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Endogenous and Exogenous Elemental Speciation in Human Hair by Various Washing Procedures and Determination by Flame and Electrothermal Atomic Absorption Spectrometry

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Human hair is a suitable biomarker for determination of trace elements absorbed into the body. However, it is necessary to differentiate between the part which is adsorbed on the hair from environment (exogenous) and that part originated from the body of the host (endogenous). Hence to remove exogenous elements the hair sample must be washed before digestion. In the present work, scalp human hair was exposed to sweat, spiked with certain trace elements and then washed with different solutions to desorb the adsorbed elements. Investigations of 10 various washing procedures to remove external contaminants show that in exposed hair samples cadmium, cobalt, copper, iron, nickel and zinc are significantly removed from hair using a 1 % (w/v) sodium diethyl-dithiocarbamate (DDTC) wash, with 77, 35, 79, 40, 41 and 70 %, respectively being washed-off. The removal of nickel and zinc from exposed hair is, however, more efficient with 0.1M HCl, with 65 and 78 % of each element, respectively being washed-off. After determination of the desorbed elements, the washed hair samples were digested in a mixture of HNO_3 and H_2O_2, for the determination of endogenous elements. Both the exogenous and endogenous elements were determined by ELAAS or FAAS. In order to show the effective removal of adsorbed elements, the trace elements content of hairs of two groups of people were determined, living in a village and our test group were mine workers.

Key Words: Hair analysis, FAAS, Trace elements, Exogenous, Endogenous.

INTRODUCTION

A useful classification of the elements, according to their importance to living organisms is into essential and non-essential categories. Essential trace elements are Co, Cu, Cr, Fe, Mn, Mo, Ni, Se, Si,V and Zn whereas Al, As, Cd, Sb, Sn, Pt, Hg, Pb, Bi, *etc.* are considered potentially toxic trace elements[1].

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It is recognized that such trace element are involved in biogeochemical cycles, as well as in anthropogenic activities that cause their mobilization, resulting in environmental pollution and human exposure. Since essential trace elements are clearly implicated in numerous biochemical processes, there is an increasing interest in the study of their health effects.

Trace element analysis on hair samples has been widely used to assess wildlife and human exposure to different contaminants present in the environment or at the work place[2,3]. Several advantages were mentioned for the use of this biological material in monitoring studies, namely: 1) the less invasive character of hair collection procedures that avoid venin-pucture; 2) the stability of hair, as a biological material, which facilitates the storage and transport processes; 3) the higher concentrations of residues usually found in hair samples, when compared to those on blood and urine and 4) the capacity of hair to accumulate metals during extended periods, reflecting by this way, at least 1 year of exposure[4,5].

However, before trace element levels in scalp hair become an acceptable indicator of exposure, normal values have to be established, along with the standardization of sample collection and sample preparation. In addition, trace elements incorporated in human hair are classified with respect to their origin: i) elements originated from the body of the host (endogenous) and ii) those from the external environment (exogenous)[5,6]. This external contamination complicates the interpretation of the results of hair analysis making it difficult to determine endogenous elemental levels. The aim of hair washing is to remove externally bound contaminants and thus, allow the determination of internally bound analytes. Up to this time there are no agreed washing procedure for hair, instead it is reflected in different recommendations[7-9].

A number of studies have commented that there is a need for more standardized methodology, reference ranges and a correlation between element ranges in hair and other matrices. In order to make hair analysis a reliable and reproducible technique, certain aspects such as sample pre-treatment and analytical procedures need to be investigated and standardized[8,10,11].

The international atomic energy agency (IAEA) method for washing hair involves sequential washing with acetone, water and acetone again. The IAEA protocol recommends that the hair must be soaked completely in washing solvent with constant stirring. The hair is washed successively with reagent grade acetone, 3 times in high purity deionized water and once more with acetone. The sample should then be allowed to stand at room temperature or oven-dried. This method is probably adequate for the removal of dirt and dust, but there is no evidence that it will remove exogenously bound trace elements from the hair[12,14].

