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Biomonitoring of cadmium, chromium, nickel and arsenic in general population living near mining and active industrial areas in Southern Tunisia

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Abstract The human health impact of the historic and current mining and industrial activities in Tunisia is not known. This study assessed the exposure to metals in the population of Southern Tunisia, using biomonitoring. The aim of this pilot study was to evaluate metal exposure on 350 participants living near mining and active industrial areas in the South of Tunisia. Blood specimens were analyzed for metals (Cd, Cr, As, and Ni) by Atomic Absorption Spectrometer equipped with Zeeman background correction and AS-800 auto sampler by graphite furnace and graphite tubes with integrated L'vov platform. The sample population was classified according to different age groups, sex, smoking habit, sea food and water drinking consumption, occupational exposure, amalgam fillings and place of residence. The blood As, Cd, Cr and Ni values expressed as mean±SD were 1.56 ±2.49, 0.74±1.15, 35.04±26.02 and 30.56±29.96 μg/l, respectively. Blood Cd and Ni levels in smokers were 2 and 1.2 times, respectively, higher than in non-smokers. Blood Cd levels increase significantly with age (p=0.002). As, Cd and Ni were significantly correlated with gender and age (p<0.05). Cd level in blood samples of subjects occupationally exposed was 1.3 times higher than that of non-exposed. Blood metals were not significantly affected by amalgam fillings, place of living and sea food and drinking water consumption. This first biomonitoring study of metal exposure in the South of Tunisia reveals a substantial exposure to several metals. The pathways of exposure and health significance of these findings need to be further investigated.

Keywords Human biomonitoring · Blood · Arsenic · Cadmium · Chromium · Nickel

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Introduction

During the preindustrial period of human civilization, a heavy load of toxic metals being discharged into the biosphere was generally typical for endemic regions only. Environmental exposure to metals remains a worldwide public health problem and mining is a significant contributor to metal pollution in air, soil and water (EPA 2007; Greenberg et al. 2003). Currently, the majority of the world's population is exposed to toxic chemical elements from increasing anthropogenic pollution. Toxic metals present a health threat for

populations living near mining and hazardous industrial objects.

In the case of Tunisia, published data about environment heavy metal contamination showed a relatively high content of these elements (Moldenhauer et al. 2008; Achiba et al. 2009; Klay et al. 2010; Gargouri et al. 2010; Ghannem et al. 2011; Houda et al. 2011; Serbaji et al. 2012). The South of Tunisia is considered as a relatively polluted area. In fact, elevated concentrations of heavy metals in the Tunisia country environment can originate from a variety of industrial processes, including metal plating, fertilizer production, mining, metallurgy, battery manufacturing and textile dyeing, among others (Zaghden et al. 2007; Barhoumi et al. 2009; Messaoudi et al. 2009; Eloussaief and Benzina 2010; Gannouni et al. 2011; Alaya-Ltifi et al. 2012; Garnit et al. 2012). Moreover, a phosphate treatment plant is now being developed along the coasts of Southeastern Tunisia, exposing coastal waters and marine organisms to increasing contamination (Zairi and Rouis 1999; Hamza-Chaffai et al. 2000, 2003). Crude phosphate mining has started in Tunisia in four regions of Gafsa City since 1899 (in Metlaoui, Redeyef, Moulares and Mdhilla), whereas phosphate treatment activity has been existing in Sfax City since 1952 (Thyna and Skhira) and in Ghannouch-Gabes City since 1972 (TPI: http://www.gct.com.tn/). During the first 20 years following the installation of these activities, 50 million tons of phosphogypsum have been released in the environment (Guillaumont et al. 1995). Heavy metals and metalloids, especially Cd, Cr, Ni and As, are waste products of industrial processes associated with smelting operations of phosphates (Sarbaji 2000; Azri et al. 2002a, b). In addition, South of Tunisia receives heavy metals from other sources including atmospheric fallout deposition (Sarbaji 2000) and outfall of untreated domestic sewage and wastewater (Tarchouna-Gharbi et al. 2010; Belaid et al. 2012). Moreover, the waters deriving from the treatment of a mix of industrial and domestic effluents have been used in Sfax City for forage crop irrigation for 17 years. The long-term use of this wastewater often results in the build-up of metals content in soils (Tarchouna-Gharbi et al. 2010; Belaid et al. 2012). As a matter of fact, the health of inhabitants living in these areas and chronically exposed to such contaminants for a long time could be affected. Therefore, the global burden of exposure to potentially heavy metals is unknown, and information concerning health risks to human exposed to metals in the Tunisia Country environment is scarce and remains an area to be studied.

In this context, several recent Tunisian studies have been conducted to document the contamination levels of heavy metals in various environmental samples such as sediment (Kharroubi et al. 2012; Serbaji et al. 2012), sedimentary phosphatic samples (Garnit et al. 2012), fish and mollusks (Barhoumi et al. 2009; Bellassoued et al. 2012) and plants (Kachout et al. 2012). However, only one study (of our research group) is available regarding the impact of arsenic on the population of Tunisia (Feki-Tounsi et al. 2013a, b). On the other hand, no data is available regarding the impact of Cd, Ni and Cr on humans in Tunisia. Therefore, this study represents the first human biomonitoring of Cd, Ni and Cr exposure in the Tunisian population. The present study aimed to determine the levels of Cd, Ni, Cr and As in whole blood of randomly selected Tunisian residents; assess the significance of the metal exposure and parameters including place of residence, gender and age; and compare the determined metal/metalloid reference values of Tunisian residents with those from other countries.

