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Elemental Profiling: Its Role and Regulations

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1. Introduction

The commercial significance of medicinal plants was known since millennia but its popularity has grown remarkably in terms of herbal drugs, herbal cosmetics and nutraceuticals during the last few decades. The primary reason being increased interest of developed countries in herbal medicines for safer and natural health care system. This has intensified research on medicinal plants of developing and third world countries for safer and effective drugs for chronic diseases. In fact, more than 60% of the new anti-cancer drugs approved since 1983 were derived from plants. Hence, countries in Asia, Africa and Latin America see greater scope in earning valuable foreign exchange through export of their plant wealth to the western countries. It has been observed, since the last two decades, the interest in the developed countries for complementary and alternative medicines has increased by 60%. In the US, consumer use of herbal products rose to about 50% in 2004 from 5% in 1991. According to the WHO estimate, the world market for herbal medicines and herbal products is worth US\$ 62 billion and would hit US\$ 5 trillion by 2050. The market is growing @7% per annum. US is the major market for essential oils and herbal tea. Leading markets for herbal products in Europe are Germany, France, UK and Italy with Germany having the largest herbal extraction industry in Europe.

Since many of the traditional herbs do not conform to the perceptions and norms of the western countries as they correspond to a different system (allopathy) and concept, the mismatch may create problem. The latest impact of these factors is the ban imposed by the Canadian government on 'unapproved' Ayurvedic drugs from India, on the plea of hazardous heavy metal concentration in them. The agency banned products manufactured by herbal giants like Dabur, Zandu, Himalaya and Hamdard, although some of these products (like Dabur's 'Shilajit' which is actually a natural rock extract) are claimed by their manufacturers to be free from metals. The manufacturers are of the view that they would take measures like policy advocacy on this issue. However, the ban is not applicable for those Ayurvedic products which have been authorized for sale in Canada. To prevent recurrence of similar types of incidents, the WHO has issued certain guidelines to promote traditional medicines in its traditional medicine strategy (2004-05). The organization has advocated for regulations and other arrangements (like public awareness program) so as to ensure safe utilization of the traditional medicines. If the advice is implemented in a

coordinated manner so as to avoid any kind of mismatch, then the objective of providing safer drugs to the people may be fulfilled.

2. Elements in nature

According to the position of metals in the Periodic Table, the metals are named alkali metals, alkaline earth metals, transition metals, and rare earth metals. Four elements (nitrogen, carbon, hydrogen and oxygen) account for 96% of living matter. About 50 of the known elements occur in measurable concentrations in the living systems. In humans and other mammals, 23 elements have known physiological activities (macro nutrients and micro-nutrients). The macronutrients are sodium, calcium, magnesium, potassium, chlorine, etc., which are required in larger quantities by living organism while microelements are 11 in numbers and are classified as “**trace elements**” because of their essentiality at very limited quantity in humans (less than 100 mg/day). Out of these 11 trace elements, eight are in the period IV of the Periodic table (manganese (Mn), iron (Fe), cobalt (Co), copper (Cu), vanadium (V), chromium (Cr), zinc (Zn) and molybdenum (Mo) and three are non-metals selenium (Se), fluorine (F) and iodine (I). Transition metals that are trace elements of significance for human physiology are, Cobalt (Co), molybdenum (Mo), chromium (Cr) and vanadium (V). In biological systems, trace elements are mostly present as metalloproteins (bound to proteins), or to smaller molecules, such as phosphates, phytates, polyphenols and other chelating compounds. Most of the metals in metalloproteins are part of enzymatic systems and have structural functions or use the protein to be transported to their target site in the organism (Mokdad et al., 2004). Research has indicated association of cancer, diabetes and cardiovascular diseases with diet which has prompted increased consumption of fiber, fatty acids, phytochemicals, and trace elements (Willett, 2002). The role a metal plays, depends on its chemical structure, as well as on the molecule that is chelating the metal (Halliwell & Gutteridge, 1999). For example, Zn, as with other group XII elements, has no unpaired electrons when in the state Zn^{2+} , preventing its participation in redox reactions but Zn has been recognized to act as an antioxidant by replacing metals that are active in catalyzing free radical reactions, such as Fe (Oteiza et al., 2004; Zago & Oteiza, 2001). In enzymes, the metals participate in catalytic processes in any of the following ways:

1. Constituents of enzyme active sites.
2. Stabilizers of enzyme tertiary or quaternary structure.
3. Associates in forming weak bonding complexes with the substrate.
4. Stabilizing charged transition states.

Based on the increased knowledge of the biological mechanisms ruling life we have made a good progress in increasing the life expectancy. However, this has led to increased incidence of chronic and degenerative diseases, one of the reasons of which could be increasing amount of toxic substances in our body. To deal with this essentiality/toxicity duality, biological systems have developed the ability to recognize a metal, and deliver it to the target without allowing the metal to participate in toxic reactions (Luk et al., 2003). Proteins are primarily responsible for such recognition and transport of these elements thereby making them safe for body. However increase in the intake of certain nutrients as therapeutics or through food may lead to high concentrations of these elements resulting toxicity in the body.

Trace elements are essential components of biological structures, but at the same time they can be toxic beyond the concentration needed for their biological functions. The toxicity

can be extended to other non-essential elements of very similar atomic characteristics that can mimic the reactivity of a trace element.

The presence of trace elements in foods is often determined by the availability of metals in the soil. Thus, within a geographical region with soils deprived/excess of trace elements, its population is at a risk thereby resulting into trace elements deficiency/toxicity. Unfortunately, in recent years the avalanche of uncontrolled supplementation with trace elements has put some trace elements on the border of toxicity in several populations. Thus, it is a crucial priority to define the requirements for trace elements, based on essentiality and health promotion, and the limits for toxicity. Then it becomes necessary either to supplement the basic food by adding the appropriate trace elements (milk, flour, etc.) or counteract/dilute the element in excess (de Romana et al., 2005; Hurrell et al., 2004). These supplements sometimes becomes necessary in several disease treatments, e.g. anemic conditions in kidney dialysis (Locatelli et al., 2004) and physiological conditions, e.g. extensive blood loss during menstruation (Munro, 2000). There are other factors to consider that can define the requirements for essential elements beyond their presence in foods (Table 1):

