Biological Trace Element Research (2020) 193:348–356
https://doi.org/10.1007/s12011-019-01722-6

Investigation on the 2D-Distribution of Metallic Elements after Hair Dyeing

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Received: 24 February 2019 / Accepted: 5 April 2019 / Published online: 24 April 2019

Abstract

Long-term use of hair dyes has potential effects on metal content in hair. However, little research dissects the specific distribution and composition variations of the metal after dyeing. In this study, we investigated the morphological change and metallic elements content variation after dyeing. The results showed that the concentration of essential metal elements decreased, among which the Ca, K, and Na decreased sharply even above 50%. As for the heavy metal, the most significant observation is that Pb increased almost by five times after dyeing. Besides, it revealed, using scanning electron microscope coupled with energy-dispersive X-ray spectroscopy (SEM-EDS), that Pb concentrated at the outer layer of the hair. In addition, two-dimensional proton-induced X-ray emission (2D-PIXE) was applied to analyze the distribution of metallic elements along the longitudinal and cross section of the hair. The results showed that Ca and Zn distributed evenly in the hair along the longitudinal and cross section. It is the first time that 2D-PIXE is applied to analyze the metallic distribution in the hair. This method exhibits high sensitivity and can be widely used in the environmental and medical field to analyze the distribution of metallic elements.

Keywords Human hair · Hair dye · Metallic element · 2D-PIXE · Element distribution

Introduction

As the quality of life improves, people are increasingly focusing on their appearance by dying their hairs into different colors, especially for women. It is pointed out that hair colorants are a fast-growing industry with more than $ 7billion around the world. [1] There are three kinds of hair dyes, including temporary dyes, semi-permanent dyes, and permanent dyes. [2] Permanent dyes are the most widely used type of hair colorants, which contains metallic dyes, plant dyes, and oxidative dyes. [3] Various hair dyes may have different side effects on human health, resulting from the different chemical compounds and metallic elements in various hair dyes. [4] A recent study reported that people who are always working in the barber shop may be allergic to the compounds in the dyes. [5–8]

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A large number of researches have focused on the side effects of metallic elements in hair dyes since they cannot be degraded or digested. It has been proven that metallic elements can enter cells and tissues through several ways, such as inhalation, ingestion, and skin absorption, resulting in various degrees of damage. [9, 10] Some metallic elements, such as arsenic (As), cadmium (Ca), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), cobalt (Co), copper (Cu), and zinc (Zn), were found in human hair by using inductively coupled plasma-optical emission spectroscopy (ICP-OES). [11, 12] As the component of catalytic proteins and enzymes, they are essential trace elements for the human body, such as Fe, Zn, and Cu. However, it is harmful to human once the concentration exceeds the safe value. [13–15]. For instance, Fe and Cu, found by using the inductively coupled plasma-mass spectrometry (ICP-MS), can be accumulated after frequent dyeing. [16] Besides, chronic Fe intoxication is frequently associated with genetic and metabolic diseases. Other heavy metal elements, such as Ti and Pb, are poisonous in spite of at low concentration. [17–19] So many works have paid attention to analyze the content change of metallic elements in hair. For example, the results of ICP-OES and ICP-MS showed that the content of Cr and Ti would increase in hair after eating too much highly processed food. Besides, exposure to a high

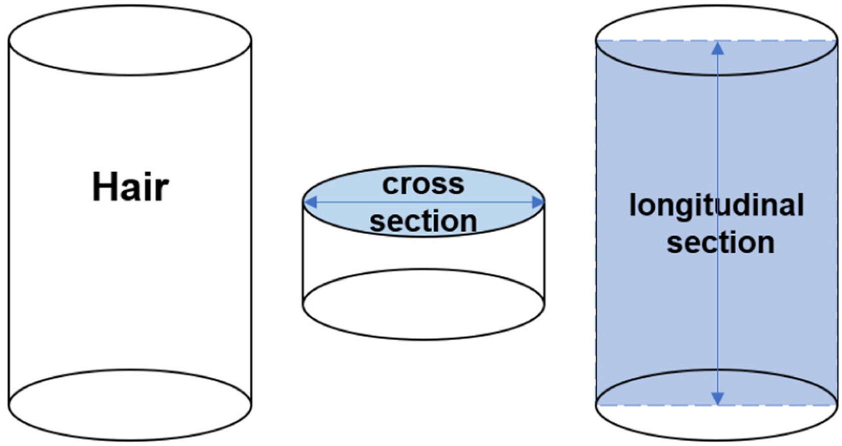

Fig. 1 The sketch map about the cross and longitudinal section of hair

content level of Pb in childhood results in irreversible damage to the neurological system. [20] Despite numerous researches have concentrated on investigating the overall variation of metallic elements content, few studies devoted to analyze the element distribution along the longitudinal and cross section of the hair. [21, 22]