Materials and methods

Study areas and subjects

In the present study, 350 healthy unrelated individuals (257 men and 93 women) from Tunisia were enrolled after approval from the Ethics Committee of the Institute. In this essentially exploratory study, the sampling of subjects was not performed according to a predefined strategy that would provide an epidemiologically valid representation of the exposure of the population of the area. Rather, the choice of study areas was based on anticipated degrees of high or low exposure, as well as on pragmatic considerations, such as accessibility. Figure 1 shows localization of the study areas in South Tunisia and distribution map of mines and industrial types. A total of 265 subjects lived in areas situated very close to mines or industrial activities (0.5–10 km). Areas were located more than 10 km from mines or industrial activities was supposed to be less intense, even in subjects (n=85) from these areas with supposedly low exposure. In these areas, local residents were simply approached and asked (after receiving background explanation about the study) if they were willing to provide blood samples and respond to a simple questionnaire about social habits and health problems of each individual. Subjects were interviewed to collect detailed information on their demographic (age, gender, and

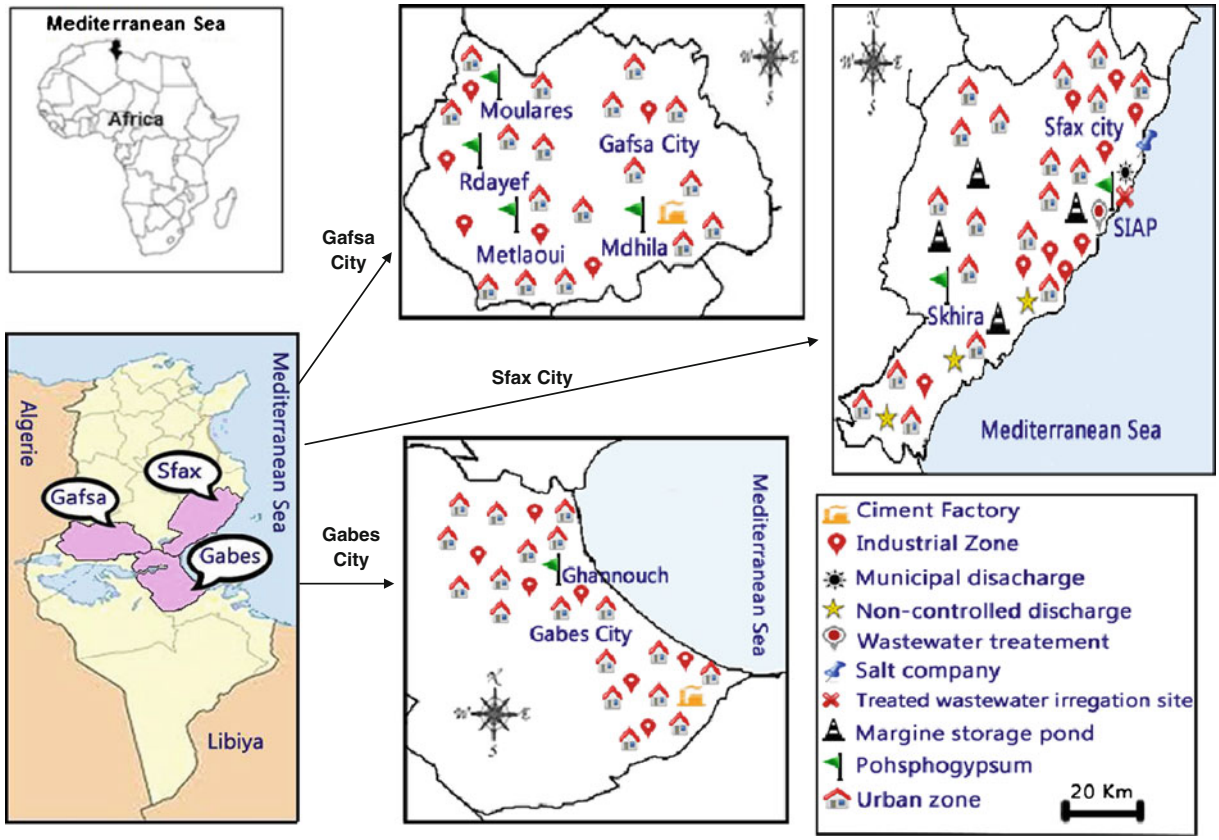

Fig. 1 Localization of the study areas in South of Tunisia and distribution map of participants and mines and industrial types

residential history), smoking habit, amalgam fillings, occupation and occupational exposure, as well as other lifestyle characteristics including water drinking and fish consumption. Lifetime consumption of tobacco smoking was collected. The average number of cigarettes smoked per day and the total number of years of smoking were used to calculate the cumulative smoking dose as "pack-years" (PY=[cigarettes per day/20]×years smoked). Lifetime of occupational exposure was also collected. The following occupational exposures were assessed (the number of subject ever exposed is given in parentheses): builders (23), painters (18), farmers (18), phosphogypsum (8), wood fumes (6), and others (16).

Positive responses were obtained from more than 98 % of those approached. Whole blood samples where As, Cr, Cd and Ni were measured, respectively, were also collected from all the selected subjects who lived in these areas. Because (1) the important industrial and mining activities which caused environmental pollution (Serbaji et al. 2012) and (2) the high contamination of fish species were collected from the Sfax and Gabès coasts (Barhoumi et al. 2009; Bellassoued et al. 2012), we have tried to study the association between Cd, Ni, Cr and As blood levels in the general population living in these polluted areas and fish consumption and tap water drinking. Additionally, because of the high number of subjects continuously exposed to chemical compounds (such as heavy metals) via tobacco smoking, occupational exposure and dental filling, we have also tried to determine the levels of these four metals in whole blood of the selected Tunisian subjects.

Collection of blood samples

For all studied subjects, 3 ml venous blood samples were drawn by nurses, stored into BD Vacutainer tubes with ethylenediaminetetraacetic acid (EDTA) anticoagulant. Samples were immediately transported in a cool box to the laboratory and then stored in a freezer at −20 °C for further analysis. Two metals were detected in these blood samples: Cr and Ni. To analyze these two metals, the blood samples were first digested with

66 % HNO_3 (Sigma-Aldrich, Germany) at 80 °C with subsequent heating to a soft boil for 8 h. After mineralization, the solution was diluted with doubly distilled water to a final volume of 5 ml. It is clear that chemical digestion in appropriate conditions is required before blood analysis. Mineralization in a closed system — where the contamination problems are significantly reduced, can be recommended for such samples.

