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Microchemistry of Single Hair Strands Below and Above the Scalp: Impacts of External Contamination on Cuticle and Cortex Layers

Jennie R. Christensen[1] · Geriene O. LaBine[1]

Abstract

External contamination of hair is the most significant challenge to it becoming an accepted matrix for monitoring endogenous metal exposure and nutritional deficiency. Here we use laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to quantify elemental concentrations in hair strands below and above the scalp in the cuticle and cortex layers to determine the extent of external contamination in a reference population. Evidence of hair strand contamination occurred for barium, calcium, iron, magnesium, and strontium in both the outer cuticle and the inner cortex layers, with increasing concentrations from root to tip. Aluminum, boron, copper, lead, and manganese showed significant contamination in the cuticle layer only, suggesting some protection of the inner cortex. Phosphorus and potassium decreased outside the scalp suggesting loss by washing, while chromium, mercury, selenium, sodium, titanium, and zinc showed no evidence of loss or external contamination above the scalp. The results clearly show that for most elements, hair chemistry above the scalp is unreliable for use in interpretation of endogenous exposures or deficiencies, and that the below-scalp portion provides a more accurate monitoring tool. This is the first paper to provide a reference range of elemental hair chemistry that is not impacted by the external environment.

Keywords Mercury · Lead · Zinc · Cortex · Cuticle · LA-ICP-MS

Introduction

Hair strands are comprised of α-keratin with a helical structure and comprised of an outer cuticle layer and an inner cortex layer. Dry hair is comprised of mainly proteins (90–97%), lipids (2%), and the remainder being made up of nucleic acids, carbohydrates, and inorganic substances [1]. Dominant elemental composition is approximately 50% carbon, 7% hydrogen, 22% oxygen, 16% nitrogen, and 4–5% sulfur by weight [1].

Due to the high sulfur (S) content in hair, and thus high binding affinity for many elements, hair has been used to quantify trace elements, in terms of potential deficiency of nutritional elements or toxic exposure to heavy metals [2–6]. Hair is an attractive matrix to assess nutritional or environmental exposure to toxic metals, as: (1) it is easy to sample and transport; (2) there are no preservation requirements; (3) the material is non-biohazardous; (4) it is relatively painless to collect; (5) some elemental concentrations are higher in hair compared to blood [7]; (6) hair provides a stable signal over time, as opposed to blood that can be highly variable [8]; and (7) hair has the potential to provide a good link to trace element levels following exposure and absorption into the body [9, 10], since the elements bind to the hair root from the blood supply during growth [11]. Depending on their utility for bodily function or their toxic effects, deficiency or excessive levels of these elements can induce health problems [2, 12–14], so finding fast, easy, non-invasive, and accurate techniques to monitor their levels in the human body is essential.

To date, conventional hair monitoring for trace elements has used bulk hair cut from the scalp, digested, and analyzed using atomic absorption spectrometry (AAS) [15, 16], inductively coupled plasma mass spectrometry (ICP-MS) [7, 17–19], inductively coupled plasma atomic emission spectrometry (ICP-AES) [20], and inductively coupled plasma optical emission spectrometry (ICP-OES) [21–23].

Additionally, standard reference ranges of elemental concentrations in a healthy reference population relying on bulk

✉ Jennie R. Christensen
  jennie.christensen@trichanalytics.com

1 TrichAnalytics Inc., #207, 1753 Sean Heights, Saanichton, BC V8M 0B3, Canada

analysis are currently limited to the specific study population for research or the commercial laboratory conducting hair mineral analysis to the public as a service [24, 25]. The variability in concentration for individual elements within presumably healthy reference populations is high [7, 8, 17–20, 26–28]. This is due to several variables that could include different washing techniques, analytical equipment, calibration standards, hair sampling methods, and potentially, physiological differences (e.g., gender, age, hair color, ethnicity) [7, 18, 25, 27]. The hair reference ranges calculated to date have been used to characterize diets [10, 29], evaluate nutritional deficiencies in individuals or populations [30, 31], toxic metal exposures [6, 9, 32, 33], or to monitor exposure in people living near anthropogenic contamination (e.g., smelter) [34–36]. Hair elemental chemistry has even been used to characterize and correlate to health issues [37], such as diabetes [38], autism [39], kidney disease [40], hypertension [41], and cancer [42, 43]. To interpret hair results as it pertains to an individual's health status would therefore rely entirely on appropriate reference ranges, and accuracy is integral [8].

Unfortunately, the major challenge of hair analysis is the inability to differentiate between trace metals incorporated into the hair matrix through endogenous (through blood, internal physicochemical processes) and exogenous (environmental contamination, air, water, hair treatments) routes [9, 44, 45]. While monitoring endogenous trace metals is the ultimate goal of hair analysis, once hair emerges from the scalp, it is exposed to hair treatments, air quality, dust particles, and water, all of which can have potentially significant impact on elemental chemistry of the hair being analyzed. Since almost all analysis of hair to date is conducted on hair collected from above the scalp, every study, reference range, comparison, and interpretation in the current literature has been potentially compromised by external contamination. Some researchers and laboratories wash the hair, and although there are a variety of techniques that provide varying success, not all elements or contamination is typically removed [46], and in some cases, some elements endogenously deposited are removed through washing [47, 48]. While most studies suggest some caution should be applied due to potential contamination of hair, there is a clear lack of literature quantifying the extent of contamination of hair strands above the scalp, either when contamination is expected (e.g., occupational worker) or not (e.g., reference population).

