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Title: Effects of sex on the levels of metals and metalloids in the hair of healthy group of Spanish adolescents (13 to 16 years old)

Peña-Fernández A. [a,b], Lobo-Bedmar M.C. [c], González-Muñoz M.J. [b]

[a] Faculty of Health and Life Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK.

[b] Universidad de Alcalá, Unidad de Toxicología, Departamento de Ciencias Biomédicas, Crta. Madrid-Barcelona Km, 33.6, 28871 Alcalá de Henares, Madrid, Spain.

[c] IMIDRA. Departamento de Investigación Agroambiental. Finca el Encín, Crta. Madrid-Barcelona Km, 38.2, 28800 Alcalá de Henares, Madrid, Spain.

Corresponding author: Peña-Fernández Antonio

School of Allied Health Sciences, De Montfort University, The Gateway, Leicester LE1 9BH, UK.

Email: Antonio.Pena-Fernandez@dmu.ac.uk

Telephone: +44 116 201 3859

Abstract

Human biomonitoring can be a reliable tool to protect the health of the citizens of major urban environments. Human hair may be an invaluable specimen to determine chronic environmental exposure to contaminants in the individual, especially in the young population. However, different factors including the lack of studies that have established reference values for metals and metalloids (trace elements) in human scalp hair, make the use of this matrix controversial. A monitoring study was performed to establish possible normal or tentative reference values of Al, As, Be, Cd, Cr, Cu, Hg, Mn, Pb, Sn, Ti, Tl and Zn in adolescents' hair aged 13-16 years that live since they born in Alcalá de Henares, Madrid region (Spain). A strict inclusion criteria was followed to study the effect of sex in the hair metal content; and the levels of the above contaminants was also studied in Alcalá de Henares's parks topsoils. Scalp hair samples were collected in 96 healthy adolescents (28 boys and 68 girls) and reference values were calculated following the recommendations of the International Union of Pure and Applied Chemistry. The levels of Cd, Cu, Pb, Sn and Zn in hair of Alcalá de Henares's adolescents have shown sex dependency, being significantly higher female participants. Sex should be a factor to take into account when developing future reference values and hair metal content. Soil metal contamination was not correlated with the levels found in hair. To conclude, the values of trace elements here analysed and discussed could be considered as tentative reference values for Spanish adolescents aged 13-16 years resident in the Madrid Region, and may be used to identified exposed adolescents in this Spanish region.

Keywords:

Biomonitoring, metals and metalloids, human hair, adolescents, coverage intervals, Spain.

1. INTRODUCTION

Urban contamination by metals and metalloids (trace elements) is becoming a public health risk to a global scale because of the high presence of these contaminants, the impact in the morbidity and mortality of the general population, the deleterious effect on child development even at a low exposure to different elements, and the challenges for recovery and remediation urban environments due to their characteristics (Peña-Fernández, 2011; Peña-Fernández et al., 2014a; Varrica et al., 2014). Therefore, environmental and biomonitoring programmes should be implemented in major cities to protect the health of the population.

There is an increased interest in the development of large scale Human Biomonitoring (HBM) studies in the European Union (Schulz et al., 2009, Becker et al., 2013; Casteleyn et al., 2015). HBM has been described as a useful tool for informing government policy and recommending changes to legislation. For example, there has been a significant reduction in the levels of lead in blood following the ban on lead in compounds used as anti-knocking agents in petrol and other fuels (Schumacher et al., 1996; Aelion et al., 2009).

Human hair is considered a good specimen to study environmental exposure to some trace elements of toxicological concern in humans (Callan et al., 2012; Demetriades et al., 2010). However, different factors including the lack of studies that have established reference values for trace elements in human scalp hair, make the use of this matrix controversial (Kordas et al., 2010). Other possible limitations have been previously discussed comprehensively elsewhere such as the necessity of performing an appropriate washing procedure to minimise external contamination (Peña-Fernández et al., 2014a, 2014b; Molina-Villalba et al., 2015).

Reference values are an appropriate tool to identify individuals with higher exposures to any contaminant such as metals and metalloids. However, a proper and complete understanding of the excretion and incorporation of trace elements into hair is required to develop appropriate reference values for these substances in human hair. Our group has developed a strict inclusion criteria and methods that could enable the determination of reference values for some elements in human hair as well as using this matrix in HBM surveys (Peña-Fernández et al., 2014a).

In Spain there is limited information available on the use of human hair in HBM studies performed on the general population, as most studies have been conducted in highly specific groups to analyse occupational or industrial exposures, or were focussed only on adults (Molina-Villalba et al., 2015). Additionally, very little HBM studies have focussed in Spanish's young population (infants and children) despite their high sensitivity to environmental pollutants. To cover this gap in the literature, a comprehensive HBM study was undertaken in Alcalá de Henares (Madrid Region, Spain) to determine the levels of metals of toxicological concern in the hair of school children and adolescents. The possible reference values for a battery of trace elements in children's hair were comprehensively discussed previously (Peña-Fernández et al., 2014b); however, to our knowledge, normal or tentative reference values have not been provided for Spanish adolescents. Our results could help to the future development of comprehensive reference values for metals and metalloids in hair for the Spanish population, which are being demanded in the EU (Casteleyn et al., 2015).

This study had the following aims: a) to determine the possible normal or reference values of aluminium (Al), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), manganese (Mn), lead (Pb), tin (Sn), titanium (Ti), thallium (Tl) and zinc (Zn) in the scalp hair of healthy adolescents (13-16 years) from Alcalá de Henares (Spain); b) to study the possible effects of sex on the presence of these pollutants in adolescents' hair; and c) to determine whether the metal content from urban parks' topsoils from Alcalá de Henares was a significant source for these contaminants for adolescents.

2. MATERIALS AND METHODS

2.1. Study design and recruitment

Hair samples were collected between April and May of 2001 from healthy Caucasian adolescents aged 13-16 years who have permanently lived in Alcalá de Henares, Madrid Region, Spain. Alcalá de Henares (latitude: 40° 28' 49'' N - longitude: 3° 22' 9''), is close to Madrid city and is one of the most densely populated city of this region. All public and private schools in Alcalá de Henares (a total of eight schools and secondary schools) were screened for recruitment of participants. Written consent was gained from the schools' directors as well as the parents or legal guardians after personal meetings. Data regarding sex, age and life-style habits was also recorded for all participants in form of a questionnaire. Information about their health status, use of medication, dental appliances and hair care treatment. The methodology and strict inclusion criteria developed by our group (Peña-Fernández, 2011; Peña-Fernández et al., 2014a) was followed to recruit participants and collect samples, briefly: participants were restricted to healthy non-smoker students with dark hair (main natural colour in Spain) who did not use any hair care products and beauty treatments (stains, fixers, permanents waves, etc.) and who have been living in Alcalá de Henares since birth. Adolescents who have occasionally smoked, followed a medication programme, had amalgam fillings, or had any metal implants (e.g. titanium plates if they a history of broken limbs), were excluded from the study. These methods and strict selection criteria were designed to minimise the possible effect of different confounding factors for elemental analysis of human hair (Peña-Fernández, 2011). Under such strict selection criteria, 96 adolescents (13 to 16-years-old), 28 boys and 68 girls only met the requirements for participation in this study after monitoring all Alcalá de Henares' private and public schools (506 adolescents in 8 different schools). The number of participants recruited was enough to be representative of the population of Alcalá de Henares. A small scalp hair sample 1-2 cm long was cut close to the occipital region. The guidelines of the Helsinki Declaration were followed to perform this study.