Two-dimensional proton-induced X-ray emission (2D-PIXE) is an excellent method to perform micro-analysis with high resolution and non-destructive characters. [23–25] 2D-PIXE has been widely used in the cultural heritage objects, medical and environmental fields. [26–28] However, there is little research about this technology applying to hair analysis. [29] 2D-PIXE is favorable in hair research because it can probe multiple elements at one time without damaging samples. [25, 27] In addition, it allows us to explore the distribution of metallic elements along the longitudinal and cross sections for further investigating the effects of hair dye.

Therefore, this paper aimed to present a holistic understanding of element change in the hair after hair dyeing with different hair dye products. We not only explored the change of hair after dyeing by using SEM and ICP, but investigated the specific distribution of metallic elements in hair with 2D-PIXE.

Experimental

Sampling and Pretreatment

Hair Dyes Selection

We chose the natural brown 4-0 of Guang Ming (domestic brand) and Schwarzkopf (German brand) hair dye products. These two products were bought from Walmart (Shanghai,

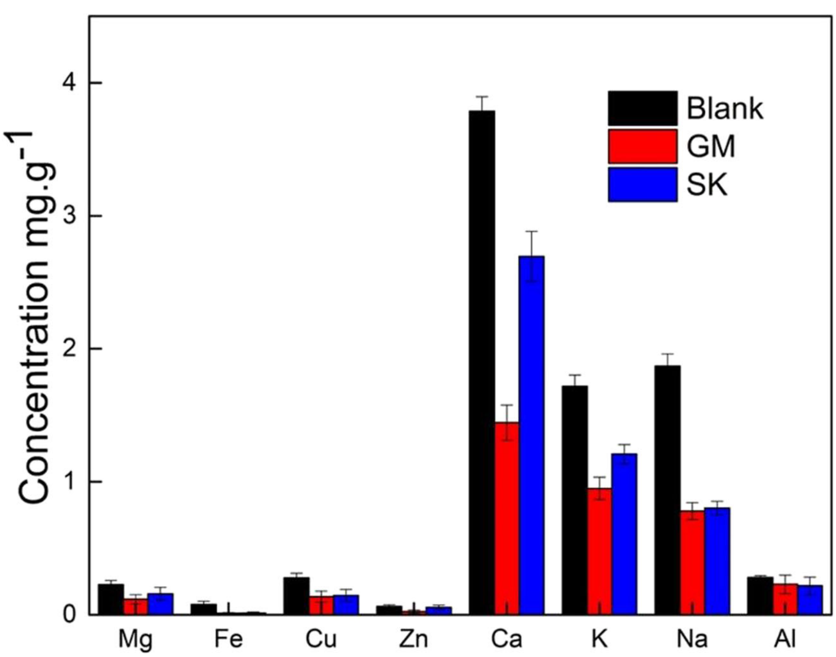

Fig. 2 The total concentration of metallic elements in the hair using ICP-OES

Table 1 The total concentration of metallic elements in the hair using ICP-OES ($mg.g^{-1}$ mean ± SD)

Metallic elements	Blank hair group	GM-dyed hair group	SK-dyed hair group
Mg	0.225 ± 0.031	0.117 ± 0.035	0.158 ± 0.047
Fe	0.078 ± 0.024	0.011 ± 0.005	0.013 ± 0.008
Cu	0.276 ± 0.034	0.136 ± 0.041	0.145 ± 0.044
Zn	0.062 ± 0.013	0.024 ± 0.011	0.056 ± 0.017
Ca	3.786 ± 0.109	1.443 ± 0.133	2.695 ± 0.188
K	1.717 ± 0.086	0.950 ± 0.085	1.206 ± 0.072
Na	1.868 ± 0.093	0.779 ± 0.064	0.802 ± 0.051
Al	0.279 ± 0.014	0.228 ± 0.068	0.216 ± 0.065

China). The hair dyed by Guang Ming and Schwarzkopf hair dye was named as the GM-dyed hair group and SK-dyed hair group, respectively.