Element	Antagonists restricting absorption, utilization or retention	Synergists promoting absorption, utilization or retention
Zinc	Phytate with high calcium intake High iron intake Heterologous milks (infants only) [High zinc status; aging]	Low calcium intake, animal proteins Homologous milk (infants only) [Late pregnancy; lactation] [Low zinc status]
Copper	High iron, high zinc intakes [High copper status] High molybdenum with high sulphur intake	High protein intake [Late pregnancy; lactation?] [Low copper status]
Iodine	Elevated goitrogen intake [Low selenium status]	- -
Selenium	Elevated heavy-metal intake	-
Chromium	Oxalates, high iron intake [High chromium status]	[Low chromium status]
Manganese	High calcium intake (infant formulae) [High manganese status]	- -
Cadmium	High calcium intake	Low iron intake, low calcium intake
Lead	Phytate with high calcium intake	Low iron, low calcium and phosphorus intakes

Table 1. Antagonists restricting and synergists promoting absorption, utilization or retention of trace elements in humans

1. Interaction among nutrients, e.g. interactions between iron and other metals (Aschner, 2000);

2. The presence of certain compounds in the diet, that can impair metal absorption, e.g. phytates bind Zn, preventing absorption (Greger, 1999; Lestienne et al., 2005);
3. Genetic defects, e.g. Zn absorption is decreased in acrodermatitis enteropathica (Wang et al., 2004);
4. drug-nutrient interactions, e.g. penicillamine used in the treatment of Wilsons disease causes Zn deficiency (Schilsky, 2001).

The important variables that should be considered when the levels of trace elements are increased in the body are the effects genetic and individual differences in the targeted population, life-style, nutra-genetic interactions, and other individual factors that can determine the effects of the nutrient on the disease.

3. Role of trace elements

Metals are non-uniformly distributed in the soil and environment. Several factors like industrialization, traffic density, and indiscriminate use of chemical fertilizers, pesticides and eco-geological conditions play deciding role in their quality and quantity. Out of the thirty five metals associated with us, due to residential or occupational exposure, twenty three of them are toxic metals (Punz & Seighardt, 1993). Metals can be accumulated /absorbed by our body as in exposure to sunrays/X-rays/ beverages/ air/water and food products. Bioaccumulation of these elements beyond the safety limits results in various malfunctions leading to several abnormalities. Based on their biological effects, they can be categorized into two types (i) non essential /toxic metals (Pb, Cd, Hg, As) and essential /beneficial metals which includes microelements like Cu, Zn, Mn and macro elements like P, Ca, K, Mg (Abdul -Wahabet al., 2008). Literature cites several reports advocating tendency of plants to absorb and accumulate heavy metals in their tissues (Bunzl et al., 2001; Yusuf et al., 2002). Heavy metal contamination is especially harmful for infants and pregnant woman as it affects the central nervous system, kidney, gastro-intestinal and reproductive systems and joints (Tong et al., 2000). However, plants harbor not only toxic metals but beneficial metals as well. They play a vital role as structural and functional components of metallo-protein and enzyme in the living cell in low doses. The role of these elements are presented very briefly in table 2.

S No.	Metal	Functions	Dietary sources/ Presence	Potential toxicity	References
1	Iron	Hemoglobin myoglobin, catalase, cytochromes Fe-sulfur enzymes proteins for Fe storage and transport and other Fe-containing or Fe-activated enzymes	Hemoglobin and myoglobin from animals. Cereals, seeds of leguminous plants, fruits, vegetables, and dairy products.	Anemia. Fe poisoning	Fraga & Oteiza, 2002; Zimmermann & Kohrle, 2002
2	Copper	Connective tissue, nerve coverings, and bone. Fe and energy metabolism. Reductant in the enzymes and several oxidases that reduce molecular oxygen	Liver and other organ meats, oysters, nuts, seeds, dark chocolate, and whole grains. Transported in the organism by ceruloplasmin. Soluble forms of copper are absorbed from the intestine (40-60%)	Normocytic, hypochromic anemia, leucopenia neuropenia, and inclusive osteoporosis in children. Liver damage	Underwood, 1977; Mason, 1979; King et al., 1978; Hoadley & Cousins, 1988

S No.	Metal	Functions	Dietary sources/ Presence	Potential toxicity	References
3	Zinc	Supports normal growth and development in pregnancy, childhood, and adolescence. Zn is involved in Zn-fingers and activity of about 100 enzymes.	Red meat and poultry, beans, nuts, seafood (oysters are extremely rich in Zn), whole grains, fortified breakfast cereals, and dairy products. Zn is mainly transported by cerulo plasmin. Small intestine is the site of maximum absorption.	Common in underdeveloped countries leading to malnutrition, affecting the immune system, wound healing, impairing DNA synthesis.	Baer et al., 1984; Hess et al., 1977
4	Selenium	Selenoproteins, glutathione peroxidase, thioredoxins,	Grains, cereals, red meats, nuts and seafood. Very efficiently absorbed by humans.	Weakened immune system, nutritional, biochemical or infectious stresses, gastrointestinal upsets, hair loss, white blotchy nails, garlic breath odor, fatigue.	Combs et al., 1986; Levander et al., 1987
6	Molybdenum	Electron transfer agent in enzymes such as oxidase and sulphite reductase xanthine dehydrogenase/oxidase, aldehyde oxidase and sulfite oxidase share a common cofactor, molybdo-pterin, a substituted pterin to which molybdenum is bound by two sulfur atoms.	Conversion of tissue purines to uric acid.	Xanthinuria, low xanthine dehydrogenase activities High intake leads to molybdenosis spontaneous fractures, and mandibular exostoses osteogenesis.	Rajagopalan, 1984; Ostrom et al., 1961; WHO, 1973
7	Chromium	Potentiates insulin action and thus influences carbohydrate, lipid and protein metabolism. Affects the ability of the insulin receptor to interact with insulin.	Processed meats, whole grain products, pulses and spices are the best sources of chromium blood plasma.	Include impaired growth, elevated serum cholesterol and triglycerides, increased incidence of aortic plaques, cornea lesions and decreased fertility and sperm count.	Anderson, 1988; Okada et al., 1981; Tuman & Doisy, 1977; Offenbacher et al., 1986
8	Manganese	Activator and a constituent of several enzymes	Unrefined cereals, nuts, leafy vegetables and tea will be high in manganese. Manganese absorption is independent both of body manganese status and of dietary manganese content.	Impaired growth, skeletal abnormalities, disturbed reproductive function, ataxia of the newborn, defects in lipid and carbohydrate metabolism, impaired iron metabolism and altered brain function	Hurley, 1987; Weigand et al., 1986

