

## Hair Trace Elements Concentration to Describe Polymetallic Mining Waste Exposure in Bolivian Altiplano

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**Abstract** Severe polymetallic contamination is frequently observed in the mining communities of Bolivian *Altiplano*. We evaluated hair trace elements concentrations at the population level to characterise exposure profile in different contexts of contact with mining and metallurgical pollution. We sampled 242 children aged 7 to 12 years in schools from five Oruro districts located in different contexts of potential contamination. Hair trace elements concentrations were measured using ICP-MS (Pb, As, Hg, Cd, Sb, Sn, Bi, Ag, Ni, Se, Cu, Cr, Mn, Co and Zn). We compared concentration according to school areas and gender. Concentrations were markedly different depending on school areas. Children from schools near industrial areas were far more exposed to non essential elements than children from downtown and suburban schools, as well as the rural school. The most concentrated non-essential element was Pb (geometric means (SD): 1.6 (1.3)  $\mu\text{g/g}$  in rural school; 2.0 (2.3)  $\mu\text{g/g}$  in suburban school; 2.3 (3.0)  $\mu\text{g/g}$  in downtown school; 14.1 (2.7)  $\mu\text{g/g}$  in the mine school and 21.2 (3.3)  $\mu\text{g/g}$  in the smelter school). Boys showed higher levels for all non-essential elements while girls had higher levels of Zn. Hair trace elements concentrations highlighted the heterogeneity of exposure profiles, identifying the most contaminated districts.

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## Introduction

Severe polymetallic contamination of the environment is frequently observed in the *Altiplanic* (highland) mining communities [1–4]. Although industrial activity is mainly centred on gold, silver, tin and zinc exploitation, lead, cadmium, arsenic and many other elements are frequently disseminated in the environment along with mining wastes and acid mine drainages.

For political and historical reasons, the population and administrative authorities do not have a clear consciousness of the sanitary risk, especially for children. This is mainly due to the lack of scientific data on biological exposure evaluation.

Assessment of human exposure to different environmental contaminants primarily relies on the analysis of body fluids such as blood or urine. Alternative biomarkers based on noninvasively collected tissue; i.e. hair, nail or saliva, have received growing interest since last decades. Hair is a biological tissue that is easily, inexpensively, and noninvasively collected. Moreover, it can be easily stored and transported to the laboratory for analysis. These characteristics make hair an attractive material in a setting like Altiplano mining communities with no availability of specialized laboratory resource and with reluctance of the population towards blood sampling.

Hair analysis has been widely used to assess human exposure to different contaminants. For example, hair mercury concentration was shown to be a reliable biomarker for methylmercury exposure [5]. Other elements have been also measured in hair and references values have been proposed in specific populations [6–22]. However, the use of hair analysis has several limitations. One problem relies in the difficulty to distinguish between endogenous (absorbed into the blood and incorporated into the hair) and exogenous contamination (derived from external contamination) [23, 24]. Other difficulties come from the absence of reliable background reference ranges for the different populations due to lack of reproducibility. These limitations are of critical relevance when hair is used to assess individual exposure to contaminants or to assess nutritional status [25–27].

Nevertheless, hair analysis may be a useful screening tool in understanding environmental exposure at a population level, if toxicological interpretation is avoided.

The present study aimed to provide a preliminary assessment of polymetallic mining waste exposure in a Bolivian mining city. We compared levels of hair trace elements concentration between school-aged children living in five different environmental contexts, in order to characterize profiles of exposure in these different areas of the city.

## Methods

### Population

The study took place in Oruro city (17° 58' S–67° 06' W), a 200,000 inhabitants mining town, situated at 3700 meters above sea level in the centre of Bolivian *Altiplano* and in Caracollo (17° 38' S–67° 13' W) a rural village 30 km North from this city. We included children from third grade (8 years of theoretical age). These children were selected from the

Department of Oruro Public Schools in five different contexts: a school near a large mine in exploitation, mainly attended by miners' children ("Mine School"), a school near an important tin smelter ("Smelter School"), a school downtown attended by children from middle class ("Downtown School"), a suburban school far from industrial centres, attended by children whose families are not involved in the mining process ("Suburban School") and a rural school in the village of Caracollo ("Rural School"), 30 km North from Oruro. Both Mine School and Smelter School are considered "Industrial Areas Schools".

In this context, third grade children do not participate in either industrial or mining activities, so their exposure is the result of environmental contact with the potential contaminants.

Previous informed written consents were obtained from parents after public meetings organized with the participation of schools teachers. This study received ethic clearance from the National Bioethics Committee of Bolivia.

