Human predisposition to neurodegenerative diseases and its relation with environmental exposure to potentially toxic elements

Marina M.S. Cabral Pinto[1*], A. Paula. Reis[2], Agostinho Almeida[3], Carlos M. Ordens[4], Maria M. V. Silva[5], Sandra Freitas[6], Mário R. Simões[7], Paula I. Moreira[8], Pedro A. Dinis[9], M. Luísa Diniz[2], Eduardo. A. Ferreira da Silva[2] M. Teresa Condesso de Melo[10]

[1] Geobiotec Research Centre and Centre for Neuroscience and Cell Biology (CNC); Department of Earth Sciences, University of Coimbra, 3030-790 Coimbra, Portugal

[2] Geobiotec Research Centre, University of Aveiro, Department of Geosciences, 3810-193 Aveiro, Portugal

[3] LAQV/REQUIMTE, Department of Chemical Sciences, Laboratory of Applied Chemistry, Faculty of Pharmacy, Porto University, 4050-313 Porto, Portugal

[4] University College London Australia, Adelaide, 5000 South Australia, Australia

[5] CEMUC; Department of Earth Sciences, University of Coimbra, 3030-790 Coimbra, Portugal

[6] Centre for Neuroscience and Cell Biology (CNC) & Centro de Investigação do Núcleo de Estudos e Intervenção Cognitivo Comportamental (CINEICC), University of Coimbra, Portugal

[7] Faculdade de Psicologia e de Ciências da Educação da Universidade de Coimbra (FPCE-UC), Portugal. Centro de Investigação do Núcleo de Estudos e Intervenção Cognitivo Comportamental (CINEICC), University of Coimbra, Portugal

[8] Centre for Neuroscience and Cell Biology (CNC); Faculty of Medicine, University of Coimbra, 3030-548 Coimbra, Portugal

[9] MARE - Marine and Environmental Sciences Centre; Department of Earth Sciences, 3030-790 Coimbra, Coimbra, Portugal

[10] CEris, DECivil, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal.

* Corresponding author: mcp@uc.pt

Abstract

New lines of evidence suggest that less than 10% of neurologic diseases have a strict genetic aetiology while their majority has an unknown origin. Environmental exposures to potentially toxic elements appear to be a risk factor for Parkinson's, Alzheimer's and sclerosis' diseases. This study proposes a multi-disciplinary approach combining neurosciences, psychology, and environmental sciences while integrating socio-economic, neuropsychological, environmental and health data. We present the preliminary results of a neuropsychological assessment carried out in elderly residents of the industrial city of Estarreja. A battery of cognitive tests and a personal questionnaire were administered to the participants. Multivariate analysis and multiple linear regression analysis were used to identify potential relationships between the cognitive status of the participants and environmental exposure to potentially toxic elements. The results suggest a relationship between urinary PTEs levels and the incidence of cognitive disorders. They also point towards water consumption habits and profession as relevant factors of exposure. Linear regression models show that aluminum (R^2=38%), cadmium (R^2=11%) and zinc (R^2=6%) are good predictors of the scores of the Mini Mental State Examination cognitive test.Median contents (µg/l) in groundwater are above admissible levels for drinking water for aluminum (371), iron (860), manganese (250), and zinc (305). Whilst the World Health Organization does not provide health-based reference values for aluminum, results obtained from this study suggest that it may have an important role in the cognitive status of the elderly. Urine proved to be a suitable biomarker of exposure both to elements with low and high excretion rates.

Keywords: neurodegenerative diseases, environmental exposure, potentially toxic elements, urine, groundwater

1. Introduction

Soils and waters are the vehicles which link the inorganic environment to life by supplying the essential macro and micronutrients to living organisms, and particularly to humans. Variations in the chemical composition of soils and waters cause metabolic changes, favouring the occurrence of endemic diseases, related either to deficient or excessive intake, such as gout, fluorosis and Keshan's disease or arsenicosis (Komatina 2004). Until the last decade, little attention was given from the neuroscience community to the neurometabolism of potentially toxic elements (Zatta et al. 2003). However, the neurobiology of the potentially toxic elements (PTEs) is now receiving growing interest, since it has been linked to major neurodegenerative diseases (Zatta et al. 2003; Forte et al. 2004; Gupta et al. 2005; Bocca et al. 2006; Gomes & Wittung-Stafshede 2010; Hozumi et al. 2011; Exley & House 2012; Zhang et al. 2013; Ashok et al. 2015; Ahlskog 2016). Recent studies have been suggesting that no more than 10% of neurologic diseases have a strict genetic etiology, while the majority of cases have unknown origin (Monnet-Tschudi et al. 2006; Kozlowski et al. 2009; Johnson & Atchison 2009). A gene-environmental interaction provides a plausible explanation for the other ~ 90% of cases (Johnson & Atchison 2009). Occupational and environmental (chronic) exposure to specific

PTEs (manganese [Mn], copper [Cu], lead [Pb], iron [Fe], mercury [Hg], zinc [Zn], aluminium [Al], cadmium[Cd]) has been suggested as a possible cause of neurodegenerative disorders, such as manganism, Parkinson's disease (PD), Alzheimer's disease (AD) and sclerosis' (Gorell et al. 1999; Cerpa et al. 2005; Gupta et al. 2005; Maynard et al. 2005; Moreira et al. 2005; Bocca et al. 2006; Moreira et al. 2006; Yokel 2006; Bressler et al. 2007; Fabrizio et al. 2007; Kozlowski et al. 2009; Johnson & Atchison 2009; Exley 2012; Exley & House 2012; Ferrer 2012; Cabral Pinto et al. 2013; Forte et al. 2014; Ashok et al. 2015; Cabral Pinto et al. 2015; Ahlskog 2016). Alzheimer's disease is the most common condition of dementia among the elderly. However, it is important noting that dementia is not an inevitable consequence of ageing but often has a concealed cause (Ferrer 2012). The development of other neurodegenerative diseases, such as PD or Amyotrophic Lateral Sclerosis (ALS), is also accompanied by cognitive disorders, like Mild Cognitive Impairment (MCI) and several dementia levels (Lemos et al. 2014), at the level of global cognitive status and cognitive domains. Currently, epidemiological studies often use urine, hair, and toenail as biological material of exposure because they are less invasive and the samples are easy to obtain in large populations (Reis et al. 2015). The information provided by each biological matrix is rather different. Urine generally reflects recent exposures (days/few weeks), and hair and nails reflect exposures occurring in the last weeks/months (Coelho et al. 2012). However, this distinction is not straightforward for some elements. The half-life, which characterizes the elimination of metals from the body, varies widely between PTEs. It can be larger than 10–12 years for Cd and Pb, with inter-individual variability of about 30%, 4 days for arsenic (As), 60 days for Hg and 0.5 to 1 year for uranium (Dorne et al. 2011). Most PTEs are excreted via the kidney in the urine, and to a much lesser extent by the gastrointestinal tract (Dorne et al. 2011).

Since increasing lines of evidence suggest that environmental exposures may be prevalent in the development of neurodegenerative disorders, studying the impact of exposure to environmental PTEs such as Mn, Cu, Pb, Fe, Hg, Zn, Al, Cd, As on the cognitive functioning of elderly people requires further attention. Hence, we propose a multi-disciplinary approach combining neurosciences, psychology, and environmental sciences, while integrating socio-economic, neuropsychological, environmental and health data.

The Estarreja Chemical Complex (ECC), located in Estarreja, central Portugal, have an intense industrial activity with negative impacts on air, soils, sediments, surface water and groundwater since the early 1950's, while having a population that historically relies on groundwater as a source of water supply for human, cattle and agricultural uses. Ground and surface water, soils and sediment contamination, has been extensively reported for the Estarreja region (Leitão 1996; Pereira et al. 2009; Van der Weijden & Pacheco 2006; Ordens 2007; Inácio et al. 2014). Such contamination has been linked to the industrial activities, enhanced by a natural vulnerability to contamination due to a combination of factors such as high permeability of the sandy soils, shallow aquifers, flat topography and high rates of groundwater recharge (Ordens 2007). Thereupon, the surrounding area of the ECC was classified by the Portuguese Environmental Agency as a priority site for land remediation (APA 2016). During the 1990's, several remediation actions resulted in an important reduction of the negative environmental legacy, but soil and waters still contain high levels of some PTEs, such As, Mn, Hg, Cu (Ordens et al. 2007; Cachada et al. 2012; Inácio et al. 2014). Consequently, Estarreja provides an ideal study area for multidisciplinary studies such as the one hereby described.

