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Insights into mixed contaminants interactions and its implication for heavy metals and metalloids mobility, bioavailability and risk assessment

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Abstract:

Mobility of heavy metals at contaminated sites is mainly influenced by the soil physicochemical properties and environmental conditions, therefore assessing heavy metals (HMs) and metalloids fractionation can provide insights into their potential risk and the mechanisms that regulate bioavailability. A 12-months mesocosms experiment was setup to investigate the effect of physicochemical factors (pH, moisture, and temperature) and weathering (time) on HMs and metalloids fractionation in three different multi-contaminated soil matrices (low, medium, and high contamination) collected from a soil treatment facility located in the United Kingdom, and two rural contaminated soil samples. The study demonstrates that even though Pb and Zn were found associated with the exchangeable fraction in the soil with the highest contamination (total average Pb 3400 mg/kg, and total average Zn 2100 mg/kg in Soil C), neither the condition applied nor the weathering caused an increase in their mobility. Although it was expected that lower pH (4.5) would favours the dissociation of HMs and metalloids, no significant differences were observed, potentially due to the initial alkaline pH of the genuine-contaminated soil samples. The results show that even though total concentration of Pb, Cu, and Zn exceed the soil standards and guideline values, HMs were predominantly associated with the non-exchangeable fraction, while only 5% were dissolved in the pore water fraction (potentially bioavailable). In addition, the

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mobility and bioavailability of HMs remained constant over the 12 months monitoring, suggesting that these soils pose negligible risk to the environment.

Keywords: Chemical mixtures, Fractionation, Mobility, Ageing, Risk Assessment

1. Introduction

Anthropogenic activities such as mining, waste disposal, combustion of leaded fuels, the use of fertilizers and pesticides, and petrochemical spills all contribute to the presence, accumulation, and persistence of heavy metals (HMs) in soil (Tóth et al., 2016; Suresh et al., 2012, Wuana and Okieimen, 2011). While organic contaminants might be degraded as they persist in the environment, inorganic contaminants, such as heavy metals and metalloids, are non-degradable and display long-term persistence in soils (Lu et al., 2017), which can potentially cause risk for plants, animals, and humans (Bolan et al., 2014).

European environmental regulatory frameworks, to manage HMs pollution, define risk based on the total extractable concentration of metals in soil. This approach does not consider how likely an HM is to be bioavailable, which can lead to an over/under estimation of risk (Cipullo et al., 2018). In relation to contaminated land risk assessment and remediation, bioavailability can be interpreted as the fraction of contaminant that is freely available in the environment (not sorbed or sequestrated), and mobile (extractable by mild extraction), thus the most likely to lead to receptor exposure (Adedigba and Semple, 2015; Dean and Scott, 2004).

Sorption and desorption are the main processes controlling bioavailability of HMs (Caporale and Violante, 2016); in particular soil components responsible for the sorption includes; amorphous materials, silicates, clay minerals, , carbonates, and organic matter (Leleyter et al., 2012). How a HM interacts with the different soil compartments will influence its bioavailability, and it is bioavailability that can inform the likelihood that a HM might leach

into the broader environment (Ashraf et al., 2012). For example, HMs that are dissolved in pore water can be easily mobilized, and are considered readily available for uptake by plants (Chang et al., 2014) or available for interaction with biological systems (Hodson et al., 2011), while those dissolved in labile fractions are potentially bioavailable, if physico-chemical conditions were to change (e.g pH decrease) (Di Bonito et al., 2018). Many physico-chemical factors such as soil pH, composition, organic carbon content, and redox potential, can impact partitioning between soil-solid phase and pore water, which will consequently have an impact on HMs bioavailability (Islam et al., 2015; Venegas et al., 2016). In contrast, HMs associated with non-exchangeable or non-mobile soil fractions are characterized by a stronger binding (weaker reversibility), therefore unlikely to leach into the surrounding environment.

Despite the recent shift toward a risk-based approaches for assessing contaminated sites, risk characterization remains a conservative approach (Harmsen and Naidu, 2013; Naidu et al., 2015; Ortega-Calvo et al., 2015), because it relies on total contaminant concentration, rather than assessing the fraction of the total (bioavailable) that can potentially interact with biological and environmental targets. For bioavailability to be implemented and support regulatory decisions, the bioavailable estimation should rely on standardized methods, however, to date there exists no systematic method of assessment (Alvarez et al., 2011; R. Y. Kim et al., 2015). A number of techniques have been developed over the past two decades, and are still used to estimate HMs bioavailability in soil; including diffusive gradient in thin films (Agbenin and Welp, 2012; Menegário et al., 2017; Parker et al., 2016; Ren et al., 2015), ion exchange (Ge et al., 2005; Qian and Schoenau, 2002), single-step extractions (R. Y. Kim et al., 2015; Pinto et al., 2015; Sakan et al., 2016), and sequential extractions (Cox et al., 2013; Fernández-Ondoño et al., 2017; Palumbo-Roe et al., 2013; Reis et al., 2014; Sungur et al., 2014). Sequential extractions, in particular, are simple low cost methods, that can be applied to different soil types (Rosado et al., 2016), and can help understanding HMs and

metalloids leachability, solubility, and mobility (Kaakinen et al., 2015), providing the most information about the fate, transport, and behaviour of HMs in soil. However, most studies focus on (1) assessing effects and toxicity of one contaminant in isolation (Cui et al., 2005), (2) using sequential chemical extraction on synthetic models or spiked samples (R.-Y. Kim et al., 2015; Ma et al., 2015) rather than genuine contaminated soil samples (Ma et al., 2015). Limitations associated with these approaches include; metal transfer among phases (Bermond, 1992) when performing sequential extractions resulting in an overestimation of metals concentration and risk , and the inability of an artificial contamination to reproduce the actual geochemistry encountered in real site conditions (Ribeiro and Mexia, 1997). While it is challenging to establish a one-size fits all approach for assessing HMs behaviour in contaminated soil, the choice of procedure should be based on a more realistic prediction of elemental mobility and characterisation of their association with the soil matrix.

In this study a modified version of a non-specific sequential extraction method coupled to chemometric analysis known as the Chemometric Identification of Substrates and Element Distributions (CISED), was used and applied to five different genuine contaminated soils. Our objectives were (1) to apply a sequential extraction technique in genuine-contaminated soil samples and identify the common soil phases, (2) to evaluate the spatial distribution of HMs/metalloids and potential changes over time in order to ascertain the bioavailability of HMs/metalloids and potential risk, (3) to determine the influence of different physico-chemical factors on HMs/metalloids solid phase distribution and bioavailability, and (4) to evaluate the effect of co-occurrence of hydrocarbons on HMs/metalloids partitioning in soil samples. The novelty of this study lies in the fact that it highlights the importance of taking into account the effects of a range of environmental stressor conditions (pH, moisture, and temperature) and weathering (time), on HMs/metalloids potentially labile fractions; including both dissolved elements (pore water), and exchangeable fraction in genuine co-contaminated

soil samples. A special emphasis on the effectiveness of this protocol with multi-contaminated samples of different nature (with and without stabilisation treatment), origins (industrial and rural), and with a wide range of HMs contents has also been verified. This information can be used as additional lines of evidence to support risk-based decisions about endpoint remediation and to evaluate potential reuse of remediated soil.

2. Materials and methods

2.1. Sample collection and preparation

Since soil contaminants are often present in the environment in a complex mixture, including both inorganic and organic compounds as by-products of industrial activities (Wawra et al., 2018), this study investigates five multi-contaminated soil samples. Three genuine contaminated soils, denoted as Soil A, Soil B (treated), and Soil C, were collected from a soil treatment facility located in the United Kingdom. Information regarding original location of the soil samples collected, and specific details regarding the treatment applied (soil B), were not disclosed to maintain anonymity and confidentiality. Two additional samples were collected from a rural site contaminated by HMs/metalloids and diesel range organic (DRO) compounds (nC_{10} - nC_{24}) (Soil D), and HMs/metalloids mineral oil range organic (MRO) compounds (nC_{22} - nC_{34}) (Soil E). The mutual presence of organic and inorganic contaminants in these soil samples could potentially enhance (or inhibit) HMs transport by competitive sorption, where metal-organic complexes are formed, limiting their capacity to interact with soil-surfaces (Wuana et al., 2014). Information about the soil matrix and type of contamination is provided in Table 1.

All samples were collected randomly by disturbing sampling soil, up to a depth of 30 cm and immediately stored at 4°C to minimise biological transformation and other chemical reactions. Soil A was a sandy loam soil heavily contaminated with tar and HMs (Total

Petroleum Hydrocarbons (TPHs) > 1000 mg/kg of soil, HMs > 800 mg/kg of soil); Soil B was similar to Soil A except that former was stabilised with a cement-binder mixture. Soil C was a sandy loam soil presenting low petroleum hydrocarbon content (TPHs < 1000 mg/kg) but high concentration of HMs (HMs > 6200 mg/kg of soil). In addition, two different rural soils contaminated with HMs and diesel (Soil D), and HMs and mineral oil (Soil E) (TPHs < 500 mg/kg of soil, HMs > 800 mg/kg of soil) were used.

2.2. Mesocosms experimental design

Duplicate soil mesocosms were set up for each condition studied using a 10 L polypropylene bucket. Each bucket was filled with approximately 5 kg of loosely packed soil, and amended with buffer or moisture as according to the experimental conditions described in Table 2. All experiments were tested over a 12 month period. Different temperature conditions were simulated by storing samples in controlled temperature rooms at 20°C and 4°C. Those experiments treated under outdoor conditions were placed outside and subject to seasonal UK temperature variations. Soil samples were amended with a mixture of sulphuric acid and water to achieve different pH conditions. Redox reactions are a relevant aspect of soil chemistry as they can affect speciation and solubility of heavy metals and metalloids in soil, altering the biochemistry of soils (Kuhlbusch TA.J. and Crutzen PJ., 2018; Tuor, 1990) . Therefore this experiment was conducted in presence of atmospheric O_2 for all the soil samples and all the condition tested. Moisture content was maintained by adding deionized water up to 20 and 70% of the soils' maximum water holding capacity (WHC) and moisture content was re-assessed bi-monthly. The moisture content for Condition 6, which was kept outdoors, was not altered. Soil samples were taken from each mesocosm at 0, 6, and 12 months' time. All samples were analysed for pseudo-total and bioavailable HMs content.

2.3. Physico-chemical characterisation

Soil samples were sieved using a 2 mm mesh to separate large particles (e.g. roots, stems, and pebbles). Each soil samples were divided and processed for analysis in the following way: (1) 5 g of sample was used for dry matter and water content analysis, (2) a volume of 225 cm^3 of sample was used for water holding capacity measurement. Additionally, a large aliquot of each soil sample, approximately 500 g, was air dried for 7 days to perform multiple analysis where individual air-dried samples were used as follow: (1) 10 ml of sample (measured with 10 ml brass scoop) was used for pH analysis, (2) 10 ml of sample was used for particle size distribution, (3) 5 g of sample was used for loss of ignition, (3) 0.001 mg was used Total Nitrogen (TN) and Total carbon (TC), (4) 5 g of sample was used for Total phosphorous (TP) and available phosphorous (AP).

For dry matter and water content analysis, 5 g of fresh soil samples were weighted in a crucible and dried at 105°C ±5°C for 24hrs; the difference in mass of an amount of soil before and after drying was used to calculate the dry matter and water contents on a mass basis. Maximum water holding capacity was determined according to ISO 11274 (1998). Soil samples were air-dried and then flooded on a wetting-up bath for 7 days; the mass recorded was used to determine the moisture content at saturation.

Soil pH was measured using a pH meter. Samples were prepared by adding distilled water to create a slurry (1 part soil: 5 parts water). Samples were shaken for 60 min and allowed to equilibrate for an additional 30 min before pH was measured (ISO 10390:2005).

Particle size distribution was determined by the sieving and sedimentation method. In short, soil organic matter was discomposed with hydrogen peroxide and the resulting slurry dispersed with a buffered sodium hexametaphosphate solution, then the different particle size fractions was determined by a combination of sieving and sedimentation (ISO 11277:2009).

