TRACE ELEMENT CONCENTRATIONS IN THE TISSUES OF *PROTEUS ANGUINUS* (AMPHIBIA, CAUDATA) AND THE SURROUNDING ENVIRONMENT

BORIS BULOG[1]*, KATARINA MIHAJL[1], ZVONKA JERAN[2]
and MIHAEL J. TOMAN[1]
[1] *Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, 1001 Ljubljana, Slovenia;* [2] *Department of Environmental Sciences, Jožef Stefan Institute, Jamova 39, 1000 Ljubljana, Slovenia*
(author for correspondence, e-mail: boris.bulog@uni-lj.si, fax: + 386 1 2573390)*

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Abstract. The concentrations of some essential (Cu, Zn, Se) and some toxic elements (Hg, As) were determined in tissues (liver, kidneys, integument, and muscle) of the endemic cave salamander, *Proteus anguinus* Laurenti 1768 and *Proteus anguinus parkelj* ssp.n. captured in the Planina Cave, Kompolje Cave, and Jelševnik, all situated within the Dinaric Karst in Slovenia. The highest amounts of selenium and mercury were found in the liver of *Proteus anguinus* specimens from all three localities. Some of the animals from the Planina Cave showed higher concentrations of copper in the liver and muscle. Pigmented subspecies of *Proteus* from Jelševnik showed increased values of arsenic in the integument and of zinc in the liver and integument. The liver of the animals contained the highest amounts of the metals analysed and therefore may be considered as a target organ. Metal levels were also measured in the cave water and sediments. The waters were not polluted with metals. However, the metals were relatively high in the sediments, but not to levels considered contaminated. In addition to our study of metal concentrations in *Proteus* tissues and habitats, several water quality parameters were measured in the water of each locality. The main differences between the three localities concerned dissolved oxygen concentration and saturation, while all other physical and chemical parameters were characteristic of the aquatic environment in the calcareous Karst region.

Keywords: Amphibia, cave habitat, karst, *Proteus anguinus*, tissues, trace elements

1. Introduction

Proteus anguinus is the sole species of the European Cave salamander and one of the most remarkable cave dwellers of the underground waters in the Dinaric Karst. Owing to its troglomorphic characteristics, especially the regressed eyes and depigmentation, *Proteus* is the world-famous model for troglobionts. Christiansen introduced the phrase troglomorphy to specify those phenotypic features that are typical of cave animal evolution and serve to distinguish cave-adapted organisms (Christiansen, 1992).

Proteus has an extended, narrow body and a reduced number of digits that is three on the anterior and two on the posterior legs. *Proteus* is an obligate neotene; the major reason for cessation of its metamorphosis, like in other obligate neotenes,

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probably lies in the insensitivity of its tissues to thyroid hormones. There is regression of its somatic development as compared with its normal sexual development. It retains neotenic characteristics throughout its life, for example, three pairs of outer gills, two pairs of gill slits, an integument with many larval characteristics, and typical visceral skeletal elements. Pigmented specimens of *Proteus* were discovered a few years ago and their gross morphological characteristics have been presented elsewhere (Istenič, 1987). The dark examples of *Proteus* diverge from the pigmentless specimens in their eyes and many other morphological differences (Bulog, 1993). The black, non-troglomorphic race has been attributed to a subspecies (Sket and Arntzen, 1994). However, allozyme analysis revealed the probability of speciation within this genus.

A large part of Slovenia, situated between the Ljubljana Marsh and the Adriatic Sea, is the classical Karst area. It is worth mentioning that almost seven thousands cave exist in this area. They are of considerable geographical and biological importance (Aljančič *et al.*, 1993). Water resources in the karst are extremely sensitive to all kinds of pollution. The contamination of karst underground waters is due to the large number of waste disposal sites leached by rainwater, as well as to the accidental overflow of various liquids (e.g. traffic accidents involving cisterns). The reflection of such pollution in the karst underground waters depends on the type and quantity of pollutants, and on the rock structure through which the waters penetrate (Kogovšek, 1995). The rocks control the course and velocity of infiltration and the potential for oxidation-degradation processes. These also control the self-purification of these waters. Self-purification processes in the underground waters are not completely understood, but they are quite different from those in surface waters (Sket and Velkovrh, 1981). During the dry seasons surface streams may often disappear completely and underground waters may be limited or inaccessible. As a consequence, there may be a higher concentration of pollutants.

Among the most serious chemical pollutants are chlorinated hydrocarbon pesticides, polychlorinated biphenyls (PCBs), and metals such as mercury, lead, cadmium, and arsenic. 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 appreciable quantity. Elements essential for animal life include sodium, calcium, phosphorus, sulphur, potassium, magnesium, manganese, iron, copper, cobalt, iodine, zinc, molybdenum, and selenium. The last six are poisonous to animals if excessive amounts are ingested.

