ANALYSES OF SOME MICROELEMENTS IN THE TISSUES OF *Proteus anguinus* (Amphibia, Caudata) AND IN ITS HABITAT

ANALIZE NEKATERIH MIKROELEMENTOV V TKIVIH MOČERILA (*Proteus anguinus,* Amphibia, Caudata) IN V NJEGOVEM HABITATU

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Boris Bulog: Analize nekaterih mikroelementov v tkivih močerila (*Proteus anguinus*, Amphibia, Caudata) in v njegovem habitatu

Članek predstavlja preliminarne ekološke raziskave na endemični jamski dvoživki. V dosedanjih analizah so določali koncentracije As, Cu, Zn, Hg, Cd in Se v tkivih in koncentracije Cu, Zn, Hg, As in Cd v vodi in sedimentih v Planinski jami. Koncentracije Hg v rečnih sedimentih so štirikrat manjše kot v tkivih. Hg v tkivih močerila ni dosegel vrednosti pri drugih dvoživkah iz nekontaminiranih habitatov. Močeril bi lahko akumuliral znatne količine posameznih mikroelementov, če upoštevamo dolgo življenjsko dobo, v onesnaženih vodah pa celo letalne doze.

Ključne besede: biologija, speleobiologija, jamska favna, Amphibia, Proteus anguinus, mikroelementi

Abstract: UDC 574.2:597.9(497.4)

Boris Bulog: Analyses of some microelements in the tissues of *Proteus anguinus* (Amphibia, Caudata) and in its habitat

This article presents preliminary ecological studies of endemic cave salamander *Proteus anguinus*. Recently we also determined the concentrations of As, Cu, Zn, Hg, Cd and Se in the tissues and the concentrations of Cu, Zn, Hg, As and Cd in the water and sediments in the Planina Cave. The concentrations of Hg in river sediments are four times smaller than in their tissues. Hg in tissues of Proteus did not reach the values of other amphibians from the uncontaminated habitats. Owing to its long life span, Proteus could accumulate a considerable quantity of the individual microelementss and in polluted waters even a lethal dose of these elements.

Key words: biology, speleobiology, cave fauna, Amphibia, Prouteus anguinus, microelements

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INTRODUCTION

Proteus anguinus is the only species of the European cave salamander and the most famous troglobiont of our underground waters in the Dinaric Karst. Christiansen introduced the phrase troglomorphy to specify those phenotypic features which were typical for cave animal evolution and served to distinguish a cave adapted organisms (Christiansen 1992). A large part of the Slovenian territory situated between the Ljubljana Marsh and the Adriatic sea is a classical Karstic area. Caves and underground rivers are features of the karst topography. It is worth mentioning that three thousands caves exist in this area, of considerable geographic and biological interest (Cave register).

The purpose of this paper is to present our recent ecological studies of the endemic cave salamander and, particularly, to report on the preliminary studies of accumulation of the individual metals and other microelements as potential toxic substances in its tissues. The water resources in the Karst area are extremely sensitive to all kind of pollution. Our Karstic areas have relatively rare sources of useful water. During the dry seasons the surface streams may often disappear completely and the underground waters may be limited or not quite accessible. Consequences of this may be also higher concentrations of pollutants in the underground waters. Self purification processes in the underground waters are not clear enough and are quite different from these in the surface waters (Sket and Velkovrh 1981).

Among the most serious chemical pollutants are the chlorinated hydrocarbon pesticides (DDT), aldrin, and dieldrin; the polychlorinated biphenyls (PCBs), which are used in a variety of industrial processes and in the manufacture of many kinds of materials; and such metals as mercury, lead, cadmium, arsenic, and beryllium. All of these substances persist in the environment, being slowly, if at all, degraded by natural processes; in addition, all are toxic to life if they accumulate in any substantial quantity. Minerals essential for animal life include common salt (sodium chloride), calcium, phosphorus, sulfur, potassium, magnesium, manganese, iron, copper, cobalt, iodine, zinc, molybdenum, and selenium. The last six of these are toxic to animals if excessive amounts are eaten.

