Ann Ist Super Sanità 2013 | Vol. 49, No. 1: 24-33
DOI: 10.4415/ANN_13_01_06

Human biomonitoring for Cd, Hg and Pb in blood of inhabitants of the Sacco Valley (Italy)

Sonia D'Ilio[a], Francesco Forastiere[b], Assunta Draicchio[c], Costanza Majorani[d], Francesco Petrucci[d], Nicola Violante[d] and Oreste Senofonte[d]

[a] *Centro Nazionale Sostanze Chimiche, Istituto Superiore di Sanità, Rome, Italy*
[b] *Azienda Sanitaria Locale Roma E, Dipartimento di Epidemiologia del Servizio Sanitario Regionale, Rome, Italy*
[c] *Ministero dell'Economia e Finanze (MEF), Rome, Italy*
[d] *Dipartimento di Ambiente e Prevenzione Primaria, Istituto Superiore di Sanità, Rome, Italy*

Abstract

Introduction. The Sacco Valley (Lazio, Italy) is characterized by high density population and several industrial chemical productions that during the time had led to a substantial amount of by-products. The result was a severe environmental pollution of the area and in particular of the river Sacco. In 1991, the analysis of water and soils samples of three industrial landfills revealed the presence of organochlorine compounds and heavy metals. A research project named "Health of residents living in Sacco Valley area", coordinated by the regional Department of Epidemiology, was undertaken and financed to evaluate the state of health of the population living near those polluted areas.
Materials and methods. Cd, Hg and Pb were quantified in 246 blood samples of potentially exposed residents of the Sacco Valley by quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS).
Results. Individuals who agreed to be sampled did not exhibit high levels of the elements. The distance from the river does not seem to be directly connected with the elements levels in blood. The contribution of these contaminants to the total intake due to ingestion of food was difficult to evaluate. The unclear trend of data would require a characterization of the polluted site with environmental sampling of different matrices.

Key words
- human biomonitoring
- trace elements
- blood
- Sacco Valley (Italy)

INTRODUCTION

Cadmium (Cd), mercury (Hg) and lead (Pb) are naturally occurring constituents in the environment with varying in concentrations across geographic regions. They can be commonly found in the environment as pollutants as a result of human activities. The environmental chemistry of these elements influences their fate and the potential effects on the population. The toxicokinetics and toxicodynamics depend on the chemical form of the element that can be regulated or stored in the organism. For these reasons, it is important to understand and distinguish among naturally occurring levels, current background levels (*i.e.,* natural and anthropogenic sources) and contributions from specific activities of concern [1]. The toxic effects of Cd exposure in humans are well known and documented. Numerous studies indicate that kidney is the primary target organ of cadmium toxicity following extended oral exposure [2]. The health effects of Pb on human health are widely treated in literature. Lead can travel in the blood to soft tissues and organs such as liver, kidneys, lungs, brain, spleen, muscles and heart. After several weeks, most of Pb moves into bones and teeth, however, blood remain an effective means of assessing a possible exposure [3]. Both elemental Hg and methylmercury are toxic, nervous system and kidney are likely to be affected by mercury. Exposure to Hg compounds at hazardous sites is more likely to occur from handling contaminated soil, drinking well-water or eating fish from contaminated waters near those sites. But not all waste sites containing mercury have had releases of mercury to the air, water or surface soils [4, 5].

A recent and, to some extent past exposure to Cd, Hg and Pb by all routes (including ingestion, inhalation and dermal absorption) could be reflected by their levels in blood, nevertheless, their presence does not by itself mean causing diseases or adverse effects [6, 7]. In order to evaluate a possible exposure to organic and inorganic

Address for correspondence: Sonia D'Ilio, Centro Nazionale Sostanze Chimiche, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy. E-mail: sonia.dilio@iss.it.

contaminants blood is commonly employed in human biomonitoring, nevertheless it does not trace the bioaccumulated chemicals [8]. Human biomonitoring is defined as the direct measurement of people's exposure to environmental contaminants by measuring substances or their metabolites in blood, urine, or other specimens [9]. The great strength of biomonitoring is that it provides unequivocal evidence that both exposure and uptake have taken place [6, 10]. Results from biomonitoring studies are particularly useful when they are combined with classical epidemiological observations and/or animal data [11]. At present, many biomonitoring investigations have been done at international level in population-based surveys designed as a basis for protecting public health [12-24].

The region of river Sacco valley is located in the central part of Italy (Lazio). This valley is characterized by high density population and several industrial settings, including chemical plants. For a long time, it has been an important industrial area involving chemicals production that led to a considerable amount of by-products, thus representing a problem for their disposal and treatment. Furthermore, the presence of illegal dumping sites has been a matter of concern for the population. In 1991, a technical survey on the substances in water and soils of three industrial landfills revealed the presence of organochlorine compounds and heavy metals. The concentration of these contaminants in soil showed a descending order with increasing distance of the river. It was therefore suggested that the land nearest the river was contaminated and that the river served as a vehicle for the contamination [25]. In 2005, the analysis of one raw milk sample of a dairy farm of Gavignano revealed a high value of β-hexachlorocyclohexane [25], a synthetic chemical classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC) [26]. Subsequent investigations on raw milk and feed sampled from different farms have confirmed the contamination of the territory. The river Sacco turned out to be severely polluted as well due to the discharge of toxic and uncontrolled waste of industrial origin. Therefore, there was the need of investigating a possible impact on human health of inhabitants living near the river due to this extensive environmental pollution.

