Heavy metal in hair samples of 109 non-industrial (miners) population in Katanga

Abstract
Introduction: This survey aims at proposing reference values of metals in the hair, in the copper-belt (Province of Katanga). Materials and methods: We ran a descriptive study, in an etiologic perspective, on a sample of non-industrial population, constituted by students of the University of Lubumbashi, healthy and without medical treatment ($n = 109$). Hair samples were analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Results: Twenty metals were identified and quantified: aluminium, antimony, silver, barium, cadmium, calcium, cerium, chromium, cobalt, copper, tin, iron, magnesium, manganese, mercury, molybdenum, nickel, lead, vanadium, and zinc. Discussion: Regarding similar surveys ran upon european non-industrial populations, our results are ranging in the same percentiles. They spread out those percentiles only when our results were too close to quantification limits (silver, tin, mercury, nickel and vanadium). For certain metals, in particular aluminium, cadmium, chromium, cobalt, copper, manganese, molybdenum and lead, we have obtained higher contents. Several reasons can explain this specificity: ICP-MS sensitivity, analysis and preparation technique difference, diet difference or simply public health issues. Conclusion: Our results are close to others surveys’ values. They can be validated as reference values for Katanga general population and used to highlight occupational exposure.

Key words: analysis technique; Congo; hair; metals; toxicology.

Résumé
Teneurs des métaux lourds dans 109 échantillons de cheveux d’une population non industrielle au Katanga

Introduction : la matérialisation des expositions professionnelles aux métaux, chez les mineurs artisanaux de la province du Katanga, suppose l’existence de valeurs de référence. Cette étude vise à proposer ces valeurs à travers une analyse du biomarqueur-cheveu. Matériels et méthodes : il s’agit d’une étude descriptive à visée étiologique, portant sur un échantillon de la population générale, non industrielle, constituée d’étudiants de la faculté de médecine, sains et sans traitement médical, résidents sur le campus de l’université de Lubumbashi ($n = 109$). Les prélèvements de cheveux ont été analysés par spectrométrie d’émission en plasma induit couplée à la spectrométrie de masse (ICP-MS). Résultats : 20 métaux ont été identifiés et quantifiés, soit aluminium, antimoine, argent, baryum, cadmium, calcium, cérium, chrome, cobalt, cuivre, étain, fer, magnésium, manganèse, mercure, molybdène, nickel, plomb, vanadium et zinc. Discussion : le rapprochement de ces résultats de ceux d’autres études, portant sur des populations non industrielles européennes, montre que nos teneurs en métaux se situent dans des percentiles communs. Elles ne s’en éloignent que lorsque nos valeurs ont été trop proches des limites de quantification (argent, étain, mercure, nickel et vanadium). Plusieurs facteurs, parmi lesquels on peut citer la différence de technique d’analyse et de préparation ainsi que les régimes alimentaires, peuvent expliquer les valeurs élevées obtenues pour certains métaux (aluminium, cadmium, chrome, cobalt,
Introduction

Survey context

The analysis of hazards and risk factors in non-industrial mining of the Katanga province revealed toxicological risks generated by exposure to the chemical components of the mineralised gangue extracted. The materialisation of this exposure requires the use of reference values and normal contents of various metals in a non-exposed population. The lack of standards for metal contents in the hair bio-tracer we used for these analyses justifies the present survey, which tends to establish reference values for metal traces in a non-industrial population living in the Katanga province.

Hypothesis and purpose

The specific objective of this survey is to settle reference values for metal contents in the hair of a non-exposed population. To achieve this, we need first to identify and quantify metals in the hair of the general non-industrial population of Katanga, then to compare the values obtained with those of other non-exposed populations.

Tools and methods

Survey material

Our surveyed population is a sample of the general non-industrial population, composed of medical students living on the Lubumbashi University campus (n = 5,000). The following participation criteria have been settled:

- to have never worked in the mining sector – industrial or not;
- For the detection and quantification of metal traces, we chose hair as bio-tracer. Several authors showed this as a good indicator of environmental pollution [1, 2] and chronic exposure. Compared to other bio-tracers such as, blood or urine, hair also gives the opportunity of easier sample taking, conservation and transport. It also allows retrospective reproduction of identical samples. Nevertheless, we must point out that in African culture, nails, epidermis and particularly hair are considered as individual attributes through which spells could be cast by means of fetishist practices. This culture made sample taking more difficult in our surveyed population. This type of sample taking is also made difficult because most men wear their hair close-cropped.
- it enables distinction and specification and proportions of isotopes of the same element.

However, despite these advantages, the use of this technique requires some precautions justified by the physical and chemical effects generated by the matrix, such as isobaric differences which must be deleted, interpretation problems about the contents of oligoelements and some metals, or problems related to the elimination of external contaminants in a professional environment.

The use of this technique required a thorough preparation of the hair samples, achieved in three steps: washing, mineralisation (or digestion), and finally, the analysis itself by means of ICP-MS.