Determination of metals

Levels of metal compounds were determined by a Perkin-Elmer Analyst 800 Atomic Absorption Spectrometer (Perkin Elmer, Norwalk, USA) equipped with Zeeman background correction and an AS-800 auto sampler by graphite furnace and graphite tubes with integrated L'vov platform (Perkin Elmer). Appropriate matrix modifiers were used for the selected heavy metal studied and prepared in 0.2 % (v/v) nitric acid and 0.1 % Triton X-100. The limits of detection (LOD) were 0.03, 0.19 and 0.24 μg/l for Cd, Cr and Ni, respectively. Calibration graphs were linear until 7, 30 and 15 μg/l (for more details, see Olmedo et al. 2010). For arsenic, a direct flow-injection atomic absorption spectrometric technique (FI-HGAAS) was used to measure the levels of total arsenic. The arsenic contained in standard solutions (calibration curve 0, 0.5, 1.5 and 2.5 μg/l) or blood samples were reduced to As^{3+} prior to analysis with a mixture of potassium iodide and ascorbic acid. To 1 ml of the sample or reference solution, 1 ml of concentrated HCl and 1 ml of 5 % (w/v) KI-ascorbic acid were added. After 45 min at room temperature, the mixture was diluted to 10 ml with water.

The reducing agent was an aqueous solution of 0.2 % (w/v) $NaBH_4$ in a 0.05 % (w/v) NaOH solution freshly prepared and filtered. Standard addition was required. The parameters for As were wavelength of 193.7 nm, integration time of 15 s, smoothing of 19 points or 0.5 s and temperature cell of 900 °C. An electrode less discharge lamp was used. The limit of detection (LOD) for this metalloid was 0.03 μg/l, and calibration graphs were linear until 5 μg/l (range of linearity from 0 to 5 μg/l) (Gil et al. 2006). All samples were analyzed at least twice, and in all the cases the concordance between both repetitions was not over 15 %.

Reference materials

The analytical method was controlled by using external certified reference materials (CRM). Reference samples for whole blood (three levels, refs. 201505, 201605 and 201705) were supplied by Seronorm (Billingstad, Norway). As they were supplied freeze dried, they were reconstituted by adding 5 ml of water and were run during the validation of procedures. The accuracy of CRM Seronorm As, Cd, Cr and Ni were 1.33 %, 0.45 %, 0.28 % and 5.26 %, respectively (Gil et al. 2006; Olmedo et al. 2010).

Statistical analysis

The basic statistics of data relating to 350 subjects included percentiles, mean, standard deviation (SD) and range. Differences in mean levels of metal compounds as well as the potential influence of classical confounders (tobacco, water drinking, amalgam fillings, fish consumption and gender) on these levels were assessed by using the Mann–Whitney test.

The blood metals means, SD, medians and quartiles were calculated. Statistical comparisons between age groups were carried out with one-way analysis of variance (ANOVA). Statistical calculations were performed by the statistics software SPSS for Windows, Version 13.0. A probability value (p) less than 0.05 was considered as significant.

Results

The demographic data of the subjects is presented in Table 1. Of the total, 73.4 % were males and 26.6 were females. Their age ranged from 17 to 95 years, with a mean±SD of 53.75±16.81. Regarding residence locations, 55.4 % of participants were from Sfax City, while 44.6 % were from the South of Tunisia, especially Gafsa (20.8 %) and Gabes (22.7 %) cities (Fig. 1). Three-quarters of the subjects lived in areas situated very close to mines and/or industrial activities (0.5–10 km). Overall, 71.7 % of participant consumed fish more than one time per week. The main sources of drinking water for our population are tap water and rain water. A total of 147 (42.0 %) subjects consumed tap water; 17.7 % of participants were current smokers and reported more than 20 PY of smoking. Moreover, 25.4 % of subjects were occupationally exposed and most (13.7 %) of them were exposed for more than 15 years. The subjects were classified into six categories according to occupational exposure (5.14 %, 5.14 %, 6.57 %, 1.71 %, 2.28 % and 4.57 % were

Table 1 General characteristics of the study population

Characteristic	*n* (%)
Gender	
Males	257 (73.4)
Females	93 (26.6)
Age	
Mean±SD	53.7±16.8
Min	17
Max	95
Place of residence	
Sfax	149 (55.4)
Outside	156 (44.6)
Residential areas (km) from mining and industrial activities	
0.5–10	265 (75.7)
>10	85 (24.3)
Dental implants	
No	302 (86.3)
Yes	48 (13.7)
Smoking (PY)	
0	233 (66.6)
≤20	55(15.7)
>20	62 (17.7)
Occupational exposure (Y)	
0	261 (74.6)
≤15	41 (11.7)
>15	48 (13.7)
Occupation	
Exposure to pesticides (Farmers)	18 (5.1)
Exposure to dyes (Painters)	18 (5.1)
Exposure to cement dust (Builders)	23 (6.6)
Exposure to wood fumes	6 (1.7)
Exposure to phosphogypsum dust	8 (2.3)
Other occupation	16 (4.6)
Water drinking	
Rain	203 (58.0)
Tap water	147 (42.0)
Fish consumption	
No	2 (0.6)
Yes 1/month	97 (27.7)
>1/month	251 (71.7)

exposed to dyes, pesticides, cement dust, wood fumes, phosphogypsum dust and other occupational exposure, respectively).

The distribution of As, Cd, Cr and Ni levels in whole blood in our study population are shown in Table 2. Because values did not follow a normal distribution the medians, ranges and 5–95th percentiles of the data for each element are presented in addition to mean and SD. As, Cd, Cr and Ni values in our general population were 1.56, 0.74, 35.04 and 30.56 μg/l, respectively. Mean values are given to make comparisons possible with other studies.

Table 3 presents data for the distribution of blood As, Cd, Cr and Ni levels in relation to gender. No significant differences were found between males and females for metal levels in blood sample analyzed.

The distribution of blood levels of these four elements in relation to age are shown in Table 4. Subjects were classified into seven groups by age. Blood Cd levels were found to increase significantly with age (p=0.002). Contrary to Cd, Ni levels were found to decrease significantly with age (p<0.001). We found a positive Spearman's correlation coefficient between blood Cd and age (p<0.001) (Table 5), which means that blood Cd accumulates with age. However, no association was found between age and As and Cr (p>0.05) (Table 4). Spearman's correlation for categorical variables shows that As, Cd and Ni were significantly correlated with gender and age (p<0.05).

