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Weekly and Longitudinal Element Variability in Hair Samples of Subjects Non-Occupationally Exposed

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Featured Application: Knowledge of weekly and longitudinal variability of metals in the hair of non-professionally exposed people is of particular importance for understanding the levels/effects of chemicals on workers.

Abstract: Hair is an ideal tissue for tracing the human health conditions. It can be cut easily and painlessly, and the relative clinical results can give an indication of mineral status and toxic metal accumulation following long-term or even acute exposure. Different authors have found outdoor pollution phenomena, such as the levels, significantly alter metal and metalloid hair contents. This paper investigates the element concentration variability in hair samples collected from a not-exposed teenager, neither environmentally nor professionally. The sampling was carried out for one week, and the samples were collected from different locations on the scalp. A nuclear analytical methodology, i.e., the Instrumental Neutron Activation Analysis, is used for determining about 30 elements. Some differences have been found among the samplings as well as between the proximal and distal sections. A deep comparison with other similar studies worldwide present in the literature has been performed for evidencing the relationships and the differences due to different ethnical origins, lifestyles, diets, and climates among the different young populations.

Keywords: hairs; variability; week; longitudinal; element; metals; INAA; occupational exposure; unexposed subject

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1. Introduction

During these last few decades, the human biomonitoring through biological fluids (blood, urine) or tissues (hair, nails) has been largely used for the assessment of health effects following an occupational or environmental exposure [1–9], particularly for the absorbed element content. Basically, the researchers have focused their attention on identifying baseline element values in different population samples, living in different areas characterized neither by air/water/soil contamination nor by exposure to chemicals. Different countries conducted large-scale surveys to assess the exposure profile of different populations and better understand serious environmental public health problems [10–14] and to establish the baseline ranges of trace elements in their populations. Other examples are the analysis of trace elements in the U.S population by the U.S. National Health and Nutrition Examination Survey (NHANES) [15], in Canada [16] and in European countries [17–21]. This topic is considered relevant both to obtain finger-print data related to a certain area [22] and, in forensic studies, to the provenance of subjects in a specific site [23–26]. Hairs are also important because they can be used to understand the element variation in case of prolonged intoxication [27–29]. Metals are integrated into hairs during their growth: in this sense the element composition of hairs is the signature of the living

area and the lifestyle of each person [30,31]. Although there is in literature a great deal of papers regarding the determination of the elemental content in hairs [32–35], to the authors' knowledge, no paper is focused on the element variability during a week. On the other hand, a few studies concern the longitudinal element distribution along human hairs: Yukawa et al. [36] reported the variation of the trace element concentration in long human hairs, showing the profiles according to the distance from the scalp; Kempson and coauthors [37,38] discussed how the exogenous contamination does not influence the levels of some elements (for instance, Zn); on the contrary, Kempson and Skinner [39] report that some other elements, such as Al, are subjected to being accumulated during pollution events and such as Ca, which was demonstrated to be sensitive to endogenous and exogenous contributions [40]; Park et al. discussed the longitudinal association between toenail zinc levels and the incidence of diabetes among American young adults [41]. Finally, Maurice et al. [42] reported a forensic case of poisoning by thallium: the authors developed an analytical method in order to determine the Tl profile all along the hair. The present authors agree with the consideration reported in a recent paper focused on the variation of longitudinal concentration of trace elements in elephant and giraffe hairs [43]. In here, it is highlighted that an important role for this concentration is played by the animals' behavior traits, which suggests that these traits have to be considered also in the study of human hair. Such studies are necessary for having knowledge of endogenous and exogenous roles of hair elements.

Starting from these considerations, this paper would like to investigate the element concentration variability in hair samples collected from a not-exposed teenager, neither environmentally nor professionally. The sampling was carried out for one week, and the samples were collected from different locations on the scalp. Each hair sample was divided into two parts in order to study the section closest (proximal) to the scalp and the most distant (distal) from it. The analyses were performed by Instrumental Neutron Activation Analysis (INAA), and about 30 elements were determined. The use of such analytical technique allowed minimizing the pre-treatment of the samples and hence the relative positive/negative artifacts and performing a multi-elemental determination simultaneously [44–46].

2. Materials and Methods

2.1. Sample Collection and Storage

Hair samples were taken over the course of a week from a female young person (10 years old) chosen among the primary school students. The samples were taken in the right, central, and left nape area on alternate days, with strands of about 10 cm (Figure 1a). All samples were taken by cutting the hair in the chosen area as close as possible to the root (Figure 1b). The cut was made by means of stainless-steel scissors, with zero release of elements [12], in order to avoid any possible contamination caused by the friction between the blades of the scissors and the surface of the hair. The hair is light brown in color, and it is not brittle. In the period preceding the sampling, the subject did not wash her hair, did not use any type of treatment, and did not bathe in the sea. The subject is a non-smoker and was not subjected to second-hand smoke; she lives in a medium-small suburban center (9000 inhabitants) of Central Italy, where there are no industrial settlements, whereas farms that employ biological agriculture are far from the site, and the vehicle traffic is limited except on Saturday morning for the weekly food market.

Immediately after collection, the wisp of hair was placed in high-purity Kartell nuclear grade containers. In laboratory, the containers with the wisp of hair were immediately placed in a silica gel dryer and kept in the dark in an environment with a temperature between 20 and 27 °C, until the time of pre-treatment and subsequent weighing.

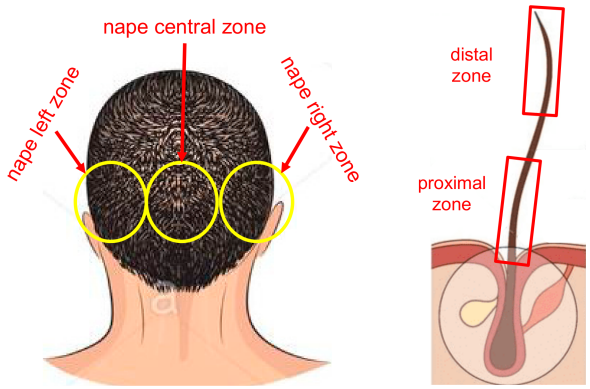

Figure 1. Scheme of the sampling area (**a**) and representation of the proximal and distal hair zones analyzed (**b**).

