Assessing Oxidative Stress Resulting from Environmental Exposure to Metals (Oids) in a Middle Eastern Population

Ata Rafiee[1], Juana Maria Delgado-Saborit[2, 3, 4,5], Noel J. Aquilina[6], Hoda Amiri[7] and Mohammad Hoseini[8*]

[1] Department of Medicine, University of Alberta, Edmonton, AB, Canada

[2] Universitat Jaume I, Perinatal Epidemiology, Environmental Health and Clinical Research, School of Medicine, Castellon, Spain

[3] ISGlobal Barcelona Institute for Global Health, Barcelona Biomedical Research Park, Barcelona, Spain

[4] Population Health and Environmental Sciences, Analytical Environmental and Forensic Sciences, King's College London, United Kingdom

[5] Division of Environmental Health & Risk Management, School of Geography, Earth & Environmental Sciences, University of Birmingham, Birmingham, United Kingdom

[6] Department of Chemistry, University of Malta, Msida, MSD 2080, Malta

[7] Environmental Health Engineering Research Center, Kerman University of Medical Sciences, Kerman, Iran

[8] Research Center for Health Sciences, Institute of Health, Department of Environmental Health, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

[*]**Corresponding** author: **Mohammad Hoseini**

Assistant Professor

Department of Environmental Health Engineering,

School of Public Health,

Shiraz University of Medical Sciences, Shiraz, Iran

Address: Razi blvd, Kuye Zahra Street, School of Public Health, Shiraz University of Medical Sciences, Shiraz, Iran.

Tell: +98071-37251001 E-mail: mohhoseini@sums.ac.ir

Abstract

Concentrations of metals and metalloids derived mainly from anthropogenic activities have increased considerably in the environment. Metals might be associated with increase reactive oxygen species (ROS) damage, potentially related to several health outcomes. This study has recruited 200 adult participants, including 110 males and 90 females in Shiraz (Iran), to investigate the relationship between chronic exposure to metals and ROS damage by analyzing malondialdehyde (MDA) and 8-Oxo-2'-deoxyguanosine (8-OHdG) concentrations, and has evaluated the associations between chronic metal exposure and ROS damage using regression analysis. Our findings showed that participants are chronically exposed to elevated As, Ni, Hg, and Pb levels. The mean urinary concentrations of 8-OHdG and MDA were 3.8±2.35 and 214±134 µg/g creatinine, respectively. This study shows that most heavy metals are correlated with urinary ROS biomarkers (R ranges 0.19 to 0.64). In addition, regression analysis accounting for other confounding factors such as sex, age, smoking status, and teeth filling with amalgam highlights that Al, Cu, Si and Sn are associated with 8-OHdG concentrations, whilst an association between Cr and MDA and 8-OHdG is suggested. Smoking cigarettes and water-pipe is considered a significant contributory factor for both ROS biomarkers (MDA and 8-OHdG).

Keywords: Biomonitoring, Heavy metals, MDA, Oxidative damage, 8-OHdG.

Introduction

Anthropogenic activities such as manufacturing of metal products, industrial activities, combustion of fossil fuel, fireworks (Lin, 2016), and generation of electronic waste (Venugopal et al., 2016) have dramatically increased during recent decades on a global scale (Zhu et al., 2018). As a consequence, the levels of metals and metalloids elements derived mainly from these activities have increased considerably in the environment (Liu et al., 2015; Zhu et al., 2018). Two sources of comparable contribution to metals and metalloids-laden fine and coarse particulate matter, especially in cities with trafficked urban areas, are motor vehicle tailpipe exhaust and non-exhaust emissions (Al Hanai et al., 2019; Dahl et al., 2006; Gietl et al., 2010; Harrison et al., 2001; Lenschow et al., 2001; Querol et al., 2004). The latter are associated with brake wear, road wear, tyre wear and road dust resuspension (Amato et al., 2014; Amato et al., 2016; Boogaard et al., 2011; Denier van der Gon et al., 2013; Keuken et al., 2013; Querol et al., 2007; Thorpe and Harrison, 2008). Whilst the tailpipe exhaust typically contains Pd, Rh and Pt, the non-exhaust emitted particles contain a complex mixture of metals and metalloids and some of them are used as markers. Zn is typically associated with tyre wear, Fe, Sb, Sn, Cu, Ba are dominant in brake wear, while Fe, Ti, Ca, K, Cs, Al, Rb, Cs are commonly found in re-suspended dust (Kwak et al., 2013; Lawrence et al., 2013; Varrica et al., 2013). Some essential elements play an important role in human health at specific concentrations, including transport of proteins, improvement of enzymes' activity and structure, and other biological system functions (Amaral et al., 2008). Selenium (Se), for example, serves as a fundamental trace element in children’s growth and in protecting cells against oxidative damage (Fábelová et al., 2018; Ventura et al., 2005). Zinc (Zn) is an intracellular element engaged in DNA repair and genetic stability, and copper (Cu) is involved in the immune system and the development of collagen's neuronal connections and synthesis (Amaral et al., 2008). On the other hand, some metals and metalloids are toxic and accumulate in internal organs (Gil and Hernandez Jerez, 2009; Molina-Villalba et al., 2015). Cadmium (Cd) is a known carcinogenic (Group I) heavy metal, and chronic exposure is toxic to the kidneys (Satarug and Moore, 2004). Arsenic (As) and lead (Pb) are ranked at the top of the list of the Agency for Toxic Substances and

Disease Registry (ATSDR, 2013). Studies revealed that heavy metals are neurotoxic and associated with impairment of the central nervous system (Chen et al., 2016). Moreover, some studies reported the association between metals exposure with detriments in attention and executive function (Bowler et al., 2007; Rafiee et al., 2020; Soetrisno and Delgado-Saborit, 2020).

The mechanisms mediating metals' toxic effects are multifactorial, but oxidative stress is suggested as one important pathway (Almeida Lopes et al., 2017). Metals generate reactive oxygen species (ROS) such as hydroxyl radicals (OH^-) and non-radical species such as hydrogen peroxide (H_2O_2) (Halliwell and Cross, 1994) through Fenton reactions (Domingo-Relloso et al., 2019a). Metal-induced ROS might lead to an imbalance in the antioxidant system, subsequently causing lipid peroxidation and cellular injury (Farmand et al., 2005). Early biological effects of ROS might be detected by increased levels of malondialdehyde (MDA), a marker of lipid peroxidation, (mainly associated with cellular membrane damage) and 8-Hydroxydeoxyguanosine (8-OHdG), also known as 8-Oxo-2'-deoxyguanosine (8-oxo-dG), a marker of DNA damage (Domingo-Relloso et al., 2019a; Teichert et al., 2009).

Human biomonitoring (HBM) has been widely used as a reliable approach in human exposure assessment in the general population and occupational settings (Hoseini et al., 2018; Rafiee et al., 2018a; Rafiee et al., 2018b). Several biological matrices have been used in biomonitoring studies such as blood (Richmond-Bryant et al., 2014), urine (Hoseini et al., 2018; Rafiee et al., 2018a; Rafiee et al., 2018b), serum (Alimonti et al., 2007; Skalny et al., 2017), plasma (Vural et al., 2010), nails (Carneiro et al., 2011; Coelho et al., 2013), and hair (Fábelová et al., 2018; Luo et al., 2014; Rafiee et al., 2020; Soetrisno and Delgado-Saborit, 2020; Varrica et al., 2014b; Zhu et al., 2018).

Because of some advantages over urine and blood, such as the high capacity to accumulate metals and metalloids over long-time periods, using hair allows researchers to monitor past and ongoing exposure to these pollutants (Amaral et al., 2008; Luo et al., 2014; Pan and Li, 2015). Moreover, hair is considered a non-invasive matrix and its transport, preparation, and analysis are more convenient than blood or urine (Drobyshev et al., 2017; Sazakli and Leotsinidis, 2017a; Zhu et al., 2018). Numerous biomonitoring studies have been investigating the exposure of the general population to metals and metalloids using hair, and in different countries such as Pakistan (Eqani et al., 2018), Russia (Skalny et al., 2015a; Skalny et al., 2015b), China (Li et al., 2014), Nigeria (Nnorom et al., 2005), Iran (Rafiee et al., 2020) and Egypt (Saad and Hassanien, 2001). Some studies have focused on children and adolescents as sensitive population sub-groups (Drobyshev et al., 2017; Fábelová et al., 2018; Molina-Villalba et al., 2015; Peña-Fernández et al., 2014; Soetrisno and Delgado-Saborit, 2020; Varrica et al., 2014b). In addition, some studies have investigated the impact of environmental metals and metalloids exposure, such as living in mining areas (Barbieri et al., 2010; Pan and Li, 2015), industrialized areas (Nnorom et al., 2005) and near e-waste facilities (Soetrisno and Delgado-Saborit, 2020). Some other studies have investigated the effect of diet and lifestyle as potential factors affecting the variability of metals and metalloids concentrations in the human scalp hair (Shao et al., 2017; Zhu et al., 2018). In addition, some studies have investigated the association between short-term exposures and oxidative stress (Almeida Lopes et al., 2017; Bortey-Sam et al., 2018; Domingo-Relloso et al., 2019a), but studies on the effect of chronic exposure to metals and metalloids and oxidative stress biomarkers in the general population are scarce, and even more in the general population in low and middle-income countries. With this in mind, the present study aimed to:

1. characterize exposure to metals and metalloids in scalp hair samples from residents of Shiraz, Iran;
2. investigate factors that could have a potential impact on metals and metalloids levels in hair, including sex, age as well as environmental and lifestyle factors; and
3. assess the relationship between the levels of oxidative stress biomarkers, including malondialdehyde (MDA) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) in the urine with the corresponding metals and metalloids levels in the hair of Shiraz city residents.

Materials and methods

Study area description

The present cross-sectional study was implemented in Shiraz, the capital of Fars province and the sixth most populous city in Iran, with approximately 1.8 million inhabitants living in an area of about 240 km^2. The city is enclosed by the mountains on its North, East, and Southeast sides, restricting air circulation. In addition, there is an industrial park on the Southside of the city, which along with traffic congestion, has contributed to elevating air pollution in the city (Mirzaei et al., 2018; Shahsavani et al., 2017).

Subjects recruitment

The cohort in this study comprised 110 males and 90 females, aged between 15 to 70, who were randomly selected to participate if they satisfied a set of criteria. Detailed information on the study recruitment are provided in (Rafiee et al., 2020). Briefly, the inclusion criteria required the subjects to be healthy with no chronic conditions, not occupationally exposed to metals and metalloids and must have been living in Shiraz’s urban area for the last decade. In addition, given the study's nature, the subjects were required to have hair longer than 5 mm. Pregnant and breastfeeding women were excluded.

Following approved written consent to take part in this study, scalp hair samples were collected from participants to be analyzed for metals and metalloids concentrations and urine samples to be analyzed for markers of oxidative stress, including MDA and 8-OHdG. The Ethical Committee approved the present study at the Shiraz University of Medical Sciences (IR.SUMS.REC.1398.320).

Hair sampling and metals and metalloids identification

Hair sampling was performed in June 2019 during the subjects’ visits to the hairdresser salons for haircuts. Around 2.5 g of hair from 1-1.5 cm of each subject’s scalp in both groups was collected using stainless-steel scissors (Rafiee et al., 2020). Hair samples were then stored in labelled polyethylene zip bags and sent to the laboratory for further analysis. Hair samples cleaning was performed based on the procedures developed by the International Atomic Energy Agency (IAEA). The method included dispersing hair samples in acetone for 10 minutes, followed by washing with ultra-pure water (Milli-pore, MA, France) and then washed with acetone. Detailed information on sample preparation and the digestion method was described in our previous work (Rafiee et al., 2020). Briefly, nitric acid 65% and perchloric acid 70% (Merck, Darmstadt Germany) were used for digestion. 100 mg sample was microwave digested using a nitric acid-perchloric acid V/V 3:1 solution and then diluted with 18 MΩ cm demineralized water. The microwave digestion program is given in Table S1 (Supplementary information).