### Measurements and Analysis

We sampled hair strands from the occipital region from every child using stainless steel scissors. No matter the length of the hair, for boys as well as for girls, only the five proximal centimetres were sent to the laboratory, in order to evaluate the same exposure period. The following trace elements were measured using ICP-MS: Pb, As, Hg, Cd, Sb, Sn, Bi, Ag and Ni considered as non-essential elements and Se, Cu, Cr, Mn, Co and Zn as essential elements.

### Sample Analysis

The washing procedure of hair samples was based on the International Atomic Energy Agency protocol (IAEA/RL/50): in a clean Pyrex beaker each sample was submitted to 10 min contacts (using a mechanical shaking) with 15 ml portions of, successively, acetone, water, water, and acetone, decanting off the wash liquid after each 10-min wash. After washing the samples were air-dried at room temperature in a dust free laboratory. The reagents used were: ultrapure water, 18 $\Omega$ , produced in a Millipore Super-Q system (Millipore<sup>®</sup>, Milford, MA, USA), nitric acid (65%) and H<sub>2</sub>O<sub>2</sub> (30%) from Merck<sup>®</sup>, Darmstadt, Germany. The acid was purified by sub-boiling distillation in a quartz still. Stock standard solutions were prepared from 1,000  $\mu$ g/g stock solutions of each element of interest (Spex Industries Inc<sup>®</sup>, Edson, New Jersey, USA). All standard solutions were made up in 2% HNO<sub>3</sub>.

The samples digestion method was based on ultrasonic assisted acid digestion by using an ultrasonic cleaner Tornton<sup>®</sup>, Model GA 1,000, 1,000 W, programmable for temperature ranging from 0°C to 90°C. Approximately 100 mg of all samples were weighted directly into clean polypropylene autosampler tubes (15 ml capacity) with 1 ml of concentrate HNO<sub>3</sub> and 0.1 ml of H<sub>2</sub>O<sub>2</sub>. The flasks were stood for 15 min at room temperature. After this period the flasks were immersed into ultrasonic water bath pre-heated at 75°C and remained in this condition for 60 min. After sonication and cooling to room temperature, approximately 1 g of In solution (10 ng/l), used as internal standard, was added. The final volume of 10 ml was made with deionized water by weight.

All samples preparations were carried out in a controlled area specially designed for biological material manipulation.

The quantification was performed by external calibration with seven standard solutions in concentrations from 1 to 70 ng ng/g, with 1 g of In internal standard solution.

Measurements were done using a sector field inductively coupled plasma mass spectrometer (SF-ICPMS) ELEMENT 1—Finnigan Mat®, Bremen, Germany using a low flow (1 ml/min) concentric nebulizer (Meinhard®, Santa Ana, CA, USA), a Scott-type spray chamber cooled to 5°C. Isotopes were acquired in E-scan mode in low and medium resolution. Three replicate analyses were performed on each sample after a 60-s uptake and 60-s stabilization period.

### Statistical Analysis

Statistical analyses were performed using SAS statistical package software version 8.1 (SAS Institute, Cary, NC). For statistical analysis, we log transformed concentrations to fit when possible a normal distribution. In order to obtain an exposure “profile” allowing comparisons between elements and areas, we standardized geometric means concentrations using Z-scores. We conducted factor analysis using principal component analysis (PROC FACTOR) in order to identify subsets (clusters) of correlated variables. This procedure attempts to reduce overall dimensionality of the linearly correlated data by using a smaller number of new independent variables, called principal components (PC). The components with eigenvalue greater than 1.0 were retained in the analysis. We applied a varimax orthogonal rotation to identify distinct latent factors and to facilitate interpretation. Interpretation is based on the pattern of correlations between the factors, or PCs, and the original variables; these correlations are called loadings. We considered loadings greater than  $\pm 0.3$  to interpret the data. We also conducted multivariate regression analyses (PROC GLM) for a subset of trace elements to compare concentration levels between schools with adjustment for age and gender.

### Results

The ages of the 242 children included in the study ranged from 7 to 12 years with a mean of 7.9 years. Mean ages were similar between genders, but differed between schools with younger children in Downtown and Mine Schools ( $P < 0.01$ ). Sex ratio differed between schools ( $P < 0.01$ ); the proportion of boys was higher in Downtown and Industrial Areas Schools (Table 1).