The magnitude of the correlation between metal concentrations in human whole blood and potential confounders were evaluated using Spearman's correlation for categorical variables (Table 5). As and Cd were significantly and positively correlated with PY number (r=0.190 and 0.304, respectively, p<0.001). Moreover, a significant correlation was seen with the seafood consumption rate for Cr and Ni (r=−0.115 and −0.117, respectively, p<0.05). A positive Spearman's correlation coefficient between amalgam fillings and blood Cr and Ni (r=0.134 and 0.137, respectively; p<0.05) was observed. Cd levels in blood samples had a stronger correlation with occupational exposure (r=0.106, p<0.05). Furthermore, the correlation between metal concentrations in human whole blood showed that the metals As–Cd, Cr–As and Ni–Cr were statistically correlated (p<0.05). There is any relationship between blood toxic element concentrations and the place of residence and water drinking in our population (Table 5).

Table 6 presents data for the bivariate analyses among As, Cd, Cr and Ni concentrations in blood as well as the major potential confounders in the study population. A positive association of Cd and Ni levels with smoking was found (comparison smoker with non-smoker) (p<0.001 and p<0.05, respectively). Moreover, blood Cd levels

Table 2 As, Cd, Cr and Ni levels (μg/l) in blood samples from the study population

Metals	Percentiles				Mean±SD	Range	Median
	5th	50th	75th	95th			
As	0.01	0.17	2.28	6.79	1.56±2.49	0.02–18.15	0.17
Cd	0.01	0.39	1.19	2.31	0.74±1.15	0.02–12.40	0.39
Cr	1.29	29.41	44.50	84.02	35.04±26.02	0. 42–108.00	29.41
Ni	0.11	22.99	49.58	83.03	30.56±29.96	0.30–97.96	22.99

SD standard deviation

among subjects exposed occupationally (0.88 μg/l) were significantly higher than those non-exposed (0.69 μg/l). However, no association between metal blood levels and amalgam filling, water drinking and residential areas of in Tunisian population was found (Table 6).

Analysis of metal levels according to the types of some occupations is presented in Table 7. Very high blood As levels among subjects exposed occupationally to pesticides (3.57 μg/l) and wood fumes (3.07 μg/l) are shown; they were also significantly higher than those non-exposed (1.53 μg/l) ($p<0.001$). Moreover, blood Cd levels among subjects exposed occupationally to dyes (1.47 μg/l) were significantly higher than those of non-exposed subjects (0.69 μg/l) ($p<0.01$). Additionally, very high blood levels of Cr among builders exposed to cement dust were found (60.14 μg/l) in comparison with those of non-exposed subjects (35.37 μg/l) ($p<0.001$).

Discussion

So far, the overall measure of exposure to multiple metals in the Tunisian population has not been well characterized, and data concerning metals contamination are insufficient. Our study is a pioneer investigation which provides much needed information regarding human exposure to Cd, Cr, Ni and As in Tunisia. In contrast with previous heavy metals monitoring Tunisian studies, our study was directly based on measurements of blood metal, which represents a measure of internal dose and not on environmental monitoring as metals in the sediment or in drinking water. Blood was validated as a biomarker of recent and chronic exposure to metals (Morton and Dunnette 1994; Gil and Pla 2001; Hall et al. 2006). Consequently, whole blood is the most widely used and accepted matrices for biomonitoring heavy metal exposure in environmental toxicology (Wilhelm et al. 2004; Gil and Pla 2001; Gil and Hernández 2009; Wang et al. 2011). Therefore, blood metal levels represent the most human biomonitoring of As, Cd, Ni and Cr exposure in our study population.

Blood metal levels in relation to gender and age

In the present study, blood As, Cd, Cr and Ni levels in our general population were 1.56, 0.74, 35.04 and 30.56 μg/l, respectively (Table 2). No significant differences were found between males and females for As, Cd, Cr and Ni levels in the blood samples analyzed (Table 3). Our results for Cd blood are an agreement

Table 3 Blood Cd, As, Cr and Ni levels (μg/l) in relation to gender

	Males				Females			
	Percentiles 5–95th	Mean±SD	Range	Median	Percentiles 5–95th	Mean±SD	Range	Median
As	0.00–6.19	1.60±2.48	18.15	0.39	0.00–7.63	1.46±2.52	13.10	0.00
Cd	0.00–2.50	0.80±1.18	12.40	0.42	0.00–1.87	0.57±1.07	8.72	0.08
Cr	1.44–80.75	34.00±25.91	108.00	27.62	1.00–97.36	37.92±26.90	100.20	33.60
Ni	0.00–89.38	29.73±31.72	97.96	17.28	0.39–74.72	32.84±24.46	79.40	32.66

SD standard deviation

Table 4 Blood Cd, As, Cr and Ni levels (mean±SD, μg/l) in relation to age

Age (years)	N	As	Cd	Cr	Ni
<30	34	1.33±2.73	0.27±0.44	32.98±28.09	44.04±37.41
31–40	49	1.48±2.24	0.59±0.82	34.42±31.81	36.88±32.50
41–50	71	1.31±2.62	0.66±1.17	34.47±21.95	34.98±28.69
51–60	73	1.40±2.27	0.66±0.97	43.09±30.55	35.34±25.52
61–70	57	2.13±2.96	0.86±1.09	32.02±22.44	24.34±29.97
71–80	48	1.90±2.45	0.96±0.85	34.34±21.93	13.36±19.99
>80	18	1.14±1.10	1.67±2.81	21.95±16.19	16.23±25.93
p value		NS	0.002	NS	< 0.001

NS non-significant ($p>0.05$), *SD* standard deviation

with those observed by Gil et al. (2011), Nkolika and Benedict (2009) and Mortada et al. (2002) in Spanish, Nigerian and Egyptian populations, respectively. However, Liu et al. (2001), Huang et al. (2011) and Forte et al. (2011) reported that the Cd value was significantly higher in females than in males in Japanese, Korean and Italian populations, respectively.

