic risks were under the threshold levels for every soil (CR < 10^{-4}), with Cr and As being the dominant contributors to risk. Furthermore, children were more susceptible to PTE toxicity than adults and urban area soils posed a higher risk than peri-urban ones. Therefore, this elements, and especially Pb pollution in the urban soils still need more attention, and the necessary soil remediation activities are needed to reduce the risks of human, especially children, exposure to PTE.

4. Soil particle size fraction and Potentially Toxic Elements bioaccessibility: a review

Li Yan[a*], Padoan Elio[a], Ajmone-Marsan Franco

[a] University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco, 10095, Italy

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Abstract

In the last decade, extensive studies have been conducted to quantify the influence of different factors on potentially toxic elements (PTE) bioaccessibility in soil; one of the most important is soil size fraction. However, there is no agreement about the size fraction and the methods to investigate bioaccessibility, as very few review articles are available on soil PTE bioaccessibility and none addressed the influence of particle size on PTE bioaccessibility. This study provides a review of the relations between PTE bioaccessibility and soil particle size fractions. The available research indicates that PTE bioaccessibility distribution across different size fractions varies widely in soil, but a general trend of higher bioaccessibility in finer size fraction was found. The different elements may exhibit different relationships between

bioaccessibility and soil size fraction and, in some cases, their bioaccessibility seems to be more related to the source and to the chemico-physical form of PTE in soil. Often, soil pollution and related health risk are assessed based on PTE total concentration rather than their bioaccessible fraction, but from the available studies it appears that consensus must be pursued on the methods to determine PTE bioaccessibility in the fine soil size fractions to achieve a more accurate human health risk assessment.

4.1 Introduction

Soils are a source and a sink of various inorganic and organic compounds, natural or anthropically-derived, depending on the soil type, soil use, parent material and anthropic pressure, e.g due to industrial and mining activities, traffic, waste disposal and others causing contamination and related environmental and health impacts (Alloway, 2013).

Soil contamination from potentially toxic elements (PTE) has become of great concern worldwide in the past few decades as PTE, contrarily to most organic pollutants, cannot be degraded and are difficult to remove, persisting for a long time in the soil environments (Ma et al., 2009; Frohne et al., 2014; Padoan et al., 2020a).

PTE may occur naturally in soils; however additional contributions come from anthropogenic activities such as mining, smelting, fertilization, and urbanization, leading to their build up and gradual release into the environment. In these cases, soils could become a sink of PTE, causing various degrees of pollution in urban, agricultural, and industrial contexts (Li et al., 2014b; Cai et al., 2016; Ajmone-Marsan et al., 2019; Mehta et al., 2019; Mokhtarzadeh et al., 2020).

Most studied PTE include the xenobiotic and highly toxic lead (Pb), arsenic (As), chromium (Cr), cadmium (Cd) and mercury (Hg), as well as some essential elements, such as copper (Cu), zinc (Zn), cobalt (Co), manganese (Mn) and nickel (Ni) that can become toxic when accumulated at high concentrations in soils (Alloway et al., 2013; Li et al., 2018). These studies rise from the fact that human exposure to PTE in soils is a major public concern and is associated with serious risks to human health. PTE in soil can exert their toxicity to human and plants when they encounter the receptor or are absorbed. Human exposure scenarios to PTE in soil are, therefore, inhalation, inadvertent oral ingestion, and dermal contact (Shi et al., 2011, Ruby and Lowney, 2012). Even though exposure routes are manifold, inadvertent oral ingestion has been considered, and calculated, to be the main exposure path in most health risk assessment studies, especially through outdoor hand-to-mouth activities by children (Oomen et al., 2002; USEPA, 2011; Pelfrêne et al., 2012).

Risk estimation due to oral ingestion of contaminated soil particles should be defined considering potential exposure, thus quantity and quality of ingested particles (USEPA, 2011), and potential absorption of PTE after ingestion (Paustenbach, 2000). Since not all the ingested PTE are easily released from the soil matrix, and thus absorbed from the body, methods for the assessment of bioaccessible concentrations have been developed. The *bioaccessibility* of an element represents the fraction of the element/compound that is soluble in the gastrointestinal tract and, therefore, available for absorption (Ruby et al., 1999). Soil PTE absorption can be assessed using *in vivo* methods, to evaluate the bioavailability of soil PTE alone or in comparison with a PTE-salt reference dose, or by *in vitro* methods, using digestion models based on human physiology that have been developed to reduce the difficulty and the costs of *in vivo* studies (Oomen et al., 2002; Juhasz et al., 2009).

Although in the last years many studies investigated PTE bioaccessibility in different soils from different areas with different contamination histories (Cui et al., 2018; Valido et al., 2018; Li et al., 2020a; Mehta et al., 2020), the factors controlling bioaccessibility, such as elemental speciation and soil physical properties, have seldom been investigated.

In this regard, an important soil property which influences PTE bioaccessibility appears to be the soil particle-size distribution since PTE partitioning in various soil particle sizes can affect both exposure and PTE extractability. For example, as the ingestion of contaminated soil is largely the result of hand-to-mouth contact, we have to consider the size fraction adhering to human hands, especially of children, as for adults soil ingestion is considered to be lower (Yamamoto et al., 2006; USEPA, 2011). Various studies assessed the soil particle size of relevance, although a definitive upper size cutoff has not yet been defined (Yamamoto et al., 2006; Siciliano et al., 2009; Ruby and Lowney, 2012). Since soil texture and organic matter directly influence dust adherence to hands, different studies achieved different cutoff sizes, ranging from 50 to 250 µm in diameter (Driver et al., 1989; Edwards et al., 1999; Yamamoto et al., 2006; Ruby and Lowney, 2012). Moreover, fine soil particles are normally more concentrated in clay minerals and organic matter, influencing the total concentration and the extractability of PTE in the finer soil fraction (Ajmone-Marsan et al., 2008; Madrid et al., 2008).

Many studies in recent years started investigating the behaviour of PTE in different soil size fractions but methods, soil fractions investigated and purposes of the studies have been quite dissimilar, making it hard to draw some general conclusions on PTE bioaccessibility and a possible unified method. Up to now, research groups have tested *in vitro* PTE bioaccessibility in different soil size fractions, however they may not be comparable as no *in vivo* data was obtained in different soil fractions.

This review is aimed to: i) compare the most commonly used methods to estimate PTE bioaccessibility in soil particles, to identify the optimal size for soil bioaccessibility and risk assessment; ii) discuss the variation of PTE bioaccessibility within particle sizes; iii) compare the efficacy of the methods to estimate metals and metalloids bioaccessibility.

4.2 PTE Bioaccessibility methods

The *in vitro* bioaccessibility is defined as the fraction of an element that is soluble in simulated biological conditions such as, for oral bioaccessibility, the gastric and intestinal juices (Ruby et al., 1999; Juhasz et al., 2009). The determination relies on the extraction of the elements with solutions mimicking the chemical conditions encountered in the human stomach or intestines (Turner, 2011). The bioaccessible fraction measured using *in vitro* methods is usually greater than the actual bioavailable fraction (Paustenbach, 2000) and its use provide a conservative measure of bioavailability. These tests have been mostly developed using soil contaminated with one or few elements, but they have been extended to various PTE for which they have not yet been demonstrated to be good analogues of *in vivo* conditions.

The most used methods include the Solubility/Bioavailability Research Consortium method (SBRC), the Simple Bioaccessiblity Extraction Test (SBET), the In Vitro Gastrointestinal method (IVG), the Physiologically Based Extraction Test (PBET), the Deutsches Institutfür Normunge V method (DIN), and the Unified BARGE Method (UBM) which is based partly on the previously developed Dutch National Institute for Public Health and the Environment method (RIVM) (Ruby et al., 1996; Rodriguez et al., 1999; Oomen et al., 2002; Drexler and Brattin, 2007; Juhasz et al., 2009; Wragg et al., 2011). The SBET method consists, however, only in the gastric phase extraction of the SBRC method and, thus, in this manuscript, will be called as SBRC gastric to diminish the acronyms.

In brief, soil aliquots are mixed with solutions simulating the gastric and intestinal environments, incubated for a particular time with a specific soil/solution ratio and then extracted PTE concentrations are quantified (Figure 4.1).

In vitro PTE relative bioaccessibility (RBA) can be calculated as follows:

$$PTE\ RBA\ (\%) = \frac{in\ vitro\ concentration}{total\ concentration} \times 100$$

All the solutions simulating the gastrointestinal fluids have a physiologically-based composition, except the SBRC gastric phase, and they are mainly composed of salts to control pH and competing ions, complexants as organic acids, enzymes, and proteins. The differences between these physiologically driven methods are mostly variations in the gastrointestinal fluid composition and extraction parameters as the incubation time in the gastric and small intestinal phases or the solution pH. For example, in the gastric phase, the soil residence time is 1 h for PBET, SBRC, UBM, and IVG but 2 h for DIN. In the small intestinal phase, the extraction time is 1 h for IVG, 2 h for UBM, 4 h for PBET and, 6 h for DIN (Li et al., 2020c).

A scheme of the main parameters and of the differences involving SBRC, PBET, IVG, DIN, and UBM methods is reported in Table 4.1.

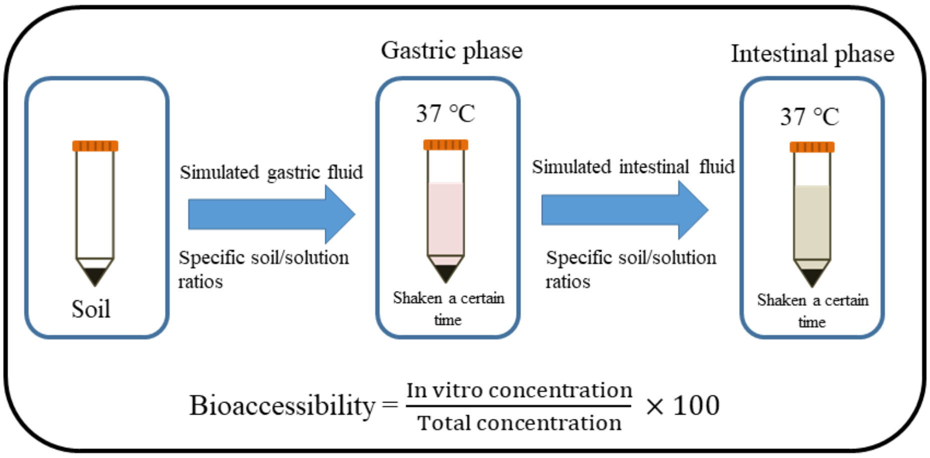

Figure 4.1. Schematic diagram of in vitro soil PTE bioaccessibility test procedure

Table 4.1 Composition of gastrointestinal fluids and operational parameters of SBRC, PBET, IVG, DIN, and UBM methods.

Method	Phase	Fluid composition (L^{-1})	pH	Soil:Solution Ratio	Duration(h)	Reference
SBRC	Gastric	30.03 g glycine	1.5	1:100	1	Drexler and Brattin, 2007
	Intestinal	1.75 g bile, 0.5 g pancreatin	7.0	1:100	4	
PBET	Gastric	1.25 g pepsin, 0.5 g malate, 0.5 g citrate, 0.42 mL lactic acid, 0.5 mL acetic acid, 8.77 g NaCl	2.5	1:100	1	Ruby et al., 1996
	Intestinal	1.75 g bile, 0.5 g pancreatin	7.0	1:100	4	
IVG	Gastric	10 pepsin, 8.77 g NaCl	1.8	1:150	1	Rodriguez et al., 1999
	Intestinal	3.5 g bile, 0.35 g pancreatin	5.5	1:150	1	
DIN	Gastric	1 g pepsin, 3 g mucin, 2.9 g NaCl, 0.7 g KCl, 0.27 g KH_2PO_4	2.0	1:100	2	Juhasz et al., 2009
	Intestinal	9.0 bile, 9.0 g pancreatin, 0.3 g trypsin, 0.3 g urea, 0.3 g KCl, 0.5 g $CaCl_2$, 0.2 g $MgCl_2$	7.5	1:100	6	

Method	Phase	Fluid composition (L^{-1})	pH	Soil:Solution Ratio	Duration(h)	Reference
UBM	Saliva	0.45 g KCl, 0.44 g NaH_2PO_4, 0.1 g KSCN, 0.28 g Na_2SO_4, 0.15 g NaCl,0.1 g urea, 0.15 g amylase, 50 mg mucin, 15 mg uric acid, NaOH	6.5	1:15	0.25	Wragg et al., 2011
	Gastric	1.85 g NaCl, 0.163 g NaH_2PO_4, 0.41 g KCl, 0.2 g $CaCl_2$, 0.15 g NH_4Cl, 0.32 g glucose, 20 mg glucuronic acid, 40 mg urea, 165 mg glucosaminehydrochloride, 1.0 g BSA, 3.0 g mucin, 1.0 g pepsin, HCl	1.2-1.7	1:37.5	1	
	Intestinal	Duodenal: 3.5 g NaCl, 2.8 g NaH_2PO_4, 40 mg KH_2PO_4, 0.28 g KCl, 25 mg $MgCl_2$, 50 mg urea, 0.2 g $CaCl_2$, 1.0g BSA, 3 g pancreatin, 0.5 g lipase Bile: 2.6 g NaCl, 2.9 g $NaHCO_3$, 0.18 g KCl, 0.12 g urea, 0.2 g $CaCl_2$, 1.8 g BSA, 6.0 g Bile, HCl	6.3	1:97.5	4	

These methods have been established starting from one or a limited number of key soil contaminants, chosen in view of their toxicity to humans and of their presence in most of contaminated sites. All the five procedures have been tested for As and Pb using a large number of contaminated soils in different studies, while for Cd, compared to As and Pb, only 4 studies provided paired bioaccessibility and bioavailability data (Li et al., 2020a). *In vitro* assays were tested to assess the correlation between *in vivo* and *in vitro* results, the most important result for the validation of the methodology, along with its repeatability and reproducibility (Wragg et al., 2011).

After their first publication the methods have been stretched and utilized for a wide variety of elements for whom the methods had not been yet validated, counting on the fact that the reliability of the results for Pb, Cd and As could be extended to elements having different chemical reactivity.

In recent years, some studies assessed the comparability of the different *in vitro* methods using a range of contaminated or reference soil samples, and results varied significantly depending on target element, and contamination source (Juhasz et al., 2015; Li et al., 2020a). Generally, gastric phases were better correlated with *in vivo* data, with SBRC and UBM methods, the simplest one (SBRC) and the most complex one (UBM) of the assays, producing the highest bioaccessibility values, providing more conservative results. Both methods were also being reported as the best assays for As, Pb and Cd bioaccessibility measurement in contaminated soils (Li et al., 2020a), with the gastric phase of SBRC also accepted by USEPA to estimate Pb bioaccessibility (USEPA, 2013). For this review, the above-depicted *in vitro* methods will be considered as comparable, to focus on the soil-dependent factors affecting the results.

4.2.1 Factors affecting PTE bioaccessibility

Beside the chemical nature of the analytes, and of the solubility and the extractability of elements in the specific conditions adopted by the different tests, it is well known that the bioaccessibility of PTE is also strongly dependent from soil characteristics, such as pH, organic matter and texture (Petruzzelli et al., 2020). For instance, previous studies have shown that the bioaccessibility of several PTE (Cu, Cd, Cr, Co, Pb, Zn, and Ni) in soil was significantly correlated with the total elemental content and soil texture (Mendoza et al., 2017; Cai et al., 2016). Other studies have attempted to identify the effect of specific soil properties on PTE bioaccessibility, revealing, not surprisingly, that pH is one of the most important parameters governing it (Li et al., 2003). Particularly, most PTE increase their bioaccessibility when the pH decreases (Du et al., 2020), which may be due to its influence on the chemical speciation and to the competition of H^+ ions for the same adsorption sites on the soil surfaces (Basta et al., 1993; Petruzzelli et al., 2015). Moreover, proton-promoted dissolution of Fe/Al/Mn oxyhydroxides, which could be a sink of PTE, also contribute to the increased bioaccessibility when pH decrease (Fu et al., 2010). Other important soil components influencing PTE bioaccessibility are soil organic matter (SOM), as well as Fe, Al and Mn oxides. Mostly, oxides can increase the stability of PTE in soils via inner and outer sphere complexation reactions, leading to a decrease in bioaccessibility, while SOM could also enhance the dissolution of PTE due to complexation from acid functional groups of low molecular weight SOM components (Palmer et al., 2014; Cai et al., 2017; Yin et al., 2015).

The effect of SOM on PTE bioaccessibility has been recently investigated directly in the simulated gastrointestinal (GI) fluids from Liu et al. (2019b). They depicted the Pb speciation and the role of organic complexes on Pb bioavailability. The authors stressed that increasing SOM content could increase Pb bioaccessibility due to the presence of Pb-humic complexes in the GI fluid.

4.2.2 Soil size fractionation

Soil texture and, more specifically, the size of particles investigated, may also have a considerable impact on bioaccessibility results.

All the extraction methods have been firstly developed using bulk soils, sieved at < 2 mm, and have been then validated using the < 250 µm soil fraction, historically chosen because of its likely representation of the soil particles that would be expected to adhere to children’s hands (Ruby and Lowney, 2012).

However, the choice of a specific particle size fraction is of crucial importance for the evaluation of PTE bioaccessibility. Different soil size fractions may exhibit different properties and compositions, affecting the behavior of pollutants in soil microenvironments (Acosta et al. 2011). Previous studies have shown that fine soil fractions accumulated higher PTE concentrations than coarser ones (Ajmone-Marsan et al., 2008; Luo et al., 2011). The fact has been attributed to the higher specific surface area, content of clay minerals, organic matter, and Fe, Al, and Mn oxides of the fine fraction compared to the coarser ones (Hardy and Cornu, 2006; Ajmone-Marsan et al., 2008; Luo et al., 2011; Liu et al., 2018). All these components present a variety of adsorption sites absent in coarser particles, enriched in harder minerals such as quartz, affecting thus PTE stability and bioaccessibility.

Up to now, research groups tested PTE bioaccessibility in different soil fractions, depending on the scope of the work, with or without a direct comparison between different particle sizes. Thus, the investigated particles commonly vary from clays (< 2 µm particles) to particles of environmental relevance, such as the < 10 µm fraction, which could represent a possible source of atmospheric particulate matter (Thorpe and Harrison, 2008), up to the < 250 µm fraction.

In the studies where a single particle size fraction is analyzed, and in most of the studies considering a range of sizes, usually the < 250 µm size fraction is considered (Wang et al., 2018; Yin et al., 2017). This size has been used since the first investigations, and most of the methods have been developed against in vivo models using this size fraction. However, in recent years, findings demonstrated that fine fractions (e.g. < 150 µm or < 63 µm) adhere more easily to children’s hands and, in addition, are more likely to be dissolved, increasing their possibilities to traverse the gastric mucosa (Ruby and Lowney, 2012; Yutong et al., 2016).

