

Scanning Electron Microscopes/Silicon Drift Detector-Energy Dispersive Spectroscopy: An Analytical Tool to Identify Waterborne Microminerals Pickup in Human Scalp Hair Cuticle due to Water Quality

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Abstract

Background and Significance: Still vast majority of the population in urban and rural set up using varied water quality for their day today washing of their hair in many parts of the countries may suffer or experience rough hair or uncombable syndrome. The quality of maintaining healthy hair may be impaired due to salty ions from water sources, dyes, or pollutants etc., We have identified a well-known instrumental technique SEM/SDD-EDS method to identify and estimate the waterborne minerals in the scalp hair cuticle in a non-destructive way. **Materials and Methods:** Identify volunteers of urban and rural folks using treated or untreated water for their routine hair wash at least for two consecutive years and examine their scalp hair cuticle using SEM/SDD-EDS. **Results:** Water borne minerals calcium and aluminium pick up were distinctly identified in the scalp hair cuticle as reflected by the quality of water used in urban and rural set up. Further restoration of lipid layer through prior application of coconut oil and or usage of conditioners prevents calcium pick up. Thereby, SEM/SDD-EDS is one of the suitable techniques to estimate the concentration of waterborne minerals in the scalp hair cuticle.

Keywords: Aluminum, calcium, human scalp hair cuticle, scanning electron microscope/silicon drift detector-energy dispersive X-ray spectrometry, water quality

INTRODUCTION

The human scalp hair fully grown during the embryonic stage is an intricate, biological composite material which has two main parts; one under the dermis called follicles and other the hair fiber shaft that extends above the skin surface. The hair fiber shaft primarily contains keratins of nearly 18 types of amino acids, water (moisture), lipids (free and structural), trace elements that are generally not free, but combined chemically with side chains of protein groups or fatty acid groups of sorbed or bound lipid and pigments. The outer hair shaft projecting outside the scalp comprised of multiple cellular biological structures composed of cuticle, cortex, and medulla. The scalp hair grows ~10 mm/month and its width varies from 50 to 100 μm with an average diameter of 70 μm with its multicellular structure; it is essentially made of specific chemical compositions but found variance due to various factors, such as ethnicity, environment, and

biological.^[1,2] The cuticle layers covering the cortex are generally made up of little scales of overlapping multiple cellular layers of 5–10 progressing from root to distal end longitudinally, such as shingles on a roof, and each cuticle layer thickness is ~0.5 μm and overlaps 5 μm , and the details are shown in schematic diagram by Fraser *et al.* (CSIRO) in Figure 1a and schematic diagram in Figure 1b.^[3] The cuticle's multiple layers are complicated in biological nature, each layer is formed by one cell of rectangular shape, and the overlap is such that only ~16% of the length is exposed outside followed by three major layers, the A-layer, the exocuticle, and the

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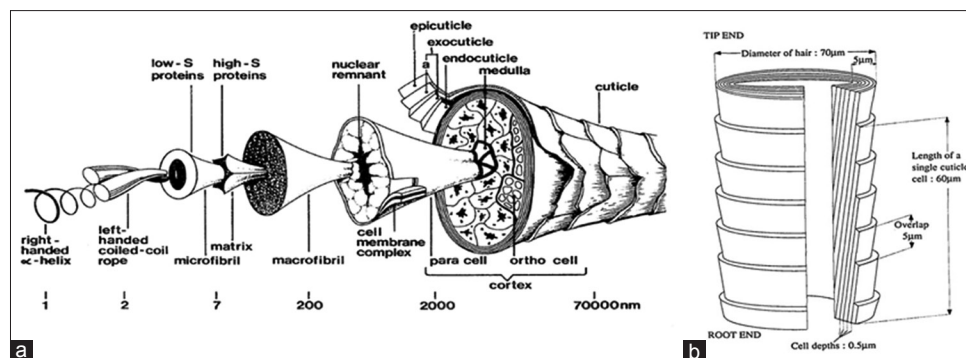


Figure 1: (a) Schematic diagram of a human hair fiber showing the major structural features except pigment granules which are normally found in the cortical cells. (b) Schematic diagram showing details of scalp hair cuticles without cortex and medulla

endocuticle having very different chemical compositions in proteins, lipids, or mineral content.^[4] The topmost cell of each layer of the native hair is covered by an important lipid called 18-methylisanoic acid (18-MEA) which is covalently attached to the protein via thioester bonds to the cysteine residues.^[5,6] This topmost surface layer is the most affected part due to influences by chemical treatments and daily-life routines such as washing, combing, heat, soil, dust particles emanating from different sources, and hair care products of various nature with a direct impact on cuticle quality by oxidizing the proteins and removing the 18-MEA layer, and this cuticle layer is subject to rapid change in chemical composition.^[7,8]

The human scalp hair contains very low mineral element content ~1% or less, and it is difficult to estimate whether this inorganic matter is derived through during fiber synthesis – endogenous or exogenous sources. It is well known that many metals of human hair exist as an integral part of the fiber structure, such as salt linkages or coordination complexes with side chains of proteins or pigments, although the possibility of mineral deposit or compound deposits as in soap deposition also exists. Hair trace element analysis provides an opportunity to investigate abnormal intake of trace elements to select the influence of single or more trace elements due to various considerations such as ethnic, water quality, geochemical, environmental, biological, or hair coloring.^[9-12]

The quality of water used in routine hair washing, rinsing using soaps, shampoos, conditioners, etc., and grooming habits using hair substances by hairstylists on the hair including dyeing and perming not only may have an impact on physical appearance but also show changes in chemical composition.^[9-11,13,14] Variation in water hardness and pH of water obtained from different geo locations of Europe and North America indicated that the uptake of calcium and magnesium by the scalp hair from water was very prominent.^[9,13,14] Total dissolved solids in water are an indication of water quality in urban and rural areas, and this source of water used for routine hair washing over long periods and also the dry climate prevailing in some parts of rural India may impact the hair shaft cuticle scales stand up, which makes the hair feel rough and tangled.

