Chapter 8

Ion Beam, Synchrotron Radiation, and Related Techniques in Biomedicine: Elemental Profiling of Hair

Karen J. Cloete

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.72076

Abstract

Elements play an imperative role in the physiological and metabolic processes of the human body. When elemental levels deviate from physiologically accepted levels due to for example poor nutrition, the body's intricate elemental and metabolic balance is disturbed. Over time, disease may develop as a result of elemental dyshomeostasis or alternatively, disease may trigger elemental dyshomeostasis as an adaptive metabolic response to an unhealthy environment. There is now a growing interest in screening human tissue to identify and quantify elemental changes as biomarkers of disease or alternatively, as outcomes of disease. The unique properties of human hair brand it the ideal substrate for the quantitative identification of elements in the body. Hair bioaccumulates elements, provides a historical overview of elemental status depending on length, and is easy and economical to sample and store. The fundamental outcome and application of hair elemental screening, however, are strongly influenced by a range of factors, including choice of analytical method. This chapter will provide a background summary of ion beam and synchrotron radiation techniques and its diverse applications for unraveling the elemental signature of hair in various fields.

Keywords: biomedicine, elemental screening, hair, ion beam analysis, synchrotron radiation

1. Introduction

The human body harbors a plethora of mineral compounds that form the lifeline of our multifaceted biological system [1, 2]. To illustrate, major and minor elements play a critical role in metabolic pathways and physiological processes of the human body. However, when toxic elements or other xenobiotic compounds from the occupational or natural environment enter the body, elemental dyshomeostasis ensues that adversely affects the aforementioned

processes. Poor nutrition is another contributing factor to elemental dyshomeostasis that results in a modified metabolism.

Besides its effect on metabolic processes, elemental dyshomeostasis in the body may also indirectly accelerate the germination of diseases such as neurological disorders and cancers that in turn may indirectly alter metabolic processes and elemental levels within tissues. A growing number of studies now highlight the complex association between elemental dyshomeostasis and biological disorders [3, 4]. Several reports also highlight the importance of monitoring elemental levels as a measure to treat elemental imbalances and prevent the onset of disease or alternatively, as an outcome of disease [5]. Applying such disease intervention through biological tissue elemental screening is however complicated and requires a more in-depth analysis of the full elemental signature of human tissues in the pre- and postdisease stage.

Elemental profiling of biological tissues now finds diverse applications in the fields of biomedicine, pharmacology, toxicology, and forensic science [6–9]. Of the biological tissues used in elemental profiling studies, hair is gaining increasing popularity for quantitative profiling of elements in the body. Since the elemental content in human hair fibers is generally less than 1%, accurate and sensitive analytical techniques are a necessity for studies focused on quantitative elemental profiling in hair fibers [10].

Studies related to hair elemental screening has mostly relied on conventional analytical techniques such as atomic absorption spectroscopy, inductively coupled plasma mass spectrometry, and inductively coupled plasma atomic emission spectrometry [11, 12]. Sample preparation for these aforementioned techniques presents a major pitfall in that elements may be lost or contaminants introduced during chemical processing of samples. A proposed extraction method may also yield high recoveries for some elements and low recoveries for others [13]. In essence, sample processing for these techniques is destructive in that samples from various donors have to be pooled and chemically processed that effectively destroys historical and spatial information of elements preserved within the length of a single hair strand [14]. Besides sample preparation, the capabilities and limitations of the testing instrument also warrant careful scrutiny. For example, some techniques may only reveal the presence of specific elements due to the instability of the analyte under imperfect experimental conditions or the low sensitivity of the technique [15, 16].

One of the most versatile and sensitive analytical techniques for elemental microanalysis of unprocessed biopsy tissues in a manifold of multidisciplinary fields fall under the umbrella of ion beam techniques [17, 18]. The continual development of the components of the beam setup such as the beam optics and scanning systems, as well as the detection and data acquisition devices, has now firmly placed ion beam techniques at the forefront of elemental analysis research in the biomedical and other fields involving hair elemental screening [19]. Besides ion beam techniques, synchrotron radiation techniques are also becoming increasingly popular in biomedical research related to hair elemental analysis.

This chapter aims to provide an overview of why hair is a popular testing substrate for elemental screening, how elements are incorporated into hair, how to prepare hair for ion beam

http://dx.doi.org/10.5772/intechopen.72076

and synchrotron radiation analysis, and an overview of the versatile applications of ion beam and synchrotron radiation techniques in hair elemental profiling in biomedical studies.

2. Why hair?

Hair is a metabolic end product that incorporates minerals from the blood supply into its keratinous matrix during the growth process [14, 20]. Over time, a temporal profile of normal or abnormal metabolic activity or alternatively, exposure to xenobiotics such as heavy metals is created. Depending on the length of the hair shaft, segmental analysis may then reveal a pattern of exposure that enables a retrospective screening of short term or chronic exposure to chemical compounds [14, 21]. Other unique characteristics of hair include its ability to store higher concentrations of minerals than blood or urine for a number of years [22]. Furthermore, mineral levels in hair do not fluctuate in response to changing physiological and/or environmental conditions such as in blood or urine [23]. Most importantly, hair samples from regions such as the scalp can be collected noninvasively and under close supervision, minimizing the risk of manipulation and cross-contamination, and is easy to handle, transport, and store [14].

Hair has become a popular tool for screening and quantifying for example elemental changes within the body [14, 24]. Alternatively, hair testing may find important applications in clinical medicine such as to (1) determine medicinal or chronic doping, (2) confirm gestational drug use, or (3) assess exposure to toxins and pollutants in the workplace or environment [25, 26]. Hair analysis has also become popular in forensic science to (1) assess drug use history, (2) criminal liability of drug users, and (3) intentional or unintentional poisoning in postmortem toxicology [27–29]. Unfortunately though, there is a lack of reports describing the mineral profile of intact, unprocessed hair in its natural physical and chemical state [14]. Fundamental information on the spatial distribution of elements within hair tissues is also lacking, which is particularly important when distinguishing for example exposure to ingested xenobiotics originating from blood feeding the inner hair tissues or exposure to environmental pollutants that accumulate in the outer hair tissues. Furthermore, how elements and drugs are absorbed and incorporated into hair, either biogenically or diagenetically, are also still poorly understood. These unanswered questions may, however, be probed by techniques such as ion beam analysis and synchrotron radiation that allows quantitative elemental spatial data that may also assist in understanding hair elemental uptake mechanisms.

3. Hair elemental uptake mechanisms and factors that affect hair elemental levels

Understanding hair elemental uptake or incorporation mechanisms and the factors that may influence elemental levels in hair is crucial before correctly interpreting ion beam analysis and

synchrotron radiation data [14]. Scientists have proposed three models explaining elemental uptake in hair [30]. The first model proposes that elements may either actively or passively diffuse into the hair shaft from the bloodstream feeding the dermal papilla cells. The second model proposes that elements may diffuse from sweat or other excretions into the hair shaft. Alternatively, powders and vapors may also diffuse into the hair shaft, as proposed by the third model.

Besides the aforementioned routes of entry, the incorporation of elements and their levels in hair may be significantly influenced by a range of other variables. These may include the dose of the chemical exposed to and the origin of the chemical [31]. Hair is often treated with a range of cosmetics such as mineral-based dyes, paints, and bleach for attractive appeal, while various shampoos such as antidandruff formulations that contain zinc and selenium are also frequently used for hygiene purposes [14, 32]. When hair is wetted before applying these formulations, the hair fiber swells and the cuticle cells lift [32], causing the hair fiber to be more permeable to cosmetic agents that are known to contain a variety of chemicals as well as tap water that may contain calcium and lead from plumbing. Bleaching may further increase the permeability of hair in that it destroys hair disulfide bonds that cause hair to be more vulnerable to aqueous solutions containing a variety of chemicals.

Other than cosmetic agents and washing that contributes to endogenous hair elemental levels, heavy metals deposited on the hair surface from atmospheric dust and pollution may also contribute to both exogenous and endogenous hair elemental levels [14, 33]. Interestingly, it has been shown in miners that particulates from mined metals may be deposited on the hair surface [34]. The surface contains distinct regions of varying chemical composition and is a unique site with varying binding affinities for different metals [31, 35]. These binding affinities may, however, be affected by hair acidity and hair cosmetic treatments that add negatively and positively charged ionizable groups to hair. Binding of metals to hair may also alternatively be influenced by the levels of eu- and pheomelanin polyanionic polymers. These compounds have been shown to bind metal cations in vivo or in vitro via electrostatic forces [14, 35].

Once elements are incorporated into hair, various other factors may affect their levels in hair. UV exposure, aging, nutritional deficiencies, morbidity, medication use, and acute metal poisoning has been described to cause natural deterioration and hair structural changes that affect the ability of hair to retain elements [14, 36, 37]. Specifically in disease, the levels of elements in the body may not be optimal and indirectly affect hair growth and hence hair structure. Besides the aforementioned variables, gender and age, ethnicity, and genetic polymorphisms may also influence the structure of hair and hence its ability to retain elements [14, 38–40]. For example with ethnicity, the structure, shape, and melanin levels of hair from different races may affect permeability and hence elemental levels within hair [14, 41]. Ethnicity may also strongly link with cultural habits related to washing and hair treatment regimens, as well as dietary differences that indirectly affect hair elemental levels [42].

http://dx.doi.org/10.5772/intechopen.72076

4. Hair sampling and preparation

Before hair samples are sourced, it is important to first obtain ethical clearance for working with human tissues. Another important consideration is to obtain informed consent from participants who will be donating their hair. Ethical approval from an institutional ethical board is a necessity. Research institutions usually provide comprehensive information on application and approval processes as well as submission deadlines on their webpages related to the ethical clearance of studies. Ethical clearance of studies involving human tissue may take up to 1 month, depending on the institutional review times. Alternatively, one may also request an expedited review in the cover letter accompanying the submission, since research involving human hair constitutes minimal risk research.

