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TRACE METAL CONTAMINATION OF THE DYJE RIVER STRETCH BETWEEN ZNOJMO AND NOVÉ MLÝNY, CZECH REPUBLIC

T. Vítek, J. Hedbávný, J. Mareš, P. Spurný

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Abstract

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The analyses of seven trace metals (Hg, Zn, Ni, Cr, Cu, Pb, Cd) in the water, bottom sediments, macroinvertebrates and fish tissues were conducted at two localities on the Dyje River, Czech Republic, at different distances downstream from the Znojmo Reservoir (at 12 and 31 km) to evaluate a contamination level. The maximum admissible limits were exceeded in Hg in the water and in the case of Ni in bottom sediments at both localities and also in Hg in zoobenthos sample at locality further from the reservoir. In regard to trace metal contamination of fish tissues, it was found that safe limits for mercury, cadmium and lead were not exceeded. Mercury and cadmium contents have declined markedly since the 1990s, but the amount of lead has increased. Human consumption of fish caught in the Dyje River is most limited by concentration of Hg in the fish muscle. The maximum tolerated intake was set as high as 3.80 kg per week.

water quality, sediments, mercury, cadmium, lead, chub (*Squalius cephalus*), fish consumption

The substances of high chemical stability tend to accumulation in the ecosystem and help to make it ecologically instable. Trace metals most often do not have primary lethal effect on hydrobionts, but they cause a long-term environmental chronic stress. The bioaccumulation in food webs cause negative impacts especially on top predators of the ecosystem, which are in the case of river ecosystems a predaceous fish species and a piscivorous birds and mammals including human (Houserová *et al.*, 2005; Houserová *et al.*, 2007).

The sources of trace metals are most often the waste waters from industry (mining, paper-mills, metallurgy, chemical plants) and agriculture (fungicides). In polluted areas trace metals are stored in the sediments, from which they are released to the water (Zhou *et al.*, 2008). In the case of fragmented rivers, trace metals are settled and fixed in the reservoir sediment (Kulahci & Dogru, 2006; Ozdemir *et al.*, 2010); thus the water environment in the river below the dam might be less contaminated and the health hazard for top predators connected with the bioaccumulation of such substances might be reduced (Makovský *et al.*, 2010). The store function of the sediment depends upon the physico-chemical stability of the water. Changes in such parameters, namely pH, often cause the release of trace elements (Simpson *et al.*, 2010) and their transportation to the biota through the biofilm (Farag *et al.*, 2007). Among fishes, chub *Squalius cephalus* (L.) belongs to the most studied species due to its broad occurrence in various ichthyocenoses in a broad range of environmental conditions (Spurný *et al.*, 2002, 2009; Kružíková *et al.*, 2009). In the point of view of human health risk, the mercury and its species, which is detected in higher amounts especially in the fish muscle, represents the most danger (Houserová *et al.*, 2006; Havelková *et al.*, 2008). In the past, the Dyje River was a significantly contaminated stream in the Czech Republic, especially contaminated by mercury and cadmium (Spurný & Mareš, 1995; Spurný *et al.*, 1997).

The main aims of this study were 1) to determine the present state of trace metal contamination of the Dyje River stretch between reservoirs Znojmo and Nové Mlýny 2) to evaluate changes in trace metal contamination of fish fauna in the above-mentioned stretch by comparing the recent situation from 2008 with the situation from the 1990s and 3) to evaluate the risk of human consumption of fish from the Dyje River.

