Investigating relationships between biomarkers of exposure and environmental copper and manganese levels in house dusts from a Portuguese industrial city

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Abstract

This study reports on data obtained from a pilot survey focusing on house dust and toenail metal(loids) concentrations in residents living in the industrial city of Estarreja. The study design hereby described aims at investigating relationships between human toenails and both copper and manganese levels in house dusts. A total of 21 households and 30 individuals were recruited for the pilot study: 19 households corresponding to 27 residents living near the industrial complex, forming the exposed group, plus 2 households and 3 residents from residential areas with no anticipated environmental contaminants that were used for comparison. Factorial analysis was used for source apportionment purposes. Investigation on the potential influence of environmental factors over copper and manganese levels in the toenails was carried out via questionnaire data and multiple correspondence analysis. The results show that copper concentrations are more elevated in the indoor dusts while manganese concentrations are more elevated in the outdoor dust samples. The geometrical relationships in the datasets suggest that the backyard soil is a probable source of manganese to the indoor dust. Copper and manganese contents in the toenail clippings are more elevated in children than in adults but the difference between the two age groups is not statistically significant ($p > 0.05$). Investigation of environmental factors influencing the exposure-biomarker association indicates a probable relationship between manganese contents in indoor dust and manganese levels in toenail clippings, a result that is partially supported by the bioaccessibility estimates. However, for copper no relationship was found between indoor dusts and the biomarkers of exposure.

Keywords: house dust, toenail clippings, copper, manganese, factorial analysis

1. Introduction

Copper (Cu) and manganese (Mn) are naturally occurring and ubiquitous in the environment. Humans are exposed to these elements via contaminated water, food, soil and dust. Although Cu and Mn are required elements in human nutrition, at elevated levels they are potentially harmful elements (PHE). Therefore, intake, uptake, Cu and Mn levels in the environment, as well as their bioavailability, are important subjects of discussion. Currently, no formal recommended dietary allowance (RDA) for Mn has been established, but the Scientific Committee for Food of the EU has estimated 1-10 mg day^{-1} as an acceptable range of intake (SCF, 2000). A considerably lower intake value (1.1 mg day^{-1}) was established for Cu in 1992 (SCF, 2003). Although balance studies and excretion data indicate that low gastrointestinal absorption and rapid elimination of Mn limits its toxicity following the ingestion of high doses, it is well established that exposure to environmental Mn can result in elevated tissue Mn levels (Aschner & Aschner 2005; Santamaria 2008). Likewise, recent studies report elevated Cu levels determined in human biomarkers collected from people exposed to environmental Cu (Ndilila et al. 2014). Manganese-induced neurotoxicity from excess respiratory or dietary exposures is well documented (Aschner & Aschner 2005). For Cu, acute symptoms of exposure to Cu include excessive salivation, epigastric pain, nausea, vomiting, and diarrhea (Araya et al. 2001).

Several characteristics suggest that contaminated indoor house dust may pose a greater risk to humans than contaminated exterior soil. Such characteristics are (i) particle size distribution; (ii) the concentration of contaminants in indoor dust relative to exterior soil; (iii) fine particle enrichment and, (iv) bioaccessibility of the PHE in dust relative to exterior soil. Indoor dust contains a higher proportion of small particles (Paustenbach et al. 1997) that makes it more mobile (easily re-suspended) and therefore more probable to be inhaled. Also, indoor dust has better skin adherence properties than exterior soils (Kissel et al. 1996), which enhances the probability of being ingested, especially by children that exhibit a typical hand-to-mouth behaviour. Therefore, dust particles come in contact with humans more readily than soil particles. The levels of contaminants in indoor dust are usually more elevated than the ones in the exterior soil, and probably more elevated in the finest particles (Ibanez et al. 2010; Niu et al. 2010). Moreover, contaminants in fine particles are more soluble in the gastrointestinal fluids and therefore exposure to contaminated indoor dust may result in an increased ingested dose relative to exposure to contaminated soils (Niu et al. 2010; Reis et al. 2014a). Experimental lines of evidence have shown that 50% or more of the soil ingested by children (the most probable receptor) on a daily basis is composed of indoor dust (Stanek and Calabrese, 1992), which reinforces the assumption that dust may be a major pathway of exposure to many environmental contaminants. A probable reason, which is more related with behavioral characteristics of the receptor rather than with characteristics of the indoor dusts, is that location (indoor or outdoor) is important for hand-to-mouth frequency, which is higher indoors than outdoors (Bierkens et al. 2011). But another key question concerns the degree to which the metal load of the indoor dust originates from the transport of outdoor dusts and soil into the interior of a home. Paustenbach et al. (1997) discuss various studies that use different approaches and deliver estimates ranging from ≈ 5 to >80% for the contribution of soil and exterior house dust to the indoor dust.

It has been demonstrated that bioaccessibility, and therefore bioavailability, depends on the chemical form of the contaminant (Rasmussen et al. 2008; Demetriades et al. 2010; Beauchemin et al. 2011; Reis et al. 2014b) and that the chemical form of the contaminant changes over time, usually resulting in a decreasing bioaccessibility (Tang et al. 2008). But the ageing processes that are effective at outdoor removal of contaminants do not operate, or they operate at reduced efficiency, indoors compared to outdoors, because contaminants in indoor dusts are protected from sunlight, rain, temperature extremes, and microbial action, in particular dust trapped in carpets and drapes (Paustenbach et al. 1997; Bierkens et al. 2011).

One promising biomarker that continues to gain attention as a possible index of long term trace element status in humans (Cottingham et al. 2013; Brockman et al. 2014) as well as a routine biomarker of exposure to environmental contaminants (Button et al. 2009; Grashow et al. 2014; Ndilila et al. 2014) is toenails. Toenails are preferred markers for assessment of long term exposure and as measures of absorption, because they are simple to collect, easy to store and handle, relatively simple to analyse and have a potential for less external contamination compared with hair or fingernails (Brockman et al. 2014). Elements deposited into the nail matrix are not subject to additional metabolic processes and many elements are present in the nail at substantially larger concentrations than in urine or blood (Brockman et al. 2014). Moreover, nails from different toes vary in time between formation and clipping, and toenails collected from all ten toes are likely to reflect an integrated exposure over the previous 6–12 months (Longnecker et al. 1993).

A number of studies have been published dealing with relationship between human biomarkers and environmental data of Pb, arsenic (As), chromium (Cr) or cadmium (Cd) (Hinwood et al., 2003; Hughes, 2006; Were et al., 2008; Button et al., 2009; Oyoo-Okoth et al., 2010). Far less information is currently available on the relationships between toenail contents and long-term low-dose exposure to Mn and Cu. However, it is well established that environmental exposure to these PHEs can result in elevated levels of these metals in human tissues.

Within the group under study, Cu and/or Mn contents of some toenails samples were elevated and statistically classified as outliers, a result that triggered the present study. In this regard, this study aims at: (i) investigating Cu and Mn levels in toenails of residents in the industrial city of Estarreja, (ii) assessing environmental factors that may influence the exposure-biomarker association, (iii) identifying relevant metal sources both at the indoor and outdoor environments of the house. Although Cu and Mn are the elements of interest in the present study, the identification of significant elemental associations in the house dust samples may indicate probable sources. In this regard, relationships between Cu, Mn, and a batch of 53 chemical elements were investigated.

2. Study design

Ethical approval for this study was obtained from the National Committee for Data Protection (Proc. nº 1241/2013). All participants gave written, informed consent to the study.

2.1 Study site

Estarreja is a small industrial city located in the district of Aveiro, North of Portugal (Fig. 1). The area is classified as a Special Protection Zone (ZPS) because of its salt-marshes that are habitats for a number of animal and plant species. The urban area is surrounded mainly by agricultural fields but is also near an important industrial complex, the chemical complex of Estarreja (CCE). The industrial plants currently produce a number of chemicals such as aniline and derivatives, chlor-alkali or aluminum salts, synthetic resins and fertilizers. But in the past, huge volumes of sulphides were used to produce sulphuric acid for the fertiliser industry. Pyrites and other sulphides that were extracted from Portuguese mines in the south of the country were used as raw material. During the past decade, a part of the stockpiles of industrial wastes containing hazardous materials such as pyrite ashes, Hg-enriched muds from the chlor-alkali plant and organochlorine compounds, were sealed and buried near the complex. Although technological upgrades associated with remediation measures implemented in the last decade by the industry have reduced the environmental burden on the city, the CCE is still regarded as the major polluter of the region.

2.2 Study group

The study reports on data obtained from a pilot survey designed to collect house dust and toenail samples from households within the urban area of Estarreja. A total of 21 households and 30 individuals were recruited for the study: 19 households corresponding to 27 residents living near the industrial complex, forming the exposed group, plus 2 households and 3 residents from residential areas with no anticipated environmental contaminants that were used for comparison. Residents were recruited through informal interviews. An information pack containing instructions for sample collection, a polythene container and a questionnaire was provided to the participants. Residents were asked to allow toenails to grow for at least two weeks prior to sample collection.

Since the exposure–biomarker association is subject to variation due to factors that are related with the behaviours and characteristics of the receptor, as well as to environmental factors, investigation on such factors was carried out via questionnaire data. Therefore, the questionnaire was intended to provide information on features such as gender, age, homegrown foodstuff consumption and smoking habits as well as about the general conditions of the physical environment of the house.

2.3 House dust collection and analysis

In this study, house dust sample is a general term that includes dust samples collected from indoor (indoor dust) and outdoor (outdoor dust) areas of the house. Participants were requested to abstain from cleaning floor surfaces for a period of 7 days before the scheduled house dust sampling. A composite indoor dust sample was collected in each home using the High Volume Small Surface Sampler (HVS3) vacuum sampler specified to capture 99% of all particles 0.5 µm and larger (Byrne 2000). The indoor dust was collected from different house compartments and connecting areas excluding potentially wet areas to protect the integrity of the sample. As children under the age of 5 years old are more likely to ingest dust and spend most of their time indoors, usually playing on the floor or on carpets (Paustenbach et al. 1997; Davis and Mirick 2006), dust particles were collected from both surfaces. Carpets and rugs are probably the most efficient "traps" for dust and normal cleaning does not remove the dust load that rapidly accumulates. However, the HSV3 sampler enables the user to control vacuum flow and pressure during operation, which makes it a very efficient dust sampler on worn carpets (Svendsen et al. 2006). This vacuum sampler was developed according to the US EPA recommendations for the collection and analysis of Pb and pesticides in residential floor dust, and has been included for use in the ASTM D5438-94 (ASTM, 1994) method for the collection of carpet dust for chemical analysis (Byrne 2000, Svendsen et al. 2006). Outdoor dust was collected from different areas outside the house with a small brush and a plastic shovel. Preferred areas have included patios, garden paths and driveways.

