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EXPERIMENTAL INVESTIGATION OF HEAVY METAL ACCUMULATION IN TISSUES OF STONE LOACH *Noemacheilus barbatulus* (L.) AND RAINBOW TROUT *Oncorhynchus mykiss* (WALBAUM) EXPOSED TO A MODEL MIXTURE (Cu, Zn, Ni, Cr, Pb, Cd)

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Abstract. During experiment, fish were exposed to a heavy metal model mixture (Cu, Zn, Ni, Cr, Pb, Cd) for 14 days. Heavy metal concentrations corresponded to Maximum Permitted Concentrations (MPC) in surface waters. The amount of heavy metals in fish tissues was determined using atomic absorption spectrophotometry (AAS). Though both investigated fish species accumulated heavy metals with similar general intensity, the stone loach did it in the sequence gills > liver > muscle, and the rainbow trout - conversely: muscle > liver > gills. Ni concentration in the stone loach muscle made 0.607 mg/kg and Pb concentration was 0.21 mg/kg, whereas Cd concentration in the rainbow trout muscle reached 0.45 mg/kg and exceeded the MPC indicated in the Lithuanian hygiene standard. These results coincide with the data of the heavy metal research into fish species from natural water bodies. Pb and Cd concentrations in fish tissues are the highest and frequently exceed the MPC. These findings urge for the constant control of the amount of heavy metals in fish.

Keywords: fish, toxicity, trophic link, experimental investigation, hygiene standard.

1. Introduction

A great variety of natural processes occur throughout the Earth every minute. Though seeming very chaotic and unpredictable, they follow a certain order. A human as all other living organisms has a great effect on the whole natural mechanism of the Earth. Being able to perform actions impacting the environment, and understanding the order of its processes and their nature a human is enabled to be aware of the harm that could be inflicted by him or vice versa.

The mankind having polluted the environment for many years was not aware of ecological problems it was going to face. The pollutants discharged into water have a direct and indirect effect on humans. Contamination with oil products, heavy metals, phenols, fertilizers, pesticides and other pollutants make the smell, taste and other water qualities considerably worse. Especially harmful to human health is drinking water with the increased amount of heavy metal (lead, zinc, cadmium, nickel, chromium, mercury) salts. Lead also penetrates into human organism with water and food. Heavy metal ions are extremely toxic, while increased amounts of heavy metal compounds have a carcinogenic effect on human organism, and are mutagenic.

Heavy metals having penetrated into human organism through food chains (one of its links could be fish), might cause various disturbances or serious diseases (Fig. 1), thus, research of this type is of practical importance, the results of which could be used in the fields of environmental protection and health care.

Technogenic pollution of natural ecosystems poses a serious threat the consequences of which could be hardly assessed. One of such problems is the emergence of toxic compounds (heavy metals, pesticides, etc.) in food chains of ecosystems which induces the increasing mutability level in natural animal populations. 75% of chemicals penetrating into environment in great amounts have not yet been sufficiently evaluated (EEUCE 1999).

Fish are the final trophic link of hydroecosystems and the final biological water production which most easily accumulates pollutants (Čepanko *et al.* 2006).

Especially harmful are heavy metal radioactive isotopes (radionuclides) which enter human organism through food chains causing internal irradiance (Idzelis *et al.* 2007).

This is likely to be caused by higher accumulation and biomagnification of pollutants in hydrobionts (water invertebrates and fish), and, consequently, ecotoxic effects being more easily recorded (EE 1998).

Heavy metals are most often detected in polluted ecosystems including natural and anthropogenic sources (SCORECARD 2005).

The list of heavy metals comprises a number of elements which are necessary for living organisms: iron, cobalt, zinc, copper, manganese, molybdenum, etc. However, high concentrations of these metals are harmful to living organisms (Mažvila 2001).

