

**STUDY OF THE DISTRIBUTION OF METALS IN MUSCLE TISSUE OF BROOK CHARR
AND BROOK TROUT FROM THE TROUT STREAM**

Milena Bušová

Department of Biochemistry, Chemistry and Biophysics, University of Veterinary and Pharmaceutical
Sciences Brno, Str. Palackého 1/3, 612 42 Brno, Czech Republic

Abstract

*Breeding salmonid fishes is in the Czech Republic widespread in areas with mountain climatic conditions. Our study is focused on the metals Cd, Pb and Hg in muscle of brook charr (*Salvelinus fontinalis*) from the fish breeding in location of the upper trout stream of the Králícký potok. The area is located above the urban agglomeration and industrial premises, where we did not anticipate any ecological burden of contaminating metals. The results are compared with the contents of metals in muscle of the naturally occurring brook trout (*Salmo trutta m. fario* L.) from the lower part of the same stream from the site of under the industrial complex and urban agglomeration. The results of the metal content in fish muscle from both locations are compared with the hygienic limit valid in the Czech Republic.*

The average values of all the analyzed metals in muscle of the brook charr did not exceed the hygienic limits for these metals ($Cd < 0.003$ mg/kg, $Pb = 0.063$ mg/kg, $Hg = 0.283$ mg/kg $\pm$). In three samples of the brook charr the limit was exceeded for mercury ($Hg \leq 1.21$ mg/kg). In the muscle of brown trout, the average concentrations of all analyzed metals complied to the hygienic limits ($Cd < 0.003$ mg/kg, $Pb = 0.14$ mg/kg, $Hg = 0.19$ mg/kg $\pm$). In none of the sample was the limit for metals exceeded, only in one case, Pb from location L2. Higher found values for mercury in the brook charr may be related to the earlier environmental burden, other character of the locality and higher age of the fish.

Key words: fish muscle tissue, heavy metals, cadmium Cd, lead Pb, mercury Hg, stream

1. INTRODUCTION

Contamination of water by heavy metals is one of the serious problems of water flows in areas with anthropogenic sources of these contaminants. Character of the stream, its location, topography and terrain elevation difference from the source area to the confluence and also the presence of contamination sources, their nature and location on the basin are significant factors affecting the level of contamination of the streams. Metals are getting into the aquatic environment mainly through the contaminated wastewater from industrial production. In addition, to contamination of flows also partially contributes dust fallout in areas of high population density and a high frequency of automobile traffic. From the contaminated water the metals are getting into the aquatic organisms in the form of simple or complex ions and compounds. Metals can sorb at the water sediments and through small aquatic animals enter the food chain (Spurný et al 2002, Svobodová et al, 2002, Kiurski et al, 2010). They may accumulate in some tissues and organs of fish and aquatic organisms. If the input of the contaminant metals does not stop significantly and the fish stays in the contaminated aquatic environments for a longer period of time, the metals accumulate in the organs and tissues, which may induce significant changes in the organs leading even to health disturbance. Most metals are bound with amino acids and -SH groups of proteins and can thus act as enzyme poisons. The negative feature is the ability of accumulation in tissues and organs (Yilmaz, 2006).

Fish living longer time in contaminated aquatic environment need not show signs of intoxication and yet their organs or muscle may contain toxic metals in concentrations above the limit. The risk of eating fish from such an environment is there especially for lovers of sport and recreational fisheries. Their catches are not subject to hygiene controls. The contaminants can thus get to the human body through fish as food. Some metals are at low concentrations required for proper functioning of the organs and metabolic processes. In higher concentrations, however, they can cause serious disorders, particularly if their supply to the organism is repeated. Some metals are even associated with the development and carcinogenesis. With regard to the hazardous effects of some metals to aquatic organisms and humans there are set limits on their concentrations in fish for consumption. The limits are given in Czech Republic by the Commission regulation (EC) No. 1831/2003 for muscle of freshwater fish (EC Commission Regulation, 2006).