In 2016, the US Environmental Protection Agency (USEPA) reviewed recent studies on soil adherence to hands, summarizing the relationship between particle size and adherence, and endorsing the use of the < 150 µm size fraction for human health risk assessment (USEPA, 2016). From a former study (Ikegami et al., 2014), emerged that using this fraction would allow to consider an average of 98% of particles adhering to children’s hands. However, other researchers pointed out that finer particles (e.g. < 63 µm or < 100 µm) are more likely transferred into the human body, thus the use of a coarser fraction may underestimate the risk of direct soil contaminant intakes (Yamamoto et al, 2006; Siciliano et al., 2009).

4.3 Elemental bioaccessibility in size fractions

In most studies, different PTE exhibited different relationships between soil particle size and bioaccessibility, indicating that the influence of particle size fraction on oral bioaccessibility is metal(loid)-dependent (Ma et al., 2019; Van der Kellen et al., 2020).

The summary of the different size fractions investigated and *in vitro* methods used in recent studies dealing with PTE bioaccessibility is reported in Table 4.2.

Table 4.2 Summary of PTE bioaccessibility studies addressing different soil size fractions

Method (*in vitro*)	Soil provenance	Particle size (µm)	Element(s)	Reference
IVG	8 soil samples, chromated copper arsenate contaminated area	<250 <90	As	Girouard and Zagury, 2009
SBRC gastric	29 soil samples: 6 railway corridor soils, 6 mining soils, 10 cattle-dip soils, and 7 geogenic soils	<2.5; 2.5-10 10-100; 100-250	As	Smith et al., 2009
UBM	72 sidewalk dust samples, urban area	0.3-5; 5-50 50-150; 150-250 250-1000; >1000	Cd, Pb	Pelfrêne and Douay, 2018
UBM	21 dust samples, urban area	<38; 38-63 63-250	Cu, Fe	Patinha et al., 2015
SBRC gastric	10 soil samples, urban area	<2; 2-10 10-22; 22-50; >50	Cr, Cu, Ni, Pb, Zn	Madrid et al., 2008

SBRC gastric	18 soil samples and 29 road dust, peri-urban area	<2.5; 2.5-10 10-200; 200-2000	Fe, Mn, Cu, Cd, Cr, Ni, Pb, Sb, Zn	Padoan et al., 2017
SBRC gastric	33 soil samples, different mining areas	<10; 10-200	Fe, Mn, Cd, Cr, Cu, Co, Ni, Pb, Zn	Padoan et al., 2020a
PBET	8 surface soils, highly urbanized area	<2; 2-10 10-50; 50-100 100-280; 280-2000	Cu, Zn, Pb, Ni, Co, Cr, Mn	Luo et al., 2011
PBET	27 soil samples, gold mining area	<45; <150 <250	As	Meunier et al., 2011
SBRC gastric	Soil samples in urban school and kindergarten from 3 cities	<63; 63-150 150-250	Al, Fe, Mn, Cd, Cr, Cu, Ni, Pb, Zn	Ma et al., 2019
SBRC	16 soil samples, including 2 shooting ranges sites, 3 incinerator sites, 2 urban sites, 8 mining sites and 1 gasworks site	<50; <100 <250; <2000	Pb	Juhasz et al., 2011

UBM, PBET, SBET, SBRC, IVG	20 soil samples, urban area	<1; 1-5 5-50; 50-250	As, Ba, Co, Cr, Mn, Ni, Pb, Zn	Li et al., 2020c
SBRC gastric	4 reference soils, mining and smelting area	<150; <250	Pb, As	Karna et al., 2017
UBM	2 composite soil dust samples, smelting and mining zone	<2; 2-20 20-50; 50-200 200-2000	As, Cd, Cu, Pb, Sb, Sn, Zn	Goix et al., 2016
UBM	2 soil samples, residential area	<2, 2-63 63-125, 125-250	Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	Qin et al., 2016
SBRC	5 street dusts and 5 soil samples, urban area	<10; 10-70 70-105; 105-250	Cu, Pb, Zn	Dehghani et al., 2018
IVG	10 soil samples, chromated copper arsenate contaminated area	<20; 20-90 90-250	As, Cu, Cr, Pb, Zn	Van der Kallen et al., 2020
SBRC gastric	30 playground soil samples, urban area	<50; <150	As, Bi, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Sb, Sn, V, Zn	Hiller et al., 2020
SBRC gastric	38 soil samples, gardens	<10; <63	As, Cd, Pb	Manjón et al., 2020

The reviewed studies used in an equal manner the different methods for bioaccessibility estimation (Figure 4.2), with a slight preference for the simpler and rapid extraction: the gastric phase SBRC (also called SBET), which is also the one endorsed from U.S. E.P.A. (USEPA, 2013).

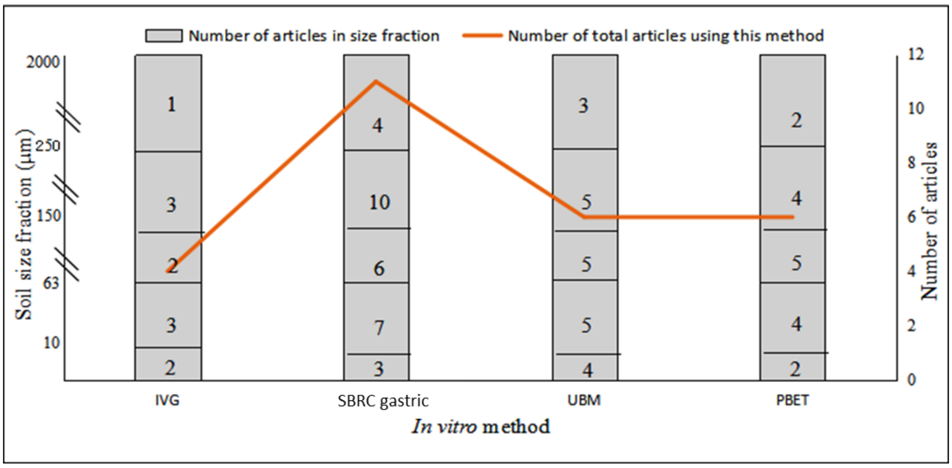

Figure 4.2. Summary of studies published in the 2000-2020 period addressing PTE bioaccessibility in different soil size fractions divided according to the used method.

4.3.1 Arsenic

Arsenic is a widely distributed metalloid, occurring in rocks, soil, water, and air (Jarup, 2003). However, its concentration in soils may rise due to anthropogenic activities (Morin and Calas, 2006). In these cases, the exposure to As is a major public concern due to the serious risks posed to human health (Yin *et al*., 2015).

Previous studies have shown that soil total As concentrations increase with decreasing size fraction (Lombi et al., 2000; Smith et al., 2006), while some researchers also reported that the bioaccessible As concentrations increased with decreasing size fraction, due to a higher total As content, while the RBA (expressed as bioaccessible % of the total content of the element in the fraction) generally increased (Ruby 2004; Girouard and Zagury, 2009). Smith et al. (2009) investigated the bioaccessibility of As in the < 2.5, 2.5-10, 10-100, and

100-250 µm soil size fractions in 50 contaminated soils. The results indicated that As was evenly distributed across fractions apart the < 2.5 µm fraction, presenting elevated As concentrations associated with a marked increase in the iron content. Arsenic RBA increased with decreasing particle size and varied depending on As origin; anthropogenic sources were more bioaccessible than geogenic ones.

Conversely, Meunier et al. (2010), working on a small number of soil samples (n=9) reported no systematic variation of As bioaccessibility within particle size fractions (< 250µm, < 150µm, < 45µm). In a successive study, Meunier et al. (2011), found the highest bioaccessible As concentrations in soil particles <45µm in size due to higher total As concentrations, while no systematic variation of RBA was observed between < 250µm, < 150µm and < 45µm particle size fractions. Results arising from these studies may be due to the different As speciation in the different soils, as a lower As bioaccessibility was associated with encapsulated As, whereas the highest As bioaccessibility resulted, according to the authors, from the presence in soils of pentavalent As in amorphous minerals on the surface of the particles or coating the grains (Meunier et al., 2011).

4.3.2 Lead

Lead is one of the most common environmental pollutants (Weber, 2014). Its exposure via ingestion and inhalation is a global public health hazard, especially for children (< 6 year of age), for whom Pb poisoning via hand-to-mouth exposure is of critical concern (Gao et al., 2020). Several studies looked at Pb total concentrations in soil size fractions and found that Pb concentration increased with decreasing particle size (Tawinteung et al., 2005; Weiss et al., 2006; Momani, 2006). Conversely, one long-term study, analyzing soils weathered for 70 years since the end of the contamination, noticed a decreasing concentration trend with decreasing particle size (Bright et al., 2006).

Among the studies, a lower number focused on the bioaccessibility in different particle size fractions. Juhasz et al. (2011) evaluated the influence of soil particle size fractions on Pb bioaccessibility and reported that, in 6 out of 16 tested soils, RBA significantly increased with decreasing particle size. Pelfrêne and Douay (2018) also found the highest RBA in the finest particles (0.3-5 µm) and a decrease when the particle size increased (5-50, 50-150, 150-250 µm), suggesting a stronger bound of Pb to coarse particles, in agreement with previous studies (Luo et al., 2011; Karna et al., 2017; Qin et al., 2016). Other studies found contrasting results; Dehghani et al. (2018) found the highest Pb bioaccessibility in 10-70 µm soil size fraction rather than the finest size fraction (< 10 µm) and Padoan et al. (2017) found an almost constant Pb RBA in all size fractions. Lead bioaccessibility vary differently among the size fractions in the different gastrointestinal phases. Yu et al. (2006), assessed the relationship among Pb bioaccessibility in three particle size fractions (< 75, 75-150, 150-250 µm). Changes in Pb RBA as a function of particle size fractions were not significant in the gastric phase while they were significant in the intestinal one. The intestinal bioaccessibility of the < 75 µm size fraction was significantly lower than 75-150 µm and 150-250 µm (same for these two size fractions), indicating that Pb was dissolved uniformly in the gastric phase but it can be quickly reabsorbed onto the surface of smaller particles after the addition of the artificial intestinal juice.

4.3.3 Cadmium and copper

Cadmium, one of the most toxic PTE, is an element naturally occurring in soils, with high background values in many regions. However, most of the anthropogenic emissions are due to mining and smelting, together with irrigation with sewage waters, phosphate fertilizers and other agricultural amendments, which essentially make Cd as a worldwide contaminant of soils (Ajmone-Marsan and Biasioli, 2010; Alloway, 2013).

Due to its low concentrations, Cd bioaccessibility has been mostly studied on bulk soil, while when it was assessed in different particle size fractions, its concentration decreased below the detection limit, for example in Padoan et al. (2017).

Most of the selected studies found that the RBA of Cd-containing particles was high in coarse sized particles (Goix et al., 2016; Qin et al., 2016; Ma et al., 2019), but, in some samples, Goix et al. (2016) and Pelfrêne and Douay (2018) found the highest RBA in the finest studied fractions. These differences between Cd RBA in the studies were most probably due to the different chemical speciation of Cd in the diverse soils and sampling areas (such as its adsorption onto clay minerals, oxides, its presence as a constituent of minerals or as a coprecipitate onto the surface of particles), thus to the different sources of soil contamination, as industrial sources such as batteries production, coating and plating, or agricultural ones.

Copper is also a widespread contaminant, mostly released by industrial and manufacturing activities together with traffic (Hu et al., 2020). Some researchers have found that total Cu concentration tends to be higher in finer fractions than in coarser ones (Ajmone-Marsan et al., 2008; Acosta et al., 2009; Li et al., 2014b), and smaller particles are likely more ingestible than coarser ones. Recently, several researchers determined Cu bioaccessibility in different particle sizes. Ma et al. (2019) observed a lower RBA in the fine particles (< 63 and 150 µm) than in coarser ones (150-250 and 250-2000 µm), which has been associated with high Cu affinity to organic matter, more present in fine soil fractions such as silt and clay (Kadum et al., 2017). Differently, Dehghani et al. (2018) tested Cu bioaccessibility in different size fractions (Table 2), finding higher RBA in the finest one (< 10 µm).

4.3.4 Other PTE

Ma et al. (2019) found that Pb and Zn generally exhibited the highest RBA in the coarsest particle sizes (250-2000 µm); contrarily, the highest RBA of Ni occurred in the finest sizes (< 63 µm). The other studied elements (Cd, Cr and Cu) did not exhibit any obvious relationships with size fraction. Li et al. (2020c) evaluated the bioaccessibility of As, Ba, Co, Cr, Cu, Mn, Ni, Pb, and Zn in different size fractions (< 1, 1-5, 5-50, 50-250 µm). The results showed that elemental RBA in finer particles was higher than in larger ones and that RBA in bulk soil was mostly correlated to soil properties whereas in fine fractions was possibly associated to the diverse pollution sources and their chemical forms. Goix et al. (2016) considered soils contaminated from smelting and mining activities. In the smelting area, all elements exhibited the same pattern, with a diminution of RBA when particles diameter decreased from 200 to 2 µm and an increase in the finest fraction (2-20 µm). Arsenic, Cd, Pb, Sb and Sn showed the maximum RBA in the 50-200 µm fraction, whereas Cu and Zn were more bioaccessible in the finest fraction. In the mining area, the minimum RBA of all the metals was found for 20-50 µm particles while the highest in 2-20 µm and < 2 µm fractions. Padoan et al. (2017), examining urban soils, pointed out that the RBA of Fe, Mn, Cr, Ni and Zn increased in the fine fractions (< 2.5 and 2.5-10 µm), with significantly higher values at traffic than at background sites, consistently with the idea of a lithogenic origin of the larger particles and a more anthropic, and bioaccessible, input of fine particles for these metals. Conversely, Cu, Pb, Sb, and Zn seem to have almost constant RBA in all size fractions.

Also, Qin et al. (2016) found that size had significant effects on the RBA of landfill contaminated soils, with a clear trend showing that As, Al, Cd, Cr, Cu, Mn, Ni, Pb, and Zn RBA increased with decreasing soil particle size, and the < 2 µm soil fraction presented also a higher concentration of the elements, as compared to coarser soil fractions.

Hiller et al. (2020), observed significantly higher RBA in < 50 µm particles than in < 150 µm for six metals, namely Co, Cu, Pb, Sn, Mn and Zn. However, no differences between fractions RBA were found for As, Cr, Ni and Cd. This could have been due to multiple factors; the primary explanation of the authors was the greater contact area of fine particles available for extracting solutions. Nevertheless, some other factors, as different physicochemical, geochemical and mineralogical properties among the grain sizes, also contribute. The bioaccessibility of Cd, Cr, Cu, Pb, Sn, Zn, and Ni showed a positive correlation with the total concentration, while As, Sb and Mn bioaccessibility was negatively correlated with Fe oxides. Correlation results also indicated a negative correlation between As, Co, Cr, Mn, Pb and Ni bioaccessibility and clay and silt contents and a weak positive correlation of As, Cd, Cu, Pb and Zn with TOC.

4.4 Discussion and health risk assessment

Generally, PTE contaminated soils can directly harm humans via oral ingestion, inhalation and dermal contact and people exposure to polluted soils may lead to serious health problems. In most cases, health risk assessment has been based on total concentration of PTE. However, not all PTE in soils are available to absorption (Huang et al., 2018; Han et al., 2020) and the use of total contents would somewhat overestimate the risk comparing with the use of bioaccessible contents.

The presented results highlighted differences in PTE distribution among different soil particle sizes, in bioaccessible concentrations and in RBA between the various PTE and sources of contamination.

Fine soil particles generally contained higher amounts of PTE, as previously reported, thus they contained, giving the same RBA between fine and coarser particles, higher bioaccessible amounts of elements, increasing the health risk associated to fine fraction.

Until now, we found only one article reporting the PTE bioaccessibility of nano-sized particles in soils. Dang et al. (2018) showed that the bioaccessibility of silver nanoparticles (0.5 – 10.9 %), was significantly lower than that of

$AgNO_3$ particles (4.7 – 14.4 %), as a result of the lower adsorption of nanoparticles to soil residues during the digestion process. Conversely, many studies investigated the bioaccessibility of PTE in different nanoparticles, such as Niu et al. (2010) on nanoparticles in atmospheric particulate matter. The results emphasized a general trend of bioaccessibility increase from coarse (1000-10000 nm) to fine (100-1000 nm) and nano (57-100 nm) fractions. Böhmert et al. (2020), described that nanoparticles can reach the intestinal epithelial cells after ingestion with only a slight reduction in their cytotoxic potential. Therefore, further studies should start to also investigate soil nanoparticles and their related health risks.

Although the number or studies is still limited, PTE relative bioaccessibility seemed to be more related to the source, and to the chemico-physical form of the PTE emitted from the source, than to specific soil parameters.

For diffuse sources, such as Pb in urban soils or PTE from smelting activities and in landfill soils, clay fraction (< 2 µm) had a higher RBA for most of the elements, due to the higher amount of adsorption sites, organic matter or oxides, accumulating PTE in a more reactive form, thus in a more bioaccessible form. In the case of point or natural contamination, the size of the particles concentrated in PTE depended strongly on the source.

Very few investigations compiled a risk assessment study using data from different particle sizes, allowing a comparison of the calculated risks. This is very important, as the choice of an incorrect size would underestimate or overestimate the risk. Li et al. (2020b) recommended to qualify the exposure risk from PTE using the fraction < 1 µm, the most toxic one in the case of the article. With the same idea, Goix et al. (2016) recommended to sieve dust/soil samples at < 50 µm before analysis to limit risk underestimation. Ma et al. (2019) used < 150 µm and < 250 µm size fractions for bioaccessibility estimation, concluding that their use could result in an underestimation of the carcinogenic risk, while the use of the < 63 µm fraction yielded carcinogenic risks close to the results obtained including detailed calculations of the proportions of particle sizes that adhere to hands. However, the use of such a small size would alter the results in case of contamination due to the addition of coarser particles, as, for example, in the case of soils in mining areas were very coarse debris could be added to nearby soils (Mehta et al., 2020).

The choice of the optimal size of particles for the bioavailability estimation and risk assessment is thus greatly dependent on the contamination source, but the < 150 µm size is generally a good compromise between easiness of sample preparation, as only a dry sieving is needed, and trustworthy and conservative bioaccessibility results.

4.5 Conclusions

The quest for a more accurate definition of PTE bioaccessibility has led to a plethora of methods and procedures for different elements and particle sizes. In fact, there is growing evidence that there is a strong relationship between PTE bioaccessibility and soil particle size. A general trend of higher PTE bioaccessibility in finer fraction was found, which is related to total concentration, element chemical form, and anthropogenic influence. However, it does not mean that the highest PTE bioaccessibility is always found in the finest size fraction due to the considerable variation in the distribution of pollutants across soils; also, different elements may exhibit different relationships between bioaccessibility and soil particles. Nevertheless, remediation of contaminated soils requires an assessment of the risk to human health and the environment and that should be based on more and more accurate measurement of the actual threat posed by PTE. Consensus should be sought towards a measure of bioaccessibility that is rapid and convenient.

