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ORIGINAL RESEARCH PAPER

CORRELATIONS BETWEEN MORPHOLOGICAL CHARACTERISTICS AND HEAVY METALS CONCENTRATION IN THREE SPECIES OF FRESHWATER FISH

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Abstract: This study is based on length-weight relationship for three species of freshwater fish (*Carassius gibelio*, *Rhodeus sericeus amarus* and *Scardinius erythrophthalmus*), collected from a section of Siret River, Galbeni Accumulation, Romania. Fish specimens were sampled from the infiltration channel and from the old riverbed, in a randomly way and were identified using keys and descriptions. Total length and weight were measured using standard methods. To find the mean lengths and weights of the classes were used data analysis. The link between the environment and life cycle of the fish was highlighted by calculating the allometric factor (length-weight ratio LWR), the results showing strong correlations between this factor and the accumulation of metals in the tissues examined (gills, muscles and scales), as well as a positive influence of the sampling site upon fish development.

Keywords: *condition factor, fish species, heavy metals, length-weight relationship, morphological characteristics*

INTRODUCTION

Fishes represents a high quality food, because of its protein contents; it's also reach in vitamins and also, contains variable quantities of fat and minerals with a lot of benefits for human health [1]. Fish oil contains vitamins A, D, E and K which have been successfully used in controlling various affections, like: coronary heart diseases, arthritis, asthma, atherosclerosis, cancer and autoimmune deficiency diseases [2]. In the study of the fisheries biology is very important to know the length-weight relationship (LWR) and the condition factor (K) of fishes. The condition factor in fish can be use like an indicator of physiological state of the fish in relation to its welfare [3] and also can provides information in the moment of comparing two populations living in certain feeding density, climate and other conditions [4]. So, the condition factor is important for understanding the fish species life cycle and it contributes to adequate management of the species, hence, maintaining the equilibrium in the ecosystem [5].

Length-weight relationship is very important too, in any fishery venture, because it gives information on size increment, stock composition, growth patterns and wellbeing of the fish [6].

This study aimed to determine length-weight relationship and condition factor analyses for three species of freshwater fish making the correlation between morphological characteristics and heavy metals concentration.

MATERIAL AND METHODS

Description of sampling area

The studied area is located in the southern part of the city of Bacău, Romania, and is represented by a section of the Siret River, downstream of the Galbeni Dam (N: 46 ° 26 '744"; E: 26 °56 '932"). Samples were collected in October 2012 from two investigated sites: the old riverbed and the infiltration channel. The infiltration channel subjected to our study lies at the confluence of the Bistriţa River and Siret River, in an area influenced by the industrial platform of the city of Bacău. Thus, this platform includes a chemical plant (chemical fertilizers), a heating plant, sewage plants, urban waste landfills and agricultural lands (Figure 1).

The three analyzed species were: *Rhodeus sericeus amarus*, *Carassius gibelio* and *Scardinius erytrophthalmus*.

Length and weight measurements

Length measurement

A measuring board with an accuracy of 0.1 cm was used for length measurement. The total length (L) of the specimens was measured from the tip of the snout with mouth closed to the tip of the longest caudal fin ray.

Also, the length without tail (l); length of head (h); the height (H) and width (w) were recorded.

