Environ Monit Assess (2008) 144:15–22
DOI 10.1007/s10661-007-9627-0

Heavy metal concentration in fish tissues inhabiting waters of "Buško Blato" reservoar (Bosnia and Herzegovina)

Elizabeta Has-Schön · Ivan Bogut · Gordana Kralik · Stjepan Bogut · Janja Horvatić · Ivan Čačić

Received: 6 September 2006 / Accepted: 22 January 2007 / Published online: 7 March 2007

Abstract Heavy metals concentration (mercury, lead, cadmium, arsenic, copper, zinc and chromium) in tissues (muscles, liver, kidney and gonads) of Dalmatian barbelgudgeon, the nase, the souffie and brown trout, inhabiting waters of Buško Blato reservoir in Bosnia and Herzegovina, has been determined by atomic absorption spectrophotometry. The meat of the tested fish sorts does not contain elevated concentration of most analyzed heavy metals with exception of lead (higher than MAC in Italy, Germany and Denmark) and mercury (in muscles of brown trout higher than MAC in most countries). The lowest level of all heavy metals is always detected in gonads, with higher values in fry compared to milt for copper, zinc, chromium and arsenic. The highest copper concentration is observed in the liver from the souffie which is suggested as a suitable biomonitor for copper intoxication. In muscles of all fish sorts, lead was always present in much higher concentration than cadmium, while in kidneys of most fish sorts, lead and cadmium concentrations were similar. We showed that bioaccumulation of some heavy metals in the fish sorts analyzed is tissue and sex dependent. Also, we concluded that the small water exchange in reversible shallow reservoir does not induce elevated concentration of heavy metals in fish tissues inhabiting Buško Blato.

Keywords Heavy metals · Brown trout (*Salmo trutta*) · The souffie (*Leuciscus turskyi*) · The nase (*Chondrostoma phoxinus*) · Dalmatian barbelgudgeon (*Aulopyge hügeli*) · Buško Blato

E. Has-Schön (✉) · J. Horvatić
Department of Biology, J.J. Strossmayer University,
Trg Lj. Gaja 6, 31000 Osijek, Croatia
e-mail: elschon@yahoo.com

I. Bogut · G. Kralik
Department of Animal Husbandry, Faculty of Agriculture,
J.J. Strossmayer University,
Trg Sv. Trojstva 3, 31000 Osijek, Croatia

S. Bogut
Health Centre Grude,
Kraljice Katarine 8, Grude, Bosnia and Herzegovina

I. Čačić
Faculty of Food Technology, J.J. Strossmayer University,
F. Kuhača 18, 31000 Osijek, Croatia

Introduction

Buško Blato reservoir is located in Bosnia and Herzegovina, at the frontier with Croatia (Fig. 1). It was built in the year 1972 and covers the largest area in Europe (57 km^2, containing ca. 790 million m^3 of water). It is relatively shallow, with a mean depth of 5 m. The central position and the terrain morphology of this area make possible water storage from the wider catchments of the Cetina River in Croatia (Štambuk-Giljanović 2001). The reservoir water is used directly for the Orlovac hydroelectric power plant (HE) and indirectly for the plants in downstream