5. Bioaccessibility of Pb in health-related size fractions of contaminated soils amended with phosphate

Li Yan[a], Giordano Annapaola[a], Ajmone-Marsan Franco[a], Padoan Elio[a*]

[a] University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco, 10095, Italy

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Abstract

Lead (Pb) contamination is one of the most significant exposure hazards to human health. Contaminated soil particles may be eroded and transferred either to the atmosphere (< 10 µm) or to streams; or they may be incidentally ingested (< 200 µm). Among strategies for the long-term management of this risk, one of the most cost-effective is the reduction of Pb mobility and bioavailability via amendment with phosphorus-containing materials.

To clarify the effectiveness of P amendment in reducing Pb mobility and bioaccessibility in different soil size fractions, an experiment was performed by adding a soluble P compound to a historically contaminated urban soil (RO), a

mining soil (MI), and an uncontaminated spiked soil (SP) at different P: Pb molar ratios (2.5:1, 5:1, and 15:1).

In the < 10 µm fraction of soils, P addition reduced bioaccessible Pb only in the SP soil at the highest dose, with little to no effect on RO and MI soils. Similarly, in the coarse fraction, Pb was immobilized only in the SP soil with all three P doses. These results were probably due to the higher stability of Pb in historically contaminated soils, where Pb dissolution is the limiting factor to the formation of insoluble Pb compounds.

The bioaccessible proportion of Pb (using SBET method) was higher than 70% of the total Pb in all soils and was similar in both fine and coarse particle fractions. Due to the enrichment of Pb in finer particles, this implies possible adverse effects to the environment or to human health if these particles escape from the soil. These results call for increasing attention to the effect of remediation activities on fine soil particles, considering their significant environmental role especially in urban and in historically low or moderately contaminated areas.

5.1 Introduction

Lead (Pb) is a ubiquitous contaminant and is a major health issue throughout anthropized areas due to the legacy of leaded gasoline, Pb-based paints, industrial contamination, and mining and smelting activities (Ajmone-Marsan and Biasioli, 2010). It is one of the most relevant elements for the non-carcinogenic risk posed to humans, due to its negative impact on neurological and cognitive development in children (Du et al., 2020; Li et al., 2021a; Zhou et al., 2019).

The two major pathways of human exposure to soil Pb are the incidental ingestion of soil and the inhalation of dust (Kastury et al., 2019; Zahran et al., 2013). The magnitude of risk is mainly related to the primary source (industrial processes, gasoline, mining), to the size of the particle and to the chemical form (speciation) of Pb in soil. Speciation affects Pb availability to plants and to the environment, and the bioaccessibility of Pb, which is defined as the fraction of the element potentially soluble in simulated biological conditions (Ruby et al., 1999).

Lead particle size distribution influences its mobility and transfer through ecosystems. Fine Pb-carrying particles (i.e. those < 10 μm in diameter) can be easily detached from soil by erosion and anthropogenic activities and can then enter the respiratory system (Kastury et al., 2018; Li et al., 2020c) or be transferred to a water body where Pb may become more available. Meanwhile, coarser particles (e.g., < 150 μm) could more likely be ingested, especially by children during their frequent hand-to-mouth activities (Ruby and Lowney, 2012).

One of the most cost-effective remediation strategies for the long-term reduction of the risk posed by Pb is chemical immobilization through the addition of phosphorus-containing compounds, the goal of which is the transformation of soil Pb into poorly soluble Pb-phosphates (i.e., pyromorphite or tertiary Pb-phosphate), leading to a decrease of the element mobility and bioaccessibility (Scheckel et al., 2013). Available examples of field application of P report a variety of P concentrations and addition methods but were, in general, applied to highly contaminated soils. Their effectiveness was mostly measured using *in vitro* methods mimicking the gastric environment at pH 1.5, evidencing a decrease in bioaccessibility from 10 to 20 % in urban and residential sites (Scheckel et al., 2013; Obrycki et al., 2016) to up to a 100 % decrease in highly Pb-contaminated sites (Bosso et al., 2008).

This method has rarely been applied to soils with low or moderate contamination (e.g., according to the Italian legislation, above 100 and below 1000 mg/kg of Pb soils are considered contaminated in residential areas while accepted in industrial areas). In urban soils, for example, although Pb is a common contaminant deriving from diffuse sources such as leaded gasoline, P treatment is seldom studied, probably because historically contaminated soils present less labile Pb forms than in highly contaminated areas (McBride, 2016); nonetheless, in urban areas, the use of P amendments may be a cost-effective and socially acceptable method to achieve a reduction of risk.

The formation of insoluble Pb-P compounds does not seem, however, to fully answer the need of environmental protection, because Pb-P contaminated particles can still be removed from the soil and transferred to other compartments (e.g., water or the human body), where they could experience different conditions, such as a different redox potential or pH, leading to an increased Pb mobility and availability in time.

Particularly, some researchers have found that, in natural environments, defective Pb-P compounds may form after P addition, with solubility products four orders of magnitude higher than the published Ksp (Yang et al., 2001), while different studies found that the performance of phosphate-induced in situ lead immobilization were affected by the overall site biogeochemistry (Xie and Giammar, 2007) or from specific conditions present, for example, at the soil-root interface, where the effect of microbial activity is not fully understood (Debela et al., 2010).

If the particles transfer to different compartments such as anoxic sediments, after erosion for example, some studies hypothesized that soluble organic matter as low molecular weight organic ligands or humic acids could dissolve Pb from phosphate minerals (Sauvé et al., 1998; Martinez et al., 2004). The fine particles (e.g., < 10 µm) are ones that more probably could undergo to such processes due to their mobility.

Thus, an important research gap is the assessment of this method’s immobilization efficacy on particles removable through wind or water erosion (< 10 µm) or relevant for incidental ingestion. This necessity is more evident in areas of diffuse, moderate contamination, as in most cases these are close to populations and not managed. Here, soil particles can freely spread to adjacent areas and increase health risk.

Fine particles are usually more mobile, reactive and enriched in metals (Ajmone-Marsan et al., 2008), and frequently have higher organic matter (SOM) and Fe-Mn oxides; if Pb is present in a fraction associated with SOM or Fe-Mn oxides, this may limit its reactivity with P (Debela et al., 2013; Wei et al., 2014).

The amount and type of P best added to moderately contaminated soils is ill-defined (Obrycki et al., 2017). Amendments from 4:1 to 15:1 P: Pb molar ratio have been proposed as effective in reducing bioaccessible Pb (Ryan et al., 2004); however, parameters for P addition vary within sites, as very large P quantities and acidic P amendments, such as phosphoric acid, could pose their own significant environmental concerns including P runoff, possible eutrophication and the possibility of an enhancement leaching of oxyanions such as Arsenic (As) (Dermatas et al., 2008; Weber et al., 2015). Furthermore, in urban areas the use of acid treatments may not be allowed by municipal governments, due to low public acceptance. Also, in case of large areas affected by diffuse contamination, it is important to consider variability in the chemical characteristics of soils, including soil pH, organic matter and Ca content, and Pb speciation, all of which can impact the effectiveness of soil amendments.

In this study, three different soils representative of Pb contamination cases were used to evaluate the fate of Pb in different soil size fractions when P is added as an amendment. All of the soils had a similar Pb concentration common in diffuse-contaminated areas in Europe. Lead availability and bioaccessibility were assessed using various extraction methods on two particle sizes that are generally considered to be representative of erosion and ingestion after amendment at different P: Pb rates.

5.2 Materials and methods

5.2.1 Soils characterization and amendment

The soils used for the experiments were: i) a roadside soil with a history of Pb contamination (RO) from the urban area of Torino (Italy); ii) a contaminated soil from a historical mining area (MI) and iii) a non-contaminated rural soil, spiked with Pb to simulate a contamination episode (SP). We selected RO from previous studies, as a roadside soil representative of average urban Pb pollution (Padoan et al., 2017); due to its position near a high traffic road in the peri-urban area, the most important source of Pb in this sample was presumably leaded gasoline. MI was sampled near the Pb and Zn mining area of Montevecchio (Sardinia, Italy) and, in contrast to the other soils, Pb here is mainly in the form of sulphide minerals (Garau et al., 2022). As the RO and MI soils have a neutral or basic pH, the SP soil was chosen for its acidic pH, which may influence Pb dynamics.

The SP soil was spiked with 500 mg/kg of Pb, similar to the concentration of RO and MI soils. Lead was added as $PbCl_2$ solution to promote chloropyromorphite formation (Yang et al., 2002). After Pb addition, the soil was homogenized through repeated mixing, watered to reach 60% of the soil's water holding capacity (about 20% by mass) and incubated in dark plastic containers at room temperature for one year.

The soils were sieved to < 2 mm and then characterized using the official Italian analytical methods for soils (Colombo & Miano, 2015). Soil texture was determined by the pipette method, pH was determined in deionized water at a 1:2.5 soil/solution ratio, total carbon and nitrogen (TC and TN) with an elemental analyzer (Elementar UNICUBE), and carbonates by a volumetric method (Padoan et al., 2021b).

A portion of each sample was further ground to 0.15 mm for aqua regia (HCl: HNO_3, 3:1) microwave digestion (Milestone Ethos D) according to ISO 11466.

On the extracts, Ca, Cu, Cr, Fe, Mn, Ni, Pb, and Zn were determined by flame atomic absorption spectrometry (FAAS) (Perkin-Elmer AA-400).

The three soils (RO, MI and SP) were amended with P and incubated for a further six months. Phosphorus was added as KH_2PO_4 at 2.5:1 (P2.5), 5:1 (P5), and 15:1 (P15) P: Pb molar ratios, which are commonly used proportions in remediation operations (Kastury et al., 2019; Obrycky et al., 2016). After the mixing of phosphate, triplicates of each P treatment plus an untreated control soil (CTR) were prepared. One hundred and fifty grams of soil were placed in dark plastic containers, watered to achieve 20% gravimetric water content (to ensure wet aerobic conditions) and incubated at room temperature. After defined intervals and at the end of the experiment, an aliquot of the soil was sampled from each container, air-dried, mixed, and analysed.

In the original and P-added soils total P was determined by H_2SO_4-$HClO_4$ digestion and malachite green colorimetric measurement (Ohno and Zibilske, 1991) and plant-available P was determined using the Olsen method (Colombo and Miano, 2015).

5.2.2 Pb availability and bioaccessibility assessment

Lead availability to the environment and to plants was estimated in amended soils using two extractants: i) 0.11 mol/L acetic acid (AA), which corresponds to the first step of the BCR procedure for chemical fractionation (Rauret et al., 1999); ii) EDTA, with a 0.02 mol/L EDTA solution containing 0.5 mol/L CH_3COONH_4 in 2.5% acetic acid brought to pH 4.65 ± 0.05. Five grams of soil were added to 25 mL of extractant, the bottles were shaken for 30 min, centrifuged at 3000 rpm for 10 min, and the supernatant filtered through cellulose filter (Whatman N°4) and analyzed by FAAS (Colombo and Miano, 2015).

No validated bioaccessibility methods are available for use with P-treated soils, as some studies have showed that both sequential extraction methods and, to some extent, *in vitro* bioaccessibility methods can cause the formation of pyromorphite during the assay, and thus creates artifacts in the results (Scheckel et al., 2013). To overcome this problem, Pb bioaccessibility was assessed using four different methods: US EPA Method 1340 (Ruby et al., 1999; US EPA, 2017) as written, and three modifications (i.e., with or without glycine at pH 1.5 and 2.5). The extraction at pH 2.5 was used, although it could cause an underestimation of the efficacy of the amendment due to artifacts (Scheckel et al., 2013), as earlier investigations suggested that this phenomenon should be limited in the presence of glycine (Barnett et al., 2011; Obrycki et al., 2016). Extracting solutions without glycine were used to assess the effect of the glycine buffer, as in the literature other methods using different organic buffers have been proposed for P-treated soils (Hettiarachchi et al., 2001). A 0.4 mol/L glycine solution (ReagentPlus, Sigma-Aldrich) was prepared, and the pH adjusted (to 1.5 ± 0.05 or 2.5 ± 0.05) with HCl. Solutions without glycine were prepared with deionized water and adjusted to pH 1.5 ± 0.05 or 2.5 ± 0.05, with HCl.

Duplicate extractions were performed by mixing 0.5 g of sample with 50 mL of extracting solution in 100 mL plastic bottles and shaking for 1 h at 37°C in a thermostatic shaker. The suspension was then centrifuged, and the supernatant filtered through cellulose filter (Whatman, N°4).

Selective sequential extraction (SSE) was conducted according to the modified BCR procedure (Community Bureau of Reference) before and after the experiment, to determine the effect of P addition on the Pb chemical fractions. In brief, metals are separated in four fractions through progressively stronger sequential extractions: i) exchangeable, acid and water-soluble; ii) reducible; iii) oxidisable; iv) residual (Rauret et al., 1999).

At the end of the P incubation, an aliquot of 50 g of soil was collected from each plastic container and size-fractionated. Bulk soils were first sieved to < 200 µm and partitioned into two fractions (< 10 and 10-200 µm). The particles were separated by repeated sedimentation and decanting as in Ajmone-Marsan et al. (2008); the process was repeated until the supernatant was clear.

Following separation, the fractions were air-dried and analyzed for total and bioaccessible Pb and P as described for the bulk soils.

5.2.3 Statistical analyses and quality assurance

All reagents used were of ultrapure or analytical grade. All analyses were made in duplicate, and results accepted when the coefficient of variation was within 5%. Blanks were below the detection limit in each batch of analyses (0.01 mg/L). Aqua regia results were checked using CRM 141R certified soil (Community Bureau of Reference, Geel, Belgium) in each batch of analyses; average recovery was 98.6 % (94-102%). For Pb extractions and accessibility assays, spiked matrix samples were included, and the average recovery was 98 ± 5%.

Statistical analyses, including analysis of variance (ANOVA), HSD and Tukey’s tests were conducted with SPSS software (v.23, IBM Corporation, Armonk, NY) and Microsoft Excel (v. 2016, Redmond, WA).

5.3 Results and discussion

5.3.1 Chemico-physical characterization

The soils were characterized for their main properties and elemental contents (Table 5.1 and Appendix. Table S3).

The SP soil was acidic (4.7), low in carbon and not contaminated, with an initial Pb concentration of 17 mg/kg. After the spiking, aqua regia extracted almost all the added Pb, with an extractable concentration of 476 mg/kg (Table 5.1). The RO soil was a typical roadside soil from the Torino area, with basic pH (8.3), a sandy loamy texture with Pb concentration of 451±15 mg/kg (Table 5.1) markedly above legislative limits for green and residential areas (100 mg/kg), most probably as a result of the use of Pb in gasoline and the consequent diffuse contamination (Li et al., 2021a). The MI soil was slightly acidic (6.4) and contaminated with Pb due to mining activities (522±9 mg/kg). All the soils had

Table 5.1 Selected soil physical and chemical properties, with standard deviations (SP: Spiked, RO: Roadside, MI: Mining).

	pH	Total C	Carbonates	Texture	Particle-size fractions (%)		Available P	Total P	Fe	Pb	Ca	Mg	K
		%	%		<10	10-200	mg/kg	mg/kg	%	mg/kg	%	%	%
SP*	4.7	0.61±0.02	1.54±0.64	Sandy Loam	29	61	15±0.4	510±28	1.98±0.17	476±17	0.62±0.03	0.94±0.02	1.66±0.12
RO	8.3	3.37±0.51	0.86±0.32	Loamy Sand	14	48	12±0.2	590±5	3.53±0.06	451±15	1.77±0.11	1.98±0.03	1.03±0.14
MI	6.4	3.47±0.38	0.81±0.21	Clay loam	26	48	4±0.4	377±3	3.47±0.5	522±9	0.17±0.03	0.33±0.02	0.35±0.01

* after Pb spiking and 12 months of incubation

average total P concentration prior to addition, with P: Pb mass ratios slightly higher than 1:1 for the SP and RO soils, with lower values for the MI soil.

5.3.2 Pb spiking and aging

After Pb addition, the SP soil was incubated for 12 months prior to P amendment. During this period, Pb extractability was monitored, and the results are reported in Figure 5.1. Aging is a process that leads to the stabilization of the freshly added Pb, and to a reduction of its availability over time, attributed primarily to surface precipitation, occlusion, or diffusion into micropores (Ma et al., 2006; Strawn and Sparks, 2000; Tang et al., 2006).

Lead availability was monitored using AA and EDTA extractions. For both indices, soluble Pb increased during the first 8 weeks after its addition, with a stabilizing trend visible after the third month of incubation. At the end of the incubation period, weakly bound (AA) Pb decrease was statistically significant ($p < 0.01$), while EDTA-extractable Pb concentrations were stable. This could indicate that aging increased the fraction of Pb adsorbed to soil amorphous oxides, which are extractable by EDTA but not by AA, as well as the fraction of Pb precipitated as poorly soluble salts.

The quantification of Pb distribution in the particle fractions of the three soils gave results consistent with those of recent studies (Padoan et al., 2017; Li et al., 2021b; Wang et al., 2021); Pb concentrated in the fine fraction, with higher total values in the < 10 µm particles than in the bulk soil (Table 5.2). The spiked soil had the highest share of Pb in the finest fraction, with a concentration nearly one order of magnitude higher than in the coarser and in the bulk soil, confirming the higher reactivity of this fraction that retained a large part of the added Pb.

When these concentrations were calculated over the proportion of each size fraction in the whole soil, the SP and MI soils confirmed that the < 10 µm fraction was the main Pb carrier, containing, respectively, the 81% and 60% of the total Pb; whereas in the RO soil the highest absolute amount of Pb was in the 10-200 µm fraction (Table 5.2). This could be due to the mixing of the fine fraction with extraneous, Pb-free, technogenic material, which is likely for a roadside soil as observed in other soils of urban areas by Madrid et al. (2008).

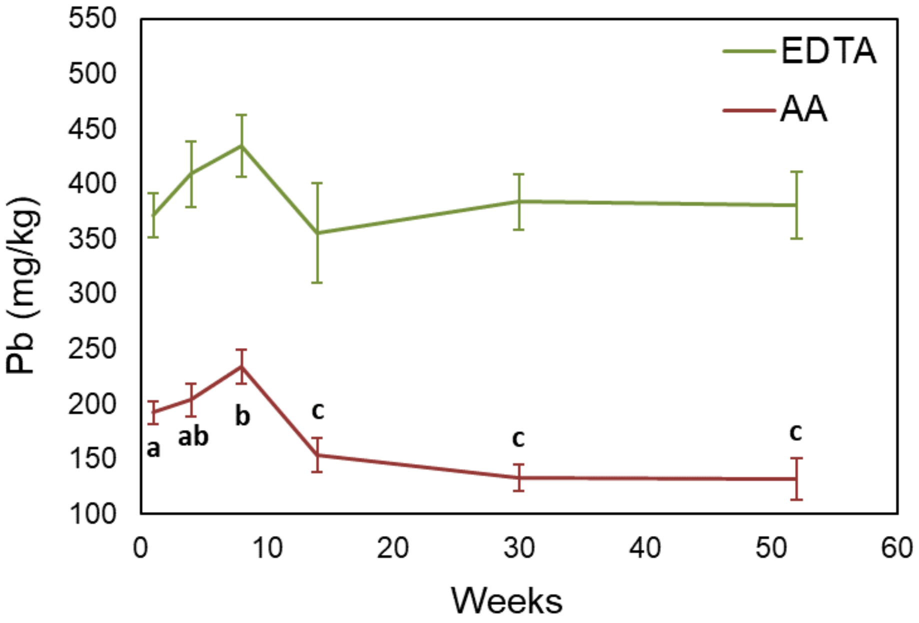

Figure 5.1. Variation of EDTA and AA extractable Pb concentration during incubation. Letters represent significant differences between the time points using Tukey HSD test ($p < 0.05$).

