

ORIGINAL ARTICLE

# A study of Zinc Contained in Yellow and Black Discolored Nails by X-ray Fluorescence and X-ray Absorption Fine Structure Analyses

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## Synopsis

The elements in discolored nails were assessed by X-ray fluorescence analysis (XRF), and the chemical state of Zn in the nail samples was assessed by X-ray absorption fine structure (XAFS) analysis. Compared to the normal nail, a part of the yellow nail contained a considerably high amount of Ca, whereas the black nail contained higher amounts of Ca and Zn. The chemical state of Zn in the yellow nail was similar to that in the normal nail. The Zn in the black nail was in a slightly different chemical state, suggesting the existence of different chemical species of Zn in the black nail.

**Key words:** nail, zinc, elemental analysis, X-ray absorption fine structure (XAFS)

## Introduction

The nail is a keratinous skin appendage that contains a high concentration of cysteine residues. The nail plate also contains minerals such as magnesium (Mg), calcium (Ca), iron (Fe), and zinc (Zn) [1]. The shape and color of the nail reflects the systemic health conditions, and black, yellow, green, or white discoloration of the nails is seen clinically [2]. Black nail reportedly occurs because of an increase in nail matrix melanocytes, an infectious pathology, malignant melanoma, etc [2-4]. Yellow nail syndrome is caused by nutritional deficiency, respiratory manifestations, infection of nails, and jaundice [2, 5].

Zn is one of the main minerals in the nail

plate. The content of Zn in nails is higher in males than in females, and is related to nail discoloration and its treatment. One of the causes of black discoloration of nails is the production of the black mineral sulfide from the hydrogen sulfide derived from the microbes causing infections [3]. Arroyo et al. reported that oral zinc supplementation with ZnS improved the discolored nail [6]. These minerals have the possibility of the nano particle combined with nail proteins. Therefore, analysis of the concentration and chemical state of Zn is important to determine the causes of nail discoloration.

In this study, the elemental minerals in the yellow and black nails were assessed by performing X-ray fluorescence (XRF) analysis. In

addition, the chemical states of Zn in these nail samples were estimated by performing X-ray absorption fine structure (XAFS) analysis.

## Materials and Methods

### 1. Specimens

Yellow discolored nails were obtained from a 71-year-old man, and black discolored nails were obtained from a 54-year-old man. The yellow nails consisted of 2 parts: a slightly yellow part (A) and a considerably yellow part (B). In addition, normal nail specimens were collected from a healthy volunteer for reference purpose. These specimens were applied to the following analyses.

### 2. XRF analysis

The elements in the nail specimens were assessed by using a fluorescence X-ray spectrometer (XGT-2000V; Horiba Co. Ltd., Kyoto, Japan). Incident X-rays generated from an Rh anode under conditions of 50 kV and 1 mA were irradiated into the center of the specimens through an X-ray guide tube (XGT) having a diameter of 100  $\mu\text{m}$ . The analysis time for each specimen was 1,200 sec. The composition of the detectable elements (S, K, Ca, Fe, and Zn) was quantitated by using the fundamental parameter method (standardless method) [7].

### 3. XAFS analysis

The XAFS spectra were measured at beamline 12C in the Photon Factory at the High Energy Accelerator Research Organization (KEK-PF). The electron storage ring was operated at 2.5 GeV with 450 mA. Synchrotron radiation was monochromatized with a Si(111) double-crystal monochromator. The incident X-ray was focused on an area of 1 mm in diameter by using two bent conical mirrors, and the specified areas of the specimens were irradiated. The Zn K-edge X-ray absorption near-edge structure (XANES) spectra of the specimens were measured using the fluorescent XAFS method with a 19-elements solid-state detector (SSD; Camberra, Meriden, U.S.A.). The  $I_0$  signals were monitored using an  $\text{N}_2$ -filled ionization chamber. The metallothionein that contains Zn and Cd (Sigma-Aldrich, St.Louis, U.S.A.) was also

subjected to XRF and XAFS analyses as the reference of Zn contained in a metalloprotein. The XANES spectra of reference materials (Zn foil, ZnO, and ZnS) were measured using an ordinary transmission method.