Different relevant studies report estimates for the contribution of soil to indoor dust that range from 8 to >80%, depending on a wide variety of site-specific factors and methodological approaches (Bierkens et al. 2011). Indoor dusts are generally composed of finer particles than soil, and according to Bierkens et al (2011) particles with a diameter exceeding 150 µm represent 80% of external soil, but only 50% of indoor dust. In addition, fine particles adhere more effectively to the skin, thus increasing the potential for exposure through ingestion (Sheppard and Evenden 1994; Calabrese et al. 1996; Kissel et al. 1996), which is the route under investigation in this study. Therefore, house dusts were dry sieved to provide the <150 µm size fraction, even though the <250 µm fraction is usually considered to be the fraction of interest for oral bioaccessibility studies (Calabrese et al. 1996). The samples were oven-dried at 40º C for 24 h, sieved through nylon mesh, and stored in polyethylene containers at ambient temperature. Dust samples were digested in Aqua Regia at 90 °C in a microprocessor controlled digestion block for 2 hours and the analysis of 55 chemical elements, including Cu and Mn, was carried out by Inductively Coupled Plasma - Mass Spectrometry (ICP-MS) at the ACTLABS Analytical Laboratory, Canada. Each sample batch prepared for ICP-MS analysis included dust samples, duplicates, blanks and standard reference materials. Results of method blanks were always below detection limits, which are of 1 mg kg^{-1} and 0.01 mg kg^{-1} for Mn and Cu, respectively. Values for precision (expressed as RSD %) are < 10 % for all elements.

In this study, contaminant bioaccessibility is defined as "the fraction of a contaminant that is extracted from the solid matrix in the gastrointestinal tract and is therefore available for absorption" (Rodriguez et al. 1999; Ruby et al. 1999), which is specifically referred to when in vitro assessment protocols are used.

The bioaccessibility of the PHE was determined by subjecting a sub-set of indoor dust samples to the Unified BARGE Method (UBM), developed by the Bioaccessibility Research Group of Europe (BARGE). The UBM simulates the leaching of a solid matrix in the human GI tract (Wragg et al. 2011) and is a two-stage *in vitro* simulation that represents residence times and physicochemical conditions associated with the gastric tract (G stage) and the gastrointestinal tract (GI stage). The methodology has been validated against a juvenile swine model for As, Cd and Pb in soils (Denys et al. 2012), as they are potentially harmful to human health and the most common elements undergoing bioaccessibility research (Wragg et al. 2011). The lack of *in vivo* data is probably the major constraint to current research on the oral bioaccessibility of less common elements such as Cu and Mn. Although the UBM is not validated for the elements of interest in this study, estimating its oral bioaccessibility (which can be regarded as a measure of potential exposure) in these house dust samples seemed important given the concomitant study on biomarkers. The bioaccessible extracts were analysed by ICP-MS at the ACTLABS Analytical Laboratory, Canada. Duplicate samples, blanks and the bioaccessibility guidance material BGS 102 were extracted with every batch of UBM bioaccessibility extractions, for quality control purposes. The blanks always returned results that were below the detection limit. For the sub-set of indoor dust samples under study, mean repeatability (expressed as %RSD) in the G stage is 1.8% for Cu and 4.6% for Mn, while in the GI stage is 7.0% for Cu and 9.4% for Mn.

Dust samples were also collected from two houses from residential areas with no anticipated environmental contaminants. The protocols used for sampling, sample treatment and analysis were the ones used for the exposed group.

2.4 Toenail collection and analysis

Toenail clippings were provided by the study participants that clipped all 10 toenails. Clippings without adequate toenail growth (n=1) or having traces of nail polish (n=1) were excluded from the study. Samples were washed thoroughly, rinsed repeatedly in Milli-Q water (Millipore Corp., Billerica, Mass.) and methanol, dried, weighed and stored in polyethylene containers for proper shipment. At the ACTLABS Analytical Laboratory, Canada, toenail clippings were acid digested with 4 ml of HNO_3 and 1 ml of H_2O_2, and a total of 36 chemical elements, including Cu and Mn, were analysed by HR ICP-MS. The number of chemical elements analysed for toenails is smaller than the one analysed for dust samples (n=53) since the analytical package for biological samples requested to the laboratory includes a reduced number of chemical elements. Quality control measures performed in the laboratory include method reagent blanks and certified reference material (NIST SRM 1575a). The recoveries obtained for Cu and Mn in the certified reference materials are always equal to 104% for Cu and vary between 97-98% for Mn, within acceptable ranges. Mean repeatability (expressed as %RSD) is 2.6% for Cu and 1.7 for Mn.

Metal contents in toenail clippings of the non-exposed group were obtained from the same protocols used for the exposed group.

2.5 Statistical analysis

As the exposure–biomarker association is subject to variation due to factors such as age and gender, significant differences of Cu and Mn contents in the toenail clippings were investigated. A paired *t*-test was performed to assess the statistical difference between datasets (women and men, adults and children). Statistical significance was considered at $p < 0.05$ (Goodpaster et al. 2010). The small size of the non-exposed group does not allow its use as a control group or identify significant differences between concentrations determined in toenails of exposed and non-exposed groups. Nevertheless, it was used for comparison purposes.

Relationships between the chemical elements determined in indoor and outdoor dust samples were investigated using a method of factorial analysis. Principal Component Analysis (PCA) is a mathematical technique adapted to quantitative variables that transforms n possibly correlated variables into a (smaller) number of uncorrelated variables referred to as principal component (Benzécri 1980; Jolliffe 2002). Analysing the correlation of each original variable with the first (and more important) PCA components it is possible to visualize the correlations between the n geochemical variables in simple bi-plots. Variables used to compute the components are known as active variables. New variables usually referred to as supplementary variables, can be displayed as supplementary points in the previously calculated PCA components. Although these supplementary variables are not accounted to derive the principal components, their geometrical relations with the active variables can be seen in the bi-plots (Reis et al. 2004, 2010).

Investigation on the influence of external factors over the exposure-biomarker relationship was carried out using multiple correspondence analysis (MCA), which is a method of factor analysis that uses categorical (or discrete) variables. The method was designed to describe a two-way contingency table N (Benzécri, 1980; Greenacre, 1984), in which we find, at the intersection of a row and a column, the number of individuals that share the characteristics of the row and that of the column. Like other factorial methods, MCA aims at reducing the dimensionality of the data input and detecting geometrical relationships between the variables. MCA defines a measure of distance (or association) between two points, which are the categories of the discrete variables. Benzécri (1980) used a distributional distance known as the χ^2. Such distance can be graphically displayed in simple bi-plots by projecting the categories on the few first factorial axes, which express most of the original n-dimensional variance (or inertia) of the dataset.

3. Results

3.1 Elemental concentrations in house dust samples

Results for the determination of pseudo-total Cu and Mn concentrations in the <150 μm size fraction of indoor (n = 19) and corresponding outdoor (n= 18) dust samples are presented in Table 1. It was not possible to collect outdoor dust from one household due to bad weather conditions during the sampling survey. Data in Table 1 are described using arithmetic means, standard deviations and medians. Actual data are provided in the form of supplementary material (Table S1).

Pseudo-total Cu concentrations are more elevated in the indoor dusts (median values are 210 and 95 mg kg^{-1} for indoor and outdoor dust, respectively) while pseudo-total Mn contents are more elevated in outdoor dust samples (median values are 173 and 246 mg kg^{-1} for indoor and outdoor dust, respectively). Concentration differences between indoor and outdoor samples are statistically significant ($p < 0.05$). A similar distribution for Cu and Mn in house dusts is reported by Rasmussen et al. (2001). The concentrations in dusts collected from Estarreja households are similar to the ones determined in houses from areas with no anticipated environmental contaminants (Table 1). A comparison between the elemental concentrations summarized in Table 1 and some published data indicates that Cu contents in house dusts from Estarreja are higher than the ones reported for Canadian house dusts (Rasmussen et al. 2001, 2008), for non-mining town houses in Zambia (Ndilila et al. 2014) and for Sydney house dusts (Chattopadhyay et al. 2003), but lower than the ones reported for USA house dusts (Tong 1998). Manganese levels in the Estarreja house dusts are lower than the ones reported by Rasmussen et al. (2001, 2008).

3.2 Source identification

Information on total concentrations is useful to identify metal sources, and significant elemental associations in the house dust samples may indicate common indoor/outdoor sources. In this study, such associations were investigated through a method of factorial analysis, the PCA. Since the number of variables is too large for the number of samples available, the original dataset was divided in two sub-sets: one for elements not typically considered to be PHE (not-PHE) (Fig. 2) and another for PHE (Fig. 3). Each data matrix used for PCA analysis is composed by 38 house dust samples (indoor and outdoor dust samples) and 18 chemical elements, not-PHE or PHE (Salminen et al. 2005), depending on the dataset under analysis. All elements in the original dataset are used as active variables with the exceptions of Cu and Mn that are projected as supplementary points in the factorial planes. The aim was to identify the relationships between the elements without the influence of the variables under investigation.

Figure 2a shows the projections of the properties (chemical elements) while Figure 2b shows the projections of the individuals (dust samples) on the 1st PCA factorial plane. From the total of 18 components produced by the analysis carried out for data on not-PHE, the first two account for ca. 52% of the total variance and were therefore investigated. Loadings of the PCA components under investigation are provided in the form of supplementary material (Table S2). From the total of the considered not-PHE, tungsten (W), calcium (Ca), bismuth (Bi), magnesium (Mg) and zirconium (Zr) are not well represented in this factorial plane. The 1st component (PCA1) clearly separates Cu concentrations from Mn concentrations (Fig. 2a). Surprisingly, Cu is projected associated to sodium (Na), gold (Au) and silver (Ag) (dashed rectangle in Fig. 2a) that are elements more typical of mineral deposits than of house environments. The 1st component (PCA1) also separates indoor (negative semi-axis of PCA1) from outdoor dust samples (Fig. 2b), which points towards distinctive geochemical compositions for outdoor and indoor dusts. However, indoor and outdoor dust samples collected from the house nº 3 (dashed rectangle in Fig. 2b) are projected very close, probably due to similar geochemical compositions in terms of not-PHE. The association of Cu to Na, Au and Ag in this factorial plane seems to be primarily due to elemental concentrations in the indoor dust (Figs 2a and 2b), which is also an unexpected result.

From the total of 18 PCA factors produced by the analysis carried out for data on PHE (Fig. 3), the first two account for ca. 51% of the total variance. Loadings of the two PCA components are provided in the form of supplementary material (Table S2). From the total of the considered PHE, only barium (Ba), antimony (Sb) and zinc (Zn) are not well represented in this factorial plane. Again, the 1st component (PCA1) clearly discriminates Cu concentrations from Mn concentrations (Fig. 3a).). Copper is projected associated to tin (Sn), nickel (Ni), Cr, Cd and mercury (Hg), and such association seems to be related to the geochemistry of the indoor dusts (Figs 3a and b). Manganese does not correlate with any PHE. The PCA results for PHE confirm that the geochemical composition of indoor and outdoor dust samples collected from the house nº 3 is similar (dashed square in Fig 3b). Possibly, the contribution of exterior soil to the interior dust, which is subject of large variation in published data (Paustenbach et al. 1997), is more important for this indoor dust sample.

Relationships between Cu and Mn levels in the dust and the physical environment of the house were further investigated through MCA analysis, separately for indoor and outdoor dust samples. The following characteristics of the house environment were considered: area of the house (AREA), age of the house (AGE), number of years since the last interior wall painting (IWP), number of years since the last exterior wall painting (EWP), type of heating systems used in the house (HEAT), the occurrence of recent repairs (less than 1 year) in the house

(REPAIRS), the existence of garden (GD) and the existence of backyard (BY). The geochemical variables (Cu and Mn) as well as variables AREA, AGE, IWP and EWP, were divided in two classes of equal frequency. Label 1 always identifies classes of less elevated values while label 2 identifies classes of more elevated values. The variable "heating" has 5 categories: open-fireplace (OPEN_FP), closed-fireplace (CLOSED FP), coal-wood heating (COAL), natural gas heating (GAS), gasoil heating (GASOIL) and electric heating (ELECTRIC). Other categorical variables are binary (questions of yes and no).