The main aims of the study are: (i) determining urinary levels of PTEs in a group of Estarreja inhabitants with more than 55 years of age; (ii) presenting preliminary results of the neuropsychological assessment of the participants that was carried out at the global cognitive status and cognitive domains (i.e. memory, executive functions, visuospatial skills, language, orientation and attention); (iii) investigating relationships between PTEs urine levels and the neuropsychological diagnosis; (iv) determining concentrations of PTEs in groundwater around the ECC and compare with maximum permissible levels established in the Portuguese guidelines; (v) ascertaining the efficacy of the selected biomarker to provide complementary information on environmental exposure to PTEs.

2. Study area

Estarreja is a municipality within the Aveiro district (central Portugal) with 26,997 inhabitants (INE 2012). The city of Estarreja is located in a low altitude (10 - 70 m), gentle slope (< 2 %) area that comprises several wetlands and shows intense agriculture, fisheries and industrial activities (Figure 1a&b).

The geology is characterized by the predominance of Quaternary unconsolidated sands and clays deposited in dune, beach and lagoon environments (Figure 1a). These sedimentary units dip gently to the west and cover Proterozoic metamorphic rocks and Mesozoic siliciclastic formations (Teixeira and Assunção 1963). The principal watercourse crossing the city of Estarreja is the Antuã river, a tributary of the Vouga river (Figure 1).

The ECC is located close to the city and has been working since the thirties of the XX century, although its development was mainly triggered by the II world war. This complex produces aniline and derivatives (nitric acid, sulfnilic acid, cyclohexilamine, cyclohexanol and nitrobenzene), chlorine-alkalis (hydrochloric acid, chlorine, sodium hypochlorite, caustic soda), aluminium sulfate and polychloride), sodium and chlorate compounds from salt through electrolysis using Hg (mercury) cathodes (Costa & Jesus 2001), polyvinyl chloride resins and polymeric methyl diphenyl isocyanate (PMDI), among others. In the past, ammonium sulphate and ammonium nitrate were also produced (Costa & Jesus 2001), as well as the production of sulphuric acid from arsenopyrite roasting, which has led to a large volume of toxic solid wastes and liquid effluents, piled-up or discharged in areas not prepared for such purpose. The aniline, benzene and its compounds, As, Hg, Zn and Pb-containing liquid effluents were discharged without any previous treatment into manmade, permeable, water channels (Costa & Jesus 2001), contaminating agricultural fields, rivers and groundwater. The solid wastes comprised sludges containing pyrite, calcium hydroxide, mercury and arsenic (Costa & Jesus 2001).

3. Study design

The aim of this study was to identify potential links between exposure to environmental PTEs and data from the neuropsychological assessment of a group of elderly, in order to assess potential factors influencing the predisposition to cognitive impairment. The study involved 103 permanent residents from the city of Estarreja (> 55 years old), who were recruited to participate through Private Institutions of Social Solidarity. All inhabitants (or their families) were clearly informed of the aims of the study and those who agree to participate gave their written consent. Urine samples were collected and analysed to determine the levels of selected PTEs. The health status of the participants was assessed by means of a complete socio-demographic questionnaire and through cognitive screening tests, which aim at the early detection of dementia and allow the identification of individuals in preclinical stages. Ethical approval for this study was obtained from the National Committee for Data Protection (Proc. No. 1241/2013). The questionnaires allowed to obtain individual information regarding clinical health status, daily habits, medical record, education level, and factors directly associated to exposure, such as agriculture practices, the sources of water for consumption and irrigation, and crop consumption. The survey instrument collected information on 29 symptoms typically associated with PTEs body burden and/or deficiency. The Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA) and Clinical Dementia Rating scale (CDR) were used to assess the cognitive performance of the study group. The test scores were categorized and used in the statistical analysis (Methods section). The Geriatric Depression Scale (GDS) was used to assess depressive symptoms in older adults.

The data made available by the cognitive screening tests and by the biomarkers determination were coupled in order to study the effect of human exposure to environmental PTEs on the predisposition to develop dementia. Additionally, PTE levels in groundwater samples collected from wells and boreholes were used to assess the importance of water ingestion as a potential exposure pathway for the population of Estarreja.

4. Methods

4.1 Neuropsychological assessment

The criteria for participation in this study were: (i) to have resided in the study area over at least the 5 previous years and (ii) to have more than 55 years of age. The status of the participants was assessed by means of a complete socio-demographic questionnaire and through cognitive assessment, which targeted the early detection of dementia and allowed the identification of individuals in preclinical stages. The following instruments were administered (in this fix order), by a experimented neuropsychologist, for the overall assessment of each participant, which had the duration of at least 1hour per participant:

1) Socio-demographic questionnaire: a complete questionnaire was administered during a personal interview with each participant. This questionnaire was used to obtain information regarding age, marital status, weight, height, nationality, education level, crop consumption, the period of time working in agriculture, pesticide application methods and duration of use, use of personal protective equipment, home-grown foodstuff consumption, irrigation water source and origin of drinking water. The survey instrument collected information on 29 symptoms typically associated with PTEs poisoning and deficiency;

2) An inventory of current clinical health status, past habits, and medical record, usually known as General Health Questionnaire (GHQ) (Goldberg et al. 1997; Fabrizio et al. 2007). The GHQ is designed to cover four identifiable elements of distress: depression, anxiety/insomnia, social impairment and hypochondriasis/somatic symptoms;

3) Mini-Mental State Examination (MMSE) (Folstein et al. 1975; Freitas et al. 2013; Freitas et al. 2015). The MMSE is the most used brief cognitive screening test for detecting cognitive deficits, allowing assessing the global cognitive status, and is not described in detail here. This measure of cognitive function allows comparisons to be made with international studies. The MMSE score ranges from 0-30 and the following categories were used in the statistical analysis described hereunder (0-25: dementia, 26-29: mild cognitive impairment (MCI), 30: normal).

4) Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005; Simões et al., 2008, Freitas et al., 2011). The MoCA is a very sensitive brief cognitive screening test. It cannot be used in illiterate participants. It is a one-page test with a maximum score of 30 points that assesses six cognitive domains: executive functions; visionspatial abilities; short-term memory; language; attention, concentration and working memory; and temporal and spatial orientation. The following categories were used in the statistical analysis that was carried out (0-16: dementia, 17-21: MCI, 22-30: normal).

5) Clinical Dementia Rating scale (CDR) (Hughes et al 1982; Morris 1993; Garret et al. 2008; Santana et al. 2015). CDR is a global staging tool for dementia that is based on the assessment

of cognitive function and functional capacity, and comprises six cognitive-behavioural categories: memory; orientation; sense and problem solving; community activities; home activities and hobbies; and personal care. The scale is administered to the adult/elderly patients and an informant through a semi-structured interview. The CDR score ranges from 0-4, and the following categories were used in the statistical analysis: 0- normal, 0.5- MCI, 1-mild dementia, 2- severe dementia.

6) Geriatric Depression Scale (GDS) (Yesavage et al. 1983; Pocinho et al. 2009; Simões et al., 2015). The GDS is a brief scale to assess depressive symptoms in older adults, composed of 30 dichotomous questions that assess emotional and behavioural symptoms of depression. The test score ranges from 0-30 and the following categories were used in the statistical analysis (0-10: absence of depressive symptoms, 11-20: mild depressive symptoms, 21-30: moderate to severe depressive symptoms).

4.2 Urine samples and analysis

Epidemiological studies using biomonitoring data often rely on urine analysis because involves a less invasive sample collection procedure and it is easy to obtain in large populations (Marchiset-Ferlay et al. 2012). First morning urine samples were collected in polyethylene containers and stored at −20 °C until analysis. All reagents used were of trace analysis grade or equivalent. All aqueous solutions were prepared using ultrapure water (>18.2 MΩ.cm).