Hair Samples Collection

Hair samples were collected from three volunteers (female, 30 years old, denoted as A, B, and C) in Anyang, Henan province. All the volunteers have not dyed their hair in the recent 3 years. For each volunteer, 100-g hair samples (20 cm long) were obtained. [30]

Hair Samples Pretreatment

Hair samples pretreatment was conducted by researchers in the laboratory. Hair samples were firstly washed with acetone and deionized distilled water and dried at 50 °C to a constant weight. To conduct hair dyeing, a section of hair from volunteer A was dyed with different hair dyes (Guang Ming or Schwarzkopf) for 1 h. After hair dyeing, the hair samples were washed with shampoo and deionized distilled water to remove the dye residues. Hair samples were then dried at 50 °C until the weight stays constant. Finally, hair samples were cut into approximately 1 cm for further use. As for volunteers B and C, hair samples were conducted following the same procedure.

For ICP-OES, hair samples were collected in Teflon tanks for digestion. The digestion process was conducted by adding 4 mL concentrated nitric acid to 0.1 g hair samples and stored for 2 h at room temperature. Then, 2 mL of 20% H_2O_2 was added into the vessel and kept at 60 °C in a constant temperature bath until the solvent was reduced to 1 mL. After cooling, the digest was diluted to 10 mL by ultrapure water. For ICP-MS tests, hair samples were digested in sealed Teflon tanks. Exactly 0.1 g hair samples were carefully weighted and then put into a Teflon tank. Four milliliters concentrated nitric acid and 2 mL 20% H_2O_2 were subsequently added into the vessel and kept the temperature at 60 °C for 4 h. After cooling, the digest was diluted to 10 mL by ultrapure water. [31–33]

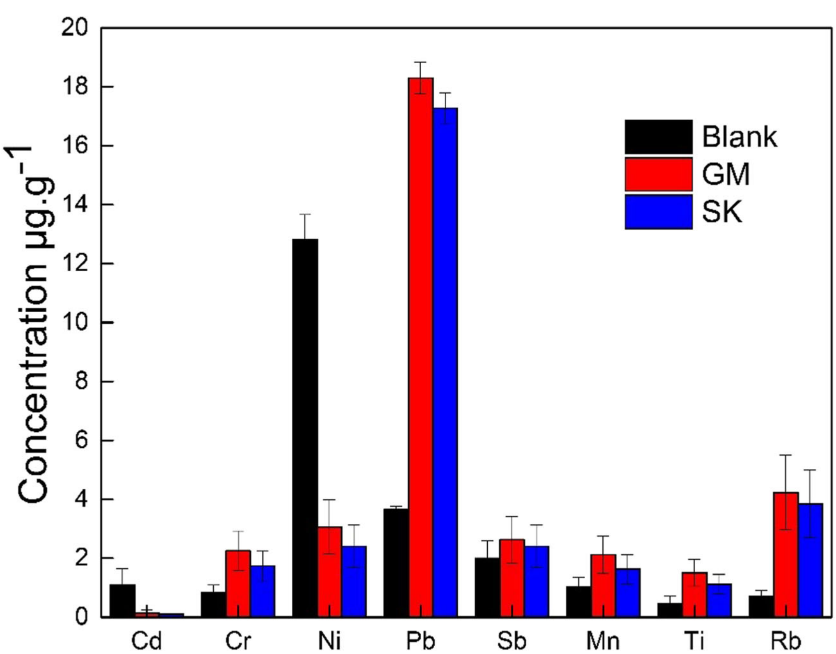

Fig. 3 The total concentration of metallic elements in the hair using ICP-MS

Table 2 The total concentration of metallic elements in the hair using ICP-MS (μg.g^{-1} mean ± SD)

Metallic elements	Blank hair group	GM-dyed hair group	SK-dyed hair group
Cd	1.11 ± 0.53	0.14 ± 0.12	0.11 ± 0.01
Cr	0.84 ± 0.25	2.25 ± 0.67	1.74 ± 0.52
Ni	12.81 ± 0.84	3.06 ± 0.91	2.40 ± 0.72
Pb	3.65 ± 0.11	18.29 ± 0.54	17.28 ± 0.52
Sb	1.98 ± 0.60	2.63 ± 0.79	2.40 ± 0.72
Mn	1.03 ± 0.31	2.12 ± 0.64	1.63 ± 0.49
Ti	0.47 ± 0.24	1.51 ± 0.45	1.12 ± 0.34
Rb	0.70 ± 0.21	4.23 ± 1.27	3.85 ± 1.15

Inductively Coupled Plasma-Optical Emission Spectroscopy and Inductively Coupled Plasma-Mass Spectrometry

Digested hair samples were used to analyze the total content of metallic elements (Mg, Fe, Cu, Zn, Ca, K, Na, Al) with ICP-OES (Hitachi Z-5000). [32] Due to the limited sensitivity of the ICP-OES, the low content metallic elements (Cd, Cr, Ni, Pb, Sb, Mn, Ti, and Rb) were determined by ICP-MS (NexION ×300).

