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The Application of the Potentiometric Stripping Analysis to Determine Traces of M(II) Metals (Cu, Zn, Pb and Cd) in Bioinorganic and Similar Materials

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1. Introduction

The development and application of new technologies in all spheres of life and work carries with it the ever-increasing pollution of the environment through harmful and toxic substances. Pesticides and heavy metals are among some of the more prominent pollutants of the environment. Heavy metals significantly contribute to human environment pollution due to the impossibility of their biodegradation, and because some of them have cumulative toxic properties. Sources of contamination by means of metals are numerous, the most important ones being combustion products in the chemical industry and metallurgy, industrial waste waters and landfills, agrochemicals, and exhaust gasses of motor vehicles. People are, therefore, exposed to toxic metals that act both directly through the contaminated air and drinking water, and indirectly through the soil, underground waters and poisoned plants and animals found in food, the pharmaceutical and cosmetic industry.

Copper and zinc are essential bioelements which, in addition to their biological role and their importance for the development of the human body, also have a toxic effect when found in amounts higher than normal in the human body. Lead and cadmium are highly toxic metals, even when found only in traces (Goyer, 1997; Goyer & Klaassen, 1995).

Copper is one of the essential biometals necessary for the growth, development and normal functioning of the human body, for the synthesis of hemoglobin, melanin, and the mineralization and development of bones. The lack of copper can lead to serious illnesses. Nevertheless, its presence in the human body in values greater than 10^{-6} mol/dm^3 inhibits certain enzymes, which hinders the bonding of other essential microelements, or even leads to bonding with certain cofactors. The increased content of copper in the human body leads to coronary and vascular disease, arteriosclerosis, hypertension and various forms of damage to the central nervous system (Uauy, et al., 1998; Hart, et al. 1928; Chapman, 2008).

Zinc is an essential oligoelement which is found in significant amounts in the human body (0.02 – 0.03 g/kg of body weight). It is necessary for the synthesis of proteins and nucleic acids, DNA replication, the human reproductive ability, and maintaining high level healthy immune function. A shortage of zinc in the human body can lead to the harmful effect of pancreatic enzymes, anemia, pulmonary disease, neurological disorders and the occurrence of certain types of cancer (Walsh et al., 1994).

The necessary amounts of these elements for the normal functioning of the human body are introduced through water and food of plant or animal origin. Recommended amounts of zinc in various products range from 0.1 to 80 mg/kg, and of copper from 2 to 100 mg/kg (Goyer & Klaassen, 1995).

Lead is a toxic metal with a cumulative effect, which competes with the essential metals in the human body (Ca, Fe, Cu, Zn). A relatively low content of lead has a negative effect on the heart, blood vessels, kidneys, liver, and respiratory system. Based on its physical-chemical characteristics, Pb(II)- ions can replace Ca (II) - ions isomorphically as part of hydroxyapatite, which leads to the accumulation of this metal in mineral tissue – the teeth and bones. During physiological processes of bone tissue remodeling, part of the Pb (II)- ions, by migration through the oral and other biological fluids, reach other remote organs – the brain, kidneys, and the liver (Pocock et al., 1994; Banks et al., 1997; Vig & Hu, 2000).

Cadmium is considered one of the most dangerous occupational and environmental poisons. It is presumed that excessive amounts of this metal in the human body are undesirable. The basis of cadmium toxicity is its negative influence on the enzymatic systems of cells, owing to the substitution of other metal ions (mainly Zn^{2+} and Cu^{2+}) in metalloenzymes and its very strong affinity to biological structures containing –SH groups. Excessive Cd exposure may give rise to renal, pulmonary, hepatic, skeletal, reproductive effects and cancer. The major effects of this type of metal poisoning are found in the lungs, kidneys and bones. Obviously, the monitoring of the cadmium level at trace level in different environment matrices which are directly related with human health is of great importance. The World Health Organization (WHO, 1996) reported tolerable weekly intakes of cadmium of 0.007 mg/kg body weight, for all groups of humans. Briefly, it is considered that this metal can have a dangerous effect human health even at ultra trace concentrations.

Due to the harmful and toxic effects of copper, zinc, lead and cadmium, it is necessary to determine and monitor their content in water, soil, food, pharmaceutical and cosmetic products, packaging. For medicinal-diagnostic purposes it is sometimes necessary to monitor the contents of these metals in clinical-biological material. Data regarding the deposits and transport mechanisms of Cu, Zn, Pb and Cd in the body can be obtained through an analysis of biopsy material both of human and animal origin (Brzoska & Moniuszko-Jakoniuk, 1998; Florianezyk, 1995).

Due to the high toxicity and stability of Pb, Cd, Zn and Cu it is necessarity to determinate their content in materials, food, water and other samples.

In order to determine the content of the aforementioned metals in the analyzed samples, an electroanalytic technique was used – the potentiometric stripping analysis (PSA). The PSA is a highly-sensitive, selective microanalytic technique for determining heavy metal traces, including metals such as lead, cadmium, copper and zinc (Vydra et al., 1976; Suturović, 2003). The advantage of this technique in relation to other current, more unavailable and costly techniques is also its low exploitation and instrumentation cost, ease of use, the ability to simultaneously determine a greater number of metals in the same sample, as well as the infinite number of analyses of the same sample, even though it has previously been analyzed (Kaličanin, 2006).

The results involved in determining micro amounts of Cu, Zn, Pb and Cd within samples of various types and origin (water, soil, packaging, dental-prosthetic material, beauty products, teas, biopsy material) by using the PSA method have been outlined in this paper, and are in agreement with the data found in the literature in regards to the detection limits of other analytic techniques. This technique can successfully be used in the quality control of bioinorganic and similar material and the analysis of biopsy material for the presence of heavy metals, considering the high values of result reproduction (Danielsson et al., 1981).

2. The electrochemical stripping analysis (ESA)

In order to determine the content of toxic heavy metals in real samples, where even element amounts lower than 1 μg/dm^3 can be significant, the proper selection of the appropriate analysis techniques is also necessary. The analytical methods used for measuring concentrations of traces of M (II) metals (Cu, Zn, Pb and Cd) in bioinorganic and similar materials include atomic absorption spectrometry (AAS), neutron-activation analysis (NAA), inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma optic emission spectroscopy (ICP-OES) and electrochemical stripping analysis (ESA). The success as well as the frequency of the abovementioned techniques is different; they depend on the detection limit, selectivity and reproducibility of the given technique, the rapidity and simplicity of the method as well as the price of the device and its exploitation (Vydra et al., 1976; Jagner, 1979; McKenzie, 1988; Brainina & Neyman, 1993).

The electrochemical stripping analysis (ESA) has the greatest sensitivity (10^{-11}mol/dm^3) coming second to the neutron activation analysis (10^{-21} mol/dm^3). Besides, the cost of its application and exploitation is much lower than with the other above-mentioned techniques while the procedure for carrying out the analysis is relatively simple and fast (Suturović, 2003; Kaličanin et al., 2002).

2.1 Characteristics of the ESA

The electrochemical stripping analysis (ESA) as a highly sensitive and selective instrumental microanalytic technique is used for the quantitative determination of metals, that is metal ions, but in the last few years it has increasingly been used to determine micro-amounts of organic compounds and anions. Bearing in mind the possibilities and the demands of the ESA, we could say that it can fulfill the very rigorous general and specific micro-analytical demands to a significant extent. The most significant features of this technique include, in addition to exceptional sensitivity, very good analytical selectivity:

- The ability to determine a great number of elements simultaneously,
- The ability of unlimited repeated analyses of the same solution,
- The small size of the instrumentation,
- The ability of carrying out analyses outside of the laboratory, “on the spot“.

The sample being analyzed with the help of the ESA has to be in a re-solvent condition. If the sample is in liquid form and if its content (matrix) is not complex (as is the case with water, for example), the preparation of the sample usually requires only the addition of an auxiliary electrolyte which primarily provides the necessary conditions for the ESA, but is often used as a de-complexing agent for the studied substance. When the liquid sample has a more complex matrix, the interfering influence of the matrix can significantly be reduced by means of the dilution of the sample, with the addition of the auxiliary electrolyte. This type of preparation is possible due to the high sensitivity of the ESA.

If the sample is in solid form, it has to be dissolved or extracted. Samples in liquid and solid form, which contain high amounts of organic substances, must be prepared for analysis by means of some of the procedures for the destruction of organic matter (Bock, 1979).

The analysis of gaseous samples requires a previous concentration (adsorption) of the analyzed material on suitable filters, and then their degradation by means of concentrated acids or though annealing, with subsequent dissolution. The preparation of the sample (solution) for the ESA includes the addition of the so-called auxiliary, indifferent electrolyte, with a concentration of 0.1-0.5 mol/dm^3. Most often these include salt solutions (chlorides, nitrates), mineral acids, bases or buffer systems (acetate or citrate). The role of the auxiliary electrolyte is to enable the maximum utilization of the electrochemical depositing, the conductivity of the solution, and to minimize the electrical current which enables migration and set the appropriate pH value (Vydra et al., 1976).