When sampling human hair, it is important to consider the location of sampling. Although scalp hair may be exposed to external contaminants, scalp hair is often the preferred sampling choice [43]. Pubic hair may present the ideal alternative to scalp hair as it is less exposed to contaminants [42]. However, pubic hair differs in morphological and structural properties from scalp hair that may impact on elemental uptake mechanisms. Furthermore, there is a large possibility of participants manipulating samples when not supervised during sampling.

The hair should be sampled as close to the skin as possible with stainless steel or Teflon-coated scissors. Hair may also be plucked so as to extract the hair bulb that may be less exposed to contaminations. Furthermore, the hair bulb is the most active metabolic area of hair and may be particularly interesting to study [44]. For retrospective analyses, it is recommended that long hair be sampled as segmental analyses may reveal a pattern of exposure that enables a retrospective screening of short-term or chronic exposure to chemicals [45, 46]. The hair should not be handled with plastic gloves as polydimethylsiloxane contaminants may be transferred to the hair. These compounds negatively influence data retrieved using certain analytical applications such as scanning ion mass spectrometry (SIMS) [47]. Once the hair samples have been properly processed, it may be stored in paper envelopes.

To produce credible results, hair that is free of surface contaminants should be analyzed. To eliminate surface contaminants such as particulates from pollution, smoking, chemical powders, and metals from mining practices, most studies wash their hair samples [14]. With that said, the question of how specific external contaminants contribute to endogenous content still remains to be more rigorously investigated. Furthermore, if certain external contaminants were to become embedded in hair, distinguishing contribution from endogenous versus exogenous elements then becomes very challenging, rendering washing of hair in essence a negligible step. Nevertheless, specific fields such as drug analysis for forensic purposes place strict emphasis on hair washing protocols as drugs in the form of powder or vapor has been shown to contaminate hair and result in false positives [48]. Alternatively, in pollution or toxicology studies, washing hair samples may simply not be necessary since determining the kind of matter deposited on hair and possibly entering the body is important.

A multitude of washing methods has been described in the literature, of which the most popular is described below [14]. The most popular of these methods include washing hair in an ultrasonic bath with acetone, deionized water, and 0.5% Triton X-100 solution. This process is followed by washing with ultrapure water and air drying. The International Atomic and Energy Agency, however, proposes washing hair with a nonpolar solvent such as double-distilled acetone followed by washings in a polar solvent such as deionized water and acetone. Each washing step should be performed for 10 minutes. Authors may also adapt their hair washing protocol to be most suitable and efficient for their specific type of analysis.

There is, however, a number of concerns related to hair washing protocols. First, no standard washing procedure is currently available in the literature that unknowingly introduces variation in hair elemental data and that has been confirmed after testing different washing protocols [49]. Second, authors may not always provide sufficient information on their washing protocol such as whether samples were sonicated and the specific solvents employed. Thirdly, insufficient washing methods may not remove surface contaminants, while washing methods that are too abrasive may cause damage to the hair sample and cause elements to either leach or diffuse into the hair, which often further complicates the assumption of elements either being of biogenic or diagenetic origin. Alternatively, no contamination may be present then representing [14]. In essence, a standard washing procedure that preserves internal elemental content and washes away external contaminants remains an urgent requirement.

Besides washing protocols for distinguishing between endogenous and exogenous hair elemental content, ion beam techniques have shown great promise for spatial distribution analysis of elements in hair tissues [14, 50]. When preparing hair for ion beam analysis, the hair may either be washed or left untreated. However, exposing the internal hair tissues is critical to assist with spatial distribution mapping of tissues and for interpretation of elemental maps. Longitudinal sectioning of hair may be employed to expose the internal tissues of the hair that facilitates distinguishing elements present at the borders of the hair that may possibly link to contamination or alternatively, biogenic elements occurring in the hair medulla [51]. For this purpose, the hair can be sectioned using a stainless steel metal plate (60 × 110 mm) manufactured with 5–mm-wide grooves of depths ranging between 20 and 80 μm [52]. A single hair is laid in a groove of fitting depth, secured, and sectioned longitudinally with a stainless steel razor blade (**Figure 1**). The sectioned hair may be mounted on a silicon wafer with carbon tape (**Figure 2**) in the case of SIMS at MeV energies [53]. Alternatively, the hair

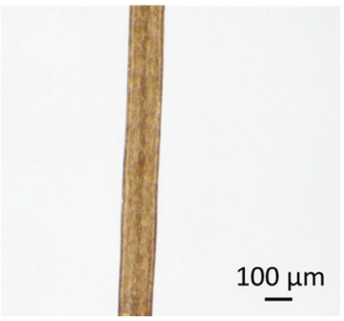

Figure 1. Light micrograph of a longitudinally sectioned scalp hair fiber (unpublished data).

http://dx.doi.org/10.5772/intechopen.72076

may also be mounted intact in special holders, allowing analyses along the length of a hair. Longitudinal sections of hair are particularly useful for the retrospective assessment of exposure to chemicals.

Some studies also cross section hair and analyze these cross sections with an ion beam technique termed microproton-induced X-ray emission spectrometry [50]. Cross sectioning of hair tissues may be performed using cryosectioning [54] in which hair fibers are fixed to a small piece of adhesive tape, which is embedded in 2% carboxymethyl cellulose and flash frozen in liquid nitrogen for 2 minutes. The frozen specimens are subsequently sectioned using a cryostat set at a temperature of −20°C. Cryosections of approximately 5 μm thickness can be prepared using a cryostat microtome (**Figure 3**). Data from a cross section of hair may well complement data from a longitudinal section in that more information becomes available on the elemental distribution in an area of hair containing different hair tissues.

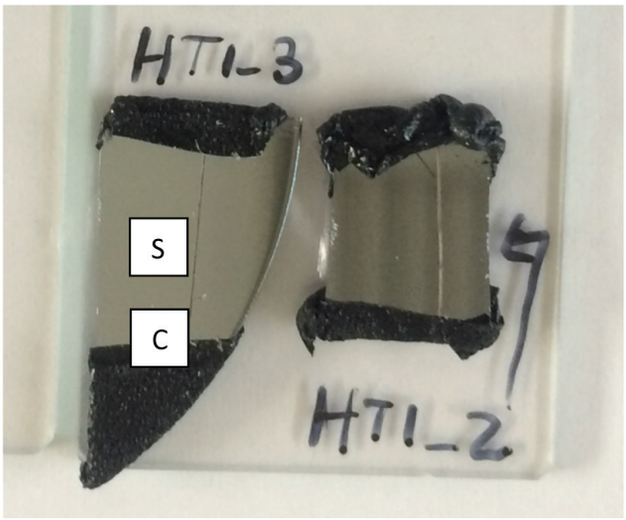

Figure 2. Longitudinally sectioned scalp hair strands secured on silicon wafers (S) with carbon tape (C). The incised surface should be positioned to face the primary ion beam.

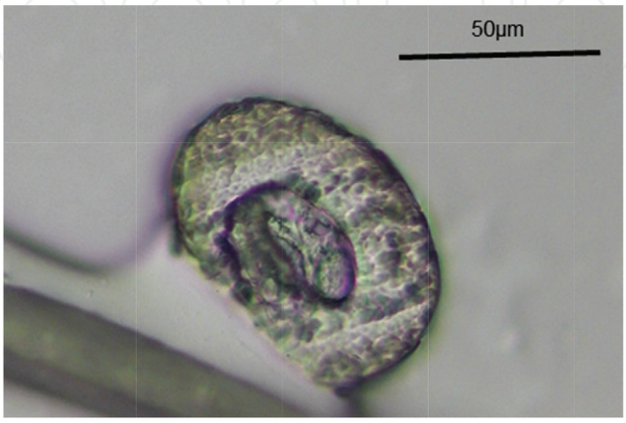

Figure 3. Light micrograph of an animal hair fiber sectioned under cryoconditions (unpublished data).

Such analysis may also effectively aid in determining elements distributed at the root sheath or cuticle cells and medulla of the hair and hence distinguish external contaminants from biogenic elements.

The aforementioned sectioning techniques may only be relevant when screening small sample sizes. However, for population studies, it is more advisable to analyze hair samples from a large number of participants that have been homogenized and pelleted [55]. This approach, however, has several disadvantages. Sampling requires a high number of participants, retrospective analysis will not be possible, information from specific individuals is lost as hair samples are homogenized, and contaminants may be introduced during sample processing. Sampling processing involving bulk processing is, however, fairly simple and traditionally involves homogenizing the hair samples into a powder using the brittle fracture technique. Approximately 2 g of hair are homogenized in a Teflon container and ball, which has been cooled with liquid nitrogen for 3 minutes. The container containing the hair and Teflon ball is vibrated for 2 minutes at 3000 cycles per min using a "micro-dismembrator." The longer the procedure is repeated, the finer the hair powder obtained. The fine powder should be carefully mixed, stored at room temperature, and pelleted before analysis. Graphite (1% pure reactor grade) may also be added to the mixture to reduce charging of samples. Homogeneity and uniformity of the sample are extremely important, particularly when using ion beam analysis techniques where the spot size of the beam is smaller than the sample to be irradiated.

5. Ion beam methods and synchrotron-based techniques in hair elemental profiling

Hair analysis has to date mostly exhausted conventional techniques that require digestion and hence destruction of a relatively large amount of hair. Such bulk analyses destroy information on the localized concentrations of minerals in a tissue. Ion beam and synchrotron radiation techniques have proven to be a solution to this dilemma. Numerous publications now exist that demonstrate the versatility of ion beam and synchrotron radiation analysis to map the quantitative distribution of minerals in hair cross sections or show the longitudinal distribution of elements in hair tissues [56, 57].