The purpose of washing is the decontamination of the samples from dust and other environmental pollutants that could impregnate the samples. The same washing technique is used for all the samples. It is achieved in three steps as shown in Table 1.

The analyses were achieved by means of the detection and quantification technique, emission spectrometry in induced plasma coupled to mass spectrometry, ICP-M [4]. This analytical technique presents the following advantages:

- this method is quick, sensitive, specific and has a fair range of linearity [5, 6];
- the analysis was also performed in three steps, achieved in three steps: washing, mineralisation (or digestion), and finally, the analysis itself by means of ICP-MS.

The product obtained from this mineralisation will be analysed to determine the contents of metals and metalloids.

Survey methodology

The hair samples were taken from the neck, according to the recommendations of the Society of hair testing [3].

The analyses were achieved by means of the detection and quantification technique, emission spectrometry in induced plasma coupled to mass spectrometry, ICP-M [4]. This analytical technique presents the following advantages:

- this method is quick, sensitive, specific and has a fair range of linearity [5, 6];
- it enables distinction and specification and proportions of isotopes of the same element.

The lack of standards for metal contents in the hair bio-tracer we used for these analyses justifies the present survey, which tends to establish reference values for metal traces in a non-industrial population. To achieve this, we need first to identify and quantify metals in the hair of the general non-industrial population of Katanga, then to compare the values obtained with those of other non-exposed populations.

Tools and methods

Survey material

Our surveyed population is a sample of the general non-industrial population, composed of medical students living on the Lubumbashi University campus (n = 5,000). The following participation criteria have been settled:

- to have never worked in the mining sector – industrial or not;
- For the detection and quantification of metal traces, we chose hair as bio-tracer. Several authors showed this as a good indicator of environmental pollution [1, 2] and chronic exposure. Compared to other bio-tracers such as, blood or urine, hair also gives the opportunity of easier sample taking, conservation and transport. It also allows retrospective reproduction of identical samples.

However, despite these advantages, the use of this technique requires some precautions justified by the physical and chemical effects generated by the matrix, such as isobaric differences which must be deleted, interpretation problems about the contents of oligoelements and some metals, or problems related to the elimination of external contaminants in a professional environment.

The use of this technique required a thorough preparation of the hair samples, achieved in three steps: washing, mineralisation (or digestion), and finally, the analysis itself by means of ICP-MS.

The purpose of washing is the decontamination of the samples from dust and other environmental pollutants that could impregnate the samples. The same washing technique is used for all the samples. It is achieved in three steps as shown in Table 1.

After washing, the samples were mineralised by means of acid treatment in a microwave oven Anton Paar made, Model MW 2000 with a six closed teflon reactor-carousel. Washing was also performed in three steps, as shown in Table 2.

The product obtained from this mineralisation will be analysed to determine the contents of metals and metalloids.

Survey methodology

The hair samples were taken from the neck, according to the recommendations of the Society of hair testing [3].

The analyses were achieved by means of the detection and quantification technique, emission spectrometry in induced plasma coupled to mass spectrometry, ICP-M [4]. This analytical technique presents the following advantages:

- this method is quick, sensitive, specific and has a fair range of linearity [5, 6];
- it enables distinction and specification and proportions of isotopes of the same element.

However, despite these advantages, the use of this technique requires some precautions justified by the physical and chemical effects generated by the matrix, such as isobaric differences which must be deleted, interpretation problems about the contents of oligoelements and some metals, or problems related to the elimination of external contaminants in a professional environment.

The use of this technique required a thorough preparation of the hair samples, achieved in three steps: washing, mineralisation (or digestion), and finally, the analysis itself by means of ICP-MS.

The purpose of washing is the decontamination of the samples from dust and other environmental pollutants that could impregnate the samples. The same washing technique is used for all the samples. It is achieved in three steps as shown in Table 1.

After washing, the samples were mineralised by means of acid treatment in a microwave oven Anton Paar made, Model MW 2000 with a six closed teflon reactor-carousel. Washing was also performed in three steps, as shown in Table 2.

The product obtained from this mineralisation will be analysed to determine the contents of metals and metalloids.

Table 1. Hair washing procedures.

<table>
<thead>
<tr>
<th>Steps</th>
<th>Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20mg of hair + 5mL acetone, vortexed 3 seconds and settled down</td>
</tr>
<tr>
<td>2</td>
<td>H₂O - ultra pure at 40 °C, vortexed 30 seconds then settled down</td>
</tr>
<tr>
<td>3</td>
<td>Drying in a sterilizer at 40 °C</td>
</tr>
</tbody>
</table>
Detection and quantification of metals and metalloids were achieved by means of emission spectrometry in induced plasma coupled with mass spectrometry (ICP-MS) on a Thermo-Optek apparatus, of ELEMENT 2 type. The calibration of this apparatus is outlined in Table 3. We used ultra pure acids from Merck, ultra pure H2O2 from Merck and demineralised water, treated in Sartorius ARIU 611 DI, in order to obtain water at 18.2 megaΩ. To control the mineralisation procedures we used reference material (certified reference material NCZC 81002 human hair from the China National Center). These analyses allowed us to identify and quantify simultaneously concentrations of 20 mineral elements.

