X-Ray Fluorescence to Determine Zn in Bolivian Children using Hair Samples

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As a first step in the evaluation of nutritional levels in Bolivian children (8–13 years-old), we carried out X-Ray Fluorescence measurements in hair samples of children belonging to different social classes and living either in rural areas or in cities. The aim of this study is to contribute to health policies tending to improve the global health of children and consequently avoid malnutrition. Our method intends to have maximum reliability and at the same time be as simple as possible from an experimental point of view. Additionally, we use this method to determine some other elements such as Fe, Cu, Pb, As and Hg, the latter three considered as contaminants that could be present in children living in areas which neighbor mines and industries. This work will be complemented by some biological and medical tests.

I. INTRODUCTION

X-ray fluorescence (XRF) is based on the irradiation of a sample by a primary X-ray beam. The individual excited atoms emit secondary X-rays that may be detected and recorded in a spectrum. In this work, we use total reflection X-ray fluorescence (TXRF) and related methods. The advantage of this method is that it allows detection of trace elements (ppb) in thin liquid samples [1].

Several elements ensure a correct functionality of the respiratory, digestive and neurovegetative systems. Among the principal elements are Zn, Cu, and Fe. In particular, Zn plays an important role in the child growth and in general, it is related with processes such as protein synthesis and cellular repair. The Zn concentration levels in hair may be used to detect malnutrition in children. Values comprised in the range of 100–150 mg/kg are considered normal. On the contrary, values below 60 mg/kg indicate severe malnutrition [2].

Due to large socioeconomic differences existing in Bolivia, it is common to find children showing some features that could be related to malnutrition. It occurs, in particular, in the poorest regions of the country; especially, in the suburban peripheries. With the aim of identifying quantitatively children suffering malnutrition, we propose a TXRF method to quantify the Zn and other elemental concentrations using children hair samples.

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The paper is structured as follows: in Section II, we describe the steps of the experimental method: hair obtention, sample preparation, calibration and measurements. In Section III, we show the principal results and discuss them, providing at the end some perspectives of the work.

II. EXPERIMENTAL METHOD

Firstly, the hair was collected in children of both sexes with ages between 8 and 13 years old. The locations where the hair was obtained are situated near the La Paz high plateau: Viacha (Aprox. 3876 masl) and El Alto (Aprox. 4070 masl), corresponding to suburban peripheries. The obtention of the hair has been made with the consent of the children and parents. Accompanying the obtention of hair, we have taken measures of weight and height and also taken notes about the school grade and the age of each of the children.

Then, by means of a linearity test [1], we found the best value of the XR tube current intensity and at the same time, we performed the calibration of the tube (Counts/second vs. Current intensity). With this, we have found the limit of detection (LOD) for different elements: Fe, Ni, Zn and Co, using the relationship

$$LOD_i = \sqrt{\frac{I_i(BG)}{t}} \cdot \frac{C_{Ga}}{I_{Ga} S_i} \tag{1}$$

where $I_i(BG)$ is the intensity in counts per second of the element i in the sample; C_{Ga} is the internal standard concentration (in our case, gallium) expressed in ppm; I_{Ga}

intensity in counts per second of Ga; and S_i is the relative sensitivity for the element i [4]. In order to determine concentrations of Zn, we complemented the calibration process using Zn standards of 0.5, 1, 3 and 4 ppm all of them prepared using 10 ppm of Ga (internal standard). The software AXIL and OXAS are used to obtain the spectral areas of the K_α lines corresponding to Zn and Ga and consequently the calibration line shown in Fig. 1.

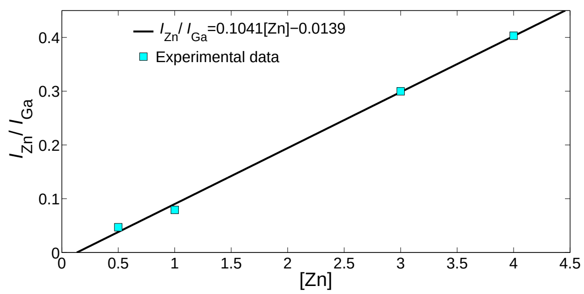

FIG. 1: Calibration curve for Zn, using concentrations of 0.5, 1, 3, and 4 ppm.