Daily routine hair wash activity using hard water may affect hair shaft and in fact may accentuate the pickup of alkaline earth metals such as calcium and magnesium. In this context, it is pertinent to investigate the uptake of alkaline metal ions such as calcium, magnesium, and other mineral salts dissolved in water and/or hair dyeing affecting the hair shaft.^[9,10] Since the hair is tangled and rough, it is more difficult to rinse out all of the soap, shampoo, or conditioners as many rural folks use soap to wash their hair. Soap is less effective in very hard water because it reacts with waterborne minerals such as calcium or magnesium salts. These are not easily soluble in water and can result in soap film. Washing of hair in soft water will have a different result because it leaves fewer insoluble deposits on the hair.^[9]

Urban water supply commonly uses organic or inorganic coagulants such as aluminum or ferric salts in water treatment process, and if estimated quantity of salts used is not related to pH of water, there is a possibility of aluminum salts remain with water which may cause pick up in the hair shaft.^[15-17] Another impact on hair is its exposure to hot dry climate with low moisture or sunny high humid area with high water vapor combined with changing ambient quality of air due to various micropollutants, sweat, and hazardous industrial environs, and continuous usage of consumer personal care products such as body spray due to urban life style change may also contribute to change in mineral elements or metal burden in human hair.^[18]

Investigation and estimation of mineral elemental profile change in the human scalp hair cuticle using scanning electron microscope-energy dispersive X-ray spectrometry (SEM-EDS) led to identify weathering of dead human scalp hair after burial and identification of linking the change in mineral elements to illness conditions, such as kidney stone, cholelithiasis, hypertension, and diabetes, as an attempt in medical diagnosis is the recent research trend.^[18,19] Identification of animal hair elemental profile using EDS spectra for the purpose of investigating poaching in the forest has also shown that SEM-EDS could be a suitable tool.^[18-20] Further, SEM micrograph of the shingled nature of hair cuticle offers large surface area for binding and access to metals.^[21] Therefore, understanding of trace elements in hair due to endogenous

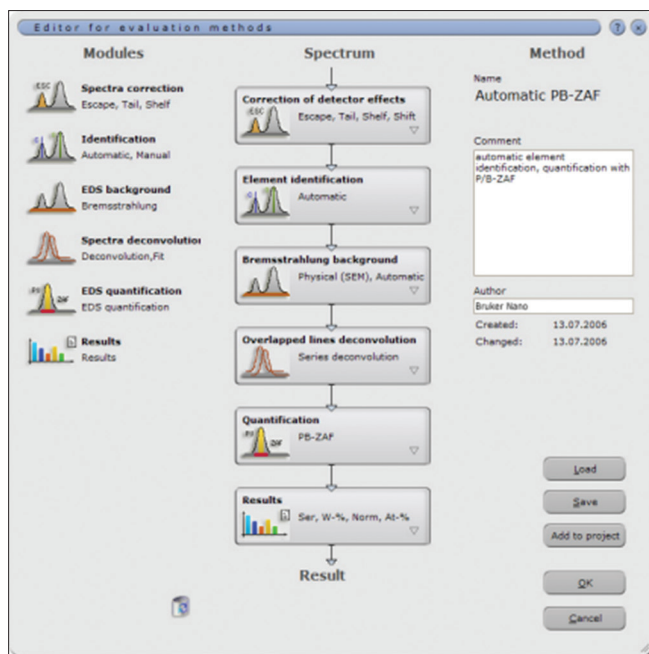


Figure 2: The ESPRIT method for spectra evaluation

or exogenous assumes importance in research as they could provide meaningful information due to change in environs or illness issues. This study involves identifying and estimating number of waterborne mineral elements such as calcium and/or magnesium along with aluminum in human scalp hair within quantitative detection limits attainable at the surface of cuticles, which is a visible part of the hair and not the whole hair shaft. SEM/silicon drift detector (SDD)-EDS is a nondigestive technique and the sample shall be preserved as it is also a nondestructive method of microanalysis of elements.

MATERIALS AND METHODS

Hair sampling and preparation

Human scalp hair samples were collected from four male Indian volunteers two each hailing from urban area Chennai, Tiruchirapalli, and Watrap, Srivilliputtur, a village/town close to the University, a rural area in southern part of India. Care was taken in sampling of hair for all the experiments through four volunteers. Two living in urban and rural area respectively were chosen reflecting the water quality used in day-to-day routine washing of hair during bathing or cleaning of hair by these respective volunteers and further at least 2 years of continuous use before the date of sampling. Sufficient quantity in adequate length (3–5 cm) of sample hair was obtained from nape of the scalp utilizing the sterilized stainless steel pairs of scissors, one each for urban and rural volunteers cutting approximately 2 mm from the scalp. All sample hairs were washed with deionized water and dried before storing in a transparent plastic pouch with zip log and placed them in desiccators maintained at 25°C air-conditioned room. Volunteers' information such as age, hair color, living area, type of water supply, dyeing of hair, soap, and shampoos was collected. Microanalysis of triplicate samples of scalp hair from

each volunteer (two each from different regions representing urban and rural areas) revealed assurance that the differences among samples reflected individual volunteer differences.