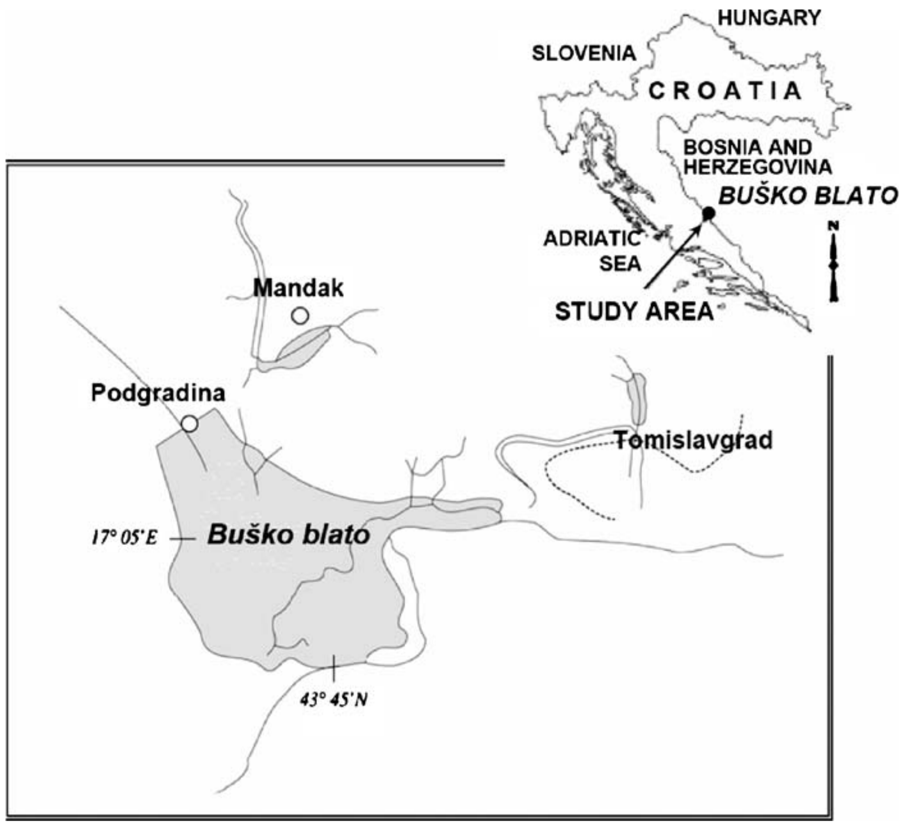

Fig. 1 The location of Buško Blato reservoir in relation to Croatia

sections: HE Djale, HE Kraljevac and HE Split (Božičević 1992), all of them located in Croatia. The area where Buško Blato reservoir is located is not densely populated and no waste water is directly released into the reservoir (Štambuk-Giljanović 2001). The pollution sources are the torrents which wash off the agricultural areas and the groundwater which is polluted by waste water from distant settlements (Štambuk-Giljanović 1998). Already mentioned study of Štambuk-Giljanović (2001) showed a satisfactory water quality of Buško Blato reservoir, what has been explained by intensive auto-purification processes, assisted by heavy winds that blow on the Buško Blato surface directly mixing the sediment with the reservoir water.

The aim of our study was to find out if the small water exchange in this reversible shallow reservoir covering large area influences the heavy metal content in fish sorts inhabiting Buško Blato water. Most of them (apart from brown trout) are endemic, what increased our curiosity concerning heavy metal content in different tissues of these fish sorts. Also, our idea was to find out if any fish sort analyzed, as well as some of its particular tissue(s) can be selected as a suitable biomonitor for certain heavy metal.

Materials and methods

Four fish sorts (Dalmatian barbelgudgeon-*Aulopyge hügeli*, the nase-*Chondrostoma phoxinus*, the souffie-*Leuciscus turskyi*, and brown trout-*Salmo trutta*) inhabiting waters of Buško Blato were analyzed for heavy metal content (mercury *Hg*, lead *Pb*, cadmium *Cd*, arsenic *As*, copper *Cu*, zinc *Zn* and chromium *Cr)* in selected tissues (muscles, liver, kidneys and gonads). Analyzed fish differ according to their eating habits: the souffie and Dalmatian barbelgudgeon are typical omnivora, while brown trout belongs to carnivora group. Finally, the nase is a typical herbivor, preferring algae as the main food constituent.

Fish were angled using fishing net in the middle part of the reservoir on several occasions during the summer (July and August) of the year 2005. Each fish

Table 1 Concentrations of *Hg*, *Pb*, *Cd*, *Cu, Zn,Cr* and *As* (mg·kg^{-1} of body mass) in muscles (*M*), liver (*L*), kidneys (*K*), and gonads (Gon)-fry (*f*) and milt (*m*) of Dalmatian barbelgudgeon, the nase, the souffie and lake trout, expressed by mean value±standard deviation and range, *N*=10

