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Fish fin-clips as a non-lethal approach for biomonitoring of mercury contamination in aquatic environments and human health risk assessment

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HIGHLIGHTS

- Use of fish fin-clips is a suitable non-lethal approach for mercury monitoring.
- THg muscle concentration and muscle/fin-clip THg ratio are negatively correlated.
- Precise prediction of THg muscle concentrations in bream and chub is possible.

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ABSTRACT

Muscle tissue and pectoral fins of two important indicator fish species, frequently used in biomonitoring programs, were sampled and analysed for total mercury content (THg) at six localities within the Czech Republic. The relationship between mercury concentration in muscle and in fin-clips was described. Mean values of THg fin-clip concentration correlate significantly ($p < 0.01$) with those measured in muscle of indicator fish. Concerning comparison among localities, a coefficient of determination (r^2) of 0.85 and 0.91 was found between studied approaches in the case of chub (*Squalius cephalus*) and bream (*Abramis brama*), respectively. THg muscle concentrations (mean, $n = 10$) varied from 0.181 to 0.491 μg g^{-1} wet, depending on indicator species and locality. A concentration-dependent relationship between muscle and fin-clip THg content was found in both species. Based on this finding, a novel method for the prediction of muscle THg concentration from fin-clips analysis was developed. The difference between measured and predicted muscle concentration was below 10% in both indicator species at most sampling sites. Use of fish fin-clips was found as an appropriate nonlethal approach for the evaluation of mercury contamination in aquatic environments as well as for human health risk assessment.

1. Introduction

Many human activities cause some type of pollution that results in surface or underground water contamination. Certain xenobiotics, such as toxic metals remain in the environment for a very long time, as these are not degradable under natural conditions. Mercury is considered as the most dangerous toxic metal, due to its neurotoxicity and levels found in the aquatic environment (Noel et al., 2013; Cerveny et al., 2014; Asefi and Zamani-Ahmadmahmoodi, 2015). Water sediments, soils and vegetation present an important sink for mercury, which results in its presence in food chains at localities where there have been no sources of pollution for many years (Vanhattum et al., 1993; Abel, 1996; Nguetseng et al., 2015).

After being released into the air, mercury returns to the ground through precipitation and enters the aquatic environment; thus, atmospheric deposition is a dominant source of mercury (Lepom et al., 2012). Mercury is neurotoxic in both its organic and inorganic forms (Atchison and Hare, 1994; Fretham et al., 2012), and the commonly encountered form of mercury, methylmercury (MeHg), is the most toxic form affecting aquatic biota (Lasorsa and Allengil,

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1995; Maceda-Veiga et al., 2012). MeHg is primarily responsible for bioaccumulation in the muscle tissue of fish with a methylmercury fraction of 83–90% of the-total mercury concentration (Lasorsa and Allengil, 1995; Kannan et al., 1998; Marsalek et al., 2005; Kruzikova et al., 2008).

Because of these mentioned characteristics, mercury has become one of the most monitored xenobiotics in aquatic environments. Use of aquatic organisms, especially fish, for monitoring of mercury is necessary as concentrations of mercury in water are usually very low. Furthermore, water is not a relevant matrix for assessing human health risks (Orban et al., 2007; Cerveny et al., 2014). No biodegradation of Hg and ability to enter the food web results in its bioaccumulation in various animal species along the trophic chain, especially in fish that are at the top of the food pyramid in an aquatic ecosystem (Dusek et al., 2005; Li et al., 2015).

As Hg is listed as a priority substance in the field of water policy and it is regulated under the Water Framework Directive, an Environmental Quality Standard (EQS) of 0.02 $\mu g\ g^{-1}$ (wet weight) for water biota was set by the Directive of the European parliament and Council (2013). Furthermore, Maximum Level (ML) of 0.5 $\mu g\ g^{-1}$ for fish muscle and fishery products intended for human consumption was set by European Commission (2008).

Fish consumption represents the main exposure pathway for mercury contamination in humans (Cuadrado et al., 1995; Cerveny et al., 2014), thus muscle tissue of adult fish is of interest to researchers and regulatory bodies all over the world. This approach is appropriate in the case of marketable fish, but in the case of wild fish, it can induce a conflict of interests with the internationally accepted guidelines for the protection of human subjects and animal welfare. These principles are also included in the EU Directive on the protection of animals used for scientific purposes (European parliament and Council, 2010). Moreover, due to the valid legislation, fish that are sampled for scientific or biomonitoring purposes cannot be used as a human food or for feeding of livestock, thus it ends as a veterinary waste.