Due to possible accumulation of toxic metals in fish, it is important to monitor the quality of water streams and reservoirs and monitor the quality of fish muscle in terms of toxic metals content.

For the actual study was selected the basin of Králícký stream, which is located in the piedmont region of northern Moravia and flows into the [Tichá Orlice](#) river. By this area also passes through one of the main [European watershed](#) of the [Black Sea](#) and the [North Sea](#). In this area also rises the significant flow of the Morava river. The Králícký stream rises in the forest at an altitude of 665 m above sea level below the the Veselka peak. After passing the town it takes on several tributaries. In the middle of the stream between the source and the confluence with the Tichá Orlice is located a former factory building on the urban territory, which is presumed to be contaminated with mercury and other metals from the industrial production.

Cadmium (Cd) is regarded as one of the most serious environmental contaminants of all heavy metals. Due to its effects on living organisms, their metabolism and particularly because of the potential carcinogenicity and teratogenicity, the use of cadmium is in many countries completely prohibited. A serious problem is the input path of cadmium into the food chain. In addition to wastewater from industrial production, this metal is emitted into the air and via the dust fallout it gets into the soil, water and sediments of water reservoirs and streams (Bušová & Čelechovská, 2013). Cadmium is capable of accumulating in biomass and reservoir sediments in a considerably high concentration. In water it occurs together with zinc and other metals that are usually found obviously in lower concentrations. Like zinc, cadmium is toxic to aquatic organisms, zooplankton and fish already at a concentration of units to tens of µg/l (Pitter, 2009).

For toxic effects in the organism is important the transfer of toxic metals into the food chain. One of the input paths of cadmium into the food chain may be fish and other aquatic animals from contaminated aquatic environment.

Toxicity of Cd after entering the organism is related to its carcinogenic effects. In the experimental conditions were demonstrated direct effects of cadmium in connection with the deregulation of cell growth and negative influence of cadmium on apoptosis of cells (Hartwig, 2010). Effects of cadmium are associated with disruption of nucleotide excision repair, base excision repair and mismatch repair. Cadmium can stimulate cell proliferation, inactivate negative growth stimuli and such as p53 (the tumor suppressor protein) and can cause preventing the process of apoptosis (Hartwig, 2012)). These multiple mechanisms can lead to higher degree of genomic instability, tumor initiation and later in tumor development (Hartwig 2010, Hiroshi et al, 2002).

Besides that, cadmium has also other adverse health effects. Dietary cadmium exposure has a demonstrable impact on the risk of osteoporosis, bone demineralization, decreased bone mineral density and increased fracture risk in a population of women (Engström et al, 2012).

In a number of other studies were shown the effects of toxic metals including Cd interaction with protein p53 with DNA *in vitro* and *in vivo* experiments. Inhibition of bind were demonstrated for all tested metals (cadmium, zinc, nickel) in this experiment (Brázdová et al 2012).

Although the main source of cadmium in the diet are mainly vegetables and cereals (bread, root vegetables and potatoes), fish can be a significant source of risk not only in terms of cadmium, but also other toxic metals.

In connection with a high degree of expansion of industrial production and widespread use of mercury (Hg) in various technologies, it has infiltrated into all environmental segments. Mercury is among the elements whose effect on the human organism is clearly negative. Mercury is - as similarly behaving cadmium - a cumulative poison. It is excreted from the organism very slowly and with difficulty. Most of the mercury that gets into the organism is concentrated mainly in the kidneys and to a lesser extent, in the liver and spleen. It has been proven that mercury can remain in the kidney even for decades. In cases of chronic mercury poisoning the kidneys are most at risk.