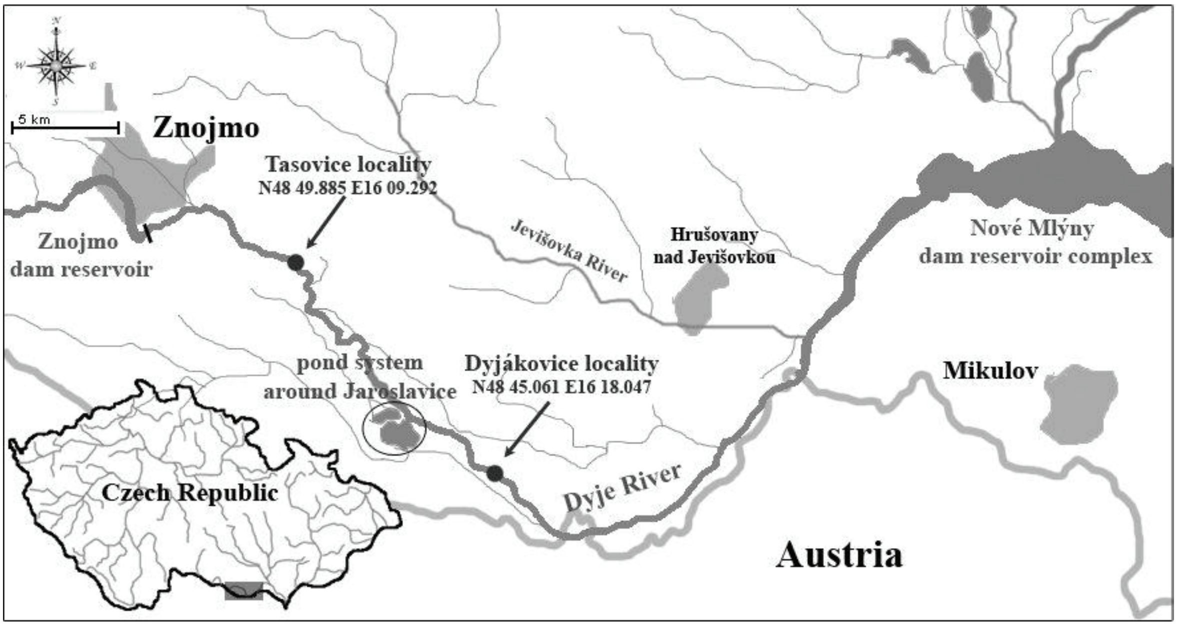
MATERIALS AND METHODS

Study area

The Dyje River constitutes an important right side tributary of the Morava River with a length of 305.6 km, a drainage area of 13,419 km^2 and a mean annual discharge at the stream mouth of 43.8 m^3/s. It flows near the Czech–Austrian border crossing it several times in the upper part. The study was conducted on the middle part of the river between two dam reservoirs: Znojmo (with a surface area of 53.3 ha, a dam height of 17 m, and a mean annual discharge of 10.25 m^3/s, river km 132.5) and Nové Mlýny (a complex of three reservoirs of total surface area 3,296 ha, a dam height from 5 to 6.7 m, river km 46–58). The observation was conducted at two localities, each at a different distance from the Znojmo reservoir (Tasovice 12 km, Dyjákovice, 31 km; Fig. 1). The Tasovice locality (river km 120.5) represents a relatively straight stretch with a depth mostly up to 0.85 m, a stream channel of approximately 20 m in width, and a mean flow velocity between 0.4–0.8 m/s. The Dyjákovice locality (river km 101.5) also represents a straight part. Shallow parts with a depth of about 0.4 m alternate with deeper ones with a depth of up to 1 m, especially near the banks. Flow velocity is mostly up to 0.5 m/s. The characteristics of sampling sites with respect to the habitat and fish assemblage structure are given in Table I as reported in more details in the study of Vítek *et al.* (2012).

Sampling

In June 2008, seven adult individuals of chub (319–405 mm TL) were captured at each locality by the method of electro-fishing (pulsating DC, 240–300 V, 2.5 A, frequency 50 Hz), killed and stored individually in plastic bags. The river water (in the amount of two l), bottom sediments (up to one l) and zoobenthos (approximately 100 ml) were also sampled at the same localities. The samples were undertaken according to Government Decree No. 23/2011 and were stored in the plastic enclosures. All samples were transported to the laboratory in a cooling box.