Microelemental analysis using scanning electron microscopy/silicon drift detector-energy dispersive X-ray spectrometry

Microanalysis is the easiest method for analyzing microscopic samples and the human scalp hair of ~7–10 mm length is easily accommodated on a double-sided conductive adhesive tape previously glued it on an aluminum stub of 12.5 mm diameter. It is sensitive to low concentrations – minimum detection limits (MDLs) are below 0.1% of mass concentration in the best cases and typically <1% – and its dynamic range runs from the MDL to 100%, with a relative precision of <1%–5% throughout the energy range. Furthermore, the technique is practically nondestructive and chemical digesting of the sample is eliminated with greatest advantage of minimal sample preparation. Since the emitted X-ray levels are analyzed at a depth of less than ~5 μm level, it is ideally suited to reveal the hair cuticle elemental profile and possibility of revealing the impact of external contamination by salty ions penetrating the multicellular surface cuticle layers.

All experiments were carried out using Bruker XFlash® 6 | 30 SDD EDS detector attached to Carl Zeiss EVO 18 SEM combines. (Bruker Nano GmBH, Berlin, Germany; Carl Zeiss Microscopy, GmBH, 07745 Jena, Germany) All spectra, mapping and hair cuticle topology were obtained using larger solid angle of a 30 mm² active area and spectral and mapping acquisition using the built-in quantification analysis provided by the inbuilt software ESPRIT 2.

The combination of SEM imaging with EDS came into being as a scientific tool in 1960 for microanalysis has become popular over the years and now is more powerful after the introduction of modern SDD technology. Thereby, analysis of acquiring the entire spectrum is much simplified with much more precise, accurate, and reliable method for elemental microanalysis. The huge advantage of modern detectors are as follows: (1) all elements in the unknown are measured simultaneously, except H and He elements, thus by minimizing the dose to the specimen, (2) capability to retain the tested sample without destruction for future reference, (3) variable pressure mode (VPM) enables microelemental analysis of nonconducting material without conductive coating on the surface of the sample aid in retaining as received samples intact without surface modification of the original material, and (4) “standard-less analysis” enables using remote elemental analysis of pure elements data stored in the software with the relevant spectral lines and their intensities to be accessed simultaneously and display of spectra of elements present in the unknown sample.

Standard-less spectrum analysis

QUANTAX EDS provides advanced true standard-less spectrum analysis based on the P/B-ZAF method. The self-calibrating P/B-ZAF analysis provides absolute (not normalized) results without standardizing or reference

measurement, and it is optimally suited for remote spectrum analysis. ESPRIT 2 software attaches all the necessary analysis automatically to the spectra, making QUNATAX spectra completely self-contained. The P/B-ZAF routine is extraordinarily tolerant to vary acquisition conditions and sample states (e.g., rough surfaces) yielding useful quantitative results even with unprepared samples.

Automatic and interactive spectrum analysis

QUANTAX performs automatic spectrum analysis during or after spectrum acquisition and supports batch processing for multiple spectra. Interactive spectrum analysis, which gives full control over all critical spectrum analysis steps, is provided for in-depth examination of special samples such as human hair shaft with biological multiple cellular structures.

Method editor

Customized analysis strategies can be configured for repeated use or routine analysis. The method editor provides control over all analysis steps, including identification, peak deconvolution, and quantification strategies. A number of predefined methods for common tasks shall be performed easily and routinely as shown in Figure 2.

ESPRIT software's corrective features include (1) spectrum mathematics (addition, subtraction, and linear combinations), (2) automatic spectra correction for all known detector artifacts (escape, shelf, tail, shift, and pile-up), and (3) true standard-less P/B-ZAF correction for rough and polished surfaces and true standard less $\Phi(\rho z)$ with XPP correction. A single-strand hair of four volunteers was placed on adhesive mount conductive carbon tape having both sides on an aluminum stub and firmly secured and was stronger enough to withstand the moderate vacuum used in VPM.

ESPRIT 2 software uses the most advanced processing for configuration and quantification of elemental peak intensities with X-ray photon energies combined with deconvolution algorithm software. Extensive database from the standard library for all elements are available as a reference for the

acquired entire range of characteristic X-ray energy with background continuum energy level-corrected spectral energy. The EDS spectrum acquires complete photon energy of all characteristic X-ray peaks, and the X-ray continuum background displays all possible elements present in the hair cuticle with different color marking of individual elemental peaks. The clearly differentiated peak intensities were quantified as un normalized weight %, normalized weight % and normalized atomic weight % of elemental analysis within 3σ error accuracy of the obtained results show that estimated confidence level is precise and accurate.^[22]

RESULTS

Scanning electron microscope analysis of human hair cuticle

The human scalp hair samples were placed on a double-sided sticky conductive carbon tape fixed onto the aluminum sample stub (12.5 mm in diameter). Uncoated samples were examined using excitation potential of 18 kV under VPM maintained at 20–40 Pa. The sample stubs were kept in SEM measurement chamber to highlight the typical topography.