Quantification of 17 selected metals and metalloids, including Al, As, Cd, Cr, Hg, Fe, Mn, Cu, Pb, Zn, B, Ni, Be, Sb, Ba, Li, and Sn was performed using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) with EOP flared end torch 2.5 mm (SPECTRO Analytical Instruments Inc. Germany). Details of the ICP-OES operating setup, as well as quality assurance and quality control protocols, are presented in Table S2 and S3, respectively.

Determination of oxidative stress markers in urine samples

The levels of MDA and 8-OHdG were determined in urine samples collected from the participants to investigate the relationship between environmental metals and metalloids exposure and markers of oxidative stress. Spot urine samples were collected in the middle of the day from subjects who donated hair samples. Urine samples were collected in polypropylene bottles, wrapped in foil, and stored at 4 °C in a portable fridge and transferred immediately to the laboratory for further analysis. At the laboratory, urine samples were first centrifuged at 1500 rpm for 10 minutes, and then supernatants were collected. Both markers were corrected for creatinine, determined by the Jaffé reaction method (Butler, 1975). The MDA equivalents were quantified from the formation of thiobarbituric acid reactive substances due to the lipid peroxidation in urine according to the method described in (Chatziargyriou and Dailianis, 2010). In summary, about 500 µL of urine was exposed to phorbol-myristate acetate (PMA) (10 $\mu g mL^{-1}$). The samples were then centrifuged at 1200 rpm for 10 minutes at 4 °C and the supernatant was removed. After vortexing for 5 s, butylated hydroxytoluene (BHT) at a concentration of 0.02% was added to prevent further lipids' peroxidation. In the last stage, the samples were incubated at 90-100 °C for 15 min and cooled at room temperature, centrifuged at 10,000 rpm for 10 min and measured spectrophotometrically at 535 nm.

Oxidative stress ELISA kit (Zell Bio, GmbH., Germany) was used to determine the concentration of 8-OHdG in the urine based on the method described elsewhere (Ściskalska et al., 2014). In brief, 100 µL of conjugate 8-OHdG/ bovine serum albumin (BSA) were added to each of the 96-well plates of the ELISA kit and incubated overnight at 4 °C and washed with water, followed by 200 µL blocking buffer and incubated for 1 hour at room temperature. 50 µL of samples and 50 µL 8-OHdG standards were added, and after 10 minutes of incubation, 100 µL of monoclonal anti-8-OHdG was added and incubated for 1 hour at room temperature, then washed three times by the addition of secondary antibody conjugated to 100 µL of horseradish peroxidase, followed by 1-hour incubation at room temperature. Next, 100 µL of substrate for peroxidase was added to the plate and incubated for 20 minutes. Then, 100 µL of reaction stop solution was added. Absorbance was spectrophotometrically measured at a wavelength of 450 nm. The amount of 8-OHdG was calculated by comparison with a standard curve determined from standards treated similarly to the samples.

Questionnaire to assess potential confounders

The subjects were given a questionnaire to identify possible confounders of chronic metals and metalloids exposure based on a previous study (Rafiee et al., 2020) to self-report their demographic (age, sex, height, weight, BMI), socioeconomic and health (types of diet, use of health supplements) status. In addition, lifestyle characteristics that influence their environmental exposure (cigarette and water-pipe smoking, environmental tobacco smoke, amalgam tooth filling, traffic density near home, frequency of hair product use, insecticide use) were also collected.

Statistical approach

In this study, SPSS 21.0 package software (SPSS Inc. Chicago, IL) and Graph Pad Prism software 8.0 were used to perform statistical analysis on metals and metalloids data in hair samples and oxidative stress markers in urine samples. The normality of the data distribution was performed using the Kolmogorov–Smirnov test. Sociodemographic and environmental differences according to sex were assessed with an independent t-test for numeric variables and the Chi-squared test for categorical ones. Mann–Whitney U test was employed to assess differences in metals and metalloids levels in hair and markers of oxidative stress among studied groups. Multiple linear regression analysis was applied to evaluate the association between concentrations of oxidative stress biomarkers and individual metals and metalloids levels in hair samples as well as covariate factors including sex, age, smoking status, and teeth filling with amalgam. Oxidative stress biomarkers concentrations were treated as continuous variables (MDA per 1 μmol/mol creatinine and 8-OHdG per 1 ng/mmol creatinine). Individual metals and metalloids concentrations in hair samples were considered continuous variables (per 1 μg/g). Covariates were considered categorical for sex (male/female), cigarette smoking (no/yes), waterpipe smoking (no/yes), and teeth filling with amalgam (no/yes). Age was considered a continuous variable (per 1 year). Collinearity between variables was analyzed by conducting the spearman correlation test. The pairwise correlation coefficient (r) of < 0.5 was used as the indicator for introducing variables in the model.

Results

Participants' characterizations

Results of the socio-demographic characterization, health status, and lifestyle habits of the studied men and women participants are shown in Table 1. Significant differences were observed among males and females regarding demographic characterizations such as age, height, weight and BMI ($P<0.05$). All participants were educated, and 84% of them held tertiary education degrees. According to the information gathered from the questionnaires and face-to-face interviews, 44% of subjects were smokers (cigarettes), and 29% were water-pipe smokers, with a higher prevalence among males ($p<0.05$). In addition, 34% of subjects were classified as passive smokers, with a higher prevalence among women ($p<0.05$) (Table 1). No sex differences were observed for teeth filling with amalgam ($p>0.05$), but it was considerably higher among smokers than non-smokers in both males and females participants ($p<0.05$). The largest proportion of subjects (58%) lived in houses with medium residential traffic levels, but more women lived in areas with low residential traffic, compared to men participating in this study ($p<0.05$). Sex differences were also observed according to hair cosmetic products usage, with 70% of women reporting to use them every day or once per two days, while only 30% reported cosmetic usage with that frequency ($p<0.05$). No sex differences were observed for taking health supplements, with 57% taking these, nor was observed for fish consumption, with 58% of subjects reporting eating fish ($p>0.05$).

Distribution of metals and metalloids concentrations among participants

Results of selected metals and metalloids levels in the hair samples of studied subjects are given in Table 2. The highest and lowest mean metals and metalloids levels were observed for B and As, respectively. The order of mean metals and metalloids levels in the hair samples of the studied subjects was as follows: B> Zn> Al> Fe> Si> Ba> Cr> Hg> Pb> Mn> Cu> Ag> Cd> Ni> Sn> As. The mean metals and metalloids concentrations were significantly different between men and women participants ($p<0.05$) for some metals. The mean levels of Ag, Ba, and Cd were considerably higher in the hair samples of females than males (Mann-Whitney U test, $p<0.05$). On the other hand, the mean concentrations of Cr, Fe, Ni, Pb, and Si of males' hair samples were significantly higher than the corresponding values in females (Mann-Whitney U test, $p<0.05$). No significant differences were observed for Al, As, B, Cu, Hg, Mn, Sn, and Zn levels between the studied groups (Mann-Whitney U test, $p>0.05$). Levels of Hg and Ag in the hair of subjects who had dental amalgam fillings were significantly higher than those with no teeth fillings with amalgam ($p<0.05$, Fig 1). Figure 2 shows the results of metals and metalloids levels in the hair of smokers and non-smokers subjects. Significant differences in the levels of metals and metalloids, Cr, Cu, Fe, Hg, Zn, and Pb, were observed between smokers and non-smokers subjects in both studied groups (Mann-Whitney U test, $p<0.05$). However, no significant differences were observed for Cd, As, Ba, Mn, and Sn levels between smokers and non-smokers (Mann-Whitney U test, $p>0.05$).

Urinary MDA and 8-OHdG in studied groups

Results of MDA and 8-OHdG levels in the urine of studied subjects are provided in Table 3 and Figures 3 and 4. Significant differences were observed between men and women regarding urinary MDA and 8-OHdG levels (Mann-Whitney U test, $p<0.05$). Levels of MDA and 8-OHdG in the urine of smoker subjects were significantly higher than in non-smokers in both groups (Mann-Whitney U test, $p<0.05$). The median

MDA and 8-OHdG in smokers' urine were 5 and 5.5 times higher than the corresponding values in non-smokers, respectively.

Association between exposure to metals and metalloids and oxidative stress biomarkers

Total metals and metalloids concentrations correlated with the levels of 8-OHdG and MDA were inversely correlated with age (Table S4). The biomarker of oxidative stress (8-OHdG) is correlated with Al, B, Cr, Cu, Fe, Hg, Ni, Pb, Si ($p<0.001$) and Zn ($p<0.05$). Likewise, MDA is correlated with Ag, Al, B, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Si ($p<0.01$) and with As, Ba, Zn ($p<0.05$). Age is inversely correlated with As, Si, Sn ($p<0.01$) and Ag, Al and Ba ($p<0.05$).

Table 4 presents the result of regression analysis to evaluate the effects of exposure to metals and metalloids on oxidative stress biomarkers considering sex, age, smoking status, and teeth filling with amalgam as covariates. The levels of Si had significant positive associations with MDA concentration, while Sn showed a negative association (p-value<0.05). An association between Cr, Cu, Fe with MDA was also suggested ($p<0.10$).

Regarding the 8-OHdG, higher concentrations were significantly associated with increased levels of Al, Cu, Si ($p<0.05$) and Cr ($p<0.1$), whereas higher levels of Ba, Sn, and Zn in hair samples were significantly associated with lower levels of 8-OHdG ($p<0.05$). In addition, sex was also found to be significantly associated with higher levels of 8-OHdG, with males showing higher 8-OHdG concentrations.

Significant associations were observed for cigarette and water-pipe smoking for both MDA and 8-OHdG biomarker levels, with higher biomarker concentrations in smokers.

Discussion

Concentrations of metals and metalloids in hair samples

The concentrations of metals and metalloids measured in the current study are compared with values reported in the literature from Tehran, a nearby megacity (Rafiee et al., 2020), as well as with concentrations reported elsewhere. The levels of Ag, Al and As were very similar to those reported from Tehran subjects (Rafiee et al., 2020), while concentrations of As in this study were higher than those reported in Portugal (Coelho et al., 2014), Greece (Sazakli and Leotsinidis, 2017b) and Italy (Varrica et al., 2014a). Thus, exposure to As in Iran appears to be associated with drinking water and dietary intake (Alamdar et al., 2019; Derakhshan et al., 2018; Rahmdel et al., 2018; Torghabeh et al., 2019). Concentrations of Ni are higher than those reported in Portugal, Greece and Italy (Coelho et al., 2013; Sazakli and Leotsinidis, 2017a; Varrica et al., 2014b), although lower than concentrations reported in Taiyuan (China) (Zhu et al., 2018). Concentrations of Hg were higher than those reported in Tehran (Rafiee et al., 2020), China and Spain (Molina-Villalba et al., 2015; Zhu et al., 2018). A similar trend was observed for Zn (Rafiee et al., 2020; Tamburo et al., 2016; Varrica et al., 2014b; Zhu et al., 2018), Ni (Rafiee et al., 2020; Zhu et al., 2018) and Pb (E et al., 2016; Peña-Fernández et al., 2014; Zhu et al., 2018). Although the concentration of Pb in the hair in Shiraz are lower than those reported in Tehran (Rafiee et al., 2020). The study in Shiraz reported Fe levels higher than those previously reported in Tehran (Rafiee et al., 2020) and Sant' Antioco (Italy) (Varrica et al., 2014b), although lower than those measured in Taiyuan (China) (Zhu et al., 2018).