Mercury danger lies in its toxicological properties. Toxic effect of mercury and its compounds is largely due to a reaction of mercuric ion with SH-groups of biomolecules with subsequent change in permeability of cell membranes and damage of intracellular hepatic enzymes. There are significant differences in the metabolism and toxic effects of elemental mercury and its inorganic and organic compounds. Elemental mercury is rapidly oxidized in the organism to Hg²⁺, phenylmercury or metoxyethylmercury are easily cleaved in the organism and ions of Hg²⁺ are released, methylmercury may be demethylized. Inorganic mercury compounds may be methylized by the effects of the intestinal microbial flora. The transformation of mercury forms occurs in the body unevenly quickly and at varying degrees. Alkyl-compounds of mercury with short chain (methylmercury) are relatively more resistant to biotransformation unlike aryl-compounds of mercury, phenylmercury. From carcinogenicity is suspected methylmercury (Mlčoch, 2008).

A significant route of entry of mercury into the food chain may be contamination of the aquatic environment of the seas, oceans and freshwater reservoirs. In sediments of water reservoirs the inorganic mercury undergoes a process of methylation and in this form it can contaminate the aquatic organisms (Cibulka, Mader & Sova, 1986). Via small aquatic organisms and plankton the mercury can easily penetrate into fish. Through such contaminated fish it can easily get into the human organism. In the form of methylmercury, mercury is absorbed into the blood. The liver is the organ accumulating mercury. From methylmercury is in the liver partially released loose inorganic mercury, which is excreted via the bile into the intestine. Only a third of mercury detained by the liver is re-excreted via the bile into the intestine getting up to the kidneys and subsequently excreted. The remaining two thirds of mercury are again absorbed back into the blood (Cibulka, Mader & Sova, 1986).

In the past, there has been extensive methylmercury poisoning in Minamata region in Japan. The source was the fish from waters contaminated by waste from industrial production (Urban, 2006).

Together with mercury and cadmium, it is also lead (Pb), that contributes to contamination of the environment. Although, lead poisoning from the food is exceptional, its health effects are undesirable. It occurs as a supporting element for the contamination of the environment with cadmium and mercury, although at lower concentrations. Its occurrence in food is under 1mg/kg. Lead poisoning mainly occurs in connection with occupational exposure, which may result in health consequences in the form of damage to the central nervous system (CNS), peripheral nerves, disorders of hematopoiesis and impaired renal function (Koplík, 2013).

Fish, in addition to other aquatic animals, can be an important indicator of contamination of the aquatic environment by various xenobiotics and toxic metals.

On the quality of fish from open waters in the Czech Republic are focused monitoring studies. According to their results it is clear that in our country and in neighbouring countries the freshwater fish from some locations may contain in their tissues and organs heavy metals in concentrations exceeding health limits.

Monitoring studies dealing with the distribution of metals in the river ecosystem showed slightly increased concentrations of mercury and lead in muscle tissue of the freshwater chub (*Leuciscus cephalus*); increased concentrations of cadmium and lead occurred in the skin of the fish (Spurný et al, 2002). Stráňai and Andreji (2007) observed contents of heavy metals in tissues of different species of fish in the river Nitra. There were interspecific differences in the content of cadmium and lead in fish muscle. Only 11.5% of the analysed muscle samples did not exceed the hygienic limits for cadmium valid in the Czech Republic $Cd = 0.050 \text{ mg/kg}$ (EC Commission Regulation, 2006). Most of cadmium was present in the muscle of the common carp (*Cyprinus carpio*, L., 1758) and the silver crucian carp (*Carassius gibelio*), the least in the European catfish (*Silurus glanis*). In comparison with this finding, the results of analyses of fish from the Danube river show a balanced concentration of cadmium in the muscle of predatory and non-predatory fish species (Stráňai et Andreji, 1999). The study results of the common carp from ponds in the Czech Republic confirmed that the concentration of cadmium was in all the samples of muscle below the limit. Multiple increase in the concentration of cadmium, however, was found in the kidneys of these fish (Čelechovská et al, 2007). A number of other studies confirm the presence of heavy metals in organs and muscle tissue of fish from different locations from free water as well as from the breeding reservoirs. According to the study (Svobodová et al, 2002), the Cd content in common carp from selected ponds in southern and western Bohemia ranged in all muscle samples below the hygienic limit; in liver the values were higher, however, the limit (set out just for muscle) was not exceeded. According to this study, the highest concentration of cadmium was found in kidneys.