## Results and Discussions

The fluorescence X-ray spectra of the normal, black, and yellow discolored nails are shown in Fig. 1. The elemental composition estimated using the standardless method is presented in Table 1. XRF analysis cannot detect the light elements (e.g., H, C, N, and O); hence, the elemental composition in Table 1 shows the weight ratio of the 5 detected elements. The normal nail had a very strong peak assigned to sulfur (S), which was derived from the keratin of the nail. In addition, clear peaks derived from K, Ca, Fe, and Zn were also observed. The S content was higher than 80wt%, and Ca, K and Fe concentrations were also measured. Zn was clearly detected, but its content was less than 1wt%. The Ca:S ratio was approximately 0.1, and the Zn:S ratio was quite low (0.008). Thus, the normal and healthy nail consists of keratin and small amount of minerals. The slightly yellow nail (A) showed an elemental composition similar to the normal nail,

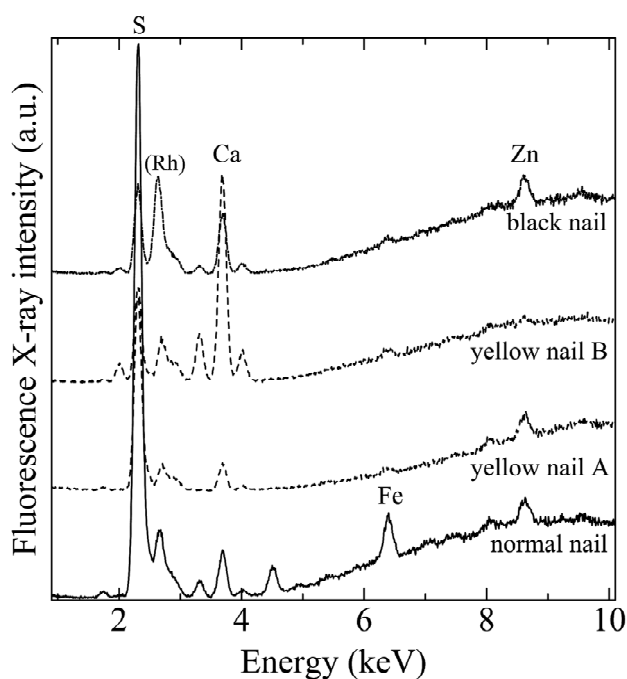


Fig.1 XRF spectra of the normal, yellow, and black nails

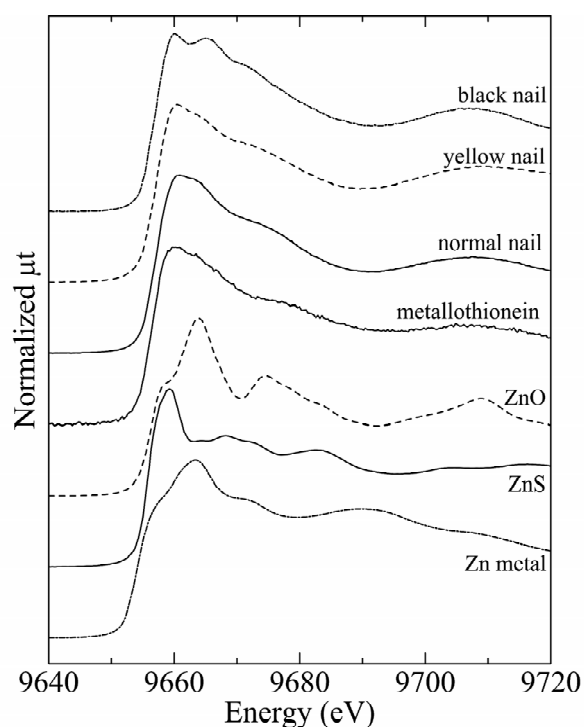
**Table 1** Element composition of nail specimens (wt%).