1: *Map of the study area showing the locations of sampling sites (geographical co-ordinates in WGS-84)*

I: *Basic characteristics of sampling sites of the Dyje River (mean values based on study by Vítek et al., 2012)*

sampling site	discharge (m^3/s)	pH	oxygen saturation (%)	conductivity of water (mS/m)	No of fish species	relative abundance of *S. cephalus* (%)	character of bottom substrate
Tasovice	5.94	7.65	88.50	32.70	10.00	34.45	boulders pebbles sand
Dyjákovice	2.25	7.65	86.00	39.71	10.00	59.55	gravel mudd

II: *Content of trace metals in particular compounds of the Dyje River ecosystem in 2008 (mean concentrations in mg/kg of wet matter of mixed sample, in the case of water in μg/l)*

locality	sample	Pb	Cd	Zn	Ni	Cr	Cu	Hg
Tasovice	water	0.20	0.05	41.00	10.00	2.02	11.00	0.55
	zoobenthos	0.14	0.01	16.32	2.30	0.45	9.99	0.02
	sediments	3.39	0.02	32.63	5.23	6.61	4.53	0.03
Dyjákovice	water	1.00	0.02	33.00	8.00	1.94	11.00	0.64
	zoobenthos	0.26	0.07	18.25	1.52	0.45	8.38	0.02
	sediments	3.14	0.08	23.30	6.16	19.68	5.24	0.02

Laboratory analyses

Several scales were taken from each fish to determine the age of sampled fish. The dorsal muscle of approximately 5 g in weight and a whole liver were subsequently dissected from the fish body and stored beside water, sediments and zoobenthos samples in the refrigerator at a temperature of about −15 °C for further analyses of trace metals assessed as follows. The total content of mercury (Hg) was determined using the AMA 254 single-purpose analyzer (Altec Inc., Prague, Czech Republic). This method is based on measurements of mercury vapor absorbance after thermal release from a gold amalgamator (widely described in Houserová *et al*., 2006). The limit of detection was 0.36 μg/kg. The contents of lead (Pb), cadmium (Cd), zinc (Zn), nickel (Ni), chromium (Cr) and copper (Cu) were assessed after wet mineralization. The samples of fish tissues, sediments and zoobenthos in the amount of 1.0, 1.5 and 0.4 g respectively were put into the 100 ml Teflon enclosures together with 5 ml of concentrated HNO_3, 2 ml of 30% H_2O_2 and 5 ml of deionised water and processed as program controlled in an ETHOS SEL micro-wave oven (Milestone Inc., Monroe, USA). After the cooling the samples were transferred to the 25 ml volumetric flask and subsequently Flame AAS (atomic absorption spectrometry) was used (AA-3000 device, Perkin Elmer, USA) to detect the concentrations of Cu and Zn (detection limits of 25, 40 and 60 μg/kg for Cu in fish tissues, sediments and zoobenthos respectively and 250, 400 and 600 μg/kg for Zn; detection limits of 2.0 μg/l for Cu and 20 μg/l for Zn in water). The remaining metals were analysed using ETA-AAS (electrothermal atomisation, atomic absorption spectrometry) on the SPECTR AA-30 device containing the GTA-96 atomizer (Varian Inc., Australia) with the following detection limits in fish tissues, sediments and zoobenthos: 0.1, 0.2 and 0.3 μg/kg for Cd, 2.5, 3.8 and 6.3 μg/kg for Pb, 5.0, 7.7 and 12.5 μg/kg for Cr and 2.0, 3.1 and 5.0 μg/kg for Ni. Detection limits in water were 0.01 μg/l for Cd, 0.2 μg/l for Pb, 0.4 μg/l for Cr and 0.2 μg/l for Ni). These methods are more precisely described in Spurný *et al.* (2009). All detection limits were assessed as the threefold standard deviation of the blank. To verify the analyses, standard reference material DORM-2 (dogfish muscle) was used.

Data processing and statistical analyses

Data from analyses of chub tissues were processed as a small sample analysis according to Horn (1983) using QC-Expert software (TriloByte Ltd., Czech Republic). The outputs were compared to valid legislation such as Government Decree No. 23/2011 Coll. and EC No. 1881/2006 setting the following maximum levels of contaminants in fish meat: mercury 0.50 mg/kg, cadmium 0.05 mg/kg, and lead 0.30 mg/kg. Differences between localities were tested by means, applying the t-test in ADSTAT software (TriloByte Ltd., Czech Republic). According to the results of exploratory data analysis (EDA), the classical Student t-test or t-test for modified skewness was used ($p < 0.05$).