With the demand for higher fish consumption, which in the Czech Republic is still under the values recommended by the WHO, the attention is focused on the production of quality fish and compliance with the hygiene standards in fish and fish products. According to the Commission Regulation (EC) No. 1881/2006 (2006) is for freshwater fish muscle meat set limit for Cd 0.050 mg/kg , Pb 0.30 mg/kg and 0.50 mg Hg/kg .

Due to the negative effects and toxicological properties of these toxic metals and cumulative nature of cadmium (Cd), lead (Pb) and mercury (Hg) in organs and the way of persistence in the human body associated with the more difficult detoxifying pathways and in particular on the potential risk of carcinogenesis, our study was focused on the content of these metals in fish muscle from the natural environment.

2. MATERIAL AND METHODS

For this study were selected basin Králícký stream. Králícký stream is considered one of the smaller streams, the catchment area is $10,260 \text{ km}^2$, the flow length 5 km. It is an important right-side tributary of the Tichá Orlice river. N-yearly flow parameters of the Tichá Orlice river on the Lichkov profile are: $Q_1 = 16.8 \text{ m}^3/\text{s}$, $Q_{100} = 102.0 \text{ m}^3/\text{s}$. After passing the town, the Králícký stream takes on several tributaries. In the middle of the stream between the source and the confluence with the Tichá Orlice is located a former factory building on the urban territory, where is presumed environmental contamination of the surrounding area by mercury, cadmium and other metals from the industrial production - Figure 1.

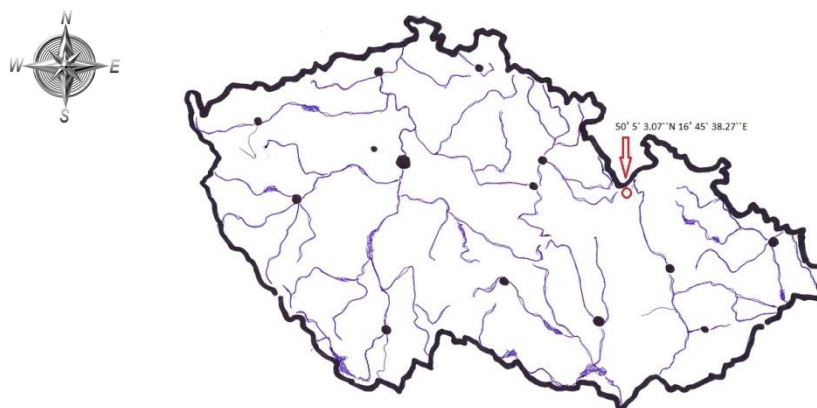


Fig. 1. Czech Republic – hydrologic map

In the autumn on the Králícký stream was carried out a catch of fish of the Salmonidae family. The first location L1 was chosen over an urban agglomeration in the natural environment without point sources of municipal and industrial pollution.

At this location is on the stream flow creation of a small flow through pond with an established breeding of brook charr (*Salvelinus fontinalis*). Fish take natural food and are fed with granulated feed. The fish fry comes from their own livestock and the fish lives from the beginning stage spawn to their market size in the same aquatic environment.

The second location L2 was chosen below an urban agglomeration in the bottom of the stream after flowing through the area with industrial production – Figure 2. The city has a sewage treatment plant.