To prevent potential external contamination, samples were washed with 1% v/v Triton X-100 in ultrapure water [Milli-Q (resistivity ≥ 18.2 MΩcm)] in an ultrasonic bath as described by Granero et al. (1998). Non-ionic surfactants do not affect the metal content of the hair, and are appropriate for washing hair for diagnostic purposes (Schuhmacher et al., 1991).

2.2. Metals and metalloids levels in hair

The levels of Al, As, Be, Cd, Cr, Cu, Hg, Mn, Pb, Sn, Ti, Tl and Zn were monitored by inductively coupled plasma-optical emission spectrometry (ICP-OES, Thermo Jarrel Ash ICAP 61), after nitric mineralisation of the sample (Schumacher et al., 1991). Quality-assurance procedures and precautions were followed. These precautions included: use of Milli-Q water (resistivity ≥ 18.2 MΩcm) in all the study's steps and only use of reagents of analytical grade. Teflon bombs were thoroughly cleaned after every run. A reference standard was analysed every ten samples (Lobster hepatopancreas, NRC Canada, TORT-2) as previously described (Ferré-Huguet et al., 2009; Peña-Fernández et al., 2014a). The mean recovery rates ranged between 78% and 110%. Limits of detection for each trace element are provided in Tables 3 to 5.

2.3. Reference levels of metals and metalloids in hair

Reference values describe the upper margin (95th percentile) of the background exposure of the population to a particular environmental contaminant at a given time. These values are calculated under strict quality control measures in a sample population large enough to be representative (Ewers et al., 1999; Poulsen et al., 1997; Schulz et al., 2009). Reference values have different applications such as the detection of individuals that have suffered a high exposure to a contaminant (Ewers et al., 1999) or to minimise confounding factors in the hair metal content such as exogenous contamination (Tsanaclis and Wicks, 2008).

Reference values for Al, As, Be, Cd, Cr, Cu, Hg, Mn, Pb, Sn, Ti, Tl and Zn were determined in the hair of healthy Alcalá de Henares's adolescents aged 13-16 years following the indications established by the International Union of Pure and Applied Chemistry (IUPAC) (Poulsen et al., 1997). This study also calculated reference values for male and female adolescents separately.

2.4. Environmental pollution sources

The trace elements monitored in this study were also determined in topsoils from urban parks across Alcalá de Henares to stablish whether topsoils were a source of elements for the studied population. Soil samples were collected and analysed in July 2001 and can be found described in Peña-Fernández et al. (2014c).

In addition to soils, the possible relationship among baseline levels of trace elements in adolescents' hair and the main environmental pollution sources (food, water, tobacco, air) was also determined as described by Avino et al. (2013).

2.5. Statistical analysis

The statistical significance of the data was computed by one-way analysis of variance (ANOVA). The Kolmogorov–Smirnov test was used to confirm that the values were normally distributed, while homogeneity of the variances was assessed using Snedecor's F-test. In addition, the Fisher's least significant difference (LSD) test was used to determine which means differed significantly from the others using a significance level of 0.05% or less.

A Pearson correlation analysis was performed to investigate possible common contamination sources of metals and metalloids by identification of significant correlations between the different elements monitored in hair (Aelion et al., 2009; Gong et al., 2010), and to establish if soils were a significant source of these contaminants. Finally, another correlation study was undertaken to study the possible relationship between the total elemental hair concentration monitored and the reference doses of those elements in the following environmental compartments: air and smoke, food and water, following the methodology described by Avino et al. (2013). The statistical package SPSS 15.0 was used to perform all the calculations.

3. RESULTS

Tables 3, 4 and 5 present the levels of Al, As, Be, Cd, Cr, Cu, Hg, Mn, Pb, Sn, Ti, Tl and Zn in hair of general, male and female adolescent healthy population that lives in Alcalá de Henares, respectively. Levels of Pb were analysed in a preliminary study performed in the same city (Peña-Fernández et al., 2014a). Baseline values or tentative reference values for trace elements in hair are suggested for general, male and female healthy adolescents (Tables 3-5).

Cd ($p<0.001$), Cu ($p<0.001$), Pb ($p<0.001$), Sn ($p<0.01$) and Zn ($p<0.001$) have shown to be sex-dependent, with concentrations greater in the female participants. In the same way, the presence of the other elements analysed were slightly higher in the female participants, except for Hg (Tables 4 and 5).

The results of the Pearson correlation study for Al, Cr, Cu, Hg, Mn, Pb, Sn, Ti and Zn analysed in the collected hair is shown in Table 6. Data for Cu, Hg and Mn were logarithmically transformed to generate distributions that were close to a normal distribution. Only Cu, Mn and Ti showed a significant correlation. Cu was positively but poorly correlated with most variables in this study: Mn ($r= 0.231$; $p<0.05$), Pb ($r= 0.358$; $p<0.001$), and Sn ($r= 0.257$; $p<0.05$). Mn was positively correlated with Al ($r= 0.379$; $p<0.01$), and Pb ($r= 0.358$; $p<0.001$). Finally, Ti was only positively correlated with Zn ($r= 0.227$; $p<0.05$).

Furthermore, the levels of trace elements found in adolescents' hair (Al, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Zn) were subjected to a Pearson correlation study with those monitored in topsoils collected in different public parks across Alcalá de Henares (Peña-Fernández et al., 2014c). All the samples analysed and compared here were collected during the same time period (*i.e.* spring-summer of 2001). The results were: Al ($r =0.069$; $p =0.662$), Cr ($r = -0.054$; $p =0.643$), Cu ($r =-0.115$; $p =0.322$), Mn ($r =-0.132$; $p =0.259$), Pb ($r =-0.015$; $p =0.899$), Sn ($r =0.077$; $p =0.505$), Ti ($r =0.156$; $p =0.159$) and Zn ($r =0.032$; $p =0.783$). This study did not show any significant correlation.

