Major Analytical Methods for Determining Lead in Environmental and Biological Samples

Jozef Sabol

Abstract Lead (Pb) is an element which is found in nature where out of its known 49 isotopes the most abundant are four: ^{208}Pb (52%), ^{206}Pb (24%), ^{207}Pb (22%) and ^{204}Pb (less than 2%). The increased concentration of lead in the environment is mainly due to some human activities. This includes use of petrol in transport vehicles and releases from industrial and other installations and facilities. From the contaminated environment where soil, water, air, animals and plants always contain certain concentration of lead, the nuclide can find a way into the human organism by inhalation and ingestion. This results in some health effects which, in the case of higher intake, may be extremely poisoning and dangerous. Chronic lead intoxication has been linked to Alzheimer's disease. Lead, like many heavy elements, tends to accumulate in bone. Therefore, analysis of the lead presence in environmental and biological samples is an important prevention measure against harmful consequences which must be minimised and in accordance with the set standards and limits. The chapter deals with some specific methods recommended for determining lead in various samples. Special attention is paid to the description of XRF and PIXE methods and especially methods based on atomic spectroscopy, namely absorption and emission spectroscopy as well as atomic fluorescence methods. While the first two methods are essentially considered as non-destructive, the atomic spectroscopy method falls into the category of destructive methods.

Keywords Lead (Pb) · Health effects · Determination · Atom absorption spectroscopy · Atomic emission spectroscopy · Atomic fluorescence spectrometry · Environmental samples · Biological samples

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J. Sabol (✉)
Department of Crisis Management, Faculty of Security Management, PACR,
Prague, Czech Republic
e-mail: sabol@polac.cz

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1 Introduction

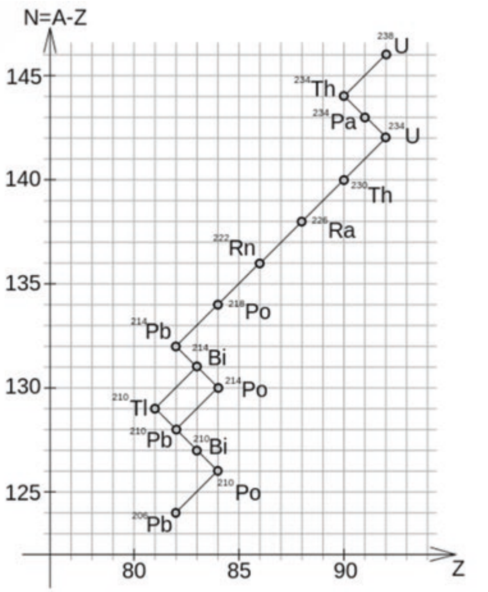
Lead (Pb) is a blue-gray malleable metal found in Group 14 (IV A) periodic table of elements. Natural lead is a mixture of predominantly four stable isotopes: ^{208}Pb, ^{206}Pb, ^{207}Pb and ^{204}Pb. Altogether, there are 49 known lead isotopes, the occurrences of the four of them mentioned above are most abundant: ^{208}Pb (52%), ^{206}Pb (24%), ^{207}Pb (22%) and ^{204}Pb (less than 2%). Of these four isotopes of lead, the only ^{204}Pb is non-radiogenic (it is not the product of radioactive decay, it originates outside the decaying series) and its presence on Earth is on Earth is unvarying. The other three are radiogenic final isotopes of the disintegration series: ^{206}Pb is the final disintegration product of ^{238}U (uranium ^{238}U decay chain), ^{207}Pb is the final product of ^{235}U (uranium ^{235}U decay chain), and ^{208}Pb is the ending product of ^{232}Th (Table 1). It is possible to distinguish with different isotopic composition whether it is a natural or anthropogenic source. This is possible if all pollution sources are characterised by their ratio of lead isotopes and pollutants therefore have their own specific isotopic composition. For example, lead released during combustion processes shows a different isotopic composition of ^{206}Pb/^{207}Pb from other sources of pollution. Also, the isotopic composition of lead emitted into the atmosphere in metallurgical processes corresponds to the isotopic composition of the original materials.

