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## Hair As An Indicator Of Trace Elements Bioaccumulation In Human Body: Optimization Of A Washing Procedure



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### ABSTRACT

For many years, hair analysis has played an important role in the monitoring of toxic risks. Concentrations of trace elements in hair reflect their mean level in the human body. At the beginning of the XXth century, the use of hair was limited to forensic toxicology, for instance, to detect arsenical intoxications. Thereafter, its application was extended to other fields, such as anthropology, archaeology and history. Thus, interesting a posteriori revelations on the possible toxic etiology of the deaths of certain famous characters have been made. Measuring the concentrations of harmful trace elements (Pb, Cd and Hg) in hair can be used to monitor a population's exposure to pollution or to estimate intake from contaminated foodstuffs. A further application is the biomonitoring of personnel occupationally exposed to heavy metals. To ensure the significance and reliability of results, the quality of the analytical procedure is paramount. Indeed, when measuring trace element levels in hair only the internal (endogenous) fraction should be taken into account, hence, the importance of the washing procedure used prior to analysis. The objective of this study was to test the effectiveness and aggressiveness of seven washing procedures to compare removal of endogenous elements and external contaminants. The procedure finally adopted was the one that most effectively removed all external contaminants without being too aggressive and removing the elements incorporated into the hair matrix during its growth, as this fraction alone reflects the accumulation of trace elements in the organism.

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### KEYWORDS

Washing procedure;  
Trace elements;  
Effectiveness;  
Aggressiveness.

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## INTRODUCTION

In toxicological studies, various biological indicators such as blood, urine, nails, milk can be used to estimate the rate of accumulation of trace metals in the human organism, an indication that can only be made more accurate by measurement in blood.

The difficulties of collecting and preserving blood complicate this method and make it impractical in many cases.

The current approach is to use a biological material which is easy to collect and to preserve hair<sup>[13,15-18,27]</sup>. Mineral analysis of hair is an inexpensive, non-invasive technique, which makes it possible to detect and quantify the presence of toxic metals (such as mercury, lead, cadmium, arsenic) in the human organism and thus reveal any exposure to these pollutants<sup>[14,34]</sup>. The analysis of other elements (such as calcium, magnesium, sodium) makes it possible to appreciate, prevent and overcome possible health problems and to highlight the use of particular medicines<sup>[12,19,34]</sup>.

Hair precisely reflects the mineral content of the body accumulated over recent months, rather than an analysis of blood or urine which reflects mineral content at the time of sampling. Furthermore, hair is easily transportable (by post) compared to blood which is often too problematic to transport.

However, the measurement of heavy metals in hair is not without its own inherent problems. In fact, analysis must only take into account the internal (endogenous) fraction<sup>[12,27]</sup> which highlights the importance of removing the external (exogenous) contaminant fraction coming from the metal-rich dust deposited on the hair<sup>[27]</sup>. To adopt hair as a reliable and reproducible means for the determination of trace metals in the human organism as a reflection of their accumulation and thus of the level of exposure of the organism to them, certain aspects such as sample preprocessing and analytical procedure have been studied<sup>[1,12,20,25,37]</sup>.

Authors emphasize the importance of the hair-washing procedure used prior to trace element analysis. The objective of this study was to compare a number of washing procedures to select the most effective method in fully or almost fully removing

the trace elements of external origin, that is, those settling on the hair, without effecting the hair matrix and removing the internal fraction which alone reflects trace metal accumulation in the organism.

## MATERIAL

Throughout this study, 5ml graduated polystyrene crystal tubes, with polyethylene corks were used<sup>[27,30]</sup>.

Prior to use, all tubes and corks were cleaned by immersion in 10% HCl for 24h, thoroughly rinsed with distilled water and then dried safe from dust in a desiccator on calcium chloride<sup>[27]</sup>.

### Preparation of samples

#### Contamination

This phase initially consisted in simulating voluntary artificial contamination of the hair<sup>[2,5,21,30]</sup>; elimination by the tested washing procedures was then measured. To this end, the hair<sup>[30]</sup> was completely immersed for 24h in a solution of eight elements (Al, As, Cd, Cu, Ni, Pb, and Zn), 1% each, in demineralized water of pH=6.3<sup>[27]</sup>. All tests were conducted on a homogeneous pool of hair. Each treatment was repeated six times.

A pool of non-contaminated hair served as a control for the different hair-washing methods.

The goal of this study was to test several hair-washing procedures intended for eliminating deposited metals<sup>[1,11,20,25,27]</sup>.