Most probably, the routes of exposure to Pb in Shiraz are similar to those identified in Tehran, mainly being dietary, associated with the use of lead water pipes (Rabin, 2008) and drinking contaminated underground water (because of electronic waste management) (Li and Achal, 2020). Other routes might be inhalation of re-suspended contaminated soil (due to wastewater irrigation) (Qishlaqi et al., 2008) and road dust (e.g. from disk brakes) (Sanderson et al., 2014; Sanderson et al., 2016). Finally, dermal exposure from the application of hair and cosmetic products (Iwegbue et al., 2016; Salama, 2016) and dietary intake (Galal-Gorchev, 1993) could also contribute to Pb exposure in the studied population. Pb in hair was higher in males than in females (Table 2), consistent with some studies (Ashraf et al., 1995; Rafiee et al., 2020; Sanna et al., 2003; Szynkowska et al., 2015), whereas other studies observed higher Pb concentrations in females (Peña-Fernández et al., 2014; Tamburo et al., 2016). The elevated concentrations of Pb in men are most likely because of the use of hair dyes that contain Pb (Zhu et al., 2018). Exposure to Hg could be attributed to participants having teeth filling with a Hg amalgam (Rafiee et al., 2020), dietary exposures, e.g. from canned tuna (Zhu et al., 2018), and industrial activities (Zhu et al., 2018). Zn exposure could be attributed to males' use of health supplements (Rafiee et al., 2020). Exposure to Zn through inhalation of PM_{10} (Parvizimehr et al., 2020) and re-suspended soil particles (Jahandari, 2020; Keshavarzi et al., 2015; Moghtaderi et al., 2020; Moghtaderi et al., 2019; Torghabeh et al., 2019) cannot be ignored, as well as from dietary intake (Ahmadi and Ziarati, 2015; Derakhshan et al., 2018; Rahmdel et al., 2018; Sepehri et al., 2018). The intake of Fe could be attributed to the use of health supplements, inhalation of particulate matter or dietary intake of kebab and grilled sheep's liver rich in iron and popular with males (Rafiee et al., 2020).

Markers of oxidative stress

The concentrations of MDA and 8-OHdG in the present study (Table 3) are higher than concentrations measured in the general population in Ghana (Bortey-Sam et al., 2018). MDA concentrations in Shiraz are also higher than those reported in a rural population in China (Yang et al., 2015). Similarly, 8-OHdG were higher than those reported for university staff in Verona (Italy) (Zanolin et al., 2015) and a Korean population living at different distances from an oil spillage in Korea (Kim et al., 2017). On the other hand, concentrations of 8-OHdG are similar to those reported in school children in Taiwan (Wong et al., 2005), college students in China (Lu et al., 2016) and the general population in Japan (Kimura et al., 2006) and Turkey (Basaran et al., 2020).

Association between metals and metalloids exposure and markers of oxidative stress

No associations are observed between chronic exposure to Ag, B, Mn, and any of the two oxidative stress biomarkers studied. No associations were observed either for Ag with MDA in a group of subjects exposed to Ag from local e-waste activities (Li et al., 2020b). A study in a Turkish population neither found an association between B and 8-OHdG (Basaran et al., 2020). Results of our study are consistent with results reported in a study in a pregnant population in Bangladesh, in which chronic Mn exposure was not associated with 8-OHdG (Engstrom et al., 2010).

This study shows that chronic Fe exposure is associated with MDA but not with 8-OHdG. A randomized controlled trial study supplementing Fe in lactating mothers found neither an association between Fe exposure and 8-OHdG (Jorgensen et al., 2017). On the other hand, a study conducted on asthmatic children found a significant association between Fe and MDA concentrations (Kocyigit et al., 2004).

Chronic Al exposure was associated with increased levels of 8-OHdG, but not MDA, which is consistent with results reported in a Japanese study for short-term Al exposure, although in their case, the association was suggestive ($p>0.10$) (Kimura et al., 2006). On the other hand, the Japanese study shows a positive association for short-term As exposure with 8-OHdG, consistent with He et al (2020) and other study of chronic As exposure in pregnant women in Bangladesh (Engstrom et al., 2010), and at odds with our observations. In contrast, a study conducted among school children in Taiwan, neither found an association between short-term exposure and 8-OHdG (Wong et al., 2005), consistent with our observations.

This study shows an inverse association between chronic Ba exposure and 8-OHdG, but not for MDA. Domingo-Relloso et al (2019) did not find any association with MDA either, but found a significant positive association between short-term Ba exposure and 8-OHdG (Domingo-Relloso et al., 2019b).

Chronic Cd exposure did not show any association with MDA or 8-OHdG in this study. Whilst Domingo-Relloso et al (2019) did not observe any association for 8-OHdG either, they report an association with MDA for short-term Cd exposure (Domingo-Relloso et al., 2019b). On the other hand, a positive association was reported between short-term Cd exposure and 8-OHdG levels in a study in Wuhan (He et al., 2020), Korea (Eom et al., 2017) and chronic exposure in rural Bangladesh (Engstrom et al., 2010).

This study shows a suggestive ($p<0.10$) association between chronic Cr exposure and urinary MDA and 8-OHdG concentrations. This is consistent with observations for short-term Cr exposure and 8-OHdG in Taiwanese school children (Wong et al., 2005) and the Japanese general population (Kimura et al., 2006), which show a positive association ($p<0.05$). On the other hand, no associations were reported for short-term Cr exposure with MDA or 8-OHdG in the Spanish Hortega study (Domingo-Relloso et al., 2019b).

Chronic Cu exposure shows an association with 8-OHdG, whilst the association is only suggestive (p>0.10) for MDA. In contrast, no associations were reported between short-term Cu exposure and 8-OHdG nor MDA in the Spanish Hortega study (Domingo-Relloso et al., 2019b), nor was it found for 8-OHdG in a Japanese population study (Kimura et al., 2006).

No association was reported between chronic Hg or Ni exposure and MDA or 8-OHdG levels (Table 4), consistent with results for short-term exposure in a Japanese study, although in their case, the association with Ni was suggestive (p>0.10) (Kimura et al., 2006).

He et al (2020) showed a significant dose-dependent relationship between urinary Pb with 8-OHdG. In this study, whilst Pb concentrations in hair show a significant correlation with 8-OHdG (Table S1); results of the regression model that take into account the effect of other covariates, do not show any dose-dependent association (Table 4). Our results are consistent with observations in a Japanese study (Kimura et al., 2006).

This study shows a positive association between chronic Si exposure and MDA and 8-OHdG concentrations. On the other hand, chronic Sn exposure is inversely associated with MDA and 8-OHdG concentrations, which is at odds with a study assessing the effect of metals on AHDH in children, which reported positive correlations instead (Li et al., 2020a).

An inverse association between chronic Zn exposure and 8-OHdG, but not with MDA, is at odds with the Spanish Hortega study results, which report positive associations between short-term Zn exposure and both MDA and 8-OHdG (Domingo-Relloso et al., 2019b), whist no associations were reported in a Japanese population study (Kimura et al., 2006).

The differences observed between observations of the current study with the literature might be related to the fact that different matrices were used to characterize metal exposure (e.g. hair vs urine or blood); to the fact that our study captures chronic exposure, whilst the other studies reflect short-term exposure. E.g. As, Cr, Ni tend to be excreted quickly from the body through urine. Therefore, urinary concentrations represent short-term exposure (Kuo et al., 2003). It can also be related to the covariates used to adjust each model, which although most of them try to capture socioeconomic factors, the variables themselves might vary among the different studies presented. Finally, although these studies examine the effect of exposure to metals on oxidative stress biomarkers, no information on other environmental exposures is considered in these studies, which might be (at least partially) driving the associations observed. All these factors could be important determinants explaining the differences observed.

Age has not been identified as an important covariate in the regression analysis in this study (Table 4), despite showing an inverse correlation in the Pearson analysis (Table S1). In the study by Bortey-Sam et al. (2018), only Co correlated statistically with age, but age was not identified as a significant covariate for any of the other metals analyzed in the Ghanaian study (Bortey-Sam et al., 2018), consistent with our results. The link between the ageing factor and 8-OHdG has been debatable (Miwa et al., 2004b; Toraason et al., 2003; Wong et al., 2005).

Sex has been identified as a significant covariate for the 8-OHdG analysis only, but not for the MDA. Differential sex effects have been reported when comparing both oxidative biomarker concentrations in the present study (Table 3). The effect of sex on oxidative stress levels warrants further investigation, as conflicting results are reported in the various studies examined (Bortey-Sam et al., 2018; Eom et al., 2017; Kimura et al., 2006; Wong et al., 2005; Zanolin et al., 2015). This might indicate that the sex differences observed in this study might not truly reflect sex inherent differences but could be attributed to other factors, e.g. socioeconomic or exposures to other environmental factors not considered in the present study.

Figures 3 and 4 show that smokers have higher levels of oxidative stress biomarkers 8-OHdG and MDA, respectively. Consistent with this observation, the regression analysis highlights smoking cigarettes and water-pipe as significant contributing factors to increased ROS damage, with increased levels of MDA and 8-OHdG (Table 4). This is consistent with results in several studies that report significantly higher concentrations of these ROS biomarkers in smokers (Tagesson et al., 1996; Yang et al., 2015; Zanolin et al., 2015). In contrast, no smoking effect was observed in 8-OHdG concentrations collected from a sample of general population in Japan (Kimura et al., 2006). The complexity of cigarette type, quality of tobacco and compounds produced during passive smoking might explain the difference across studies.

Amalgam filling has not been identified as a significant factor explaining elevated MDA or 8-OHdG concentrations in the present study (Table 4). This is consistent with the study by de Almeida Lopes et al

(2017), in which the correlation between blood levels of Pb, Cd and Hg was not statistically significant with MDA in environmentally exposed Brazilian adults, even though the Hg levels were higher in subjects having amalgam fillings.

Limitations and strengths

Other factors that might be associated with increase oxidative stress might have not been captured in the current study design. For instance, urinary 8-OHdG levels are susceptible to environmental pollutant exposure, such as diesel exhaust emissions, electronic waste dismantling (Wang et al., 2010), and cooking fumes (Ke et al., 2009). Such factors might also contribute to the high oxidative stress biomarker concentrations obtained in this study. Another limitation of our study was using spot urine samples to assess the association between ROS markers with metals and metalloids. Ideally, 24-h pooled or fasting urine samples should be employed to assess biomarkers of oxidative stress, especially when including smokers in the population under study (Zanolin et al., 2015). However, Miwa et al (2004) studied the diurnal variability of 8-OHdG and concluded that a morning spot urine sample could be used to measure 8-OHdG instead of inconvenient 24-h sampling, despite the inherent variability in the biomarker excretion across the day (Miwa et al., 2004a).

This study reports chronic exposure to metals in an urban population from a middle-income country in the Middle East, providing valuable information about metal exposures for a population where such evidence is scarce. In addition, it has also analyzed levels of two different oxidative stress biomarkers and analyzed the associations between chronic metal exposures and oxidative stress. Most of the available studies focus on short-term exposures, whilst information on such associations on chronic exposures is very limited, even more from low and middle-income countries in the Middle East or elsewhere. Therefore, this study provides valuable evidence to evaluate chronic exposure to metals in this region of the world and their effects on oxidative stress.

Conclusion

The carcinogenic effects due to chronic exposure to metals is not well characterized. However, it is generally assumed that the concept of additivity applies when the exposure is associated with low-level chemical mixtures (Hartwig and Schwerdtle, 2002). This study shows that urban residents of Shiraz, one of the main urban centers in the Middle East, are chronically exposed to elevated concentrations of As, Ni, Hg and Pb. Concentrations of MDA are lower than concentrations reported in other studies, whilst concentrations of 8-OHdG are intermediate. Results of regression analyses assessing the association between urinary ROS markers with metals and metalloids, adjusted for other confounders, highlight that Al, Cu, Si and Sn are associated with 8-OHdG concentrations, while Cr is suggestive of an association with both MDA and 8-OHdG. In addition, smoking cigarettes and water-pipe is considered a significant risk factor increasing the concentrations of both ROS biomarkers (MDA and 8-OHdG).

Given that several metals have been significantly associated with ROS biomarkers and considering that ROS might be a significant factor in the etiology of many health diseases, further research of the health effect of metal in dose-effect relationships of environmentally exposed subjects should be implemented.