Table 5.2 Pb and Total Carbon (TC) concentrations in soil size fractions and, in the case of Pb, absolute concentrations calculated using the proportion of each fraction in the corresponding soil.

	Total Pb (mg/kg)		TC (%)	
	<10 µm	*10-200 µm*	*<10 µm*	*10-200 µm*
SP	1330 ± 13	145 ± 44	1.13 ± 0.01	0.14 ± 0.01
RO	710 ± 2	361 ± 16	5.00 ± 0.39	2.65 ± 0.18
MI	803 ± 17	288 ± 13	4.92 ± 0.01	2.75 ± 0.27

5.3.3 P addition and monitoring

After the addition of the P amendment, concentrations of Pb and P were measured at intervals. As expected, the available P concentration, which is a good proxy for the reaction of this element with the soil matrix, decreased with time, even at the highest dose (P15, reported in Figure 5.2).

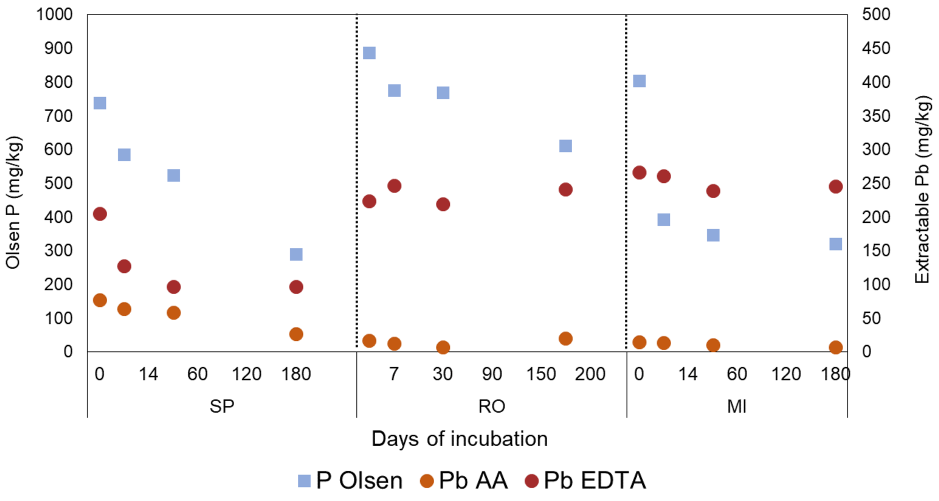

Figure 5.2. Variation of available P and Pb during the 6 months of incubation in the three soils for the P15 trial.

At the same time, Pb availability (as measured by AA and EDTA) showed a decreasing trend with a drop in correspondence of the maximum P addition, indicating the Pb reactivity reduction due to the P amendment (complete data are reported in Appendix. Figure S4 and S5). Extractable Pb decreased in the first weeks, but after 3 months both available P and extractable Pb concentrations stabilized for the remaining incubation time. At the end, almost all P added in the P2.5 and P5 trials had reacted, while the extra P loading added in P15 trial remained almost completely available in the RO soil, while in the SP and MI soils the increase in available P was the half of the incremental addition. This could indicate, probably, that the amount of P added was higher than the available adsorption or reactions sites.

5.3.4 Pb availability in the fine fractions

The availability of Pb in the < 10 µm and 10-200 µm fractions, measured by extraction with AA and EDTA after 6 months from the P addition, is presented in Figure 5.3. AA extraction is assumed to extract the readily exchangeable and acid soluble Pb fractions. The percentage of extractable Pb in the < 10 µm particles was lower than 5% of the total concentration for all soils and treatments, contrasting with the coarser size fraction. Due to these very low quantities, no statistically significant effects were seen in this fraction. The share of Pb bound to Fe-Mn oxides and SOM in fine fractions compared to coarser ones could have contributed to the limited Pb extractability (Sharifi and Renella, 2015; Landrot and Khaokaew, 2018; Xu et al., 2019b).

On the contrary, a significant Pb amount was AA-extractable from the 10-200 µm fraction – and consequently from the bulk soil – indicating that, with increasing particle size, Pb retention from soil surfaces is reduced. Differences emerged between the soils: in the SP soil, P addition was efficient at Pb fixation, and a decrease of extractability was observed with increasing P doses, from 34% of the total Pb with P2.5 to 23% with P15. A similar trend, less pronounced but still statistically significant, was observed in the bulk soil fraction in the RO soil whereas in the MI soil extractable Pb was low regardless of treatment.

The results are explainable considering that the treatment efficacy on Pb immobilization is rate limited by the release of Pb from the soil matrix. The SP soil was the most acidic and thus a higher Pb release was expected; this was further expected considering the higher lability of Pb in the spiked soil due to the limited incubation. In urban soils, conversely, Pb is primarily associated with Al and oxyhydroxide or crystalline FeO (Attanayake et al., 2015; Entwistle et al., 2020), which may retain Pb and limit the amount of Pb available to react with P. Similarly, in the case of MI soil, the limited dissolution of Pb compounds such as galena (PbS), cerussite ($PbCO_3$) or anglesite ($PbSO_4$) may have contributed to the low treatment efficacy.

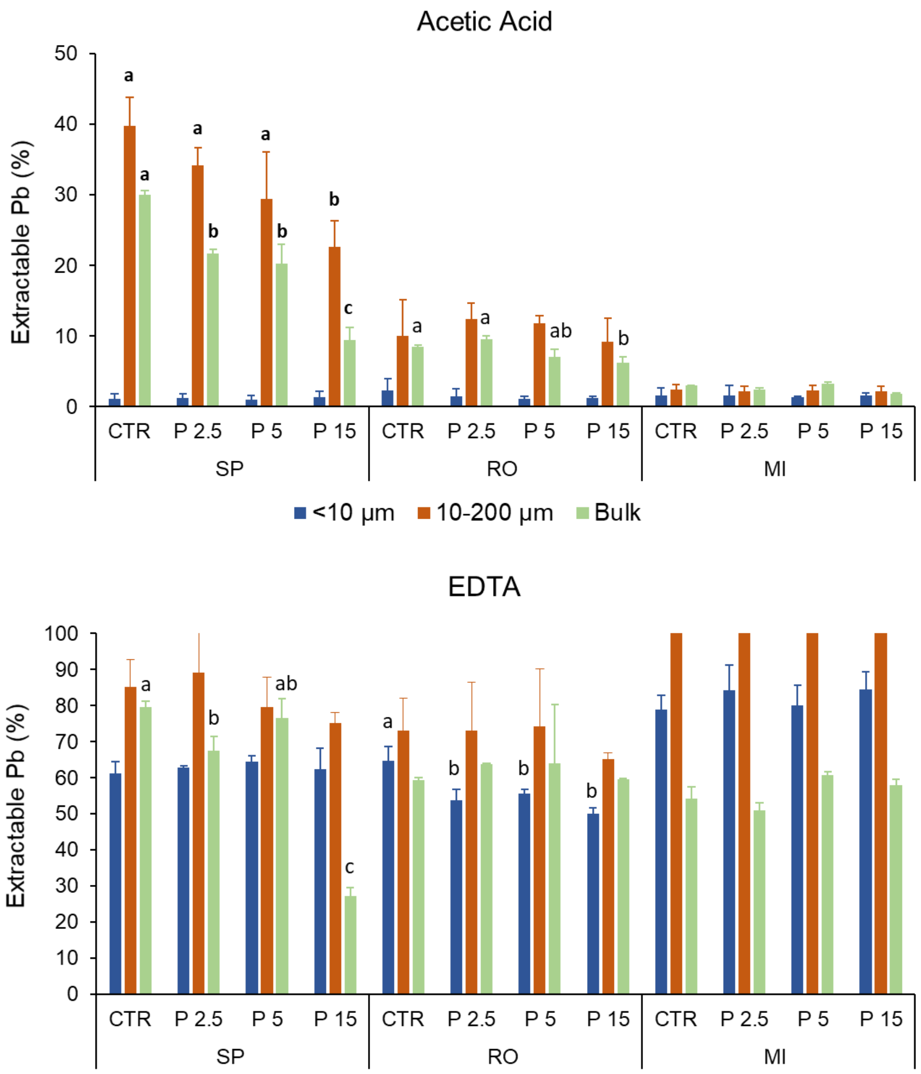

Figure 5.3. Pb availability (% of *aqua regia* Pb content) in the particle-size fractions (bulk = whole soil) six months after P addition. P2.5, P5, P15 are the P doses; CTR is the untreated control. If present, letters represent significant differences within the same soil for the different treatments (Tukey's HSD test, $p < 0.05$).

The extraction with EDTA removed, as expected, a higher proportion of Pb from all soils and size fractions than did AA. In general, this suggests that Pb stabilization by P is not very efficient if a chelating agent is in the system (e.g., fulvic acid low molecular weight organic ligands or humic acids) (Sauvé et al., 1998; Martinez et al., 2004). In general, stabilization, i.e., the inverse of EDTA extractable Pb, should be higher in the finest particles than in the 10-200 µm fraction in view of their larger specific area and general reactivity. However, fixation was obtained only in the fine fraction of RO soil, where the EDTA extractable Pb was significantly reduced from 65% (in the CTR) to 50% (in P15). A possible partial explanation of this result lays in the high Pb and organic matter concentrations in this fraction, which could have limited the efficiency of the EDTA extraction (high Pb lowers the EDTA: Pb ratio), thus artificially increasing the determined efficacy of the P amendment, as noted by Kim et al. (2003).

Considering the Pb content of the two particle fractions, the ratio was higher in the coarse (65 – 100%) than in the fine fraction (50 – 84%), promoting a more efficient extraction. In the SP soil an increase in stabilization with increasing P dose was evident in the 10-200 µm fraction and in the bulk soil, where P2.5 and P5 amendments determined a slight immobilization, while the higher addition (P15) lowered the EDTA-extractable Pb from 80% to 27 % of total Pb. This difference could be due to the different forms of Pb existing in the soil, or to the interference of Ca, Mg, K, Fe and other components that could react with P forming (in the case of soluble Ca) hydroxyapatite or being incorporated into the formed Pb-phosphates, destabilizing their structure (Mavropoulos et al., 2002). In this case, a very high amount of P may overcome this phenomenon resulting in a higher efficacy (Porter et al., 2004).

This fixation effect was not apparent in the other soils. This could be due also to the presence of other cations, Fe especially (Table 5.1), that would compete with Pb for EDTA chelation and decrease its efficiency (Kim et al., 2003). Conversely, in the coarse fraction of the MI soil, the high EDTA: Pb ratio and the low amount of competing cations explains the complete extractability of Pb.

From this data, P stabilization of Pb appears to be poor when measured with a complexing agent, especially in the coarse fraction, regardless of the type of soil or Pb source.

5.3.5 Pb bioaccessibility in particle fractions after P addition

The aim of P addition to Pb contaminated soils is to decrease the general availability of the metal, thus reducing the probability that the latter migrates to other environmental compartments. A major concern, however, is the possibility that small soil particles act as Pb carriers and, after escaping from the soil, end up in an environment where the metal is then released.

Six months after P addition, Pb bioaccessibility was evaluated in the < 10 and 10-200 µm size fractions using the USEPA 1340 method, at two pH levels (1.5 and 2.5) to ensure comparability with previous investigations, as it has been indicated that the pH can significantly affect results in P-amended soils (Obrycki et al., 2016; Ryan et al., 2004; Scheckel et al., 2013). It has also been suggested that the most suitable method for measuring Pb *in vitro* bioaccessibility would be a modification of the USEPA 1340 method, i.e., an extraction at pH 2.5 without glycine (Obrycki et al., 2016).

Our results for the bulk soils (Appendix. Table S4) showed that at pH 2.5 a smaller fraction of Pb was extracted than at pH 1.5 but considering the possibility of the precipitation of part of the Pb during the assay due to the extraction conditions, this does not necessarily imply better Pb stabilization and a consequent reduction of the harmful effect of released Pb.

The results for the two size fractions for the three doses of P addition are illustrated in Figure 5.4.

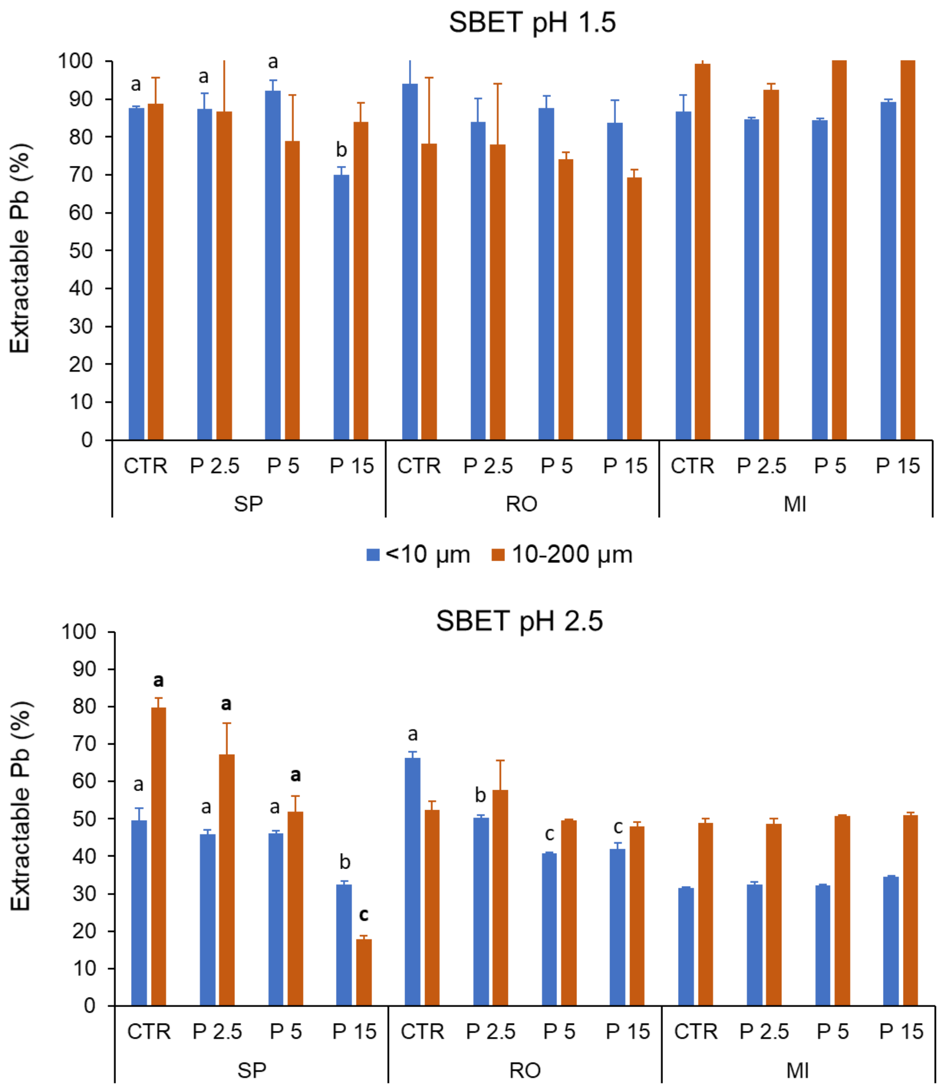

Figure 5.4. Pb bioaccessibility measured on the different size fractions after P amendment, reported as percentage of the aqua regia content control. P2.5, P5, P15 are the P: Pb doses. If present, letters represent significant differences within the same soil and size fraction for the different treatments (Tukey’s HSD test, $p < 0.05$).

Both extractions revealed that the P amendment had little effect in decreasing the bioaccessibility of Pb. At pH 1.5, original USEPA method 1340, which was validated by showing a strong *in vivo - in vitro* correlation, the extracted Pb in the < 10 µm fraction of SP soil decreased significantly with the P15 amendment, being 930 mg/kg in this fraction (the 70% of the total Pb), far higher than the trigger level of 100 mg/kg. A pH value of 1.5 represents a fasting pediatric gastric pH (Ruby et al., 1996) to produce a conservative estimate for risk assessment, but in these conditions any ingested soil particle would release, in our cases, between the 70 and the 100% of its Pb load. By contrast, in the 10-200 µm fraction, Pb stabilization was not evident. Contrasting results were obtained for the RO and MI soils: while in the former most of the Pb (more than 80%) was extracted from the < 10 um fraction (with a small tendency of stabilization visible), in the latter almost all Pb, which was supposed to be P-stabilized, was extracted from the 10-200 µm particles. No differences were observed with any amendment ratio in these two soils.

When Pb was extracted at pH 2.5, a decline in its bioaccessibility was observed for both size fractions in the SP soil, with the highest decrease in the 10-200 µm fraction, where extractable Pb decreased 4-fold in P15 compared to the control. In the RO soil a stabilization was significant in the < 10 µm fraction while in MI soil the results were comparable to ones found at the lower pH, without a stabilization effect. In general, bioaccessibility was higher in the 10-200 µm fraction than in the < 10 µm.

Using the original SBET pH 1.5 method, the majority of Pb species were solubilized, implying that the formation of chloropyromorphite and tertiary Pb phosphate was not achieved, as these compounds do not dissolve at pH 1.5 (Yan et al., 2020). This is also evident when comparing the results with some recent works using a different immobilization method (i.e. the formation of plumbojarosite), where Pb bioaccessibility was reduced by 77–97% after the treatment and spectroscopic results confirmed the presence of the target compound in treated soil (Karna et al., 2018; Kastury et al., 2021).

These results are in accordance with the EDTA results (Figure 5.3) and with previous works that observed a higher Pb bioaccessibility in the coarser fraction

(Li et al., 2021b; Ma et al., 2019). Kastury et al. (2019) investigated the effect of P additions on Pb in soil size fractions and found a decrease in Pb bioaccessibility and bioavailability in the < 250 µm and < 10 µm size fractions upon addition of P. The differences can be attributed to the different chemical speciation of the element, as Pb bioaccessibility in the bulk soil is more related to soil properties while in the fine particles it is dependent on the different pollution sources and associated chemical forms (Yang et al., 2001; Ma et al., 2019).

Cai et al. (2017) reported results on Pb bioaccessibility in urban soil amended with different P-containing materials, and Xu et al. (2019b), after potassium dihydrogen phosphate addition. The formation of Pb phosphates, particularly pyromorphite, is efficient at low pH, combined with very soluble phosphate and Pb forms (Chrysochoou et al., 2007).

