

## **Applicability of Human Hair as a Bioindicator for Trace Elements Exposure**

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**Abstract**—Hair is a useful bio-indicator to estimate human exposure to trace elements, because it is easy to collect and can reflect relatively long term exposure. However, trace elements in hair do not always reflect the levels arising only from endogenous uptake due to the possible contamination by exogenous materials such as soil, dust, and water that might be adhering to it. The objective of this study is to evaluate the suitability of hair as a bio-indicator of trace elements exposure using (i) determination of relative affinity of various metals by multi elements adsorption experiment, and (ii) elemental mapping of hair sections using synchrotron radiation micro beam X-ray fluorescence ( $\mu$ -SXRF). Relative affinities of 11 metals and metalloids determined by adsorption experiment were:  $\text{Hg}^{2+} > \text{Pb}^{2+} > \text{Cu}^{2+} > \text{Cr}^{3+} > \text{Cd}^{2+} > \text{As}^{(\text{III})} > \text{Se}^{(\text{IV})} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Sb}^{(\text{III})} > \text{Ni}^{2+}$ . Clearly higher affinity for soft metals suggested that soft ligands act as the adsorption sites for these metals, and also adsorbed soft metals cannot be removed by simple washing because of the existence of covalent bonding between the elements. Our results indicate that careful consideration is necessary while using hair to assess human exposure to trace elements.

Keywords: hair, imaging, adsorption

### INTRODUCTION

Human scalp hair provides useful information on the occupational and environmental exposure to trace elements (Katz, 1979). It is a useful tool in the diagnosis of certain diseases relating to trace elements pollution and can also provide information on the effects of drug usages (Batzevich, 1995). However, before accepting trace element levels in scalp hair as reliable indicators of exposure, standard values have to be established, along with the standardization of sample collection and preparation. In addition, trace elements incorporated into hair from the body (endogenous) must be differentiated from contamination by external (exogenous) sources.

In order to assess the suitability of hair as an indicator of trace elements exposure, we focused on the mechanism of contamination by exogenous matter. In our earlier study, we found extremely high concentration of Pb from a resident in a lead-acid battery recycling site, but it was suspected that the major source of hair contamination was exogenous rather than endogenous matter (Noguchi *et al.*, 2010). Synchrotron X-ray fluorescence elemental mapping ( $\mu$ -SXRF) was employed to assess the contribution of exogenous matter. Additionally, multi element adsorption experiment was carried out for systematic understanding of metals/metalloids affinity at the hair surface. Relative affinities of various elements can indirectly provide information on chemical binding at the hair surface. Through these approaches, implications on the suitability of hair as a bio-indicator can be deduced. The aim of this approach is to evaluate relative affinity of 11 trace elements (Cr, Co, Ni, Cu, Zn, As, Se, Cd, Sb, Hg, Pb) to the surface of human hair by adsorption experiment to clarify the binding mechanism of exogenously bound trace elements. This approach can enable us to clarify the characteristic of each trace element as indicator of human exposure.

#### MATERIALS AND METHODS

Hair sample was obtained from a 22-year old student living in Ehime, Japan. The hair was black in color and collected from the top of the head. The hair unaffected by any cosmetic treatment was selected. Hair was collected by plucking from the scalp using tweezers and stored in plastic bag at room temperature.

Approximately 0.1 g of hair sample was weighed into 12 mL metal spiked artificial sweat solution with pH adjusted to 6.0 (Morton *et al.*, 2002). Three levels of metal spiked solutions containing 0.1, 1, and 10 mg/L each of Cr, As, Se, Cd, Sb and Hg (group 1) and Pb, Cu, Ni, Co and Zn (group 2) were prepared. The hair was allowed to react for 12 hours by shaking at 120 rpm in a reciprocating shaker. After this, reversibly adsorbed fraction was removed by shaking with metal free artificial sweat solution for 12 hours, and then washed three times by the same solution.