RESULTS

In regard to the contamination of particular compounds of the Dyje River (Table II), important differences between Tasovice and Dyjákovice were found in concentrations of Pb in the water, Pb and Cd in the zoobenthos and Cd and Cr in the bottom sediments, when Dyjákovice locality was more contaminated than Tasovice. Contamination gradients in particular compounds of both localities were set as follows: sediment >> zoobenthos >> water in Pb and Cr; sediment > zoobenthos >> water in Cd, Ni and Zn; zoobenthos > sediment >> water in Cu and sediment ≥ zoobenthos >> water in the case of Hg.

The maximum admissible concentrations in the water, set by Government Decree No. 23/2011 Coll., were not exceeded, but with the exception of mercury. The concentration of this metal at both localities ten times overreached the limit. The limits of trace metal contamination of biota were exceeded only in the case of Hg in the zoobenthos at Dyjákovice, which was slightly above the limit. The limits in sediments were markedly exceeded in Ni at both localities. The concentrations of remaining metals (Pb, Cd and Hg) were below the limit.

With respect to the contamination of fish tissues by trace metals (Table III) in the point of view of human health, the safe limits for fish muscle prescribed by EC No. 1881/2006 were not exceeded in principle. Detected mean concentrations of mercury and cadmium in chub muscle were far below the limit. In the case of lead, the limiting value was reached in Dyjákovice, but not in Tasovice.

III: *Content of trace metals in chub tissues (in mg/kg of wet matter, median ± standard deviation based on median) from the Dyje River in 2008 (age in years, TL - total length in mm, w - weight in g, all given as min.-max.)*

locality	n	age	TL	w	tissue	Hg	Zn	Ni
Tasov	7	5+ – 7+	323–405	411–767	muscle	0.093 ± 0.017	5.200 ± 2.594	0.109 ± 0.046
					liver	0.039 ± 0.007	33.49 ± 13.62	0.091 ± 0.034
Dyjak	7	5+ – 9+	319–392	395–628	muscle	0.057 ± 0.016	3.769 ± 0.617	0.165 ± 0.033
					liver	0.028 ± 0.007	22.760 ± 3.867	0.095 ± 0.160
locality		**tissue**		**Cr**		**Cu**	**Pb**	**Cd**
Tasov		muscle		0.034 ± 0.016		0.231 ± 0.085	0.087 ± 0.200	0.002 ± 0.011
		liver		0.028 ± 0.023		14.700 ± 5.219	0.523 ± 0.289	0.066 ± 0.067
Dyjak		muscle		0.034 ± 0.011		0.221 ± 0.097	0.311 ± 0.160	0.001 ± 0.000
		liver		0.023 ± 0.020		12.505 ± 4.198	0.190 ± 0.061	0.039 ± 0.011

IV: *Limits for human consumption of fish caught in the Dyje River with respect to the trace metal contamination according to JECFA (2007) recommendation (values in kg of wet matter, PTWI – provisional tolerated weekly intake, PMTDI – provisional maximum tolerated daily intake, limits were set for 60 kg human consumer)*

trace metal	Pb	Cd	Zn	Cu	Hg
limit (mg/kg)	0.025	0.007	1.000	0.500	0.005
	PTWI	PTWI	PMTDI	PMTDI	PTWI
intake (kg)					
Tasovice	6.30	140.00	12.55	129.87	3.80
Dyjákovice	4.85	420.00	14.67	140.19	4.17

V: *The above – limit concentrations of trace metals in particular compounds of various rivers in the Czech Republic (concentrations in the water are given in µg/l, remaining in mg/kg of wet matter, the limiting concentrations by Government Decree No. 23/2011 Coll.)*

River	No. of sites	metals above limit (detected concentrations)		
		water (µg/l)	zoobenthos (mg/kg)	sediments (mg/kg)
Jihlava (Makovský *et al.*, 2010)	2	Hg (0.99–2.00)	Pb (25.08)	Pb (120.21)
			Cd (0.18–0.20)	Ni (32.06–90.66)
Jihlava (Spurný *et al.*, 2002)	3	-	Pb (1.92–4.49)	Cd (0.62–0.70)
			Cd (0.21)	Ni (6.40–8.28)
Jihlava (Spurný *et al.*, 2009)	4	-	Pb (1.12–1.42)	Ni (12.29–36.68)
			Cd (0.22–0.57)	
			Hg (0.02–0.07)	
Bečva (Spurný & Mareš, 2005)	4	-	Pb (1.43–3.79)	Ni (12.26–20.23)
			Hg (0.03–0.05)	
Loučka (Vítek *et al.*, 2007)	4	Cu (20.00)	Cd (0.26)	Cd (0.25–0.30)
		Hg (0.1–0.7)	Hg (0.02–0.03)	Ni (13.15–24.98)
Dyje (this study)	2	Hg (0.55–0.64)	Hg(0.02)	Ni (14.36–21.30)

No significant differences between localities were found in chub muscle and liver at $p < 0.05$.

