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# Metals and metalloids in hair samples of children living near the abandoned mine sites of Sulcis-Inglesiente (Sardinia, Italy)

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ABSTRACT

The Sulcis-Iglesiente district (SW Sardinia, Italy) is one of the oldest and most important polymetallic mining areas in Italy. Large outcrops of sulfide and oxide ores, as well as the products of the long-lasting mining activity, are present throughout the district releasing significant quantities of metals and metalloids into the surrounding environment. Here are reported concentrations of 21 elements determined in scalp hair samples from children (aged 11–13 years) living in different geochemical environments of southwestern Sardinia: Iglesias, hosting several abandoned mines, and the island of Sant'Antioco, not affected by significant base metal mineralization events. Trace element determinations were performed by ICP-MS. Statistically significant differences ($p < 0.01$) in elemental concentration levels between the two study sites were found. Hair of children from Iglesias exhibited higher concentration values for Ag, Ba, Cd, Cr, Ni, Pb, Rb, Sb, U, V, and Zn. Rubidium, V and U resulted more abundant at Sant'Antioco. Hair samples from Iglesias showed gender-related differences for a larger number of elements (Ag, Ba, Cd, Co, Cu, Ni, Sr, U and Zn) than at Sant'Antioco, where only U was significantly different. The above elemental concentrations in females were always higher than in male donors. Robust Principal Component Analysis operated on log-transformed elemental concentrations showed components indicative of a) sulfides ore minerals (PC1) reflecting the influence of the diffuse mineralization covering the entire study area, b) the presence of some bioavailable As sources (PC2) as As-rich pyrite and Fe-containing sphalerite and c) other sources of metals overlapping the diffuse mineralizations, as carbonate rocks and coal deposits (PC3). The results provided evidence of a potential risk of adverse effects on the health of the exposed population, with children living at Iglesias being greatly exposed to several metals and metalloids originated in mining tailings, enriched soils, waters and food.

## 1. Introduction

Mining areas may release various metals and metalloids into the surrounding environment, contaminating air, ground and surface waters, plants and soils and also causing the risk of health problems among local residents. These adverse effects persist long after the mining activities have ceased. In Italy, the risk from inactive or abandoned mines is particularly present in Sardinia where 427 mine sites have been listed (MATT, 2006). The Sulcis-Iglesiente district (SW Sardinia), one of the oldest and most important polymetallic mining areas in Italy, was intensively exploited for valuable zinc, lead, silver and barium deposits from pre-Roman times to the end of the last century. Nowadays, large outcrops of sulfide and oxide ores, as well as the products of the long-lasting mining activity, are present throughout the district and are responsible for unusually large concentrations of metals and metalloids. The largest concentration of mine wastes in Sardinia occurs in the San Giorgio valley (Sulcis-Iglesiente district, SW Sardinia), close to the town of Iglesias. This area includes the mines of Monteponi, San Giovanni, Campo Pisano, Monte Agruxau and Monte Scorra. About 11 million cubic meters of hazardous mine wastes have been stored in dams, dispersed into the nearby San Giorgio river bed and into the Sa Masa lagoon. The Italian Government, through Italian Decree no. 334/1999, declared the mineralized areas of Sardinia at high risk of environmental crisis and a possible threat for the inhabitants' health. The impact of metal mining in Sardinia was described by Cidu and Fanfani (2002). More recently, deterioration of waters quality in the Sulcis-Iglesiente area, caused by mine drainage and the weathering of mining-related

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wastes, has been documented in several reports by Cidu et al. (2007, 2009 ), Cidu (2011) and Medas et al. (2012a, 2012b), which found high concentrations of Zn, Cd and Pb in local surface and groundwaters. Stream sediments showed significant Zn–Mn–Pb–Cu–Ag anomalies as well as a wide diffusion of pollutant elements like Cd, As, Hg and Sb not directly exploited from the ores (Boni et al., 1999; De Vivo et al., 1997; 1998; 1999; 2006). Chemical determinations on local soils also revealed very high levels of heavy metals distributed in all the size classes and particularly in the finer fractions (Ciccu et al., 2003). Exceptionally high levels of Pb, Zn, and Cd were recognized in vegetation samples, with Pb easily absorbed by plant roots and translocated to foliage (Leita et al., 1989). Mine activity in the San Giorgio valley ended a few decades ago after intense industrial exploitation. The processes by which plant and animal life adapt to these heavy metals are reported for microbes in Podda et al. (2014) and De Giudici et al. (2014), and for plants in Sprocati et al. (2014) and Bacchetta et al. (2012).

Information on the possible adverse impact of metal pollution on residents of Sulcis-Iglesiente area can be deduced from the epidemiological study of Biggeri et al. (2006) that detected regional variations in the incidence of diseases among the Sardinian population. The study documented a statistically significant higher incidence of respiratory diseases, pneumoconiosis, and malignant lung tumors in individuals living close to the mining sites compared to other parts of the island. Biomonitoring studies have reported higher concentrations of lead in hair from children living in the Sulcis-Iglesiente area than those from children residing in unexposed geographical areas of southern Sardinia (Sanna et al., 2008).

The goal of our study was to gain insights about the potential exposure to metal and metalloids of the population living within the Sulcis-Iglesiente mining district. It was pursued through the following objectives:

- to determine the levels of 21 elements (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, U, V, and Zn) in scalp hair samples from children (aged 11–13 years) living in different geochemical environments of southwestern Sardinia: Iglesias and the island of Sant'Antioco. This latter site, located southwest of Iglesias, was not subjected to mining and it was not affected by significant base metals mineralization events (Morra et al. 1994; Lustrino et al., 2013);
- to verify, from a statistical point of view, whether significant differences in hair metal concentrations exist between the selected groups of children and, therefore, to determine whether the degree of human exposure to trace elements is subject to changes in local environmental factors.

The proposed approach was based on the assumption that human biomonitoring constitutes a powerful tool for risk assessment from natural and anthropogenic exposure to toxic metals and metalloids. Human scalp hair, being a detoxification system for trace elements, constitutes an optimal matrix in this type of survey for their ability to accumulate trace elements, superior to that of other biological matrices. Studies on use of hair for assessing exposure to metals of people living close to mining areas, although not abundant, may be found in the literature (Pereira et al., 2004; Rapant et al., 2006; Bao et al., 2009; Barbieri et al., 2011; Coelho et al., 2014).