Finally, another Pearson's correlation study was performed between the levels of As, Cd, Cr, Cu, Hg, Mn, Ni, Pb, Sn, and Zn monitored in the adolescent population's hair with the reference doses reported for each trace element in mayor pollution/environmental sources (air, water, food and cigarrete smoke) by Sabbioni et al. (1981), following the methodology described by Avino et al. (2013). The relative regression coefficients (R^2) are collected in Table 7, for the general adolescent population. The element average composition of scalp hair shown in Table 7 was calculated individually for each participant considering 70 kg as the standard weight for human adults (Sabbioni et al., 1981). The average weight determined in Alcalá de Henares's adolescents participants was 63.55 ± 12.2 kg for males and 58.24 ± 10.1 kg for females, which is similar to those reported in comprehensive studies in the literature for adolescents aged 13 to 16 years old living in the Madrid Region (Martínez-Gómez et al., 2009). The environmental element reference doses proposed by Sabbioni et al. (1981) were calculated using animal *in vivo* metabolic studies considering three different administration routes: oral, inhalation and injection. The results of our study show very good correlation between elements and food (R^2=0.942), water (R^2=0.876), and good correlation with air (R^2=0.676). A very poor relative regression coefficient was observed among elements in hair and cigarette smoke (R^2=0.016). The same correlation study was performed for male and female participants, as sex may affect the presence of metals in human hair. Similar relative regression coefficient (R^2) results were obtained for male and female participants, as follows for males: water (R^2=0.864), air (R^2=0.676), food (R^2=0.937) and smoke (R^2=0.016); and female participants: water (R^2=0.880), air (R^2=0.675), food (R^2=0.943) and smoke (R^2=0.016).

4. DISCUSSION

4.1. Concentrations and reference values of metals and metalloids in adolescents' hair in Alcalá de Henares

Baseline levels or tentative reference values (95% confidence interval for 95 population percentile or CI-PP95) defined here (Tables 3-5) may be used to determine if any adolescent that lives in the Madrid Region has been significantly exposed to any of the trace elements described with considerations. Adolescents living in this Spanish region have similar life-style and dietary habits and would be exposed to similar environmental sources of metals and metalloids. However, these reference values should be carefully used as the establishment of them in human hair is controversial (Kilic et al., 2004). A similar study should be performed in the future to re-define these reference values if the environmental background of these contaminants and/or the life-style changes (Ewers et al., 1999).

It has been reported that the reference values depend on the background exposure (Ewers et al., 1999), and the levels of metals and metalloids in hair would be site specific (Tamburo et al., 2015), fact that will limit the use of the reference values described in a different region and country. However, to identify possible mistakes undertaken during the calculation of the reference values in the recruited Spanish adolescents, these values were compared with the reported in other similar studies carried out on young population in Germany (Seifert et al., 2000) and Czech Republic (Beneš et al., 2003).

The levels of Al, Cd, Cr, Cu, Pb and Zn in the hair of the monitored group in Alcalá de Henares were compared with the reference values proposed by Seifert et al. (2000) for a German healthy general population of 6 to 14 years (Table 1). The arithmetic means of these pollutants were at or below the average reference, except for Cr, which was higher in Alcalá de Henares's population (0.50 vs. 0.11 μg/g).

The presence of the metals Cd, Cr, Cu, Hg, Pb and Zn in the hair of the Alcalá de Henares's adolescent population were similar to those levels proposed as a reference in Czech population with an age average of 9.9 years old (Beneš et al., 2003; Table 2), except for Cr (0.50 vs. 0.40 μg/g), Hg (0.55 vs. 0.27 μg/g) and Zn (148.25 vs. 128.0 μg/g), which were higher in Alcalá de Henares (Table 3).

Furthermore, the levels of metals and metalloids found in the hair of the adolescents (Table 3) were compared with those reported in other similar studies, both national and international. In general, the levels of these substances have been similar or lower in the samples studied. A brief description of each element is described below.

The levels of As, Be, and Tl, were lower than the limit of detection (LOD) in all hair samples monitored in the adolescent group (Table 3). Moreover, the presence of Cd was detected in very few samples: 15 of the 96 adolescents who participated in the study (Table 3). These results are in agreement with those described by Nadal et al. (2005a), who did not detect levels of As, Be, Cd and Tl in any of the samples monitored on 134 boys of ages 12-14 years that lived in Tarragona (Spain). These authors observed the same result in a further study conducted on the same group in 2007 (Ferré-Huguet et al., 2009).

The presence of Al in hair was compared with other studies despite the limitations that have been described for the analysis of this metal in human hair (Peña-Fernández et al., 2014b). Thus, its presence was lower in the group studied than that reported by Unkiewicz-Winiarczyk et al. (2009) in a general population aged 20 to 30 years from Lublin, Poland, in both men (4.86 vs. 49.8 µg/g), and women (5.45 vs. 45.4 µg/g), although it is a group of very different ages. Also, the geometric mean of this metal has also been much lower than that obtained in the general population of Indonesia 17-60 years (4.43 vs. 146.5 µg/g) (Tommaseo-Ponzetta et al., 1998). However, it is necessary to analyse the appropriateness of monitoring Al in hair as humans are easily exposed to this neurotoxic from different sources, mainly dietary. Thus, this metal is used for the treatment of drinking water and in a variety of food products to produce emulsions and change textures. Moreover, Al is present in kitchen equipment and utensils and is used in the pharmaceutical industry in antacids and in skincare and beauty products such as deodorants (Pérez-Granados and Vaquero, 2002; Peña-Fernández, 2011).

The range of Cd found in the hair of Alcalá de Henares' adolescents (15 samples out of 96; 0.02 to 0.52 µg/g, Table 3), has been of the same order as that observed in the adolescent population living in Tarragona (11 samples out of 134; <0.03 to 0.26 µg/g) (Nadal et al, 2005a). The detection of this carcinogen in a few samples could be attributed to the fact that only non-smoker adolescents were included in this study. Smoking is a main source of Cd in humans (Afridi et al., 2015). In addition, the arithmetic concentration of Cd found in the hair of female participants in our study was lower than that indicated in a similar study carried out on 760 students aged 11 to 13 years in Istanbul, Turkey (0.11 vs 53.38 µg/g) (Özden et al., 2007). These authors explain the high presence of this metal in the hair because Istanbul is a heavily industrialized city with high traffic volumes.

The levels of Cr found in the Spanish adolescent group were lower than those determined in an Italian population aged 12-16 years in both the males (0.47 vs. 1.3 µg/g) and females (0.51 vs. 1.1 µg/g) (Tables 4 and 5, respectively). However, the average concentration of this metal (0.50 µg/g; Table 4) was greater than that described by Granero et al. (1998), in 11-13 years subjects from Tarragona (0.22 µg/g). However, these authors observed that the presence of Cr increased from 0.22 to 0.97 µg/g, from 1998 to 2007 in population between 12-14 years from Tarragona (Ferré-Huguet et al., 2009), a concentration of Cr that was much higher than that described in the present study (0.50 µg/g).