Urine samples were defrosted 24h hours before the analysis and diluted 10-fold diluted with 1% v/v HNO_3 for elemental analysis of 11 chemical elements using a Thermo X-series inductively coupled plasma-mass spectrometry (ICP-MS) instrument. Samples with concentrations higher than 200 µg/L were reanalysed after further 10-folds dilution. Samples with extremely high concentrations were also analysed by inductively coupled plasma-mass spectrometry-optical emission spectrometry (ICP-OES), using a Horiba JobinYvon Activa M instrument, but the results weren’t significantly different.

Freeze-dried human urine Seronorm™ Trace Elements was used in experimental studies on the validation of the analytical procedure used for PTEs quantification in urine samples. This material was also analysed during each analytical run as a quality control (QC) sample. Results were well within the acceptable range for all the metals, excepting Fe.

Urinary data are usually adjusted to a constant creatinine concentration to correct for factors unrelated to exposure, particularly the variable dilutions among spot samples (ref.[a]: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1277864/). Results of urine samples were therefore adjusted and reported as µg/g creatinine.

4.3 Groundwater sampling and analyses

The groundwater sampling was part of a larger project aiming at characterizing the contamination of the shallow aquifer in the surroundings of the ECC, as well as developing a conceptual model of contamination and attenuation processes (Ordens 2007; Ordens et al. 2007; Condesso de Melo & Marques da Silva 2008). A total of 31 samples were collected in the phreatic zone of the shallower aquifer. The sampling procedure included measurements of physicochemical field parameters (temperature; pH; electrical conductivity (EC); redox potential (Eh); dissolved oxygen (DO) concentration; and alkalinity) using HANNA instruments. After the stabilization of these parameters, water samples were collected and filtered through a 0.45 µm membrane. A 100 mL volume was titrated for on-field alkalinity analysis with a HACH Alkalinity kit.

The water samples were analysed for major and trace elements by ICP-MS at the Activation Laboratories (Ontario, Canada). Analytical blanks and potential instrumental drifts were carefully monitored, and instrument standardization and reproducibility were performed with Certified Standard Reference Materials.

4.4 Statistical analysis

Relationships between PTEs concentrations in urine samples and the preclinical stages of dementia, as determined by the different tests, were obtained through a method of factor analysis that uses categorical (or discrete) variables and is known as multiple correspondence analysis (MCA). Other variables such as water consumption, dietary habits or the number of years living in the city, were also included in the study to investigate relationships between environmental factors, PTEs concentrations in the urine and the preclinical stages. MCA was designed to describe a two-way contingency table N (Benzécri 1980; Greenacre 1984). MCA defines a measure of distance (or association) between two points, which are the categories of the discrete variables (χ^2). The analysis was performed using the AnDad (v. 7.12) free software package. Given that MCA uses categorical variables, all quantitative variables in the dataset were previously categorised (Reis et al. 2007, 2015). Variables used to compute the MCA factors are known as active variables. New variables usually referred to as supplementary variables can be displayed as supplementary points in the previously calculated MCA factors. Although these supplementary variables are not accounted to obtain the MCA factors, their geometrical relations with the active variables can be seen in the bi-plots (Reis et al. 2004, 2010).

Multiple linear regression (MLR) analysis is an approach for modelling the relationship between a scalar dependent variable (y) and various explanatory variables (or independent variables - X). In linear regression, the relationships are modelled using linear predictor functions whose unknown model parameters are estimated from the data. Such models are called linear models. In this study, stepwise MLR analysis was performed using the IBM®SPSS (v. 21) software and aimed at modelling the relationship between MMSE scores and quantitative variables such as PTEs contents in urine samples. The criteria for stepwise MLR were: probability of F to enter ≤ 0.05 and probability of F to remove ≥ 0.1. The Durbin-Watson test assures the absence of first-order linear autocorrelation in our multiple linear regression data. Residuals plots were used to assess whether residuals were approximately normally distributed.

5. Results and Discussion

5.1 PTEs levels in urine samples and neuropsychological assessment data

From an overall analysis of the socio-economic data, it was found that most of the subjects are female (78.6%) with an education level of 4 years (mode of the population), and mainly falling under the marital status of widowed (60.2%). Considering the neuropsychological assessment results obtained from MMSE, MoCA and CDR tests, 40.2% of the subjects had a normal performance on these tests, 18.3% showed a mild cognitive impairment compatible with the MCI conditions (considering the cut-offs for MCI established in Portuguese validation studies and CDR = 0.5) and 36.6% had a cognitive performance suggestive of dementia condition (CDR≥1 and MMSE and MoCA scores below the respective thresholds). The scores of the GDS (Mode =3) reflect an absence of depressive symptoms in most subjects of the study group. The average results of the MMSE (Mode= 29, Median=22, Standard-Deviation=8) suggest the absence of cognitive impairment in the overall sample.

Urinary contents of PTEs are used as indicators of recent exposure (via ingestion or inhalation) because urine is presumed to be the main route of excretion of most trace metal species. Studies of industrial workers and populations exposed to high levels of environmental contaminants have shown that there is a relationship between urinary levels of a few PTEs and estimates of their exposure via ingestion, inhalation or dermal contact (Marchiset-Ferlay et al. 2012; Kuiper et al. 2014). However, to the best of our knowledge, there are few neurological studies using urine samples as biological matrices of exposure to environmental contaminants.

Summary statistics for quantitative variables (MMSE scores and elemental concentrations in urine samples) are shown in Table 1. Given the wide ranges in element concentrations in urine samples reported from studies in different parts of the world (Kazi et al. 2008; Kuiper et al. 2014), table 1 shows the ranges of concentration (P5-P95) available from the study of Goullé et al. (2005) for healthy people. It can be observed that, on average, urinary levels of PTE for the participants exceed those reported in the literature for healthy people. However, looking to the median values it is of note that, in most cases, the values fall within the range of values reported by Goullé et al. (2005). The exceptions are Al, Cd, Mn, and Zn which show median values above the values available from the literature (Goullé et al. 2005; Kazi et al. 2008; Kuiper et al. 2014).

5.2 Relationships between social-behavioral factors, cognitive tests and PTEs

Relationships between PTEs levels in urine samples and neuropsychological assessment data were investigated through MCA where elemental concentrations were used as active variables and the results of the neuropsychological tests were projected as supplementary variables. Since PTEs levels in urine are quantitative variables, these were previously categorized in classes of concentration where category 1 represents low levels and correspond to the interval [minimum value of the dataset - median value obtained from the literature for healthy people[, category 2 represents average levels and correspond to the interval [median value obtained from the literature for healthy people – 95th percentile value obtained from the literature for healthy people[and category 3 represents high levels and correspond to the interval [95th percentile value obtained from the literature for healthy people – maximum value of the data set].

For the cognitive tests, MMSE and MoCA variables were divided into three categories: 0-dementia, 1-MCI, 2-normal, while CDR variable was divided into five categories: 0- normal, 1-MCI, 2- low dementia state, 3 – moderate dementia and 4- severe dementia state. The variable DIA, which results from the overall diagnostic evaluation, has three categories: 0: Normal, 1: Dementia, 2: MCI.

The first two factors produced by the MCA account for ca. 60 % of the total variance and were therefore investigated. The coordinates of the categories in the first two MCA factors are provided in the form of supplementary material (Table S1). In order to enhance clarity of the figures, projections of active and supplementary variables are displayed in different plots, although such projections result from the same MCA and correspond to the same factorial plane.

The projections in the first factorial plane (Figure 2) of the categories defined for PTEs contents in urine samples (active variables) shows that factor 2 separates high values (categories 3) from medium and low values (categories 2 and 1, respectively). The exceptions are Al and As, where categories 1 and 3 (extreme values) are both associated to the positive semi-axis of factor 2. The first factor separates high contents of Hg+Ni+Pb+Fe+Cu from high contents of Cr+Mn+Se+Cd, suggesting a different behaviour between these elements. Low Al content (Al1) and average Cr concentrations (Cr2) show important contributions to the first factor (Figure 2 and Table S1).