mg·kg^{-1}	Tissue		Dalmatian barbelgudgeon	The nase	The souffie	Lake trout
Hg	*M*		0.493±0.119 (0.311–0.711)	0.482±0.094 (0.314–0.641)	0.494±0.135 (0.324–0.728)	0.668±0.096 (0.510–0.798)
	L		0.208±0.038 (0.138–0.24)	0.209±0.042 (0.147–0.275)	0.225±0.039 (0.147–0.289)	0.284±0.070 (0.148–0.368)
	K		0.108±0.21 (0.059–0.131)	0.133±0.013 (0.118–0.157)	0.123±0.010 (0.110–0.141)	0.192±0.043 (0.097–0.234)
	Gon		0.099±0.016 (0.079–0.121)	0.068±0.018 (0.048–0.11)	0.101±0.012 (0.084–0.12)	–
Pb	*M*		0.844±0.275 (0.505–1.234)	0.735±0.218 (0.413–1.121)	0.685±0.227 (0.412–1.147)	0.885±0.126 (0.671–1.106)
	L		0.893±0.167 (0.699–1.202)	0.835±0.136 (0.621–1.005)	0.795±0.281 (0.421–1.251)	1.146±0.376 (0.694–1.797)
	K		0.865±0.099 (0.721–1.041)	0.877±0.109 (0.684–0.994)	0.796±0.080 (0.658–0.879)	0.779±0.092 (0.651–0.931)
	Gon		<0.05	<0.05	<0.05	–
Cd	*M*		0.031±0.010 (0.018–0.049)	0.044±0.012 (0.029–0.061)	0.029±0.015 (0.012–0.066)	0.042±0.08 (0.025–0.52)
	L		0.830±0.307 (0.074–1.202)	0.070±0.008 (0.058–0.088)	0.076±0.013 (0.058–0.098)	0.144±0.191 (0.069–0.687)
	K		0.622±0.089 (0.489–0.724)	0.815±0.106 (0.661–0.963)	0.637±0.097 (0.412–0.741)	0.116±0.025 (0.079–0.144)
	Gon		0.004±0.002 (0.001–0.007)	0.005±0.001 (0.003–0.007)	0.005±0.002 (0.002–0.008)	–
Cu	*M*		0.574±0.098 (0.398–0.714)	0.627±0.064 (0.541–0.734)	0.550±0.159 (0.269–0.821)	0.547±0.075 (0.418–0.652)
	L		21.4±7.619 (11.90–34.10)	23.18±8.729 (10.90–36.2)	7.101±3.294 (2.470–12.71)	43.97±4.970 (38.4–51.5)
	K		4.14±1.002 (2.170–5.88)	5.6±1.039 (4.45–7.54)	4.339±0.954 (2.210–5.57)	3.499±0.827 (2.56–5.21)
	GON	*F*[a]	1.11±0.023 (1.069–1.124)	1.094±0.029 (1.057–1.121)	0.838±0.412 (0.108–1.097)	–
		M[a]	0.264±0.443 (0.057–1.057)	0.048±0.009 (0.038–0.059)	0.089±0.016 (0.068–0.108)	–
Zn	*M*		13.203±2.003 (10.756–16.112)	0.121±0.024 (0.094–0.167)	13.526±6.30 (9.149–31.022)	41.82±10.823 (22.4–63.7)
	L		74.93±6.614 (66.40–87.4)	0.096±0.032 (0.034–0.135)	66.88±12.518 (45.7–87.2)	59.9±16.816 (39.4–88.4)
	K		171.91±23.125 (132.7–210.5)	0.071±0.043 (0.009–0.131)	149.9±16.196 (121.6–177.7)	148.1±18.328 (122.5–184.5)
	Gon	*F*[a]	100.00±3.926 (94.1–104.5)	0.066±0.031 (0.021–0.113)	97.04±2.418 (94.1–100.5)	–
		M[a]	10.58±1.746 (8.10–12.4)	0.040±0.033 (0.002–0.112)	10.6±1.632 (8.1–12.4)	–
Cr	*M*		0.111±0.018 (0.087–0.141)	0.134±0.027 (0.085–0.174)	0.122±0.030 (0.57–0.165)	0.189±0.036 (0.139–0.247)
	L		0.149±0.028 (0.099–0.188)	0.119±0.011 (0.099–0.134)	0.151±0.021 (0.120–0.178)	0.142±0.028 (0.098–0.198)
	K		0.088±0.018 (0.054–0.109)	0.113±0.013 (0.098–0.132)	0.111±0.028 (0.074–0.166)	0.225±0.319 (0.064–1.124)
	Gon	*F*[a]	0.062±0.009 (0.051–0.074)	0.120±0.012 (0.11–0.147)	0.071±0.016 (0.058–0.098)	–
		M[a]	0.03±0.005 (0.018–0.031)	0.096±0.021 (0.054–0.121)	0.027±0.008 (0.019–0.039)	–
As	*M*		0.133±0.025 (0.098–0.164)	0.067±0.037 (0.01–0.105)	0.122±0.013 (0.107–0.146)	0.152±0.015 (0.124–0.174)
	L		0.057±0.009 (0.042–0.071)	0.133±0.027 (0.102–0.185)	0.059±0.016 (0.034–0.084)	0.076±0.014 (0.047–0.091)
	K		0.146±0.049 (0.091–0.294)	0.282±0.064 (0.187–0.384)	0.122±0.011 (0.110–0.147)	0.139±0.044 (0.099–0.256)
	Gon	*f*[a]	0.066±0.005 (0.059–0.071)	0.271±0.064 (0.2–0.374)	0.075±0.006 (0.068–0.084)	–
		M[a]	0.037±0.007 (0.031–0.047)	0.099±0.029 (0.054–0.154)	0.042±0.006 (0.034–0.051)	–