2.2. Hair Pre-Treatment

The sample pre-treatment was performed by means of a standard procedure, already adopted in previous samplings [12], following a protocol suggested by the International Atomic Energy Agency (IAEA) [47]: washing the samples in 25 mL of ultrapure acetone for 10 min and subsequently rinsing them with ultrapure deionized H_2O (resistivity 18 MΩ × cm), repeated three times.

After drying in an oven at 40 °C for 15 min, all the samples were placed back in the desiccator, brought back to room temperature, and weighed in containers of nuclear-grade polythene for neutron irradiation. The weighing was carried out by means of the AE 160 analytical balance (Mettler-Toledo GmbH, Greifensee, Switzerland) having a sensitivity of ±0.1 mg.

2.3. Neutron Irradiation and Gamma Measurements

The neutron irradiation of the samples was carried out in the rotating rack of the TRIGA Mark II research nuclear reactor in the ENEA Casaccia Research Centre. The irradiation time was 24 h, and the neutron flux was $\Phi = 2.34 \times 10^{12}$ n × cm^{-2} × s^{-1} (corresponding to fluence $F = 1.997 \times 10^{17}$ n × cm^{-2}). These parameters were decided in order to detect those elements that, once activated, produce radionuclides whose half-life was between 3 h and several years [48,49].

After the irradiation in the rotating rack, two sets of measurements were carried out: the first one was after 20 h of decay and lasted between 40 min and 1.5 h; the second one was after 15 days of decay and lasted between 20 and 72 h. The position of the samples in the two sets of measurements was vertical at a distance of 4 cm from the detector for the first set and vertical but in contact with the detector for the second set (Figure 2).

The measurements were carried out by an HPGe coaxial Canberra detector with a resolution (FWHM) of 1.88 keV at 1332.5 keV, a relative efficiency of 42.1, and a Peak-to-Compton ratio of 65.8:1. The system has a Canberra multichannel analyzer with 8192 channels.

The energy and efficiency calibrations for the different counting geometry were carried out respectively with a source of ^{137}Cs and ^{60}Co and with a source of ^{152}Eu, whose activities were certified by the Centre Communitaire de Référence (CEA).

Primary standards and secondary reference materials (SRMs) were used: as primary standards, single-element solutions at a concentration of 1000 µg mL^{-1}; as SRM material with similar composition to the investigated matrix, the BCR CRM 397 (human hair), just used in a previous study [50], as well as the NIST1515 (Apple Leaves) for an overall checking.

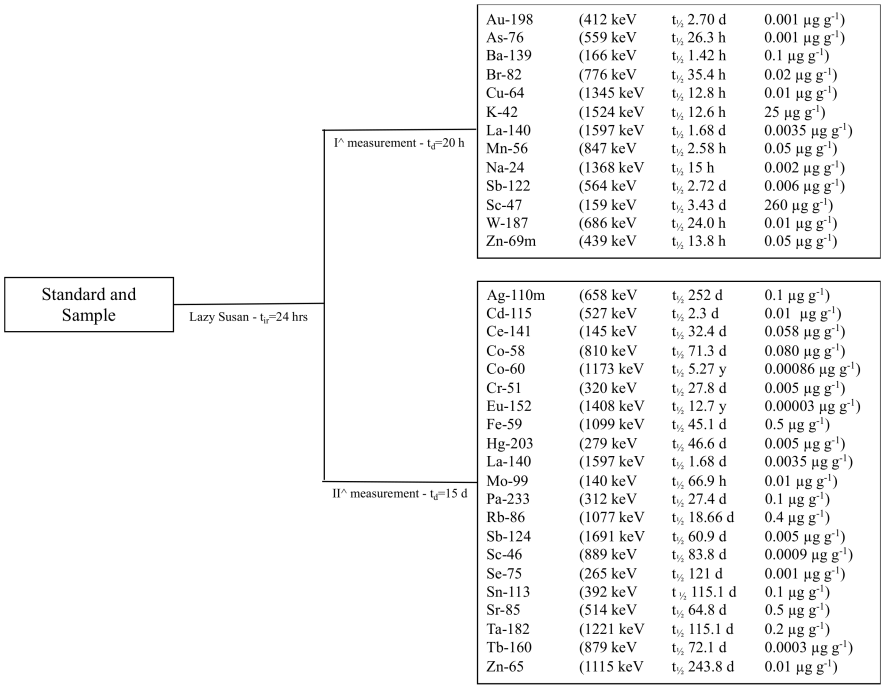

Figure 2. Instrumental Neutron Activation Analysis (INAA) master scheme (t_{ir} irradiation time; t_d decay time; $t_{\frac{1}{2}}$ half-life time). In brackets for each product, nuclides are reported as well as some nuclear analytical chemistry parameters, i.e., peak energy (keV), half-life time (m minute, h hour, d day, y year), and limit of detection (LOD).

3. Results and Discussion

3.1. Quality Control and Quality Assurance (QC/QA)

The environmental studies regarding the correlation between pollutants and biological tissues, such as heavy elements in human hair, need very sensitive and accurate analytical techniques in order to determine contaminants at trace and ultra-trace levels [51]. Among the different possibilities (e.g., spectroscopy, electrochemistry, etc.), the nuclear methods are still the main available techniques to address such stringent requirements. Their high accuracy and precision and the very low limits of detection (LODs) allow investigating a matrix deeply [52]. Furthermore, the possibility of avoiding chemical–physical treatments or performing radiochemical separations is of fundamental importance for achieving these results. Even if INAA is considered a primary analytical technique—i.e., it is possible to analyze the sample just knowing all the nuclear parameters (such as all the nuclear cross-sections of each radionuclide and the nuclear reactor data)—the easier way to analyze a sample is by comparing its activity with that of a standard irradiated in the same conditions. The comparison between the data analysis obtained by INAA and the certified values is the first step for assessing the quality assurance and the quality control (QA/QC). For this aim, standard reference materials (SRMs) such as BCR CRM 397 (Human Hair) and NIST 1515 (Apple Leaves) were chosen according to the matrix similarity and biological origin. Table 1 reports the differences (Δ expressed as %) between our data and the certified values of both the SRMs for each element.