Washing with acetone (IAEA): this procedure involved sequential washing of hair samples 3x with acetone, 3x with water and finally 3x with acetone. During this process, the hair must be completely immersed in the decontamination solution<sup>[21,25,30]</sup>. Several studies have used this washing procedure as a routine method<sup>[6,8,36,30]</sup>. Others have used procedures with organic solvents such as isopropanol<sup>[10,23]</sup>. Some studies have shown that this method may reduce the original hair lipid content by more than 10%<sup>[33]</sup>. It is considered the most efficient method to free hair of the contaminants and dust which settle on it.

Further methods use ionic surfactants such as sodium-lauryl-sulphate (SLS)<sup>[23,26]</sup>, nonionic surfactants<sup>[1,28,32]</sup> such as triton X-100<sup>[1,8,22,29]</sup>, acids such as hydrochloric acid 1%<sup>[1,3,11,30]</sup> or nitric acid 1%<sup>[1,30]</sup>. A

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combination of different washing procedures may be preferred, such as organic washing followed by washing with a surfactant<sup>[7,14,31,35]</sup> or even a combination of a chemical method and a physical method (sonification, agitation)<sup>[37]</sup>.

The procedure finally adopted was the one that most effectively removed all external contaminants without being too aggressive and removing the elements incorporated into the hair matrix during its growth, as this fraction only reflects the accumulation of trace elements in the organism<sup>[21]</sup>.

### Digestion

A precisely weighed 50 mg<sup>[27]</sup> test specimen of hair (contaminated or non-contaminated) was carefully washed and dried at constant temperature to constant weight. This specimen was introduced into a polystyrene tube, and 1ml nitric acid 8N was added<sup>[21,24]</sup>.

The corked tube was preserved at ambient temperature for 24h. During this preliminary phase, most of the sample dissolved in the acid. The liquid recovered was yellow, more or less dark, depending on hair color<sup>[9]</sup> and had a viscous consistency. For per-

fect dissolution, the corked tubes were placed for 1h in a boiler at 60°C<sup>[21]</sup>. Corks were maintained in place by pressure (plate plus weight). The result was a clear, yellowish liquid.

### Analysis

The recovered liquids were diluted in a suitable amount of demineralized water for trace element analysis.

Trace metal concentrations were determined by flame atomic absorption spectroscopy for nickel and zinc, and by graphite furnace atomic absorption spectroscopy for aluminium, arsenic, cadmium, copper, lead and selenium. Metal concentrations were expressed as mean individual values  $\pm$  standard deviation.

## RESULTS AND DISCUSSION

### Study of effectiveness

TABLE 1 shows the concentrations of trace elements in contaminated and non-contaminated hair before and after washing with seven different methods:

**TABLE 1: Comparison of the concentrations of eight trace elements (Al, As, Cd, Cu, Ni, Pb, and Zn) in contaminated (C) and non-contaminated hair (UC)**