The soil organic content was determined by loss of ignition (LOI); air-dried soil was dehydrated at 105°C then ashed at 450°C, loss on ignition is expressed as a percentage of the dehydrated sample (British Standard BS EN 13039:2000).

Total carbon and total nitrogen in soil material were determined by heating to a temperature of at least 900°C in the presence of oxygen gas, the amount of nitrogen and carbon is then measured by a thermal conductivity detector (TCD) (British Standard BS EN 13654-2:2001).

Total phosphorous was measured with a hydrochloric/nitric acid mixture extraction; the phosphorus content was then determined by a spectrometric measurement in solution (ISO 11047:1998). Available phosphorous was measured by treating the soil with a 0.5 mol L^{-1} sodium hydrogen carbonate solution at pH 8.5, the extract is then analysed by a spectrometric method (ISO 11263:1994).

2.4. Extraction and quantification of total petroleum hydrocarbons

The method used to determine total petroleum hydrocarbons (TPHs), including both aliphatic (ALKs) and aromatic (PAHs) fractions in soil, was based on the Risdon et al. (2008) procedure. Briefly 2.5 g of soil were weighted and chemically dried with 2 g anhydrous sodium sulphate. At the same time as weighing samples for extraction, moisture content was measured to provide the appropriate correction factors. The chemically dried samples were extracted for TPHs content with a mixture of 10 ml of dichloromethane: hexane sonicated for 20 min at room temperature, and shaken at 150 g for 16h. On the following day, samples were again sonicated for 20 min at room temperature and centrifuged at 2000 g for 10 min to separate the solid and liquid fractions. The liquid fraction was then cleaned onto a 6 ml SPE DSC-Si silica tubes, concentrated to dryness (on ice) under a gentle stream of nitrogen, and re-suspended in 0.5 ml DCM:Hex (1:1) with addition 0.5 ml of internal standards comprised of a deuterated alkanes mix ($C10^{d22}$, $C19^{d40}$ and $C30^{d62}$) and deuterated PAH mix (1,4-

dichlorobenzene ^{d4}, naphthalene ^{d8}, anthracene ^{d10}, chrysene ^{d12} and perylene ^{d12}) at 10 µg ml^{-1} each, respectively. Concentration of petroleum hydrocarbons were identified and quantified by gas chromatography-mass spectrometry (GC-MS) using an Agilent gas chromatograph coupled to a Turbomass Gold mass spectrometer operated at 70 eV in positive ion mode. The column used was a Restek fused silica capillary column (30 x 0.25 mm internal diameter) coated with RTX®-5MS (0.25 µm film thickness). Splitless injection with a sample volume of 1 µl was applied. The oven temperature was increased from 60°C to 220°C at 20°C min^{-1} then to 310°C at 6°C min^{-1} and held at this temperature for 15 min; for a total run time of 38 min. The mass spectrometer was operated using the full scan mode (range m/z 50-500) for quantitative analysis of target aliphatic and aromatic hydrocarbons. For each compound, quantification was performed by integrating the peak at specific m/z. External multilevel calibrations were carried out using alkane (standard solution (C_8-C_{40}) Sigma Aldrich, Dorset, UK) and PAHs (EPA 525 PAH Mix A; Sigma Aldrich, Dorset, UK) standards, the concentration of which ranged from 2.5 to 50 µg ml^{-1} respectively. For quality control, blank controls and a 500 µg mL^{-1} diesel standard solution (ASTM C_{12}-C_{60} quantitative, Supelco) were analyzed every 20 samples. The variation of the reproducibility of extraction and quantification of soil samples were determined by successive injections (n=7) of the same sample and estimated to ± 8%. In addition, duplicate reagent control and reference material were systematically used. The reagent control was treated following the same procedure as the samples without adding soil sample. The reference material was an uncontaminated soil of known characteristics, and was spiked with a diesel and mineral oil standard at a concentration equivalent to 16,000 mg kg^{-1}. Relative standard deviation (RSD) values for all the soils was <10%.

2.5. Modified sequential extraction and pseudo-total element digestion

Assessing metal partitioning through the non-specific sequential extraction with Chemometric Identification of Substrates and Element Distributions (CISED) can (1) limit the re-adsorption and re-distribution of elements among phases during extraction, often happening in genuine contaminated samples, (2) overcome problems linked with "operational speciation", where soil phases (operationally defined metal forms) are identified strictly based on their response to the extraction reagents, which not necessarily reflects the behaviour of natural samples (Adamo and Zampella, 2008).

In this work, a modified procedure for sequential extraction was conducted similar to that described in Cave et.al (2004). Soil samples of approximately 2 g were consecutively extracted by addition of 10 mL of an extraction solution (Table 3) which contained an increasing concentration of nitric acid (i.e. from 0 to 5 M). After adding 10 ml of extraction solution, samples were mixed on an end-over-end shaker for 10 minutes, and the liquid phase was recovered via centrifugation (4350 g for 5 min) and used for analysis; the soil pellet was resuspended again with the following extraction solution. Each extraction solution (7 solutions) was used twice to obtain a total of 14 extracts (10 ml). As highlighted in Table 3, in the last 8 extractions (E_7 to E_{14}) increasing amount of H_2O_2 were added to the extraction solutions to enhance degradation of organic matter and favour the dissociation of Fe–Mn oxides (Filgueiras et al., 2002). However addition of H_2O_2 caused a high release of gas in the genuine contaminated soil samples, rendering the centrifugation and separation phase not possible without losing significant amount of soil material. We hypothesised that this was due to both (1) high calcium and phosphorus content typical of the content of cement-based stabilisers(Saeed, 2012) ,(2) the high reactive organic content soils caused by the presence of co-contamination (petroleum hydrocarbons), often observed in multi-contaminated soil matrix, such as the industrially-polluted soils used in this study. Therefore in our approach we

implemented a modified version of the Cave et.al (2004) extraction procedure, which required the inclusion of an additional step. Hence, when 10 ml of solution 4 (9.75 ml of 0.10 M HNO_3, and 0.25 ml of H_2O_2 100 volumes >30% w/v) were added to the samples, tubes were placed in a water bath for 30 min at 70ºC, to favour the reaction and limit the gas production. This additional step was sufficient to reduce the re-mixing of the solution allowing a proper separation when centrifuging.

The pseudo-total element digestion was performed according to the ISO 11047 method with aqua regia (ISO 11047:1998). Briefly, 0.5 g of soil was extracted with 8 mL hydrochloric/nitric acid mixture using a microwave digestion system. The extract was then filtered with 0.45µm 25mm nylon syringe filters and made up to 50 mL volume with water.

All pseudo-total and sequential solutions extracted were filtered with 0.45µm 25mm nylon syringe filters and diluted 4 times with 1% HNO_3 before analysis by inductively coupled plasma mass spectrometry (ICP-MS A NexION® 350D ICP-MS, Perkin Elmer). The ICP-MS was calibrated using a mixture of both major (Ca, Fe, K, Mg, Mn, Na, S, Si, P) and trace (Al, As, Ba, Cd, Co, Cr, Cu, Hg, Li, Mo, Ni, Pb, Sb, Se, Sr, V, Zn) elements. The concertation ranges were 1, 5, 15, 20, 40 µg/mL for major elements and 0.01, 0.1, 0.5, 1, 2 µg/mL for trace elements. In both cases, working standards were prepared in matching sample matrix solutions (nitric acid 1%). Calibration standards and samples extracts were spiked with the following mix of four internal standards: Sc, Ge, Rh, and Bi. ICP–MS was calibrated after each sample (14 sequential extracts). Limits of detection (LOD) were estimated as the concentrations corresponding to three times the standard deviation of measurements of analytes in a series of blank solutions (MilliQ water with 1% HNO_3 and buffer solution) (n = 40) treated the same way as the samples. The results are given in Table A **2**. Additionally, acid blanks (1% nitric acid), digestion blank, and guidance materials (BGS_{102}) were analysed every batch of 7 samples along with an adequate rinse time

programmed in between samples; to monitor blank contamination, sensitivity, operating conditions, and extraction's accuracy. For the quantitative analyses, no blank correction was necessary as the calibration standards and samples were treated exactly in the same way adding the same amount and concentration of HNO_3. The blank value was therefore taken into account in the calibration curve.

Mean repeatability of guidance materials (BGS_{102}) (expressed as relative standard deviation %) was lower than 6 and 8 % for sequential and aqua regia digestion respectively. All elements' concentrations have been converted into mg/kg extracted from the soil-solid matrix. Descriptive statistics for the metals and metalloids concentrations (expressed in mg/kg) is presented in

Table **A1**.

Soil samples extraction recoveries obtained with the CISED method compared with pseudo-total metal concentration averages were lower. The reason these extraction recoveries are not 100% is that the CISED extraction protocol mainly targets the easily soluble surface coatings without attacking the silicate matrix of soil. However, by assessing the pore-water, carbonates, and oxides fractions, it is sufficient to make assumption on HMs and metalloids fractionation as in contaminated soil the input of HMs (anthropogenic contamination) is mostly provided by non-silicate bound forms (Wuana et al., 2014).

2.6. Modelling

Data obtained from the HMs/metalloids sequential extraction were analysed using MatLab (Version R2015a) following the protocol developed by Cave et al. (2004). The non-specific sequential extraction method named Chemometric Identification of Substrates and Element Distributions (CISED) assumes that the chemical composition data for each extract is made up from different proportions of the physicochemical components in the soil. Since the

algorithm is designed to identify the number of components based on principal component analysis and by Varimax rotation (Giacomino et al., 2011), for the purpose of the modelling, the soil samples extracted were grouped according to soil matrix type (Soil A, Soil B, Soil C, Soil D, and Soil E) and metals concentration (low, medium, and high contamination), in order to derive a more homogeneous data matrix for processing. Data processing of the sequential extraction was carried out on 5 multiple data matrices, each comprising the elemental extraction data (25 elements) for the 14 extracts for each test soil, over 7 conditions at 3 sampling times (294 rows of data per matrix). The data were processed using a self-modelling mixture resolution (SMMR) algorithm in MatLab (Cave et al., 2004). The algorithm output is based on three main data matrixes: profile (PRF), distribution (DST), and composition (CMP). The PRF of each modelled soil component is calculated as the overall amount extracted (mg/kg) in each of the 14 extractions. The DST expressed in mg/kg represents the concentration of each element across the different soil components identified by the model. The CMP data is expressed as a percentage of each element present in the identified component. Both PRF and CMP are then used to calculate the single element concentrations (mg/kg).

2.7. Cluster analysis and complex associations between variables

Modelled soil components and element distribution data, obtained from the MatLab algorithm, have been post-processed in RStudio to create a matrix, which has been further categorised using a clustering methodology, and visualised in a heatmap as previously described by Wragg et al., (2014) and Cox et al., (2013).

The SMMR algorithm produced distinct sets of physico-chemical phases for each of the 5 multiple data matrices analysed. Briefly, representative samples for each soil were selected and arranged in a data matrix containing on the left side the elements composition (CMP) (Na, Mg, Al, Si, P, S, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Li, Mo, Cd, Sb, Sr, Ba, Pb,

Se) expressed as percentage, and on the right side the extraction profile (PRF) of each soil under investigation (expresses in mg/kg). The matrix was then imported in RStudio and subjected to hierarchical clustering where the data were mean centred and scaled with Euclidean distance and linkage using Ward's method (Ward, 1963) and the 'Agnes' function in the cluster package (Maechler et al. 2012) in RStudio (v.3.4.1). Clustering results were visualized using a heatmap (Figure 1) created using ggplot2, reshape2, grid, and ggdendro packages (Wickham 2007, Kahle and Wickham, 2013), where each row represents a physicochemical soil components found for a given soil. Soil name is indicated by the letter previously used in Table 1 (Soil A, Soil B, Soil C, Soil D, and Soil E), followed by the elements name (e.g Ca, Ca-K-Si, Fe-Mg) that make up > 10% of the physicochemical component composition. The hierarchal clustering obtained was used in parallel with chemical profile to provide interpretations and classify the components into common, distinct soil phases pore water (readily available or bioavailable), carbonates (potentially available with time) and oxides (bounded, non-available) and to assess the partitioning and bioavailable concentrations of HMs/metalloids in soil.