Heavy metals are a crucial form of aquatic pollution because they are difficult to remove by any natural process. The effects of metals on aquatic organisms are difficult to determine as many physical and chemical properties contribute to the outcome (Chang and Cockerham, 1994).

Preliminary studies (Dermelj *et al.*, 1984; Bulog, 1994; Bulog, 1996; Bulog, 1997) on depigmented *Proteus anguinus* specimens from the Planina Cave (central part of Slovenia) showed that the highest amounts of mercury, arsenic, copper, selenium, and zinc were found in the liver of the animals. The concentrations of arsenic, copper, and zinc in the sediments of the rivers Pivka and Rak, both flowing through Planina Cave, were higher than in *Proteus* tissues, while mercury concentrations were approximately four times lower (Bulog, 1996).

The aim of the present study was to extend our research to two new localities of *Proteus*, the Kompolje Cave and Jelševnik, situated in the south-eastern part of Slovenia. In addition, we also made some new measurements of metal concentrations in the tissues of *Proteus* from the Planina Cave (central part of Slovenia) and in its underground habitat (water and sediments). We evaluate the concentrations of some essential (Cu, Zn, and Se) and some toxic (Hg and As) elements in the tissues of depigmented *Proteus anguinus* specimens from the Kompolje Cave, and the pigmented subspecies *Proteus anguinus parkelj* from Jelševnik and we compare them: (a) with elemental levels measured in the water and sediments of these two habitats, (b) with the results of our previous and present studies from the Planina Cave, and (c) with data on other vertebrates.

2. Materials and Methods

2.1. STUDY AREA AND SAMPLING

The study area comprised three localities where *Proteus anguinus* is found, all situated within the Dinaric Karst in Slovenia: (1) the Planina Cave at Planina, near Postojna; (2) the Kompolje Cave in the Dolenjska region, near Kompolje; (3) the springs at Jelševnik in the south-eastern part of Slovenia, near Črnomelj (Figure 1). At Jelševnik, there is a permanent limnocrene spring (called Jezero), and two associated groups of temporarily active ‘boiling holes’; the southern group of holes (called Jamnice) and the northern group of holes (called Na Trati) are about 150 m apart. Among the northern group of boiling holes there are two holes with permanent water, while all the other holes eject water only a few times a year after heavy rains (Sket, 1997). The Planina and Kompolje Cave are habitats of the depigmented *Proteus anguinus* (white subspecies), while at Jelševnik the dark pigmented *Proteus anguinus parkelj* was found (Sket and Arntzen, 1994; Sket, 1997).

Planina Cave waters and sediments were sampled in the rivers Pivka and Rak, both flowing through the Cave, while all the animals were captured in the Pivka river. Water samples at Jelševnik were collected in the two holes with permanent water (referred to as Na Trati 1 and Na Trati 2). Because no sediments were found in these two holes, sediment samples were collected in the Jezero spring, while both animals were captured in the hole referred to as Na Trati 2. The two holes and the Jezero spring are part of the same underground karst system and located close together so we can assume that the composition of the sediment is identical.

Water and sediment samples were collected several times during the study period in clean polypropylene bottles (water samples for Zn, Cu, and As determination