Scanning Electron Microscope Coupled with Energy-Dispersive X-Ray Spectroscopy

The morphology of the hair was observed by scanning electron microscope (MAIA3, TESCAN). Field-emission scanning electron microscope (FESEM, S-4800, Hitachi, Corp.) coupled with energy-dispersive X-ray spectroscopy (EDS) was performed to observe the element distribution of the dyed and undyed hair treated by different cosmetics. [34] The samples were coated with gold layer (80–100 Å) before measurement to reduce electrostatic charging of the SEM. [10, 32, 35]

Two-Dimensional Proton-Induced X-Ray Emission

We used a Si (Li) detector (Sirius80, Gresham Ltd.) installed at 135 °C to collect proton-induced characteristic X-rays. This detector has an 80-mm^2 active area and energy resolution of 150 eV, and the final resolution of the proton beam was nearly 20 μm. [29] Data analyses were done by using the OMDAQ 2007 software.

The 1-cm sample of clean hair was immersed in 100% paraffin wax at 37 °C for 1 h and embedded in a plastic dish filling with new 100% paraffin wax at low temperature. Samples were sliced to serial 8-μm-thick paraffin sections using LEICA RM2135 and investigated along the cross and longitudinal sections (Fig. 1). Paraffin sections were picked up on the mylar membrane after floated on the surface.

Results and Discussion

ICP-OES and ICP-MS

In order to observe the element content change in hair after dyeing, we investigated the content of metals by ICP-OES. The element content of three volunteers shows no significant difference, so we only present the results of volunteer A. Three parallel experiments were conducted for each of the ICP experiments. As can be seen in Fig. 2, Ca, K, and Na have higher quantities of 3.786, 1.717, and 1.868 mg.g^{-1} in the blank hair group. However, the content of Mg, Fe, Cu, Zn, and Al was relatively low, with 0.225, 0.078, 0.276, 0.062, and 0.279 mg.g^{-1}, respectively. After dyeing, the contents of all metallic elements in the hair

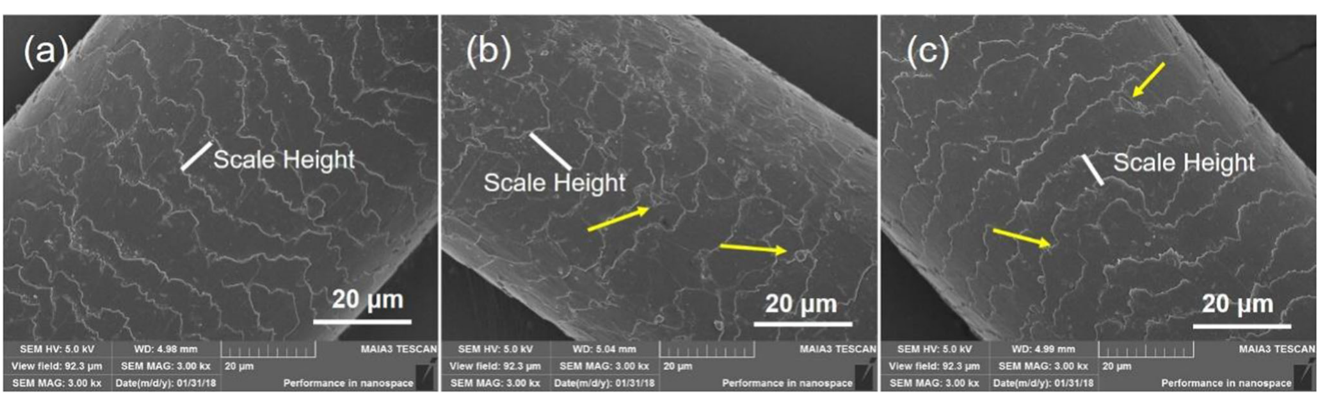

Fig. 4 The morphology of the outer layer of the hair by SEM (5.0 kV, ×3000). **a** Blank hair. **b** GM-dyed hair. **c** SK-dyed hair

