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Metallic contamination from a mining site and its accumulation by human

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ABSTRACT

Both blood and hair samples from the scalp were used in a preliminary assessment of heavy metals exposure (Cd, Cu, Pb and Zn) of the human population living near Drâa Lasfar mine (Marrakech-Morocco). In parallel with the collection of samples, individuals from a different community at the South of the mine area answered a questionnaire designed to obtain information about potential exposure pathways to these elements. The questionnaire allowed data collection about the most frequently consumed foodstuffs, drinking water sources, smoking habits, alcohol consumption and health condition. Higher concentrations, and subsequently higher ranges, of Cd, Cu, Pb and Zn were recorded in individuals living near the mine, in opposition to individuals living several kilometers apart. Additionally, the concentrations recorded in both blood and hair of individuals from the studied population were above the reference values for non-exposed (rural) individuals, suggesting their enhanced exposure. However, significant differences were found for the average concentration of these elements between the two populations. Men from the studied population presented higher concentrations of Cd, Cu and Zn in scalp hair, and higher concentrations of Cu and Zn in blood. Our results suggest that the population of Drâa Lasfar mine area may be exposed to some of the elements analysed namely Pb. In conclusion, humans living chronically exposed to Drâa Lasfar mine waste show high concentrations of essential and non-essential trace metals in both blood and scalp hair, and is suggested that this type of exposure may be as harmful as living close to industrial facilities.

KEYWORDS

Metallic trace elements;
Human blood, hair;
Exposure;
Mine area.

INTRODUCTION

In recent years, there has been an increased awareness in the several countries about the health effects of toxic and other trace elements in relation to nutritional disorders, environmental or occupational exposure, pathological status and medical therapy[14,61].

A variety of human activities, notably industrial and mining process have been responsible for the wider diffusion of heavy metals into the environment.

Several toxic metals, notably Cd and Pb, have been released in large quantities into the environment[56] in industrial areas, and large population groups may be exposed to toxic metals from the polluted environment, as is the case in the district of DARÄA LASFAR, located in the North-West of the Mrabtine zone, approximately 10 km to the west of Marrakech city (Morocco) (Figure 1).

Thus, exposure to these metals, and other environmental pollutants, are considered as severe environmental pollution and occupational hygiene problems with possible short- and long-term adverse effects on human health.

The threat of heavy metals to human health is aggravated by their long-term persistence in the environment[16], they may be transferred and accumulated in the bodies of animals or human beings through food chain, which will probably include teratogenesis, anomalies in reproduction, cause DNA damage and carcinogenic effects by their mutagenic ability[16,78].

Biological monitoring of the general population and of groups considered at risk provides essential information for both the evaluation of exposure and risk characterization. In this study we measured the concentrations of Cd, Cu, Pb and Zn in blood and hair of 'healthy' subjects (men, women, girls and boys) using analytical methods based on electro-thermal atomic absorption spectrometry.

In this paper, we present the preliminary results of this survey and evaluate them in the light of data obtained in a group of healthy subjects (controls) from a rural area, and data previously reported in the literature for the general population living in other countries.

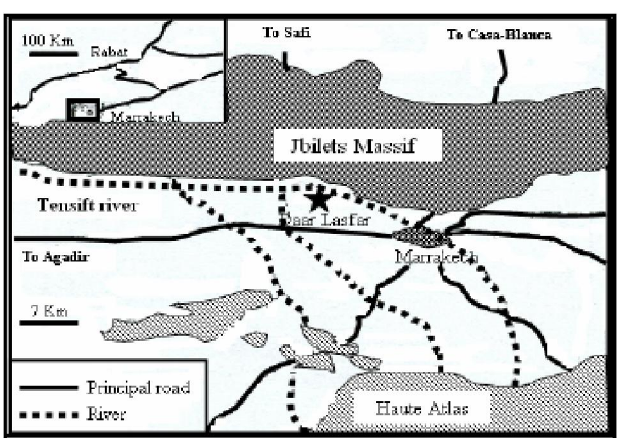

Figure 1: Drâa Lasfar mine localisation in Marrakech region.