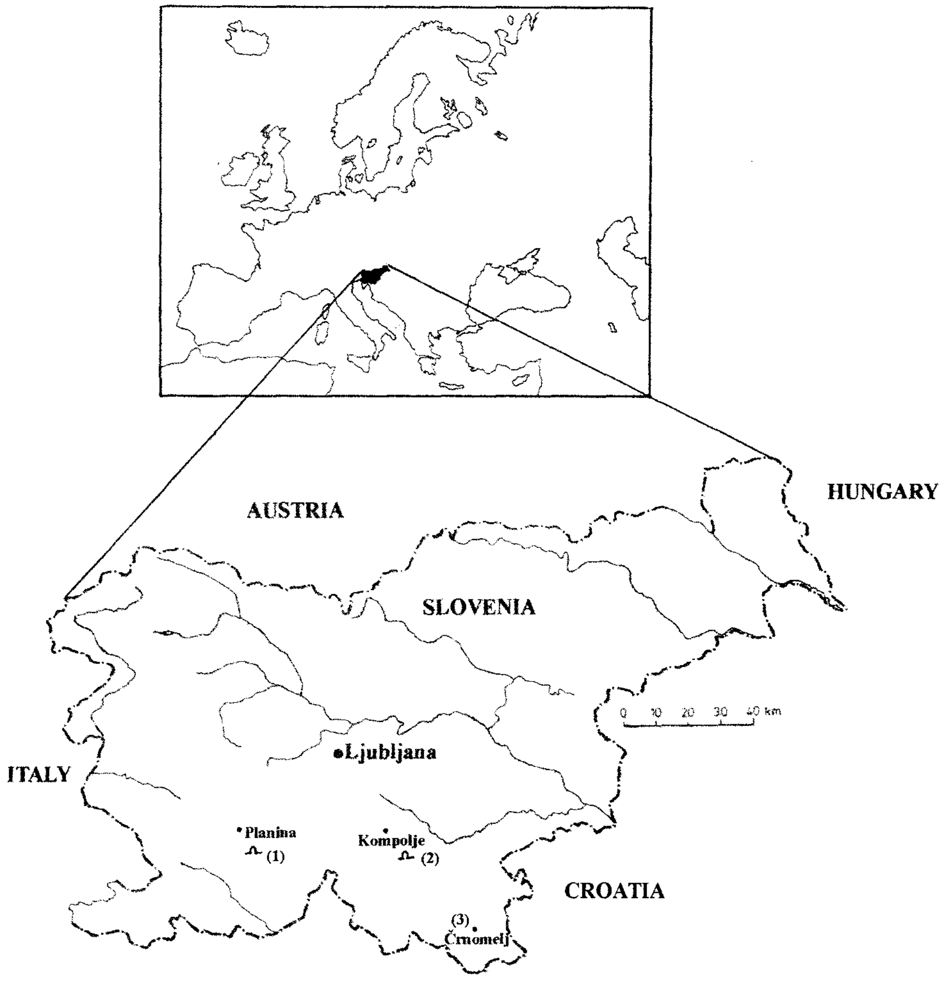

Figure 1. Map of Slovenia with sampling sites as follows: (1) Planina Cave, (2) Kompolje Cave, and (3) springs at Jelševnik, near Črnomelj.

were acidified by addition of HNO_3 immediately after sampling). Water samples for mercury determination were collected in glass bottles, filled with 1% HCl until sampling. In the laboratory all water and sediment samples were kept at 4 °C until metal analysis.

Animals were captured with a net, transported to the laboratory and transferred in tanks with the original, field water. Approximately one week after capture animals were prepared for analysis. Each animal was anaesthetised with 0.3% ethyl 3-aminobenzoate methane sulphonic acid salt (MS-222), decapitated and dissected. Tissue samples were lyophilised, homogenised and divided into three parts for three separate radiochemical procedures. Only a small number of *Proteus anguinus* specimens were available for our studies owing to the very strict enforcement of natural conservation laws; namely five animals from the Planina Cave, three animals from the Kompolje Cave, and two animals from Jelševnik were captured. All animals were adults.

2.2. ANALYSIS OF ANIMAL TISSUES

Due to the very small amount of animal tissue (especially liver and kidney) available for analysis, radiochemical neutron activation analysis as a very sensitive and accurate analytical method was used for determination of As, Cu, Hg, Se and Zn. After irradiation of samples in polythene or quartz ampoules (for Hg) for 20 hr in the carousel facility of the TRIGA Mark II reactor, IJS, at a thermal neutron flux of 1.1×10^{12} ncm^{-2} s^{-1}, three different separation schemes were used as follows: (1) simultaneous determination of Hg and Se based on pyrolysis (Byrne and Kosta, 1974; Dermelj *et al.*, 1979); (2) determination of As after wet digestion of the sample and selective solvent extraction of the iodides in toluene (Byrne and Vakselj, 1974) and (3) simultaneous Cu and Zn determination via wet digestion and carbamate extraction (Dermelj *et al.*, 1979).

For quality control of the methods used, suitable certified reference materials were also analysed, such as BCR CRM-186, Pig Kidney.

2.3. ELEMENTAL LEVELS IN SEDIMENTS

The k_0-standardization method of instrumental neutron activation analysis (Smodiš *et al.*, 1992; Smodiš *et al.*, 1993) was applied for elemental analysis of sediments. About 100–150 mg of lyophilised sediment samples were packed in polyethylene ampoules and then irradiated 20 hr together with an Al – 0.1% Au alloy serving as comparator and fluence rate monitor in the carousel facility of the TRIGA Mark II reactor at a thermal flux of 1.1×10^{12} ncm^{-2} s^{-1}. Measurements were performed on absolutely calibrated HP Ge detectors connected to Canberra Series 90 or S 100 multichannel analysers. The gamma spectra were evaluated by a PC version of the SAMPO 90 program. The effective solid angles and element concentrations in the measured samples and monitors were calculated by the KAYZERO/SOLCOI program Ver.4a (KAYZERO/ SOLCOI).

The accuracy of the results obtained was confirmed by the analysis of suitable certified reference materials (BCR CRM-277, Estuarine Sediment; BCR CRM-320, River Sediment; IAEA SL-1, Lake Sediment).