The ultimate control of pollution will presumably involve the decision not to allow the escape into the environment of the substances that are harmful to life, the decision to contain and recycle those substances that could be harmful if released into the environment in excessive quantities, and the decision not to release into the environment substances that persist and are toxic to living things. Essentially, therefore, pollution control does not mean an abandonment of existing productive human activities but their reordering so as to guarantee that their side effects do not outweigh their advantages.

MATERIALS AND METHODS

We used a small number of specimens for our studies owing to the very strict enforcement of natural conservation laws. We established these studies on ten specimens that we captured in the Pivka branch of the Planina Cave near the sampling point three and four. Animal tissue metal contents were measured by neutron activation analysis. Planina Cave waters and sediments have been sampled since 1994 on sampling points one and two (Rak branch) and on sampling points three and four (Pivka branch).

Metal levels in water and sediment samples were determined by one of the following methods: neutron activation analysis, cold vapour atomic absorption spectrometry, atomic fluorescence spectrometry, or X-ray fluorescence (Byrne and Kosta 1974, Horvat et al. 1989, Fajgelj 1993, Wobrauschek 1993). Metal contents have been determined on Institute "Jožef Stefan" - The laboratory for the environmental chemistry.

RESULTS

We have established the contents of the copper, zinc, arsenic, selenium cadmium, and mercury in the liver, kidneys, integument, and muscles of Proteus. The metal contents of animal tissue were measured by neutron activation analysis. They were also established in the rivers Pivka and Rak, both streaming through the cave, and their sediments (Dermelj et al. 1984, Bulog in prep.).

Recent preliminary studies showed that the concentration of Hg in the individual tissues did not exceed a mean value of approximately 0.7 $\mu g/g$ of fresh tissue (Table 1.). The largest concentrations of mercury and arsenic were found in the liver of Proteus, a smaller one in muscle, and the smallest in the kidneys and the integument (Table 1, Figs. 1-4). The largest concentrations of Cd were established in the integument and the largest concentrations of Cu, Zn and Se were determined in the liver.

The concentrations of As, Sb, Cu, Zn, Cd, and Co in the river sediments exceed these in the tissues of Proteus. The concentrations of Hg in the river sediments are four times smaller than in tissues.

Table 1 presents the recent analyses of concentrations of six microelements in the tissues of Proteus and Table 2 the concentrations of five microelemnts in the water and sediment of its habitat.

	LIVER						MUSCLES						KIDNEY						INTEGUMENT					
	As	Cu	Zn	Hg	Cd	Se	As	Cu	Zn	Hg	Cd	Se	As	Cu	Zn	Hg	Cd	Se	As	Cu	Zn	Hg	Cd	Se
1	/	1.4	10.9	0.19	/	6.6	/	0.13	6.0	0.10	/	1.0	/	0.42	10.5	0.05	/	2.6	/	0.23	9.1	0.04		1.3
2	/	3.4	10.5	0.54	/	7.0	/	0.11	4.1	0.17	/	0.6	/	0.40	10.5	0.07	/	1.6	/	0.23	5.7	0.17		0.6
3	/	14.6	16.6	1.30	/	7.7	/	/	/	/	/	/	/	2.80	/	0.09	/	/	/	0.40	16.7	0.01		1.7
4	/	1.8	9.3	0.44	/	2.7	/	0.60	6.2	0.48	/	0.5	/	0.70	16.6	0.11	/	2.0	/	0.30	14.3	0.06		0.2
5	/	1.5	9.5	0.29	/	2.4	/	0.30	6.9	0.37	/	0.4	/	0.50	12.7	0.13	/	1.2	/	0.60	9.3	0.06		3.5
6	/	18.7	8.1	0.60	/	5.2	/	0.35	6.2	0.40	/	0.5	/	0.50	14.5	0.13	/	1.4	/	0.45	13.1	0.06		0.5
7	/	3.0	8.5	0.75	/	4.9	/	0.13	3.3	0.54	/	0.4	/	0.69	12.8	0.22	/	1.0	/	0.19	8.0	0.11		0.5
8	/	3.3	10.9	0.81	/	6.4	/	0.23	3.7	0.38	/	0.8	/	0.72	8.9	0.14	/	2.9	/	0.49	10.5	0.07		0.5
9	0.34	2.6	12.9	0.43	0.25	6.4	0.13	0.47	7.3	0.40	$<10^{-2}$*	0.6	0.05	0.77	15.6	0.06	$<10^{-2}$*	1.6	0.09	0.32	6.8	0.13	0.67	0.5
10	/	2.8	7.3	1.21	0.07	8.8	/	2.43	4.3	0.46	$<10^{-2}$*	0.7	/	/	/	0.20	/	4.2		0.15	7.6	0.09	$<10^{-2}$*	0.8
x	**0.34**	**5.31**	**10.5**	**0.66**	**0.16**	**5.81**	**0.13**	**0.53**	**5.33**	**0.37**	**$<10^{-2}$***	**0.61**	**0.05**	**0.83**	**12.8**	**0.12**	**$<10^{-2}$**	**2.06**	**0.09**	**0.34**	**10.1**	**0.08**	**<0.67**	**1.01**