| Washing         | Direct | IAEA               | HNO <sub>3</sub><br>(1%) (3x) | HNO <sub>3</sub><br>(10%) (3x) | HNO <sub>3</sub><br>(1%) (4x) | HNO <sub>3</sub><br>(10%) (4x) | HCl<br>(1%) (3x)  | HNO <sub>3</sub><br>(10%) and<br>ultrasound<br>(3x) |                   |
|-----------------|--------|--------------------|-------------------------------|--------------------------------|-------------------------------|--------------------------------|-------------------|---|-------------------|
| <b>Elements</b> |        |                    |                               |                                |                               |                                |                   |   |                   |
| Al              | C      | 19.03 $\pm$ 0.88   | 17.88 $\pm$ 2.63              | 9.35 $\pm$ 2.9                 | 12.45 $\pm$ 2.05              | 9.01 $\pm$ 3.47                | 6.27 $\pm$ 0.26   | 7.79 $\pm$ 0.91                                     | 5.76 $\pm$ 1.18   |
|                 | NC     | 16.92 $\pm$ 0.4    | 16.69 $\pm$ 3.56              | 8.68 $\pm$ 3.71                | 10.71 $\pm$ 0.87              | 8.71 $\pm$ 2.39                | 6.18 $\pm$ 0.63   | 6.29 $\pm$ 1  | 5.64 $\pm$ 0.77   |
| As              | C      | 120.37 $\pm$ 14.69 | 7.04 $\pm$ 0.92               | 3.85 $\pm$ 0.78                | 3.37 $\pm$ 2.40               | 2.98 $\pm$ 3.64                | 1.40 $\pm$ 2.56   | 3.18 $\pm$ 2.73                                     | 1.40 $\pm$ 2.58   |
|                 | NC     | 6.6 $\pm$ 0.95     | 3.4 $\pm$ 1.81                | ND                             | ND                            | ND                             | ND                | 1.8 $\pm$ 0.9                                       | ND                |
| Cd              | C      | 12.7 $\pm$ 2.65    | 11.47 $\pm$ 3.4               | 5.8 $\pm$ 1                    | 4.3 $\pm$ 0.6                 | 6.7 $\pm$ 0.9                  | 2.25 $\pm$ 0.2    | 6.6 $\pm$ 0.1                                       | 2.6 $\pm$ 0.3     |
|                 | NC     | 3.7 $\pm$ 0.5      | 3.6 $\pm$ 0.2                 | 3.2 $\pm$ 1                    | 3.1 $\pm$ 0.7                 | 3.1 $\pm$ 0.4                  | 3.0 $\pm$ 0.3     | 3.1 $\pm$ 0.5                                       | 1.35 $\pm$ 0.2    |
| Cu              | C      | 14.79 $\pm$ 0.85   | 15.99 $\pm$ 5.78              | 13.25 $\pm$ 0.76               | 8.47 $\pm$ 1.28               | 9.39 $\pm$ 2.06                | 6.38 $\pm$ 0.834  | 8.61 $\pm$ 1.97                                     | 6.89 $\pm$ 1      |
|                 | NC     | 13.09 $\pm$ 2.18   | 15.8 $\pm$ 0.91               | 13.13 $\pm$ 0.5                | 8.16 $\pm$ 0.51               | 9.07 $\pm$ 1.94                | 6.28 $\pm$ 1.05   | 7.28 $\pm$ 0.82                                     | 6.85 $\pm$ 0.92   |
| Ni              | C      | 10.4 $\pm$ 1.93    | 8.33 $\pm$ 0.5                | 5.63 $\pm$ 1.18                | 4.04 $\pm$ 1.49               | 5.24 $\pm$ 2.37                | 3.58 $\pm$ 0.83   | 6.42 $\pm$ 1.53                                     | 3.05 $\pm$ 1.6    |
|                 | NC     | 5.97 $\pm$ 3.97    | 5.65 $\pm$ 0.62               | 4.84 $\pm$ 0.68                | 3.48 $\pm$ 1.53               | 4.62 $\pm$ 1.94                | 3.52 $\pm$ 1.8    | 5.47 $\pm$ 1.59                                     | 2.87 $\pm$ 1.03   |
| Pb              | C      | 8.27 $\pm$ 0.28    | 4.34 $\pm$ 0.28               | 3.12 $\pm$ 0.45                | 1.68 $\pm$ 0.2                | 2.79 $\pm$ 0.42                | 1 $\pm$ 0.08      | 2.28 $\pm$ 0.41                                     | 0.98 $\pm$ 0.08   |
|                 | NC     | 1.05 $\pm$ 0.12    | 1.01 $\pm$ 0.18               | 1.04 $\pm$ 0.14                | 0.64 $\pm$ 0.02               | 1 $\pm$ 0.09                   | 0.64 $\pm$ 0.07   | 0.85 $\pm$ 0.07                                     | 0.41 $\pm$ 0.1    |
| Se              | C      | 23.59 $\pm$ 6.19   | 10.29 $\pm$ 1.27              | 13.11 $\pm$ 1.85               | 9.23 $\pm$ 0.63               | 12.01 $\pm$ 1.34               | 7.02 $\pm$ 10.37  | 14.17 $\pm$ 2.09                                    | 6.73 $\pm$ 7.47   |
|                 | NC     | 5 $\pm$ 0.58       | 3.22 $\pm$ 1.1                | 3.17 $\pm$ 0.14                | 2.66 $\pm$ 0.57               | 3.19 $\pm$ 0.63                | 3.64 $\pm$ 0.35   | 4.91 $\pm$ 0.47                                     | 3.65 $\pm$ 2.46   |
| Zn              | C      | 153.03 $\pm$ 15.32 | 142.42 $\pm$ 34.51            | 136.82 $\pm$ 20                | 97.4 $\pm$ 13.09              | 109.05 $\pm$ 22.24             | 82.55 $\pm$ 19.15 | 125.58 $\pm$ 11.16                                  | 92.93 $\pm$ 3.66  |
|                 | NC     | 129.41 $\pm$ 5.11  | 129.64 $\pm$ 4.79             | 130.59 $\pm$ 9.77              | 93.25 $\pm$ 14.33             | 102.91 $\pm$ 11.73             | 82.06 $\pm$ 15.44 | 98.12 $\pm$ 15.2                                    | 91.66 $\pm$ 12.51 |

m  $\pm$   $\sigma$  (n = 6 samples)

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- Acetone/water/acetone(IAEA): 10 minutes each (reference method) (A)<sup>[27]</sup>.
- 1% nitric acid: 3×10 min (B)
- 10% nitric acid: 3×10 min (C)
- 1% nitric acid: 4×10 min (B4)
- 10% nitric acid: 4×10 min (C4)
- 1% hydrochloric acid: 3×10 min (E)
- 10% nitric acid with ultrasound: 3×10 min (D)

TABLE 1 shows the concentrations of trace elements(Al, AS, Cd, Cu, Ni, Pb, Se and Zn) in contaminated and non-contaminated hair washed with the various procedures described above. It shows that all hair-washing procedures decrease the concentration of trace elements in the hair matrix both in contaminated and in non-contaminated hair. It seems that trace element concentration is dependent on the washing method used<sup>[24]</sup>.