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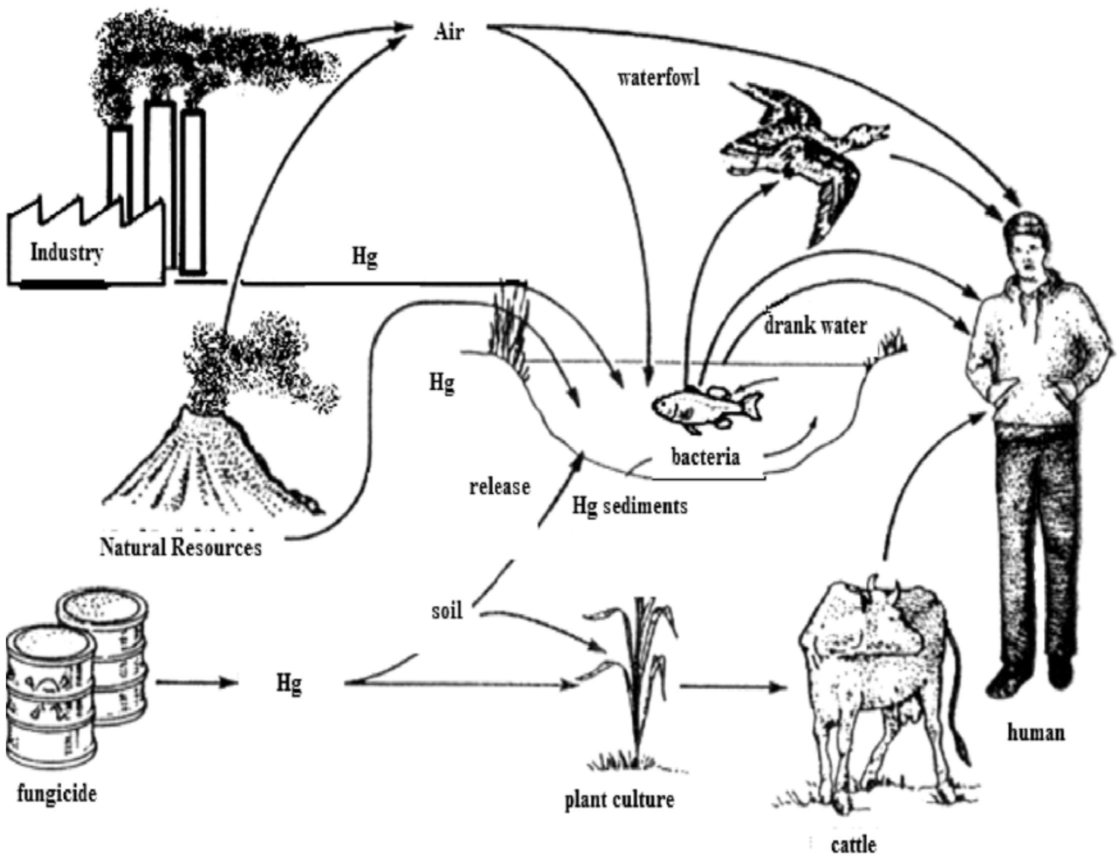

Fig. 1. Penetration of heavy metals into human organism through food chains

In natural unpolluted waters, the amounts of heavy metals and other chemical elements hazardous to biota are negligible, whereas in zones of intensive pollution, due to altered geochemical conditions and intensive introduction of contaminants, the established natural balance is disturbed, the amounts of elements change as well as their geochemical interrelations (Institute of Geology 2001).

The effect of heavy metals on organism depends not only on their concentration in the environment but also on their interrelation, migration form and what amount of them is easily assimilated. Now heavy metals are considered to be durable pollutants. While most organic pollutants and photooxides decompose in nature, the aforementioned metals are not disintegrated and destroyed in the natural environment (Short Ecological Glossary 1992).

During the studies of fish in Lithuania, the types of heavy metals and fish tissues most frequently accumulating them were determined (Metals in the Environment 2006). If the water is contaminated with various pollutants including heavy metals, fish behaviour is the key indicator of the pollution level. Under effect of high concentrations of contaminants, the fish perish (Scherer 1992).

In Lithuania, heavy metal monitoring has been periodically conducted since 1993 in water bodies of different type and eutrophication level: the Baltic Sea, the Curonian Lagoon, specially selected lakes (Dusia, Tauragnas, Plateliai, Lukštas, Žuvintas, etc.) and rivers (Žeimena, Būka, Skroblus). For the control of population parameters, the following core species of fish communities were selected: in the Baltic Sea – Baltic herring and flounder, in the Curonian Lagoon, lakes and rivers – roach and perch, and in streams – brown trout and bullhead (Kesminas *et al.* 1998).

In Lithuania, such investigations were carried out for the determination of the accumulation of heavy metals in tissues of fish species of different ecological groups. However, there are no data obtained by experimental investigation.