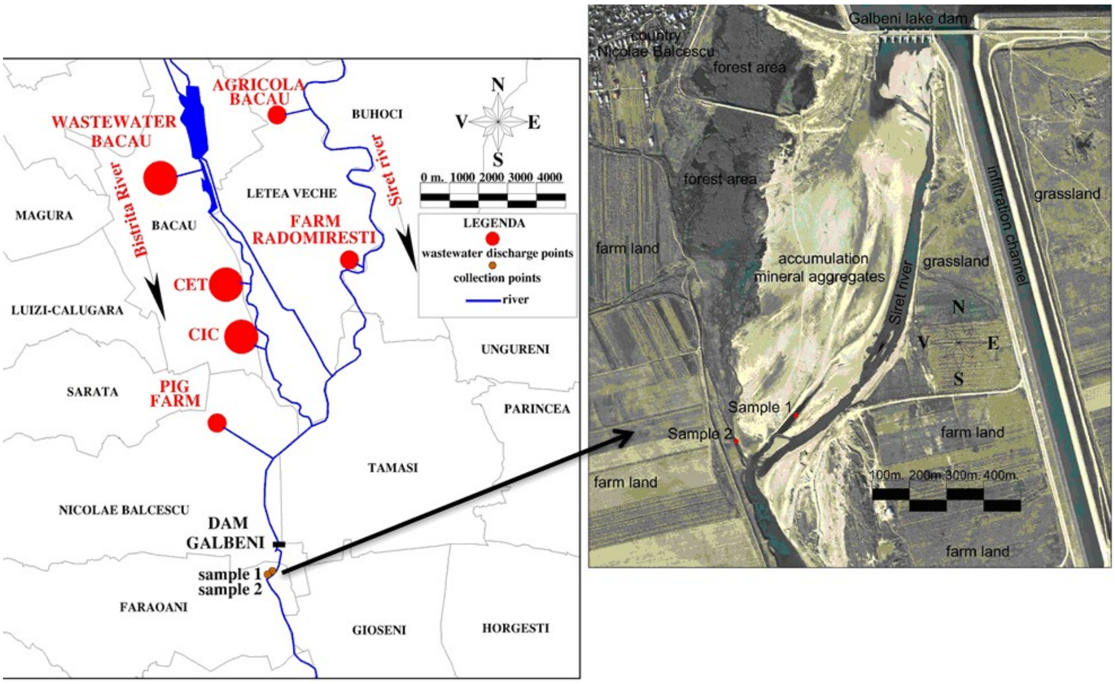


Figure 1. Map of the investigated area, the sampling sites and the main sources of pollution located near the Bistriţa and Siret Rivers

Body weight measurement

Before the body weight measurements the specimens were drying with a filter paper. All the data were recorded using a weighing balance with an accuracy of 0.1 g.

Parameters of length-weight relationships (LWR)

The LWR are very useful for the evaluation of the condition of the habitat where the fish live [7], modelling aquatic ecosystems [7], reproduction history [8], estimate fish biomass through the length frequency [9], life cycle [8] and the general health of fish species in order to draw morphological [8], abundance and occurrence comparisons [10] between species and populations [11].

Length-weight relationships were calculated using the regression equation 1 [9, 12]:

$$W = aL^b \qquad (1)$$

where W (g) is the weight of fish; L (cm) is the total length of fish; a is the allometric constant; b is the allometric scaling.

The values for the allometric coefficient and scaling were obtained using a power regression formed by length and weight of fish [7]. The correlation (Adj R^2) represents the degree of the association between length and weight [7].

Determination of heavy metals in tissues

Samples preparation before the analysis of heavy metals was based on the digestion of scales, gills and muscles for *Carassius gibelio* and *Scardinius erytrophthalmus species*. For the protected species *Rhodeus sericeus amarus* only scales digestion was performed.

Before digestion, the fish tissues (scales, gills, muscles) were washed with double-distilled water, then were placed in Petri dishes and dried in the oven at 75 °C until reaching a constant weight. After drying, each tissue was converted to a powder by grinding.
The digestion was performed by mixing 0.5 g of dry powder with a mixture of nitric acid and hydrogen peroxide (35 %) in a ratio of 2 : 1, and then the sample was placed in an autoclave at a temperature of 120 °C for 30 minutes. The obtained sample was filtered in a 25 mL flask that was filled to the mark with double-distilled water.
All chemical reagents were purchased from Sigma Aldrich.
Heavy metals (lead - Pb and cadmium - Cd) analysis was carried out using VARIAN AA 240F Atomic Absorption Spectrometer.

Statistical analysis

All the descriptive statistical analyses data were generated separately for water, sediment and fish. Using IBM SPSS Statistic version 20 and The Unscrambler® X version 10 software was made the whole set of statistical analysis.