This decrease could be due to the removal of the contaminants bound onto the hair shaft and also of the fraction incorporated into the hair matrix. Decrease varies from one product to another.

The results show that 3 washes with acetone and hydrochloric acid (1%) had difficulty in removing certain trace elements. Thus, acetone removed 43.7% aluminium, 95.8% arsenic, 37.5% cadmium, 88.5% copper, 39.5% nickel, 53.9% lead, 62.3% selenium and 46.4% zinc. Hydrochloric acid eliminated only 28.9% aluminium, 83.8% copper, 50.6% selenium and 22.8% zinc although it seemed to be more effective than IAEA in removing 98.8% arsenic, 72.4% cadmium, 78.5% nickel and 80.3% lead.

In the majority of cases, 3 washes with 1% nitric acid appeared less effective than 4 washes with 1% nitric acid. This observation was also valid for 10% nitric acid, which points to a relationship between the effectiveness of the washing method in removing trace elements and the number of washes carried out. This approach indicates that the result is directly related not only to the washing method(the product used) but also to the washing conditions (length, number of baths).

Three washes with 1% nitric acid gave good results but these were less interesting than those obtained using 4 washes with 1% nitric acid. Three washes with 1% nitric acid removed the majority of trace elements at a rate above 68% except for sele-

mium which was eliminated at a lower rate of 47%. Four washes with 1% nitric acid was more successful, eliminating all the elements at a higher percentage than 3 washes with 1% nitric acid, even for copper which was eliminated at a rate exceeding 52%.

Three washes with 10% nitric acid compared to 3 washes with 1% nitric acid gave good results in the majority of cases. This was also observed when comparing 4 washes with 10% nitric acid and 1% nitric acid. This result testifies the dependence of the effectiveness of hair washing on the concentration of the washing agent.

Four washes with 10% nitric acid efficiently removed the majority of trace elements: aluminium 95.7%, arsenic 98.8%, cadmium 94%, copper 98.8%, nickel 98.7%, lead 94.9%, selenium 82% and zinc 97.9%.

Three washes with 10% nitric acid and combined ultrasound improved the elimination of trace elements from hair compared to all other procedures except for 4 washes with 10% nitric acid. This improvement is due to the physical disturbances generated by ultrasound when hair is exposed to its radiations leading to a rise in temperature and a local variation in pressure<sup>[4]</sup>. There is a triple-combined effect on washing: concentration-temperature-pressure.

Study of the effect of the number of washings on the effectiveness of washing only the two procedures giving the best results above were included in this study: 10% nitric acid alone and combined with ultrasound.

This second study was carried out to determine the effect of the number of washes on the effectiveness of 10% nitric acid alone and combined with ultrasound to remove the trace elements bound to the hair. The principle consisted in monitoring trace element concentrations in artificially contaminated and non-contaminated hair after washing with nitric acid 10% alone and combined with ultrasound for 2, 3, 4, 5 and 6×10 min cycles.

The resulting concentrations for 8 trace elements are shown in TABLES 2 and 3.

The data from the various analyses show that the concentration of trace elements in hair tends to decrease with the increase in the number of washes although no overall generalization can be made as

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**TABLE 2: Comparison of the concentrations of eight trace elements (Al, AS, Cd, Cu, Ni, Pb, and Zn) in contaminated hair (C) and non-contaminated hair (NC) washed with HNO<sub>3</sub> (10%) alone**