A comprehensive research project entitled "Health of residents living in Sacco Valley area", coordinated by the regional Department of Epidemiology, was undertaken and financed to deeply evaluate the state of health of the population living near those polluted areas. The study was divided in four different parts, each unit has dealt with a particular aspect of the project:

a) epidemiological surveillance and evaluation of the effects on human health with analysis of mortality, morbidity and reproductive health area;

b) survey on population with cross-level characterization of the accumulation of organic contaminants and metals with the study of biological markers, disease in adults and reproductive health;

c) cohort studies of people living with enrollment of a cohort to study the relationship between residence in the area and causes of death and hospitalization, and assess the risk among farmers of farms located along the course of the river Sacco;

d) analysis of mortality of the cohort of workers with mortality study of the cohort of workers engaged in the chemical processing of the former industrial complex of Colleferro and railway carriages.

In the framework of this project, a human biomonitoring programme was carried out on the levels of Cd, Hg and Pb in blood of 246 potentially exposed residents in the area as a part of point b) of the analysis. Blood was previously acid assisted digested and the elements were all quantified by means of quadrupole inductively coupled plasma mass spectrometry (Q-ICP-MS). This paper presents and discusses the outcome of this study.

MATERIALS AND METHODS

Areas

In this study, nine small municipalities were involved, three of them were located near the industrial pole of Rome territory (Colleferro, Segni and Gavignano) and the remaining six were of Frosinone's province (Paliano, Anagni, Ferentino, Sgurgola, Morolo and Supino).

Four areas were selected on the basis of the distance from the different pollution sources (industrial area and river Sacco), as follows:

Area 1: area of Colleferro (1 km from the industrial area);

Area 2: areas of the centers of Colleferro, Segni, Gavignano, Sgurgola and Morolo (1 km from the river);

Area 3: other areas of Colleferro and a rural area of Gavignano and Segni;

Area 4: inhabited areas of Gavignano and Segni.

Area 1 and 2 have 31 000 and 57 000 inhabitants, respectively.

Selection of population and data collection

The subjects to be eligible for the study must be Italian citizen, aged in the range 25-70 years, had been living for a continuative period of at least 15 years in one of the mentioned four zones. Candidates (all Italian) were asked to answer a questionnaire in order to verify any possible additional exposure through ingestion of food, smoking, use of medicine, and other. This information turned particularly useful in the evaluation of final data, especially in case of very high values that require a close examination of questionnaires to exclude confounding factors.

A number of 509 questionnaires were distributed among residents living in the areas. Four hundred and four people were considered eligible for the study with only 240 blood samples. Sampling of blood (10 ml from each participant) was done in the Transfusion Centers of the hospitals of Colleferro and Anagni. After sampling, blood specimens were stored in a freezer at -20 °C in BD Vacutainer® blood collection tube with heparin (Becton, Dickinson and Company, NJ, USA). The population participating in this study was considered representative of the inhabitants of the Sacco valley due to their specific location.

Table 1
Characteristics of the subjects with a direct interview

	Residence areas %				Sum
	1	2	3	4	
Sex					
male	31	116	47	20	**214**
female	60	142	70	23	**295**
Age (years)					
20 to 34	14	62	22	5	**103**
35 to 44	27	68	26	13	**134**
45 to 54	20	49	26	10	**105**
55 to 70	30	79	43	15	**167**
House distance from the river					
less than 500 m (d1)	6	70	0	0	**76**
from 500 m to 1 km (d2)	43	138	10	0	**191**
from 1 to 2 km (d3)	42	42	84	0	**168**
from 2 to 5 km (d4)	0	7	19	0	**26**
more than 5 km (d5)	0	1	4	43	**48**
Ever smoking					
yes	51	116	62	19	**248**
no	40	142	55	24	**261**
Domestic use of well water					
yes	0	54	2	1	**57**
no	91	204	115	42	**452**
Use of well water for cooking					
yes	3	163	12	0	**178**
no	88	95	105	43	**331**
Personal washing use of well water					
yes	1	179	11	0	**191**
no	90	79	106	43	**318**
Other use of well water					
yes	1	7	1	1	**10**
no	90	251	116	42	**499**
Total number of responders	**91**	**258**	**117**	**43**	**509**

Ethical considerations

The Department of Epidemiology of the Local Health Authority informed the subjects about the objectives of the investigation. All participants were asked to give their informed consent in order to contribute to the research. The local guidelines for human investigations were adopted for this human biomonitoring study.