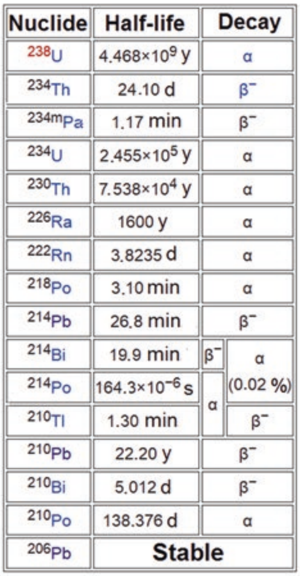
Lead was known and used since prehistoric times. Exposure to lead has been consequently increased mainly because the environment is more and more contaminated by this element. Although acute lead poisoning has become sporadic, chronic exposure to low levels of lead is still considered to be a public health issue (Shilu et al. 2000). Lead intake can lead to a variety of adverse health impacts All over the word; the relevant standards for lead emissions have become increasingly stringent because of new findings about its possible health impacts. The associated regulations require monitoring the situation in order to keep the level and lead concentration below the limits and action levels set by national regulatory authorities.

Lead had previously been mainly introduced into the waters by road and vegetation flushes from the immediate vicinity of busy roads. The exhaust gas contained lead as the decomposition products of tetra-alkyl, which was a common anti-knock additive for gasoline. To a lesser degree lead and lead alloy plants also contribute to contamination. It also gets into the water from the lead pipe. The tetra-alkyl compounds are very volatile and easily pass into the atmosphere when aerating the water. It accumulates in bones and other tissues with age. It interferes with red blood cell enzymes and may cause death at higher doses, causing mental retardation. Often enough lead (200 mg kg^{-1}) in the grass along the highways could even kill cattle.

The nuclides produced as results of the decay of natural radionuclides with very long half-life such as ^{238}U (half-life 4.5 10^9 years), ^{235}U (half-life 0.7 10^9 years) and ^{232}Th (half-life 14 10^9 years) are radioactive and continue to form more radioactive nuclides until a non-radioactive nuclide is formed. A uranium-radium decay chain begins with ^{238}U and ends with the stable ^{206}Pb after going through 18 intermediate steps. Uranium-235 is at the beginning of the uranium-actinium decay chain leading via 15 radionuclides to ^{207}Pb. With ten intermediate states, the thorium decay chain

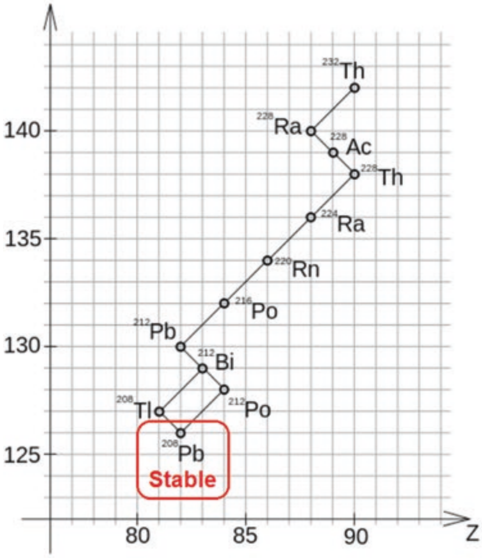
Table 1 Three main decay chains (or families) are observed in nature, commonly called the thorium series, the radium or uranium series, and the actinium series, representing three of these four classes, and ending in three different, stable isotopes of lead (*A*, mass number corresponding to number of protons and neutrons; *Z* atomic or proton number; *N* number of neutrons)