It should be noted that the Human Hair SRM shows a Δ below 6.5%: Se and Zn show high precision and accuracy as well as the indicative or informative element values (i.e., As, Co, Cr, Cu, Fe, Hg, and Mn). The comparison profile for the Apple Leaves is slightly different: among the certified elements, good Δ (below 16%) are achieved for Ba, Ca, Fe, K, Mn, Na, Rb, Sr, and Zn, as also reported in previous paper [53], whereas Hg and Ni show quite relevant differences such as −48.4% and −21.9%, respectively. For Ni, the reason is due to the poor INAA sensitivity. Mercury shows two different results: for Human Hair SRM, the difference between measurements is 5.7%, whereas for Apple Leaves, the SRM is −48.4%. According to our evaluation, the cause has to do with the different Hg levels in

the two SRMs. In the first SRM, Hg is at 12.3 μg g^{-1}, whereas the authors find 13.0 μg g^{-1} with a coefficient of variation (CV%, defined as the ratio between standard deviation and mean value × 100) of 6.1, a good value according to the references [49,54,55]. In the second SRM, the certified Hg value is 0.0432 μg g^{-1} (or 43.2 ng g^{-1}), whereas the authors find 0.0223 μg g^{-1} with a CV% of 45.7, which is an unsatisfactory value according to the ref [54]. The second SRM shows an Hg content almost 300 times lower than the first SRM: this occurrence justifies the high Δ between ours and the certified data in the second SRM.

Table 1. Analytical standard comparison (mean ± s.d.; μg g^{-1}) between our data and certified values: BCR CRM 397 (human hair) and NIST-SRM 1515 (Apple Leaves).

Elem.	Nucl. [1]	BCR CRM 397			NIST-SRM 1515		
		Found	Certified	Δ	Found	Certified	Δ
As	^{76}As	0.29 ± 0.02	(0.31 ± 0.02) [a]	−6.5	n.d.	-	-
Ba	^{131}Ba	n.d.	-	-	51.0 ± 8.0	48.8 ± 2.3	4.3
Br	^{82}Br	n.d.	-	-	3.1 ± 0.7	(1.8) [b]	41.9
Ca	^{47}Ca	n.d.	(1560 ± 40) [b]	-	15267 ± 676	15250 ± 100	0.3
Ce	^{141}Ce	n.d.	-	-	3.6 ± 0.3	(3) [b]	20.0
Co	^{60}Co	0.56 ± 0.02	(0.55 ± 0.03) [b]	1.8	0.10 ± 0.01	(0.09) [b]	10.0
Cr	^{51}Cr	93 ± 9	(91 ± 33) [b]	2.2	0.4 ± 0.1	(0.3) [b]	33.3
Cu	^{64}Cu	113 ± 7	(110 ± 5) [a]	2.7	n.d.	5.69 ± 0.13	-
Fe	^{59}Fe	575 ± 4	(580 ± 10) [b]	−0.9	72 ± 1	82.7 ± 2.6	−12.9
Hg	^{203}Hg	13.0 ± 0.8	12.3 ± 0.5	5.7	0.0223 ± 0.0102	0.0432 ± 0.0023	−48.4
K	^{42}K	n.d.	-	-	16035 ± 717	16080 ± 210	−0.6
La	^{140}La	n.d.	-	-	20.4 ± 0.5	(20) [b]	2.0
Mn	^{56}Mn	10.9 ± 0.6	(11.2 ± 0.3) [b]	−2.7	54.4 ± 1.6	54.1 ± 1.1	0.6
Na	^{24}Na	n.d.	-	-	28.3 ± 0.7	24.4 ± 2.1	16.0
Ni	^{58}Co	n.d.	(46.0 ± 1.4) [a]	-	0.731 ± 0.080	0.936 ± 0.094	−21.9
Rb	^{86}Rb	n.d.	-	-	9.4 ± 0.9	10.2 ± 1.6	−7.8
Sc	^{46}Sc	n.d.	-	-	0.028 ± 0.001	(0.03) [b]	−6.7
Se	^{75}Se	2.1 ± 0.1	2.00 ± 0.08	5.0	n.d.	-	-
Sm	^{153}Sm	n.d.	-	-	2.9 ± 0.1	(3) [b]	3.3
Sr	^{85}Sr	n.d.	-	-	28.0 ± 3.0	25.1 ± 1.1	11.6
Zn	^{65}Zn	196 ± 4	199 ± 5	−1.5	12.50 ± 0.11	12.45 ± 0.43	0.4

[1] Product nuclide; [a] indicative values expressed as μg g^{-1}; [b] informative values expressed as μg g^{-1}; n.d.: not detected; Δ: difference (%) between our mean values calculated and certified one as $\frac{(ourvalue - certifiedvalue)}{certifiedvalue} \times 100$. The standard deviation is calculated among five replicas.

3.2. Element Content in Human Hair Samples

Before performing the analysis, the authors worried about the effects of cleaning and cutting hair before sampling. The problem of hair cleaning is largely discussed as well as the effect of washing using different procedures, as reported in the literature [56–62]. The IAEA recommends a cleaning procedure for hair [47]. Frequent head washing does not affect (the significance limit greater than 0.05) the content of some elements (Br, Co, Cu, Mn, Se, Zn) or of some pollutants (Ni, Hg); on the other hand, As does get washed out but with no such great amount (lower by 1.7 times) [39,47,63]. The hairs were cut by metallic scissors: before that, the oxides were carefully removed. Any effect of the friction between blades and hair shaft were limited in order to prevent possible sample contamination.

Table 2 shows the level of 24 elements measured in the samples investigated along with the minimum and maximum values and the CV%. First, two important elements, i.e., As and Hg, considered hazardous elements for the human health and of exogenous origin, are below the corresponding LODs: this is a preliminary confirmation that the subject is not exposed to sources of As and Hg. Other considerations could be drawn about essential elements, i.e., Cr, Cu, Fe, Mn, Se, and Zn: their variability, expressed as CV%, is below 40% except for Cr and Cu, which have 66.2% and 68.8%, respectively.