Several studies have been published that deal with non-lethal approaches for the evaluation of mercury contamination in aquatic environment using fish as bioindicators. Some of them evaluated different biopsy techniques (Schmitt and Brumbaugh, 2007; Ackerson et al., 2014), and some were using scales or fin-clips for analysis and prediction of muscle tissue mercury concentrations (Gremillion et al., 2005; Ryba et al., 2008; Cervenka et al., 2011; Piraino and Taylor, 2013). The aim of the present study is to contribute to the discussion and extend the knowledge of the relationship between muscle and fin-clips mercury concentration in freshwater fish. Muscle and fin-clip mercury concentrations analysed in the two most used indicator fish species from six localities within Czech Republic are presented.

2. Material and methods

2.1. Monitored sites

Six sampling sites were chosen within Czech Republic. These sites were chosen according to occurrence of indicator fish species and they were supposed to cover both areas with low and higher mercury contamination. Sampling site Neratovice lies on the Elbe River in an area, where important source of pollution – the chemical plant Spolana Neratovice is located. Another three sampling sites were chosen in the vicinity. Kostelec and Obristvi are located upstream and downstream, respectively, from the Neratovice, and Mlekojedy is a sandpit without connection to the Labe River watercourse close to the Neratovice. The remaining two sampling sites are located in the southern part of the Czech Republic. Hnevkovice and Rimov are the dams located on Vltava and Malse River, respectively.

2.2. Fish sampling and sample preparation

European bream (*Abramis brama*) and European chub (*Squalius cephalus*) were chosen as the indicator fish species to be caught at all experimental sampling sites. Both bream and chub are native species of Czech Republic and they are not artificially stocked. These species are most often used as fish bioindicators in the central Europe monitoring programs. Fish were caught by an electrofishing device, gillnets and angling. Both indicator species were caught at all sampling sites except for Mlekojedy, where only bream was caught.

Ten specimens of both fish species were caught at each sampling site. All of the sampled fish were measured and weighed. Muscle tissue from the mid-dorsal part of the body and the fin-clip of pectoral fin were obtained from all individuals. The samples were placed into 2-ml Eppendorf tubes, cooled down and stored at 4 °C during transport to the laboratory, where they were kept frozen (−20 °C) until analysis was performed. Fish sampling was realized from April to June 2015 and analyses were completed in July of the same year. All experimental animals were handled in accordance with the national and institutional guidelines for the protection of human subjects and animal welfare (European parliament and Council, 2010).

2.3. Mercury analysis

The total mercury (THg) content was determined directly in the sample units by a selective mercury analyser (Advanced mercury analyser, AMA-254, Altec) based on atomic absorption spectroscopy (AAS). This method is based on thermal decomposition of a sample in a flow of oxygen, the capture of mercury by a gold amalgamator, and measurements of the mercury vapour absorbance after thermal release from the amalgamator. Briefly, 100–200 mg of thawed muscle/fin-clip was loaded in nickel boat and analysed. No pre-treatment of muscle samples was made, and fin-clips were only washed with deionized water prior to analysis. Final concentration of the sample was calculated as a mean of two independent measurements and it is expressed as wet weight (ww) concentration. For calibration of the instrument, MERCK calibration solution–CertiPUR was used. To demonstrate quality assurance/quality control performance of the analytical method, blank samples and certified reference material BCR-422 (lyophilized cod muscle) were used. THg concentrations in blank samples were below the limit of detection. Based on BCR–422, the method uncertainty of 3.54% was found and expressed as a relative standard deviation (RSD) of seven measurements (at $0.559 \pm 0.016\ \mu g\ g^{-1}$ Hg). Recovery for the same reference material was from 100% to 105% ($n = 7$).

2.4. Prediction of THg muscle concentration from fin-clips analysis

Individuals of each species from all sampling sites were divided into three groups according to the THg concentration measured in fin-clips. A median of muscle/fin-clips THg ratio (quotient) was then calculated for each of these three groups and it was indicated as a "prediction factor" (PF). The prediction factors and ranges of fin-clip THg concentrations that were used for the calculation are given in Table 1. Concentrations of THg measured in fin-clips were transferred into predicted muscle concentration using appropriate PF in all individuals at all sampling sites. The mean predicted muscle concentration was then calculated for each locality from individually acquired values in both indicator species.