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Assessment of Landfill Pollution Load on Hydroecosystem by Use of Heavy Metal Bioaccumulation Data in Fish

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***Abstract*—**Landfill leachates contain a number of persistent pollutants, including heavy metals. They have the ability to spread in ecosystems and accumulate in fish which most of them are classified as top-consumers of trophic chains. Fish are freely swimming organisms; but perhaps, due to their species-specific ecological and behavioral properties, they often prefer the most suitable biotopes and therefore, did not avoid harmful substances or environments. That is why it is necessary to evaluate the persistent pollutant dispersion in hydroecosystem using fish tissue metal concentration. In hydroecosystems of hybrid type (e.g. river-pond-river) the distance from the pollution source could be a perfect indicator of such a kind of metal distribution. The studies were carried out in the Kairiai landfill neighboring hybrid-type ecosystem which is located 5 km east of the Šiauliai City. Fish tissue (gills, liver, and muscle) metal concentration measurements were performed on two types of ecologically-different fishes according to their feeding characteristics: benthophagous (Gibel carp, roach) and predatory (Northern pike, perch). A number of mathematical models (linear, non-linear, using log and other transformations) have been applied in order to identify the most satisfactorily description of the interdependence between fish tissue metal concentration and the distance from the pollution source. However, the only one log-multiple regression model revealed the pattern that the distance from the pollution source is closely and positively correlated with metal concentration in all predatory fish tissues studied (gills, liver, and muscle).

***Keywords*—**Bioaccumulation in fish, heavy metals, hydroecosystem, landfill leachate, mathematical model.

I. INTRODUCTION

URBAN waste landfills are one of the main point sources which, are associated with negative effects on the environment and human health, causing soil and groundwater pollution [1]-[8]. Landfills naturally generate leachate, which usually have steady composition and contains numerous persistent inorganic (heavy metals) and organic pollutants, which are non-biodegradable and commonly extremely toxic to aquatic life [3]-[7]. Landfills could remain long-term pollution sources (for many hundreds of years) following the closure [9]. Majority of closed landfills do not fully comply with the requirements of the Landfill Directive (99/31/EC) [10]. When released unchecked into the aquatic environment leachates change the chemical composition of the water and sediment, disturb the biological balance of the self-cleaning processes, which can lead to unpredictable changes in the environment [11]. Furthermore, persistent pollutants migrate from one biological system to another and accumulate in aquatic organisms and ecosystems [12].

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Heavy metals (Zn, Cu, Ni, Cr, Pb, Cd) are assigned to priority hazardous substances (pollutants) in many countries due to their toxicity, non-degradation, indeterminate persistence, and affinity for bioaccumulation (Directive 2008/105/EC 2008; US EPA 2009) [13], [14].

Furthermore, such heavy metals as zinc, copper, nickel and hexavalent chromium were selected as toxic indicators of general ambient water quality [15].

Fish (many of them top-consumers of the food chain) bioaccumulate heavy metals in organs/tissues through two main mechanisms: via the direct uptake from water by gills (bioconcentration) and via the consumption of contaminated food (biomagnification) [18]. Bioconcentration and biomagnification are capable of leading to toxic effects of metals in fish, even at low exposure levels as they integrate into important protein synthesis reactions and as a result disturb vital processes [16].

Heavy metal bioaccumulation in fish depends on a complexity of a lot abiotic and biotic factors such as: physicochemical characteristics of the water, chemical speciation of the metal and its bioavailability, metal concentration in water, sediment and food-objects, fish species, its trophic level and feeding habits, size, age and gender, species-specific differences in sensitivity to various metals and accumulative-metabolic activity, contaminant uptake route, etc. [20], [21].

Due to the property to accumulate persistent pollutants, fish are an excellent bioindicators reflecting the relative health of aquatic ecosystems [17]. Tissue concentrations of chemicals are a function of uptake, storage, and excretion and therefore, are excellent indicators of the environmental load of a specific toxicant [19].