Figure 3 shows projections of the categories defined for the cognitive tests and variable DIA (supplementary variables) in the same factorial plane. Projections displayed in Figures 2 and 3 can, therefore, be combined and jointly interpreted to infer relationships between PTEs contents in urine samples and the neuropsychological status of the study group, which was the main aim of the study. In the plots, the quadrant defined by the positive semi-axis of factor 2 and negative semi-axis of factor 1 shows the association of MOC0 (dementia) and CDR4 (severe dementia) to high levels of As, Al, Hg, Fe, Ni, Pb, Cr and Zn, and low levels of Al in the urine samples. The quadrant defined by the positive semi-axes of factor 2 and factor 1 shows the association of CDR2 (mild dementia) and MMS0 (dementia) to high contents of Cr, Mn, Cd, and Se. Category DIA1 (dementia) is projected in the positive semi-axis of factor 2 and further supports the association between the diagnosis of dementia and high PTEs contents in urine samples.

Involvement of PTEs in the risk of developing neurological disorders has been suggested in several studies (Zatta et al. 2003; Perl and Moalem 2006; Monnet-Tschudi et al. 2006; Rodella et al. 2008; Johnson & Atchison 2009; Gomes & Wittung-Stafshede 2010; Breydo & Uversky 2011; Hozumi et al. 2011; Exley 2012; Ashok et al. 2015; Ahlskog 2016), and although controversial, increasing lines of evidence point towards the existence of an actual link. Neurodegenerative diseases constitute a set of pathological conditions originating from the slow, irreversible, and systematic cell loss within the various regions of the brain and/or the spinal cord. Depending on the affected region, the outcomes of the neurodegeneration are very broad and diverse, ranging from dementia to movements disorders (Breydo & Uversky 2011). The aetiology of these diseases is still unclear. A genetic vulnerability seems likely, but additional factors like endo- and exotoxins are proposed to contribute to the induction and, in some cases, possibly the acceleration of the neurodisorders (Gaenslen et al. 2008). Age and dietary habits, as well as environmental and occupational factors, favour the onset of neuropathologies while less than 1% of Parkinson disease cases seem to have a genetic origin (Forte et al. 2004). High PTE levels in urine samples of the participants suggest exposure to them, and Figures 2 and 3 show an association between the diagnosis of dementia or cognitive impairment and high urinary PTE levels. However, for Al, high and low urine levels appear to be associated with neurodegenerative disorders. Bocca et al. (2006) and Forte et al. (2014) found lower concentrations of Al in the urine of neurological patients than in control groups, which is in agreement with our study. However, other authors found a link between high contents of Al and neurological disorders (Roberts et al. 1998; Polizzi et al. 2002), which is also in agreement with our study.

Main kinetic characteristics of Al are low intestinal absorption, rapid urinary excretion, and slow tissue uptake. Neurons may be the cells most liable to accumulation (Ganrot 1986; Van der

Voet 1992). According to the authors, Al may cause or contribute to some specific diseases, most of them related to aging (Ganrot 1986). Whereas high levels of a few PTEs and low levels of Al in the urine of some participants seem associated to cognitive impairment, this can be explained by probable chemical competition/substitution phenomena, in a similar way to competition/substitution that occur in nature and in crystals. Ions such as Si, Fe, Ca and Cr compete with Al (Ganrot 1986) for binding sites and many of the participants in this study used to take Fe, Zn and Ca supplements, which substantiates the hypothesis of such chemical interactions in the human body, particularly in the absorption process at the gastrointestinal tract..

Whereas MCA also aimed at identifying relationships between the health data and socio-economic and environmental factors likely to influence a potential association between PTEs levels in urine samples and the neuropsychological assessment of the subjects, other relevant variables were projected in the same factorial plane. The following characteristics of the participants and their life-habits were considered: a) record of neurological health problems in the family (ANT), which is a binary variable (1-yes, 2-no); b) the number of years living in Estarreja (variable AR), which was divided into three categories of equal amplitude; c) professional occupation (variable PRO), which was divided into four categories, Pro0 (agriculture), Pro1 (services and trade), Pro2 (industry), Pro3 (housewife); d) the education level, which was divided into the categories “ana”(illiterate), “4º”, “9º” and “12º”; e) type/origin of water used in irrigation (variable REG), which was divided into the categories: “well” (Reg1), “borehole” (Reg2), “stream” (Reg3) and tap water (Reg5); f) the origin of drinking water (variable PRV), which was divided into four categories: Prv1 (tap water), Prv2 (well), Prv3 (bottle), Prv4 (tap water and bottle); g) the variable “Cha”, which is binary (1-yes, 2-no) and describes drinking tea habits; h) the consumption of home-grown foodstuffs (Veg), which is also a binary variable (1-yes, 2-no).

Figure 4 shows the projections of the categories previously established to assess relationships between environmental factors, cognitive tests (Figure 3) and PTE levels in urine samples (Figure 2). Comparing the three bi-plots, it is of note that Reg5 (tap water is used for irrigation) is associated to average Cr concentration and low As, Fe and Cu levels in urine (Figure 2), while Reg3 (stream water is used for irrigation) is associated to dementia (Dia1, CDR2, MMS0 in Fig. 1b) and high Cr, Mn, Cd and Se levels in urine samples. PRV2 (well water is used to drink) is associated to severe dementia (CDR4 in Figure 3) and high levels of As, Al, Ni, Pb and Hg, as well as to low Al contents in urine samples (Figure 2a). Category PRV4 (tap and mineral water) is associated to low PTEs levels in urine samples (Figure 2). The results indicate that, from all the environmental factors under investigation, water used either to drink or for irrigation is probably the most important exposure pathway. From the above, urine appears to be a suitable specimen to assess exposure to environmental PTEs through different pathways. Although acknowledging that factors other than drinking water may influence urine concentrations, Karagas et al. (2001) found a significant correlation between urinary and drinking water As concentrations. Also Lin et al. (2010) found a correlation between As levels in drinking water and urine. Kasper-Sonnenberg et al. (2011) reported a positive association between Ni in ambient air and urinary Ni in a subgroup of 6-yr-old children living near a steel mill. Afridi et al. (2008) state that the association of urinary excretion rates with renal Hg content and functional status suggests that urinary porphyrin profiles may serve as a useful biomarker of mercury accumulation and nephrotoxicity during prolonged Hg exposure through drinking water. The studies of Forte et al. (2004) and Roberts et al. (1998) successfully used urinary PTEs contents as biomarkers for neurological pathologies. Hence, a wide number of studies have used urine to confirm exposures and assess health effects. Whilst a direct relationship between PTE levels in drinking water and urine cannot be established in the present study, the results obtained so far indicate a relationship between urinary PTE levels and water consumption habits of the participants. Furthermore, both factors seem to be related to the incidence of cognitive disorders.

Figure 5 shows the projections, in the same factorial plane of MCA, of socio-economic factors considered to be potentially relevant. In this study group, it is not obvious a relationship between the education level and the neuropsychological assessment of the subjects. However, a high number of years of residence in Estarreja (AR3 in Figure 5) seems associated to a diagnosis of dementia (Figure 3) and to high PTE concentrations in urine samples (Figure 2). Individuals who have worked either in agriculture (Pro0) or in industry (Pro2) tend to have higher PTE levels in urine and the results of their neurological tests indicate a state of dementia. Individuals with a family record of neurodegenerative conditions (ANT1) are mainly associated to a diagnosis of “normal” (MMS1-MCI, MOC2-normal, CDR0-normal, dia 0-normal) and low levels (category 1) of PTEs in urine. Although recent studies investigating relationships between PTEs levels in toenail clippings or human hair and socio-economic factors are available from the literature (Cabral Pinto et al. 2013; Ndilila et al. 2014; Cabral Pinto et al. 2015; Hao et al. 2015; Reis et al. 2015), to the best of our knowledge this has not yet been attempted using urine samples as specimen to measure biomarkers of environmental exposure and its impact on the health status of the population.

The statistical analysis of this multidisciplinary and complex dataset allowed establishing a relationship between high PTE levels in the urine of the participants and their neuropsychological condition. Whilst several environmental factors can be associated to increased PTE levels and to a diagnosis of dementia, no relationship could be established between the genetic burden of the individuals and a tendency to neurodegenerative disorders (Figure 5). Although water ingestion arises as the most likely exposure pathway to environmental contaminants, other environmental and social factors such as profession or the number of years living in the city seem to be relevant, and further studies are necessary to investigate other potential pathways of exposure.