[a] *N*=5

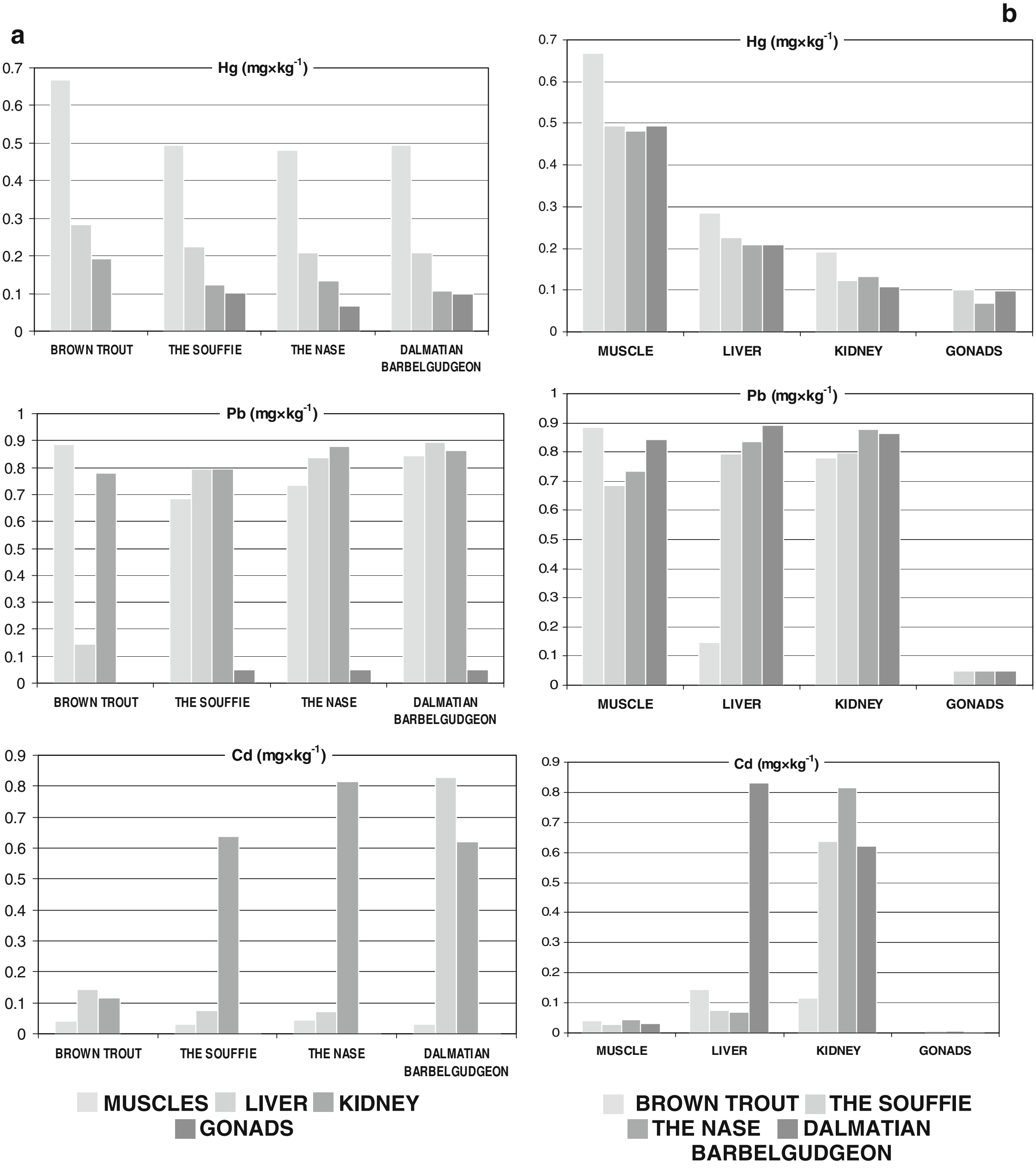

Fig. 2 Comparison of the mean values for the same heavy metal (*Hg*, *Pb* and *Cd*) between different fish species **a**, and between different tissues of the same fish species **b**

sort was represented by 10 individuals. Fish age was determined by scales monitoring, applying the method of Holčik and Hensel (1972). For the metal concentration analysis, fish samples 3–4 years old were selected. Fish organ samples (muscles, liver, kidney and gonads) were taken and stored in