2.4. ELEMENTAL LEVELS IN WATER

For water samples the following methods were used: flame atomic absorption spectrometry for Zn determination, electrothermic atomic absorption spectrometry for Cu determination, cold vapour atomic absorption spectrometry for Hg determination (Horvat *et al.*, 1986), and neutron activation analysis for As determination (Byrne and Vakselj, 1974).

2.5. PHYSICAL AND CHEMICAL PARAMETERS

The water quality parameters pH, conductivity, total dissolved solids (TDS), temperature, dissolved oxygen, saturation, total and Ca hardness, $NO_3{}^-$, $NO_2{}^-$, NH^{4+}, and $PO_4{}^{3-}$ were measured in the water of each locality. The last six parameters were determined according to the 'Hatch' method (Hatch Company, U.S.A.).

3. Results

3.1. PHYSICAL AND CHEMICAL PARAMETERS

The main differences between the three localities and their freshwater systems (Table I) concerned dissolved oxygen concentration and saturation. The oxygen concentration during late summer (August, September) in the water source Na Trati 1 from Jelševnik was almost zero, saturation between 4 and 10% (anoxic conditions), whereas the oxygen concentration and saturation in water source Na Trati 2 was 96 mg L^{-1} and 62%. The distance between these two water sources is approximately 50 m.

All other physical and chemical parameters of waters at all the sites investigated (Planina Cave system, Kompolje Cave and Jelševnik springs) are characteristic of the aquatic environment in the calcareous Karst region. Nutrient concentrations were very low, mainly orthophosphate. In some sampling sites conductivity was higher than 400 $\mu S\ cm^{-1}$ due to the high concentration of Ca ion.

3.2. METAL LEVELS IN ANIMAL TISSUES

The concentrations of trace elements in the integument, muscle, liver, and kidneys of each individual animal of the *Proteus anguinus* specimens from the three sampling locations are presented in Table II. As evident from this Table, the variation of the element concentrations, especially for As in all organs, Cu in liver and Zn for some tissues of *Proteus* species from the same sampling location are relatively high, which made the comparison of elemental composition in particular tissues of animals from different localities difficult.

Pigmented subspecies of *Proteus* from Jelševnik showed increased values of arsenic and zinc in the individual tissues. The concentration of arsenic in the integument of the animal first analysed was 14.90 $\mu g\ g^{-1}$ wet weight, which was about 65-times higher than the mean value in the integument of the animals from the Kompolje Cave, and about 22-times higher than the mean value in the integument of the animals from the Planina Cave. The concentration of arsenic in the muscle of the first analysed pigmented animal (1.51 $\mu g\ g^{-1}$ wet weight) was also elevated,

TABLE I

Physical and chemical characteristics of water from the Pivka River of Planina Cave, Kompolje Cave, and springs Na Trati 1 and Na Trati 2 of Jelševnik. *n*: number of samples, X: mean value, R: range

	Planina Cave – Pivka			Kompolje Cave			Jelš. – Na Trati 1			Jelš. – Na Trati 2		
Variable	*n*	X	R	*n*	X	R	*n*	X	R	*n*	X	R
pH	9	7.6	(7.1–8.0)	16	7.6	(6.8–8.3)	18	7.6	(6.6–8.1)	19	7.4	(6.6–7.8)
T (°)	11	9.0	(4.8–12.5)	16	9.0	(7.6–10.4)	20	11.4	(9.4–15.8)	22	11.2	(10.2–14.9)
[Cond.](μS cm^{-1})	10	370	(288–418)	16	373	(221–460)	19	345	(241–446)	20	421	(334–586)
TDS (mg L^{-1})	10	186	(149–210)	16	190	(118–231)	19	174	(120–223)	20	212	(167–294)
[Oxy.] (mg L^{-1})	8	11.2	(8.5–12.7)	15	10.5	(7.9–14.3)	19	7.6	(0.3–11.7)	21	9.3	(6.5–12.7)
[Sat.] (%)	4	91	(80–103)	15	92	(75–121)	17	68	(2–106)	19	82	(60–100)
Ca + Mg (mg L^{-1})	8	192	(166–217)	14	217	(184–240)	16	191	(131–241)	15	223	(192–294)
Ca (mg L^{-1})	8	169	(153–187)	14	167	(157–198)	16	147	(104–187)	15	187	(152–279)
NO_3^- (mg L^{-1})	9	5.0	(0.9–9.7)	15	3.3	(0.4–9.7)	16	–	(< 0.1–4.4)	18	4.7	(0.9–10.6)
NO_2^- (mg L^{-1})	10	0.04	(0.01–0.10)	15	0.04	(0.01–0.08)	17	0.04	(0.01–0.08)	19	0.06	(0.01–0.55)
NH^{4+} (mg L^{-1})	10	–	(< 0.01–0.25)	15	–	(< 0.01–0.09)	17	–	(< 0.01–0.58)	19	–	(< 0.01–0.16)
PO_4^{3-} (mg L^{-1})	6	0.36	(0.19–0.56)	13	0.23	(0.12–0.37)	16	0.27	(0.03–0.53)	16	0.24	(0.12–0.50)

T: temperature, [Cond.]: conductivity, TDS: total dissolved solids, [Oxy.]: dissolved oxygen, [Sat.]: saturation, Ca + Mg: total hardness, Ca: Ca hardness.

being more than 15-times higher than the mean value in muscle of the Planina Cave animals.