The analysis of the samples, which was carried out by means of the electrochemical stripping analysis, takes place in an electrochemical cell, which is made up of three electrodes (a working electrode, a reference electrode and an auxiliary electrode), a reaction zone and a solution mixer. The working electrode is a thin-layer mercury electrode which is obtained by adding a thin layer of mercury, 10 to 1000 nm thick (Jagner, 1982; Suturović, 2003), to the surface of the inert carrier made of vitreous carbon. Electrodes made of vitreous carbon are especially suitable due to their chemical inertness and relatively wide interval of varying potentials, ranging from -0.75 V to + 1.0 V (Konvalina et al., 2000). Electrodes made of vitreous carbon, despite their high chemical inertness, should not be exposed to the effects of concentrated solutions of potent oxidizing agents and acids, such as hydrogen-peroxide, nitric acid and sulfuric acid. In addition, these electrodes must not be exposed to overly positive potentials $E > 2$ V (ZKE), since this could lead to their irreversible destruction (Kaličanin, 2006).

Thin layers of mercury are deposited using a special mercury (II)- ion solution, whose value must be smaller than 2, at a constant electrical current (galvanostatic conditions), since it enables one to obtain the required thickness of the layer of mercury, irrespective of the resistance in the electrochemical cell. The reference electrode in the ESA is a silver-silver chloride electrode, while the auxiliary (counter) electrode is solely a platinum electrode.

The sensitivity of the ESA is in great part dependent on the intensity of the mixing. The mixing is usually done with a stirring stick mechanical mixer with good control of the number of rpms, constant geometry of the electrochemical cell and other conditions necessary for the analysis (Wang, 1985).

2.2 Processes within the ESA

The electrochemical stripping analysis is a specific analytical technique which is carried out in four successive steps. The first step is the culonometric process during which the determined material is concentrated either on or in the working electrode, under controlled hydrodynamic conditions and during a precisely specified period of time. The concentration of analytes can be carried out by means of an electrolysis, which is most often the case, or by unspecific adsorption or specific chemical reactions. The sensitivity of the ESA is directly dependent on the effectiveness of this step, while the precision is dependent on the determination of the degree of reproducibility of the conditions under which it takes place (Wang, 1985).

The factors which affect the effectiveness of the concentration of the analytes by means of electrolysis include: the potential of the electrolysis, the duration of the concentration, the value of the pH, the conditions of mass transport and the features of the amalgam.

The potential of the electrolysis is the most important factor in electrolysis concentration, as it affects the amount of the separated deposit, and thus the sensitivity and reproducibility of the determination. The value of the electrolysis potential must as a rule range from 300 to 500mV more negative than the polarographic half-wave potential of the determined element (Bard et al., 1985).

The time needed for the analyte electrolysis depends on its concentration. In the case of more diluted solutions, longer electrolysis time is needed, while for solutions of greater concentrations, the duration of the electrolysis is shorter. During the ESA, the usual duration of the electrolysis ranges from 60 to 900 s, where the utilization is from 5-10 %.

The pH value of the analyzed solution affects the chemical state of the analyte, that is, the amount of electroactive ions and in general the analyte ion form in the solution. If the environment is alcaline, the determination of most of the metals is not possible due to the deposition of metal ions in the form of their hydroxides. An overly acidic environment influences the creation of chemical disturbances and also does not enable the determination of certain analytes (organic compounds, elements with a negative redox potential), due to the extraction of hydrogen to the surface of the electrode (Suturović, 2003). The optimum pH values, auxiliary electrolytes and dilution potentials of certain metals in the ESA are shown in Table 1.

Element	pH	Auxiliary electrolyte	Dilution potential (V)
Cu	1-2	HCl	-0.15
Zn	4.6	acetate buffer	-1.00
Pb	4.6	acetate buffer	-0.46
Cd	4.6	acetate buffer	-0.57

Table 1. Optimum pH values, auxiliary electrolytes and dilution potentials of certain metals in the ESA

The effectiveness of the concentration of the studied solution in the ESA improves considerably due to mass transfer through convection. Mass transport through convection is achieved by mixing the solution, using a stirring stick or the rotation of a reaction vessel (Jagner, 1982).

The viscosity of the amalgam influences the diffusion of the metals dissolved in the mercury, or the speed of the electrochemical reactions on the amalgam electrodes.

The second phase of the ESA is the reduction in the velocity of the flow of the solution which lasts from 15 to 30 s and which provides the necessary conditions for the diffusion mass transfer in the next step, and the homogenization of the deposit in the working electrode.

The first two steps are identical for all the “stripping” techniques, while the techniques differ in the third analytic step.

The techniques which include the use of chemical oxidation or less reductive means in the analytic step of the ESA are **potentiometric techniques**.

The fourth step is necessary in the case of the repeated analysis of the same solution, and requires the stirring of the solution for a period of 5 s, so that it could become homogenous and the deposit could dissolve completely.

The content of the analyte is determined through some of the relative methods, most commonly through the method of standard addition or the calibration curve. This is why it is important that the experimental conditions in all four steps of the ESA be very reproducible.

2.3 The potentiometric stripping analysis

The potentiometric stripping analysis (PSA) is the youngest of all the stripping techniques, and was first presented in 1976 (Jagner, 1976). Due to its simplicity and the work of Jagner et al., the PSA very soon gained substantial practical significance (Jagner, 1979, 1982, 1993).

The most frequently used oxidizing agents in the PSA are Hg^{2+}- ions, which are used to determine elements with a more negative redox potential than mercury, or the MnO_4^- or $Cr_2O_7^{2-}$ ions to determine mercury or more precious metals (Jagner, 1979). The concentration of the oxidizing agent, which is added to the sample, must always be high enough to enable the oxidation of all of the analytes, but not too high, as in that case it speeds up the oxidation (it reduces the sensitivity of the determination) and increases the contamination of the sample. (Jagner et al., 1981).

In 1979 Jagner proved the possible uses of the diluted oxygen as an oxidizing agent in the PSA (Jagner, 1979). This specific modification of the PSA with oxygen as the oxidizing agent has a great advantage, since oxygen is already present in the solution and does not require the use of deaeration, which significantly lengthens the duration of the analysis and represents a risk in the sense of the contamination of the solution, damage to the thin-layer electrode and the blocking of the active surface of all three electrodes.

As part of this technique, great care must be taken regarding the negative influence of some of the products of its reduction, as well as in terms of its direct (non-electrical) influence. Thus, due to the reduction of oxygen during electrolysis, the hydroxyl ions will be separated in the vicinity of the working electrode, which in turn can cause the hydrolysis of certain metal ions, while the effect is more pronounced the closer the pH value is to neutral. This effect can be prevented by adjusting the pH value with the help of the appropriate buffers. In addition, the presence of oxygen in this modification of the PSA can contribute to the separation of calomel on the mercury electrode if the solution contains chloride ions. In order to avoid this problem, it is necessary to separate the working electrode and the analyzed solution at the end of the analysis.

The formation of analyte deposits, in the PSA with oxygen as the oxidizing agent, is carried out during the electrolysis procedure. Once the electrolysis is completed, the potentiostatic control is discontinued and a change in the potential of the working electrode is registered, which occurs during the chemical oxidation of the formed deposit. Thus, due to the oxidizing effect of the oxygen, the potential of the working electrode increases and shifts towards the more positive values. The potential increases until it becomes the same as the potential for the dissolution of the most negative of the deposited metals (E_R) and remains constant until the metal with the smallest redox potential is oxidized. i Following that, there is a sudden increase in potential, until the oxidation potential of the following positively deposited metal is reached. The moment the most positive deposited metal on the electrode that can be oxidized by means of oxygen is dissolved, the potential of the working electrode increases to a borderline value, which is in the function of the pH solution, and then remains constant (at around +0,1 V, ZKE) (Fig. 1). This is, at the same time, an indication of the completion of the analytic step of the PSA (Suturović, 2003).

2.3.1 Interference in the potentiometric stripping analysis

The most frequently occurring interference which can take place during stripping analysis is a result of the presence of organic compounds, due to the formation of intermetal compounds and the overlap between the dilution potential of the determined elements (Suturović, 2003).