Statistical analyses

To describe our survey population and the use of the results of this analysis of hair samples by ICP-MS, we made a descriptive study with an exploratory purpose, through usual descriptive statistics: the average, 5th and 95th percentiles. We used SPSS version 17.0.

Results

Socio-demographic description of the sample used

The age of our population samples varies from 18 to 40 years of age, with an average of 25 years. Eighty-eight percent of the individuals are men, 43% live in family, and 50% of them are smokers, while 63% drink alcohol, beer or local-made alcohol.

Presentations of the metal-trace contents in hair

Twenty elements have been simultaneously quantified in hair: aluminium, antimony, silver, barium, cadmium, calcium, cerium, chromium, cobalt, copper, tin, iron, magnesium, manganese, mercury, molybdenum, nickel, lead, vanadium and zinc. Table 4 shows the results, which enabled us to validate the use of the
ICP-MS method for analysing the hair samples taken.

This table demonstrates that the variation margin (CV = 100*DS/average, DS = Standard Deviation) of the repeatability, such as calculated on the Chinese CRM (NCS ZC 81002) provided by the China National Analysis Center for Iron and Steel, show a low dispersion. Dispersions observed with the metal contents in hair of the Katanga population would then be due to their nature and structure. The linear regression line relying the concentrations and signals measured is of 0.99. Reproducibility is better than 15%, except for tin (19.4%) and lead (17.4%). For these 20 metals underscored in the hair of our sample population, table 5 shows quantified values.

**Discussion**

The comparison of the results above with those obtained from other surveys shows some differences, which may be due to different preparation and analysis techniques.

Actually, after having sent several samples of identical hair to various laboratories, the American Agency for Toxic Substances and Substances and Disease Registry (ATSDR) found out that the results given by these various laboratories presented some variability, which could not be due to the samples of hair [7]. This is why we propose a comparison between our results and those of Goulle et al. [8] and Kintz [9] who also proceed to preliminary washing the hair samples.
Table 5. Values obtained in our survey.
Tableau 5. Résultats obtenus dans notre enquête.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Average (µg/g)</th>
<th>Dispersion 5th and 95th percentile (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>380.38</td>
<td>244.92-698.73</td>
</tr>
<tr>
<td>Antimony</td>
<td>0.08</td>
<td>0.03-0.134</td>
</tr>
<tr>
<td>Silver</td>
<td>0.15</td>
<td>0.05-0.93</td>
</tr>
<tr>
<td>Barium</td>
<td>2.76</td>
<td>1.93-5.24</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.32</td>
<td>0.12-0.82</td>
</tr>
<tr>
<td>Calcium</td>
<td>517.86</td>
<td>258.16-1187.24</td>
</tr>
<tr>
<td>Cerium</td>
<td>0.42</td>
<td>0.31-0.71</td>
</tr>
<tr>
<td>Chromium</td>
<td>1.16</td>
<td>0.62-4.20</td>
</tr>
<tr>
<td>Cobalt</td>
<td>1.67</td>
<td>0.80-2.02</td>
</tr>
<tr>
<td>Copper</td>
<td>49.86</td>
<td>16.45-224.16</td>
</tr>
<tr>
<td>Tin</td>
<td>0.21</td>
<td>0.10-0.79</td>
</tr>
<tr>
<td>Iron</td>
<td>235.25</td>
<td>138.92-498.80</td>
</tr>
<tr>
<td>Magnesium</td>
<td>64.89</td>
<td>42.27-240.76</td>
</tr>
<tr>
<td>Manganese</td>
<td>8.90</td>
<td>4.43-23.87</td>
</tr>
<tr>
<td>Mercury</td>
<td>0.34</td>
<td>0.19-0.60</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>0.12</td>
<td>0.08-0.21</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.49</td>
<td>0.25-0.96</td>
</tr>
<tr>
<td>Lead</td>
<td>17.77</td>
<td>6.82-35.19</td>
</tr>
<tr>
<td>Vanadium</td>
<td>0.79</td>
<td>0.44-1.40</td>
</tr>
<tr>
<td>Zinc</td>
<td>52.19</td>
<td>35.95-80.23</td>
</tr>
</tbody>
</table>

Conclusions and recommendations

In so far as the differences observed between metal contents presented in this survey and those established in other surveys about identification and quantification of elements and traces in hair by ICP-MS in other regions, are justified by inter-group variability and the extreme sensitivity of the method used, we think that the values presented in our survey may be considered as reference values for the general population of the Katanga province. These results may then be used to evaluate the possible effectivity of an exposure, for example of a...
professional type, or for the survey of an environmental sector supposed to be particularly exposed.

Acknowledgements and other statements
Financing: none; conflicts of interests: none.

References