The highest amounts of zinc (46.30 and 52.04 μg g^{-1} wet weight) were found in the livers of the pigmented *Proteus* specimens from Jelševnik, which were approximately 7-times higher than the mean value in the liver of the animals from the other two localities. Also the integument of this pigmented *Proteus* showed higher concentrations of zinc (15.60 and 43.95 μg g^{-1} wet weight) in comparison to those found in *P. anguinus* from the Kompolje and Planina Cave, where the mean values were 10.19 (Planina Cave) and 7.44 (Kompolje Cave) μg g^{-1} wet weight.

The highest mean concentration of Hg was found in the liver of animals from Planina cave (0.63 μg g^{-1}wet weight). They have also the highest mean concentration of Hg in muscle (0.33 μg g^{-1} wet weight) in comparison with the animals from other two localities.

Animals from Planina cave also had the highest mean concentration of copper (8.61 μg g^{-1} wet weight) and selenium (7.95 μg g^{-1} wet weight) in the liver respectively. One specimen from the Planina Cave showed copper concentrations in the liver up to 16.10 μg g^{-1} wet weight.

3.3. METAL LEVELS IN WATER AND SEDIMENT

The concentrations of elements in the waters and sediments of the Planina Cave, Kompolje Cave, and Jelševnik are presented in Tables III and IV. As seen from Table III, where the results for elemental concentrations in water are presented as mean values and their range, these levels were very low, usually below or slightly above the detection limit of the methods used. These results are also in agreement with the results of a similar investigation of Dermelj *et al.* (1984) in Planina cave (Table VI).

As evident from Table IV, the levels of Cu, Se and Hg were also low in sediments from all three sampling localities, usually below the detection limit for Cu and Se. They were somewhat higher only for Zn and As concentrations at some locations. The highest amounts of zinc with mean values of 117 and 113 μg g^{-1}, respectively, were found in sediments from the Pivka river from Planina Cave and in the Jezero spring at Jelševnik, while in Kompolje Cave levels above 100 μg g^{-1} were measured only in the period between January-March. The highest amounts of arsenic were found in the Jezero spring at Jelševnik, at the locality of the pigmented *Proteus*, where the mean concentrations were approximately twice as high as in the sediments of the other localities.

4. Discussion

The pathways of metals through the body of *Proteus* are unknown. We may assume the ingestion of metals occurs by: (a) consumption of sediment particles, prey,

TABLE II

Metal concentrations ($\mu g\ g^{-1}$ wet wt) in the liver, muscle, kidney, and integument of *Proteus anguinus* specimens from the River Pivka of the Planina Cave (P1-P5), Kompolje Cave (K1-K3), the spring Na Trati 2 of Jelševnik (J1-J2), and their mean values (x)