A number of studies have used the IAEA method as a matter of routine[3,15-19], however, others have departed from this protocol and have used different organic solvents, such as isopropanol or ethyl ether. It has also been argued that detergent and organic washes can diminish the lipid content of hair by up to 10 % of the original content[20].

Other methods use shampoos[21], ionic surfactants such as sodium dodecyl hydrogen sulphate (SDS)[22], non-ionic surfactants such as octyl phenoxy polyethoxyethanol (Triton-X-100), wash with an acid such as hydrochloric acid or nitric acid[8] and wash with strong complexing agents such as EDTA[11,23]. One such investigation of hair washing found that 0.1 M HCl could remove 87, 73 and 5 % of cadmium, lead and mercury, respectively[22].

The main goal of this study was to develop a procedure for hair pre-treatment and analysis that can be used for determination of cadmium, cobalt, copper, iron, nickel and zinc. Investigation of different washing solutions were carried out to find out to remove exogenously bound contaminants from the hair and thus allow the endogenous trace element content to be determined.

The washing solutions investigated in this study included the use of acetone, SDS, isopropanol, EDTA, sodium diethyldithiocarbamate (DDTC), 8-hydroxyquinoline (oxine), Triton-X-100, dilute nitric acid, dilute hydrochloric acid and dilute ammonium hydroxide. The optimized hair washing procedures were used to determine the levels of trace elements in the hair of non-exposed persons and occupationally exposed mine workers.

EXPERIMENTAL

All determinations were carried out using a Varian model spectra AA-220 atomic absorption spectrometer equipped with a GTA-100 graphite furnace atomizer, deuterium lamp as a background corrector and a Varian programmable sample dispenser. Details of the operating conditions are summarized in Table-1. A mechanical shaker platform used to shake the samples.

TABLE-1
SPECTROMETER OPERATING CONDITIONS AND TECHNIQUES

Elements	Wavelength (nm)	Spectral bandwidth (nm)	Lamp current (mA)	Technique
Cd	228.8	0.5	4.0	ETAAS
Co	240.7	0.2	7.0	ETAAS
Cu	324.8	0.5	4.0	FAAS
Fe	248.3	0.2	5.0	FAAS
Ni	232.0	0.2	4.0	ETAAS
Zn	213.9	1.0	5.0	FAAS

EAAS = Electrothermal atomic absorption spectrometry; FAAS = Flame atomic absorption spectrometry.

Suprapure Merck HNO_3, H_2O_2 and HCl were used in the treatment of hair samples and preparation of standard solutions. All reagents used in the present study were analytical grade and deionized water of high purity (from a Millipore ultra pure water system). Cd, Co, Ni and Zn stock standard solutions at a concentration of 1.000 g L^{-1} were prepared from cadmium nitrate 4-hydrate, cobalt nitrate 6-hydrate, nickel nitrate 6-hydrate and zinc nitrate 4-hydrate, respectively (Merck). Copper and iron stock standard solutions at a concentration of 1.000 g L^{-1} were prepared from copper and iron metal strip, respectively (Merck). Additional chemicals used in the washing procedures were EDTA and oxine, HPLC grade acetone and isopropanol, Triton-X-100 and ammonium hydroxide from Merck. SDS and DDTC from Fluka were also used.

The simulated sweat solution was composed of calcium sulphate, sodium chloride, potassium sulphate, sulphur and urea (Merck) dissolved in deionized water.

Glassware, nalgene bottles and sealed plastic bags were all cleaned in 5 % (v/v) nitric acid before use.

Because certified reference materials for hair were not available for us, the validation of outlined procedure was performed by analysis of spiked samples. For this purpose, appropriate amounts of metal standard solutions were added to hair samples before digestion. Average recovery from 5 spiked hair samples were 96 % for Cd and Cu, 92 % for Co, 102 % for Fe, 99 % for Zn and 101 % for Ni.