RESULTS AND DISCUSSIONS

Morphological characteristics of the investigated species

The accumulation of heavy metals in varying degrees may be influenced by the morphological features from different species of fish. For example, based on weight or the total length of the fish, the metals can accumulate in concentrations above or below, also, the length of the fish is associated with the age of the fish. Experts claiming that, according with a theoretical point of view, the larger fish builds up larger quantities of heavy metals in comparison with the younger fish [13].
All data on the morphological characteristics of the species *Carassius gibelio*, *Rhodeus sericeus amarus* and *Scardinius erythrophthalmus* were processed and summarized in Tables 1 - 3. The processing of data taken into account were the maximum, minimum, mean and standard deviation for both collection points.
The data from Table 1 reveals that for the species *Rhodeus sericeus amarus*, individuals from the two sections of the river had different morphological features. Thus, the average values for each morphological feature to the species collected from the infiltration channel are higher than the values for specimens collected from the old riverbed.
For the other two fish species investigated, the mean of the values of the morphological characteristics are as follows:
- For all morphological characteristics, for the individuals of *Carassius gibelio* from the infiltration channel the results are lower than those obtained for specimens that were sampled from the old riverbed (Table 2).
- In the case of the species *Scardinius erythrophthalmus* from the infiltration channel, the values obtained are higher than the values obtained for the fish sampled from old riverbed (Table 3).

Table 1. *Morphological characteristics of Rhodeus sericeus amarus fish species collected from the two investigated areas*

Sampling area	No. of samples	Parameters	L [cm]	l [cm]	h [cm]	H [cm]	w [cm]	W [g]
Old riverbed	14	Minimum	4.6	3.7	0.7	1.0	0.6	1.5
		Maximum	5.7	4.7	0.9	1.5	1.7	2.9
		Mean	**5.1**	**4.2**	**0.7**	**1.2**	**0.7**	**2.1**
		Std. Deviation	0.4	0.4	0.1	0.2	0.3	0.6
Infiltration channel	14	Minimum	5.4	4.3	0.7	1.3	0.6	2.1
		Maximum	5.9	4.8	0.9	1.6	0.6	3.5
		Mean	**5.7**	**4.6**	**0.8**	**1.4**	**0.6**	**2.6**
		Std. Deviation	0.1	0.1	0.1	0.1	0	0.3

L - total length; l - length without tail; h - length of head; H - height; w - width; W - weight

Table 2. *Morphological characteristics of Carassius gibelio fish species collected from the two investigated areas*

Sample	No. of samples	Parameters	L [cm]	l [cm]	h [cm]	H [cm]	w [cm]	W [g]
Old riverbed	14	Minimum	5.6	4.5	1.3	1.5	0.5	3.9
		Maximum	12.4	10.4	2.3	4.2	1.6	37.0
		Mean	**7.9**	**6.4**	**1.7**	**2.5**	**1**	**12.5**
		Std. Deviation	2.1	1.8	0.3	0.9	0.4	10
Infiltration channel	5	Minimum	5.4	3.9	1.1	1.7	0.6	2.8
		Maximum	8.5	7.0	1.9	2.6	1.3	12.8
		Mean	**7.1**	**5.7**	**1.5**	**2.2**	**0.9**	**7.4**
		Std. Deviation	1.2	1.2	0.3	0.4	0.3	4.0

L - total length; l - length without tail; h - length of head; H - height; w - width; W - weight

Table 3. *Morphological characteristics of Scardinius erythrophthalmus fish species collected from the two investigated areas*

Sample	No. of samples	Parameters	L [cm]	l [cm]	h [cm]	H [cm]	w [cm]	W [g]
Old riverbed	6	Minimum	4.6	3.8	0.7	1.0	0.4	1.3
		Maximum	5.9	4.8	1.0	1.4	0.6	2.5
		Mean	**5.4**	**4.4**	**0.9**	**1.2**	**0.5**	**2**
		Std. Deviation	0.5	0.4	0.1	0.1	0.1	0.4
Infiltration channel	14	Minimum	6.9	5.5	1.2	1.5	0.6	3.4
		Maximum	8.8	7.0	1.8	2.1	0.8	7.6
		Mean	**7.6**	**6.0**	**1.4**	**1.8**	**0.8**	**5.2**
		Std. Deviation	0.6	0.5	0.2	0.2	0.1	1.3