METHOD AND MATERIAL

Sampling

Blood samples were collected from 67 subjects. The majority of them was born and/or lived at least one year on the spot. They are distributed as following:

- 26 men of more than ten years.
- 18 women of more than ten years.
- 16 boys of less than ten years.
- 8 girls of less than ten years.

All the subjects receive the same food ration composed especially on local animal foodstuffs and vegetables irrigated from the same source: the Tensift River receiving the Drâa Lasfar mine wastewater.

Control blood samples were collected from 30 subjects from a rural area. The rural area selected was at approximately 35Km in the southern part of the Marrakech region (Amzmiz region), to minimize the possible influence of atmospheric deposition from the industrial region (the prevailing wind direction in Marrakech is from the northwest).

Before sampling blood, a questionnaire was managed with both population groups in order to collect variables relating to the individual factors likely to influence the rate of the elements traces in blood (and hair) and the variables relating to the various factors of studied risks.

Sampling blood was carried out by puncture after careful disinfection using the cotton soaked with surgical alcohol. Approximately 5 ml of blood were collected aseptically with needles of single use and stored in heparinised tubes. Tubes are numbered and placed in a refrigerator.

Approximately 10 g of hair of each subject were taken on from the nape of the neck then placed in plastic bags and numbered.

Preparation of samples

All samples obtained from the smokers and from the alcohol drinkers were eliminated. The literature has reported that these two factors can intervene in the evolution of the metal concentrations.

Blood

Approximately 2 ml of blood sub-samples were dried to constant weight at 85°C[6,42,43,46,52,65,66]. Dried samples were cold digested in 1.5 ml of concentrated nitric acid overnight[52].

Hair

The measurement of trace elements in hair is not without its own inherent problems. In fact, analysis must only take into account the internal (endogenous) fraction[59] which highlights the importance of removing the external (exogenous) contaminant fraction coming from the metal-rich dust deposited on the hair[59].

The hair procedure washing was carried out as it's described by several authors: nitric acid (10%) 4 x 10 min.

Approximately 2g sub-samples were dried to a constant weight at 80°C. A precisely weighed 50 mg test specimen was carefully dried at constant temperature to constant weight. This specimen was introduced into a polystyrene tube, and 1ml nitric acid 8N was added[42].

The corked tube was preserved at ambient temperature for 24h. During this preliminary phase, most of the sample dissolved in the acid. To perfect dissolution, the corked tubes were placed for 1h in a boiler at 60°[42]. Corks were maintained in place by pressure (plate plus weight).

The recovered liquids were diluted in a suitable amount of bi-demineralised water for trace element analysis.

Method validation

Since the quality of analytical results is an essential pre-requisite for the assessment of health risks, methods were validated in-house according to the EURACHEM guideline[25] and the requirements of ISO/IEC 17025[32] and ISO 15189[31], including estimates of uncertainty of measurement according to the EURACHEM/ CITAC Guide[26].

Analysis

Trace metal concentrations were determined by flame atomic absorption spectroscopy for Copper and zinc, and by graphite furnace atomic absorption spectroscopy for cadmium and lead. Metal concentrations were expressed as mean individual values ± standard deviation.

Results

155 samples of hair and 67 samples of blood were analyzed.

However, all the results were not exploitable, so we eliminated some values those were obviously too high and were apart from an acceptable zone of variation and as a consequent were representing either a particular exposure to metals or a contamination during the preparation of the samples.

Results (TABLE 1 and 2) show that higher concentrations of Cd, Cu, Pb and Zn in both blood and hair were recorded in individuals living near the mine, in opposition to individuals living several kilometers apart in a rural area.

TABLE 1 : Metalic trace elements concentration in hair of the two studied population.