In the MI soil, the slow dissolution of Pb-bearing primary and secondary minerals may have had an influence, as suggested by the results of Li et al. (2020b), who found the exchangeable and carbonate fractions to contribute almost 50% of the bioavailable Pb in two smelting-contaminated soils, and the Fe/Mn associated fraction to account for 76% of bioavailable Pb in a mining-contaminated soil. Also, high concentrations of Pb have been associated with the increase of organic carbon, Fe, Mn and Al oxides and clay minerals with decreasing soil size fraction (Li et al., 2020b) that may also limit the amount of available Pb to react with P; analogously, McBride (2016), on a number of urban soils, found that extractable Pb negatively correlated with SOM in bulk soils, but not with extractable P, suggesting that the binding of Pb by the SOM may play a more important role in limiting Pb lability than P. However, the long-term stability and mechanisms of SOM-associated Pb are still unclear. In our study, we found a significantly higher carbon content in the fine (< 10 µm) particle fraction than in the coarse (10-200 µm) (Table 5.2), which may imply a higher retention of Pb in fine particles.

5.3.6 BCR sequential extraction of Pb

Lead speciation was investigated using BCR sequential extraction in the bulk soils pre- and post-treatment and incubation. These protocols have been criticized as a tool to infer the exact speciation of a metal (Calmano et al., 2001; Scheckel et al., 2013) but they are useful in combination with other extractions. The extraction was not performed on the size-fractionated samples because of the water separation method could change the results due of a partial solubilization of the metal.

The soils displayed a major part of Pb in the second (Fe/Mn oxyhydroxides) fraction (Figure 5.5) but while in the SP soil 27% of Pb was present in the readily exchangeable fraction, this percentage was only 4% in the RO and 3% in the MI soils, and nearly 25% of Pb was present, in both soils, in the oxidizable or the residual fractions.

In the SP soil a decrease in the exchangeable fraction of Pb (from 27% to 5%) and an increase in the reducible (63% to 83%) and of the residual (5% to 8%) fractions was noted with increasing P dose. A similar result was reported by Sanderson et al. (2016) for Pb in shooting range soils. In the RO soil, by contrast, relevant changes in fractionation were between the oxidizable and the residual fractions, with an apparent stabilization of Pb. This stabilization, however, was not associated with a decrease in Pb availability, as seen above, thus the result could have been partly due to the formation of pyromorphite induced by the extraction procedure itself, as observed by Scheckel and collaborators (2013).

The results of the BCR extraction scheme corroborates the previous outcomes, with a lower reactivity of the Pb contained in RO and MI when compared to the SP soil.

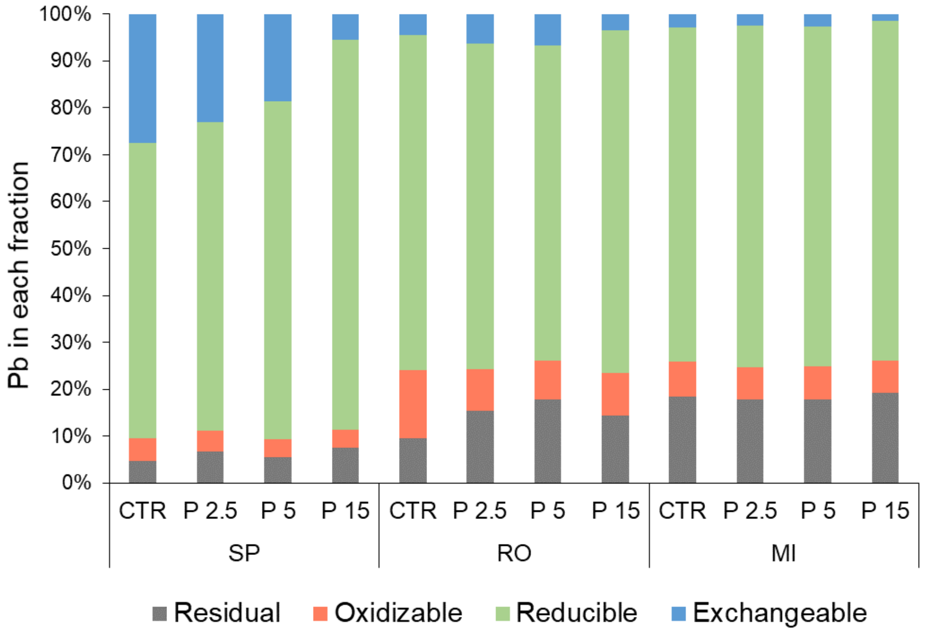

Figure 5.5. Pb fractionation in soils as percentage of the total concentration. Results are reported for control and amended soils.

5.4 Conclusions

Three different soils, representative of moderate levels of Pb contamination (a rural soil spiked and incubated for 1 year, an urban roadside soil and a soil from a mining area) were studied after amendment with a soluble P compound. We tested the amendment's effectiveness at promoting Pb immobilization in different size fractions representative of the inhalable (< 10 µm) and ingestible (< 200 µm) fraction using, as a measure, the decrease of the Pb availability and bioaccessibility.

In the bulk soil, the P amendment was effective only on SP soil, reducing Pb availability (by AA extraction) to almost 70% of the pseudo-total pool.

In the finest fraction of the soil, the P addition was effective on the spiked soil, where the highest P dose slightly reduced the bioaccessible fraction, while a small effect was visible in the RO soil and no effect was observed in the MI soil. This was probably due to a higher stability of the Pb phases in the two long-term contaminated soils, containing more organic matter in the fine particles, Fe oxides and Ca, all concurring to a stabilization of the Pb compounds.

The coarser, ingestible, fraction was more reactive to P amendments, and in SP soil an immobilization was evident with all three P doses and with different extractants. Also in this case, the effects on the RO and MI soils were low or not evident.

In general, P addition was poorly effective or ineffective on the historically contaminated roadside and mining soils and in reducing *in vitro* bioaccessible Pb due, probably, to low Pb solubility, and reactivity, in such soils. Thus, different P amendment types and application rates should be considered, taking into account the potential downsides of high P application, such as cost and eutrophication risks.

The Pb bioaccessible fraction was similar in both the fine and coarse particles, with a much higher concentration in the < 10 µm fraction implying possible adverse effects to the environment or to human health if these particles escape from the soil or are subjected to different environmental conditions.

To better reduce the risk of Pb in urban soils, it is important to consider that the variation in quality and composition of urban soils, including soil pH, Ca content, SOM, and chemical forms of Pb present in soil, can all impact the effectiveness of soil amendments. At the same time, more attention should be paid to the effect of remediation in fine soil size fractions for their environmental role.

6. Combining DGT with bioaccessibility methods as tool to estimate potential bioavailability and release of PTE in the urban soil environment

Li Yan[a*], Ajmone-Marsan Franco[a], Padoan Elio[a]

[a] University of Turin, Department of Agricultural, Forest and Food Sciences, Grugliasco, 10095, Italy

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Abstract

Potentially toxic elements (PTE) in urban soil environments pose a noticeable risk to both ecosystem and human health; however, only a fraction of the elemental content is available for biota. To better know the potential risk of PTE in the urban soil environment, geochemical fractionation, bioaccessibility, and potential bioavailability of four PTE (Cd, Cu, Pb, and Zn) were investigated by the combined use of different methods. The results showed that a high non-residual chemical fraction is related to a high bioavailability of the selected elements. The ranges of labile concentration of Cu, Zn, Cd and Pb in all sampling sites measured by diffusive gradients in thin films (DGT) were 3.5-

18.0, 14.2-26.5, 0.09-1.0, and 1.8-15.7 µg/L, respectively. The high non-residual contents pointed out a serious hazard to the urban environment. The bioaccessible concentrations in gastric and lung phases were closely positively correlated with DGT-measured content ($r = 0.63$-0.99, $p < 0.05$), suggesting the potential use of DGT for the prediction of PTE risk to human health. Moreover, the correlation of DGT results with the soluble and reducible fractions of PTE may allow DGT use for quick screenings of the PTE fraction potentially mobilizable during flooding events in urban soil environments. Our study suggests that combing DGT, bioaccessibility and biogeochemical fractionation could provide a more accurate assessment of the urban environmental quality and be helpful for pollution control and urban planning.

6.1 Introduction

In the last decades, the rapid industrialization and expansion of urban areas led to the dispersal of numerous metals and metalloids, commonly referred to as potentially toxic elements (PTE). In the urban environment, the soil, generally regarded as a sink of pollutants from different anthropogenic activities, has been contaminated to varying degrees and PTE are among the most hazardous pollutants due to their non-degradability, persistency, and high toxicity (Frohne et al., 2014; Padoan et al., 2020a).

Review studies of the available data showed that many cities around the world are contaminated by PTE (Ajmone-Marsan and Biasioli, 2010; Yu et al., 2012) as they tend to accumulate in soils. In urban environments, where soils are the recipients of large amount of pollutants from a variety of sources, such as industrial wastes and vehicle emissions, this may lead to the deterioration of the soil ecosystem, threaten human health, and cause other environmental problems (Li et al., 2021a; Qing et al., 2015). Therefore, it is particularly significant to monitor the presence and health risk of PTE in urban environment, especially in industrial cities.

It is well known that the toxicity and bioaccumulation of PTE are primarily related to their bioavailable fraction, the portion of elements that actually enters a receptor and cause effect on the organism (Naidu et al., 2008), rather than their total concentration (Paustenbach., 2000; Padoan et al., 2017; Han et al., 2020). Recent research has shown that the mobility of PTE in soils are inextricably associated with their geochemical fractionation (Khadhar et al., 2020; Kim et al., 2014). Thus, this study is essential to understand PTE mobility, bioavailability, and potential threats to the urban soil environment. To address this, sequential extraction methods (e.g., the three-step BCR; Rauret et al., 1999) had been widely used to determine the chemical speciation of different soil elements and their potential mobility. In addition, in an attempt to better characterize the reactivity of PTE, a variety of *in vitro* methods have been developed to assess PTE bioaccessibility in soils, which is the portion dissolving in a biological fluid, hardly determined by the BCR. These techniques include the physiologically based extraction test (PBET) (Ruby et al., 1996) for oral gastrointestinal bioaccessibility and the modified Gamble's solution (Wragg and Klinck, 2007) for lung bioaccessibility.

To complement these methods, the performance of the in situ technique of diffusive gradients in thin films (DGT) to predict elements bioavailability has been investigated in response to the increasing need to consider contaminant bioavailability in soil quality studies and risk assessments (Davison and Zhang, 1994; Sun et al., 2021). Numerous studies have reported that DGT measurement provides a reliable estimate of PTE uptake by plants (e.g. Tandy et al., 2011; Wen et al., 2020) and, in addition, DGT might also serve in PTE bioaccessibility studies (Camusso and Gasparella, 2006; Zhang and Davison, 2015). For instance, the results from Bade et al. (2012) showed that DGT measurement can predict Pb, Cu, and Zn bioavailability to earthworms in industrial-contaminated soils. Roulier et al. (2010) also found a significant correlation ($r^2 = 0.89$) between DGT-measured and total concentrations of Cu and Pb in Chironomus riparius in freshwater sediments. Further, the application of DGT to assess the bioavailability and potential human health risk in soils has received great attention, and it has been revealed that DGT technique is effective in prediction of the PTE bioavailability in contaminated soils and sediments (Bade et al., 2012; Ren et al., 2015). More recently, the human health risk due to PTE in urban park soils has been assessed by using the DGT method (Sun et al., 2021) while no studies addressed a range of urban soils contaminated from different anthropic activities.

Turin is the second-largest metropolitan area in north Italy and has a long industrialization history. This has led to heavy pollution of its soils (Biasioli et al., 2006; Li et al., 2021a). PTE contamination in urban areas may arise from point or diffuse sources and has specific characteristics, such as marked spatial variability, the coexistence of different contaminants and possible inclusion of anthropic materials, leading to changes of the soil physical and chemical characteristics (Biasioli and Ajmone-Marsan 2007; Ajmone-Marsan et al., 2019). Given the complexity of urban soil properties, the widespread PTE presence in the urban environment and the proximity of people to urban soils, a knowledge of PTE mobility and bioavailability is an important aspect of environmental evaluation. In this study, we combined BCR, *in vitro* oral and lung bioaccessibility, and DGT methods to: i) examine the geochemical fractionations, mobility, bioaccessibility and potential bioavailability for PTE; and ii) explore the correlation for different methods to predict the bioavailability of PTE in urban soils.

6.2 Materials and methods

6.2.1 Soil samples selection and characterization

Eight urban soil samples were selected from the sample dataset of a previous investigation in the wider Turin area (Fig. 6.1), whose goal was to assess the health risk due to PTE in the urban and peri-urban area (Li et al., 2021a). The soil samples were selected along a North-South directory, as the city has spread along this directory in the last 150 years; sampling areas were located within the main historical industrial areas of the city, some of them are now residential areas. The criterion for sample selection was the presence of PTE total concentration higher than the legislative limit (MATTM, 2006). Samples were taken in spring 2020 at a 0–10 cm depth and a composite soil sample at each site was obtained by mixing and homogenize three sub-samples taken 1 m away from each other. The collected samples were put in plastic bags, air dried at room temperature and sieved through a 2 mm plastic before further analyses.

The pH of soil samples was measured in 1:2.5 soil/water suspensions; total carbon (TC)and nitrogen (TN) were measured by an element analyser (CE Instruments, NA2100 Elemental Analyzer, ISO 10694), carbonates were analysed by the volumetric method (ISO 10693). Particle size distribution and fraction below 10 μm were measured and collected via the hydrometer method (Padoan et al., 2017). Briefly, 50 g of the air-dried soil (< 2 mm) was placed into a shaking bottle, add 200 mL of deionised water and 20 mL of Na-hexametaphosphate. Place the bottle on an end-over-end shaker and shake for 16 hours (overnight) at 15 rpm. Then transfer it into 25-cm decanting cylinders, after an appropriate time calculated by the Stokes' Law. The supernatant was siphoned off at a fixed depth below the surface and collected. After the deposit was resuspended in water, this procedure was repeated until the supernatant was clear at the sampling time. The deposits (< 10 μm) were air-dried, weighted, and stored for further analysis. Pseudo-total PTE were measured according to Ajmone-Marsan et al. (2019) using aqua regia microwave digestion and then determined by inductively coupled plasma mass spectrometry (ICP-MS, PerkinElmer NexION® 350D). Complete details on samples collection, and analytical methods are reported in the previous study (Li et al., 2021a).

A summary of PTE concentration and physicochemical properties for the selected samples is presented in Table 6.1.

6.2.2 BCR extraction

Selective sequential extraction was conducted on soil samples according to the modified BCR procedure (Community Bureau of Reference; Rauret et al., 1999). In brief, PTE were divided in four fractions through sequential extractions:

- Exchangeable, acid and water-soluble (F1, acetic acid extraction, 40 mL of 0.11 mol/L acetic acid solution);
- Reducible (F2, 40 mL of 0.5 mol/L hydroxylamine hydrochloride solution at pH 1.5);
- Oxidisable (F3, 10 mL of 30% w/v H_2O_2 twice then 50 mL of 1 M ammonium acetate at pH 2);
- Residual (F4, microwave digestion with aqua regia).

All the extracted solutions were centrifuged (3000 rpm, 15 min), filtered through cellulose filter (Whatman N°4), and analyzed by ICP-MS (PerkinElmer NexION® 350D). Before and after each extraction step, samples were weighed, as in case of a soil loss the result is an underestimation of the elemental contents in the subsequent steps (Padoan et al., 2020b).

6.2.3 In vitro gastrointestinal and pulmonary bioaccessibility

Gastrointestinal bioaccessibility of PTE in soil samples was determined using the physiologically based extraction test (PBET) (chemical composition and quantities are reported in the Table 6.1) (Ruby et al., 1996) on the < 250 µm fraction, chosen in view of its likely representation of the soil particles that would be adhere to children’s hands (Ruby and Lowney, 2012). In brief, 0.3 g of soil were weighted in 50 mL polypropylene conical centrifuge tubes, then mixed with 30 mL of the gastric extraction fluids. The solution pH was adjusted to 2.5 using concentrated HCl and the tubes shaken in an orbital shaker at 37°C for 1 hour. After the gastric extraction, the suspension was centrifuged at 3000 rpm for 15 min and the supernatant was filtered through a 0.45 µm membrane filter. Following the differences between the gastric and the intestinal phases, the simulated intestinal pH was adjusted to 7 using solid $NaHCO_3$ and bile and pancreatin were added to the extracting solution. After 4 h of agitation at 37°C, the soil slurry was centrifuged and filtered as before.

Lung bioaccessibility determination was performed using the optimized Gamble solution on the < 10 µm fraction of the soils (Li et al., 2021a) as this is the fraction potentially resuspendable through mechanical processes as erosion or during anthropic activities (Padoan et al., 2021a). The fraction was obtained by repeated wet sedimentation and decanting according to the pipette method as in Ajmone-Marsan et al. (2008). The < 10 µm samples (0.3 g) were weighted and mixed with the Gamble solution at a solid: solution ratio of 1:1000. The mixture was shaken at 37 °C for 24h, then centrifuged and filtered as above. All the samples were stored at 4 °C before analysis.

PTE bioaccessibility was calculated as a percent of the aqua regia content in the considered soil fraction.

6.2.4 DGT deployment and measurement

The DGT device (ZrO-Chelex, EasySensor Ltd) is composed of a filter membrane (0.80 mm thick), a diffusive gel (0.80 mm thick) and a binding gel (0.40 mm thick). The DGT was deployed in accordance with the procedure described by Ding et al. (2016). Soil samples (approximately 50 g) were weighted, placed in a 100 mL beaker, and mixed with ultrapure water to reach 80% of the water holding capacity (Gu et al., 2020). The well stirred soil was then covered with plastic wrap to prevent water evaporation and incubated for 48 h at a constant ambient temperature (20 ± 2°C). Then a small amount of rehydrated soil was homogenized transferred by a plastic spoon, shaking gently to make sure the soil settled fully and was on contact with the surface of the filter membrane. After that, more soil was added and gently pressed to ensure filling of the cavity entirely. The DGT device was placed into a plastic, semi-opened bag with some deionized water, to maintain moisture, and placed under constant temperature for 24 h.

After incubation, DGT core was retrieved from soil and unscrewed by using another base unit. The surface of the filter membrane was rinsed with deionized water immediately, the binding gel was retrieved and placed in a 4 mL centrifuge tube and eluted with 1.8 mL of 1 M HNO_3 for 24 h. The PTE concentration in the DGT extract liquor were converted into PTE mass (M) and calculated based on Eq. (1)

$$M = \frac{c_{e(V_e+V_g)}}{f_e} \quad (1)$$

Where C_e is the concentration of PTE in the extract liquor; V_e is the extract liquor volume (1.8 mL); V_g is the gel volume (2 mL); f_e is the extraction rate (Table 6.2). The DGT labile-concentration (C_{DGT}) was then calculated using Eq. (2)

$$C_{DGT} = \frac{M\Delta g}{DAt} \quad (2)$$

Where Δg is the diffusive layer thickness, which was 0.90 mm in our study; *D* is the PTE diffusion coefficient (Table 6.3); A is the DGT-device exposed surface area (3.14 cm^2 in our study); t is the deployment time (48 h).