L - total length; l - length without tail; h - length of head; H - height; w - width; W - weight

Concentration of heavy metals of the investigated species

The values of the lead and cadmium concentrations in scales, muscles and gills are presented in Tables 4 - 6 for the three species collected from the old riverbed and from the infiltration channel.

Thus, even if the three species live in the same environmental conditions, differences were found regarding the accumulation of metals. These differences can be attributed to the diet of species, their trophic levels and contamination gradients. As can be seen from the results, the accumulation of the two heavy metals in the investigated fish fluctuates from one species to another, and within the same species, from one organ to another.
In the case of *Rhodeus sericeus amarus*, which is a protected species, only the accumulation of the two heavy metals in scales is reported (Table 4). Therefore, for the specimens from the old riverbed, both cadmium and lead accumulated in higher concentrations comparable to those found in the scales of the other two species (Tables 5 and 6).

Table 4. *Heavy metals concentration for Rhodeus sericeus amarus*

Sample		**Scales**	
		Pb [μg·g^{1}]	Cd [μg·g^{-1}]
Old riverbed	Minimum	5.879	0.367
	Maximum	22.625	2.462
	Mean	**10.468**	**1.098**
	Std. Deviation	5.346	0.575
Infiltration channel	Minimum	0.0264	0.0118
	Maximum	0.3864	0.0921
	Mean	**0.1583**	**0.0413**
	Std. Deviation	0.0881	0.0190

Table 5. *Heavy metals concentration for Carassius gibelio*

Sample		**Gills**		**Muscles**		**Scales**	
		Pb [μg·g^{1}]	Cd [μg·g^{-1}]	Pb [μg·g^{1}]	Cd [μg·g^{-1}]	Pb [μg·g^{1}]	Cd [μg·g^{-1}]
Old riverbed	Minimum	0.390	0.0175	0.0097	0.9524	0.0990	0.0111
	Maximum	5.259	0.1909	1.7867	8.2812	2.4068	0.2179
	Mean	**1.981**	**0.0873**	**0.8639**	**3.4889**	**0.4940**	**0.0578**
	Std. Deviation	1.316	0.0610	0.5822	1.9612	0.6224	0.0668
Infiltration channel	Minimum	1.0117	0.2422	0.8829	3.1538	0.0167	0.0156
	Maximum	6.3583	1.2667	2.3514	11.8627	0.1744	0.0583
	Mean	**2.8077**	**0.5909**	**1.3616**	**5.8223**	**0.0555**	**0.0262**
	Std. Deviation	2.1282	0.4124	0.6273	3.5908	0.0674	0.0180

Table 6. *Heavy metals concentration for Scardinius erythrophthalmus*

Sample		**Gills**		**Muscles**		**Scales**	
		Pb [μg·g^{1}]	Cd [μg·g^{-1}]	Pb [μg·g^{1}]	Cd [μg·g^{-1}]	Pb [μg·g^{1}]	Cd [μg·g^{-1}]
Old riverbed	Minimum	4.546	0.2100	1.422	0.5357	0.1869	0.0207
	Maximum	10.475	0.7375	3.328	8.7500	4.0750	0.5773
	Mean	**6.277**	**0.4718**	**1.955**	**4.3482**	**2.0729**	**0.2639**
	Std. Deviation	2.207	0.2170	0.710	2.8653	1.5969	0.2160
Infiltration channel	Minimum	1.7022	0.5283	0.5341	0.3636	0.0564	0.0220
	Maximum	8.4429	1.9000	2.1366	7.9268	0.2332	0.0750
	Mean	**4.4078**	**0.9276**	**1.1449**	**2.4383**	**0.1305**	**0.0361**
	Std. Deviation	1.9323	0.3764	0.4214	2.2009	0.0628	0.0154