2.8. Data analysis for descriptive statistics

In the context of this research, PERMANOVA was used to investigate the significance and relationship between conditions tested (Cond 1, Cond 2, Cond 3, Cond 4, cond 5, Cond 6, and Cond 7), and TPHs concentration (high, medium, low) on (1) pore water, (2) exchangeable, and (3) non-exchangeable fractions of inorganic contaminants in the soil samples. Permutational Multivariate Analysis of Variance (PERMANOVA) is a Multivariate ANOVA with permutations, it was applied by using "adonis" function of the vegan library in R Studio (v.3.4.1, R) (Oksanen et al., 2011).

Descriptive statistics for the metals and metalloids concentrations in the different fractions and the 40 blank measurements and limit of detection (LOD) are provided in Tables A1-A3.

To establish a direct or indirect correlation between HMs, which might be indicative of similar elements behaviour in multi-contaminated soil, univariate linear regression analysis was used by applying Pearson correlation coefficient with the "corrplot" package in R Studio (Oksanen et al., 2011). The output returned a correlation matrix for each soil (available in Appendix ; Tables A4 to A8) which allows assessment of relationships between HMs.

3. Results and discussion

3.1. Soils characteristics and pseudo-total HMs and TPHs content

All soil samples physicochemical properties, HMs/metalloids pseudo-total concentrations, and hydrocarbons total content are summarised in Table 4.

Soils A, B, and C are representative of industrial sites with low total nitrogen (700, 800 and 1200 mg/kg) and phosphorus (450, 430 and 500 mg/kg) contents as well as alkaline pH, conditions often found in urban settings (Vodyanitskii and Savichev, 2017). Soil samples collected at the treatment facility (Soil A, B, and C) belonged to a manufacturing gas plant, where often in addition to co-presence of PAHs and heavy metals, the coal ash and wood are generally characterised by alkaline pH (Hatheway and Speight, 2017). Soil B had high calcium and phosphorus content typical of the content of cement-based stabilisers (Saeed, 2012). For the majority of metals alkaline conditions can potentially increase the adsorption of HMs (Horváth et al., 2015), which reduces HM mobility and thus limits risk of exposure. However, some metals (Cr(VI), Mo(V)) and metalloids (e.g. As and Se) are mainly present in stable oxyanions forms (e.g. arsenate, selenite, vanadate, Cr(VI) chromate, and molybdate under alkaline pH. Oxyanionic species are negatively charged and can be more mobile compared to the cationic species due to their high solubility and lack of adsorption on the surface of soil minerals (Cornelis et al., 2008).

Soil D and Soil E texture was clay loam (sand content < 35%), and pH 7.0 - 8.0, and presented a higher nutrient content overall. In these soils the presence of soil particles smaller than 0.002 mm, such as clay, could contribute to increase the HMs retention capacity due to the larger specific surface area (Ander et al., 2011).

The C/N ratio of Soil A and B was more than 5 times higher than for the rural contaminated soils (Soil D and E), because of the larger amount of hydrocarbons present in the industrial contaminated soil. The high organic content might also be responsible for higher HMs retention, (Almeida et al., 2008; Millward et al., 2004). For all the soils investigated, the pseudo-total metal concentrations of Pb and Zn exceeded 8 times and 4 times the UK Soil Guideline Values (SGVs) and the European Directive 86/278/EEC; with an average pseudo-total concentration of respectively 3400 mg/kg (Pb) and 2130 mg/kg (Zn) for the most contaminated sample (Soil C).

Descriptive statistics for the total concentration of aliphatic, PAHs, and TPHs compounds are provided in Table 4 where total maximum concentrations values in rural contaminated soil samples were half compared to industrial samples; 500, 180, 460, 430, 260 mg/kg ALKs, 2700, 1100, 244, 400, 360 mg/kg PAHs, for Soil A, Soil B, Soil C, Soil D and Soil E, respectively.

3.2. HMs Solid phase distribution

Soil samples were subjected to the CISED sequential extraction procedure to determine the physicochemical soil components (substrates) being extracted from the soil (e.g carbonates, clays, exchangeable phases); and the solid phase distribution of HMs/metalloids between each identified soil component. Figure 1 indicates the presence of 10 distinct physicochemical clusters (blocks) which have been further grouped as: (1) Pore water, (2) Carbonates (low and high carbonates) and (3) oxides (Al-oxides, Mn-Oxides, and Fe-oxides) (Figure 2). HMs chemical speciation results, obtained by sequential extraction, are essential

to understand their mobility; the results obtained show that the extractable amounts obtained from each fraction can vary widely (Figure 1 and Figure 2). The first physicochemical cluster (Figure 1, clusters 3, 5, and 8) is dominated by the presence of Na, Ca, S, and K, and was extracted by the initial step of CISED, when deionized water (E_1-E_2) or low acid concentration (HNO_3 0.01 M, E_3-E_4) was used. This step was used to extract elements that are soluble, highly mobile, and most likely associated with the pore water fraction. The second physicochemical cluster, Ca dominated, is well identified in these samples (in particular Soil B) and mainly composed of Ca and, to lesser extent, of K, Si, and S (Figure 1, clusters 1, 2, 7, and 9). The elevated presence of Ca in this fraction is linked to the fact that common binders are calcium-based. This fraction can be divided into low carbonate (extracted with low acid strength HNO_3 0.05 – 0.1 M, E_5-E_6, E_7-E_8) and high carbonate (extracted with HNO_3 0.5 M, E_9-E_{10}). The third physicochemical cluster identified through the modelling corresponds to oxides including Mn-oxides, Al-oxides, and Fe-oxides (Figure 1, clusters 4, 6, and 10). This cluster was associated with elements (e.g. Mn, Al, and Fe) released after H_20_2 addition and dissolved by the concentrated HNO_3 (E_7 to E_{14}). These elements were extracted with very strong acid concentrations (E_9-E_{14}) and likely associated with the clay components of the soil, therefore being overall immobile under natural environmental conditions.

3.3. Relationship between HMs and metalloids distribution and bioavailability

The compositional data and distribution of HMs and metalloids, for all soil samples in these fractions, were obtained by transforming each original raw concentration (i.e. mg/kg) into proportions of the total (100%) and are shown in Figure 2**.** Concentrations have been averaged across time and conditions in order to provide an overview of the overall metal behaviour in the five soils types (soil A, soil B, soil C, soil D, and soil E) (see for details Table A3). The most mobilised elements in the exchangeable fraction were the following: Hg and Se for Soil A; Cd and Se for Soil B; Cd and Hg for Soil C; Cd for Soil D, and Cd, Cu for

Soil E. Conversely, As and Cr showed the least mobility. The order of mobility of the metals in the exchangeable fraction was as follows: Hg > Se > Ni > Cr > Cd > As > Zn > Cu > Pb (Soil A); Se > Cd > Cr Hg > Ni > As > Cu > Zn > Pb (Soil B); Cd-Hg-Zn > Pb > Ni > Cu > Se > Cr >As (Soil C); Cd > Hg > Ni–Zn > Cu > As > Se > Pb > Cr (Soil D); and Cd > Cu > Pb-Se > Zn > Ni > As > Cr > Hg (Soil E). Interestingly As was the least mobile, while Cd was very mobile at pH > 9 in the industrial soil samples (Soil A, Soil B, and Soil C). Previous literature showed that As adsorption tend to decrease under alkaline conditions (pH > 9) due to the presence of negatively charged H_2AsO_3, HAsO3 , and AsO_3 (forming soluble species of As(III)) (Dias et al., 2009). However in these samples, As was found mostly associated with the non-exchangeable fraction (Mn-Al and Fe oxides). Therefore we can assume that the majority of As was present as inorganic oxyanion As(V) forming H_2AsO_4 and $HAsO_4^{2-}$, which is known to strongly interact with oxides (positively charged) in both un-contaminated and contaminated soils (Lin and Puls, 2000; Sarkar, 2002).

In soil C, D, and E samples, Cd sorption was limited as the element was predominantly found in the exchangeable fraction which is likely due to the influence of the soil-solid particle distribution on Cd behaviour. Previous studies highlighted that Cd binding on clay minerals is weaker compared to binding to organic matter (Janssen, 1997; Prokop et al., 2003) which was the case for these soils.

In Soil A, both Mn and Fe were below detection limit in the pore water fraction. Changes in Mn and Fe concentrations in the pore water was negligible for Soil B and Soil C. In contrast for Soil E, both Fe and Mn concentrations decreased overtime in the pore water, suggesting that pore water Fe(II) was oxidised to insoluble Fe(III).

The partitioning, mobility and distribution of HMs and metalloids assessed in these soil samples can provide different level of information, such as (i) information on the origin of the

contamination, (ii) the effectiveness of cement-stabiliser and potential reuse of soil material, (iii) the limitation often associated with guideline values thresholds. HMs/metalloids partitioning can provide information on the origin of the contamination, where often HMs from anthropogenic sources usually bind to the exchangeable fractions (Frentiu et al., 2008; Hu et al., 2006; Iwegbue, 2015); as observed for Soil A where 90, 50, and 25% of Hg ,Se and Ni were distributed in the exchangeable fraction. Over 33, 28, and 20% of Se, Cd, and Cr (Soil B), and over 80% of Cd, Hg, Pb, and Zn (Soil C) were found in the exchangeable fraction; while Soil D, E showed lower values below 50% for Zn and Ni. Nevertheless, HMs and metalloids present in the exchangeable fraction can also become mobilised over time (Baran and Tarnawski, 2015) and should therefore be considered for a more complete assessment of the entire pool of mobilisable elements. However data regarding the soil origin and underline geology must also be considered in order to estimate the weight of the effect of geogenic or (anthropogenic) contribution on HMs/metalloids bioavailability (Borgese et al., 2013). Being the soil samples provided anonymously from a treatment facility, no further information on the geology, location, or origins of the contamination were provided. The potential of re-using soil that has been treated or remediated is a viable and sustainable strategy (Mehta et al., 2018), however concerns regarding safety of the re-used material and the possible further spreading of contaminants still exists. Results obtained from sequential extraction highlighted that even though Soil A, and B showed a similar HMs mobility pattern, where some of the less mobile elements including Cr, As, and Zn were significantly more associated with the non-exchangeable fraction of Soil B (treated with stabiliser). The presence of the cement stabiliser was able to reduce HMs solubility, adsorption, and incorporation to the porous surfaces, as previously observed in the literature (Jiang et al., 2006; Johnson, 2004). Since no information was available on the type of cement stabiliser used in Soil B, it was not possible to draw further conclusion on the mechanism dominating

the fixation of HMs. Ultimately, whilst providing information on the target HMs metalloids for risk assessment, total concentration cannot provide sufficient information about elements mobility and bioavailability in soil; highlighting that soil guideline values (SGVs) are useful, but their application in the detailed quantitative risk assessment is limited. Sequential extraction instead provided specific information on the solid-phase fractionation of HMs/metalloids in soil (Cox et al., 2013; Palumbo-Roe et al., 2013; Reis et al., 2014), therefore allowing relevant stakeholders and regulators to make informed assumptions on bioavailability for risk assessment (Kaakinen et al., 2015).

3.4. Behaviour of exchangeable metal fraction over time

Average HMs/metalloids content and distribution across the three fractions (pore water, exchangeable, and non-exchangeable) in the five soil samples for the 7 conditions tested are presented in Figure 3. In Soil A, no changes were found for Cd, Cr, Cu, Ni, Pb, and Zn over time. On the other hand, As and Se concentrations changes can be explained by the good As-Se-metals correlation (Tables A4 to A6) suggesting that As, Se and metals could come from sulphides. The mobility and toxicity of As and Se depends on their redox state. In neutral to alkaline soils, As and Se mobility increases because of the formation of arsenate ($H_2AsO_4^-$) and selenate (SeO_4^{-2}) ions (Soukup, 2013), which weakly bond to oxides and other minerals.