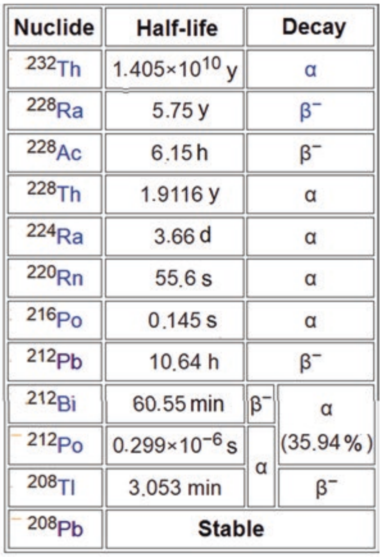




Nuclide	Half-life	Decay	
^{238}U	4.468×10^{9} y	α	
^{234}Th	24.10 d	β$^-$	
^{234m}Pa	1.17 min	β$^-$	
^{234}U	2.455×10^{5} y	α	
^{230}Th	7.538×10^{4} y	α	
^{226}Ra	1600 y	α	
^{222}Rn	3.8235 d	α	
^{218}Po	3.10 min	α	
^{214}Pb	26.8 min	β$^-$	
^{214}Bi	19.9 min	β$^-$	α (0.02 %)
^{214}Po	164.3×10^{-6} s	α	
^{210}Tl	1.30 min		β$^-$
^{210}Pb	22.20 y	β$^-$	
^{210}Bi	5.012 d	β$^-$	
^{210}Po	138.376 d	α	
^{206}Pb	Stable		

Uranium series



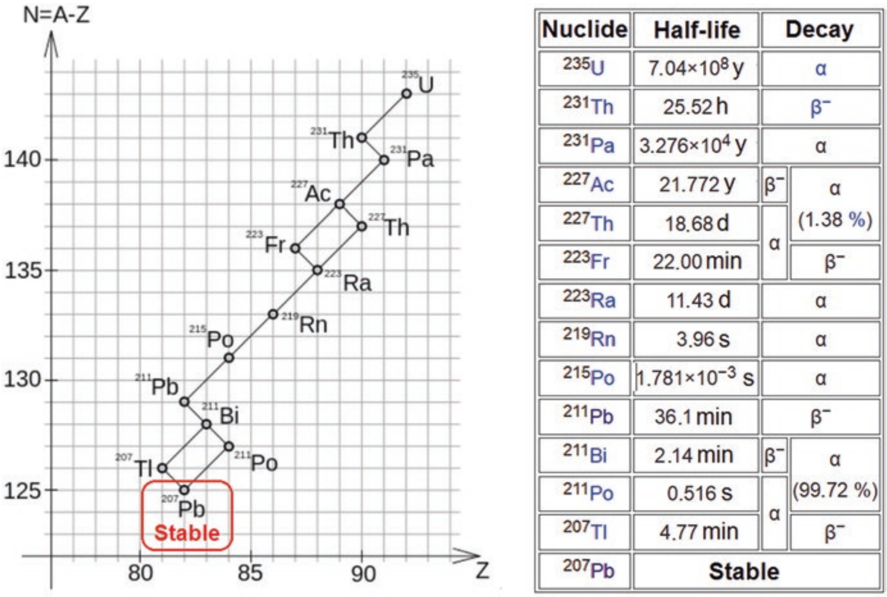


Nuclide	Half-life	Decay	
^{232}Th	1.405×10^{10} y	α	
^{228}Ra	5.75 y	β$^-$	
^{228}Ac	6.15 h	β$^-$	
^{228}Th	1.9116 y	α	
^{224}Ra	3.66 d	α	
^{220}Rn	55.6 s	α	
^{216}Po	0.145 s	α	
^{212}Pb	10.64 h	β$^-$	
^{212}Bi	60.55 min	β$^-$	α (35.94 %)
^{212}Po	0.299×10^{-6} s	α	
^{208}Tl	3.053 min		β$^-$
^{208}Pb	Stable		

Thorium series

(continued)

Table 1 (continued)



Nuclide	Half-life	Decay	
^{235}U	7.04×10^{8} y	α	
^{231}Th	25.52 h	β⁻	
^{231}Pa	3.276×10^{4} y	α	
^{227}Ac	21.772 y	β⁻	α (1.38 %)
^{227}Th	18.68 d	α	
^{223}Fr	22.00 min		β⁻
^{223}Ra	11.43 d	α	
^{219}Rn	3.96 s	α	
^{215}Po	1.781×10^{-3} s	α	
^{211}Pb	36.1 min	β⁻	
^{211}Bi	2.14 min	β⁻	α (99.72 %)
^{211}Po	0.516 s	α	
^{207}Tl	4.77 min		β⁻
^{207}Pb	Stable		

Uran-actinium series

starting with ^{232}Th and ending at ^{208}Pb is the shortest. The daughter nuclides arising from the disintegration of naturally occurring ^{238}U, ^{235}U and ^{232}Th are all radioactive and therefore disintegrate until the last one which is stable lead, namely ^{206}Pb, ^{207}Pb and ^{208}Pb, respectively.

