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# Fish as a bioindicator of heavy metals pollution in aquatic ecosystem of Pluszne Lake, Poland, and risk assessment for consumer's health

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ABSTRACT

Heavy metals content (Zn, Cu and Hg) were measured in gills, liver, gonads and muscles of perch, *Perca fluviatilis* (L.) and roach, *Rutilus rutilus* (L.) from Lake Pluszne (north-eastern Poland). Correlations between heavy metals levels and total length, weight, HSI, GSI and FCF were examined. As expected, muscles contained the significantly highest values of Hg ($P \leq .05$). The concentrations of Zn were significantly higher in gills of roach and gonads of perch ($P \leq .05$), while the liver of fish accumulated significantly more Cu than other organs ($P \leq .05$). In all organs of perch the higher content of mercury was found ($P \leq .05$). The value of Zn and Cu was highest in organs of roach ($P \leq .05$) (with the exception of Zn in muscles $P > .05$). Sequence of metals in both species was Zn > Cu > Hg. Only in muscle tissue, Hg was significantly positive correlated with weight of roach ($r = 0.811$, $P = .045$) and perch ($r = 0.652$, $P = .041$), and total length of roach ($r = 0.806$, $P = .005$). A positive relationship was also observed between Zn concentration in gills of perch and their weight ($r = 0.634$, $P = .049$). In contrary, Zn in gills of roach decreased with weight ($r = -0.693$, $P = .026$)) and length ($r = -0.668$, $P = .035$). Cu concentration in liver of perch was statistically positively correlated with HSI ($r = 0.717$, $P = .020$), whereas Hg content in muscle tissue of roach with FCF ($r = 0.643$, $P = .045$). There was negative relationship between Hg in perch gonads and GSI ($r = -0.808$, $P = .005$). Metal pollution index (MPI) in gills, liver, gonads and muscles of roach was 7.68, 7.24, 6.77 and 3.13, respectively, whereas in these organs of perch was 3.25 (gills), 4.75 (liver), 5.84 (gonads) and 4.44 (muscles), therefore the contamination of each tissue ranged from very low contamination to low contamination. The concentration of mercury was lower than the maximum acceptable limit estimated by the Commission Regulation (EC) No 629/2008 of 2 July 2008. The values of HI and THQ were below 1, which means that consumption of these fish is not hazardous to the consumer.

## 1. Introduction

Living organisms, including fish, require heavy metals occurring in trace amounts to survive. Excessive amounts of some metals can be detrimental to the organism, while mercury, lead and cadmium do not matter on organisms, and during prolonged exposure to the bodies can cause illness or death (Azaman et al., 2015). Heavy metals belong to the group of elements that they have density at least 5 times higher the density of water. Their toxicity among others depends on exposition route, nutritional status of exposed individuals, chemical species or age, genetics and gender (Tchounwou et al., 2012). Aquatic organisms, including fish, absorb the pollutants from water (directly) and from food chains (indirectly). Some of the toxic effects of heavy metals on fishes and aquatic invertebrates lead to reduction of the developmental growth, increase of developmental anomalies or to reduction of fish survival (especially at the beginning of exogenous feeding) and to extinction of the whole population in polluted reservoirs (Khayatzadeh and Abbasi, 2010). The metals get into the aquatic animals mainly with food. In fish, they can also come via mechanical capture of suspended particles of hydroxides in gills, as well as chemical absorption of ions on the mucous membrane (Shesterin, 2010). Yancheva et al. (2015) reported that research, i.e. bioaccumulation, histological and biochemical analyses or other investigated biomarkers mainly used are the respiratory organs – the gills and parenchymal organs – liver and kidney, whereas in terms of human health the most appropriate tissue are the muscles of fish. In biomonitoring studies on the influence of pollutants present in the aquatic environment before death and/or disease in biota as bioindicators were used fish (Dalzochio and Gehlen, 2016). About the fact that the fish may be used as bioindicators of environmental pollution is mentioned in many publications (Fatima et al., 2014; Govind and Madhuri, 2014; Authman et al., 2015; Awheda et al., 2015; Jaćimović et al., 2015; Salamat et al., 2015; Yancheva et al., 2015;

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Nwabunike, 2016). This is in accordance with definitions of bioindicator that says that is an organism or part of an organism and a community of organisms containing a series of information on the quality of the environment or its parts (Markert, 2008). Fish may be applied as biomarkers in order to elucidate the aquatic behavior of environmental pollutions, and to assess exposure of aquatic organisms (Van der Oost et al., 2003). So called biomarker response can be measured in organs, tissues, cells or body fluids. That response is a reaction on xenobiotics and is restricted to physiological, biochemical, cellular or even molecular changes (Lam and Gray, 2003, Facey et al., 2005). Biomarkers, parameters measurable at the sub organismic level, can change structurally or functionally. These changes indicate the influence of the environment, particularly the action of environmental pollutants (Markert et al., 2003). The tools for monitoring fish, as well as providing important information on environmental conditions, including pollution, may be indicators calculated on the basis of hepatosomatic index (HSI), gonadosomatic index (GSI) and condition factor (CF) (Kreitsberg, 2014). HSI provides an indication on status of energy reserve in an animal, this has been observed in fish because in a poor environment with less energy reserved in the liver, fish usually have a smaller liver (Lee et al., 2012). HSI value provides many information about the healthy condition of fish and also about the quality of aquatic ecosystem, because higher value of HSI means fish are growing rapidly and have a good environment in which they live and if value of HSI is less it means fish do not grow well and it is facing unhealthy environmental problems (Kareem et al., 2015). The condition factor FCF of different populations of the same species may indicate food availability, timing and duration of breeding process (Zamri et al., 2016). In addition, these indicators may indicate changes in fish health due to stress (Parente, Hauser-Davis, 2013). GSI as an indicator of the state of maturity and gonadal development (Hama et al., 2015) is a convenient biomarker because depending on the severity of exposure to xenobiotics, the sublethal effects can be to reduce growth and impair reproduction as well as can restrict physiological capacity. You can say that GSI as a biomarker of exposure to toxicants and that histopathology represents a useful tool to assess the degree of contamination (Pieterse, 2004). A number of the literature cites examples of applications of above biomarkers to do the assessment of contamination aquatic ecosystem and fish living in it (Van der Oost et al., 2003; Facey et al., 2005; Patino et al., 2012; Liebel et al., 2013; Çiftçi et al., 2015; Sabullah et al., 2015; Dalzochio and Gehlen, 2016). It is known that accumulation of metals in fish organs also depends on environmental conditions (i.e. pH, water temperature, hardness, etc.), exposure duration, feeding habits and species-specific living (Zeitoun and El-Sayed, 2014). According to Jezierska and Witeska (2006) and Jakimska et al. (2011), the bioaccumulation of metals also depends on other abiotic (distribution of metals in its environment, salinity and interactions with other metals) and biotic factors (species, size, age, sex, feeding type and position in the trophic chain).