Table 2. Element levels and single daily concentrations (μg g^{-1}) determined in all the investigated independent samples (five independent measurements).

Element	Concentration	Concentration (Mean ± s.d. [1])		
	x (Min–Max; CV%)	1st Day	2nd Day	3rd Day
Ag	0.740 (0.34–1.2; 36.6)	0.630 ± 0.354	0.855 ± 0.417	0.735 ± 0.134
As	<LOD	<LOD	<LOD	<LOD
Au	0.022 (0.008–0.37; 63.6)	<LOD	0.010 ± 0.03	0.028 ± 0.013
Ba	1.44 (1.20–1.69; 15.9)	1.20 ± 0.18	1.58 ± 0.31	1.50 ± 0.27
Br	26.5 (22.9–32.1; 13.2)	25.0 ± 3.0	24.1 ± 0.6	30.4 ± 2.3
Ca	2010 (1363–2601; 25.4)	1982 ± 875	2264 ± 468	1784 ± 303
Co	0.041 (0.031–0.066; 31.7)	0.032 ± 0.002	0.038 ± 0.006	0.054 ± 0.017
Cr	0.086 (0.002–0.180; 66.2)	0.086 ± 0.006	0.081 ± 0.007	0.092 ± 0.128
Cu	15.1 (2.30–27.8; 68.8)	19.9 ± 9.0	21.2 ± 9.4	4.17 ± 2.70
Eu	0.001(0.001; N/A)	<LOD	<LOD	0.001
Fe	17.7 (13.5–28.4; 33.3)	14.1 ± 0.1	14.3 ± 1.1	24.6 ± 5.4
Hg	<LOD	<LOD	<LOD	<LOD
K	249 (178–364; 26.5)	195 ± 23	228 ± 8	323 ± 58
La	0.957 (0.288–1.54; 61.8)	0.915 ± 0.886	0.805 ± 0.641	1.35 ± 0.12
Mn	3.75 (1.91–5.22; 33.0)	3.10 ± 1.70	3.20	4.66 ± 0.69
Na	109 (88.2–141; 16.5)	95.1 ± 9.8	109 ± 6	122 ± 27
Rb	0.269 (0.105–0.540; 60.9)	0.117 ± 0.017	0.319 ± 0.070	0.370 ± 0.241
Sb	0.075 (0.045–0.120; 37.3)	0.055 ± 0.013	0.076 ± 0.029	0.096 ± 0.034
Sc	0.007 (0.002–0.011; 71.4)	0.007 ± 0.007	<LOD	0.007 ± 0.006
Se	0.413 (0.035–0.050; 13.3)	0.390 ± 0.057	0.455 ± 0.064	0.395 ± 0.049
Tb	0.020 (0.020; N/A)	<LOD	<LOD	0.020
Th	0.009 (0.000–0.036; 200)	0.00033	0.00043	0.018 ± 0.025
W	0.019 (0.012–0.043; 63.1)	0.012 ± 0.001	0.015 ± 0.003	0.031 ± 0.017
Zn	52.3 (66.3–92.5; 37.2)	77.1 ± 12.0	89.7 ± 3.9	72.1 ± 8.2

[1] s.d. standard deviation.

Br, Ca, K, and Na, the last three considered labile elements (because they are strongly influenced by washing [64]) show a low CV% below 27%, confirming that the hair was not washed. Silver and gold are present at low concentrations, 740 and 22 ng g^{-1}, respectively: their presence could suggest a previous use of shampoos containing nanoparticles of these two elements into the composition for antimicrobial activity [65,66]. Other elements such as La, Rb, Sc, Th, and W can be considered of environmental origin: their CVs% are high, above 60%, especially Th, up to 200%. Finally, Ba, Br, and Sb show low CVs%: their presence and levels could be due to anthropogenic pollution and particularly to airborne particulate matter, as just evidenced by authors in previous papers [46,49].

Table 3 shows the concentration trend (the number of samples per nuclide is too low—only three scalp locations and two longitudinal positions—to obtain any actual reasonable trend that could be considered real and not obtained by chance) in the areas of the nape where the sampling was carried out (left, center, and right) as well as the longitudinal variation of the concentrations along the hair.

If the trends of Br, Ca, K, and Na from Tables 2 and 3 are taken into account, similar concentrations are noted for Br both during the days and in correspondence with the sampling zones and in the longitudinal variation: mean value 26.5 ± 3.46 μg g^{-1}; 1st day 25.0 ± 3.0 μg g^{-1}; 2nd day 24.1 ± 0.6 μg g^{-1}; 3rd day 30.4 ± 2.3 μg g^{-1} (Table 2); left zone 27.1 ± 2.1 μg g^{-1}; central 23.7 ± 0.45 μg g^{-1}; right 28.8 ± 1.7 μg g^{-1}; hair proximal section 26.5 ± 2.6 μg g^{-1}; distal 26.5 ± 4.9 μg g^{-1}. The same stability in the concentration trend is evident for Na, Ca, and K. These four elements, i.e., Br, Ca, K, and Na, are ubiquitous in the environment; besides, Ca, K, and Na are among the fundamental components of the tissues and biological fluids in the human body, and K and Na are also present in the body secretions (exudate, etc.). In the samples, Br, K, and Na show an increasing concentration trend between the first and third day (Table 2). Similarly, the same trend is shown, but in a much more marked way, by elements of environmental origin such as La, + 47.5%

increase in concentration on the third day compared to the first; Rb, + 216%; Th, + 98%; and W + 158%. The authors would like to underline that a fundamental requirement of this study was to have unwashed hairs, in order to understand the natural element levels in human hair. Confirming this statement (i.e., unwashed hair), a similar trend is found for all the elements mentioned above, with the exception of bromine which remains constant, whereas greater increases are found for the elements of environmental origin.

Table 3. Element concentrations (μg g^{-1}) in the nape different areas and along the hair (proximal and distal zones), (five independent measurements).