In the case of cadmium, the data presented in Table 5 shows that the highest concentration (5.822 $\mu g \cdot g^{-1}$) has accumulated in the muscles of *Carassius gibelio* for the infiltration channel samples.
For the fish collected from the old riverbed, the highest concentration of lead (6.277 $\mu g \cdot g^{-1}$) was accumulated in the gills of *Scardinius erythrophthalmus* (Table 6).
Between the species collected from the infiltration channel, the highest concentration of lead (4.407 $\mu g \cdot g^{-1}$) was determined also in the gills, in *Scardinius erythrophthalmus* (Table 6).
For both sampling sites, it can be observed that the two species (*Scardinius erythrophthalmus* and *Carassius gibelio*) accumulate different concentrations of heavy metals, confirming the theory that each body accumulates heavy metals in a unique way [14].
The data from Table 5 and 6 indicates that *Scardinius erythrophthalmus* accumulates lead in a higher concentration than *Carassius gibelio.* In the case of cadmium, the data indicate a greater ability of the *Carassius gibelio* to accumulate it, especially in the muscles.
These results show that *Scardinius erythrophthalmus* can be considered an indicator of lead pollution of the fresh waters and *Carassius gibelio* can be used to monitor a freshwater aquatic ecosystem in terms of cadmium pollution.

Correlations between morphological characteristics of the fish and heavy metals concentration in fish

The accumulation of two heavy metals selected for investigation (Cd and Pb) in various tissues belonging to three different species of fish (*Carassius gibelio*, *Scardinius erythrophthalmus*, *Rhodeus sericeus amarus*) may vary depending on a number of variables, such as: the sampling site, the investigated fish species, the morphological characteristics of the species and the organs investigated. We further present the results obtained after the data processing for the three fish species investigated, for each separate organ.

Scales
Regarding the Pb accumulated in scales, the results reveal that at the species *Scardinius erythrophthalmus*, the accumulation is closely linked to the morphological characteristics of the species, an affirmation reinforced by the high value of the Pearson correlation coefficient (R) higher than 0.5 (0.872) (Table 7). The close relationship between the two variables (concentration of lead and morphological sizes) is explained by the high value of determination coefficient $R^2 = 0.760$, indicating that 76 % of the accumulation of lead in scales has been accounted for, being influenced by the morphological sizes of the species (Figure 2A).

Table 7. *The degree of correlation between lead and cadmium accumulation and the morphological characteristics of Scardinius erythrophthalmus species*

Heavy metals	R	R Square	Adjusted R Square	Std. Error of the Estimate
Pb	0.872	0.760	0.700	0.548
Cd	0.830	0.688	0.610	0.624

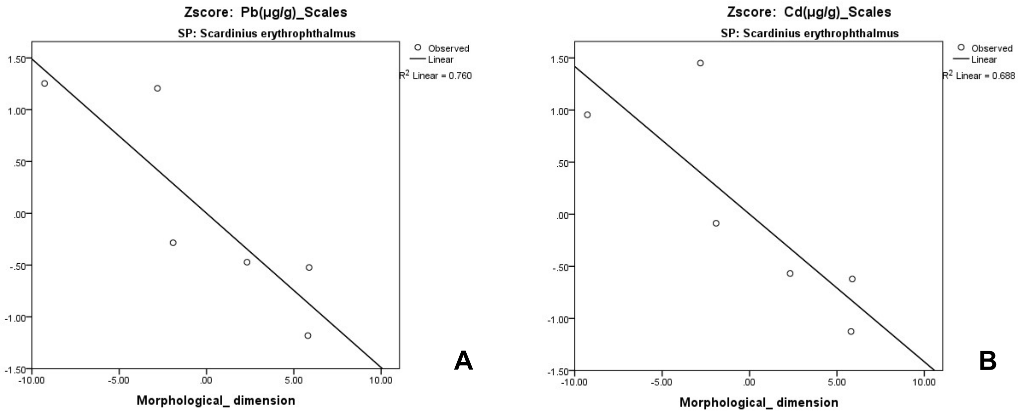


Figure 2. *The dependency curve between the value of the Pb and Cd concentration in the scales and the standardized morphological dimensions for Scardinius erythrophthalmus species*