Fish are freely-swimming organisms. Therefore, they must be in physiological harmony with the surrounding environment [22]. Sensitive salmonid fishes are able to detect and avoid heavy metals even at environmentally-relevant concentrations [23]. Meanwhile, other eurytopic, pollution-resistant species can prefer contaminated sites, apparently, due to the motivation to occupy suitable biotopes.

Heavy metal spread in the aquatic ecosystem is one of the most important factors deciding its ecotoxicological state [24].

The distance from the pollution source plays a key role in metal migration, dispersion, and distribution in an ecosystem, especially in that of hybrid type (river-pond-river). It would be logical to assume that the farther the biotope is located from the pollution source the less metals fish will accumulate in the tissues. Moreover, a number of investigations confirm this suggestion: fish under metal pollution gradient conditions accumulate different content of heavy metals in various sites [25]-[29].

Metal content in fish tissues, perhaps, could be a perfect indicator of metal dispersion, and distribution in an ecosystem. Therefore, it is very important to determine and evaluate the relationship between the distance from the pollution source and fish tissue metal concentration as well as metal accumulation patterns in fish from different ecological groups according to their feeding properties (bethophagous or predatory) under metal pollution gradient conditions, the significance of metal bioaccumulation data in establishing and predicting pollutant dispersion and distribution in biota and aquatic ecosystem in general.

In [30], correlation analysis (Pearson *R*) between different heavy metals, their concentrations in tissues and various external and internal biotic and abiotic factors showed that there is no single rule in patterns of heavy metal bioaccumulation in fish. Particular metal accumulation is more affected by physicochemical parameters of the water, while others by biological parameters (condition factor, gill and liver somatic index, general water toxicity, etc.). No significant correlation between the distance from the pollution source and fish tissue metal concentration was established. The only one indisputably pattern was identified concerning zinc and mercury bioaccumulation: bentophagous fish accumulate more zinc than predatory fish; meanwhile predatory fish accumulate more mercury than bentophagous fish. Zinc was accumulated mostly in the gills while mercury in the muscle.

The objectives of the present study were: (1) to determine whether the relationship between the distance from the pollution source and fish tissue metal concentration in a hybrid type (river-pond-river) aquatic ecosystem permanently polluted by closed landfill leachate really exists using more complex mathematical methods; (2) to establish the patterns of metal bioaccumulation in fish from different ecological groups according to their feeding properties (benthophagous and predatory) along a metal pollution gradient; (3) to assess the significance of metal bioaccumulation data in establishing and predicting metal dispersion and distribution in fish and aquatic ecosystem in general.

II. Materials and Methods

A. Study Area

The Kairiai landfill is located 5 km east of the Šiauliai City (55°55'42.7", 23°23'42.81", WGS). The landfill began performance in 1960 and was closed in 2007. During the landfill performance, a huge quantity of household and industrial waste containing toxic substances was deposited in it. Still today, the landfill continues to seep leachate, and the flow is channeled to two isolated holding reservoirs, maintained under open air conditions. In our opinion, the landfill leachate penetrates through permeable soils from holding reservoirs and pollutes neighbouring water bodies. Existence of the pollution gradient was confirmed in the previous study because water quality parameters and general toxicity were completely depended on the distance from leachate holding reservoirs [31]. The hydroecosystem incorporated in the landfill is a representative hybrid type (river-pod-river) aquatic ecosystem. It consists of a nameless drainage channel surrounding the landfill, which flows for 1.5 km into the Ginkūnai pond (of 1.1 km^2 area). The channel supplies the pond with water. In turn, Švedė Creek flows out of the pond. In total, six sampling sites (No. 0, 1, 2, 3, 4, 5) were set at increasing distances from the leachate-holding reservoirs in the drainage channel, the pond and the Creek, the distances being about 10, 800, 1300, 2200, 2900 and 3200 meters along the water flow from the reservoirs, respectively (Fig. 1).