The 1st two factors produced by the MCA analysis carried out for outdoor dust samples account for ca. 52% of the total variance and were therefore investigated. The absolute contributions of the variables to the first two MCA components are provided in the form of supplementary material (Table S3). The projection of the categories in the 1st factorial plane (Fig. 4a) shows that Cu2 and Mn1 oppose to Mn2 and Cu 1, suggesting an opposite behaviour between Cu and Mn in the outdoor dusts. More elevated Cu concentrations are associated to older (AGE2) houses that have backyard while more elevated Mn levels seem to be influenced by the occurrence of repairs in the house. Figure 4b shows the projections of the samples in the same factorial plane (MCA1/MCA2), identified according to the existence or not of a backyard. Samples corresponding to houses with backyard cluster together in the quadrant corresponding to positive MCA1 and negative MCA2 loadings. Looking at the projections of samples and variables (Fig.4), it is apparent that more elevated concentrations of Cu and lower Mn levels in the outdoor dusts occur mainly in houses that have backyard.

Projections on the two first factors of the MCA analysis carried out for the dataset corresponding to indoor dust samples were investigated since these factors account for ca. 60% of the total variance. The absolute contributions of the variables to the first two MCA components are provided in the form of supplementary material (Table S3). MCA2 separates categories 1 (less elevated concentrations) from categories 2 (more elevated concentrations). While Cu2 is projected associated to younger houses (AGE1) that have closed fireplaces (or stoves), more elevated concentrations of Mn are associated to houses that have backyard and closed fireplaces (Fig.5a). Figure 5b shows the projections of the samples in the same factorial plane (MCA1/MCA2), identified according to the existence or not of a backyard. Houses with backyard cluster together in the quadrant corresponding to positive MCA1 and MCA2 loadings (Fig. 5b), corresponding to more elevated Mn concentrations (Fig. 5a). Such clustering is not obtained for any other characteristics of the physical environment of the house (not shown), which suggests that the existence of a backyard is a key factor influencing Mn concentrations in the indoor dusts.

3.3 Levels of Cu and Mn in toenail clippings

Results for Cu and Mn contents in the toenail clippings are presented in Tables 2 and 3, respectively, divided by age group (adult or child) and by gender.

Copper contents in toenails are higher in children than in adults (median value is 3.99 and 4.84 mg kg^{-1}, for adults and children respectively) but the difference is not statistically significant ($p > 0.05$). No significant differences were found between Cu contents in toenails of women and men (median value is 4.11 and 4.00 mg kg^{-1}, for women and men respectively). Copper contents in toenails of the exposed group are similar to the ones determined in toenails of the non-exposed participants (Table 2). Two participants have Cu concentrations in the toenails that are outliers within the dataset, which is testified by the difference between the 3rd quartile and the maximum value of the dataset (Table 2). Moreover, the two values are above the upper adjacent values of the dataset and were therefore statistically classified as outliers. The two participants were identified as a couple having different occupations. The man is professionally retired and his current activity is connected to politics while the woman is a housewife. These type of daily activities points towards a dietary or a domestic source for the intake of Cu rather than an occupational exposure. Average Cu levels in toenails summarised in Table 2 are lower than the ones found in some residents in Kano, Nigeria (Ayodele & Ajala 2009), but similar to values reported for 63 individuals recruited to a nutritional study carried out in Columbia Missouri, and for a mining community in the Copperbelt mining region of Zambia (Ndilila et al. 2014).

Manganese concentrations in toenail clippings (Table 3) are higher in children than in adults (median value is 0.24 and 0.32 mg kg^{-1}, for adults and children respectively) but the difference is not statistically significant ($p > 0.05$). In general, men have more elevated Mn contents in their toenails (median value is 0.19 and 0.4 mg kg^{-1}, for women and men respectively), but again, the difference is not statistically significant. Manganese contents in toenails of the exposed group are similar to the ones determined in toenail clippings of the non-exposed participants (Table 3). Moreover, median Mn levels in toenails reported here are lower than toenail Mn levels measured in Portuguese miners (Coelho et al. 2013) and US welders (Grashow et al. 2014).

Trends within the dataset were examined through principal components analysis (PCA) of the measured metal concentrations. Since the number of chemical elements under study is too large for the number of samples available (27 toenail samples), the original dataset was divided into two sub-sets, one for not-PHE (Figure 6) and another for PHE (Figure 7). Figures 6a and 7a show the projections of the variables in the 1st factorial plane for not-PHE and PHE, respectively, while Figures 6b and 7b show the projections of the individuals (toenail clippings) discriminated according to gender. Copper, Mn and the participant #28 were projected as supplementary points in the bi-plots. The participant identified as #28 is clearly an outlier in the dataset, as is shown in the inset (c) included in Figures 6b and 7b, and was therefore excluded from the original data matrix.

Elements with toenail contents below the detection limit of the analytical method were removed from the multivariate analysis. For toenail samples, concentrations of light rare earth elements (LREE, sum of concentrations of lanthanum, cerium, praseodymium, neodymium, samarium and europium) and heavy rare earth elements (HREE, sum of concentrations of gadolinium, terbium, dysprosium, holmium, erbium, thulium, ytterbium and lutetium) were summed (ΣREE is represented by REE in figure 6a), because the corresponding projections in the factorial plane are overlapped. The variables Cu and Mn were not used to produce the PCA components and were only projected as supplementary points in the factorial planes. Therefore, the new data matrices used for PCA analysis (datasets for not-PHE and PHE) contain 27 samples and 14 chemical elements. The first two PCA components for not-PHE account for ca. 59% of the total variance. Loadings of the PCA components under investigation are provided in the form of supplementary material (Table S4). From the elements under study, strontium (Sr), Bi and Zr are not well represented in the factorial plane (Figure 6a). Copper is projected close to Ag and iron (Fe), while Mn is associated to gallium (Ga) and Fe. All these elements are positively correlated with PCA1. There is a fairly good discrimination between women and men (Figure 6b), and the negative correlations between female samples and PCA1 suggests that women have lower elemental concentrations in their toenails than men.

The first two components produced by the PCA analysis carried out using data on PHE, account for ca. 59% of the total variance and were therefore investigated. Loadings of the PCA components under investigation are provided in the form of supplementary material (Table S4). Elements such as Cr, Hg, U, cesium (Cs), Sb and Cd are not well represented in the plane (Figure 7a). Manganese is correlated with PCA1 but is not associated to any chemical element. Copper is projected close to vanadium (V), molybdenum (Mo), Pb, cobalt (Co) and Ni, which are strongly correlated with PCA1. For the PHE, none of the PCA components discriminates between male and female participants (Figure 7b). Participants identified with labels #26, #27, #28 and #30 are outliers within the dataset due to more elevated toenail contents of elements that are correlated with PCA1 (e.g Cu, Pb, Mo and V). Participant #24 is also an outlier in the dataset, but in this case due to more elevated contents of Zn, Se, As and Cd, which are correlated with PCA2 (Figure 6a).

3.4 Factors influencing the exposure-biomarker association

The relationships between concentrations of Cu and Mn in toenail clippings and environmental factors were further investigated using MCA. In the new dataset, the categorical variables are: gender, “Cu contents indoor dust”, “Mn contents indoor dust”, “Cu contents in outdoor dust“,“Mn contents in outdoor dust“, “consumption of homegrown food stuffs (HGFS)”, “drinking water” and “cooking water” that were considered to be potential influencing factors of exposure. The professional occupation is an important factor influencing exposure assessment at the home environment since it can clarify about a potential occupational exposure. However, this factor was not considered in this study as only one participant works in the industry. Copper and Mn concentrations in dusts are labeled as CuI, MnI, CuO and MnO, for samples collected respectively indoors and outdoors. Variable "drinking water" is divided in 3 categories, which are spring water (SPRING), tap water (TAP) and mineral water (MINERAL), while the variable "cooking water" is divided in the categories well water (WELL) and tap water (TAP_COOK). Copper and Mn concentration in the toenails (and dusts) are divided in 2 classes (less elevated values: identified by label 1; more elevated values: identified by label 2) of equal frequency. However, Cu and Mn contents in the house dusts were projected as supplementary points in the factorial planes to prevent disrupting the relationships between contents in the toenails and other environmental factors under study.

The absolute contributions of the variables to the first two MCA components are provided in the form of supplementary material (Table S5). Figure 8 shows the projections of variables (Fig. 8a) and samples (Figs. 8b and 8c) in the 1st MCA factorial plane (MCA1/MCA2), which accounts for ca. 80% of the total variance of the dataset. In figure 8b, the samples are identified according to the gender of the residents, while in figure 8c the separation is carried out according to the participant status on the consumption of homegrown food stuffs. The 1st factor discriminates more elevated metal concentrations in toenail clippings associated to the consumption of homegrown food stuffs and ingestion of spring and well water (semi-positive axis of MCA1), from less elevated metal concentrations and ingestion of tap water (semi-negative axis of MCA1). The same factor also discriminates participants that consume homegrown food from those who do not (Fig. 8c). Therefore, ingestion of homegrown food stuffs, well water and spring water seem to be related with more elevated Cu and Mn contents in the toenails. The 2nd factor opposes Mn2 to all classes of concentration and separates men from women (Fig.8b), most likely due to the fact that men have higher Mn contents in their toenails than women (Table 2). Looking at the projections of the supplementary points (black squares in Figure 8a), which correspond to metal concentrations in the house dusts, the most relevant result is probably the association of more elevated Mn levels in toenail clippings to more elevated Mn concentrations in the indoor dusts. Therefore, at this point, the indoor house dusts remain a probable source of Mn to the residents. More elevated Cu contents in toenail clippings are associated to less elevated concentrations in the house dusts, suggesting that the dusts are not a probable major pathway of exposure to Cu.

3.5 Oral bioaccessiblity of Cu and Mn in indoor dust samples

One of the purposes of the present study is to estimate the bioaccessibility of Cu and Mn in the house dust. Therefore, a sub-set of eleven indoor dust samples corresponding to the exposed group was selected to carry out the bioaccessibility testing. In this study, bioaccessibility is defined as the concentration of metal extracted from dust in the G stage, which is the UBM stage that provides higher extracted concentrations (Reis et al. 2014b) and better repeatability values (see section 2.3).

Bioaccessible concentrations of Cu range from 44 to 198 mg kg^{-1}, with a correspondent median value of 82 mg kg^{-1}, while bioaccessible concentrations of Mn in the indoor dust vary between 57 and 197 mg kg^{-1} and have a median value of 98 mg kg^{-1}. The bioaccessible fraction (BAF), which stands for the proportion of the total metal content that is extracted in the G stage of the UBM protocol, varies between 13 - 37% for Cu and between 46 - 69% for Mn. Average BAF values are 30% and 61%, for Cu and Mn respectively. Thus, large fractions of Mn in the indoor dusts are in forms that are soluble in the synthetic G fluids: In contrast, large proportions of the total Cu concentrations may not be available for absorption in the gastrointestinal tract following incidental dust ingestion.