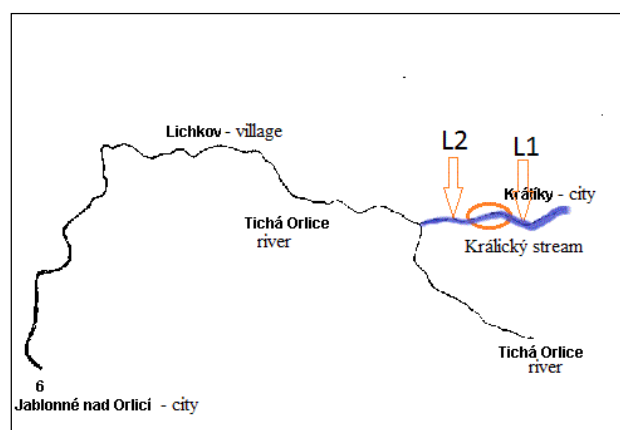


Fig. 2 Overview of investigated localities on Králícký stream

In the first location L1 were caught a total of 12 pieces of brook charr (weight $204,15 \pm 48,23$ g). From the second site L2 were caught here naturally occurring fish species brook trout (*Salmo trutta m. fario*, L.1758) at the number of 10 pieces of (weight $97,15 \pm 32,62$ g). Of all the caught fish was after killing taken samples of dorsal muscle, put under ice and after transportation to the laboratory were frozen at $- 28$ °C until the analysis. The muscle was weighed, homogenized, and mineralised by

boiling in concentrated nitric acid (HNO_3). Measuring the concentration of Cd and Pb was performed by the AAS method (atomic absorption spectrometry) on the Spectra AA-220FS device (Varian). Calibration and standardization of the method was performed using standard Astasol materials and Merck chemicals. The resulting concentrations of analysed metals were after conversion expressed in mg/kg of the wet muscle tissue. Measuring the concentration of Hg was performed on the mercury analyser AMA-254. The resulting concentrations were expressed after conversion v mg/kg of the wet muscle tissue.

3. RESULTS AND DISCUSSION

All samples of brook charr from the site L1 and brown trout from L2 under the city were analysed for the content of Cd, Hg and Pb in the dorsal muscle. The results of the analysis of metals in fish from the mountain stream of Králický stream are listed (Tab. 1.)

Table 1. Results of analysis of muscle tissue samples from location L1 a L2

Location - fish	weight of fish $\pm$ SD (g)	$\bar{O}$ Cd (mg/kg) $\pm$ SD	$\bar{O}$ Pb (mg/kg) $\pm$ SD	$\bar{O}$ Hg (mg/kg) $\pm$ SD
L1 - Brook charr	97,150 $\pm$ 32,62	DL	0,0621 $\pm$ 0,0264	0,2825 $\pm$ 0,3149
L2 - Brook trout	204,015 $\pm$ 68,50	0,0023 $\pm$ 0,0018	0,1425 $\pm$ 0,2415	0,1920 $\pm$ 0,0686
Hygienic limit	--	0,05	0,30	0,50

Note: $\bar{O}$ concentration of metals in mg/kg of wet material , DL – concentration below the detection limit

SD = standard deviation

3.1 Evaluation of cadmium

The average concentration of Cd in all muscle samples of the brook charr from the site L1 was below the limit of the method quantification, Cd <0.003 mg/kg of muscle. The concentration of Cd in rainbow trout from the site L2 was in case of half of the fish samples (5 samples) below the limit of the method quantification (Cd < 0.003 mg/kg of muscle), in the remaining 5 samples of rainbow trout the concentration of Cd was = 0.004 mg/kg.

3.2 Evaluation of lead

For both species were set concentrations of Pb in muscle. The average concentration of Pb in brook charr from the L1 site was Pb = 0.0621 mg/kg $\pm$ 0,0264 , in brook trout from the site L2 Pb = 0.1425 mg/kg $\pm$ 0,2415. Of the total of 12 samples from L1 the hygiene limit was not exceeded in any of the samples. In L2, the majority of samples was deeply below the limit, of the total of 10 samples exceeded the limit only 1 muscle sample (Pb = 1.1 mg/kg).