**Table 1** Age and Sex Distribution According to School

| Schools  | Number | Age       |         |          |           |          |           | Sex-ratio M/F |
|----------|--------|-----------|---------|----------|-----------|----------|-----------|---------------|
|          |        | Total     |         | Girls    |           | Boys     |           |               |
|          |        | Mean (SD) | Min-Max | <i>n</i> | Mean (SD) | <i>n</i> | Mean (SD) |               |
| Rural    | 33     | 8.2 (1.0) | 7–12    | 20       | 8.1 (0.8) | 13       | 8.4 (1.2) | 0.39          |
| Suburb   | 52     | 8.2 (0.8) | 7–10    | 30       | 7.9 (0.7) | 22       | 8.5 (0.9) | 0.42          |
| Downtown | 71     | 7.6 (0.5) | 7–9     | 25       | 7.7 (0.6) | 46       | 7.6 (0.5) | 0.65          |
| Mine     | 60     | 7.9 (1.0) | 7–2     | 19       | 7.7 (0.6) | 41       | 7.9 (1.1) | 0.68          |
| Smelter  | 26     | 8.1 (0.8) | 7–11    | 10       | 8.2 (0.6) | 16       | 8.1 (0.8) | 0.62          |

Basic distribution parameters for trace elements concentrations in the five different schools are given in Tables 2, 3 and illustrated in Fig. 1, using standard deviations from the mean (*Z*-scores).

A strong variation of mean concentration levels was observed between areas of school attendance for every trace element measured (Table 2, 3, Fig. 1). Basically, children from the Industrial Areas Schools showed higher mean levels for non-essential elements than the other areas. The lowest levels for non-essential elements were observed in the Rural School.

Mean levels of non-essential elements in children from the Mine school were at least twice to twelve times higher when compared to children from the Rural School. On the contrary, the concentrations of essential elements such as Zn, Cu and Mn were quite similar between these two areas. For Cobalt, 46 samples had values below the detection limits of ICP-MS technique (19%), while the rest of the samples showed a log normal distribution. Unexpectedly, all these low values were found in the Mine School ( $n=32$ ) and the Smelter School ( $n=14$ ).

In these two schools, the distributions of trace elements were found to be more dispersed and with very low and high outlier values (Fig. 1). For example, Zn distribution ranged from 32 to 357  $\mu\text{g/g}$  in the Mine School, while it ranged from 85 to 210  $\mu\text{g/g}$  in the Rural School. The same dispersion pattern was observed for Ni, Co, Mn, Se and Cu (Fig. 1).

We conducted PCA using the 15 metal concentrations measured in 242 children (Table 4). Three Principal Components (eigenvalues $>1$ ) emerged and accounted for 62% of the total variance. Factor 1 was positively correlated with almost all non-essential metals, showing maximum loadings for As, Pb, Sn, Ag and Bi with significant contribution of Hg and Sb, suggesting polymetallic contamination at the individual level. Unexpectedly, Se was also correlated with the first component with a loading of 0.67, whereas a negative correlation was found for Co. Factor 2 was clearly associated with essential elements,

**Table 2** Trace Elements Concentration in the Rural, Suburban and Downtown Schools

|    | Rural school        |            | Suburb school       |            | Downtown school     |            |
|----|---------------------|------------|---------------------|------------|---------------------|------------|
|    | Geometric mean (SD) | P5–P95     | Geometric mean (SD) | P5–P95     | Geometric mean (SD) | P5–P95     |
| Pb | 1.57 (1.33)         | 1.10–2.64  | 2.03 (2.29)         | 0.39–8.05  | 2.32 (2.98)         | 0.33–10.19 |
| As | 0.07 (6.73)         | 0.00–0.44  | 0.42 (2.5)          | 0.11–1.74  | 0.39 (2.31)         | 0.11–1.24  |
| Cd | 0.05 (1.53)         | 0.02–0.12  | 0.08 (1.95)         | 0.03–0.35  | 0.08 (2.11)         | 0.03–0.19  |
| Hg | 0.13 (1.93)         | 0.07–0.90  | 0.13 (1.48)         | 0.08–0.21  | 0.15 (3.03)         | 0.05–0.50  |
| Sn | 0.05 (1.68)         | 0.02–0.09  | 0.08 (1.75)         | 0.03–0.17  | 0.12 (2.20)         | 0.04–0.38  |
| Sb | 0.02 (9.47)         | 0.00–0.08  | 0.09 (1.57)         | 0.04–0.21  | 0.08 (3.75)         | 0.02–0.35  |
| Ni | 0.18 (3.13)         | 0.05–1.05  | 0.52 (1.85)         | 0.24–2.11  | 0.13 (6.42)         | 0.00–0.85  |
| Bi | 0.01 (3.73)         | 0.00–0.18  | 0.02 (3.12)         | 0.01–0.11  | 0.05 (3.98)         | 0.01–0.51  |
| Ag | 0.05 (1.48)         | 0.03–0.11  | 0.12 (2.28)         | 0.02–0.51  | 0.07 (3.31)         | 0.01–0.76  |
| Mn | 1.26 (2.14)         | 0.27–5.48  | 1.53 (1.93)         | 0.48–3.86  | 0.56 (6.33)         | 0.00–2.81  |
| Cr | 0.05 (1.69)         | 0.02–0.12  | 0.09 (1.57)         | 0.04–0.14  | 0.07 (2.81)         | 0.01–0.32  |
| Cu | 7.76 (1.29)         | 4.04–11.76 | 7.40 (1.34)         | 4.54–11.20 | 6.89 (1.62)         | 3.53–15.44 |
| Co | 0.01 (2.05)         | 0.00–0.05  | 0.02 (1.72)         | 0.01–0.04  | 0.01 (1.79)         | 0.00–0.03  |
| Se | 0.57 (1.58)         | 0.33–1.21  | 1.10 (1.96)         | 0.45–3.77  | 0.90 (2.12)         | 0.34–3.89  |
| Zn | 145.40 (1.31)       | 85.1–210.1 | 105.87 (1.75)       | 35.1–190.3 | 85.28 (1.57)        | 32.9–146.3 |