The unifying characteristics of these techniques include their sensitivity, selectivity, quantitative multielemental character, and speed of analysis [58]. Furthermore, depth profiling without physical sectioning is often possible with ion beam techniques, while the ion microprobe may allow μm or lower spatial resolution for diverse applications. Analysis of intact samples without any chemical processing or dissolution is also possible, whereas damage to analyzed samples may be minimal, allowing for downstream analyses with other techniques.

Ion beam and synchrotron radiation methods are based on the interaction of nuclear particles with atomic nuclei. Chemical bonding information is, however, not available as the electronic

http://dx.doi.org/10.5772/intechopen.72076

shell of the atom does not contribute to the physical process. Chemical speciation information is also not available with certain ion beam techniques, although results are not affected by the chemical form of the element. Furthermore, only surface near regions may be analyzed in most cases because of the short range of ions in matter. Perhaps the most significant downside of these techniques is the expensive equipment required and the fact that access to nuclear analytical facilities and expertise may not be accessible in some developing regions of the world.

Important considerations when performing nuclear analysis include the type of standard to use, the thickness of the sample, as well as the effect of irradiation on the sample [59]. The use of standards may vary depending on the type and aim of the study, while it is imperative to analyze relatively thin material (few nm to 10 μm) due to the energy loss of the charged particles. For irradiation effect, there are some detailed studies on whether analysis with ion beams lead to sample damage. To illustrate, in secondary ion mass spectrometry analysis at MeV energies (MeV-SIMS) and at low beam fluences—meaning the number of primary ions hitting the target area unit—the analysis should be nondestructive [60]. Over time, the yield of secondary molecules should also exponentially decrease as a function of beam fluence. The slope of this exponential fall is determined by the damage cross section, which explains the damage induced on the specimen surface by one primary ion. Experiments at the Jožef Stefan Institute in Slovenia utilizing a 5.8 MeV $^{35}Cl^{6+}$ primary ion beam reported damage cross section values of approx. 2 nm^2 for the amino acids arginine and leucine. A fluence of 10^{12} ions/cm^2 is the commonly accepted static limit of MeV-SIMS corresponding to 3 hours of measurement that agrees to approximately 8% of the target surface undergoing chemical alteration due to ion-induced damage. Since hair sample measurements is normally 1 hour, the hair chemical environment and morphology should remain unchanged. Scanning electron micrograph images further confirmed that the morphology of the analyzed samples remained unaltered after 1 hour of measurement with MeV-SIMS [53]. With particle-induced X-ray emission, however, a clear change in the sample's physical appearance can be noted when measuring for a longer time frame.

Besides sample damage, volatile analytes may also be lost during sample irradiation as a function of their thermal and radiation stability [61]. Adjusting the beam intensity and irradiation time may assist with alleviating this problem. Alternatively, another ion beam technique may be chosen to avoid this problem. For example, in-air proton-induced X-ray emission lends the advantage of performing measurements in air, resulting in negligible charging of the insulating targets, reduced radiation damage to the specimen due to the cooling effect of the air, and ultimately reduced loss of volatile elements [62]. Representative spectra obtained from a single hair fiber with in-air proton-induced X-ray emission is represented in **Figure 4**.

Another concern includes charge buildup on samples. For hair samples, charge buildup has been observed during bombardment with protons [55]. Many factors may influence this phenomenon, but most importantly, the thickness of the hair. Charge buildup and episodic discharging from samples lead to high background levels in the spectra and should be avoided at all cost. This may be achieved by blending thick targets with graphite or, alternatively, by coating thin samples with a layer of carbon or other appropriate conducting materials.

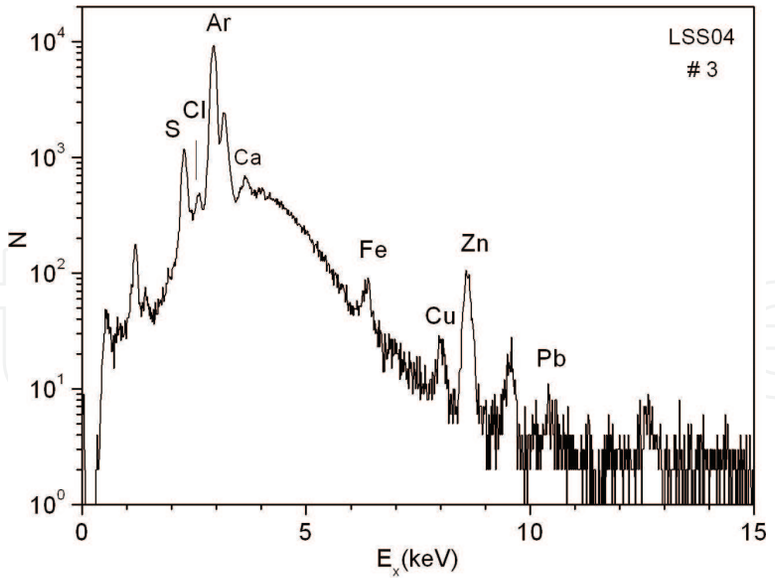

Figure 4. Representative in-air PIXE spectra from a single scalp hair, with the x-axis representing characteristic X-ray emission energy (KeV) and the y-axis representing X-ray emission intensity (counts) (unpublished data).

In the subsequent paragraphs, the focus will be placed on specific ion beam and synchrotron radiation analytical methods used in hair analyses. It should be borne in mind that techniques such as neutron activation analysis allow analysis of an entire sample, while specific ion beam techniques allow analysis of only the surface of a sample.

5.1. Neutron activation analysis

Neutron activation analysis has been described as a versatile method with a low detection limit for sample analysis of over 60 elements (major and trace elements) [63]. Samples do not require chemical preparation in which volatile elements may be lost. In addition, laborious sample homogenization procedures are not required for when a large amount of samples representative of a population need to be analyzed. The technique further requires only a small amount of sample weighing from 1 μm to hundreds of grams. Samples to be analyzed may also vary in form and shape. However, this need to be taken into consideration as sample geometry may affect results and also influence the choice of standard. A schematic overview of the technique is provided in **Figure 5**.

Instrumental neutron activation analysis is a popular analytical tool for the determination of elements in hair specifically for forensic applications [64]. Interestingly, instrumental neutron activation analysis has been applied in the forensic analysis of Napoleon's hair [65]. Napoleon died at the age of 51 on 5 May 1821, with his death officially ascribed to stomach cancer. Extensive investigations exploiting hair analysis, however, tried to dispute the officially declared cause of death as stomach cancer. Radiochemical neutron activation analysis showed that Napoleon's hair contained 10.38 ppm of arsenic. Longitudinal hair analysis to assess retrospective exposure to arsenic further showed that the arsenic was unevenly distributed along the hair length. Additional investigations with instrumental neutron activation analysis showed that Napoleon's hair also contained mercury, and abnormal levels of

http://dx.doi.org/10.5772/intechopen.72076

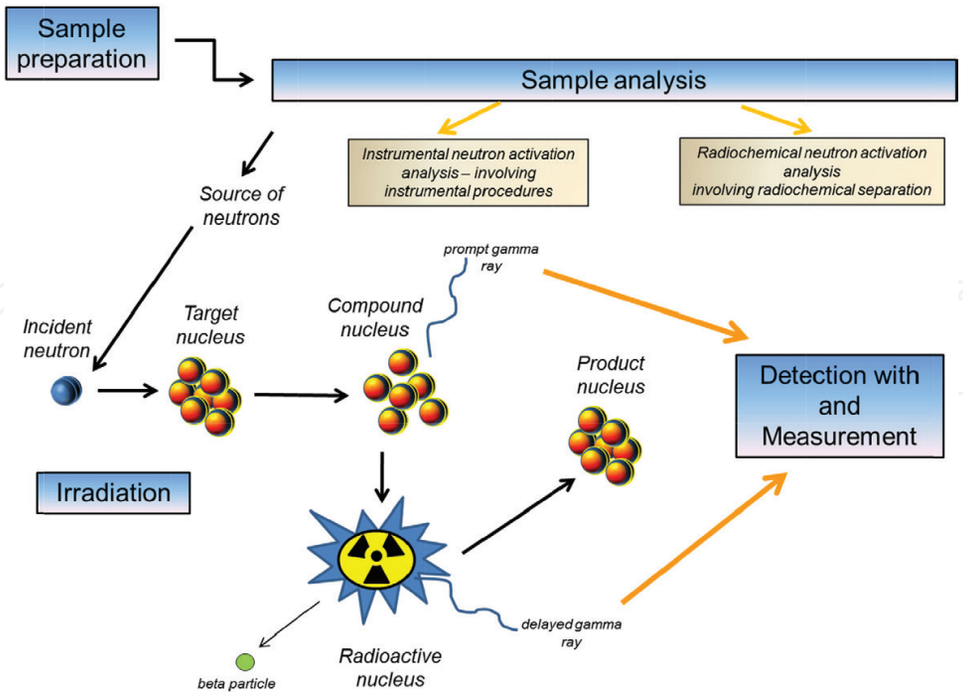

Figure 5. Schematic overview of the basic principle of neutron activation analysis.

chromium, antimony, and zinc that may have entered the body via food or medication. For example, Napoleon consumed a great deal of calomel that contains mercury to relieve constipation and thirst, while antimony was a component of tartar emetic, which was given to him shortly before his death as an agent to inhibit vomiting that would have expelled the poisons from his body.

Doubt, however, still exists whether the concentration of arsenic in Napoleon's hair was of endogenous or exogenous origin as exogenous contaminants may also be evenly distributed along the length of the hair. Hair may absorb arsenic from external contaminants that include wallpaper, coal smoke, water, cosmetics, and preservatives. The absorbed arsenic from either endogenous origin appearing in the medulla or exogenous origin appearing at the hair surface regions may remain in the hair structure even after washing. The conundrum of differentiating exogenous from endogenous exposure in hair can perhaps be more clearly understood through the use of ion beam imaging techniques such as particle-induced X-ray emission.