We found a positive Spearman's correlation coefficient between blood Cd and age ($p<0.001$) (Table 5), which means blood Cd accumulates with age ($p=0.002$) (Table 4) and confirms that blood Cd traduces both recent and chronic exposures (Jarup et al. 1983, 1997). Our findings are in agreement with several other published data (Forte et al. 2011; Huang et al. 2011; Bjermo et al. 2013). This is a reasonable finding in view of this metal's well-known tendency to accumulate in the human body with increasing age (Friberg et al. 1974). Cd is not biotransformed. It is the most toxic elements, and many accumulating evidence points to negative health effects from cumulative lower level exposure (Hu 2002). These negative effects on human's health are due to its low excretion rate (half-life in tissue as long as 15–20 years and in blood is about 2.5 months) (Jin et al. 1998) and its accumulation in the organism. Hence, we can explain that the high Cd levels in the blood among older subjects can be due to their cumulative effects. Contrary to Cd, Ni blood levels were found to decrease with age ($p<0.001$). While cadmium is a ubiquitous environmental pollutant whose concentrations in blood increase with the age as consistently found in the literature (Forte et al. 2011; Huang et al. 2011; Bjermo et al. 2013), Ni concentrations in blood are mainly affected by occupational exposure and decline rapidly when exposure is reduced or stopped (Von Burg 1997). Accordingly, it could be possible to observe that nickel blood levels decrease with age since a 40-year-old individual occupationally exposed to nickel could display higher nickel blood concentrations than another 60-year-old individual who is non-exposed.

Comparison between values in blood of metals selected in this study and reported in the literature

Mean values of our study are given to make comparisons possible with other studies. As, Cd, Cr and Ni are

Table 5 Spearman correlation analyses of heavy metal concentrations in blood sample with potential confounders

	As	Cd	Cr	Ni
Cd	0.136*			
Cr	0.115*			
Ni			0.290**	
Gender	−0.113*	−0.153**		0.112*
Age	0.194**	0.264**		−0.318**
Place of residence				
Smoking [a]	0.190**	0.304**		
Occupational exposure [b]	0.106*			
Amalgam fillings			0.134*	0.137*
Water drinking				
Seafood consumption [c]			−0.115*	−0.117*

[a]Tobacco smoking (pack-years)
[b]Occupational exposure (years)
[c]Consumption/month
*$p<0.05$; **$p<0.001$

Table 6 Associations between heavy metal concentrations (mean±SD, μg/l) in blood samples and potential confounders

	Metals (mean ± SD, μg/l)			
	As	Cd	Cr	Ni
Smoking				
No	1.40±2.43	0.57±1.07	36.55±27.90	28.19±25.76
Yes	1.88±2.60	1.07±1.40**	32.02±22.21	35.26±36.59*
Amalgam fillings				
No	1.57±2.55	0.76±1.18	33.15±23.75	29.41±30.04
Yes	1.21±2.14	0.59±0.99	46.87±37.04	37.79±28.68
Water Drinking				
Mineral	1.59±2.54	0.73±1.30	32.55±23.09	29.74±28.33
Tap	1.54±2.46	0.74±1.04	36.84±28.15	31.15±31.14
Fish consumption				
1/month	2.23±3.20	0.66±0.72	38.70±27.84	32.15±29.29
>1/month	1.28±2.08	0.77±1.28	33.43±25.46	29.62±30.14
Place of residence				
Sfax	1.47±2.50	0.60±0.73	35.54±27.23	30.74±29.51
South Tunisia	1.67±2.48	0.91±1.51	34.74±24.91	30.33±30.60
Residential areas (km) from mining and industrial activities				
0.5–10	1.57±2.56	0.90±1.12	31.86±21.95	28.46±27.92
>10	1.59±2.30	0.69±1.16	38.15±50.93	31.15±30.58
Occupational exposure				
No	1.53±2.52	0.69±0.98	35.37±25.37	30.35±25.36
Yes	1.66±2.40	0.88±1.55*	33.87±28.74	31.51±33.35

SD standard deviation

*$p<0.05$; **$p<0.001$

the metal compounds more widely studied in blood although large variations in their levels have been reported (Table 8). Mean concentrations are between 0.11 and 8.78 μg/l, 0.15 and 9.81 μg/l, 0.44 and 248 μg/l and 0.06 and 10.4 μg/l for As, Cd, Cr and Ni, respectively. The highest concentration of blood Cd

Table 7 Blood metal variation with types of occupational exposure

Occupational exposure	Metals (mean±SD, μg/l)			
	As	Cd	Cr	Ni
Not occupationally exposed	1.53±2.52	0.69±0.98	35.37±25.37	30.35±25.36
Exposure to pesticides (Farmers)	3.57±4.97***	0.82±0.95	24.44±20.81	33.01±34.76
Exposure to dyes (Painters)	1.36±2.20	1.47±2.83**	41.98±25.39	33.12±32.71
Exposure to cement dust (Builders)	1.53±1.55	0.56±0.70	60.14±127.33***	30.18±36.24
Exposure to wood fumes	3.07±7.38***	0.32±0.41	15.34±14.40	5.76±5.57
Exposure to phosphogypsum dust	2.51±3.56	0.63±0.73	32.61±15.07	31.48±22.02
Other occupational exposure	2.57±3.11*	0.68±0.74	38.21±21.88	42.77±24.96**

SD standard deviation

*$p<0.05$, **$p<0.01$ and ***$p<0.001$: subjects occupationally exposed compared with non-exposed

Table 8 Values in blood of As, Cd, Cr and Ni (mean±SD, μg/l) selected in this study and reported in the literature