Table 6.2 Extraction rate (f_e) of different elements (Wang et al., 2016)

Elements	Mn	Co	Ni	Cu	Zn	Cd	Pb
f_e	0.967	0.975	1.05	1.03	0.88	0.938	0.955

Table 6.3 Diffusion coefficient (*D*, 20 °C) of different elements ($10^{-6}cm^{-2}s^{-1}$) (Wang et al., 2016)

Elements	Mn	Co	Ni	Cu	Zn	Cd	Pb
D	5.14	5.68	5.69	5.74	6.00	5.55	7.32

6.2.5 Quality control and quality assurance

All extracting solutions were freshly prepared daily; all the extractions were carried out in duplicate including blanks; certified reference materials (CRM 141R) were included in the study. Relative standard deviations based on the mean values obtained for each replicate were accepted if lower than 5%.

6.2.6 Statistical analysis

Statistical analysis was performed using SPSS 20.0 software (IBM, U.S.), and plotting using Origin 2021 software (OriginLab Inc., U.S.). The correlations between DGT-measurement, BCR-fractions, soil properties and bioaccessibility in this study were evaluated using Pearson correlation analysis, with a significance level at $p < 0.05$ or $p < 0.01$.

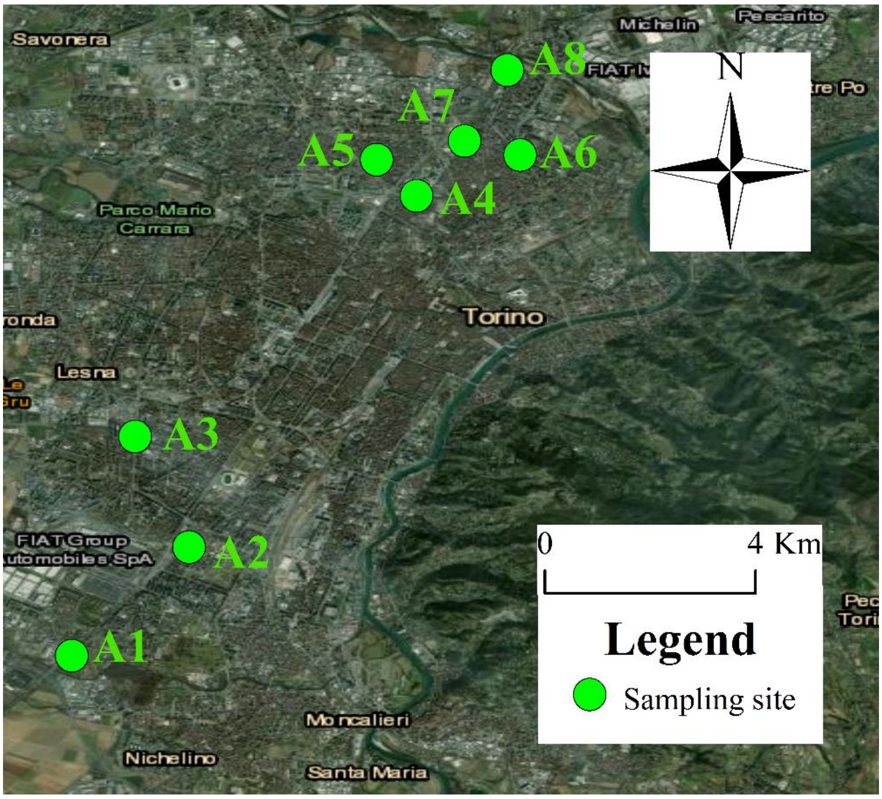

Fig 6.1. Sampling sites of urban soils in Turin

6.3 Results and discussion

6.3.1 Soil PTE total and DGT-labile concentration

The physicochemical properties of soil samples and their total PTE concentrations are presented in Table 6.1. Soil pH was neutral to alkaline, which may derive from historical inclusion in the soil of extraneous materials, such as concrete (Biasioli et al., 2006). Soil samples were rich in sand, with the content ranging from 52.3% to 84.7%. The total concentrations of PTE in all 8 samples were 84-258 mg/kg (Cu), 179-551 mg/kg (Zn), 0.6-7.9 mg/kg (Cd), and 130-1174 mg/kg (Pb). In comparison with the current Italian legislative limits (MATTM, 2006), part or all the elements in the soil samples exceeded the guidelines for residential and green areas, indicating a serious soil pollution in all the samples. Total organic carbon (TOC) contents were high in all the samples probably because all the sampled soils were under a tree line or grass-covered (Padoan et al., 2017). However, the significant correlation between Pb and TOC contents ($r = 0.73$, $p < 0.05$) could point to a carbon enrichment due to exogenous organic pollutants derived from road traffic in the samples, all sampled in roadside areas.

The ranges of the labile-DGT concentrations of Cu, Zn, Cd and Pb in all sampling sites are reported in Figure 6.2. Zinc was the element with the highest labile-bioavailable concentrations, with values ranging from 14 to 26 µg/L, while Cd had the lowest, in accordance with its lower total concentrations. Considering the ratio between DGT-labile and total PTE concentration, conversely, Cd had the highest DGT-labile ratio, while Pb had the lowest. These labile concentrations were, however, substantially higher than values reported in other studies on urban soils, with, for example, all the samples showed Pb concentrations higher than those reported by Xu et al. (2019a) for Shenzhen (China) soils and by Sun et al. (2021) for park soils in Beijing (China). As for other elements, like Cd, Cu, and Zn concentrations were higher than those reported for roadside samples in Melbourne, Australia (De Silva et al., 2020).

Our results indicate a high bioavailability of the PTE in the urban environment, probably because of the long industrial history of the city, leading to anthropic PTE contamination. In urban areas it has been shown that elements from anthropogenic sources are generally more soluble and bioavailable than natural ones (Padoan et al., 2017; Hernández-Pellón et al., 2018).

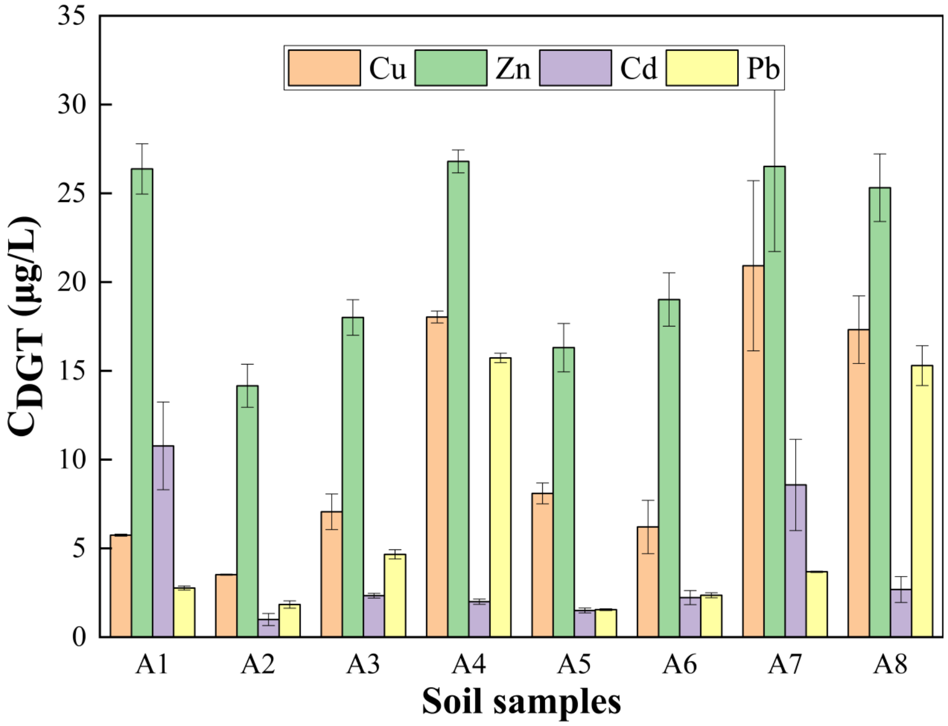

Figure 6.2. Labile-DGT measured concentrations (average and standard deviation bar) of PTE in the urban soil samples (Cd concentrations have been multiplied x10 to enhance visibility).

Table 6.1 Physicochemical properties and total concentration of PTE in soils.

Soil	pH	TOC (%)	Soil Texture (%)			Total concentration (mg/kg)			
			Clay	Silt	Sand	Cu	Zn	Cd	Pb
A1	6.3	4.59 ± 0.05	3	29	68	94 ± 1	322 ± 6	4.6 ± 0.06	196 ± 1
A2	7.8	3.02 ± 0.08	5	20	75	81 ± 2	179 ± 7	0.6 ± 0.05	130±5
A3	7.6	3.56 ± 0.13	3	23	74	116 ± 3	287 ± 13	1.8 ± 0.05	487±19
A4	7.3	5.77 ± 0.09	1	17	82	193 ± 5	467 ± 12	2.3 ± 0.02	1174±15
A5	7.5	2.50 ± 0.07	6	38	56	89 ± 3	249 ± 14	1.3 ± 0.06	118±5
A6	7.6	3.50 ± 0.08	5	35	60	112 ± 3	341 ± 6	1.8 ± 0.05	216±6
A7	7.4	4.10 ± 0.15	7	41	52	257 ± 7	508 ± 16	7.9 ± 0.25	317±10
A8	7.6	4.23 ± 0.07	0	15	85	183 ± 3	551 ± 12	3.1 ± 0.04	1070±22
Legislative limit for residential areas [a]						120	150	2	100

a: MATTM, 2006

6.3.2 Geochemical fractionation of PTE

The results of the modified BCR sequential fractionation of Cd, Zn, Cu and Pb in the urban soils are summarized in Figure 6.3. The F1 fractions of Cd, Zn, Cu and Pb encompassed the 13-26%, 11-19%, 1-6% and 0.6-6% of the total concentrations, respectively. As the F1 represents the most mobile and potentially bioavailable fraction of PTE, Cd and Zn appear to pose a higher risk to the urban soil environment than Cu and Pb, in agreement with the results from DGT measurements, where Cd and Zn were the elements with the highest DGT-labile fraction.

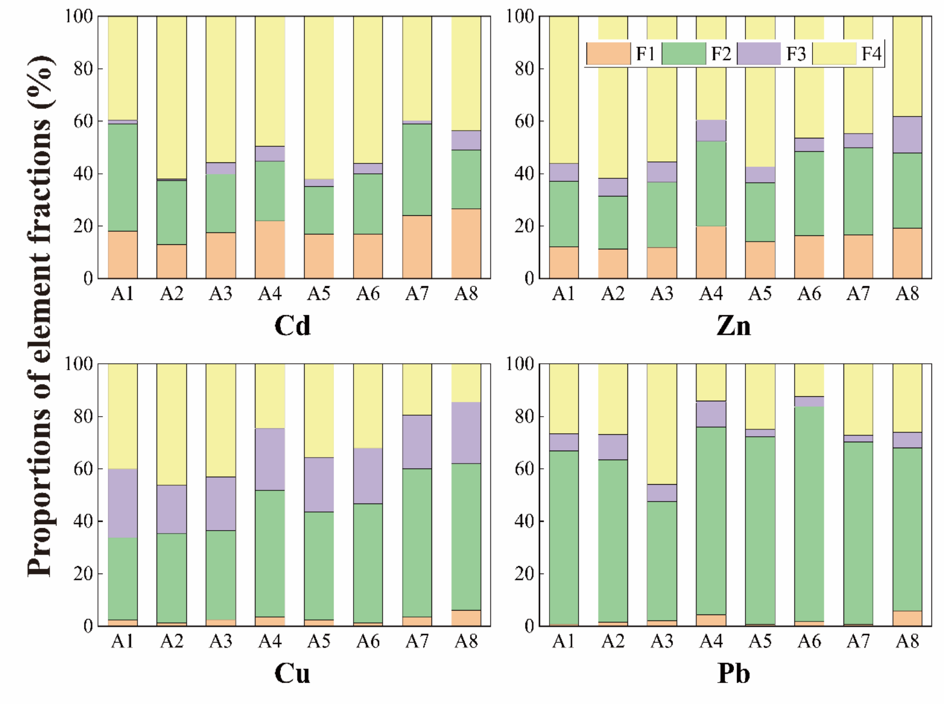

Figure 6.3. Geochemical fractions (% of total content) of PTE in the urban soil samples.

The F2 fraction accounted for the 18-41% (Cd), 20-33% (Zn), 31-56% (Cu) and 45-82% (Pb) of total PTE concentrations. These relatively high portions indicate that Fe-Mn oxideshave an important influence on PTE stability in soils, particularly Pb, an element widespread in urban areas. As oxides are redox sensitive and could release the metals under reducing conditions, this is an important information in the case of Turin, lying at the confluence different rivers, where the flooding of some areas is a growing problem due to the increase of extreme climatic events (Ajmone-Marsan et al., 2019).

The F3 fraction was less important for Cd, Zn and Pb, while not for Cu, due to the affinity of this element for the soil organic matter, as described also in previous studies in different urban areas of the world (Ajmone-Marsan et al., 2019; Mokhtarzadeh et al., 2020). The sum of the non-residual fractions (F1+F2+F3) accounted for the 38-60% (Cd), 38-62% (Zn), 54-85% (Cu), and 54-88% (Pb). Considering that these fractions are more mobile and sensitive to changes in the soil environment, they can become potentially bioavailable in response to human-induced or environmental changes, thereby posing a serious hazard to the urban environment.

6.3.3 In vitro bioaccessibility of PTE

Gastrointestinal bioaccessibility

The PBET method was used to measure the bioaccessibility of PTE in the gastric and intestinal phases (Figure 6.4). The average bioaccessible fraction of PTE in the urban soils decreased in the order Cu (52.9%) > Pb (39.9%) > Cd (32.8%) > Zn (26.2%) in the gastric phase and Zn (20.2%) > Pb (16.1%) > Cd (10.8%) > Cu (6.7%) in the intestinal phase. All the elements had a higher bioaccessibility in the gastric than in the intestinal stage, which is consistent with previous studies (Monneron et al., 2020; Sun et al., 2021). Lead, Cu and Cd displayed the largest decrease between the two stages, on average. This was expected in view of the higher pH of the intestinal phase and the consequent lower solubility of the elements (Du et al., 2020; Li et al., 2021a). For example, by precipitation as relatively insoluble carbonates, sulphates and phosphates, or due to re-adsorption on the particle surface of oxides and clay minerals when they entered into the carbonate enriched intestinal environment (Liu et al., 2021). Complexation by organic matter as pH rises, leading to insoluble complexes, may explain the marked decrease of Cu bioaccessibility from the gastric to the intestinal phase. Candidates for complexants include the bile acid anions chenodeoxycholate and hyodeoxycholate, the malate ion from malic acid added, and soil organic matter. The addition of bile salt has been looked as a discriminating factor, as Cu complexes and aggregates have been shown to be more stable than Zn and Cd ones (Turner and Ip, 2007).

The presence of different PTE sources in industrial urban areas also contribute to the differences in bioaccessibility. When compared to samples coming from mining or smelting areas, as it has been observed that, in urban and periurban areas, elements deriving mainly from anthropogenic sources, such as the four PTE studied, have, generally, a higher bioaccessible fraction than in soils where they derive only from natural processes (Hernández-Pellón et al., 2018).

Lung bioaccessibility

PTE tend to concentrate in fine soil particles, thus posing potential health risks by directly entering in the lungs and then possibly in the blood system via inhalation of the resuspension soil particles (Li et al., 2021a). The soil fraction prone to suspension due to wind or mechanical disturbances, and likely susceptible to enter the lungs is the <10 µm fraction, the so-called thoracic fraction of particulate matter. Total PTE concentration in the <10 µm particles are reported in Table 6.4 while bioaccessibility results are shown in Figure 6.5 as percentage of total PTE concentration (<10 µm).

Determined *aqua regia* concentrations were higher than ones found in the bulk soils (up to threefold), a trend also observed in previous studies and due to different phenomenon, such as the increase of sorption due to the high specific surface of fine particles or the anthropic emission of PTE associated with fine particulate matter (Ajmone-Marsan et al., 2008). On average, the increase was more pronounced for Pb (2.5 times higher) and Zn (2.3) while less for Cu (1.9) and Cd (1.8). Also, it was higher in some of the more contaminated soils, such as the A3 and the A8, both roadside sites from trafficked avenues.

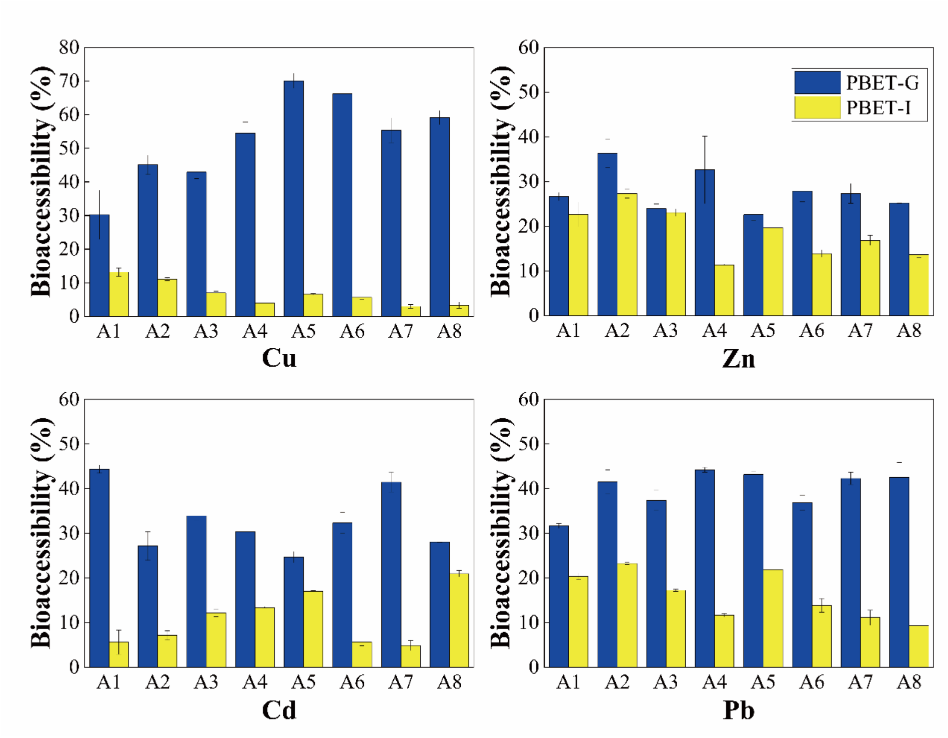

Figure 6.4. *In vitro* gastric and intestinal bioaccessibility of PTE in urban soil environment.

Table 6.4 Total concentration of PTE in < 10 µm fraction of soils.