## 2. Material and methods

### 2.1. Study areas

The area under investigation is in the southwestern part of Sardinia. (Fig. 1). Sardinia belongs to the Gondwana-derived Iberian-Armorican microplate assem-

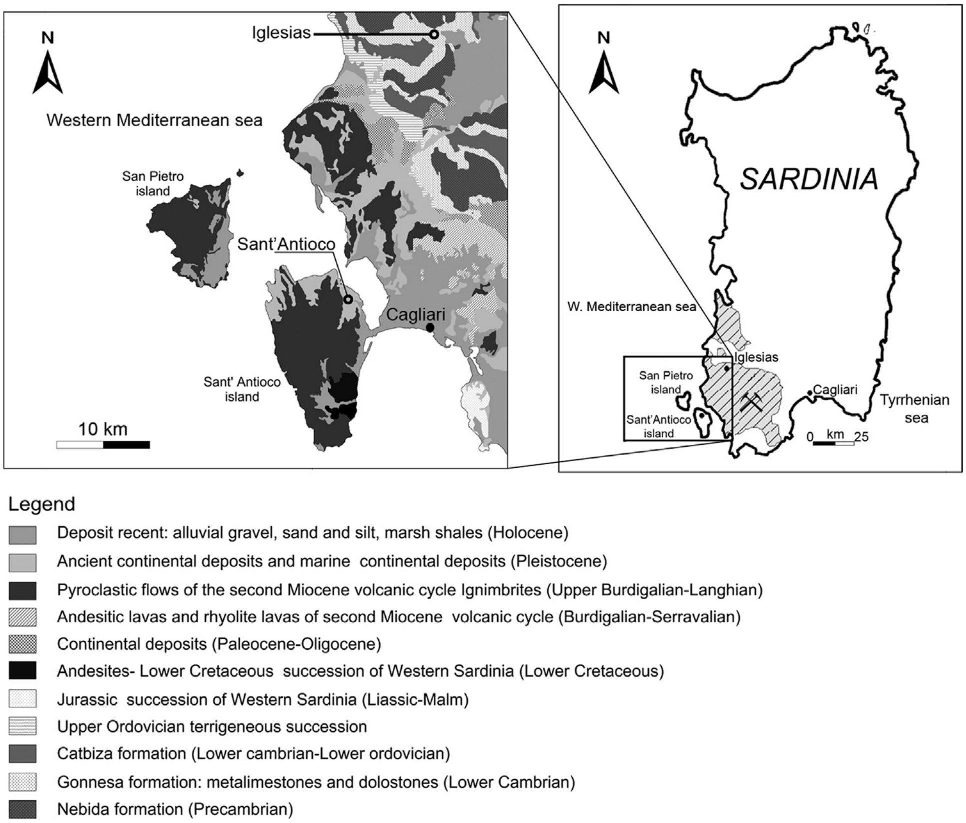

**Fig. 1.** Location and geological sketch map of the study area (SW Sardinia), modified from Carmignani et al. (2012). The shaded area indicates the mining areas.

blage (Crowley et al., 2000), and forms a small segment of the southern flank of the Variscan orogen (Arthaud and Matte, 1977), linked to the 'Armorica' fold zone by a narrow suture (Carmignani et al., 2001). The Sardinian Paleozoic basement generally shows strong tectono-stratigraphic and metallogenic features. In the Iglesiente-Sulcis area, considered as the external zone of the 'Sardinian' Variscides, the geology is largely dominated by Paleozoic lithotypes, of sedimentary and igneous origin. Cambrian sedimentary rocks prevail: part of the succession, namely the Gonnesa Group carbonates, host the largest Mississippi Valley Type (MVT) deposits in Italy (Boni et al., 2002). The metals for the stratabound ores originated from a crustal source (Bechstädt and Boni, 1994). Lead isotope analysis showed that this metal belongs to the same isotopic province as that of the mineral deposits in the Southern Alps, Austro-Alpine nappes, Southern France and Spain (Arribas and Tosdal, 1994). The San Giorgio valley hosts several mines belonging to the so-called "metalliferous ring", notably the most important are Campo Pisano, San Giovanni, Monteponi, Monte Agruxau and Monte Scorra. Commonly, the upper parts of these ores have been subject to supergene processes leading to calamine formation. In the Iglesiente area, main metal sulfides in the ores are galena, sphalerite, pyrite and chalcopyrite. Main sulfate minerals are barite, anglesite and chalcanthite. Metal carbonates include cerussite, smithsonite, malachite, and azurite. Another important Zn mineral that was produced is hemimorphite. In addition Cd minerals commonly are found, particularly the Cd oxide Monteponite. Fosgenite and native silver are other rare minerals typical of this area.

Sant'Antioco Island has different geologic and environmental features, and is characterized by a different intensity of anthropogenic impact. Tertiary rocks of Sant'Antioco Island were not affected by significant base metal mineralization events, and the geochemical background of Sant'Antioco Island is not anomalous in heavy metals (Morra et al., 1994; Lustrino et al., 2013). Sant'Antioco Island is made of pyroclastic volcanic rocks originating along the Sardinian Trough, a N–S oriented graben developed during the Late Oligocene–Middle Miocene (Assorgia et al., 1990; Lecca et al., 1997; Cherchi et al., 2008).

The Portovesme Harbor is only 12 km from the town of Sant'Antioco. It was built in 1870 to ship ores (sphalerite, galena and pyrite) from the historical mines of the Sulcis-Iglesiente district and it received major inputs from industrial discharges until a few decades ago. In addition, when the Portoscuso-Portovesme industrial district began to develop and expand its activities, the metallurgical Portovesme industrial plants were operating from the 1960s until a few years ago. An additional source of heavy metals, close to the Portovesme harbor, is a dam built to store red mud residues of a bauxite treatment plants. The industrial activities in the area suffered a profound crisis since the end of the last century, as part of the general economic crisis period, and they are no longer active.

### 2.2. *Climate*

The climate of the study area is classified as Mediterranean. The mean annual temperature of the Iglesiente and Sant'Antioco areas is 16° and 17 °C, respectively. The annual average rainfall ranges between 800 and 1100 mm depending on elevation for the Iglesiente area, while the average value for Sant'Antioco Island is 500 mm/year. Rain events occur mainly in fall and spring, while summer is typically a dry period. Year-round, winds from N and/or N-W are dominant in both areas, and these winds represent 40% of the yearly events for wind speed higher than 13.5 m $s^{-1}$. It is worth noting that, during the dry summer, insolation and wind deflation result in drying of the soil surface. This effect is particularly important for the mine wastes where vegetation is typically scarce and is not a limiting factor for wind erosion and transport. Further technical notes and climatic data of Sardinia are available at the website of the Sardinia Environment Protection Agency (ARPAS, http://www.sar.sardegna.it/pubblicazioni/notetecniche/nota2/index.asp).