In this study, stepwise multiple linear regression (MLR) analysis was used to identify which PTEs are best predictors of MMSE scores. Since the results of MCA associate the number of years residing in Estarreja to high PTEs levels in urine, this variable was also included in the stepwise MLR analysis. The linear models obtained are shown in Table 2. R^2 indicates the proportion of the variance in MMSE scores accounted for by each regression model. All regression models are statistically significant ($p < 0.005$).

Although Al is clearly showed to be the best predictor of MMSE scores (R^2= 38%), Cd and Zn are also relevant predictors as demonstrated by the significant increase in R^2 (17% increase). Whereas model 4 is also statistically significant, the 2% increase in the R^2 value indicates that the number of years residing in Estarreja is not as relevant to predict MMSE scores as the urinary levels of Al, Cd and Zn.

Polizzi et al. (2002) applied neuropsychological tests to dust-exposed workers to Al and to an unexposed population, and their findings lead them to suggest a possible role of the inhalation of Al-dust in pre-clinical mild cognitive disorder which might prelude Alzheimer’s disease (AD) or AD-like neurological deterioration. Rogers and Simon (1999), studying the link between dietary Al intake and risk of Alzheimer's disease, shown that the past consumption of foods containing large amounts of Al additives differed between people with Alzheimer's disease and controls, suggesting that dietary intake of Al may affect the risk of developing this disease. Albeit an important number of studies have linked exposure to environmental Al, either through inhalation or through ingestion, to neuropsychological disorders, the underlying mechanisms are still largely unknown and further studies are warranted to corroborate or refute these findings.

Unlike Al, Cd has a long biological half-life mainly due to its low rate of excretion from the body. Thus, prolonged exposure to Cd will cause toxic effects due to its accumulation over time in a variety of tissues, including kidneys, liver, central nervous system and peripheral neuronal systems. However, mechanisms underlying Cd neurotoxicity are not yet completely understood (Wang &Du 2013). Viaene et al. (2000) observed slowing of visuomotor functioning on neurobehavioural testing and increase in complaints consistent with peripheral neuropathy, complaints about equilibrium, and complaints about concentration ability that were dose-dependent to urinary Cd. They further found that age, exposure to other neurotoxicants or renal function status could not explain these findings.

Different studies on the role of Zn have come to very contrasting conclusions. Excess Zn in senile plaques and vascular amyloid deposits may initiate amyloid deposition affecting polymerized microtubule stability. On the other hand Zn may counter oxidative stress and neurotoxicity, thereby preventing neurodegeneration and cognitive impairment, in a process of potential therapeutic use (Yegambaram et al. 2005). Further confirmatory studies are required to fully understand the role of Zn either in the development or in the prevention of neurodegenerative diseases.

5.3 Groundwater PTEs concentrations

Given the results obtained in the statistical analysis, which indicated a relationship between urinary PTEs levels and a diagnosis of dementia, and further suggested water ingestion as an important probable pathway of exposure to the environmental contaminants, concentrations of major, minor and trace elements in groundwater samples were investigated.

Figure 6 shows the concentrations (µg/L) of the PTEs under study in groundwater samples collected from wells and boreholes in ECC's surroundings. Values form the WHO guidelines (2011) and Maximum Admissible Values (MAV) established in Portuguese legislation (Portuguese Decree-Law 1998, 2007) for drinking water are also shown and used as thresholds of water quality. Obtained PTEs concentrations were well above WHO (2011) and MAV in water for human consumption, except for Cr and Se. Means and maximum values were often several orders of magnitude higher than the admissible values, namely for Al, As, Cd, Fe, Hg, Mn and Zn, which showed very high concentrations (contamination). However, when looking at the median values, it is reassuring to see that, in most cases, the median concentrations fall below the international and Portuguese legislation. The exceptions are Al, Fe, Mn, and Zn. It is worth noting that the large difference between the mean and median concentration found for some PTEs suggests variable patterns of contamination. Further studies are required to identify possible PTEs sources, transport of contaminants within the aquifer and fate of the elements of concern, as all this factors modify human exposure risk.

Urinary median concentrations of Al, Cd, Mn and Zn of the participants in this study were elevated relative to the typical ranges that have been reported in studies performed in other parts of the world (Goullé et al. 2005; Kazi et al. 2008; Kuiper et al. 2014). Furthermore, the LRM models show that Al, Cd and Zn urinary levels are good predictors of MMSE scores. In addition, the MCA suggests an association between the consumption of well and stream water and high urinary contents of Al, Cd, Mn, Zn and other PTEs. Finally, the analysis of groundwater samples collected from wells and boreholes located near the ECC indicates unacceptable levels of Al, Fe, Mn and Zn for drinking purposes (Figure 6). Although current median Cd in groundwater samples was below the MAV, a long-term exposure to Cd-contaminated waters could still be reflected in urine samples, because of Cd long biological half-life (accumulation over time in the human body). Thereupon, we can assume that the elevated urinary concentrations of Al, Cd, Mn and Zn are likely related to the ingestion of contaminated groundwater. Martyn et al. (1989) carried out a large survey across England and Wales and found that the risk of AD was 1.5 times higher in districts where the mean Al concentration exceeded 0.11 mg/l than in districts where the concentrations were lower than 0.01 mg/l, which is in good agreement with the present study. Furthermore, the authors have not found any other probable cause for the incidence of the disease. However, in our study, the results suggest that both low and high levels of Al can be related to cognitive impairment. Considering that Al does not play any metabolic role in the human body, an Al deficiency in the participants cannot cause any deleterious health effect. Instead, the high contents of other PTEs such as Cd (and probably Zn), that in some participants are associated to low urinary Al levels, may be the ones actually causing the health effects. Unlike the findings of Martyn and co-authors, the results of the study hereby described suggest that, besides Al, also environmental Cd is related to the cognitive disorders found in the participants.

6. Conclusions

This study describes a multi-disciplinary approach combining neurosciences, psychology and environmental sciences, while integrating socio-economic, neuropsychological, environmental and health data.

For some PTEs, the urinary levels of the study group, elderly residents in Estarreja (a Portuguese industrial city in the Centre of Portugal), are generally above the ranges reported for healthy populations in other parts of the world.

The multivariate statistical analysis (MCA) of the multidisciplinary dataset indicates a relationship between high PTE levels in the urine of the participants and their neuropsychological impairment. It further suggests an association between high urinary PTEs levels and water consumption habits, the number of years residing in the city and the professional. Workers in agriculture and industry have higher PTE levels and lower scores on neuropsychological tests, suggesting that not only environmental but also occupational exposure has to be considered. No relationship was found between the genetic burden of the individuals and a tendency to neurodegenerative impairment.

Multiple linear regression models show that the cognitive evaluation results (MMSE scores) can be predicted by Al, Cd and Zn levels in urine and, to some extent, by the number of years living in the city.

The groundwater concentrations in ECC's surroundings are often above the Portuguese maximum allowed levels in water for human consumption, particularly in the case of Al, Fe, Mn and Zn. Whereas the WHO (2011) does not provide health-based reference values for PTEs such as Al, this study suggests that long-term exposure to this PTE may have a deleterious effect in the cognitive abilities of the elderly residents. Although median Cd in groundwater samples was below the permissible values, a long-term exposure to Cd-containing waters could still be reflected in urine samples because Cd has a long biological half-life and accumulates over time in the human body. Considering the contrasting behavior of Al and Cd in the human gut that often requires using different biological matrices to assess exposure to these environmental contaminants, in this study urine proved to be a suitable specimen to evaluate long-term exposure, both for elements with low and high excretion rates.

The results of the statistical analysis indicate that the time of residence in Estarreja is positively associated with high urinary levels of PTEs, which suggests a long-term body burden of the participants through the ingestion of drinking water. Although technological upgrades associated with remediation measures implemented in the last decade by the industry have reduced the environmental burden of the city, the Estarreja Chemical Complex is still regarded as the major polluter of the region. Residents of Estarreja were exposed to a highly-contaminated environment for decades, which may explain the positive association between high PTEs levels in urine and low MMSE scores.