Country	Note	*n*	As	Cd	Cr	Ni	Reference
Tunisia	General population	350	1.60±2.48	0.80±1.18	34.00±25.91	29.73±31.72	This study
Pakistan	Psoriasis patients living in the vicinity of cement factory						Afridi et al. 2011a
	Male	49		9.81±2.54	238.0±16.9	4.72±1.36	
	Female	55		9.35±1.39	187.0±13.3	6.82±2.12	
	Controls exposed						
	Male			3.3±0.5	83.5±6.7	1.3±0.4	
	Female			3.1±1.4	79.6±6.6	1.6±0.5	
Pakistan	Paralysed steel mill workers	42	4.07±0.38	6.9±0.90			Afridi et al. 2011b
	Exposed referent	62	2.56±0.30	5.16±0.63			
Pakistan	Smoker hypertensive patients	297		8.9±1.3		5.85±1.2	Afridi et al. 2010
	Controls	186		4.24±1.27		1.95±0.56	
India	Gallbladder cancer	9	0.13±0.03		1.02±0.20	1.74±0.40	Rautray et al. 2009
	Controls	10	0.11±0.03		1.07±0.20	1.13±0.30	
Sri Lanka	Cases with endometriosis	50		0.7 (0.7–0.9)		2.6 (1.9–3.3)	Silva et al. 2013
	Controls	50		0.8 (0.6–1.0)		0.8 (0.7–0.9)	
France	Mild steel welders (σ)	30			1.8 (2.3)		Edmé et al. 1997
	Stainless steel welders (σ)	112			3.6 (2.3)		
France	French cadavers (5th–95th percentile)	54				4.13 (1.21–11.50)	Goullé et al. 2007
France	French population (nmol/l)	–			2.01±0.77		Chappuis et al. 1992
Germany	Healthy subjects (range)	73		0.99 (0.40–20)			Jung et al. 1993
Germany	Stainless steel welders, After work (range)	7			2.0 (0.4–8.3)	0.8 (0.8–3.3)	Stridsklev et al. 2004
Germany	Stainless steel welders, After work (range)	7			0.4 (0.3–1.3)	0.9 (0.8–3.3)	Stridsklev et al. 2007
Spain	General population: All	178		0.49±0.61	1.31±3.01	0.96±3.68	Gil et al. 2011
	Male	–		0.49±0.61	1.31±3.05	0.80±2.95	
	Female	–		0.44±0.67	1.42±1.99	4.22±10.94	
	Smoker	–		0.89 ±0.69	1.53±4.22	1.00±3.90	
	Non-smoker	–		0.15±0.18	1.14±1.28	0.94±3.56	
Spain	Healthy subjects (nmol/l)	243			3.01 ±1.45		Torra et al. 1999

Table 8 (continued)

Country	Note	*n*	As	Cd	Cr	Ni	Reference
UK	Patients after metal-on-metal hip arthroplasty (median, nmol/l)	199			40.3		Newton et al. 2012
UK	Stainless steel venipuncture needles	10			2.43±1.55	10.40±4.69	Hodnett et al. 2012
Serbia	Smoker/non exposed (range)	123				0.01–0.42	Stojanović et al. 2004
	Non-smoker/non exposed (range)	123				0.01–0.26	
Sweden	Subjects with ventricular hypertrophy (nmol/l)	1016			11.80	90.60	Lind et al. 2012
Sweden	Males	300		0.39			Wennberg et al. 2006
	Females	299		0.56			
Norway	(nmol/l, range)	–			1–3		Brune et al. 1993
Japan	High-exposure area (GM)	563		24.10			Wang et al. 2011
	Low-exposure area (GM)	589		1.87			
Taiwan	Oral cancer patients	79			0.79 (0.26)		Chiang et al. 2011
	Controls	641			0.44 (0.39)		
Taiwan	Oral cancer patients	101			0.83±0.31	1.61±0.58	Yuan et al. 2011
	Smokers				0.81±0.30	1.62±0.60	
	Non-smokers				1.04±0.32	1.45±0.33	
	Controls	104			0.61±0.29	1.00±0.73	
	Smokers				0.64±0.34	1.01±0.60	
	Non-smokers				0.57±0.21	0.98±0.89	
Taiwan	Workers in electroplating factories (range)	521			0.067–6.47		Chang et al. 2006
Korea	Residents near municipal waste incinerators	841		1.7			Lee et al. 2012
Korea	Residents near abandoned metal mines (GM)	248		0.80			Kim et al. 2011
Belgium	Polluted area (GM)	1679		0.36			Schroijen et al. 2008
Belgium	General population (range)	68		0.69 (0.10–1.90)			Roels et al. 1993
Russia	Welders	38			10.6±2.2	10.4±3.2	Ivanenko et al. 2012
	Controls	24			4.3±1.3	4.2±1.5	
Australia	Patients with hip resurfacing prosthesis (nmol/l, median)	29			23.13		Walter et al. 2008
Denmark	Danish population (nmol/l)	23			3.5±2.0		Christensen et al. 1992
	Danish population (nmol/l) (range)	35				13.6–32.4	Christensen and Kirchhoff 1985

Table 8 (continued)

Country	Note	*n*	As	Cd	Cr	Ni	Reference
	Danish population (range)	–			0.12–0.34		Christensen et al. 1993
Canada	Pregnant Canadian women (GM)	93		0.46		2.1	Foster et al. 2012
	Pregnant foreign women (GM)	20		0.59		2.4	
Canada	Arctic populations (GM; nmol/l): All	493		26.0			Fontaine et al. 2008
	Males	209		25.5			
	Females	284		26.5			
Brazil	Orthodontic patients allergic to nickel	96				0.1±0.1	Pazzini et al. 2009
USA		39				1.26±0.33	Sunderman et al. 1984
Italy	Central Italy: All (range)	434		0.1–3.4			
	Smoker (median)	163		1.1			dell'Omo et al. 1999
	Non-smoker (median)	271		0.5			
Italy	Rome (GM): All	252	0.82	0.26	0.40	0.92	Pino et al. 2012
	Males	112	0.84	0.25	0.41	0.96	
	Females	140	0.80	0.27	0.39	0.99	
Italy	Rome (GM)	215		0.53			Forte et al. 2011
Italy	Residents near abandoned metal mines (GM)	265		0.79			Madeddu et al. 2012
Venezuela	(nmol/l)	–			0.77–6.7		Granadillo et al. 1994
Prague	General population (GM)	397		0.6			Batáriová et al. 2006
Nigeria	Urban population in Enugu (range, ppm)	205		0.007–0.293			Nkolika and Benedict 2009
Egypt	General population : All	93		2.07±1.10			Mortada et al. 2002
	Smokers	25		2.67±1.21			
	Non-smokers	43		1.37±0.45			
Egypt	Exposed chromoplating	33			4.19±0.14		EL-Shafei 2012
	Non-exposed	42			1.41±0.26		
Tunisia	Bladder cancer patients	86	8.78±24.18				Feki-Tounsi et al. 2013a, b
	Controls	196	3.31±6.57				

