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ABSTRACT

Fly ash engender significant environmental and human health problems due to enhanced contents of potentially harmful trace elements (TrElems). This study aims to evaluate human exposure to TrElems via a combined ingestion (i.e., oral bioaccessibility) and inhalation (i.e., respiratory bioaccessibility) pathway. Five fly ash samples were collected from power plants operating in the main lignite basins of Greece, while the ingestible (<250μm) and inhalable (<10μm) particle size fractions were utilized. The Unified Bioaccessibility Method (UBM) was utilized to assess the oral bioaccessibility, while the respiratory bioaccessible fractions were extracted using the Artificial Lysosomal Fluid (ALF). All studied FAs exhibited significantly higher contents in Ba, Cr, Ni, V and Zn. Cadmium was presented relative enriched in the finer size fraction (<10μm), while Ba, Co, Cr, Cu, Mn, Ni and V were depleted. The UBM-extractable concentrations fluctuated greatly among the studied FAs, while notably lower bioaccessible contents were recorded in the gastrointestinal phase. On the other hand, ALF-extractable concentrations were surprisingly higher than the corresponding UBM-extractable ones in the gastric phase. The oral bioaccessibility of the studied TrElems ranged from 12.5 to 100%, while respiratory bioaccessibility presented high values exceeding 45% on average. A significant effect of fly ash type on human bioaccessibility was revealed. Thus, high-Ca FAs exhibited significantly higher bioaccessibility of the studied TrElems via ingestion, while a relatively higher bioaccessibility via inhalation was observed for high-Si FAs. Regarding non-carcinogenic health risk via ingestion and inhalation, Cr and Co exhibited the highest HQ_{ing} and HQ_{inh} values, however there were significantly lower than safe level (HQ < 1). On the contrary, Cr was the dominant contributor to carcinogenic risk with CR values being well above threshold or even tolerable risk levels.

Keywords: human bioaccessibility; fly ash; trace element; health risk; cancer risk

1. INTRODUCTION

Coal-fired power plants are still predominant energy suppliers worldwide trying to fulfill the growing global energy demands (Izquierdo et al. 2013). Fly ash (FA) as the primary by-product of coal-based energy supply, present increased production rates which reach 100 Mt in the European Union every year (ECOBA, 2016). Fly ash suitability in a variety of engineering and industrial applications has been proposed through continuous research efforts, however under 50% of world's fly ash production is presently utilized (ECOBA, 2016). Thus, large volumes of fly ash are still stockpiled, dumped in landfills sites or lagooned around the world causing potential environmental hazards.

Fly ash is considered by many researchers (Izquierdo and Querol 2012; Jankowski et al. 2006; Ram et al. 2015; Singh et al. 2011) as extremely contaminating due to enhanced levels of potentially harmful or health-hazardous trace elements (TrElems). The content/distribution of TrElems in fly ash depends on several factors such as elements volatilization, the modes of occurrence of TrElems in coal, the coal source(s), the combustion conditions i.e. operation parameters of boilers, the particle size of the ash, and pollution control equipment (Gong et al. 2018; Mardon et al., 2008; Mokhtar et al., 2014; Vassilev and Vassileva, 2007). The determination of TrElems in FAs is of great importance in consideration of their hazards and thereby a substantial volume of bibliographic data report on the physicochemical properties of fly ash (Dai et al., 2011; Georgakopoulos et al. 1994; Gong et al. 2018; Kostakis et al. 2009; Kostova et al. 2016; Koukouzas et al. 2006; Medina et al. 2010; Meij and Winkel, 2009; Tian et al. 2013; Vassilev and Vassileva 1996; Vassilev 1994; Zhao et al. 2018). On the other hand, the total TrElems contents are not a reliable indicator of the potential environmental hazards providing overestimated predictions of the risk levels. Thus, leaching characteristics of FAs provide a more realistic estimation of TrElems availability and mobility and has attracted substantial attention (Belviso et al. 2015; Flues et al. 2013; Georgakopoulos et al. 2002a, b; Ibrahim 2015; Izquierdo et al. 2011; Jankowski et al. 2006; Nyale et al. 2014; Sandeep et al. 2016; Yilmaz 2015).

On the other hand, human exposure to fly ash particles is a health concern being linked with pulmonary and cardiovascular diseases (Borm 1997; Huang et al. 2006; Sichletidis et al. 2005; Smith et al. 2006). Although particulate matter emissions from coal-fired power plants are well controlled through the operation of electrostatic precipitators, fly ash emissions stay, in a regional-scale, an important proportion of the total particulate exposure for employees in power plants and residents of significantly affected adjacent areas. Moreover, in the absence of specific occupational limit for fly ash emissions, exposure to fly ash particles is not properly regulated. Thereby, accurate calculations of exposure to potentially harmful or health-hazardous TrElems via ingestion and inhalation of fly ash are required.

While the traditional approaches in exposure assessment is based on the determination of intake dose (the amount of a chemical ingested or inhaled by an individual) based on total elemental contents, the trend in currently shifting towards the adsorbed dose which result in more adverse health effects. Thus, information regarding bioaccessible fractions i.e. the fractions soluble in the conditions of the target compartment, i.e., the gastrointestinal tract for oral bioaccessibility (Oomen et al. 2002; Ruby et al. 1996) and respiratory tract for respiratory bioaccessibility (Kastury et al. 2017), after coming into contact with simulated biological fluids are crucial in health risk assessment.