Element Concentration (Mean ± s.d. [1])					
	Scalp Location			Longitudinal Variability	
	Left Zone	Central Zone	Right Zone	Proximal	Distal
Ag	0.882 ± 0.250	1.15 ± 0.295	0.830 ± 0.095	0.953 ± 0.172	0.527 ± 0.133
Au	<LOD	<LOD	0.037 ± 0.009	0.037	0.015 ± 0.006
Ba	<LOD	<LOD	1.30 ± 0.19	1.30	1.49 ± 0.26
Br	27.1 ± 2.1	23.7 ± 0.5	28.8 ± 1.7	26.5 ± 2.6	26.5 ± 4.9
Ca	1363 ± 619	1933 ± 331	1998 ± 214	1765 ± 349	2255 ± 594
Co	0.033 ± 0.001	0.034 ± 0.004	0.042 ± 0.012	0.036 ± 0.005	0.046 ± 0.018
Cr	0.081 ± 0.004	0.086 ± 0.005	0.092 ± 0.090	0.056 ± 0.047	0.116 ± 0.058
Cu	26.3 ± 6.4	27.8 ± 1.8	6.12 ± 1.91	20.1 ± 12.1	10.1 ± 6.8
Eu	<LOD	<LOD	0.00070 ± 0.00035	0.01	<LOD
Fe	14.2 ± 0.1	13.5 ± 0.8	20.8 ± 3.8	16.2 ± 4.0	19.2 ± 8.0
K	211 ± 16	234 ± 6	282 ± 41	242 ± 36	255 ± 97
La	0.288 ± 0.626	0.351 ± 0.453	1.35 ± 0.67	0.663 ± 0.595	1.40 ± 0.20
Mn	4.30 ± 1.21	3.20 ± 1.61	4.20 ± 0.48	3.90 ± 0.61	3.52 ± 2.30
Na	102 ± 7	105 ± 4	103 ± 19	103 ± 1	114 ± 26
Rb	0.129 ± 0.011	0.270 ± 0.049	0.200 ± 0.170	0.199 ± 0.070	0.338 ± 0.219
Sb	0.064 ± 0.009	0.096 ± 0.014	0.072 ± 0.019	0.077 ± 0.017	0.073 ± 0.040
Sc	0.012 ± 0.005	<LOD	0.007 ± 0.004	0.008 ± 0.007	0.007 ± 0.006
Se	0.352 ± 0.004	0.414 ± 0.045	0.430 ± 0.035	0.397 ± 0.042	0.430 ± 0.070
Tb	<LOD	<LOD	0.020 ± 0.010	0.020	<LOD
Th	<LOD	<LOD	0.001 ± 0.017	0.001	0.012 ± 0.020
W	0.012 ± 0.000	0.013 ± 0.002	0.019 ± 0.012	0.015 ± 0.04	0.024 ± 0.016
Zn	68.6 ± 4.1	87.0 ± 2.7	66.3 ± 5.8	74.0 ± 11.3	85.3 ± 7.3

[1] s.d. standard deviation.

A similar trend is shown by the daily trend (the number of samples per nuclide is too low—only 3 days—to obtain any actual reasonable trend that could be considered real and not obtained by chance) of some elements considered essential, such as Cr, Fe, and Mn; for the last two, the increase is significant: + 74.5% and + 50.3% respectively. Cr and Fe increase their level in the distal area with respect to the proximal one, and a similar trend is also shown by Se and Zn, which, instead, have a daily trend with a maximum on the second day.

However, there are elements that maintain a constant concentration both with regard to the daily trend (Table 2) and in the nape sampling areas and in the distal and proximal ones (Table 3), as highlighted by the coefficient of variation (CV%) calculated taking into account all the concentrations obtained. These elements are the ones present either in the body's tissues or in the biochemical systems, such as Ca, K, Na, and Br (CV% 15.2%, 16.6%, 7.76%, and 8.64%, respectively), and those considered essential such as Mn, Se, and Zn (15.7%, 7.73%, and 11.4%, respectively) and those considered pollutants of anthropogenic origin: Ba, Sb, and Co (10.7%, 18.7%, and 19.2%, respectively). For the last ones, it is possible to hypothesize a hair contamination deriving from their levels in the environment, whereas the level stability of the essential elements is due to regular biochemical systems/reactions. This occurrence allows defining basal elements concentration levels in the hair analysis both in

good health conditions and in "anomalies" caused by occupational and/or environmental exposure.

On the other hand, the concentration of elements of environmental origin (i.e., La, Rb, Sc, Th, and W) is less stable: their CVs vary between 38.4%, tungsten, and 126.9%, thorium. The hypothesis that can be advanced is that their presence in the environment, and therefore their levels in the hair, is connected to the variation of local climatic conditions: wind direction, wind speed, temperature, humidity, etc.

A trend similar to that of the elements of environmental origin is also shown by two chemicals considered essential, i.e., Cr and Cu, and, albeit to a lesser extent, by Fe (CV% 45%, 53.2%, and 23.5%, respectively). For these three elements, the hypothesis of a double origin could be put forward; i.e., there is an overlapping between the element basal levels (i.e., the concentration levels naturally present in the human body) and the concentrations deriving from environmental pollution. This hypothesis can be confirmed because these three elements (i.e., Cr, Cu, and Fe) significantly increase their concentrations in the distal area of the hair (i.e., the section more in contact with the environment) compared to the proximal area (Table 3), even if their average concentration level agrees with hair data reported for Rome and Italy (Table 4). Finally, it is interesting to note that some elements of environmental origin but coming from anthropogenic pollution and considered particularly toxic such as As, Hg, and Ni were not revealed by INAA, i.e., they show levels below the LOD (Figure 2). Therefore, it can be assumed that As, Hg, and Ni are < 1 ng g^{-1}, < 5 ng g^{-1}, and < 80 ng g^{-1}, respectively. Very low levels of Eu and Tb, considered Rare Earth Elements (REEs) of environmental origin, were measured just in one hair sample at concentrations of 1 and 20 ng g^{-1} respectively (LOD for Eu 0.03 and for Tb 0.3 ng g^{-1}).

Table 4. Concentration (μg g^{-1}) comparison between these data and those found in other Italian and international studies.