	Liver					Muscle					Kidney					Integument				
	As	Cu	Zn	Hg	Se	As	Cu	Zn	Hg	Se	As	Cu	Zn	Hg	Se	As	Cu	Zn	Hg	Se
P1	0.79	12.42	13.30	0.72	10.83	0.17	0.10	3.90	0.39	0.32	–	0.50	12.30	0.09	1.41	2.65	0.15	9.00	0.13	0.82
P2	0.17	3.45	8.29	0.46	6.96	0.03	0.16	5.67	0.17	0.43	0.19	–	–	–	–	0.24	0.23	12.70	0.05	1.76
P3	0.17	16.10	0.22	0.44	4.95	0.12	0.12	0.21	0.33	0.62	0.02	0.35	1.02	–	–	0.11	0.22	1.26	0.04	0.63
P4	0.51	2.35	3.80	–	–	0.08	0.06	2.30	–	–	0.79	0.40	2.60	–	–	0.20	0.16	17.80	–	–
P5	0.02	–	–	0.90	9.04	0.06	–	–	0.41	0.52	0.02	–	–	0.12	1.93	0.14	–	–	0.11	1.01
x	**0.33**	**8.61**	**6.40**	**0.63**	**7.95**	**0.09**	**0.11**	**3.02**	**0.33**	**0.47**	**0.26**	**0.42**	**5.31**	**0.11**	**1.67**	**0.67**	**0.19**	**10.19**	**0.08**	**1.10**
K1	0.16	4.27	5.78	1.11	4.96	1.01	0.13	6.70	0.29	0.17	–	0.43	7.84	0.27	0.77	0.07	0.15	4.42	0.13	0.85
K2	0.23	1.42	10.10	0.24	4.65	0.67	0.10	3.80	0.11	0.33	–	0.48	13.40	0.16	1.76	0.50	0.11	9.12	0.03	0.85
K3	1.15	1.25	8.80	0.29	3.48	0.07	0.03	3.57	0.34	0.71	0.02	0.34	7.27	–	–	0.13	0.12	8.78	0.07	0.65
x	**0.51**	**2.31**	**8.23**	**0.55**	**4.36**	**0.58**	**0.09**	**4.69**	**0.25**	**0.40**	**0.02**	**0.42**	**9.50**	**0.22**	**1.27**	**0.23**	**0.13**	**7.44**	**0.08**	**0.78**
J1	0.47	2.20	46.30	0.30	3.75	1.51	0.10	2.73	0.24	0.17	–	0.68	7.91	0.12	0.65	14.90	0.12	15.60	0.08	0.24
J2	0.03	0.77	52.04	–	–	0.08	0.46	8.35	–	–	0.01	0.34	9.23	–	–	0.11	0.25	43.95	–	–
x	**0.25**	**1.49**	**49.17**	**0.30**	**3.75**	**0.80**	**0.28**	**5.54**	**0.24**	**0.17**	**0.01**	**0.51**	**8.75**	**0.12**	**0.65**	**7.51**	**0.19**	**29.78**	**0.08**	**0.24**

TABLE III

Metal concentrations in water from the Pivka River of Planina Cave, Kompolje Cave, and springs Na Trati 1 and Na Trati 2 of Jelševnik. *n*: number of samples, x: mean value, R: range

Metal	Planina Cave – Pivka			Kompolje Cave			Jelš. – Na Trati 1			Jelš. – Na Trati 2		
	n	X	R	*n*	X	R	*n*	X	R	*n*	X	R
Zn (μg L^{-1})	4	–	< 5.0	10	–	< 5.0	12	–	(< 5.0–5.6)	12	–	(< 5.0–14.6)
Cu (μg L^{-1})	4	–	(< 0.5–3.6)	10	–	(< 0.5–17.2)	12	–	(< 0.5–2.6)	12	–	(< 0.5–9.8)
Hg (ng L^{-1})	1	3.17	–	5	2.29	(0.36–5.86)	6	1.54	(0.29–5.05)	5	0.55	(0.08–0.72)
As (μ g L^{-1})	2	0.51	(0.25–0.76)	5	0.28	(0.15–0.42)	6	–	(< 0.01–1.20)	6	–	(< 0.01–1.00)

TABLE IV

Metal concentrations in sediments from the Pivka River of Planina Cave, Kompolje Cave, and the spring Jezero of Jelševnik. *n*: number of samples, x: mean value, R: range, * RNAA

	Planina Cave – Pivka			Kompolje Cave			Jelš. – Jezero		
Metal	*n*	X	R	*n*	X	R	*n*	X	R
Zn (μg g^{-1})	5	116.9	(92.6–140.0)	10	93.0	(39.3–287.0)	8	113.4	(78.2–136.0)
Cu (μg g^{-1})	5	–	< 20	10	–	< 20	8	–	< 20
Hg (μg g^{-1})*	2	0.095	(0.090–0.100)	2	0.145	(0.140–0.150)	1	0.128	–
As (μg g^{-1})	5	8.41	(6.85–12.30)	10	10.84	(6.62–19.60)	8	19.61	(10.90–24.30)
Se (μg g^{-1})	5	–	< 1.0	10	–	< 1.0	8	–	< 1.0

or water; (b) direct absorption from the surrounding water by the integument and through the gills; (c) absorption from the air during lung respiration. *Proteus* spends most of its lifetime in close contact with sediments in its underground habitat, and for this reason it is potentially exposed to elements bound to sediments. It has been observed by Persaud *et al.* (1987) that bottom dwellers have higher metal levels in tissues than those which live in the water column.

Our investigations of the nutrition of *Proteus* confirmed its predatory nature. It has been found that its nutrition varies according to the season. The main organisms eaten are crustaceans and snails. In the summer, the diet is supplemented by insects (unpublished observations). Essential as well as also toxic elements could probably enter *Proteus* with prey consumption, but considering its very low metabolic rate and survival for longer periods without food we may anticipate that the uptake of elements through the food chain is less crucial.