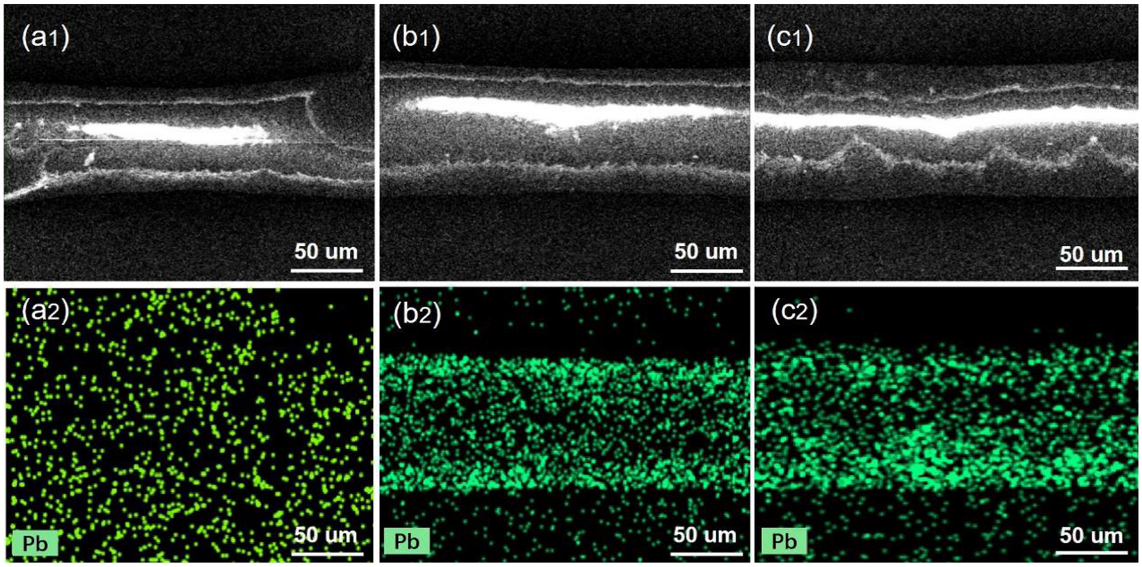

Fig. 5 SEM images and the corresponding EDS-mapping results. (15 kv, ×500). (a1)–(a2) blank hair, (b1)–(b2) hair dyed by GM, and (c1)–(c2) hair dyed by SK

decreased (Table 1). Note that the content of Ca decreased from 3.786 to 1.443 $mg.g^{-1}$ for the GM-dyed hair group and to 2.695 $mg.g^{-1}$ for the SK-dyed hair group, which were nearly 61.89 and 28.82% reductions, respectively. Besides, the decline ratio of Fe was 85.83% for the GM-dyed hair group and 83.65% for the SK-dyed hair group.