	Our Data	Literature Values				
		Rome [50]			Italy [67]	International [68–76]
		Female	Male + Female	Light Brown		
Ag	0.740 ± 0.271	0.23 ± 0.42	0.40 ± 1.58	0.29 ± 0.30	0.83 ± 1.96	0.005–9.9
Au	0.022 ± 0.014	0.06 ± 0.08	0.09 ± 0.16	0.10 ± 0.12	0.036 ± 0.038	0.0005–591
Ba	1.44 ± 0.23	0.33 ± 0.94	0.57 ± 1.50	0.87 ± 1.96		0.12–29
Br	26.5 ± 3.5	9.63 ± 15.2	8.66 ± 13.8	5.54 ± 4.92	12.9 ± 10.1	0.15–490
Ca	2010 ± 512	812 ± 580	1120 ± 1060	1776 ± 1776	750 ± 1150	7–10887
Co	0.041 ± 0.013	0.17 ± 0.26	0.17 ± 0.25	0.12 ± 0.17	0.145 ± 0.133	0.002–15
Cr	0.086 ± 0.057	0.15 ± 0.10	0.17 ± 0.15	0.17 ± 0.09	0.234 ± 0.155	0.026–65.3
Cu	15.1 ± 10.4	9.56 ± 4.78	10.5 ± 7.11	11.6 ± 8.91		3.68–72.5
Eu	0.001					-
Fe	17.7 ± 5.9	16.8 ± 26.4	16.5 ± 23.9	17.5 ± 19.3	13.5 ± 6.2	3–2400
K	249 ± 66	335 ± 272	314 ± 274	258 ± 280	940 ± 1110	0.94–2370
La	0.957 ± 0.592	0.03 ± 0.04	0.03 ± 0.03	0.03 ± 0.03	0.038 ± 0.031	<0.003–37
Mn	3.75 ± 1.24	0.40 ± 0.33	0.42 ± 0.32	0.41 ± 0.24		0.03–81.5
Na	109 ± 18	647 ± 545	581 ± 521	476 ± 426	1180 ± 1260	0.04–3500
Rb	0.269 ± 0.164				3.20 ± 5.06	0.01–0.14
Sb	0.075 ± 0.028	0.06 ± 0.06	0.06 ± 0.05	0.07 ± 0.05	0.047 ± 0.034	0.007–38
Sc	0.007 ± 0.005	0.0010 ± 0.0003	0.0010 ± 0.0002	0.0011 ± 0.0008	0.0023 ± 0.0025	0.0004–550
Se	0.413 ± 0.055	0.96 ± 1.55	0.99 ± 1.56	1.17 ± 1.62	0.64 ± 0.28	0.002–66
Tb	0.020					-
Th	0.009 ± 0.018				0.0011 ± 0.0014	-
W	0.019 ± 0.012					-
Zn	52.3 ± 19.5	178 ± 46	189 ± 82	240 ± 144	139.9 ± 65.1	<1–1770

3.3. Comparison with Studies on Adult and Teenager Population

Table 4 shows a comparison between these data and similar levels found in a previous study performed in Rome [50].

First, the presence of elements such as Ag, Au, and Co can be due to cosmetics and/or personal hygiene products. In particular, Ag shows concentrations comparable with the data of the Rome group (0.740 ± 0.271 μg g^{-1} versus 0.40 ± 1.58 μg g^{-1}) [50] and the Italian population (0.83 ± 1.96 μg g^{-1}) [67] as well as Au (0.022 ± 0.014 μg g^{-1} vs. 0.036 ± 0.038 μg g^{-1} for the Italian population) [50], whereas Co shows decidedly lower levels than the Italian population (0.041 ± 0.013 μg g^{-1} vs. 0.145 ± 0.133 μg g^{-1}), but they fall within the international data (0.002–15 μg g^{-1}) [68–76].

Levels of Ca, K, and Na are in line with the data of the Rome and Italy groups. In particular, Ca and K show similar levels to the light brown hair in the Rome group [50] (Ca: 2010 ± 512 μg g^{-1} vs. 1776 ± 1776 μg g^{-1}; K: 249 ± 66 μg g^{-1} vs. 258 ± 280 μg g^{-1}) with a much lower standard deviation. Similar considerations can be also put forward for K, which are compatible with the relevant standard deviation of both Rome and Italian data. Br is instead at concentration levels equal to more than double the concentration data of all the Rome and Italian samples, even though it is slightly above the variability of the Italian data (26.5 ± 3.5 μg g^{-1} vs. 12.9 ± 10.1 μg g^{-1}) but it falls within the international data range (0.15–490 μg g^{-1}).

Among the essential elements, Cr, Cu, Fe, Se, and Zn show good agreement with the data of Rome and Italy groups, whereas Co presents lower levels than those of Rome and Italy groups and Mn higher than the ones of the Rome samples.

As for the elements of environmental origin, the levels of Rb, Sc, and Th are in full agreement with the data reported in Table 4, whereas W is absent in all the other comparison samples, and La is at significantly higher levels. For La, as well as for Mn and Br, all the concentration data are higher than the comparison values [50]: 0.957 ± 0.592 μg g^{-1} vs. 0.038 ± 0.031 μg g^{-1} in the Italian reference group, i.e., 25 times higher; Mn 3.75 ± 1.24 μg g^{-1} vs. 0.42 ± 0.32 μg g^{-1} in the entire series of the Rome samples, 9 times higher; Br 26.5 ± 3.5 μg g^{-1} versus 12.9 ± 10.12 μg g^{-1} in the Italian group, 2 times higher. This anomaly can be explained by environmental contamination, since these three elements are present in the urban atmosphere, especially in airborne and dust depositions [77,78]. In previous papers [79,80], the authors report data on the elements in PM_{10} and $PM_{2.5}$ airborne sampled in downtown Rome along with the trends during the last three decades: both La and Mn and Br show significant levels (particularly, La 170 ± 101 pg m^{-3} in PM_{10}; Mn 60 ± 44 ng m^{-3}, Br 17.1 ± 13.9 ng m^{-3} in $PM_{2.5}$).