| Procedure       | Direct | HNO <sub>3</sub><br>(10%)(2x) | HNO <sub>3</sub><br>(10%)(3x) | HNO <sub>3</sub><br>(10%)(4x) | HNO <sub>3</sub><br>(10%)(5x) | HNO <sub>3</sub><br>(10%)(6x) |             |
|-----------------|--------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------|
| <b>Elements</b> |        |                               |                               |                               |                               |                               |             |
| Al              | NC     | 13.46 ± 2.11                  | 6.61 ± 1.23                   | 4.43 ± 0.71                   | 3.65 ± 1.05                   | 3.62 ± 1.04                   | 3.29 ± 0.87 |
|                 | C      | 21.77 ± 5.17                  | 10.76 ± 1.28                  | 4.32 ± 1.04                   | 3.7 ± 0.9                     | 3.69 ± 0.68                   | 3.08 ± 1.16 |
| As              | NC     | 17.03 ± 2.67                  | 8.54 ± 1.55                   | 5.73 ± 0.9                    | 4.72 ± 1.33                   | 4.67 ± 1.31                   | 4.25 ± 1.11 |
|                 | C      | 27.54 ± 6.54                  | 13.89 ± 1.62                  | 5.58 ± 1.32                   | 4.78 ± 1.14                   | 4.76 ± 0.86                   | 3.97 ± 1.47 |
| Cd              | NC     | 1.79 ± 0.28                   | 0.91 ± 0.16                   | 0.61 ± 0.09                   | 0.51 ± 0.14                   | 0.50 ± 0.14                   | 0.46 ± 0.12 |
|                 | C      | 2.9 ± 0.69                    | 1.49 ± 0.17                   | 0.60 ± 0.14                   | 0.51 ± 0.12                   | 0.51 ± 0.09                   | 0.43 ± 0.15 |
| Cu              | NC     | 10.46 ± 1.64                  | 5.83 ± 1.14                   | 3.61 ± 0.55                   | 2.98 ± 0.82                   | 2.95 ± 0.81                   | 2.68 ± 0.68 |
|                 | C      | 16.92 ± 4.02                  | 8.76 ± 0.99                   | 3.52 ± 0.81                   | 3.01 ± 0.82                   | 3.00 ± 0.81                   | 2.51 ± 0.9  |
| Ni              | NC     | 7.36 ± 1.15                   | 3.68 ± 0.67                   | 2.47 ± 0.39                   | 2.03 ± 0.58                   | 2.01 ± 0.57                   | 1.83 ± 0.48 |
|                 | C      | 11.9 ± 2.83                   | 5.99 ± 0.7                    | 2.41 ± 0.57                   | 2.06 ± 0.48                   | 2.05 ± 0.37                   | 1.71 ± 0.63 |
| Pb              | NC     | 5.85 ± 0.92                   | 2.99 ± 0.54                   | 2.00 ± 0.31                   | 1.65 ± 0.46                   | 1.63 ± 0.54                   | 1.47 ± 0.38 |
|                 | C      | 9.47 ± 2.25                   | 4.86 ± 0.56                   | 1.95 ± 0.46                   | 1.67 ± 0.39                   | 1.67 ± 0.3                    | 1.39 ± 0.51 |
| Se              | NC     | 10.69 ± 2.62                  | 8.63 ± 1.52                   | 5.79 ± 0.88                   | 4.77 ± 1.31                   | 4.72 ± 1.29                   | 4.30 ± 1.08 |
|                 | C      | 27 ± 6.41                     | 14.04 ± 1.59                  | 5.64 ± 1.3                    | 4.83 ± 1.12                   | 4.81 ± 0.85                   | 4.01 ± 1.44 |
| Zn              | NC     | 113.80 ± 17.85                | 19.21 ± 3.57                  | 12.88 ± 2.06                  | 10.61 ± 3.06                  | 10.51 ± 3.01                  | 9.56 ± 2.54 |
|                 | C      | 184.09 ± 43.71                | 31.24 ± 3.72                  | 12.56 ± 3.03                  | 10.75 ± 2.62                  | 10.71 ± 1.98                  | 8.93 ± 3.37 |

m ± σ (n = 6 samples)

**TABLE 3: Comparison of the concentrations of eight trace elements (Al, AS, Cd, Cu, Ni, Pb, and Zn) in contaminated hair (C) and non-contaminated hair (NC) washed with HNO<sub>3</sub> (10%) combined with ultrasound**