Several in vitro methods have been developed which mimic the solubilization process during digestion and inhalation, however to the best of our knowledge the literature contains only limited data related to TrElems released from various fly ash materials in simulated gastric and lung fluids (Bourliva et al. 2017; Jin et al. 2013; Lokeshappa et al. 2014; Twining et al. 2005). For example, Twining et al. (2005) reported that bioaccessibility of metals was ranging between 7 % for Pb to 100 % for Cu in fly ash from coal-fired power plants, as well as fly ash from tire and coal co-combustion. Moreover, Lokeshappa et al. (2014) determined the oral bioaccessible fractions of As, Cr, Pb, Se and Zn in Ca-rich and Si-rich fly ashes using a physiologically based extraction test (PBET). As reported, more than 40 % of As was in bioaccessible form, while Se was very bioaccessible in Ca-rich fly ashes. Meanwhile, Pb was found to be insignificantly bioaccessible in the studied fly ashes. On the contrary, Jin et al. (2013) focused on As bioaccessibility in simulated gastric and intestinal solutions and reported that 33-61% of the total As was in bioaccessible form. Finally, our previous investigation (Bourliva et al. 2017) investigated oral bioaccessibilty of fly ash-derived magnetic components where TrElems exhibited enhanced bioaccessibility varying between 11.3 (for Cr) and 83.6 % (for As) in the fly ash magnetic fractions. Following on from our prior studies and taking into consideration that little data is available on the bioaccessibility of TrElems in the particle size-associated fractions of fly ash and on human exposure to TrElems through ingestion (i.e., oral bioaccessibility) and inhalation (i.e., respiratory bioaccessibility) pathways, the specific objectives of this study was to (i) investigate the TrElems distribution in ingestible and inhalable size fractions of fly ashes, (ii) assess the bioaccessibility of selected TrElems via ingestion and inhalation of fly ash, (iii) evaluate the influence of fly ash type on bioaccessibility, and (iv) calculate the potential health risk for on-site workers and people living around fly ash disposal sites.

2. MATERIALS AND METHODS

2.1. Materials and sample preparation

Sample selection was aiming to cover different FA chemical types. Thus, five fly ash (FA) samples produced in coal-fired power plants operating in the main lignite basins of Greece, were selected. In particular, three FA samples (AD, KD, AF) were from power plants installed in the Ptolemais-Amynteo lignite basin, while the rest samples (MG1, MG2) originated from power plants operating in the Megalopolis basin. All fly ash samples were composite samples of three different subsamples from each power plant. Sample pretreatment included homogenization, air-drying and grain/particle sizing by dry sieving. The particle sizes utilized were representative of the fraction that could be ingested (size fraction <250μm) or inhaled (<10μm). Fly ash samples were sealed in plastic zip locks bags, and stored at 4 °C until extraction and analysis. Details of the power plants and the main physicochemical characteristics of the bulk FAs are presented elsewhere (Bourliva et al. 2017). In brief, FAs originating from Kozani-Ptolemais power plants (samples AD and KD) exhibited a large proportion of CaO (>35%) and simultaneously relatively lower SiO_2 (around 20%) contents. Inversely, FAs originating from Megalopolis power plants showed low CaO contents (11.79-13.56%) in combination with enhanced SiO_2 (>35%) and Fe_2O_3 (almost 10%) contents. In contrast, Amynteo FA (AM) exhibited elevated CaO and SiO_2 contents. In any case, the studied FAs did not meet exactly the Class C and Class F classification requirements and therefore they are mentioned as *high-Ca* (AD, KD and AM) and *high-Si* (AM, MG1 and MG2) FAs, hereafter.

2.2. Analytical Methods

2.2.1. Mineralogical and morphological characterization

Mineralogical composition of bulk FAs was identified by X-ray powder diffraction (XRPD) using a water-cooled Rigaku Ultima+ diffractometer with CuKa radiation, a step size of 0.05° and a step time of 3 s, operating at 40 kV and 30 mA. The size and the morphology of the fly ash particles were examined by scanning electron microscopy (SEM) on a JEOL JSM-840A microscope operating at 20 kV connected with an X-ray energy dispersive spectrometer-EDX (INCA 300). SEM observations (randomly selected fields of view) were made on carbon-coated representative portions of each grain size fraction onto a double-sided aluminum tape mounted on a SEM stub.

2.2.2. Elemental analysis

Near-total elemental contents of the examined FA samples were determined, after an *aqua regia* digestion, using inductively coupled plasma mass spectrometry (ICP-MS) at ActLabs Analytical Laboratories Ltd., Canada (for ingested size fraction <250μm) and GeoBioTec Laboratory, University of Aveiro, Portugal (for inhaled size fraction <10μm). Quality assurance and quality control (QA/QC) included reagent blanks, analytical replicates, sample duplicates and analyses of in house reference materials. The results of the blanks were always below detection limits. The recovery rates were estimated within ±10 % of the certified value, and analytical precision (expressed as RSD%) was <10 % for all elements.