Limits for human consumption of fish caught in the Dyje River according to the recommendation of Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2007) with respect to the contamination by trace metals are given in Table IV. The mercury was the limiting metal at both localities, which allowed maximum tolerated weekly intake of fish muscle up to 3.80 kg in Tasovice and 4.17 kg in Dyjákovice.

DISCUSSION

Compared to the situation in the other rivers of the Czech Republic, the Dyje River belongs to less contaminated streams, as documented in Table V, even though the limits for mercury set by the Government Decree No. 23/2011 Coll. were exceeded in the water and also in the biota. Concentrations of mercury in the water were markedly higher for example in the Jihlava River (Makovský *et al.*, 2010). Beside mercury, the above – limit concentration of copper was found in the water of the Loučka River (Vítek *et al.*, 2007). In the case of zoobenthos, the other authors reported higher concentrations of lead and cadmium, namely in the Jihlava River (Makovský *et al.*, 2010; Spurný *et al.*, 2002, 2009), the Bečva River (Spurný & Mareš, 2005) and the Loučka River (Vítek *et al.*, 2007). The above – limit contents of nickel in the bottom sediments of the Dyje River assessed in this study were similar to those documented in the Bečva River by Spurný & Mareš (2005) and in the Loučka River by Vítek *et al.* (2007), but were lower in comparison with the Jihlava River (Makovský *et al.*, 2010; Spurný *et al.*, 2009).

Chub *S. cephalus* is explicitly mentioned as an indicator in Government Decree No. 23/2011 Coll. In comparison with such legislation, the concentrations of Hg in the tissues of chub exceeded the limits, but those limits are set for the mixed samples of whole fish. Thus our results were compared to EC No. 1881/2006, valid in EU member countries, which sets admissible concentrations directly in the fish muscle. Such comparison revealed the above-limit concentration only in the case of lead in the locality of Dyjákovice.

Comparing the obtained trace metal concentrations in chub tissues to studies from the 1990s, the Dyje River could be considered to have become less contaminated in recent years. Whereas Spurný & Mareš (1995) found mean concentrations of mercury in chub muscle as high as 0.15 mg/kg in Tasovice and 0.17 mg/kg in Hevlín (close to Dyjákovice), the mean values in this study did not exceed 0.1 mg/kg. Even the content of cadmium in our study (0.001–0.003 mg/kg) was ten times lower compared to the data from Hevlín. Spurný *et al.* (1997) also documented higher concentrations of mercury in comparison with our study: 0.13 mg/kg in Tasovice and Hevlín, 0.21 mg/kg in the upstream locality of Podhradí, and even 0.44 mg/kg in the downstream locality of Nové Mlýny. This is in conformity with the general improvement in the levels of water pollution documented in various rivers in the Czech Republic, e.g. the Elbe (Dušek *et al.*, 2005; Žlábek *et al.*, 2005), the Jihlava (Spurný *et al.*, 2002; Peňáz *et al.*, 2005; Houserová *et al.*, 2006; Spurný *et al.*, 2009), and the Morava (Valová *et al.*, 2010), but with the exception of lead. Increased concentrations of this metal exceeding the safe limit for fish muscle were mentioned by Vítek *et al.* (2007). In this study the analysed amount of lead in chub muscle (0.087 mg/kg in Tasovice and 0.311 mg/kg in Dyjákovice) was markedly higher compared to the results of Spurný & Mareš (1995; 0.01–0.05 mg/kg), although it was below the safe limit. As in the case of the Jihlava River, described by Makovský *et al.* (2010), reservoirs might contribute to the decline in trace metal contamination, but the contaminants are stored in the reservoir sediment and could potentially be released in large amounts in the future, especially as a consequence of the deterioration in the chemical parameters of the water.