### 2.3. *Sampling design and analytical methods*

A total of 144 hair samples (namely SAR, for Sardinia) discussed in the present study were collected from children, 11–13 years old, of both genders, without colored or treated hair. A total of 59 (32 females and 27 males) were from children residing around Iglesias (IG) and 85 samples (52 females and 33 males) from Sant'Antioco (SAN). The SAR population set includes IG+SAN. Children were selected for this study because they represent non-occupationally exposed subjects. They are more sensitive to poisoning than adults, have a high metabolic index and may experience different degrees of exposure from air, water, or food compared with adults. Hair samples were obtained according to the local existing legislation on privacy protection. All the children's legal representatives signed consent forms authorizing sample collection. All donors were interviewed to obtain only information on dietary habits and lifestyle. According to their responses it was supposed that they share the same lifestyle and partially the same diet, with some differences due to the locally produced food (fruits and vegetables, milk, cheese and meat). Personal data were entered in an anonymous format. Hair samples 1–2 cm long were cut with stainless steel scissors from the nape of the neck close to the occipital region, and kept in plastic bags. Once in the laboratory, the samples were cut into smaller pieces with a sterile surgical scalpel, to enhance the washing procedure, carried out as recommended by the International Atomic Energy Agency (Ryabukin, 1978; Subramanian, 1996; Sela et al., 2007), following the sequence acetone–water–water–water–acetone. More specifically, hair samples were immersed in 20 mL of acetone (Fisher, analytical reagent grade) and 20 mL of 18 MΩ cm demineralized water and stirred, each time, in an ultrasonic bath for 15 min. The washed samples were individually placed in glass beakers and dried for 24 h at 40 °C in a drying oven, and then weighed. A total of 3 mL of $HNO_3$ (Suprapur, Merck) was added to about 150 mg of washed hair sample and digested for 24 h in Teflon vessels, at room temperature. Digestion was then completed by adding 500 μL of $H_2O_2$ (Suprapur, Merck) for an additional 24 h. After digestion, the solutions were diluted by the addition of 18 MΩ cm demineralized water to reach a volume of 25 mL. Trace element determinations were carried out at the Dipartimento di Scienze della Terra e del Mare (DiSTeM), University of Palermo. The 21 elements (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Sb, Se, Sr, U, V, and Zn) were measured by inductively coupled plasma mass spectrometry (ICP-MS, Perkin-Elmer, Elan 6100 DRC-e) after the addition of Re–Sc–Y as internal standards. For As, Cr, Fe, Se and V, the ICP-MS was operated in DRC (Dynamic Reaction Cell) mode with $CH_4$ as reaction gas. All standard solutions were prepared with 18 MΩ cm demineralized water, the ICP Multi Element Standard Solutions XXI CertiPUR (Merck), and the Mo and Sb CertiPUR standards (Merck). To minimize matrix effects, the standard addition technique was used for all metal determinations. Sample blanks were also analyzed, and the operational limit of detection (LOD) for each element was calculated as 3 times the standard deviation of the analyte concentration in blank samples. Values below the detection limit were set at one-third the detection level and treated as real values in the statistical analyses. Analytical precision, estimated from triplicate analyses of every tenth sample, was in the range 5–10% for all analyzed elements, except As and Cr, in which the range was ±15%. The validity of the analytical procedure was checked by the standard reference material QMEQAS08H-02 (hair collected from a single unexposed donor, spiked with selected analytes) of the Institut National de Santé Publique – Center de Toxicologie, Québec (Canada). The metal recovery rates of certified elements in the reference material ranged between 86% and 109% (Table 1).

**Table 1**
Comparison of measured and reported concentrations and metal recovery of certified elements in the standard reference material QMEQAS08H-02. Data expressed in μg/g. LOD is 3 times the standard deviation of the analyte concentration in blank samples. n.c. – not certified.

| | QMEQAS08H-02 | This study | Recovery% | LOD |
|---|---|---|---|---|
| Ag | 1.27 ± 0.27 | 1.3 ± 0.1 | 100 | 0.004 |
| Al | 31.9 ± 7.8 | 27.4 ± 1.1 | 86 | 0.027 |
| As | 3.45 ± 0.51 | 3.6 ± 0.1 | 103 | 0.006 |
| Ba | 3.58 ± 0.58 | 3.8 ± 0.1 | 105 | 0.01 |
| Cd | 3.54 ± 0.48 | 3.6 ± 0.1 | 102 | 0.001 |
| Co | 4.45 ± 0.51 | 4.4 ± 0.2 | 98 | 0.001 |
| Cr | 0.67 ± 0.289 | 0.7 ± 0.02 | 101 | 0.005 |
| Cu | 77.4 ± 7.2 | 74.2 ± 3.4 | 96 | 0.022 |
| Fe | n.c | 11 ± 0.6 | | 0.063 |
| Li | n.c | 0.1 ± 0.01 | | 0.002 |
| Mn | 1.87 ± 0.29 | 1.6 ± 0.1 | 87 | 0.001 |
| Mo | 1.01 ± 0.13 | 1.1 ± 0.1 | 106 | 0.004 |
| Ni | 5.12 ± 0.67 | 4.9 ± 0.7 | 96 | 0.002 |
| Pb | 13.2 ± 1.7 | 13.1 ± 0.8 | 99 | 0.001 |
| Rb | n.c | 0.06 ± 0.007 | | 0.002 |
| Sb | 0.973 ± 0.164 | 1.0 ± 0.04 | 98 | 0.0006 |
| Se | 1.20 ± 0.24 | 1.0 ± 0.1 | 86 | 0.017 |
| Sr | n.c | 2.16 ± 0.1 | | 0.003 |
| U | 0.242 ± 0.053 | 0.2 ± 0.01 | 99 | 0.002 |
| V | 1.10 ± 0.17 | 1.2 ± 0.04 | 109 | 0.011 |
| Zn | 413 ± 60 | 449 ± 6.9 | 109 | 0.038 |

### 2.4. *Statistical analysis*

Data were analyzed using the free R software (http://www.r-project.org) and the related compositions, Hotelling, moments, klaR, FactoMineR, and rrcov packages. All the tests in this study were considered significant at $p < 0.01$. Because most of the variables exhibited positively skewed distribution, the data were log transformed and subjected to parametric statistics. Data transformation improved normality of the distributions as demonstrated by the skewness values which approached to zero. The geometric mean was therefore preferred to arithmetic mean or median for assessing the central tendency or typical value of all elements.

## 3. Results and discussion

The descriptive statistical parameters of trace elements in scalp hair from children residing in the study areas of Sardinia are

**Table 2**
Basic statistical parameters of trace element contents in scalp hair of children from Sardinia. SAR (entire dataset), IG (area: Sulcis-Iglesiente), SAN (area: Sant'Antioco). Concentration data expressed as μg g$^{-1}$ (dry weight basis). Notes: GM – geometric mean, Min. – minimum value, Max. – maximum value, $Q_5$ and $Q_{95}$ indicate the 5th and 95th percentiles, respectively. $Q_{25}$ and $Q_{75}$ indicate the first (25th percentile) and third (75th percentile) quartiles, respectively.