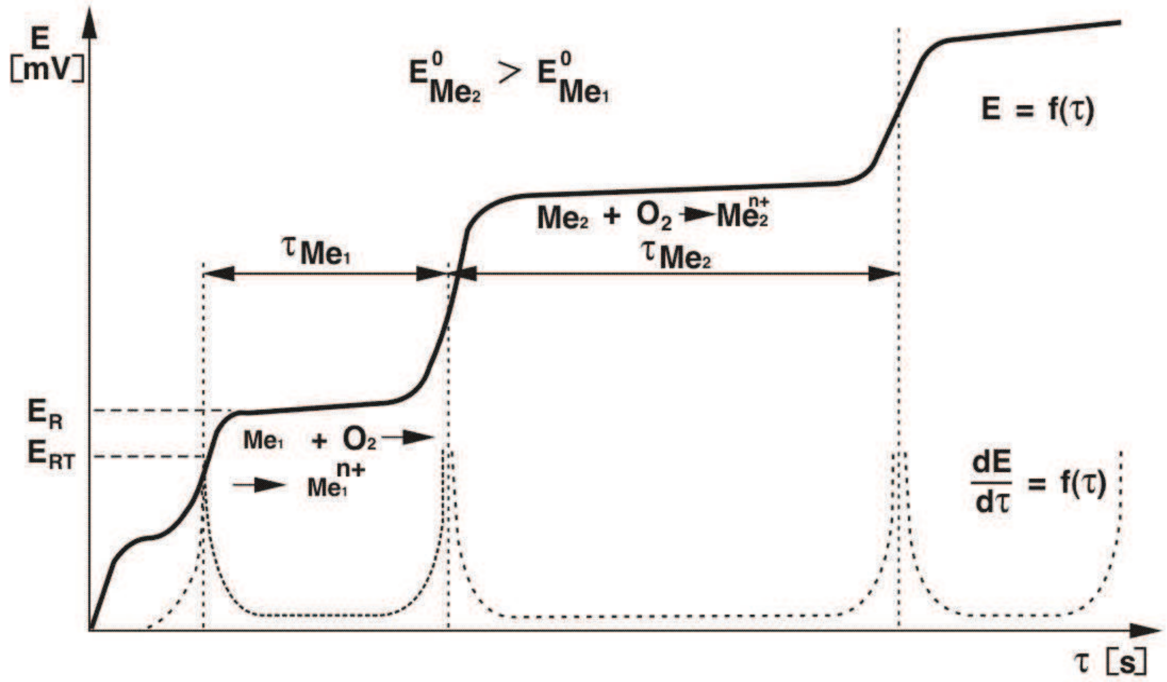

Fig. 1. Response signals in the PSA

Surface active agents such as polysaccharides, alcohols, salts of fatty acids, proteins and the like, cause interference in the PSA by adsorbing to the surface of the working electrode and influencing the mechanism of mass transfer, increasing the value of the dilution potential and decreasing the sensitivity of the determination. These difficulties can be eliminated through the use of a destruction procedure of the organic material, such as microwave radiation, UV radiation, ozone oxidation, dry or wet destruction procedures and the like (Suturović, 2003).

Humic and fulvic acid, in addition to being surface active agents, are also complexing agents, and so their presence can doubly interfere with the electrolytic and analytic steps of the PSA (Suturović, 2003). In this case, the pH value of the studied solution must be adjusted, so that the increase in the pH value will replace the degree of adsorption, but on the other hand, hinder the de-complexing of the analyte and facilitate the creation of organic-metal complexes.

Intermetal compounds can be formed between the metals concentrated in the mercury electrodes or between the electrode material (solid electrodes) and the deposited metals. Intermetal compounds are usually formed with copper and zinc, considering the fact that both metals are present in most of the real samples. These disturbances are mostly eliminated by adding a third element which builds more stable intermetal compounds with the elements which cause an interference, or with the addition of the auxiliary electrolyte, which will form a complex with the *interfering* element (Tyszczuk et al., 2006; Wang, 1985).

The overlap between the dilution potential of certain elements can have a significant influence on the PSA only in the case of elements which have similar dilution potentials (Sn and Pb, Cd and Tl, Bi and Sb). These interferences can be eliminated through the selected communication of selectively statement the potential of the deposit or the use of computerized equipment, which enables the reduction of the analytical signal of the interfering element from the overall signal.

3. Heavy metals

Heavy metals significantly contribute to the increase in the pollution of the human environment, due to the fact that they cannot be biologically decomposed, and the cumulative-toxic effect that some of them have. The sources of metal contamination are numerous, while the most significant are the products of combustion in the chemical industry and metallurgy, industrial waste waters and landfills, agrochemical products and exhaust fumes from motor vehicles. Special attention needs to be focused on contamination due to lead, cadmium, mercury and thallium, since these metals have a cumulative-toxic effect, and are also deposited in the human body (Goyer & Klaassen, 1995).

Essential metals, such as copper, zinc, nickel and others can lead to serious illnesses when they are present in the human body in insufficient amounts, but can also have a harmful and toxic effect if found in doses higher than the recommended ones.

Metals, like all the chemical substances which make their way into the human body, with their physical-chemical features can cause changes and numerous structural or functional damage to one or more of the organs or system of organs.

The penetration of metals from the outside environment into places where they could have a negative effect and the manifestation of toxic effects represents a process which includes the exposition phase (contact in the outside environment), the toxicokinetic phase (absorption, distribution, deposition, disintegration, transformation and elimination) as well as the toxicodynamic phase.

Metals display an affinity towards various organic molecule ligands, especially those that contain S, N and O and which are electron donors: -OH, -COOH, -SH, $-NH_2$ and others.

The affinity, and thus the toxicity of divalent metal ions, according to the appropriate ligands is:

- for the –SH group: Hg > Ag > Pb > Cd > Zn;
- for the –COOH group: Cu > Ni > Co >Mn;
- for the $-NH_2$ group: Hg > Cu > Ni >Pb > Zn > Co > Cd >Mn > Mg

3.1 Copper

Copper is an essential biometal necessary for the proper growth, development and normal functioning of the human body. It takes part in the metabolic acceleration, the increase in the oxidation of glucose, the strengthening of tissue respiration, the mineralization and development of bones, contributes to the resorption of iron in the digestive tract, and catalyzes the biosynthesis of hemoglobin by aiding the inclusion of iron into the hem. Along with calcium, copper takes part in the metabolism of phosphorus (Uauy, et al., 1998).

The biological significance of copper for humans was practically discovered in the work of Hart et al. (Hart, et al. 1928), who have shown that copper plays a very important role in the process of erythropoiesis, that is, the production of red blood cells.

The afore mentioned various roles of copper in the human body are made possible due to its polyvalence on the one hand, and propensity for the formation of stable complex compounds, on the other. The copper (I)- ion bonds with lingands via the –SH groups of proteins. The copper (II)-ion reacts with aminoacids and amino groups of proteins, and with nitrogen in the DNA and RNA molecules. Metabolic disorders involving copper are related to many illnesses, such as diabetes, Wilson's disease, acute and chronic hepatitis, cirrhosis of the liver, cardiovascular disease, osteoporosis.

The daily requirements of an adult range from 2-3 mg of copper (Goyer & Klaassen, 1995).

3.1.1 The toxicity of copper

Copper is a biometal essential for human life. Nevertheless, at concentrations of 10^{-6} mol/dm^3 copper inhibits certain enzymes (acid phosphatase), preventing the bonding of other essential microelements or bonds to certain cofactors, such as glutation (Ahasan et al., 1994).

The world health organization (WHO, 1996) stated that 10-12 mg/per day can be the minimum amount of safe daily intake. Nevertheless, if approximately 2 mg of copper salts are introduced into the body, copper-induced hemolytic anemia and kidney failure could ensue.

Copper found in drinking water or beverages, in amounts of 8 ppm $CuSO_4$ (0,022 mg Cu/kg) (Gotteland et al., 2001) causes nausea, vomiting, abdominal pain, and diarrhea. Amounts of 1-2 g can cause severe poisoning symptoms, and lead to hemolysis, destructive changes to the brain tissue and liver, which could be terminal (Ahasan et al., 1994).

The use of agrochemical substances based on copper, can also lead to an increased intake of copper via food which is produced on soil that has been exposed to it.

The increased content of copper in the body can stem from food or drinking water, which are in immediate contact with copper. Acidic foods or drink can dissolve milligrams of copper, which are sufficient enough to cause acute toxicity and symptoms (Goyer & Klaassen, 1995). Increased contents of copper in the human body can have a harmful effect on the cardiovascular system, leading to coronary disease, and high blood pressure. The toxicity of copper is usually a consequence of excessive intake or small amounts of other necessary nutrients. Small amounts of copper in the food lead to an increase in the content of copper, as they compete for absorption in the gastrointestinal tract (Uauy, et al., 1998). This indicates the necessity of monitoring copper content in various samples, including soil, water, food, and air since copper and other metals are involved in the circulation of matter in nature.

The content of copper in the human body can be determined on the basis of blood work, urine samples, nails or teeth.