2 Health Effects

While the use of lead has been greatly reduced in developed countries, it is still used widely in developing countries because it has some unique useful properties suitable and required in a number of various applications. The continued use of lead has caused its levels to raise worldwide, posing serious threats not only to the environment but also to the humans living in it. Lead can cause some damage in every organ and tissue in human body, Exposure to high lead levels can severely affect the brain and kidneys and eventually cause death. High levels of exposure can lead to miscarriage in pregnant women. Lead is also widely thought to be cancerogenic. Toxic effects of lead have been observed especially on the renal, reproductive and nervous system. Therefore, some the techniques were developed for treating lead toxicity. Some more information about the recent progress in this area is given in the review

Table 2 Toxicology of lead (adopted from Das and Grewal 2011)

Form entering body	Major route of absorption	Distribution	Major clinical effects	Key aspects of mechanism	Metabolism and elimination
Inorganic lead oxides and salts	Gastrointestinal, respiratory	Soft tissues; redistributed to skeleton (>90% of adult body burden	CNS deficits; peripheral neuropathy; anaemia; nephropathy; hypertension; reproductive toxicity	Inhibits enzymes; interferes with essential cations; alters membrane structure	Renal (major); faeces and breast milk (minor)
Organic (tetraethyl lead	Skin, gastrointestinal, respiratory	Soft tissues, especially liver, CNS	Encephalopathy	Hepatic de alkylation (fast) tri alky metabolites (slow) dissociation to Pb	Urine and faeces (major); sweat (minor)

(Wani et al. 2015). An overview of possible health effects caused by lead which entered human body through various routes is given in Table 2.

In general, it has been widely accepted that lead is a probable human carcinogen. Lead can affect every organ and system in the body. Exposure to high lead levels can severely damage the brain and kidneys and ultimately cause death. In pregnant women, high levels of exposure to lead may cause miscarriage. Internationally for lead regulatory limits have been introduced corresponding to15 parts per billion (ppb) in drinking water and 0.15 μg per cubic meter in air.

Since lead is an element that occurs naturally in the earth, trace amounts of lead may occur in the foods we eat and the water we drink. Regulatory authorities in most countries are trying to control population exposure to lead from various sources. The main sources of lead entering human body include the following:

- Inhalation of lead dust which originates from lead-based paint and lead-contaminated soil.
- Touching by hands other objects contaminated with lead dust and then putting them into mouths.
- Consuming food, candy or water contaminated by lead.
- By means of dishes or glasses that contain lead.
- From colour additives in paints and cosmetics (special case may be children playing with toys that contain lead paint).

In addition to occupationally related exposure by some workers, the most sensitive group among population represent children, which may be affected by lead due to many different situations (Fig. 1).

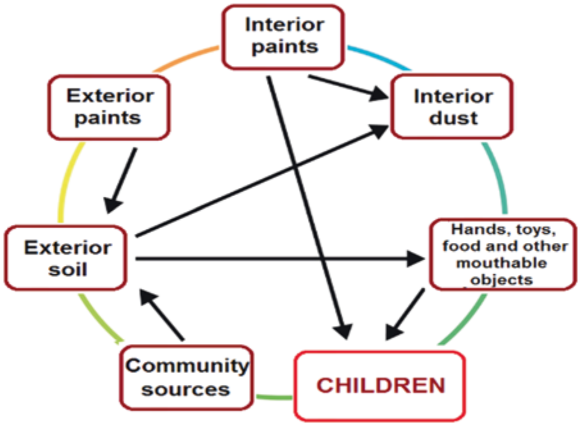


Fig. 1 The main routes of lead entering children's bodies

3 Overview of Basic Analytical Methods for Determining Lead

There are several methods used for the monitoring and identification of the occurrence of lead in analysed samples. The techniques differ as to sample preparation and treatment as well as to instrumentation used.

3.1 X-Ray Fluorescent Spectroscopy and PIXE Method

The principles of both methods are similar. In case of X-ray fluorescence (XRF) method, an incident X-ray photon removes an orbital electron leaving a hole which is filled by an electron from an outer shell which results in the emission of a photoelectron and a characteristic photon with an energy inherent to the type of the atom of a nuclide under examination. The PIXE (particle induced X-ray emission) method is essentially the same, only instead incident X-ray photons charge particles (in most cases protons) are used. The process is illustrated in Fig. 2.