		Cd (µg/g)		Cu (µg/g)		Pb (µg/g)		Zn (µg/g)	
		M	SD	M	SD	M	SD	M	SD
Mine population	Boys	1,76	0,86	24,78	3,26	15,01	7,18	161,06	27,46
	Men	2,03	0,77	26,24	5,90	19,36	5,65	159,56	57,94
	Women	1,91	0,64	25,36	1,86	22,44	6,37	157,48	37,55
	Girls	1,62	0,41	22,13	3,43	17,06	5,67	158,68	48,20
Rural population	Boys	0,37	0,13	12,87	1,09	5,74	1,57	96,77	9,06
	Men	0,43	0,12	13,10	1,56	5,33	1,18	117,40	35,69
	Women	0,40	0,15	12,90	1,78	6,11	1,26	104,24	17,20
	Girls	0,35	0,06	12,04	2,54	6,02	2,03	90,05	9,97

M : Mean; SD : Standard deviation

TABLE 2 : Metalic trace elements concentration in blood of the two studied population.

		Cd (µg/l)		Cu (µg/l)		Pb (µg/l)		Zn (mg/l)	
		M	SD	M	SD	M	SD	M	SD
Mine population	Boys	1,28	0,81	1591,7	335,5	194,00	36,46	3,37	0,87
	Men	1,67	0,83	1328,8	479,2	194,68	75,39	3,97	1,06
	Women	1,95	1,11	1551,1	418,2	262,31	57,27	3,70	0,85
	Girls	1,86	1,06	1463,7	266,4	236,17	28,41	3,21	0,87
Rural population	Boys	0,69	0,45	716,7	298,9	37,4	2,2	2,1	0,42
	Men	0,71	0,41	544,8	104,2	40,0	4,9	2,3	0,33
	Women	0,73	0,36	603,3	130,9	58,1	2,3	2,2	0,33
	Girls	0,70	0,32	488,4	93,6	52,0	2,0	2,0	0,47

M: Mean; SD: Standard deviation

Additionally, significant differences were found for the average concentration of these elements between the two areas suggesting enhanced exposure of Drâa Lasfar mine group.

Descriptive statistics on blood and hair concentrations of cadmium, copper, lead and zinc analysed in 67 subjects living near Drâa Lasfar mine and in the 30 subjects living in a rural area are shown in

TABLES 3, 4, 5 and 6.

Trace elements in hair

Cadmium

Statistical analysis of results shows that men (2,03±0,77 µg/g) and boys (1,76±0,86 µg/g) living in the mine area present higher Cd concentration in hair than women (1,91±0,64 µg/g) and girls (1,62±0,41 µg/g). Moreover, student test (t) doesn't show any influence of age ($H_{0,1}$ and $H_{0,2}$: TABLE 3) and sex ($H_{0,9}$ and $H_{0,10}$: TABLE 4) on Cd accumulation in mine population hair.

Several authors have made different observations for the populations living in the neighbourhood of deserted mines. In their study, Zumkley et al..[80] have found that Cd cadmium contents are higher in men (8,2 µg/g) than in women (6,4 µg/g). Same has been noted by Medeiros and Pellum[50] 9,5 µg/g vs 7,9 µg/g in the USA and Nishiyama and Nordberg[55] 6,9 µg/g vs 4,5 µg/g in Japan. The Cd concentrations obtained in our study are less than to those noted in USA and in Japan, but are higher than to those noted by Pereira et al..[58] in Portugal for the population living in the vicinity of an unused Cu mine (0,89 µg/g in men and 0,62 µg/g in women), by Barlow and Al,[2] 0,22 µg/g observed in general population in Great Britain and by Leotsinidis and Kondakis[44] 0,19 µg/g in Greece. Remembering that the contents level noted in our study remain inferior to the reference values cited in by Iyenger and Woittiez[34] of the order of 2.4 µg/g.

Copper

Statistical analysis of results shows that men (26,24 ± 5,9 µg/g) and boys (24,78 ± 3,3 µg/g) of the mine area present higher Cu concentration in their hair than women (25,36 ± 1,9 µg/g) and girls (22,13 ± 3,4 µg/g). Additionally, Student test (t) doesn't show any influence of age ($H_{0,3}$ and $H_{0,4}$: TABLE 3) and sex ($H_{0,11}$ and $H_{0,12}$: TABLE 4) on Cu accumulation in hair.

