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Identification of exposure to toxic metals by means of segmental hair analysis. A case report of alleged chromium intoxication

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Abstract

Hair mineral analysis has become an interesting diagnostic tool in biomonitoring of exposure to toxic elements, in the assessment of health and nutritional status. The most inconvenience of this matrix is the lack of sufficient information to define normal ranges of metal levels in a general healthy population. In this study, segmental hair analysis was used to depict a chronological scheme of exposure to arsenic, cobalt, cadmium, chromium, copper, manganese, nickel and lead in a 16-year-old girl showing signs of potential intoxication. The quantitative results obtained from consecutive segments of hair proved the exposure to chromium. In particular, segment A (0-6 cm), approximately reflecting the last 6 months of exposure, resulted in the chromium level at 5.60 µg/g. The technique of segmental analysis allowed us to establish "intra-individual" physiological variation ranges for each heavy metal hair concentration. As a consequence, these "confidence" intervals could be used as

individualized references to highlight the occurrence of atypical metal levels in any specific hair segment, possibly identifying a period of anomalous exposure and/or intoxication.

Keywords. Metals, Intoxication, Hair, Segmental analysis, Atomic Absorption Spectrophotometry

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1 Introduction

2

3 Hair analysis currently represents a reliable and well-established means of clinical
4 and forensic investigation [1]. The memory property of hair due to sequential
5 accumulation of chemicals in its inner structure, together with the opportunity of
6 conducting retrospective analysis, accounted for its success in several application
7 contexts such as the confirmation of drug-facilitated crimes, the assessment of drug
8 consumption history in addiction treatments, the environmental and occupational
9 exposure to pollutants [1, 2-4]. Also workplace drug testing, driving re-licensing,
10 withdrawal control, **postmortem** toxicology, **prenatal** exposure to drugs, and doping
11 control extensively apply hair analysis for screening and confirmation purposes [5-
12 13]. **Upon investigating** extended time-windows, as opposed to biological fluids, hair
13 analysis **has been recognized as** the most powerful tool in the assessment of chronic
14 consumption or exposure to various chemicals. The relatively constant head hair
15 growth, with an estimated average rate of about 1.0 cm/month, allows to trace the
16 chronological exposure profile by segmental hair analysis [14, 15]. As a matter of
17 fact, segmental hair analysis has been repeatedly used to ascertain occasional abuse
18 of drugs, alcohol and doping agents [1], verify the compliance of enforced abstinence
19 [8], and outline a chronological sequence of drug exposure [16]. Lastly, hair analysis
20 has been used to estimate the nutritional status of individuals and to assess poisoning
21 and environmental intoxication of exposed subjects [17-19] from a variety of organic
22 and inorganic substances, including heavy metals. From another point of view,
23 human hair can be considered as a secondary excretion vehicle of toxic substances
24 from the body. For example, the concentrations of heavy metals in hair are up to 10-
25 times higher than in blood and urine [20-21]. Heavy metals, such as chromium, lead,
26 mercury, cadmium, and arsenic – whenever biologically available - are extremely
27 toxic to most living organisms even at very low concentrations. The presence of
28 heavy metals in human hair usually reflects their bioavailability and therefore is
29 generally found at ultra-trace **levels**. Significant excess of these elements in human