X-ray photons or charged particles excite inner electron shells of the sample material. The ejected electron leaves the atom of the target element as a photoelectron. Other electrons fill the gap and give off large amounts of energy in the form of characteristic X-rays, which are detected, their energy can thus identify the element, and the intensity of X-rays identifies concentration of the element in the sample. In general, incident photons may be produced either by an X-ray tube or a radionuclide emitting soft gamma radiation.

As a source of primary radiation, the XRF analysers can use either an X-ray tube (Fig. 3a) or a suitable radioactive excitation source (Fig. 3b) such as ^{57}Co and ^{109}Cd.

In addition to laboratory XRF analysers, usually equipped with radioactive sources, there are several various types of portable analysers (Fig. 4) designed to

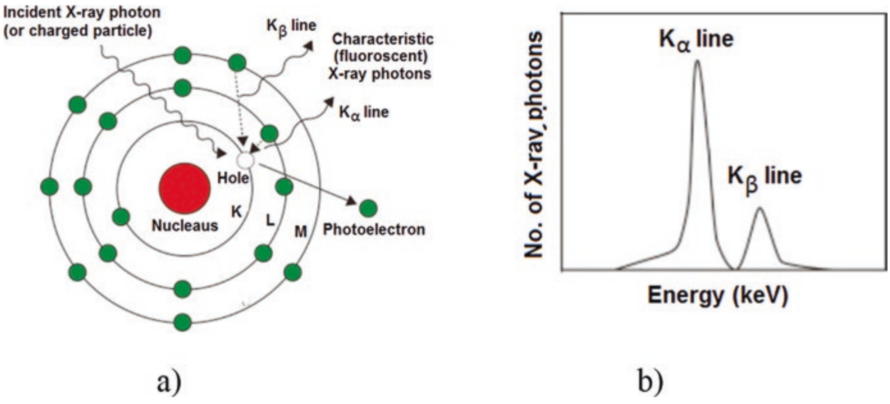


Fig. 2 The principle of the mechanism of XRF (and PIXE) method; (**a**) the generation of characteristic photons; (**b**) their energy distribution (Based on Motohiro et al. 2015)

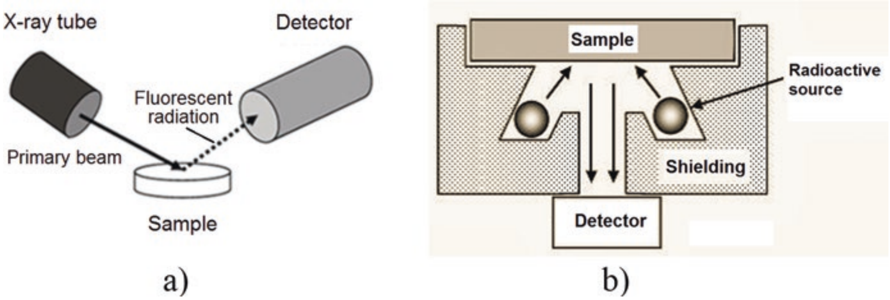


Fig. 3 Basic arrangement of an XRF analyser using (**a**) an X-ray tube and (**b**) a radioactive source

monitor the detection of heavy metals (including Pb) in soil, paint, toys, and so on. These handheld XRF analysers can also be used for specific material identification and hazardous material analysis, and for metal alloy identification, consumer goods screening, compliance screening and many other analysis needs. These analysers serve also for checking compliance with the standards and limits introduced by the relevant regulatory authorities,

An X-ray spectrographic technique known as the *PIXE method*, can be used to analyse solid, liquid or aerosol filter samples in a non-destructive, simultaneous elemental way. The X-ray photons are initiated when energetic protons excite target atoms in the inner shell of electrons. When these inner shell electrons are subsequently expelled, they produce X-rays whose energies are emitted when the resulting vacancies are again filled. These vacancies are unique to the sample elements being analysed, with the number of X-rays emitted being proportional to the mass of the corresponding sample element.