Accordingly, the only portion of a hair strand that is not subject to direct external contamination lies below the scalp within the follicle: the root portion. As conventional hair analysis requires anywhere from 0.1 to 1 g of bulk hair, plucking enough hairs to obtain enough root sample for bulk analysis is prohibitive. This may explain why there is such a lack of studies on the concentrations of trace elements in the root portion of the hair strand. Analysis of hair at a microscale using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) may provide a solution, as this technique can allow for smaller tissue volumes, such as a single hair strand, and has the potential to focus analysis on sub-portions within a single hair strand [9, 10, 49–54].

There is an obvious gap in the collective scientific realm regarding reference concentrations of all elements in an uncontaminated setting (i.e., below the scalp), and conversely, a lack of quantification and differentiation of the exogenous contamination that impacts hair chemistry interpretation [9, 52]. To improve understanding of where elements accumulate in the hair and to differentiate between endogenous and exogenous routes of incorporation, the main objectives of this paper include as follows: (1) determine which elements are consistently detected in hair below and above the scalp; (2) quantify the differences between concentrations in the cuticle and cortex below the scalp to assess natural binding affinity; (3) evaluate the level of contamination from external sources through comparisons of hair regions below the scalp (protected) and those above the scalp (near and distal), including if the contamination impacts the protected inner cortex layer; and (4) provide reference ranges of trace elements, free from external contamination, for the first time.

Methods

Sample Collection

Hair samples from the scalp (with root bulb intact) were collected from 61 adult (> 18 years old) individuals following informed, written, and signed consent, and approved by the ethics board at the University of Saskatchewan (BIO17-55). All aspects of the project follow the ethical guidelines for human research [55]. Hair color ranged from light brown to dark brown/black. Other demographic information (e.g., age, ethnicity, gender) regarding participants was unavailable due to the anonymous nature of the project.

Different Regions of Hair

Three regions of the hair strand from each individual were analyzed separately to represent different conditions surrounding potential external contamination. Region 1 was selected at 1 to 2 mm from the root bulb ("below scalp") and represents the keratinized portion of hair not exposed to any form of external contamination. This region represents the "reference" by which the other two regions were compared. Region 2 was selected at 10 to 11 mm from the root bulb ("near scalp") and represents the keratinized portion of hair above the scalp and exposed to external contamination for

a short period of time (less than 2 weeks). Region 3, 39 to 40 mm from the root bulb ("distal scalp"), represents the portion of hair above the scalp and exposed for a longer period (approximately 3.5 to 4 months) to external contamination. Exposure time periods are based on an average hair growth rate of 1 cm per month.

LA-ICP-MS and Standards, Double Ablation

Hair strands were washed with acetone and distilled water to remove the outer root sheath, oils, sweat, and any loosely adhered particles/dust. They were then air-dried and staged on glass microscope slides using double-sided adhesive tape. Samples were analyzed for 26 elements (Table 1) using an iCAP RQ series ICP-MS (ThermoFisher Scientific, Canada), connected to an NWR-266 (Elemental Scientific Lasers, USA) laser ablation system with helium gas at 800 mL/min, located at the ISO 17025:2017 accredited TrichAnalytics Inc. (Saanichton, British Columbia, Canada) laboratory.

Analysis was performed using a double ablation technique, meaning hair strands and reference material were ablated with the laser twice. Therefore, each of the three regions described above was ablated: (1) first, as a "burn" which had a larger spot size and lower power and represents the outermost layer of the hair, namely the cuticle; and (2) second, as a "double ablation" which had a smaller spot size within the line scan of the initial "burn," and at a higher power, and represents the inner layer of the strand, namely the cortex. Accordingly, LA-ICP-MS settings were as follows: burn — 60-µm spot size, power at 2 J/cm^2, 50 µm/s laser speed, and a frequency of 20 Hz; double ablation — 30-µm spot size, 10 J/cm^2 power, 50 µm/s laser speed, and 20-Hz frequency.

Test samples were run in sample sets that included the certified reference material (CRM) for calibration, namely DORM-4 (National Research Council of Canada). The CRM was analyzed alongside all hair samples to facilitate conversion of elemental concentrations from signal intensities, calculate detection limits, and determine accuracy and precision. All elements were corrected to an internal standard, sulfur (4%), to account for any variability in ablated tissue volumes. Mass spectrometer signal intensity was acquired using Qtegra software (ThermoFisher Scientific, Version 2.8.3170.309).

Data Quality

Using the CRM to assess accuracy and precision, the average accuracy ranged from 95 to 110% for reporting the cuticle layer and from 101 to 120% for the cortex layer. Precision ranged from 3.2 to 27% in the cuticle layer, with the lowest precision for titanium (Ti), which was at a concentration close to the detection limit. Similarly, precision ranged from 5.2 to 25% for reporting the cortex layer, with the lowest precision for cadmium (Cd), as the concentration was close to the detection limit.

Statistical Analysis

Analysis of variance (ANOVA) with repeated measures was used to test for differences in each elemental concentration among regions (the repeated measure: below scalp, near scalp, and distal scalp in each of the cuticle and cortex layers). Concentrations below detection were converted to values equal to the detection limit prior to statistical analysis. Data were $\log_{10}$ transformed prior to statistical analysis due to non-normal distribution and unequal variances in most elements. For all statistics, alpha was set to 0.05, but was corrected using Bonferroni correction due to the large number of elements tested. Reference intervals were calculated for each element in each hair layer as the median, interquartile range (IQR; 75th percentile minus 25th percentile), and 5th and 95th percentile in the below scalp portion of the cuticle and cortex. Statistics were conducted using XLSTAT (version 2022.3.2). Standard box plots were employed to visually present the elemental concentrations, where the box represents the interquartile range (IQR; first and third quartile), the line within the box denotes the median, the *X* in the box is the mean, the bars represent the minimum and maximum, and with circles representing outliers.