3.2 Zinc

Zinc is an essential oligoelement, which can be found in significant amounts in the human body, approximately 0.02-0.03 g/kg of body weight of it. Of the overall amount of zinc in the human body, 20 % of it can be found in the skin, and it can be found in significant amounts in the pancreas, teeth, bones, blood, liver, kidneys, and nervous system (Walsh et al., 1994). Zinc is a necessary microelement for the lives of humans, animals, plants and microorganisms. It influences growth and development, bone formation, blood, the metabolism of nucleic acids, proteins, and carbohydrates. Participation in these processes is bound to the effect of enzymes, of which zinc is an important component or activator.

Among patients with diabetes, the amount of zinc in the pancreas is approximately half of what is found in healthy people.

Zinc also plays a certain role in the stabilization of the cell membrane, as well as in the functioning of certain nervous structures under whose control we find the senses of taste and sight. Zinc has an extremely significant, but insufficiently studied function in immunological processes. Zinc ions, along with Cu(II) and Co(II) ions improve the body's immune system.

Through a normal, varied diet, man daily intakes from 10 to 15 mg of zinc. The human body *stores* zinc and in the case of a lack of zinc, its excretion is reduced (Walsh et al., 1994).

3.2.1 The harmful effect of zinc on the human health

The lack of zinc in the human body can occur due to reduced intake, imbalanced absorption, increased excretion and the body's increased need for zinc. This lack of zinc leads to pathological states which are manifested in the occurrence of dermatitis, diarrhea, alopecia, mental disturbances, mental lethargy, stunted growth and development, loss of appetite, reduced neuropsychological functions, the occurrence of infantilism and slow wound healing.

On the other hand, the significant concentrations of zinc salts, such as chlorides, can have a harmful effect on the human body, to the extent that they could even damage tissue epithelials. A high content of zinc can have a harmful effect on the storage of iron (Walsh et al., 1994). Toxic amounts of zinc in the food can reduce the life span of red blood cells and can lead to anemia, since the use of iron is much faster. (Goyer, 1997).

Zinc as an element is necessary for the normal exocrine and endocrine functioning of the pancreas. Its concentration in this tissue is many times greater than in the plasma and is an important means of zinc elimination. Studies carried out *in vivo* (Chobanian, 1981) have shown that a high zinc content (800 mg/per day) causes a considerable increase in amylase and lipase in the serum, and an increase in blood sugar levels.

The increased zinc content can be connected to the occurrence and development of neurological disease. The significant increase of zinc in the human body can lead to a disturbance in neurological functions and the occurrence of multiple sclerosis among workers involved in production processes where zinc is the basic ingredient. Zinc contents ranging from 6.54 to 16.35 mg/dm^3 lead to minor damage, while amounts exceeding 16.35 mg/dm^3 are neurotoxic (Choi et al., 1988).

The recommendation is that zinc should be taken with dairy products since milk contains picolinic acid with which zinc builds chelating complex compounds which are best absorbed in the intestines.

3.3 Lead toxicity

Lead belongs to a group of the most toxic of elements, with a cumulative-toxic effect. (Vig & Hu, 2000). Lead is not an essential metal, but is present in all of the tissues and organs of mammals, and can mostly be found in mineral tissue – bones and teeth (over 90 % of the overall amount of this element) (Gulson, & Gillings,1997). If it is constantly introduced into the human body, even in small amounts, lead partially replaces calcium in the tertiary calcium-phosphate bone skeleton, where its toxic effect is gradually increased.

Daily amounts of lead which a human normally absorbs mostly through food and drink, can range up to 0,3 mg, but this amount does not cause poisoning, since lead is excreted in approximately the same amount daily from the human body (Goyer, 1997).

Lead intake can occur in different ways. Lead bound in tetraethyl lead, as an addition of gasoline, through its combustion is transferred into the atmosphere and reaches the human respiratory system. Part of the lead is absorbed by plants and animals alike, so that it is introduced into the human body by means of food. The innards used in the human diet (especially the liver and kidneys) contain high concentrations of lead. Nevertheless, it has been proven that only 3 % the lead in the innards is absorbed into the human body.

Besides food, lead can be introduced into the body by mans of water, which lead reaches via the air, soil or pipelines. Studies have shown that lead from the water or other beverages is reabsorbed to a greater extent than that from food. In addition, lead introduced into the body between meals is absorbed to a greater extent than the lead introduced during a meal, while the greater frequency of food intake minimizes the absorption of lead. It has been proven that 50 % of lead is absorbed from water, following an overnight fast (Vig & Hu, 2000). Professional exposure to lead, in factories and workshops leads to severe and prolonged illnesses as the gravest of professional illnesses.

According to the American Center for Disease Control (CDC) (Goyer & Klaassen, 1995) a lead content in the blood of less than 240 μg/dm^3 is *normal*, 250-490 μg/dm^3 belongs to the *moderate risk* category, 500-690 μg/dm^3 to the *high risk* category and contents of over and amounts that exceed 700 μg/dm^3 fall into the *urgent risk* category. Nevertheless, prolonged exposure to low-level toxicity (< 240 μg/dm^3) can lead to various psychological disorders and learning disabilities among children. Naturally, these symptoms can occur among children even in the case of lead amounts of less than 50 μg/dm^3 (Banks et al., 1997).

Lead competes with essential metals (Ca, Fe, Zn, Cu) for various important functions in the human body.

The intake of smaller amounts of iron and vitamins C and D, as part of our diet, can increase the lead content in the blood, absorption from the intestines and can lead to the accumulation of lead in the body (Pocock et al., 1994).

Lead competes with iron for the binding spots in the ferritin transport protein Fe^{3+}-ion and can block the active center of the ferritin, building lead-sulfide which can only be dissolved with difficulty.

According to some of its physical-chemical features, lead is chemically similar to calcium, and so in the body it behaves like calcium and can be found in calcified tissue (bones and teeth), in blood plasma bound to proteins or in an ionized form, as well as in the form of compounds with various biomolecules (such as citrates) (Kaličanin et al., 2004).

Naturally, the very important affinity of lead towards mineral tissue (bones, teeth) where it accumulates over time, has been confirmed in the works of many authors. Lead in the bones contributes to the development of osteoporosis, the reduction of bone tissue, changes in the structure of bone structure and increases the resorption of bones tissue among the elderly (Gulson, & Gillings, 1997).

3.4 Cadmium toxicity

Cadmium is one of the most dangerous poisons of the working and living environment, which can be introduced into the body by means of air, food or drinking water. Thus, the lack of iron can significantly increase the accumulation of cadmium, and sufficient amounts of iron in the blood inhibit the accumulation of cadmium. In addition, increased doses of vitamin D act as an antidote to cadmium poisoning. The cadmium content in the human body has a value of $1 \cdot 10^{-4}$ % of the overall body mass (Danielsson et al., 1981).

Cadmium poisoning can be acute and chronic. Acute poisoning occurs due to inhalation of the fumes of particles of cadmium salts (oxides, chlorides, sulfides, sulfates, carbonates and acetates) whose concentration in the air is approximately 1mg/m^3 (Goyer & Klaassen, 1995).

The toxic effect of cadmium to a great extent depends on the intake of calcium. Low calcium intake leads to higher cadmium absorption, the retention, accumulation and increased toxic effect of this metal. The consequences of this include kidney and bone damage (osteomalacia) as well as hypertension and anemia. (Brzoska & Moniuszko-Jakoniuk, 1998).

The presence of cadmium in the air originates from the combustion of oil derivatives, coal and plastic mass, and is found in cigarette smoke. The absorption of cadmium from the air mostly takes place through breathing, and to a lesser extent through the gastrointestinal tract, and in trace amounts via the skin.

Once it enters the body, cadmium is transported into the blood by means of red blood cells and a highly molecular blood protein – albumin. The normal level of cadmium in the blood of adults is less than 1 μg/dm^3. Even though cadmium circulates via the blood throughout the entire body, the greatest accumulation (from 50 to 60 % of the body's cadmium load) can be found in the kidneys and liver (Florianezyk, 1995).

As is the case with other metals, cadmium also participates very little or not at all in the direct metabolic exchange, but is bound to various biological components, such as proteins, thiol (-SH) groups and anion groups of various macromolecules. The basis of the toxicity of cadmium is its negative influence on the enzyme system of the cells, due to the exchange of other metal ions (mostly Zn^{2+} and Cu^{2+}) (Kaličanin, 2006).

The toxic effect of cadmium to a significant extent depends on nutritive factors. A protein-free diet with insufficient amounts of calcium, vitamin D along with a zinc, manganese, copper and selenium deficiency in the body increases while vitamins C and E reduce the toxicity of cadmium. (Deng et al., 2004).