The obtained Cu levels in our study are lower than those found by Suzuki et al.[70] (46 µg/g) in exposed female population in Japan and those noted by Jamall and Allen[35] (30,7 µg/g) in a total population in Pakistan.

However, they are lower than those published by Gibson[29] (14,9 µg/g) in a general population) and by Leotsinidis and Kondakis[44] (10,6 µg/g in women and 10,5 µg/g in men).

Lead

Statistical analysis of results shows contrary to Pb and Cu that higher Pb concentrations were recorded in women and girls hair living in Drâa Lasfar mine area. Thus, Pb contents noted respectively in women and girls (22,44 ± 6,4µg/g and 17,06 ± 5,8 µg/g) are higher than those recorded in men and boys (19,36 ± 5,7 µg/g and 15,01 ± 7,2 µg/g) respectively. Moreover, student test (t) doesn't show any influence of age ($H_{0,5}$ and $H_{0,6}$: TABLE 3) and sex ($H_{0,13}$ and $H_{0,14}$: TABLE 4) on Pb accumulation in mine population hair.

Pb contents noted in this study are higher than those published by Waqar and al.[76] in Pakistan in a total population (10,6 µg/g) and by Leotsinidis and Kondakis[44] for the population in Greece (4,4 µg/g). However, they are lower than those noted by other authors; in the United States, 54 µg/g in women and 23 µg/g in men[50], in Venezuela, 49,9 µg/g in a total population exposed to Pb[10] and in Pakistan (31,7 µg/g) in a total population[35].

Zinc

Statistical analysis of results shows that men (159,6 ± 57,9 µg/g) and boys (161,06 ± 27,5 µg/g) living near Drâa Lasfar mine present higher Zn concentration in their hair than women (157,48 ± 37,6 µg/g) and girls (158,68 ± 48,2 µg/g). Moreover, Student test (t) doesn't show any influence ($p<0,05$) of age ($H_{0,7}$ and $H_{0,8}$: TABLE 3) and sex ($H_{0,15}$ and $H_{0,16}$: TABLE 4) on Zn accumulation in mine population hair.

Absence of a sex effect on the accumulation of zinc in hair was noted by several authors[64]. In this study,

TABLE 3 : Cd, Cu, Pb and Zn hair levels in mining population and statistical study of influence of age on their accumulation.

Element	Sex	AP		IP		F	test	Z	t	Conclusion
		M	SD	M	SD					
Cd (µg/g)	F	1.91	0.64	1.62	0.41	2.46	T	0.40	2.02	$H_{0,1}$
	M	2.03	0.77	1.76	0.86	1.23	T	1.42	2.02	$H_{0,2}$
Cu (µg/g)	F	25.36	1.86	22.13	3.43	3.41	NA	1.26	2.13	$H_{0,3}$
	M	26.24	5.90	24.78	3.26	3.27	T	0.12	2.02	$H_{0,4}$
Pb (µg/g)	F	22.44	6.37	17.06	5.76	1.26	T	1.34	2.02	$H_{0,5}$
	M	19.36	5.67	15.01	7.18	1.61	T	1.17	2.02	$H_{0,6}$
Zn (µg/g)	F	157.48	37.55	158.68	48.20	1.65	NA	0.13	2.02	$H_{0,7}$
	M	159.56	57.94	161.06	27.46	4.45	T	0.07	2.01	$H_{0,8}$

IP: Infantile population; AP: Adult population
F: Females; M: Males

TABLE 4 : Cd, Cu, Pb and Zn hair levels in mining population and statistical study of influence of sex on their accumulation.