Sample collection and hair washing methods: A control group of natural hair samples were collected from Tehran and Takab province (Northwest of Iran). Individuals were invited to answer a small questionnaire designed to obtain information about age, sex, food ingestion (home grown fruits and vegetables, fish and animals, breeding cattle and canned food), source of drinking water, smoking habits, alcohol consumption, health condition, medication and workplace. The occupational samples from the zinc mine workers were cut from the nape of the neck with stainless steel scissors. All samples were stored individually in sealed plastic bags at room temperature.

Ten different washing solutions were investigated. The data obtained for metal content of washed hair compared to those from unwashed hair samples. The washing solutions were 1 % (w/v) SDS, 1 % (v/v) Triton-X-100, 1 % (w/v) EDTA, 1 % (w/v) DDTC, isopropanol, 0.1 M HNO_3, 0.1 M HCl, 1 % (w/v) oxine and 0.1 M NH_4OH. The IAEA procedure was also tested.

To optimise the washing procedure, each hair sample was weighed directly into a conical sample tube, 25 mL of the washing solution was added and the tube capped. The container was shaken on a mechanical shaker for 4 h at 20 °C. Then the supernatant was pipetted-off and kept for further analysis. The residue was treated 2 time, each time with 40 mL of deionized water, shaken for 4 h and the water was pipetted-off. The hair sample was oven- dried at 70 ° C for 8 h and then digested the next day.

Hair digestion procedure: All washed hair samples were digested by weighing the sample directly (about 1 g) into teflon bombs and 10 mL of concentrated HNO_3 (65 %) was added. The samples were heated on a hot plate (initially at 80 °C for 1 h and then at 100 °C for 45 min). After dissolution, the solution was allowed to cool and 5 mL of H_2O_2 (30 %) was added. The mixture was heated (at 130 °C for 2 h).

After digestion, the sample was diluted to final volume with deionized water. Standard solution of each element was prepared in a mixture of HNO_3, H_2O_2 and deionized water. Detections were carried out using FAAS or ETAAS.

Simulated sweat experiment: Twelve portions of the same hair sample (each *ca.* 1 g) were weighed and each one transferred to a wide mouth bottle. To each bottle 25 mL of a mixed spike solution of Cd, Cu, Fe, Ni and Zn added. The concentration of each element in the spike solution was 0.5 ppm dissolved in sweat solution. The bottles were capped and shaken for 1 h, then left for 24 h, after which the spiked sweat solution was decanted and the hair samples were left to dry overnight. Ten out of 12 samples were washed with washing solution; two sample with each solution, 1 % (w/v) DDTC, 1 % (w/v) oxine, with 1 % (w/v) EDTA, 1 % (w/v) SDS 0.1 M HCl. The rest were left unwashed. All samples were then digested and analyzed by FAAS or ETAAS.

RESULTS AND DISCUSSION

Preliminary study of 10 washing solutions: Ten washing solutions were investigated. The results are listed in Table-2. Statistical paired t-tests were carried out between all the results of washed and unwashed hair samples and were used at 95 % confidence intervals. This was to determine whether a significant amount of the trace element was removed from the hair when washed with a solution or not. Initial studies of the various washing solutions showed that acetone did not remove most elements from hair samples. Although isopropanol could be used instead of acetone, but the result was almost the same as acetone. Triton X-100 and 0.1 M nitric acid could not significantly remove most of the elements from the hair matrix.

Inspection of the cadmium levels listed in Table-1 indicates that, cadmium could be removed by all washing solutions except NH_4OH. Iron could be efficiently removed by all tested solutions except 0.1 M HNO_3. About 25, 22 and 19 % of iron was removed by solutions 1 % (w/v) SDS, 1 % (w/v) DDTC and 1 % (w/v) EDTA, respectively. In contrast, cobalt was not removed significantly from the hair by any of the washing solution at this stage.