Results and Discussion

Elemental Detectability

Low detectability ($<50\%$) for arsenic (As), cadmium (Cd), cobalt (Co), molybdenum (Mo), nickel (Ni), uranium (U), and vanadium (V), occurred in both cuticle and cortex in the 61 hairs (Table 1). This suggests that these elements do not readily accumulate in hair, the individuals tested have experienced no exposure to these elements, or the detection limits at these laser settings were too high. This is particularly evident for arsenic (As), where there were no detections, and molybdenum (Mo) where there was only one detection, occurring in the cuticle layer. Other studies have obtained detectable concentrations in human hair for these elements [7, 17–21, 26, 28], and some of their reported concentrations are lower than the detection limits in this study. Therefore, using a higher resolution ICP-MS, or increasing the laser power, and/or spot size may improve detection limits of these elements moving forward. Due to the low detectability of these elements, they were not explored further in this study.

Calcium (Ca), chromium (Cr), copper (Cu), magnesium (Mg), mercury (Hg), phosphorus (P), strontium (Sr), sulfur

Table 1 Percentage of detectable concentrations of each element in the cuticle and cortex layers of hair below, near and distal scalp regions. Detectable concentrations for sulfur (S) was 100% in all layers and regions; not presented here as it was used as the internal standard

Element	Layer/Region							
	Cuticle				Cortex			
	Below (%)	Near (%)	Distal (%)	Trend: below to distal	Below (%)	Near (%)	Distal (%)	Trend: below to distal
Aluminum (Al)	98	100	100	-	5	8	11	⟶
Arsenic (As)	0	0	0	-	0	0	0	-
Barium (Ba)	61	90	100	⟶	10	28	49	⟶
Boron (B)	100	100	100	-	64	89	92	⟶
Cadmium (Cd)	5	11	11	⟶	0	0	3	-
Calcium (Ca)	100	100	100	-	100	100	100	-
Chromium (Cr)	100	100	100	-	100	100	100	-
Cobalt (Co)	2	10	30	⟶	0	0	2	-
Copper (Cu)	100	100	100	-	100	100	100	-
Iron (Fe)	100	100	100	-	67	75	74	⟶
Lead (Pb)	97	100	100	-	46	61	70	⟶
Magnesium (Mg)	100	100	100	-	100	100	100	-
Manganese (Mn)	56	84	95	⟶	5	16	39	⟶
Mercury (Hg)	92	92	93	-	90	87	90	-
Molybdenum (Mo)	2	2	0	-	0	0	0	-
Nickel (Ni)	10	34	61	⟶	0	0	3	-
Phosphorous (P)	100	100	100	-	100	100	100	-
Potassium (K)	100	93	98	-	72	41	54	⟵
Selenium (Se)	93	92	95	-	72	72	57	⟵
Sodium (Na)	100	92	97	-	77	44	54	⟵
Strontium (Sr)	100	100	100	-	100	98	100	-
Titanium (Ti)	100	100	100	-	89	85	79	-
Uranium (U)	11	21	31	⟶	0	0	0	-
Vanadium (V)	2	15	23	⟶	0	0	3	-
Zinc (Zn)	100	100	100	-	100	100	100	-

Note: Gray cells denote < 50% detection. Arrows indicate the direction of a trend in detection

(S), titanium (Ti), and zinc (Zn) were almost always detected in both layers, particularly below the scalp, indicating that there is a strong binding affinity and/or incorporation of these elements into the structure of the growing hair through endogenous sources at detectable levels.

Percent detections increased in the cuticle layer for barium (Ba), cadmium (Cd), cobalt (Co), manganese (Mn), nickel (Ni), uranium (U), and vanadium (V) further along the hair strand towards the distal scalp region compared to below scalp. The increasing detectability of elements towards the distal scalp in the cuticle layer suggests contamination of the hair strand is increasing further away from the protection of the scalp. In the cortex layer, there were also some increases in detectable elements from root towards the distal scalp: aluminum (Al), barium (Ba), boron (B), iron (Fe), lead (Pb), and manganese (Mn). Conversely, potassium (K) and sodium (Na), and to some extent, selenium (Se) and titanium (Ti), had lower detectability beyond the scalp, particularly in the cortex.

Cuticle and Cortex Elemental Chemistry

The cuticle consistently had higher concentrations for most elements compared to the cortex in all regions ($p < 0.0028$), except for chromium (Cr) (i.e., the only element higher in the cortex), and manganese (Mn), mercury (Hg), selenium (Se), and titanium (Ti), which were relatively uniform between layers (Supplementary Information Table S1, "Cuticle vs Cortex"). Kempson and Skinner [47] also showed aluminum (Al), calcium (Ca), copper (Cu), sodium (Na), iron (Fe), magnesium (Mg), and potassium (K) concentrations were higher in the cuticle compared to the cortex. In ancient Chilean mummies, Bartkus et al. [56] used LA-ICP-MS to analyze cross sections of the hair strands and found that lead (Pb) was higher in the cuticle, as opposed to the cortex which had virtually undetectable concentrations. Where mercury (Hg) was uniform between the hair layers in this study, Stadlbauer et al. [46] reported a higher mercury concentration in the cuticle layer compared to the cortex; however, their mercury concentrations were > 40 mg/kg in the cuticle and ~ 10 mg/kg in the cortex, compared to those in this study that were 5- to 20-fold lower, respectively. This may suggest higher exposure to mercury endogenously may increase the differentiation between cuticle and cortex concentrations, or that in their study there was considerable external contamination from a mercury source. Abad et al. [57] clearly demonstrated that inorganic mercury vapor can adsorb permanently to the hair cuticle causing higher concentrations in the cuticle relative to the cortex.