2.2.3. In vitro oral and respiratory bioaccessibility

The oral and respiratory bioaccessibility determinations of trace elements of environmental concern were carried out in GeoBioTec, University of Aveiro, Portugal. Oral bioaccessibility was determined in the < 250μm fraction, using the Unified Bioaccessibility Method (UBM) validated against an in vivo model (Denys et al. 2012). The UBM protocol, fully described by Wragg et al. (2011), is a two stage in vitro procedure which simulates the leaching of a solid matrix in both the Gastric (G phase) and the GastroIntestinal tract (GI phase) by using synthetic digestive solutions according to physiological transit times. Respiratory bioaccessibility determinations were performed in the inhalable size fraction (<10μm) using an artificial lysosomal fluid (ALF) solution (pH 4.5) which simulates the intracellular conditions in the lungs and extracts higher concentrations of metals than other commonly used simulated lung fluids (Pelfrêne et al. 2017). ALF solution is a complex medium with high concentration of organic complexing agents and low pH, which may have contributed to the dissolution of these elements. 0.05g (± 0.0001) of the inhaled size fraction <10μm was weighed into 85 mL polycarbonate centrifuge tubes and 50 mL of the simulated fluid (ratio - 1:1000) added. The samples were shaken at 37 ºC on an end-over-end shaker for 24 h and the particles were separated from the solution by centrifugation at 4500g for 15 min. All simulated biological fluids (SBFs), both digestive and lung, were freshly prepared prior extraction. Analyses of oral and pulmonary bioaccessible concentrations (mg kg^{-1}) in the extracts were performed by ICP-MS. Blanks, duplicate samples and bioaccessibility guidance materials have been obtained with each batch of bioaccessibility extractions for quality control. The blanks always returned outcomes below detection limits, while mean repeatability (expressed as RSD%) for the gastric phase was <5 % for all studied TrElems except of Pb with an uncertaintly of <10%. In the absence of certified reference materials for bioaccessibility determinations, materials providing bioaccessibility guidance values were used for quality control in combination with analytical duplicates and blanks. In particular, the UBM-specific certified reference soil BGS102 (an ironstone soil from Lincolnshire, UK), which provides UBM guidance values (Wragg et al. 2009, 2011; Hamilton et al. 2015) along with the standard reference material BCR-723 which respiratory bioaccessibility values are reported (Pelfrêne et al. 2017), were used in order to validate the uncertainty of the extraction protocols. For BGS102 recovery rates were ranging between 100.8% (Cd) and 118.6% (Cu) for gastric phase, while a range of 72.2% (Cd) – 117.5% (Ni) was found for gastrointestinal phase. For BCR723 the recovery rates were ranging between 70.9% (Cd) and 184.3% (Ni).

2.3. Statistical analysis

Relationships between bioaccessible TrElems and FA type were investigated via statistical analysis including principal component analysis (PCA) and Pearson’s correlation. PCA with a VARIMAX rotation was applied for interpretation of the principal components (PCs) and factors with eigenvalues greater than unity were retained in the analysis. Moreover, Pearson’s correlation was additionally adapted to evaluate relationships between the two characteristics (chemical composition and bioaccessibility) in order to support the interpretation of PCA results. Moreover, analysis of variance (ANOVA) was used to identify significant differences among different variables. All statistical determinations were conducted by means of SPSS v.25 software (IBM Corporation, Armonk, NY).

2.4. Health risk assessment

2.4.1. Exposure assessment

In order to evaluate the potential health risk, mainly for onsite workers, through the exposure to fly ash particles the models developed by the United States Environmental Protection Agency (U.S.E.P.A., 2002) were employed. Specifically, the non-carcinogenic and carcinogenic health risk via ingestion and inhalation was estimated for several TrElems of environmental concern i.e. Cd, Co, Cr, Cu, Ni, Pb and Zn. For the exposure assessment model, Chronic Daily Intake via incidental ingestion (CDI_{ing}, mg kg^{-1} d^{-1}) and direct inhalation (CDI_{inh}, mg kg^{-1} d^{-1}) of fly ash particles were calculated according to the following formulas:

$$CDI_{ing} = \frac{C_{(Gastric)} \times IngR \times ED \times EF}{BW \times AT} \times 10^{-6} \quad (1)$$

$$CDI_{inh} = \frac{C_{(Pulmonary)} \times InhR \times ET \times ED \times EF}{PEF \times BW \times AT \times 24} \quad (2)$$

where $C_{(Gastric)}$ and $C_{(Pulmonary)}$ are the bioaccessible concentrations (mg kg^{-1}) in simulated gastric (G phase) and lung fluids, respectively; $IngR$ is the ingestion rate (mg day^{-1}); $InhR$ is the inhalation rate (m^3 day^{-1}); ET is the exposure time (h day^{-1},); EF is the exposure frequency (days year $^{-1}$,); ED is the exposure duration (years); BW is the average body weight (kg); AT is the averaging time (days); PEF is the particle emission factor (m^3 kg^{-1}). Exposure data were adapted from US Environmental Protection Agency guidelines (U.S.E.P.A. 2014), however regional or individual differences may introduce some uncertainties in the procedure. The adopted parameter values are given in Table S1 (Supplementary Material).

2.4.2. Risk characterization

The Hazard Quotient (HQ) and the Cancer Risk (CR) were quantified for non-carcinogenic and carcinogenic health risks, respectively. Non-carcinigenic health risk via ingestion (HQ_{ing}) and inhalation (HQ_{inh}) were calculated by the following formulas (U.S.E.P.A., 2007)

$$HQ_{ing} = \frac{CDI_{ing}}{RfD_o}$$

$$HQ_{inh} = \frac{CDI_{inh}}{RfC_i}$$

where RfD_o is the oral and inhalation reference dose (mg kg^{-1} d^{-1}) and RfC_i is the inhalation reference concentrations (earlier terminology was "inhalation reference dose-RfD", converted to mg kg^{-1} d^{-1}), which correspond to the maximum allowable daily oral and inhalation dose that is not likely to cause any deleterious effects on human health. HQ < 1 indicates no adverse health effects, while HQ > 1 indicates likely adverse health effects (Man et al., 2010; Bourliva et al. 2017b).

On the other hand, the CR posed by the elements was obtained for both ingestion (CR_{ing}) and inhalation (CR_{inh}) using the following formulas:

$$CR_{ing} = CDI_{ing} \times SF_o$$

$$CR_{inh} = CDI_{inh} \times IUR$$

where SF_o is the oral slope factor (mg kg^{-1} day^{-1}) and IUR is the inhalation unit risk (μg m^{-3}) respectively. For the regulatory purposes, a CR value below 1×10^{-6} was considered acceptable (Li et al., 2017; Megido et al., 2017). The RfD_o, RfC_i, SF_o, and IUR values for each element were found in the screening level (RSL) tables provided by USEPA (USEPA, 2016).