Element	Sex	F M	F SD	M M	M SD	F	test	Z	t	Conclusion
Cd (µg/l)	AP	1.91	0.64	2.03	0.77	1.48	t	1.24	2.02	$H_{0,9}$
	IP	1.62	0.41	1.76	0.86	4.47	NA	0.35	2.02	$H_{0,10}$
Cu (mg/l)	AP	25.36	1.86	26.24	3.26	10.09	NA	0.24	2.03	$H_{0,11}$
	IP	22.13	3.43	24.78	5.90	1.11	t	2.01	2.07	$H_{0,12}$
Pb (µg/l)	AP	22.44	6.37	19.36	5.67	1.27	t	1.71	2.02	$H_{0,13}$
	IP	17.06	5.76	15.01	7.18	1.60	t	0.47	2.07	$H_{0,14}$
Zn (mg/l)	AP	157.48	37.55	159.56	57.94	2.38	NA	0.14	2.02	$H_{0,15}$
	IP	158.68	48.20	161.06	27.46	3.08	t	0.10	2.07	$H_{0,16}$

IP: Infantile population; AP: Adult population
F: Females; M: Males

Zinc levels were lower than those publishes by Waqar and al.[76] in Pakistan (254,4 ± 16,6 µg/g in men and 236,1 ± 22,5 in women) and by Leotsinidis and Kondakis[44] (182,5 µg/g in men and 187,5 µg/g in women) in Greece.

Trace elements in blood

Cadmium

Contrary in hair, blood cadmium levels (CdB) recorded in women (1,95 ± 1,1 µg/l) were higher than in men (1,67 ± 0,8 µg/l), but the difference between these two values was not significant (P. < 0,05).

For infants, higher Cd level was noted in girls blood (1,86± 1,1 µg/l) in opposition to boys (1,28 ± 0,8 µg/l). No significant difference was noted between these levels (p<0,05).

Student test (t) doesn't show any influence (p<0,05) of age ($H_{0,17}$ and $H_{0,18}$: TABLE 5) and sex ($H_{0,23}$ and $H_{0,24}$: TABLE 6) on Cd accumulation in mine population blood.

The obtained Cd levels in this study were lower than those published by Maravelaias et al..[47] in an exposed Greek population (3,7 µg/l in men and of 3,4 µg/l in women), by Bergomi et al..[4] in an Italian general population (4,7 µg/l), by Senft et al..[67,68] (CdB = 5,9µg/l) in a total population in Czech, and by Koreckova[37] in the same population (2,36 ± 0,26 µg/l). However, our values were higher than those noted by Kowal et al..[40] in USA population (1,1 µg/l in men and 0,7µg/l in women). Remembering that the contents level noted in our study remain inferior to the reference values (0,3-0,7 µg/dl) cited in by Iyenger and Woittiez[34].

Copper

Normal values of copper in blood (CuB) vary between 0,8 to 1,4 mg/l[33].

Statistical analysis of the obtained results shows that men and boys present higher Cu concentration (1,55 ± 0,4 mg/l and 1,59 ± 0,3 mg/l) than those in women and girls (1,33 ± 0,5 mg/l and 1,46 ± 0,3 mg/l). No significant difference was noted by the statistical test. Moreover, Student test (t) doesn't show any influence (p<0,05) of age ($H_{0,19}$ and $H_{0,20}$: TABLE 5) and sex ($H_{0,25}$ and $H_{0,26}$: TABLE 6) on Cu accumulation in mine population blood.

These CuB values are higher than those reported by Cui et al.[16] in two Chinese exposed populations (1,06 mg/l and 0,89 mg/l in men, 1,02 mg/l and 0,90 mg/l in women), by Dlhopolcek and Laurincova[21] in a total population in Slovak (0,94 mg/l), by Vlcek et al.[73] in Czech (1,3 mg/l) and by Burguera et al.[10] (1,0 mg/l in women and 1,4 mg/l in men) in a population in Venezuela.

Lead

In the balance state, blood lead (PbB) represents 2 % of the total lead quantity present in the organism[12,15].

Contrary to the results of many studies (higher PbS in men than in women), our results confirmed the reverse, with respectively 262,31 ± 57,3 µg/l and 236,17 ± 28,4 µg/l in women and girls blood, whereas PbB noted in men and boys are only 194,68 ± 75,4 µg/l and 194,00 ± 36,5 µg/l respectively.

Statistical analysis of these results highlighted that only sex ($H_{1,2}$ and $H_{1,3}$: TABLE 6) has an effect on the accumulation of this element in blood contrary to age ($H_{0,21}$ and $H_{0,22}$: TABLE 5).