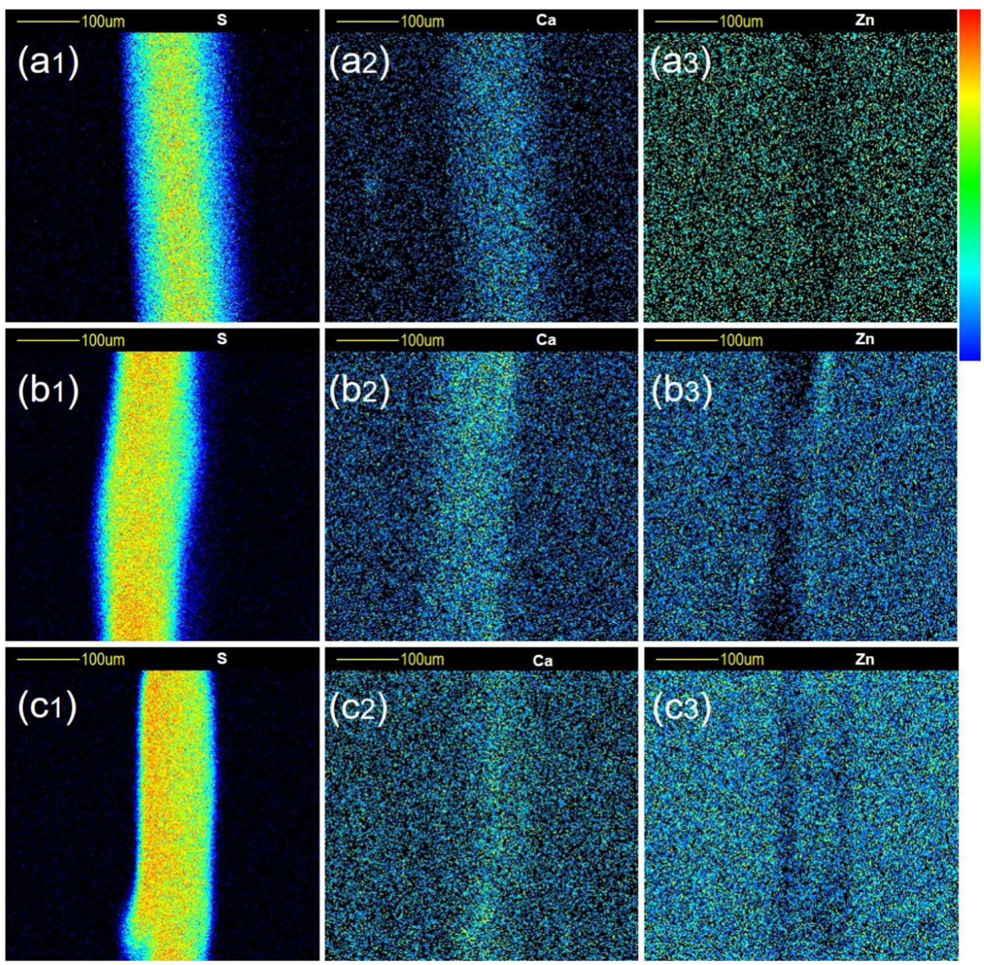

Fig. 6 The elemental distribution along the longitudinal section at the end of the hair. (a1)–(a3) blank hair, (b1)–(b3) hair dyed by GM, and (c1)–(c3) hair dyed by SK. In the color bar, the dark region represents the count of zero, and the red region represents the highest count

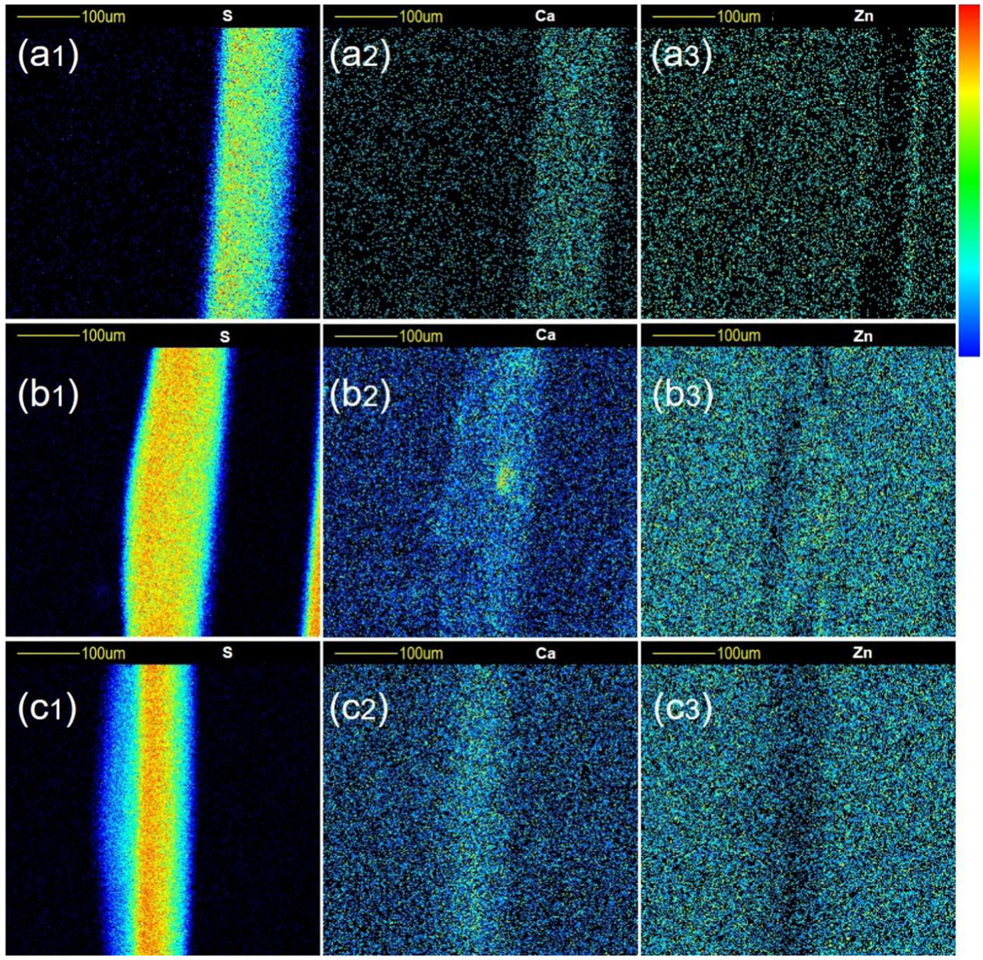

Fig. 7 The elemental distribution along the longitudinal section in the middle of the hair. (a1)–(a3) blank hair, (b1)–(b3) hair dyed by GM, and (c1)–(c3) dyed hair by SK. In the color bar, the dark region represents the count of zero, and the red region represents the highest count