3. RESULTS AND DISCUSSION

3.1. Mineralogy and microstructural characteristics of FAs

The mineralogical composition of the studied FAs is given in Table S2 (Supplementary Material), while the XRD patterns are illustrated in Fig.1. As shown, the sampled FAs included both amorphous and crystalline mineral phases. The broad humps observed in the baseline between 10 and 40^o in the XRD patterns of the FAs (Fig.1) verify the presence of an amorphous glassy phase. The amorphous matrix was not calculated however the broader humps shaped in the high-Si Megalopolis FAs indicated higher amorphous contents compared to the high-Ca FAs. A range of 20-50% for the amorphous phases in the Greek FAs was reported from previous researchers (Koukouzas et al. 2006). The crystalline fraction consists mainly of quartz and a variety of Ca-bearing mineral phases, while minor amounts of hematite, maghemite, K-feldspars, plagioclase and clays were detected. Notable differences were observed among the studied FAs. Specifically, the high-Ca FAs were dominated by lime (CaO, range of 24-32%), while the high-Si Megalopolis FAs (samples MG1 and MG2) were mainly consisted of quartz (33-34%) but with substantially lower contents of Ca-bearing species (i.e. lime and calcite) and the abundance of hematite and maghemite due to higher Fe contents in the FAs. Insignificant differences were found among the studied FAs regarding the presence of secondary Ca-bearing phases i.e. Ca-sulphates (i.e. anhydrite) and Ca-hydrated silicates such as gehlenite with both being detected in high amounts in all FAs.

Figure 1

SEM observations on both separated size fractions (<250μm and <10μm) of the studied FAs were performed and different types of fly ash particles were revealed to dominate the sampled FAs as shown in Figure 2. The same magnification (x500) was used in all images for comparison reasons. Regarding the ingestible fraction (<250μm) notable differences in the morphological properties among different FA samples were observed. Sample KD is composed mainly of irregular shaped Si-rich particles and angular Ca-rich agglomerates in a wide range of sizes (Fig.2). Moreover, traces of spherical shaped particles with high Si contents and low Ca, Mg, K and Fe contents were detected by the EDS (Fig.2). Furthermore, irregular shaped agglomerates with high Ca and S contents were detected being probably anhydrite determined by XRD. On the other hand, the structural characteristics of AM and MG1 samples were notably different from KD sample exhibiting multiplicity of spherical particles. Specifically, sample AM presented Si-rich and Ca-rich spherules, while amorphous aluminosilicate glass spheres and Ca-Al-silicate spherical shaped particles were also detected by EDS. Conversely, although the morphological characteristics of sample MG1 look alike with those of sample AM, more well structured, individual spherules and of higher particles sizes were observed (Fig.2). In particular, Si-rich spherules with lower contents of Ca, Al, Mg and Fe were detected. Moreover, a large amount of Fe-rich spherules were revealed verifying the high Fe-contents in the FAs originating from Megalopolis power plants. As regards the inhalable fraction (<10μm), sample KD was dominated by formless Ca-rich agglomerates (Fig.2). Unlike, sample AM presented spherical shaped agglomerates with elevated Ca contents and amorphous Ca-rich spheres. Moreover, needle anhydrite crystals were detected on the surface of Ca-rich agglomerates and spheres. On the contrary, sample MG1 was dominated by well structured, distinct spherules (Fig.2). Specifically, aluminosilicate glass spheres and ferrospheres were detected, while needle anhydrite crystals were also present.

Figure 2

3.2. Concentration of TrElems in FAs

The near total concentration of TrElems in the different particle sizes (<250μm and < 10μm) of the studied FAs are given in Table 1. As shown, TrElems presented mean concentration values following a decreasing order as: Ba > Ni > V > Cr > Zn > Cu > Mo > Pb > Co > Sn > Be > Sb > Cd in the <250μm size fraction, while in the finer fraction (<10μm) the order was modified as Zn > Ba > Ni > V > Cr > Cu > Pb > Co > Cd > Be. Generally, in both studied size fractions, all samples presented significantly higher concentrations in a number of trace elements, most notably Ba, Cr, Ni, V and Zn. Specifically, the aforementioned elements exhibited mean concentrations of 368.4 mg kg^{-1}, 182.6 mg kg^{-1}, 206.2 mg kg^{-1}, 192.2 mg kg^{-1}, 116.6 mg kg^{-1}, respectively in the <250μm size fraction and 232.1 mg kg^{-1}, 136.6 mg kg^{-1}, 173.1 mg kg^{-1}, 167.9

mg kg^{-1}, and 245.1 mg kg^{-1}, respectively in the finer fraction (<10μm). In contrast, the contents of Be, Cd and Sb were the lowest presenting mean concentrations <3 mg kg^{-1}.