In the case of cadmium, the close relationship between the accumulation of heavy metal in the scales and the morphological characteristics of the studied individuals is evident also at the species *Scardinius erythrophthalmus* where the Pearson correlation coefficient has a value of 0.830 (Table 7), thus well above 0.5. Also, the strong correlation is confirm by the coefficient of determination R^2 whose value is 0.688, which explains the fact that 68.8 % of the cadmium concentration accumulated in scales is influenced by the morphological characteristics of the studied specimens (Figure 2B).
For cadmium, a slightly lower correlation occurs in relation to the scales of the species *Carassius gibelio*, where the Pearson correlation coefficient is 0.622 (Table 8). Thus, even if the correlation coefficient is above 0.5, the value of the determination coefficient R^2 is only 0.387 (Figure 3), which shows a slight dependence between the two categories of variables (concentration of cadmium in scales and morphological characteristics). The correlation for lead in the case of *Carassius gibelio* scales is similar to cadmium (data nor shown).

Table 8. *The degree of correlation between cadmium accumulation and the morphological characteristics of Carassius gibelio species*

R	R Square	Adjusted R Square	Std. Error of theEstimate
0.622	0.387	0.335	0.815

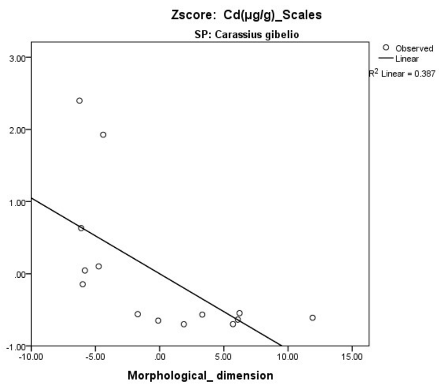


Figure 3. *The dependency curve between the value of the Cd concentration in the scales and the standardized morphological dimensions of Carassius gibelio species*

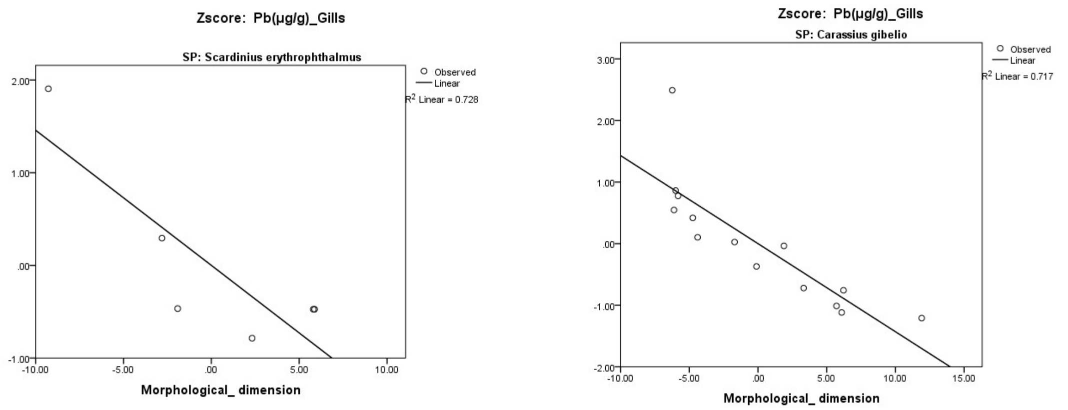
Gills

Given that from the species *Rhodeus sericeus amarus*, which is a protected species, there were collected only scales, in the case of gills, the accumulation of the two metals only at the species *Carassius gibelio* and *Scardinius erythrophthalmus* was reported. Thus, the best accumulation of Pb in the gills was registered at individuals of *Scardinius erythrophthalmus* (Table 9). The Pearson correlation factor between the dependent variable, represented by the lead concentration in the gills, and the independent variable (the morphological characteristics of the analysed individuals) has a high value, 0.853, which indicates a strong correlation between the two variables. This idea is supported by the determination coefficient, R^2 = 0.728 (Figure 4A), indicating that 72.8 % of the lead concentration accumulated in the gills is influenced by the morphological characteristics of the individuals studied.