Influence of External Contamination on Cuticle and Cortex Elemental Concentrations

The concentrations of five elements along the strand showed significant contamination above the scalp (Supplementary Information Table S1, "External Influence") in both the cuticle and the cortex, namely barium (Ba), calcium (Ca), iron (Fe), magnesium (Mg), and strontium (Sr) ($p < 0.0001$; Fig. 1). Barium showed the greatest affinity for contamination of the hair strand outside the scalp, where the distal portion was 51-fold and 84-fold higher in the cuticle and cortex, respectively, compared to below the scalp (Fig. 1). The strontium (Sr) had the second highest affinity, followed by magnesium (Mg), calcium (Ca), and iron (Fe). The near scalp portion has consistently lower contamination than the distal portion for these elements suggesting either some protection nearer the scalp, or that the distal portion has been exposed to the environment for a longer period of time. The contamination of both hair layers suggests that there was penetration of these elements into the hair matrix. As hair is porous, these elements may have been integrated from a more aquatic, exogenous source (e.g., water).

Five elements, aluminum (Al), boron (B), copper (Cu), lead (Pb), and manganese (Mn) exhibited significant contamination in the cuticle layer only ($p < 0.0001$), with no significant contamination in the cortex (Fig. 2). This was indicated by a significant region × layer interaction ($p < 0.0001$), with the concentration difference between the cuticle and cortex layers increasing towards the distal scalp compared to below scalp (boron, B, did not have a significant interaction, $p = 0.633$). This result not only suggests contamination by these elements, particularly in the cuticle, but that the cuticle acts to protect the cortex layer from these contaminants to some degree. When dosed with arsenic (As) and mercury (Hg) in solution, the cuticle provided some protection from accumulation in the cortex as indicated by elevated concentrations in the cuticle compared to the cortex [58]. Here, lead (Pb) had the greatest contamination effect with the distal region 13-fold and 5.2-fold higher in the cuticle and cortex, respectively, relative to the below scalp region. Manganese (Mn) had the second highest contamination, followed by copper (Cu), aluminum (Al), and boron (B) with no contamination evident in the cortex. As the cortex appears mostly protected from accumulation of these elements, their exogenous contamination source may be more airborne (e.g., dust), and thus not infiltrating the cuticle to the same extent as those other five elements that readily accumulate in both layers.