Table 1

In order to evaluate the effect of particle size on elemental contents and to define the enrichment/depletion in the finer size fraction of the studied FAs, the Relative Enrichment Factor (REF) was used (Gong et al. 2018; Zhou et al. 2015). REF, defined as the content of trace elements in finer fractions (< 10 μm) compared to that in coarser fractions < 250 μm was calculated as follows:

$$REF = \frac{C_{i,<10\mu m}}{C_{i,<250\mu m}}$$

where, C_i is the concentration of element *i* in the fine (<10μm) and the coarse (<250μm) size fraction and the results are illustrated in Figure S1 (Supplementary Material). As shown, Cd exhibited the highest REFs with ranges of 0.92-4.40, respectively, indicating a relative enrichment in the finer fraction as previously reported by several researchers (Gong et al. 2018; Tang et al. 2013; Zhou et al. 2015). On the other hand, REFs for elements such as Ba, Co, Cr, Cu, Mn, Ni and V were lower or approximate to 1, suggesting a relative depletion in the finer fraction. Furthermore, noteworthy were the differences of REFs among studied FAs with low-Ca FAs (samples MG1 and MG2) samples presenting higher values, most notably for Cd, Pb and Zn. Specifically, REFs for Pb and Zn were approximate to or exceeding 1.5 for low-Ca FAs from Megalopolis power plants (samples MG1 and MG2), in contrast with the corresponding values of high-Ca FAs from Ptolemais-Amynteo power plants (AD, KD and AF) ranging between 0.42 and 0.69 for Pb and between 0.52 and 0.82 for Zn. As the content of Fe was significantly higher in the Megalopolis samples (range of 7.2-7.8%) in the finer fraction (<10μm), it could be supposed that the statistically significant REFs values in Megalopolis FAs are probably relative to Fe in the fine particles. Besides, trace elements are easily bonded to Fe oxides in fly ash (Zhou et al. 2015).

By comparing the obtained values with the reported average values for European fly ashes (Moreno et al. 2005) and also with values reported for different FAs worldwide (Silva et al. 2012; Vassilev et al. 2003; Yang et al. 2014), the studied FA samples were presented relatively enriched in Cr and Ni, while noteworthy enrichment in V and Zn was observed in Megalopolis FAs (Table 1). On the other hand, Mo in Megalopolis FAs was observed with levels more than 10 times greater than the respective average values recorded for the European FAs. In particular, elevated Mo concentrations of 201 mg kg^{-1} and 123 mg kg^{-1} were recorded for samples MG1 and MG2, respectively, in contrast to a range of 5-22 mg kg^{-1} reported by Moreno et al. (2005). On the contrary, the studied FAs were depleted in a number of elements of environmental concern such as Pb and Sb. In particular, Pb exhibited a diminished range of concentrations of 22.1-51.4 mg kg^{-1} compared to the corresponding ones reported for European FAs (80 mg kg^{-1}, Moreno et al. 2005) and Chinese FAs (69.7 mg kg^{-1}, Yang et al. 2014).

3.3. Oral and respiratory bioaccesibility

The bioaccessible concentrations (mg kg^{-1}) of the studied TrElems in the studied FAs are presented in Table S3 (Supplementary material), while bioaccessible fractions (BAF %) are illustrated in Figure 3.

Figure 3

3.3.1. Bioaccessibility in gastrointestinal tract

The average UBM-extractable contents of the studied TrElems in the gastric phase (G-phase) exhibited a decreasing order as follows: Cr (61.07±38.86 mg kg^{-1}) > Ni (51±24.64) > Zn (39.51±11.62) > Cu (26.04±6.34) > Pb (9.91±2.12) > Co (6.47±1.51) > Cd. (0.61±0.26). Notably lower bioaccessible contents were recorded in the gastrointestinal phase (GI-phase) probably due to lower solubility under higher pH conditions (pH=6.3) with those of Cr, Cu, Pb and Zn being statistically significant ($p < 0.01$, ANOVA). Besides, the pH dependency of bioaccessibility has been previously reported (Reis et al. 2013). Specifically, the mean bioaccessible contents in the GI-phase were 0.38±0.19 for Cd, 5.47±1.45 for Co, 6.33±7.80 for Cr, 12.26±7.35 for Cu, 51.17±27.27 for Ni, 0.21±0.14 for Pb and 8.47±3.04 for Zn showing a decreasing order of Ni > Zn > Cu > Cr > Co > Cd > Pb. The observed pattern of constantly lower bioaccessibility contents (mg kg^{-1}) in the GI phase has been previously reported in a number of studies (Zia et al., 2011; Reis et al., 2013; Patinha et al. 2015; Bourliva et al. 2017; Pelfrêne and Douay 2018).

When expressing UBM-extractable contents as a percentage with respect to the total elemental contents in FAs (Fig.3), a particularly different trend is presented indicating that the highest determined bioaccessible fractions do not match to the highest bioaccessible contents. The bioaccessible fractions (BAF%) of the studied TrElems ranged from 12.5 to 100% indicating that some elements are highly bioaccessible, while others not. Specifically, the mean BAF% exhibited a decreasing order of Cd > Zn > Cu > Cr > Pb > Co > Ni in the gastric phase, while it was formulated as follows: Cd > Ni > Co > Cu > Zn > Cr > Pb in the GI-phase. In any case, the BAF% of all studied elements, fluctuated greatly among the studied samples. Specifically, BAF% exhibited ranges of 48.3-100% for Cd, 14.9-36.3% for Co, 13.7-80.3% for Cr, 21.6-46.1% for Cu, 12.5-37.5% for Ni, 25.4-41.5% for Pb and 24.7-50% for Zn in the G phase and 31.5-55.7% for Cd, 11.7-32% for Co, 0.8-8% for Cr, 3.6-30.2 % for Cu, 10.3-39.4% for Ni, 0.3-0.9% for Pb, and 5.8-11.3% for Zn in the GI-phase.

Moreover, correlations among bioaccessible concentrations (mg kg^{-1}) / fractions (%) and total concentrations were searched in the studied FA samples. There were no important correlations between total and bioaccessible concentrations (G phase) of the studied TrElems (data not shown) except for Zn (R^2= 0.975, p < 0.01), suggesting that total concentrations of TrElems could not be a good indicator of oral bioaccessibility. On the contrary, significant correlations appeared in GI phase for Cr (R^2= 0.908, p <0.05), Pb (R^2= 0.942, p <0.05) and Zn (R^2= 0.961, p <0.01). Similarly, no significant correlations were recorded (data not shown) among total contents and bioaccessible fractions (%), except for Co which exhibited a negative strong correlation in both G (R^2= -0.892, p < 0.05) and GI (R^2= -0.951, p < 0.05) phase.