*Table 1. Microelement concentrations in the organs of ten specimens of Proteus and their mean values, in μg/g and *ng/g of fresh tissue.*
*Tabela 1. Koncentracije mikroelementov v organih 10 primerkov močerila in njihove srednje vrednosti, v μg/g in *ng/g svežega tkiva.*

	Cu		Zn		Hg		As		Cd	
Sampling points in Planina Cave	**water**	**sediment**	**water**	**sediment**	**water**	**sediment**	**water**	**sediment**	**water**	**sediment**
1	1.35	36	6.08	107	0.27	0.11	0.13	9.5		0.65
2	1.44	41	8.40	140		0.16	0.14	11,3		0.64
3	2.23	28	13.70	100	0.29	0.04	0.12	6.5		0.31
4	2.27	20	14.80	76	0.29	0.03	0.15	4.3		0.12
x	**1.82**	**31.25**	**10.75**	**105.75**	**0.28**	**0.085**	**0,14**	**7.9**		**0.43**

Table 2. Microelement concentrations in water and sediments from the Planina Cave, in μg/l (in water) and in μg/g of dry sample (in sediments) and their mean values.
Tabela 2. Koncentracija mikroelementov v vodi in sedimentih Planinske jame, v μg/l (v vodi) in v μg/g suhe mase vzorca (v sedimentih) ter njihove srednje vrednosti.

Fig. 1. Microelements concentrations in the liver of ten specimens of *Proteus* and their mean values
Sl. 1. Koncentracije mikroelementov v jetrih desetih primerkov močerila in njihove srednje vrednosti

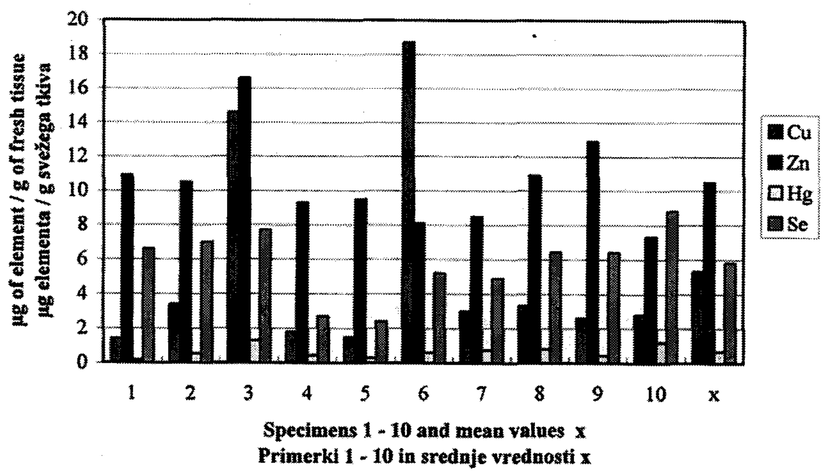


Fig. 2. Microelements concentrations in the muscles of ten specimens of *Proteus* and their mean values
Sl. 2. Koncentracije mikroelementov v mišicaah desetih primerkov močerila in njihove srednje vrednosti