S No.	Metal	Functions	Dietary sources/ Presence	Potential toxicity	References
9	Nickel	Typical nickel-containing enzymes found in plants and microorganisms, namely urease, hydrogenase, methyl coenzyme M reductase and carbon-monoxide dehydrogenase	foodstuffs and simple substances, including milk, coffee, tea, orange juice, ascorbic acid and ethylenediaminetetraacetate depress this high absorption	No report for deficiency of nickel	Thauer, 1985; Hausinger, 1987; Walsh & Orme-Johnson, 1987; Nielsen, 1982, 1984
10.	Boron	Steroid hormone metabolism	Fruits, leafy vegetables, nuts and legumes are rich sources. Wine, cider and beer are also high in boron. Boron is distributed throughout the tissues, organs and bones of animals and humans.	Induce secondary hyperparathyroidism affects steroid hormone metabolism in humans and animals.	Nielsen et al., 1982, Hunt, 1981, Nielsen , 1988
11.	Vana-dium	Regulation of Na ⁺ /K ⁺ -exchanging ATPase, phosphoryl-transfer enzymes, adenylate cyclase and protein kinases. enzyme cofactor, and in hormone, glucose, lipid, bone and tooth metabolism	Whole grains, seafood, meats and dairy products. Spinach, parsley, mushrooms and oysters.	no significant report of deficiency of vanadium is available	Byrne & Kosta 1978; Nielsen, 1982; Bennet, 1984
12.	Cadmium	Cadmium metallothionein	Rock, soil, mining	kidney and possibly the skeleton.	Punz & Seighardt, 1993
13.	Lead	Pesticides, Industrial waste	Pesticides, geochemical mineralization and industrial waste. --	nervous system of infants and children is particularly sensitive to lead toxicity.	National research council, 1989
14.	Arsenic	Soil, food and water	Soil, food and water --	increased incidence of keratinization and pigmentation of the skin, together with an increased risk of skin cancer.	Kovalskij, 1977; National Academy of Sciences, 1980
15.	Mercury	Pesticides, food, water and soil	Fishes, pesticides, food, water and soil --	Kidneys central nervous system brain	Bennet, 1984; WHO, 1989, 1976, 1990

Table 2. Functions, Dietary source, Biochemistry and Potential toxicity of some of the metals.

In general, all medicinal plants/herbs/spices/food/water/soil and its products (for human and animals use) must meet regulatory guidelines for quality, safety and efficacy. Monitoring of these metals, using advanced techniques, in the plant is important for protecting public against the hazards of metal toxicity and also in creating awareness towards its nutritional qualities.

4. Elemental profiling

Elemental profiling specially of trace elements can be divided into three subgroups:

1. Easy to determine routinely by several techniques (e.g. iron and zinc);
2. Not always easy to assay, particularly at low concentrations (e.g. arsenic, selenium and tin); and
3. Expert handling (e.g. cadmium, chromium, lead, manganese, mercury, molybdenum and nickel) which require a high level of analytical expertise because of the low concentrations present, detection limit problems, matrix interferences, incomplete recoveries and related methodological difficulties. At low concentrations, the analysis of dietary material presents considerable difficulties, depending on whether the matrix is simple (e.g. drinking-water and beverages) or complex (dairy products). Meat and a few other food products contain some trace elements at very high concentrations and are generally easy to analyse. The implications of these various conditions are important when single foods are analysed, something that may present an array of problems. For example, moisture content of foods varies widely, ranging from 94% in leafy vegetables to 60% in meat, 20% in grains and cereals, and up to 10% in oils and fats. Fat content of foods can cause difficulty, e.g. a high-fat product such as cheese will present a real challenge, if dissolution steps are involved. (Oxidation of samples rich in fats and oils with perchloric acid should be avoided because of explosion risks). The concentrations of several trace elements vary considerably even in foods belonging to similar groups belonging to different groups. The problem of trace element analysis can be overcome by freeze-drying of mixed diets as this may lead to six fold enrichment of the component (Benramdane, 1999; Aceto, 2002).

5. Analytical techniques

Analytical techniques such as atomic absorption spectrophotometry (AAS) (flame and flameless), atomic emission spectroscopy (direct-current and inductively coupled plasma), chemical and electro-analytical methods, gas and liquid chromatography, mass spectrometry (in different modes) nuclear-activation techniques and X-ray fluorescence offer sufficiently low detection limits to make them suitable for investigating a variety of biomatrices.

Low detection limits alone are not sufficient to answer all the questions as analytical data on trace elements are mostly regarded with skepticism. Ignorance of various interferences, e.g. matrix-related problems, flaws in sample and standard preparation and inadequate calibration procedures all contribute to this regrettable situation. The analyst is therefore by far the most important component of any analytical system (Ma, 2004; Hiefje, 2000).

5.1 Choice of type of assay

The analyst is faced with the choice between multielement and single-element assays, which is affected by a number of factors. Thus, even though sometimes only partly quantitative, multielement assays are useful in obtaining simultaneous elemental composition profiles of a given specimen. For example, the non-destructive procedures offer the possibility of generating data simultaneously (including repeated determinations on the same test portion) for several elements for purposes of comparison. They also offer the possibility of internal quality control so that unusual situations involving any specific element can be

evaluated. Moreover, in a carefully designed study, multielement assays can provide very useful information at relatively low cost. However, some elements must be determined alone because of serious analytical problems. Clinical, environmental and nutritional laboratories dealing with specific elements frequently need single-element assays. In a laboratory performing a wide range of analyses, therefore, a combination of both single- and multi element capability may be essential for effective functioning.

5.2 Choice of analytical technique

The choice of an analytical technique depends on a number of factors, including:

1. susceptibility to matrix effects;
2. range of elements covered;
3. detection limits; and
4. suitability for the matrix of interest.