3.3.2. Bioaccessibility in respiratory tract

In the present study, respiratory bioaccessibility of TrElems was measured in the sampled FAs using ALF as a simulated lung fluid. The respiratory bioaccessible concentrations exhibited ranges of 0.31-2.20 mg kg^{-1} for Cd, 42.67-83.36 mg kg^{-1} for Cr, 4.40-9.13 mg kg^{-1} for Co, 20-60.86 mg kg^{-1} for Cu, 41.31-85.65 mg kg^{-1} for Ni, 8.50-34.06 mg kg^{-1} for Pb and 29.37-127.09 mg kg^{-1} for Zn. The average ALF-extractable contents presented a descending order of Zn (86.46 mg kg^{-1}) > Ni (71.90 mg kg^{-1}) > Cr (63.64 mg kg^{-1}) > Cu (38.43 mg kg^{-1}) > Pb (19.47 mg kg^{-1}) > Co (6.86 mg kg^{-1}) > Cd (1.23 mg kg^{-1}). The ALF-extractable concentrations were surprisingly higher than the corresponding UBM-extractable ones in the gastric phase for most TrElems (except Cr and Co) indicating the aggressiveness of the selected simulated lung fluid (Wiseman and Zereini 2014; Pelfrêne et al. 2017; Potgieter-Vermaak et al. 2012).

The values of the respiratory bioaccessible fractions (%) of TrElems were also expressed as a percentage of the total contents (Fig.3). All elements exhibited a resembling trend where their solubility in the ALF lung fluid was significantly high recording values >45% on average. In particular, the average respiratory bioaccesibilty presented a decreasing order as follows: Cu (76.8±21.9 %) > Pb (68.5±23.8 %) > Cd (63.5±16.6%) > Zn (60.4±29.6%) > Cr (56.9±24.6%) > Co (46.8±15.9%) > Ni (45.9±14.5%). Moreover, the ratio of bioaccessible to total contents ranged from 44.4 to 79.8% for Cd; 28.9 to 77.3% for Cr; 27.9 to 62.4 for Co; 47.5 to 96.1% for Cu; 28.6 to 61.3% for Ni; 42.2 to 91.5% for Pb and; 28.1 to 90.8% for Zn exhibiting a substantial variability among FA samples suggesting that the solubility in the applied lung fluid (ALF) was influenced by the heterogenic composition of the fly ash particles and also on the chemical status of the elements.

Finally, in contrast to oral bioaccessibility, a significant (p<0.01) correlation among total and ALF-extractable concentrations (data not shown) was noticed for all TrElems (except Cr and Ni) indicating the strong control of the total contents on the extractable contents in the lung. On the other hand, no correlation was observed among total contents and respiratory bioaccessible fractions.

3.3.3. Effect of fly ash type on bioaccessibility

As aforementioned, the bioaccessibility fluctuated greatly among the studied FAs. In order to elucidate possible relations between bioaccessibility and fly ash type, principal component analysis (PCA) was performed and the results are given in Figure 4 and Table S4 (Supplementary Material). The datasheet used to PCA included the oral (G phase) and respiratory BAF% values of all TrElems under study in all sampled FAs along with the major oxides i.e. SiO_2, Al_2O_3, CaO, Fe_2O_3, which proportions and sums could classify FAs into different chemical types.

Figure 4

The PCA results revealed two principal components (PCs) explaining almost 95% of the total variance (Fig.4 and Table S4, Supplementary Material). The results revealed that the oral bioaccessibility cluster composed of Cr, Co, Cu, Ni, Pb, Zn and to a lesser extent Cd UBM-extractable fractions (BAF%, G phase) were associated with total Ca contents. In that case, as illustrated in Figure 5, the gastric extractable fractions of all TrElems in the high-Ca FAs i.e. samples presenting CaO contents above 30% (samples AD, KD and AM), were notably higher (with those of Cu, Ni, Pb and Zn being statistically significant, $p<0.01$) compared to the corresponding ones in FAs with low CaO contents (below 15%, samples MG1 and MG2). Thus, the studied TrElems were presented relatively more bioaccessible in the high-Ca FAs, while the low-Ca FAs presented notably lower BAF values (detailed data in Table S3, Supplementary material). Specifically, Cr was highly to moderately bioaccessible (30.2-80.3) in the high-Ca FAs (samples AD, KD, AM), while it was almost not available to humans (13.7-15.5%) in the FAs with low Ca contents from Megalopolis (samples MG1 and MG2). Likewise, Ni was moderately bioaccessible (32.5-37.5 %) in the high-Ca FAs, while it was almost not available (12.5%) in the Megalopolis FAs. Exception was the case of Cd, where besides the notable differences among the different studied FAs types, it was presented highly bioaccessible (48-100%) in all FAs. Lokeshappa et al. (2014) also reported significant differences in oral bioaccessibility among fly ash types. In particular, Pb was reported as highly bioaccessible in the Ca-rich FAs, while in the case of Si-rich FAs it was almost unavailable (5-6% in bioaccessible form) in the humans. On the contrary, for the Ca-rich ashes, the bioaccessible fractions of Cr were much lower (10-20 %) than the Si-rich ashes (43-59 %), but the different analytical protocol should be noted.