Table 5 shows a large comparison among our data and data collected from teenagers (boys and girls) worldwide [81–91]. The table also reports some reference data from Korean [92], Canadian [93], and American [94] teenagers hair values. First of all, it could be seen that our data are broader and more accurate than those determined in other studies, considering the high performance of the analytical method used, i.e., INAA. This occurrence allows drawing only few significant considerations on the content of some metals: Ca (from 2 to 10 times) and Mn (up to 10 times; ours and some other data are similar, an exception is present in the data collected from girl teenagers in Rome, i.e., 69.2) are higher than those reported in the other studies [81–91] and in the reference values contained in [92–94], whereas Cr and As are always less than those reported in the literature. Finally, Ag, Cu, Fe, Se, and Zn show levels close to those reported worldwide.

For a better correlation and significance of this comparison, the authors have reported the correlations in Table 6 and the relative plots in Figure 3 between our data and those determined in girl teenagers hair worldwide [81–91]. As it can be seen, there is high correlation (above 0.9) with the data found, especially in West Europe (different Italian locations, Spain), and low correlation (ranging between 0.1 and 0.6) with those determined in Artic area (Arkhangelsk) and Korea, for this latter also for boys. This occurrence can be interpreted considering the different ethnical origins, lifestyles, diets, and climates (including the presence of different and massive anthropogenic and/or natural sources) among the different young populations.

Table 5. Comparison of element levels (μg g^{-1}) found in hair samples of teenagers worldwide.

(**a**)

	Our	Pakistan [81]		Russian Federation [82]				Italy [83]		Korean [84]		Brazil [85]		Italy [86]		East Aral Sea [87]			
				North Eur.		Arkhangelsk		Rome						Palermo		Kazalinsk		Zhanakorgan	
	G [1]	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G	B	G
Ag	0.74			0.25	0.25	0.17	0.16												
As	<LOD [2]							0.08	0.2	8.99	8.56	0.004	0.01	0	0	0.66	0.61	0.78	0.58
Au	0.022																		
Ba	1.44			0.5	0.5	2.45	2.19			0.33	0.32			1.06	1.5				
Br	26.5															7.37	10.38	7.56	5.79
Ca	2010	567	577.4	1125	850	478.58	1496.76	347	630	198.31	227.75					806	904	887	1170
Co	0.041	1.925	0.881	0.5	0.6	0.2	0.19	0.48	0.19	0.01	0.01	0.008	0.007	0.26	0.16	0.73	1.03	1.41	0.96
Cr	0.086	1.128	2.952	1	1	0.73	0.62	3.24	1.73	0.48	0.45			0.21	0.08	0.52	0.43	0.52	0.7
Cu	15.1	8.06	22.12	11.5	11	10.61	9.42	9.2	16.7	15.09	15.96			18.88	24.44	12.4	12.8	25.8	20.8
Eu	0.001																		
Fe	17.7	35.21	77.4	31	32.5	57.4	27.75	38.5	14.5	12.4	12.86					47.5	43.8	56	44.8
Hg	0.005									0.49	0.51	0.22	0.09			0.87	1.51	0.88	0.88
K	249			530	800	1410	93.04			32.71	35.59					387	714	838	819
La	0.957																		
Mn	3.75	2.172	4.651	1.75	1.75	2.86	2.2	0.28	69.2	0.3	0.27	0.35	0.29	0.34	0.22	3	3.59	2.76	2.97
Na	109									27.8	26.42					619	782	401	492
Rb	0.269													0.03	0.01				
Sb	0.075											0.005	0.005	0.04	0.02				
Sc	0.007																		
Se	0.413			1.9	1.9	0.85	0.88	0.51	0.48	0.75	0.74	0.12	0.12	0.59	0.41	0.91	0.85	0.69	0.85
Sr	0.5			2.75	2.75	1.93	8.12	1.02	2.32					4.05	7.56	10.12	11.7	8.34	14.34
Tb	0.02																		
Th	0.009																		
W	0.019																		
Zn	52.3	117.4	225.1	187.5	187.5	155.59	184.76	153	162	72.53	67.25			137.12	197.2	199.6	175	197.6	193.7

Table 5. *Cont.*

(b)															
	Italy [88]		**Spain [89]**		**Poland [90]**		**Italy [91]**						**Reference Values**		
	Rome		**Madrid**				**Sicily (Urban)**		**Sicily (Rural)**		**Sicily (Ind.)**		**Korean**	**Canadian**	**American**
	B	**G**	**B**	**G**	**B**	**G**	**B**	**G**	**B**	**G**	**B**	**G**	[92]	[93]	[94]
Ag			0.194	0.335											
As	0.096	0.09	0.07	0.07			0.0003	0.0003	0.06	0.03	0.06	0.03	0.18–0.24	<1	0.10–0.20
Au															
Ba			0.3	0.6			0.91	1.49	0.21	1.44	0.53	1.39	0.4–0.6	<0.6	0.00–2.60
Br															
Ca	447.9	374.6			186	252							270–430	190–738	220–970
Co	0.954	0.052	0.0112	0.02183			0.07	0.08	0.17	0.2	0.12	0.16	0.01–0.01	0.01–0.05	0.01–0.03
Cr	0.559	0.427	0.4	0.4			0.17	0.06	0.24	0.28	0.14	0.11	0.80–0.90	0.23–0.80	0.20–0.80
Cu	21.74	10.5	16.2	40.3	10	11	18.16	20.83	14.81	18.93	10.18	12.85	13–16	3.9–23.3	9–39
Eu															
Fe	19.48	12.8	18.2	18.1	9	9							11–13	11–24	5–16
Hg													0.5–0.7	<1.5	0.09–0.18
K													770–1490	5–40	20–240
La															
Mn	0.426	0.27	0.34	0.398			0.22	0.31	0.32	0.41	0.28	0.37	0.23–0.32	0.20–0.80	0.10–1.30
Na													340–650	18–85	40–360
Rb							0.03	0.01	0.1	0.11	0.14	0.12			
Sb							0.03	0.01	0.05	0.05	0.03	0.04			
Sc															
Se	0.838	0.45	0.4	0.4			0.44	0.39	0.72	0.71	0.39	0.32	0.80–0.90	0.50–2.00	0.30–1.80
Sr			0.57	1.9			3.43	7.87	0.8	3.53	5.31	24.35			
Tb															
Th															
W															
Zn	159.1	144.5	101	90.6	138	163	132.41	197.21	199.16	257.51	168.7	173.06	50–60	180–220	100–210

[1] B: boys, G: girls; [2] LOD: limit of detection.