Our results are higher than those published by Koreckova and Skopkova[37] in a Czech population exposed to metal risks (respectively 71,3 and 42,7 µg/l in men and women), by Bittnerova[5] in a Czech population (165,8µg/l) and by Roels and Lauwerys[63] in a Belgian population (163 µg/l at the men and 114 µg/l among women).

However, our results are less than those noted by Vysckocil et al.[74] in a Czech population (296,3 µg/l), by Kriz and Kodl[41] 267 µg/l in boys and 255,9 µg/l in girls respectively.

Zinc

Our results showed that women and girls present lower Zn levels in blood (ZnB) (3,70 ± 0,9 mg/l and 3,21 ± 0,9 mg/l respectively) than in men and boys (3,97 ± 1,1 mg/l and 3,37 ± 0,9 mg/l respectively).

Moreover, Student test (t) doesn't show any influence (p<0,05) of age ($H_{0,23}$ and $H_{0,24}$: TABLE 5) and sex ($H_{0,30}$ and $H_{0,31}$: TABLE 6) on Zn accumulation in mine population blood.

In this study, ZnB were largely higher than those published by Cui et al.[16] in two exposed populations in China (1,18 and 1,12 mg/l in men and 1,11 and 1,14 mg/l in women), by Burguera et al.[10] (ZnB = 0,98 mg/l) in a total population in Venezuela, by Lepsi et al..[45] (ZnB = 1,1 Mg / L) in a male population in Czech, by Derzsiova et al.[19] (ZnB = 1,1 mg/l) in a total population living in a mine zone in Slovak, by Kosut[39] (ZnB = 1,1 mg/l) in an exposed total population in Czech. Dastych et al..[17] have noted higher values for the same population, but remain lower than those noted in our study (ZnB = 1,4 mg/l). However, theese are lower than those found by Astrug et al.[1] (6,9 ± 3,7 mg/l), Djingova[20] (8,5 ± 0,8 mg/l), Dover et al.[22] (6,6 mg/l) and by Sedki[65] (6,2 ± 2,5 mg/l in women and 8,5 ± 3,2 mg/l in men).

TABLE 5: Cd, Cu, Pb and Zn blood levels in mining population and statistical study of influence of age on their accumulation.

Element	Sex	IP		AP		F	test	Z	t	Conclusion
		M	SD	M	SD					
Cd (µg/l)	F	1.86	1.11	1.95	1.06	1.09	t	0.25	2.02	$H_{0,17}$
	M	1.28	0.81	1.67	0.83	1.07	t	1.56	2.07	$H_{0,18}$
Cu (mg/l)	F	1.46	0.27	1.55	0.48	1.55	t	0.47	2.02	$H_{0,19}$
	M	1.59	0.34	.33	0.42	3.24	t	0.08	2.02	$H_{0,20}$
Pb (µg/l)	F	236.17	28.41	262.31	57.27	3.66	NA	1.27	2.02	$H_{0,21}$
	M	194.00	36.46	194.68	75.39	4.27	NA	0.34	2.07	$H_{0,22}$
Zn (mg/l)	F	3.21	0.87	3.70	0.85	1.05	t	0.55	2.02	$H_{0,23}$
	M	3.37	0.86	3.97	1.06	1.50	t	1.14	2.07	$H_{0,24}$

IP: Infantile population; AP: Adult population
F: Females; M: Males

TABLE 6 : Cd, Cu, Pb and Zn blood levels in mining population and statistical study of influence of sex on their accumulation.