3.3 Evaluation of mercury

Mercury was found in the muscle of brook charr from locations L1 at an average concentration of 0.2825 mg/kg $\pm$ 0,3149. In 3 charr samples from locations L1 the concentrations exceeded the hygienic limit (Hg <1.21 mg/kg); the other samples from this site were safely below the limit. In

contrast to this finding, the average concentration of Hg in muscle of brook trout from the site L2 placed under the urban agglomeration was found lower, $\text{Hg} = 0.1920 \text{ mg/kg} \pm 0,0686$. In the muscle of this fish the hygienic limit for mercury was not exceeded in any of the analysed samples.

Graphical evaluation of the analyses compared with the hygienic limit in force in the Czech Republic is given (Fig. 3, Fig. 4 and Fig. 5).

Hygienic limit - Commission Regulation (EC) No. 1881/2006 (2006) is for freshwater fish muscle meat set limit for **Cd 0.050 mg/kg, Pb 0.30 mg/kg and Hg 0.50 mg /kg**.

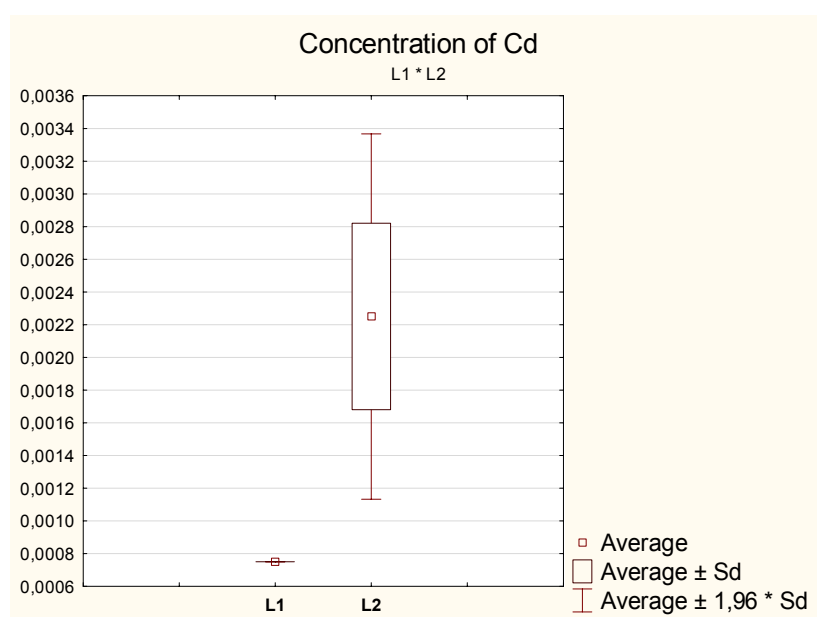


Fig 3. Concentration of Cd in muscle in mg/kg of wet material. L1, L2 – location.

Hygienic limit: Cd = 0,05 mg/kg of wet material.

After comparing the results of our analyses from locations L1 and L2 were found higher concentrations of mercury in muscle tissue of brook charr from locations L1. When compared with those published by other authors (Žlábek & Randák, 2007), these authors stated higher findings of mercury in muscle tissue of predatory fish. The findings in the asp (*Aspius aspius*) from the Elbe catchment area exceeded the hygienic limit ($\text{Hg} = 0.896 \text{ mg/kg}$), the concentration in pike (*Esox lucius*) was just above the limit ($\text{Hg} = 0.509 \text{ mg/kg}$). The authors Čelechovská et al (2007) in a study dealing with the distribution of metals in tissues and organs, state the highest mercury concentrations in muscle tissue of the common carp, while in the kidneys and liver the values are in the same fish lower. Their measured values found in the common carp from ponds in the Czech Republic are low and comply with the hygienic limit. According to other authors (Spurný et al, 2002) the findings of mercury in muscle tissue of freshwater chub (*Leuciscus cephalus*) from the Jihlava river are low and meet the hygienic limit ($\text{Hg} \leq 0.139 \text{ mg/kg}$).