The susceptibility of an analytical technique to matrix effects depends on the sample composition. With some matrices, these effects are of major importance, but others can be avoided by a modification of the technique. The usefulness of an analytical method for trace-element analysis, also depends on the range of elements covered and the order of magnitude of its detection limits for the elements at the top and bottom of its sensitivity range. Detection limits will not be the same for all elements, so that simultaneous multielement determination will require compromises in experimental conditions that will affect the accuracy and precision of at least a few elements. Even when there is a method of choice for the analysis of a particular element, its performance will depend on the concentration of the element in question and that of others in the matrix (Toelg, 1988). Concentration ranges also vary widely between different types of biomaterials and foods (Kumpulainen, 1980). These changes in relationships between elements may necessitate modifications to the technique for specific applications in order to maintain optimum performance and prevent any decline in detection limits.

The most important criterion of the suitability of a method, however, is whether it is appropriate for the matrix of interest. Using a set of four representative biological matrices, namely bovine liver, porcine muscle, Bowen's kale and human serum, an advisory group designated by the International Atomic Energy Agency evaluated the performance of different analytical techniques (Cornelis, 1980). For elements such as copper, iron and zinc, several methods were suitable. Thus, for zinc, many methods can generate results with a 1% CV. On the other hand, for elements such as fluorine, iodine, tin and vanadium, the choice was limited. The analytical techniques studied nevertheless reached detection limits below the ng/g level for chromium, manganese and vanadium, i.e. the level at which these elements are expected to occur in some specimens (Jones, 1992; Watson, 1998; Bernazzani, 2001).

6. Introduction of atomic absorption spectrometry

The atom is a nucleus surrounded by electrons which travel around the nucleus in discrete orbitals. Every atom has a number of orbitals in which it is possible for electrons to travel. Each of these electron orbitals has an energy level associated with it. In general, the further away from the nucleus an orbital, the higher its energy level. When the electrons of an atom are in the orbitals closest to the nucleus and lowest in energy, the atom is in its most preferred and stable state, known as its *ground state*. When energy is added to the atom as the result of absorption of electromagnetic radiation or a collision with another particle

(electron, atom, ion, or molecule), one or more of several possible phenomena take place. The two most probable events are for the energy to be used to increase the kinetic energy of the atom (*i.e.*, increase the velocity of the atom) or for the atom to absorb the energy and become excited. This later process is known as *excitation*.

When an atom becomes excited, an electron from that atom is promoted from its ground state orbital into an orbital farther from the nucleus and with a higher energy level. Such an atom is said to be in an *excited state*. An atom is less stable in its excited state and will thus decay back to a less excited state by losing energy through a collision with another particle or by emission of a "particle" of electromagnetic radiation, known as a *photon*. As a result of this energy loss, the electron returns to an orbital closer to the nucleus.

If the energy absorbed by an atom is high enough, an electron may be completely dissociated from the atom, leaving an ion with a net positive charge. The energy required for this process, known as ionization, is called the ionization potential and is different for each element. Ions also have ground and excited states through which they can absorb and emit energy by the same excitation and decay processes as an atom. The difference in energy between the upper and lower energy levels of a radiative transition defines the wavelength of the radiation that is involved in that transition (Frank, 1998; Skoog, Holler & Nieman, 1998). The phenomenon of atomic absorption observed in 1802 with the discovery of the Fraunhofer lines in the sun's spectrum. It took more than half century to utilize observed lines for quantitative chemical analysis. Atomic absorption analysis involves measuring the absorption of light by vaporized ground state atoms and relating the absorption to concentration governed by Beer's law.

7. Atomic Absorption Spectroscopy (AAS)

The basic aim of analytical atomic absorption spectroscopy is to identify elements and quantify their concentrations in various media. The procedure consists of three general steps: atom formation, excitation, and emission. For UV and visible spectroscopy, the input energy must be sufficient to raise an electron from the ground state to the excited state. Once the electron is in the excited state, the atom emits light, which is characteristic of that particular element. Before excitation, an element that is bound in a specific matrix must be separated from that matrix so that its atomic emission spectra are free from interferences.

The elements present in a sample are converted to gas phase atoms in the ground state. The UV-Vis absorption of these gas phase atoms are then measured by irradiation of light at a highly specific wavelength causing transition of some of the gas phase atoms to a higher energy level. The extent to which light is absorbed is related to the original concentration of ground state atoms. This situation is completely analogous to the Beer-Lambert law in conventional liquid UV-Vis absorption spectrophotometry. Conversion of the sample from its native state to the atomic state can be achieved using a flame (flame-AAS) or an electric furnace (electro-thermal or graphite furnace AAS). The later will be studied herein. In the furnace, the sample undergoes a number of pretreatment steps prior to analysis.

1. Sample is dried by evaporating the solvent (in this case the water).
2. The organic matrix is decomposed by heating of the sample $\geq 1000^{\circ}\text{C}$. (taking care not to lose any of the analyte through evaporation processes).
3. Furnace is rapidly heated to temperatures around 2400°C to produce vaporized neutral atoms.

This method provides both sensitivity and selectivity since other elements in the sample will not generally absorb the chosen wavelength and thus, will not interfere with the measurement. However, molecular species may also be formed during the atomization step. Which can alter the spectral characteristics of the analyte metal or can cause spectral interference at the wavelength being monitored. To reduce background interference, the wavelength of interest is isolated by a mono-chromator placed between the sample and the detector. Additional techniques such as D2 or Zeeman background correction may also be used for complex matrices such as beer.

The most common instrument components consist of a hollow cathode lamp source, a pneumatic nebulizer for an atomizer, a conventional grating mono-chromator and photomultiplier tube detector. The hollow cathode lamp is made of a glass envelope with a quartz window filled with an inert gas at slightly above atmospheric pressure. The cathode is made of the pure metal of interest. The pneumatic nebulizer aspirates and nebulizes the liquid sample solution when the sample is sucked through a capillary tube. The grating mono-chromator eliminates much of the background light from the flame and the photomultiplier tube detector detects that light from the hollow cathode lamp which passes through the flame.

Atomic absorption analyses are most commonly and routinely performed on solutions. Therefore a sample must be converted to liquid form prior to analysis using a microwave to digest the sample, leaving a solution that can then be analyzed. However this method has the following limitations (Ingle & Crouch, 1988; Lajunen, 1992; Frank, 1997; Skoog et al., 1998).

7.1 Limitations

1. Elemental range is limited to metals and metalloids.
2. Sample preparation is tedious and time consuming.
3. The sample is destroyed by the analysis.
4. Only one element at a time can be measured.