The width/diameter of scalp hair samples of four volunteer's hair shaft was measured using SEM images obtained using 20/30- μm scale, and age, hair color, living area, type of water supply, hair washing habits, use of soaps, shampoos, conditioners, and dye application were obtained from the respective volunteers are provided in Table 1.

Figures 3 and 4 show the SEM images of human scalp hair of rural and urban volunteers, respectively, and the cuticle multilayer scales are visible at 20- μm scale or $\times 2000$ magnification. From Table 1 and Figures 3, 4, it is apparent that hair shaft width or diameter of the volunteers varies from 59 μm to 92 μm and the cuticle shows surface variations in appearance. The cuticle surface looked rough with increased in width or diameter indicated a swelled hair shaft in case of a rural volunteer 2 and a possibility of damaged cuticle

Table 1: Volunteers profile and washing habits

Volunteers	Age	Living area/residence	Type of water supply	Hair color	Washing habits	Other habits	Hair width/diameter (μm)
Rural 1	43	Watrap 9.6357° N 77.6393°	Borewell Untreated	Black Dyed	Daily once	No shampoo/conditioner	74.41
Rural 2	36	Srivilliputhur 9.5121° N 77.6341° E	Mixed Bore well (untreated) and river (treated)	Black	Daily once	Soap	92.28
Urban 1	32	Tiruchirapalli 10.7905° N 78.7047°/Madurai 9.9252° N 78.1192° E	River Treated	Black	Daily once	Shampoo	74.53
Urban 2	66	Chennai 13.0551° N 80.221° E	Treated	Grey	Daily once	Apply coconut oil before bath and shampoo/conditioner	59.97

surface due to ultraviolet (UV) radiation and subsequently increased surface pore volumes which is likely to absorb more moisture.^[11] However, urban volunteers' cuticle surface not only looked smoother but also revealed vanishing top lipid layer (fatty acids) or 18-MEA, and thinning down of hair-width/diameter was observed in urban volunteer 2 exposing underneath cuticle layers due to aging.^[7]

Microelemental profile of the hair cuticle surface obtained from acquisition of photon energy dispersive X-ray spectra of four volunteers are given in Figures 5a, b and 6a, b. It was very essential to optimize EDS operating parameters such

as accelerating voltage (18 keV), sample distance to beam detector (9.5–10 mm), pulse throughput (30 kcps), acquisition real time (300 s), dead time ~ 9%, and algorithm-based acquisition of data combined with efficiently acquired and statistically significant spread of spectral elemental peaks in the entire range of acquired spectra to get meaningful quantification. The acquired spectra using optimized excitation potential of 18 keV in the entire range of 4–5 keV show that all the peak photon energy levels emitted from within the sample by adopting area scan covering 30 μm × 100 μm repeatedly. While analyzing sulfur content of human scalp hair using SEM/microscopy X-ray microanalysis, it has been shown that critical excitation potential range of 15.602–20.608 keV will be required to penetrate the sample depth of 4.750–7.715 μm.^[23] EDS spectral peak identified elements were of low atomic weight elements, namely carbon, nitrogen, oxygen, aluminum, sulfur, and calcium with characteristic X-ray photon energy peaks of the respective elements within MDLs as shown in Figures 5a, b and 6a, b. Table 2 shows the consolidated elemental composition in unnormalized, normalized, and atomic weight all normalized to 100% weight % with 3σ error statistically significant accuracy of the data of each element's individual counts. The acquired data are indeed from characteristic X-ray energy levels emitted by the respective elements. It is pertinent to observe that aluminum, sulfur, and calcium elements have statistical significance with very high level of accuracy and confidence on the estimated quantitative elemental profile showing 3σ error <1%.

DISCUSSION

The ability of SEM/SDD-EDS method that adequately samples the cuticle layers of 8–10 layers up to a depth of ~5 μ has been well recognized.^[23] The scalp hair cuticle

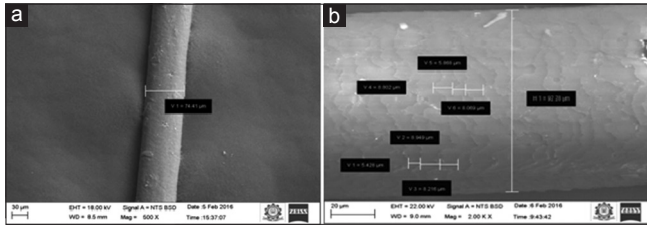


Figure 3: Scanning electron microscope images – Volunteers (a) rural 1 and (b) rural 2 scalp hair

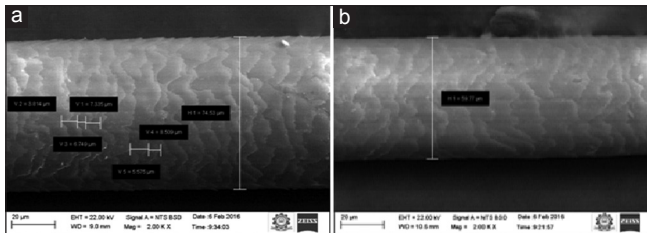


Figure 4: Scanning electron microscope images – Volunteers (a) urban 1 (b) and urban 2 scalp hair