30 hair with respect to the expected population's average may reflect the degree of body
31 exposure to these poisons, either from environmental pollution, workplace or food
32 chain [17,18,22]. Hair mineral analysis has also become an interesting diagnostic tool
33 in biomonitoring the exposure to toxic elements and the health and nutritional status
34 assessment. Likewise organic substances and drugs, trace metal analysis on hair
35 material presents several advantages **over** biological fluids, because it may provide an
36 historical overview on the individual exposure to these elements, recognize acute vs.
37 chronic intoxication, and monitor the nutritional status of the investigated subject
38 over extended periods of time [23]. On the other hand, hair analysis also presents
39 some limitations, including the lack of well-defined and generally accepted reference
40 concentration ranges [23]. This uncertainty arises from the large differences existing
41 in the elements' level as a function of sex, age, residence area, ethnicity, hair color,
42 dietary habits, and individual physiological variability [24]. The present study was
43 addressed to the evaluation of arsenic (As), cobalt (Co), cadmium (Cd), chromium
44 (Cr), copper (Cu), manganese (Mn), nickel (Ni), and lead (Pb) concentrations in the
45 human scalp hair collected from a subject showing signs of potential intoxication,
46 allegedly arising from exposure to toxic metals. Segmental hair analysis was
47 performed to obtain information about the history of the patient's exposure,
48 approximately in the preceding 3 years. Taking into account that (i) considerable
49 interest exists in the toxicological perspectives opened by hair analysis toward the
50 confirmation of heavy metal poisoning, and (ii) insufficient data are available in the
51 literature about heavy metals acceptable **especially for** physiological hair
52 concentrations, we decided to validate the whole analytical method and to establish
53 an independent hair reference range for these metals in **scalp** hair, based on specimen
54 collected from laboratory personnel volunteers ($n=10$). Furthermore, the technique of
55 segmental analysis allowed us to establish "intra-individual" physiological variation
56 ranges for each heavy metal hair concentration. As a consequence, these
57 "confidence" intervals could be used as individualized references to highlight the

58 occurrence of atypical metal levels in any specific hair segment, possibly identifying
59 a period of anomalous exposure and/or intoxication.

60

61 Materials and methods

62

63 Case history

64

65 A 16-year-old girl came to our laboratory to verify the possible past exposure to toxic
66 metals by means of hair analysis. The girl had spent the previous 9 months abroad,
67 where she lived, spent most of her time, and took her meals mostly inside a college.
68 During this period, she presented severe symptoms, including metabolic disorders,
69 skin irritation, nose bleeding, bronchitis and dysentery. Furthermore, recent blood
70 tests evidenced sub-standard levels of glycemia (63 mg/dL) and increased potassium
71 level at 5.94 mEq/L (range 3.5-5.5). The patient's parents suspected that the disorders
72 presented by their daughter were to be attributed to a possible exposure to toxic
73 substances arising either from the environment or the food, not only to the change in
74 eating habits and climatic conditions.

75

76 Sample preparation

77

78 Two locks (diameter approximately 0.5 cm) of patient's hair (color: blond) were
79 sampled in its entire length (about 40 cm) from the vertex region of the head [25],
80 using stainless steel scissors and then stored at room temperature in a plastic box. The
81 hair was segmented as follow: starting from the skull extremity, we considered six
82 segments of 6 cm each and a final segment of 4 cm. The analyzed weight for each
83 segment was in the range 400-500 mg. The hair aliquots were washed to remove
84 potential external contaminations using the method proposed by Ohmori [26],
85 consisting of three consecutive steps with 3 ml each of acetone, water and again

86 acetone. After decontamination, the samples were dried at room temperature
87 overnight. The procedure adopted for hair digestion was based on the method
88 commonly adopted in several literature studies [27-29]. Briefly, the dried hair
89 aliquots were digested with a mixture of 65 % nitric acid (6 mL) and 67-72%
90 perchloric acid (1 mL) at 70-80°C, until the hair was completely dissolved and the
91 solution became clear (about 25 min). Lastly, each sample solution was diluted to 50
92 mL with demineralized Milli-Q water.

93 Standard solutions for calibration were prepared from a 1000 mg/L ICP Multielement
94 solution (Merck, Milan, Italy), ranging from 0.10 to 10 µg/g. (0.50 to 50 µg/g for
95 Cu).

96

97 Instrumentation

98

99 All analyses were performed using a Perkin-Elmer Analyst 800 atomic absorption
100 spectrophotometer (Perkin Elmer, Norwalk, USA) equipped with an AS-800
101 autosampler and THGA graphite tubes with end caps (Perkin-Elmer). The
102 instrumental parameters are described in Table 1.