Fig. 4 Some examples of portable XRF analysers, (**a**) Oxford X-MET5000 Handheld XRF Analyzer (Oxford 2019) and (**b**) Thermo scientific XRF analyser (Thermo 2019)

The PIXE method for the most part uses protons to generate X-rays in a sample, the probability of which depends on the proton energy (in MeV) as well as on the total number of incident protons. This number can be expressed as proton current (in mA): the greater the proton current, the greater the probability for X-ray production. As the proton energy changes, the probability for X-ray production also changes. Both of these factors must be accurately known in order to perform a correct quantitative analysis. When protons interact to produce X-rays, each collision in turn transfers kinetic energy from the mobile proton to the immobile target atom. While each collision produces a small amount of energy, as the collisions and resulting energy increase, they eventually reduce the proton's energy as well as its ability to generate X-rays, and in the end the proton becomes immobile. The instrument calibration is carried out at specific proton energy, Data on proton energy loss is necessary to calibrate instruments for performing accurate quantitative analysis. Since instrument calibration is carried out at specific proton energy, information of the proton energy loss is indispensable for quantitative analysis. The PIXE technique has been used in the monitoring of lead and other metals. The range of Pb concentrations in human rib bone was found to be in the range of 1.4–11.5 $\mu g\ g^{-1}$ for the trabecular surface by PIXE (Deibel et al. 1995).

3.2 *Atomic Spectroscopy*

In general, atomic spectroscopy (spectrometry) represents the determination of elemental composition based on the evaluation of electromagnetic radiation absorbed and emitted. The analysis of the electromagnetic spectrum of elements, called

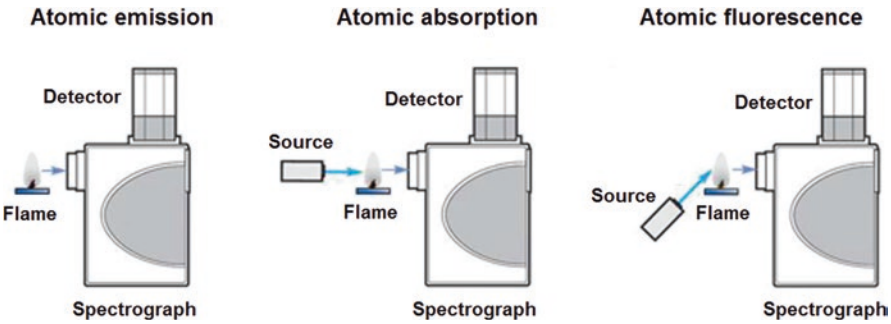


Fig. 5 The illustration of all three techniques of atomic spectroscopy based on atomic emission, atomic absorption and atomic fluorescence

optical atomic spectroscopy, includes three techniques: atomic absorption, atomic emission and atomic fluorescence versions (Fig. 5). The excitation and decay to the ground state is involved in all three fields of atomic spectroscopy. Either the energy absorbed in the excitation process, or the energy emitted in the decay process is measured and used for analytical purposes.

Atomic absorption measures the amount of light of a certain (resonant) wavelength which is absorbed as it penetrates through a cloud of atoms; the greater the number of atoms the greater the amount of light absorbed. This measurement can give a quantitative determination of the amount of analysed element in the sample. The specific light source emitting photons of suitable wavelength can determine quantitatively individual elements among other elements. This technique is fast and accurate, making atomic absorption a popular method for determining the amount of lead as well as other metals in any given substance.

Atomic emission spectrometry is a technique in which a high energy, thermal environment such as an electrical arc, a flame, or even plasma is applied to a sample in order to produce excited atoms which can emit light. The resulting emission spectrum consists of the discrete wavelengths (emission lines) which can also be used for qualitatively identifying an element.

The third field of atomic spectroscopy is *atomic fluorescence*. This method incorporates aspects of both atomic absorption and atomic emission modality. Like atomic absorption, ground state atoms created in a flame are excited by focusing a beam of light into the atomic vapor. Instead of looking at the amount of light absorbed in the process, however, the emission resulting from the decay of the atoms excited by the source light is measured.

Atomic fluorescence spectrometry takes characteristics from atomic absorption as well as atomic emission. Like atomic absorption, a beam of light is shone into atomic vapour, exciting ground state atoms created in a flame. Unlike it, however, the emission from the decay of the atoms excited is measured.