5.2. Ion beam imaging

Ion beam imaging has become a very popular tool for understanding the distribution of minerals in biological tissues. For hair elemental screening, particle-induced X-ray emission has emerged as one of the most popular and powerful ion beam techniques allowing quantitative elemental mapping in tissues at micrometer or lower resolution that well complements data obtained using other techniques such as synchrotron radiation X-ray fluorescence (XRF). The only disadvantage of the technique is that not all countries have robust facilities hosting the technique.

The technique falls under a broad family of X-ray emission techniques for the quantitative distribution mapping of low Z elements ($20 < Z < 35$ and $75 < Z < 85$) [66]. Particle-induced X-ray emission is based on the irradiation of a sample with a high-energy ion beam (typically 1–2 MeV of H or He) that excites the inner electron shells of an atom, inducing characteristic X-rays to be emitted by de-excitation of the atom. As negligible overlapping of characteristic X-rays for different elements occurs as the energy of an emitted X-ray is characteristic for a target element, multielemental detection becomes possible. X-rays are measured by an energy-dispersive (for example Si[Li]) detector placed at an angle of 135° relative to the beam direction to minimize the Bremsstrahlung background. When correcting for absorption and X-ray yields, quantitative data for elements may be obtained at detection limits of orders of magnitude around 10 ppm for thick samples. Since the physical processes involved in the generation of X-rays are well studied, no standards are required for producing quantitative elemental imaging with proton-induced X-ray emission. Elemental imaging in samples is obtained by scanning the ion beam microprobe over the surface of the specimen with a penetration depth of up to 100 μm.

Variants of particle-induced X-ray emission such as microproton-induced X-ray emission and in-air proton-induced X-ray emission offer supplementary benefits. For example, microproton-induced X-ray emission offers the added advantage of spatially resolved multielemental analysis of micrometer resolution [66], while in-air proton-induced X-ray emission lends the added advantage of performing measurements in air resulting in negligible charging of the insulating targets, reduced radiation damage and loss of volatile elements due to the cooling effect of the air, and simple handling and changing of samples [62]. In essence, ion beam techniques such as proton-induced X-ray emission provide nondestructive, fully quantitative, and multielemental mapping at micrometer-scale or lower spatial resolution and μg/g-level sensitivity of samples that require minimal processing [67]. Examples of applications of ion beam imaging using particle-induced X-ray emission for hair research will be discussed in Section 6. It is also recommended that the readers familiarize themselves with the numerous literatures describing the experimental setup, analysis, and data processing for the technique.

5.3. Time-of-flight MeV secondary ion mass spectrometry

Another technique that may well complement particle-induced X-ray emission is time-of-flight SIMS. Particle-induced X-ray emission may be used to quantify the elemental fingerprint of intact human scalp hair fibers, whereas time-of-flight SIMS may give an indication of both organic and inorganic compounds present in longitudinally sectioned hair. Time-of-flight SIMS is currently one of the most sensitive surface analysis techniques for chemical mapping, providing micrometer or lower resolution for depth profiling of the first one or two surface monolayers in an intact sample [47]. In addition, it allows parallel analyses and imaging of multiple elements, isotopes, and molecules in complex samples without exhaustive sample preparation such as labeling to provide information on the spatial localization of organic and inorganic compounds [68]. The main drawback of the technique is, however, the inability to quantify secondary ions due to the effect of the chemical composition of the matrix on the yield of secondary ions, also commonly referred to as the matrix effect [69].

http://dx.doi.org/10.5772/intechopen.72076

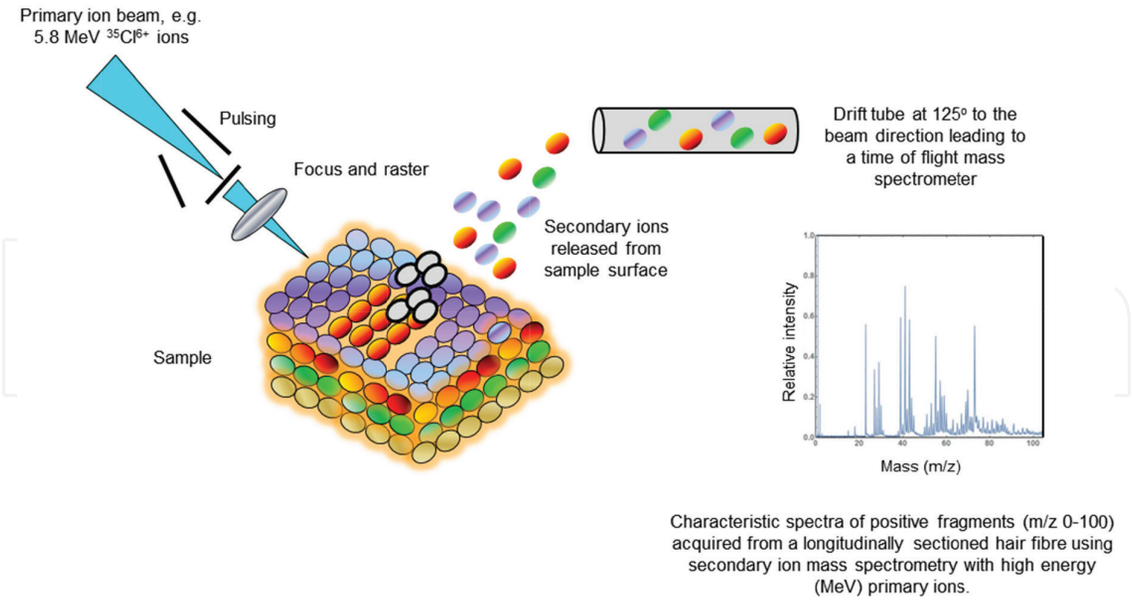

Figure 6. Schematic overview of the basic principle of time-of-flight secondary ion mass spectrometry with high-energy (MeV) primary ions.

In time-of-flight SIMS analysis, the sample is traditionally bombarded with ions in the energy range of 5–25 keV [68]. Recently, the technique has, however, also been tested with primary ions at MeV energies in a method termed MeV-SIMS [60, 70]. A schematic overview of the technique is provided in **Figure 6**. In MeV-SIMS, the interactions between primary ions and sample molecules are based on electronic energy loss, which creates a softer sputtering process and a 1000 times greater yield of larger and intact ionized molecules than conventional SIMS in the keV energy range. A pulse of specific primary ions at MeV energy—depending on the molecules of interest—is focused and rastered over the sample surface under ultra-high vacuum to generate position-dependent mass spectra [60, 70]. Various charged ions, also termed secondary ions, are released by the impact of the MeV primary ions with the sample surface. These secondary ions are of equal kinetic energy and enter a drift tube in which they are separated according to their mass-to-charge ratio. The masses of the secondary ions depend on the chemical composition of the sample scanned at each point, while the intensity of the secondary ion signal depends on the concentration of the particular compound in the area sampled as well as the ionization yield of the compound. The yield of secondary ions in turn depends on the incident angle, nature, and energy of the primary ion beam as well as the chemical properties of the target matrix.

The technique allows (1) parallel counting of secondary ions matched with preselected masses obtained from the analyzed points and (2) simultaneous building of chemical images [47]. The technique further allows for mass spectra to be extracted from an image region and vice versa to display the distribution of a secondary ion within the tissue. Interestingly, the technique is also able to analyze isotopic composition of light elements in a matrix such as hair [53]. The reader is, however, strongly recommended to first familiarize themselves with the experimental setup and a range of available detectors, the mechanism involved in

secondary ion formation and the effect of the matrix composition on yield, as well as data processing and multivariate statistics deployed in SIMS before tackling this robust and challenging technique.

5.4. Synchrotron radiation

When electrons travel near the speed of light and are forced to change direction by a magnetic field, electromagnetic radiation is emitted that is also commonly described as synchrotron radiation [58, 71]. Today, synchrotron radiation has become one of the most powerful methodological tools for understanding the properties of matter in various scientific fields. The distinctive properties of synchrotron light include its ability to provide an energy tunable source of X-rays that can be highly linear, circular, or elliptically polarized; have a high brightness and collimation; have a wide energy spectrum with energies ranging from infrared light to hard X-rays; and provide nanosecond long light pulses that permit time-resolved studies.

Synchrotron facilities are characterized by a storage ring from which synchrotron radiation is emitted in a forward direction in a narrow cone that is at a tangent to the electron's orbit [71]. The width of this cone of electrons is affected by the speed of the electrons, which further affects the spectrum of the radiation, shifting it toward shorter wavelengths with increasing electron energy. The storage ring of so-called third-generation light sources is further equipped with magnetic insertion devices termed undulators and wigglers that are used to generate linear or circular polarized light by generating magnetic fields that drive electrons into an oscillating or spiral trajectory. These third-generation facilities may specialize in short-wavelength (hard X-rays), long-wavelength (soft X-rays), or vacuum-ultraviolet X-rays.

To focus the X-rays on the sample at micro- or nanometer resolution, different types of X-ray optics are used at various synchrotron facilities [72]. Some of these systems such as the Kirkpatrick-Baez mirror system use two glancing-angle, bowl-shaped mirrors with a curve designed to demarcate the beam profile at a focal point. Other systems include refractive lenses or Fresnel zone plates. These optical systems permit hard X-rays to be focused to 30–150 nm spot sizes that are injected to the sample. Ideally, the energy of the X-ray beam should be selected over a specified energy range. X-ray spectra are recovered subsequent to step- or raster scanning of the sample in the x and y planes at 45° to the beam path. More than one detector may be used to capture the spectra and deliver data with the beam x,y coordinates for each incident beam point. Conventional detectors for various applications that are common include Si(Li) detectors, intrinsic Ge detectors, or silicon drift detectors.