Our findings of mercury are contrary to expectations higher in location L1, which is situated above the industrial area. In case of the L1 location is not expected any point source of pollution. Contamination of fish muscle from this area could come from an earlier ecological burden of the entire area by

industrial emissions with the use of mercury in the production technology. Through the dust fallout the mercury could be emitted into the environment.

Difference is also in the nature of the bottom. Location L1 is a flow pond, where part of the fallout can be accumulated in sediments. In contrast L2 location is a stream with a stony bottom.

The cadmium content in muscle tissue from location L2 were significantly higher ($p < 0,05$) compared to the cadmium content in muscle tissue of fish from location L1. On the contrary, mercury content in muscle tissue of fish from L1 were significantly higher ($p < 0,05$) compared to the mercury content in muscle tissue of from L2. Insignificant difference was found between lead content in muscle tissue of fish from both locations.

To the above findings should be also noted the differences in the age of the tested fish from both groups. From the L1 location were caught the fish of market size at the age of 2,5 years. In contrast, from the L2 location were caught naturally occurring brook trouts whose weight was average $97,15 \pm 32,62$ grams and the age of 1,5 years. It is also related with lower fish stocking density of the stream and sporadic occurrence of brook trouts in natural conditions in these waters in recent years. This assumption is consistent with the findings of Spurný et al (2002), whose results of mercury in fish muscle of younger age are lower ($Hg \leq 0.078$ mg/kg), than in case of older fish ($Hg \leq 0.139$ mg/kg).

Brook charr mercury contamination from the complementary granulated feed is not expected, because the used feed is intended for breeding fish and its composition including the certificate for foreign substances is declared.

Positive effect on the water Kralický stream can have its own wastewater treatment plants.

Our study has confirmed the results of monitoring on the Králický stream and Tichá Orlice river in the given area, which was carried out by the Forest Management Institute (FMI), in Czech “ÚHÚL”, a government organization established by the Ministry of Agriculture of the Czech Republic. According to the FMI data of the metal content in fish muscle (lead, cadmium, nickel, copper, zinc, chromium and aluminium) of fish caught in the Tichá Orlice river area and its tributary – the Králický stream – with the exception of nickel in the muscles of the brook trout from the locality of the Tichá Orlice – Lichkov, the valid hygienic limits for the content of hazardous elements in fish were not exceeded (Forest Management Institute, 2007). From their results analysed in the years 1995 - 1997 implies that the source of metals contamination of fish is not only the manufacturing facility in the town, but some metals are found in higher concentrations even in fish from the Králický stream from the area above the industrial area. These findings are entirely consistent with our findings in this study and demonstrate a persistent environmental burden of the area even after 20 years.

4. CONCLUSION

Our study was located on the northern edge of our Republic to the natural area between Orlické hory mountains and the massif Jeseníky. Near the Králický stream is located production facility which in the past engaged in the manufacture of lamps. Production related to environmental contamination especially by metals. Our study focused on the aspect of safety for consumers.

Compared with the original approach did not show highly significant content of metals in fish from the site of the industrial complex and urban agglomeration. The average concentration of metals in fish meat tissues did not exceed occupational exposure limits for freshwater fish.

Fish are an important part of our diet. In terms of food safety, it is important to protect and monitor the quality of the environment in which the fish lives.

ACKNOWLEDGEMENTS

This study was supported by Project MSM No. 6215712402 Veterinary Aspects of Food Safety and Quality.