4. Experimental conditions for the determination of Cu, Zn, Pb and Cd by using the PSA

As a carrier of the layered mercury electrode, during PSA, a disc electrode made of vitreous carbon was used in a standard electrochemical cell (Suturović, 2003, Kaličanin, 2006).

In all of the analyses, the layered mercury electrode was created from a special solution of mercury(II)-ions (with a content of 100 μg/dm^3), which was made acidic y means of hydrochloric acid (pH ~ 1.65). The mercury was deposited by means of electrolysis at to a constant electrical current of –50 μA, for a period of 240 s. The thickness of the level of mercury formed in this way was approximately 130 nm.

The initial volume of all of the analyzed solution models and samples was constant (25 cm^3). Hydrochloride acid was used as an auxiliary electrolyte, due to the fact that HCl is suitable for de-complexing most of the metals in real samples (Kaličanin, 2001a). In some cases 4 % CH_3COOH was used as the auxiliary electrolyte, which is at the same time in some of the experiments it was used for the extraction of Cu, Zn, Pb and Cd from various samples.

In order to determine the content of the soluble metals (Pb, Cu, Zn and Cd) the simplest modification of the PSA was used, the PSA with oxygen as the oxidizing agent.

4.1 Optimization of the conditions for the PSA of Cu, Zn, Pb and Cd

PSA as an exceptionally sensitive and selective microanalytic technique enables us to concurrently determine a large number of elements. Nevertheless, considering the fact that in the samples we determined the contents of Cu, Zn, Pb and Cd, due to the possible interferences in the PSA as a result of the formation of intermetal compounds of Cu-Zn, Cu and Pb from the same solution were usually determined together, while Zn and Cd were determined together in a different series of analyses, with the addition of a complexing agent, most often galium, which would form complexes with the Cu and enable the unhindered determination of Zn.

For the PSA of the cited metals, optimization was carried out, and the most optimum values of the parameters of analysis were selected: the potential of the electrolysis, the duration of the electrolysis, and solution stirring speed. The value of the pH model solution and samples ranged around 2.6.

The optimized experimental conditions for determining Cu, Pb, Cd and Zn are shown in Table 2. The duration of the electrolysis depends on the contents of the determined metal in the studied analyte and varied from 60 to 600, that is 900 s.

Parameters	Pb, Cu	Cd , Zn
Electrolysis potential Ag/AgCl/KCl (3.5 mol/dm^3) (V)	-0.962	-1.522
Final potential Ag/AgCl/KCl (3.5 mol/dm^3) (V)	0	-0.15
Sample volume (dm^3)	0.025	0.025
Duration of the break (s)	15	15
Solution mixing speed (rpm)	4000	4000
Electrolysis duration (s)	60-600	60-900

Table 2. The conditions for determining Pb, Cu, Cd and Zn with the help of PSA

4.2 The linearity of the analytical signal in the PSA of PSA Cu, Zn, Pb and Cd

After the optimization of the determination conditions, both the linearity and the reproducibility of the analytical signal were defined.

The linearity of the analytical signal of each of the heavy metals (Cu, Zn, Pb and Cd) was studied on model dilutions of specific mass concentrations.

Under the defined optimum values of the electrolysis potential (-0.702 V for Cu, -1.31 V for Zn, -0.962 V for Pb i –1.10 V for Cd), the stirring speed (4000 min^{-1}) and the duration of the electrolysis (300 s, 480 s, 360 s i 600 s), the linearity of the analytical signal in the PSA was studied for the cited metals.

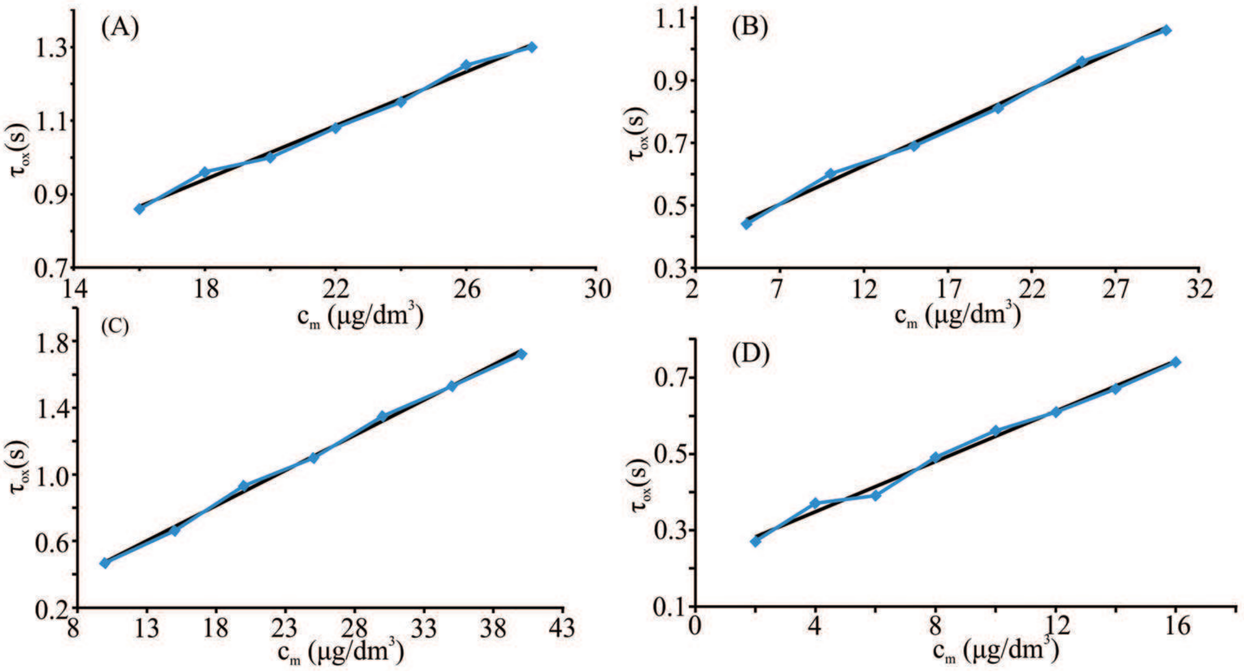

Fig. 2. The linearity of the analytical signal in PSA: A) Cu; $\tau_{ox} = 0.2804 + 0.0366 \cdot c_m$; r = 0.9964; B) Zn; $\tau_{ox} = 0.33 + 0.0246 \cdot c_m$; r = 0.9976; C) Pb; $\tau_{ox} = 0.0532 + 0.0422 \cdot c_m$; r = 0.9987; D) Cd; $\tau_{ox} = 0.2168 + 0.0329 \cdot c_m$; r = 0.9957

Using the method of the smallest squares , for each of the studied metals, the values were calculated for the intercept size (a), the direction coefficient (b) and correlation coefficient (r) in the linear function $\tau_{ox} = a + b \cdot c_m$. The results of the study are given in figure 2, as the dependence of the duration of oxidation in the function of the concentration.