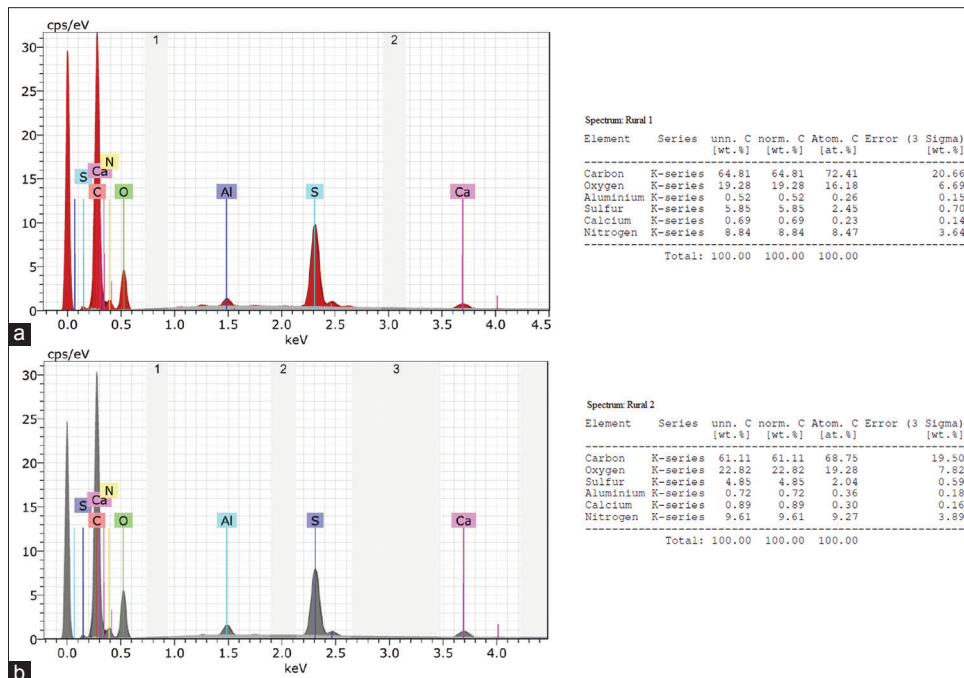


Figure 5: (a) Energy dispersive X-ray spectrometry spectra – Volunteer (a) rural 1 and (b) rural 2 scalp hair

average sulfur content of 5.4% by weight has been reported beyond 1.1 μ m is considered cumulative concentration of whole of the cuticle though A-layer reflecting more sulphur concentration level. It compares well with standard protein samples containing sulfur concentration of 5.6% of physically isolated cuticle layer.^[23] Therefore, method validation and detection limit are said to compare with our observation and our experiment [Tables 2a and b] using SEM/SDD-EDS.

Rural volunteer's scalp hair cuticle showed significant levels of calcium pickup of 0.69% and 0.89% and aluminum pickup of 0.52% and 0.72%, respectively, from the emitted X-ray peak intensity levels as shown in Tables 2a and b which is also statistically significant in the estimated elemental analysis. Our study of rural volunteers' scalp hair and EDS spectra of calcium accumulation due to routine hair washing on the cuticle surface is very distinct and shows substantial pickup from water and also oxidative surface damage. Thus, observed estimation of higher calcium accumulation in the scalp hair is due to routine washing of hair with untreated water supply from deep groundwater sources used at homes. The initial and final measurement of waterborne minerals calcium and magnesium of water sample analyzed influenced the uptake of calcium and magnesium many folds in the scalp hair.^[13] Smart *et al.* have also shown that scalp hair-dyed samples washed several multiple times with tap water deposited high concentration of calcium found its way into sulfonate-rich region of exocuticle using advanced method, nanoscale ion-mass spectrometric study.^[9,10]

The study undertaken using scalp hair surface roughness, wetting, charge characteristics, etc., provides understanding of physical characteristics such as shine and combability of the

scalp hair.^[4,9,24] These studies provide details of cross-linkages of disulfide bonds association, peptide chains and side salt chains hosting trace minerals through histological process, and exogenous activities such as routine hair washing, grooming, application of soaps, shampoos, conditioners, dyeing, and other hair styling using chemical, mechanical, and heat process.^[9,24,25] Therefore, increased uptake levels of calcium accumulation at the cuticle surface are the result of absorbed water content due to alkalinity, increased hardness, and pH of water. The permissible limits of total solids in water, particularly in the rural area, are far exceeding the limit as observed through various studies conducted by the Government of India, Ministry of Water Resources, Central Ground Water Board, and South Eastern Coastal Region for Virudhunagar district, where rural area Watrap and Srivilliputhur town is located. The high hardness combined with beyond high pH level observed in their GROUNDWATER BROCHURE-technical report series released in May 2009 provides ample evidence of untreatable water quality. Typical tested water quality sample result is shown in Table 3. also proved that groundwater-borne minerals are higher. Therefore, uptake of metal ions found to vary markedly depends on high initial total hardness and/or high initial pH in general and specific to this study, resulting in more adsorption of these alkaline earth metals calcium.