REFERENCES

1. Brázdová M, Adamik, M., Navrátilová, L., Tichý, V., Polasková, A., Helma, R., Hromnešová, L., Holacká, K., Ballová, L., Babula, P., Busová, M., Fojta, M. 2012.: Influence of heavy metals on p53 family tumor supresor and mutant p53 oncogenic behavior in vitro and in glioblastoma cells. *Interdisciplinary Toxicology*, vol.5 (Suppl.1), p.29.
2. Bušová, M. & Čelechovská, O. (2013) Study of cadmium occurrence of common carp *Cyprinus carpio* L. *Proceedings of the 39th Food Quality and safety Conference*. Ingr's Days 2013. Mendel University in Brno, Czech Republic, pp.36-42.
3. Cibulka, J., Mader, P., & Sova, Z., 1986. Pohyb olova, kadmia a rtuti v zemědělské výrobě a biosféře. VŠZ Praha, 157 p.
4. Čelechovská, O., Svobodová, Z., Žlábek, V., Macharáčková, B. 2007. Distribution of Metals in Tissues of Common Carp (*Cyprinus carpio* L.). *Acta Vet. Brno*, vol 76, pp. 93-100.
5. EC Commission Regulation, 2006. *Nařízení komise (ES) č.1881/2006, kterým se stanoví maximální limity některých kontaminujících látek v potravinách*. Úřední věstník Evropské unie. In Czech
6. Engström, A., Michaëlsson, K., Vahter, M., Julin, B., Wolk, A., Åkesson, A. 2012. Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. *Bone*, vol 50, pp. 1372-8.
7. Forest Management Institute (FMI), 2007. *Tichá Orlice River and its Králícký Stream*.
<<http://www.uhul.cz/mcl/mon97retez/5.2.php>> viewed 14 April 2013
8. Hartwig, A. 2010. Mechanism in cadmium-induced carcinogenicity: recent insights. *BioMetals*, vol 23, pp.951-60.
9. Hiroshi, K., Hideaki, K., Masahiko, S., Chiharu, T. 2002. Low dose exposure to cadmium and its health effects. Genotoxicity and carcinogenicity. *Journal of Hygiene* 57, vol 3, pp. 547-55.
10. Kiurski, J., Vasic, M.V., Aksentijevic, S., Luburic, U.K., Miloradov, M.V. 2010. Metals distribution and particle size analysis in water and sediment of the Djetinja river and Dragica spring (Serbia) *Chem Ind & Chem Eng Quart*, vol 16 (4), pp. 363-372.
11. Koplík, R. 2013. *Toxické prvky v životním prostředí a v potravinách*. viewed 22 April 2013
< http://web.vscht.cz/koplikr/Toxick%C3%A9%20prvky_4r.pdf>
12. Mlčoch, Z., 2008: *Otravy rtutí, projevy, příznaky, léčba, amalgám*, viewed 22 April 2013
<<http://www.zbynekmlcoch.cz/informace/medicina/nemoci-lecba/otrava-rtuti-priznaky-projevy-lecba-prevence-amalgam>>
13. Pitter, P. 2009. *Hydrochemie*. VŠCHT Praha. 592 pp.
14. Spurný, P., Mareš, J., Hedbávný, J., Sukop, I., 2002. Heavy metal distribution in the ecosystems of the upper course of the Jihlava River. *Czech J. Anim. Sci.* vol 47(4), pp. 160-7.

15. Stráňai, I. & Andreji, J. 2007. Heavy metals (Co, Ni, Cr, Pb, Cd) content in tissues of Nitra river fishes. *Slovak J. Anim. Sci.*, vol 40, (2), p.97-104.
16. Svobodová, Z., Žlábek, V., Čelechovská, O., Randák T., Máchová, J., Kolářová, J., Janoušková, D. 2002. Content of metals in tissues of marketable common carp and in bottom sediment of selected ponds of South and West Bohemia. *Czech J. Anim. Sci.*, vol 47, (8), pp. 339-350.
17. Urban, P. 2006. Aktuální problémy neurotoxicity rtuti. *Neurol.prax*, vol 5, pp.251-3.
18. Yilmaz, F. 2006. Bioaccumulation of heavy metals in water, sediment, aquatic plants and tissues of *Cyprinus carpio* from Kizilirmak, Turkey. *Fresenius Environ Bull* 15, pp. 360-9.
19. Žlábek, V & Randák T .2007. *Kontaminace ryb z volných vod*. viewed 12 November 2008.
<http://www.bezpecnostpotravin.cz/UserFiles/Deutsch/Kontaminaceryb2006_1__1_.pdf>