A microwave digestion (MWD) method has been used to prepare the liquid samples as suggested in Ref. [3]. For the preparation of each sample, we have used 200 mg of hair using a 3 ml mixture composed by HNO_3 and H_2O_2 with concentrated ratio (2:1, v/v) as oxidant; this mixture rested for five minutes before starting the MWD process by means of an Anton Paar (Instruments) microwave digester. Two steps were followed for the digestion program: (i) 100 W of power for five minutes and (ii) 800 W of power for 15 minutes. The digestion conditions were 300°C as the maximum temperature and 75 bar of pressure. When the MWD process was finished, we added 0.25 mg of Ga in order to have 10 ppm for the internal standard concentration.

The last part of the experimental method is the analysis using TXRF techniques in order to quantify the Zn concentration in the samples. We used an X-Ray generator ITAL STRUCTURES COMPACT 3K5 model with voltage and amperage ranging from 10 to 60 kV, and 1 to 50 mA, respectively. The output power of the device is 3 kW and it uses a molybdenum target. Quartz reflectors are used for the sample lectures. In order to avoid contamination and consequently biased results, the reflectors must be washed carefully using some acidic solutions. When the reflectors are completely cleaned, a sample aliquot of 10 μl is placed on the reflector, the liquid sample undergoes an evaporation dry process with an infrared lamp, and finally is measured in the XR tube.

A summary of the experimental method in the form of a flowchart is shown in Fig. 2.

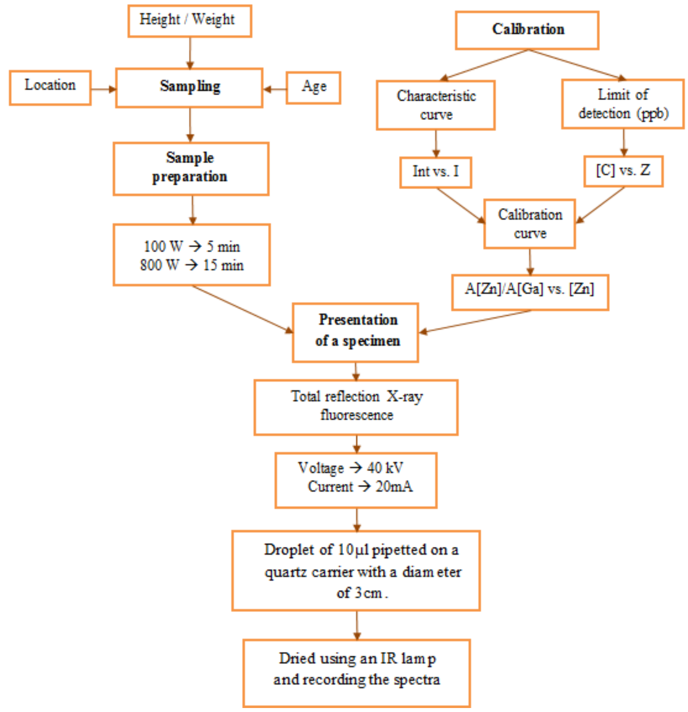

FIG. 2: Flow chart summarizing the experimental method.

III. RESULTS AND DISCUSSION

The technical part corresponding to this project is finished and now we are devoting our efforts to interpret the obtained measurements in order to gain some medical, biological and even socioeconomic aspects from them. First, we performed a simple statistical analysis of the Zn, Fe and Cu concentrations. The medians and the quartiles are shown in Fig. 3.