8. Techniques based on atomic spectrometry

In *atomic absorption spectrometry* (AAS), light of a wavelength characteristic of the element of interest is shone through this atomic vapor. Some of this light is then absorbed by the atoms of that element. The amount of light that is absorbed by these atoms is then measured and used to determine the concentration of that element in the sample.

In *optical emission spectrometry* (OES), the sample is subjected to temperatures high enough to cause not only dissociation into atoms but to cause significant amounts of collisional excitation (and ionization) of the sample atoms to take place. Once the atoms or ions are in their excited states, they can decay to lower states through thermal or radiative (emission) energy transitions. In OES, the intensity of the light emitted at specific wavelengths is measured and used to determine the concentrations of the elements of interest.

One of the most important advantages of OES results from the excitation properties of the high temperature sources used in OES. These thermal excitation sources can populate a large number of different energy levels for several different elements at the same time. All of the excited atoms and ions can then emit their characteristic radiation at nearly the same time. This results in the flexibility to choose from several different emission wavelengths for

an element and the ability to measure emission from several different elements concurrently. However, a disadvantage associated with this feature is that as the number of emission wavelengths increases, the probability also increases for interferences that may arise from emission lines that are too close in wavelength to be measured separately.

In *atomic fluorescence spectrometry* (AFS), a light source, such as that used for AAS, is used to excite atoms only of the element of interest through radiative absorption transitions. When these selectively excited atoms decay through radiative transitions to lower levels, their emission is measured to determine concentration, much the same as in OES. The selective excitation of the AFS technique can lead to fewer spectral interferences than in OES. However, it is difficult to detect a large number of elements in a single run using AFS, as the number of spectral excitation sources and detectors that can be used at one time is limited by the instrument (Smith et al., 1995; Bernazzani & Paquin, 2001).

Another technique, called *atomic mass spectrometry*, is related to three atomic spectroscopy techniques described above. Instead of measuring the absorption, emission or fluorescence of radiation from a high temperature source, such as a flame or plasma. Mass spectrometry measures the number of singly charged ions from the elemental species within a sample. Similar to the function of a monochromator in emission/absorption spectrometry that separates light according to wavelength, a quadrupole mass spectrometer separates the ions of various elements according to their mass-to-charge ratio in atomic mass spectrometry (Jones, 1992).

8.1 Atomic Emission Spectroscopy (AES)

Atomic emission spectroscopy (AES) is one of the most important techniques of elemental analysis. One of its advantages over atomic absorption is the capability for simultaneous multi-element analysis. It can be used for the analysis of major components of the sample as well as for trace analysis, because calibration curves are linear over several orders of magnitude. As a result of these advantages AES technique are popular in analytical laboratories. Over the past decade, a new generation of spectrometer configurations based on charge coupled device (CCD) detectors has appeared (Hiefje, 2000; Bernazzani & Paquin, 2001) Some of them are simple and low-cost. The perfect atomic emission source would have the following characteristics:

1. Easy to operate.
2. Inexpensive to purchase and maintain.
3. A source that can handle a range of solvents, both organic and inorganic in nature.
4. A source that is adjustable to handle solids, slurries, liquids, or gases.
5. Complete removal of the sample from its original matrix thus minimum interferences.
6. Complete atomization but minimum ionization of all elements to be analyzed.
7. A controllable energy source for excitation, which allows the proper energy needed to excite all elements without appreciable ionization.
8. An inert chemical environment, which prohibits the formation of undesirable molecular species (e.g. oxides, carbides, etc.) that may affect the accuracy of the measurement.
9. No background radiation from the source (unwanted atomic or molecular emission that could interfere with the analytical wavelengths).

Every element has its own characteristic set of energy levels and has unique set of absorption and emission wavelengths. This property of the element makes atomic spectrometry useful for element-specific analytical techniques. The ultraviolet (UV)/visible region (160 - 800 nm) of the electromagnetic spectrum is the region used for analytical

atomic spectrometry. This is also the region of the electromagnetic spectrum. The main reasons for the popularity of analytical techniques that use the UV/visible region are that these techniques are accurate, precise, flexible and relatively inexpensive compared to techniques which use other regions, such as gamma ray spectrometry and X-ray spectrometry (Watson et al., 1998; DeGraff et al., 2002).

8.2 Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)

One of the largest volume uses for ICP-MS is in the medical and forensic field, specifically, toxicology. A physician may order a metal assay for a number of reasons, such as suspicion of heavy metal poisoning, metabolic concerns, and even hepatological issues. Depending on the specific parameters unique to each patient's diagnostic plan, samples collected for analysis can range from whole blood, urine, plasma, serum, to even packed red blood cells. Another primary use for this instrument lies in the environmental field. Such applications include water testing for municipalities or private individuals all the way to soil, water and other material analysis for industrial purposes. This technique has been utilized in food, herbs, herbal drug analysis for safety and efficacy. This technique is also widely used the field of radiometric dating, in which it is used to analyze relative abundance of different isotopes. ICP-MS is more suitable for this application than the previously used Thermal Ionization Mass Spectrometry, as species with high ionization energy such as Osmium (Os) and Tungsten (Hf-W) can be easily ionized (Vladimir, 2007, Eliot, 2007).

8.3 Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES)

An inductively coupled plasma spectrometer is a tool for trace detection of metals in solution, in which a liquid sample is injected into argon gas plasma contained by a strong magnetic field. The elements in the sample become excited and the electrons emit energy at a characteristic wavelength as they return to ground state. The emitted light is then measured by optical spectrometry. This method, known as inductively coupled plasma atomic emission spectrometry (ICP-AES) or inductively coupled optical emission spectrometry (ICP-OES). ICP Spectrometers can be used for the analysis of environmental samples, contaminants in food or water, metalloproteins in biological samples, and similar studies. Most ICP-AES instruments are designed to detect a single wavelength at a time (mono-chromator). Since an element can emit at multiple wavelengths, it is sometimes desirable to detect more than one wavelength at a time. This can be done by sequential scanning or by using a spectrometer that is designed to capture emissions of several wavelengths simultaneously (poly-chromator). Detection limits typically range from parts per million (ppm) to parts per billion (ppb), although depending on the element and instrument, it can sometimes achieve even less than ppb detection.

8.3.1 Interferences

Any chemical or physical process that adversely affects the measurement of the radiation of interest can be classified as interference. Interferences in ICP-AES may start in the sample preparation stage and extend to the plasma operating conditions (Montaser & Golightly 1988).