103

104 Validation of analytical methods

105

106 The characteristic validation parameters for the analytical methods were determined
107 from the analysis of blank water and standard solutions at different concentrations for
108 each metal. These parameters, following the recommendations of ISO/IEC
109 17025:2005 international standard and others guidelines [25,30], included the limit of
110 detection (LOD), limit of quantification (LOQ), linear range, precision (as CV %) and
111 accuracy (as bias %). The linearity interval was evaluated by checking the linear
112 regression coefficient (r^2) of the calibration curve. The linearity was considered
113 acceptable when $r^2 > 0.995$. The linear calibration model was checked by analyzing
114 (two replicates) blank water spiked with the working solution at five concentrations

115 levels in the range of 0.10-10 µg/g (0.50 to 50 µg/g for Cu). The LODs and the LOQs
116 were extrapolated by Hubaux and Vos approach [31]. For all elements, intraday
117 precision (expressed as percent variation coefficient, CV%) and accuracy (expressed
118 as bias %) were evaluated by spiking blank solution at low and high concentration
119 levels at 0.10 µg/g (0.50 µg/g for Cu only) and 10 µg/g (50 µg/g for Cu only).
120 Intraday precision was satisfactory when CV% values were below 15%. Satisfactory
121 accuracy was achieved when the experimentally determined average concentration
122 lied within ±15% from the expected value.

123

124 Results and discussion

125

126 Validation parameters

127

128 All the validation results are reported in Table 2. Each element showed a coefficient
129 of determination higher than 0.995 indicating good fit and linearity for the calibration
130 curves (0.10-10 µg/g for all elements, except for Cu, 0.50-50 µg/g). LOD values
131 ranged from 0.012 µg/g for Pb to 0.35 µg/g for Cu, while LOQ values lied between
132 0.021 for Mn µg/g and 0.70 for Cu µg/g. Intraday precision and accuracy were
133 satisfactory for most but not all analytes. In particular, at the lowest calibration level,
134 accuracy (as bias %) for As, Cd, Cu, and Mn exceeded the accepted values, while at
135 high concentration level (10 µg/g), only Cd exceeded the accepted values. At last,
136 intraday precision (as CV %) exceeding the accepted interval of ±15% was observed
137 for Co, Cr, Mn, and Pb at low concentrations, while modest deviation from 15% was
138 observed at high concentration for As.

139

140

141

142 Analysis of real hair samples

143

144 Physiological reference values and ranges for all heavy metals in hair were
145 determined on ten healthy volunteers, four females and six males. For women's hair,
146 segmental hair analysis was used whenever possible, but no concentration difference
147 was found among the segments arising from the same subject. Table 3 reports the
148 median and reference ranges obtained thereby. Table 3 also reports the expected
149 concentration ranges of trace metals in the hair of healthy subjects, as were
150 determined within a systematic ICP-MS study reported in the literature ($n=45$) [32].
151 These values can be compared with those obtained within the present study from
152 young laboratory personnel hair ($n=10$, six of which were browns, two blondes and
153 two blacks) using electro-thermal atomization AAS. While the lower limits of the
154 reference concentration ranges appear to be similar in the two studies, the same did
155 not apply to the upper limits of Co, Cr, and Mn, that are 4-8 times higher in the
156 present study than in the one previously published [32], despite the lower number of
157 subjects involved in the present study. For these metals, also the median values are
158 appreciably higher. The results obtained on the hair sample from our case in question
159 are summarized in Table 4 and visually represented in Fig. 1. The visual examination
160 of the data obtained from the seven segments reveals that the level of Cr in segment
161 A (0-6 cm), approximately reflecting the last 6 months of exposure, is much higher
162 than in the preceding segments. In fact, segments B-G have an average Cr
163 concentration of $0.74 \pm 0.39 \mu\text{g/g}$; a Student t -test made with the level found in
164 segment A ($5.6 \mu\text{g/g}$) produces a residual probability $\alpha < 10^{-20}$. Analogous t -tests
165 calculated with respect to the reference Cr levels of both the present and Goullé et al.
166 [32] studies (see Table 3) show a highly significant difference for segment A ($\alpha <$
167 10^{-12}). Although no literature data are available on the Cr scalp hair concentration in
168 patients suffering from subchronic Cr intoxication, the exceedingly high Cr
169 concentration found in hair segment A - corresponding to the period that the patient
170 spent abroad - is consistent with the patient's symptoms in that period and the