| | | **GM** | **Min** | **Max** | **Median** | **$Q_5$** | **$Q_{25}$** | **$Q_{75}$** | **$Q_{95}$** | **GENDER** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | **GM-Female** | **GM-Male** |
| **Sardinia (SAR, *N*=144)** | ***Ag*** | 3.6E-02 | 1.4E-03 | 9.5E-01 | 3.1E-02 | 5.0E-03 | 1.1E-02 | 1.2E-01 | 3.7E-01 | 4.0E-02 | 3.0E-02 |
| | ***Al*** | 4.0E+00 | 1.4E+00 | 1.5E+01 | 4.2E+00 | 2.1E+00 | 3.2E+00 | 5.2E+00 | 6.7E+00 | 3.7E+00 | 4.3E+00 |
| | ***As*** | 3.9E-02 | 2.3E-03 | 3.2E-01 | 4.2E-02 | 9.5E-03 | 2.8E-02 | 6.4E-02 | 1.4E-01 | 3.8E-02 | 4.1E-02 |
| | ***Ba*** | 8.6E-01 | 1.3E-02 | 5.9E+00 | 8.6E-01 | 2.3E-01 | 4.7E-01 | 1.8E+00 | 3.0E+00 | 1.1E+00 | 6.2E-01 |
| | ***Cd*** | 1.9E-02 | 3.1E-04 | 1.9E+00 | 1.5E-02 | 9.3E-04 | 4.4E-03 | 1.3E-01 | 4.3E-01 | 2.1E-02 | 1.8E-02 |
| | ***Co*** | 4.7E-02 | 8.1E-03 | 3.8E+00 | 3.1E-02 | 1.3E-02 | 1.9E-02 | 7.9E-02 | 3.5E-01 | 5.4E-02 | 3.8E-02 |
| | ***Cr*** | 1.2E-01 | 1.7E-02 | 2.0E+00 | 1.3E-01 | 4.3E-02 | 8.3E-02 | 1.8E-01 | 3.6E-01 | 1.2E-01 | 1.3E-01 |
| | ***Cu*** | 1.5E+01 | 6.9E+00 | 1.2E+02 | 1.3E+01 | 8.0E+00 | 1.0E+01 | 2.0E+01 | 4.5E+01 | 1.6E+01 | 1.4E+01 |
| | ***Fe*** | 1.1E+01 | 4.6E+00 | 7.2E+01 | 1.0E+01 | 5.5E+00 | 8.1E+00 | 1.5E+01 | 2.6E+01 | 1.2E+01 | 1.1E+01 |
| | ***Li*** | 1.5E-02 | 1.4E-03 | 1.2E-01 | 1.6E-02 | 3.4E-03 | 9.2E-03 | 2.5E-02 | 4.2E-02 | 1.4E-02 | 1.7E-02 |
| | ***Mn*** | 1.9E-01 | 5.0E-03 | 1.6E+00 | 2.0E-01 | 6.3E-02 | 1.4E-01 | 3.0E-01 | 4.9E-01 | 2.1E-01 | 1.7E-01 |
| | ***Mo*** | 3.3E-02 | 9.5E-04 | 2.0E-01 | 3.8E-02 | 1.1E-02 | 2.5E-02 | 4.7E-02 | 9.0E-02 | 3.9E-02 | 2.6E-02 |
| | ***Ni*** | 1.9E-01 | 3.0E-02 | 1.7E+00 | 1.7E-01 | 7.5E-02 | 1.2E-01 | 3.4E-01 | 6.8E-01 | 2.4E-01 | 1.4E-01 |
| | ***Pb*** | 5.9E-01 | 7.2E-02 | 1.5E+01 | 5.1E-01 | 1.2E-01 | 2.4E-01 | 1.4E+00 | 3.6E+00 | 5.8E-01 | 6.0E-01 |
| | ***Rb*** | 6.0E-03 | 4.8E-04 | 1.1E+00 | 6.3E-03 | 1.2E-03 | 3.8E-03 | 1.0E-02 | 2.1E-02 | 5.9E-03 | 6.1E-03 |
| | ***Sb*** | 1.3E-02 | 1.6E-04 | 6.0E-01 | 1.2E-02 | 4.0E-03 | 7.2E-03 | 2.4E-02 | 5.1E-02 | 1.2E-02 | 1.5E-02 |
| | ***Se*** | 8.4E-01 | 3.4E-01 | 2.5E+01 | 7.9E-01 | 4.6E-01 | 6.3E-01 | 1.0E+00 | 1.6E+00 | 7.7E-01 | 9.5E-01 |
| | ***Sr*** | 1.5E+00 | 1.8E-01 | 7.7E+00 | 1.9E+00 | 3.7E-01 | 8.6E-01 | 2.7E+00 | 4.4E+00 | 2.2E+00 | 9.1E-01 |
| | ***U*** | 1.6E-02 | 6.2E-04 | 2.9E-01 | 1.8E-02 | 2.8E-03 | 7.9E-03 | 3.6E-02 | 6.1E-02 | 2.2E-02 | 9.8E-03 |
| | ***V*** | 1.5E-01 | 1.6E-02 | 1.0E+00 | 1.5E-01 | 5.2E-02 | 9.1E-02 | 2.5E-01 | 5.3E-01 | 1.7E-01 | 1.4E-01 |
| | ***Zn*** | 2.5E+02 | 1.3E+02 | 1.1E+03 | 2.3E+02 | 1.6E+02 | 2.0E+02 | 3.0E+02 | 4.9E+02 | 2.7E+02 | 2.2E+02 |
| **Iglesias (IG, *N*=59)** | ***Ag*** | 1.3E-01 | 3.2E-03 | 9.5E-01 | 1.5E-01 | 2.9E-02 | 7.6E-02 | 2.4E-01 | 4.7E-01 | 1.7E-01 | 8.6E-02 |
| | ***Al*** | 4.2E+00 | 1.6E+00 | 1.5E+01 | 4.3E+00 | 2.1E+00 | 3.2E+00 | 6.0E+00 | 7.8E+00 | 3.8E+00 | 4.7E+00 |
| | ***As*** | 3.9E-02 | 2.3E-03 | 3.2E-01 | 4.1E-02 | 8.8E-03 | 2.8E-02 | 6.8E-02 | 1.6E-01 | 3.5E-02 | 4.6E-02 |
| | ***Ba*** | 1.4E+00 | 1.3E-02 | 5.9E+00 | 1.9E+00 | 2.9E-01 | 7.6E-01 | 2.7E+00 | 4.0E+00 | 2.6E+00 | 6.9E-01 |
| | ***Cd*** | 1.6E-01 | 1.5E-02 | 1.9E+00 | 2.0E-01 | 3.2E-02 | 7.8E-02 | 3.0E-01 | 7.6E-01 | 2.5E-01 | 9.2E-02 |
| | ***Co*** | 4.6E-02 | 1.1E-02 | 2.9E+00 | 3.5E-02 | 1.4E-02 | 2.1E-02 | 7.2E-02 | 3.9E-01 | 7.4E-02 | 2.6E-02 |
| | ***Cr*** | 1.6E-01 | 3.9E-02 | 2.0E+00 | 1.5E-01 | 5.2E-02 | 1.2E-01 | 2.3E-01 | 9.0E-01 | 1.8E-01 | 1.5E-01 |
| | ***Cu*** | 1.8E+01 | 7.4E+00 | 1.2E+02 | 1.6E+01 | 8.2E+00 | 1.1E+01 | 2.4E+01 | 4.8E+01 | 2.2E+01 | 1.3E+01 |
| | ***Fe*** | 1.3E+01 | 5.3E+00 | 7.2E+01 | 1.2E+01 | 6.2E+00 | 9.5E+00 | 1.8E+01 | 4.0E+01 | 1.2E+01 | 1.3E+01 |
| | ***Li*** | 1.6E-02 | 2.9E-03 | 1.2E-01 | 1.6E-02 | 3.3E-03 | 9.8E-03 | 2.6E-02 | 7.5E-02 | 1.4E-02 | 1.9E-02 |