3.2.1 Atomic Absorption Spectroscopy

Atomic absorption spectroscopy (spectrometry), AAS, is a spectrometric analytical method used to determine both trace and significant concentrations of individual elements in an environmental or biological sample. The method can analyse over 60 elements of the periodic table with sensitivity from about 0.01–100 ppm. The greatest boom was recorded in the 1960s and 1980s, when it was one of the most sensitive and widely used instrumental analytical techniques. In forensic chemistry, it is mainly used to detect heavy metals and ammunition residues.

The analytical sample solution is fogged, and the resulting aerosol is introduced into a flame or graphite atomiser, where the solution is immediately evaporated and the chemical bonds in the molecules of the present compounds are broken. At the same time, the atomisation conditions are chosen so that the largest possible population of measured atoms remains in the neutral state and does not ionise to form charged particles.

A beam of light passes through a flame from a special discharge lamp whose photons are absorbed when they meet the atoms of the analysed element and the atom of the element passes into the relevant excited state. This leads to a decrease in the intensity of the transmitted light, and the loss is given by the Lambert–Beer law in the following form

$$I = I_0 e^{-(k.n.l)}$$

where I_0 is the intensity of the exciting radiation, I is the intensity of the radiation after passing through the absorbing environment (flame), k is the atomic absorption coefficient for the specified absorption line, n is the number of atoms of the analysed element in the volume unit and l is the length of the absorption layer.

In practice, the logarithm of the attenuation of light energy called absorbance (A) is used as the measure corresponding to the concentration of the nuclide monitored. The absorbance is expressed by the relation

$$A = \log \frac{I_0}{I} = 2.303\left(k.n.l\right)$$

A very simple linear dependence on the atomic concentration of the measured element is then valid for absorbance. Therefore, all AAS spectrometers indicate the measured results in terms of absorbance units reflecting the actual measured light transmittance. Individual parts of a common atomic absorption spectrometer are shown in Fig. 6.

The atomic emission spectrometry is suitable for the assessment of lead concentration in various waters. Results of some measurements in Iran are presented in Table 3 (Dadfarnia et al. 2001). Another table (Table 4) illustrates the use of atomic absorption spectrometry in determining lead concentration in tea leaves, mixed Polish herbs and oriental tobacco leaves (Chwastowska et al. 2008). The same authors (Chwastowska et al. 2008) measured also lead concentration in soil, street dust and grass with the results of 67.6, 21.3 and 2.0 μg g^{-1}, respectively.

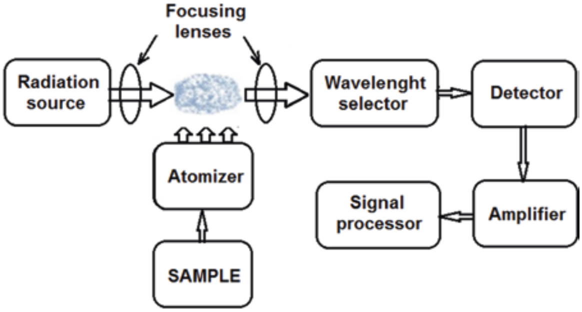


Fig. 6 Block diagram of a typical atomic absorption spectrometer (Based on Alshana 2007)

Table 3 Determination of lead in natural waters; sample volume: 1000 mL (pH = 3); eluent: 5 mL of HNO3 (4 M)

Samples	Concentration ($\mu g\ L^{-1}$)
Tap water	2.4 ± 0.1
River water	2.8 ± 0.1
Well water	2.0 ± 0.1
Spring water	3.2 ± 0.1

Table 4 Results of analysis of the concentration of lead together with Cu and Cd determined in three different samples

Sample	Pb ($\mu g\ g^{-1}$)	Cu ($\mu g\ g^{-1}$)	Cd ($\mu g\ g^{-1}$)
Tea leaves	1.78 ± 0.24	20.4 ± 1.5	0.030 ± 0.004
Mixed Polish herbs	2.16 ± 0.23	7.77 ± 0.53	0.199 ± 0.015
Oriental tobacco leaves	4.91 ± 0.80	14.1 ± 0.50	1.12 ± 0.12

3.2.2 Atomic Emission Spectroscopy

Atomic emission spectroscopy relies on the principle that when light or heat is applied to a molecule, it gets excited and moves to a higher energy level, making it unstable. The excited molecule then jumps to a lower energy level, thereby emitting photons of characteristic energy. The emitted wavelengths are then recorded in the emission spectrometer.