Since Mn in indoor dust samples is bioavailable to humans, the relationships between Mn contents in dusts and toenail clippings were further investigated using Pearson's product moment correlation coefficient (r). A paired sample t-test is used to establish the statistical significance of r that was considered at $p< 0.01$. Correlation coefficients were calculated to the entire sub-set selected for the bioaccessibility testing, as well as to male and female residents from the same sub-set. The results presented in Table 4 indicate that, for the sub-set of samples under study, there are no significant correlations between total Mn concentrations in the dust and Mn contents in toenail clippings. At a first glance, there is also a lack of correlation between Mn levels in toenail clippings and the bioaccessible concentrations in the indoor dust. However, the correlation calculated using exclusively data for women is strong and statistically significant ($r= 0.99$, $p< 0.01$). A simple linear regression analysis (Fig.9) shows that more elevated Mn contents in the toenails of women correspond to increasing proportions of bioaccessible Mn in the indoor dust.

3.6 Discussion

The PCA results point towards distinctive geochemical compositions for outdoor and indoor dusts. Copper is projected associated to elements such as Hg, Ni, Na, Au and Ag. This group of elements could have easily be found in the waste materials that until the beginning of the century were dumped or discharged through artificial channels to the nearby lagoon, sites that were never remediated. Such materials included pyrite ashes (enriched in Cu, Ni, As and other metals), Hg-enriched muds from the chlor-alkali plant and organochlorine compounds. Finer particles of these materials can easily be blown by the wind from the old channels into the urban area. However, Cu is also correlated with a number of elements such as Cr and Sn that are commonly found in higher concentrations indoors than in the outdoor dust, and such concentrations are usually related with indoor sources (Rasmussen et al. 2001; Ibanez et al. 2010). In fact, the elemental associations found for Cu are strongly supported by elemental concentrations in indoor samples. Therefore, the results obtained so far do not point to a specific metal source. For Mn, the element does not correlate with any PHE under study but correlates with elements such as Fe, rubidium (Rb), lithium (Li), LREE and HREE, which are usually originated from soil (Ibanez et al. 2010). Moreover, such correlations are mainly derived from elemental concentrations in outdoor dust samples. Therefore, it is reasonable to assume that the source of Mn in probably geogenic and related with the soil component brought into the house.

In this study, the relationships between Cu and Mn levels in dust samples and the physical environment of the house were investigated using MCA. Such relationships indicate that houses with a backyard have more elevated Cu concentrations and lower Mn levels in the outdoor dusts (Fig. 4). But houses with backyards are also associated with elevated Mn concentrations in the indoor dusts (Fig. 5), which is in good agreement with the results of the PCA (Fig. 2) that indicate the exterior soil as a probable source of Mn. Yet, more elevated Mn concentrations are also associated to open fireplaces and wood-coal heating systems. According to data published by the U.S. Environmental Protection Agency (1995), the combustion and pyrolysis of wood in fireplaces and stoves produce atmospheric emissions of, among others, several mineral residues such as potassium (K), Na, Cd, Cr and Mn. Thus, wood combustion as an indoor source of Mn is a likely scenario that should not be discarded.

Investigation of environmental factors influencing the exposure-biomarker relationship indicates that, among the participants in the survey, usual consumers of homegrown food, spring water and well water have more elevated Cu concentrations in the toenails (Fig. 8a). However, the house dusts seem to be an unlikely source of Cu. Mean dietary Cu intakes from food of adults in different European countries have been estimated with a range of 1.0-2.3 mg day^{-1} for males and 0.9-1.8 mg day^{-1} for females (Van Dokkum, 1995), which are lower than the tolerable upper intake level (UL) of 5 mg day^{-1} derived by the European Scientific Committee for Food (SCF, 2003). Unless foods consumed are contaminated with Cu, they do not represent a common cause of acute toxicity. Likewise, recent published data (SCF, 2006) indicates that only a very small proportion of consumers take dietary supplements containing Cu. Thus, for the subjects under study, intakes of well water and homegrown products are probable pathways of exposure to environmental Cu.

For Mn, the relationships between environmental factors and the biomarkers of exposure suggest that the dust collected from indoor areas is a probable source of Mn to the residents. But, if emission of mineral residues following wood combustion in fireplaces is a probable source of Mn to the house dust, is also a probable pathway of exposure through inhalation to the residents. In order to investigate the probable influence of wood combustion on Mn levels in toenail clippings, the categorical variable “heating” was added to the dataset and a new MCA was carried out. The results, displayed in Figure 10, show that more elevated Mn contents in the toenail clippings are associated to more elevated Mn concentrations in indoor dust samples and to wood-coal heating systems. But, Mn is a normal component of human diet that accumulates in body tissues such as toenails, which complicates establishing a sound relationship between the biomarkers of exposure and environmental factors. However, for the study group of Estarreja there is some evidence that wood combustion for purposes of domestic heating may influence Mn levels in the toenails of the residents, either through inhalation or through the ingestion of Mn containing house dust. Exposure to Mn by inhalation is neurotoxic. Oral intake of Mn despite its poor absorption in the GI tract (about 3-8%, (WHO, 1996)) has also been shown to cause neurotoxic effects. However, at this point no UL value was yet set for EU countries due to limitations of the human data and non-availability of NOAELs for critical endpoints from animal studies (SCF, 2006). Further investigation is required to assess the health effects of human exposure to environmental Mn.

Results of the oral bioaccessibility testing indicate that a large proportion of the total Cu content in the indoor dusts is in forms that are not bioaccessible and therefore, are potentially unavailable for the residents. Moreover, such low estimates for the BAF (29% on average) suggest that Cu in the dust is associated to resistant mineral phases that are hardly dissolved by the acidic solutions used in the stomach stage of the UBM protocol. If so, the waste materials of the CCE are more likely sources of Cu than the indoor sources. Mineralogical and solid-phase distribution studies are critical to identify the actual sources of the metal and such studies are now being carried out. However, at this point such data are not yet available.

The relationships found between bioaccessibility estimates and Mn contents in toenail clippings show that increasing Mn contents in female toenails correspond to increasing proportions of Mn in the dust that may become available for absorption in the gastrointestinal tract following incidental dust ingestion. Evidently, this result has to be interpreted with extreme caution due to the small number of samples available. Moreover, Mn is a normal dietary component and is present in all human tissues and fluids, which limits interpretation in terms of exposure to environmental Mn. Also, a variety of potentially confounding factors such as vitamin intake are not being considered in this evaluation of exposure-biomarker association. However, there is increasing evidence that health effects of toxic metals differ in prevalence or are manifested differently in men and women (Vahter et al. 2007; Lee & Kim 2014), which is in the line of the results obtained in this study. Moreover, gender differences in the absorption of Mn are well documented, with men absorbing significantly less Mn compared to women depending on their Fe status (Finley et al. 1994; Aschner & Aschner 2005), which is also in good agreement with the results presented here.

4. Conclusions

The main aims of the present study were identifying outdoor and indoor sources of PHE in settled house dust, and investigating relationships between concentrations of Cu and Mn in toenail clippings and indoor dusts collected.

The results of multivariate analysis suggest that Cu and Mn levels in dust samples are probably related to backyard soil. Moreover, Mn contents in indoor dust samples seem to be associated to wood-coal combustion used for house heating.

Investigation of environmental factors influencing the relationships between toenails and house dusts indicates that, among the participants in the survey, usual consumers of homegrown food and well-water have more elevated Cu concentrations in the toenails. Yet, the house dusts seem to be an unlikely source of Cu. Therefore, for the ingestion pathway, homegrown food stuffs and well water are probable routes of exposure to environmental Cu. No relationship was identified between Cu contents in settled house dusts and levels of Cu in the toenails of the residents. For Mn, the relationships between environmental factors and the toenails suggest that the indoor settled dusts are a probable route of exposure to the residents. Moreover, the backyard soil is a probable source of Mn to the indoor settled dust.

A strong and significant correlation was found between bioaccessibility estimates and Mn contents in toenail clippings of female participants. Despite the number of confounding factors that were not considered, the results suggest that, for the dataset under study, bioaccessible Mn is a suitable indicator of exposure to environmental Mn.

Investigating relationships between environmental Mn and levels in toenail clippings needs complementary information to understand causal associations between environmental and health data, namely in what regards the influence of oral bioaccessibility on human exposure to environmental Mn. The results achieved in this study show the necessity of further investigation on these relationships to better understand the health impact of exposure to environmental contaminants.

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Supplementary Material

Pseudo-total concentrations of Cu and Mn in outdoor and indoor settled dust samples are provided in the form of Supplementary Material (Table S1). Also PCA loadings and MCA contributions can be found in the Supplementary Material (Tables S2–S5).

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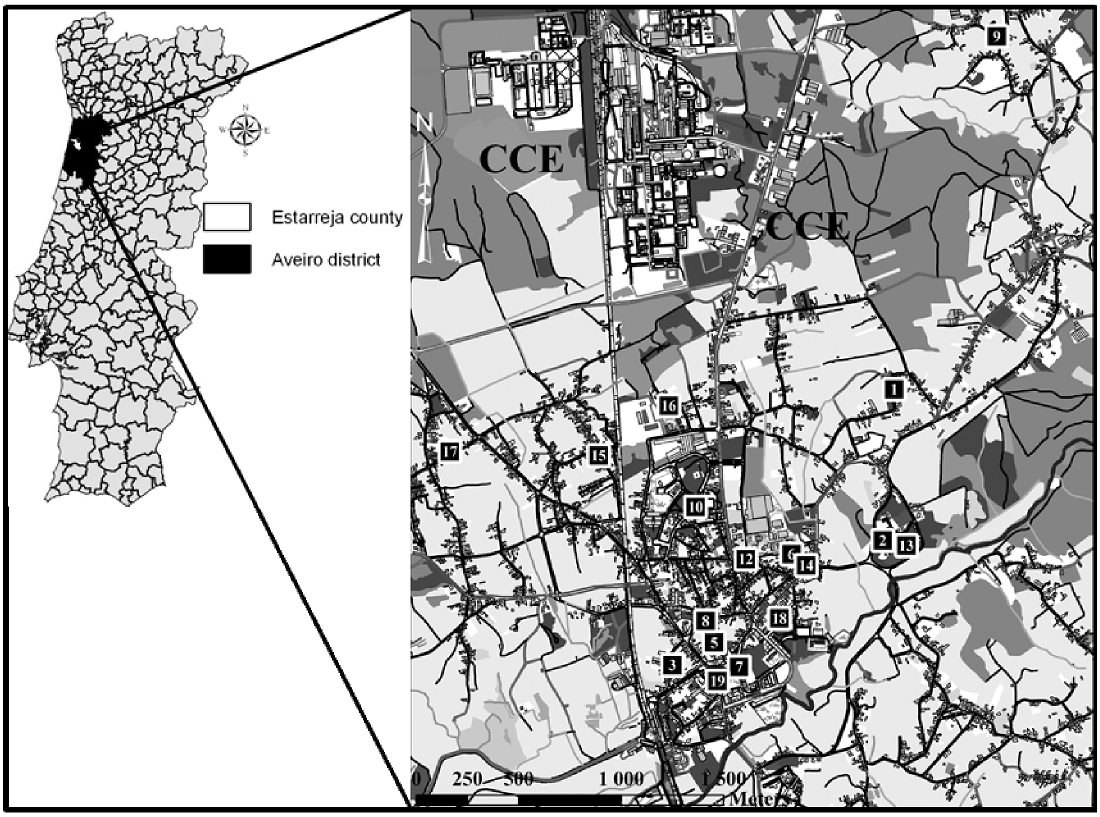


Fig1. Map of Estarreja showing the location of the 19 households (numbered black squares) that were recruited for the pilot study; the outset sets the city within the district of Aveiro; the label CEC identify the location of the chemical complex.