Making a comparison of maximum tolerated human intake with respect to the fish muscle contamination by trace metals of the Dyje River with the other studies of other Czech rivers, the Dyje River might be considered as less threatened. Makovský *et al.* (2010) documented in the Jihlava River the limit of intake between 1.84–3.15 kg per week, Vítek *et al.* (2007) reported even 1.5 kg in the case of the Loučka River. With respect to the amount of caught fish, on the other hand, the Dyje River represents a potential of intoxication in anglers' families. The anglers' catch per unit of effort (CPUE), calculated according to evidence data of Moravian Anglers' Union in last five years, ranged between 0.22–0.44 kg in Tasovice and from 0.46 to 0.68 kg in Dyjákovice. It means that an average angler is able to catch up to 3.11 kg of fish per week in Tasovice and up to 4.76 kg in Dyjákovice. As reported by Vítek *et al.* (2011), an average angler in the Czech Republic catches 25.6 kg of fish per season in 11–50 visits that means CPUE between 0.5–2.3 kg and a potential to catch up to 16 kg per week. Having in mind the bag limit as high as 7 kg per day, the situation could potentially become more important, because the effort is expected to increase (Vítek *et al.*, 2011). In order to fulfil the limit of health hazard, one would have to consume more than 0.5 kg of fish every day, which is very unusual even in the most successful anglers in the Czech Republic conditions. Therefore the risk of intoxication by heavy metals stays rather theoretical.

CONCLUSIONS

A considerable contamination of the ecosystem of a human altered lowland river was found in the case of the Dyje River. The maximum admissible limits newly adopted by the Czech authorities were exceeded in Hg in the water and zoobenthos and in the case of Ni in the bottom sediments. Regarding the concentrations in fish tissues, the contamination by Hg and Cd has decreased since 1990s, but increased in the case of Pb. Human (especially anglers and their families) are only minimally threatened by trace metal contamination, even when caught fish are consumed regularly.

SUMMARY

The analyses of seven trace metals (Hg, Zn, Ni, Cr, Cu, Pb, Cd) in the water, bottom sediments, macroinvertebrates and fish tissues were performed at two localities on the Dyje River, Czech Republic, at different distances downstream from the Znojmo Reservoir (at 12 and 31 km). The main aims were to determine the present state of trace metal contamination, to evaluate changes in the

contamination of fish fauna by comparing the recent situation with the situation from the 1990s, and to evaluate the risk of human consumption of fish.

The maximum admissible limits, newly adopted in the valid Czech legislation, were exceeded in Hg in the water (assessed concentrations were between 0.55–0.64 µg/l) and in the case of Ni in bottom sediments at both localities (5.23–6.16 mg/kg of wet matter) and also in Hg in zoobenthos sampled at locality further from the reservoir. The important differences between localities were found in concentrations of Pb in the water, Pb, and Cd in the zoobenthos and Cd and Cr in the bottom sediments, when the further downstream locality was more contaminated.

In regard to trace metal contamination of fish tissues, it was found that safe limits for Hg (determined content in the fish muscle ranged between 0.06–0.09 mg/kg of fresh matter), Cd (0.001–0.002 mg/kg) and Pb (0.09–0.31 mg/kg) were not exceeded. No significant differences between localities were found. The contents of Hg and Cd have declined markedly since the 1990s, but the amount of Pb has increased.

Human consumption of fish caught in the Dyje River is most limited by concentration of Hg in the fish muscle. The maximum tolerated intake was set as high as 3.80 kg per week. Such limit represents a theoretical risk of contamination in anglers and their families, since their catch can obviously reach up to 4.8 kg, but the real risk is relatively low.

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Address

Ing. Tomáš Vítek, Ph. D., doc. Dr. Ing. Jan Mareš, prof. Ing. Petr Spurný, CSc., Ústav zoologie, rybářství, hydrobiologie a včelařství, Mendelova univerzita v Brně, Zemědělská 1, 613 00, Brno, Česká republika, RNDr. Josef Hedbávný, Ústav chemie a biochemie, Mendelova univerzita v Brně, Zemědělská 1, 613 00, Brno, Česká republika, e-mail: gabon@centrum.cz, mares@mendelu.cz, fishery@mendelu.cz, hedbavny@mendelu.cz