171 hypothesis of her intoxication by Cr exposure, which can produce the severe health
172 effects actually observed (metabolic disorders, severe skin irritation, and dysentery).
173 Indeed chromium is an essential nutrient in our diet that helps insulin to maintain
174 normal glucose level [33]. **Because** Cr^(III) is poorly absorbed by any route, the toxicity
175 of chromium is mainly attributable to the Cr^(VI) form. It can be absorbed by the lung
176 and gastrointestinal tract, and even to a certain extent by intact skin. **It is known** that
177 **Cr toxicity is commonly** associated with stomach upsets, ulcer, **and kidney and liver**
178 **damages** [17]. Inhaling high levels of Cr can cause irritation to the lining of the nose,
179 nose ulcers and breathing problems. Long term exposure can cause **damages to the**
180 **liver and kidney, and can cause** circulatory and nerve disorders, as well as severe skin
181 irritation [34]. For the remaining metals (As, Cd, Co, Cu, Ni, Pb), the concentrations
182 found in the patient's hair segments are consistently within the expected reference
183 ranges (see **Tables 3 and 4**). Only the Mn level is somehow higher than the published
184 [32] upper reference limit, but this **result is not** considered significant because the Mn
185 hair concentration was found approximately constant throughout the seven segments
186 and lower than the upper reference limit observed in the present study (Table 3).

187

188 Conclusions

189

190 The human exposure to toxic metals is generally monitored by determining their
191 concentrations in conventional body fluids such as blood and urine. Just recently,
192 other non-conventional matrices, in particular scalp hair, are gaining importance in
193 the investigation of possible excessive exposure to toxic metals. Unlike conventional
194 matrices, human scalp hair, and particularly segmental analysis **on long hair**, provide
195 historical and chronological information on trace **element concentrations in the body**,
196 that portrays a unique profiling of exposure. **Compared to the screening for drugs in**
197 **the keratin matrix, the detection of (heavy) metals is less influenced by the**
198 **redistribution along the hair shaft and less affected by washing out effects. Therefore,**
199 **a segmental hair analysis is much more effective in order to obtain an over months**

200 **chronological information about poisoning/exposure with/to heavy metals.** In
201 reporting an interesting real case of alleged intoxication by Cr, the present study
202 demonstrates that segmental hair analysis allows to compare the heavy metal
203 exposure **during** a specific period of time with that **during** other time intervals,
204 possibly corresponding to different external conditions (i.e., different environmental,
205 occupational, or domiciliary exposure, or even deliberate poisoning). This unique
206 intra-individual comparison is referred to the subject under study, and proves to be
207 more specific than any comparison made with a generic reference population. The
208 patient under examination presented severe symptoms possibly associated to heavy
209 metal intoxication, and indeed the concentration of Cr in her hair segment grown
210 during the period when she changed her domicile and living habits was found to be
211 exceedingly high, **as compared with those in** the hair segments grown in preceding
212 time periods. Moreover, the most severe symptoms that she accused (i.e.
213 hypoglycemia, diarrhea and erythema), were consistent with the hypothesis of sub-
214 chronic intoxication by Cr. The present study is likely to contribute to the scientific
215 knowledge about the relationship existing between exposure to toxic metals and their
216 expected concentration in the scalp hair grown in the corresponding period.

217

218 **Conflict of Interest.** The authors declare that they have no conflict of interest.