Similarly, to atomic absorption spectroscopy, the sample should be transformed into free atoms. This is normally achieved using a high-temperature excitation source. Liquid samples are dispersed and led in the excitation source by a flowing gas. Solid samples can be introduced into the source by slurry or by laser ablation of the solid sample in a gas stream. Solids can also be directly vaporised and excited by a spark between electrodes or by a laser pulse. The excitation source must desolvate, atomise, and excite the analyte atoms. The excitation sources include flame, inductively coupled plasma, laser-induced plasma, direct-current plasma, microwave-induced plasma and spark or arc. The basic scheme of a standard atomic emission spectrometer is in Fig. 7.

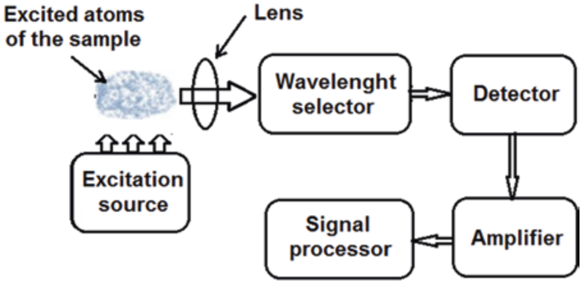


Fig. 7 Principal arrangement of an atomic emission spectrometer

3.2.3 Atomic Fluorescent Spectroscopy

An X-ray region in the electromagnetic spectrum is that which lies between the wavelengths of 0.01 and 10 nm. X-rays are produced, for example, in elementary reactions particles, in the decay of radioactive elements and other processes. Secondary X-rays are also produced by exposing the sample with appropriate (primary) X-rays. This phenomenon is called X-ray fluorescence and is the basis for qualitative and quantitative X-ray fluorescence spectroscopy (XRF—X-ray fluorescence) analytical techniques. Upon absorption of the primary photon by the electron in the inner shell of the atom, the release of this electron (photo effect) and the formation of an electron hole occur. This hole is filled with an electron jumping from higher energy level. In doing so, the secondary (fluorescent) X-rays are released, the spectrum of which is of a line character and characteristic of the element being analysed.

As a source of the fluorescent radiation, usually an X-ray tube or suitable radioactive sources are used. The X-ray source consists of an evacuated tube, tungsten fibre cathode, water-cooled target anode (Ca, Rh, Pd, Ag, W), supplied voltage source (5–80 kV) and a beryllium window. A radioactive source is an appropriate radionuclide emitting gamma radiation which is usually of lower intensity compared to X-ray tube. These sources are especially useful in smaller and portable spectrometers. Characteristic parameters of some suitable radionuclides used in XRF spectrometers are presented in Table 5.

4 Conclusion

Lead is clearly among the toxic elements. Historically, overuse of lead is one of the factors that have contributed to the extinction of the Roman Empire. The main contribution to the excessive intake of lead was due to the use of lead acetate as a sweetener. At present, lead is a ubiquitous environmental contaminant due to the use of lead in drinking water installations, in the production of paints, as an additive in gasoline and its other industrial applications. Lead penetrates into the body mainly in the bone, and a certain amount is found in the blood. Even traces of lead in the environment and food can lead to subsequent severe illnesses, as lead is accumulated in the body. Therefore, the instrumentation for the measurement and monitoring of

Table 5 Radionuclides suitable for the use in XRF spectrometry

Radionuclide	Half-life	Photon energy (keV)	Elements excited
Fe-55	2.7 years	5.9	Na up to V
Cd-109	453 days	22, 88	Cu up to Mo (K) Sn up to U (K)
Am-241	433 years	59.5	Sn up to Tm (K)
Co-57	272 days	122	Ta up to U (K)

lead concentration in specific environmental and biological samples is so important. For the time being, there are some gaps in controlling lead and in adoption of consistent regulations and standards where the situation must be improved.

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