8.3.2 Applications

Pharmaceutical industries (metals in wine (Aceto, 2002), arsenic in food (Benramdane, 1999), and trace elements bound to proteins Biological samples (Ma, 2004). Precious metal

estimation at low level, Heavy metal estimation at sub ppm level Rock, Soil, Fly ash (Complete analysis), Environmental sample analysis (Water, Air, Soil, sediments, etc.) and Polymer industries.

In plasma mass spectroscopy (MS), the inductively coupled argon plasma (ICP) is once again used as an excitation source for the elements of interest. However in contrast to OES, the plasma in ICP-MS is used to generate ions that are then introduced to the mass analyzer. These ions are then separated and collected according to their mass to charge ratios. The constituents of an unknown sample can then be identified and measured. ICP-MS offers extremely high sensitivity to a wide range of elements.

8.4 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES Analysis)

ICP technology has evolved significantly since its inception in 1960's, when the first ICP prototypes emerged to provide better analytical sensitivity and capacity than Atomic Absorption Spectrometry. During the course of time this technique showed significant improvements in technology, capability and price. Nowadays, ICP-OES is commonly found in contract analytical laboratories and academic facilities where routine analysis needing high sensitivity throughput.

Plasma is an ionized gas that in addition to atoms also contains electrons and ions. After ignition with a Tesla spark the energy transfer is via the high frequency field in the coil that is surrounding the plasma. Free electrons are accelerated and heat the plasma by collision with argon atoms. We distinguish between ionization, electron and excitation temperature, which are different at different locations of the plasma. The sample aerosol is introduced through the center of the plasma flow without affecting its stability and equilibrium.

In the plasma the atoms and particularly the ions are excited to emission. After spectral dispersion of the emitted radiation in a powerful optical system the element-specific wavelengths are used for identification and quantification. Samples are introduced into the plasma in a process that desolvates, ionises, and excites them. The constituent elements can be identified by their characteristic emission lines, and quantified by the intensity of the same lines. The method has the following advantages (Bafley, 1989):

- High sample throughput enabling the efficient analysis of large batches
- Simultaneous determination of multiple elements in each sample
- Complementary analysis to techniques like XRF
- Large dynamic linear range
- Low chemical and matrix interference effects.

9. High-Performance Inductively-Coupled Plasma Optical Emission Spectrometry (HP-ICP-OES) through exact matching

Using high-performance inductively coupled plasma optical emission spectrometry (HP-ICP-OES), relative expanded uncertainties on the order of 0.2 % can be routinely achieved. Nevertheless, analysis results can be improved by implementing "exact matching" with the HP-ICP-OES protocol. This should be very careful matching of the analyte mass fractions, internal standard element mass fractions, and solution matrices of the calibration solutions to the samples. The analytical benefits of this approach are being systematically investigated. Results show that the primary benefit is mitigation of the deleterious effects of nonlinearities of the ICP-OES instrument responses to the analyte and/or internal standard element mass fractions.

10. Regulatory and dietary recommendations

The side effects against heavy metals have built up over time. Close examination by toxicologists studying cases of poisoning from heavy metals has revealed that only in cases of high exposures, visible clinical symptoms are likely. At lower but still unacceptable levels of exposure, as in consumption of certain foods, effects may be restricted at physiological or biochemical level only, (Hutton, 1987). Hamilton (1988) noted that the first legislation to control the adulteration of food or drink occurred in Britain in 1860, in response to long use of heavy metal salts as coloring matter in food. Regulatory agencies in most countries now seek to protect public health by exercising control limits over the chemical composition of specific food types. The process typically involves setting appropriate standards for potentially toxic chemicals in foods which by law should not be exceeded. These or similar agencies were setup to overview random and/or periodic chemical testing of appropriate samples to ensure compliance of the law. For trace elements, given the absence of metabolization of the metal or nonmetal, it is possible to establish clear separations among essentially, health benefits and toxicity. Many countries and regions have defined the requirements and limits of supplements for trace elements. Walker (1988) has summarized international food standards for cadmium applicable in 1986. 19 countries had set regulatory limits for cadmium in foods, but with some exceptions. Australia, Denmark, the Netherlands and Hungary had set limits for cadmium in particular foods.

11. Initiation for scientific and regulatory bodies

The report of the WHO Expert Committee on Trace Elements in Human Nutrition that met in 1973 ended with six important general recommendations for future national and international activities in trace-element nutrition. They are briefly given as under:

1. Need to obtain reliable information on the trace-element content of foods, especially milk
2. To monitor contents in relation to future changes in agricultural and industrial practices.
3. Trace-element requirements should be taken into account in food standards and especially in those for formulated foods designed for infants and young children.
4. Need for international centers for the study of trace elements in humans and for international analytical reference laboratories.
5. Further review of new findings in trace-element nutrition in order to update the recommended levels of intake.

The two dietary standards -the 1990 version of the RNIs and 1989 RDAs, did not differ much in the described derivations of the recommended intakes but differences remain about how intended uses are described, resulting in some confusion for the users of both reports. The answer was sought in the joint U.S. and Canadian development of the new Dietary Reference Intakes/Recommended dietary Allowances (DRIs/RDAs). The Food and Nutrition Board of the United States National Academy of Sciences has taken responsibility for establishing guidelines on what quantities of the various nutrients should be eaten by human males and females at various ages. These were called RDAs (for Recommended Dietary Allowances, and often referred to as Recommended Daily Allowances). They provide the data on which food labels are based. To avoid any further confusions, the terms used in the guidelines were explained explicitly.

12. Needs of individuals requirement

This is the lowest continuing level of nutrient intake that, at a specified efficiency of utilization, will maintain the defined level of nutrient in the individual.

12.1 Basal requirement

This refers to the intake needed to prevent pathologically relevant and clinically detectable signs of impaired function attributable to inadequacy of the nutrient.

12.2 Normative requirement

This refers to the level of intake that serves to maintain a level of tissue storage or other reserve that is judged by the expert consultation to be desirable. The essential difference between the basal requirement and the normative requirement is that the latter usually facilitates the maintenance of a desirable level of tissue stores. For most trace elements, metabolic and tissue-composition studies indicate the existence of discrete stores which, by undergoing depletion at times of reduced intake or high demand, can provide protection for a certain period against the development of pathological responses to trace-element deficiency. Since higher levels of intake are needed to maintain these reserves the normative requirement is necessarily higher than the basal requirement.