Up to 2002, permitted pollution levels of heavy metals in Lithuania were regulated by standards set in 1989. Since 2002, the hygiene standard HN 54: 2001 has become effective. This hygiene standard regulates the Maximum Permitted Concentration (MPC) (mg/kg) of heavy metals in muscle meat of fish from Lithuanian waters and its products. The Lithuanian hygiene standard HN 54: 2001 designates the Maximum Permitted Heavy Metal Pollution Level, i. e. such level of heavy metals when its short- or long-term effect could not cause diseases or health disturbances and makes 10 mg/kg for copper, 40 mg/kg for zinc, 0.5 mg/kg for nickel, 0.3 mg/kg for chromium, 0.2 mg/kg for lead and 0.05 mg/kg for cadmium.

2. Material and methods

The aim of this study was experimental determination of regularities of heavy metal accumulation in tissues of stone loach *Noemacheilus barbatulus* (L.) and rainbow trout *Oncorhynchus mykiss* (Walbaum) exposed to a model mixture (Cu, Zn, Ni, Cr, Pb, Cd).

Stone loach was collected during experimental catches in the Gauja (Šalčininkai district) and Siesartis (Ukmergė district) Rivers in May-June of 2007. The randomly selected collecting sites are indicated in Figs. 2 and 3. The needed number of fish was collected during three experimental fishing using a special catcher and electrofishing equipment.

Rainbow trout were obtained from Žeimena Fish Farm (Švenčionys District).

Fig. 2. Stone loach collecting sites in the Gauja stream near Dieveniškiai in Šalčininkai district in 2007

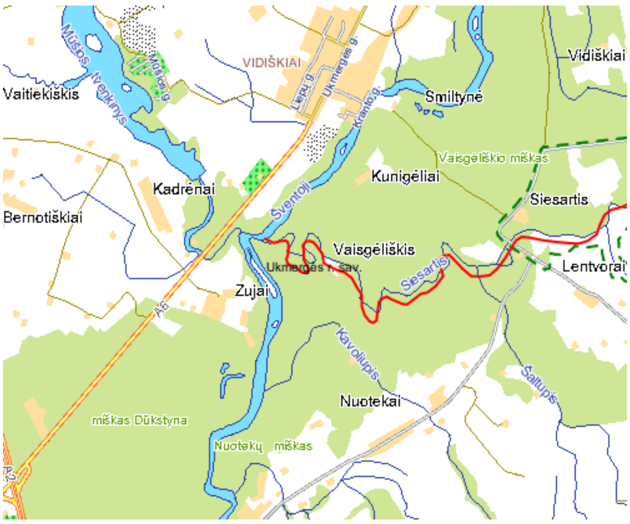

Fig. 3. Stone loach collecting sites in the lower reaches of the Siesartis River in Ukmergė district in 2007

5 to 7-year-old stone loach of approximately the same size (average weight of 10 g) and yearlings of rainbow trout (average weight of 10 g) were selected for experimental investigation.

The tests were conducted in the Laboratory of Ecology and Physiology of Hydrobionts of the Institute of Ecology of Vilnius University. Test fish were allowed to acclimate to laboratory conditions for two weeks. They were kept in flow-through aerated holding tanks and fed daily with bloodworms.

The tests were started when fish behaviour and feeding became normal. They were transferred into 30 litre tanks. One fish group was kept in the tank with clean water (control group), and the fish in other tank were exposed to a model mixture of the Maximum Permitted Concentrations (MPC) of the following heavy metals: copper, zinc, nickel, lead, cadmium and chromium (test group). The stock solution was prepared in distilled water using conventional in water toxicology chemically pure metal salts. The final concentration was recalculated according to the amount of heavy metal ion. The concentration of the stock solution was 2000 times higher comparing to the final MPC, and it was added to the tank in the amount of 10 ml.

Deep-well water was used for dilution. The average hardness of dilution water was 284 mg/l as $CaCO_3$, alkalinity was 244 mg/l as HCO_3^-, pH was 7.9–8.1, the temperature ranged from 10.5°C to 11.5°C, and the oxygen concentration varied from 8 to 10 mg/l.

During investigation, fish behaviour was observed. They were fed with bloodworms. Clean water and heavy metal solutions were renewed every other day. The whole procedure was carried on for 14 days. Heavy metal concentrations to which test fish were exposed are presented in Table 1.