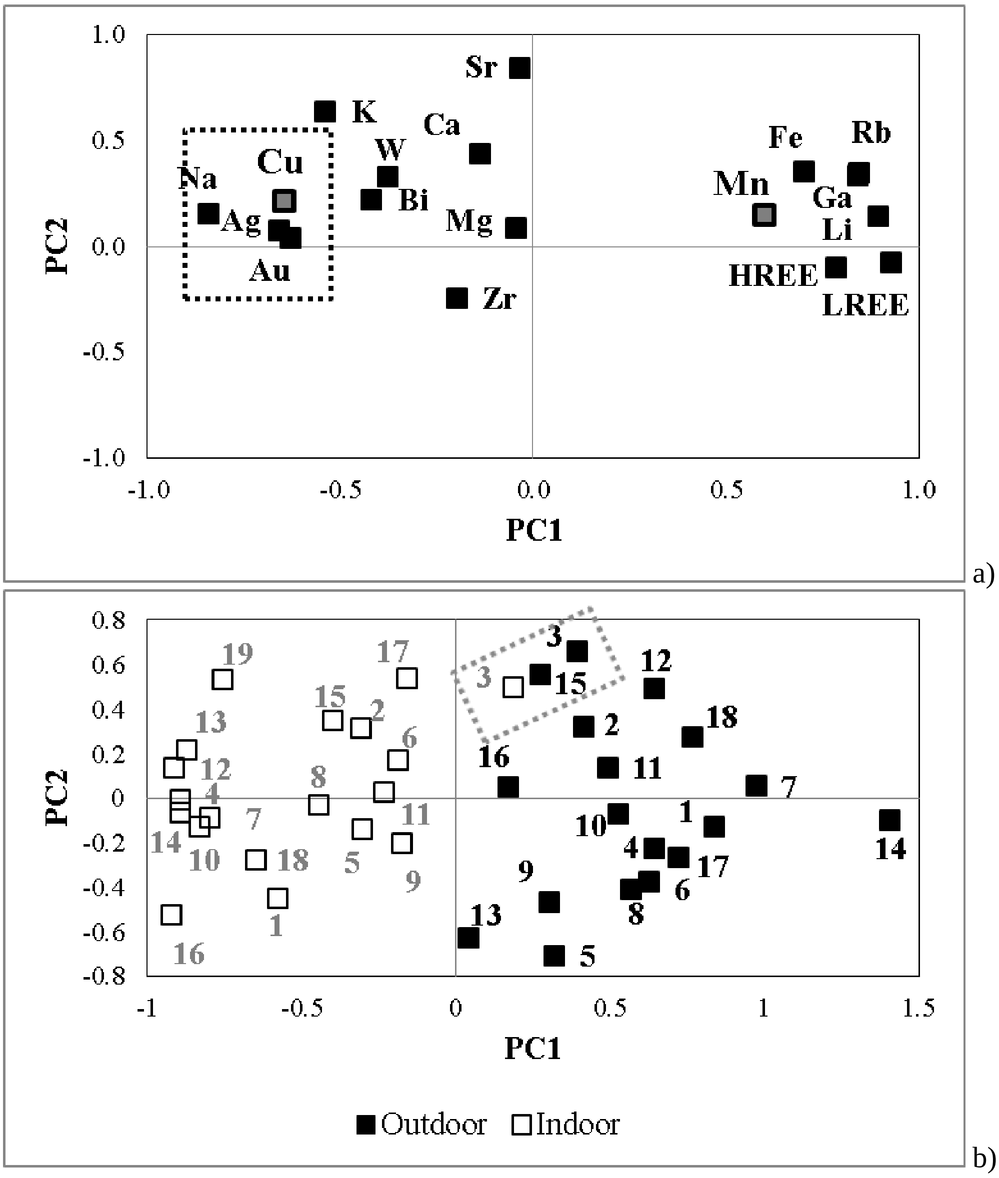


Fig2. Projections in the 1st factorial plane (PCA1/PCA2) of PCA analysis carried out for geochemical data on not-PHE in house dusts: plot 2a shows projections of properties (chemical elements); plot 2b shows projections of individuals (dust samples); Cu and Mn are projected as supplementary variables; Key: LREE, light rare earth element; HREE, heavy rare earth element.

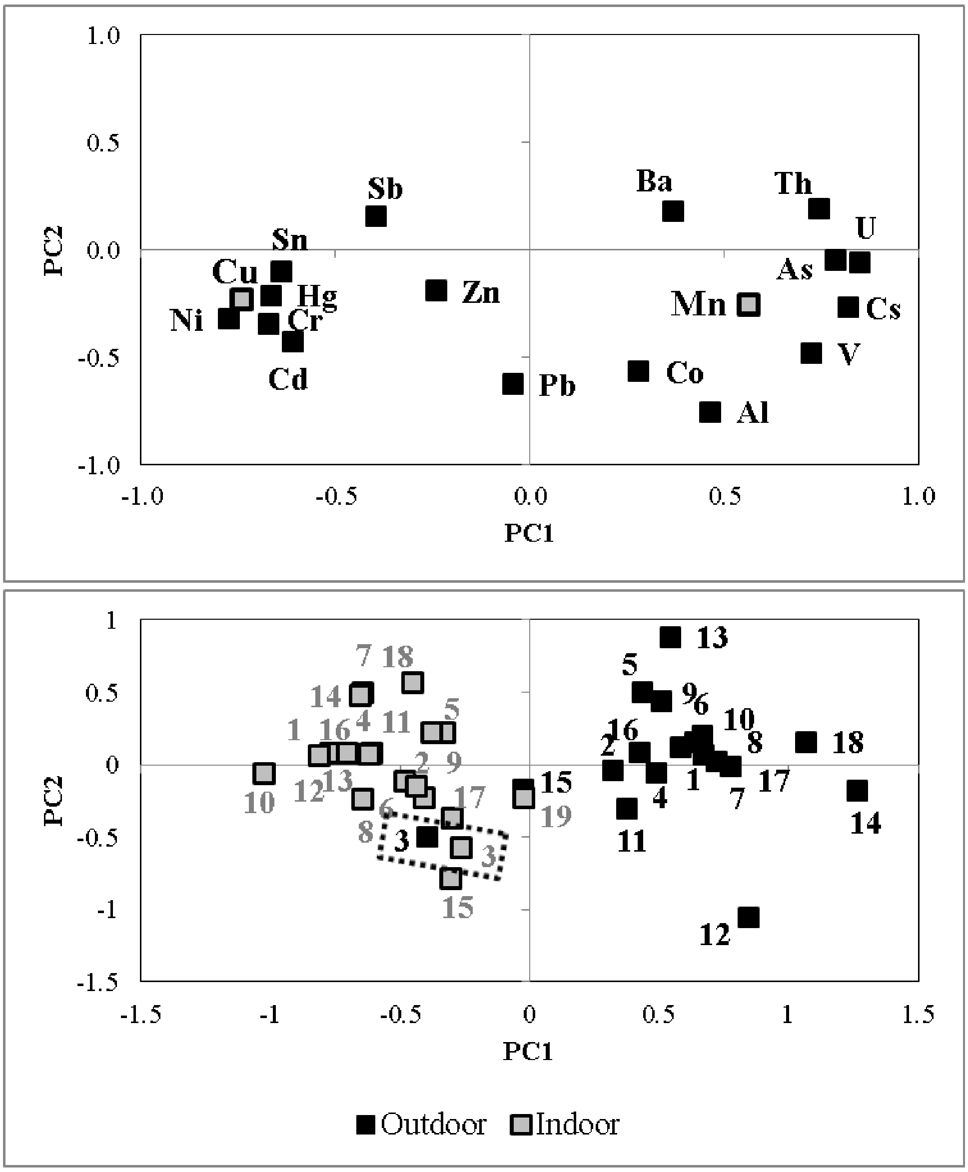
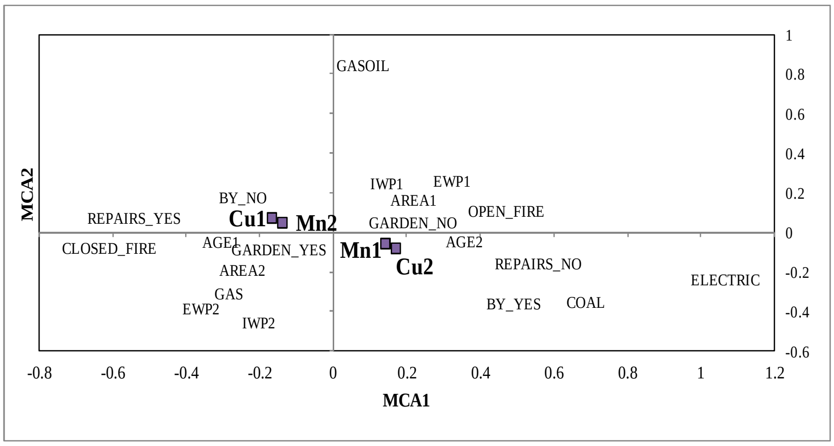
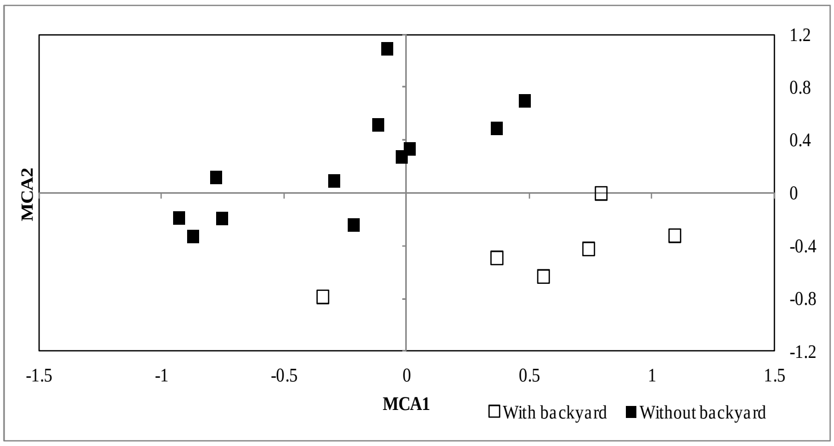


Fig3. Projections in the 1st factorial plane (PCA1/PCA2) of PCA analysis carried out for PHE in house dusts: plot 3a shows projections of properties (chemical elements); plot 3b shows projections of individuals (dust samples). Cu and Mn are projected as supplementary variables.