Paired t-tests confirmed that there were significant differences between the copper levels of unwashed hair and hair washed with solutions of 1 % (w/v) DDTC or 1 % (w/v) oxine. About 29 % of Ni was removed with HCl. An increase in nickel concentrations was observed when the hair was washed in 0.1 M NH_4OH, possibly because of the contamination by NH_4OH (analysis of the ammonium hydroxide used in these experiments showed considerable concentrations of magnesium and nickel).

Inspection of the zinc levels indicates that up to 32 % (with 0.1 M HCl) of the zinc was removed. There is also significant difference between the zinc levels in the unwashed hair and those in the hair washed with 0.1 M NH_4OH, 1 % (w/v) oxine, or 1 % (w/v) DDTC.

These preliminary results showed that hair washing can decrease the concentrations of zinc, nickel, cadmium and iron after being washed with 0.1 M HCl. Because of the solubility of the above metals chlorides and their formation in contact with dilute HCl, they could be removed significantly. Nickel and most of the elements

TABLE-2
COMPARISON OF EFFICIENCY OF TEN WASHING SOLUTIONS BY THE AMOUNT OF EACH ELEMENTS CONCENTRATION

Element	Elements concentrations (µg g^{1})										
	Un-washed	Washed in acetone	Washed in EDTA	Washed in DDTC	Washed in Oxine	Washed in SDS	Washed in HCl	Washed in HNO$_3$	Washed in NH$_4$OH	Washed in Triton	Washed in Iso-propanol
Cadmium											
Hair 1	0.70	0.67	0.50	0.55	0.60	0.68	0.59	0.65	0.77	0.62	0.66
Hair 2	1.14	0.98	0.79	0.88	0.94	0.88	0.90	0.98	1.09	1.02	0.90
Hair 3	1.31	1.12	1.00	1.04	1.04	1.03	1.03	1.17	1.29	1.19	1.08
Cobalt											
Hair 1	2.96	2.86	2.31	2.85	2.52	2.76	2.76	2.94	3.00	3.08	2.85
Hair 2	6.90	6.86	4.75	4.48	5.15	5.70	6.45	6.92	5.25	6.49	6..51
Hair 3	2.29	2.25	1.86	2.02	2.91	2.02	2.21	2.28	1.98	2.13	2.14
Copper											
Hair 1	10.42	8.82	8.25	7.59	7.74	10.30	8.31	6.06	10.81	9.84	11.13
Hair 2	7.35	7.07	6.35	6.00	5.95	7.55	6.28	5.80	7.40	7.30	7.25
Hair 3	10.54	10.18	10.46	8.54	8.80	10.43	9.90	8.73	11.12	9.35	9.65
Iron											
Hair 1	14.68	11.52	11.87	11.52	12.25	10.56	11.75	15.88	12.66	12.36	12.25
Hair 2	12.75	12.58	10.38	9.08	10.90	9.65	11.39	14.01	11.99	10.90	11.75
Hair 3	10.26	9.18	8.09	8.54	9.31	7.94	8.35	9.73	9.31	10.26	9.44
Nickel											
Hair 1	2.65	2.52	1.96	1.65	2.27	3.05	2.06	2.49	2.70	2.36	2.16
Hair 2	1.55	1.43	1.12	1.00	1.34	1.27	1.00	1.12	2.12	1.25	0.93
Hair 3	4.00	4.02	2.93	3.42	3.19	3.91	3.21	4.90	3.89	3.36	2.25
Zinc											
Hair 1	187.30	149.29	132.02	139.01	149.28	171.15	114.71	144.13	105.95	187.35	185.62
Hair 2	132.77	123.50	109.54	109.54	106.48	135.69	95.02	128.88	89.34	115.44	109.09
Hair 3	161.56	158.00	100.82	134.84	135.96	151.41	124.09	141.58	124.09	175.97	136.92

were also removed when the hairs were washed with EDTA, DDTC and oxine, possibly as a result of complex formation. Hence, the 4 mentioned solutions and SDS were chosen for further investigations.