Elevated calcium and their accumulation at the cuticle edges as given in mapping for elemental profile are clearly shown in Figure 7 and found that the richest region of calcium is in the cuticle edges.^[24] The increased uptake of these metals can have a detrimental impact on fiber properties, for example, reducing shine and causing a poor wet and dry feel.^[4] The preferential locations of calcium, showing a high concentration of calcium in the cuticle region of colored hair, specifically in the sulfur-rich regions (A-layer and exocuticle) though calcium is not directly taking part in color chemistry its affinity in sulfur-rich region is well established.^[10,24] It is expected that fiber damage mechanism is due to reactive oxidative damage to hair proteins and lipids as a result of reaction between hydrogen peroxide redox metals such as copper from water. It has been demonstrated that calcium uptake increases as the hair undergoes multiple treatments from an oxidative colorant and water. It has been hypothesized that this is due to the enhanced number of metal binding sites that are formed in the hair during the oxidation process.^[9,10,24]

However, EDS spectra of urban volunteer's hair cuticle show lower pickup of alkaline metal/waterborne minerals, calcium of

Table 2a: Consolidated - energy dispersive X-ray spectrometry microanalytical elemental profile of four volunteer's hair

Volunteers	Weight percentage					
	Carbon	Oxygen	Nitrogen	Sulfur	Aluminum	Calcium
Rural 1	64.81	19.28	8.84	5.85	0.52	0.69
Rural 2	61.1	22.82	9.61	4.85	0.72	0.89
Urban 1	70.15	18.65	4.20	5.76	1.03	0.21
Urban 2	62.73	18.89	11.40	5.15	1.60	0.24

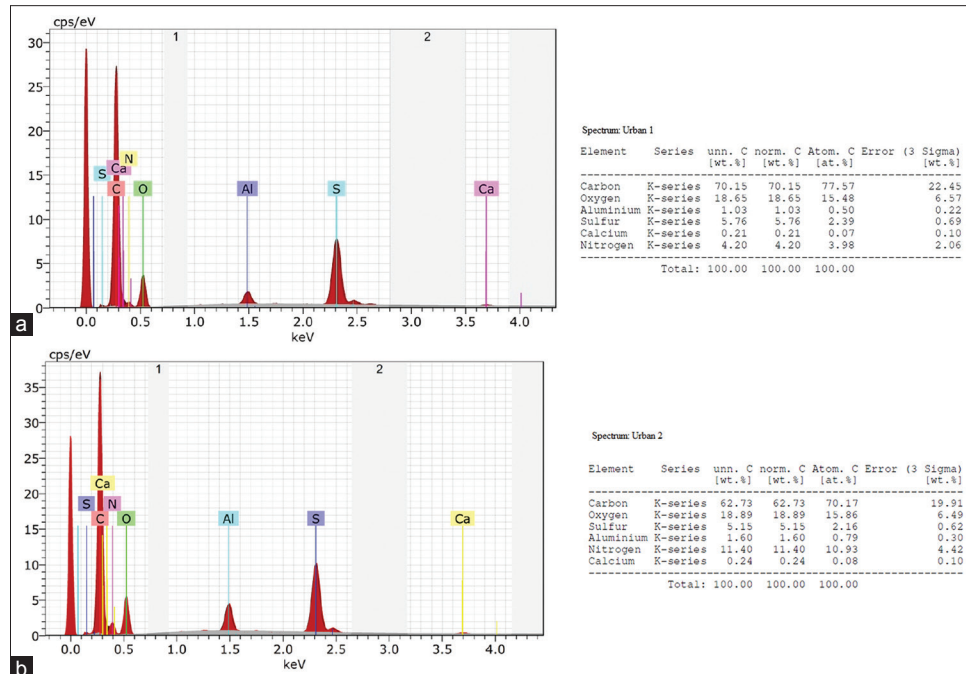
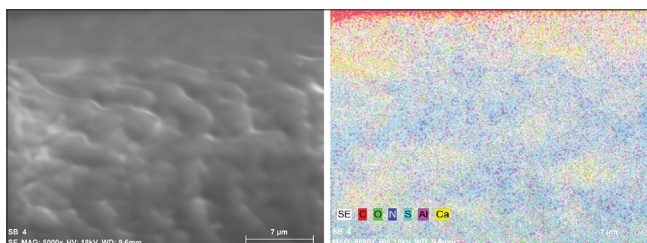
EDS optimized parameters: 18 kV, 30 kcps throughput, 5 kcps input, live time 300 s, total counts 1,500,000 cycles, dead time: 9%, Spectral elemental error 3 σ error weight percentage: Sulfur, aluminum, and calcium \leq 1%; Nitrogen \leq 4%, oxygen \leq 7%, carbon \leq 20%. EDS: Energy dispersive X-ray spectrometry

Table 2b: Statistical analysis of elemental estimation of hair cuticles

Volunteers	Weight percentage					
	Carbon	Oxygen	Nitrogen	Sulfur	Aluminum	Calcium
Rural 1	66.23 \pm 1.84	19.75 \pm 1.22	8.8 \pm 0.67	6.03 \pm 0.2	0.56 \pm 0.03	0.72 \pm 0.06
Rural 2	62.03 \pm 1.75	22.60 \pm 0.95	9.72 \pm 0.88	4.96 \pm 0.13	0.73 \pm 0.06	0.96 \pm 0.08
Urban 1	71.41 \pm 1.1	17.76 \pm 1.2	4.53 \pm 0.33	5.96 \pm 0.09	1.01 \pm 0.11	0.25 \pm 0.05
Urban 2	63.27 \pm 1.46	17.43 \pm 1.42	11.95 \pm 0.72	5.01 \pm 0.08	1.66 \pm 0.12	0.28 \pm 0.03