Results in microgram per gram of hair

**Table 3** Trace Elements Concentration in the Mine and the Smelter Schools

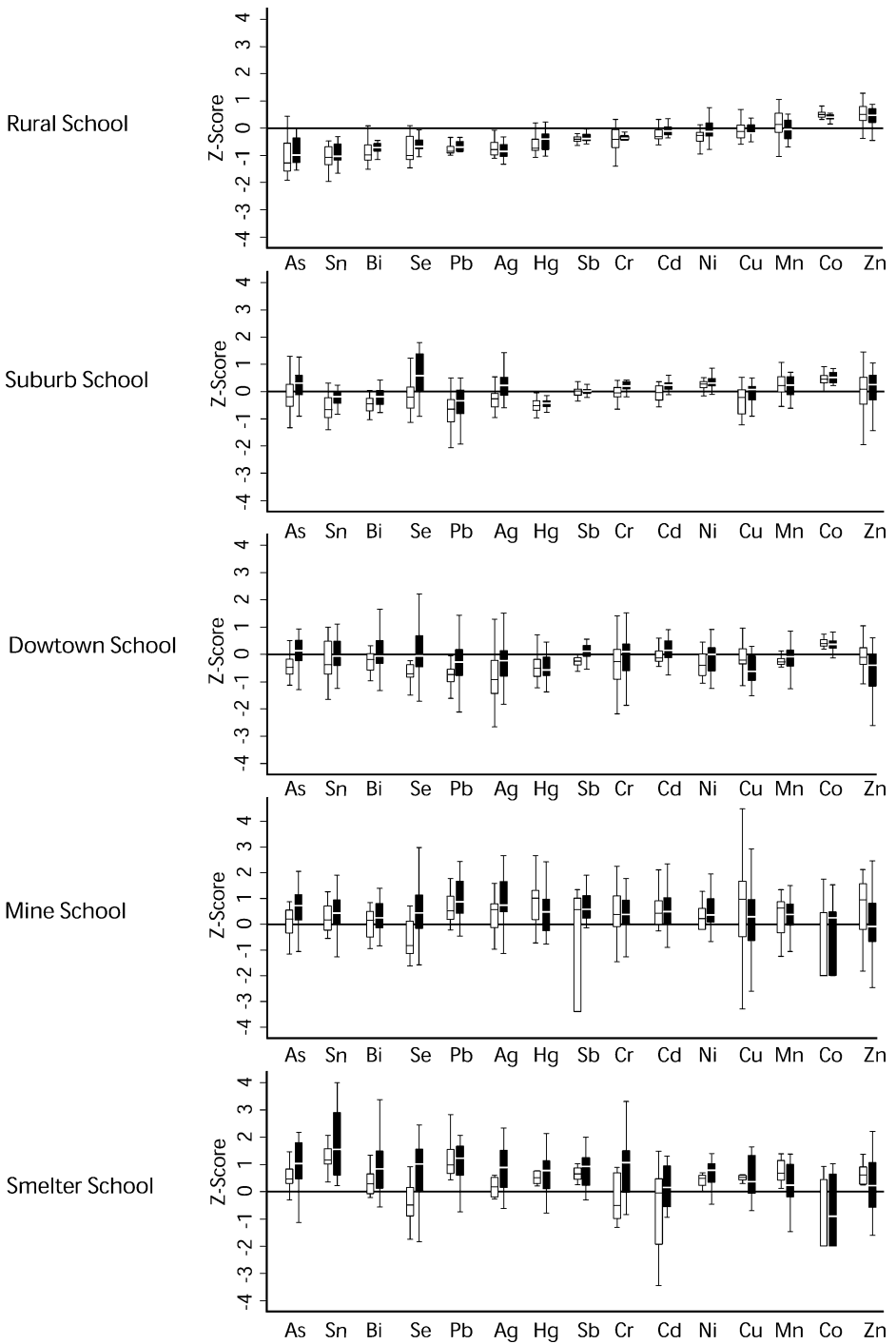
|    | Mine school         |             | Smelter school      |            |
|----|---------------------|-------------|---------------------|------------|
|    | Geometric mean (SD) | P5–P95      | Geometric mean (SD) | P5–P95     |
| Pb | 14.08 (2.72)        | 3.24–65.4   | 21.22 (3.30)        | 2.67–208.8 |
| As | 0.79 (2.79)         | 0.10–3.30   | 1.32 (3.01)         | 0.28–5.55  |
| Cd | 0.07 (16.72)        | 0.00–2.03   | 0.03 (15.93)        | 0.00–0.65  |
| Hg | 0.49 (3.68)         | 0.09–8.44   | 0.50 (2.57)         | 0.13–2.55  |
| Sn | 0.20 (2.20)         | 0.05–0.67   | 0.72 (3.15)         | 0.18–4.86  |
| Sb | 0.10 (56.16)        | 0.00–4.14   | 0.62 (4.02)         | 0.08–5.44  |
| Ni | 0.36 (9.55)         | 0.00–6.94   | 0.64 (4.26)         | 0.12–2.82  |
| Bi | 0.06 (3.41)         | 0.02–0.72   | 0.11 (3.77)         | 0.02–0.88  |
| Ag | 0.33 (3.54)         | 0.04–3.11   | 0.29 (2.87)         | 0.08–2.04  |
| Mn | 1.34 (6.75)         | 0.03–8.18   | 2.41 (2.91)         | 0.63–9.58  |
| Cr | 0.12 (4.12)         | 0.00–0.83   | 0.11 (5.51)         | 0.00–0.69  |
| Cu | 11.37 (2.57)        | 3.17–44.89  | 11.77 (1.47)        | 6.69–22.26 |
| Co | 0.00 (35.77)        | 0.00–0.08   | 0.00 (36.45)        | 0.00–0.05  |
| Se | 1.08 (2.39)         | 0.28–5.23   | 1.27 (2.51)         | 0.24–5.84  |
| Zn | 123.66 (2.14)       | 31.47–357.3 | 142.38 (1.76)       | 57.3–385.4 |