With respect to Cu, its presence in the hair of the population studied was higher than levels described in an Italian adolescent group (11-16 years), both boys (7.81 vs. 6.2 µg/g) and girls (13.71 vs. 8.4 µg/g) (Tables 4 and 5, respectively) (Perrone et al., 1996).

Human hair is a good specimen to determine exposure to Hg (Peña-Fernández, 2011). In general, the presence of this potent neurotoxic agent was lower in the hair of the study participants than concentrations described in national studies. Thus, the arithmetic mean concentration of total Hg was lower than that indicated in subjects 11-13 years from Tarragona (0.55 vs. 0.90 µg/g) (Granero et al., 1998). This group of researchers has observed, in a subsequent study of temporal variation, a decrease in the levels of this metal from 1998 to 2007, finding an arithmetic mean of 0.63 µg/g in 2007 (Ferré-Huguet et al. 2009), a value which is still higher than that described in our study.

The geometric mean of total Hg determined in the hair of the adolescents recruited in this study (0.43 µg/g; Table 3) shown to be several units lower than that described by Gonzalez et al. (1985) in the general population of Madrid (7.96 µg/g), although this could be attributed to the higher age range studied by these authors. It was also lower than the geometric mean reported in individuals of 14 years old of the Faroe Islands, Denmark (0.43 vs. 0.96 µg/g) (Budtz-Jørgensen et al., 2004).

On the other hand, the presence of total Hg (0.09 to 2.41 µg/g, Table 3) was lower than the threshold limit proposed by WHO (<10 µg/g), above which is likely to develop neurotoxicity by exposure to this metal (WHO, 1990). Moreover, the average of total Hg found in the male adolescent group was lower than that described by Liu et al. (2008), from which the risk of the incidence of cardiovascular disease in the adult population (2 µg/g) would increase, although it is a proposed range for adult men. However, the content of total Hg found in the hair of 15 of the 96 adolescents monitored exceeded the level related to cognitive and neurological damage in the individual of 1 µg/g (NRC, 2000; Freire et al. 2010). However, prior to take any public health intervention in this Spanish region, the different source(s) of this neurotoxic metal in individuals living in Alcalá de Henares should be carefully studied and identified. Food consumption, specifically fish and seafood, should be carefully monitored in this population as it has been described as one of the main sources of Hg for humans, as shown in other studies (Castaño et al., 2015). The exposition to this metal in the Alcalá de Henares's population could be considered as minimal as the presence of this element in the topsoils of the city was smaller than the detection limit in all samples taken (Peña-Fernández et al., 2014c).

With respect to Pb, its presence in the hair monitored was lower than that reported in other similar studies as described comprehensively in Peña-Fernández et al. (2014a).

Contrarily to the other metals and metalloids studied here, the presence of Sn in the hair of the Alcalá de Henares's adolescents were much higher than those determined in adolescents of Tarragona in 2002 (1.52 vs. 0.13 µg/g) (Table 3) (Nadal et al., 2005a). This difference could be attributed to the higher presence of Sn in urban soils of Alcalá de Henares (0.31 µg/g; Peña-Fernández et al., 2014c) than in Tarragona (0.17 µg/g; Nadal et al, 2005b). However, the environmental presence of Sn in soils of Alcalá de Henares was not correlated with those determined in hair, after conducting a study of correlation between the two matrices (soil-hair), results from the soil analysis are described below.

With respect to Ti, its range in the hair of the Spanish group studied (0.48 to 1.42 µg/g; Table 3) was lower than that reported in general population from 17 to 60 years from Indonesia (0.95 to 17.3 µg/g) by Tomasseo-Ponzetta et al. (1998). The arithmetic mean of Ti was also lower than that found in hair of patients with implants based on this metal (0.90 to 23.5 µg/g) (Kasai et al., 2003).

Finally, the level of Zn found in the hair of the Alcalá de Henares's adolescent group was lower than the level monitored in 41 Polish adolescents aged 11 to 15 years (148.25 vs. 187.0 µg/g), but similar ranges were shown between both groups of population (120.0 to 298.0 vs. 101.0 to 235.12 µg/g) (Lech, 2002). The concentration of Zn analysed in the female segment was also lower than that described by Wang et al. (2008), in hair of 148 girls aged 15-17 years in northern Taiwan (154.66 vs. 166.9 µg/g). These authors observed a significant positive relationship between the presence of Zn in the hair with academic achievement, *i.e.* they determined higher hair concentrations of this essential element in those students with a higher educational performance. The beneficial effect of Zn could be considered in future studies of multi-elemental biomonitoring carried out in schoolchildren and young student populations.

4.2. Effect of sex on levels of metals and metalloids in adolescents' hair in Alcalá de Henares

Cd, Cu, Pb, Sn and Zn in hair of Alcalá de Henares's adolescents have shown sex dependency, being significantly higher in the hair collected on female adolescents. The effect of the sex on the metal and metalloid content in hair is controversial as different effects and trends have been reported in the literature. Therefore, baseline or tentative reference concentrations here reported are described for the general, male and female adolescent groups (Tables 3-5). Thus, Tamburo et al. (2016) have recently reported that sex would be a confounding element when determining reference values of metals and metalloids in human hair. Most of the authors have found significantly elevated levels of trace elements in the hair of the female population (Pereira et al, 2004; Khalique et al., 2005; González-Muñoz et al., 2008). Contrarily, studies performed on Spanish adolescents have reported sex-differences only in the levels of As (females; $p<0.05$) in the hair of adolescents living in Constanti, Tarragona, after monitoring different elements (As, Cd, Cr, Hg, Mn, Ni, Pb and Sn) in hair of 124 children from 11 to 13 years old. These authors have reported similar results due to sex in further studies performed in adolescents from Tarragona in 2002 (134 adolescents aged 12 to 14 years; Nadal et al., 2005a), and in 2007 (96 samples of adolescents of 12-14 years old; Ferré-Huguet et al., 2009), although none of them detected levels of As.

Cd is the trace element that presented the greatest influence due to sex of all the elements monitored in the hair of Alcalá de Henares's adolescent population, being only detected in the hair of the female adolescents (Tables 4 and 5). Other studies have reported higher concentrations of Cd in hair of girls (Wolfsperger et al, 1994; Vienna et al., 1995; Beneš et al., 2003). So, Bosque et al. (1991) have reported significant higher levels of Cd in hair of females ($p<0.05$), in a study conducted on 226 children aged 6-14 years in the province of Tarragona, Spain.