– undefined, *GM* geometric mean, *SD* standard deviation

and blood Cr is in Pakistan (Afridi et al. 2011a) and that for blood Ni is found in Russian and British populations (Ivanenko et al. 2012; Hodnett et al. 2012). However, for blood As, the highest concentration is in Tunisia (Feki-Tounsi et al. 2013a, b). When we compare our findings with those represented in previous studies (Table 8), we observed that our results for blood Cd among healthy individuals (0.74 ±1.15 μg/l) agree with those of other published data in different localities; 0.99 (0.4–2) μg/l in Germany (Jung et al. 1993), 0.69 (0.1–1.9) μg/l in Belgium (Roels et al. 1994); and 0.1–3.4 μg/l in Italy (dell'Omo et al. 1999). Also, the results obtained in our study for blood As agree with those of Afridi et al.'s (2011b) study in Pakistan (1.7±0.4 μg/l). Despite the higher blood Cr levels in our study in comparison with other populations, blood Cr levels in the Pakistani population (Afridi et al. 2011a) were 2-fold higher than our Cr values. However, our Ni values were far higher than those found by Ivanenko et al. (2012) and Hodnett et al. (2012). Cr and Ni high concentrations in blood samples of the Tunisian inhabitants might be appropriate to reflect their long-time environmental exposure to metals despite of the relatively short biological half-lives of these metals in human body. Therefore, it is reasonable to assume that the blood Cr and blood Ni levels of the study subjects were relatively consistent over a long period and could be reflected by the current metal levels in blood samples. Therefore, it might be appropriate to use the relative relationships between current blood Cr and blood Ni levels to describe the long-term relationships between blood Cr and blood Ni levels in our population.

In the literature, several epidemiologic studies indicate that a general population living near an industrial zone is a major risk factor for exposure to metals such as Cd, Cr, Ni and As (Banza et al. 2009; Chiang et al. 2010; Wang et al. 2011; Afridi et al. 2011a; Kim et al. 2011; Pino et al. 2012; Lee et al. 2012; Madeddu et al. 2012; Erraguntla et al. 2012). However, in the case of Tunisia, only one study of our research group (Feki-Tounsi et al. 2013a, b) is available regarding the impact of arsenic on the population of Tunisia. Regarding residence locations, a normal distribution of metal levels in the whole blood was found between the areas studied (Table 6). Moreover, inhabitants living in Southern Tunisian areas (Sfax, Gabes and Gafsa Cities) for years might be constantly exposed to and affected by such environmental metal pollutions. Previous studies have shown that Southern Tunisia area was highly affected by heavy metals pollution due to many industrial activities (Fig. 1), especially crude phosphate treatment industries (Azri et al. 2002a, b; Ben Amor-Magouri 2007; Elouear et al. 2008; Ghannem et al. 2011; Gargouri et al. 2010; Serbaji et al. 2012; Garnit et al. 2012; Kharroubi et al. 2012). The phosphate industry represents a potentially serious soil pollution hazard, with deposited contaminants being potentially hazardous to plants and groundwater. Phosphorus species were the principal carriers of heavy metals in soils (Tayibi et al. 2009; Garnit et al. 2012; Kassir et al. 2012; Al-Attar et al. 2012). Therefore, it is reasonable to assume that the blood-metal levels of the study subjects were relatively consistent over a long period of time and could be reflected by the current metal levels in blood samples. Additionally, with long-term exposure, blood metal can receive inputs from recent exogenous exposure such as diet, tap water drinking tobacco smoking and occupational exposure.

Associations between heavy metal concentrations in blood samples and potential confounders

In order to identify the factors influencing blood As, Cd, Cr and Ni levels, we looked for associations or correlations between these levels and occupational exposure, amalgam fillings, cultural habits such as smoking, fish consumption and the type of water consumed.

Tobacco smoking

The most outstanding finding in the current study is the positive association of Cd and Ni levels with smoking ($p<0.001$ and $p<0.05$, respectively) (Table 6). Moreover, As and Cd were significantly and positively correlated with PY number ($p<0.001$) (Table 5). Previous studies reported that smoking constitutes an important source of chronic exposure to numerous xenobiotics, including heavy metals such as As (NRC 1999), Ni (Carter et al. 1997; Samet et al. 1997), Cr (IARC 1990; Chiba and Masironi 1992; Bernhard et al. 2005; Borgerding and Klus 2005) and in particular Cd in ionic form (Jin et al. 1998; Abshire et al. 1996; Joseph et al. 2001; Shih et al. 2003). Smoking 20 cigarettes/day has been estimated to result in an inhalation of about 2 to 4 μg Cd. Assuming a 25–50 % pulmonary absorption of this amount, smoking 20

cigarettes/day would result in a daily retention of about 1 to 2 μg Cd (Friberg et al. 1974; Elinder et al. 1983). Until now, exposure to heavy metals via smoking has been estimated based on the measurements of their concentrations in the blood, tissues or urine of smokers compared to non-smokers (Mortada et al. 2002; Stojanović et al. 2004; Batáriová et al. 2006; McKelvey et al. 2007; Son et al. 2009; Afridi et al. 2010; Gil et al. 2011; Forte et al. 2011). Therefore, our results reflect that smoking could be another major exposure source to these elements in our population and may in part explain the high variability in the blood metal levels. However, this well-known exposure source could not explain the high blood levels of metals due to exposure via tobacco smoking, suggesting that some other unknown exposure sources might be involved.