For further investigating the trace metallic elements, we tested the contents of Cd, Cr, Ni, Pb, Sb, Mn, Ti, and Rb by ICP-MS, considering that it has a lower detection limit and higher sensitivity. Figure 3 shows that most trace metallic elements increased after the hair dye process, except for Cd and Ni. As shown in Table 2, the content of Pb increased to 18.29 and 17.28 $\mu g.g^{-1}$ from 3.65 $\mu g.g^{-1}$ for the GM- and SK-dyed hair group, respectively. Also, the content of Rb rose to 4.23 from 0.70 $\mu g.g^{-1}$ for the GM-dyed hair group and to 3.85 $\mu g.g^{-1}$ for the SK-dyed hair group. Apart from Pb and Rb, Mg exhibits slight accumulation after dyeing. The content of Ni decreased acutely from 12.81 to 3.06 $\mu g.g^{-1}$ for the GM-dyed hair group and it decreased to 2.40 $\mu g.g^{-1}$ for the SK-dyed hair group.

Therefore, the similarity of the change in metal concentration was the accumulation of Cd, Cr, Pb, Sb, Mn, Ti, and Rb and the reduction of Fe, Zn, Mg, Ca, Al, K, and Na. Note that there were also differences between these two groups. The extent of accumulation of heavy metals and the reduction of essential metallic elements were higher in the GM-dyed hair group than in the SK-dyed hair group. We can conclude that different dyes result in different degrees of metallic concentration change, although the overall trend is consistent.

SEM-EDS

The outer layer of the hair was characterized by SEM to have an overall view about the physical influence of the hair dye on the hair fibers. [36, 37] The representative SEM images were chosen from three fibers of each sample. As shown in Fig. 4a, the untreated hair has intact smooth cuticle edges and the scale height was almost 8–10 μm. However, visible changes can be observed after dyeing at the edge of the cuticle and the scales appear to be fragmented and thinner [Fig. 4b, c]. The hair scale height is about 10–17 μm for the GM-dyed sample while it is approximately 8–15 μm for the SK-dyed hair group. This observation implies that the dyeing procedure possibly affected the structure of cross-linking proteins. [38] Moreover, the hair dyed by GM exhibits a thinner cuticle and more severe fragmentation of the cuticle cells than the SK-dyed sample. Therefore, the obtained result revealed that hair dye may have different degrees of impact on the outer layer of the hair.

The hair specimens were studied by EDS in order to further acquire the additional information of the metallic element change after dyeing. [39] As shown in Fig. 5, there was almost no Pb in the original hair or too little to observe. On the contrary, we can see a clear profile of Pb distribution in Fig. 5(b2), (c2). Meanwhile, other metallic elements were not detected. This observation may be associated with the fact that the content of Pb concentrated at the outer layer of the hair after dyeing treatment, and other metallic elements with large quantity may distribute elsewhere in the hair.

2D-PIXE

In order to investigate the element distribution in the hair, the longitudinal and cross sections of the hair were analyzed by using 2D-PIXE. Figure 6 shows the element distribution along the longitudinal section at the end of the hair. A clear outline of S and Ca is observed for the blank hair, implying that the content of S and Ca is relatively high. However, the profile of Ca becomes vague after dyeing. This observation suggests that the content of Ca in hair decreases after dyeing, which is consistent with the ICP-OES results. Besides, the profile of Zn can hardly be observed due to the severe background interference. In order to identify whether there were differences in various parts of the hair, the middle part of the hair was also scanned and the results are shown in Fig. 7. S, Ca, and Zn exhibit similar profiles in the middle and the end part of the hair. Therefore, the obtained results confirmed that element distribution exhibits no apparent differences between the end and the middle part of the hair. In addition, the distribution of Ca and Zn was uniform along the hair and not accumulated in a specific part.

After probing the distribution in the longitudinal, we further investigated the element distribution in the cross section and the results are shown in Fig. 8. It can be seen that the longitudinal profile of Zn was clear in the blank hair, but it became vague after dyeing (Fig. 8(a3)–(c3)). This observation was consistent with the results in the longitudinal section. As to Fe, the blurry profile became even worse after dyeing, implying its content further decreased after dyeing (Fig. 8(a4)–(c4)). In contrast, a fairly high content of Al was observed, which was caused by the pollution from the blade in the machine (Fig. 8(a1)–(c1)). To eliminate this unconformity, we changed the tool to a ceramic knife to cut off the thin slice, and the results showed that the content of Al was low in the hair. Therefore, we can conclude that the metallic elements were homogeneously distributed along the cross and longitudinal sections, and that the content of Zn and Fe are higher in the cross section than in the longitudinal section.