| | ***Mn*** | 2.3E-01 | 9.6E-03 | 1.6E+00 | 2.5E-01 | 6.2E-02 | 1.8E-01 | 3.2E-01 | 5.2E-01 | 2.3E-01 | 2.2E-01 |
| | ***Mo*** | 3.1E-02 | 9.5E-04 | 1.9E-01 | 3.1E-02 | 1.1E-02 | 2.1E-02 | 4.1E-02 | 1.5E-01 | 3.9E-02 | 2.3E-02 |
| | ***Ni*** | 2.4E-01 | 4.4E-02 | 9.9E-01 | 2.5E-01 | 7.5E-02 | 1.2E-01 | 4.6E-01 | 9.0E-01 | 4.3E-01 | 1.2E-01 |
| | ***Pb*** | 1.5E+00 | 1.7E-01 | 1.5E+01 | 1.6E+00 | 2.4E-01 | 1.0E+00 | 2.7E+00 | 5.9E+00 | 1.7E+00 | 1.3E+00 |
| | ***Rb*** | 4.3E-03 | 4.8E-04 | 3.2E-02 | 4.2E-03 | 1.1E-03 | 2.2E-03 | 8.6E-03 | 2.1E-02 | 3.5E-03 | 5.6E-03 |
| | ***Sb*** | 2.2E-02 | 1.8E-03 | 6.0E-01 | 2.0E-02 | 6.4E-03 | 1.3E-02 | 3.8E-02 | 9.9E-02 | 1.6E-02 | 3.0E-02 |
| | ***Se*** | 9.2E-01 | 3.4E-01 | 2.5E+01 | 8.7E-01 | 4.6E-01 | 6.9E-01 | 1.1E+00 | 2.3E+00 | 7.7E-01 | 1.2E+00 |
| | ***Sr*** | 1.3E+00 | 1.8E-01 | 6.6E+00 | 1.7E+00 | 3.0E-01 | 5.4E-01 | 2.6E+00 | 3.8E+00 | 2.5E+00 | 5.5E-01 |
| | ***U*** | 8.7E-03 | 6.2E-04 | 4.5E-02 | 8.8E-03 | 2.6E-03 | 4.5E-03 | 1.8E-02 | 3.1E-02 | 1.2E-02 | 5.7E-03 |
| | ***V*** | 9.9E-02 | 1.6E-02 | 4.1E-01 | 9.7E-02 | 4.1E-02 | 6.9E-02 | 1.4E-01 | 2.8E-01 | 9.7E-02 | 1.0E-01 |
| | ***Zn*** | 2.9E+02 | 1.3E+02 | 1.1E+03 | 3.1E+02 | 1.6E+02 | 2.1E+02 | 3.8E+02 | 5.5E+02 | 3.7E+02 | 2.2E+02 |
| **Sant'Antioco (SAN, *N*=85)** | ***Ag*** | 1.5E-02 | 1.4E-03 | 5.9E-01 | 1.3E-02 | 3.8E-03 | 6.2E-03 | 2.8E-02 | 6.9E-02 | 1.6E-02 | 1.3E-02 |
| | ***Al*** | 3.8E+00 | 1.4E+00 | 7.0E+00 | 4.0E+00 | 2.1E+00 | 3.2E+00 | 4.8E+00 | 6.0E+00 | 3.7E+00 | 4.0E+00 |
| | ***As*** | 3.9E-02 | 4.8E-03 | 1.5E-01 | 4.2E-02 | 1.1E-02 | 3.0E-02 | 6.1E-02 | 1.0E-01 | 4.0E-02 | 3.7E-02 |
| | ***Ba*** | 6.1E-01 | 8.2E-02 | 2.9E+00 | 6.7E-01 | 1.5E-01 | 3.9E-01 | 9.8E-01 | 1.8E+00 | 6.3E-01 | 5.8E-01 |
| | ***Cd*** | 4.5E-03 | 3.1E-04 | 7.8E-02 | 5.1E-03 | 9.0E-04 | 2.0E-03 | 9.0E-03 | 3.9E-02 | 4.4E-03 | 4.8E-03 |
| | ***Co*** | 4.7E-02 | 8.1E-03 | 3.8E+00 | 3.0E-02 | 1.3E-02 | 1.9E-02 | 1.0E-01 | 3.5E-01 | 4.5E-02 | 5.1E-02 |
| | ***Cr*** | 1.0E-01 | 1.7E-02 | 1.3E+00 | 1.0E-01 | 3.7E-02 | 7.2E-02 | 1.5E-01 | 2.4E-01 | 9.3E-02 | 1.2E-01 |
| | ***Cu*** | 1.4E+01 | 6.9E+00 | 7.2E+01 | 1.3E+01 | 8.0E+00 | 1.0E+01 | 1.6E+01 | 3.7E+01 | 1.4E+01 | 1.4E+01 |
| | ***Fe*** | 1.0E+01 | 4.6E+00 | 6.1E+01 | 9.6E+00 | 5.4E+00 | 7.8E+00 | 1.3E+01 | 2.5E+01 | 1.1E+01 | 9.5E+00 |
| | ***Li*** | 1.5E-02 | 1.4E-03 | 8.5E-02 | 1.7E-02 | 3.9E-03 | 9.0E-03 | 2.4E-02 | 3.8E-02 | 1.4E-02 | 1.5E-02 |
| | ***Mn*** | 1.7E-01 | 5.0E-03 | 1.4E+00 | 1.7E-01 | 6.6E-02 | 1.3E-01 | 2.4E-01 | 4.0E-01 | 1.9E-01 | 1.4E-01 |
| | ***Mo*** | 3.5E-02 | 9.3E-03 | 2.0E-01 | 4.0E-02 | 1.1E-02 | 2.7E-02 | 4.8E-02 | 6.9E-02 | 3.9E-02 | 2.9E-02 |
| | ***Ni*** | 1.7E-01 | 3.0E-02 | 1.7E+00 | 1.6E-01 | 7.5E-02 | 1.1E-01 | 2.2E-01 | 5.1E-01 | 1.7E-01 | 1.5E-01 |
| | ***Pb*** | 3.1E-01 | 7.2E-02 | 3.3E+00 | 3.0E-01 | 9.6E-02 | 1.8E-01 | 5.3E-01 | 1.4E+00 | 3.0E-01 | 3.3E-01 |
| | ***Rb*** | 7.5E-03 | 8.8E-04 | 1.1E+00 | 7.6E-03 | 2.0E-03 | 5.1E-03 | 1.1E-02 | 2.1E-02 | 8.2E-03 | 6.6E-03 |
| | ***Sb*** | 9.3E-03 | 1.6E-04 | 4.5E-02 | 9.6E-03 | 3.1E-03 | 5.9E-03 | 1.5E-02 | 3.2E-02 | 1.0E-02 | 8.3E-03 |
| | ***Se*** | 7.9E-01 | 3.7E-01 | 2.0E+00 | 7.4E-01 | 4.7E-01 | 6.0E-01 | 9.9E-01 | 1.4E+00 | 7.7E-01 | 8.1E-01 |
| | ***Sr*** | 1.7E+00 | 4.6E-01 | 7.7E+00 | 2.0E+00 | 5.4E-01 | 1.0E+00 | 2.9E+00 | 4.6E+00 | 2.0E+00 | 1.4E+00 |
| | ***U*** | 2.4E-02 | 1.7E-03 | 2.9E-01 | 3.1E-02 | 3.6E-03 | 1.3E-02 | 4.2E-02 | 8.0E-02 | 3.2E-02 | 1.5E-02 |
| | ***V*** | 2.1E-01 | 3.8E-02 | 1.0E+00 | 2.2E-01 | 8.4E-02 | 1.4E-01 | 2.8E-01 | 6.3E-01 | 2.3E-01 | 1.7E-01 |
| | ***Zn*** | 2.2E+02 | 1.3E+02 | 4.0E+02 | 2.2E+02 | 1.7E+02 | 2.0E+02 | 2.4E+02 | 3.3E+02 | 2.3E+02 | 2.2E+02 |