Sample treatment and analysis

An open acid digestion procedure was adopted by means of a high throughput (48 positions) digestion system (ModBlock™ Digestion System, CPI International, Amsterdam, The Netherlands) in decontaminated disposable metal-free polypropylene sample tubes (50 ml ± 0.25 ml). A portion of 1 ml of blood was transferred in each tube with 2 ml of 67-69% Super Pure Nitric Acid (Romil, Cambridge, Great Britain), 1 ml of 30% super pure hydrogen peroxide (Romil, Cambridge, Great Britain) and 1 μg/l of Rh. These were subsequently digested at 60 °C for three hours. The final digested solutions were quantitatively transferred in 15 ml Falcon® tubes (Becton Dickinson Labware, Franklin Lakes, NJ, USA) with ultrapure deionized water (specific resistivity of 18 MΩ cm, Easy Pure, PBI International, Milan, Italy) up to a volume of 10 ml. These solutions were stored at -20 °C and no further dilution was necessary for the analysis. Single element 1000 mg/l stock standard solutions of Cd, Hg, Pb and Rh in 2% (v/v) HNO_3 were properly diluted for the analysis (Spex Industries Inc., Edison, NJ, USA).

Each cycle of digestion contained three blanks (1 ml of deionized water for each Vacutainer® blood tube) in

Table 2
Characteristics of the 240 subjects (blood withdrawal)

	Residence area (%)				Sum
	1	2	3	4	
Sex					
male	51.1	51.8	49.1	56.7	**51.7**
female	48.9	48.2	50.9	43.3	**48.3**
Age					
20 to 34	12.8	23.6	22.6	13.3	**20.0**
35 to 44	29.8	25.5	22.6	30.0	**26.3**
45 to 54	19.1	21.8	24.5	20.0	**21.7**
55 to 70	38.3	29.1	30.2	36.7	**32.1**
House distance from the river					
less than 500 m (d1)	0.0	3.6	11.3	73.3	**13.3**
from 500 m to 1 km (d2)	8.5	3.6	41.5	16.7	**14.6**
from 1 to 2 km (d3)	36.2	36.4	32.1	3.3	**31.3**
from 2 to 5 km (d4)	40.4	30.0	9.4	0.0	**23.8**
more than 5 km (d5)	14.9	26.4	5.7	6.7	**17.1**
Ever smoking					
yes	59.6	33.6	54.7	53.3	**45.8**
no	40.4	66.4	45.3	46.7	**54.2**
Domestic use of well water					
yes	4.3	75.5	13.2	3.3	**38.8**
no	95.7	24.5	86.8	96.7	**61.3**
Cooking use of well water					
yes	4.3	61.8	13.2	0.0	**32.1**
no	95.7	38.2	86.8	100.0	**67.9**
Personal washing use of well water					
yes	0.0	70.9	11.3	0.0	**35.0**
no	100.0	29.1	88.7	100.0	**65.0**
Other use of well water					
yes	0.0	2.7	0.0	3.3	**1.7**
no	100.0	97.3	100.0	96.7	**98.3**
Total of replies	**100.0**	**100.0**	**100.0**	**100.0**	
Percent composition	**19.6**	**45.8**	**22.1**	**12.5**	**100**

order to evaluate possible residue contamination from heparin. These solutions were left to stand for 24 h, subsequently subjected to the same digestion procedure and analyzed. Their concentrations were always below the instrumental limit of quantification (Cd 0.043 µg/l, Hg 0.334 µg/l, Pb 0.101 µg/l).

The trueness of the method was assessed by the reference material (RM) Clinchek® whole blood level I (RECIPE Chemicals, Munich, Germany), certified for the content of Cd (1.3 ± 0.4 µg/l), Hg (3.8 ± 1.2 µg/l) and Pb (81 ± 15 µg/l). Results for trueness (expressed in terms of percent deviation) were: -2.6% Cd, +10% Hg and -5% Pb.

The quantification of Cd, Hg and Pb was performed by Q-ICP-MS (Elan DCR II, Perkin Elmer SCIEX, Norwalk, CT, USA) applying the published in-house validated method [27]. Matrix effects were compensate by the addition calibration approach that uses digested blood added with a known amount of the element for calibration curve, in this way any fluctuation of the analytical signal was corrected by Rh (internal standard).