The possible involvement of environmental factors in the aetiology of aging related diseases such as AD, PD and ALS unveils new perspectives for prevention and treatment. Whereas genetic factors cannot always be controlled, if environmental factors causing human exposure can be identified, such information will provide invaluable support to any protective measures considered to be relevant to assure the well-being of the population.

Whereas no direct relationships could be established in this study, the relationship between water ingestion, high urinary Al, Cd and Zn levels largely justifies the need for further studies on the water ingestion pathway and potential long-term effects on the cognitive abilities of the inhabitants. Increased understanding on the underlying causal relationships between environmental exposure and health effects is decisive for effective remediation and science-based decision making.

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References

APA (2016). Programa Operacional Temático Valorização do Território Eixo Prioritário III. *Recuperação do Passivo Ambiental. Documento Enquadrador*, 26 pp. https://poseur.portugal2020.pt/media/38027/01_docenq_passivoambiental.pdf

Ahlskog, J. E. (2016). New and Appropriate Goals for Parkinson Disease Physical Therapy. *JAMA neurology*, 1-2.

Ashok, A., Rai, N. K., Tripathi, S., & Bandyopadhyay, S. (2015). Exposure to As-, Cd-, and Pb-mixture induces Aβ, amyloidogenic APP processing and cognitive impairments via oxidative stress-dependent neuroinflammation in young rats. *Toxicological Sciences*, kfu208.

Cabral Pinto, M.M.S., Almeida, A., Pinto, E., Freitas, S., Simões, M., Diniz, L., Moreira, P., Silva, M. M. V. G., Ferreira da Silva, E., Condesso de Melo, T. (2015). *Occupational and environmental exposure to Mn in manganese mining areas (South Portugal) and the occurrence of dementia*. 25th Alzheimer Europe Conference "Dementia: putting strategies and research into practice"

Cabral Pinto, M.M.S., Freitas, S., Simões, M., Moreira, P.I., Dinis, L., Ferreira da Silva, E.A. (2013). *Neurodegenerative diseases in the Estarreja (Central Portugal) inhabitants and their potential relationship with trace elements in the environment – preliminary results*. 5º International Conference on Medical

Geology. 25-29 Aug,2013, Virginia, USA.

Coelho, P., Costa, S., Silva, S., Walter, A., Ranville, J., Sousa, A. C., ... & Laffon, B. (2012). Metal (loid) levels in biological matrices from human populations exposed to mining contamination—Panasqueira Mine (Portugal). *Journal of Toxicology and Environmental Health,* 75, 893-908.

Benzecri, F. (1980). Introduction à l'analyse des correspondances d'après un exemple de données médicales. *Les cahiers de l'analyse des données*, 5(3), 283-310.

Birchall, J. D., & Chappell, J. S. (1988). The chemistry of aluminum and silicon in relation to Alzheimer's disease. Clinical Chemistry, 34(2), 265-267.

Bocca, B., Alimonti, A., Senofonte, O., Pino, A., Violante, N., Petrucci, F., ... & Forte, G. (2006). Metal changes in CSF and peripheral compartments of parkinsonian patients. *Journal of the Neurological Sciences*, 248(1), 23-30.

Breydo, L., & Uversky, V. N. (2011). Role of metal ions in aggregation of intrinsically disordered proteins in neurodegenerative diseases. Metallomics,3(11), 1163-1180.

Bressler, J.P., Olivi, L., Cheong, J.H., Kim, Y., Maerten, A., & Bannon, D. (2007). Metal transporters in intestine and brain: their involvement in metal-associated neurotoxicities. *Hum. Exp. Toxicol*, 26, 221-229.

Cachada, A., Pereira, M. E., Ferreira da Silva, E., & Duarte, A. C. (2012). Sources of potentially toxic elements and organic pollutants in an urban area subjected to an indus-trial impact. *Environmental Monitoring and Assessment*, 184, 15–32.

Cerpa, W., Varela-Nallar, L., Reyes, A. E., Minniti, A. N. & Inestrosa, C. (2005). Is there a role for copper in neurodegenerativediseases? *Molecular Aspects of Medicine*, 26, 405–420.
Coelho, P., Costa, S., Silva, S., Walter, A., Ranville, J., Sousa, A. C., ... & Laffon, B. (2012). Metal (loid) levels in biological matrices from human populations exposed to mining contamination—Panasqueira Mine (Portugal). *Journal of Toxicology and Environmental Health*, 75, 893-908.
Condesso de Melo, M.T. & Marques da Silva, M.A. 2008. The Aveiro Quaternary and Cretaceous aquifers. In: Edmunds, W.M. & Shand, P. (ed.). *The natural baseline quality of groundwate*r. Blackwell Publishers. Oxford.

Costa, C., & Jesus-Rydin, C. (2001). Site investigation on heavy metals contaminated ground in Estarreja-Portugal. Engineering Geology, 60, 39-47.

Dorne, J. L., Kass, G. E., Bordajandi, L. R., Amzal, B., Bertelsen, U., Castoldi, A. F., ... & Scaravelli, E. (2011). Human risk assessment of heavy metals: Principles and applications. *Met Ions Life Sci*, 8, 27-60.

Duarte, H., Menezes Pinheiro, L., Bernardes, C., Teixeira, F. C., Bouriak, S., & Monteiro, J. H. (2005). High Resolution Seismic Stratigraphy of the Ria of Aveiro (Portugal). *Iberian Coastal Holocene Paleoenvironmental Evolution – Coastal Hope* 2005 – Proceedings, 52-53.

Ferrer, I. (2012). Defining Alzheimer as a common age-related neurodegenerative process not inevitably leading to dementia. *Progress in Neurobiology*, 97(1), 38-51.

Exley, C., & House, E. R. (2012). Aluminium in the human brain (pp. 95-101). Springer Vienna.

Fabrizio, E., Vanacore, N., Valente, M., Rubino, A., & Meco, G. (2007). High prevalence of extrapyramidal signs and symptoms in a group of Italian dental technicians. *BMC Neurol*., 3, 7- 24.

Folstein, M., Folstein, S., & McHugh, P. (1975). Mini-Mental State: A practical method for grading the cognitive state of patients for the clinician. *Journal of Psychiatric Research*, 12(3), 189-198.

Forte, G., Deiana, M., Pasella, S., Baralla, A., Occhineri, P., Mura, I., ... & Carru, C. (2014). Metals in plasma of nonagenarians and centenarians living in a key area of longevity. *Experimental Gerontology*, 60, 197-206.

Freitas, S., Simões, M. R., Alves, L., & Santana, I. (2011). Montreal Cognitive Assessment (MoCA): Normative study for the Portuguese population. *Journal of Clinical and Experimental Neuropsychology*, 33(9), 989-996.

Freitas, S., Simões, M. R., Alves, L., & Santana, I. (2013). Montreal Cognitive Assessment (MoCA): Validation study for Mild Cognitive Impairment and Alzheimer´s Disease. *Alzheimer Disease and Associated Disorders*, 27(1), 37-43.

Freitas, S., Simões, M. R., Alves, L., & Santana, I. (2015). The relevance of sociodemographic and health variables on MMSE normative data. *Applied Neuropsychology: Adults*, 22(4), 311-319.

Gaenslen, A., Unmuth, B., Godau, J., Liepelt, I., Di Santo, A., Schweitzer, K. J., ... & Berg, D. (2008). The specificity and sensitivity of transcranial ultrasound in the differential diagnosis of Parkinson's disease: a prospective blinded study. *The*

Lancet Neurology, 7(5), 417-424.

Garret, C., Santos, F., Tracana, I., Barreto, J., Sobral, M., & Fonseca, R. (2008). *Avaliação Clínica da Demência [Clinical Dementia Rating Scale*]. In Grupo de Estudos de Envelhecimento Cerebral e Demências [Study Group on Brain Aging and Dementia] (Ed.), Escalas e testes na demência [Scales and tests in dementia] (pp. 17-32). Lisbon: GEECD.

Goldberg, D. P., Gater, R., Sartorius, N., Ustun, T., Piccinelli, M., Gureje, O., & Rutter, C. (1997). The validity of two versions of the GHQ in the WHO study of mental illness in general health care. *Psychological Medicine,* 27(01), 191-197.