Table 6. Linear regression and coefficient of determination (R^2) between our data and each dataset for different girl teenagers hair determined worldwide [81–91].

Country	Sex	Linear Regression	R^2
Pakistan			
	girls	y = 4.3399 x − 8.841	0.9596
	boys	y = 2.2490 x − 5.702	0.9486
Russian Federation			
North Europe	girls	y = 3.2442 x − 6.0669	0.9962
	boys	y = 2.1688 x + 2.6307	0.9770
Arkhangelsk	girls	y = 0.4252 x + 16.754	0.2921
	boys	y = 5.6558 x − 26.022	0.9887
Italy			
Rome (2000)	girls	y = 2.6415 x − 8.3798	0.9097
	boys	y = 2.8262 x − 5.304	0.9436
Korean			
	girls	y = 0.1558 x + 8.2496	0.3186
	boys	y = 0.1489 x + 8.7558	0.2621
Brazil			
	girls	y = 0.0685 x + 0.0381	0.8539
	boys	y = 0.0768 x + 0.0630	0.6464
Italy			
Palermo	girls	y = 3.6786 x − 3.6842	0.9690
	boys	y = 2.5653 x − 2.4715	0.9739
East Aral Sea			
Kazalinsk	girls	y = 3.4555 x + 9.0975	0.7891
	boys	y = 2.1209 x + 21.810	0.6089
Zhanakorgan	girls	y = 3.4786 x − 4.0669	0.9684
	boys	y = 3.4353 x − 6.7967	0.9882
Italy			
Rome (1992)	girls	y = 2.7462 x + 2.2731	0.7651
	boys	y = 2.9344 x − 7.3893	0.9395
Spain			
Madrid	girls	y = 1.7397 x − 0.6410	0.9506
	boys	y = 1.8624 x − 3.0717	0.9646
Poland			
	girls	y = 4.2421 x − 59.335	0.9945
	boys	y = 3.5639 x − 48.761	0.9952
Italy			
Sicily—urban	girls	y = 3.6786 x − 3.6842	0.9690
	boys	y = 2.4794 x − 2.5041	0.9741
Sicily—rural	girls	y = 4.7754 x − 6.3693	0.9532
	boys	y = 3.7000 x + 5.1878	0.9541
Sicily—industrial	girls	y = 3.1540 x − 1.8459	0.9286
	boys	y = 3.1144 x − 4.0461	0.9461

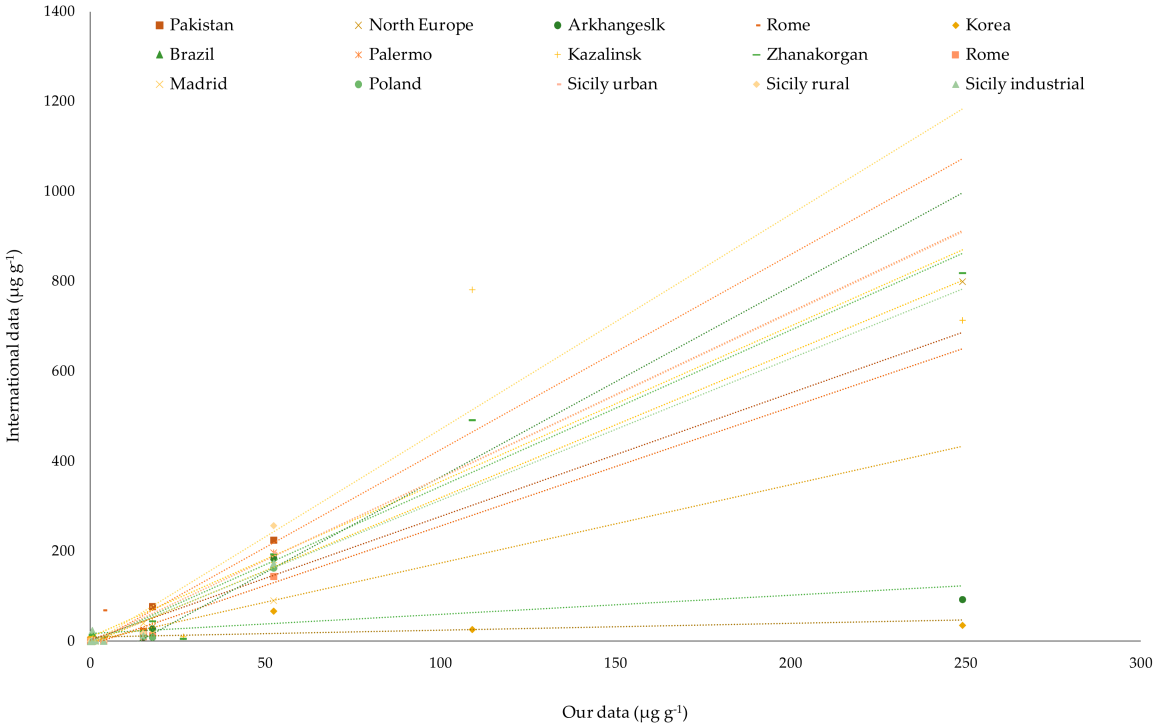

Figure 3. Correlation plots among our data and similar data determined in girl teenagers' hair worldwide [81–91].

4. Conclusions

The data determined in this study are a first tentative study of the element profile both in different hair sections and in different days of a week, also in relationship to the anthropogenic and/or natural sources present in an area, specifically in a suburban area in Central Italy. The element content in the hairs of a girl is compared with studies reporting the relative levels in hairs collected from girl teenagers worldwide: the diverse correlations found with West Europe and East Europe/Central Asia values highlight differences depending on habits, lifestyles, and nutritional diets.

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