Table 1. Heavy metal concentrations in the mixture to which test fish were exposed

Heavy metal	Source	MPC mg/l
Cu	$CuSO_4 \cdot 5H_2O$	0.01
Zn	$ZnSO_4 \cdot 7H_2O$	0.1
Ni	$NiSO_4 \cdot 7H_2O$	0.01
Cd	$Cd(CH_3COOH)_2 \cdot 2H_2O$	0.005
Pb	$Pb(NO_3)_2$	0.005
Cr	$K_2Cr_2O_7$	0.01

After the experiment, test fish (of control and test groups) were scarified and stored frozen. Later they were used for the removal of needed tissues. In total, 28 individuals of each fish species were investigated.

The amounts of heavy metals in fish tissues were determined using the atomic absorption spectrophotometer (Buck Scientific, model 210 VGP) in the Laboratory of Chemical Methods of Vilnius Gediminas Technical University. This method is based on the measurement of the element concentration in a sample using the flame atomic absorption spectrometry.

The following fish tissues were selected for the determination of the amounts of heavy metals in fish:

- muscle without scales,
- liver,
- gills.

Having performed the morphometric analysis of fish, muscle, liver and gills were separated from the bone tissue.

The accuracy of the determination of heavy metals greatly depends on the appropriate sampling of specimens and their treatment. Following the methodological requirements, tissue specimens contaminated with heavy metals should be sent to the laboratory for the examination just after their removal from fish, otherwise they should be frozen.

0.5–5 g sample was dried in temperature of 105 °C for 2 hours. The procedure was repeated in the same temperature for 30 min. Then it was cooled in the desiccator and 0.5–1.5 g weighed to accuracy of 0.0001 g. The weighed specimen was placed into a plastic container and examined with the spectrophotometer.

As heavy metals were detected in tissues of both control and exposure fish groups, the intensity of accumulation was described by a relative value – bioconcentration coefficient (BC):

BC = E/C, where

E – amount of heavy metal in tissue of exposed fish

C – amount of heavy metal in tissue of control fish.

When BC = 1, heavy metal balance in fish tissues is observed; when BC > 1, heavy metal accumulation (catabolism), and when BC < 1, heavy metal release (metabolism) are witnessed.

3. Results and discussion

Results of experimental investigation are presented graphically (Figs. 4–15). Test fish species of two different ecological groups: stone loach (freshwater reophile, benthophage) and rainbow trout (diadromous reophile-linmophile, euriphage) are compared.

Copper concentrations (mg/kg) in tissues of stone loach and rainbow trout are presented in Figs. 4 and 5.

Copper bioconcentration coefficient (BC) in stone loach tissues was as follows: in muscle – 2.66, liver – 6.55 and gills – 0.93.

Copper BC in rainbow trout tissues was as follows: in muscle – 2.01, liver – 9.39 and gills – 5.80.

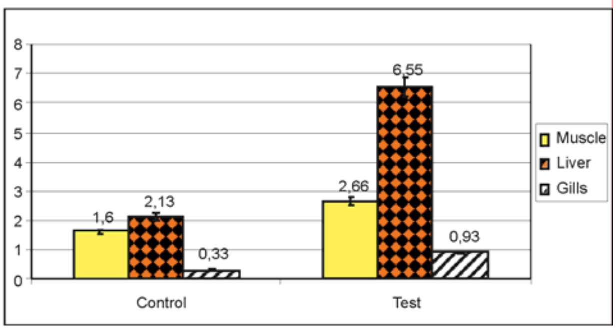

Fig. 4. Copper concentration (mg/kg) in tissues of stone loach

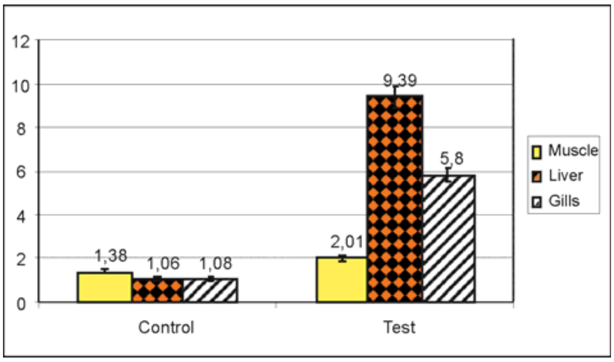

Fig. 5. Copper concentration (mg/kg) in tissues of rainbow trout

In both cases the Maximum Permitted Concentration (MPC) indicated in the Lithuanian hygiene standard (10 mg Cu/kg) was not exceeded.

Lead concentrations (mg/kg) in tissues of stone loach and rainbow trout are presented in Figs. 6 and 7.