Table 3: Water analysis report

BIS 10500:2012	Acceptable limit	Permissible limit in the absence of alternate source	Result
Turbidity NT units	1	5	1.2
Total dissolved solids (mg/L)	500	2000	1873
pH	6.5-8.5	6.5-8.5	7.2
pH alkalinity as CaCO ₃ (mg/L)	-	-	0
Total alkalinity as CaCO ₃ (mg/L)	200	600	465
Total hardness as CaCO ₃ (mg/L)	200	600	576
Calcium as Ca (mg/L)	75	200	159
Magnesium as Mg (mg/L)	30	100	43

**Figure 6:** (a) Energy dispersive X-ray spectrometry spectra – Volunteer (a) urban 1 (b) and urban 2 scalp hair**Figure 7:** Energy dispersive X-ray spectrometry elemental mapping – Volunteer (urban 2) scalp hair

0.21% and 0.24% respectively. However, other metal aluminum pickup is high 1.03% and 1.60%, respectively, for two urban volunteers as regularly treated water supply to home is effected by respective water boards of the city claiming (in their website) that treated water supply meets the standard norms and hence assumed to meet the water characteristics of normal water. Hence, it is expected that normal water with controlled pH and/or soft water supply in cities may not impact calcium pickup at the cuticle surface^[9] as compared to rural volunteers.

Although water treatment process for drinking and bathing purposes maintains that pH of water ranges between 6.5 and 8.5, it is not clear how water treatment process is adopted. There is a possibility of uncalculated quantity of coagulants using alum cakes (aluminium salts – one of the inorganic salts) instead of effectively calculating alum solution required and without adjusting to initial pH of water quality or practice of using aluminium gel as flocculants may also give rise to dissolved fine particles of floc containing dissolved aluminium ions remaining in the water after filtration and sedimentation.^[15,17] Therefore, there is a possibility of treated water containing dissolved fine particles of aluminum salts left in the treated water supply due to inefficient treatment to homes of urban volunteers cannot be ruled out. As a net result, calcium pickup in the cuticle may not occur in this case but may impact dissolved fine particles of aluminum salts entering through large surface pores of damaged surface cuticle as surface pores volume will enable good amount of water absorption as a result of coagulation/flocculation process. This cyclic activity of the water washing or weathering of

hair using inefficient treatment of water may also damage cuticle surface and thought to affect aluminum pickup and extremely high content of aluminum 1.03% and 1.60% found in case of urban volunteers 1 and 2, respectively, compared to rural volunteers. It has been observed that very high level of aluminum found in hair shaft may also be due to high exposure to external environment sources such as antiperspirant and other cosmetic usage. Aluminum entering through dietary components right from hair bulb part to all regions has also been evidentially observed.^[18]

The observed calcium pickup at the cuticle surfaces of rural volunteer's hair shaft affected by routine hair washing due to alkaline groundwater quality supply in their individual homes has resulted in damaged hair shaft cuticles, and upkeep of their hairstyle without adequate precautionary measures or personal care may continue to damage the hair shaft cuticles. Hair care products play an important role in improving the hair manageability of day-to-day combing, grooming, and styling activities of one's personal care of scalp hair.^[4,6,7] The surfactant-based chemical products such as shampoos, conditioners, or oil-based grooming products may enhance one's hair appearance as modern life style is embraced world over. Most of the shampoos are primarily used to cleanse the hair, but in the process, they may also remove natural oils from the hair surface. Hair conditioners found to act by way of the opposite process with ingredients adhering to hairs surface and add shine to the hair.^[25] SEM morphological studies on the hair shaft of female volunteers show that styling conditions using cosmetics, dyeing or bleaching, and grooming styles could lead to hair shaft damage, but control group practicing using oil either pre- or post-application of hair wash show no damage.^[26] It is

not clear whether this control group used treated or untreated water for routine hair washing. Coconut oil, but not sunflower or mineral oil, can penetrate the hair shaft and reduce protein loss.^[25] Surface wetting characteristic study using AFM shows that hydrocarbon liquids such as oil preferentially wet the cuticle edges but water has seen to penetrate the bulk of the hair.^[27] Similar observation in our study also shows that coconut oil penetrates the hair shaft resulting in six times reduced pickup of calcium to 0.11%wt % compared to 0.69%, thus preventing the water to penetrate the hair surface. This was evident from rural volunteer 1 practicing prior application of natural unrefined coconut hair oil to scalp hair before hair wash for 6 months and continued his routine hair wash using the same untreated bore well water supply to his home. EDS spectra of the scalp hair cuticle taken after this experiment in case of rural volunteer 1 are shown in Figure 8a. It is well known that excreted sebum from the sebaceous gland is a naturally produced oily substance which acts as a conditioner for the hair and coats ~35 mm of hair shaft from the root due to capillary action. As the hair shaft grows, there is a tendency for sebum secretion not reaching the full length of hair, making it look dry devoid of fatty acids on the top layer. It is the process of combing that helps distribute the sebum distally and coat the hair evenly. However, damage to hair shaft may occur due to either non-secretion of adequate lipids or normal wear and tear of the cuticle surface edges by combing or grooming, chemical bleaching, and UV radiation which ultimately affecting the keratinous bonds. In this context, it is pertinent to observe that the practice of using hair oil in India since ancient times is akin to using conditioners today. The morphologically significant multi-layered bundle of cuticle cells of the human scalp hair devoid of lipid layer will expose the proteinous components of differing compositions may