Statistical analysis

The statistical analysis used the information from the questionnaires gathered from the interviews and

Table 3
Reference values from literature of Cd, Hg and Pb in blood (μg/l)

	Cd	Hg	Pb
All	0.15-2.04[35] ; 1.6[36]; 3[37]; 0.1-1.97[25]	0.94-8.13[35].; 4.9[36]; 1.7-9.9[24]	11.4-62.8[35] ; 42[36];75[37]; 12.8 - 276[24]
Female	3[37]	4.4[36]; 4.0[37]	70[33]; 80[34]; 35[36]; 65[37]
Male	3.5[37]	5.4[36]; 3.1[37]	90[33]; 90[34]; 48[36]; 95[37]
Smokers	4.5[37]	-	-
Non-smokers	1.0[33]; 1.2[34]; 1.1[37]	-	-

Table 4
Mean concentrations and standard deviation for Cd, Hg and Pb in blood (μg/l) *vs* distance from the river Sacco

Element	Distance from the river				
	<500 m (d1)	from 500 m to 1 km (d2)	from 1 km to 2 km (d3)	from 2 km to 5 km (d4)	>5 km (d5)
Cd	0.418 ± 0.265	0.659 ± 0.752	0.502 ± 0.588	0.464 ± 0.395	0.356 ± 0.318
Hg	3.82 ± 2.32	3.08 ± 1.46	3.26 ± 2.32	3.72 ± 2.42	3.81 ± 2.27
Pb	34.4 ± 25.2	29.1 ± 19.3	30.0 ± 19.2	31.6 ± 26.8	39.1 ± 30.3

Table 5
Characteristics of the interviewees

	Residence area				Total
Classes in the areas (years)	d1	d2	d3	d4	
0 - 5 (y1)	2.2	1.9	2.9	3.3	**2.5**
6 - 10 (y2)	8.2	7.1	8.1	7.7	**7.7**
11 - 15 (y3)	12.8	12.9	13.3	11.5	**12.7**
> 15 (y4)	40.1	30.8	38.9	41.1	**35.1**
Total	**25.8**	**26.0**	**30.4**	**25.0**	**26.8**

linked life styles and possible conditions of exposure to the polluted zones. Sex, age, home distance from the source of pollution (identified by the river Sacco), smoking habits, use of well water and residence area were the eligible variables.

As the first step, a comparison by the information of all questionnaires (509 answers) and the results of the instrumental analysis (240 answers) was adopted to evaluate the effect of the self-selection on experimental data: individuals choose whether or not to participate in sampling of blood, that choice could be correlated with their life style and/or the awareness of a personal exposure *(Tables 1-2)*. This possibility is called the self-selection problem [28]. The Kolmogorov-Smirnov test [29] compares the area distribution of sampled participants (240) versus the area distribution of the total people interviewed (509) by analyzing each single eligible variables: if the test null hypothesis of identical sample distribution is rejected it is possible consider the presence of the blood sample selection effect.

As a second step, the cluster analysis was applied. This kind of analysis considers different measurement definitions for observations data comparison; the aim is to assign objects into group or clusters in such a way that two objects from the same cluster are more similar than two object from different clusters. The similarity is defined by the distance measurement used and by the information – variables considered [30].

Statistical treatment of data was performed by means of the Elaboration Software SAS (ver. 9.1.3 SAS Institute Inc., Cary, NC, USA). Outliers were removed from this statistical treatment and data was checked for duplications.

Table 6
Interviewees characteristics in four clusters by considering the three quantitative variables

	Cluster*[40]				Total
	1	2	3	4	
Metals concentration, age and number of years in polluted areas, average values of 4 clusters selected					
Average blood level (µg/l)					
Cd	0.45958	0.46250	0.42100	0.53893	**0.50203**
Hg	3.89021	4.62333	3.71250	3.92491	**3.93776**
Pb	30.55938	29.28417	42.65150	32.83634	**33.06750**
Average age	44.6	42.0	49.6	45.6	**45.5**
Average number of years in polluted areas	28.9	25.8	31.9	25.9	**27.3**
Observations distributions for: 4 selected clusters, sex, age classes, years lived in polluted areas, smoking habits and use of well water					
Sex					
male	54.2	58.3	65.0	48.2	**52.1**
female	45.8	41.7	35.0	51.8	**47.9**
Age					
20 to 34	27.1	25.0	10.0	23.2	**22.9**
35 to 44	25.0	33.3	20.0	25.9	**25.5**
45 to 54	18.8	33.3	30.0	20.5	**21.9**
55 to 70	29.2	8.3	40.0	30.4	**29.7**
Home distance from river					
less than 500 m	12.5	8.3	15.0	13.4	**13.0**
from 500 m to 1 km	10.4	16.7	5.0	18.8	**15.1**
from 1 to 2 km	37.5	58.3	35.0	25.0	**31.3**
from 2 to 5 km	22.9	8.3	30.0	25.9	**24.5**
more than 5 km	16.7	8.3	15.0	17.0	**16.1**
Ever smoking					
yes	41.7	33.3	55.0	50.9	**47.9**
no	58.3	66.7	45.0	49.1	**52.1**
Domestic use of well water					
yes	45.8	8.3	45.0	37.5	**38.5**
no	54.2	91.7	55.0	62.5	**61.5**
Cooking use of well water					
yes	41.7	8.3	30.0	30.4	**31.8**
no	58.3	91.7	70.0	69.6	**68.2**
Personal washing use of well water					
yes	41.7	0.0	40.0	34.8	**34.9**
no	58.3	100.0	60.0	65.2	**65.1**
Other use of well water					
yes	4.2	0.0	0.0	0.9	**1.6**
no	95.8	100.0	100.0	99.1	**98.4**
Total of replies	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

(*) Average linkage cluster method results for standardized data.