Consequently, the aim of this study was to estimate whether metals content related to fish species, different organs (muscles, liver, gonads and gills) and the factor condition, body weight or total length of fish from Lake Pluszne. At the same time they attempted to determine whether the fish and these organs can be a good indicator of pollution of the freshwater reservoir, although today the water has chemical good status. This study also evaluated health risk for humans consuming these fish by using THQ and HI.

## 2. Material and methods

### 2.1. Sampling and sample preparation

Ten individuals of each species (roach, *Rutilus rutilus* L. and perch, *Perca fluviatilis* L.) were collected from lake Pluszne included in the Olsztyn Lake District (Poland). The body weight ( ± 0.01 g) and total length ( ± 0.01 cm) of each fish are presented in Table 1. Sample preparation for further analysis is described in an earlier publication (Łuczyńska et al., 2016)

### 2.2. Element analysis

For the determination of mercury sample preparation is described in an earlier publication (Łuczyńska et al., 2016).

For the determination of zinc and copper samples were initial dried at 65–70 °C in quartz tests, then were dried at 105 °C to achieve constant weight. Muscle tissue were dry-digested at 450 °C using laboratory furnaces (Nabertherm, Germany). After obtaining the white ash, it was dissolved in 1 M $HNO_3$ (Suprapur-Merck, Darmstadt, Germany) and transferred with deionized water (Merck-Millipore Elix Advantage 3, USA) into a volumetric flask of volume 25 mL. Liver, gills and gonads samples were weighed and placed into boro-silica glass tube. The prepared sample was treated with a nitric and perchloric acid mixture (Merck, Darmstadt, Germany, 3:1, v/v) at a temperature of 190 °C and wet mineralized using heating block with a programmable temperature and digestion time (DK 20, VELP Scientifica, Italy). The resulting solution was transferred to flasks with a volume of 25 mL and using deionized water.

### 2.3. Instrumental analysis and quality control

The total mercury was measured by atomic absorption thermal decomposition using Milestone DMA-80 with dual-cell (Italy). Parameters of the method used for mercury determination are described in an earlier publication (Łuczyńska et al., 2016). The detection limit (LOD) was 0.02 μg $kg^{-1}$.

Samples were prepared in two parallel wells. Four blanks and four standards were analyzed with each batch of samples. Concentrations of these elements were measured using the flame atomic absorption spectrometry (iCE 3000 Series AAS, Thermo Scientific, England) equipped with a correction deuterium lamp. The absorptions wavelength was as follows: 213.9 nm for zinc and 324.8 nm for copper. The calibration curves were prepared using four solution standards (1000 μg $L^{-1}$) with 0.1 M $HNO_3$ supplied by J.T.Baker® (Netherlands). The calibration curves were linear within the range of metal concentration (regression coefficients $R^2 \geq 0.999$). The detection limits (LOD) were 0.1 mg $kg^{-1}$ for Zn, and 0.05 mg $kg^{-1}$ for Cu, and the sensitivity were 0.05 mg $^{-1}$ L and 0.02 mg $L^{-1}$, respectively.

The quality control of methods was tested using the elements in reference material: BCR CRM 422 (muscles of cod *Gadus morhua* (L.)) with a certified value of zinc, copper and mercury. The recovery rates of Zn, Cu and Hg were: 105.0%, 103.0% and 100.2%, respectively.

1. *FCF - Fulton's condition factor* (Table 1) (Hamid, Łuczyńska et al., 2015, 2016)
   The fish condition was calculated as follows:

   $$FCF = 100 \times W/L^3$$

   where: W – total body weight of fish (g), L – total length of fish (cm). The relationship of total length-weight of each fish shows by the formula (Le Cren, 1951; Datta et al., 2013)

   $$W = a\ L^b$$

   and was expressed in its logarithmic form of linear equation as:

   $$\log W = \log a + b \log L$$

   where parameters: log W – (y); log L – (x)
2. *HSI - Hepatosomatic index and GSI - Gonadosomatic index* were estimated according to the following pattern (Sadekarpawar and Parikh, 2013) (Table 1)

   $$HSI = 100 \times (\text{Liver weight/Fish weight}),$$
   $$GSI = 100 \times (\text{Gonad weight/Fish weight}).$$