For Soil B, all the HMs/metalloids showed little or no difference in distribution across the three sampling events (T0, T6, and T12). Most of HMs/metalloids were almost entirely found in the non-exchangeable fraction. Such behaviour can be explained because either (1) the addition of the stabilisers was effective in retaining the contamination over time, as highlighted in previous paragraph, or (2) these metals were mainly associated with clay related elements (e.g. Mn, Al, and Fe) released after H_20_2 addition and dissolved by the concentrated HNO_3. This suggests that Pb, Cu, and Zn are unlikely to become available with time. For example, Pb quantities present in the exchangeable fraction (Soil B) fell within the

range of the median concentration for UK urban topsoil G-BASE data (48-128 mg/kg), with a 75th percentile of 253 mg/kg (Ander et al., 2011) (Table A3).

Similarly, As, Cd, Cr, and Zn in Soil B were not affected by ageing. In particular As (93%) and Cr (81%) were almost exclusively present in the non-exchangeable fraction. Pb, Cu, Ni, and Se distributions barely changed overtime being exclusively in the exchangeable fraction. Even though Pb is one of the main contaminant of concern with high concentration in exchangeable fraction (mean 1500 mg/kg), its concentration persisted over the 12-month incubation. This is probably due to the formation of insoluble Pb compounds such as phosphates, carbonates, and oxides typically formed when the pH is above 6 (Wuana and Okieimen, 2011). Zn was almost entirely associated with the exchangeable fraction: it is well known to generally display strong affinity to the non-residual fraction of the soil (Naji et al., 2010).

Soil D and E presented a very similar distribution with the exception of Cu,Hg, Pb, and Se, which were more exchangeable in Soil E. In both soils and similarly to Soil B, As, Cd, Cr, Cu, Pb, and Se were not affected by ageing. The increase of Zn concentration in the exchangeable fraction observed for Soils D and E during mesocosms incubation was attributed to Zn affinity for hydroxides and carbonates, which can promote remobilization of this element in soil (Kumar, 2016). Ni also showed trends similar to Zn in metal release and its concertation increased in mobile fraction after incubation.

Ageing, has been previously identified as a main driver for leachability of metals in soil. However, an inverse relationship between time of residence in soil and amount of metal leachable exists: the shorter the time, the larger is the amount that can be released (Kumar, 2016). Regarding the time when the contamination occurred, there was no information associated to the soil samples collected. Nevertheless, it was assumed that contamination in Soils D and E was more recent when compared to Soils A, B, and C.

3.5. Influence of the environmental parameters on HMs and metalloids behaviour and fate

Sequentially extracted fractions were compared to gain a mechanistic understanding on how measurements varied when different conditions were applied at the different times. After evaluating the concentration and distribution of the HMs/metalloids in the 5 soils, a detailed investigation of the 3 metal pool fractions behaviour under the 7 mesocosms conditions was carried out. No significant differences were found for the same soil samples exposed to different pH (Conds 1 and 2), different moisture content (Conds 3 and 4) and different temperature (Conds 6 and 7) (data not shown).

PERMANOVA was used to investigate the significance and relationship between conditions tested (Cond 1-7), and TPHs concentration (high, medium, low), on (1) pore water, (2) exchangeable, and (3) non-exchangeable fractions of inorganic contaminants in the soil samples. For all industrial contaminated samples (Soil A, Soil B, and Soil C) no significant effect ($p > 0.5$) of conditions, nor TPHs concentrations on pore water, exchangeable, and non-exchangeable fractions were recorded. This confirms that difference observed in HMs partitioning among different fractions, in different soil samples was minimal, and that these soil materials potentially pose low risk to the environment. For both Soil D and E no significant effect ($p > 0.5$) of conditions, TPHs concentrations on pore water concentrations was observed. However, in Soil D only, a significant effect of TPHs concentrations on exchangeable ($p = 0.001$) and non exchangeable ($p = 0.003$), fractions were found; suggesting that additional factors may play a role in contaminant concentration changes (e.g degradation of organic contaminants, volatilisation, and interaction with soil organic matter), rather than the condition applied, which did not cause a significant difference between groups. The co-presence of TPHs increased the HMs/ metalloids redistribution into the exchangeable fraction for Zn, Pb, Ni, As, and Cu, while no changes were observed for Ce, Cr, Hg, and Se.

While individual compounds in a complex chemical mixture are assumed to have independent sorption behaviour, at high concentrations co-presence of contaminants can influence sorption as a results of changes in the soil-solution equilibrium (Gao et al., 2006). Thus, co-presence of mixed contaminants such as petroleum hydrocarbons and heavy metals may influence/change mobility, behaviour and bioavailability of HMs. The degree and type of combined effect obtained from mixtures is highly dependent on both concentration and time of persistence in soil (Wuana and Okieimen, 2011). Most studies report a negative effect of PAHs-HMs co-occurrence, due to the negative influence of HMs on soil microbial community which can hamper the biodegradation. However some other studies highlighted the positive interaction of heavy metals and PAHs; Saison et al. (2004), Gao et al. (2006), and Zhang et al. (2011) observed an increase in adsorption of phenanthrene in presence of HMs, additionally a positive interaction between Zn, Cd, and phenanthrene towards microbial enzyme activity was observed in Shen et al (2005) study. Some examples of studies assessing effects of co-contamination are present in literature (Ding et al., 2017; Iwegbue, 2015; Lin et al., 2008), however mechanisms that regulates sequestration, displacement, and partitioning of HMs in complex contaminated sites is still poorly understood.

Since pH governs trace metal solubility (low pH decreases sorption and increases bioavailability and mobility), a greater variation in HMs/metalloids distribution associated to Conds 1 and 2 were expected: this was not observed in any of the soils tested. Different authors have previously reported that pH has less or no effect on Cu, Pb and Zn sorption (De Matos et al., 2001; Gomes et al., 2001; Katyal and Sharma, 1991). Such behaviour suggests that soil chemical properties (e.g. pH) are not the only parameters affecting HMs remobilisation. This finding can be attributed to the addition of mild acidic sulfuric acid solutions (Conds 1 and 2) which were not sufficient to permanently modify the soils' pH. This acid addition could just cause a transient decrease in pH and thus revealed the strong pH

buffer capacity of these soils. Our finding were consistent to previous results in the literature which highlighted the minor effect on HMs leaching behaviour affected by mildly acidic and neutral pH soil values (Du et al., 2014). The lack of changes in the HMs stability was more remarkable in samples with higher sorption capacity owing to the presence of porous material such as high clay content (Soil D) or presence of cement stabiliser (Soil B), which could have played a role in increasing the retention of soluble HMs. A higher interstitial water (Cond 4) can cause a decrease in soil redox potential and change HMs oxidation states (Mukwaturi and Lin, 2015). This influences the fate and transport of metals and, combined with pH, could also control solubility or the formation of coordination complexes. Changes in pH and moisture content (Conds 3 and 4) did not affect however, the behaviour and transformation pathways of the metals in the soils tested. Among the factors that could influence metal distribution, temperature (Conds 6 and 7) has previously been found not to exert any measurable effects on Pb solubility (Kalbasi et al., 1978) or Cd leaching (Basta and Tabatabai, 1992). A similar trend was observed for moisture on paddy soil (Liu et al., 2016).

3.6. Descriptive statistics

Descriptive statistics were conducted on the data to identify relationships between HMs and metalloids, details are provided in Appendix . A positive correlation coefficient among HMs suggests that those elements may share common sources, mutual dependence, and have identical behaviour during the transport (Kennou et al., 2015; Suresh et al., 2012, 2011). Information on potential sources and pathways of HMs can be obtained based on inter-element relationships (Altan et al., 2016).

Zn, As, Cd and Pb were positively correlated for Soil A, whereas Cu did not relate with any of the studied metals and Se was only correlated with Hg (Table A4). Similarly, a significant

positive correlation was also detected between Zn, As, Cd, Pb and Se for Soil B (Table A5). In Soil C, a significant positive correlation was also observed for Zn, Cd and Pb (Table A6).

Table A7 and Table A8 further showed high Pearson's correlation coefficients between Cu, Zn, As, Pb, and Se in Soil D and between Ni, Cu, Zn, Cd, Pb, and Se in Soil E.

Cu, Zn, and Cd in particular belong to the groups 12 and 13 of the periodic table and share similar physico-chemical characteristics therefore behaving similarly. Absence of correlation between Hg-Cu (-0.052) or Pb-Hg (-0.080) for Soil A, Cr-Hg (0.304) Soil B, Ni-Cr (0.436) , Pb-Hg (0.273) for Soil C, Cd-Cr (-0.296), Cd-As (-0.270) for Soil D, and Cr-Hg (-0.067), Cu-Cr (0.125) for Soil E suggests that these metals behave very differently and their fate and distribution are not controlled by a single common factor (Kennou et al., 2015).

4. Conclusions

Assessing the partitioning of HMs and metalloids in soil is a more suitable tool to understand distribution and fate, rather than total concentration and the generic guideline values which commonly assumes that 100% of the contaminant of concern is bioavailable. The solid phase distribution highlighted the following: (1) while pseudo-total concentration shows that Pb, Cu, and Zn exceed the guideline values, only a negligible fraction of these HMs were dissolved in pore water, which confirm that these metals were not readily-available;(2) the concentration of Zn and Pb in the mobile fractions (exchangeable) was higher than those in the non-mobile fraction (non-exchangeable), both fractions remained stable during weathering and under the different treatment applied; (3) a clear difference was observed between Soil A and Soil B, where HMs were significantly more bounded in Soil B, a fact confirming that the stabilisation was a successful technique to minimize element's mobility. We assessed the behaviour of exchangeable metal fraction over time, results showed that HMs were stable and, similar behaviours were observed for both industrial contaminated soils (Soil A, B and C), and rural contaminated soil (Soil C and E) at 0, 6 and 12 months. In addition, the conditions applied such as different pH (Cond 1 and Cond 2), different moisture

(Cond 3 and Cond 4) and temperature (Cond 6 and Cond 7) did not have a clear pattern/effect on metals concentration over time. This finding can be attributed to both the soils having a strong soil pH buffer capacity, and the initial alkaline pH of the soil samples. Lastly, the Pearson's correlation coefficients showed similarities between investigated HMs/metalloids and their observed distribution pattern among the three phases (pore water, exchangeable, and non-exchangeable), and helped to classify these HMs in groups. Overall, the limited changes in metal fractionation in these soil samples, including limited removal from more recalcitrant fractions, suggest that stable soil-complexes and interaction with the soil matrix were formed and may render the elements less mobile over time, therefore reducing environmental risk. In conclusion, standard guidelines values can provide initial information on the target HMs for risk assessment, but they are not sufficient to understand the role of metal speciation and soil properties on metal bioavailability and their potential effects (risk). Using sequential extraction to measure the HMs concentration allows site specific assessment criteria to be determined and refined, providing a better estimate of the HMs/ metalloids potential bioavailable concentration.