Overall, 10 elements show significant contamination from external sources once the hair emerges from the scalp, particularly in the outer cuticle layer (Figs. 1 and 2). Kempson
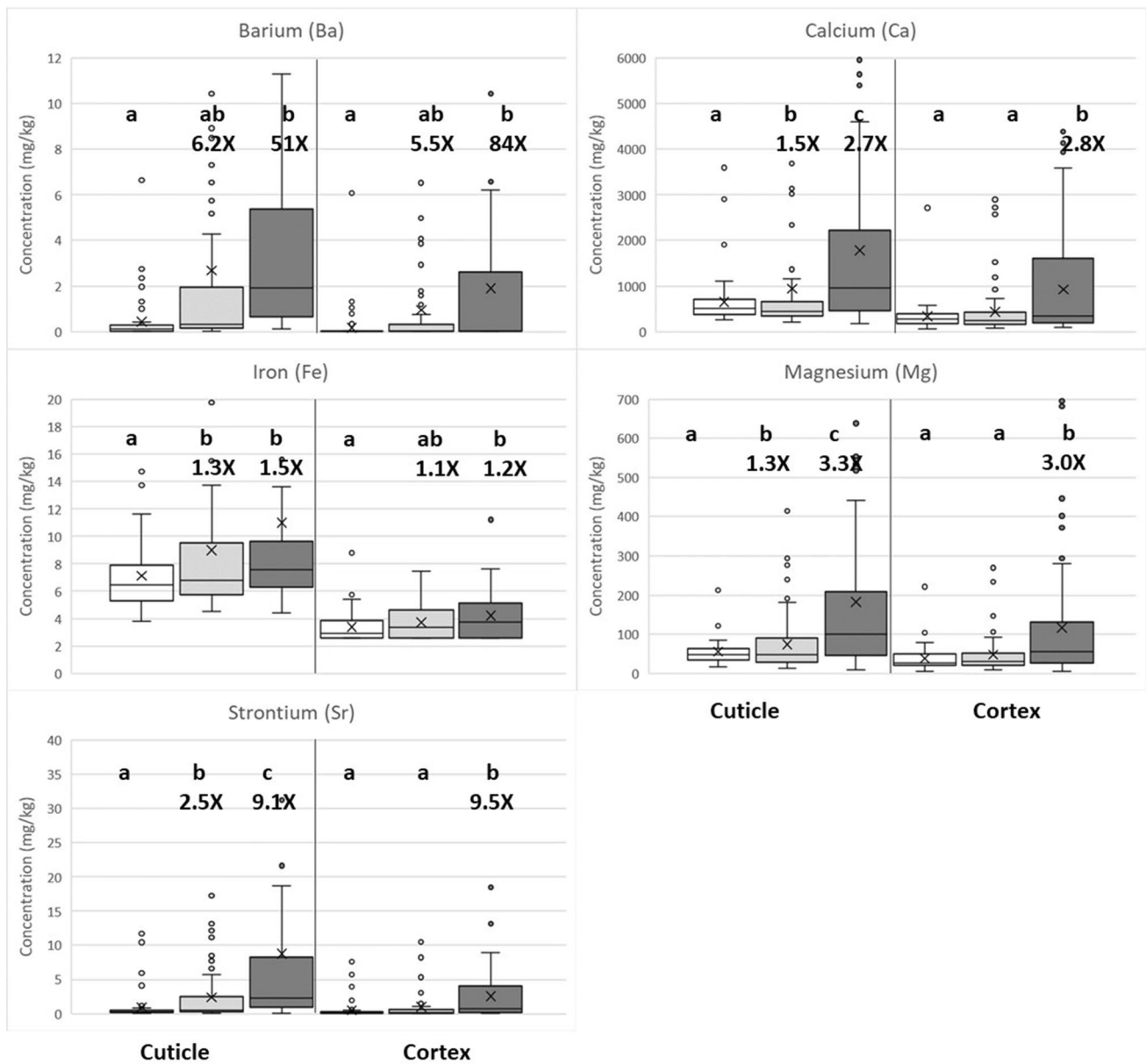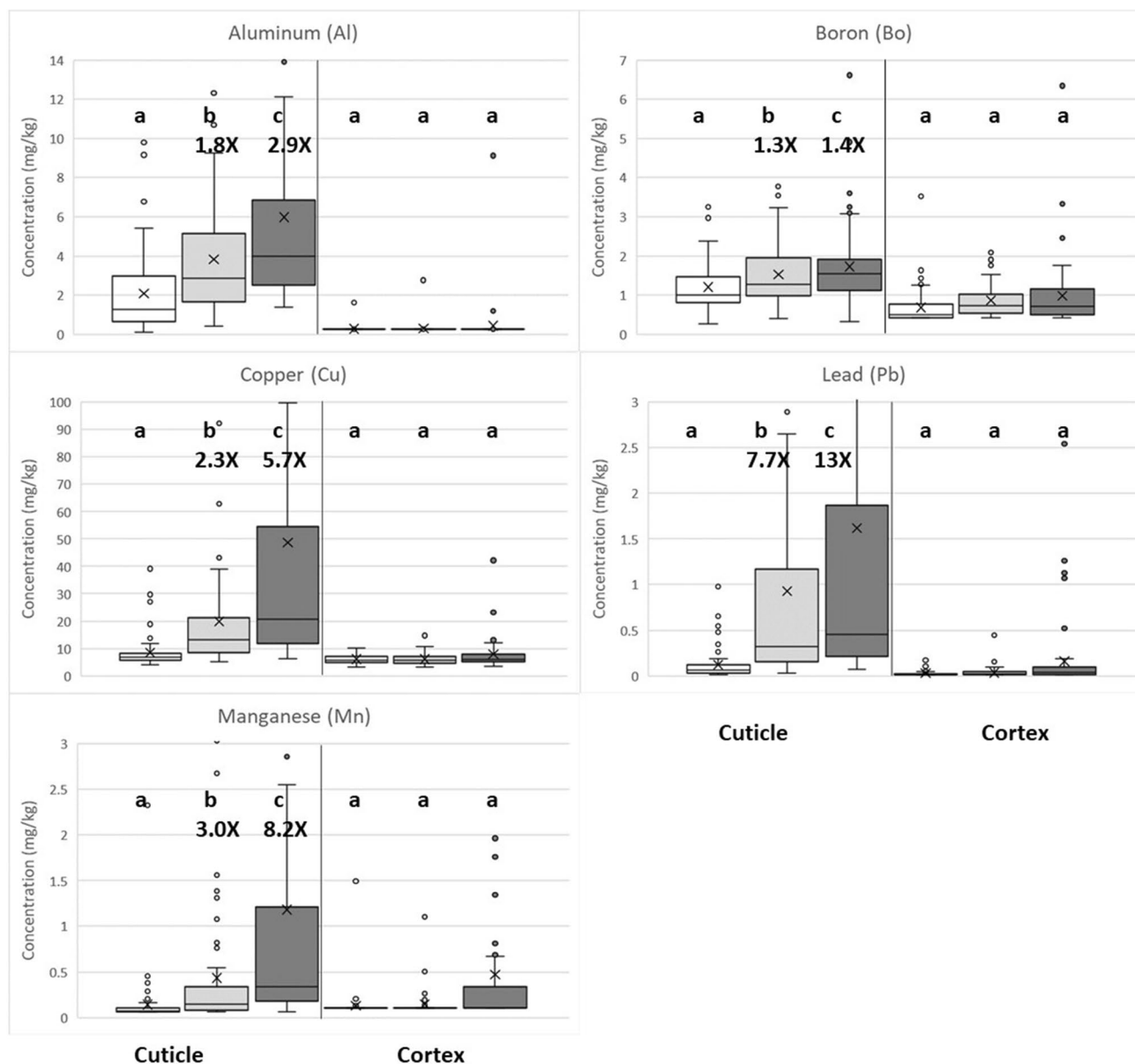
Fig. 1 Elements showing significant contamination in both the cuticle and cortex layers above the scalp (near = light gray; distal = dark gray), relative to the protected below-scalp region (white). Significant differences among hair regions within the cuticle and cortex layers are depicted by letters (a, b, c) above the boxplots. Numerical values above box plots represent the ratio of near scalp and distal scalp concentrations relative to below-scalp concentrations, within each layer

and Skinner [47] also reported increasing concentrations from the root towards the distal tip for aluminum (Al), calcium (Ca), iron (Fe), magnesium (Mg), and titanium (Ti). Christensen et al. [9] reported significant external lead (Pb) contamination in both occupationally-exposed and reference individuals in portions of hair above the scalp. Bos et al. [59] presented similar results for calcium (Ca). Using the synchrotron X-ray fluorescence, Kempson et al. [44] determined calcium (Ca), iron (Fe), and lead (Pb) to be significant external contaminants in hair exposed to the environment,

but not copper (Cu) or zinc (Zn). Trunova et al. [60] concluded that calcium (Ca), chromium (Cr), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), potassium (K), and strontium (Sr) were unreliable in hair due to contamination, but considered copper (Cu), selenium (Se), titanium (Ti), and zinc (Zn) acceptable in hair.