Declaration

Funding:

The present study was financially supported by the Shiraz University of Medical Sciences (grant no.17569)

Authors thank all the subjects who participated in this study.

Conflict of Interest:

Authors declare that they do not have any competing financial interests in relation to this study.

Availability of data and material

Authors d Not applicable.

Code availability

Not applicable.

Authors' contributions

Ata Rafiee: Conceptualization, Methodology development, Formal Analysis, Writing- Original draft preparation, Reviewing and Editing. **Juana Maria Delgado-Saborit**: Writing- Reviewing and Editing. **Noel J. Aquilina**: Writing. **Hoda Amiri**: Data curation. **Mohammad Hoseini**: Funding acquisition and Project administration.

Ethics approval

The Ethical Committee approved the present study at the Shiraz University of Medical Sciences (IR.SUMS.REC.1398.320).

Consent to participate

All subjects were informed about study’s objectives and gave written consent to participate in this study.

Figures legend:

Figure 1. Ag and Hg levels in hair of subjects who are smoker/non-smokers and with/without amalgam teeth fillings (µg/g)

Figure 2. Sum of metals and metalloids, Pb, and Cd levels in hair of smoker and non-smoker subjects (µg/g)

Figure 3: Comparison of urinary 8-OHdG levels (µg/g creatinine) in (a) men and women and (b) smokers and non-smokers

Figure 4: Comparison of urinary MDA levels (µg/g creatinine) in (a) smokers and non-smokers and (b) men and women

References

Unknown article.

Ahmadi A, Ziarati P. Chemical Composition Profile of Canned and Frozen Sweet Corn (Zea mays L.) in Iran. Oriental Journal of Chemistry 2015; 31: 1065-1070.

Al Hanai AH, Antkiewicz DS, Hemming JDC, Shafer MM, Lai AM, Arhami M, et al. Seasonal variations in the oxidative stress and inflammatory potential of PM2.5 in Tehran using an alveolar macrophage model; The role of chemical composition and sources. Environment International 2019; 123: 417-427.

Alamdar R, Kumar V, Moghtaderi T, Naghibi SJ. Groundwater quality evaluation of Shiraz City, Iran using multivariate and geostatistical techniques. Sn Applied Sciences 2019; 1.

Alimonti A, Ristori G, Giubilei F, Stazi MA, Pino A, Visconti A, et al. Serum chemical elements and oxidative status in Alzheimer's disease, Parkinson disease and multiple sclerosis. NeuroToxicology 2007; 28: 450-456.

Almeida Lopes ACB, Urbano MR, Souza-Nogueira Ad, Oliveira-Paula GH, Michelin AP, Carvalho MdFH, et al. Association of lead, cadmium and mercury with paraoxonase 1 activity and malondialdehyde in a general population in Southern Brazil. Environmental Research 2017; 156: 674-682.

Amaral AFS, Arruda M, Cabral S, Rodrigues AS. Essential and non-essential trace metals in scalp hair of men chronically exposed to volcanogenic metals in the Azores, Portugal. Environment International 2008; 34: 1104-1108.

Amato F, Cassee FR, Denier van der Gon HAC, Gehrig R, Gustafsson M, Hafner W, et al. Urban air quality: The challenge of traffic non-exhaust emissions. Journal of Hazardous Materials 2014; 275: 31-36.

Amato F, Favez O, Pandolfi M, Alastuey A, Querol X, Moukhtar S, et al. Traffic induced particle resuspension in Paris: Emission factors and source contributions. Atmospheric Environment 2016; 129: 114-124.

Ashraf W, Jaffar M, Anwer K, Ehsan U. Age- and sex-based comparative distribution of selected metals in the scalp hair of an urban population from two cities in Pakistan. Environmental Pollution 1995; 87: 61-64.

ATSDR. Summary Data for 2013 Priority List of Hazardous Substances. Department of Health and Human Services, Public Health Service Atlanta, 2013.

Barbieri F, Cournil A, Souza Sarkis JE, Bénéfice E, Gardon J. Hair Trace Elements Concentration to Describe Polymetallic Mining Waste Exposure in Bolivian Altiplano. Vol 139, 2010.

Basaran N, Duydu Y, Bacanli M, Anlar HG, Dilsiz SA, Ustundag A, et al. Evaluation of oxidative stress and immune parameters of boron exposed males and females. Food and Chemical Toxicology 2020; 142: 6.

Boogaard H, Kos GPA, Weijers EP, Janssen NAH, Fischer PH, van der Zee SC, et al. Contrast in air pollution components between major streets and background locations: Particulate matter mass, black carbon, elemental composition, nitrogen oxide and ultrafine particle number. Atmospheric Environment 2011; 45: 650-658.

Bortey-Sam N, Ikenaka Y, Akoto O, Nakayama SMM, Asante KA, Baidoo E, et al. Association between human exposure to heavy metals/metalloid and occurrences of respiratory diseases, lipid peroxidation and DNA damage in Kumasi, Ghana. Environmental Pollution 2018; 235: 163-170.

Bowler RM, Nakagawa S, Drezgic M, Roels HA, Park RM, Diamond E, et al. Sequelae of fume exposure in confined space welding: A neurological and neuropsychological case series. Neurotoxicology 2007; 28: 298-311.

Butler AR. The Jaffe reaction. Identification of the coloured species. Clinica Chimica Acta 1975; 59: 227-232.

Carneiro MFH, Grotto D, Batista BL, Rhoden CR, Barbosa Júnior F. Background values for essential and toxic elements in Children's nails and correlation with hair levels. Biological Trace Element Research 2011; 144: 339-350.

Chatziargyriou V, Dailianis S. The role of selenium-dependent glutathione peroxidase (Se-GPx) against oxidative and genotoxic effects of mercury in haemocytes of mussel Mytilus galloprovincialis (Lmk.). Toxicology in Vitro 2010; 24: 1363-1372.

Chen P, Miah M, Aschner M. Metals and Neurodegeneration. F1000Research 2016; 5.

Coelho P, Costa S, Costa C, Silva S, Walter A, Ranville J, et al. Biomonitoring of several toxic metal(loid)s in different biological matrices from environmentally and occupationally exposed populations from Panasqueira mine area, Portugal. Environ Geochem Health 2014; 36: 255-69.

Coelho P, Costa S, Costa C, Silva S, Walter A, Ranville J, et al. Biomonitoring of several toxic metal(loid)s in different biological matrices from environmentally and occupationally exposed populations from Panasqueira mine area, Portugal. Vol 36, 2013.

Dahl A, Gharibi A, Swietlicki E, Gudmundsson A, Bohgard M, Ljungman A, et al. Traffic-generated emissions of ultrafine particles from pavement–tire interface. Atmospheric Environment 2006; 40: 1314-1323.

Denier van der Gon HAC, Gerlofs-Nijland ME, Gehrig R, Gustafsson M, Janssen N, Harrison RM, et al. The Policy Relevance of Wear Emissions from Road Transport, Now and in the Future—An International Workshop Report and Consensus Statement. Journal of the Air & Waste Management Association 2013; 63: 136-149.

Derakhshan Z, Mahvi AH, Faramarzian M, Dehghani M, Salari M, Fakhri Y, et al. Data on heavy metal concentration in common carp fish consumed in Shiraz, Iran. Data in Brief 2018; 21: 1890-1894.

Domingo-Relloso A, Grau-Perez M, Galan-Chilet I, Garrido-Martinez MJ, Tormos C, Navas-Acien A, et al. Urinary metals and metal mixtures and oxidative stress biomarkers in an adult population from Spain: The Hortega Study. Environment International 2019a; 123: 171-180.

Domingo-Relloso A, Grau-Perez M, Galan-Chilet I, Garrido-Martinez MJ, Tormos C, Navas-Acien A, et al. Urinary metals and metal mixtures and oxidative stress biomarkers in an adult population from Spain: The Hortega Study. Environment International 2019b; 123: 171-180.

Drobyshev EJ, Solovyev ND, Ivanenko NB, Kombarova MY, Ganeev AA. Trace element biomonitoring in hair of school children from a polluted area by sector field inductively coupled plasma mass spectrometry. Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS) 2017; 39: 14-20.

E T, D V, G D. Gender as a key factor in trace metal and metalloid content of human scalp hair. A multi-site study. Science of The Total Environment 2016; 573: 996-1002.

Engstrom KS, Vahter M, Johansson G, Lindh CH, Teichert F, Singh R, et al. Chronic exposure to cadmium and arsenic strongly influences concentrations of 8-oxo-7,8-dihydro-2 '-deoxyguanosine in urine. Free Radical Biology and Medicine 2010; 48: 1211-1217.

Eom SY, Seo MN, Lee YS, Park KS, Hong YS, Sohn SJ, et al. Low-Level Environmental Cadmium Exposure Induces Kidney Tubule Damage in the General Population of Korean Adults. Archives of Environmental Contamination and Toxicology 2017; 73: 401-409.

Eqani S, Tanveer ZI, Qiaoqiao C, Cincinelli A, Saqib Z, Mulla SI, et al. Occurrence of selected elements (Ti, Sr, Ba, V, Ga, Sn, Tl, and Sb) in deposited dust and human hair samples: implications for human health in Pakistan. Environmental Science and Pollution Research 2018; 25: 12234-12245.

Fábelová L, Vandentorren S, Vuillermoz C, Garnier R, Lioret S, Botton J. Hair concentration of trace elements and growth in homeless children aged <6years: Results from the ENFAMS study. Environment International 2018; 114: 318-325.

Farmand F, Ehdaie A, Roberts CK, Sindhu RK. Lead-induced dysregulation of superoxide dismutases, catalase, glutathione peroxidase, and guanylate cyclase. Environmental Research 2005; 98: 33-39.

Galal-Gorchev H. Dietary intake, levels in food and estimated intake of lead, cadmium, and mercury. Food Addit Contam 1993; 10: 115-28.

Gietl JK, Lawrence R, Thorpe AJ, Harrison RM. Identification of brake wear particles and derivation of a quantitative tracer for brake dust at a major road. Atmospheric Environment 2010; 44: 141-146.

Gil F, Hernandez Jerez A. Significance of Biochemical Markers in Applied Toxicology. 2, 2009.

Halliwell B, Cross CE. Oxygen-derived species: Their relation to human disease and environmental stress. Environmental Health Perspectives 1994; 102: 5-12.

Harrison RM, Yin J, Mark D, Stedman J, Appleby RS, Booker J, et al. Studies of the coarse particle (2.5–10μm) component in UK urban atmospheres. Atmospheric Environment 2001; 35: 3667-3679.

Hartwig A, Schwerdtle T. Interactions by carcinogenic metal compounds with DNA repair processes: toxicological implications. Toxicology letters 2002; 127: 47-54.

He Y, Zou L, Luo W, Yi Z, Yang P, Yu S, et al. Heavy metal exposure, oxidative stress and semen quality: Exploring associations and mediation effects in reproductive-aged men. Chemosphere 2020; 244: 125498.

Hoseini M, Nabizadeh R, Delgado-Saborit JM, Rafiee A, Yaghmaeian K, Parmy S, et al. Environmental and lifestyle factors affecting exposure to polycyclic aromatic hydrocarbons in the general population in a Middle Eastern area. Environmental Pollution 2018; 240: 781-792.

Iwegbue CMA, Emakunu OS, Obi G, Nwajei GE, Martincigh BS. Evaluation of human exposure to metals from some commonly used hair care products in Nigeria. Toxicology Reports 2016; 3: 796-803.

Jahandari A. Pollution status and human health risk assessments of selected heavy metals in urban dust of 16 cities in Iran. Environmental Science and Pollution Research 2020; 27: 23094-23107.

Jorgensen JM, Yang ZY, Lonnerdal B, Chantry CJ, Dewey KG. Effect of iron supplementation during lactation on maternal iron status and oxidative stress: A randomized controlled trial. Maternal and Child Nutrition 2017; 13: 11.