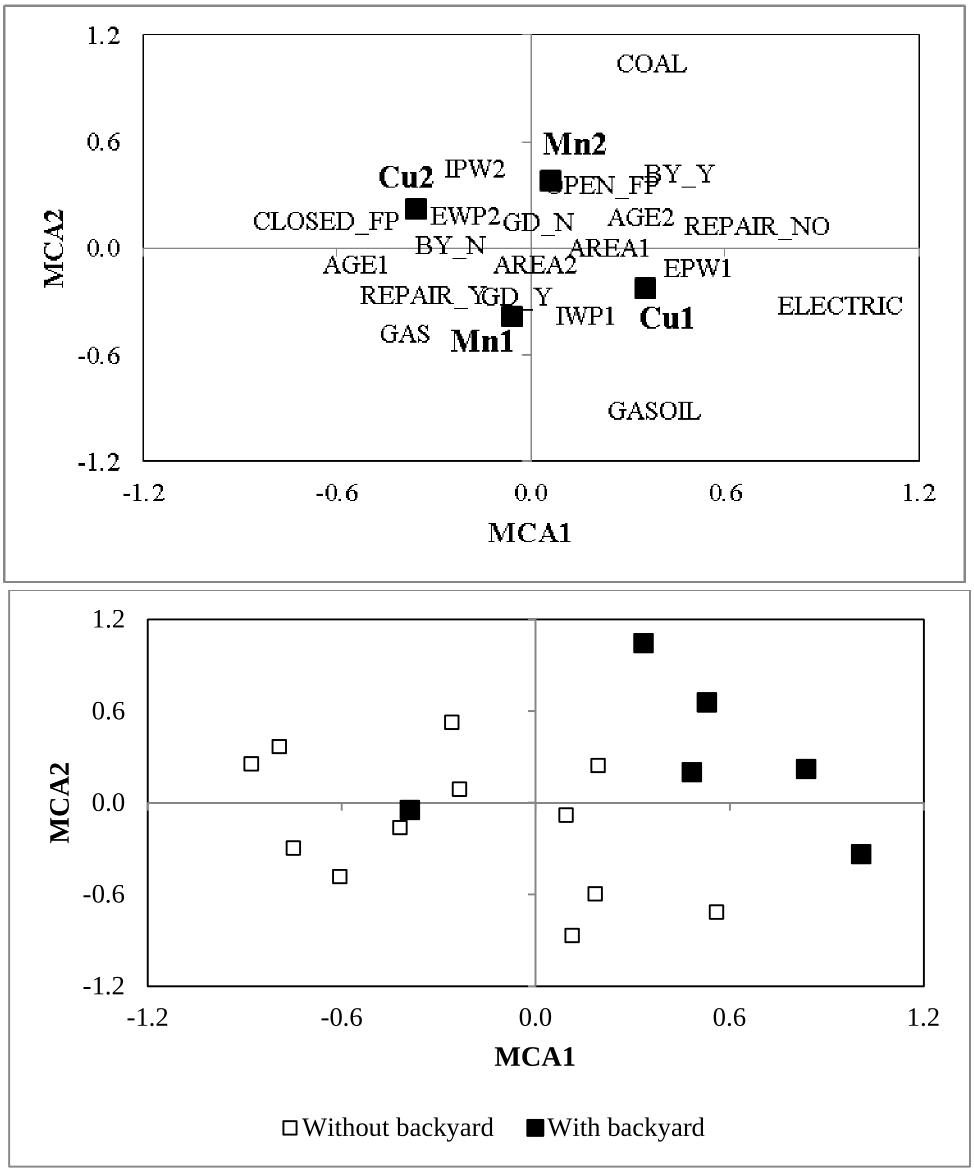


a)



b)

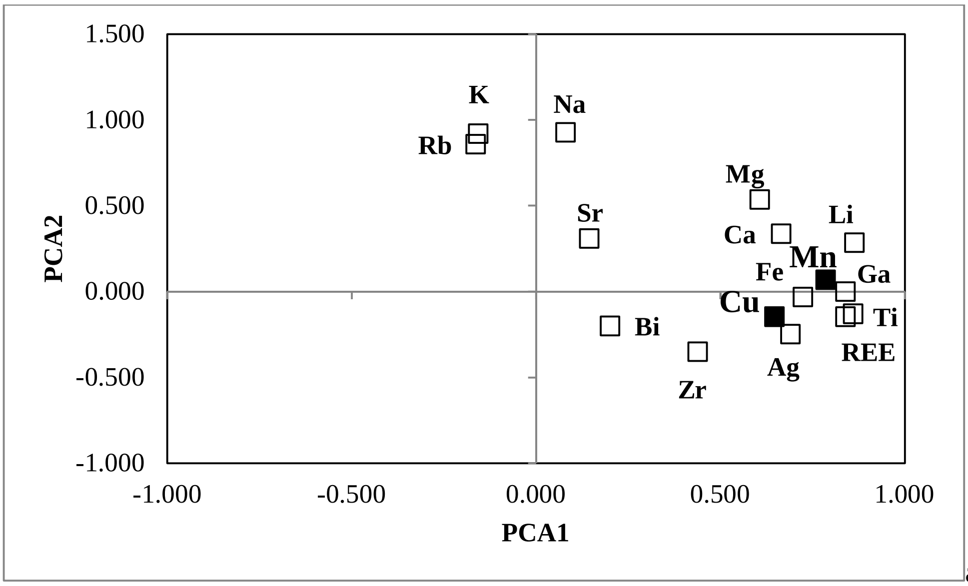
Fig4 Projections in the 1st factorial plane (MCA1/MCA2) of the MCA carried out for the outdoor dust dataset: plot 4a shows projections of the categories; plot 4b shows projections of the samples, discriminated according to the existence of a backyard; Key: REPAIRS_YES, houses that have had recent repairs (<1 year); REPAIRS_NO, houses that have not had recent repairs <1 year); CLOSED_FIRE, fireplace is closed; OPEN_FIRE, fireplace is open; GASOIL, heating system operated with gasoil; COAL, heating system operated with coal or wood; GAS, heating system operated with natural gas; ELECTRIC, electric heaters; AGE, age of the house; AREA, indoor area of the house; GARDEN_YES, the house has got garden; GARDEN_NO, the house hasn't got garden; BY_YES, the house has got backyard; BY_NO, the house hasn't got backyard; EWP, number of years since the last wall painting in exterior areas of the house; IWP, number of years since the last wall painting indoors.



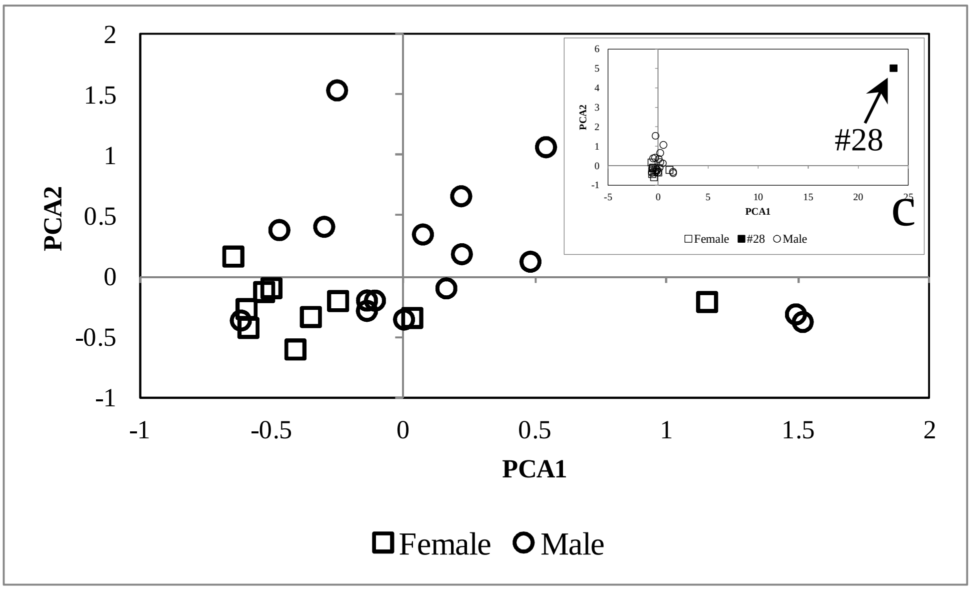
a)

b)

Fig5. Projections in the 1st factorial plane (MCA1/MCA2) of the MCA carried out for the indoor dust dataset: plot 5a shows projections of the categories; plot 5b shows projections of the samples, discriminated according to the existence of a backyard; Key: REPAIRS_YES, houses that have had recent repairs (<1 year); REPAIRS_NO, houses that have not had recent repairs <1 year); CLOSED_FIRE, fireplace is closed; OPEN_FIRE, fireplace is open; GASOIL, heating system operated with gasoil; COAL, heating system operated with coal or wood; GAS, heating system operated with natural gas; ELECTRIC, electric heaters; AGE, age of the house; AREA, indoor area of the house; GD_YES, the house has got garden; GD_NO, the house hasn't got garden; BY_YES, the house has got backyard; BY_NO, the house hasn't got backyard; EWP, number of years since the last wall painting in exterior areas of the house; IWP, number of years since the last wall painting indoors.



a)



b)

Fig6. Projections in the first plane of the PCA analysis carried out for not-PHE: plot 6a shows projections of properties (chemical elements); plot 6b shows projections of individuals (toenail clippings) and an inset (C) of the same factorial plane containing the projection of the outlier sample (#28). Copper, Mn and sample #28 are projected as supplementary points; Key: REE, sum of rare earth elements.