Removal of exogenously bound elements from unexposed hair samples: Ten different hair samples were chosen. Each sample was divided into 6 portions. The first portion was not washed. The remaining each hair sample washed with one solution namely 1 % (w/v) DDTC, 1 % (w/v) EDTA, 1 % (w/v) oxine, 1 % (w/v) SDS and 0.1 M HCl, respectively. Another 5 hair samples were chosen and each divided into two portions. The first portion was not washed and the second was washed with 1 % (w/v) DDTC. All samples were digested and the trace elements concentrations determined by FAAS. The results are tabulated in Table-3. Paired t-tests were used to establish whether the differences between the results of washed and unwashed samples were statistically significant or not.

TABLE-3
TRACE ELEMENT CONCENTRATIONS DETERMINED IN THE NORMAL GROUP HAIR SAMPLES AFTER WASHING WITH DIFFERENT SOLUTIONS

Element	Unwashed [a] (μg g^{-1}; n = 15[b])	Washed in EDTA (μg g^{-1}; n = 10)	Washed in DDTC (μg g^{-1}; n =15)	Washed in Oxine (μg g^{-1}; n = 10)	Washed in HCl (μg g^{-1}; n = 10)	Washed in SDS (μg g^{-1}; n = 10)
Cd	1.28 [0.48-2.48]	1.14 [0.51-1.99]	0.98 [0.38-2.18]	1.31 [0.46-2.19]	1.34 [0.59-2.10]	1.22 [0.50-1.96]
Co	1.94 [0.57-4.98]	2.02 [0.61-3.24]	1.58 [0.49-4.18]	2.26 [0.84-4.01]	2.39 [0.75-4.48]	2.33 [0.78-4.01]
Cu	8.66 [4.75-17.22]	7.57 [4.36-12.75]	6.69 [2.37-13.32]	6.97 [3.99-12.22]	8.16 [4.86-14.74]	8.40 [4.10-16.86]
Fe	15.66 [8.28-21.75]	12.82 [7.36-16.52]	12.36 [7.57-17.67]	11.93 [6.24-15.98]	13.73 [8.53-18.47]	12.41 [6.73-18.95]
Ni	1.36 [0.15-3.87]	1.13 [0.61-2.25]	1.00 [0.15-2.58]	1.63 [0.62-3.87]	1.23 [0.57-1.98]	1.62 [0.63-3.98]
Zn	114.13 [53.30-196.49]	99.08 [66.89-166.15]	92.80 [52.05-178.41]	93.23 [44.12-147.37]	94.09 [50.63-152.66]	119.41 [70.25-178.41]

[a] Mean value in bold and the range of values in square brackets; [b] Number of replicate determination.

The cadmium levels were significantly reduced when hair was washed with 1 % (w/v) EDTA, 1 % (w/v) DDTC, 1 % (w/v) SDS, 1 % (w/v) oxine and 0.1 M HCl, which is in agreement with the preliminary study. The cobalt levels in washed hair samples, washed with 1 % (w/v) EDTA and 1 % (w/v) DDTC did show a decrease of *ca.* 28 and 19 %, respectively, which were statistically significant. This is in contrast to the data obtained in the preliminary study and is most likely as a result of inhomogeneous samples.

It was revealed that nickel could be washed by 0.1 M HCl, 1 % (w/v) EDTA or 1 % (w/v) DDTC. Copper was removed significantly from hair by washing with 1 % (w/v) DDTC which equates well to the data obtained in the preliminary test.

Paired t-tests confirmed that there were also significant differences between the iron levels in the unwashed hair and those in the hair washed with 1 % (w/v) DDTC, 1 % (w/v) EDTA, 1 % (w/v) SDS, 1 % (w/v) oxine and 0.1 M HCl. The removal of iron as a result of hair washing with HCl is not in agreement with the obtained data in the preliminary study, which indicate that the hair samples used in this study had little exogenously bound iron. Zinc was significantly removed from the hair when the samples were washed with 0.1 M HCl, 1 % (w/v) EDTA or 1 % (w/v) oxine.