Ke Y, Cheng J, Zhang Z, Zhang R, Zhang Z, Shuai Z, et al. Increased levels of oxidative DNA damage attributable to cooking-oil fumes exposure among cooks. Inhalation toxicology 2009; 21: 682-687.

Keshavarzi B, Tazarvi Z, Rajabzadeh MA, Najmeddin A. Chemical speciation, human health risk assessment and pollution level of selected heavy metals in urban street dust of Shiraz, Iran. Atmospheric Environment 2015; 119: 1-10.

Keuken MP, Moerman M, Voogt M, Blom M, Weijers EP, Röckmann T, et al. Source contributions to PM2.5 and PM10 at an urban background and a street location. Atmospheric Environment 2013; 71: 26-35.

Kim JA, Noh SR, Cheong HK, Ha M, Eom SY, Kim H, et al. Urinary oxidative stress biomarkers among local residents measured 6 years after the Hebei Spirit oil spill. Science of the Total Environment 2017; 580: 946-952.

Kimura S, Yamauchi H, Hibino Y, Iwamoto M, Sera K, Ogino K. Evaluation of urmiary 8-hydroxydeoxyguanine in healthy Japanese people. Basic & Clinical Pharmacology & Toxicology 2006; 98: 496-502.

Kocyigit A, Armutcu F, Gurel A, Ermis B. Alterations in plasma essential trace elements selenium, manganese, zinc, copper, and iron concentrations and the possible role of these elements on oxidative status in patients with childhood asthma. Biological Trace Element Research 2004; 97: 31-41.

Kuo H, Chang S, Wu K, Wu F. Chromium (VI) induced oxidative damage to DNA: increase of urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) among electroplating workers. Occupational and environmental medicine 2003; 60: 590-594.

Kwak J-h, Kim H, Lee J, Lee S. Characterization of non-exhaust coarse and fine particles from on-road driving and laboratory measurements. Science of The Total Environment 2013; 458-460: 273-282.

Lawrence S, Sokhi R, Ravindra K, Mao H, Prain HD, Bull ID. Source apportionment of traffic emissions of particulate matter using tunnel measurements. Atmospheric Environment 2013; 77: 548-557.

Lenschow P, Abraham HJ, Kutzner K, Lutz M, Preuß JD, Reichenbächer W. Some ideas about the sources of PM10. Atmospheric Environment 2001; 35: S23-S33.

Li JQ, Cen DZ, Huang DL, Li XF, Xu JJ, Fu SL, et al. Detection and Analysis of 12 Heavy Metals in Blood and Hair Sample from a General Population of Pearl River Delta Area. Cell Biochemistry and Biophysics 2014; 70: 1663-1669.

Li W, Achal V. Environmental and health impacts due to e-waste disposal in China – A review. Science of The Total Environment 2020; 737: 139745.

Li YH, Cha CH, Lv XJ, Liu J, He JY, Pang QH, et al. Association between 10 urinary heavy metal exposure and attention deficit hyperactivity disorder for children. Environmental Science and Pollution Research 2020a; 27: 31233-31242.

Li Z, Li X, Qian Y, Guo C, Wang Z, Wei Y. The sustaining effects of e-waste-related metal exposure on hypothalamus-pituitary-adrenal axis reactivity and oxidative stress. Science of The Total Environment 2020b; 739: 139964.

Lin C-C. A review of the impact of fireworks on particulate matter in ambient air. Journal of the Air & Waste Management Association 2016; 66: 1171-1182.

Liu Y, Wang H, Li X, Li J. Heavy Metal Contamination of Agricultural Soils in Taiyuan, China. Pedosphere 2015; 25: 901-909.

Lopes ACBA, Urbano MR, de Souza-Nogueira A, Oliveira-Paula GH, Michelin AP, Maria de Fátima HC, et al. Association of lead, cadmium and mercury with paraoxonase 1 activity and malondialdehyde in a general population in Southern Brazil. Environmental research 2017; 156: 674-682.

Lu SY, Ren L, Fang JZ, Ji JJ, Liu GH, Zhang JQ, et al. Trace elements are associated with urinary 8-hydroxy-2'-deoxyguanosine level: a case study of college students in Guangzhou, China. Environmental Science and Pollution Research 2016; 23: 8484-8491.

Luo R, Zhuo X, Ma D. Determination of 33 elements in scalp hair samples from inhabitants of a mountain village of Tonglu city, China. Vol 104C, 2014.

Mirzaei S, Hashemi H, Hoseini M. Concentration and potential source identification of trace elements in wet atmospheric precipitation of Shiraz, Iran. Journal of Environmental Health Science and Engineering 2018; 16: 229-237.

Miwa M, Matsumaru H, Akimoto Y, Naito S, Ochi H. Quantitative determination of urinary 8-hydroxy-2'-deoxyguanosine level in healthy Japanese volunteers. BioFactors 2004a; 22: 249-253.

Miwa M, Matsumaru H, Akimoto Y, Naito S, Ochi H. Quantitative determination of urinary 8-hydroxy-2'-deoxyguanosine level in healthy Japanese volunteers. Biofactors 2004b; 22: 249-253.

Moghtaderi T, Alamdar R, Rodriguez-Seijo A, Naghibi SJ, Kumar V. Ecological risk assessment and source apportionment of heavy metal contamination in urban soils in Shiraz, Southwest Iran. Arabian Journal of Geosciences 2020; 13.

Moghtaderi T, Aminiyan MM, Alamdar R, Moghtaderi M. Index-based evaluation of pollution characteristics and health risk of potentially toxic metals in schools dust of Shiraz megacity, SW Iran. Human and Ecological Risk Assessment 2019; 25: 410-437.

Molina-Villalba I, Lacasaña M, Rodríguez-Barranco M, Hernández AF, Gonzalez-Alzaga B, Aguilar-Garduño C, et al. Biomonitoring of arsenic, cadmium, lead, manganese and mercury in urine and hair of children living near mining and industrial areas. Chemosphere 2015; 124: 83-91.

Nnorom IC, Igwe JC, Ejimone JC. Multielement analyses of human scalp hair samples from three distant towns in southeastern Nigeria. African Journal of Biotechnology 2005; 4: 1124-1127.

Pan Y, Li H. Trace elements in scalp hair from potentially exposed individuals in the vicinity of the Bayan Obo mine in Baotou, China. Environmental toxicology and pharmacology 2015; 40: 678-685.

Parvizimehr A, Baghani AN, Hoseini M, Sorooshian A, Cuevas-Robles A, Fararouei M, et al. On the nature of heavy metals in PM10 for an urban desert city in the Middle East: Shiraz, Iran. Microchemical Journal 2020; 154: 104596.

Peña-Fernández A, Lobo-Bedmar MC, González-Muñoz MJ. Monitoring lead in hair of children and adolescents of Alcalá de Henares, Spain. A study by gender and residential areas. Environment International 2014; 72: 170-175.

Qishlaqi A, Moore F, Forghani G. Impact of untreated wastewater irrigation on soils and crops in Shiraz suburban area, SW Iran. Environmental Monitoring and Assessment 2008; 141: 257-273.

Querol X, Alastuey A, Ruiz CR, Artiñano B, Hansson HC, Harrison RM, et al. Speciation and origin of PM10 and PM2.5 in selected European cities. Atmospheric Environment 2004; 38: 6547-6555.

Querol X, Viana M, Alastuey A, Amato F, Moreno T, Castillo S, et al. Source origin of trace elements in PM from regional background, urban and industrial sites of Spain. Atmospheric Environment 2007; 41: 7219-7231.

Rabin R. The lead industry and lead water pipes "A Modest Campaign". American journal of public health 2008; 98: 1584-1592.

Rafiee A, Delgado-Saborit JM, Gordi E, Quémerais B, Kazemi Moghadam V, Lu W, et al. Use of urinary biomarkers to characterize occupational exposure to BTEX in healthcare waste autoclave operators. Science of The Total Environment 2018a; 631-632: 857-865.

Rafiee A, Delgado-Saborit JM, Sly PD, Quemerais B, Hashemi F, Akbari S, et al. Environmental chronic exposure to metals and effects on attention and executive function in the general population. Science of the Total Environment 2020; 705.

Rafiee A, Gordi E, Lu W, Miyata Y, Shabani H, Mortezazadeh S, et al. The impact of various festivals and events on recycling potential of municipal solid waste in Tehran, Iran. Journal of Cleaner Production 2018b; 183: 77-86.

Rahmdel S, Rezaei M, Ekhlasi J, Zarei SH, Akhlaghi M, Abdollahzadeh SM, et al. Heavy metals (Pb, Cd, Cu, Zn, Ni, Co) in leafy vegetables collected from production sites: their potential health risk to the general population in Shiraz, Iran. Environmental Monitoring and Assessment 2018; 190.

Richmond-Bryant J, Meng Q, Davis A, Cohen J, Lu S-E, Svendsgaard D, et al. The influence of declining air lead levels on blood lead-air lead slope factors in children. Environmental health perspectives 2014; 122: 754-760.

Saad A, Hassanien MA. Assessment of arsenic level in the hair of the nonoccupational Egyptian population: Pilot study. Environment International 2001; 27: 471-478.

Salama AK. Assessment of metals in cosmetics commonly used in Saudi Arabia. Environmental Monitoring and Assessment 2016; 188: 11.

Sanderson P, Delgado-Saborit JM, Harrison RM. A review of chemical and physical characterisation of atmospheric metallic nanoparticles. Atmospheric Environment 2014; 94: 353-365.

Sanderson P, Su SS, Chang ITH, Saborit JMD, Kepaptsoglou DM, Weber RJM, et al. Characterisation of iron-rich atmospheric submicrometre particles in the roadside environment. Atmospheric Environment 2016; 140: 167-175.

Sanna E, Liguori A, Palmas L, Soro MR, Floris G. Blood and hair lead levels in boys and girls living in two Sardinian towns at different risks of lead pollution. Ecotoxicology and Environmental Safety 2003; 55: 293-299.

Satarug S, Moore MR. Adverse health effects of chronic exposure to low-level cadmium in foodstuffs and cigarette smoke. Environmental health perspectives 2004; 112: 1099-1103.

Sazakli E, Leotsinidis M. Hair biomonitoring and health status of a general population exposed to Nickel. Journal of trace elements in medicine and biology : organ of the Society for Minerals and Trace Elements (GMS) 2017a; 43: 161-168.

Sazakli E, Leotsinidis M. Hair biomonitoring and health status of a general population exposed to Nickel. J Trace Elem Med Biol 2017b; 43: 161-168.

Ściskalska M, Zalewska M, Grzelak A, Milnerowicz H. The influence of the occupational exposure to heavy metals and tobacco smoke on the selected oxidative stress markers in smelters. Biological trace element research 2014; 159: 59-68.

Sepehri M, Zokaei M, Rezvani M, Zarei A. Comparison of the concentration of heavy metais in some vegetables (celery, broccoli and lettuce). Amazonia Investiga 2018; 7: 324-334.

Shahsavani S, Hoseini M, Dehghani M, Fararouei M. Characterisation and potential source identification of polycyclic aromatic hydrocarbons in atmospheric particles (PM10) from urban and suburban residential areas in Shiraz, Iran. Chemosphere 2017; 183: 557-564.

Shao L, Chen H, Zhang C, Huo X. Effects of Major Grassland Conservation Programs Implemented in Inner Mongolia since 2000 on Vegetation Restoration and Natural and Anthropogenic Disturbances to Their Success. Sustainability 2017; 9: 466.

Skalny AV, Klimenko LL, Turna AA, Budanova MN, Baskakov IS, Savostina MS, et al. Serum trace elements are interrelated with hormonal imbalance in men with acute ischemic stroke. Journal of Trace Elements in Medicine and Biology 2017; 43: 142-147.

Skalny AV, Skalnaya MG, Tinkov AA, Serebryansky EP, Demidov VA, Lobanova YN, et al. Reference values of hair toxic trace elements content in occupationally non-exposed Russian population. Environmental Toxicology and Pharmacology 2015a; 40: 18-21.