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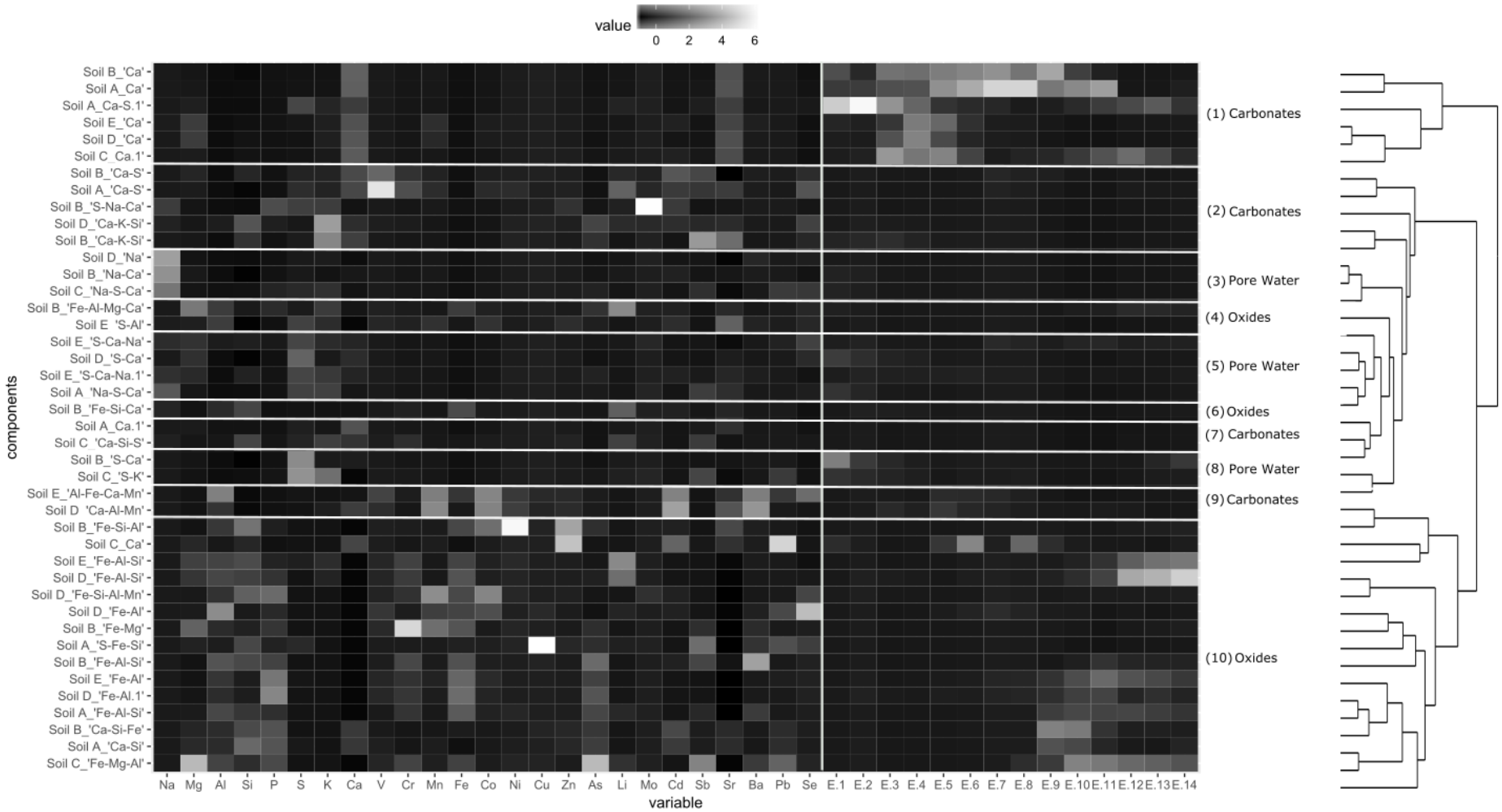

Figure 1: Heatmap and associated clustergram for CISED extraction data for a selection of test soils. The horizontal white lines divide the heatmap into the 10 different clusters, The vertical white line divides the elements composition data on the left side (e.g Na, Mg, Al) from the extraction number data (E_1 to E_{14}) on the right side. A high proportion of each component and an indication of its composition are shown by a white or pale grey colouration with a low proportion as dark grey or black

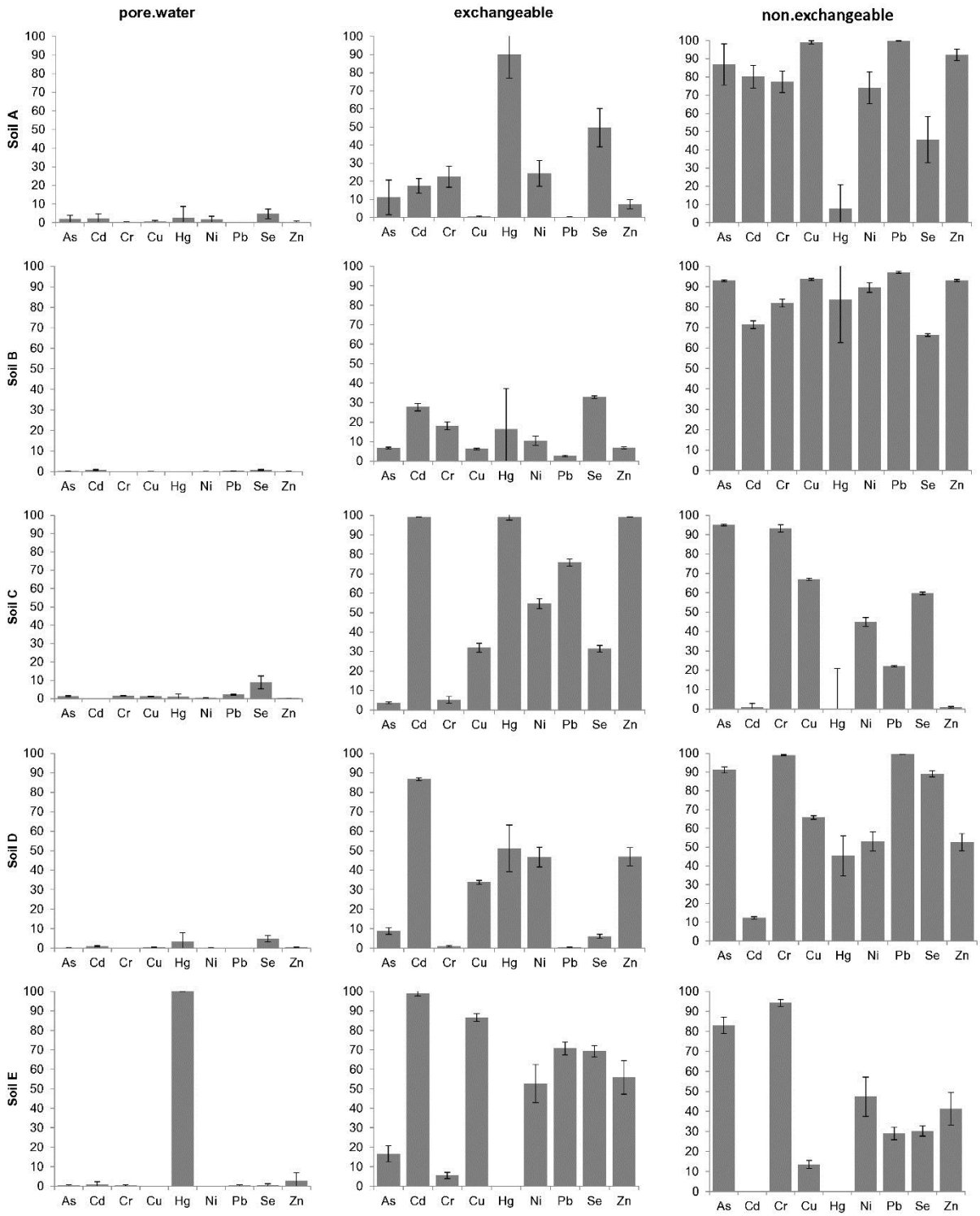

Figure 2: Overall HMs/ metalloids distribution for Soil A, Soil B, Soil C, soil D, and Soil E across the 7 conditions for all sampling times (T0, 6, and 12 months) expressed as percentage. Concentrations have been averaged across time and conditions in order to provide an overview of the overall metal behaviour.

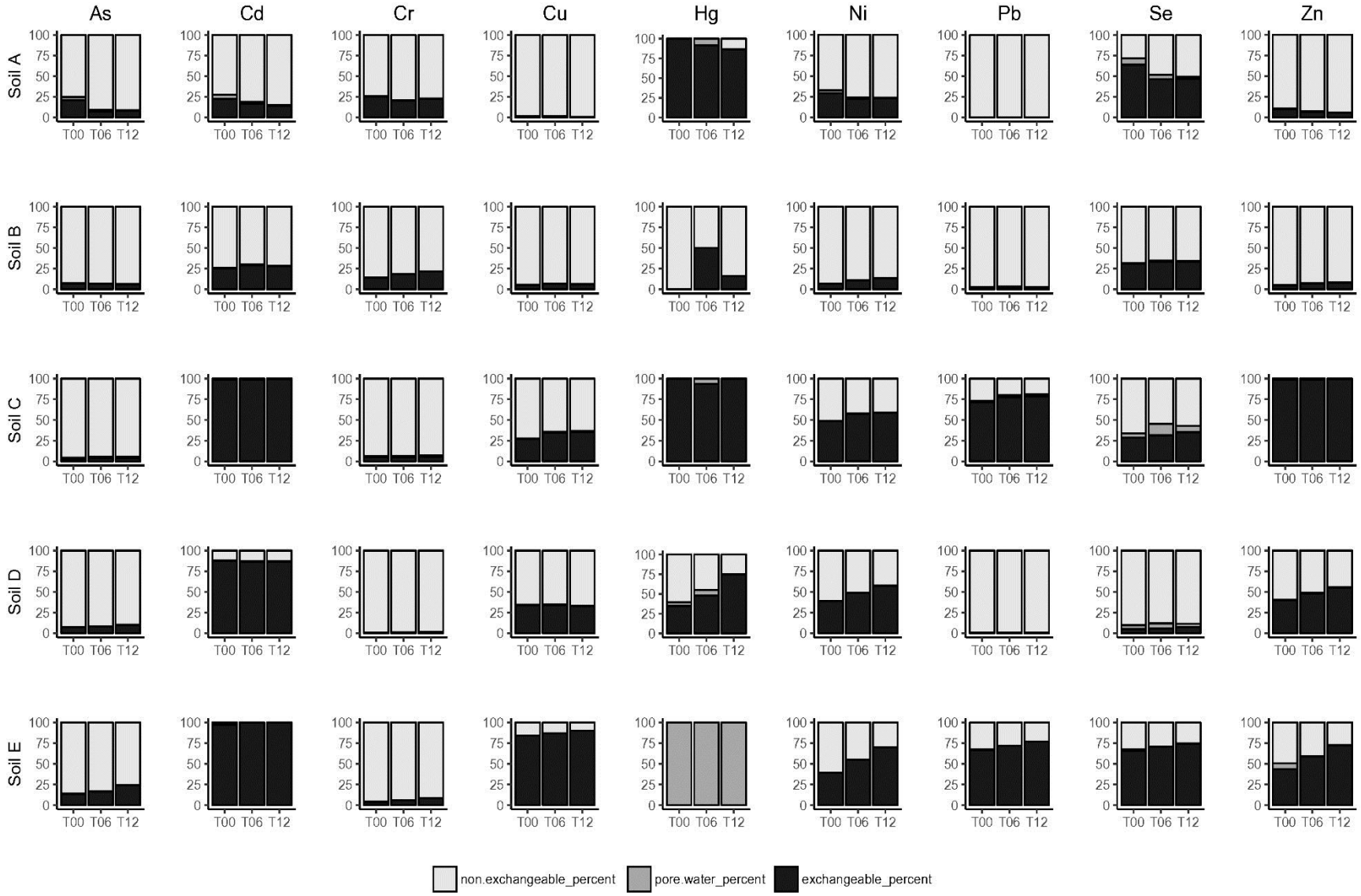

Figure 3 : Heavy metals and metalloids distribution expressed as percentage (pore water, exchangeable, and non-exchangeable), within the 5 soil samples under the 7 mesocosm conditions tested at T0, 6 and 12 months.