Not all elements displayed impacts from external contamination, but rather were lost above the scalp. Potassium (K) concentrations were significantly lower outside the scalp compared to below scalp in the cuticle layer (Fig. 3),
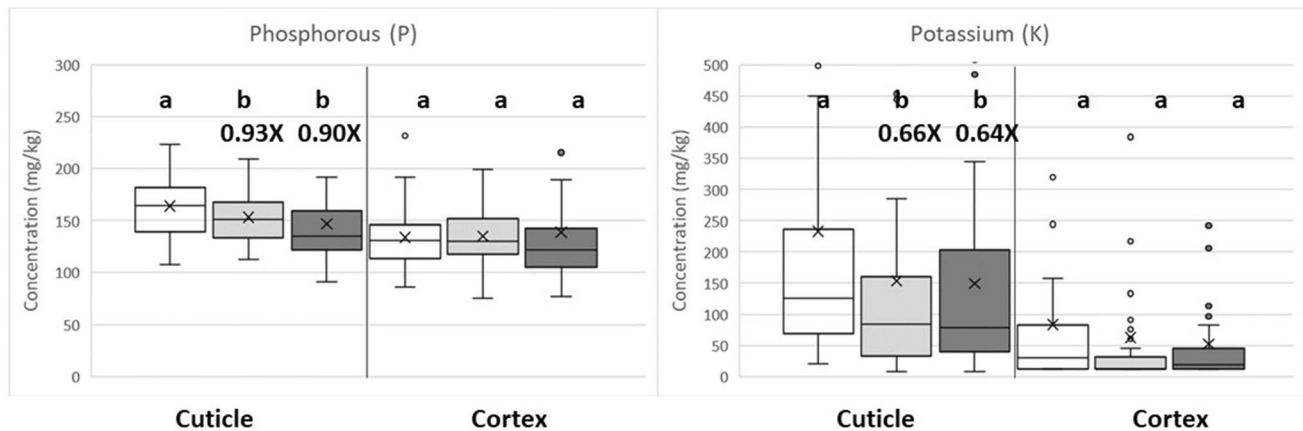
Fig. 2 Elements showing significant contamination in the cuticle layer only (cortex layer protected to some extent) above the scalp (near = light gray; distal = dark gray), relative to the protected below-scalp region (white). Significant differences among hair regions within the cuticle and cortex layers are depicted by letters (a, b, c) above the boxplots. Numerical values above box plots represent the ratio of near scalp and distal scalp concentrations relative to below-scalp concentrations, within each layer

suggesting water may leach this element from the exposed strand through washing. In this case, concentrations above the scalp would provide an underestimation of the individual's endogenous levels. Kempson and Skinner [47] also determined that potassium (K) concentrations in hair decreased following emergence from the scalp, most likely from washing. Phosphorous (P) also showed a decrease outside the scalp in the cuticle layer only, which may suggest there was some cuticle loss or damage.

There were no indications of contamination or loss for chromium (Cr), mercury (Hg), selenium (Se), sodium (Na), titanium (Ti), or zinc (Zn) (Fig. 4) with concentrations rather steady in both the outer cuticle and inner cortex layers. These elements may not be present as an exogenous source in a reference population, as other studies suggest at least some of these elements will contaminate the hair if present [58, 60]. Noguchi et al. [58] dosed hair with various elements including chromium (Cr), mercury (Hg), selenium (Se), and zinc (Zn), with all showing high binding affinity to the exposed hair, with mercury having the highest binding affinity of all elements tested.

Fig. 3 Elements showing significant loss in the cuticle layer above the scalp (near = light gray; distal = dark gray), relative to the protected below-scalp region (white). Significant differences among hair regions within the cuticle and cortex layers are depicted by letters (a, b) above the boxplots. Numerical values above box plots represent the ratio of near scalp and distal scalp concentrations relative to below-scalp concentrations, within each layer

The considerable number of elemental concentrations in the hair strand impacted outside the scalp in a reference population highlights the ubiquitous nature of the elements in the environment of the general public. It clearly demonstrates that at least for 12 elements — aluminum (Al), barium (Ba), boron (B), calcium (Ca), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), and strontium (Sr), including phosphorus (P) and potassium (K) that decrease outside the scalp — analysis of any portion of hair above the scalp should not be used to infer endogenous sources.

The cuticle layer (approximately 0.3 to 0.5 µm) of the hair strand is comprised of overlapping scale-like cells, and the cortex is comprised of elongated cortical cells of approximately 100-µm long, made up of macrofibrils [61]. In addition to structural differences between the cuticle and cortex, there are chemical differences, particularly associated with amino acid composition [1]. The cuticle layer contains over two-fold the amount of the amino acid cysteine [1], which is thought to be the main contributor to elemental binding due to its sulfur (S) content. The types of keratin are also different: there is a myriad of members of the keratin family in hair, each of which has localized placements that can differ between the hair layers [62]. While many keratin types are shared between the cuticle and cortex, only the cuticle layer contains K32, K40, and K82 keratins [62]. These keratin types may have higher binding affinity for some elements, and may explain the significant differences between cuticle and cortex elemental concentrations for so many elements. Differences in elemental concentrations between cuticle and cortex hair layers were examined using double ablation LA-ICP-MS by Bowman et al. [63] in reference adult males and found that 16 elements had higher concentrations in the cuticle layer, including aluminum (Al), arsenic (As),

barium (Ba), cadmium (Cd), calcium (Ca), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), nickel (Ni), potassium (K), sodium (Na), strontium (Sr), and zinc (Zn), while five elements were similar between layers: chromium (Cr), phosphorus (P), mercury (Hg), selenium (Se), and sulfur (S). They also documented contamination of the inner cortex layer in hairs from individuals that were submerged in seawater and sediment for > 170 years for aluminum (Al), arsenic (As), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), mercury (Hg), nickel (Ni), strontium (Sr), and vanadium (V), although this type of contaminant exposure in the environment for hair strands is extreme, and the hair strands were highly degraded making the cuticle layer less protective [63].