Elements	Normal	Yellow (A)	Yellow (B)	Black
S	84.5	81.7	23.4	48.3
K	4.4	0.4	12.6	6.3
Ca	8.6	15.6	62.7	40.8
Fe	1.8	0.6	0.9	1.2
Zn	0.7	1.7	0.4	3.4
Ca:S	0.10	0.19	2.7	0.84
Zn:S	0.008	0.02	0.017	0.070
Ca:Zn	13	9.0	170	12

but the considerably yellow nail (B) showed an extremely strong Ca peak. The Ca:S ratio of the considerably yellow nail (B) was 2.7; this value is quite higher than that of the normal nail (0.10), suggesting the presence of a large amount of calcified matter in this nail type. In contrast, the Zn content in this considerably yellow nail was about half of that in the normal nail. The black nail showed a clear Zn peak, and the Zn content was found to be 5 times higher than that in the normal nail. Karita et al. have reported the Ca, Mg, and Zn levels in fingernails [8]: the Ca:Zn ratio was reported to be 0.6~28.4 (median = 5.7). In our study, the yellow nail (B) showed a Ca:Zn ratio of more than 170, which is much higher than that reported by Karita et al. In case of the black nail, the Ca:Zn ratio was in the normal range, but the Zn:S ratio (0.07) was higher than that in the other nail specimens, indicating that the Zn content was quite higher than the S (keratin) in the black nail.

Fig. 2 shows the Zn k-edge XANES spectra of the normal, yellow, and black nails. The XANES spectrum of the normal nail showed a good resemblance to that of metallothionein, which is a type of cysteine-rich protein that can bind to heavy metals such as Zn, Cu, and Cd through a thiol group of cysteine residues. Metallothionein is suggested to play a role in the detoxification of heavy metals and regulation of essential trace metal elements. Thus, Zn in the nail possibly exists as a protein complex, similar to metallothionein. The yellow nail showed a XANES spectrum that was quite similar to that of the normal nail, then the chemical state of Zn in the yellow and normal nails would be similar.

The edge energy of the black nail was the same as that of the other nails; however, the shape of the spectrum was slightly different from the other spectra and it showed a small peak at around 9666eV. This result suggested that existence of different chemical species in the black nail. One of the causes of the black nail reported by Zuehlke et al. is the production of hydrogen sulfide by microbes when they react with the metallic elements in the nail and consequently produce black metal sulfides [3]. The XANES



**Fig.2** Zn K-edge XANES spectra of the normal, yellow, and black nails

spectrum of the black nail was different from that of the normal nail and ZnS. Thus, different chemical species of Zn seem to exist in the black nail.

With regard to the XAFS study of human nails, Katsukini et al. reported the findings of extended X-ray absorption fine structure (EXAFS) analysis of Zn in human nails [9]. They assumed that Zn coordinates with the nitrogen (N) and S contained in histidine and cysteine, which are the major amino acids of nail keratin. They also reported that the coordination numbers and bond lengths of N and S varied depending on the manifestation (clubbed nail, tuberculosis, and fibrosis). In our study, we observed a definite difference in the Zn K-edge XANES spectra between the black nail and the normal nail, and we inferred the presence of different chemical species in the black nail. Thus, element analysis by XRF and the chemical state analysis by XAFS of nails would be useful for the diagnosis of conditions of human nails.

### Conclusion

The XRF and XAFS analyses were performed to assess the element composition and the chemical state of the discolored nails. XRF analysis revealed that the normal nail contained a higher concentration of S derived from nail keratin but a lower concentration of Ca and Zn. A part of the yellow nail showed considerably higher Ca content, and we therefore inferred that the yellow nail contains a good proportion of calcified matter. Compared to the other nail samples, the black nail contained a higher concentration of Ca and much higher concentration of Zn. The chemical state of Zn, as estimated by XAFS, in the yellow nail was similar to that in the normal nail. The Zn present in the black nail showed a slightly different chemical state, and therefore, we inferred the existence of different chemical species of Zn in the black nail.

### Acknowledgements

The XAFS measurements were conducted after receiving approval of the Photon Factory Advisory Committee (Proposal No. 2010G022). This work was supported by a Grant-in-Aid for Challenging Exploratory Research No. 21659449, from the Ministry of Education, Culture, Sports,

Science and Technology, Japan.

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(Received: November 10/  
Accepted: December 18)

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