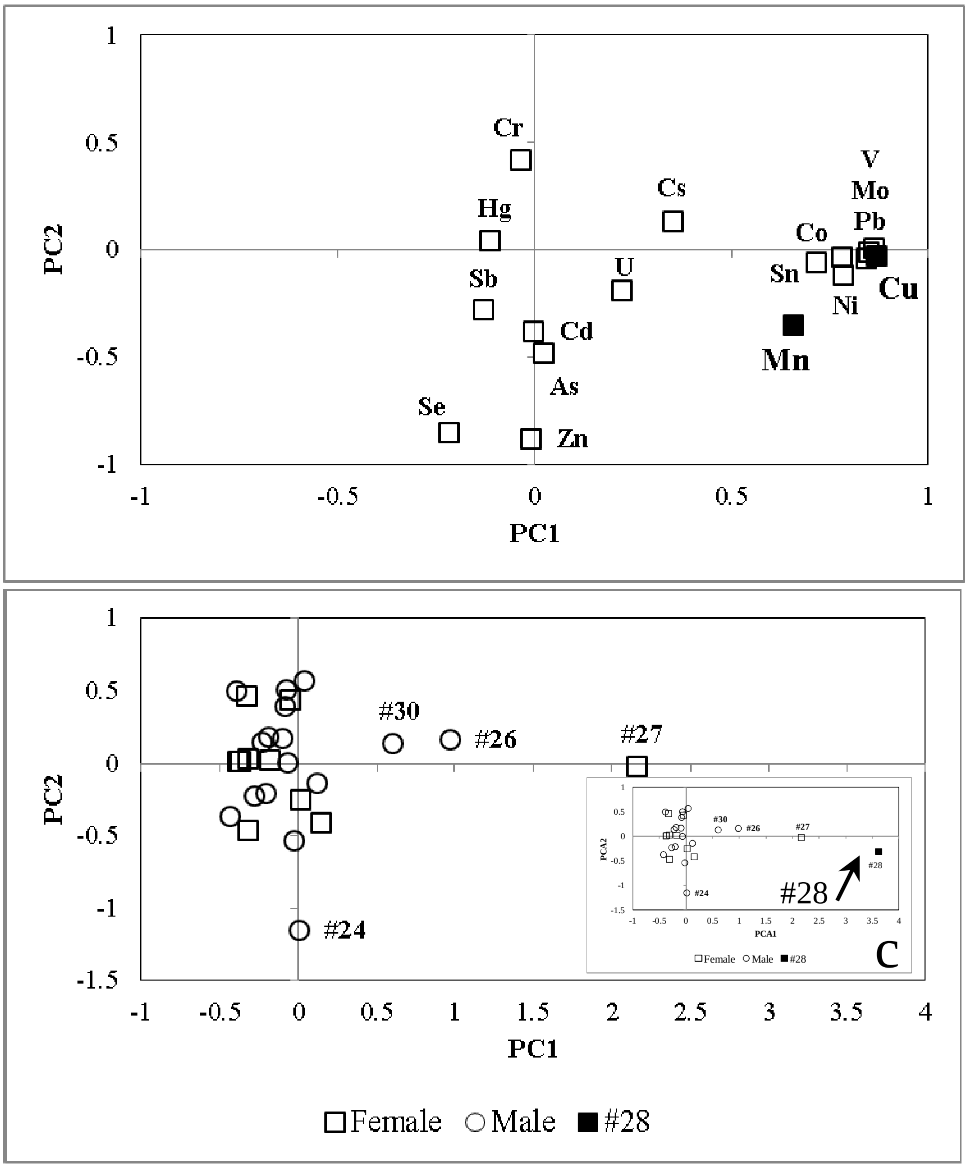
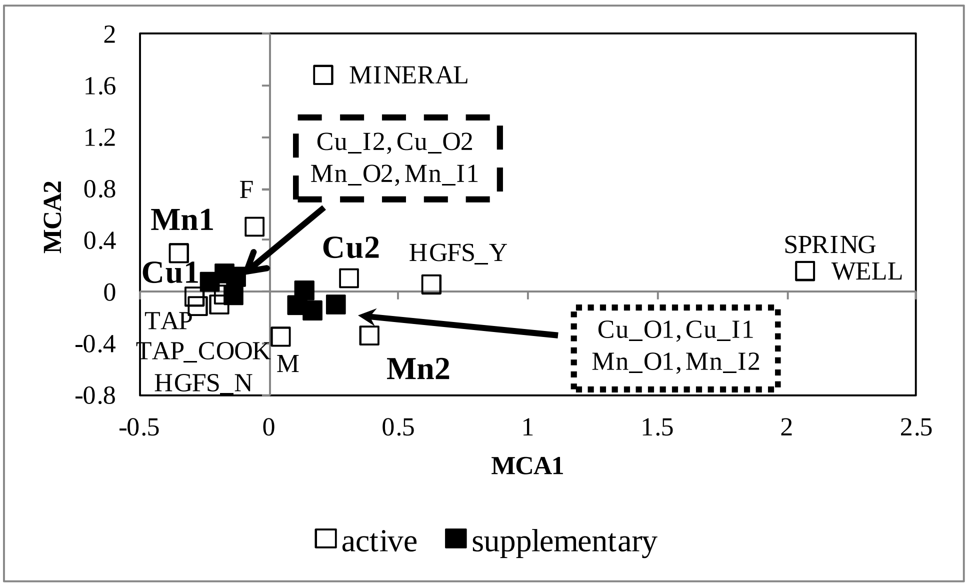
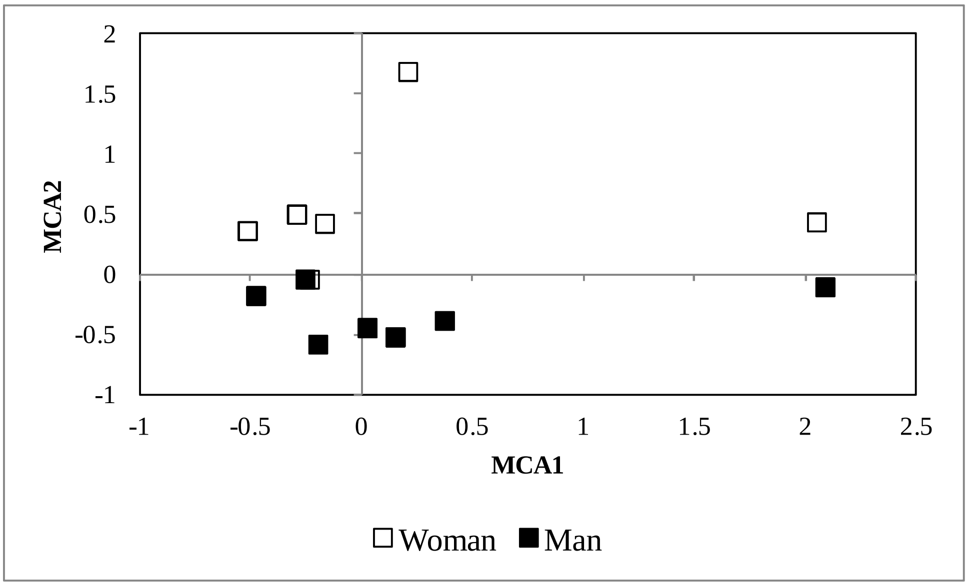


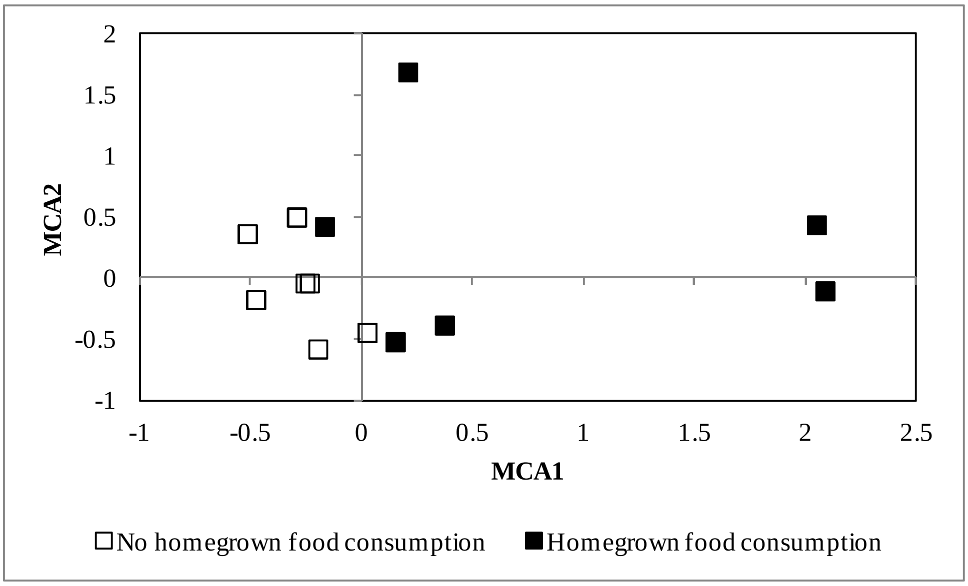
Fig7. Projections in the first factorial plane of the PCA analysis carried out for the PHE dataset: plot 7a shows projections of properties (chemical elements); plot 7b shows projections of individuals (toenail clippings) and an inset (C) of the same factorial plane containing the projection of the outlier sample (#28). Copper, Mn and sample #28 are projected as supplementary points.


MINERAL
Cu_I2, Cu_O2
Mn_O2, Mn_I1
F
Mn1
Cu1
Cu2
HGFS_Y
SPRING
WELL
TAP
TAP_COOK
HGFS_N
M
Mn2
Cu_O1, Cu_I1
Mn_O1, Mn_I2
MCA2
MCA1
active
supplementary

a)


MCA2
MCA1
Woman
Man

b)


MCA2
MCA1
No homegrown food consumption
Homegrown food consumption

c)

Fig8 Projections in the 1st factorial plane of the MCA analyis carried out for toenail clippings: plot 8a shows projections of the categories; plot 8b shows projections of the samples, discriminated according to the gender of the residents; plot 8c shows projections of the samples, discriminated according to the status on consumption of homegrown food stuffs; Key:, TAP, residents drink tap water; MINERAL, residents drink mineral water; SPRING, residents drink spring water; WELL, residents drink water from their private well; TAP_COOK, residents cook use tap water to cook; HGFS_Y, the residents consume homegrown food stuffs; HGFS_N, the residents do not consume homegrown food stuffs; F, female; M, male; Cu_O, Cu contents in outdoor dust samples; Mn_O, Mn contents in outdoor dust samples; Cu_I, Cu contents in indoor dust samples; Mn_I, Mn contents in indoor dust samples.

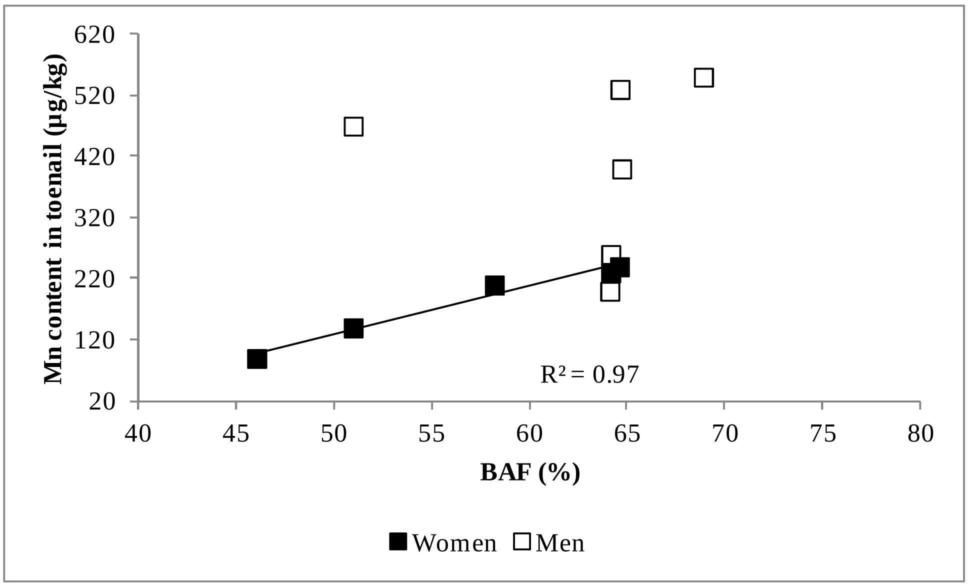
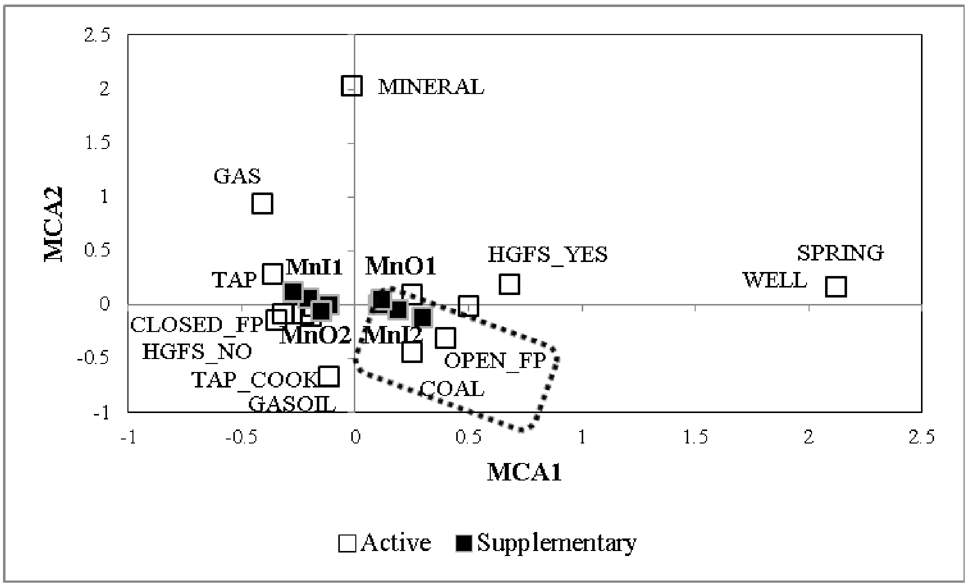


Fig9. XY graph showing the relationship between the bioaccessible fraction (BAF %) and Mn levels in toenail clippings of men and women; for women only, the quality of the linear model is assessed by the R^2 value, measuring its goodness of fit.



a)

Fig10. Projections of the categories in the 1st factorial plane of the MCA analysis carried out for toenail clippings; elemental concentrations in house dusts are projected as supplementary points; Key:, TAP, residents drink tap water; MINERAL, residents drink mineral water; SPRING, residents drink spring water; WELL, residents drink water from their private well; TAP_COOK, residents cook use tap water to cook; HGFS_Y, the residents consume homegrown food stuffs; HGFS_N, the residents do not consume homegrown food stuffs; CLOSED_FP, fireplace is closed; OPEN_FP, fireplace is open; GASOIL, heating system operated with gasoil; COAL, heating system operated with coal or wood; GAS, heating system operated with natural gas; ELECTRIC, electric heaters.