B. Fish Sampling and Analysis for Heavy Metals

Fish samples in the river-type water bodies were collected by electric fishing gear (HANS Grassl GmbH IG 200/2, Germany) (current – 540 V, frequency – 20-60 Hz, pulse duration of 2-12 m s^{-1}) and in the pond using gill-nets of various mesh sizes in April-May 2012. After the experimental fishing was completed, individuals of the same length group and approximately of the same weight were selected. Predatory fish (Northern pike and perch) and benthophagous fish (Gibel carp and roach) were pooled into two ecologically different groups (bentophagous and predatory fish). Fish were measured (total and standard length in mm), weighted (total and eviscerated weight in g), then required tissues and organs for morphological and metal content analysis were removed and also weighted (± 0.001 g): muscle without skin (~3 g), gills and liver (whole organ, respectively).

In total, from 6 to 9 individuals of mentioned-above groups were used for every sampling site. Body tissue samples were hot air oven-dried at 85ºC for 24 hours until reached a constant weight. Then they were microwave-digested in aqua regia (concentrated HNO_3 and HCl at a ratio of 1:3 v/v) for 50 minutes at a temperature ~ 180°C. Heavy metal analysis was performed by atomic absorption spectrophotometry on Varian Spectr AA 55 (USA) with a graphite furnace technique in accordance with standardized procedure ISO 11047:2004 using a graphite furnace technique [32]. Mercury analysis was performed according to ISO 16772:2004 the final concentration being expressed as mg/kg wet weight [33].

C. Statistics

We decided to improve working hypothesis that the relationship between the distance from the pollution source and fish tissue metal concentration really exists (the farther away the fish biotopes are located from the pollution source; the less heavy metals fish accumulate) using multiple regression models. For this purpose, we introduced a new variable average metal concentration in the definite fish tissue.

What means the sum of all average metal contents in the definite tissue (gills, liver or muscle) divided by a number of metals measured (in our case $N = 5$). Then we performed log-transformation of all the data. Logarithmically transforming variables in a regression model is a very common way to handle situations where a non-linear relationship exists between the independent and dependent variables. Using the logarithm of one or more variables instead of the un-logged form makes the effective relationship non-linear, while still preserving the linear model. Logarithmic transformations are also a convenient means of transforming a highly skewed variable into one that is more approximately normal. (In fact, there is a distribution called the log-normal distribution defined as a distribution whose logarithm is normally distributed – but whose untransformed scale is skewed) [34].

Prior to multiple regression model construction, we performed simple pairwise comparisons (Pearson correlation) between all four possible variable combinations of transformations involving logarithms: the linear case with no transformations, the linear-log model, the log-linear model, and the log-log model [34].

The distance from the pollution source (m) was assumed as the dependent variable, while fish tissue metal concentrations were assumed as independent variables. During multiple regression model construction, we used mixed-effect models (average metal in parallel with a single metal concentration in the definite fish tissue) all possible variable combinations, un-logged and log-transformed variables [35], [36]. Multiple regression model construction was performed until more significant beta (β) coefficients at $p < 0.05$ were obtained.

All statistical calculations were performed using STATISTICA 7.0 (StatSoft Inc., Tulsa, Oklahoma, USA) software.