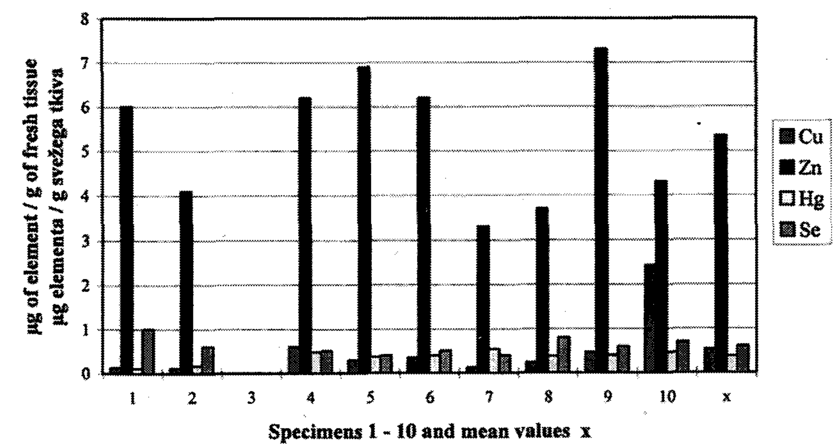
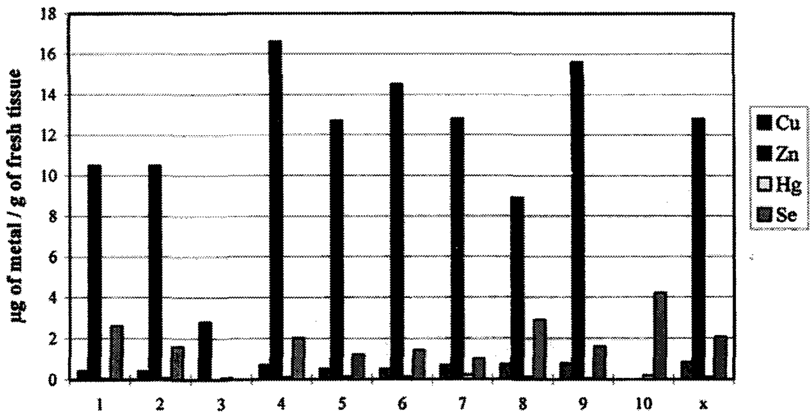
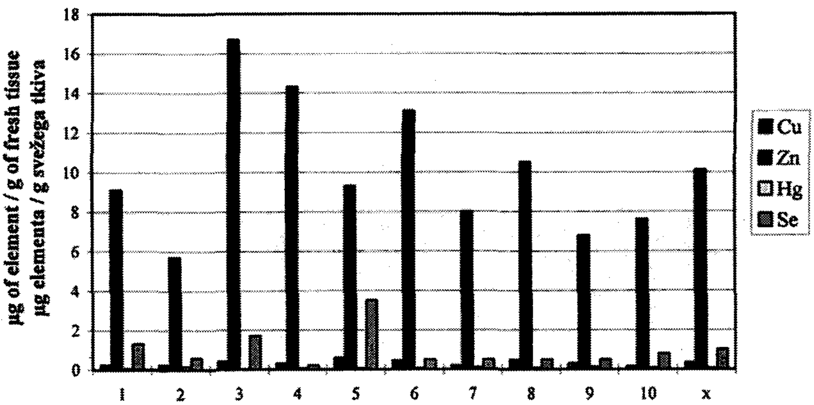


Fig. 3. Metals concentrations in the kidneys of ten specimens of *Proteus* and their mean values
Sl. 3. Koncentracije mikroelementov v ledvicah desetih primerkov močerila in njihove srednje vrednosti