Skalny AV, Skalnaya MG, Tinkov AA, Serebryansky EP, Demidov VA, Lobanova YN, et al. Hair concentration of essential trace elements in adult non-exposed Russian population. Environmental Monitoring and Assessment 2015b; 187.

Soetrisno FN, Delgado-Saborit JM. Chronic exposure to heavy metals from informal e-waste recycling plants and children's attention, executive function and academic performance. Science of the Total Environment 2020; 717.

Szynkowska MI, Marcinek M, Pawlaczyk A, Albińska J. Human hair analysis in relation to similar environmental and occupational exposure. Environmental Toxicology and Pharmacology 2015; 40: 402-408.

Tagesson C, Källberg M, Wingren G. Urinary malondialdehyde and 8-hydroxydeoxyguanosine as potential markers of oxidative stress in industrial art glass workers. International archives of occupational and environmental health 1996; 69: 5-13.

Tamburo E, Varrica D, Dongarrà G. Gender as a key factor in trace metal and metalloid content of human scalp hair. A multi-site study. Science of The Total Environment 2016; 573: 996-1002.

Teichert F, Verschoyle RD, Greaves P, Thorpe JF, Mellon JK, Steward WP, et al. Determination of 8-oxo-2′-deoxyguanosine and creatinine in murine and human urine by liquid chromatography/tandem mass spectrometry: application to chemoprevention studies. Rapid Communications in Mass Spectrometry 2009; 23: 258-266.

Thorpe A, Harrison RM. Sources and properties of non-exhaust particulate matter from road traffic: A review. Science of The Total Environment 2008; 400: 270-282.

Toraason M, Butler MA, Ruder A, Forrester C, Taylor L, Ashley DL, et al. Effect of perchloroethylene, smoking, and race on oxidative DNA damage in female dry cleaners. Mutation Research/Genetic Toxicology and Environmental Mutagenesis 2003; 539: 9-18.

Torghabeh AK, Jahandari A, Jamasb R. Concentration, contamination level, source identification of selective trace elements in Shiraz atmospheric dust sediments (Fars Province, SW Iran). Environmental Science and Pollution Research 2019; 26: 6424-6435.

Varrica D, Bardelli F, Dongarrà G, Tamburo E. Speciation of Sb in airborne particulate matter, vehicle brake linings, and brake pad wear residues. Atmospheric Environment 2013; 64: 18-24.

Varrica D, Tamburo E, Milia N, Vallascas E, Cortimiglia V, De Giudici G, et al. Metals and metalloids in hair samples of children living near the abandoned mine sites of Sulcis-Inglesiente (Sardinia, Italy). Environ Res 2014a; 134: 366-74.

Varrica D, Tamburo E, Milia N, Vallascas E, Cortimiglia V, De Giudici G, et al. Metals and metalloids in hair samples of children living near the abandoned mine sites of Sulcis-Inglesiente (Sardinia, Italy). Environmental Research 2014b; 134: 366-374.

Ventura MG, Freitas MDC, Pacheco AM. Selenium levels in mainland Portugal. Water, Air, and Soil Pollution 2005; 166: 167-179.

Venugopal P, Halog A, Dubey BK. Editorial Special Issue on E-waste Management: E-waste: An Urgent Need to Act. Management and Labour Studies 2016; 41: vii-ix.

Vural H, Demirin H, Kara Y, Eren I, Delibas N. Alterations of plasma magnesium, copper, zinc, iron and selenium concentrations and some related erythrocyte antioxidant enzyme activities in patients with Alzheimer’s disease. Journal of Trace Elements in Medicine and Biology 2010; 24: 169-173.

Wang H, Zhang Y, Liu Q, Wang F, Nie J, Qian Y. Examining the relationship between brominated flame retardants (BFR) exposure and changes of thyroid hormone levels around e-waste dismantling sites. International Journal of Hygiene and Environmental Health 2010; 213: 369-380.

Wong RH, Kuo CY, Hsu ML, Wang TY, Chang PI, Wu TH, et al. Increased levels of 8-hydroxy-2 '-deoxyguanosine attributable to carcinogenic metal exposure among schoolchildren. Environmental Health Perspectives 2005; 113: 1386-1390.

Yang QY, Qiu XH, Li R, Ma J, Li KQ, Li G. Polycyclic aromatic hydrocarbon (PAH) exposure and oxidative stress for a rural population from the North China Plain. Environmental Science and Pollution Research 2015; 22: 1760-1769.

Zanolin ME, Girardi P, Degan P, Rava M, Olivieri M, Di Gennaro G, et al. Measurement of a urinary marker (8-hydroxydeoxy-guanosine, 8-OHdG) of DNA oxidative stress in epidemiological surveys: a pilot study. International Journal of Biological Markers 2015; 30: E341-E345.

Zhu Y, Wang Y, Meng F, Li L, Wu S, Mei X, et al. Distribution of metal and metalloid elements in human scalp hair in Taiyuan, China. Ecotoxicology and Environmental Safety 2018; 148: 538-545.

Table 1. Socio-demographic characteristics and health status of the participants stratified by sex

Variables	**Men (n=110)**	**Women (n=90)**	**Total (n=200)**	**Group differences p-value**
Age (years)	38 ± 10	29 ± 12	35 ± 14	**<0.0001[a]**
Height (cm)	179 ± 6	160 ± 8	172 ± 8	**<0.0001[a]**
Weight (kg)	78 ± 11	61 ± 9	70 ± 13	**<0.0001[a]**
BMI (kg/m^2)	26 ± 4	24± 5	24 ± 4	**<0.0019[a]**
Education (%)				0.9156[b]
High school Diploma	17	15	16	
Bachelor	45	45	45	
Master	38	40	39	
Cigarette smoking (%)				**0.0006[b]**
Yes	55	31	44	
No	45	69	56	
Water-pipe smoking (%)				**0.0389[b]**
Yes	31	27	29	
No	69	73	71	
Environmental Tobacco Smoke (%)				**0.0161[b]**
Yes	25	41	34	
No	75	59	66	
Teeth filling with amalgam (%)				0.4335[b]
Yes	74	69	72	
No	26	31	28	
Traffic situation near the place of residence (%)				**0.0219[b]**
Low	20	35	26	
Medium	60	55	58	
High	20	10	16	
Hair cosmetic products usage (%)				**<0.0001[b]**
Everyday	20	25	21	
Once per two days	10	45	26	
Once a week	15	20	18	
Seldom	55	10	35	
Taking health supplement (%)				**0.0318[b]**
Yes	60	53	57	
No	40	47	43	
Fish consumption (%)				0.4744[b]
Yes	55	60	58	
No	45	40	42	

a) t-student independent means test; b) Chi-squared test

Table 2. MM levels in hair of the participants (μg/g) stratified by sex

Metal	Total (N=200)		Men (N=110)		Women (N=90)	
	Mean ± S.D (Min-Max)	Geometric mean (95% CI)	Mean ± S.D (Min-Max)	Geometric mean (95% CI)	Mean ± S.D (Min-Max)	Geometric mean (95% CI)
Ag	3 ± 2.14 (0.5-9)	2.05 (1.85-2.27)	1.7 ± 0.9 (0.4-5)	1.5 (1.3-1.6)	**4 ± 2.5** **(0.7-9)**	3.1 (2.65-3.6)
Al	97 ± 40 (19.6-215)	89 (83.7-94.6)	101 ± 42.5 (20-215)	92 (92-100)	93 ± 37 (28-215.6)	85.5 (78.3-93.3)
As	0.3 ± 0.18 (0.1-1)	0.27 (0.25-0.3)	0.32 ± 0.18 (0.1-1)	0.29 (0.27-0.32)	0.3 ± 0.18 (0.1-1)	0.26 (0.23-0.29)
B	271.5 ± 139 (8-613)	224 (203-248)	288.6 ± 142.9 (44.5-613)	245 (217-276)	250.5 ± 132 (8-454.5)	202 (238.4)
Ba	24 ± 24 (3-112)	17 (14.9-18.6)	15 ± 13 (3-88)	12 (11-13.5)	**35 ± 29** **(7-112)**	24.6 (20.5-29.5)
Cd	2.2 ± 3 (0.2-13)	1.3 (1.1-1.43)	1 ± 1.2 (0.2-13)	0.82 (0.73-0.9)	**3.7 ± 4** **(0.5-13)**	2.14 (1.7-2.7)
Cr	21 ± 18 (2-79.5)	14.6 (12.9-16.5)	**26.3 ± 21.5** **(2-79.5)**	18 (14.9-21)	14 ± 8 (3-27)	11.4 (10-13.4)
Cu	4.9 ± 3 (0.9-19)	4 (3.7-4.4)	5 ± 3 (0.9-19)	4.2 (3.7-4.65)	5 ± 3 (0.9-14)	3.9 (3.4-4.5)
Fe	69 ± 37 (8-215)	59.5 (55-64.5)	**76 ± 35** **(23-215)**	69 (64-75)	61 ± 38 (8-177.5)	49.5 (43.1-57.04)
Hg	16.5 ± 11 (1.5-48.5)	12 (10.45-13.5)	18 ± 12 (1.6-48.5)	12.3 (10-15)	14.8 ± 10 (2-37)	11.4 (9.6-13.4)
Mn	5 ± 4.3 (1-23)	3.6 (3.3-4)	5 ± 3.3 (1-17)	3.6 (3.1-4.2)	5 ± 5.2 (1-23.3)	3.7 (3.1-4.3)
Ni	2 ± 1.9 (0.2-11)	1.35 (1.2-1.5)	**2.5 ± 2.24** **(0.5-11)**	1.8 (1.5-2.1)	1.36 ± 1.1 (0.2-3.5)	1 (0.82-1.17)
Pb	7.6 ± 5 (0.7-24)	6 (5.5-6.7)	**9 ± 5.2** **(0.7-24)**	7 (6.2-8.3)	6 ± 3.7 (1-18)	5 (4.4-5.65)
Si	34.5 ± 12 (12.6-67.5)	32.5 (31-34)	**37 ± 11** **(12.5-66)**	35 (33-37)	32 ± 13 (15-67.4)	30 (27.5-32.2)
Sn	0.4 ± 0.19 (.03-1)	0.32 (0.2-0.38)	0.35 ± 0.16 (0.03-0.8)	0.3 (0.2-0.3)	0.4 ± 0.21 (0.1-0.8)	0.34 (0.3-0.4)
Zn	199 ± 93 (79-1002)	183 (173-193)	206 ± 107 (79-1002)	187 (173-203)	190 ± 73 (114-336)	178 (165-191.5)

Bold represents the highest concentration between the two sexes ($p<0.05$).