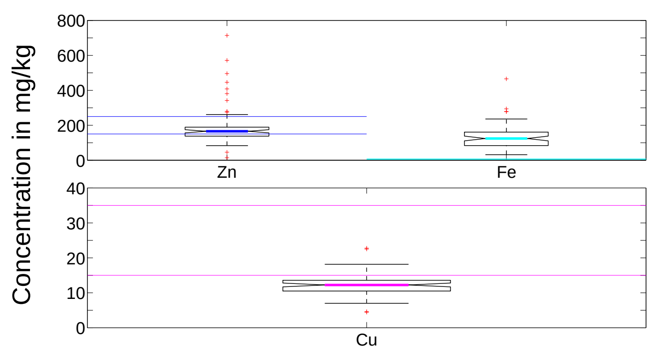

FIG. 3: Medians and quartiles for the Zn, Fe (top) and Cu (bottom) concentrations. The colored horizontal lines are associated to the tolerance ranges for the concentration of each element.

We have performed quadratic discriminant analysis, a classification method used in statistical classification and in machine learning [5] that we have used in order to classify the children using their body mass index (BMI) and the [Zn]. Despite the good classification, it is hard to affirm that the BMI is related to the malnourished children (Fig. 4). Finally, we introduce a factor that is the ratio [Zn]:[Cu] with the aim of improving the classi-

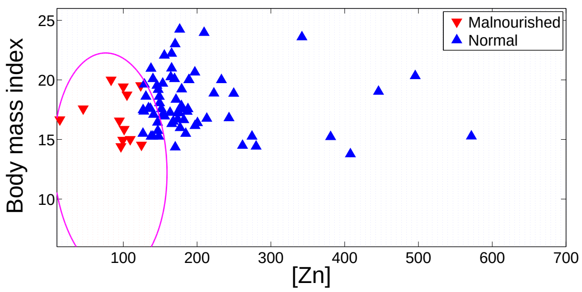

FIG. 4: Quadratic discriminant analysis between [Zn] and BMI.

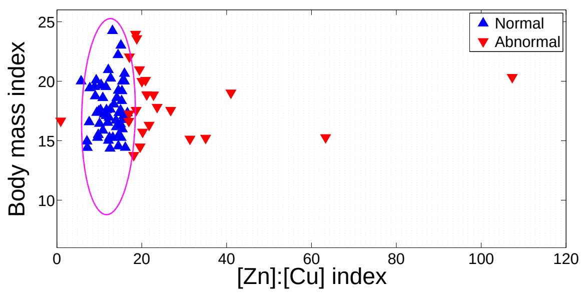

FIG. 5: Classification analysis between the ratio [Zn]:[Cu] and BMI.

fication of normal ([Zn]:[Cu]=[4,12]:1) [6] and abnormal individuals (Fig. 5). Certainly, other indices should be defined in order to obtain better and more significative classifications [7]. The relationships between the concentration of the elements are undoubtedly a feature that we must further explore. It could be interesting to apply additional methods such as the recognition of clinical signs that could be related to severe illness [8] to complement our results.

IV. CONCLUSIONS

We have verified the usefulness of the TXRF method detecting elements in samples even for small concentrations. The results allow us to classify normal and abnormal individuals based on anthropometric parameters and measurements of Zn and Cu concentrations. Nevertheless, we have not found a real correlation between these parameters and measurements. Further measurements, increasing the number of tested individuals, and obtaining additional parameters such as the eating and nutritional habits could lead to find the correlation with the Zn concentration.

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[1] R. Klockenkamper, *Total reflection x-ray fluorescence analysis*, John Wiley & Sons, (1997).
[2] C.W. Weber, G. W. Nelson, M. Vasquez, P. B. Pearson, J. Trop. Pediatrics **36**, 230 (1990).
[3] A.R. Memon *et al.*, Clin. Chim. Acta **379**, 66 (2007).
[4] V.F. Nascimento Filho *et al.*, Biol. Trace. Elem. Res. **71**, 423 (1999).
[5] T. Hastie, R. Tibsharini, J. Friedman, *The elements of statistical learning: data mining, inference, and prediction*, Springer, (2008).
[6] D.L. Watts, Trace. Elem. News. **21**, 1 (2010).
[7] Certain clinical laboratories consider a ratio [Zn]:[Cu]=[4.3,16.7]:1 for their hair analysis ratio worksheet.
[8] E. Mazzi *et al.*, J. Trop. Pediatrics **56**, 307 (2010).