RESULTS AND DISCUSSION

Human exposure is commonly assessed by the use of human biomonitoring (HBM) data or reference values (RV), which are also recommended by the German Human Biomonitoring Commission (GHBC). HBM values are derived on the basis of toxicological and epidemiological studies by expert judgment [31]. According to the German Commission two different levels can be identified: HBM I and HBM II. The former represents the concentration below which there is neither any risk of adverse health effects nor any need for action, while the latter represents the concentration above which an increased risk of adverse health effects exists and intervention and action are needed (reducing exposure and providing advice for individual biomedical care); additional analysis is needed at any concentration level between the aforementioned levels. RVs are statistically derived values that indicate the upper margin of background exposure to a given pollutant in a given population at a given time [31]. The GHBC provided for blood a value of 5 µg/l (HBM I) and 15 µg/l for Hg (HBM II), 150 µg/l (HBM I) and 250 µg/l for Pb (HBM II), 1.0 µg/l for Cd (RV for non-smoking adults), 2.0 µg/l for Hg (RV), 70 µg/l (female) and 90 (male) µg/l for Pb (RV with an analytical uncertainty of 20 µg/l) [31]. In 2009, following to new findings, the GHBC suspended the HBM values (HBM I and HBM II) for Pb in blood for all groups of people; therefore, levels above the RV can be considered as coming from a specific source [32].

Table 3 summarizes RVs and/or ranges of values for Cd, Hg and Pb in blood as obtained from the international literature. These concentrations were selected for this study as arbitrary threshold values by evaluating the recommended HBM, RVs and the information collected from literature: 5 µg/l for Cd, 9 µg/l for Hg and 100 µg/l for Pb. A higher value for Cd was selected to take smokers into account.

Table 4 displays the mean concentrations of Cd, Hg and Pb in blood classified by the distance from the river Sacco. An unexpected scenario emerges from the data, for the levels of the Cd, Hg and Pb do not decrease along with the distance from the river. Indeed, when the data values are organized in an ascending order, the distance from the pollution source does not show a proportional or a direct relation with the elements level. In fact, Cd is higher in d2 and lower in individuals living far from the river (d5). Mercury and Pb do not seem to display a trend and, surprisingly, their levels are relatively high in individuals living more than 5 km from the river (d5). Apparently, the recorded Hg and Pb levels are always higher in the farthest and the nearest areas, while no association can be derived for Cd. For a more specific evaluation of this outcome, an additional characterization of the polluted site would be worth achieving, including sampling of environmental samples.

A comparison between the relative frequency distribution of the areas containing the 509 interviewed people (17.9% belong to area 1; 50.7% to area 2; 23.0% to area 3; 8.4% to area 4) and the relative frequency distribution of the 240 participants who gave a blood sample (19.6% belong to area 1; 45.8% to area 2; 22.1% to area 3; 12.5% to area 4) reveals that interviews of areas 1 and 4 are the most interesting, in particular: 19.6% of participants versus the 17.9% of people interviewed in area 1 and 12.4% of the participants versus the 8.4% of the interviewed people in area 4; the same can be said for men, non-smokers and users of well water of all areas.

The Kolmogorov-Smirnov test is capable of giving a statistical measure of the difference between the two distributions of data, and has indicated as statistically significant the differences of distributions in the five items of the distance from the river and the four items of the areas between the 509 and the 240 subjects. This outcome is important because the definition of the area reflects the aspects of the pollution and the administrative constraints, and it highlights the action of the self-selection process among the areas taken in consideration (for the five distances of *Table 4*, starting from the nearest to the river test values for null hypothesis rejection were 0.88, 0.72, 0.26, 0.64 and 0.86). The test has showed other differences regarding the distribution of data for the two following groups: smokers (null hypothesis rejection test value 0.08) and people who do not use well water at home (null hypothesis rejection test value 0.16). This result may indicate that inhabitants participating in this survey are aware of a possible exposure to environmental pollution.

Other key information may be derived by evaluating the distance from the river (d) as a function of the elements concentration. The three elements resulted quite characterized; more specifically, no sample exceeds the assigned value for Cd (most smokers belonged to the area d1), while Hg and Pb presented three and four high values, respectively. However, these findings do not add useful details about a possible exposure of the subjects enrolled in this monitoring study.

The same approach may be adopted to investigate other characteristics, such as gender and smoking habits. Two different studies reported higher levels of Cd in the blood of smokers belonging to the Italian general population of the Umbria region and in Prague's homeless population [38, 39]. The same result seems to be confirmed by two other investigations, specifically a German biomonitoring survey [33] and a Czech biomonitoring project on general population that found the median value of Cd in blood of smokers to be about 3 times higher than the non-smokers one [37]. However, it is still difficult to evaluate to which extent food ingested by the population contributes to the total intake of these elements. Although in this study the data pertaining to Cd never exceeds the threshold value, there still exists a difference in the distribution of the results between smokers and non-smokers interviewees. The average values are shifted towards higher values for smokers, supporting the hypothesis that the habit of smoking can affect the levels of Cd in blood [36]. The remaining two elements do not exhibit any particular trends related to smoking habits. Gender differentiation revealed that Cd is higher in male specimens, who present a different frequency when compared to female specimens, while in female specimens Hg increases with the distance from the river and a similar relation appears to apply to Pb in male specimens.