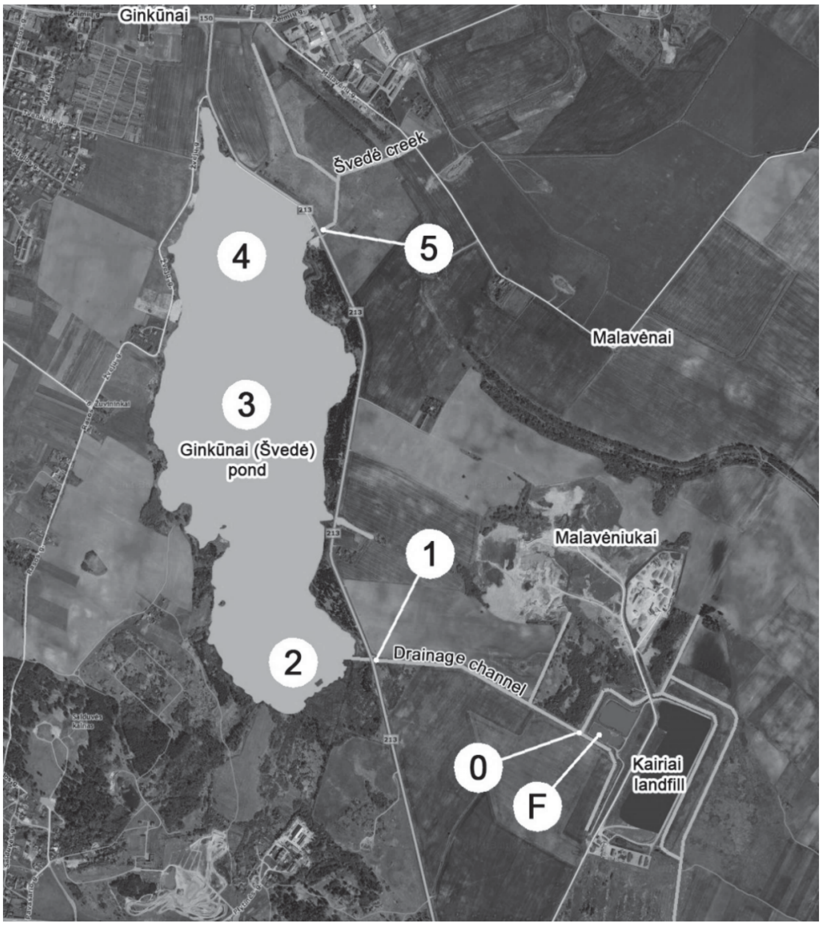

Fig. 1 The scheme of the study area and sampling sites (s/s): landfill leachate holding reservoir (F), drainage channel (site No. 0 and 1), Ginkūnai pond (site No. 2, 3, and 4) and Švedė creek flowing out of the pond (site No. 5)

III. Results

Pairwise interdependence comparison (Pearson correlation) between variables using all possible log-transformations showed that only in one case marginally significant non-linear relationship between the logarithm of the distance from the pollution source and average heavy metal or average zinc content in the gills of predatory fish exists (Tables I and II).

Similarly, we managed to construct only two significant models of non-linear multiple regressions: using average heavy metal and average zinc concentrations in body tissues of predatory fish. These models included log-transformed distance from the pollution source as depended variable and non-logged fish tissue metal concentration as independent variables (log-linear multiple regression) (Tables III and IV).

TABLE I
CORRELATION (PEARSON *R*) BETWEEN DISTANCE TO FROM POLLUTION SOURCE (M) AND AVERAGE HEAVY METAL CONCENTRATION (MG/KG OF W/W) IN BODY TISSUES OF FISH FROM DIFFERENT ECOLOGICAL GROUPS. ALL POSSIBLE COMBINATIONS OF LOG-TRANSFORMATIONS

Sampling sites	Distance from pollution source	Average heavy metal concentration in fish body tissues (mean ± SD)					
		Bentophagous			Predatory		
		Gills	Liver	Muscle	Gills	Liver	Muscle
0	10	10.4 ± 9.7	10.7 ± 6.3	2.6 ± 1.6	16.1 ± 15.6	7.5 ± 5.8	1.1 ± 1.0
1	800	18.4 ± 18.0	10.9 ± 9.8	2.2 ± 2.2	3.0 ± 3.0	4.5 ± 4.5	1.5 ± 1.5
2	1300	15.8 ± 8.5	6.3 ± 2.6	1.8 ± 0.8	3.1 ± 1.7	4.5 ± 1.9	1.0 ± 0.6
3	2200	12.7 ± 6.9	6.1 ± 2.2	1.0 ± 0.5	2.6 ± 1.4	4.6 ± 2.0	1.0 ± 0.5
4	2900	17.0 ± 9.2	5.4 ± 1.9	0.02 ± 0.01	2.7 ± 1.4	4.2 ± 1.8	0.6 ± 0.3
5	3200	13.4 ± 13.0	11.2 ± 8.9	1.6 ± 1.6	9.1 ± 7.5	7.6 ± 4.4	0.1 ± 0.1
Linear model (Y = a + b X)							
	R	0.18	0.34	0.78	0.42	0.10	0.80
	p	0.74	0.51	0.06	0.41	0.85	0.06
Logarithmic model (Y = a + b ln$X$)							
	R	0.24	0.37	0.56	0.31	0.11	0.73
	p	0.64	0.47	0.24	0.55	0.83	0.1
Log-linear model (ln$Y$ = a + b X)							
	R	0.57	0.44	0.69	0.81*	0.51	0.41
	p	0.24	0.38	0.13	0.049	0.30	0.42
Log-log model (ln$Y$ = a + b ln$X$)							
	R	0.63	0.46	0.41	0.71	0.52	0.40
	p	0.18	0.36	0.42	0.11	0.29	0.44