Results in  $\mu\text{g/g}$  of hair

especially with Zn and Cu and to a lesser extent with Mn and Cr. The third PC showed higher loadings for Cd, Co and Sb. These three elements showed similar pattern of distribution with a high dispersion of values for Industrial Areas Schools compared with the other areas (Fig. 1).

A scatter plot of loadings on the two first PCs by School and gender revealed a spatial and gender pattern (Fig. 2). As expected, Smelter and Mine Schools showed higher loadings on the first factor compared to the other areas, especially in comparison with the Rural School. In each school, boys tended to have higher values for the first axis, suggesting “higher risk” for polymetallic contamination. Differences between schools were less marked for Factor 2, indicating that overall, children exhibited more similar levels of essential elements like Zn, Cu, Mn and Cr. However, contrary to the first factor, girls tended to exhibit higher levels of essential elements compared with boys.

After this description of the exposure profile by school and gender, we conducted multivariate regression analyses to compare concentration levels between schools and between genders. Only three elements were selected for these analyses to restrain the number of statistical inferences. We selected Pb, As and Zn because they exhibited the highest loadings; Pb and As with the first component (non-essential elements) and Zn with the second component (essential elements). Comparison of mean levels of Pb, As and Zn between the schools showed significant differences. Two-by-two comparisons with Bonferroni correction indicated that Pb levels were similar in Smelter and Mine Schools on one hand and in Suburban, Downtown and Rural Schools on the other hand. As expected, the children from the Industrial Areas Schools had significantly higher levels of Pb concentration compared to the other three ( $P < 0.0001$  for all comparisons). Mean levels of As were also significantly higher in the Industrial Areas Schools, except for the comparison between Mine and Suburban schools ( $P = 0.26$ ). Arsenic levels in the children from the Rural School were also significantly lower compared to all other areas ( $P <$



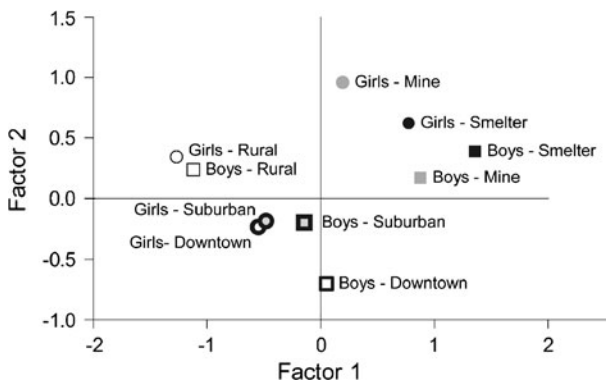
**Fig. 1** Trace elements profile, by sex, according to school district. *Black boxplots* for boys; *white boxplots* for girls