Table 3. Urinary levels (µg/g creatinine) of stress oxidative markers in the studied groups stratified by gender

Markers	**Total**		**Men**		**Women**		**Group differences p-value**
	Mean ± S.D (Min-Max)	Geometric mean (95% CI)	Mean ± S.D (Min-Max)	Geometric mean (95% CI)	Mean ± S.D (Min-Max)	Geometric mean (95% CI)	
8-OHdG	3.8 ± 2.35 (0.35-8.35)	2.9 (2.45-3.34)	4.97 ± 2.03 (1.12-8.35)	4.47 (3.92-5.1)	2.6 ± 2.03 (0.35-7.12)	1.83 (1.45-2.31)	**<0.0001**[a]
MDA	214 ± 134 (20.6-522)	167 (145-192.5)	261 ± 126 (57.5-522)	230 (200-263.7)	167 ± 125 (20.6-518)	122 (97.5-152)	**<0.0001**[a]

Table 4. Association between oxidative stress biomarkers and chronic exposure to MM and other potential confounders

MM Levels and covariates	**MDA (µmol/mol creatinine)**			**8-OHdG (ng/mmol creatinine)**		
	Estimate	**[95% Conf.**	**Interval]**	**Estimate**	**[95% Conf.**	**Interval]**
Ag	0.193	-0.250	1.547	0.079	-0.805	1.607
Al	0.171	-0.008	0.069	**0.226**	**0.010**	**0.112**
As	-0.019	-8.862	7.014	0.008	-10.093	11.209
B	-0.066	-0.015	0.008	-0.081	-0.021	0.008
Ba	-0.161	-0.121	0.019	**-0.203**	**-0.190**	**-0.003**
Cd	0.082	-0.435	0.810	.038	-0.704	0.967
Cr	*0.200*	*-0.008*	*0.156*	*0.176*	*-0.012*	*0.209*
Cu	*0.207*	*-0.072*	*1.181*	**0.355**	**0.593**	**2.275**
Fe	*0.275*	*-.008*	*0.129*	0.209	-0.023	0.161
Hg	0.051	-0.087	0.151	0.090	-0.074	0.245
Mn	0.000	-0.421	0.422	-0.012	-0.597	0.534
Ni	-0.134	-1.259	0.349	-0.080	-1.485	0.672
Pb	-0.124	-0.492	0.131	-0.052	-0.532	0.305
Si	**0.322**	**0.032**	**0.341**	**0.358**	**0.106**	**0.520**
Sn	**-0.329**	**-20.249**	**-4.347**	**-0.393**	**-32.746**	**-11.409**
Zn	-0.097	-0.017	0.004	**-0.142**	**-0.029**	**-0.001**
Sex (m/f)	-0.100	-4.573	1.715	**-0.212**	**-8.790**	**-0.353**
Age	0.024	-0.069	0.094	-0.030	-0.134	0.086
Cigarette Smoking (n/y)	**0.402**	**0.396**	**2.008**	**0.382**	**0.154**	**0.542**
Waterpipe Smoking (n/y)	**0.302**	**0.117**	**2.859**	**0.281**	**0.182**	**0.908**
Teeth filling with amalgam (y/n)	-0.024	-3.448	2.693	0.025	-3.534	4.706

Bold type font in grey cell represent regression coefficients with p-value <0.05. Italic font represents coefficients with p-value<0.1. Red font represents positive associations; blue font represents negative associations.

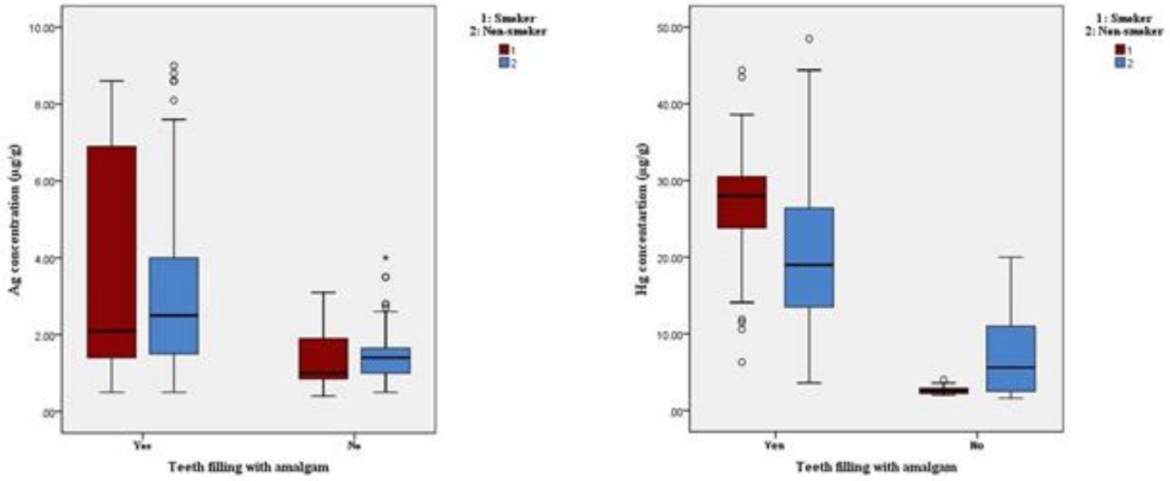

Fig. 1 Ag and Hg levels in hair of subjects who are smoker/non-smokers and with/without amalgam teeth

fillings (lg/g)

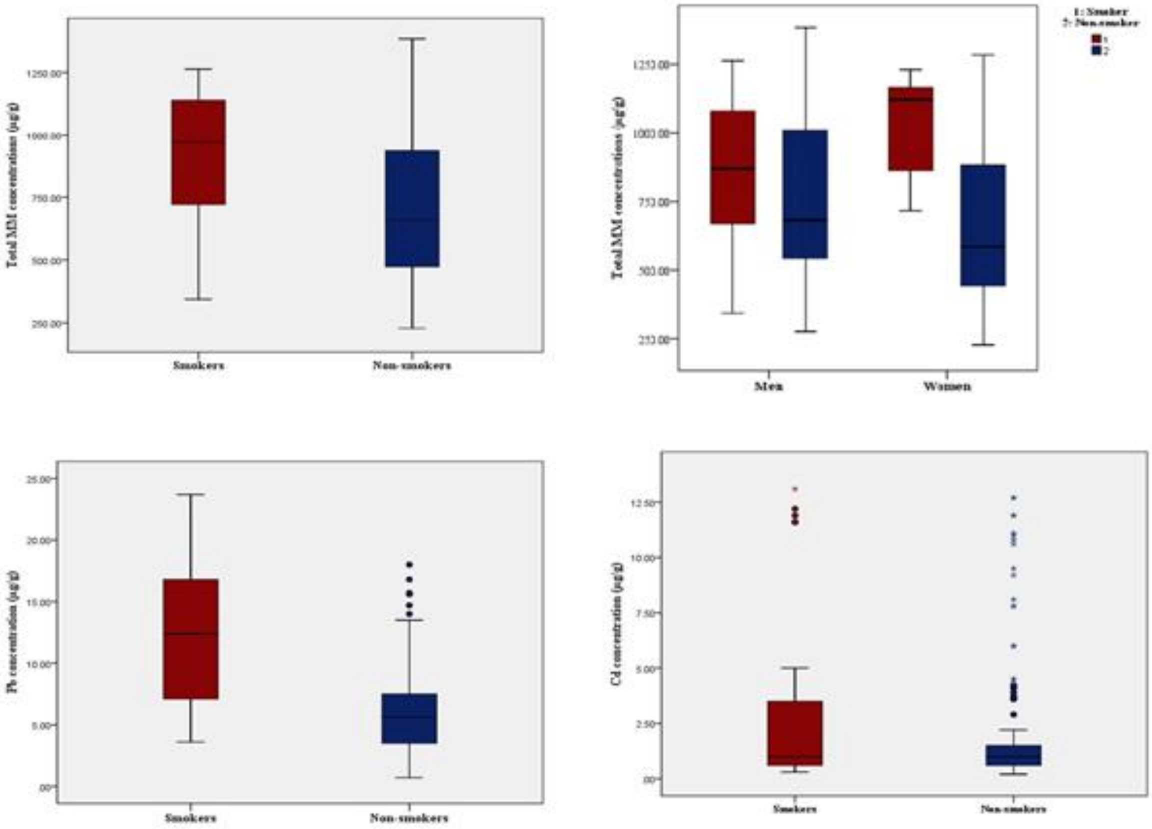

Fig. 2 Sum of metals and metalloids, Pb, and Cd levels in hair of smoker and non-smoker subjects (lg/g)

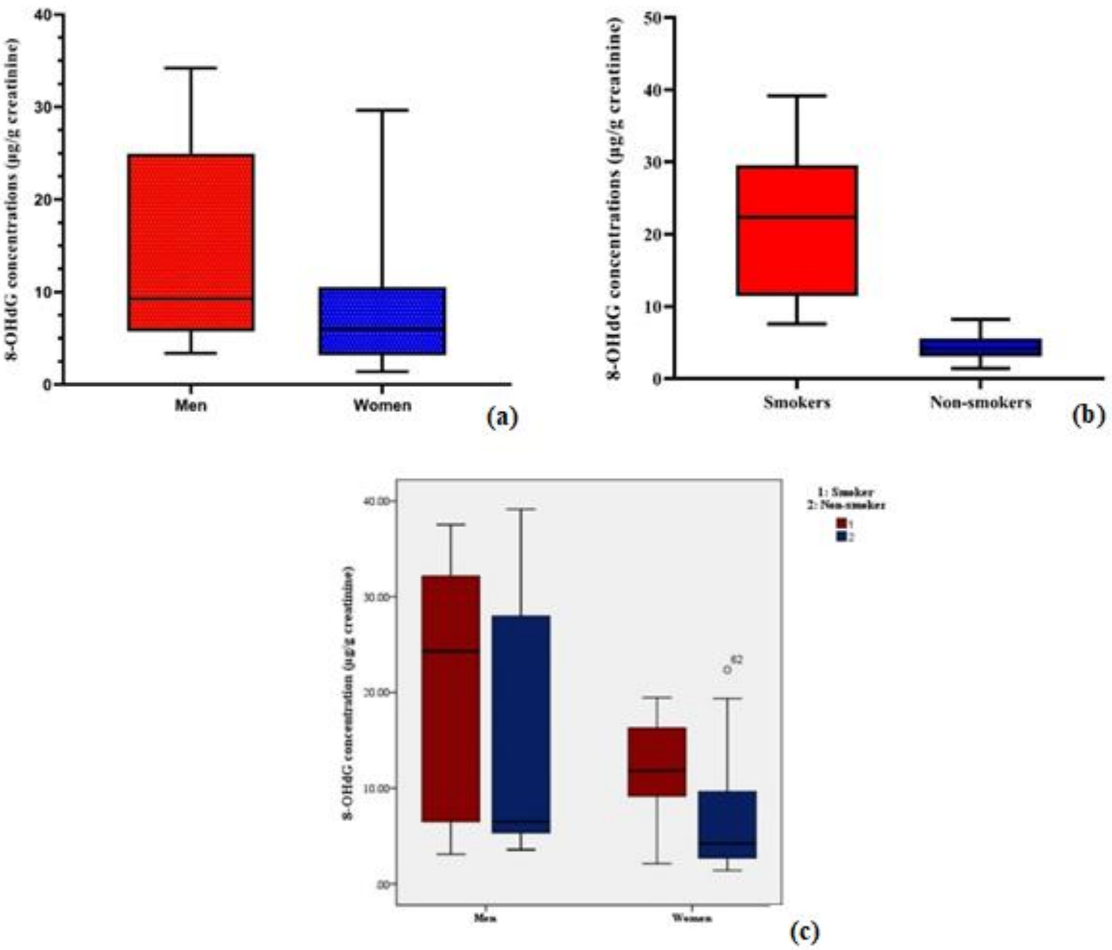

Fig. 3 Comparison of urinary 8-OHdG levels (lg/g creatinine) in a men and women and b smokers and non-smokers