| Procedure       | Direct | HNO <sub>3</sub><br>(10%)+US(2x) | HNO <sub>3</sub><br>(10%)+US(3x) | HNO <sub>3</sub><br>(10%)+US(4x) | HNO <sub>3</sub><br>(10%)+US(5x) | HNO <sub>3</sub><br>(10%)+US(6x) |             |
|-----------------|--------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|-------------|
| <b>Elements</b> |        |                                  |                                  |                                  |                                  |                                  |             |
| Al              | NC     | 13.46 ± 2.11                     | 5.20 ± 1.55                      | 4.65 ± 0.97                      | 4.04 ± 0.47                      | 3.96 ± 0.69                      | 2.38 ± 0.48 |
|                 | C      | 21.77 ± 5.17                     | 9.36 ± 2.51                      | 4.40 ± 0.73                      | 4.09 ± 1.07                      | 4.06 ± 0.85                      | 1.73 ± 0.62 |
| As              | NC     | 17.03 ± 2.67                     | 6.44 ± 1.97                      | 5.76 ± 1.22                      | 5.00 ± 0.93                      | 4.91 ± 0.87                      | 2.14 ± 0.61 |
|                 | C      | 27.54 ± 6.54                     | 11.85 ± 3.17                     | 5.45 ± 0.92                      | 5.07 ± 1.35                      | 5.03 ± 1.07                      | 2.94 ± 0.78 |
| Cd              | NC     | 1.79 ± 0.28                      | 0.67 ± 0.21                      | 0.6 ± 0.13                       | 0.52 ± 0.10                      | 0.51 ± 0.09                      | 0.30 ± 0.06 |
|                 | C      | 2.9 ± 0.69                       | 1.20 ± 0.33                      | 0.56 ± 0.10                      | 0.53 ± 0.14                      | 0.52 ± 0.11                      | 0.22 ± 0.08 |
| Cu              | NC     | 10.46 ± 1.64                     | 3.84 ± 1.021                     | 3.43 ± 0.75                      | 2.98 ± 0.57                      | 2.93 ± 0.53                      | 1.75 ± 0.38 |
|                 | C      | 16.92 ± 4.02                     | 6.91 ± 1.95                      | 3.25 ± 0.57                      | 3.02 ± 0.83                      | 3.00 ± 0.86                      | 1.28 ± 0.48 |
| Ni              | NC     | 7.36 ± 1.15                      | 2.67 ± 0.85                      | 2.39 ± 0.53                      | 2.07 ± 0.40                      | 2.03 ± 0.37                      | 1.22 ± 0.26 |
|                 | C      | 11.9 ± 2.83                      | 4.80 ± 1.37                      | 2.26 ± 0.4                       | 2.10 ± 0.58                      | 2.08 ± 0.58                      | 0.89 ± 0.34 |
| Pb              | NC     | 5.85 ± 0.92                      | 2.11 ± 0.68                      | 1.89 ± 0.42                      | 1.64 ± 0.32                      | 1.61 ± 0.30                      | 0.96 ± 0.21 |
|                 | C      | 9.47 ± 2.25                      | 3.79 ± 1.10                      | 1.78 ± 0.32                      | 1.66 ± 0.47                      | 1.65 ± 0.37                      | 0.70 ± 0.27 |
| Se              | NC     | 10.69 ± 2.62                     | 5.92 ± 1.93                      | 5.29 ± 1.20                      | 4.59 ± 0.91                      | 4.51 ± 0.85                      | 2.7 ± 0.60  |
|                 | C      | 27 ± 6.41                        | 10.64 ± 0.90                     | 5.01 ± 0.90                      | 4.66 ± 1.32                      | 4.62 ± 1.05                      | 1.97 ± 0.76 |
| Zn              | NC     | 113.80 ± 17.85                   | 13.75 ± 4.52                     | 12.29 ± 2.81                     | 10.68 ± 2.14                     | 10.48 ± 1.99                     | 6.28 ± 1.40 |
|                 | C      | 184.09 ± 43.71                   | 24.73 ± 7.28                     | 11.63 ± 2.11                     | 10.82 ± 3.10                     | 10.73 ± 2.46                     | 4.58 ± 1.79 |

m ± σ (n = 6 samples)

## Review

the concentrations remain almost stable between 4 and 5 washes with 10% nitric acid before decreasing to minimal values with 6 washes. Two washes with 10% nitric acid alone removed 61.3% aluminium, 62.2% arsenic, 62.7% cadmium, 63.3% copper, 63.8% nickel, 64% lead, 64.5% selenium and 87.9% zinc from non-contaminated hair whereas 57% aluminium, 57.9% arsenic, 58.6% cadmium, 59.2% copper, 59.7% nickel, 60% lead, 60.1% selenium and 86.6% zinc were removed from artificially contaminated hair.

These percentages increased when 3 cycles were used before reaching almost stable values for the 4<sup>th</sup> and 5<sup>th</sup> cycles. Thus, for non-contaminated hair, 4 washes with 10% nitric acid alone removed 70% aluminium, 70.6% arsenic, 71.1% cadmium, 71.5% copper, 71.9% nickel, 72% lead, 72.5% selenium and 90.6% zinc whereas for 5 washes, 70.6% aluminium, 71.2% arsenic, 71.6% cadmium, 72% copper, 72.4% nickel, 72.5% lead, 73% selenium and 90.8% zinc were removed.

After this plateau, the percentage of trace elements eliminated increased abruptly to maximum values after 6 washes (figures 1 and 2).

The same observations were made for 10% nitric acid combined with ultrasound (Figure 3 and 4). This increased loss could be explained by the fact that using 6 washes with 10% nitric acid becomes so aggressive that it removes the endogenous fraction of trace elements from the hair shaft.