Table 1: Soils samples and soil characteristics used in the mesocosms experimental setup

Soil	Treatment	Soil type	Contamination type	Soil matrix
Soil A	Pre- treatment*	Industrial	TPHs > 1000 mg/kg (high), HMs > 800 mg/kg	Sandy loam
Soil B	Post- treatment**			
Soil C	No treatment	Industrial	TPHs <1000 mg/kg (medium), HMs > 6200 mg/kg	Sandy loam
Soil D	No treatment	Rural	TPHs < 500 mg/kg (low), HMs > 800 mg/kg	Clay loam
Soil E	No treatment			

TPHs: total petroleum hydrocarbons, HMs: heavy metals

* No stabiliser, ** application of cement stabiliser

Table 2: Experimental design, conditions applied to all soils

Condition	pH	Temperature	Moisture content
Cond 1	buffered to 6-7	20°C	As received
Cond 2	constant acid rain simulation (pH 4.5)	20°C	As received
Cond 3	As received	20°C	20% WHC
Cond 4	As received	20°C	70% WHC
Cond 5	As received	20°C	As received
Cond 6	As received	Outdoor	As received
Cond 7	As received	4°C	As received

Cond: condition, WHC: Water Holding Capacity

Table 3: Sequential extraction steps

Extraction number	Solution number	Concentration (M)	Deionised water	Volume HNO_3 (ml)	Volume H_2O_2 (ml)	Total volume (ml)
E_{1-2}	Sol 1	0.00	10.0	0.00	0.00	10.00
E_{3-4}	Sol 2	0.01	0.00	10.00	0.00	10.00
E_{5-6}	Sol 3	0.05	0.00	10.00	0.00	10.00
E_{7-8}	Sol 4	0.10	0.00	9.75	0.25	10.00
E_{9-10}	Sol 5	0.50	0.00	9.50	0.50	10.00
E_{11-12}	Sol 6	1.00	0.00	9.25	0.75	10.00
E_{13-14}	Sol 7	5.00	0.00	9.00	1.00	10.00

Table 4: Physicochemical properties of the five soil samples including pseudo-total heavy metals/metalloids and total petroleum hydrocarbon concentrations

		Industrial			Rural	
Characteristics	**Analysis**	Soil A	Soil B	Soil C	Soil D	Soil E
Nutrients	Total N (%)	0.07	0.08	0.12	0.23	0.25
	Total C (%)	4.00	4.14	3.87	2.39	2.78
	C / N Ratio	57.28	52.86	31.71	10.44	10.93
	Total P (mg/kg)	453.37	433.73	499.60	798.59	801.12
	available P (mg/kg)	31.55	30.55	42.18	35.22	36.72
	70% of WHC_{max} (% m/m)	21.92	20.37	19.64	39.21	40.02
	20% of WHC_{max} (% m/m)	6.26	5.99	5.61	11.20	12.00
	Dry matter content W_{dm} (%)	78.40	76.37	79.88	68.24	68.28
	Water content (%)	27.55	30.94	25.19	46.53	46.46
	pH	9.71	9.56	9.22	7.99	7.54
	LOI (%)	4.28	3.97	5.44	5.99	6.49
Stone/gravel content	% > 5.5 mm	25.97	23.47	20.13	0.00	0.00
	% 5.5 mm< > 2 mm	24.54	27.00	38.73	24.69	23.57
	% < 2 mm	49.48	49.54	41.15	75.31	76.43
Particle size	% 600 µm (Coarse Sand)	11.88	13.65	16.86	3.55	4.36
	% 212 µm (Medium Sand)	29.86	33.41	34.58	14.90	14.46
	% 63 µm (Fine Sand)	30.37	27.04	20.24	11.70	11.29
	Overall sand content	72.11	74.10	71.68	30.16	30.10
	% 0.002 mm-0.063 mm (Silt)	19.67	16.70	16.14	40.57	36.07
	%< 0.002 mm (Clay)	8.22	9.20	12.17	29.28	33.83

Heavy metals and metalloids pseudo-total concentrations (mg/kg) *	As	1.38 - 22.05	4.13 - 22.4	3.31 - 46.99	3.95 - 25.89	5.88 - 33.29
	Cd	0.08 - 3.6	0.26 - 2.29	0.27 - 1.9	0.05 - 0.4	0.08 - 0.33
	Cr	3.08 - 44.02	8.7 - 99.99	5.2 - 51.23	7.7 - 85.17	19.93 - 61.81
	Cu	5.19 - 169.82	10.42 - 99.08	9.25 - 128.08	4.01 - 34.28	8.47 - 30.49
	Hg	0 - 1.63	0 - 5.68	0 - 0.24	0 - 0.06	0 - 0.04
	Ni	2.16 - 29.76	6.39 - 34.88	4.1 - 36.54	7.04 - 49.14	10.64 - 34.44
	Pb	18.49 - 794.1	9.21 - 672.67	337.38 - 6603.57	11.51 - 66.85	20.44 - 59.73
	Se	0.42 - 45.24	0.61 - 44.95	0.55 - 4.11	0.81 - 4.08	0.97 - 3.97
	Zn	15.42 - 272.17	66.2 - 281.63	277.81 - 3527.2	30.03 - 156.62	44.57 - 130.83
Petroleum hydrocarbons total concentrations (mg/kg)*	TOT ALKs	82.45 - 505.22	49 - 187.78	82.91 - 460.78	79.22 - 432.51	78 - 258.48
	TOT PAHs	195.55 - 2770.11	122.84 - 1146.95	8.44 - 244	0.36 - 401.9	0.6 - 363.37

*across all condition tested and rime points analysed

N: nitrogen, C: carbon, P: phosphorous, WHC: water holding capacity, LOI: loss of ignition, As: arsenic, Cd: cadmium, Cr: chromium, Cu: copper, Hg: mercury, Ni: nickel, Pb: lead, Se: selenium, Zn: zinc, HMs: heavy metals, ALKs: alkanes, PAHs: Polycyclic aromatic hydrocarbons..

Appendix - Insights into mixed contaminants interactions and its implication for heavy metals and metalloids mobility, bioavailability, and risk assessment – Cipullo et al.

Table A1: Descriptive statistics for metals and concentrations (expressed in mg/kg), obtained from replicates CISED sequential extraction of guidance materials (BGS_{102})**.**

Elements	min	max	range	sum	median	mean	SE.mean	CI.mean.0.95	var	std.dev	coef.var
Al	244.59	379.45	134.87	1682.34	342.91	336.47	24.65	68.45	3039.10	55.13	0.16
As	0.39	0.81	0.43	2.49	0.43	0.50	0.08	0.22	0.03	0.18	0.36
Ba	8.45	10.38	1.93	47.71	10.18	9.54	0.44	1.23	0.98	0.99	0.10
Ca	1108.26	1535.07	426.81	6375.74	1221.39	1275.15	72.74	201.95	26454.26	162.65	0.13
Cd	0.02	0.05	0.03	0.16	0.03	0.03	0.00	0.01	0.00	0.01	0.33
Co	1.37	1.83	0.46	8.13	1.72	1.63	0.09	0.24	0.04	0.20	0.12
Cr	0.37	3.45	3.08	11.46	2.85	2.29	0.54	1.51	1.48	1.22	0.53
Cu	0.73	1.24	0.51	5.12	1.12	1.02	0.09	0.26	0.04	0.21	0.20
Fe	319.69	857.41	537.72	3392.79	762.15	678.56	94.22	261.59	44383.96	210.68	0.31
K	63.99	107.52	43.54	435.88	81.22	87.18	8.39	23.30	352.23	18.77	0.22
Li	0.24	0.60	0.36	2.04	0.46	0.41	0.07	0.19	0.02	0.15	0.37
Mg	42.30	77.94	35.64	319.76	63.17	63.95	6.47	17.95	209.06	14.46	0.23
Mn	364.95	442.46	77.51	1996.96	404.15	399.39	13.99	38.85	979.00	31.29	0.08
Mo	0.04	0.38	0.34	0.95	0.20	0.19	0.05	0.15	0.02	0.12	0.64
Ni	1.17	2.04	0.87	8.60	1.84	1.72	0.16	0.45	0.13	0.36	0.21
P	43.34	84.58	41.24	317.31	63.71	63.46	6.56	18.22	215.22	14.67	0.23
Pb	2.42	4.07	1.65	16.28	3.29	3.26	0.36	1.00	0.65	0.81	0.25
S	19.19	53.78	34.59	162.83	33.81	32.57	6.14	17.06	188.78	13.74	0.42
Se	0.24	0.58	0.33	1.62	0.27	0.32	0.06	0.18	0.02	0.14	0.44
Si	178.17	294.16	115.99	1204.00	266.29	240.80	23.00	63.87	2645.73	51.44	0.21
Sr	2.49	3.03	0.54	14.19	2.97	2.84	0.11	0.31	0.06	0.25	0.09
V	1.43	2.62	1.19	10.73	2.25	2.15	0.23	0.62	0.25	0.50	0.23
Zn	2.79	5.04	2.26	20.93	4.36	4.19	0.37	1.04	0.70	0.84	0.20

Standard error on the mean (SE.mean), confidence interval of the mean (CI.mean) at the p level, variance (var), standard deviation (std.dev) and variation coefficient (coef.var) defined as the standard deviation divided by the mean.

Table A 2: Descriptive statistics of 40 blank measurements (expressed in counts per second), and limit of detection (LOD, expressed in µg/L) of the analytical method applied.

Elements	min	max	range	sum	median	mean	SE.mean	CI.mean.0.95	std.dev	coef.var	stdev	stdev*3	slope	LOD
Al	116.00	322.00	206.00	1029.09	148.55	171.52	30.86	79.33	75.59	0.44	51.60	154.80	136.78	1.130
As	1.00	6.00	5.00	19.26	3.13	3.21	0.70	1.81	1.72	0.54	1.15	3.45	318.13	0.010
Ba	1961.00	3850.00	1889.00	14543.48	2192.99	2423.91	290.26	746.14	710.99	0.29	411.49	1234.48	66104.02	0.020
Ca	2.00	12.00	10.00	38.02	6.01	6.34	1.41	3.61	3.44	0.54	2.13	6.38	4.66	1.370
Cd	5.00	26.00	21.00	59.79	7.40	9.97	3.24	8.33	7.94	0.80	3.48	10.45	6069.74	0.002
Co	4.00	448.00	444.00	497.65	7.50	82.94	73.09	187.87	179.02	2.16	81.32	243.96	7670.48	0.030
Cr	30.00	1450.00	1420.00	1681.42	43.50	280.24	234.05	601.65	573.30	2.05	214.74	644.23	3768.54	0.170
Cu	145.00	835.00	690.00	1783.92	201.00	297.32	108.43	278.72	265.59	0.89	151.06	453.18	5688.58	0.080
Fe	2606.00	30202.00	27596.00	44906.37	2922.75	7484.40	4545.59	11684.81	11134.37	1.49	4171.68	12515.05	2943.85	4.250
Hg	79.00	159.00	80.00	644.52	101.51	107.42	11.26	28.95	27.59	0.26	16.86	50.58	6151.55	0.010
K	7777.00	10162.00	2385.00	54500.85	9138.67	9083.47	310.91	799.21	761.56	0.08	298.63	895.89	299.74	2.990
Li	391.00	713.00	322.00	3080.93	487.97	513.49	47.47	122.03	116.28	0.23	96.14	288.42	81705.34	0.004
Mg	168.00	832.00	664.00	1826.76	205.75	304.46	105.88	272.18	259.36	0.85	110.08	330.24	486.38	0.680
Mn	8.00	363.00	355.00	433.07	14.50	72.18	58.20	149.60	142.55	1.98	53.42	160.26	1685.35	0.100
Mo	14.00	48.00	34.00	162.38	24.94	27.06	4.65	11.94	11.38	0.42	6.98	20.94	13962.55	0.001
Ni	19.00	663.00	644.00	830.95	31.25	138.49	105.07	270.09	257.36	1.86	128.73	386.19	2241.95	0.170
Na	4654.00	11725.00	7071.00	37876.17	5386.84	6312.70	1101.63	2831.83	2698.43	0.43	1631.17	4893.50	1139.94	4.290
P	161.00	197.00	36.00	1086.09	182.05	181.02	4.98	12.81	12.20	0.07	8.64	25.93	8.73	2.970
Pb	776.00	3336.00	2560.00	7708.58	904.50	1284.76	411.16	1056.93	1007.14	0.78	384.69	1154.08	88169.82	0.010
S	2597.00	3306.00	709.00	18408.34	3124.92	3068.06	99.74	256.39	244.31	0.08	114.11	342.33	3.06	111.890
Sb	23.00	86.00	63.00	271.53	41.77	45.26	8.90	22.87	21.79	0.48	12.52	37.56	19894.58	0.000
Se	2.00	11.00	9.00	39.51	6.76	6.59	1.23	3.16	3.01	0.46	2.11	6.33	24.32	0.260
Si	34440.00	41612.00	7172.00	236813.42	40173.71	39468.90	1037.49	2666.97	2541.33	0.06	1046.54	3139.62	116.55	26.940
Sr	732.00	1548.00	816.00	5612.91	840.45	935.48	124.52	320.08	305.00	0.33	153.70	461.11	64139.65	0.010
V	1.00	10.00	9.00	23.28	3.14	3.88	1.30	3.33	3.18	0.82	1.69	5.06	2962.43	0.002
Zn	39.00	491.00	452.00	791.95	65.75	131.99	72.04	185.19	176.46	1.34	74.68	224.05	494.81	0.450

Standard error on the mean (SE.mean), confidence interval of the mean (CI.mean) at the p level, variance (var), standard deviation (std.dev) and variation coefficient (coef.var) defined as the standard deviation divided by the mean.