5. Application of PSA for the determination of Cu, Zn, Pb and Cd in different samples

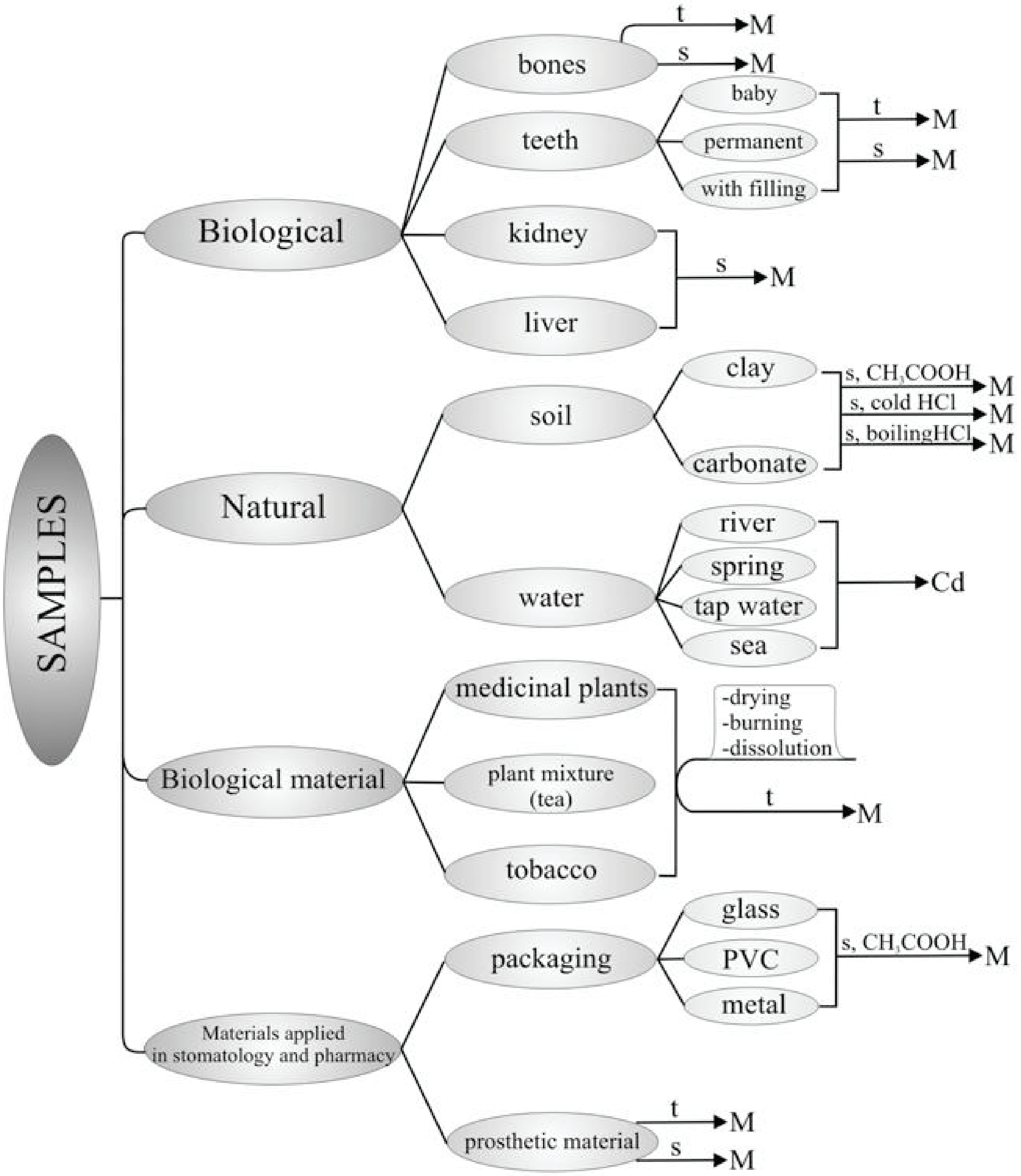

Fig. 3. The experimental design of the PSA analysis of heavy metals in various sample types: M – Cu, Zn, Pb and Cd; t- the total content of M in a sample dissolved in HNO_3, HCl and H_2O, at 130°C for a period of 2 hours; s – the content of soluble metals in the given mediums

5.1 Determining the content of Cu, Zn and Pb in natural human teeth

The overall content of Cu, Zn and Pb was determined in baby teeth, permanent intact teeth and teeth from which the amalgam filling, which had been a part of the teeth for years, had been mechanically extracted. These teeth were also used to determine the content of metal which is leached in a 4 % CH_3COOH solution, over a period of 24 hours.

Metals	Content (µg/g)	Sample		
		Permanent tooth	Baby tooth	Tooth with removed filling
Cu	t*	1.44 - 3.20	2.75 - 4.07	18.20 - 87.26
	s*	1.01 - 2.27	2.50 - 3.76	11.60 - 66.27
Cu leached	(%)	70.14- 73.93	87.74 – 92.38	53.72 – 75.94
Zn	t*	116.16 - 153.20	128.50 - 142.32	1505.20 - 5684.74
	s*	63.70 - 84.20	67.53 - 72.23	565.64 - 919.92
Zn leached	(%)	54.84 – 58.27	50.75 – 52.55	11.38 – 37.58
Pb	t*	2.20 - 5.41	1.52 - 2.17	2.70 - 6.73
	s*	0.82 - 1.99	0.97 - 1.40	1.52 - 3.90
Pb leached	(%)	35.26 – 37.27	63.81 – 64.52	56.13 – 57.94

Table 3. The overall and diluted content of copper, zinc and lead from human teeth

Over time heavy metals accumulate in the mineral tissue of the teeth, including Cu ranging from 1 to do 4 µg/g, Pb ranging from 1.5 to do 5.5 µg/g and Zn ranging from approximately 110 to do 150 µg/g. From the teeth which had an amalgam filling, a significantly higher content of the cited metals was noted. It was determined that the mobility of these metals in the 4 % CH_3COOH, over a period of 24 is almost identical to that in the permanent and baby teeth in the case of zinc, while there is significantly more copper and lead in the baby teeth. The greater mobility of lead from these teeth is probably a consequence of imperfect structure, low hardness and the frequent occurrence of caries (Kaličanin & Nikolić, 2008, 2010; Kaličanin et al., 2003, 2004; Nikolić et al., 2004).

The lead ions are, due to their size (r = 0.132 nm), more dominant over the smaller ions, and from the surface of the dentin, where they arrive from food, water, air and amalgam fillings, they transfer to an acidic solution, which increases their migration (Oehme et al., 1978; Gulson & Gillings, 1997).

5.1.1 The release of toxic metals from teeth under the influence of soft drinks

In today's day and age more and more refreshing soft drinks and energy drinks are being consumed by the younger population. Most of them contain carbon-dioxide, artificial sweeteners, preservatives and other additives and supplements, which enhance their taste. Frequent and constant consumption of these drinks, as well as of sour-tasting food, can have harmful effects on the mineral structure of teeth (Cairns et al., 2002; Attin et al., 2003; Ehlen et al., 2008). This analysis was carried out with the aim of identifying the effects of consuming sour-tasting food and refreshing drinks on the bone tissue of teeth among teenagers. The cumulative effect of a year-long exposure of teeth bone tissue to the erosive effects of a model system of acidic mediums (citric acid, lactic acid, acetic acid, apple vinegar, lemonade, the soft drink Sprite, mineral water) was studied. The effects were registered by means of the amount of released biometal ions, of zinc and copper, and toxic lead, during a period of 24 hours at room temperature, using the potentiometric stripping analysis.

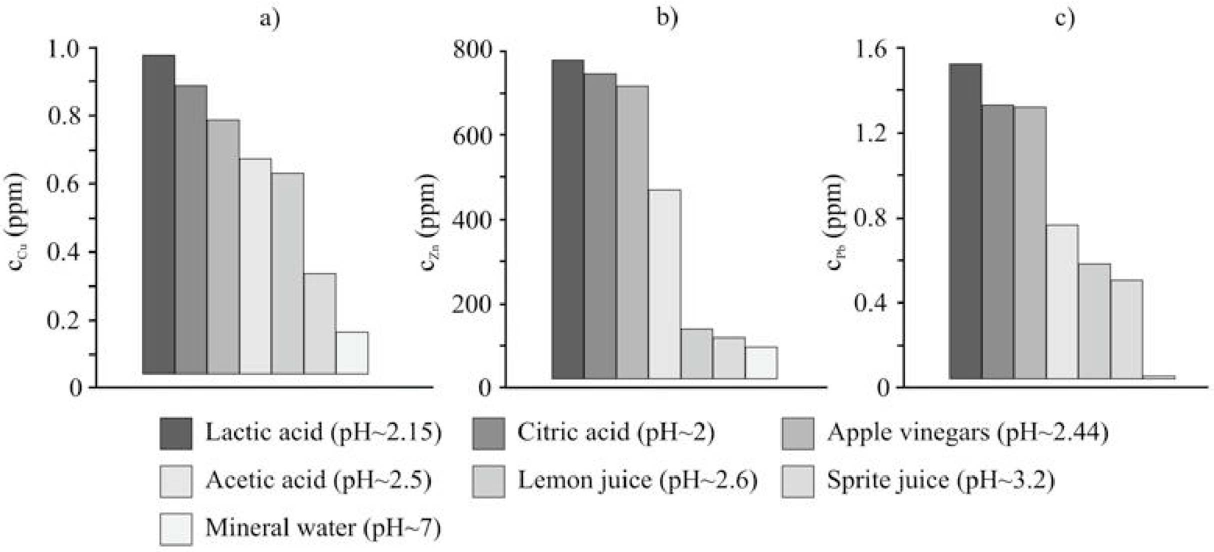

Fig. 4. The graphic dependence of the contents of released a) copper; b) zinc and c) lead (in ppm) depending on the studied system

In the given time span, 75 to 750 ppm of zinc, from 0.1 to 1.0 ppm of copper, and up to 1.5 ppm of lead was released from the dental matrix, while the release of cadmium was below the level of detection.

5.2 The application of the PSA in the analysis of pathohystological material (the kidneys and the liver)

The PSA method has been used to monitor the toxic effect of cadmium and lead from prosthetic implants, to which these metals have been added during their manufacture (5 μg/g of body weight), in the experiment model involving rats of the Spague Dolly kind, all of them female, 6-8 weeks old (Kaličanin et al., 2008). The content of Pb and Cd was determined in the kidney tissue and liver tissue, after a period 12 and 24 weeks during the experiment.