The amount of each element determined in the 15 tested samples exhibit a wide range of concentration and hence need a larger control group to ensure the representative average values.

It has previously been reported that EDTA and HCl can leach chromium and cadmium from hair samples[15,22]. The use of DDTC, EDTA and HCl for removal of copper, cobalt, iron, nickel and zinc have not been reported previously, in the literature according to the best of our knowledge.

Washing spiked samples: Beside the use of the mentioned solutions for washing hair samples to differentiate between the exogenously and endogenously bound elements, spiked hair samples were also investigated. Since hair is in contact with sweat, the spiked solutions were prepared in a simulated human sweat for use in this experiment. The pH of the simulated sweat solution used in this study was 6.5. The results obtained from the simulated sweat spikes samples are tabulated in Table-4.

TABLE-4
ELEMENTAL CONCENTRATIONS DETERMINED IN HAIR SAMPLES SPIKED WITH A STANDARD OF ELEMENTS IN SWEAT SOLUTION AND WASHED WITH FIVE SOLUTIONS, 1 % (w/v) EDTA 1% (w/v) DDTC, 1 % (w/v) OXINE, 0.1 M HCl AND 1 % (w/v) SDS, RESPECTIVELY

Method	Cd	Co	Cu	Fe	Ni	Zn
Concentrations determined in unwashed, unspiked hair (μg g^{-1})	0.70	2.96	10.42	14.68	1.78	187.30
Concentrations determined in spiked hair (μg g^{-1})	78.81	10.69	35.64	56.72	6.10	1250.79
Concentrations determined in hair spiked and washed EDTA (μg g^{-1})	4.64	7.16	19.98	17.37	1.78	390.12
Concentrations determined in hair spiked and washed DDTC (μg g^{-1})	1.95	6.90	11.15	15.10	3.28	698.45
Concentrations determined in hair spiked and washed oxine (μg g^{-1})	14.50	8.20	21.40	14.88	2.65	582.94
Concentrations determined in hair spiked and washed HCl (μg g^{-1})	2.10	9.06	18.24	19.34	2.14	214.82
Concentrations determined in hair spiked and washed SDS (μg g^{-1})	16.19	10.88	26.46	15.62	5.29	862.50

Table-4 indicates that all mentioned elements adsorb to the hair strongly. The adsorbed cadmium was removed completely when washed with 1 % (w/v) DDTC, 0.1 M HCl or 1 % (w/v) EDTA (over 98, 97 and 94 %, respectively, of the spiked amount). Copper was partially washed-off, that means the amount of it was reduced by 69 % when washed with 1 % (w/v)DDTC solution. Almost 33 % of the adsorbed cobalt washed-off by 1 % (w/v) EDTA or 1 % (w/v)DDTC solution.

Iron and zinc were also removed by all 5 washing solutions. The best washing solution for zinc is HCl that reduces the amount of adsorbed zinc by 83 % average.

In a similar experiment carried out in 2002, it was found that exogenously bound chromium, cadmium and lead were removed after washing with 0.1 M HCl[22]. The result for cadmium is in agreement with those of this study.

This study showed that it is possible to differentiate between exogenously and endogenously bound cadmium, copper, iron, nickel and zinc. Also experiments with simulated sweat spiked with each of these elements show that exogenously bound elements are removed after washing with 1 % (w/v) DDTC or 0.1 M HCl.

Occupational zinc samples: It is observed that the cadmium, cobalt, copper, iron, nickel and zinc can be removed from unexposed hair samples by washing with 1 % (w/v) DDTC. It seemed necessary to carry out similar experiments on hair samples where the hair has been exposed to heavy metals. This would also gives more reliable results compared to the case when endogenous elemental concentrations of exposed hairs are determined. Ten hair samples were collected from workers of zinc mine located in Takab province which has a cold climate even during summer times. The normal group hair samples were collected from the city of Takab. The hair samples were cut from the nape of the neck and placed directly into a plastic sample bag. The samples were washed with 0.1 M HCl or 1 % (w/v) DDTC for determination of the trace elements.