Probably the most important aspect of the analysis is optimizing the absorption of the X-rays in the samples to be analyzed [72]. The absorption of different types of X-rays produced in a synchrotron is controlled by the photoelectric effect. However, absorption may also be influenced by the chemical environment of the sample and element oxidation state. When an X-ray penetrates a sample and its energy is lower than the binding energy of the core electrons in the element of significance, its atoms do not absorb the X-rays. When the energy of the incoming X-rays and the binding energy of the core level electrons of an element are equal, X-ray photons are absorbed. Photoelectrons are produced since the incident X-ray electrons excite

http://dx.doi.org/10.5772/intechopen.72076

the core electrons. For the atom to return to a ground state, an electron needs to fill the empty position of the ejected photoelectron. This transition energy or wavelength is released as fluorescence or as an Auger electron for lighter elements. The number or intensity of X-ray photons is positively correlated with atomic abundance and hence individual element quantities. This is however mostly true for thin samples in which self-absorption within the sample is negligible. The net X-ray intensities of the elemental makeup are obtained via spectral deconvolution methods.

The absorption of X-rays into matter outlined above forms the backbone of a versatile synchrotron technique, X-ray absorption spectroscopy (XAS). The latter technique provides detailed information on elemental chemistry such as oxidation state and molecular geometry [72, 73]. Based on the energy region of interest, XAS can be further differentiated into X-ray absorption near-edge structure, extending from the pre-edge region to approximately 50 eV above the absorption edge and extended X-ray absorption fine structure (EXAFS), extending from about 50 to 1000 eV beyond the edge. Since electron transitions may occur from a partly bound and excited state in the pre-edge region, certain chemical features may become visible in this region. For EXAFS, the physical processes giving rise to the signal can be modeled by selected computer programs that allow accurate assessment of the identity of the surrounding atoms, bond distances, and coordination numbers.

Another technique based on the absorption of X-rays in matter described above is XRF [72, 73]. XRF permits quantitative elemental mapping. The physics of the photon interaction with matter is also well understood, simplifying quantification of data. Although XRF is similar to proton-induced X-ray emission and scanning electron microscopy with energy dispersive X-ray spectroscopy, synchrotron XRF is more sensitivity due to a high photon flux, weak scattering, and the availability of a tunable beam. Currently, XRF is one of the most sensitive imaging techniques offering spatially resolved down to 100 nm and quantitative topographical maps for a range of elements at submicron resolution. Depth resolution, however, depends on the elements of interest and sample nature, although high penetration depths of up to 1000 μm is possible that allows imaging of thicker samples or in-situ experiments.

Since this section only summarizes the background to synchrotron radiation and applicable techniques for analysis of hair samples, it is recommended that the readers familiarize themselves with the numerous literature sources available on specific synchrotron radiation techniques applicable to their samples and familiarize themselves with the beamlines and experimental setup at a chosen synchrotron facility. The latter may include the type of source device (bending magnet, wiggler magnet, or undulator), mirrors used to deflect or focus the X-rays, monochromators used for sorting incoming X-ray energies, glitch avoidance (double diffractions occurring in monochromator cryst detectors), detectors, availability of true imaging microscopes, electronics, and most importantly, data collection and statistics. Furthermore, it is important to familiarize oneself with an optimal sample preparation and calibration methodology for a specific synchrotron technique, elements accessible with the technique, effect of external parameters such as temperature on sample analysis, and nonscientifically, the culture of the facility and its researchers. Most importantly, beamtime application procedures also warrant careful attention.

6. Biomedical applications of ion beam and synchrotron analysis of hair

The amount of literature on hair chemical and specifically elemental analysis has grown substantially in recent years and with the specific focus on elemental analysis, it has been shown that almost any macro, trace, and xenobiotic elements can be quantitatively measured and mapped in hair tissues. The predominant amount of work with ion beam and synchrotron analyses has, however, focused on analyzing the distribution and content of elements in the hair of diseased patients versus healthy controls or those exposed to environmental pollutants. In the subsequent sections, examples of selected studies that employed ion beam and synchrotron radiation analysis to link elemental content with morbidity and toxicology will be discussed.

6.1. Dermatology

There is an alarming lack of fundamental studies describing the role of elements in the growth and physiology of hair or how elemental levels vary during the different growth stages of hair. Variation in elemental levels in hair at different stages of growth is also an important confounding variable in hair elemental research. The first researchers that tried to assess the quantitative elemental distribution in organ-cultured hair follicles during the anagen and catagen growth phases were from the Jožef Stefan Institute in Slovenia and ion microprobe facility ATOMKI in Debrecen [74]. Combined ion beam analysis exploiting proton-induced X-ray emission and scanning transmission ion microscopy were used to quantify elemental content in hair follicles during the catagen and anagen growth phases. The results showed that elemental concentrations were similar in selected parts of the hair follicle in both growth stages. However, the outer/inner root sheath keratinocyte layers of hair follicles in the catagen growth phase contained four times more calcium than the same regions in hair follicles in the anagen growth phase. These layers are known to express the receptor for capsaicin, TRPV1, which function as a calcium-permeable channel [75]. During the catagen growth phase of hair, an increase in the intracellular calcium concentration inhibits the proliferation of keratinocytes that triggers the induction of apoptosis [76]. In summary, this study showed the promise of ion beam techniques such as proton-induced X-ray emission and scanning transmission ion microscopy to improve our understanding of changes in elemental levels during hair growth.

6.2. Pediatrics

The deleterious effect of environmental pollutants such as lead on human health cannot be over emphasized [77]. The outcomes of lead exposure in children are particularly concerning due to its irreversible effects on one of the most sensitive organs to lead exposure, the developing brain [78]. In particular, children exposed to lead may have cognitive and behavioral effects that persist into adulthood [79]. To assess daily lead absorption during the prenatal phase, the longitudinal distribution of lead in fetal and parental hair was studied using synchrotron radiation micro-XRF [80]. The technique proved very successful in mapping lead along a longitudinal length that reflected a retrospective profile of lead exposure and absorption during the prenatal phase.

Hair elemental profiles may also find application as risk factors for disease. For example, intraindividual variations of 32 hair elemental levels as epidemiological risk factors for atopic dermatitis in infants were studied using particle-induced X-ray emission combined with rigorous regression statistics [57]. Hair samples were retrieved 1 month after birth and also at 10 months after birth with the onset of atopic dermatitis. The results showed that selenium and strontium could be used as explanatory variables in a regression model for atopic dermatitis. However, large intraindividual variations for selenium and strontium could affect the regression coefficients for strontium and selenium. Particle-induced X-ray emission was specifically employed in this study to understand intraindividual variations for selenium and strontium to correct for intraindividual variations in the previously described regression model. Such type of approach combining analytical techniques with statistical models is particularly important in promoting the application of hair elemental data in epidemiological research.

6.3. Psychiatry

Trace elements have also been shown to play an important role in psychiatric diseases. A study using proton-induced X-ray emission was conducted to assess the trace elements in the scalp hair of patients with alcohol-induced psychosis [81]. The results showed that iron and copper levels were higher in patients with alcohol-induced psychosis, whereas the concentrations of manganese and zinc were lower, compared to healthy controls.

The same approach was applied to identify and quantify elements in the scalp hair of patients with bipolar disorder based on gender [82]. For males, the concentration of copper was higher in bipolar patients compared to controls, whereas the concentrations of manganese, iron, zinc, and selenium were lower in bipolar patients of both genders compared to healthy controls. The same was observed in females, except that instead of manganese, nickel was higher in bipolar patients of both genders than controls. Furthermore, the Cu/Zn ratio was found to be higher in bipolar patients of both genders. This is not surprising as it is known that elemental dyshomeostasis triggers the formation of free radicals that may in turn affect neurotransmitter activity that plays an important role in psychiatric disorders [83]. However, treatment may also affect elemental levels in psychiatric patients, emphasizing the importance of assessing elemental levels before and after treatment [84].

6.4. Oncology

Breast cancer is a devastating cancer affecting the lives of many women and men around the world. Interestingly, hair samples have been used to assess the risk of breast cancer [85]. With XRF and X-ray diffraction techniques, it was shown that not only elemental levels but also hair structure differ between healthy controls and those with breast cancer [86]. More specifically, the data revealed that trace element levels were higher in healthy controls than in breast cancer patients and that wavelength of XRF presented with a 96% sensitivity compared to a 77% sensitivity for mammography, the gold standard for breast cancer screening.

Besides breast cancer, the effect of radiation therapy on hair trace elemental concentrations in cervical cancer patients has also been investigated with ion beam analysis; in this case, proton-induced X-ray emission [87]. Testing was done before and during radiation therapy. The concentrations of chlorine, potassium, calcium, titanium, chromium, manganese, iron, nickel, and zinc were lower and copper higher before irradiation. It was also shown that the concentration of specific elements varied during the course of radiation therapy, indicating a possible effect of this type of cancer therapy on elemental levels in the body.

6.5. Toxicology: environmental and occupational exposure to heavy metal pollution

Elemental analysis of hair samples also now finds important applications in the fields of toxicology. In this field, ion beam techniques such as particle-induced X-ray emission has been mostly used in the evaluation of toxic element pollution in various regions of the world [88]. For example, one study used particle-induced X-ray emission to assess the levels of toxic elements in the vicinity of a mining area in Mongolia, which included places of milling, grasslands, and villages [89]. Among the samples tested, human hair was also used. The average concentrations of titanium, arsenic, and strontium were found to be higher in hair samples from miners than that of control participants. Besides studies related to environmental pollution, ion beam analysis has also been applied in toxicology research. For example in toxicology studies, mercury has completely overpopulated the literature [14].