However, different authors have observed a different correlation due to sex for this metal, *i.e.*, higher concentrations of Cd in hair of the young male population (Chłopicka et al, 1998; Nowak and Chmielnicka, 2000). Thus, Dunicz-Sokolowska et al. (2006) observed significantly higher levels of Cd in the hair of boys after monitoring hair from 3420 Polish adolescents aged 10 to 20 years. Likewise, a similar study conducted by our research group in Spanish University students aged 20 to 24 years revealed a concentration of this metal significantly higher ($p<0.01$) in the hair of the male subjects (González-Muñoz et al., 2008), although this study was performed in individuals with a different range of age. Furthermore, Unkiewicz-Winiarczyk et al. (2009) did not observe significant differences in the content of Cd in the hair of non-smoking students aged 20 to 30 years from Lublin, Poland.

Different studies have described that the gastrointestinal absorption of Cd is higher in girls, especially when the iron stores in the body are lower (Rubio and al, 2006; Kippler et al, 2009), which could explain the sex dependency found in the levels of Cd in the hair of the population here monitored. However, an opposite trend was expected as it has been found that Spanish young male population consume more food per body weight than female, and therefore, more pollutants such as: As, Cd, Hg and Pb, mainly through the consumption of fish and seafood (Martorell et al., 2010). These authors have observed the same result in a recent study conducted in adolescents aged 10-19 years.

Therefore, currently there is no scientific evidence that could explain the effect of sex on the presence of Cd in the hair. In addition, numerous studies have reported that there would be no sex dependence on the content of Cd in whole blood and serum. Thus, Farzin et al. (2008) have found no differences in the presence of Cd in serum due to sex in general population of 6 to 62 years of Tehran, Iran. Meanwhile, Ferré-Huguet et al. (2009) have not observed a sex-dependence in the content of Cd in whole blood in a study conducted on population 12-14 years of Tarragona, Spain. Batáriová et al. (2006) has also indicated that there would have not been a relationship with sex after the biomonitoring of this metal in whole blood of 333 children in the Czech Republic.

In relation to Cu, Perrone et al. (1996) found significantly higher levels of this micronutrient in the hair of Italian female adolescents aged 12-16 years, which is in concordance with the population studied here: 13.71 vs. 7.81 µg/g ($p<0.001$). Bárány et al. (2002) have observed that the concentration of this essential metal was significantly higher in whole blood of teenage girls, aged 15 to 17 years, from Switzerland. However, there is controversy on the effect of sex on the excretion of Cu by this matrix. Thus, Sakai et al. (2000) have described an opposite effect due to sex in the levels of this essential metal in hair of Japanese population aged 6 months to 20 years old, *i.e.*, significantly higher levels (p<0,01) of Cu in the hair of male subjects. Meanwhile, Dunicz-Sokolowska et al. (2006) have found no sex dependence in the presence of this bioelement in hair of a Polish population aged 10 to 20 years. A similar trend was also described in the levels of Cu in serum by Arvanitidou et al. (2007), *i.e.* the levels of Cu in the serum of 105 children aged 3 to 14 years of Thrace, Greece, were not significant between both sexes.

The presence of Pb was also significantly higher in hair of Alcalá de Henares's adolescent girls (0.53 vs. 0.77 µg/g; p<0,001) being in accordance to other studies as explained previously in Peña-Fernández et al. (2014a). However, the possible effect of the sex on the Pb-levels in human hair in the literature is controversial. Thus, Ferré-Huguet et al. (2009) have reported no effect due to sex in the levels of this pollutant in the hair of adolescents of 12-14 years of Tarragona, although it was higher in whole blood of females (p<0.05). Contrarily, Chłopicka et al. (1998) found that the concentration of this metal was greater in male's hair and whole blood of 158 Polish children aged 8 to 15 years.

The presence of Sn has also shown dependence due to the sex, being significantly higher in the hair of the female adolescent population (1.62 vs. 1.27 µg/g; p<0.01). This effect was previously observed by Creason et al. (1975), who found a geometric mean of Sn significantly higher in the hair of girls in a study conducted on people aged 0-15 years living in three different neighbourhoods of metropolitan New York. However, and as reported for other metals, Ferré-Huguet et al. (2009) detected higher levels of this metal in the hair of girls aged 12-14 years of Tarragona, but without being significantly different.

Zn also show significant higher levels in hair of Alcalá de Henares's adolescent girls (154.66 vs. 132.70 µg/g; p<0,001). This is in concordance with the results reported by Dunicz-Sokolowska et al.

(2006) in Poland for girls aged 10 to 20 years. However, Sakai et al. (2000) have not found any differences for Zn due to sex in the hair of 418 Japanese people aged 6 months to 20 years.

Currently, there is no clear explanation of how sex influences the excretion of metals and metalloids in human hair (Stupar et al., 2007). Several authors have found significant differences between sexes for different trace elements (Khalique et al., 2005; Kordas et al., 2010) and others have not found any significant difference (Olivero et al., 2002, Nadal et al., 2005a; Reis et al., 2009).

Sex differences described here for Cd, Cu, Pb, Sn and Zn could not be entirely explained by possible sex differences in the food consumption and intake of metals in Alcalá de Henares's adolescents. This statement would be supported by the fact that, although food is the major source of metals for humans that are not exposed to these pollutants through the workplace, our results are not related with the dietary intakes reported for those metals in several comprehensive surveillance studies performed in the Catalonian region, Spain. Thus, higher dietary intakes of As, Cd and Pb have been reported in males for three different age groups: adolescents, adults and seniors (Martí-Cid et al., 2008; Perelló et al., 2014). This group of research has also reported that the dietary intake of Hg was higher in male adolescents in a previous study (Martorell et al., 2011). Moreover, in a more recent study published by Perelló et al. (2015), these researchers have described higher levels of daily dietary intakes in male adolescents aged 10-19 years living in Catalonia for Al, Cr, Cu, Mn, Ni, Zn and other metals not monitored in Alcala's adolescents hair in the present study such as antimony, barium, bismuth, molybdenum or selenium.

The higher daily intake of elements in male population has been also reported in other Spanish regions such as in the Madrid Region. Thus, our group reported higher dietary intakes of Cd, Hg, Mn and Sn in male and no sex differences in the daily intakes of As, Cr, Ni, Pb and Zn, after a comprehensive study performed in a population 20-24 years old living in the Madrid Region (González-Muñoz et al., 2008). However, all the comprehensive dietary surveys described above were performed in slightly different age groups, so a similar study on 13-16 years old adolescents living in this Spanish Region should be performed to support this statement.

On the other hand, Perrone et al. (1996) have pointed out that fluctuations of metals and metalloids due to sex and age are not surprising, and would be derived from the intention of obtaining a general conclusion from the analysis of biomonitoring, without having established a well-defined control group. However, we monitored a very well-defined group of population and we used strict inclusion criteria to avoid confounding factors as described before.

Although finding possible hypotheses that explain the differences found in this study is challenging and beyond the scope of this work, sex has shown to be a confounding factor in the human hair metal content that should be better explored in future human biomonitoring studies. Sex should be taken into account when establishing reference values due to the clinical relevance of these values.