In nails, also a keratin-based tissue used for monitoring elemental levels, differences between layers in elemental concentrations are also evident. Rodushkin and Axelsson [53] measured higher concentrations of most elements in the harder, surficial (dorsal) layer of the nail clippings relative to the inner layer, although they contributed these differences to external contamination. Christensen et al. [64] also recorded higher concentrations of lead (Pb) and copper (Cu) in the dorsal layer of the nail from a deceased crewman of the 1845 Franklin Expedition, but no differences for zinc (Zn) among layers.

Elemental Concentration Reference Ranges in Uncontaminated Hair

As a result of significant influence of the external environment on hair chemistry outside the scalp for most elements, the reference ranges were derived from the cuticle and cortex layers below the scalp only (Table 2). It is not possible to compare these reference values to other studies, as there are
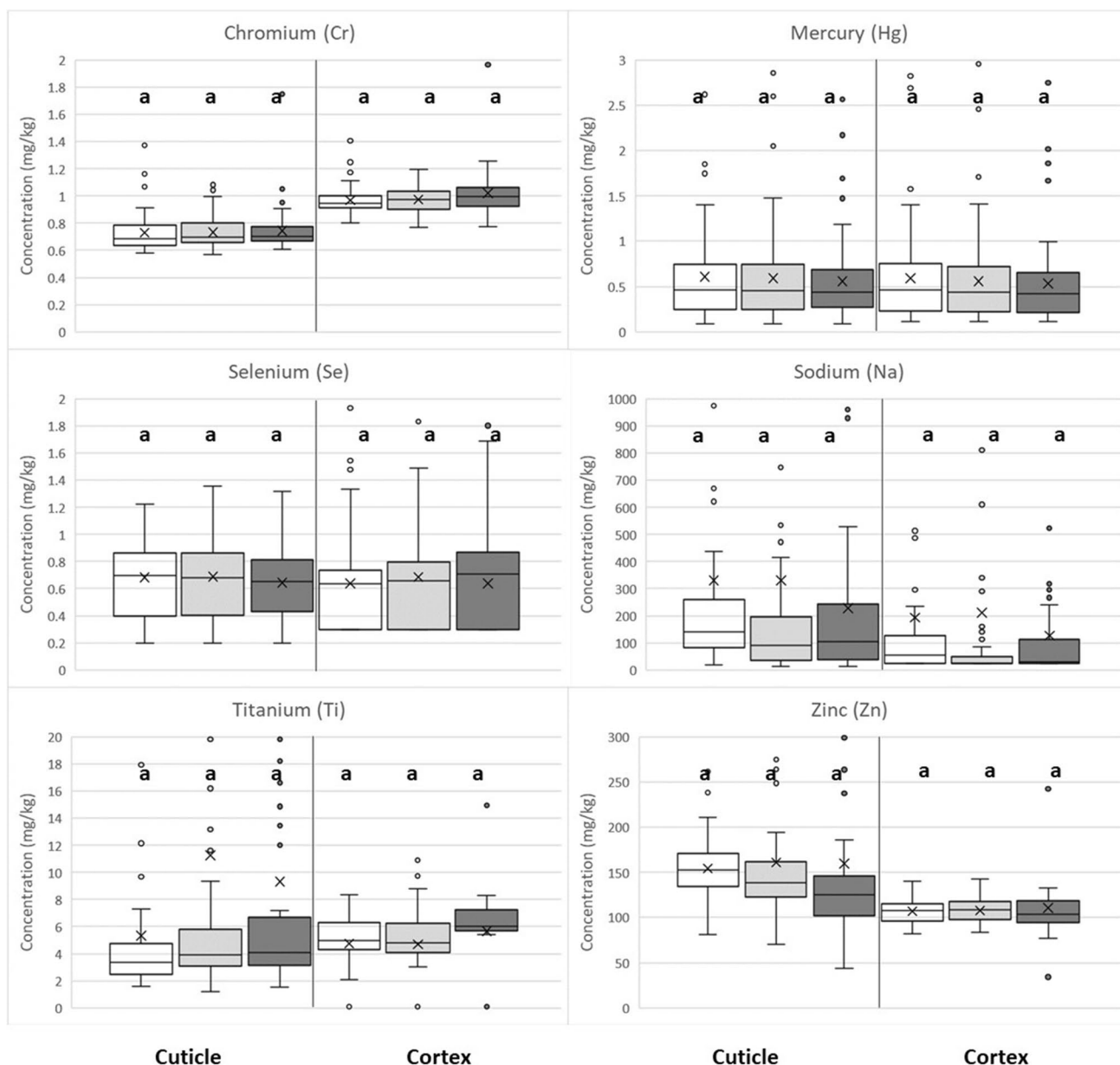
Fig. 4 Elements showing no loss or contamination in either layer above the scalp (near = light gray; distal = dark gray), relative to the protected below-scalp region (white). There are no significant dif-ferences among hair regions within the cuticle and cortex layers (depicted by letter a) above the boxplots

no other reference ranges for hair below the scalp, to the author's knowledge. Therefore, it is only possible to compare to reference ranges available from studies that have conducted hair elemental analysis using bulk hair (cut from above the scalp, homogenized, digested).