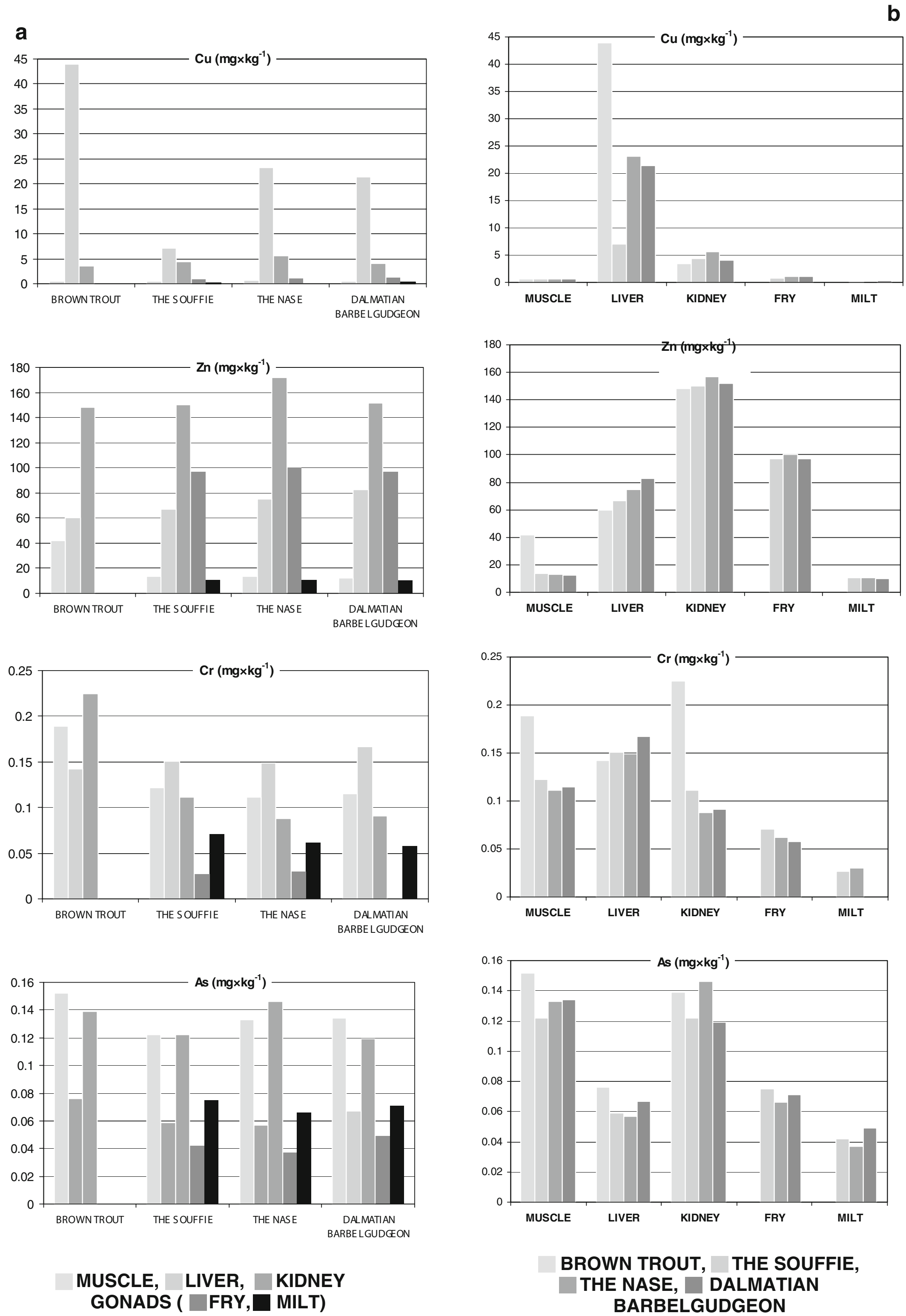

Fig. 3 Comparison of the mean values for the same heavy metal (*Cu*, *Zn*, *Cr* and *As*) between different fish species **a**, and between different tissues of the same fish species **b**

Table 2 Relationship of lead and cadmium concentrations, expressed by their ratio (Pb/Cd) in muscles, liver and kidneys of all fish sorts analyzed

Tissue	Fish sort			
	Dalmatian BarbelGudgeon	The nase	The souffie	Lake trout
Muscles	27.2	16.7	23.6	21.1
Liver	1.1	11.9	10.5	0.8
Kidney	1.4	1.1	1.3	6.7

refrigerator at −18°C until processed. For brown trout gonads were not accessible in this part of the year.

Lead, cadmium, copper, zinc, chromium and arsenic concentrations were determined by atomic absorption spectrophotometry on Perkin Elmer AAS 3100 in the Veterinary Institute Brno, Czech Republic. The external three-point calibration was performed in case of *Pb* (in the range 2.5–10 mg/l), *Cd* (in the range 0.5–1.5 mg/l) and *As* (1–4 mg/l). Mercury was measured in the same laboratory using Automatic Mercury Analyzer 254 (ALTEC s.r.o., Prague, Czech Republic), which was calibrated by manufacturer. The calibration was checked regularly using *Hg*-standard (0.1 ppm).

All standard chemicals were from Merck (Darmstadt, Germany). Each sample has been analyzed in triplicate. The results obtained are expressed as $mg \times kg^{-1}$ of fresh weight.

The validity of the procedures is tested once a year by participating in the international FAPAS proficiency tests and national tests distributed by Ekocentrum (Ostrava, Czech Republic). The quality assurance also included periodical analysis (once in two months) of Czech Certified Reference Material: Hazardous elements in horse liver (Ekocentrum, Ostrava, Czech Republic).

Results and discussion

Concentrations of *Hg*, *Pb*, *Cd*, *Cu, Zn, Cr* and *As* in tissues of analyzed fish sorts, expressed by mean value ± standard deviation and range are presented in Table 1.

Mean values of the same heavy metal concentration in tissues of different fish species are displayed in Fig. 2a (*Hg*, *Pb* and *Cd*) and Fig. 3a (*Cu*, *Zn*, *Cr* and *As*), while mean values of the same heavy metal concentration in different tissues of the same fish species are presented in Fig. 2b (*Hg*, *Pb* and *Cd*) and in Fig. 3b (*Cu*, *Zn*, *Cr* and *As*).