4.3. Relationship among baseline levels of metals and metalloids in adolescents' hair and the main environmental pollution sources

The lack of correlation among elements and tobacco smoke (R^2=0.016; Table 7) is logical as adolescents who have occasionally smoked or smoked were excluded during the sampling process. The very good correlation with food (R^2=0.942) and water (R^2=0.876) could indicate that Alcalá de Henares's adolescents were mainly exposed to the analysed metals and metalloids through the diet and would have had a minor environmental exposure to those pollutants. The overall consideration of the above results could demonstrate that the baseline levels for trace elements proposed were determined in non-exposed Spanish adolescents. Therefore, the values proposed here could be considered as preliminary baseline or reference values for adolescents living in this area.

4.4. Strengths and limitations of this study

The methodology and strict inclusion criteria described here could be used in any group of a population for determining baseline values of environmental pollutants, specifically metals and metalloids, in hair. These baseline values are useful to identify possible expositions to any of these contaminants that may result in the increment of a specific pathology (Avino et al., 2013). Moreover, this study provides for first time levels of a range of metals and metalloids in hair of Spanish adolescents from one of the most populated Spanish regions.

Limitations of this study are that subjects were only recruited in Alcalá de Henares and not in other important cities in the Madrid Region such as Madrid city. However, this would not prevent use of the baseline levels proposed here as preliminary values applicable to other adolescents living in the Madrid Region as similar life-styles and environmental exposure could be assumed due to the proximity of the communities and the relatively small area of this community. Another possible limitation is the difference between the numbers of male vs. female participants. However, this limitation is usually reported in similar studies in the literature due to the challenge of hair sampling in male participants due to their hairstyle (Mortada et al., 2002; Li et al., 2011; Avino et al., 2013). Although more comprehensive studies will be required, baseline levels have been established for male and female adolescents as sex has been described as a confounding factor for determining metals in human hair.

5. CONCLUSIONS

We have proposed baseline levels or tentative reference values for different metals and metalloids with a toxicological or nutritional interest (Al, As, Be, Cd, Cr, Cu, Hg, Mn, Pb, Sn, Ti, Tl and Zn) in the hair of Spanish adolescents aged 13-16 years for the first time. These values were extracted on a well-defined and non-exposed healthy population of adolescents that have lived since born in Alcalá de Henares, one of the mayor cities in the Madrid Region, Spain. Reference values were established following the IUPAC recommendations and may a useful clinical tool to identify adolescents that have been highly exposed to any of the trace elements considered and require further studies and follow up. These values could be included in Madrid's public health policies to protect its young population.

The presence of Cd, Cu, Pb, Sn and Zn in hair of Alcalá de Henares's adolescents has shown sex dependency, being significantly higher in female participants. Therefore, baseline levels or tentative reference values are described for the general, male and female adolescents groups. Possible hypothesis that explain the effect of sex in the presence of trace elements in hair remains unknown and require a better understanding due to the clinical significance when establishing reference values.

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References

Aelion, CM., Davis, H.T., McDermott, S., Lawson, A.B., 2009. Soil metal concentrations and toxicity: associations with distances to industrial facilities and implications for human health. Sci. Total Environ. 407:2216-2223.

Afridi, H.I., Kazi, T.G., Talpur, F.N., Brabazon, D., 2015. Evaluation of trace and toxic elements in the samples of different cigarettes and their impact on human health of Irish diabetes mellitus patients. Clin. Lab. 61(1-2):123-40.

Arvanitidou, V., Voskaki, I., Tripsianis, G., Athanasopoulou, H., et al., 2007. Serum copper and zinc concentrations in healthy children aged 3-14 years in Greece. Biol. Trace Elem. Res. 115(1):1-12.

Avino, P., Capannesi, G., Renzi, L., Rosada, A., 2013. Instrumental neutron activation analysis and statistical approach for determining baseline values of essential and toxic elements in hairs of high school students. Ecotoxicol. Environ. Saf. 92:206-14.

Bárány, E., Bergdahl, I.A., Bratteby, L.E., Lundh, T., et al., 2002. Trace elements in blood and serum of Swedish adolescents: relation to gender, age, residential area, and socioeconomic status. Environ. Res. 89(1):72-84.

Batáriová, A., Spevácková, V., Benes, B., Cejchanová, M., Smíd, J., Cerná, M., 2006. Blood and urine levels of Pb, Cd and Hg in the general population of the Czech Republic and proposed reference values. Int. J. Hyg. Enviorn. Health 209(4):359-366.

Becker, K., Schroeter-Kermani, C., Seiwert, M., Rüther, M., et al., 2013. German health-related environmental monitoring: assessing time trends of the general population’s exposure to heavy metals. Int. J. Hyg. Environ. Health 216(3):250-254.

Beneš, B., Sladká, J., Spěváčková, V., Šmíd, J., 2003. Determination of normal concentration levels of Cd, Cr, Cu, Hg, Pb, Se and Zn in hair of the child population in the Czech Republic. Cent. Eur. J. Publ. Health 11(4):184-186.

Bosque, M.A., Domingo, J.L., Llobet, J.M., Corbella, J., 1991. Cadmium in hair of school children living in Tarragona Province, Spain. Relationship to age, sex, and environmental factors. Biol. Trace Elem. Res. 28(2):147-155.

Budtz-Jørgensen, E., Grandjean, P., Jørgensen, P.J., Weihe, P., Keiding, N., 2004. Association between mercury concentrations in blood and hair in methylmercury-exposed subjects at different ages. Environ. Res. 95:385-393.

Callan, A.C., Winsters, M., Barton, C., Boyce, M., Hinwood, A.L., 2012. Children’s exposure to metals: a community-initiated study. Arch. Environ. Contam. Toxicol. 62(4):714-722.

Castaño, A., Cutanda, F., Esteban, M., Pärt, P., Navarro, C., et al., 2015. Fish consumption patterns and hair mercury levels in children and their mothers in 17 EU countries. Environ. Res. doi: 10.1016/j.envres.2014.10.029.

Casteleyn, L., Dumez, B., Becker, K., Kolossa-Gehring, M., Den Hond, E., et al., 2015. A pilot study on the feasibility of European harmonized Human Biomonitoring: Strategies towards a common approach, challenges and opportunities. Environ. Res. http://dx.doi.org/10.1016/j.envres.2014.10.028iCDC

Chłopicka, J., Zachwieja, Z., Zagrodzki, P., Frydrych, J., Słota, P., Krośniak, M., 1998. Lead and cadmium in the hair and blood of children from a highly industrial area in Poland. Biol. Trace Elem. Res. 62: 229-234.

Creason, J.P., Hinners, T.A., Bumgarner, J.E., Pinkert, C., 1975. Trace elements in hair, as a related to exposure in metropolitan New York. Clin. Chem. 21(4):603-612.