### Study of aggressiveness

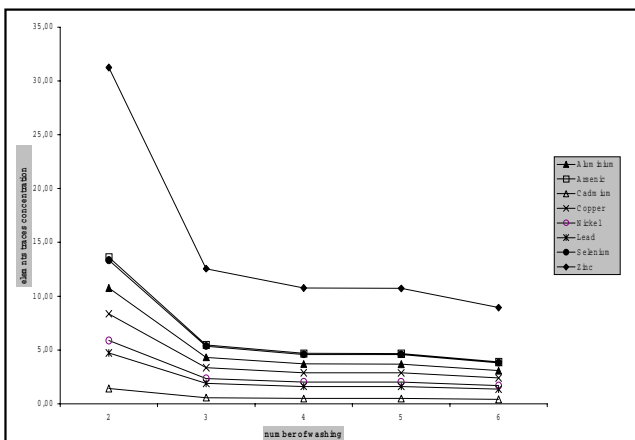


Figure 1: Evolution of trace element concentrations eliminated from contaminated hair as a function of number of washes with 10% nitric acid alone

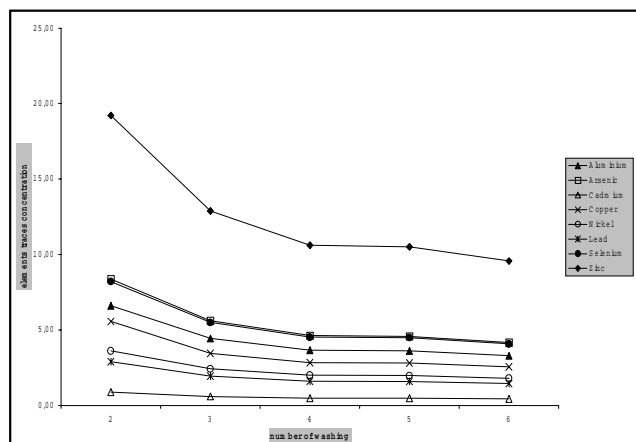


Figure 2: Evolution of trace element concentrations eliminated from non-contaminated hair as a function of number of washes with 10% nitric acid alone

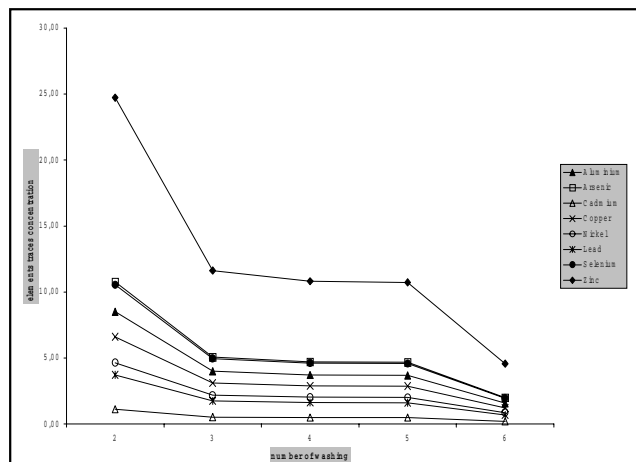


Figure 3: Evolution of trace element concentrations eliminated from contaminated hair as a function of number of washes with 10% nitric acid and ultrasound

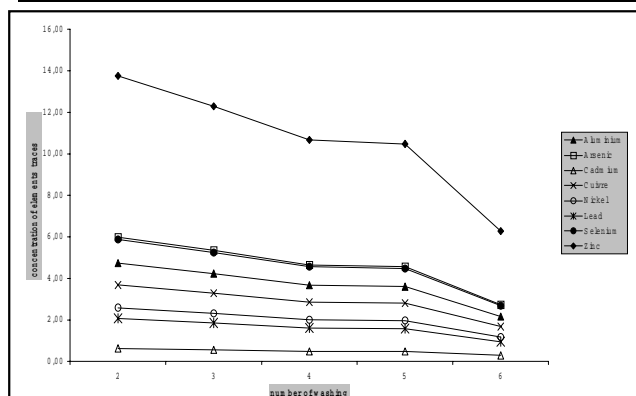


Figure 4: Evolution of trace element concentrations eliminated from non-contaminated hair as a function of number of washes with 10% nitric acid and ultrasound

**TABLE 4: Quantity of trace elements in the contamination solution before (Sc Bf) and after (Sc Af) contamination and in the different washing solutions**