summarized in Table 2. The highest geometric means were found for Zn, Cu and Fe; zinc (251 μg g$^{-1}$) resulted by far the most abundant element, one order of magnitude greater than the concentrations of Cu and Fe (15.3 μg g$^{-1}$ and 11.3 μg g$^{-1}$, respectively); this triad of elements was followed by Al and Sr which exhibited typical values of 3.98 μg g$^{-1}$ and 1.52 μg g$^{-1}$, respectively. Geometric means below

1.0 $\mu g\ g^{-1}$ were observed for all the remaining elements. More precisely, the contents of the elements in the studied hair samples decreased in the order Zn > Cu > Fe > Al > Sr > Ba > Se > Pb > Mn, Ni > V > Cr > Co > Ag, As > Mo > Cd, Li, U > Rb, Sb. However, it was also observed that for Ba, Pb and Se the 95th percentiles exceed 1 $\mu g\ g^{-1}$ (3.05 $\mu g\ g^{-1}$, 3.62 $\mu g\ g^{-1}$ and 1.58 $\mu g\ g^{-1}$, respectively).

The application of *t*-tests (with $p < 0.01$) evidenced significant differences in concentration levels between the two study sites for Ag, Ba, Cd, Cr, Ni, Pb, Rb, Sb, U, V, and Zn. Copper and Mn resulted statistically different at $p < 0.02$. Hair of children from Iglesias (IG) exhibited higher values for all of these elements (the highest geometric mean/geometric mean ratios were observed for Ag, Ba, Cd, Pb and Sb, ranging from 2.3 for Ba, Sb up to 35 for Cd with the exception of Rb, V and U which resulted more abundant at SAN than IG (from 1.7 to 2.7 times).

To reduce the dimensionality of dataset and to identify possible sources of metals in hair samples a Principal Component Analysis (PCA) was performed to group the chemical variables into independent factors accounting for the maximum explainable variance of original data. The robust-PCA approach was privileged as it is largely unaffected by the presence of extreme values. According to the minimum covariance determinant (MCD) algorithm, high breakdown robust multivariate location and scale estimator were computed and used in place of mean vector and sample covariance matrix, respectively. More details about the technical calculation procedure can be found in Rousseeuw and van Driessen (1999). The results of PCA are shown as biplots (Fig. 2) which allow to represent both observations and variables on the same plot. According to Gabriel (1971), observations and variables were scaled up and down by the square root of the number of observations. Three Principal Components were extracted, which together explained almost 58.5% of the total variance. A first group of elements, composed by Ag, Ba, Cd, Cu, Ni, Pb, Sb, V and Zn, weights negatively (with the exception of V) on the first component PC1, which accounts for 27.9% of the total variance in the data set (Fig. 3). These metals, excluding V, are indicative of sulfides ore minerals and PC1 reflects the influence of the diffuse mineralization covering the entire study area. The second component PC2, which explains almost 19.9% of the total variance, includes Se, As, Cr and Fe (negative loadings) and U (with a positive influence). Selenium, As, Cr and Fe are thought to reflect some bioavailable As sources, as As-rich pyrite and Fe-containing sphalerite, both present in the stratabound sulfides occurring in the Cambrian carbonates (Boni et al., 1999; De Vivo et al., 2006), whereas the positive loading of U is attributed to the content of this element in the calc-alkaline rocks of Sant'Antioco (De Vivo et al., 2006).The third component, with a variance of 10.7% is positively correlated with Co, Ni and Sr. It may indicate that other sources of metals are overlapping the diffuse mineralizations, such as carbonate rocks and coal deposits.

Samples from IG and SAN typically formed distinct groups (Fig. 2), with males and females properly separated at IG, but not at SAN. This is a consequence of the multiple univariate differences previously observed between IG and SAN, and between males and females, in terms of chemical profiles.

The highly significant distinctiveness of the IG and SAN groups was statistically confirmed by computing a robust one-way MANOVA ($p < 10^{-5}$). A linear discriminant analysis was then performed applying a stepwise forward variable selection using the Wilk's Lambda criterion (see Mardia et al., 1979 for more details). Cd was by far the most discriminating variable. This is not surprising as the ratio between the geometric means observed in the two groups (IG and SAN) was as high as 35. U, Se, V, Ag, Cr, Sr, Zn, Sb, Mn were also integrated to the discriminant model. Other elements, such as Pb, which were found statistically different within both groups, were not selected, because they appear highly correlated with more discriminant variables. According to a leave-one-out procedure of cross-validation, only 2 and 3 samples were misclassified for IG and SAN, respectively. The discriminating efficiency was therefore highly satisfactory with a recognition efficiency of almost 97%.

The comparison of IG results with those from SAN and the data from Sicily (SIC), where a similar study was conducted on young students of the same ages (Dongarrà et al., 2011, 2012; Varrica

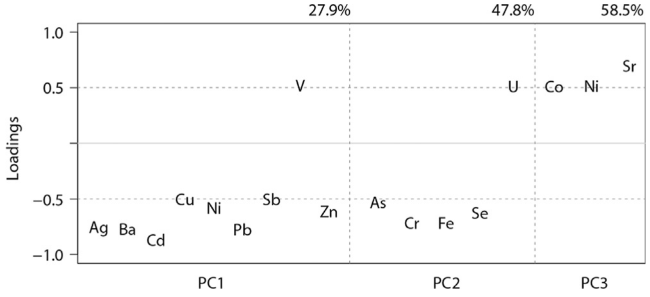

**Fig. 3.** Loading plot for the three principal components of elemental concentrations in hair samples (after log-transformation). Only elements with loadings > 0.5 or < −0.5 are reported.

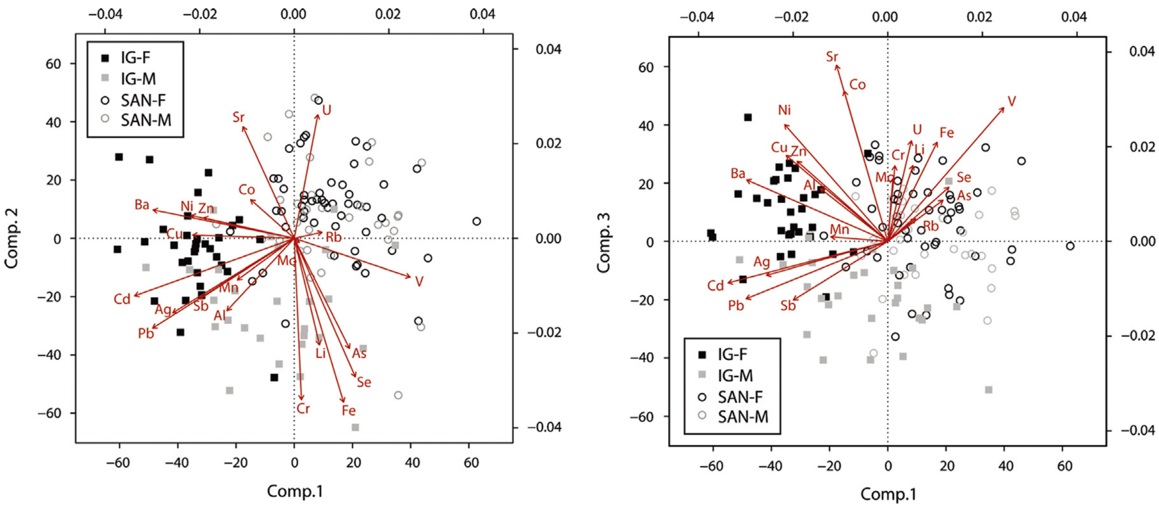

**Fig. 2.** Biplot of the 21 variables (vectors) and the 144 hair samples. PC2 vs PC1 (left) and PC3 vs PC1 (right). Note that data have been log-transformed before the analysis.