Gomes, C. M., & Wittung-Stafshede, P. (Eds.). (2010). *Protein folding and metal ions: mechanisms, biology and disease*. CRC Press

Gorell, J. M., Johnson, C. C., Rybicki, B. A., Peterson, E. L., Kortsha, G. X., Kortsha, G. G., & Richardson, R. J. (1999). Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. *Neurotoxicology*, 20, 239– 248.

Goullé, J. P., Mahieu, L., Castermant, J., Neveu, N., Bonneau, L., Lainé, G., ... & Lacroix, C. (2005). Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair: Reference values. *Forensic Science International*, 153(1), 39-44.

Greenacre, M. J. (1984). *Theory and applications of correspondence analysis*. London Eds. ISBN : 0-12-299050-1

Gupta, V. B., Anitha, S., Hegde, M. L., Zecca, L., Garruto, R. M., Ravid, R., ... & Rao, K. J. (2005). Aluminium in Alzheimer's disease: are we still at a crossroad? *Cellular and Molecular Life Sciences CMLS*, 62(2), 143-158.

Hinwood, A. L., Sim, M. R., de Klerk, N., Drummer, O., Gerostamoulos, J., & Bastone, E. B. (2002). Are 24-hour urine samples and creatinine adjustment required for analysis of inorganic arsenic in urine in population studies? *Environmental Research,* 88(3), 219-224.

Hughes, C. P., Berg, L., Danziger, W. L., Coben, L. A., & Martin, R. L. (1982). A new clinical scale for the staging of dementia. *The British Journal of Psychiatry*, 140, 566-572.

Hao, Z., Li, Y., Liu, Y., Li, H., Wang, W., & Yu, J. (2015). Hair elements and healthy aging: a cross-sectional study in Hainan Island, China. *Environmental Geochemistry and Health*, 38(3), 723–735. http://doi.org/10.1007/s10653-015-9755-3

Hozumi, I., Hasegawa, T., Honda, A., Ozawa, K., Hayashi, Y., Hashimoto, K., ... & Tanaka, Y. (2011). Patterns of levels of biological metals in CSF differ among neurodegenerative diseases. *Journal of the Neurological Sciences*, 303(1), 95-99.

Inácio, M., Neves, O., Pereira, V., & da Silva, E. F. (2014). Levels of selected potential harmful elements (PHEs) in soils and vegetables used in diet of the population living in the surroundings of the Estarreja Chemical Complex (Portugal). *Applied Geochemistry*, 44, 38-44.

Johnson, F. O., & Atchison, W. D. (2009). The role of environmental mercury, lead and pesticide exposure in development of amyotrophic lateral sclerosis. *NeuroToxicology*, 30 (5), 761–765.

Kazi, T. G., Afridi, H. I., Kazi, N., Jamali, M. K., Arain, M. B., Jalbani, N., & Kandhro, G. A. (2008). Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. *Biological Trace Element Research*, 122(1), 1-18.

Komatina, M. M. (2004). *Medical Geology – Effects of geological environments on human health*. Developments in Earth & Environmental sciences 2. Elsevier. 488 pp.

Leitão, T.B.E. 1996. *Metodologia para a reabilitação de aquíferos poluídos*. PhD Tesis. Faculdade de Ciências da Universidade de Lisboa.

Kozlowski. H., Janicka Klosb, A., Brasunb, J., Gaggelli, E., Valensinc, D., & Valensinc, J. (2009). Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation*). Coordination Chemistry Reviews*, 253, 2665–2685.

Kuiper, N., Rowell, C., Nriagu, J., & Shomar, B. (2014). What do the trace metal contents of urine and toenail samples from Qatar′ s farm workers bioindicate? *Environmental research*, 131, 86-94.

Lemos, R., Duro, D., Simões, M. R., & Santana, I. (2014). The free and cued selective reminding test distinguishes frontotemporal dementia from Alzheimer's disease. Archives of Clinical Neuropsychology, 29(7), 670-679

Marchiset-Ferlay, N., Savanovitch, C., & Sauvant-Rochat, M. P. (2012). What is the best biomarker to assess arsenic exposure via drinking water? *Environment International*, 39(1), 150-171.

Martyn, C. N., Osmond, C., Edwardson, J. A., Barker, D. J. P., Harris, E. C., & Lacey, R. F. (1989). Geographical relation between Alzheimer's disease and aluminium in drinking water. *The Lancet*, 333(8629), 61-62.

Maynard, C. J., Bush, A. I., Masters, C. L., Cappai, R., & Li, Q. X. (2005). Metals and amyloid-β in Alzheimer's disease. *International Journal of Experimental Pathology*, 86(3), 147-159.

Monnet-Tschudi, F., Zurich, M.-G., Boschat, C., Corbaz, A., & Honegger, P. (2006). Involvement of Environmental Mercury and Lead in the Etiology of Neurodegenerative Diseases. *Reviews on Environmental Health Reviews on Environmental Health*, 21 (2), 105–118.

Moreira, M. D. F. R., & Neves, E. B. (2008). Use of urine lead level as an exposure indicator and its relationship to blood lead. *Cadernos de Saúde Pública*, 24(9), 2151-2159.

Moreira, P.I., Honda, K., Liu, Q, Santos, M.S, Oliveira, C.R., Aliev, G., Nunomura, A., Zhu, X., Smith, M.A., & Perry, J. (2005). Oxidative Stress: The Old Enemy in Alzheimer's Disease Pathophysiology. *Current Alzheimer Research*, 2, 403-408.

Moreira, P.I., Zhu, X., Lee, H.-G., Honda, K., Smith, M.A., & Perry, G. (2006). The (un)balance between metabolic and oxidative abnormalities and cellular compensatory responses in Alzheimer disease. *Mechanisms of Ageing and Development*, 127, 501–506.

Morris, J. C. (1993). The Clinical Dementia Rating (CDR): Current version and scoring rules. *Neurology*, 43, 2412-2414.

Nasreddine, Z., Phillips, N. A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J. L., & Chertkow, H. (2005). The Montreal Cognitive Assessment, MoCA: A brief screening tool for Mild Cognitive Impairment. *American Geriatrics Society*, 53(4), 695-699.

Ndilila, W., Callan, A. C., McGregor, L. A., Kalin, R. M., & Hinwood, A. L. (2014). Environmental and toenail metals concentrations in copper mining and non mining communities in Zambia. *International Journal of Hygiene and Environmental Health*, 217(1), 62–69.

Ordens, C. M., Condesso de Melo, M. T., Grangeia, C., & Marques da Silva, M. A. (2007). *Groundwater–surface water interactions near a Chemical Complex (Estarreja, Portugal) – implications on groundwater quality*. Proceedings 35th Congress of International Association of Hydrogeologists, Lisbon, Portugal, 17–21 September.

Ordens, C.M. (2007). *Estudo da contaminação do aquífero superior na região de Estarreja*. Unpublished Ms thesis. Coimbra University, 149 pp. accessed in http://www.lneg.pt/download/3268/carlos_ordens.pdf on 11/03/2015

Pereira, M. E., Lillebø, A. I., Pato, P., Válega, M., Coelho, J. P., Lopes, C., Rodrigues, S., Cahada, A., Otero, M., Pardal, M.A., & Duarte A.C. (2009). Mercury pollution in Ria de Aveiro (Portugal): a review of the system assessment. *Environment Monitoring and Assessment*, 155, 39-49.

Perl, D. P., & Moalem, S. (2006). Aluminum and Alzheimer's disease, a personal perspective after 25 years. *Journal of Alzheimer's Disease*, 9 (3), 291-300.

Pocinho, M. T. S., Farate, C., Dias, C. A., Lee, T. T., & Yesavage, J. A. (2009). Clinical and psychometric validation of the Geriatric Depression Scale (GDS) for Portuguese Elders. *Clinical Gerontologist*, 32, 223-236.

Polizzi, S., Pira, E., Ferrara, M., Bugiani, M., Papaleo, A., Albera, R., & Palmi, S. (2002). Neurotoxic effects of aluminium among foundry workers and Alzheimer's disease. *Neurotoxicology*, 23(6), 761-774.