Mercury concentration is highest in muscles and lowest in gonads of all analyzed fish sorts. It does not exceed MAC value (maximal allowed concentration) in Croatia (1.0 $mg \times kg^{-1}$, Ministry of Health-MH, 1994), but in brown trout muscles it is higher than MAC in most countries (0.5 $mg \times kg^{-1}$, Máchová et al. 1991) and much higher than MAC in Bohemia for peaceful fish in all analyzed fish sorts (0.1 $mg \times kg^{-1}$, Máchová et al. 1991).

Lead concentration in all tissues apart from gonads almost reaches MAC in Croatia (1.0 $mg \times kg^{-1}$, MH, 1994) and most other countries, and is higher than MAC in Italy (0.7 $mg \times kg^{-1}$), Germany (0.5 $mg \times kg^{-1}$) and Denmark (0.3 $mg \times kg^{-1}$) (Máchová et al. 1991).

Cadmium concentration is lower than MAC in Croatia (0.1 $mg \times kg^{-1}$, Ministry of Health 1994) and Russia (0.2 $mg \times kg^{-1}$, Máchová et al. 1991) in most tissues with exception of liver in Dalmatian barbelgudgeon (0.83 $mg \times kg^{-1}$) and kidney of all fish sorts apart from brown trout. When compared with MAC in Germany and Denmark (0.05 $mg \times kg^{-1}$, Máchová et al. 1991), *Cd* liver and kidney concentrations in all fish sorts are too high. The lowest *Cd* concentration is observed in brown trout.

Copper concentration does not exceed meat Maximal Level (ML) of 10.0 $mg \times kg^{-1}$ (Web1) in all tissues apart from liver. The highest value (almost five times as high as ML) is observed in the liver from the souffie, so that souffie liver can be proposed as a suitable biomonitor fish for copper intoxication.

Table 3 Copper, zinc, chromium and arsenic distribution in females and males gonads of dalmatian barbelgudgeon, the nase and the souffie, expressed by fry to milt ratio

Heavy metal	Fish sort		
	Dalmatian BarbelGudgeon	The nase	The souffie
Cu	4.2	22.8	9.4
Zn	9.5	1.7	9.2
Cr	2.1	1.3	2.6
As	1.8	2.7	1.8

Zinc concentrations in liver, kidney and fry from all fish sorts are higher than meat ML ($50\,mg\times kg^{-1}$, Web1 2005). The highest values are detected in kidneys, being more than three times as high as ML. Of special interest is the observation that fry contains nearly 10 times as much zinc as milt.

Chromium concentrations are in all tissues from each fish sort much lower than the ML value for the whole fish ($5.5\,mg\times kg^{-1}$, Usero et al. 2004), with lowest concentration in gonads. Interestingly, too, fry *Cr* concentration is double as high as in milt.

Finally, arsenic concentrations in all tissues of all fish sorts are much lower than MAC in Croatia ($2.0\,mg\times kg^{-1}$, Ministry of Health 1994) or in most other countries ($1.0\,mg\times kg^{-1}$, Máchová et al. 1991). Again, higher concentrations are found in fry than in milt.

In our investigation we noticed also that in muscles of all fish sorts analyzed, lead was always present in much higher concentration than cadmium (Table 2). This finding is in agreement with the predicted order of metal occurrence presented by Pourang et al. (2005). Results similar to ours reported (Barak and Mason 1990) for the muscles of tench from eastern England and (Collings et al. 1996) for muscles of eel from New Brighton, England. Unfortunately, in the already mentioned extensive review by Pourang et al. (2005), no data about other fish tissues are available. In our study, *Pb–Cd* ratio in muscles of Dalmatian barbelgudgeon, the souffie, lake trout and the nase is 27.2, 23.6, 21.1 and 16.7, respectively, (Table 2). On the contrary, in other tissues analyzed, bioaccumulation of cadmium is higher (*Pb–Cd* ratio is slightly over 1, Table 2), especially in kidneys (with exception of lake trout), while in liver it depends strictly on fish sort (*Pb–Cd* ratio is 11.9 and 10.5 in the nase and the souffie, compared with 1.1 and 0.8 in Dalmatian barbelgudgeon and lake trout, Table 2).