Several studies reported the tissue concentrations of mercury in vertebrates but only few in amphibians. The distribution of mercury in individual tissues of amphibians has been measured by Byrne *et al.* (1975) who found the highest concentrations of total Hg in uncontaminated waters in general in the liver to be 2 μg g^{-1} wet weight, in the kidneys 1.5 μg g^{-1} wet weight, and in muscle 0.5 μg g^{-1} wet weight. Concentrations in animals from contaminated waters were much higher about 20 μg g^{-1} wet weight in the liver and kidneys and 2–3 μg g^{-1} wet weight in muscle and integument. The concentrations of Hg in the tissues of *Proteus* (Table II) did not reach the values of amphibians from uncontaminated waters measured by Byrne *et al.* (1975). The results also showed that the liver Hg concentrations were higher than those found in the water and sediment of the habitats.

Selenium, an essential trace element, is found as selenocysteine in the enzyme glutathione peroxidase which protects the organism against oxidative damage by lipoperoxidase or hydrogen peroxidase (Seiler *et al.*, 1988). It is well known that selenium exerts a protective effect against the toxicity of mercury through mechanisms still to be unveiled (Lindh *et al.*, 1996). Byrne *et al.* (1975) reported a possible association between selenium and mercury in amphibian liver. They measured 5-times higher selenium liver concentrations in amphibians from mercury-contaminated areas in comparison to those from uncontaminated areas. In our work the highest tissue Se concentrations were determined in the liver of *Proteus anguinus* from all three localities. It is very interesting that these concentrations exceeded the selenium liver concentrations measured by Byrne *et al.* (1975) in amphibians from mercury contaminated areas, although the mercury concentrations in the livers of *Proteus* were much lower than those reported in amphibians from mercury contaminated areas.

The concentration of arsenic in the integument of the first analysed pigmented *Proteus* from Jelševnik is more than 42000-times higher than in the underground water of its habitat (i.e. 14.9 μg g^{-1} wet weight: 0.35 μg L^{-1}) and about 65-times higher than in the integument of depigmented species of *Proteus* from the Kompolje Cave. It is well known that one of the main target organs for As is the integument. Moore and Ramamoorthy (1984) reported that fish from unpolluted or mildly contaminated waters usually contain < 0.4 μg As g^{-1} wet weight. Compared to these values, the first animal from Jelševnik contained much greater arsenic concentrations, particularly in the integument. Arsenic in the groundwater habitat of the pigmented *Proteus* is under the WHO maximum permissible limit, while in the sediments the arsenic concentrations reached a mean value of 20 μg g^{-1} which is approximately twice as high as in the sediments from Planina and Kompolje Caves. High As concentrations in the sediments may be the consequence of using arsenic-containing pesticides in agricultural processes, because there are many vineyards and fields in the close vicinity of Jelševnik.

Zinc is an essential trace element in the vertebrate body, found in high concentration in the red blood cells as an essential part of carbonic anhydrase. Byrne *et al.* (1975) found very constant zinc values in the liver of frogs, with an average of 20 μg g^{-1} wet weight for 24 specimens from different areas. Pasanen and Koskela (1974) also found very little variation in zinc content in the liver of the common frog, *Rana temporaria*, ranging from 13.0 to 22.8 μg g^{-1} wet weight. The first analysis of tissues of the pigmented subspecies of *Proteus* from Jelševnik revealed elevated concentrations of zinc in the liver (46.30 and 52.04 μg g^{-1} wet weight) and the integument (15.60 and 43.95 μg g^{-1} wet weight). Although the zinc concentrations in the tissues of depigmented animals from the Planina and Kompolje Cave were much lower in comparison with those of pigmented animals from Jelševnik, the main sediment and water Zn concentrations were almost the same for all three localities. However, the Zn sediment value from Jelševnik is probably not representative of the sediment typical of the inaccessible underground habitat of these animals. It is known that in the close vicinity the Belt factory (producing batteries) has deposited a Zn-rich sludge, and this may be the reason for higher Zn levels in the pigmented animals.

Copper is an essential trace element for all living systems, crucial for many biochemical cell activities. It is required for the activity of enzymes associated with Fe^{2+} metabolism and melanin production. The primary target organ for Cu accumulation is the liver (Seiler *et al.*, 1988). Certain specimens of the Dominican toad, *Bufo marinus*, have black livers which are found to contain copper concentrations ranging from 1248 to 2091 μg of Cu g^{-1} dry weight. In spite of these astonishingly high copper concentrations no injury to hepatocytes was detectable by light or electron microscopy (Goldfischer *et al.*, 1970). Byrne *et al.* (1975) found great variations in copper concentrations in the liver of frogs, ranging from 4 to 300 μg g^{-1} wet weight, with animals even from the same locality varying considerably. The results of our study also showed variations in Cu tissue concentrations even within animals from the same locality. Some of the animals from the Planina Cave showed increased Cu liver contents, up to 16.1 μg g^{-1} wet weight in comparison to animals from the other two localities. There is a great possibility that starvation influences the tissue copper concentrations in *Proteus*.