On the other hand, respiratory bioaccessibility cluster i.e. all TrElems ALF-extractable fractions (%), was associated with total Si contents and to a lesser extent with Fe and Al. Thus, high-Si FAs (SiO_2>30%, samples AM, MG1 and MG2) presented significantly ($p < 0.01$, ANOVA) higher ALF-extractable fractions in all studied TrElems compared to low-Si FAs (samples AD and KD) (Fig.5). Therefore, the average ALF-extractable fractions of the studied TrElems exhibited a decreasing order of Cu (92.2%) > Pb (85.2%) > Zn (80.4%) > Cd (75.4%) > Cr (74.8) > Co (58.1%) >Ni (55.8%) in the high-Si FAs and modulated as Cu (53.7%) > Cd (45.7%) > Pb (43.5%) > Ni (31.1%) > Zn (30.3%) > Cr (30.1%) > Co (29.7%) in the low-Si FAs. In that case, for example Cu, is presented highly bioaccessible (85.4-96.1%) in the high-

Si FAs, while is moderately bioaccessible (47.5-59.9%) in the low-Si FAs. On the contrary, Cr is highly bioaccessible (72.3-77.3%) in the high-Si FAs compared to the substantially lower BAF values (28.9-31.2%) in the low-Si FAs.

The PCA results were further supported with Pearson's correlation coefficients as shown in Table 2. The total Ca contents were significantly correlated with the UBM-extractable fractions of Co (r=0.901, $p<0.05$), Cu (r=0.933, $p<0.05$), Ni (r=0.901, $p<0.05$), Pb (r=0.988, $p<0.01$) and Zn (r=0.887, $p<0.05$). On the contrary, significant correlations were revealed between ALF-extractable fractions of Cd (r=0.896, $p<0.05$), Cr (r=0.942, $p<0.05$), Co (r=0.905, $p<0.05$), and Cu (r=0.889, $p<0.05$) and total Si contents. In addition, significant negative correlations were observed between the UBM-extractable fractions of the studied TrElems and the total Al and Fe contents.

Table 2

The obtained results indicated that the mineralogy of FAs along with differences in simulated biological fluids formulation and pH values could control the bioaccessibility of the studied TrElems. Specifically, the acidic solutions used in the gastric phase of the UBM protocol would partially to completely dissolve the soluble and reactive carbonates resulting in higher bioaccessibilities of the studied TrElems in the high-Ca FAs. On the contrary, the more resistant, acid stable and relatively insoluble aluminosilicates presented in the low-Ca FAs resulted in a large proportion of the studied TrElems being in a non bioaccessible form. Besides, as observed by several authors (Desboeufs et al. 2005; Schaider et al. 2007; Kastury et al. 2017; Keshavarzifard et al. 2019), an aluminosilicate matrix could lead to low metal dissolution, compared to a carbonaceous matrix which result in high dissolution. On the other hand, the complexity of ALF solution (among other simulate lung fluids) have probably resulted in higher bioaccessibilities. Moreover, in acidic conditions, the presence of chlorides in its formula could lead in the formation of metal-chloride complexes which are readily solubilized (Colombo et al. 2008). Furthermore, the complex formula of ALF containing components that may act as complexing agents could promote the metal release/dissolution. Specifically, the formation of weak organic complexes (in the presence of ligands i.e. citrate) with silanol groups at the surface could be the most plausible explanation for enhanced respiratory bioaccessibilities in high-Si FAs (Herting et al. 2014). On the contrary, the elevated contents of carbonates in the low-Si FAs could favor the formation of insoluble complexes.

3.4. Health risk assessment of TrElems in fly ashes

The non-carcinogenic and carcinogenic risk of multiple TrElems of environmental concern in the studied FAs was evaluated for onsite workers and the obtained HQs and CRs values via ingestion and inhalation are presented in Table 3. With reference to ingestion exposure pathway, Cr and Co presented the highest HQ_{ing} values among the studied TrElems, ranging from 8.3×10^{-3} to 4.0×10^{-2} (mean 2.0×10^{-2}) and from 1.7×10^{-2} to 2.9×10^{-2} (mean 2.1×10^{-2}), respectively. On the contrary, Zn exhibited the lowest HQ_{ing} values varying between 9.7×10^{-5} and 1.9×10^{-4} (mean 1.3×10^{-4}). The contribution of elements to the cumulative health risk decreased in the following order Cr > Co > Ni > Pb > Cd > Cu > Zn for sample AD, Cr > Co > Ni > Pb > Cd > Cu > Zn for sample KD, Co > Cr > Pb > Ni > Cu > Cd > Zn for sample AM, Co > Cr > Pb > Ni > Cd > Cu > Zn for sample MG1 and Co > Cr > Pb > Ni > Cu > Cu > Cd >Zn for sample MG2. The cumulative health risk via ingestion indicated no significant differences among the studied FAs (range of 2.98×10^{-2} (MG2)-6.73×10^{-2} (AD), however slight lower values were observed for Megalopolis FAs.

Table 3

Regarding inhalation route, the obtained HQ_{inh} values of all evaluated TrElems were significantly lower ($p < 0.01$, one-way ANOVA) than the corresponding ones via ingestion (HQ_{ing}). Among the evaluated TrElems, Co, Cr, and Ni exhibited notably higher HQ_{inh} values exhibiting ranges of 2.8×10^{-4}-4.9×10^{-4} (mean 3.5×10^{-4}), 1.4×10^{-4}-2.7×10^{-4} (mean 2.1×10^{-4}), and 1.5×10^{-4}-3.1×10^{-4} (mean 2.6×10^{-4}), respectively. The observed contribution of the evaluated TrElems to the cumulative health risk presented a descending order of Co > Ni > Cr > Pb > Cd > Cu > Zn for all FA samples except sample AM for which the order is modulated as follows: Co > Ni > Cr > Pb > Cu > Cd > Zn. The cumulative health risk via inhalation was significantly lower than the corresponding one via ingestion presenting a range of 7.71×10^{-4} (MG1)-9.15×10^{-4} (AD) among the studied FA samples. In any case, in both exposure pathways the obtained HQs of all TEs were significantly lower than safe level ($HQ < 1$) indicating a negative non-carcinogenic health effect by the studied TrElems via both ingestion and inhalation.