Soil	Total concentration (mg/kg)			
	Cu	Zn	Cd	Pb
A1	146 ± 3	558 ± 22	6.4 ± 0.3	318 ± 14
A2	165 ± 10	432 ± 17	1.2 ± 0.1	379 ± 16
A3	334 ± 13	967 ± 43	4.1 ± 0.2	1554 ± 53
A4	319 ± 13	1099 ± 38	3.8 ± 0.1	2980 ± 104
A5	153 ± 5	426 ± 14	2.1 ± 0.1	296 ± 9
A6	177 ± 3	571 ± 7	2.4 ± 0.1	422 ± 12
A7	391 ± 10	850 ± 16	11.8 ± 0.2	631 ± 14
A8	465 ± 16	1720 ± 39	7.2 ± 0.2	3426 ± 102

Bioaccessibility in the < 10 µm fraction varied between the different elements (Figure 6.4). Lead, Cu and Cd had a relatively high bioaccessible fraction on average (more than the 40% of the aqua regia measured content) while Zn displayed lower values (with an average 21%). This might be the result of the geological origin of the elements as well as some other anthropogenic effects. For instance, Pb in industrial regions is typically in the form of low-solubility components, such as PbS or $PbSO_4$, when compared to residential areas (Huang et al., 2018), Cd oxide and Cd chloride are easily dissolved in the lung phase, while Cd sulphide not (Pelfrêne and Douay, 2018). In addition, the presence of cysteine in the simulated lung solution provides thiol groups that strongly coordinate with Cu and Pb, could also led to the relatively high bioaccessibility of Cu and Pb (Li et al., 2021a). Contrarily, Zn solubility is less controlled from organic matter presence, while pH is the dominant factor affecting Zn solubility, greatly decreased at lungs pH (Alloway, 2013).

The lung bioaccessibility in this study is relatively higher than what has been reported by other authors (Drahota et al., 2018; Huang et al., 2014), suggesting a higher risk in the urban soil environment. In particular, PTE associated with fine soil fraction would have a higher bioaccessibility when absorbed into alveolar-interstitial region during human breathing (Guney et al., 2016; Li et al., 2021a).

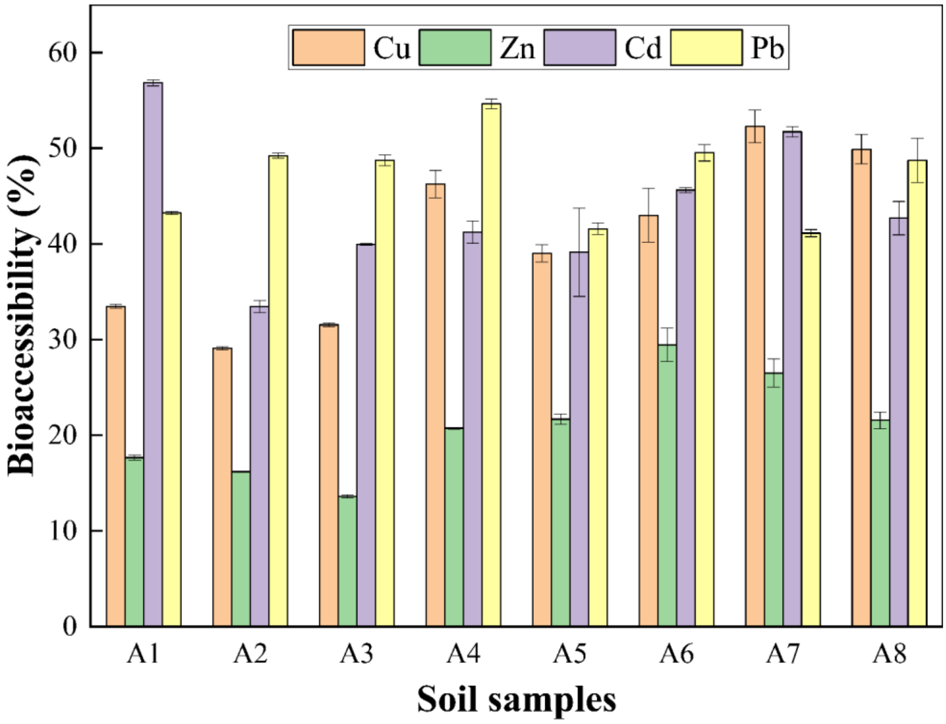

Figure 6.5. *In vitro* lung bioaccessibility (% of the total content in the < 10 μm fraction) of PTE in the urban soil samples.

6.3.4 Relationship between different measurements and soil properties

In the last years, DGT-measured concentrations have been used to predict PTE bioavailability based on the comparison of DGT results with animal models or some specific extractions (Liu et al., 2019b; Menegário et al., 2017). To better understand the mechanisms of different bioavailability assessment methods, we further explored the relationship between DGT-extractable PTE, bioaccessible concentration and BCR fractions (Figure 6). As DGT procedure more accurately reflect the mobility and bioavailability of PTE when compared to allosteric measurements, it has been proposed that the labile fractions of PTE detected by DGT can be absorbed by organisms, realistically reflecting PTE risk to humans (Sun et al., 2019). Therefore, a correlation analysis was performed to investigate the relationship between the concentration of PTE in the different fractions of soils, the bioaccessible concentration, and the amount of the PTE accumulated in the DGT (Bade et al., 2012).

DGT and bioaccessible concentration

The application of the DGT approach to predict bioavailability and toxicity relies on the assumption that labile components of PTE are able to pass through cell membranes and hence be detrimental to different organisms (Davison, 2016; Guan et al., 2021). In our study, all the elemental concentrations accumulated in the DGT were positively correlated with the bioaccessible concentration in gastric and lung phases ($r=0.63-0.99$, $p<0.05$), while only Pb-DGT values were positively correlated with the intestinal phase concentrations. Similarly, Bade et al. (2012) found that the bioavailable concentrations of Pb, Cu, and Zn estimated by DGT were correlated with the concentrations in earthworms, as well as with the gastric and intestinal concentrations estimated by PBET ($r>0.65$, $p<0.05$). Also, Ren et al. (2015) reported Zn, Ni, and Cd concentrations measured by DGT as positively correlated with the concentration extracted by PBET in the gastric and intestinal phases ($r>0.68$, $p<0.01$). Conversely, Sun et al. (2021), working on Pb bioaccessibility in urban soils, and Liu et al. (2021), investigating the bioavailability of Cr, Cu, Zn, Cd, and Pb in sediments, reported no significant correlations between DGT measurements and PBET bioaccessibility. The different results of the cited studies could be attributed to the fundamental difference in the measurement mechanism between DGT and bioaccessibility methods. DGT relies on a dynamic diffusion process over the time of the deployment (24h) whereas PBET is an exhaustive extraction (Liu et al., 2021).

These results indicate that the DGT technique can be a tool to predict the potential bioavailability of PTE in urban soils and could be a good indicator of the potential risk of soil contamination to humans, being moreover a much simpler method compared with both *in vivo* and *in vitro* techniques.

DGT and BCR

Despite its drawbacks, such as partial dissolution of a given soil fraction due to reabsorption and reprecipitation, as well as dissolving of non-target phases, sequential extraction has been routinely employed to evaluate the mobility and potential bioavailability of PTE (Li et al., 2015; Mokhtarzadeh et al., 2020). In this study, a positive correlation between DGT-measured content and the potential bioavailable fractions determined by BCR (F1+F2+F3) was observed for all the elements (Figure 6). Lead and Cu were the elements where the correlation was stronger (r>0.95, $p<0.01$) and especially with the readily exchangeable or weakly acid soluble fraction (F1) and the reducible fraction in the soil (F2). The correlation between the sum of the first two fractions and DGT-measured values was also very significant, ranging between 0.86 (for Cd) and 0.99 (for Pb). These forms are the ones easily captured by DGT due to their mobility in a wet soil, as requested from the DGT measurement method.

The correspondence between the first fractions of sequential extractions and the metals measured using DGT agreed with results reported in earlier works on sediments (Ren et al., 2015; Roulier et al., 2010; Yin et al., 2014). The authors suggested that the metals extracted in the BCR fractions were likely the main source of labile DGT uptake, with the fractions easily exchangeable, specifically sorbed to oxides or weakly bound to labile organic matter participating to the flux of resupply to the porewater that is available for DGT. This was happening in a reducing environment and thus, although the small number of samples studied in this work, we could hypothesize the use of DGT as a simple, effective, and reliable predictor of the PTE release as sum of soluble and reducible fractions from urban wet soils or under reducing conditions (e.g. occasional flooding).

Bioaccessibility and BCR

Some studies reported that PTE chemical speciation is strongly associated with their bioaccessibility (Chi et al., 2020; Li et al., 2022) as the first three fractions have a greater potential to mobilize from soils and become bioavailable when the boundary condition change, as in the case of ingestion or inhalation.

Here, the bioaccessible concentrations in gastric and lung phases had a strong positive correlation with F1 and F2 ($r > 0.84$, $p < 0.01$) (Figure 6.6), while the intestinal phase not (except for Pb). These findings further suggest that weakly-sorbed and Fe/Mn oxides-bound species were easily released in gastric fluids and might comprehend an important share of the bioaccessible fraction. These results are consistent with other studies reporting that the exchangeable fraction (F1) significantly contributes to the bioaccessible PTE (Liu et al., 2021; Ren et al., 2015; Xie et al., 2019). The reason for the insignificant correlation with the intestinal phase may be the difference in pH levels, as the BCR (F1+F2) and gastric extractions are acidic while the intestinal extraction is weekly alkaline (Ren et al., 2015) so they extract different forms of the elements. Li et al. (2020b) made a comparison of the in vivo oral bioavailability with the Tessier sequential extraction and found exchangeable and carbonate fractions contributing nearly 50% of the bioavailable Pb in two smelting-contaminated soil, while Fe/Mn fraction accounted for the 76% of the bioavailable Pb in a mining-contaminated soil, highlighting the importance of the speciation on the bioavailability.

DGT and soil properties

DGT-extractable concentration is also affected by the soil chemical properties (Liu et al., 2019b). All the elements were positively correlated with the total concentration ($r > 0.84$, $p < 0.01$), whereas no correlation was observed for pH and TOC, similarly to the results of Che et al. (2020) and Xu et al. (2018), possibly because of the narrow variation range of pH and TOC. Also, no correlation was observed with soil texture (except for Pb), consistently with Liu et al. (2021). This lack of correlation between DGT measurement and soils properties may be due to the nature of the DGT technique. Some authors have described DGT as a holistic approach, thus a technique already including the effects of different soils characteristics in the measurement (Tian et al., 2008).

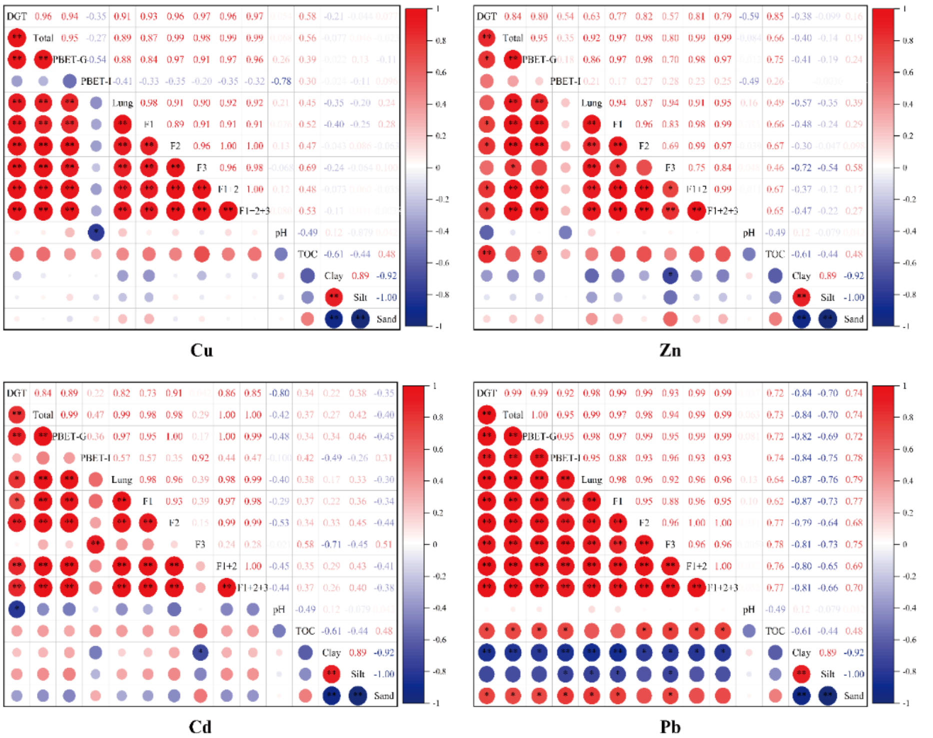

Figure 6. Correlation between DGT extraction, bioaccessible concentration in gastric (G), intestinal (I), lung phase, BCR fractions and soil properties in urban soil environment. (Red colors represent positive correlation, blue colors represent negative correlation, relativity increases as size of the circle, * $p < 0.05$, ** $p < 0.01$).

6.4 Conclusions

The geochemical fractionation, bioaccessibility, and bioavailability of PTE in the urban soil environment were investigated by BCR, PBET, and DGT methods. The PTE concentrations in almost all the selected soils exceeded the Italian guidelines for residential areas. The DGT-extracted concentration was also high, indicating a high bioavailability of the PTE to plants and potentially the environment. Considering the ratio between DGT-extracted and total PTE concentration, Cd was the most mobile elements, while Pb the lesser.

More than the 50% of Cu and Pb were in the non-residual, thus potentially mobilizable, fractions according to BCR method. The DGT-measured contents of PTE were highly correlated with the exchangeable and reducible fractions, indicating DGT as a good predictor for the PTE release from soils under reducing conditions. Further studies are needed but this would be an easy tool to predict metal quantities released from soils due to intense rain episodes or flooding.

The bioaccessible fraction was higher in the gastric than in the intestinal phase for all metals, with values up to the 50% of the total content for Cu and lower values for others, in the order Cu > Pb > Cd > Zn, indicating a serious hazard to the urban population and environment, especially for children due to inadvertent oral ingestion. The bioaccessible concentrations were closely related to the DGT extraction, suggesting that the DGT technique could be a tool to predict the potential risk of soil contamination in urban areas.

The fine fraction of the soils, enriched in PTE up to threefold, was studied by lung bioaccessibility. PTE had a relatively high bioaccessibility compared to the literature, evidencing the anthropic origin of the contaminants.

The significant relationships among DGT-measures, BCR-fractionation, PBET and lung-extraction indicate the potential use of DGT for the in situ prediction of elements bioaccessibility and bioavailability. Overall, this study provides a more accurate assessment of the urban environment quality and be helpful for the pollution control and urban planning, although more research is needed to in-depth investigate the mechanisms to predict PTE.

7. General conclusions

The work investigated the size-related bioaccessibility, potential bioavailability, and human health risk of PTE in urban soils. Soil samples were collected along a north-south transect across Turin in urban and peri-urban areas, and the results will help the comprehension of the potential risk of PTE posed to human in the urban environment and would be helpful to implement better management for soil contamination and remediation.

The observed average PTE contents and physico parameters of soils were in line with previous works in this area. Concerning PTE bioaccessible percentages, they exhibited a decreasing order of Pb > Cd, Cu > Zn > Co > As, Ni, Sb > Cr in the gastric environment and Pb > Cd, Cu > Zn > Co > As, Ni, Sb >Cr regarding lung bioaccessibility. The average bioaccessibility values suggested that PTE would more bioavailable if ingested than inhaled, and a higher solubility of Pb, Cd, Zn, and Cu was found using both methods, which may reflect a higher level of anthropogenic pollution.

Human health risk was assessed for the combined ingestion and inhalation pathways, using the bioaccessible fractions in simulated fluids. An unacceptable non-carcinogenic risk was found through ingestion exposure for children in some urban sites and Pb was the most hazardous element. Carcinogenic risks were under the threshold levels for all samples, while Cr and As being the dominant contributors to risk. Therefore, necessary soil remediation activities are needed to reduce the risks of human, especially for children, exposure to Pb.

Soil size fractions play a very important role in PTE bioaccessibility, we provide an overview of the relations between PTE bioaccessibility and soil particle size fractions. The available research indicates that PTE bioaccessibility distribution across different size fractions varies widely in soil, but a general trend of higher bioaccessibility in finer size fraction was found, which is related to PTE total concentration, chemical form, and anthropogenic influence. Our review attributed the variability to site-specific soil effects and chemico-physical forms of PTE bioaccessibility in different size fraction. Hence, further validation and intentional study of influential factors in PTE bioaccessibility, conversion of the bioaccessibility to relatilive bioavailability, and investigation into correlative influences on the bioaccessibility would lead to more robust and better information available to human health risk assessment.

As for the effectiveness of P amendment in reducing Pb bioaccessibility in different size fractions, P addition was only effective on spiked soil at the highest P does, with little to no effect on RO and MI soils in both fractions.

The Pb bioaccessible fraction was higher than 70 % and was similar in both fine and coarse particles, while with a much higher concentration in the < 10 µm fraction implies possible adverse effects to the environment or to human health if these particles escape from the soil. These results call for increasing attention to the effect of soil remediation methodology in fine size fractions for their environmental role and could induce significant cost savings, reduce the requirements for undertaking intrusive remedial or risk management work at contaminated sites.

With regard to the geochemical fractionation and potential bioavailability of PTE in urban soils, a high non-residual chemical fraction is related to a high bioavailability, according to BCR method, thus pointed out a serious hazard to the urban environment. The bioaccessible concentrations in gastric and lung phases were closely positively correlated with DGT-measured content (r = 0.63-0.99, $p < 0.05$), suggesting the potential use of DGT for the prediction of PTE risk to human health. Moreover, this study could supply a more accurate assessment of the urban environment quality and be helpful for the pollution control and urban planning, although more research in needed to in-depth investigate the mechanisms to PTE bioavailability.

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10. Appendix

10.1 Appendix 1

Table S1. Composition of Gamble's solution (g/L)

Reagents	Formula	Gamble's solution (pH=7.3)
Sodium chloride	$NaCl$	6.79
Calcium chloride	$CaCl_2$	0.022
Sulfuric acid	H_2SO_4	0.045
Sodium dihydrogen phosphate	NaH_2PO_4	0.144
Sodium hydrogen carbonate	$NaHCO_3$	2.27
Ammonium chloride	NH_4Cl	0.535
Sodium citrate	$C_6H_5Na_3O_7$	0.052
DTPA	$C_{14}H_{23}N_3O_{10}$	0.079
L-Cysteine	$C_3H_7NO_2S$	0.121
Glycine	NH_2CH_2COOH	0.375

DTPA: diethylenetriaminepentaacetic acid

Table S2. Pearson's correlation analysis of PTE concentration in the urban and peri-urban areas.