Demetriades, A., Li, X., Ramsey, M.H., Thornton, I., 2010. Chemical speciation and bioaccessibility of lead in surface soil and house dust, Lavrion urban area, Attiki, Hellas. Environ. Geochem. Health 32(6):529-552.

Dunicz-Sokolowska, A., Graczyk, A., Radomska, K., Długaszek, M., Wlaźlak, E., Surkont, G., 2006. Contents of bioelements and toxic metals in the Polish population determined by hair analysis. Part II. Young persons aged 10-20 years. Magnes. Res. 19(3):167-179.

Ewers, U., Krause, C., Schulz, C., Wilhelm, M., 1999. Reference values and human biological monitoring values for environmental toxins. Int. Arch. Occup. Environ. Health 72:255-260.

Farzin, L., Amiri, M., Shams, H., Ahmadi Faghih, M.A., Moassesi, M.E., 2008. Blood levels of lead, cadmium, and mercury in residents of Tehran. Biol. Trace Elem. Res. 123(1-3):14-26.

Ferré-Huguet, N., Nadal, M., Schuhmacher, M., Domingo, J.L., 2009. Monitoring metals in blood and hair of the population living near a hazardous waste incinerator: temporal trend. Biol. Trace Elem. Res. 128(3):191-199.

Freire, C., Ramos, R., López-Espinosa, M.J., Díez, S., Vioque, J., Ballester, D., Fernández, M.F., 2010. Hair Mercury levels, fish consumption, and cognitive development in preschool children from Granada, Spain. Environ. Res. 110:96-104.

Gong, M., Wu, L., Bi, X.Y., Ren, L.M., Wang, L., Ma, Z.D., Bao, Z.Y., Li, Z.G., 2010. Assessing heavy-metal contamination and sources by GIS-based approach and multivariate analysis of urban-rural topsoils in Wuhan, central China. Environ. Geochem. Health 32:59-72.

Gonzalez, M.J., Rico, M.C., Hernandez, L.M., Baluja, G., 1985. Mercury in human hair: a study of residents in Madrid, Spain. Arch. Environ. Health 40(4):225-228.

González-Muñoz, M.J., Peña, A., Meseguer, I., 2008. Monitoring heavy metal contents in food and hair in a sample of young Spanish subjects. Food Chem. Toxicol. 46:3048-3052.

Granero, S., Llobet, J.M., Schuhmacher, M., Corbella, J., Domingo, J.L., 1998. Biological monitoring of environmental pollution and human exposure to metals in Tarragona, Spain. I. Levels in hair of school children. Trace Elem. Electrol. 15(1):39-43.

Joas R., Casteleyn LA., Biot P., Kolossa-Gehring M., Castano A., Angerer J., Schoeters G., Sepai O., Knudsen LE., Joas A., Horvat M., Bloemen L. Harmonised human biomonitoring in Europe: activities towards and EU HBM framework. Int J Hyg Environ Health 2012; 215:172-175.

Kasai, Y., Iida, R., Uchida, A., 2003. Metal concentrations in the serum and hair of patients with titanium alloy spinal implants. Spine 28(12):1320-1326.

Khalique, A., Ahmad, S., Anjum, T., Jaffar, M., Shah, MH., Shaheen, N., Tariq, S.R., Manzoor, S., 2005. A comparative study based on gender and age dependence of selected metals in scalp hair. Environ. Monit. Assess. 104(1-3):45-57.

Kilic, E., Saraymen, R., Demiroglu, A., Ok, E., 2004. Chromium and manganese levels in the scalp hair of normals and patients with breast cancer. Biol. Trace Elem. Res. 102(1-3):19-25.

Kippler, M., Goessler, W., Nermell, B., Ekström, E.C., Lönnerdal, B., El Arifeen, S., Vahter, M., 2009. Factors influencing intestinal cadmium uptake in pregnant Bangladeshi women: a prospective cohort study. Environ. Res. 109(7):914-921.

Kordas, K., Queirolo, E.I., Ettinger, A., Wright, R.O., Stoltzfus, R.J., 2010. Prevalence and predictors of exposure to multiple metals in preschool children from Montevideo, Uruguay. Sci. Total Environ. 408(20):4488-4494.

Lech, T., 2002. Lead, copper, zinc, and magnesium content in hair of children and young people with some neurological diseases. Biol. Trace Elem. Res. 85:111-126.

Liu, X., Cheng, J., Song, Y., Honda, S., Wang, L., Liu, Z., Sakamoto, M., Liu, Y., 2008. Mercury concentration in hair samples from Chinese people in coastal cities. J. Environ. Sci. 20:1258-1262.

Martorell, I., Perelló, G., Martí-Cid, R., Llobet, J.M., Castell, V., Domingo, J.L., 2011. Human exposure to arsenic, cadmium, mercury, and lead from foods in Catalonia, Spain: temporal trend. Biol. Trace Elem. Res. 142(3):309-322.

Molina-Villalba, I., Lacasaña, M., Rodríguez-Barranco, M., Hernández, A.F., et al., 2015. Biomonitoring of arsenic, cadmium, lead, manganese and mercury in urine and hair of children living near mining and industrial areas. Chemosphere 124:83-91.

Nadal, M., Bocio, A., Schuhmacher, M., Domingo, J.L., 2005a. Monitoring metals in the population living in the vicinity of a hazardous waste incinerator. Levels in hair of school children. Biol. Trace Elem. Res. 104:203-213.

Nadal, M., Bocio, A., Schuhmacher, M., Domingo, J.L., 2005b. Trends in the levels of metals in soils and vegetation samples collected near a hazardous waste incinerator. Arch. Environ. Contam. Toxicol. 49:290-298.

Nowak, B., Chmielnicka, J., 2000. Relationship of lead and cadmium to essential elements in hair, teeth, and nails of environmentally exposed people. Ecotoxicol. Environ. Saf. 46(3):265-274.

NRC (National Research Council), 2000. Committee on the toxicological effects of methyl mercury. National Academies Press, Washington, DC.

Olivero, J., Johnson, B., Arguello, E., 2002. Human exposure to mercury in San Jorge River basin, Colombia (South America). Sci. Total Environ. 289:41-47.

Özden, T.A., Gökçay, G., Ertem, H.V., Süoğlu, Ö.D., Kiliç, A., Sökücü, S., Saner, G., 2007. Elevated hair levels of cadmium and lead in school children exposed to smoking and in highways near schools. Clin. Bio. 40:52-56.

Martínez-Gómez, D., Welk, G.J., Calle, M.E., Marcos, A., Veiga, O.L., AFINOS Study Group, 2009. Preliminary evidence of physical activity levels measured by accelerometer in Spanish adolescents: the AFINOS Study. Nutr. Hosp. 24(2):226-32.