219 **Ethical approval.** All procedures performed in studies involving human participants
220 were in accordance with the ethical standards of the institutional and/or national
221 research committee and with the 1964 Helsinki declaration and its later amendments
222 or comparable ethical standards. Informed consent was obtained from all individual
223 participants included in the study

224

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229

230 **Figure caption**

231 **Figure 1** Graphical representation of metal concentrations in each segment. The
232 segmentation (cm) is reported on x-axis. The y-scale for Cu was reduced by one order
233 of magnitude

234

235

236

237

References

238

239 1. Kintz P, Salomone A, Vincenti M (2015) Hair analysis in clinical and forensic
240 toxicology. Academic Press/ Elsevier, London

241 2. Appenzeller BMR, Tsatsakis AM (2012) Hair analysis for biomonitoring of
242 environmental and occupational exposure to organic pollutants: state of the art,
243 critical review and future needs. *Toxicol Lett* 210:119-140

244 3. Vincenti M, Salomone A, Gerace E, Pirro V (2013) Application of mass
245 spectrometry to hair analysis for forensic toxicological investigation. *Mass*
246 *Spectrom Rev* 32:312-332

247 4. Vincenti M, Salomone A, Gerace E, Pirro V (2013) Role of LC-MS/MS in hair
248 testing for the determination of common drugs of abuse and other psychoactive
249 drugs. *Bioanalysis* 5:1919-1938

250 5. Pragst F, Balikova MA (2006) State of the art in hair analysis for detection of drug
251 and alcohol abuse. *Clin Chim Acta* 370:17-49

252 6. Cone EJ (2001) Legal, workplace, and treatment drug testing with alternative
253 biological matrices on a global scale. *Forensic Sci Int* 121:7-15

254 7. Caplan YH, Goldberger BA (2001) Alternative specimens for workplace drug
255 testing. *J Anal Toxicol* 25:396-399

256 8. Kintz P, Villain M, Cirimele V (2006) Hair analysis for drug detection. *Ther Drug*
257 *Monit* 28 442-446

258 9. Kintz P (2007) Bioanalytical procedures for detection of chemical agents in hair in
259 the case of drug-facilitated crimes. *Anal Bioanal Chem* 388:1467-1474

260 10. Lozano J, Garcia-Algar O, Vall O, de la Torre R, Scaravelli G, Pichini S (2007)
261 Biological matrices for the evaluation of in utero exposure to drugs of abuse. *Ther*
262 *Drug Monit* 29:711-734

263 11. Barroso M, Dias M, Vieira DN, Lopez-Rivadulla M, Queiroz JA (2012)
264 Simultaneous quantitation of morphine, 6-acetylmorphine, codeine, 6-acetylcodeine

265 and tramadol in hair using mixed-mode solid-phase extraction and gas
266 chromatography–mass spectrometry. *Anal Bioanal Chem* 396:3059–3069

267 12. Gerace E, Salomone A, Pellegrino S, Vincenti M (2012) Evidence of Haldol
268 (haloperidol) long-term intoxication. *Forensic Sci Int* 215:121-123

269 13. Salomone A, Gerace E, Di Corcia D, Martra G, Petrarulo M, Vincenti M (2012)
270 Hair analysis of drugs involved in drug-facilitated sexual assault and detection of
271 zolpidem in a suspected case. *Int J Legal Med* 126:451-459

272 14. Beumer JH, Bosman IJ, Maes RA (2001) Hair as a biological specimen for
273 therapeutic drug monitoring. *Int. J Clin Pract* 55:353-357

274 15. Kintz P, Alvarez JC, Deveaux M, Dumestre V, Gaillard Y, Gaulier JM (2014)
275 Conflicting hair testing results can have an impact in courts: interpretation of single
276 exposure to zolpidem. *J Anal Toxicol* 38:304-305

277 16. Pirro V, Fusari I, Di Corcia D, Gerace E, De Vivo E, Salomone A, Vincenti M
278 (2014) Hair analysis for long-term monitoring of buprenorphine intake in opiates
279 withdrawal. *Ther Drug Monit* 36:796-807

280 17. Onuwa OP, Ishaq SE, Rufus S (2012) Analysis of heavy metals in human hair
281 using atomic absorption spectrometry (AAS). *Am J Anal Chem* 3:770-773

282 18. Randall JA, Gibson RS (1989) Hair chromium as an index of chromium exposure
283 of tannery workers. *Br J Ind Med* 46:171-175

284 19. Momčilović B, Prejac J, Višnjević V, Brundić S, Skalny AA, Mimica N (2016)
285 High hair selenium mother to fetus transfer after the Brazil nuts consumption. *J Trace*
286 *Elem Med Biol* 33:110–113

287 20. Bader M, Dietz MC, Ihrig A, Triebig G (1999) Biomonitoring of manganese in
288 blood, urine and axillary hair following low-dose exposure during the manufacture of
289 dry cell batteries. *Int Arch Occup Environ Health* 72:521-527.