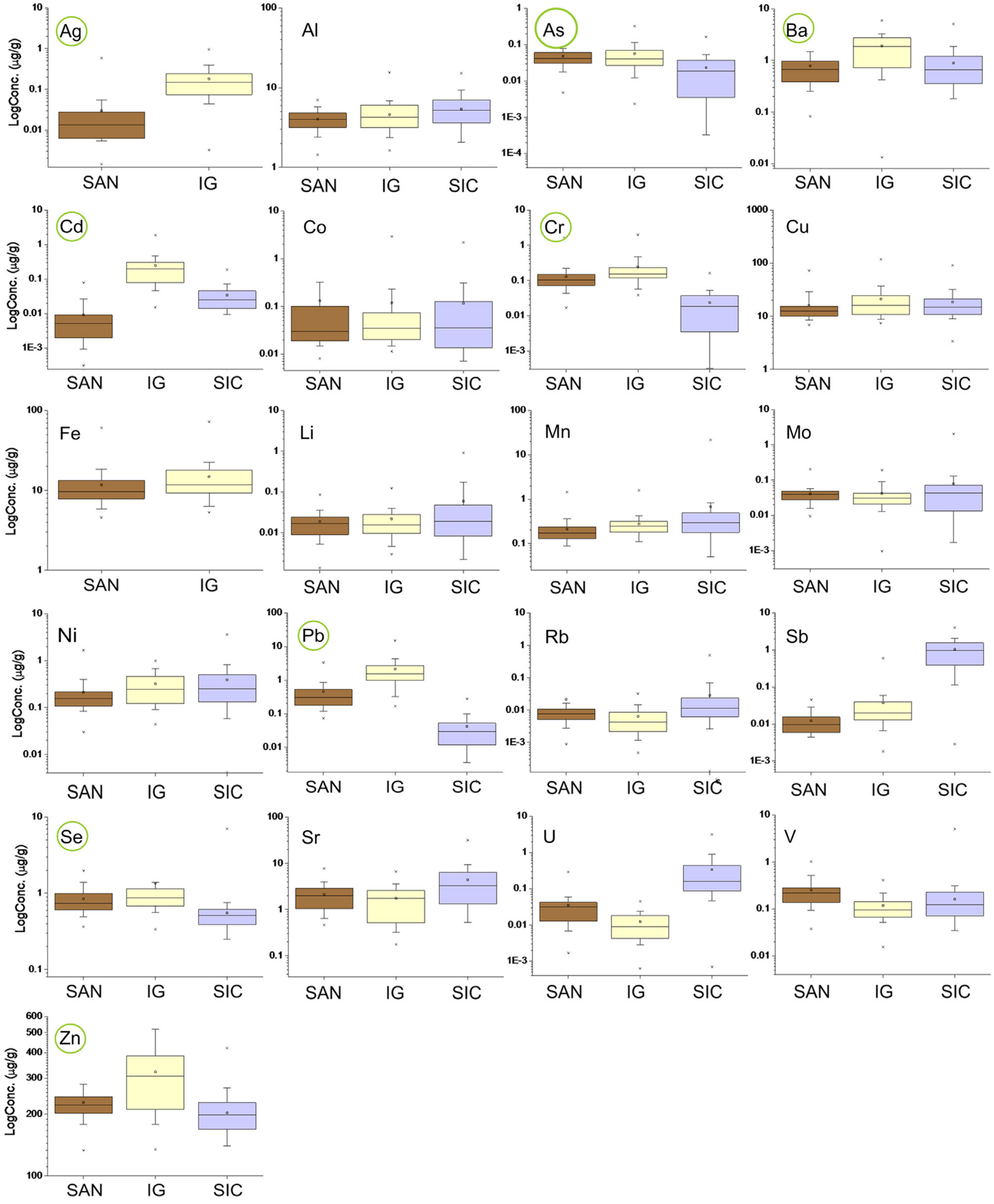

**Fig. 4.** Box and whisker plot displaying mean, median, quartiles and extremes of trace element concentrations in the analyzed human hair samples from Sant'Antioco (SAN), Iglesias (IG) and Sicily (SIC). Boxes delineate interquartile range (25–75%) with indication of median (solid line); small square inside the box marks the average value; whisker indicate 10–90% range. Data are given in μg/g.

et al., 2014) is highly significant. Data from Sicily (593 samples) include different geochemical environments, as a volcanic area (Mt. Etna, 377 samples), a large urban area (Palermo, 137 samples) and two small rural towns (Mistretta and Caltagirone, 79 samples). The comparison was possible because the chemical analyses were performed with the same procedure adopted in the present study.

Hair concentrations of children from the IG area were greater (Hotelling's *T*-squared statistic, $p < 10^{-6}$) for some elements (Ag, As, Ba, Cd, Cr, Pb, Sb, Se, and Zn) as compared to SAN and/or SIC, confirming their greater exposure to polymetallic contaminants (Fig. 4). The most pronounced exposure seemed to be Cd > Ag > Pb > Ba > Sb.

Cadmium is a teratogen, carcinogen, and possible mutagen (Eisler, 1985). Cadmium geometric mean concentration at IG is around 40 and 10 times greater than those observed at SAN and SIC, respectively. All the analyzed hair samples from children living at or around Iglesias (IG) exhibited a Cd concentration higher than the median value observed in hair samples from children of SAN. The level of hair Cd at IG is also greater than the median value reported by Chojnacka et al. (2010) regarding the population of 20-year old people, living in an urban and industrial region of Poland. The median value of 0.2 $\mu g\ g^{-1}$ found at IG is quite similar to that (0.19 $\mu g\ g^{-1}$) found in the hair from people living in Altamira (Brazil), an area with environmental impact from activities of gold mining (Carvalho et al., 2009), to the mean value observed by Li et al. (2012) in people living in villages around the Fenghuang polymetallic mine (China), and also comparable with that found in hair samples from children living near an abandoned cupric pyrite mine in Portugal (Pereira et al., 2004). Barbieri et al. (2011) obtained geometric mean values of 0.07 and 0.03 $\mu g\ g^{-1}$ for school-aged children living close to a large mine in exploitation and near an important tin smelter of the Bolivian Altiplano.

The geometric mean of hair Ag found in children from IG (0.13 $\mu g\ g^{-1}$) was around one order of magnitude higher than that found at SAN (0.015 $\mu g\ g^{-1}$), and approximately five times higher that of the population studied in several small towns located around Mt. Etna (0.03 $\mu g\ g^{-1}$, personal communication). For Ag, all of the analyzed hair samples from children living in or around Iglesias (IG) exhibited concentrations higher than the geometric mean observed in hair samples from children of SAN, for which 95% fell within the lower 5-percentile of children from IG. This high level of hair burden of Ag in such non-professionally exposed subjects is comparable, although less, with those (0.29–0.33 $\mu g\ g^{-1}$) from students of the Polymetallic Mining area in the Bolivian Altiplano (Barbieri et al., 2011).