**Table 4** Principal Component Loadings using Varimax Normalized Rotation on the Dataset of Selected Metals ( $n=242$ )

| Metal             | Factor 1 | Factor 2 | Factor 3 |
|-------------------|----------|----------|----------|
| As                | 0.827    |          |          |
| Pb                | 0.825    |          |          |
| Sn                | 0.805    | 0.307    |          |
| Ag                | 0.745    |          |          |
| Bi                | 0.723    |          |          |
| Se                | 0.673    |          | 0.343    |
| Hg                | 0.590    | 0.468    | -0.330   |
| Zn                |          | 0.880    |          |
| Cu                |          | 0.810    |          |
| Mn                |          | 0.534    | 0.463    |
| Cr                | 0.335    | 0.507    | 0.391    |
| Cd                |          |          | 0.729    |
| Co                | -0.358   |          | 0.673    |
| Sb                | 0.502    |          | 0.529    |
| Ni                |          |          | 0.465    |
| Eigen value       | 5.66     | 1.91     | 1.73     |
| % total variance  | 0.38     | 0.13     | 0.11     |
| Cumul. % variance | 0.38     | 0.51     | 0.62     |

0.0001), including Downtown and Suburban Schools. The comparisons between levels of Zn displayed a different pattern. Mine, Smelter, and Rural Schools had similar Zn concentrations, significantly higher than the concentration observed in children from the Downtown School ( $P=0.001$ ,  $P=0.0009$ ,  $P=0.0005$ , respectively). Suburban School displayed intermediate concentration levels with no significant difference with any other school.

The comparison of concentration levels between boys and girls (Table 5), after adjusting for school affiliation, indicated significant higher levels of Pb and As in boys ( $P=0.001$  and  $P<0.0001$ , respectively) whereas levels of Zn were significantly higher in girls ( $P=0.02$ ).



**Fig. 2** Scatter plot of loadings on the two first PCs by school and gender

**Table 5** Trace Element Concentration by Sex

|    | Girls               |             | Boys                |             |
|----|---------------------|-------------|---------------------|-------------|
|    | Geometric mean (SD) | P5–P95      | Geometric mean (SD) | P5–P95      |
| Pb | 2.86 (3.52)         | 0.57–30.05  | 5.69 (4.02)         | 1.02–65.47  |
| As | 0.25 (4.30)         | 0.04–1.35   | 0.62 (3.33)         | 0.08–4.11   |
| Hg | 0.20 (3.13)         | 0.07–1.54   | 0.23 (3.17)         | 0.07–2.89   |
| Cd | 0.05 (5.11)         | 0.00–0.33   | 0.08 (6.27)         | 0.00–0.65   |
| Sn | 0.10 (2.55)         | 0.03–0.48   | 0.16 (2.99)         | 0.04–2.13   |
| Sb | 0.06 (11.70)        | 0.00–1.14   | 0.12 (11.60)        | 0.00–4.06   |
| Bi | 0.03 (3.26)         | 0.01–0.21   | 0.05 (4.71)         | 0.01–0.68   |
| Ni | 0.23 (4.89)         | 0.00–1.15   | 0.32 (6.50)         | 0.00–5.50   |
| Ag | 0.08 (2.75)         | 0.02–0.44   | 0.17 (3.90)         | 0.02–2.04   |
| Se | 0.69 (1.93)         | 0.31–2.20   | 1.23 (2.18)         | 0.38–5.03   |
| Cu | 8.63 (1.77)         | 4.04–23.48  | 8.45 (1.91)         | 3.82–25.07  |
| Cr | 0.07 (3.01)         | 0.01–0.32   | 0.10 (3.05)         | 0.01–0.51   |
| Mn | 1.36 (3.33)         | 0.31–6.69   | 0.98 (5.81)         | 0.05–5.87   |
| Co | 0.0047 (13.52)      | 0.00–0.05   | 0.0039 (15.45)      | 0.00–0.04   |
| Zn | 123.85 (1.74)       | 45.43–273.5 | 102.71 (1.84)       | 32.85–255.4 |

Results in microgram per gram of hair

## Discussion

In this study, hair trace elements concentrations revealed a marked heterogeneity of children exposure to polymetallic contaminants, depending on the area of school attendance. Children from industrial districts were far more exposed to non essential elements than children from the rural district, downtown or the suburb schools. Children attending the Mine and the Smelter Schools seemed to be in contact with a complex “cocktail” composed by Pb, As, Hg, Sn, Ag, Bi, Sb and Se. In these districts, Pb was the most concentrated non essential element in hair. In the rural area, distant from mining and metallurgical activities, the levels were systematically lower for all non essential elements.