Note: Asterisks (*) denote significant *R* ($p < 0.05$)

TABLE II
CORRELATION (PEARSON *R*) BETWEEN DISTANCE FROM POLLUTION SOURCE (M) AND AVERAGE ZINC CONCENTRATION (MG/KG OF W/W) IN BODY TISSUES OF FISH FROM DIFFERENT ECOLOGICAL GROUPS. ALL POSSIBLE COMBINATIONS OF LOG-TRANSFORMATIONS

Sampling sites	Distance from pollution source	Average zinc concentration in fish body tissues of fish (mean ± SD)					
		Bentophagous			Predatory		
		Gills	Liver	Muscle	Gills	Liver	Muscle
0	10	49.2 ± 0.0	32.1 ± 0.0	8.5 ± 0.0	78.4 ± 0.0	29.9 ± 0.0	5.1 ± 0.0
1	800	90.5 ± 0.0	50.0 ± 0.0	10.9 ± 0.0	14.8 ± 0.0	20.7 ± 0.0	7.6 ± 0.0
2	1300	79.0 ± 7.7	20.4 ± 13.6	7.1 ± 2.2	15.5 ± 4.5	18.4 ± 0.8	4.6 ± 4.7
3	2200	62.5 ± 18.5	19.3 ± 2.5	3.8 ± 3.5	13.1 ± 1.9	18.6 ± 4.5	4.7 ± 0.6
4	2900	84.0 ± 20.0	16.3 ± 1.3	0.0 ± 0.0	13.2 ± 1.3	17.5 ± 2.7	2.5 ± 2.2
5	3200	65.7 ± 0.0	46.0 ± 0.0	7.9 ± 0.0	44.2 ± 44.0	26.5 ± 22.2	0.0 ± 0.0
Linear model (Y = a + b X)							
	R	0.20	0.17	0.45	0.42	0.35	0.69
	p	0.70	0.74	0.45	0.41	0.50	0.20
Logarithmic model (Y = a + b ln$X$)							
	R	0.27	0.24	0.42 (N = 5)	0.31	0.34	0.75 (N = 5)
	p	0.60	0.65	0.48	0.55	0.51	0.15
Log-linear model (ln$Y$ = a + b X)							
	R	0.59	0.13	0.32	0.82*	0.71	0.26
	p	0.21	0.81	0.60	0.048	0.12	0.67
Log-log model (ln$Y$ = a + b ln$X$)							
	R	0.66	0.22	0.34 (N = 5)	0.71	0.68	0.33 (N = 5)
	p	0.15	0.68	0.57	0.11	0.14	0.59

Note: Asterisks (*) denote significant *R* ($p < 0.05$)

TABLE III
RELATIONSHIP BETWEEN DISTANCE FROM POLLUTION SOURCE (M) AND AVERAGE HEAVY METAL CONCENTRATION IN PREDATORY FISH BODY TISSUES (MG/KG OF W/W). COMPLETE MULTIPLE REGRESSION ANALYSIS RESULTS