Lead bioconcentration coefficient (BC) in stone loach tissues was as follows: in muscle – 0.201, liver – 0.27 and gills – 0.21.

Lead BC in rainbow trout tissues was as follows: in muscle – 0.11, liver – 0.09 and gills – 0.16.

In all tissues of stone loach the MPC indicated in the Lithuanian hygiene standard (0.2 mg Pb/kg) was exceeded, whereas it was not exceeded in tissues of rainbow trout.

Cadmium concentrations (mg/kg) in tissues of stone loach and rainbow trout are presented in Figs. 8 and 9.

Cadmium bioconcentration coefficient (BC) in stone loach tissues was as follows: in muscle – 0.053, liver – 0.033 and gills – 0.009.

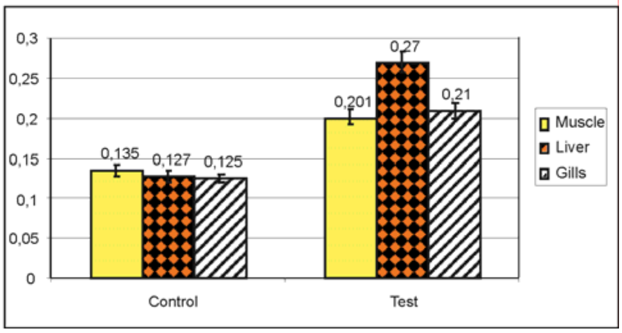

Fig. 6. Lead concentration (mg/kg) in tissues of stone loach

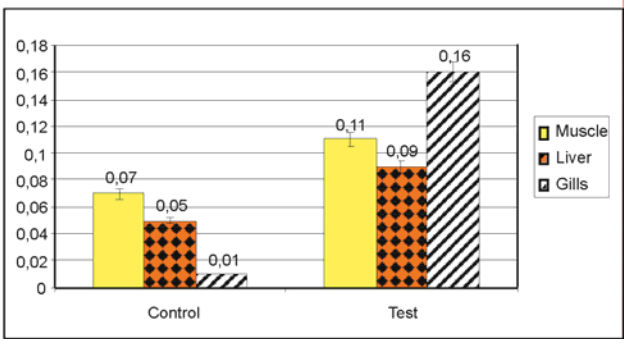

Fig. 7. Lead concentration (mg/kg) in tissues of rainbow trout

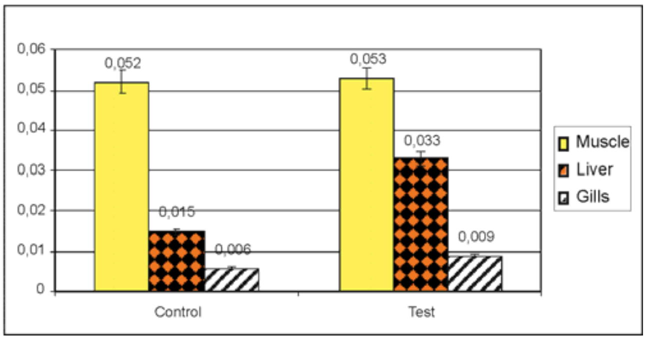

Fig. 8. Cadmium concentration (mg/kg) in tissues of stone loach

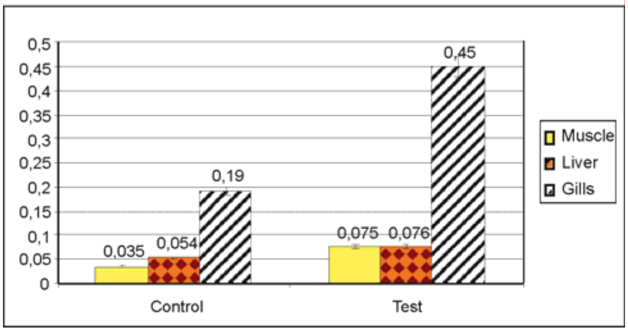

Fig. 9. Cadmium concentration (mg/kg) in tissues of rainbow trout

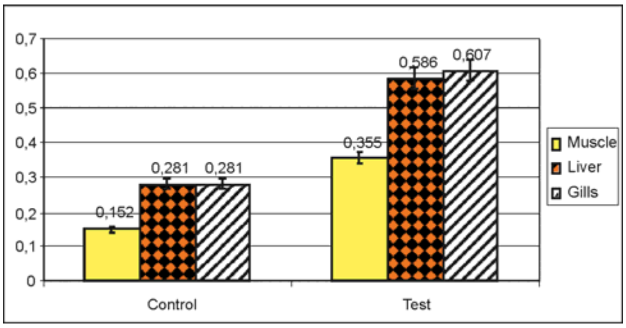

Fig. 10. Nickel concentration (mg/kg) in tissues of stone loach

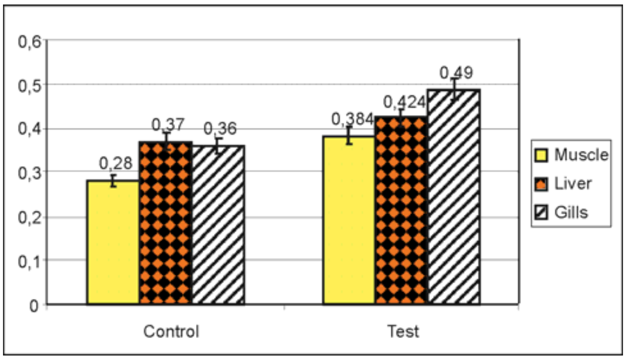

Fig. 11. Nickel concentration (mg/kg) in tissues of rainbow trout