Sample	Content of Pb (μg/g)		Content of Cd (μg/g)	
	12 weeks	24 weeks	12 weeks	24 weeks
Kidneys	27.52	48.06	11.50	30.60
Liver	5.06	10.30	1.70	2.50

Table 4. The content of Pb and Cd in the kidneys and livers of the rats

Our results indicated that lead content is higher in the kidneys than in the, liver which corresponds to the results obtained by other authors, by means of other analysis techniques.

5.3 The use of the PSA in the analysis of soil for heavy metal content

As part of the Earth's crust, we find many silicate minerals which contain Al, Fe, Ca, Mg, K, Na, and Ti. Elements such as Zn and Cu are not present to such an extent. Nevertheless, toxic metals such as Pb and Cd both in their mineral form and as "additions" of silicate can also be found.

The results of the PSA analysis of the contents (mg/kg) of toxic metals Pb, Cd, Zn and Cu in various phases of demineralization of the soil samples, show that they primarily contain kaolinite as their main component, $Al_2(Si_2O_5)(OH)_4$, as shown in Table 5.

Metal	I (CH_3COOH)	II (HCl, cold)	III (HCl, boiling)	Average soil sample content
Pb	4.20	1.14	2.20	0.5-30
Cd	-	4.57	5.15	<1
Zn	-	3.24	4.60	10-300
Cu	-	17.21	20.13	2-10

Table 5. The mean values of the toxic metal content in naturaly occurring kaolinite

Lead can be found in the soil in the form of carbonite, phosphate or sulphate. It is easily adsorbed to other minerals and so its mobility is relatively small. When the soil becomes more acidic, Pb is released and then becomes available to the plants (Kaličanin et al., 2005; Mendil et al., 2009).

The increased presence of cadmium in the soil occurs due to the use of phosphate fertilizer, and fungicides. It is found in motor oil and tires, which leads to the increased content in the soil near roads and landfills. The geo-chemical relation between Cd and Zn leads to both of these elements being present in the soil at the same time. Zinc actually has a positive effect on cadmium absorption.

Zinc can be found in the soil in approximately 10-300 ppm, depending on the soil composition, pH values, and the presence of other metal ions. The Zn^{2+} ion is easily adsorbed onto the clay minerals, but also onto organic matter (Premović et al., 2001). Cadmium builds complexes well with ligand anions, which suits its solubility and higher mobility in the environment (Anđelković et al., 2006).

Copper can be found in the soil in very large extents (2-100 ppm). It is not a very mobile ion in the soil because it forms very good complexes with organic matter. High levels of copper in the soil can cause disturbances in the absorption of other microelements (Fe, Mo and Zn). What also has an effect on the toxicity and tolerance to copper, in addition to its content, is also the presence of organic matter, the level of phosphates in the soil and pH values.

5.4 Using PSA to analyze water

A potentiometric stripping method for a direct measurement of cadmium in various natural water samples using a Stripping analyzer M1 unit has been developed (Jagner et al., 1981). In order to ascertain optimum conditions for the determination of the effect of the mercury time electro-deposition, the electrolysis potential and the solution stirring rate of the cadmium analytical signal have been examined. Linearity of the cadmium analytical signal was achieved within the wide range of the mass concentrations, from 1 $\mu g/dm^3$ to 15 $\mu g/dm^3$. A detection limit of 0.10 $\mu g/dm^3$ was obtained, with a reproducibility of 3.55% expressed as the coefficient of variation. The accuracy of the method was confirmed by parallel analyses by flameless atomic absorption spectrophotometry as the reference method (Kaličanin, 2009).

Source of water	Content of cadmium ($\mu g/dm^3$)		
	Calibration curve method (min-max)	Standard addition method (min-max)	FAAS (min-max)
Tap	0.12 - 0.36	0.13 - 0.38	0.11 - 0.38
Mineral	0.15 - 0.21	0.17 - 0.20	0.14 - 0.23
River	1.25 - 3.52	1.31 - 3.60	1.37 - 3.60
Sea	6.67 - 13.25	6.80 - 13.60	6.50 - 12.95

Table 6. Cadmium contents in the water samples determined by PSA and FAAS methods

The results of the comparative analysis showed a very good agreement between the PSA and the FAAS methods. The best results agreement was achieved with the samples with micro cadmium contents as achieved, thanks to the great sensitivity of the given method in analyzing the cadmium in the water samples. On the basis of the values of the coefficient of variation, it can be concluded that it is possible to apply the calibration curve method for obtaining more reproducible values than by the standard addition method where the deviations are somewhat greater. Hence, the calibration curve method is proposed as the standard method for the determination of cadmium in the various natural water samples.

5.5 Determining Cu, Pb and Cd in herbs and herbal tea mixtures

Mineral matter has several roles in the development and growth of plants. Microelements take part in the metabolism of plants, have an effect in very small concentrations, in a highly specific manner and in certain phases of growth and development. Cu and Zn are necessary microelements for certain metabolic processes. Nevertheless, in higher concentrations, irrespective of the physiological role, they have a toxic effect. The heavy metal content of Pb, Cd, Cr and others in herbs can be an indicator of the contamination of the area in which they are growing. The accumulation of heavy metals in certain parts depends on the plant life, mobility of the metal, soil composition, pH values, precipitation, and the presence of other ions and molecule types in the soil.

We analyzed the plan species *Thymus serpyllumm,* in order to determine its Cu, Pb and Cd content. This plant is used to make tea, and as an aromatic spice in aromatherapy (Jeftović, 2001).

Location	Pb		Cd		Cu	
	t	s	t	s	t	s
I	6.62	6.60	0.42	0.40	3.20	3.18
II	15.80	14.90	1.06	1.04	3.80	3.60
III	2.42	2.50	0.25	0.30	1.06	1.10
IV	0.84	1.00	0.06	<0.50	0.84	0.80

Table 7. The toxic metal content in the plant species *Thymus serpyllumm* from various location in the vicinity of Niš (μg/kg). I – a location near a well-known local landfill; II – a meadow off the Niš-Belgrade highway; III – a mountain meadow; IV – a field in the mountain

Our findings showed especially high levels of lead in the flower of the plants which grew 20-30 m off the highway (15.80 ppm) and near the local landfill where waste is burned

(6.60 ppm). In the soil, lead turns into relatively soluble compounds, carbonate and phosphate, from which it is released due to acidification. When present in higher concentrations it causes numerous physiological, anatomic, morphological and chemical changes (Deng et al., 2004).

Cd reaches plants through their roots, but also from the air and above-ground parts of the plant. Its ability to form complexes with Cl^- and OH^- ions also contributes to this, which leads to greater mobility in the environment and increases the possibility of altering adsorption to cations (Ca^{2+} and Zn^{2+}). The increased level of Cd in the plants which were collected near the landfill where waste is burned and next to the highway is probably the result of accumulation which was made possible through the above ground parts of the plants.

The content of copper in the flower of the plant *Thymus serpyllumm* is within the normal limits for herbaceous annual plants and vegetables. Copper can be found in the soil up to 20mg/kg, but it is not a very mobile cation, so it is easily bonded to clay minerals, adsorbed, to form complexes and so is not readily available to plants and so is more frequent in the soil.

The results of the determination of the overall content of lead in commercial plant drugs, as well as the content of lead in teas prepared according to the recommendations of the manufacturers, by means of the PSA are shown in Table 8.

Sample	Content of Pb (μg/g)		Pb leached (%)
	t	s	
Chamomillae flos	0.73-0.81	0.32-0.35	43.20-44.00
Senae folium	1.21-1.64	0.58-0.74	45.12-47.93
Theae folium	1.74-2.37	0.27-0.39	14.28-16.46
Menthae folium	1.18-1.34	0.42-0.48	35.59-36.59
Uvae ursi folium	0.92-1.13	0.27-0.30	25.66-29.34

Table 8. The overall and soluble contents of Pb in herbal drugs and tea mixtures

The obtained results indicate that plant-based drugs contain a certain amount of lead, but that the obtained contents are within the limits prescribed for this metal. The content of lead which is released from the plant-based drug into the tea was 3 to 5 times lower than the overall content of this metal. The smallest percentage of the leached lead was found in green tea, which indicates that the migration of lead from this plant-based drug into the tea is the smallest. The lead content in teas depends on how they are prepared and is higher in teas which are prepared as a decoction, and the lowest in those prepared as macerate.