Trace element analysis: Dried hair sample was digested in a Teflon vial using a microwave system (Ethos D, Milestone S.r.l., Sorisole, BG, Italy). All samples were analyzed using an inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500cx, Agilent Technologies, Tokyo, Japan).

$\mu$ -SXRF analysis: Hair sections cut into <1 mm by scissors were embedded in resin (Sakura, Tissue Tek). Clio-microtome was used to slice the hair into thin sections of 10~14  $\mu$ m thickness. Synchrotron micro X-ray Fluorescence analyses (SXRF) were performed utilizing a synchrotron light source at BL4A, photon factory, Tsukuba, Japan. Prior to the analysis, shape and distribution of hair section within the thin section were examined by optical microscopy. For micro X-ray analysis, the thin section was positioned at an angle of 45° against the incident beam, and fluorescence X-ray was recorded by a Si semiconductor. The size of incident X-ray beam was fixed at 5  $\times$  5  $\mu$ m<sup>2</sup>, which became 7 (horizontal)  $\times$  5 (vertical)  $\mu$ m<sup>2</sup> on the sample due to the angle of the sample to the incident X-

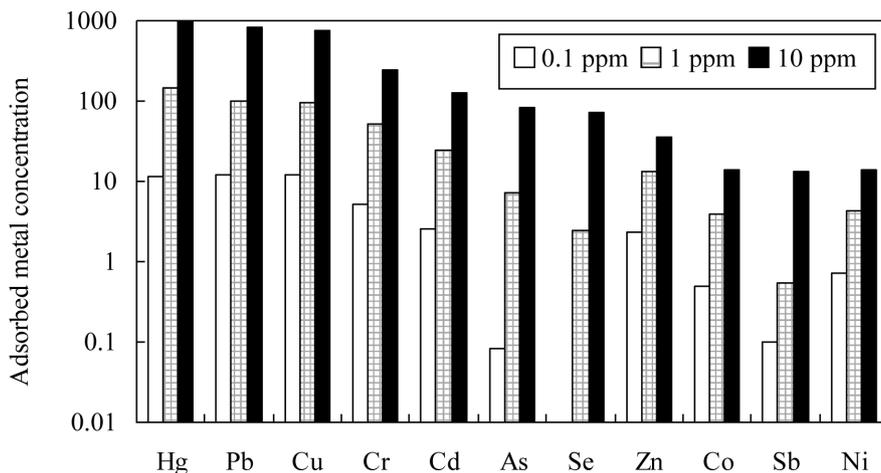


Fig. 1. Concentrations of adsorbed metals and metalloids in hair ( $\mu\text{g/g}$ ) after adsorption experiment.

ray beam. The step of the scanning was fixed at 4 and 3  $\mu\text{m}$  for horizontal and vertical scans, respectively. Intensity of incident X-ray was fixed at 14.2 keV. During scanning, the intensities of fluorescence X-ray of  $\text{S-K}\alpha$ ,  $\text{Cr-K}\alpha$ ,  $\text{Cu-K}\alpha$ ,  $\text{Zn-K}\alpha$ ,  $\text{As-K}\alpha$ ,  $\text{Se-K}\alpha$ ,  $\text{Hg-L}\alpha$ ,  $\text{Pb-L}\alpha$ , and  $\text{Pb-L}\beta$  were monitored simultaneously using single channel analyzers tuned for the energy of each fluorescence X-ray.

## RESULTS AND DISCUSSION

Results of adsorption experiment indicated that the amount of adsorbed metals/metalloids increased in accordance with increasing levels in solution (Fig. 1).

Although adsorption of each element occurs on the surface of hair, spatial distribution of each metal/metalloid was confirmed using  $\mu\text{-SXRf}$  imaging (Fig. 2). The main constituents of hair, such as S, was uniformly distributed in hair section, whereas adsorbed metals were clearly enriched on the surface of hair suggesting that adsorbed metals did not penetrate into the internal part of hair.