Cadmium BC in rainbow trout tissues was as follows: in muscle – 0.075, liver – 0.076 and gills – 0.45.

Only in stone loach muscle the MPC indicated in the Lithuanian hygiene standard (0.05 mg Cd/kg) was slightly exceeded, whereas it was significantly exceeded in all tissues of rainbow trout.

Nickel concentrations (mg/kg) in tissues of stone loach and rainbow trout are presented in Figs. 10 and 11.

Nickel bioconcentration coefficient (BC) in stone loach tissues was as follows: in muscle – 0.355, liver – 0.586 and gills – 0.607.

Nickel BC in rainbow trout tissues was as follows: in muscle – 0.384, liver – 0.424 and gills – 0.49.

In stone loach liver and gills the MPC indicated in the Lithuanian hygiene standard (0.5 mg Ni/kg) was exceeded, whereas it was almost exceeded in tissues of rainbow trout.

Chromium concentrations (mg/kg) in tissues of stone loach and rainbow trout are presented in Figs. 12 and 13.

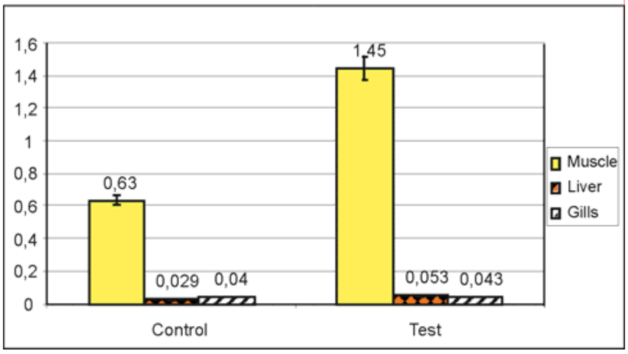

Fig. 12. Chromium concentration (mg/kg) in tissues of stone loach

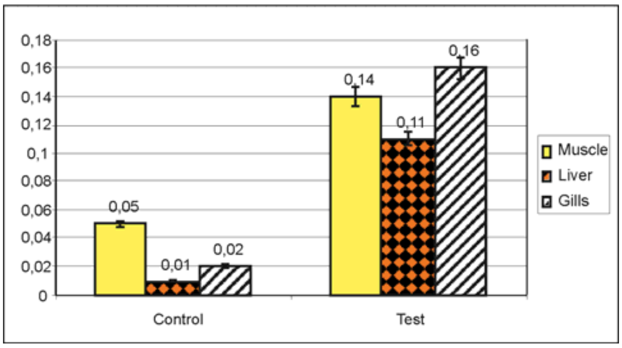

Fig. 13. Chromium concentration (mg/kg) in tissues of rainbow trout

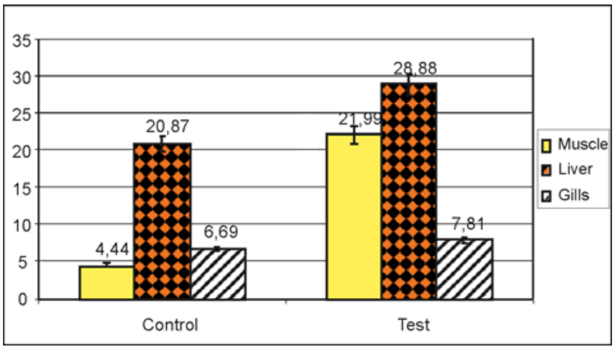

Fig. 14. Zinc concentration (mg/kg) in tissues of stone loach

Chromium bioconcentration coefficient (BC) in stone loach tissues was as follows: in muscle – 1.45, liver – 0.053 and gills – 0.043.