N=6	Regression Summary for Dependent Variable: ln(DISTANCE) R=0.99999851 R²=0.99999702 Adjusted R²=0.99998512 F(4.1)=84033, p<0.00259 Standard Error of estimate:0.00126					
	Beta	Standard Error of Beta	B	Standard Error of B	t(1)	p-level
Intercept			6.30941	0.641794	9.8309	0.010189
AM-PR-G	-1.51151	0.085807	-0.60275	0.034217	-17.6153	0.003207
AM-PR-L	0.71310	0.094256	0.97260	0.128556	7.5656	0.017026
AM-PR-M	-0.33173	0.040224	-1.48067	0.179539	-8.2471	0.014386

Variable	Variables currently in the Equation; Dependent Variable: ln(DISTANCE)			
	Partial Correlation	Semi-partial Correlation	Tolerance	R-square
AM-PR-G	-0.996793	-0.492541	0.106185	0.893815
AM-PR-L	0.982974	0.211541	0.088000	0.912000
AM-PR-M	-0.985614	-0.230597	0.483219	0.516781

Case No.	Predicted & Residual Values. Dependent variable: ln(DISTANCE)								
	Observed Value	Predicted Value	Residual	Standard Predicted value	Standard Residual	Standard Error of Predicted value	Mahalanobis Distance	Deleted Residual	Cook's Distance
1	2.302590	2.300118	0.002472	-1.98535	0.01803	0.136705	4.139467	0.454399	2.732215
2	6.684610	6.626805	0.057805	-0.01035	0.42169	0.116248	2.762564	0.205842	0.405423
3	7.170120	7.336915	-0.166796	0.31379	-1.21680	0.068038	0.398475	-0.221321	0.160554
4	7.696210	7.667018	0.029191	0.46447	0.21296	0.070280	0.480965	0.039601	0.005485
5	7.972470	7.897955	0.074514	0.56989	0.54359	0.121899	3.120685	0.356194	1.334891
6	8.070910	8.068097	0.002813	0.64755	0.02052	0.136131	4.097843	0.204388	0.548146
Minimum	2.302590	2.300118	-0.166796	-1.98535	-1.21680	0.068038	0.398475	-0.221321	0.005485
Maximum	8.070910	8.068097	0.074514	0.64755	0.54359	0.136705	4.139467	0.454399	2.732215
Mean	6.649485	6.649485	-0.000000	-0.00000	0.00000	0.108217	2.500000	0.173184	0.864452
Median	7.433165	7.501967	0.016002	0.38913	0.11674	0.119074	2.941625	0.205115	0.476785

Note: AM-PR-G, AM-PR-L and AM-PR-M denote average metal concentration in predatory fish gills, liver and muscle, respectively

TABLE IV
RELATIONSHIP BETWEEN DISTANCE FROM POLLUTION SOURCE (M) AND ZINC CONCENTRATION IN PREDATORY FISH BODY TISSUES (MG/KG OF W/W). COMPLETE MULTIPLE REGRESSION ANALYSIS RESULTS

N=6	Regression Summary for Dependent Variable: ln(DISTANCE) R=0.99908038 R²=0.99816160 Adjusted R²=0.99540401 F(3.2)=361.97 p<0.00276 Standard Error of estimate:0.14863					
	Beta	Standard Error of Beta	B	Standard Error of B	t(1)	p-level
Intercept			4.832194	0.821730	5.8805	0.027721
Zn-PR-G	-1.67620	0.110919	-0.137793	0.009118	-15.1119	0.004350
Zn-PR-L	0.81350	0.111409	0.351443	0.048130	7.3019	0.018244
Zn-PR-M	-0.51280	0.030890	-0.437062	0.026328	-16.6007	0.003609

Variable	Variables currently in the Equation; Dependent Variable: ln(DISTANCE)			
	Partial Correlation	Semi-partial Correlation	Tolerance	R-square
Zn-PR-G	-0.995650	-0.458166	0.074713	0.925287
Zn-PR-L	0.981756	0.221382	0.074058	0.925942
Zn-PR-M	-0.996391	-0.503304	0.963320	0.036680