Specimens 1 - 10 and mean values x

Fig. 4. Microelements concentrations in the integument of ten specimens of *Proteus* and their mean values
Sl. 4. Koncentracije mikroelementov v koži desetih primerkov močerila in njihove srednje vrednosti



Specimens 1 - 10 and mean values x
Primerki 1 - 10 in srednje vrednosti x

DISCUSSION

The possible pathways of metals through the body of Proteus are not known. We may expect the ingestion of metals: **1.**) With the consumption of the prey, sediment particles or with water consumption. **2.**) With the direct absorption from the surrounding water by the integument and through the gills. **3.**) With the absorption from the air during the lung respiration. The absorption of Hg in amphibians could take place with the food consumption through the skin and with the absorption through the air (Bryne et al. 1975). Simkiss and Taylor (1989) discussed about the cellular mechanisms of the metal obtainment in the aquatic animals. They supposed this may be passive process or connected with the active ionic channels for the transport of important ions. Further analyses in Proteus will deal with the control of accumulation of microelements, their uptake and transport through the animal.

The maximal end value of Hg concentrations in fishes recommended for their and our protection is 0.5 μg/g of fresh tissue (Saiki et al. 1992). The reproductive sterility in fishes was described in cases where the concentration of Se reached values > 12 μg/g of fresh tissue (Saiki et al. 1992).

Proteus' proportion of Hg in individual tissues was also found in other amphibians (Byrne et al. 1975). They measured the largest concentrations of Hg in the tissues of amphibians overall in uncontaminated waters in the liver (2 μg/g of fresh tissue), in the kidney (1.5 μg/g of fresh tissue), and in the muscles (0.5 μg/g of fresh tissue). The concentrations of Hg in tissues of amphibians in contaminated waters are much larger (about 20 μg/g of fresh tissue in the liver and kidney and 2-3 μg/g of fresh tissue in muscles and integument). Obviously, the concentrations of Hg in tissues of Proteus did not reach the values of other amphibians from the uncontaminated habitats. Preliminary studies suggest that the liver of Proteus accumulates the largest amounts of microelements and may be considered as the target organ (Table 1, Cijan 1994, Bulog 1994). Owing to its long life span, Proteus could accumulate a large quantity of the individual metals and in polluted waters even a lethal dose of these elements.

The comparison of metal concentrations in the river sediments with their natural values (after Turkeian and Wadepohtl 1961) showed that analysed microelements accumulate in the sediments of Planina Cave.

Certain modern industrial and biological processes concentrate mercury compounds to dangerous levels. Besides the danger from many consumer goods that contain potentially harmful levels of mercury, the air may be contaminated by mercury vapours, fumes, and dusts and the waters by effluent wastes containing mercury in various forms. The latter may then be converted by bacteria in the muddy sediments into organic mercurial, which may in turn be concentrated by the fishes, amphibians and other aquatic forms of life. The exact mechanism by which mercury enters the food chain remains largely unknown, and probably vary among ecosystems. We do know, however, that certain bacteria play an important early role. Studies have shown that bacteria that process sulfate in the environment take up mercury in its inorganic form, and through metabolic processes convert it to methylmercury. The conversion of inorganic mercury to methylmercury is important for two reasons: (1) methylmercury is much more toxic than inorganic mercury, and (2) organisms require considerably longer to eliminate methylmercury. At this point, the methylmercury-containing bacteria may be consumed by the next higher level in the food chain, or the bacteria may release the methylmercury to the water and then to the next level in the food chain (Miller, D.R. 1979). Depending on the type of mercury compound and the mode of contact, the symptoms of intoxication vary.