6.6. Veterinary science

Elemental analysis of hair samples has also branched into the field of veterinary sciences. Interestingly, ion beam techniques have also been exploited in animal hair analysis. For example, particle-induced X-ray emission was employed to assess the levels of elements in the main hair of horses in a study aimed at investigating the relationship between main hair elemental concentrations and the severity of second degree atrioventricular block in horses [90]. The results showed that there was a significant positive correlation between the zinc/copper ratio and calcium concentration in main hair and the drop in ventricular beats measured hourly in animals with a second-degree atrioventricular block. Receiver operating characteristic curve analysis suggested that the cut-off points for hair calcium concentration were set at 1536 μg/g and 26.0 for the zinc/copper ratio in detecting second-degree atrioventricular block in horses. It was concluded that the levels of calcium, zinc, and copper in the main hair of horses may be used as a diagnostic tool for testing susceptibility to atrioventricular block.

7. Summary

Today, more and more studies connect metabolic homeostasis and morbidity with the complicated interaction between essential and nonessential elements [91]. However, disease may also perturb elemental levels and alter the expression of other chemical compounds in the body [14]. A good example here includes the effect of arsenic exposure on the reduction of

glucose tolerance [92]. Elemental dyshomeostasis in the body will also affect hair metabolic processes and hence elemental levels within the actively growing hair.

A fundamental understanding of what for example elemental concentrations in hair actually describe still requires more rigorous research and debate. More specifically, more studies investigating the incorporation of chemicals into hair, for example how elemental levels relate to blood levels and other biomarkers of disease, and most importantly, the contribution of contaminants to biogenic hair elemental content, are required. When performing hair analysis, careful consideration of the various confounding factors in hair elemental analysis such as medication use, smoking, and seasonal diet fluctuations is also warranted [14, 93]. In essence, these considerations are imperative before the intricate link can be cemented between hair elemental concentrations and its biological significance related to morbidity or other factors.

Studies utilizing ion beam and synchrotron radiation techniques have, however, improved our understanding of the distribution of elements in hair tissue and elemental dyshomeostasis in disease [50, 80, 82]. Hair elemental analysis finds perhaps the most important application in toxicology research relating to acute versus chronic exposure to toxic metals and other xenobiotic compounds [80]. A fascinating example includes the case of the famous race horse Phar Lap in which synchrotron radiation XRF was used to confirm the cause of death [94]. Arsenic mapping in hair using synchrotron XRF and XAS not only allowed longitudinal distribution mapping of arsenic in the hair of Phar Lap, but also the retrospective changes in the metabolic incorporation of arsenic into the hair shaft. Another example includes the use of ion beam or synchrotron radiation analysis in epidemiological and etiological studies assessing exposure to toxic metals in vulnerable populations [14]. A recent exploratory study also pointed to the use of MeV-SIMS for the detection and mapping of the micronutrient lithium and its isotopes in longitudinally sectioned hair [53]. Lithium is an important psychopharmaceutical in the psychiatric community, and since it is quite challenging detecting the low Z element lithium that is present at trace levels in biological tissues, such analysis may find important application in monitoring medication adherence among psychiatric patients using hair. In summary, hair analysis remains a cost-effective baseline tool for not only more comprehensive studies linking morbidity with the chemicals and more specifically elements found in hair and vice versa, but also various other important applications in diverse fields. With the continuous development and optimization of ion beam and synchrotron radiation techniques, the future of hair analysis remains bright.

Author details

Karen J. Cloete

Address all correspondence to: kabouter cloete@gmail.com

iThemba Laboratory for Accelerator Based Sciences-National Research Foundation, Somerset West, South Africa

References

[1] Soetan KO, Olaiya CO, Oyewole OE. The importance of mineral elements for humans, domestic animals and plants: A review. African Journal of Food Science. 2010;**4**:200-222

[2] Mann J, Truswell A, editors. Essentials of Human Nutrition. 5th ed. Oxford: Oxford University Press; 2017. 720 p

[3] Prashanth L, Kattapagari KK, Chitturi RT, Baddam VR, Prasad LK. A review on role of essential trace elements in health and disease. Journal Dr. NTR University of Health Sciences. 2015;**4**:75-85. DOI: 10.4103/2277-8632.158577

[4] Zhang X-Y, Zheng L-N, Wang H-L, Shi J-W, Feng W-Y, Li L, Wang M. Elemental bioimaging of biological samples by laser ablation-inductively coupled plasma-mass spectrometry. Chinese Journal of Analytical Chemistry. 2016;**44**:1646-1651. DOI: 10.1016/S1872-2040(16)60969-6

[5] Hwang C, Hong K. Other micronutrient deficiencies in inflammatory bowel disease: From A to Zinc. In: Ananthakrishnan AN, editor. Nutritional Management of Inflammatory Bowel Diseases. Switzerland: Springer; 2016. p. 65-101. DOI: 10.1007/978-3-319-26890-3

[6] Dinis-Oliveira RJ, Carvalho F, Duarte JA, Remião F, Marques A, Santos A, Magalhães T. Collection of biological samples in forensic toxicology. Toxicology Mechanisms and Methods. 2010;**20**:363-414. DOI: 10.3109/15376516.2010.497976

[7] Takai N, Tanaka Y, Inazawa K, Saji H. Quantitative analysis of pharmaceutical drug distribution in multiple organs by imaging mass spectrometry. Rapid Communications in Mass Spectrometry. 2012;**26**:1549-1556. DOI: 10.1002/rcm.6256

[8] Norris JL, Caprioli RM. Analysis of tissue specimens by matrix-assisted laser desorption/ionization imaging mass spectrometry in biological and clinical research. Chemical Reviews. 2013;**113**:2309-2342. DOI: 10.1021/cr3004295

[9] Mercolini L, Protti M. Biosampling strategies for emerging drugs of abuse: Towards the future of toxicological and forensic analysis. Journal of Pharmaceutical and Biomedical Analysis. 2016;**130**:202-219. DOI: 10.1016/j.jpba.2016.06.046

[10] El BG, Flieger J, Huber M, Kocjan R. Examination of the elemental composition of hair in cholelithiasis, kidney stone, hypertension and diabetes by scanning electron microscopy and energy dispersive spectrometry SEM/EDS. Journal of Analytical & Bioanalytical Techniques. 2014;**5**:207. DOI: 10.4172/2155-9872.1000207

[11] Schöpfer J, Schrauze GN. Lithium and other elements in scalp hair of residents of Tokyo prefecture as investigational predictors of suicide risk. Biological Trace Element Research. 2011;**144**:418-425. DOI: 10.1007/s12011-011-9114-x

[12] Zaitseva IP, Skalny AA, Tinkov AA, Berezkina ES, Grabeklis AR, Skalny AV. The influence of physical activity on hair toxic and essential trace element content in male and female students. Biological Trace Element Research. 2015;**163**:58-66. DOI: 10.1007/s12011-014-0172-8

[13] Abbruzzini TF, Silva CA, Andrade DA, Carneiro WJO. Influence of digestion methods on the recovery of iron, zinc, nickel, chromium, cadmium and lead contents in 11 organic residues. Revista Brasileira de Ciência do Solo. 2014;**38**:166-176. DOI: 10.1590/S0100-06832014000100016

[14] Kempson IM, Lombi E. Hair analysis as a biomonitor for toxicology, disease and health status. Chemical Society Reviews. 2011;**40**:3915-3940. DOI: 10.1039/c1cs15021a

[15] Jaber JA, Holt D, Johnston A. Method development for the detection of basic/weak basic drugs in hair by LCMSMS: Comparison between methanolic and alkaline extraction on real samples. Pharmacology & Pharmacy. 2012;**3**:263-274. DOI: 10.4236/pp.2012.33035

[16] Namera A, Kawamura M, Nakamoto A, Saito T, Nagao M. Comprehensive review of the detection methods for synthetic cannabinoids and cathinones. Forensic Toxicology. 2015;**33**:175-194. DOI: 10.1007/s11419-015-0270-0

[17] Pillay AE. A review of accelerator-based techniques in analytical studies. Journal of Radioanalytical and Nuclear Chemistry. 2000;**243**: 191-197. DOI: 10.1023/A:1006748104841

[18] Nastasi M, Mayer JW, Wang Y, editors. Ion Beam Analysis, Fundamentals and Applications. Boca Raton: CRC press; 2014. 472 p

[19] Jeynes C, Webb RP, Lohstroh A. Ion beam analysis: A century of exploiting the electronic and nuclear structure of the atom for materials characterisation. Reviews of Accelerator Science and Technology. 2011;**4**:41-82. DOI: 10.1142/S1793626811000483

[20] Dong Z, Jim RC, Hatley EL, Backus ASN, Shine JP, Spengler JD, Schaider LA. A longitudinal study of mercury exposure associated with consumption of freshwater fish from a reservoir in rural South Central USA. Environmental Research. 2015;**136**:155-162. DOI: 10.1016/j.envres.2014.09.029

[21] Favretto D, Vogliardi S, Tucci M, Simoncello I, El Mazloum R, Snenghi R. Occupational exposure to ketamine detected by hair analysis: Retrospective and prospective toxicological study. Forensic Science International. 2016;**265**:193-199. DOI: 10.1016/j.forsciint.2016.03.010

[22] Preedy V, editor. Handbook of Hair in Health and Disease. The Netherlands: Wageningen Academic Publishers; 2012. 476 p. DOI: 10.3920/978-90-8686-728-8

[23] Katz SA, Chatt A. The Use of Hair as a Biopsy Tissue for Trace Elements in the Human Body, Application of Hair as an Indicator for Trace Element Exposure in Man, A Review. NAHRES-22. Vienna: International Atomic Energy Agency; 1994. 11 p