12.3 Daily intake

It is defined as the individual's average intake persisting over moderate periods of time without necessarily being present in those amounts each day. Individuals differ in their requirements, even though they may have the same general characteristic (e.g. age, sex, physiological state and body size). One may therefore speak of the *average requirement* of a group of individuals (e.g. young adult men) or of the level that marks a point in the upper tail of the requirement distribution, the level previously identified as the recommended or safe level of intake. Except where specifically indicated, the estimates refer to the maintenance of a defined level of nutritional status in individuals already in that state. They refer to healthy individuals, and the estimated requirements may be altered by disease or other conditions.

12.4 A nutrient intake value

It is estimated to meet the requirement of half of the healthy individuals in a life stage and gender group.

12.5 Recommended Dietary Allowance (RDA)

The dietary intake level that is sufficient to meet the nutrient requirements of nearly all healthy individuals in a life stage and gender group.

12.6 Adequate Intake (AI)

A recommended intake value based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of healthy people that are assumed to be adequate (used when an RDA cannot be determined).

12.7 Tolerable upper intake level (UL)

The highest level of nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population. As intakes increase above the UL, the risk of adverse effects increases.

13. UK Recommended Daily Allowance (RDA) for minerals

The **Recommended Daily Allowance (RDA)** of foods and supplements has traditionally been set by government health bodies in the UK, Europe and USA. In the UK the department of health gave the RDA for vitamins A, C, D, three of the B vitamins, and three minerals in 1979. However, in 1993 the European Union (EU) issued a directive on food labelling for its members, which included RDAs for twelve vitamins and six minerals. As a result, UK food labels are being revised to include the EU recommendations. In addition, the government of the UK issued a report in 1991 suggesting new guidelines for daily requirements of vitamins and minerals, which would replace the RDAs. These are called, collectively, Dietary Reference Values (DRVs), and consist of these terms:

Estimated Average Requirement (EAR), which should meet the requirements of half of the population.

Reference Nutrient Intake (KNI), which replaces the former RDA, is meant to represent the nutrient requirements of some 97 percent of the population. The amount recommended is higher than most people actually need. The EAR meets the requirements of half of the population, of the remaining half some need more and some need less than the EAR.

Lower Reference Nutrient Intake (LRNI), which is the nutritional requirement for those whose needs are low. Most people will need more than this amount in order to maintain their health. Anyone who is getting less than the LRNI might be in danger of nutritional deficiency. The following list describes those vitamins and minerals that should be present in the daily diet. The descriptions include the RDAs for the EU and USA. The amounts given are those that should be taken to prevent a mineral deficiency, not those needed to improve health or prevent non-deficiency diseases:

Recommended Daily Allowances / Dietary Reference Intake (Table 3-6)

In the Recommended Dietary Allowance charts below, amounts marked with a * indicate AI (Adequate Intake). Figures taken from the Dietary Reference Intakes (DRI, 1997, 98, 2000, 2001, 2003 and 2004).

Minerals	0-6 months	7-12months	1-3 years	4-8 years
Calcium	210* mg	270* mg	500* mg	800* mg
Chromium	0.2* µg	5.5* µg	11* µg	15* µg
Copper	200* µg	220* µg	340 µg	440 µg
Fluoride	0.01* mg	0.5* mg	0.7* mg	1* mg
Iodine	110* µg	130* µg	90 µg	90 µg
Iron	0.27* mg	11 mg	7 mg	10 mg
Magnesium	30* mg	75* mg	80 mg	130 mg
Manganese	0.003* mg	0.6* mg	1.2* mg	1.5* mg
Molybdenum	2* µg	3* µg	17 µg	22 µg
Phosphorus	100* mg	275* mg	460 mg	500 mg
Selenium	15* µg	20* µg	20 µg	30 µg
Zinc	2* mg	3 mg	3 mg	5 mg
Potassium	0.4* g	0.7* g	3.0* g	3.8* g
Sodium	0.12* g	0.37* g	1.0* g	1.2* g
Chloride	0.18* g	0.57* g	1.5* g	1.9* g

1µg = 1mcg = 1microgram = 1/1,000,000 of a gram; 1mg = 1milligram = 1/1,000 of a gram; 1g = 1gram

Table 3. Recommended Daily Allowances (RDA) Chart for Infants & Children

Minerals	Male 9-13 Yrs	Male 14-18 Yrs	Female 9-13 Yrs	Female 14-18 Yrs
Calcium	1300* mg	1300* mg	1300* mg	1300* mg
Chromium	25* µg	35* µg	21* µg	24* µg
Copper	700 µg	890 µg	700 µg	890 µg
Fluoride	2* mg	3* mg	2* mg	3* mg
Iodine	120 µg	150 µg	120 µg	150 µg
Iron	8 mg	11 mg	8 mg	15 mg
Magnesium	240 mg	410 mg	240 mg	360 mg
Manganese	1.9* mg	2.2* mg	1.6* mg	1.6* mg
Molybdenum	34 µg	43 µg	34 µg	43 µg
Phosphorus	1250 mg	1250 mg	1250 mg	1250 mg
Selenium	40 µg	55 µg	40 µg	55 µg
Zinc	8 mg	11 mg	8 mg	9 mg
Potassium	4.5* g	4.7* g	4.5* g	4.7* g
Sodium	1.5* g	1.5* g	1.5* g	1.5* g
Chloride	2.3* g	2.3* g	2.3* g	2.3* g

Table 4. Recommended Daily Allowances for Older Children (9 to 18 Years)

Minerals	Male 19-50 Yrs	Male >50 Yrs	Female 19-50 Yrs	Female >50 Yrs
Calcium	1000* mg	1200* mg	1000* mg	1200* mg
Chromium	35* µg	30* µg	25* µg	20* µg
Copper	900 µg	900 µg	900 µg	900 µg
Fluoride	4* mg	4* mg	3* mg	3* mg
Iodine	150 µg	150 µg	150 µg	150 µg
Iron	8 mg	8 mg	18 mg	8 mg
Magnesium #1	400/420 mg	420 mg	310/320 mg	320 mg
Manganese	2.3* mg	2.3* mg	1.8* mg	1.8* mg
Molybdenum	45 µg	45 µg	45 µg	45 µg
Phosphorus	700 mg	700 mg	700 mg	700 mg
Selenium	55 µg	55 µg	55 µg	55 µg
Zinc	11 mg	11 mg	8 mg	8 mg
Potassium	4.7* g	4.7* g	4.7* g	4.7* g
Sodium #2	1.5* g	1.3* g	1.5* g	1.3* g
Chloride #2	2.3* g	2.0* g	2.3* g	2.0* g