Arsenic is very widely distributed in the environment, and all animals are exposed to low levels of this element. For most people, food constitutes the largest source of arsenic intake (about 25 to 50 micrograms per day), with lower amounts coming from drinking water and air. Some edible fish and shellfish contain elevated levels of arsenic, but this is predominantly in an organic form that has low toxicity. Above-average levels of exposure are usually associated with one or more of the following situations: Arsenic is believed to exert its toxicity by combining with certain enzymes (the organic catalysts of the cell), thereby interfering with cellular metabolism. The amount of arsenic intake that is required to cause a harmful effect depends on the chemical and physical form of the arsenic. In general, inorganic forms of arsenic are more toxic than organic forms, and forms that dissolve easily in water (soluble forms of arsenic) tend to be more toxic than those that dissolve poorly in water. Also, toxicity depends somewhat on the electric charge (the oxidation state or valence) of the arsenic.

Zinc is an essential trace element in the vertebrate body, where it is found in high concentration in the red blood cells as an essential part of the enzyme carbonic anhydrase, which promotes many reactions relating to carbon dioxide metabolism. It is the component of numerous proteins. Zinc present in the pancreas may aid in the storage and excretion of insulin and other hormones (Hambridge et al. 1986). Zinc is a component of some enzymes that digest protein in the gastrointestinal tract. The toxicity of the metals increases sharply in the order zinc, cadmium, mercury. The toxicity of zinc is low. In drinking water zinc can be detected by taste only when it reaches a concentration of 15 parts per million (ppm); water containing 40 parts per million zinc has a definite metallic taste. Cases of fatal poisoning have resulted through the ingestion of zinc chloride or sulfide, but these are rare.

Compared with those of zinc, the toxic hazards of cadmium are quite high. It is soluble in the organic acids found in food and forms salts that are converted into cadmium chloride by the gastric juices. Even small quantities can cause poisoning, with the symptoms of increased salivation, persistent vomiting, abdominal pain, and diarrhea. Fatal cases have been reported. Cadmium has its most serious effect as a respiratory poison: a number of fatalities have resulted from breathing the fumes or dusts that arise when cadmium is heated. Symptoms are difficult or laboured breathing, a severe cough, and violent gastrointestinal disturbance.

Copper is important in synthesis of haemoglobin. It is the component of many enzymes. Excess of copper in the biological systems changes individual biochemical processes (Fajgelj, 1993). Wilson's disease, also called hepatolenticular degeneration is a hereditary defect associated with the metabolism of copper and characterized by the progressive degeneration of the basal ganglia of the brain, the development of a brownish ring at the margin of the cornea, and the gradual replacement of liver cells with fibrous tissue.

Among organic compounds, the most toxic are derivatives that contain the halogen elements (fluorine, chlorine, bromine and iodine), sulfur, selenium, tellurium, nitrogen, phosphorus, arsenic, lead, and mercury. Most organometallic compounds are toxic, while oxygen-containing derivatives of the hydrocarbons are usually less toxic.

In all biologic systems the dose of an added substance, including nutrients, determines the effect. The level at which the nutrient may exert toxic effects varies, and for some nutrients, such as vitamins A and D, iron, fluoride, selenium, and iodine, the level is much lower than for others.

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Povzetek

Kraški svet zavzema skoraj polovico ozemlja naše domovine, zaradi česar nosi Slovenija pečat ene najbolj kraških dežel na svetu. Močeril ali človeška ribica naseljuje podzemske vode Dinarskega krasa in je edini jamski vretenčar v Evropi. Razširjen je v podzemlju Dinarskega krasa od reke Isonzo-Soča v Italiji na severozahodu do reke Trebišnjica v Hercegovini na jugovzhodu. Ta neotenična dvoživka ohranja celo življenje nekatere larvalne znake v odraslem stanju. *Neotenija* je fenomen, pri katerem osebki dosežejo reproduktivno zrelost in ohranjajo zunanje znake ličinke. Po vsej verjetnosti pride do upočasnjenega razvoja somatičnih organov pri relativno normalni hitrosti dozorevanja spolnih organov. Pri rednih neotenih (*Proteus, Necturus* in *Amphiuma*) je razlog za nepovratno neotenijo v neobčutljivosti tkiv na tireoidne hormone.