[24] Rosen EP, Thompson CG, Bokhart MT, Prince HM, Sykes C, Muddiman DC, Kashuba AD. Analysis of antiretrovirals in single hair strands for evaluation of drug adherence with infrared-matrix assisted laser desorption electrospray ionization mass spectrometry imaging. Analytical Chemistry. 2016;**88**:1336-1344. DOI: 10.1021/acs.analchem.5b03794

[25] Buononato EV, De Luca D, Galeandro IC, Congedo ML, Cavone D, Intranuovo G, Guastadisegno CM, Corrado V, Ferri GM. Assessment of environmental and occupational exposure to heavy metals in Taranto and other provinces of Southern Italy by means of scalp hair analysis. Environmental Monitoring and Assessment. 2016;**188**:337. DOI: 10.1007/s10661-016-5311-6

[26] Kintz P. Forensic Science Series: Analytical and Practical Aspects of Drug Testing in Hair. Florida: Boca Raton; 2007. 382 p

[27] Pragst F, Balikova MA. State of the art in hair analysis for detection of drug and alcohol abuse. Clinica Chimica Acta. 2006;**370**:17-49. DOI: 10.1016/j.cca.2006.02.019

[28] Kegler R, Büttner A, Nowotnik J, Rentsch D. Postmortem investigation of 88-cm-long dreadlocks for drugs of abuse: An unusual case report in the northeast of Germany. Forensic Toxicology. 2016;**34**:419-424. DOI: 10.1007/s11419-016-0318-9

[29] Rasmussen KL, Kučera J, Skytte L, Kameník J, Havránek V, Smolík J, Velemínský P, Lynnerup N, Bruzek J, Velley J. Was he murdered or was he not? – Part I: Analysis of mercury in the remains of Tycho Brahe. Archaeometry. 2013;**55**:1187-1195. DOI: 10.1111/**j**.1475-4754.2012.00729.x

[30] Robbins CR, editor. Chemical and Physical Behavior of Human Hair. 4th ed.. New York: Springer; 2013. 483 p. DOI: 10.1007/b97447

[31] Gerace E, Veronesi A, Martra G, Salomone A, Vincent M. Study of cocaine incorporation in hair damaged by cosmetic treatments. Forensic Chemistry. 2017;**3**:69-73

[32] Dias MFRG. Hair cosmetics: An overview. International Journal of Trichology. 2015;**7**:2-15. DOI: 10.4103/0974-7753.153450

[33] Galliano A, Ye C, Su F, Wang C, Wang Y, Liu C, Wagle A, Guerin M, Flament F, Steel A. Particulate matter (PM's) adheres to human hair exposed to severe aerial pollution: Consequences for certain hair surface properties. International Journal of Cosmetic Science. DOI: 10.1111/ics.12416

[34] Brewer PA, Bird G, Macklin MG. Isotopic provenancing of Pb in Mitrovica, northern Kosovo: Source identification of chronic Pb enrichment in soils, house dust and scalp hair. Applied Geochemistry. 2016;**64**:164-175. DOI: 10.1016/j.apgeochem.2015.08.003

[35] Robbins CR. Chemical Composition of Different Hair Types. 5th ed. New York: Springer; 2012. DOI: 10.1007/978-3-642-25611-0_2

[36] Kanti V, Nuwayhid R, Lindner J, Hillmann K, Stroux A, Bangemann N, Kleine-Tebbe A, Blume-Peytavi U, Garcia Bartels N. Analysis of quantitative changes in hair growth during treatment with chemotherapy or tamoxifen in patients with breast cancer: A cohort study. The British Journal of Dermatology. 2014;**170**:643-650. DOI: 10.1111/bjd.12716

[37] Grosvenor AJ, Marsh J, Thomas A, Vernon JA, Harland DP, Clerens S, Dyer JM. Oxidative modification in human hair: The effect of the levels of Cu (II) ions, UV exposure and hair pigmentation. Photochemistry and Photobiology. 2016;**92**:144-149. DOI: 10.1111/php.12537

[38] Baker JA, Ayad FK, Maitham SA. Influence of various parameters on the levels of arsenic in washed scalp hair from Karbala, Iraq by using ICP-OES technique. Karbala International Journal of Modern Science. 2016;**2**:104-112. DOI: 10.1016/j.kijoms.2016.02.004

[39] Błażewicz A, Liao KY, Liao HH, Niziński P, Komsta Ł, Momčilović B, Jabłońska-Czapla M, Michalski R, Prystupa A, Sak JJ, Kocjan R. Alterations of hair and nail content of selected trace elements in nonoccupationally exposed patients with chronic depression from different geographical regions. BioMed Research International. DOI: 10.1155/2017/3178784

[40] Skalny AV, Simashkova NV, Skalnaya AA, Klyushnik TP, Bjørklund G, Skalnaya MG, Tinkov AA. Assessment of gender and age effects on serum and hair trace element levels in children with autism spectrum disorder. Metabolic Brain Disease. DOI: 10.1007/s11011-017-0056-7

[41] Velasco MVR, de Sá Dias TC, de Freitas AZ, Júnior NDV, de Oliveira Pinto CAS, Kaneko TM, Baby AR. Hair fiber characteristics and methods to evaluate hair physical and mechanical properties. Brazilian Journal of Pharmaceutical Sciences 2009;**45**:153-162. DOI: 10.1590/S1984-82502009000100019

[42] Sukumar A. Factors influencing levels of trace elements in human hair. Reviews of Environmental Contamination and Toxicology. 2002;**175**:47-78

[43] Chojnacka K, Michalak I, Zielinska A, Gorecka H, Gorecki H. Inter-relationship between elements in human hair: The effect of gender. Ecotoxicology and Environmental Safety. 2010;**73**:2022-2028. DOI: 10.1016/j.ecoenv.2010.09.004

[44] Lemasters JJ, Ramshesh VK, Lovelace GL, Lim J, Wright GD, Harland D, Dawson Jr TL. Compartmentation of mitochondrial and oxidative metabolism in growing hair follicles: A ring of fire. The Journal of Investigative Dermatology. 2017;**137**:1434-1444. DOI: 10.1016/j.jid.2017.02.983

[45] Han E, Yang H, Seol I, Park Y, Lee B, Song JM. Segmental hair analysis and estimation of methamphetamine use pattern. International Journal of Legal Medicine. 2013;**127**:405-411. DOI: 10.1007/s00414-012-0766-7

[46] Poetzsch M, Baumgartner MR, Steuer AE, Kraemer T. Segmental hair analysis for differentiation of tilidine intake from external contamination using LC-ESI-MS/MS and MALDI-MS/MS imaging. Drug Testing and Analysis. 2015;**7**:143-149. DOI: 10.1002/dta.1674

[47] Vickerman JC, Briggs D. ToF-SIMS: Materials Analysis by Mass Spectrometry. Surface Spectra. 2nd ed. Chichester: Manchester and IM Publications, Chichester; 2013. 732 p

[48] Schaffer M, Hill V, Cairns T. Hair analysis for cocaine: The requirement for effective wash procedures and effects of drug concentration and hair porosity in contamination and decontamination. Journal of Analytical Toxicology. 2005;**29**:319-326

[49] Kempson IM, Skinner WM. A comparison of washing methods for hair mineral analysis: Internal versus external effects. Biological Trace Element Research. 2012;**150**:10-14. DOI: 10.1007/s12011-012-9456-z

[50] Pineda-Vargas CA, Eisa MEM. Analysis of human hair cross sections from two different population groups by nuclear microscopy. Nuclear Instruments and Methods in Physics Research B. 2010;**268**:2164-2167. DOI: 10.1016/j.nimb.2010.02.041

[51] Kempson IM, Skinner WM. ToF-SIMS analysis of elemental distributions in human hair. Science of the Total Environment. 2005;**338**:213-227. DOI: 10.1039/c1cs15021a

[52] Kempson IM, Skinner WM, Kirkbride PK. A method for the longitudinal sectioning of single hair samples. Journal of Forensic Sciences. 2002;**47**:889-892. DOI: 10.1520/JFS15452J

[53] Cloete KJ, Jenčič B, Šmit Ž, Kelemen M, Mkentane K, Pelicon P. Detection of lithium in scalp hair by time-of flight secondary ion mass spectrometry with high energy (MeV) primary ions. Analytical Methods. DOI: 10.1039/C7AY01616F

[54] Van Baar HM, Schalkwijk J, Perret CM. Cryosectioning of hair follicles. An improved method using liquid nitrogen conduction freezing. Acta Dermato-Venereologica. 1990; **70**:335-338

[55] Valkovic V.Sample Preparation Techniques in Trace Element Analysis by X-ray Emission Spectroscopy. IAEA-TECDOC-300. Vienna: International Atomic Energy Agency; 1983. 188 p

[56] Israelsson A, Eriksson M, Pettersson HBL. On the distribution of uranium in hair: Non-destructive analysis using synchrotron radiation induced X-ray fluorescence micro-probe techniques. Spectrochimica Acta B. 2015;**108**:28-34. DOI: 10.1016/j.sab.2015.04.001

[57] Yamada T, Saunders T, Nakamura T, Sera K, Nose Y. On intra-individual variations in hair minerals in relation to epidemiological risk assessment of atopic dermatitis. In: Kitsos C, Oliveira T, Rigas A, Gulati S, editors. Theory and Practice of Risk Assessment. Vol 136. Springer Proceedings in Mathematics and Statistics. Cham.: Springer; 2015. DOI: 10.1007/978-3-319-18029-8_9

[58] da Cunha MML, Trepout S, Messaoudi C, Wub T-D, Ortega R, Guerquin-Kern J-L, Marco S. Overview of chemical imaging methods to address biological questions. Micron 2016;**84**:23-36. DOI: 10.1016/j.micron.2016.02.005