Table 9. *The degree of correlation between lead accumulation in the gills and the morphological characteristics of Scardinius erythrophthalmus and Carassius gibelio species*

Species	R	R Square	Adjusted R Square	Std. Error of the Estimate
Scardinius erythrophthalmus	0.853	0.728	0.660	0.583
Carassius gibelio	0.847	0.717	0.693	0.554

A very good accumulation of lead was found at the species *Carassius gibelio*, where the Pearson coefficient is 0.847 (Table 9), and the determination coefficient R^2 = 0.717 (Figure 4B), meaning that the morphological characteristics of *Carassius gibelio* individuals influence the accumulation of lead in their gills at a rate of 71.7 %.



A B

Figure 4. *The dependency curve between the value of the Pb concentration in the gills and the standardized morphological dimensions of Scardinius erythrophthalmus (A) and Carassius gibelio (B) species*

Whereas both species showed a good lead accumulation in gills, the situation is different in the case of cadmium. This time, the species that presented the best results was *Carassius gibelio*. The Pearson correlation coefficient (R) had a value of 0.758 (Table 10), while the determination coefficient R^2 presented a value slightly above 0.5, namely 0.574 (Figure 5A).

In the case of the species *Scardinius erythrophthalmus*, even if the Pearson correlation coefficient has a value of slightly above 0.5, namely 0.537 (Table 10), the determination coefficient has a low value, below 0.5, specifically 0.289 (Figure 5B), which indicates a weak correlation between cadmium concentration in gills of this species and its morphological characteristics.

Table 10. *The degree of correlation between cadmium accumulation in the gills and the morphological characteristics of Scardinius erythrophthalmus and Carassius gibelio species*

Species	R	R Square	Adjusted R Square	Std. Error of the Estimate
Carassius gibelio	0.758	0.574	0.538	0.679
Scardinius erythrophthalmus	0.537	0.289	0.111	0.943

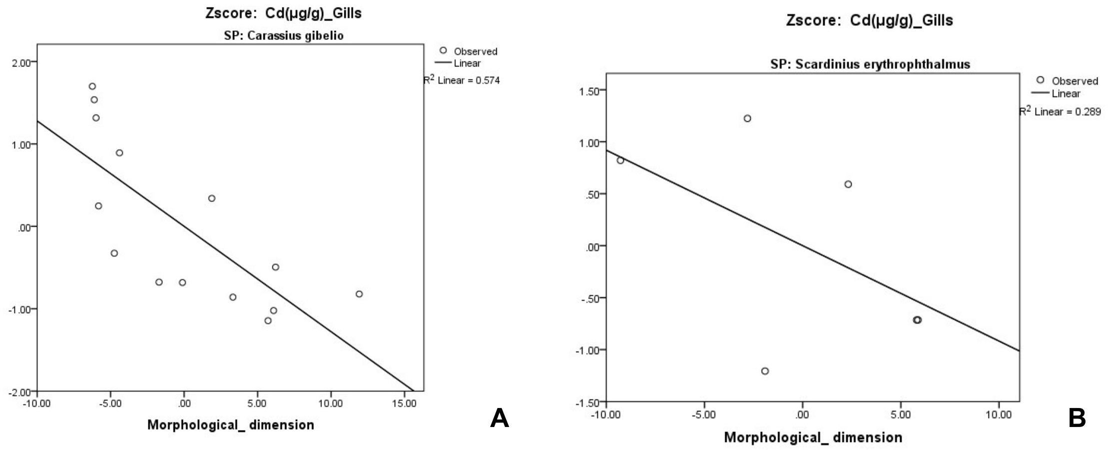


Figure 5. *The dependency curve between the value of the Cd concentration in the gills of Carassius gibelio (A) and Scardinius erythrophthalmus (B) species and the standardized morphological dimensions*

Muscles

Lead accumulation in muscles will be discussed only for the species *Carassius gibelio* and *Scardinius erythrophthalmus*.