Boys were more exposed than girls and there is no clear explanation for this observation. Hypothesis can be made that boys might have more outdoors activities, which would increase their contact with the contaminated areas whereas, for cultural reasons, girls might stay more at home.

The low levels of hair Co concentrations in children from the Mine and Smelter Schools as well as their high levels of Se were difficult to interpret. In the same way, Zn concentrations were unexpectedly lower in children from the Downtown and Mine Schools.

These findings did not provide specific information about the origin and pathway of this contamination. The potential sources are numerous, constituted by mine tailings, acid mine drainage, industrial wastelands, informal smelters, industrial smelters, etc. Most of the time these polluted areas do not have any protection barriers or access restrictions. Air pathway may contribute to the dissemination of contaminated wastes in these arid and windy areas, but water and food contamination should not be neglected. Despite the multiplicity of the sources and the likely diffusion of the contaminants through various pathways, the present study has highlighted a marked heterogeneity of

exposure between the different school areas. The overall pattern of exposure in the different areas of the city appeared to be consistent with the geographical situation of major sources of contamination, i.e. a mine and an industrial tin smelter. Thus, hair analysis appeared to be a good screening tool in providing assessment of general pattern of environmental exposure.

Meanwhile, it should be pointed out that for most substances (with the exception of methyl mercury) scientific data are insufficient to predict a health effect from the measurement of the substance in hair [28]. Hair concentration levels cannot be quantitatively qualified as low or high, mainly because of the absence of reliable reference ranges.

Comparative values have been proposed for many trace elements in hair, based on means obtained in healthy exposed and non exposed populations as well as in specific subgroups or clinical specific conditions (Table 6). Goullé et al. [14] observed in France 0.41  $\mu\text{g/g}$  of Pb and 0.05  $\mu\text{g/g}$  of As in healthy volunteers. Senefonte et al. [15] found in children from Rome (Italy) 7  $\mu\text{g/g}$  of Pb and 0.09  $\mu\text{g/g}$  of As. Pereira et al. [29] found 0.24  $\mu\text{g/g}$  of As in the vicinity of a cupric pyrite mine. Sukumar et al. [8] found 5.7  $\mu\text{g/g}$  of lead in healthy adults from New Delhi and its proximities. In the present work, focusing on school-aged children living in rural unexposed area, we found 1.6  $\mu\text{g/g}$  of Pb and 0.07  $\mu\text{g/g}$  of As.

These values brought some valuable information, but several issues need to be solved before the differences observed between these studies can be fully interpreted. One of the main issue is that hair analysis suffers a lack of inter- as well as intra-laboratory reproducibility [26, 27, 30]. Standardization of laboratory methodologies and procedures would improve comparability of concentration levels between studies. There is also a need for a better identification and understanding of the “confounding” factors which may influence level of hair trace element concentration independently of the exposure.

Confirmation of these results on blood samples is thus necessary and we are currently conducting a multidisciplinary program in this purpose.

It should be remarked that besides scientific contribution, the implementation of a study based on hair samples proved to be a worthwhile preliminary step for developing further research. Indeed, the population was quite reluctant to accept blood sampling partly because of cultural beliefs, and also because of an ambivalent feeling towards scientific research on health risks associated with mining activities. Although the inhabitants of this mining town aspire to improve their sanitary conditions and to protect their health, they are in a sensitive position because mining activities are traditional and the economic dependence remains important. In a similar context, Peruvian colleagues were thrown out from an industrial town [31]. This first study has provided arguments to develop a communication strategy with the population in order to obtain their participation in a research programme involving blood sampling.

## Conclusion

Hair trace elements analysis appeared to be a useful screening tool for epidemiological purposes, especially now that ICP-MS is more accessible. Although our results could not be fully interpreted, they have highlighted the heterogeneity of exposure according to the area of school attendance, which reflected the geographical location of the major sources of contamination.