V okviru naših ekoloških raziskav poteka tudi proučevanje kopičenja mikroelementov v naravnem okolju in tkivih močerila. Vodni viri na kraškem območju so zaradi specifičnosti zgradbe kraškega sveta izjemno občutljivi na enkratne in trajne oblike onesnaževanja. V sušnih obdobjih lahko površinski tokovi povsem presahnejo in ena izmed nevarnih posledic sušnosti je sorazmerno blago razredčenje odpadnih voda in povišane koncentracije škodljivih snovi. Samoočiščevalni procesi v podzemskih vodah niso povsem jasni in v večji meri tudi nepredvidljivi (Sket in Velkovrh 1981).

Namen prispevka je predvsem predstaviti predhodne analize kopičenja posameznih težkih kovin in drugih mikroelementov kot potencialno strupenih substanc v močerilovih tkivih. Vsebnost mikroelementov je bila merjena v podzemskih tokovih rek Pivka in Rak. in v rečnih sedimentih. Analizirana je bila vsebnost, bakra, cinka, arzena in živega srebra, v rečnih usedlinah pa še kadmija. Pri tem smo uporabili različne analitične metode (atomska absorpcijska spektrometrija hladnih par, nevtronska aktivacijska analiza, X-žarkovna fluorescenca).

Rezultati analiz vsebnosti kovin v vodah iz Planinske jame kažejo, da so njihovi koncentracijski nivoji pod maksimalnimi dovoljenimi koncentracijami (MDK - Pristov 1992). Izmerjene vrednosti v Pivki in Raku so dokaj izenačene, kljub temu, da ju napaja voda iz različnega hidrografskega zaledja. Rečne usedline kopičijo precejšnje količine mikroelementov zaradi adsorpcije, hidrolize,... in se z desorbcijo ponovno sproščajo v vodo. Primerjava koncentracij kovin v jamskih rečnih usedlinah z naravnimi vrednostmi le teh (po Turekian in Wedepohl 1961) kaže, da se analizirane kovine kopičijo v rečnih usedlinah. Kopičenje kovin v rečnih usedlinah iz Planinske jame je sicer precejšnje, vendar pa vsebnosti kovin niso tako visoke, da bi lahko govorili o močnem onesnaženju. Za realno sliko vsebnosti kovin v vodi in usedlinah bomo v naslednjih letih redno odvzemali vzorce.

V dosedanjih študijah je bila določena tudi vsebnost bakra, cinka, arzena, selena, kadmija in živega srebra v jetrih, ledvicah, koži in mišicah močerila (Tabela 1, Slike 1 - 4). Vsebnost teh mikroelementov je bila merjena z nevtronsko aktivacijsko analizo. Predhodni rezultati kažejo, da jetra kopičijo največjo količino mikroelementov (Bulog 1994).

Privzem kovin v telo močerila bi lahko potekal na več načinov: 1.) z zaužitjem plena, usedline ali vode, 2.) z absorbcijo direktno iz vodnega okolja skozi kožo in škrge in 3.) z absorbcijo iz zraka skozi pljuča in kožo. Privzem živega srebra pri dvoživkah naj bi potekal s konzumacijo hrane, preko kože in z zračno absorbcijo (Byrne et al. 1975). Upoštevajoč način prehranjevanja močerila lahko predvidevamo, da močeril sprejema precejšen del mikroelementov nakopičenih v rečnih sedimentih. Simkiss in Taylor (1989) razpravljata o načinih privzema kovin na celičnem nivoju pri vodnih organizmih in menita, da je v mnogih primerih privzem kovine v telo pasiven proces. Lahko pa je povezan z aktivnimi ionskimi črpalkami (npr. kadmij), ki služijo transportu pomembnih ionov (npr. za kalcij).

V nadaljnjih raziskavah nameravamo vključiti redno kontrolo kopičenja kovin v tkivih in naravnem okolju močerila, proučevanje možnih poti privzema v telo močerila, transporta, porazdelitve, biotransformacij in izločanja mikroelementov.