On the other hand, the carcinogenic risk for Cr via ingestion and Cd, Co, Cr and Ni via inhalation was estimated (Tab.3) and all obtained CRs were greater than the threshold value (10^{-6}). However, CR values for Cd, with a range of 2.26×10^{-6}-1.60×10^{-5}, were of an acceptable or tolerable risk (10^{-6} – 10^{-4}), while CRs for Ni were slight above the tolerable value (range 1.37×10^{-4}-2.85×10^{-4}). On the contrary, Cr was the dominant contributor to cumulative carcinogenic risk with CR values ranging between 4.17×10^{-3} and 1.98×10^{-2} via ingestion and between 4.13×10^{-2} and 8.06×10^{-2} via inhalation, well above threshold or even tolerable risk values suggesting a cancinogenic risk for onsite workers.

4. CONCLUSIONS

Fly ash is the main by-product of coal-combustion with large volumes being stockpiled, disposed of or lagooned. The enhanced contents of potentially hazardous trace elements in fly ash still attract the global scientific concern and arise significant environmental and human health problems. The aim of the present study was to evaluate human exposure to TrElems in fly ash through ingestion (i.e., oral bioaccessibility) and inhalation (i.e., respiratory bioaccessibility) pathway. Different fly ash chemical types were collected from power plants operating in the main lignite basins of Greece, while the ingestible (<250μm) and inhalable (<10μm) particle size fractions were utilized for further analysis. The Unified Bioaccessibility Method (UBM) was utilized to assess the oral bioaccessibility, while the respiratory bioaccessible fractions were extracted using the Artificial Lysosomal Fluid (ALF). The average TrElems contents exhibited a decreasing order of Ba > Ni > V . Cr > Zn > Cu > Mo > Pb > Co > Sn > Be > Sb > Cd in the <250μm size fraction and Zn > Ba > Ni > V > Cr > Cu > Pb > Co > Cd > Be in the finer fraction (<10μm). A relative enrichment of Cd was observed in the inhalable size fraction (<10μm) compared to the ingestible one, while Ba, Co, Cr, Cu, Mn, Ni and V were relatively depleted. No significant correlations were detected among total and UBM-extractable contents with the later exhibiting a decreasing order of Cr > Ni > Zn > Cu > Pb > Co > Cd in the G-phase and Ni > Zn > Cu > Cr > Co > Cd > Pb in the GI-phase. On the contrary, a strong control of total TrElems contents on the ALF-extractable concentrations was revealed with the respiratory bioaccessible concentrations being notably higher than the oral bioaccessible concentrations. Regarding the bioaccessible fractions, oral bioaccessibility of the studied TrElems ranged from 12.5 to 100%, while respiratory bioaccessibility presented high values exceeding 45% on average. In detail, average values of oral bioaccessibility decreased as: Cd > Zn > Cu > Cr > Pb > Co > Ni in the G-phase and as: Cd > Ni > Co > Cu > Zn > Cr > Pb in the GI-phase, while it was formulated as Cu > Pb > Cd > Zn > Cr > Co > Ni for respiratory bioaccessibility. A significant effect of fly ash type on human bioaccessibility was revealed. Specifically, UBM-extractable fractions exhibited a significant correlation with total Ca contents, while ALF extractable factions were highly associated with total Si contents. Thus, high-Ca FAs exhibited significantly higher bioaccessibility of the studied TrElems via ingestion, while a relatively higher bioaccessibility via inhalation was observed for high-Si FAs. Regarding non-carcinogenic health risk via ingestion and inhalation, Cr and Co exhibited the highest HQ_{ing} and HQ_{inh} values, however there were significantly lower than safe level (HQ < 1). On the contrary, Cr was the primary contributor to carcinogenic risk with CR values being up to 800 times above the threshold risk levels (1×10^{-4}). Despite the fact that in our study the associated cancer risk was evaluated based on the bioaccessible concentrations (in contrast to traditional approaches) reducing the probabilities of underestimating the health risk, epidemiological and toxicological studies remain necessary in order to draw clear and accurate conclusions.

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Figure Captions

Figure 1. XRD patterns of the studied fly ash (bulk fraction). The most intense reflections of respective minerals are labeled. A: Anhydrite, L: Lime, C: Calcite, Q:

Quartz, G: Gehlenite, Mg: Maghemite, Ht: hematite, Kf: K-Feldspars, Pl: Plagioclase, Cl: Clays

Figure 2. SEM secondary electron images of ingestible (up) and inhalable (down) of the Greek fly ash samples.

Figure 3. Oral (gastric-G and gastrointestinal-GI phases) and respiratory bioaccessibility (BAF %) of selected trace elements (TrElems) in the Greek fly ashes.

Figure 4. Loading plot of PCA analysis carried out for oral (G phase) and respiratory (L) BAF% values of all TrElems along with major oxides of the Greek fly ashes.

Figure 5. Effect of CaO and SiO_2 content in Greek FAs on the obtained oral and respiratory bioaccessibility of the studied TrElems.

Highlights

1. A relative enrichment of Cd on finer fraction was observed.
2. Oral bioaccessibility ranged from 12.5 to 100%.
3. Respiratory bioaccessibility was >45% on average.
4. An effect of fly ash chemical type on human bioaccessibility was revealed.
5. Cr was the dominant contributor to cumulative carcinogenic risk.