Case No.	Predicted & Residual Values. Dependent variable: ln(DISTANCE)								
	Observed Value	Predicted Value	Residual	Standard Predicted value	Standard Residual	Standard Error of Predicted value	Mahalanobis Distance	Deleted Residual	Cook's Distance
1	2.302590	2.304559	-0.001969	-1.98359	-0.013250	0.148196	4.137227	-0.334465	1.258465
2	6.684610	6.757268	-0.072658	0.04921	-0.488838	0.138923	3.534625	-0.574788	3.266073
3	7.170120	7.148793	0.021327	0.22795	0.143486	0.082624	0.711722	0.030864	0.003331
4	7.696210	7.536458	0.159752	0.40493	1.074798	0.075115	0.443663	0.214547	0.133035
5	7.972470	8.085944	-0.113474	0.65579	-0.763447	0.113145	2.064023	-0.269837	0.477461
6	8.070910	8.063889	0.007022	0.64572	0.047243	0.147771	4.108741	0.606111	4.109075
Minimum	2.302590	2.304559	-0.113474	-1.98359	-0.763447	0.075115	0.443663	-0.574788	0.003331
Maximum	8.070910	8.085944	0.159752	0.65579	1.074798	0.148196	4.137227	0.606111	4.109075
Mean	6.649485	6.649485	-0.000000	0.00000	-0.000001	0.117629	2.500000	-0.054595	1.541240
Median	7.433165	7.342626	0.002526	0.31644	0.016997	0.126034	2.799324	-0.119487	0.867963

Note: Zn-PR-G, Zn-PR-L and Zn-PR-M denote average zinc concentration in predatory fish gills, liver and muscle, respectively

The final model would look as follows:

$$DISTANCE = e^{[6.30941 - 0.60275\ AM\text{-}PR\text{-}G + 0.9726\ AM\text{-}PR\text{-}L - 1.48067\ AM\text{-}PR\text{-}M]} \quad (1)$$

The final model would look as follows:

$$DISTANCE = e^{[4.832194 - 0.137793\ Zn\text{-}PR\text{-}G + 0.351443\ Zn\text{-}PR\text{-}L - 0.437062\ Zn\text{-}PR\text{-}M]} \quad (2)$$

After antilogarithm calculation observed and predicted values of the distance from the pollution source are presented in Table V.

TABLE V
OBSERVED AND PREDICTED VALUES OF DISTANCE FROM POLLUTION SOURCE (M) ESTIMATED USING DIFFERENT HEAVY METAL BIOACCUMULATION DATA IN FISH

Observed value	Predicted value (Standard error of predicted value)	
	Average heavy metal concentration in predatory fish body tissues	Average zinc concentration in predatory fish body tissues
10	9.98 (1.15)	10 (1.16)
800	755.07 (1.12)	799.99 (1.15)
1300	1535.97 (1.07)	1300 (1.09)
2200	2691.77 (1.13)	2199.99 (1.08)
2900	2897.04 (1.00)	2900.01 (1.12)
3200	3191.02 (1.15)	3200.01 (1.16)

IV. DISCUSSION

Pairwise comparison (Pearson correlation) showed that metal gill content data almost duplicated each other (Tables I and II). This, apparently, could be explained by the fact that both benthophagous both predatory fish accumulate significantly much more zinc in all body tissues (gills, liver and muscle) as compared to other metals as it was established in previous study [30]. Overall, zinc constituted the largest percentage of the total metal content, representing 97.4 ± 2.6, 81.7 ± 7.4 and 80.0 ± 39.2% in the gills, liver and muscle of predatory fish (mean ± SD, respectively). Thus, gills accumulated the highest amounts of zinc as compared to the liver and muscle. These results consist with the data obtained by many researchers demonstrating that fishes in general accumulate much more zinc in comparison with other metals such as copper, nickel, chromium, cadmium or lead (from several-fold to several orders of magnitude) [26], [28], [29], [37].