290 21. Omedo P, Pla A, Hernández AF, López-Guarnida O, Rodrigo L, Gil F (2010)
291 Validation of a method to quantify chromium, cadmium, manganese, nickel and lead
292 in human whole blood, urine, saliva and hair samples by electrothermal atomic
293 absorption spectrometry, *Anal Chim Acta*. 659:60-67.

- 294 22. Queipo Abad S, Rodríguez-González P, García Alonso JI (2016) Evidence of the
295 direct adsorption of mercury in human hair during occupational exposure to mercury
296 vapour, *J Trace Elem Med Biol*, 36:16–21
- 297 23. Ozmen H, Akarsu S, Polat F, Cukurovali A (2013) The levels of calcium and
298 magnesium, and of selected trace elements, in whole blood and scalp hair of children
299 with growth retardation. *Iran J Pediatr* 23:125-130
- 300 24. Szykowska MI, Pawlaczyk A, Wojciechowska E, Sypniewski S, Paryjczak T
301 (2009) Human hair as a biomarker in assessing exposure to toxic metals. *Polish J*
302 *Environ Stud* 18:1151-1161
- 303 25. Salomone A, Tsanaclis L, Agius R, Kintz P, Baumgartner MR (2016) European
304 guidelines for workplace drug and alcohol testing in hair. *Drug Test Anal*, doi
305 10.1002/dta.1999
- 306 26. Ohmori S (1984) Hair as a monitor for environmental pollution. Variations in
307 element concentrations for different lead-processing workers and different washing
308 methods of their hair. *J Radioanal Nucl Chem* 84:451-459
- 309 27. Srogi K (2006) Determination of trace elements in the human hair. *Wiadom*
310 *Chem* 3:279-300
- 311 28. Teresa M, Vasconcelos SD, Tavares HM (1997) Trace element concentrations in
312 blood and hair of young apprentices of a technical-professional school. *Sci Total*
313 *Environ* 205:189-199
- 314 29. Chojnacka K, Górecka H, Górecki H (2006) The effect of age, sex, smoking habit
315 and hair color on the composition of hair. *Environ Toxicol Pharmacol* 22:52-57
- 316 30. Society of Forensic Toxicologists/American Academy of Forensic Sciences.
317 (2006) Forensic toxicology laboratory guidelines. Mesa/Colorado Springs
- 318 31. Hubaux A, Vos G (1970) Decision and detection limits for calibration curves.
319 *Anal Chem* 42:849–855
- 320 32. Goullé JP, Mahieu L, Castermant J, Neveu N, Bonneau L, Lainé G, Bouige D,
321 Lacroix C (2005) Metal and metalloid multi-elementary ICP-MS validation in whole
322 blood, plasma, urine and hair: reference values. *Forensic Sci Int* 153:39-44

- 323 33. Schwartz K, Mertz W (1959) Chromium(III) and the glucose tolerance factor.
324 Arch Biochem Biophys 85:292-295
- 325 34. Senofonte O, Violante N, Caroli S (2000) Assessment of reference values for
326 elements in human hair of urban schoolboys. J Trace Elem Med Biol 14:6-13
- 327

Table 1 Experimental parameters used in electro-thermal atomization for atomic absorption spectrophotometry (AAS) analyses.