Table 1. Total concentrations of Cu and Mn in house dust samples.

	Cu mg kg^{-1}		Mn mg kg^{-1}	
	Indoor (n= 19)	Outdoor (n= 18)	Indoor (n= 19)	Outdoor (n= 18)
Min	148	37	98	186
Max	585	178	304	472
Mean ± SD	261 ± 118	101 ± 45	178 ± 58	265 ± 69
Median	210	95	173	246
Non-exposed houses	220**	66**	116**	385**
Tong 1998	510*	253*	-	-
Rasmussen et al. 2001	157**	12.1**[a]	-	-
Chattopadhyay et al. 2003	103*	-	54*	-
Rasmussen et al. 2008	152**	17**[a]	267**	532**
Ndilila et al. 2014	16**	13**[a]	-	-

Min;: minimum; Max: maximum; SD: standard deviation; *value refers to the mean; **value refers to the median; a value is for garden soil; n: number of samples

Table 2. Copper contents in toenail clippings divided according to gender and age group.

copper	toenails by age group		toenails by gender	
mg kg^{-1}	adults n= 21	children n= 6	women n= 10	men n= 17
Min	2.41	3.72	2.69	2.41
Q1	3.16	4.05	3.45	3.16
Q3	4.43	6.30	5.25	4.69
Max	44.00	8.27	43.90	44.00
mean ± SD	7.58 ± 12.12	5.37 ± 1.78	8.05 ± 12.63	6.78 ± 10.39
median	3.99	4.84	4.11	4.00
Non exposed group	4.17*	-	4.47*	4.02*
Ndilila et al. 2014	4.57*	-	-	
Brockman et al. 2009	-	-	3 ± 1*	3 ± 1*
Ayodele & Ajala 2009	27.62 ± 13.29**	-	-	-

SD: standard deviation; *- value refers to the mean; **- value refers to the mean ± SD; Q3: third quartile; Q1: first quartile

Table 3. Manganese contents in toenail clippings divided by age group and gender.

Manganese mg kg^{-1}	toenails by age group		toenails by gender	
	adults n= 21	children n= 6	women n= 10	men n= 17
Min	0.09	0.14	0.09	0.12
Q1	0.14	0.16	0.13	0.17
Q3	0.53	0.88	0.26	0.61
Max	1.00	2.25	1.00	2.25
mean ± SD	0.34 ± 0.25	0.70 ± 0.83	0.26 ± 0.27	0.51 ± 0.51
median	0.24	0.32	0.19	0.4
Non exposed group	0.24*	-	0.35*	0.29*
Coelho et al. 2012	2.02 ± 0.49*	-	-	-
Grashow et al. 2014	0.81**	-	-	-

SD: standard deviation; *- value refers to the mean** - value refers to the median;

Table 4. Pearson's correlation coefficients (*r*) estimated for Mn contents in toenail clippings , total Mn contents and the BAF of Mn in the indoor dust; *r* is estimated using the all sub-set of toenail samples selected for the bioaccessibility testing, as well as for women and for men belonging to the same sub-set; significance of *r* was set at $p<0.01$.

r	Mn in toenails		
	All residents	Women	Men
Total Mn	0.67	-0.36	0.74
BAF	0.48	0.99*	-0.03

*significant at p< 0.01

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[1,2*]Reis A P, [1]Costa S, [1]Santos I, [1]Patinha C, [3]Noack Y, [4]Wragg J, [4]Cave M, [5]Sousa, A J,

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Table S1. Pseudo-total concentrations of Cu and Mn in outdoor and indoor settled dust samples, expressed as mg kg^{-1}.

Site	Outdoor Dust		Indoor Dust	
nº	Mn	Cu	Mn	Cu
1	315	68	154	299
2	253	148	193	303
3	311	173	296	339
4	314	61	220	190
5	193	42	161	166
6	186	64	205	329
7	262	76	120	210
8	247	96	173	250
9	231	37	222	153
10	222	96	138	585
11	244	175	165	186
12	359	178	98	407
13	472	71	188	391
14	255	111	106	156
15	238	140	228	158
16	214	94	126	350
17	222	86	304	155
18	226	97	113	148
19	-	-	179	175

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Table S2. Loadings of the first two PCs, extracted from two different data matrices corresponding to not-PHE and PHE concentrations in house dusts; % E.V.- percentage of the total variance explained by the PCs.

	Not PHE			PHE	
	PC1	PC2		PC1	PC2
Li	0.896	0.146	Al	0.465	-0.755
Na	-0.843	0.158	Cs	0.819	-0.269
Mg	-0.044	0.089	V	0.726	-0.485
K	-0.539	0.637	Cr	-0.672	-0.347
Ca	-0.139	0.438	Ni	-0.776	-0.321
Fe	0.701	0.355	Zn	-0.240	-0.191
Ga	0.842	0.335	As	0.789	-0.045
Rb	0.847	0.350	Cd	-0.610	-0.426
Sr	-0.035	0.848	Sn	-0.641	-0.100
Ag	-0.660	0.075	Ba	0.370	0.180
W	-0.375	0.331	Pb	-0.043	-0.628
Au	-0.630	0.038	U	0.852	-0.058
LREE	0.787	-0.100	Hg	-0.665	-0.212
HREE	0.929	-0.073	Th	0.744	0.192
Bi	-0.420	0.219	Sb	-0.396	0.156
Zr	-0.199	-0.244	Co	0.281	-0.566
Cu	-0.645	0.216	Mn	0.565	-0.256
Mn	0.601	0.152	Cu	-0.740	-0.230
Eigenvalue	6.41	1.97	Eigenvalue	6.03	2.19
% E.V.	40.1	12.3	% E.V.	37.7	13.7

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Table S3. Variables contributions to the first two components (F[1] and F[2]) that were extracted from two different data matrices, one containing data for indoor dusts and another for outdoor dust samples; % E.V.- percentage of the total variance explained by the MCA component.

	OUTDOOR DUST		INDOOR DUST	
	MCA1	MCA2	MCA1	MCA2
AGE1	4.68	0.08	6.11	0.48
AGE2	4.68	0.08	6.11	0.48
AREA1	2.92	2.00	2.10	1.67
AREA2	2.92	2.00	2.10	1.67
IWP1	1.46	9.99	3.12	5.77
IWP2	1.83	12.48	3.89	7.21
EWP1	6.27	9.86	6.23	1.13
EWP2	6.27	9.86	6.23	1.13
Mn1	1.18	0.40	0.19	13.03
Mn2	1.18	0.40	0.19	13.03
Cu1	1.71	0.84	6.71	4.49
Cu2	1.71	0.84	6.71	4.49
OPEN_FIRE	3.01	0.07	1.89	2.56
CLOSED_FIRE	9.76	0.01	7.90	1.17
GASOIL	0.09	22.88	1.33	12.52
COAL	3.07	3.06	0.66	10.90
GASOIL	1.60	4.47	1.29	4.25
ELECTRIC	7.59	0.89	5.97	1.14
REPAIR_Y	7.74	0.71	7.96	0.99
REPAIR_N	9.67	0.89	9.94	1.24
GD_Y	2.72	0.00	0.81	1.39
GD_Y	3.40	0.01	1.01	1.74
BY_Y	9.70	12.11	7.70	4.99
BY_N	4.85	6.06	3.85	2.50
Eigenvalue	0.08	0.04	0.09	0.06
% E.V.	36.40	15.20	37.40	22.10

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Table S4. Loadings of the first two PCs, extracted from two different datasets corresponding to toenail contents of not-PHE and PHE; % E.V.- percentage of the total variance explained by the PCs.

	Not-PHE			PHE	
	PC1	PC2		PC1	PC2
Ag	0.690	-0.248	As	0.022	-0.484
Ca	0.665	0.336	Cd	-0.002	-0.382
Bi	0.201	-0.201	Co	0.780	-0.033
Fe	0.724	-0.034	Cr	-0.034	0.419
Ga	0.839	-0.002	Cs	0.350	0.129
K	-0.156	0.920	Hg	-0.112	0.044
Li	0.864	0.283	Mo	0.849	-0.011
Mg	0.606	0.535	Ni	0.786	-0.121
Na	0.081	0.926	Pb	0.843	-0.044
Rb	-0.164	0.857	Sb	-0.129	-0.281
Sr	0.145	0.308	Se	-0.218	-0.855
Ti	0.861	-0.131	Sn	0.717	-0.060
Zr	0.439	-0.349	U	0.222	-0.191
ΣREE	0.840	-0.148	V	0.863	0.005
Cu	0.647	-0.148	Zn	-0.011	-0.883
Mn	0.785	0.067	Cu	0.869	-0.029
Ag	0.690	-0.248	Mn	0.658	-0.352
Ca	0.665	0.336	As	0.022	-0.484
Eigenvalue	5.017	3.275	Eigenvalue	4.165	2.220
% E.V.	35.84	23.40	% E.V.	27.77	14.8

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Table S5. Variables contributions to the first two MCA components for data on toenail clippings, % E.V.- percentage of the total variance explained by the MCA component.

	MCA1	MCA2
Cu1	3.54	0.08
Cu2	3.84	0.08
Mn1	5.63	2.00
Mn2	6.09	2.00
F	0.13	9.99
M	0.08	12.48
HGFS_Y	10.74	9.86
HGFS_N	5.06	9.86
SPRING	29.59	0.40
TAP	2.96	0.40
MINERAL	0.14	0.84
WELL	29.59	0.84
TAP_COOK	2.57	0.07
Cu_I1	1.20	0.01
Cu_I2	1.30	22.88
Mn_I1	2.46	3.06
Mn_I2	2.67	4.47
Cu_O1	0.78	0.89
Cu_O2	0.84	0.71
Mn_O1	0.53	0.89
Mn_O2	0.67	0.00
Eigenvalue	0.19	0.07
% E.V.	58.60	21.20