**Table 6** Mean Trace Elements Concentrations in Human Hair Samples from Previous Studies

| Author                               | Country  | Population                      | Conditions                 | Number               | As             | Pb           | Cd           | Hg           | Sb           | Sn           | Ni            | Al            | Cu           | Cr           | Mn           | Se           | Co             | Zn  |
|--------------------------------------|----------|---------------------------------|----------------------------|----------------------|----------------|--------------|--------------|--------------|--------------|--------------|---------------|---------------|--------------|--------------|--------------|--------------|----------------|-----|
| Senonfonte et al. 2000               | Italy    | Children (3–15 year)            | Urban                      | 137–411 <sup>a</sup> | 0.09           | 7.11         | 0.23         |              |              |              | 1.49          | 10.2          | 22.1         | 0.99         | 0.35         | 0.77         | 0.67           | 150 |
| Carneiro et al. 2002                 | Brazil   | General population (1–80 year)  | Urban female<br>Urban male | 968<br>466           | 0.07<br>0.08   | 5.91<br>7.18 | 0.17<br>0.17 | 1.28<br>1.80 | 0.02<br>0.02 | 0.54<br>0.24 | 8.76<br>7.9   | 23.5<br>20.8  | 0.66<br>0.54 | 0.02<br>0.01 | 0.02<br>0.01 | 0.02<br>0.01 | 190<br>187     |     |
| Shamberger 2003                      | USA      | Women                           | Premenstrual syndrome      | 46                   | 0.34           | 0.97         | 0.049        | 0.48         |              | 0.34         | 6.27          | 19.8          | 0.053        | 0.37         | 1.3          | 0.066        | 116.8          |     |
| Samanta et al. 2004 <sup>b</sup>     | India    | General population              | As in drinking water       | 50<br>44             | 0.22<br>3.43   | 0.74<br>8.03 | 0.035<br>0.4 | 0.22<br>0.88 |              | 0.47<br>1.59 | 22.5<br>14.76 | 0.06<br>15.48 | 0.62<br>0.87 | 1.2          | 0.104        | 120.4        |                |     |
| Razagui and Ghribi 2004 <sup>†</sup> | UK       | Mother – New born               | Mother<br>Neonate          | 82<br>82             | 7.95<br>4.56   | 0.49<br>0.57 |              |              |              |              | 18.4<br>6.7   |               |              |              |              |              | 122.5<br>146.9 |     |
| Sharma et al. 2004                   | India    | General population (6–60 year)  | Male<br>Female             | 194<br>160           | 0.013<br>0.008 | 7.3<br>8.3   | 1.3<br>1.3   | 0.73<br>0.77 |              | 6.1<br>5.6   | 9.0<br>15.6   |               |              |              |              |              | 166.6<br>177.1 |     |
| Goullé et al. 2005 <sup>c</sup>      | France   | Adults, general population      | Urban area                 | 45                   | 0.05           | 0.41         | 0.011        | 0.66         | 0.008        | 0.046        | 0.23          | 1.63          | 20.3         | 0.20         | 0.067        | 0.54         | 0.023          | 162 |
| Rapant et al. 2006                   | Slovakia | General population (10–90 year) | Rural mining area          | 71                   | 0.379          |              |              |              | 0.357        |              |               |               |              |              |              |              |                |     |

|   |                  |  |                            |             |       |      |       |       |
|---|------------------|--|----------------------------|-------------|-------|------|-------|-------|
| Duniec-Sokolowska et al. 2007             | Poland           | Adults healthy volunteers (40–60 year) | Females                    | 1,320–2,382 | 1.37  | 0.15 | 11.2  | 170.6 |
| Sukumar and Subramanian 2007 <sup>d</sup> | India            | General population                     | Males                      | >620        | 2.09  | 0.22 | 11.25 | 154.7 |
|   |                  |  | Urban women                | 82          | 8.3   | 0.5  | 40.7  | 146.5 |
|   |                  |  | Rural women                | 66          | 9.1   | 0.9  | 12.3  | 199.4 |
| Amaral et al. 2008 <sup>e</sup>           | Portugal, Azores | Male (3–89 year)                       | Volcanic contamination     | 68          | 3.5   | 0.1  | 17    | 240   |
|   |                  |  | Control                    | 90          | 1.4   | 0.02 | 11    | 210   |
| González-Muñoz et al. 2008                | Spain            | Healthy adults                         | Women                      | 200         | 0.011 | 1.46 | 23.34 | 145.8 |
|   |                  |  | Men                        | 150         | 0.00  | 0.27 | 12.47 | 161.1 |
| Bao et al. 2009 <sup>f</sup>              | China            | Children (7–16)                        | Heavy metal pollution      | 549         | 4.19  | 0.1  | 0.07  | 211.5 |
| Ferré-Huguet et al. 2009                  | Spain            | Children (12–14 year)                  | Waste incinerator exposure | 96          | nd    | 0.58 | 1.31  | 0.21  |
|   |                  |  |                            |             | 0.16  | 0.48 |       |       |

nd not detected

<sup>a</sup>Data below detection limit were not included in mean calculation

<sup>b</sup>Geometric means

<sup>c</sup>Medians

<sup>d</sup>Other subgroups are considered in this study

<sup>e</sup>Values estimated from graphs

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