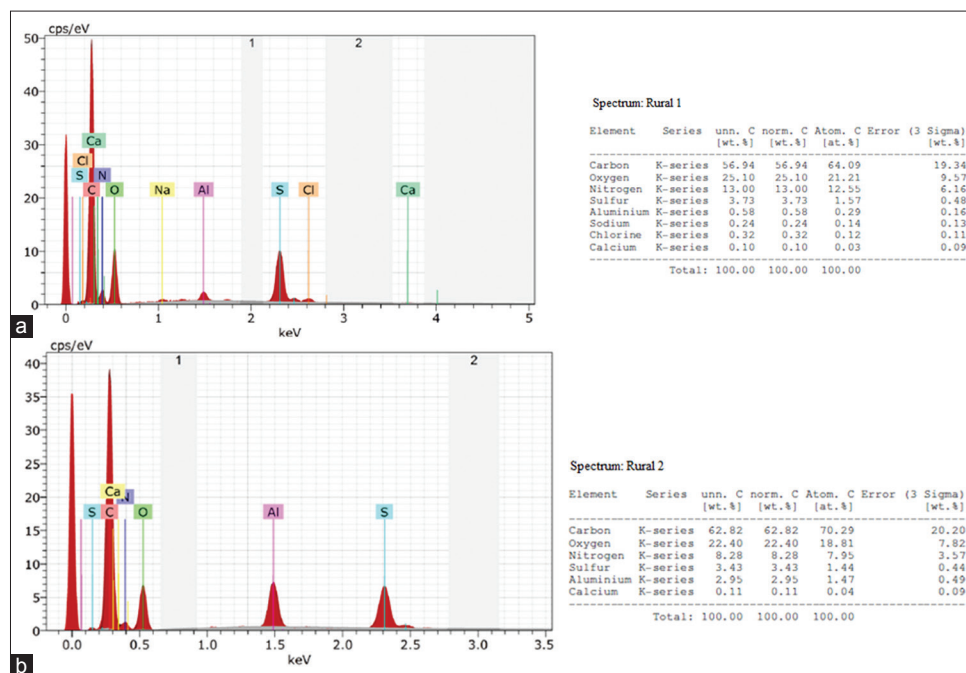


Figure 8: (a) Energy dispersive X-ray spectrometry spectra treated with coconut oil and after 8 months – Volunteer (rural 1) scalp hair. (b) Energy dispersive X-ray spectrometry spectra after 8 months – Volunteer (rural 2) scalp hair

result in losing its hydrophobicity, and as a consequence, the change of charge potential and wetting characteristics of these layers may absorb water.^[27,28] Therefore, application of coconut oil, one of the remedial measures to restore partially damaged hydrophobic and hydrocarbon material, will enhance the wetting properties and change the charge potential to protect the hair shaft from further damage. Although our experiment is limited to few male volunteers, a clear trend is observed to reduce the uptake of calcium as urban volunteers routine hair wash using shampoo/conditioner and/or oil application before bath has shown similar lower calcium pickup. EDS spectra obtained from rural volunteer 2 as shown in Figure 8b after 8 months show extremely low calcium pickup as the volunteer started receiving treated water supply to home and continue to practice using of soap for hair wash. This is not surprising as water used for routine hair wash has changed due to improved water quality with controlled pH in Srivilliputhur town where the rural volunteer 2 receives his water supply. However, the aluminum pickup in the hair seems to be varied in concentration as the treated water supply tends to bring in dissolved alum-based chemicals used in the water treatment. It is possible that aluminum has a tendency to be excessive in human hair and follows some systemic way of enriching in concentrations if such condition could exist for absorption.^[29] Ability of single-strand hair for analysis of metal burden may be sufficient.^[30] Our research approach showed that application of coconut oil before routine hair wash for 6 months even using untreated water for routine hair wash shall preferentially prevent uptake of minerals if hydrophobic effect of the cuticles, i.e., lipid content, is restored (hydrophobic materials such as oils and hydrocarbons are intact) or water used to rinse the hair routinely is of soft in nature with lower pH.^[25,28] Similarly, commercially available shampoo, conditioners, or soap wash shall prevent damage to the hair shaft cuticle and possibly restore the damage progressively, and poor water quality or waterborne minerals play a role in hair shaft damage.

CONCLUSIONS

Thus, we conclude that SEM/SDD-EDS is a suitable tool to identify and quantify the impact of waterborne minerals calcium and aluminum and its profile change due to routine hair wash with change in water quality. Further, it shall be possible to reduce the waterborne mineral like calcium ions pick up through prior application of coconut oil/conditioner on the human scalp hair for several months as analyzed by SEM/SDD-EDS. Study involved only few volunteers but shall be expanded to cover various geo locations with large number of volunteers to study the impact of waterborne minerals calcium or aluminum affecting the human scalp hair quality covering across gender, ages, ethnicity, etc., using SEM/SDD-EDS technique.

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Conflicts of interest

There are no conflicts of interest.

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