Element	Sex	F		M		F	test	Z	t	Conclusion
		M	SD	M	SD					
Cd (µg/l)	AP	1.95	1.06	1.67	0.83	1.61	t	0.36	2.02	$H_{0,25}$
	IP	1.86	1.11	1.28	0.81	1.89	t	0.97	2.09	$H_{0,26}$
Cu (mg/l)	AP	1.33	0.48	1.55	0.42	1.31	t	1.66	2.02	$H_{0,28}$
	IP	1.46	0.27	1.59	0.34	1.58	t	0.38	2.09	$H_{0,29}$
Pb (µg/l)	AP	262.31	57.27	194.68	75.39	1.92	t	2.63	2.02	$H_{1,1}$
	IP	236.17	28.41	194.00	36.46	1.65	t	2.32	2.09	$H_{1,2}$
Zn (mg/l)	AP	3.70	0.85	3.97	1.06	1.57	t	0.98	2.02	$H_{0,30}$
	IP	3.21	0.87	3.37	0.86	1.01	t	0.51	2.09	$H_{0,31}$

IP: Infantile population; AP: Adult population
F: Females; M: Males

The obtained values in our study remained in general inferior to the reference values (6 to 7 mg/l) cited by Iyengar[33].

DISCUSSION

The evaluation of the risks constitutes today a major tool for decision making regarding management of the environmental situations.

At international level, several ecotoxicological researches reported that the use of wastewater to irrigate arable lands contributes to considerable concentration of metals in grounds[11,28,78].

Several works[27,49,53] reported that surface and underground watery ecosystems those are enriched in metal contents, cause the contamination of the agricultural soils[23], of the spontaneous higher plants[72] and of local fauna: earthworms, birds, bovines and sheep[65]. The results of this work indeed shows that there can be a real transfer of mineral micropollutants through the polluted soils towards the links of the trophic chains to reach the Man finally, living sedentarily in these zones, via the food products, animals and vegetables[42]. In this context, several authors[30,43,65] underlined in their works that the impregnation of human body by the trace elements does not depend only on the transfer of these micropolluants through the polluted soils via the foodstuffs to the Man, but also on the sex of the individual, his age, his professional statute, his passive nicotinism[43,65] and on the factors related to the habitat and its situation compared to the mine[30].

The results of the trace elements measuring in hair show that the mean contents of Cd (as out of Cu) are higher in male sex than in female sex. These results perfectly coincide with those of several works[58,65]. This report can be justified by the nature of their activities (more than 63 % are farmers), of their frequent contact with the ground and wastewater and of their physical efforts required causing an increase in the organism metabolism and consequently an increase in the quantity of introduced contaminated food.

In blood, the Cd concentrations recorded in men are contrary less than in women. This light increase in women can be explained by a longer biological half-life of Cd than in the men[9]. This great capacity of conservation of blood Cd could be due to a more effective synthesis of metallothioneic proteins in women[77].

Contrary to Cd in blood, the results show that the mean contents of Cu in blood are higher in men than in women. These results Coincide perfectly with work of several authors[45,65], which justify this difference by the fact that 90 to 95 % (according to the authors) of trace elements in blood are related to red blood corpuscles.

The number and the volume of red blood corpuscles play a main role in the fluctuation within each individual. However, precisely the age and the sex induce significant modifications, either through the number of red blood corpuscles or through their mean volume. Several authors confirmed that the number of red blood corpuscles is lower by 10 % in women[65].

Contrary to Cd, the Pb content is higher in both women hair and blood. Several explanations are possible: in addition to the direct contamination due to the consumption of contamination foodstuffs and contact with wastewater, the grounds dust.., women of this zone have particular habits to treat diseases or to make up themselves. They use mineral, vegetable and animals products which are sometimes very rich in certain trace elements like henna and especially kohl (cosmetic eyepiece). This cosmetic is made of at least 65 % of Pb[36,43,65] and its preparation utilizes other minerals, vegetables or animal substances which make its use dangerous (antimony, arsenic, mercury, silver, ginger, musk, camphor, crushed cores of olive, opium, birds bile, viper extract …). The list of the products using in the preparation of kohl is very long and increasingly complex according to the product finality.

Nevertheless, this cosmetic has a great reputation within the population. Its use is very dangerous because women are accustomed to soaking ' Miroued' (instrument of make-up) by saliva before the application of Kohl, method which facilitates and permits to this metal to pass in body. This particular risk is significant because women of this zone use this cosmetic at least three times per week and since their youth.

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