The statistical analysis applied so far took the range of elements concentration in blood in consideration and gave priority to the residence areas. Following analysis are presented by adding the new variable, the number of years spent by the subjects in the investigated areas. Data were subsequently distributed in four classes: from 0 to 5 years (y1), from 5 to 10 years (y2), from 10 to 15 years (y3) and more than 15 years (y4). More than three quarters of the interviewees has lived in those areas for a long time. Then, for the quantitative new variable definition, the number of years (in classes) is reported in a continuous variable assuming a uniform distribution in the category range for the observations that have an identical age. Therefore, individuals belonging to a given class of possible years of exposure and of the same age are considered to be equally distributed. For instance, three individuals belonging to class (y1) and aged 41 in the new variable are considered to have spent 0, 2.5 and 5 years in polluted areas, respectively. Focusing on an individual age constraint of 70, three individuals of the class (y4) and aged 58 in the new variable have spent 16, 50.5 and 58 years in the polluted areas, respectively. *Table 5* shows the average values of this new quantitative variable for each area and class of exposure. The results show that the interviewees have spent on average 26.8 years of their life in polluted areas. This can be derived from the interviewees' concentration in the last classes (y4). It also appears that the average time of exposure is higher in the d3 area, as more than the 73% of the interviewees were subjected to an exposure time of 38.9 years. By considering the territorial distribution, the periods of exposure are very similar, with the only exception of the d3 area. This new quantitative variable is then used in the cluster analysis.

First of all, all available variables (quantitative and qualitative) were considered with different clustering methods; thus, by evaluating the results of different elaborations, a set of variables offering the clearest indication for the target of this study was identified. The cluster analysis, considered until now, used an average linked approach by considering only the three quantitative variables that give the elements measurement in blood.

The analysis identifies four prevalent groups *(Table 6)*, not comprehensive of all observations (192 out of 240, or 80% of the observations), which present some peculiar characteristics deriving only from data on blood: individuals belonging to the four groups identified through cluster analysis typically present higher values of Cd and lower values of Pb in blood versus the total average of interviewees, and a similar trend can be observed for Hg as well. The first group does not have distinct characteristics for the analyzed elements; participants have lived in polluted areas for more than 28 years (28.9) and their average age is 44.6; for the most part they are men showing low attention to potential risks arising from smoking and using well water. The second group presents the lower Pb values, higher Hg values and an interesting Cd value. Individuals belonging to this group are for the most part men with the lowest average age (42 years), more than half of them had lived in the polluted area; they also seem to pay attention to the domestic use of well water. The third group presents the higher average Pb value and the lower Hg and Cd values. The group is mainly composed of males (65%) with an average age of 49.6 years; most of them had lived in the polluted areas. The fourth group presents the highest level of Cd and an average age of 45.6 years; this group also presents a slight but identifiable prevalence of women who are not particularly characterized for the use of well water. No other group specific characteristics were identified, but data in table shows that the gender characterization of the second and the third group as the differences in the well water use had effects on the average presence of metals in blood.

CONCLUSIONS

From the analysis of data, it can be observed that no sample exceeds the assigned value for Cd, whereas for Hg and Pb three and four high values were highlighted. Although some gender differentiation was noticed for Cd, its content in blood is normally influenced by smoking habit as also reported by international literature, while, for non-smokers the main source of exposure remains food. As regards Pb, a rather wide range of reference concentrations was found in literature, and this turned out to be a disadvantage for the evaluation of the results, additionally, it is known that the amount of Pb increases with age. The consumption of food and potable water generally affects the level of these three trace elements in human specimens. The test of Kolmogorov-Smirnov showed differences regarding the distribution of data for people interviewed and participants, the two groups were the smokers and people who do not use well water at home.

Generally, participants did not exhibit hazardous levels of Cd, Hg and Pb in blood. These results do not add significant information on the spread of pollution with reference to these three elements. Surprisingly, the distance from the river Sacco does not seem to be directly connected with the levels of these elements in blood of residents. This biomonitoring study does not highlight specific risks or life-threatening situations for health of people living in those areas. The unclear trend of data would require a further characterization of the polluted site possibly with environmental sampling of different matrices.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study. The opinions expressed by the author A. Draicchio under no circumstances reflect the views of the Ministero dell'Economia e Finanze.

Acknowledgements

The authors thank Gianluca Finocchiaro for editorial assistance on the manuscript.

Received on 4 April 2012.
Accepted on 28 August 2012.