The mean value of the unwashed hair zinc level for the concentration operator worker and stone breaker operator were 1606.20 and 1289.32 µg g^{-1} compared with 506.77 and 309.92 µg g^{-1} for laboratory technician and manager, respectively. These results are significantly higher than the normal group average 114.10 µg g^{-1}. In this study, the concentrations of the zinc in the hairs of all workers were well above maximum level of the normal group which was 196 µg g^{-1}. However, after washing the samples with 1 % (w/v) DDTC or 0.1 M HCl the hair zinc levels were significantly reduced.

This is an indication of external contamination with the average (unwashed) hair zinc concentration being over 1081.99 µg g^{-1}. When hair samples of the zinc mine workers were washed with 0.1 M HCl, it was found that 78 % of the zinc was removed. This could suggest that the remaining 22 % of it is due to endogenous zinc in the hair.

All the other elements of interest in this study were also determined in the zinc mine workers hair samples. The determined values and the percentages of species removed by 1 % (w/v) DDTC solution were compared with those obtained for normal group shown in Table-5. The results indicate that the level of all elements are higher in the workers hairs than the levels of these elements in the normal group hairs. Two-tailed t-tests of the mean values of the elements in the zinc mine workers' hair and the mean values of the normal group confirmed that the levels of zinc, copper, cobalt, cadmium, iron and nickel differed significantly at the 95 % confidence limit. These results suggest that the zinc mine workers are exposed to higher concentrations of zinc, copper, cadmium, iron and nickel than the unexposed subjects and that this is reflected in both the average levels found and the percentage of the elements removed by washing.

Conclusion

Hair is one of the best biomarkers for speciation of trace elements entering the body. Analysis of hair after washing it with a kind of solvent or detergent dose not provides accurate results. Washing hair with certain solutions to remove external contaminants indicated that, "it is possible to differentiate between exogenously

TABLE-5
COMPARISON OF THE TOTAL CONCENTRATIONS OF Cd, Cr, Co, Cu, Fe, Mg, Mn, Ni, Mo AND Zn IN THE ZINC MINE WORKERS' HAIR COMPARED WITH LEVELS IN THE NORMAL GROUP AND THE PERCENTAGE REMOVED WHEN WASHED WITH 1 % (w/v) DDTC OF ELEMENTS OF INTEREST

Element	Element concentration in the zinc mine workers[a] (μg g^{-1}; n = 10 [b])	Percentage of element removed with the DDTC wash (n = 10)	Element concentration in the normal group (μg g^{-1}; n =15)	Percentage of element removed with the DDTC wash (n = 15)
Cadmium	6.02 [1.10-22.17]	77	1.28 [0.48-2.48]	28
Cobalt	1.96 [0.60-4.56]	30	1.94 [0.57-4.98]	19
Copper	46.66 [19.10-85.63]	79	8.66 [4.75-17.22]	21
Iron	44.77 [17.62-102.47]	40	15.66 [8.28-21.75]	20
Nickel	3.58 [1.57-6.12]	41	1.36 [0.15-3.87]	15
Zinc	1081.99 [293.45-2324.58]	59	114.13 [53.30-196.49]	16

[a] Mean value in bold and the range of values in square brackets.
[b] Number of replicate determination.

and endogenously bound species of cadmium, cobalt, copper, iron, nickel and zinc". A solution of 1 % (w/v) DDTC desorbs all the above elements which had been spiked onto the hair. Zinc is also removed almost completely with HCl.

Hair analysis of exposed workers showed that washing the hair removed a considerable amount of the elements present, more so than in the unexposed hair samples. Further studies are needed to establish the relationship between levels of elements in washed hair and serum or total body values.

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