Finally, for some heavy metals measured, it has been shown a strong tendency for bioaccumulation in female gonads (Tables 1 and 3). This was most pronounced for *Cu* in the fry of the nase, followed by *Cu* in fry of the souffie and *Zn* and *Cu* in the fry of Dalmatian barbelgudgeon (fry to milt ratio being 22.8, 9.4, 9.5 and 9.2, respectively, Table 3). We also noticed a slightly higher concentration of *Zn*, *Cr* and *As* in fry of Dalmatian barbelgudgeon, the nase and the souffie, compared to their concentration in milt (fry to milt ratio ranging from 1.3 to 2.8, Table 3).

Conclusions

- Meat (muscles) of the fish sorts inhabiting waters of Buško Blato does not contain elevated concentration of most analyzed heavy metals with exception of lead (higher than MAC in Italy, Germany and Denmark) and mercury (in muscles of brown trout higher than MAC in most countries).
- Concentration of most heavy metals is tissue and sex dependent.
- We recommend for human nutrition only the muscles of fish sorts analyzed (without inner organs and head).
- The souffie can be proposed as a suitable biomonitor fish for elevated copper concentration detection, because its tendency to cumulate this metal in liver tissue.
- Elevated cadmium concentration in kidneys and liver of all fish sorts analyzed may be the consequence of torrents which wash off the surrounding agricultural areas as well as of groundwater polluted by waste water from distant settlements.
- The small water exchange in reversible shallow reservoir does not induce elevated concentration of heavy metals in fish tissues inhabiting Buško Blato.

References

Barak, N. A. E., & Mason C. F. (1990). Mercury, cadmium and lead concentrations in five fish species of freshwater fish from eastern England. *Science of the Total Environment, 92*, 257–263.

Božičević, S. (1992). *Phenomenon karst* (pp. 28). Zagreb: School Book Zagreb.

Collings, S. E., Johnson, M. S., & Leah, R. T. (1996). Metal contamination of angler-caught fish from the Mersey Estuary. *Marine Environmental Research, 41*, 281–297.

Holčík, J., & Hensel, K. (1972). *Ichtyologicka prirucka* (*Handbook of ichthyology*) (p. 217). Bratislava: Obzor.

Máchová, J., Svobodová, Z., Hrjtmánek, M., & Hrbková, M. (1991). Control of hygienic quality of fish from the point of view of foreign substances content. In: *Diagnostic, prevention and therapy of fish diseases and intoxications, manual for international training course on fresh-water fish diseases and intoxications: Diagnostics, prophylaxis and therapy* (pp. 325–445). Vodnany, Czech Republic: Research Institute of Fish Culture and Hydrobiology.

Ministry of Health, Republic of Croatia (1994). Pravilnik o količinama pesticida, toksina, mikotoksina, metala i histamina i sličnih tvari koje se mogu nalaziti u namirnicama, te o drugim uvjetima u pogledu zdravstvene ispravnosti namirnica i predmeta opće uporabe (By-law on the allowed concentrations o*f pesticides, toxins, myco-toxins, metals, and histamine and related substances in food, and on other safety conditions of food and necessities in large-scale use*). Narodne novine no.46, pp. 30.

Pourang, N., Nikouyan, A., & Dennis, J. H. (2005). Trace element concentrations in fish, surficial sediments and water from northern part of the persian gulf. *Environm*ental Monitoring and Assessment, 109, 293–316.

Štambuk-Giljanović, N. (1998). *Waters of Neretva river and its catchment area* (p. 638). Split: Institute of Public Health Split.

Štambuk-Giljanović, N. (2001). The quality of water in the Buško Blato reservoar. *Environmental Monitoring and Assessment, 71*, 279–296.

Usero, J., Izquierdo, C., Morillo, J., & Gracia, I. (2004). Heavy metals in fish (*Solea vulgaris*, *Anguilla anguilla* and *Liza aurata*) from salt marshes on the southern atlantic coast of Spain. *Environment International, 29*, 949–956.

Web1 http://66.249.93.104/search?q=cache:UglbDsfK5MYJ:www.ospar.org/documents/dbase/decrecs/agreements/05-06e_agreement%2520Bcs.doc+Agreement+on+background+Concentrations+for+contaminants+2005-6&hl=hr&gl=hr&ct=clnk&cd=1, accessed 6.09.2006.