Gills are in direct in contact with water; therefore, the gill metal content reflects metal concentration in the fish biotope water. The high metal concentration within liver represents storage of metals from water for detoxification [38].

Teleost fish gills play an important role in ion regulation, gas exchange, acid-base balance, and nitrogenous waste excretion, thus reflecting the interface of the fish and their environment [39], [40]. The gills are assumed to be the major site of metal uptake due to their large surface area and direct contact with the aquatic environment [41]. Fish gills are very susceptible to waterborne metals, and often show various metal induced lesions [42]. Metal level in the gills depends on the absorption of metals not only in the gill tissue, but also due to their complexation with the mucus on the gill surface [43].

Multiple regression analysis results (Tables III and IV) indicate that observed and predicted dependent variable – the distance from the pollution source (m) in both cases are similar; however, the use of average zinc concentrations in predatory fish gills liver and muscle as independent variables produced much better results and, consequently, much more accurate multiple regression model than used average heavy metal concentration in predatory fish body tissues (Table V).

Zinc is classified as an essential trace mineral that is necessary in low quantities to fish, due to its involvement in protein synthesis and the ability to regulate the enzymatic reactions [18]. Intensification of anthropogenic activities often leads to an increase in the concentration of zinc in aquatic ecosystems [44]. Zinc can enter the fish's body by two routes: directly from water (via gills) and indirectly – from food (via digestive tract). It is considered that both routes are closely related; the uptake of dietborne metals can modify the uptake of waterborne metals and vice versa [45], [46]. At higher concentrations of zinc in the water, the quantity of it in the fish body is controlled by homeostasis, but too much increased content of the metal becomes toxic and induces a number of lesions [46]. It was found that in clean environments, zinc is mainly absorbed from food while in the fish exposed to increased zinc concentrations in water, the gills become the primary target organ for zinc toxicity and accumulation [18].

Water hardness is the only water quality parameter that modifies zinc toxicity in fish. Calcium cation (Ca^{2+}) competes with (Zn^{2+}) ion for the specific binding sites at the gill surface, thus reducing zinc uptake and toxicity [45]. Because of the high water pollution, zinc can seriously disturb calcium ion (Ca^{2+}) uptake into the fish's body. Calcium ion deficiency affects fish survival, growth, reproduction, egg hatching, etc. It can also lead to hypocalcaemia and ultimately mortality [43].

Natural and toxic ranges of zinc are much wider than those of copper or cadmium. In unpolluted aquatic ecosystems, zinc concentrations in surface waters amounts a few *nM* or less (*nM* = 0.065 *μg/L*) and in fewer industrialized areas, they are up to 0.75 *μM*. (*μM* = 65 *μg/L*). For example, in most differently polluted regions of Canada inland water zinc concentration ranges from 2 to 18 *μM* [47].

V. CONCLUSION

Primarily, in our case, the use of non-linear multiple regression models helped us to reveal patterns, which could not be determined analyzing relationships between variables by pairwise comparison. This is a very important scientific aspect, because now we can assuredly confirm that heavy metal bioaccumulation intensity in predatory fish body tissues (gills, liver and muscle) significantly depends on the pollution dispersion and distribution in the aquatic ecosystem (to the logarithm of the distance from the pollution source).

The second conclusion that could be drawn from this study is that there is no need to measure many metals in fish tissues because it is quite enough to measure tissue zinc concentration, and it seems it is quite appropriate to measure predatory fish gill zinc content for rapid, orientational assessment of metal load in different sites of the continuously polluted aquatic ecosystem. To obtain more reliable results all tissues (gill, liver and muscle) zinc concentration should be determined.

We think that for these purposes such species as a perch (*Perca fluviatilis*) could be useful. Of all the amount of used predatory fish in this study perch accounted for 89%. Young perches usually are benthophagous, and after they reached three years of age become perfect predators. In this study, we used adult individuals from 3 to 5 years old. Perch is a promising species as they are native to Europe; a eurytopic species, widely distributed and easy available in the rivers, lakes, ponds and even in the brackish waters.

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