[59] Verma HR. Atomic and Nuclear Analytical Methods XRF, Mössbauer, XPS, NAA and Ion Beam Spectroscopic Techniques. Berlin: Springer-Verlag; 2007. 376 p. DOI: 10.1007/978-3-540-30279-7

[60] Jenčič B, Jeromel L, Potočik LA, Vogel-Mikuš K, Kovačec E, Regvar M, Siketić Z, Vavpetič P, Rupnik Z, Bučar K, Kelemen M, Kovač J, Pelicon P. Molecular imaging of cannabis leaf tissue with MeV-SIMS method. Nuclear Instruments and Methods in Physics Research B. 2016;**371**:205-210. DOI: 10.1016/j.nimb.2015.10.047

[61] Lucarelli F, Calzolai G, Chiari M, Nava S, Carraresi L. Study of atmospheric aerosols by IBA techniques: The LABEC experience. Nuclear Instruments and Methods in Physics Research B. (in press). DOI: 10.1016/j.nimb.2017.07.034

[62] Giuntini L. A review of external microbeams for ion beam analyses. Analytical and Bioanalytical Chemistry. 2011;**401**:785-793. DOI: 10.1007/s00216-011-4889-3

[63] Witkowska E, Szczepaniak K, Biziuk M. Some applications of neutron activation analysis. Journal of Radioanalytical and Nuclear Chemistry. 2005;**265**:141-150. DOI: https://doi.org/10.1007/s10967-005-0799-1

[64] Vasconcellos MBA, Bode P, Paletti G, Catharino MGM, Ammerlaan AK, Saiki M, Fávaro DIT, Byrne AR, Baruzzi R, Rodrigues DA. Determination of mercury and selenium in hair samples of Brazilian Indian populations living in the Amazonic Region by NAA. Journal of Radioanalytical and Nuclear Chemistry. 2000;**244**:81-85. DOI: 10.1023/A:1006775006509

[65] Lin X, Alber D, Henkelmann R. Elemental contents in Napoleon's hair cut before and after his death: Did Napoleon die of arsenic poisoning? Analytical and Bioanalytical Chemistry. 2004;**379**:218-220. DOI: https://doi.org/10.1007/s00216-004-2536-y

[66] Johansson SAE, Campbell JL, Malmqvist KG. Particle Induced X-Ray Spectrometry (PIXE). New York: Wiley; 1995

[67] Campbell JL. Particle-Induced X-Ray Emission. Characterization of Materials. New York: John Wiley and Sons; 2002

[68] Liu J, Ouyang Z. Mass spectrometry imaging for biomedical applications. Analytical and Bioanalytical Chemistry. 2013;**405**:5645-5653. DOI: 10.1007/s00216-013-6916-z

[69] Alnajeebi AM, Vickerman JC, Lockyer NP. Matrix effects in biological SIMS using cluster ion beams of different chemical composition. Biointerphases. 2016;**11**:02A317. DOI: 10.1116/1.4941009

[70] Jeromel L, Siketić Z, Potočnik NO, Vavpetič P, Rupnik Z, Bučar K, Pelicon P. Development of mass spectrometry by high energy focused heavy ion beam: MeV SIMS with 8 MeV Cl^{7+} beam. Nuclear Instruments and Methods in Physics Research B. 2014;**332**:22-27. DOI: 10.1016/j.nimb.2014.02.022

[71] Willmott P. An Introduction to Synchrotron Radiation: Techniques and Applications. New York: John Wiley and Sons; 2011. 368 p. DOI: 10.1002/9781119970958

[72] Collingwood JF, Adams F. Chemical imaging analysis of the brain with X-ray methods. Spectrochimica Acta Part B. 2017;**130**:101-118. DOI: 10.1016/j.sab.2017.02.013

[73] West M, Ellis AT, Potts PJ, Streli C, Vanhoofe C, Wobrauschekc P. Atomic spectrometry update – A review of advances in X-ray fluorescence spectrometry and its applications. Journal of Analytical Atomic Spectrometry. 2016;**31**:1706-1755. DOI: 10.1039/c6ja90034h

[74] Kertesz Z, Szikszai Z, Pelicon P, Simcic J, Telek A, Biro T. Ion beam microanalysis of human hair follicles. Nuclear Instruments and Methods in Physics Research B. 2007;**260**:218-221. DOI: 10.1016/j.nimb.2007.02.025

[75] Bodó E, Bíró T, Telek A, Czifra G, Griger Z, Tóth BI, Mescalchin A, Ito T, Bettermann A, Kovács L, Paus R. A hot new twist to hair biology involvement of vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control. The American Journal of Pathology. 2005;**166**:985-998. DOI: 10.1016/S0002-9440(10)62320-6

[76] Bikle DD. Vitamin D and the skin: Physiology and pathophysiology. Reviews in Endocrine & Metabolic Disorders. 2012;**13**:3-19. DOI: 10.1007/s11154-011-9194-0

[77] Mason LH, Harp JP, Han DY. Pb neurotoxicity: Neuropsychological effects of lead toxicity. BioMed Research International. 2014. DOI: 10.1155/2014/840547

[78] Ordemann JM, Austin RN. Lead neurotoxicity: Exploring the potential impact of lead substitution in zinc-finger proteins on mental health. Metallomics. 2016;**8**:579-588. DOI: 10.1039/c5mt00300h

[79] Counter SA, Buchanan LH, Ortega F. Neurocognitive status of Andean children with chronic environmental lead exposure. Journal of Environmental and Occupational Science. 2015;**4**:179-184. DOI: 10.5455/jeos.20151029110613

[80] Tong Y, Sun H, Luo Q, Feng J, Liu X, Liang F, Yan F, Yang K, Yu X, Li Y, Chen J. Study of lead level during pregnancy by application of synchrotron radiation micro XRF. Biological Trace Element Research. 2011;**142**:380-387. DOI: 10.1007/s12011-010-8805-z

[81] Pradeep AS, Nagaraju GJ, Sarita P. Trace elements in the scalp hair of patients with alcohol induced psychosis. Journal of Radioanalytical and Nuclear Chemistry. 2012;**294**:271-276. DOI: 10.1007/s10967-011-1591-z

[82] Pradeep AS, Naga Raju GJ, Sattar SA, Sarita P, Prasada Rao AD, Ray DK, Reddy BS, Reddy SB. Trace elemental distribution in the scalp hair of bipolars using PIXE technique. Medical Hypotheses. 2014;**82**:470-477. DOI: 10.1016/j.mehy.2014.01.028

[83] Gadoth N, Göbel HH. Oxidative Stress and Free Radical Damage in Neurology. New York: Springer; 2011. 323 p. DOI: 10.1007/978-1-60327-514-9

[84] Kazi TG, Baloch S, Afridi HI, Talpur FN, Sahito OM. Variation of lithium contents in scalp hair samples of different male psychiatric patients before and after treatment with its pharmaceutical supplements. Pharmaceutica Analytica Acta. 2016;**7**:520-526. DOI: 10.4172/2153-2435.1000520

[85] Adkins-Jackson SL, PB CP, Mitchell E, Montgomery S. A review of hair product use on breast cancer risk in African American women. Cancer Medicine. 2016;**5**:597-604. DOI: 10.1002/cam4.613

[86] Maziar A, Shahbazi-Gahrouei D, Tavakoli MB, Changizi V, Non-invasive XRF. Analysis of human hair for health state determination of breast tissue. International Journal of Cancer Management. 2015;**8**:e3983. DOI: 10.17795/ijcp-3983

[87] Charles MJ, Reddy SB, Raju GJN, Ravi Kumar M, Seetharamireddy B, Mallikharjuna Rao B, Reddy TS, Murty GAVR, Ramakrishna Y, Vijayan V, Ramani A. Effect of radiation therapy on trace elemental concentrations of hair samples of cervical cancer patients — PIXE technique. X-Ray Spectrometry. 2004;**33**:410-413. DOI: 10.1002/xrs.739

[88] Ejaz S, Camer GA, Anwar K, Ashraf M. Monitoring impacts of air pollution: PIXE analysis and histopathological modalities in evaluating relative risks of elemental contamination. Ecotoxicology. 2014;**23**:357-369. DOI: 10.1007/s10646-014-1193-y

[89] Oyuntsetseg B, Kawasaki K, Watanabe M, Ochirbat B. Evaluation of the pollution by toxic elements around the small-scale mining area, Boroo, Mongolia. ISRN Analytical Chemistry. 2012. DOI: 10.5402/2012/153081

[90] Suzuki K, Yamaya Y, Asano K, Chiba M, Sera K, Matsumoto T, Sakai T, Asano R.Relationship between hair elements and severity of atrioventricular block in horses. Biological Trace Element Research. 2007;**115**:255-264. DOI: 10.1007/BF02686000

[91] Chellan P, Sadler PJ. The elements of life and medicines. Philosophical Transactions. Series A, Mathematical, Physical, and Engineering Sciences. 2015;**373**(2037). DOI: 10.1098/rsta.2014.0182

[92] Farzan SF, Gossai A, Chasan-Taber YCL, Baker E, Karagas M. Maternal arsenic exposure and gestational diabetes and glucose intolerance in the New Hampshire birth cohort study. Environmental Health. 2016;**15**:106. DOI: 10.1186/s12940-016-0194-0

[93] Afridi HI, Talpur FN, Kazi TG, Brabazon D. Assessment of toxic elements in the samples of different cigarettes and their effect on the essential elemental status in the biological samples of Irish hypertensive consumers. Journal of Human Hypertension. 2015;**29**:309-315. DOI: 10.1038/jhh.2014.87

[94] Kempson IM, Dermot HA. Titelbild: Determination of arsenic poisoning and metabolism in hair by synchrotron radiation: The case of Phar Lap (Angew. Chem. 25/2010). Angewandte Chemie. 2010;**122**:4239. DOI: 10.1002/ange.201002029

IntechOpen