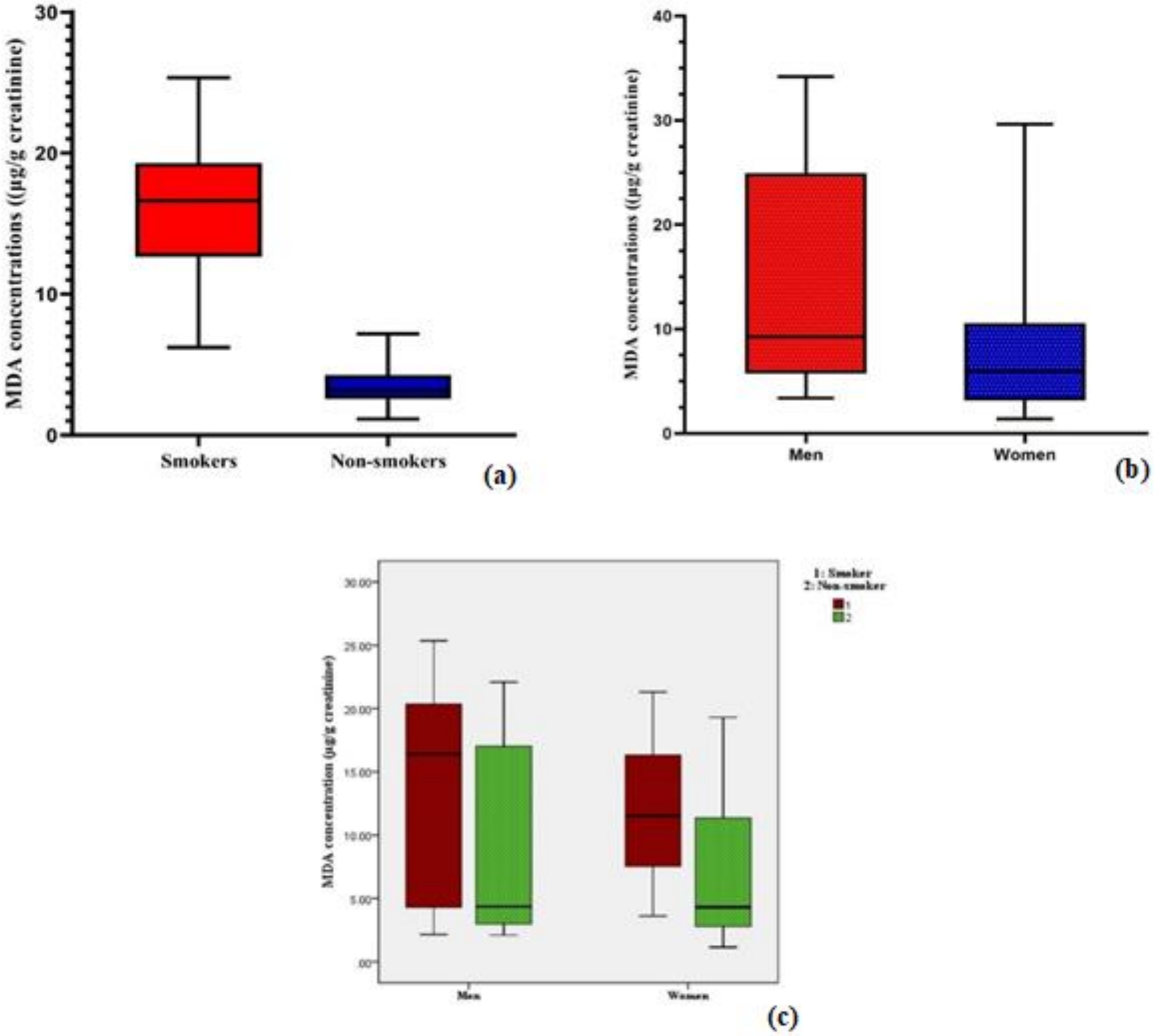

Fig. 4 Comparison of urinary MDA levels (lg/g creatinine) in a smokers and non-smokers and b men and women

Table S1. Microwave digestion program using $HClO_4$ and HNO_3 mixtures

Step	Duration, min	Temperature, ℃	Pressure, bar
1	10	100	80
2	10	130	80
3	10	180	80
4	50	Cooling	-

Table S2. Operating parameters of ICP-OES

Parameter	Setting
Sample uptake time (s)	240
Sample uptake delay (s)	0
Rinse time (s)	45
Stabilization time (s)	45
RF power (kW)	14
frequency of RF generator (MHz)	27
Nebulizer gas flow (L/min)	0.85
Plasma gas flow (L/min)	14.5
Aux gas flow (L/min)	0.9
Sample loop size (mL)	1
Bubble inject time (s)	4.5
Sample injection pump speed (rpm)	30
Pre-wash time (s)	45
Prewash pump speed (rpm)	60 (for 15 sec), 30 (for 30 sec)

Table S3. Limit of Detection and Limit of Quantification for MM in hair

Element	LOD (μg/g)	LOQ (μg/g)
Cd	0.03	0.09
As	0.06	0.18
B	0.03	0.09
Be	0.08	0.24
Co	0.04	0.12
Hg	0.02	0.06
Sb	0.01	0.03
Sn	0.04	0.12
V	0.03	0.09
Al	0.07	0.21
Ba	0.03	0.09
Cr	0.02	0.06
Cu	0.01	0.03
Fe	0.07	0.21
Li	0.02	0.06
Mn	0.03	0.09
Ni	0.04	0.12
Pb	0.01	0.03
Zn	0.01	0.03

Table S4: Correlation analysis between MM concentrations, urinary MDA and 8-OHdG (µg/g creatinine) and age of participants (N=200).

Variables	Total	8-OHdG	MDA	Ag	Al	As	B	Ba	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Si	Sn	Zn	Age
Total	1	.557**	.598**	.493**	.727**	.385**	.820**	.386**	.310**	.622**	.610**	.764**	.434**	.316**	.512**	.526**	.709**	.466**	.611**	-.218*
8-OHdG	.557**	1	.901**	.063	.573**	.174	.443**	.001	.084	.562**	.455**	.571**	.352**	.160	.397**	.405**	.592**	.074	.215*	-.084
MDA	.598**	.901**	1	.287**	.578**	.202*	.462**	.193*	.269**	.508**	.464**	.641**	.331**	.192*	.372**	.343**	.632**	.164	.233*	-.057
Age	-.218*	-.084	-.057	-.204*	-.180*	-.308**	-.163	-.233*	-.106	-.027	-.150	-.300**	-.116	-.104	-.086	-.114	-.239**	-.317**	-.073	1

** p<0.01, * p<0.05 (2-tailed).

Discussion of the observed correlations in view of existing literature

This study shows correlations between two markers of oxidative stress, namely 8-OHdG and MDA (Table S3), and chronic exposure to most metals and metalloids. This includes correlation with Ag, Al, As, B, Ba, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Si and Zn. Sn did not correlate with any of the two biomarkers measured, whereas Ag, As, Ba, Cd, Mn and Sn did not correlate with 8-OHdG. Results of regression analysis adjusted by sociodemographic (sex, age) and environmental differences (smoking status, and teeth filling with amalgam) show consistent associations between MDA and Si and Sn ($p<0.05$), as well as smoking status (cigarette and water-pipe smoking). In addition, MDA was likely associated ($p<0.1$) with Cr, Cu and Fe. On the other hand, 8-OHdG is associated ($p<0.01$) with a larger number of metals measured in hair, such as Al, Ba, Cu, Si, Sn, Zn, in addition to age, cigarette and water-pipe smoking. Cr has shown an association but not statistically significant ($p<0.1$) with 8-OHdG (Table 4).

This is the first study to the best of our knowledge to assess chronic metal exposure and oxidative stress. Published studied have generally focused on assessing the associations among short-term metal exposure (generally measured in urine or blood) and oxidative stress biomarkers. Therefore, caution should be exercised when comparing chronic exposure results with results extracted from studies using metals concentrations measured from urine or blood, which reflect short-term exposures. A detailed comparison with previous studies reporting correlations between short-term exposure to metals and metalloids and biomarkers of oxidative stress can be found hereunder.

Published studied have generally focused on assessing the associations among short-term metal exposure (generally measured in urine or blood) and oxidative stress biomarkers. Therefore, caution should be exercised when comparing chronic exposure results with results extracted from studies using metals concentrations measured from urine or blood, which reflect short-term exposures.

In a study by Lopes et al. (2017), the correlation between blood levels of Pb, Cd and Hg was not statistically significant with MDA in environmentally exposed Brazilian adults, even though the Hg levels were higher in subjects having amalgam fillings. They also reported an inverse correlation between short-term exposure to Hg and 8-OHdG, which contradicts the current observations (Lopes et al., 2017). On the other hand, short-term Hg exposure did not show any correlation with 8-OHdG in a study of college students in Guangzhou (China) (Lu et al., 2016).

Chronic Pb exposure correlates with 8-OHdG and MDA, consistent with results from short-term exposure reported in the general population in Japan (Kimura et al., 2006). On the contrary, Almeida Lopes et al (2017) did not report any correlation among short-term Pb exposure and 8-OHdG concentrations, nor was reported among college students in Guanzhou (China) (Lu et al., 2016). Bortey-Sam et al (2018) reported correlations among short-term Pb exposure and MDA, but not 8-OHdG, whilst this study found correlations between both biomarkers with chronic Pb exposure.

This study shows that chronic exposure to Cr correlates with urinary biomarkers of oxidative stress, 8-OHdG and MDA, consistent with observations with short-term Cr exposure in the general population in Japan (Kimura et al., 2006) and schoolchildren in Taiwan (Wong et al., 2005). In contrast, no correlation was observed for college students in Guangzhou (China) or in Ghana among short-term Cr exposure and

8-OHdG (Bortey-Sam et al., 2018; Lu et al., 2016), whereas, short-term Cr exposure was correlated with MDA in Ghana (Bortey-Sam et al., 2018).

Our findings revealed that chronic exposure to Cd in the general population of Shiraz correlated with MDA levels, but not with 8-OHdG concentrations. Similar findings were observed for MDA in Kumasi (Ghana)(Bortey-Sam et al., 2018), college students in Guangzhou (China) (Lu et al., 2016), participants of the Spanish Hortega cohort study (Domingo-Relloso et al., 2019b), the study population in Wuhan (He et al., 2020) and a study of the general population in Korea (Eom et al., 2017) and Brazil (Lopes et al., 2017). This study shows that chronic As exposure correlates with MDA, but not with 8-OHdG. This is at odds with several studies that report a correlation among short-term As exposure with 8-OHdG in the general population in Japan (Kimura et al., 2006), Wuhan (China) (He et al., 2020), Kumasi (Ghana) (Bortey-Sam et al., 2018), school children in Taiwan (Wong et al., 2005) and college students in Guangzhou (Lu et al., 2016). The correlation with MDA is consistent with observation in Ghanaian population (Bortey-Sam et al., 2018).

Chronic Al and Ni exposure in Shiraz correlates with both MDA and 8-OHdG, consistent with observation of short term exposure with 8-OHdG in the general population in Japan (Kimura et al., 2006) and college students in Guangzhou (Lu et al., 2016). Likewise, Ni correlated with MDA and 8-OHdG in a children study in Guangzhou (Li et al., 2020a). On the other hand, short term Ni exposure correlated with MDA, but not with 8-OHdG in a Ghanaian population (Bortey-Sam et al., 2018).

Chronic Zn exposure correlates with MDA and 8-OHdG, consistent with observations between short-term Zn exposure and MDA in the Hortega study in Spain (Domingo-Relloso et al., 2019b). On the other hand, a study of the general population in Kumasi (Ghana) reported correlation with short-term Zn exposure with MDA but not the 8-OHdG (Bortey-Sam et al., 2018).

This study found correlations among chronic exposure to Cu and Al with 8-OHdG, whilst no correlation was found for Fe or Sn, consistent with correlations found among short-term exposure in college students in Guangzhou (Lu et al., 2016). On the other hand, a study with children in Guangzhou found significant associations between Cu and Sn with MDA and 8-OHdG (Li et al., 2020a). Mn chronic exposure correlates with MDA, but not with 8-OHdG, consistent with the observation in a Ghanaian population for short-term Mn exposures (Bortey-Sam et al., 2018). On the other hand, the Ghanaian study is consistent with our results for Cu, for which correlations among short-term Cu exposure and MDA and 8-OHdG were reported (Bortey-Sam et al., 2018).

In the study by Bortey-Sam et al (2018), metals and metalloids levels in the urine of subjects living in urban areas showed a similar significant correlation ($p<0.01$) with MDA as the correlation observed in adults in a highly urbanized city like Shiraz. However, only Cu was statistically correlated with 8-OHdG in the Ghanaian study. In contrast, in this study, 8-OHdG correlates statistically with Al, B, Cr, Cu, Fe, Hg, Ni, Pb and Si ($p<0.01$) and with Zn ($p<0.05$).

The differences observed between this study and previous studies could be attributed to the fact that this study assesses chronic metal exposure, whilst the other studies assess short-term exposures. Differences could be also attributed to the different matrix from which the metals and metalloids were extracted, which could be an important determinant.