REFERENCES

1. US Environmental Protection Agency. *Framework for Metals Risk Assessment*. Washington (USA): US EPA; 2007. (EPA Document 120/R-07/001).
2. *ATSDR ToxProfiles™, Toxicological profile for Cadmium*. Washington (USA): Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services; 2008.
3. *ATSDR ToxProfiles™, Toxicological profile for Lead.* Washington (USA): Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services; 2007.
4. *ATSDR ToxProfiles™, Toxicological profile for Mercury.* Washington (USA): Agency for Toxic Substances and Disease Registry, U.S. Department of Health and Human Services; 1999.
5. World Health Organization. *Health risks of heavy metals from long-range transboundary air pollution.* Copenhagen (Denmark): Joint World Health Organization/Convention Task Force on the Health Aspects of Air Pollution, WHO Regional Office for Europe; 2007. (E91044:144, 2007).
6. Needham LL, Calafat AM, Barr DB. Uses and issues of biomonitoring. *Int J Hyg Environ Health* 2007:210:229-38. DOI: 10.1016/j.ijheh.2006.11.002
7. Paustenbach D, Galbraith D. 2006. Biomonitoring: is body burden relevant to public health? *Regul Toxicol Pharm* 21006;44:249-61. DOI: 10.1016/j.yrtph.2006.01.005
8. Esteban M, Castaño A. 2009. Non-invasive matrices in human biomonitoring: A review. *Environ Int* 2009;35:438-49. DOI: 10.1016/j.envint.2008.09.003
9. Centers for Disease Control and Prevention. *CDC Fourth National Report on Human Exposure to Environmental Chemicals.* Atlanta (USA): DC, Department of Health and Human Services; 2009.
10. Sexteon K, Needham LL, Pirkle JL. Measuring chemicals in human tissues is the "gold standard" for assessing people's exposure to pollution. *Am Sci* 2004;92:38-45.
11. European Communities.*Technical Guidance Document on Risk Assessment.* Luxembourg: Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau (ECB), Office for official publications of the European Communities; 2003 (European Commission - TGD part I).
12. Pirkle JL, Osterloh J, Needham LL. Sampson EJ. National exposure measurements for decisions to protect public health from environmental exposures. *Int J Hyg Environ Health* 2005;208:1-5. DOI: 10.1016/j.ijheh.2005.01.001
13. Reis MF, Sampaio C, Brantes A, Melim M, Cardoso L, Gabriel C, Simão F, Miguel JP. Human exposure to heavy metals in the vicinity of Portuguese solid waste incinerators - Part 1: Biomonitoring of Pb, Cd and Hg in blood of the general population. *Int J Hyg Environ Health* 2007;210:439-46.
14. Ferré-Huguet N, Nadal M, Schuhmacher M, Domingo JL. Monitoring metals in blood and hair of the population living near a hazardous waste incinerator: temporal trend. *Biol Trace Elem Res* 2009;128:191-9. DOI: 10.1007/s12011-008-8274-9.
15. Khassouani CE, Soulaymani R, Mauras Y, Allain P. Blood cadmium concentration in the population of the Rabat area, Morocco. *Clin Chim Acta* 2000;302:155-60. DOI: 10.1016/S0009-8981(00)00368-5
16. Son JY, Lee J, Paek D, Lee JT. Blood levels of lead, cadmium, and mercury in the Korean population: results from the second Korean National Human Exposure and Biomonitoring Examination. *Environ Res* 2009;109:38-744. DOI: 10.1016/j.envres.2009.03.012
17. Ikeda M, Zhang ZW, Shimbo S, Watanabe T, Nakatsuka H, Moon CS, Matsuda-Inoguchi N, Higashikawa K. Urban population exposure to lead and cadmium in east and south-east Asia. *Sci Total Environ* 2000;249:373-84. DOI: 10.1016/S0048-9697(99)00527-6
18. Bu-Olayan AH, Thomas BV. Levels of lead in blood in residents of Kuwait. *Environmentalist* 2001;21:109-13.
19. Gundacker C, Komarnicki G, Zödl B, Forster C, Schuster E, Wittmann K. Whole blood mercury and selenium concentrations in a selected Austrian population: Does gender matter? *Sci Total Environ* 2006;372:76-86. DOI: 10.1016/j.scitotenv.2006.08.006
20. McKelvey W, Gwynn RC, Jeffery N, Kass D, Thorpe LE, Garg RK, Palmer CD, Parsons PJ. A biomonitoring study of lead, cadmium, and mercury in the blood of New York city adults. *Environ Health Pers* 2007;115:1435-41. DOI: 10.1289/ehp.10056
21. Afridi HI, Kazi TG, Jamali MK, Kazi GH, Arain MB, Jalbani N, Shar GQ, Sarfaraz RA. Evaluation of toxic metals in biological samples (scalp hair, blood and urine) of steel mill workers by electrothermal atomic absorption spectrometry. *Toxicol Ind Health* 2006;22:381-93. DOI: 10.1177/0748233706073420
22. Jin T, Nordberg M, Frech W, Dumont X, Bernard A, Ye T, Kong Q, Wang Z, Li P, Lundström NG, Li Y, Nordberg GF. Cadmium biomonitoring and renal dysfunction among a population environmentally exposed to cadmium from smelting in China (ChinaCad). *BioMetals* 2002;15:397-410.
23. Cortes-Maramba N, Paciano Reyes J, Trinidad Francisco-Rivera A, Akagi H, Sunio R, Crisanta Panganiban L. Health and environmental assessment of mercury exposure in a gold mining community in Western Mindanao, Philippines. *J Environ Manage* 2006;81:126-34. DOI: 10.1016/j.jenvman.2006.01.019
24. Alimonti A, Bocca B, Mattei D, Pino A. *Biomonitoraggio della popolazione italiana per l'esposizione ai metalli: valori di riferimento 1990-2009. Rapporto 2010.* Roma: Istituto Superiore di Sanità; 2010. (Rapporti ISTISAN, 10/22).
25. Fano V, Porta D, Dell'Orco V, Blasetti F, De Felip E, Di Domenico A, Fantini F, Corbo A, D'Ovidio M, Forastiere F, Perucci CA. *Esperienza del Lazio sulla valle del fiume Sacco: studi epidemiologici in un'area contaminata da composti organoclorurati persistenti*. Roma: Istituto Superiore di Sanità; 2010. (Rapporti ISTISAN, 06/19).
26. *ATSDR ToxProfiles™, Toxicological profile for alpha-, beta-, gamma-, and delta-hexachlorocyclohexane.* Washington (USA): Agency for Toxic Substances and Disease Registry, US Department of Health and Human Services; 2005.
27. D'Ilio S, Majorani C, Petrucci F, Violante N, Senofonte O. Method validation for the quantification of As, Cd, Hg and Pb in blood by ICP-MS for monitoring purposes. *Anal Methods* 2010;2:2049-54. DOI: 10.1039/C0AY00429D
28. Wooldridge JM. *Econometric analysis of cross section and panel data*. England (Massachusetts London): The MIT Press Cambridge; 2004.
29. Piccol, D. *Statistica.* Bologna: Il Mulino; 2000.
30. Everitt BS, Landau S, Leese M, Stahl D. *Cluster analysis.* 5th Ed. USA: John Wiley & Sons Ltd; 2011.
31. Schulz C, Angerer J, Ewers U, Kolossa-Gehring M. The German Human Biomonitoring Commission. *Int J Hyg Environ Health* 2007;210:373-82. DOI: 10.1016/j.ijheh.2007.01.035
32. Wilhelm M, Heinzow B, Angererd J, Schulze C. Reassessment of critical lead effects by the German Human Biomonitoring Commission results in suspen-