Table A3: Descriptive statistics of heavy metals (HMs) and metalloids concentrations in pore water, exchangeable and non-exchangeable fraction (expressed in mg/kg) in the soil samples analysed (Soil A, Soil B, Soil C, Soil D, and Soil E).

Sample	Element	Pore water				Exchangeable				Non-exchangeable			
		Range			Median	Range			Median	Range			Median
Soil A	As	0.050	-	0.113	0.080	0.494	-	0.615	0.599	6.031	-	7.345	7.050
	Cd	0.002	-	0.005	0.003	0.042	-	0.051	0.047	0.216	-	0.259	0.234
	Cr	0.003	-	0.006	0.005	0.882	-	1.092	1.029	3.070	-	4.716	3.648
	Cu	0.056	-	0.126	0.089	0.168	-	0.213	0.193	25.918	-	41.040	33.170
	Hg	0.000	-	0.000	0.000	0.001	-	0.002	0.002	0.000	-	0.000	0.000
	Ni	0.035	-	0.079	0.056	1.256	-	1.506	1.388	4.197	-	5.605	4.984
	Pb	0.000	-	0.000	0.000	0.044	-	0.056	0.048	96.378	-	122.121	106.981
	Se	0.033	-	0.075	0.053	0.691	-	0.845	0.791	0.732	-	0.918	0.864
	Zn	0.106	-	0.238	0.168	4.769	-	5.756	5.320	70.648	-	81.294	73.908
Soil B	As	0.016	-	0.028	0.021	0.542	-	0.607	0.582	7.678	-	8.684	7.929
	Cd	0.004	-	0.008	0.006	0.214	-	0.256	0.250	0.582	-	0.705	0.656
	Cr	0.000	-	0.000	0.000	1.091	-	1.354	1.263	4.910	-	6.364	5.375
	Cu	0.024	-	0.036	0.029	2.528	-	3.079	2.867	41.476	-	46.504	43.960
	Hg	0.000	-	0.000	0.000	0.001	-	0.001	0.001	0.001	-	0.006	0.002
	Ni	0.004	-	0.005	0.004	0.874	-	0.990	0.894	5.945	-	9.796	6.501
	Pb	0.357	-	0.648	0.468	3.848	-	4.699	4.486	149.771	-	186.686	171.491
	Se	0.010	-	0.017	0.013	0.544	-	0.590	0.569	1.102	-	1.229	1.139
	Zn	0.161	-	0.315	0.214	8.978	-	11.568	10.750	131.240	-	150.798	140.395
Soil C	As	0.168	-	0.246	0.192	0.590	-	0.741	0.671	13.142	-	20.037	16.334
	Cd	0.000	-	0.000	0.000	1.129	-	1.446	1.340	0.010	-	0.015	0.012
	Cr	0.126	-	0.201	0.154	0.390	-	0.536	0.472	7.415	-	11.306	9.216
	Cu	0.389	-	0.581	0.461	12.106	-	15.287	14.005	22.493	-	34.295	27.957
	Hg	0.000	-	0.000	0.000	0.007	-	0.016	0.009	0.000	-	0.000	0.000
	Ni	0.029	-	0.043	0.036	5.545	-	7.054	6.614	4.092	-	6.239	5.086

	Pb	42.420	-	62.902	52.165	1624.888	-	2064.239	1891.046	420.330	-	640.887	522.437
	Se	0.087	-	0.126	0.104	0.437	-	0.561	0.510	0.747	-	1.139	0.929
	Zn	2.052	-	3.118	2.540	1313.302	-	1657.856	1535.797	9.358	-	14.268	11.631
Soil D	As	0.002	-	0.005	0.004	0.325	-	0.385	0.343	3.094	-	4.406	3.622
	Cd	0.001	-	0.002	0.002	0.170	-	0.204	0.187	0.024	-	0.032	0.028
	Cr	0.001	-	0.002	0.001	0.060	-	0.073	0.063	4.267	-	9.207	5.086
	Cu	0.048	-	0.090	0.069	5.286	-	6.041	5.708	10.286	-	12.040	10.661
	Hg	0.000	-	0.000	0.000	0.002	-	0.003	0.002	0.001	-	0.003	0.001
	Ni	0.002	-	0.009	0.004	2.759	-	3.327	3.047	2.028	-	5.379	2.245
	Pb	0.000	-	0.000	0.000	0.290	-	0.340	0.302	53.687	-	58.755	56.100
	Se	0.068	-	0.116	0.080	0.109	-	0.135	0.118	1.544	-	1.920	1.615
	Zn	0.124	-	0.232	0.159	15.243	-	18.038	16.736	12.100	-	28.150	13.715
Soil E	As	0.000	-	0.000	0.000	0.600	-	0.732	0.646	2.945	-	4.349	3.659
	Cd	0.000	-	0.000	0.000	0.200	-	0.231	0.216	0.000	-	0.000	0.000
	Cr	0.000	-	0.001	0.000	0.325	-	0.382	0.347	3.900	-	9.083	7.032
	Cu	0.000	-	0.000	0.000	12.187	-	14.558	13.209	1.825	-	2.550	2.113
	Hg	0.001	-	0.034	0.002	0.000	-	0.000	0.000	0.000	-	0.000	0.000
	Ni	0.000	-	0.000	0.000	3.272	-	3.777	3.530	1.583	-	5.488	3.898
	Pb	0.000	-	0.007	0.000	47.840	-	58.162	53.318	18.523	-	26.776	22.418
	Se	0.000	-	0.000	0.000	1.071	-	1.377	1.261	0.462	-	0.645	0.534
	Zn	0.001	-	0.031	0.002	18.681	-	21.512	20.115	9.521	-	22.601	17.437

Range of measurement between different sampling times (0, 6, and 12 months) and different conditions (Cond 1-Cond 7)

Table A4: Pearson's correlation matrix for the heavy metals and metalloids concentrations (Soil A)

	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb	Se
Cr	1.000								
Ni	**0.980**	1.000							
Cu	0.566	0.572	1.000						
Zn	**0.910**	**0.877**	0.722	1.000					
As	**0.965**	**0.977**	0.618	**0.936**	1.000				
Cd	**0.926**	**0.933**	0.669	**0.973**	**0.968**	1.000			
Hg	0.279	0.427	-0.052	0.022	0.346	0.199	1.000		
Pb	0.837	0.800	0.800	**0.985**	**0.878**	**0.935**	-0.080	1.000	
Se	0.456	0.600	0.086	0.195	0.502	0.383	**0.959**	0.085	1.000

Table A5: Pearson's correlation matrix for the heavy metals and metalloids concentrations (Soil B)

	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb	Se
Cr	1.000								
Ni	0.742	1.000							
Cu	**0.890**	0.775	1.000						
Zn	**0.859**	**0.918**	**0.959**	1.000					
As	**0.911**	0.786	**0.995**	**0.956**	1.000				
Cd	**0.867**	0.716	**0.945**	**0.900**	**0.932**	1.000			
Hg	0.304	0.664	0.360	0.521	0.359	0.318	1.000		
Pb	**0.864**	0.738	**0.996**	**0.944**	**0.983**	**0.936**	0.334	1.000	
Se	**0.888**	0.718	**0.895**	**0.860**	**0.899**	**0.977**	0.307	**0.869**	1.000

Table A6: Pearson's correlation matrix for the heavy metals and metalloids concentrations (Soil C)

	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb	Se
Cr	1.000								
Ni	0.436	1.000							
Cu	**0.908**	0.773	1.000						
Zn	-0.424	0.630	-0.005	1.000					
As	**0.999**	0.424	**0.902**	-0.436	1.000				
Cd	-0.423	0.631	-0.005	**1.000**	-0.435	1.000			
Hg	-0.075	0.198	0.017	0.280	-0.111	0.275	1.000		
Pb	-0.160	0.818	0.268	**0.962**	-0.174	**0.961**	0.273	1.000	
Se	**0.902**	0.752	**0.985**	-0.022	**0.897**	-0.021	0.034	0.249	1.000

Table A7: Pearson's correlation matrix for the heavy metals and metalloids concentrations (Soil D)

	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb	Se
Cr	1.000								
Ni	0.719	1.000							
Cu	0.810	0.837	1.000						
Zn	0.706	**0.998**	**0.853**	1.000					
As	**0.960**	0.669	**0.885**	0.671	1.000				
Cd	-0.296	0.442	0.172	0.465	-0.270	1.000			
Hg	0.577	**0.893**	0.689	**0.890**	0.514	0.463	1.000		
Pb	**0.891**	0.528	**0.856**	0.535	**0.969**	-0.356	0.363	1.000	
Se	**0.952**	0.613	**0.863**	0.614	**0.992**	-0.337	0.461	**0.985**	1.000

Table A8: Pearson's correlation matrix for the heavy metals and metalloids concentrations (Soil E)

	Cr	Ni	Cu	Zn	As	Cd	Hg	Pb	Se
Cr	1.000								
Ni	0.435	1.000							
Cu	0.125	**0.946**	1.000						
Zn	0.372	**0.997**	**0.966**	1.000					
As	**0.964**	0.644	0.371	0.592	1.000				
Cd	0.034	**0.914**	**0.995**	**0.940**	0.284	1.000			
Hg	-0.067	-0.068	-0.055	-0.042	-0.073	-0.040	1.000		
Pb	0.296	**0.986**	**0.985**	**0.995**	0.528	**0.963**	-0.064	1.000	
Se	0.332	**0.990**	**0.977**	**0.996**	0.560	**0.951**	-0.067	**0.999**	1.000

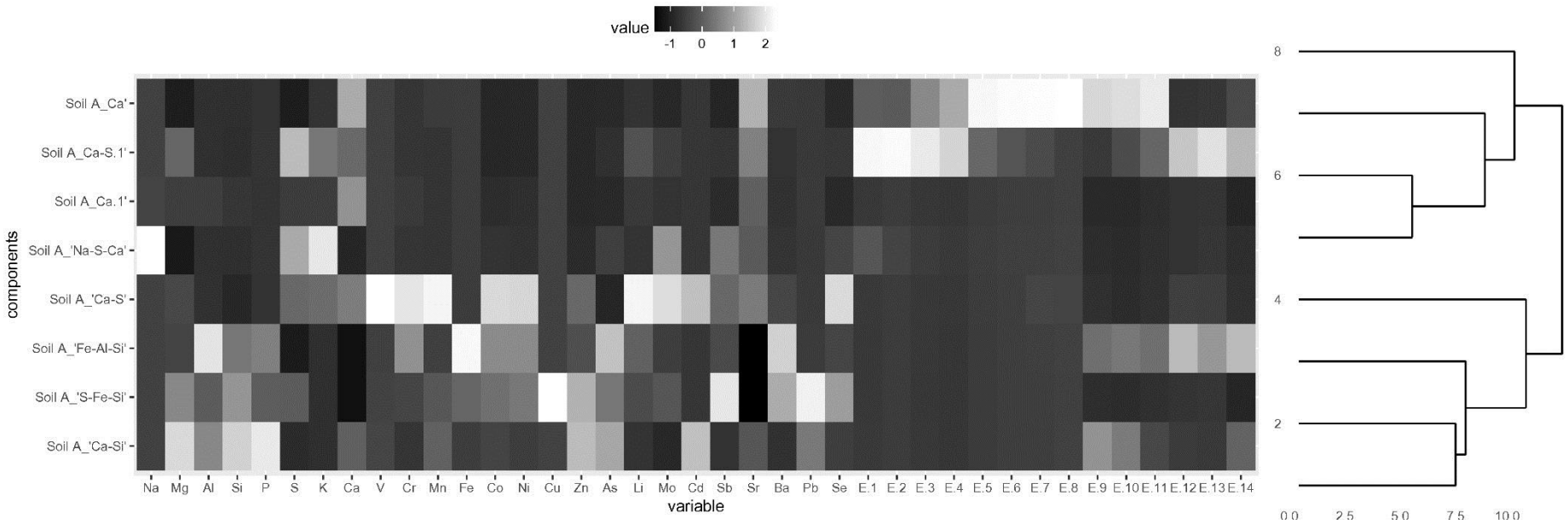

Figure A1 : Heatmap and associated hierarchical cluster for the CISED extraction data for Soil A