Portuguese Decree 236 (1998). *Portuguese legislation on water quality*. Diário da República IA, 3676-3722.

Portuguese Decree 306 (2007). Portuguese legislation on water quality. Diário da República IA, 5747-5765.

Reis, A. P., Costa, S., Santos, I., Patinha, C., Noack, Y., Wragg, J., ... & Sousa, A. J. (2015). Investigating relationships between biomarkers of exposure and environmental copper and manganese levels in house dusts from a Portuguese industrial city. *Environmental Geochemistry and Health*, 37(4), 725-744.

Reis, A. P., Patinha, C., Ferreira da Silva, E., Sousa, A., Figueira, R., Sérgio, C., & Novais, V. (2010). Assessment of human exposure to environmental heavy metals in soils and bryophytes of the central region of Portugal. *International Journal of Environmental Health Research*, 20(2), 87–113. http://doi.org/10.1080/09603120903394649

Reis, A. P., Sousa, A. J., Ferreira Da Silva, E., Patinha, C., & Fonseca, E. C. (2004). Combining multiple correspondence analysis with factorial kriging analysis for geochemical mapping of the gold-silver deposit at Marrancos (Portugal). *Applied Geochemistry*, 19(4), 623–631. http://doi.org/10.1016/j.apgeochem.2003.09.003

Reis, A.P., Menezes de Almeida, L., Ferreira da Silva, E., Sousa, A.J., Patinha, C., Fonseca, E.C., 2007. Assessing the geochemical inherent quality of natural soils in the Douro river basin for grapevine cultivation using data analysis and geostatistics. *Geoderma* 141, 370–383.

Roberts, N. B., Clough, A., Bellia, J. P., & Kim, J. Y. (1998). Increased absorption of aluminium from a normal dietary intake in dementia. *Journal of Inorganic Biochemistry*, 69(3), 171-

176.

Rodella, L. S., Ricci, F., Borsani, E., Stacchiotti, A., Foglio, E., Favero, G., Rezzani, R., Mariani, C., & Bianchi, R. (2008). Aluminium exposure induces Alzheimer' disease-like histopathological alterations in mouse brain. *Histol Histopathol*, 23-433-439.

Rogers, M. A., & Simon, D. G. (1999). A preliminary study of dietary aluminium intake and risk of Alzheimer's disease. *Age and Ageing*, 28(2), 205-209.

Santana, I., Vicente, M., Freitas, S., Santiago, B., & Simões, M. R. (2015). *Avaliação Clínica da Demência (CDR) [Clinic Dementia Rating, CDR*]. In Mário R. Simões, Isabel Santana e Grupo de Estudos de Envelhecimento Cerebral e Demência (Eds.), Escalas e Testes na Demência (3ª. edição; pp. 12-17) [Scales and Tests in Dementia, 3rd edition]. Lisboa: Novartis.

Simões, M. R., Freitas, S., Santana, I., Firmino, H., Martins, C., Nasreddine, Z., & Vilar, M. (2008). *Montreal Cognitive Assessment (MoCA): Versão portuguesa [Montreal Cognitive Assessment (MoCA): Portuguese version*]. Coimbra, Portugal: Serviço de Avaliação Psicológica da Faculdade de Psicologia e de Ciências da Educação da Universidade de Coimbra [Psychological Assessment Department, Faculty of Psychology and Educational Sciences, University of Coimbra].

Simões, M. R., Prieto, G., Pinho, M. S., & Firmino, H. (2015). *Geriatric Depression Scale (GDS-30)*. In Mário R. Simões, Isabel Santana e Grupo de Estudos de Envelhecimento Cerebral e Demência (Eds.), Escalas e Testes na Demência (3ª. edição; pp. 128-133) [Scales and Tests in Dementia, 3rd edition]. Lisboa: Novartis.

Teixeira, C. & Assunção, C. F. T. (1963). *Ovar Geological Map*, 13C. Instituto Geográfico e Cadastral, Eds. Lisboa.

Van der Voet, G. B. (1992). Intestinal absorption of aluminum. In *The vulnerable brain and environmental risks* (pp. 35-47). Springer US.

Van der Weijden, C. & Pacheco, F.A.L. (2006). Hydrogeochemistry in the Vouga River basin (central Portuhal): Pollution and chemical weathering. *Applied Geochemistry*, 21, 580-613.

Viaene, M. K., Masschelein, R., Leenders, J., De Groof, M., Swerts, L. J., & Roels, H. a. (2000). Neurobehavioural effects of occupational exposure to cadmium: a cross sectional epidemiological study. *Occupational and Environmental Medicine*, 57, 19–27.

Wang, B., & Du, Y. (2013). Review Article Cadmium and Its Neurotoxic Effects. *Oxidative Medicine and Cellular Longevity*, doi.org/10.1155/2013/898034

World Health Organisation. (2011). *Guidelines for drinking-water quality* - 4th ed. Viewed online on http://who.int/en/ on 28 April 2016.

Yesavage, J. A., Brink, T. L., Rose, T. L., Lum, O., Huang, V., Adey, M., & Leirer, V. O. (1983). Development and validation of a geriatric depression screening scale: A preliminary report. *Journal of Psychiatric Research,* 17(1), 37-49.

Yokel, R.A. (2006). Blood-brain barrier flux of aluminum, manganese, iron and other metals suspected to contribute to metal-induced neurodegeneration. *J Alzheimers Dis.* 10(2-3), 223-53.

Zatta, P., Lucchini, R., Van Rensburg, S. J., & Taylor, A. (2003). The role of metals in neurodegenerative processes: aluminum, manganese, and zinc. *Brain Research Bulletin,* 62, 15-28.

Zhang, B., Cheng, X. R., da Silva, I. S., Hung, V. W., Veloso, A. J., Angnes, L., & Kerman, K. (2013). Electroanalysis of the interaction between (−)-epigallocatechin-3-gallate (EGCG) and amyloid-β in the presence of copper. *Metallomics*, 5(3), 259-264.

Figure Captions:

Figure 1: Location, geological and land-use (Corine Land Cover of 2006) maps of the studied area.

Figure 2: Projection in the first factorial plane of the categories defined for PTEs contents in urine samples (active variables). Categories with label 1 include low PTEs levels while categories with label 2 include average PTEs levels and categories with label 3 represent high PTEs contents in urine samples.

Figure 3: Projections in the first factorial plane of the categories defined for the cognitive tests and variable DIA (supplementary variables). Key: MOC: MoCA cognitive test (MOC0: dementia, MOC1: MCI and MOC2: normal; MOC9 refers to illiterate participants that could take part in the MoCA test); MMS: MMSE cognitive test (MMS0: dementia, MMS1: MCI and MMS2: normal); CDR: CDR cognitive test (CDR0: normal, CDR1: MCI, CDR2: mild dementia, CDR3: dementia; CDR4: severe dementia, DIA: diagnosis (Dia0: normal, Dia1: dementia, Dia2: MCI).

Figure 4: Projections in the 1st factorial plane of the categories defined for environmentally relevant life-habits of the participants. Key: Reg: origin of water used in irrigation (Reg1: well water, Reg2: borehole water, Reg3: stream water, Reg5: tap water); Prv: origin of drinking water (Prv1: tap water, Prv2: well water, Prv3: bottled water, Prv4: tap water and bottled water); Cha: drinking tea habits (Cha1: yes, Cha 2: no); Veg: consumption of home-grown food (Veg1: yes, Veg2: no)

Figure 5 - Projections in the 1st factorial plane of categories established for relevant socio-economic factors. Key: Ant: record of neurological health problems in the family (Ant1: yes, Ant2: no); AR: number of years living in Estarreja (AR1: small, AR2: average, AR3: large); Pro: profession (Pro0: agriculture, Pro1: services and trade, Pro2: industry, Pro3: housewife); education level is identified by labels "ana"(illiterate), "4º" (4th grade), "9º"(9th grade), "12º (12th grade) and ">12ª" (higher education level).

Figure 6: Concentrations found in water samples in ECC's surroundings. The top of the error bars indicate one standard deviation. WHO refers to guideline values for drinking water from WHO (2011). MAV refers to Maximum Admissible Value for human consumption, according Portuguese Legislation (Portuguese Decree 1998, 2007).