As per the Food and Nutrition Board (FNB) Recommendations : Men from 31 to 50 need slightly more magnesium (420 mg) than those from 19 to 30 years old (400 mg). Women from 31 to 50 also need slightly more magnesium (320 mg) than those from 19 to 30 years old (310 mg). Adults over 70 years need slightly different levels of sodium (1.2 g) and chloride (1.8 g). Pregnant women from 31 to 50 need slightly more magnesium (360 mg) than those between 19 to 30 years old (350 mg). Women from 31 to 50 who are breastfeeding also require slightly more magnesium (320 mg) than those between 19 to 30 years old (310 mg).

Table 5. Recommended Daily Allowances for Adults (19 Years and Up)

Minerals	Pregnancy 14-18 Yrs	Pregnancy 19-50 Yrs	Lactation 14-18 Yrs	Lactation 19-50 Yrs
Calcium	1300* mg	1000* mg	1300* mg	1000* mg
Chromium	29* µg	30* µg	44* µg	45* µg
Copper	1000 µg	1000 µg	1300 µg	1300 µg
Fluoride	3* mg	3* mg	3* mg	3* mg
Iodine	220 µg	220 µg	290 µg	290 µg
Iron	27 mg	27 mg	10 mg	9 mg
Magnesium #3	400 mg	350/360 mg	360 mg	310/320 mg
Manganese	2.0* mg	2.0* mg	2.6* mg	2.6* mg
Molybdenum	50 µg	50 µg	50 µg	50 µg
Phosphorus	1250 mg	700 mg	1250 mg	700 mg
Selenium	60 µg	60 µg	70 µg	70 µg
Zinc	12 mg	11 mg	13 mg	12 mg
Potassium	4.7* g	4.7* g	5.1* g	5.1* g
Sodium	1.5* g	1.5* g	1.5* g	1.5* g
Chloride	2.3* g	2.3* g	2.3* g	2.3* g

Table 6. Recommended Daily Allowances for Pregnancy / Lactating Mothers

14. Trace minerals in human milk and in drinking water guidelines (Table 7 and 8)

In considering the consumption of drinking water by vulnerable populations, a figure of 0.75 liters per day has been used for a 5kg child and a figure of one liter per day for a 10kg

Mineral	Mature human milk (Lawrence & Lawrence, 1999)	Drinking water guidelines (WHO, 1996)	EC, SCF, 2003 (Recommended energy content: 60-70kcal/dl; based on 65kcal/dl, cow's-milk- protein based formula)
	mg/L	mg/L	mg/L
Calcium	280	--	325-910 (Ca: P=1-2)
Iron	0.40	0.3 ^b	1.95-8.45
Zinc	1.2	3.0 ^b	3.25-9.75
Copper	0.25	1.0 ^b ; 2.0* (P)	0.228-0.65
Selenium	20	10*	20-59
Fluoride	0.016	1.5 (P)	≤ 0.65
Magnesium	30	--	33-98
Sodium	180	200*	130-390
Sulphate	140 (sulphur)	250*	--
Chloride	420 (chlorine)	250*	325-1040
Manganese (µg/L)	6	100 ^b ; 500* (P)	6.5-650
Molybdenum (µg/L)	2µg/dc	70	--

* Health-based guideline value, (P): provisional; ^b Parameters in drinking water that may give rise to complaints from consumers, ^c FNB

Table 7. Trace minerals in human milk and in drinking water guidelines

	Inland surface water		Drinking water			WHO (2006)	
	BIS/CPCB	WHO	BIS	CPCB	WHO(1993)	Normal	Health based
Cd	2.0	0.1	2.0	2.0	0.003-0.005	<1 µg/l	0.003
Cu	3.0	0.05-1.5	3.0	3.0	2.0	-	2.0
Fe	3.0	0.1-1.0	-	3.0	0.2	0.5-5.0	-
Mn	2.0	0.05-0.5	0.1	0.1	0.5-0.05	-	0.4
Ni	2.0	-	-	2.0	0.02	0.02	0.07
Pb	0.1	0.1	2.0	3.0	0.01	-	0.01

Table 8. Recent standards for heavy metals (mg/l) in Inland surface and drinking water (Bharti 2007)

child. Although these figures may be applicable for standard calculations, the range of quantitative water intake observed in populations at that age might be considerable according to the Food and Nutrition Board of the Institute of Medicine.

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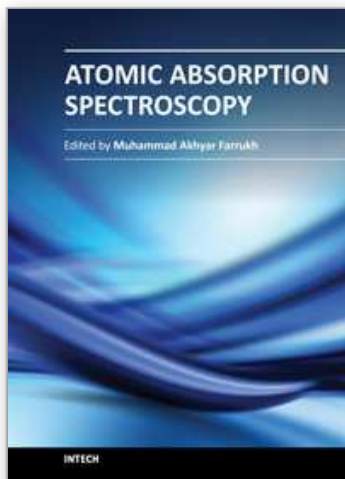
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Atomic Absorption Spectroscopy is an analytical technique used for the qualitative and quantitative determination of the elements present in different samples like food, nanomaterials, biomaterials, forensics, and industrial wastes. The main aim of this book is to cover all major topics which are required to equip scholars with the recent advancement in this field. The book is divided into 12 chapters with an emphasis on specific topics. The first two chapters introduce the reader to the subject, its history, basic principles, instrumentation and sample preparation. Chapter 3 deals with the elemental profiling, functions, biochemistry and potential toxicity of metals, along with comparative techniques. Chapter 4 discusses the importance of sample preparation techniques with the focus on microextraction techniques. Keeping in view the importance of nanomaterials and refractory materials, chapters 5 and 6 highlight the ways to characterize these materials by using AAS. The interference effects between elements are explained in chapter 7. The characterizations of metals in food and biological samples have been given in chapters 8-11. Chapter 12 examines carbon capture and mineral storage with the analysis of metal contents.

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