Chromium BC in rainbow trout tissues was as follows: in muscle – 0.14, liver – 0.11 and gills – 0.16.

Only in stone loach muscle the MPC indicated in the Lithuanian hygiene standard (0.3 mg Cr/kg) was exceeded, whereas it was not exceeded in tissues of rainbow trout.

Zinc concentrations (mg/kg) in tissues of stone loach and rainbow trout are presented in Figs. 14 and 15.

Zinc bioconcentration coefficient (BC) in stone loach tissues was as follows: in muscle – 21.99, liver – 28.88 and gills – 7.81.

Zinc BC in rainbow trout tissues was as follows: in muscle – 8.43, liver – 13.56 and gills – 32.70.

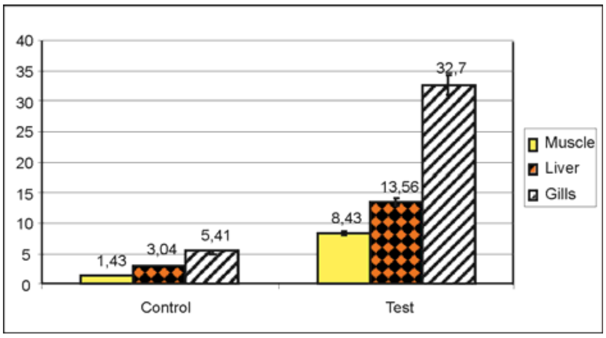

Fig. 15. Zinc concentration (mg/kg) in tissues of rainbow trout

In both cases the MPC indicated in the Lithuanian hygiene standard (40 mg Zn/kg) was not exceeded.

Having calculated the average values of bioconcentration coefficient (BC_{av}), it was ascertained that stone loach accumulated the largest amount of heavy metals in gills (BC_{av} = 9.01), then in liver ($BC_{av.}$ = 2.12) and the least – in muscle (BC_{av} = 1.34). Meanwhile, rainbow trout accumulated the largest amount of heavy metals in muscle (BC_{av} = 5.80), then in liver (BC_{av} = 4.10) and the least – in gills ($BC_{av.}$ = 3.80). Total mean BC_{av} for stone loach was 4.16 and for rainbow trout – 4.56. These findings show that both investigated fish species accumulated heavy metals with approximately the same general intensity.

It is evident that ecologically different fish species accumulate heavy metals with marked distinction. That is likely to depend on species-specific physiological-biochemical and behavioural (stone loach is a passive bottom fish species, whereas rainbow trout is an active near bottom fish species) characteristics as well as on other factors of unclear nature.

In stone loach muscle Ni concentration comprised 0.607 *mg/kg*, Pb concentration was 0.21 mg/kg, while in the muscle of rainbow trout Cd concentration reached 0.45 mg/kg and exceeded the MPC indicated in the Lithuanian hygiene standard. These results coincide with the data of the heavy metal research into fish species from natural water bodies. Pb and Cd concentrations in fish tissues are the highest and often exceed the MPC (Kesminas *et al.* 1998). The results obtained urge for the constant control of the amount of heavy metals in fish.

4. Conclusions

1. Experimental data obtained suggest that though fish underwent a 14-day exposure to the Maximum Permitted Concentrations (MPC) of Cu, Zn, Ni, Cr, Pb, Cd model mixture in surface waters, a tendency to accumulate heavy metals in fish tissues was observed.

2. The experimental investigation proved that both investigated fish species accumulated heavy metals with similar general intensity, however, the stone loach did it in the sequence gills > liver > muscle, and the rainbow trout - conversely: muscle > liver > gills.

3. Ecologically different fish species (stone loach – freshwater reophile, benthophage and rainbow trout – diadromous reophile-linmophile, euriphage) demonstrate quite different accumulation of heavy metals. We assume that it is predetermined by species-specific physiological-biochemical and behavioural (stone loach is a passive bottom fish species, whereas rainbow trout is an active near bottom fish species) characteristics as well as other factors of unclear nature.