Parameters	As	Cd	Co	Cr	Cu	Mn	Ni	Pb
Wavelength (nm)	193.7	228.8	240.7	357.9	324.8	279.5	232.0	283.3
Slit-width (nm)	0.7	0.7	0.2	0.7	0.7	0.2	0.2	0.7
Pretreatment temperature (°C)	1200	700	1400	1500	700	900	1500	1900
Atomization temperature (°C)	2000	1900	2450	2300	2250	2400	2300	2450
Sample volume (μL)	20	20	20	20	20	20	20	20

As: arsenic, *Cd*: cadmium, *Co*: cobalt, *Cr*: chromium, *Cu*: copper, *Mn*: manganese, *Ni*: nickel, *Pb*: lead

Table 2 Limits of detection (LODs), limits of quantitation (LOQs), coefficient of determination (r^2), and data of accuracy and precision for determination of As, Cd, Co, Cr, Cu, Mn, Ni, and Pb by electro-thermal atomization AAS

Parameter	As	Cd	Co	Cr	Cu	Mn	Ni	Pb
LOD ($\mu\text{g/g}$)	0.10	0.050	0.10	0.12	0.35	0.021	0.050	0.012
LOQ ($\mu\text{g/g}$)	0.20	0.10	0.20	0.25	0.70	0.042	0.10	0.024
r^2	^a 0.9988	0.9962	0.9988	0.9984	0.9972	0.9999	0.9997	0.9999
Accuracy	^b +37.3	+21.4	+7.4	+15.7	+18.8	+18.2	+1.0	+13.3
(bias %)	^c -0.04	-39.9	+0.07	-2.8	+5.5	-1.9	+3.2	-7.9
Precision	^b 8.9	2.0	21.2	31.1	13.4	22.3	11.0	26.3
(CV%)	^c 16.5	5.4	0.8	2.2	0.6	1.3	4.4	4.2

Limits of Detection and Quantification, Squared Correlation Coefficient, Accuracy and Precision data are reported.

^aThe linearity range tested was 0.10-10 $\mu\text{g/g}$ (0.50 -50 $\mu\text{g/g}$ for Cu)

^bLow concentration 0.10 $\mu\text{g/g}$ (0.50 $\mu\text{g/g}$ for Cu)

^cHigh concentration 10 $\mu\text{g/g}$ (50 $\mu\text{g/g}$ for Cu)

Table 3 Comparison of heavy metal levels in hair obtained from the healthy subjects in the present study with those reported by Goullé et al. [32]

Element	Present study (<i>n</i> =10)		Goullé, J.P. et al. [32] (<i>n</i> =45)	
	Reference range ($\mu\text{g/g}$ or ppm)	Median ($\mu\text{g/g}$ or ppm)	Reference range ($\mu\text{g/g}$ or ppm)	Median ($\mu\text{g/g}$ or ppm)
As	< 0.10	-	0.03-0.08	0.05
Cd	< 0.050	-	0.04-0.17	0.011
Co	0.10-1.1	0.19	0.004-0.14	0.023
Cr	0.12-2.4	0.34	0.11-0.52	0.20
Mn	0.022-3.8	0.35	0.016-0.57	0.067
Ni	0.05-1.9	0.66	0.08-0.90	0.23
Cu	6.5-42	11	9.0-61	20
Pb	0.013-1.4	0.42	0.13-4.6	0.41

Table 4 Analytical results for the case under study. Concentration of trace elements in the scalp hair of the patient are reported as $\mu\text{g/g}$ or ppm.

Segment (cm)		As	Cd	Co	Cr	Ni	Pb	Cu	Mn
A	0-6	< 0.10	< 0.10	< 0.10	5.6	0.29	0.38	16	1.1
B	6-12	< 0.10	< 0.10	< 0.10	0.26	0.15	0.32	17	1.1
C	12-18	< 0.10	0.26	< 0.10	1.1	0.26	0.31	17	1.1
D	18-24	< 0.10	0.15	0.29	0.55	0.37	0.56	19	1.8
E	24-30	< 0.10	0.25	0.22	0.39	0.94	0.77	23	1.7
F	30-36	< 0.10	0.25	< 0.10	1.1	0.57	1.6	27	1.9
G	36-40	< 0.10	0.52	< 0.10	1.1	0.52	1.8	25	2.0

Figure 1

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