TABLE V

Comparison of metal concentrations (μg g^{-1}) in the liver, muscle, kidney, and integument of *Proteus anguinus* specimens from the Pivka River of Planina Cave measured in 1978, 1993, and 1999

		1978[a]		1993[b]		1999	
		n	X ± SD	*n*	X ± SD	*n*	X ± SD
liver	As	–	–	1	0.34	5	0.33 ± 0.31
	Cu	8	5.96 ± 6.73	2	2.68 ± 0.11	4	8.58 ± 6.74
	Zn	8	10.54 ± 2.67	2	10.10 ± 3.96	4	6.40 ± 5.66
	Hg	8	0.62 ± 0.35	2	0.82 ± 0.55	4	0.63 ± 0.22
	Se	8	5.36 ± 1.96	2	7.63 ± 1.68	4	7.95 ± 2.55
muscle	As	–	–	1	0.13	5	0.09 ± 0.05
	Cu	7	0.26 ± 0.17	2	1.45 ± 1.39	4	0.11 ± 0.04
	Zn	7	5.20 ± 1.45	2	5.81 ± 2.11	4	3.02 ± 2.32
	Hg	7	0.35 ± 0.16	2	0.43 ± 0.04	4	0.33 ± 0.11
	Se	7	0.60 ± 0.22	2	0.64 ± 0.07	4	0.47 ± 0.13
kidney	As	–	–	1	0.05	4	0.26 ± 0.37
	Cu	8	0.84 ± 0.80	1	0.77	3	0.42 ± 0.08
	Zn	7	12.36 ± 2.64	1	15.60	3	5.31 ± 6.11
	Hg	8	0.12 ± 0.05	2	0.13 ± 0.10	2	0.11 ± 0.02
	Se	7	1.81 ± 0.72	2	2.91 ± 1.81	2	1.67 ± 0.34
integument	As	–	–	1	0.09	5	0.67 ± 1.11
	Cu	8	0.36 ± 0.15	2	0.24 ± 0.12	4	0.19 ± 0.04
	Zn	8	10.84 ± 3.61	2	7.20 ± 0.53	4	10.19 ± 6.96
	Hg	8	0.07 ± 0.05	2	0.11 ± 0.03	4	0.08 ± 0.04
	Se	8	1.12 ± 1.08	2	0.66 ± 0.17	4	1.10 ± 0.49

[a] Dermelj *et al.* (1984), [b] Bulog (1996).

It is known from previous studies (Dermelj *et al.*, 1984; Bulog, 1994; Bulog, 1996; Bulog, 1997) on *Proteus anguinus* from the Planina Cave, and from the present study, that the liver of the animals contained the highest metal concentrations and therefore may be considered as a target organ. There is no essential difference between the elemental tissue concentrations in previous and recent studies, with the exception of copper in muscle, which was approximately 5-times higher in our previous studies (Table V). Also the mean concentrations of zinc in the kidneys and copper in the kidneys and integument were twice as low as in previous studies.

TABLE VI

Comparison of metal concentrations in the water (μg L^{-1} and ng L^{-1})* and sediments (μg g^{-1}) of the River Pivka from Planina Cave measured in 1978, 1993, and 1999

	Water				Sediment			
	As	Cu	Zn	Hg	As	Cu	Zn	Hg
1978[a] (n = 1)	1.10	3.20	11.60	70.00*	13.00	44.0	120.0	0.100
1993[b] (n = 1)	0.12	2.23	13.70	0.27*	6.50	28.0	100.0	0.040
1999	0.51	< 0.50–3.60	< 5.00	3.17*	8.41	< 20.0	116.9	0.095

[a] Dermelj *et al.* (1984), [b] Bulog (1996).

The concentrations of some metals in the water and sediment of the Pivka river from the Planina Cave were also measured in previous studies in 1978 and 1993 (Dermelj *et al.*, 1984; Bulog, 1994; Bulog, 1996; Bulog, 1997) (Table VI) and the results showed no evidence of metal pollution. In the present study the waters were not polluted with metals, which however were relatively high in the sediments, but not to levels considered polluted. The concentrations of zinc and copper in the water were lower than in 1978 and 1993. Mercury concentrations in water in 1993 were lower than in the present study, while in 1978 they were much higher. In the sediments there is no essential difference between the metal contents determined in previous and recent studies.

This study forms part of efforts to provide effective protection for the endemic cave salamander and other creatures living underground, which are protected by Slovenian conservation laws. In the past Slovenian caves became famous for the animals they contained and which could not be found elsewhere. We hope to promote the proposal to include *Proteus* in the list of the most protected species of the Washington Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), which entered into force on 1 July, 1975.

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