The hair of students from IG exhibited a geometric mean value of lead (1.48 $\mu g\ g^{-1}$) approximately five times higher than those from SAN. Minimum and maximum values were 0.17 $\mu g\ g^{-1}$ and 14.7 $\mu g\ g^{-1}$ at IG and 0.07 $\mu g\ g^{-1}$ and 3.27 $\mu g\ g^{-1}$ at SAN. 95% of donors from SAN showed lead levels included within the first quartile (25%) of Pb levels at IG. Sanna et al. (2008), who studied, in 2002, lead levels in hair of 11–14 year old children, from towns in the Sulcis-Iglesiente area (Carbonia and Gonnesa) showed that they had higher mean hair lead levels (respectively, 1.86 and 0.91 $\mu g\ g^{-1}$ in males and 2.21 and 2.03 $\mu g\ g^{-1}$ in females) than those from the unexposed town of Sinnai (0.68 μg/g in males and 0.50 $\mu g\ g^{-1}$ in females). Sanna et al. (2011) reported lower levels of hair Pb also in eastern Sardinia. Vallascas et al. (2013) reported mean values of Pb in hair of 3.65 $\mu g\ g^{-1}$ in males and 2.98 μg/g in females for 11–15 year old boys and girls of central-southern Sardinia, sampled from 1998 to 2007.

The geometric mean value of lead at IG was higher than that observed in Sicily (median: 0.66 $\mu g\ g^{-1}$), in the city of Palermo, with high vehicle traffic (0.78 $\mu g\ g^{-1}$; Dongarrà et al., 2011), and also higher than the median value (0.88 $\mu g\ g^{-1}$) observed by Wilhelm et al. (2002) in hair samples from children aged 8–10 years living in the industrialized area of Dusseldorf (Germany), and comparable with that of subjects living in villages near the Panasqueira mine, Portugal (Coelho et al., 2014). However, Pb levels at IG were mostly lower than the mean concentration of Pb observed in hair samples from in people living close to the active chinese mine of Fenghuang (Li et al., 2012).

Geometric mean of Ba was two times higher at IG (1.40 $\mu g\ g^{-1}$) as compared to SAN (0.61 $\mu g\ g^{-1}$). The geometric mean value observed at SAN was within the first quartile of IG. More than 90% of the group of students from SAN had Ba content of hair below the geometric mean value calculated for IG students. The geometric mean value at IG was also higher than that observed at SIC (0.66 $\mu g\ g^{-1}$).

Antimony hair content at IG (geometric mean = 0.021 $\mu g\ g^{-1}$) is greater than at SAN (0.009 $\mu g\ g^{-1}$). Its distribution ranged from 0.002 to 0.60 $\mu g\ g^{-1}$ at IG, while it ranged from 0.0002 to 0.045 $\mu g\ g^{-1}$ at SAN, with 85% of students from IG exhibiting Sb hair content above the geometric mean value calculated for SAN students. However, the geometric mean value for IG is well below the Sb hair content found in hair samples from residents of Zlata Idka village (Slovakia), where Sb-ore mining took place over the centuries (Rapant et al., 2006).

For Zn, the geometric mean value observed at IG is higher than those at SAN, SIC, Palermo and also in the studied young population of Poland (Chojnacka et al., 2010). It is worth noting that 25% of hair samples from IG exhibited Zn concentrations exceeding 382 $\mu g\ g^{-1}$ and 5% greater than 534 $\mu g\ g^{-1}$. Such high occurrences are generally well above the published data unless peculiar sites such as the S. Domingos mine area (Portugal) and neighbor localities are considered (Pereira et al. 2004). Even though Zn is an essential element, high levels of Zn in the human body may result in adverse health effects.

### 3.1. Gender-related differences

The presence of gender-related differences was also evaluated on IG and SAN dataset, taken separately. The hair samples from IG showed statistically significant differences between males and females ($t$-test, $p < 0.01$) for a larger number of elements (Ag, Ba, Cd, Co, Cu, Ni, Sr, U and Zn) than at SAN, where only U was significantly different. The above elemental concentrations in females were always higher than in male donors.

Biological and non-biological factors may differently influence the response of men and women to the exposure to chemicals (Vahter et al., 2007). Higher concentration of Sr and U in hair samples from females was reported by Dongarrà et al. (2011, 2012) and Varrica et al. (2014). High content of Sr in hair of females was considered a common gender characteristic, independent of sampling site, due to the proven chemical similarity of Sr to Ca, a main component of bones, and young girls, having a shorter growth period than boys, complete their skeletal growth generally earlier and faster than boys. It is likely that Sr release from bone follows the general physiology of bone Ca metabolism as it occurs for Pb (Pounds et al., 1991). According to Leggett (1994) the distribution of U in the skeleton is similar to that of Ca, being deposited on the bone surface where it substitutes, as uranyl ion ($UO_2^{2+}$), for Ca. Both drinking water and food can be significant sources of U exposure in areas with naturally high concentrations in soil and bedrock (Berglund et al., 2011).

However, it is problematic to explain why Ag, Ba, Cd, Co, Cu, Ni, Sr, U and Zn levels were significantly higher in females at IG and not at SAN (except for U). A different gender-related way of assimilation and detoxifying, occurring when the metal burden increases, may be invoked but more information and further investigation are needed to reach such a conclusion. However, this finding indicates that females could be more vulnerable to exposure to trace metals than males, particularly at higher exposure. This finding is consistent with the observations of Berglund et al. (2011), who reported that females seemed to be more at risk for toxic metal exposure than males.

This issue also contradicts the common belief that young boys are more exposed than girls because they spend more time outside and therefore are more exposed to trace metals from soil and dust (Shalat et al., 2003; Freeman et al., 2005; Dongarrà et al., 2011).

## 4. Conclusions

The study brought out marked differences in hair trace element concentrations of children living near the historic mining district of Sulcis-Iglesiente, compared with boys and girls of the same age living south of this area, in the Sant'Antioco zone, or in other sites.

The elemental analyses showed, on a statistical basis, that children living at Iglesias are greatly exposed to several metals and metalloids (Ag, Ba, Cd, Cr, Cu, Mn, Ni, Pb, Sb and Zn) originated in mining tailings, enriched soils, waters and food. Similarly, the comparison of IG results with data from the literature showed that the level of exposure at IG was higher. The most pronounced exposure seemed to be to Cd, Ag, Pb, Ba, Sb and Zn.

The gender based variations indicated that at Iglesias females are likely more at risk for toxic metal exposure than males.

A multivariate statistical analysis supported these findings, which demonstrate that the elemental profile in human scalp hair is influenced by local environmental factors, such as contamination of stream water, sediments, and soils by metals and metalloids.

Nevertheless, the observed differences among the various sites reinforce the idea that considerable attention must be paid in exporting reference values as those calculated for one site may not be applicable to another site. Reference values have to be considered site-specific.

Although the findings of this survey are not intended to be diagnostic, they provide evidence of a potential risk of adverse effects on the health of the exposed population and suggest the need to go beyond these preliminary results extending this approach further by acquisition of new data from other sites of Sardinia to implement the normal surveillance activity of the public health.

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