Urban

	Mn	Cr	Ni	Cu	Zn	Pb	Co	Cd	Sb
Mn	1								
Cr	-0.269	1							
Ni	-0.277	**0.883****	1						
Cu	0.195	0.239	-0.110	1					
Zn	0.130	0.177	-0.098	**0.934****	1				
Pb	-0.129	0.119	-0.067	**0.735****	**0.836****	1			
Co	-0.104	**0.802****	**0.914****	-0.191	-0.232	-0.285	1		
Cd	0.253	0.272	-0.075	**0.908****	**0.804****	0.445*	-0.095	1	
Sb	-0.085	0.128	-0.057	**0.674****	**0.738****	**0.922****	-0.254	0.352	1

Peri-urban

	Mn	Cr	Ni	Cu	Zn	Pb	Co	Cd	Sb
Mn	1								
Cr	-0.382	1							
Ni	-0.148	**0.919****	1						
Cu	**0.805****	-0.350	-0.216	1					
Zn	0.674*	-0.152	-0.074	**0.967****	1				
Pb	0.611	-0.225	-0.178	**0.949****	**0.983****	1			
Co	0.284	0.714*	**0.887****	0.149	0.225	0.086	1		
Cd	0.553	-0.236	-0.196	**0.907****	**0.952****	**0.971****	0.027	1	
Sb	0.518	0.092	0.216	**0.836****	**0.918****	**0.892****	0.414	**0.900****	1

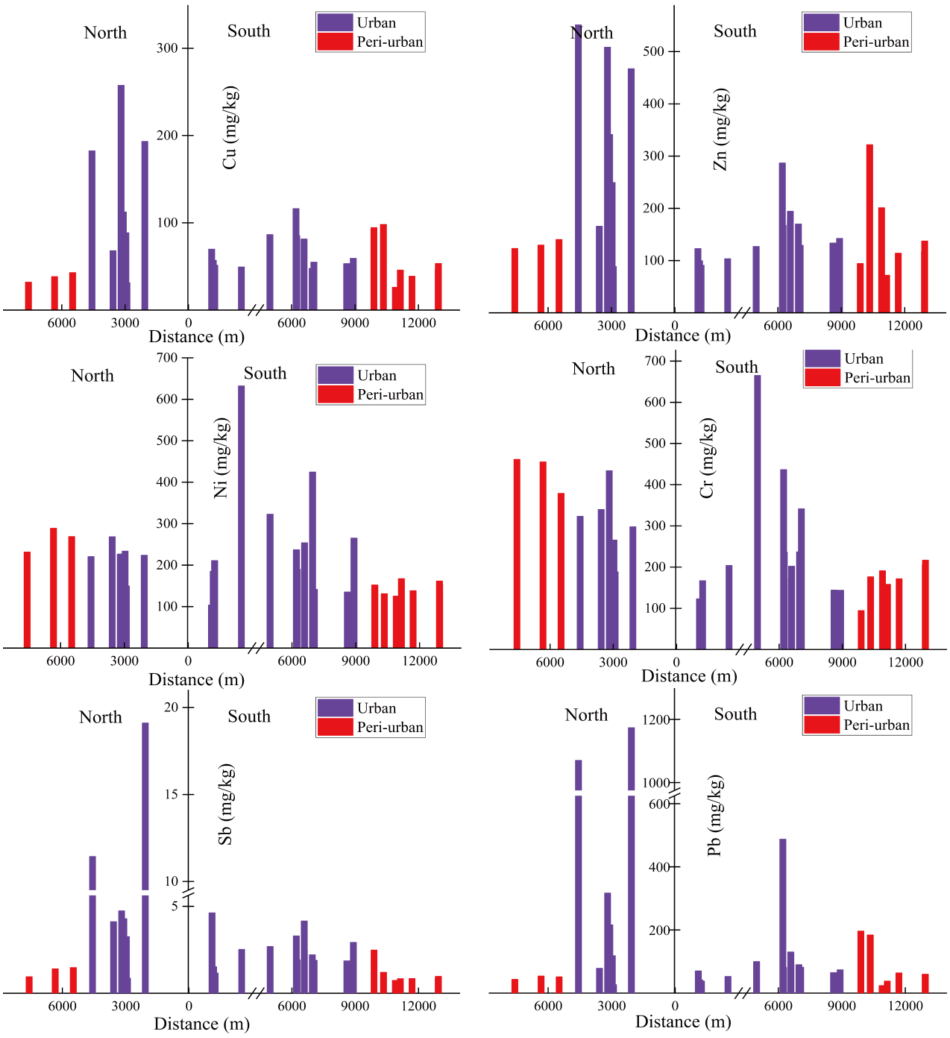

Figure S1. Variation of PTE concentrations with the increase of the distance from the city. Distances are calculated from the historical city centre of Turin (P.za Castello, Fig 3.1).

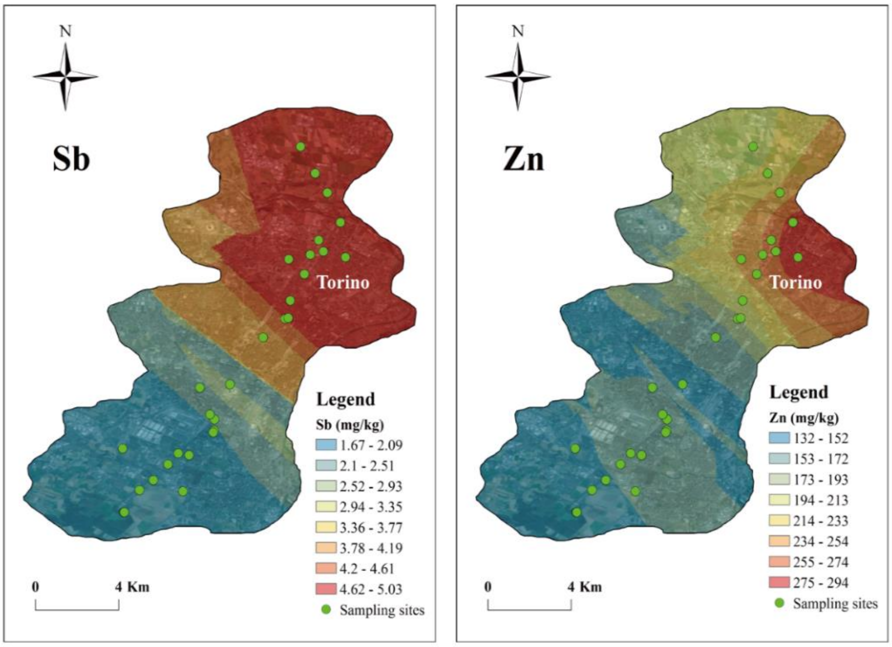

Figure S2. Spatial distribution maps of selected PTE concentration in the study area.

Although the variables do not show regionalized properties – in urban areas the sampling points do not have all the same probability of being sampled and the variation between iso-concentration lines cannot be assumed to be linear – a GIS representation can give a snapshot of the distribution of the elements.

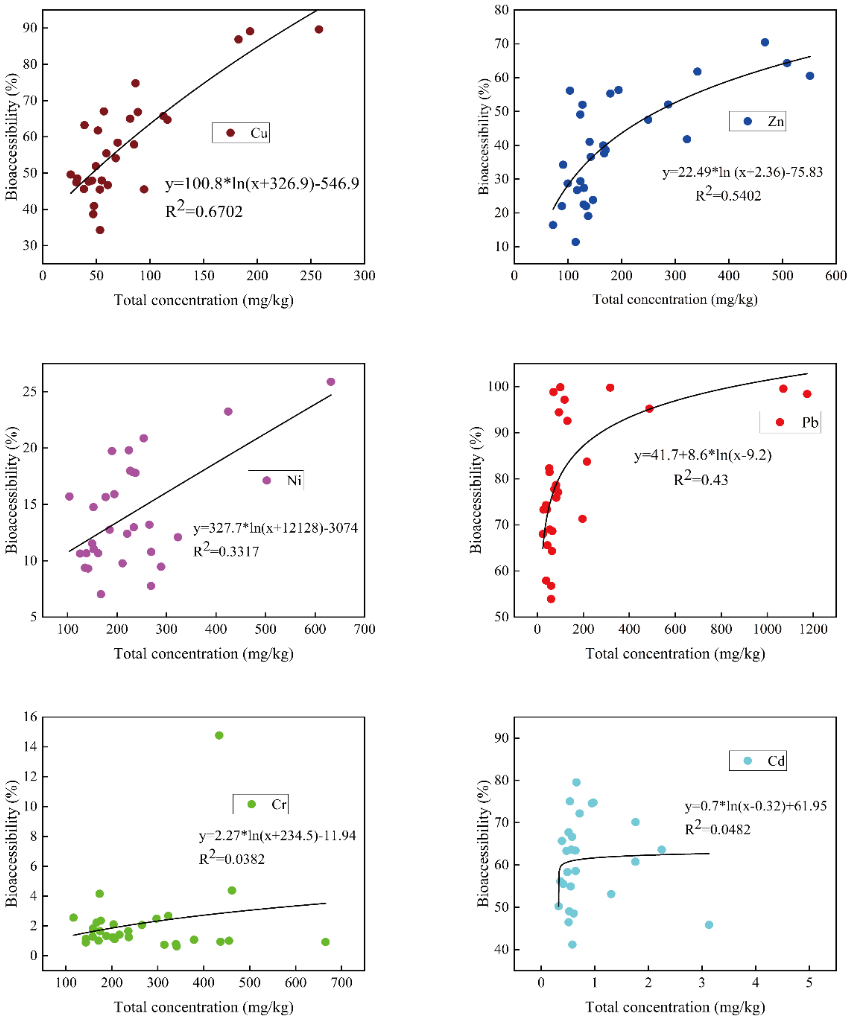

Figure S3. Correlation of total concentration and bioaccessibility of selected PTE in the soil samples.

10.2 Appendix 2

Table S3. Complete chemico-physical characterization of soils.

	pH	**TC**	**TN**	**C/N**	**Carbonates**	**Olsen P**	**Particle size distribution (%)**				
		%	%		%	mg/kg	< 2 µm	2 - 20 µm	20 - 50 µm	50 - 200 µm	200 - 2000 µm
SP	4.7	0.61	0.06	10	1.54	15	14	24	9	44	9
RO	8.3	3.37	0.25	13	0.86	12	3	11	10	37	39
MI	6.4	3.47	0.28	12	0.81	4	34	34	5	15	12
	Total P	**Fe**	**Ca**	**Mg**	**K**	**Mn**	**Ni**	**Cr**	**Cu**	**Pb**	**Zn**
	mg/kg	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
SP	510	1.98	0.62	0.94	1.66	852	27	43	237	467	65
RO	590	3.53	1.77	1.98	1.03	50	223	10	124	451	449
MI	377	3.47	0.17	0.33	0.35	721	17	37	38	522	733

Table S4. *In vitro* bioaccessibility of Pb with different treatments (bulk soil) reported as percentage of the *aqua regia* content control. Lower-case letters show significant differences ($p < 0.05$) within the same soil for the different treatments.

		HCl 2.5			**HCl 1.5**			**SBET 2.5**			**SBET 1.5**		
		Avg	S.D.		Avg	S.D.		Avg	S.D.		Avg	S.D.	
SP	CTR	30.8	1.6	a	82.6	2.3	a	65.3	0.2	a	88.0	1.1	a
	P 2.5	20.5	1.4	b	70.5	4.8	ab	52.2	2.4	ab	85.4	0.1	a
	P 5	20.3	0.1	b	71.9	7.0	ab	53.8	5.9	ab	82.0	2.6	ab
	P 15	6.7	0.6	c	63.4	6.8	b	49.6	7.8	b	78.5	3.6	b
RO	CTR	2.7	0.2		51.6	10.4		34.2	6.3	a	63.6	1.0	
	P 2.5	3.5	0.8		51.5	0.1		46.5	2.3	b	65.9	3.7	
	P 5	2.7	1.3		53.6	1.4		48.2	0.2	b	64.4	8.8	
	P 15	2.9	0.8		61.5	6.5		42.3	3.9	ab	66.0	3.7	
MI	CTR	3.7	0.1		35.5	1.0		34.6	0.3		69.6	1.5	
	P 2.5	3.7	0.1		34.1	1.8		32.3	0.9		67.9	2.2	
	P 5	3.3	0.1		31.8	0.1		32.2	0.1		63.4	0.9	
	P 15	3.2	0.5		28.4	0.1		29.8	0.5		64.0	3.2	

10.3 Appendix 3

Effect of different extracting methods on the evaluation of the efficacy of the amendment

On the bulk soils we tried then to comprehend the solubility of the formed Pb species using different modifications of the USEPA 1340 method and modifications. Extractable fractions of Pb strongly depend on the pH and glycine content of the in vitro method, as expected. The strength of the extraction method decreased in the order of: pH 1.5 with glycine > pH 1.5 without glycine > pH 2.5 with glycine > pH 2.5 without glycine. Within the same pH level, Pb bioaccessibility measured in methods using glycine was 10 to 20% greater at pH 1.5 while 30% higher at pH 2.5, as glycine act as complexing agent. A lower pH and glycine presence lead to results showing P amendments to be less effective. Amending was effective in lowering Pb bioaccessibility in the spiked soil, with a 25% decrease for P15 treatment measured using HCl (pH 2.5) while, for the historically contaminated roadside soil and mining soil, no effect was visible with any extraction method.

P addition and monitoring

Phosphate addition changed the pH of SPy soil from 4.7 to 5, 5.2 and 5.5 (for P2.5, P5, and P15 amendments, respectively); conversely, RO soil pH decreased from 8.3 to 7.8, 7.6, and 6.5, and no large changes were seen for MI soil, starting from 6.4 to 6.2, 6.1, and 6.1, respectively.

Soil pH plays an important role in P-induced immobilization of Pb, as decreasing soil pH will promote the dissolution of P (apatite, phosphate rock) and Pb (carbonate mineral phase) (Melamed et al., 2003; Wang et al., 2013) and also increase the solubility of other metals, that can interact with P, and may increase dissolved organic matter in the soil solution, which could chelate Pb (Lang and Kaupenjohann, 2003).

Available P (Olsen method) was measured to monitor phosphate reaction during incubation. Measured P concentrations decreased rapidly during the first three months in the same way in all soils, then soils reached equilibrium and concentrations remained stable or decreased slightly until the end of the incubation. At the end of the incubation, almost all P added in P3 and P6 trials reacted in SP and RO soils (Figure S5), while Olsen P was higher in MI soil, with 174 mg/kg of extractable P in P6 addition. Conversely, after the addition of the highest P concentration, the extra loading of P resulted almost all available in the case of RO soil and half of the incremental addition to SP and MI soil. This could indicate, probably, that the amount of P added was higher than the available adsorption or reactions sites.

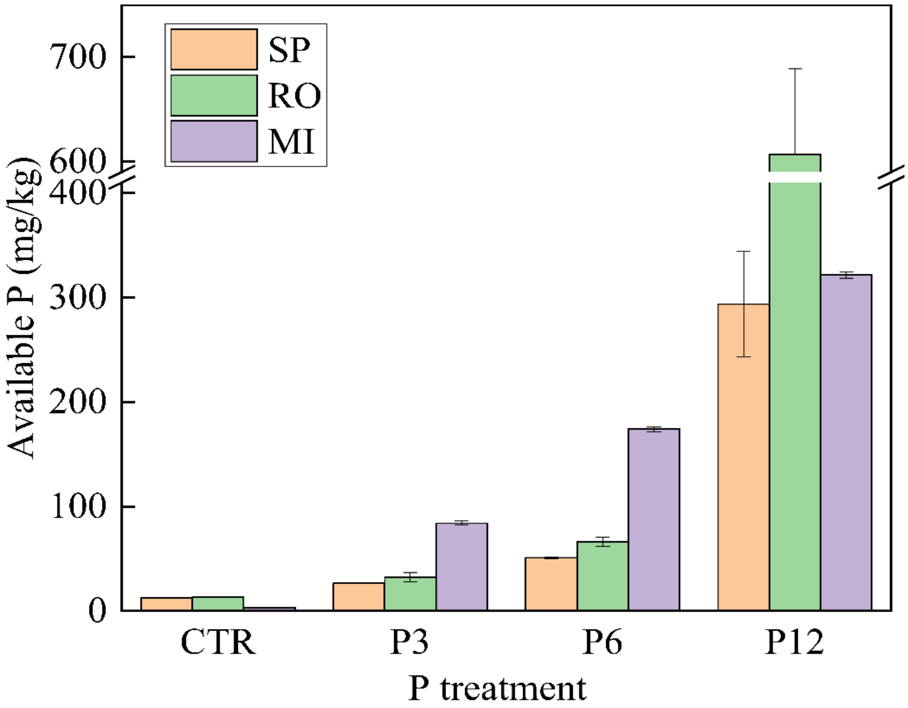

Figure S4. Available P in the different soils at the end of the incubation

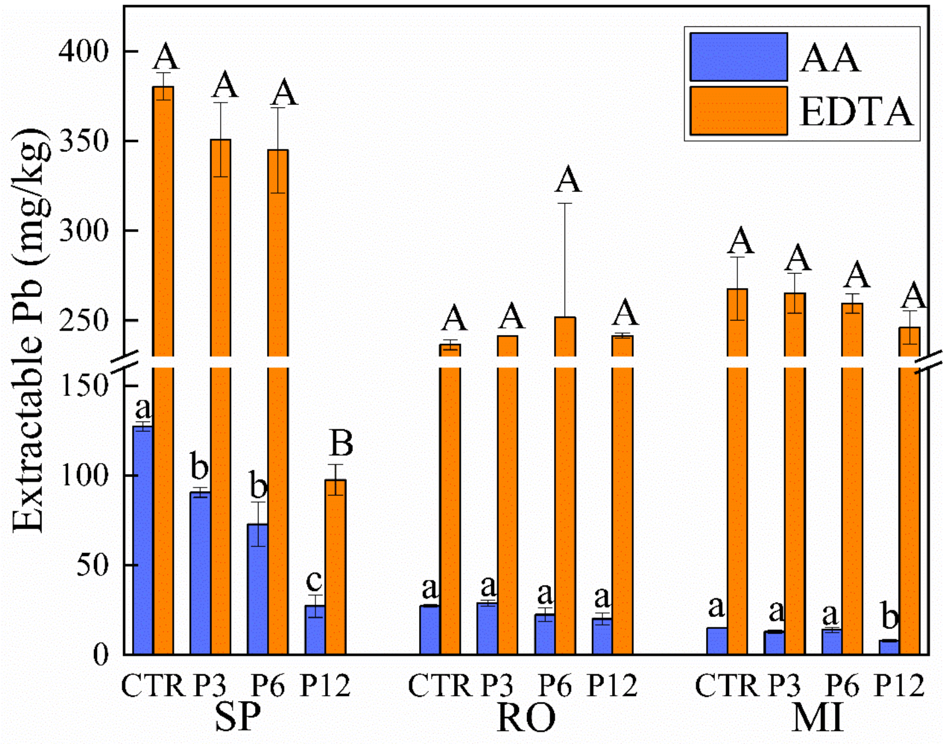

Figure S5. Extractable Pb concentrations in the different soils at the end of the incubation.