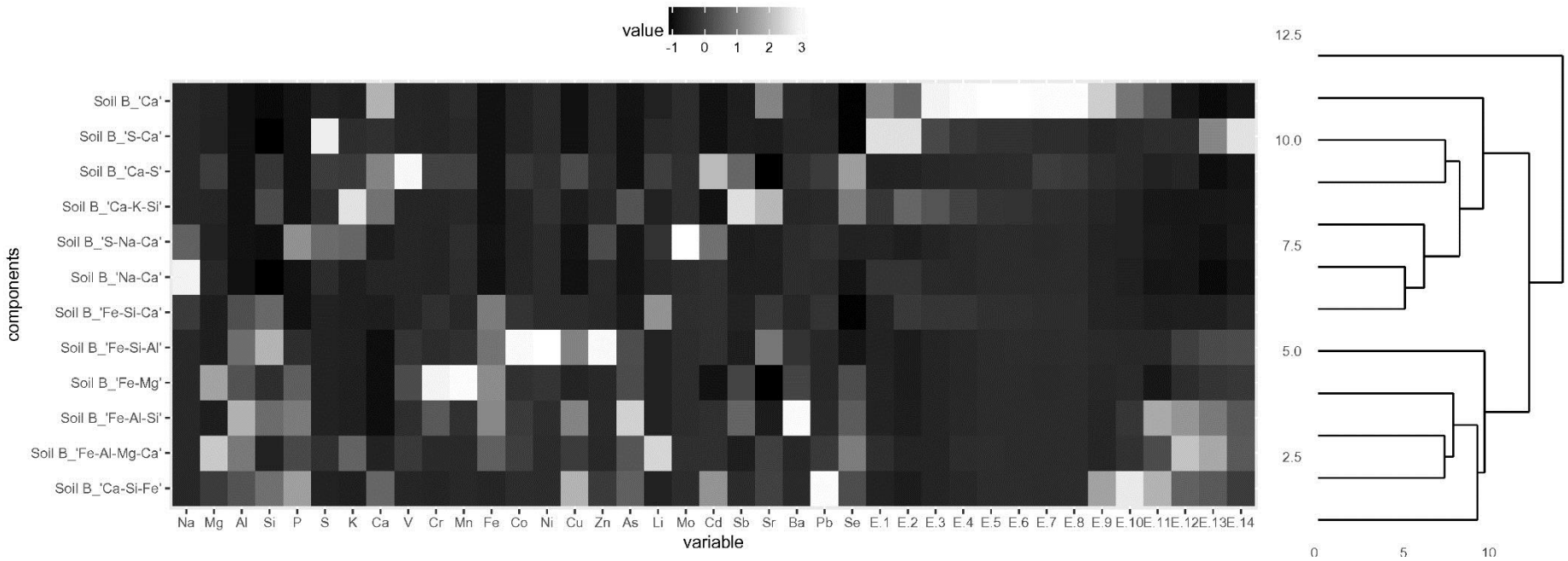

Figure A2: Heatmap and associated hierarchical cluster for the CISED extraction data for Soil B

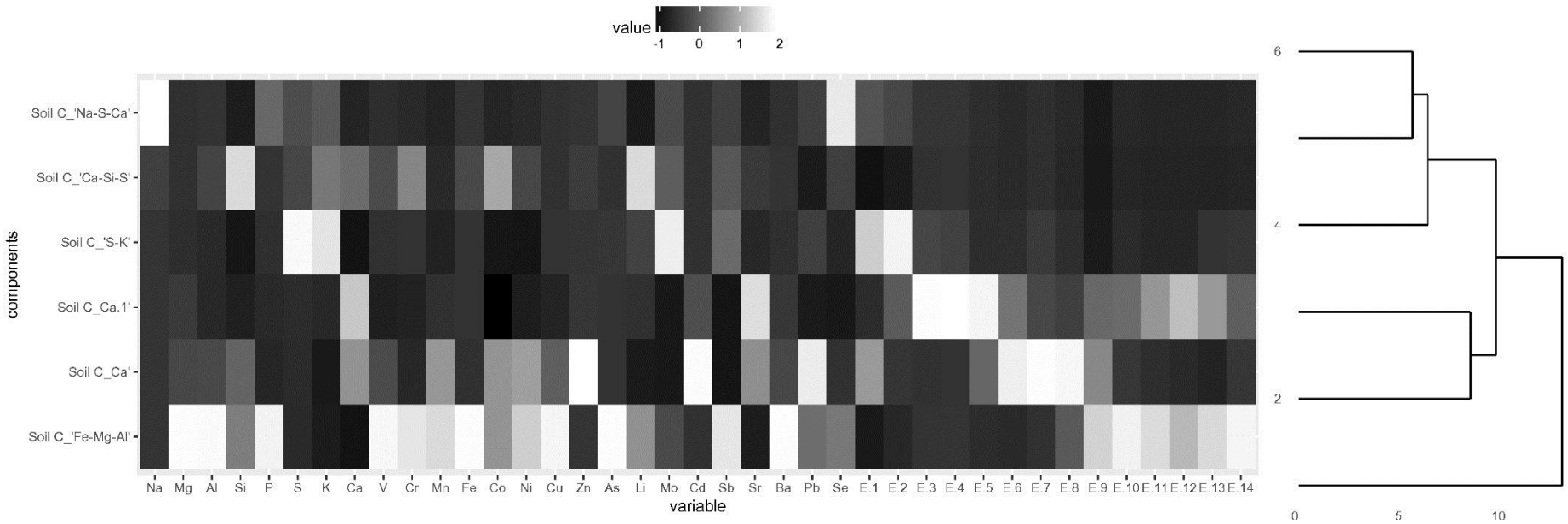

Figure A3: Heatmap and associated hierarchical cluster for the CISED extraction data for Soil C

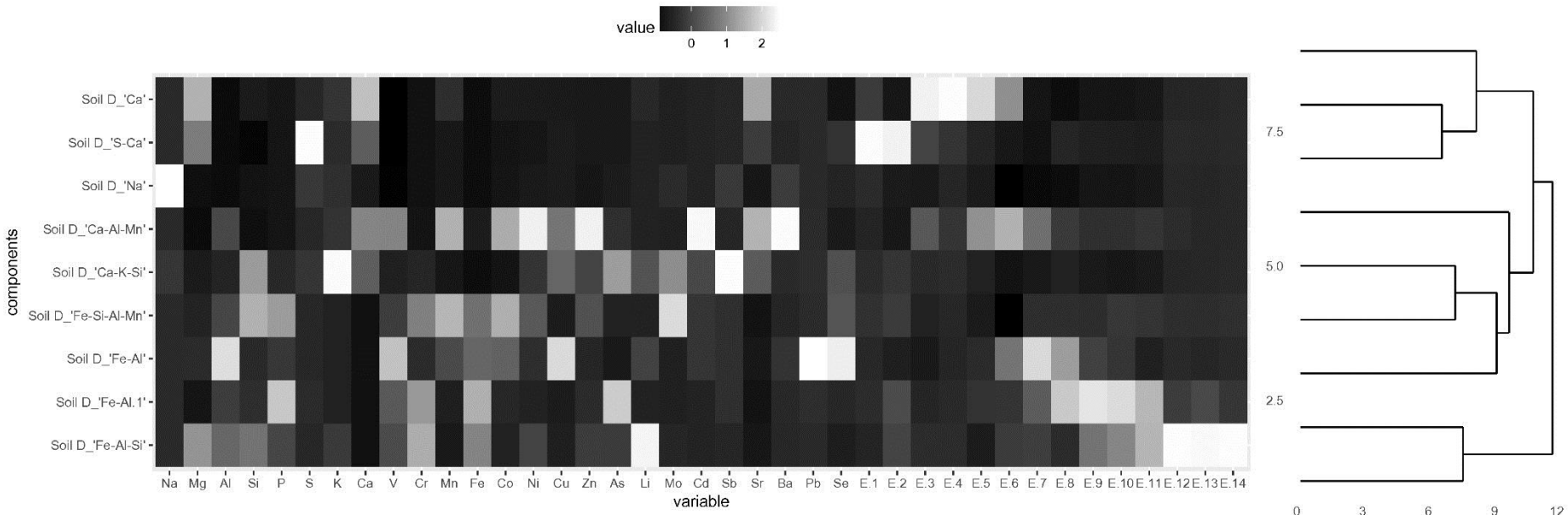

Figure A4: Heatmap and associated hierarchical cluster for the CISED extraction data for Soil D

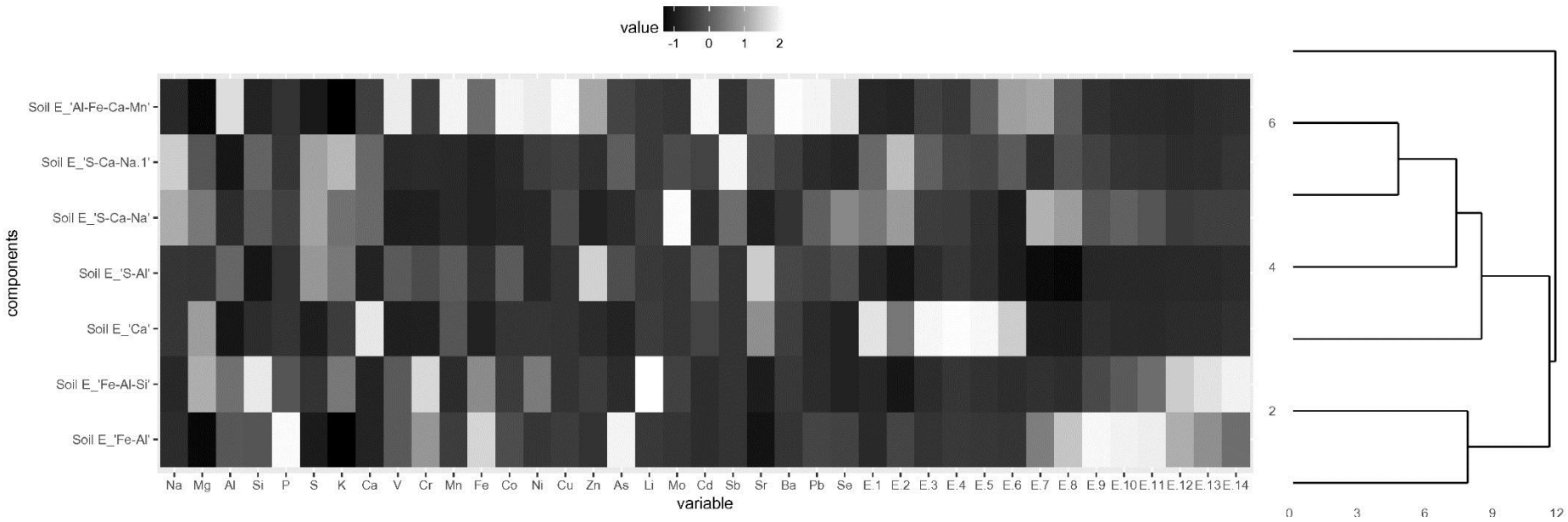

Figure A5: Heatmap and associated hierarchical cluster for the CISED extraction data for Soil E

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Insights into mixed contaminants interactions and its implication for heavy metals and metalloids mobility, bioavailability and risk assessment

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