First, there is high variability in the reference ranges from large data sets among studies for most of the elements [7, 18–21, 26, 28, 65], particularly at the higher end of the reference ranges. As the portion of hair they analyzed was always above the scalp and subject to contamination, the

higher end of their reference range is, in many cases, higher than that reported here. For example, aluminum (Al) in the cuticle layer in this study has a reference range of 0.219 to 6.91 mg/kg, where the upper range is at least twofold lower compared to the upper range of other studies: from 12.75 mg/kg [18] to 20 mg/kg [20] to 25.6 mg/kg [7]. Lead (Pb) shows even greater difference, with our reference range in the cuticle at 0.013 to 0.540 mg/kg, with the upper range in other studies sixfold to 40-fold higher: from 3 mg/kg [18, 19] to 7.26 mg/kg [7] to 19.8 mg/kg [20]. Interestingly, when

Table 2 Elemental concentration reference ranges (median ± IQR and 5th to 95th percentile) derived from below-scalp region only. Sulfur (S) was used as a correction factor in calculating elemental concentrations

Element	Cuticle			Cortex		
	Median	± IQR (50%)	5th–95th percentile	Median	± IQR (50%)	5th–95th percentile
Aluminum (Al)	1.27	2.32	0.219–6.91	<0.260	-	<0.260–0.266
Arsenic (As)	<0.350	-	<0.350	<0.400	-	<0.400
Barium (Ba)	0.118	0.276	<0.010–2.33	<0.010	-	<0.010–1.02
Boron (B)	0.994	0.674	0.451–2.99	0.508	0.331	<0.430–1.42
Cadmium (Cd)	<0.130	-	<0.130	<0.310	-	<0.310
Calcium (Ca)	510	327	316–1.831	275	205	83.8–564
Chromium (Cr)	0.686	0.150	0.582–1.15	0.944	0.090	0.840–1.20
Cobalt (Co)	<0.060	-	<0.060	<0.110	-	<0.110
Copper (Cu)	6.87	2.51	4.46–26.2	5.78	2.21	3.97–9.43
Iron (Fe)	6.47	2.56	4.25–13.5	2.94	1.26	2.60–5.35
Lead (Pb)	0.061	0.091	0.013–0.540	<0.010	-	<0.010–0.160
Magnesium (Mg)	47.2	28.6	19.5–123	26.9	30.0	10.4–78.0
Manganese (Mn)	0.070	0.040	<0.067–0.448	<0.107	-	<0.107–0.128
Mercury (Hg)	0.464	0.496	<0.088–1.84	0.460	0.521	<0.110–1.58
Molybdenum (Mo)	<0.010	-	<0.010	<0.010	-	<0.010
Nickel (Ni)	<0.170	-	<0.170–0.320	<0.360	-	<0.360
Phosphorus (P)	164	42	125–211	131	32.4	97.9–180
Potassium (K)	125	169	26.3–1.016	29.7	70.7	<12.0–312
Selenium (Se)	0.697	0.468	<0.2–1.17	0.636	0.437	<0.300 to 1.46
Sodium (Na)	139	179	33.9–944	53.2	102	<23.0–510
Strontium (Sr)	0.354	0.332	0.132–6.06	0.152	0.198	0.030–3.78
Titanium (Ti)	3.38	2.26	1.91–11.9	4.99	1.99	0.100–7.27
Uranium (U)	<0.010	-	<0.010–0.035	<0.010	-	<0.010
Vanadiun (V)	<0.03	-	<0.030	<0.054	-	<0.054
Zinc (Zn)	152	36.6	106–211	108	19.8	89.5–129

Christensen et al. [9] analyzed hair portions below the scalp only (protected from external contamination), the mean lead (Pb) concentrations in reference individuals were similar to this study, at 0.585 mg/kg. Additionally, comparing cortex layer elemental concentrations, Bowman et al. [63] reported similar results for all 21 elements they measured.

Conclusions

Bulk hair analysis has been used in thousands of research papers, but perhaps more disconcerting, results have been used as a means to link hair chemistry to human exposure to toxic metals [10, 34–36], diet [29], health and disease [37], and nutritional deficiencies [30, 31]. Our results clearly show that interpretation of hair chemistry for health reasons should focus solely on below scalp hair only, at least for most elements, in order to provide the most accurate assessment. Due to the low detectability and concentrations of some elements in the cortex, the cuticle layer appears to be more ideal in monitoring for an entire suite of elements.

However, the results clearly show that only the below scalp portion of hair provides the most uncontaminated signal for interpretation on endogenous sources of trace elements. Interestingly, the cuticle did provide some protection of the cortex for aluminum (Al), boron (B), copper (Cu), lead (Pb), and manganese (Mn) lending potential to monitor these elements beyond the scalp with caution, and only using the inner cortex layer. Using above scalp hair for monitoring chromium (Cr), mercury (Hg), selenium (Se), sodium (Na), titanium (Ti), and zinc (Zn) appears to be acceptable due to demonstration of no contamination or loss.

This is the first study to provide reference ranges for multiple elements in human hair that is, with certainty, not impacted by contamination. Future research could focus on the limitations of the study, which include increasing the number of samples, and also evaluating potential differences among geographical locations, ethnicity, age, gender, hair color, and hair treatments.

Data Availability The datasets generated during and analyzed during the current study are available upon reasonable request.

Declarations

Ethics Hair samples from the scalp (with root bulb intact) were collected from 61 individuals following informed, written, and signed consent, and approved by the ethics board at the University of Saskatchewan (BIO17-55). All aspects of the project follow the ethical guidelines for human research, from the Canadian Institute of Health Research (CIHR), national Sciences and Engineering research Council of Canada, Social Sciences and Humanities Research Council (2018) – Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans. (http://publications.gc.ca/collections/collection_2019/irsc-cihr/RR4-2-2019-eng.pdf).

Competing Interests Jennie Christensen reports a relationship with TrichAnalytics Inc. that includes as follows: board membership, employment, and equity. Geriene LaBine reports a relationship with TrichAnalytics Inc. that includes as follows: employment.

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