Occupational exposure

In the present study, 25.4 % of the participants reported exposing themselves to metal-related raw materials in the workplace. Results of the Spearman correlation and bivariate analyses indicated that Cd levels in blood samples had stronger correlation and association with occupational exposure (Tables 5 and 6) ($p<0.05$). In fact, the Cd level in blood samples of subjects occupationally exposed was 1.3 times higher than that of non-exposed (Table 6). A previous follow-up study on Cd-exposed workers showed that blood Cd was a good indicator of cumulative dose many years after the cessation of exposure (Jarup et al. 1997). In addition, the classification by type of occupation shows that As, Cd and Cr levels in the blood samples of subjects among workers exposed to pesticides and wood fumes, dyes, and cement dust, respectively, were significantly higher than those of non-exposed (Table 7). In previous studies, elevated As, Cd, Cr and Ni levels in human blood have been reported, which might be attributed to the occupational exposure source (Chang et al. 2006; Stridsklev et al. 2004, 2007; Gil et al. 2011; Afridi et al. 2011a, b; Hodnett et al. 2012; Ivanenko et al. 2012; El-Shafei 2012; Feki-Tounsi et al. 2013a, b; Caciari et al. 2012). Therefore, the elevated blood metal levels among workers occupationally exposed in this study could be ascribed to occupational exposure such as workers exposed to cement dust, wood fumes, pesticides, pigments and dyes. Hence, we can explain that the high heavy metal levels in the blood of these workers can be due to the elevated concentration of As in pesticides and wood (Quandt et al. 2010; Decker et al. 2010), Cd in pigments/dyes such as cadmium pigments (IARC 1993) and Cr in cement (Sinyoung et al. 2011).

Sea food consumption and water drinking

Because seafood is a staple in the Mediterranean diet, our study population is a moderate consumer of fish. Several studies have been conducted to document the contamination levels of heavy metals in natural populations of fish collected from the Coastal areas of Sfax and Gabes governments (Hamza-Chaffai et al. 2003; Messaoudi et al. 2009; Barhoumi et al. 2009; Bellassoued et al. 2012). In our study, the correlation of blood Cr and Ni with sea food consumption (Table 5) confirms the environmental contamination by these two metals which may be concentrated throughout the dietary chain. The bioaccessibility from food to human indicates the portion of total chemical with food digested into solution and is potentially to be assimilated to reach systemic circulation by the alimentary canal (He et al. 2010; Bjermo et al. 2013). However, the bioaccessibility may provide an excellent provision of data in enhancing human health risk assessment (Man et al. 2010; Ju et al. 2012).

There is any relationship between blood toxic element concentrations and tap water drinking in the Tunisian population (Tables 5 and 6). Therefore, drinking water was not found to be incriminated as exposure source. This result involves the safety of water drinking, although there is no direct evidence to confirm this hypothesis. Indeed, standards determined by the specialized authorities are not yet updated to the last WHO (2000) guidelines, reflecting lack of control of these elements likely due to its formerly established rarity in the Tunisian environment.

Amalgam fillings

Amalgam, which was introduced more than 150 years ago, is the most frequently used material in tooth filling restoration. Dental fillings provide a major iatrogenic exposure to xenobiotic compounds such as heavy metals (Brownawell et al. 2005). Conventional dental amalgam is a mercury (Hg) and silver-based alloy and may also contain traces of cadmium, platinum and palladium. Considering the high number of subjects continuously exposed for many years to dental filling constituents, we have determined the levels of Cd, Ni, Cr and As in whole blood of these selected Tunisian subjects. Our results

showed a positive Spearman's correlation coefficient between amalgam fillings and blood Cr and blood Ni ($p<0.05$) (Table 5). However, no association between metal blood levels and amalgam filling was found (Table 6). Our findings were in accordance with previous studies that reported the absence of metal exposure resulting from the use of dental appliances (Bishara et al. 1993; Kerosuo et al. 1997).

There are several limitations to the current study. First, the findings were based on individuals whose age ranged from 17 to 95 years. The estimate was consistent with other studies. However, it is also important to include children because they are especially susceptible to heavy metal exposure and because of their sensitivity of the developing nervous system (Jarup 2003). Second, the designed questionnaire did not allow us to cover all the potential sources of exposure to these four metals. Therefore, future studies should focus on dietary sources. Third, the findings on metals were based only on subjects who lived in polluted areas. Therefore, a future study should focus on the evaluation of metals in people who lived in unpolluted areas, to serve as the control group.

Finally, as reported in several studies (Rautray et al. 2009; Chiang et al. 2010; Afridi et al. 2010, 2011b; Yuan et al. 2011; Chhabra et al. 2012; Pasha et al. 2010; Romanowicz-Makowska et al. 2011; Wadhwa et al. 2011; Feki-Tounsi et al. 2013a), elevated metal levels in human blood, which might be attributed to metals contaminants in some polluted areas, occupational exposure, smoking and contaminated water drinking sources, could play a significant role in the development of diseases and cancer. Therefore, we consider it necessary to study the effects of metal exposure on our general population using sensitive biomarkers of acute and chronic effect.

Conclusion

In summary, the present study is the first cadmium, chromium and nickel human biomonitoring study in Tunisia; hence, it provides values that may be useful for comparisons in future studies or when addressing public and environmental health challenges associated with heavy metal contamination. The As, Cd, Cr and Ni values in our general population were 1.56, 0.74, 35.04 and 30.56 μg/l, respectively. Levels of blood Cd and As were positively correlated with age and the levels blood Cd were accumulated with age. Despite the environmental heavy metal contamination, by mining and industrial activities, in the south of Tunisia, smoking and occupational exposure seem to be the main exposure source to heavy metals. Cd blood levels were significantly correlated with PY number (of smoking). The blood Cd levels of subjects occupationally exposed was 1.3-fold higher than that of non-exposed. Additionally, analysis of metals levels according to the type of occupational exposure showed an association between the very high blood As levels and exposure to pesticides and wood fumes, blood Cd levels and exposure to dyes and also blood Cr levels and exposure to cement dust. Finally, we consider that it is particularly important to evaluate simultaneous exposure to potentially toxic metals in areas with mining and industrial activities. This strategy may allow getting a better characterization of exposure to metals in resident subjects of these areas, which will reflect more closely the "real world" of exposed subjects. This may represent an advantage for the risk assessment analyses that should consider the results of the interaction of metals in population exposed simultaneously to mixture of metals and contribute to a better interpretation on health consequences. Overall, it is clear that more biomonitoring studies are urgently needed in regard to inhabitants' health in heavy metal-polluted areas in order to provide tools for better risk assessment, but mainly for the implementation of actions to reduce exposure.

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Conflicts of interest The authors have no conflicts to report.

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