5.6 Using the PSA in the quality control of glass packaging for the food and pharmaceutical industry

Glass is a high quality packaging material, which is used in the food and pharmaceutical industry. Packaging, in addition to its basic components, can also contain metals (lead, zinc) as pollutants or as components used to achieve higher quality packaging. Considering the fact that products over a longer period of time, from packaging to use, are in contact with the packaging material, there is the possibility of ion metal migration from the packaging into the product. The international standard (ISO 7086/2) prescribes that the content of the

leached lead from glass packaging cannot exceed the prescribed limits of 5 mg/dm^3, for small hollow glass and 2.5 mg/dm^3, for large glass, under prescribed conditions.
Table 9 compares the results from a measuring of the contents of soluble lead from glass packaging for the food industry, under prescribed conditions, using different stripping analysis techniques and the AAS technique, as the referential technique prescribed by the standard (Kaličanin et al., 2001a, 2001b, 2001c; 2002).

Sample	C_{Pb} (µg/dm^3)		
	Analytical methods		
	PSA	PSA-i_R	AAS
Bottle for fruit juice, 1 dm^3 of volume, colorless	2.20	2.30	1.80
Jar, 1.5 dm^3 of volume, colorless	2.80	2.91	1.80
Jar, 0.72 dm^3 of volume, colorless	1.70	1.82	0.80
Bottle for strong alcoholic drinks, 0.7 dm^3 of volume, of green color	2.70	2.86	2.80

Table 9. Lead contents in the glassware for the food industry extracts by applying the PSA, PSA-i_R (potentiometric stripping analysis with constant inverse current in the analytic step) and AAS

These results indicate that there is proper agreement between the contents obtained through the stripping techniques and AAS technique as the referential one.
Figure 5, shows the content of the leached Pb from various packaging material, which is used in the pharmaceutical industry, during a period of 1, 5 and 7 days. Most of the lead is leached from glass packaging of brown color and of greater volume. According to our research, plastic packaging is more durable to the effects of an acidic medium.

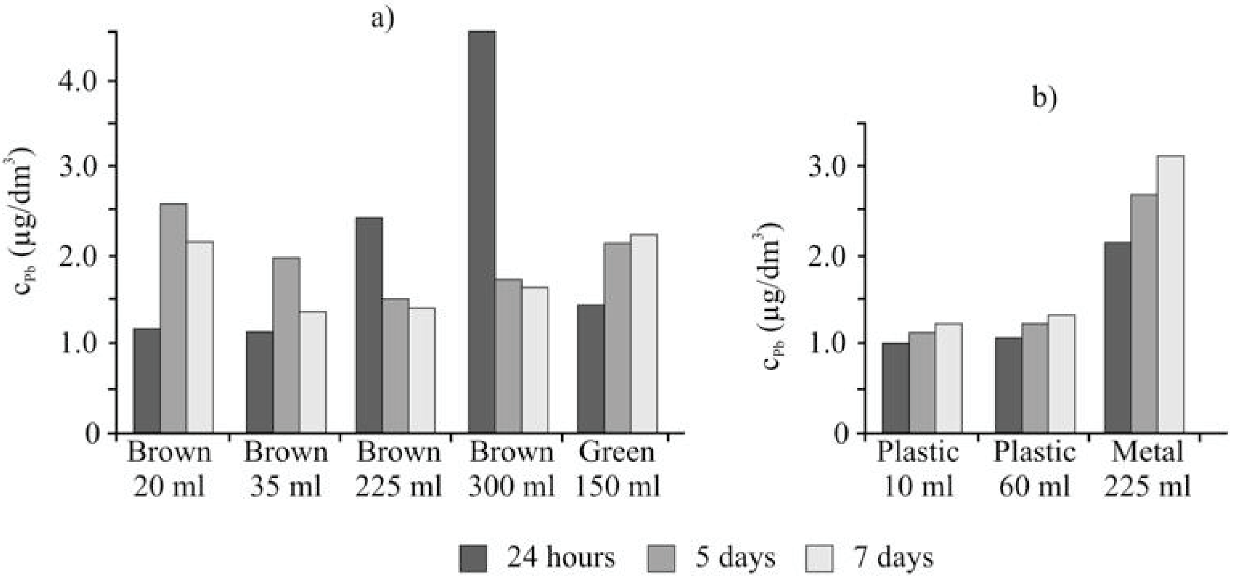

Fig. 5. The content of leached lead from packaging for the pharmaceutical industry a) glassware b) plastic and metal, depending on the duration exposure, volume and color of the packaging

5.7 The use of the PSA in the quality control of dental-prosthetic material

Dental-prosthetic material is very pure material of varying compositions. The same materials can consist of toxic heavy metals (Pb, Cd, Zn, Cu) which can be released under the influence of the corrosive effect of the oral medium or food with a high acidic taste (O'Brien, 2002). During the production phase of prosthetic implants, due to various physical-chemical processes, they are transformed into more stable units, they become less mobile, so that the finished product (metalceramic crown) limited release (Kaličanin & Ajduković, 2008; Kaličanin et al., 2007; Nikolić et al., 2001; Kaličanin & Nikolić, 2008, 2010).The results shown in table 10 indicate that these materials also contain Cu, Zn, Pb and that their traces can also be determined by means of the PSA technique.

Sample	Cu	RSD (%)	Zn	RSD (%)	Pb	RSD (%)
Dental ceramic	1.98	3.87	1.20	2.83	104.50	1.30
Ceramic color	2.05	1.93	0 .55	4.70	1.25	3.30
Cast alloys	2.60	2.14	215.95	4.39	0.33	10.60
Metal-ceramic crown	3.40	3.13	6.30	7.15	0.65	4.80
Zinc-phosphate cement	1.20	2.80	-	-	2.65	1.90
Glass- ionomer cement	0.76	1.50	1.98	2.15	0.35	1.80
Acrylic materials	n.d.	-	53.05	12.50	4.50	3.80
Hydroxyapatite	33.05	8.50	116.10	15.50	5.70	3.50

Table 10. The content (μg/g) of released copper, zinc and lead from various dental prosthetic materials under the effect of 4% acetic acid, over a period of 24 hours

6. Conclusion

The potentiometric stripping analysis is a highly sensitive and highly selective instrumental microanalytic technique for the quantitative determination of the metal ions. This technique can be used to determine low contents of heavy metals (Cu, Zn) and highly toxic metals (Pb and Cd) in samples of various origins Lead can be determined up to levels of 0.65 μg/dm^3, and cadmium up to 0.10 μg/dm^3, under the prescribed optimal conditions.

PSA fulfills very strict general and specific microanalytic demands:

- High sensitivity and proper analytical selectivity
- The possibility of determining a large number of elements at the same time
- The possibility of the unlimited re-analyses of the same solution
- The relatively small instrumentation and the possibility of “on-the-spot” analyses
- Lower cost of the instrumentation and exploitation in relation to other techniques.

The results of the determination of the content of Cu, Zn, Pb and Cd in the samples of bioinorganic and similar origin, have shown that the PSA technique with oxygen as an oxidant, as the simplest modification that can be done to this technique, can be used in the analysis and quality control of various samples with success. This technique can also be used to analyze:

- Clinical-biological material (mineral and soft tissue *in vivo* and *in vitro* analyses)
- Samples significant in quality control of the environment (water, soil)

- Plant material samples (herbs and aromatic plants, tea mixtures and spices)
- Packaging material and packaging for food and pharmaceutical products (glass, ceramics, plastic, metal)
- Highly pure bioinorganic material (dental-prosthetic materials)
- Beauty products.

7. Acknowledgment

Some results presented here are part of projects N° 45017 and 41018, which have been realized with partial financial support of the Republic of Serbia Ministry of Science and Environmental Protection.
We would also like to thank Marta Dimitrijevic for translating the original paper from Serbian into English.

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Wide Spectra of Quality Control
Edited by Dr. Isin Akyar

ISBN 978-953-307-683-6
Hard cover, 532 pages
Publisher InTech

Published in print edition July, 2011

Quality control is a standard which certainly has become a style of living. With the improvement of technology every day, we meet new and complicated devices and methods in different fields. Quality control explains the directed use of testing to measure the achievement of a specific standard. It is the process, procedures and authority used to accept or reject all components, drug product containers, closures, in-process materials, packaging material, labeling and drug products, and the authority to review production records to assure that no errors have occurred.The quality which is supposed to be achieved is not a concept which can be controlled by easy, numerical or other means, but it is the control over the intrinsic quality of a test facility and its studies. The aim of this book is to share useful and practical knowledge about quality control in several fields with the people who want to improve their knowledge.

Biljana Kaličanin and Ružica Nikolić (2011). The Application of the Potentiometric Stripping Analysis to Determine Traces of M(II) Metals (Cu, Zn, Pb and Cd) in Bioinorganic and Similar Materials, Wide Spectra of Quality Control, Dr. Isin Akyar (Ed.), ISBN: 978-953-307-683-6, InTech, Available from: http://www.intechopen.com/books/wide-spectra-of-quality-control/the-application-of-the-potentiometric-stripping-analysis-to-determine-traces-of-m-ii-metals-cu-zn-pb

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