According to the amount of metals/metalloids adsorbed, relative affinities of 11 metals and metalloids to hair surface were found as:  $\text{Hg}^{2+} > \text{Pb}^{2+} > \text{Cu}^{2+} > \text{Cr}^{3+} > \text{Cd}^{2+} > \text{As}^{(\text{III})} > \text{Se}^{(\text{IV})} > \text{Zn}^{2+} > \text{Co}^{2+} > \text{Sb}^{(\text{III})} > \text{Ni}^{2+}$ . This order is primarily controlled by hard and soft acid and base characteristics. In this theory, Hg, Pb and Cu are classified with soft acid and tend to make covalent bonding with soft bases. On the other hand, Zn, Co and Ni are classified as hard cations and tend to make ionic bonding with hard base (Ralph, 1963). Sulfur is a major constituent of hair protein originating from cysteine and methionine amino acid residues having keratinous structure. Many metals found in hair are bound to sulfur atoms in thiol (SH) groups present in other amino acids (Lenihan, 1987; Chatt and Katz, 1988). High affinity of soft cation on the surface of hair and low concentration of

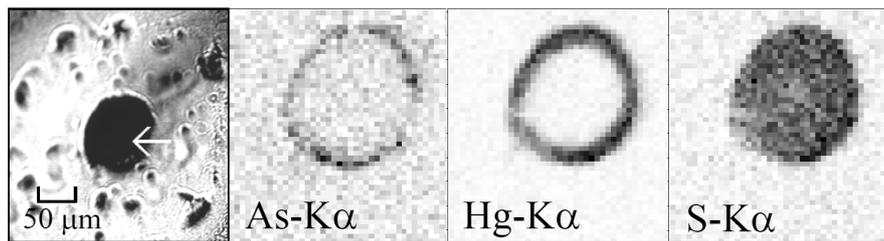


Fig. 2. Spatial distribution of As, Hg and S in the hair section after adsorption experiment at pH 6.

hard cation indicates that the binding sites of these metals should be soft ligands; probably thiol group (SH).

Two metalloids, As and Se showed higher affinity than the hard cations but lower than soft cations. Although both metalloids can form covalent bonding with soft ligands, these metalloids generally coordinated with oxygen to form oxyanion in solution. Antimony could be classified in the same group of As and Se as per the predominance of oxyanion in neutral solution, although its affinity to hair surface is much lower. These results suggest that the charge of an ion is also an important factor controlling the affinity of attachment to hair surface. Chromium is a unique example. Chromium takes trivalent charge in solution and show moderate affinity to hair surface despite its preference for hard ligand. This is possibly due to the higher charge density than other hard cations. Hence, charge of an ion may also affect the affinity for attachment to hair surface.

It has been believed that some essential metals, such as Zn, Cu, Se and S are stable in longitudinal analyses along hairs above the scalp because all these are essential elements (Sky-Peck, 1990), thus showing no detectable influence due to environmental accumulation or removal (Rodushkin and Axelsson, 2003; Kempson and Skinner, 2006). Actually, these metals/metalloids showed relatively low affinity for hair surface. Unlike these elements, the results presented here indicated that toxic metals, such as Hg, Pb, and Cd, can form irreversible bonding with hair surface which are not easy to be broken. This characteristic may give potentially unreliable results if the surrounding environment was heavily contaminated, such as at industrial areas and waste disposal sites. This result is consistent with previous findings which indicated limitation of hair as a bioindicator for Pb due to the lack of correlation between hair and blood (Bos *et al.*, 1985; Trunova *et al.*, 2003; Martin *et al.*, 2005). Unfortunately, metals having higher affinity to thiol are generally important for environmental monitoring due to their high toxicity to organisms. Despite the limitations, hair is still a superior monitoring tool since the method of collection is easy and non-invasive, enabling a preliminary screening of the human exposure to trace elements enriched around contaminated sites. To minimize these limitations upon using hair as a monitoring tool, optimized washing procedure should be carried out before analysis to prevent over or underestimation of trace element exposure.

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