sion of the human biomonitoring values (HBM I and HBM II) for lead in blood of children and adults. *Int J Hyg Environ Health* 2010;213:265-9. DOI: 10.1016/j.ijheh.2010.04.002
33. Wilhelm M, Ewers U, Schulz C. Revised and new reference values for some trace elements in blood and urine for human biomonitoring in environmental medicine. *Int J Hyg Environ Health* 2004;207:69-73.
34. Černá M, Spěváčková V, Beneš B, Čejchanová M, Šmíd J. Reference values for lead and cadmium in blood of Czech population. *Int J Occup Med Environ Health* 2001;14:89-192.
35. Goullé JP, Mahieu L, Castermant J, Neveu N, Bonneau L, Lainé G, Bouige D, Lacroix C. Metal and metalloid multi-elementary ICP-MS validation in whole blood, plasma, urine and hair. Reference values. *Forensic Sci Int* 2005;153:39-44. DOI: 10.1016/j.forsciint.2005.04.020
36. Center for Disease Control and Prevention. *Fourth National Report on Human Exposure to Environmental Chemicals.* Atlanta (USA): CDC, National Center for Environmental Health, Division of Laboratory Sciences; 2009.
37. Batáriova A, Spěváčková V, Beneš B, Čejchanová M, Šmíd J, Černá M. Blood and urine levels of Pb, Cd and Hg in the general population of the Czech Republic and proposed reference values. *Int J Hyg Environ Health* 2006;209:359-66. DOI: 10.1016/j.ijheh.2006.02.005
38. dell'Omo M, Muzi G, Piccinini R, Gambelunghe A, Morucci P, Fiordi T, Ambrogi M, Abbritti G. Blood cadmium concentrations in the general population of Umbria, Central Italy. *Sci Total Environ* 1999;226:57-64.
39. Hrnčířová D, Batáriova A, Černá M, Procházka B, Dlouhý P, Anděl M. Exposure of Prague's homeless population to lead and cadmium, compared to Prague's general population. *Int J Hyg Environ Health* 2008;211:580-6. DOI: 10.1016/j.ijheh.2007.09.006
40. Sokal RR, Michener CD. A Statistical Method for Evaluating Systematic Relationships. *Univ Kans Sci Bull* 1958;38:1409-38.