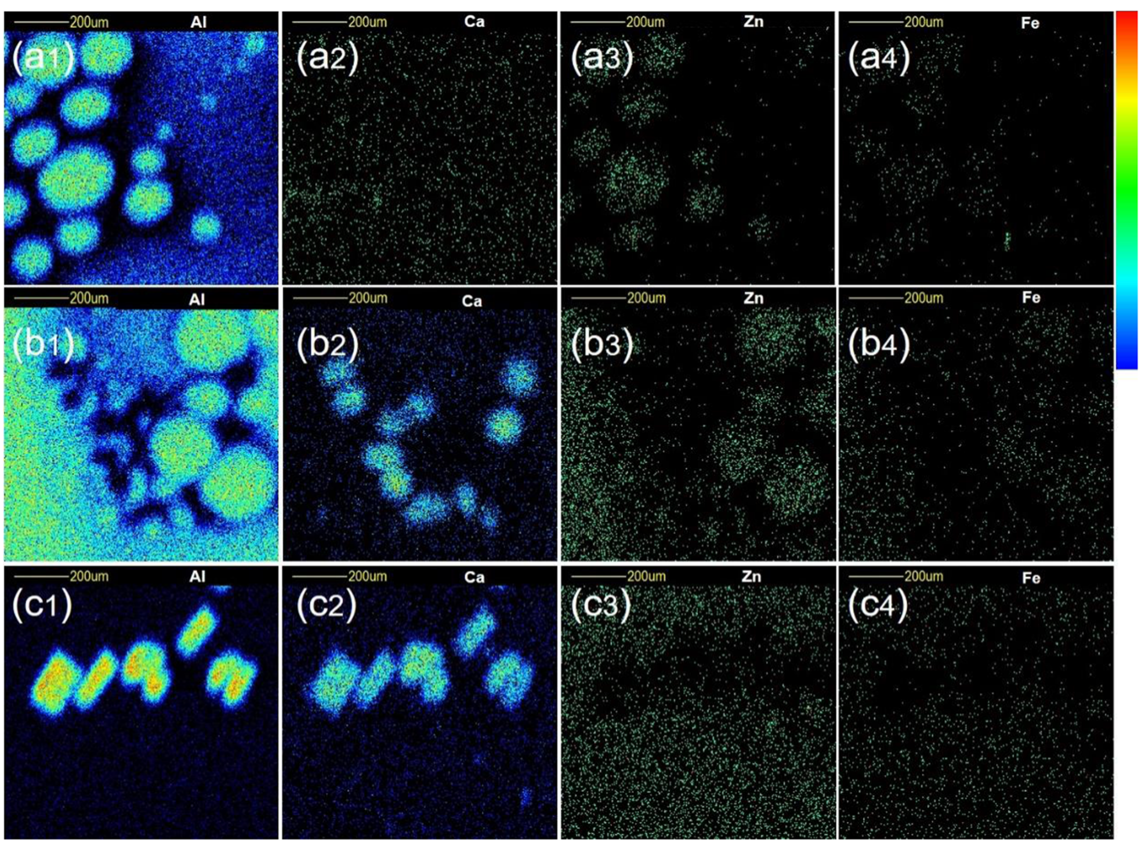

Fig. 8 The elemental distribution in the hair in the cross section. (a1)–(a4) blank hair, (b1)–(b4) hair dyed by GM, and (c1)–(c4) hair dyed by SK. In the color bar, the dark region represents the count of zero, and the red region represents the highest count

Conclusions

This work investigated the morphological change and the content variation of metals after dyeing by ICP-OES, ICP-MS, SEM-EDS, and 2D-PIXE. The ICP results showed that the content of Pb, Mn, and Rb increased after dyeing, whereas the content of Ni decreased. In addition, Pb, originating from the dye product, concentrated near the outer layer of the hair. 2D-PIXE results demonstrated that the metallic element distributed evenly along the longitudinal and cross sections of the hair for both the blank and dyed hair. Besides, the distribution of Ca and Zn was uniform, and they did not accumulate in a specific part of the hair. This study provides a method of detecting the metallic elements from different perspectives and can contribute to the research of hair dye.

Funding This study was funded by National Natural Science Foundation of China (grant No. 20945002) and Shanghai Natural Science Foundation (grant Nos. 16142202400, 09ZR1403900).

Compliance with Ethical Standards

All procedures performed in studies involving human participants were approved by the Institutional Review Board of School of Life Sciences, Fudan University (No. BE1923). All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of Interest The authors declare that they have no conflict of interest.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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