Peña-Fernández, A., 2011. Presencia y distribución medioambiental de metales pesados y metaloides en Alcalá de Henares, Madrid. Evaluación del riesgo para la población y biomonitorización de la población escolar. PhD Thesis. University of Alcalá. Available at: http://dspace.uah.es/dspace/handle/10017/9510

Peña-Fernández, A., Lobo-Bedmar, M.C., González-Muñoz, M.J., 2014a. Monitoring of lead in hair of children and adolescents of Alcalá de Henares, Spain. A study by gender and residential areas. Environ. Int. 72:170-175.

Peña-Fernández, A., González-Muñoz, M.J., Lobo-Bedmar, M.C., 2014b. “Reference values” of trace elements in the hair of a sample group of Spanish children (aged 6-9 years) - are urban topsoils a source of contamination? Pharmacol. Environ. Toxicol. 38(1):141-152.

Peña-Fernández, A., González-Muñoz, M.J., Lobo-Bedmar, M.C., 2014c. Establishing the importance of human health risk assessment for metals and metalloids in urban environments. Environ. Int. 72:176-185.

Pereira, R., Ribeiro, R., Gonçalves, F., 2004. Scalp hair analysis as a tool in assessing human exposure to heavy metals (S. Dominos mine, Portugal). Sci. Total Environ. 327(1-3):81-92.

Perelló, G., Llobet, J.M., Gómez-Catalán, J., Castell, V., Centrich, F., Nadal, M., Domingo, J.L., 2014. Human health risks derived from dietary exposure to toxic metals in Catalonia, Spain: temporal trend. Biol. Trace Elem. Res. 162(1-3):26-37.

Perelló, G., Vicente, E., Castell, V., Llobet, J.M., Nadal, M., Domingo, J.L., 2015. Dietary intake of trace elements by the population of Catalonia (Spain): results from a total diet study. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 32(5):748-55.

Pérez-Granados, A.M., Vaquero, M.P., 2002. Silicon, aluminium, arsenic and lithium: essentiality and human health implications. J. Nutr. Health Aging 6(2):154-162.

Perrone, L., Moro, R., Caroli, M., di Toro, R., Gialanella, G., 1996. Trace elements in hair of healthy children sampled by age and sex. Biol. Trace Elem. Res. 51:71-76.

Poulsen, O.M., Holst, E., Christensen, J.M., 1997. Calculation and application of coverage intervals for biological reference values – a supplement to the approved IFCC recommendation (1987) on the theory of reference values. Pure Appl. Chem. 69(7):1601-1611.

Reis, A.T., Rodrigues, S.M., Araújo, C., Coelho, J.P., et al., 2009. Mercury contamination in the vicinity of a chlor-alkali plant and potencial risks to local population. Sci. Total Environ. 407:2689-2700.

Rubio, C., Hardisson, A., Reguera, J.I., Revert, C., Lafuente, M.A., González-Iglesias, T., 2006. Cadmium dietary intake in the Canary Islands, Spain. Environ. Res. 100:123-129.

Sabbioni, E., Goetz, L., Birattari, C., Bonardi, M., 1981. Environmental biochemistry of current environmental levels of heavy metals: preparation of radiotracers with very high specific radioactivity for metallobiochemical experiments on laboratory animals. Sci. Total Environ., 17(3):257-76.

Sakai, T., Wariishi, M., Nishiyama, K., 2000. Changes in trace element concentrations in hair of growing children. Biol. Trace Elem. Res. 77:43-51.

Schuhmacher, M., Domingo, J.L., Llobet, J.M., Corbella, J., 1991. Lead in children`s hair, as related to exposure in Tarragona Province, Spain. Sci. Total Environ. 104:167-173.

Schuhmacher, M., Bellés, M., Rico, A., Domingo, J.L., Corbella, J., 1996. Impact of reduction of lead in gasoline on the blood and hair lead levels in the population of Tarragona Province, Spain, 1990-1995. Sci. Total Environ. 184:203-209.

Schulz, C., Angerer, J., Ewers, U., Heudorf, U., Wilhelm, M., on behalf of the Human Biomonitoring Commission of the German Federal Environment Agency., 2009. Revised and new reference values for environmental pollutants in urine or blood of children in Germany derived from the German Environmental Survey on Children 2003-2006 (GerES IV). Int. J. Hyg. Environ. Health 212:637-647.

Seifert, B., Becker, K., Helm, D., Krause, C., Schulz, C., Seiwert, M., 2000. The German Environmental Survey 1990/1992 (GerEs II): reference concentrations of selected environmental pollutants in blood, urine, hair, house dust, drinking water and indoor air. J. Exp. Anal. Environ. Epidemiol. 10:552-565.

Štupar, J., Dolinsek, F., Erzen, I., 2007. Hair-Pb longitudinal profiles and blood-Pb in the population of young Slovenian males. Ecotoxicol. Environ. Saf. 68:134-143.

Tamburo, E., Varrica, D., Dongarrà, G., 2015. Coverage intervals for trace elements in human scalp hair are site specific. Environ. Toxicol. Pharmacol. 39(1):70-6.

Tamburo, E., Varrica, D., Dongarrà, G., 2016. Gender as a key factor in trace metal and metalloid content of human scalp hair. A multi-site study. Sci. Total Environ. 573:996-1002.

Tommaseo-Ponzetta, M., Nardi, S., Calliari, I., Lucchese, M., 1998. Trace elements in human scalp hair and soil in Irian Jaya. Biol. Trace Elem. Res. 62:199-212.

Tsanaclis, L., Wicks, J.F., 2008. Differentiation between drug use and environmental contamination when testing for drugs in hair. Forensic Sci. Int. 176(1):19-22.

Unkiewicz-Winiarczyk, A., Gromysz-Kałkowska, K., Szubartowska, E., 2009. Aluminium, cadmium and lead concentration in the hair of tobacco smokers. Biol. Trace Elem. Res. 132(1-3):41-50.

Varrica, D., Tamburo, E., Dongarrà, G., Sposito, F., 2014. Trace elements in scalp hair of children chronically exposed to volcanic activity (Mt. Etna, Italy). Sci. Total Environ. 470-471:117-126.

Vienna, A., Capucci, E., Wolfsperger, M., Hauser, G., 1995. Heavy metal concentration in hair of students in Rome. Anthropol. Anz. 53(1):27-32.

Wang, C.T., Li, Y.J., Wang, F.J., Shi, Y.M., Lee, B.T., 2008. Correlation between the iron, magnesium, potassium and zinc content in adolescent girl´s hair and their academic records. Chang Gung Med. J. 31(4):358-363.

WHO 1990. IPCS. Environmental Health Criteria 101, Methylmercury, Geneva.

Wolfsperger, M., Hauser, G., Göβler, W., Schlagenhaufen, C., 1994. Heavy metals in human hair samples from Australia and Italy: influence of sex and smoking habits. Sci. Total Environ. 156:235-242.