| Elements | Solutions   |             |            |            |            |            |            |            |             |
|----------|-------------|-------------|------------|------------|------------|------------|------------|------------|-------------|
|          | S-Bf        | S-Af        | A          | B          | C          | D          | E          | B4         | C4          |
| Al       | 59.0 ± 34.4 | 26.3 ± 12.3 | 9.1 ± 2.3  | 15.6 ± 2.6 | 22.6 ± 3   | 29.4 ± 4.4 | 9.5 ± 2.5  | 16.8 ± 3.6 | 22.7 ± 12.5 |
| As       | 17.4 ± 0.1  | 0.2 ± 0.1   | 11.5 ± 9.9 | 11.4 ± 9.8 | 11.0 ± 9.5 | 19.9 ± 5.4 | 11.3 ± 9.7 | 11.2 ± 9.7 | 16.7 ± 1.4  |
| Cd       | 12.8 ± 1.3  | 3.0 ± 0.6   | ND         | 1.2 ± 0.7  | 1.8 ± 0.5  | 3.0 ± 0.6  | 1.0 ± 0.4  | 4.2 ± 0.4  | 5.5 ± 0.2   |
| Cu       | 5.8 ± 0.9   | 1.0 ± 0.1   | 3.0 ± 0.2  | 1.7 ± 0.4  | 1.8 ± 0.3  | 5.0 ± 4.4  | 3.6 ± 0.1  | 2.5 ± 0.8  | 4.1 ± 0.5   |
| Ni       | 49.8 ± 5.4  | 41.9 ± 3.9  | ND         | 6.5 ± 1.4  | 7.8 ± 0.9  | 8.6 ± 1.9  | 7.0 ± 1.7  | 7.1 ± 2.0  | 7.5 ± 2.6   |
| Pb       | 5.5 ± 0.4   | 1.8 ± 0.3   | 0.2 ± 0.1  | 1.2 ± 0.1  | 3.2 ± 0.1  | 4.2 ± 0.1  | 1.4 ± 0.1  | 2.3 ± 0.1  | 3.2 ± 0.1   |
| Se       | 24.0 ± 2.8  | 17.9 ± 2.2  | 1.4 ± 0.2  | 3.8 ± 0.1  | 3.5 ± 0.6  | 6.6 ± 0.5  | 5.2 ± 0.1  | 2.1 ± 0.4  | 4.7 ± 0.6   |
| Zn       | 28.7 ± 3.5  | 21.5 ± 2.6  | ND         | 4.7 ± 0.8  | 4.2 ± 0.7  | 7.9 ± 0.6  | 6.8 ± 0.2  | 4.9 ± 0.2  | 5.6 ± 0.7   |

$m \pm \sigma$  (n = 6 samples)

A study was elaborated to test the aggressiveness of the different washing procedures. The principle of the analysis was to compare the differences in quantity of contaminating elements in the hair before and after decontamination(washing), that is, a comparison between the quantity of exogenous contaminating trace elements settling on the hair and that eliminated by the different washing procedures tested.

To achieve this goal, a test specimen of 3.8g hair was contaminated for 24h at ambient temperature with a contamination solution(120ml).

The quantities (Q1) of trace elements contained in the contaminated solution are reported in TABLE 4. The solutions used for contamination were recovered to determine the remaining quantities (Q2) of trace elements after contamination. The difference (Q3) between (Q1) and (Q2) represented the quantity of trace elements artificially fixed onto the hair, which, in this study, corresponds to the exogenous fraction to be entirely eliminated by the various washing procedures tested.

After contamination, the hair was washed with the various washing procedures. The recovered washing solutions were used to determine the quantities (Q4) of trace elements eliminated by washing.

Any concentrations above the quantity fixed by contamination will testify to the aggressiveness of the washing procedure and, on the contrary, procedures giving results under this threshold will be considered non-aggressive. The concentrations measured in the various solutions are shown in TABLE 4.

The data from the various analyses made on the contamination solution before and after contamina-

tion of the hair and on the washing solutions after decontamination(washing) of contaminated hair shows that, in the majority of cases, none of the procedures affect the internal fraction of trace elements except nitric acid 10% with ultrasound which leads to a more excessive loss of certain trace elements (As, Cu, Ni, Pb, Se and Zn) than those settled by contamination. This observation can be justified by the effect of the variation of local pressure which is generated by the ultrasound and which induces a mechanical phenomenon of cavitation of the hair shaft leading thereafter to the removal mobilization of the internal fraction of trace elements in the hair matrix.

Washing four times with nitric acid 10% seems to be the most effective method to remove trace elements giving results in all cases close to the rate induced by the contamination solution, eliminating 69.4% aluminum, 96.9% arsenic, 85.4% copper, 56.1% cadmium, 94.9% nickel, 86.5% lead, 77% selenium and 77.8% zinc.

## CONCLUSION

While it has never been doubted that hair is a good medium to determine exposure to various trace elements, this study has shown that washing hair before their analysis constitutes a crucial stage to eliminate the exogenous fraction of trace elements and consequently to allow a good estimate of internal fraction which only reflects the impregnation of human organism.

The choice of a suitable hair washing procedure

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must hold in account two important parameters: effectiveness and aggressiveness of the product. The finally adopted method must be, on the one hand, the most effective one which involves the totality (or the majority) of the exogenous fraction of trace elements, and on the other hand, the least aggressive one which doesn't mobilize the internal fraction.

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