4. In stone loach muscle Ni concentration comprised 0.607 mg/kg, Pb concentration was 0.21 mg/kg, while in the muscle of rainbow trout Cd concentration reached 0.45 mg/kg and exceeded the MPC indicated in the Lithuanian hygiene standard.

5. The results obtained coincide with the data of the heavy metal research into fish species from natural water bodies. Pb and Cd concentrations in fish tissues are the highest and often exceed the MPC. These findings urge for the constant control of the amount of heavy metals in fish.

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SUNKIŲJŲ METALŲ KAUPIMOSI ŠLYŽIO *Noemacheilus barbatulus* (L.) IR VAIVORYKŠTINIO UPĖTAKIO *Oncorhynchus mykiss* (Walbaum) AUDINIUOSE EKSPERIMENTINIAI TYRIMAI VEIKIANT MODELINIU MIŠINIU (Cu, Zn, Ni, Cr, Pb, Cd)

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Santrauka

Bandomosios žuvys 14 parų buvo veikiamos modeliniu sunkiųjų metalų mišiniu (Cu, Zn, Ni, Cr, Pb, Cd). Metalų koncentracijos atitiko jų didžiausias leidžiamas koncentracijas (DLK) paviršiniuose vandenyse. Sunkiųjų metalų kiekis audiniuose buvo nustatomas atominės absorbcijos spektrofotometriniu (AAS) būdu. Nors abiejų tirtų rūšių žuvų audiniuose sunkieji metalai kaupėsi vienodu intensyvumu, šlyžio audiniuose jie kaupėsi tokiu eiliškumu: žiaunos > kepenys > raumenys, o vaivorykštinio upėtakio – atvirkščiai: raumenys > kepenys > žiaunos. Šlyžio raumenyse Ni koncentracija buvo 0,607 mg/kg, o Pb – 0,21 mg/kg ,vaivorykštinio upėtakio raumenyse Cd koncentracija siekė 0,45 mg/kg ir viršijo Lietuvos higienos normoje nurodytąją DLK. Šie rezultatai sutampa su sunkiųjų metalų nuolatinės stebėsenos kontroliuojamų rūšių žuvyse iš natūralių vandens telkinių tyrimų duomenimis. Žuvų audiniuose Pb ir Cd koncentracijos yra didžiausios ir dažnai viršija DLK. Tokie rezultatai įpareigoja nuolat kontroliuoti sunkiųjų metalų kiekį žuvyse.

Reikšminiai žodžiai: žuvys, toksiškumas, mitybinė grandis, eksperimentiniai tyrimai, higienos norma.

ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ НАКОПЛЕНИЯ ТЯЖЕЛЫХ МЕТАЛЛОВ В ТКАНЯХ ГОЛЬЦА *Noemacheilus barbatulus* (L.) И РАДУЖНОЙ ФОРЕЛИ *Oncorhynchus mykiss* (Walbaum) ПРИ ВОЗДЕЙСТВИИ МОДЕЛЬНОЙ СМЕСИ (Cu, Zn, Ni, Cr, Pb, Cd)

Р. Л. Идзялис, В. Кясминас, Г. Свяцявичюс, А. Венсловас

Резюме

Подопытные рыбы в течение 14 суток подвергались воздействию смеси тяжёлых металлов (Cu, Zn, Ni, Cr, Pb, Cd) в предельно допустимых концентрациях (ПДК), предусмотренных для внутренних водоёмов. Количество тяжелых металлов в тканях рыб устанавливали при помощи атомной абсорбции спектрофотометрическим методом. Несмотря на то, что оба вида рыб накапливали металлы с одинаковой общей интенсивностью, голец их накапливал в следующей последовательности: жабры > печень> мышцы, а радужная форель – наоборот: мышцы > печень> жабры. В мышцах гольца концентрация Ni составила 0,607 мг/кг, Pb – 0,21 мг/кг, а в тканях форели концентрация Cd составила 0,45 мг/кг и превышала ПДК. Экспериментальные данные совпадают с данными, полученными в мониторинговых водоемах. Установлено также, что концентрация Pb и Cd в тканях зачастую превышает ПДК. Полученные результаты обязывают постоянно контролировать уровень тяжелых металлов в тканях рыб.

Ключевые слова: рыбы, токсичность, пищевая связь, экспериментальный анализ, гигиеническая норма.

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