Therefore, the species *Carassius gibelio* showed the best response in terms of lead accumulation in muscles, given the high value of the Pearson correlation coefficient R = 0.921 (Table 11), and the determination coefficient R^2 = 0.849 (Figure 6A). The high value of the latter coefficient shows that lead accumulation in muscles at the *Carassius gibelio* individuals is influenced by the morphological characteristics of the species at a rate of 84.9 %.

For *Scardinius erythrophthalmus* species the Pearson correlation coefficient is above 0.5 (R = 0.749) (Table 11) and the determination coefficient R^2 = 0.561 (Figure 6B). So, the morphological characteristics of individuals influence lead accumulation in muscles at a rate of 56.1 %, in the case of this species.

Table 11. *The degree of correlation between lead accumulation in the muscles and the morphological characteristics of Scardinius erythrophthalmus and Carassius gibelio species*

Species	R	R Square	Adjusted R Square	Std. Error of the Estimate
Carassius gibelio	0.921	0.849	0.837	0.404
Scardinius erythrophthalmus	0.749	0.561	0.451	0.741

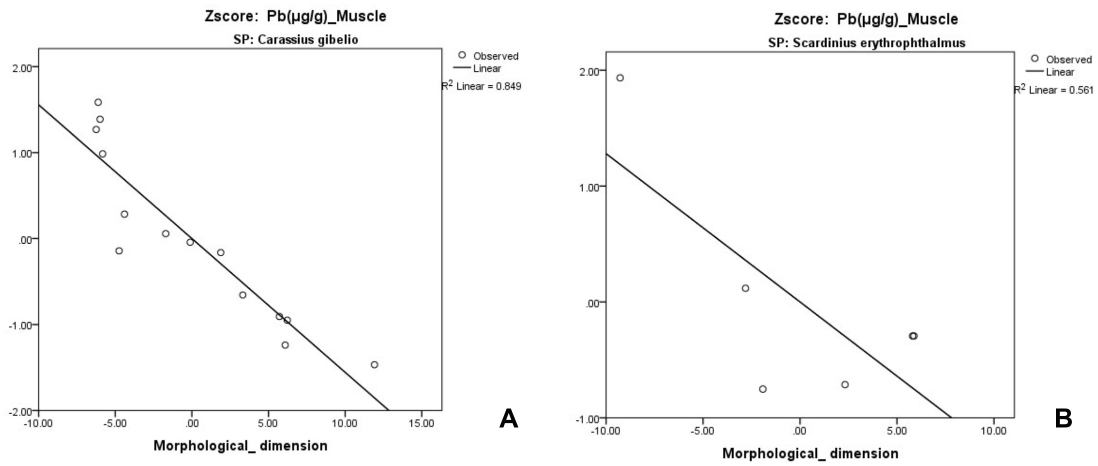


Figure 6. *The dependency curve between the value of the Pb concentration in the muscles of Carassius gibelio (A) and Scardinius erythrophthalmus (B) species and the standardized morphological dimensions*

In Table 12 and Figure 7 respectively, it can be noticed the correlation coefficient 0.515 and the determination coefficient 0.265 in case of *Carassius gibelio* muscles.

Table 12. *The correlation between the cadmium accumulation in the muscles and the morphological characteristics of the Carassius gibelio species*

R	R Square	Adjusted R Square	Std. Error of the Estimate
0.515	0.265	0.204	0.829

The results presented in the current study confirm once again that there is a strong link between the accumulation of heavy metals in fish and morphological characteristics which the scientific literature mentioned [13, 15].

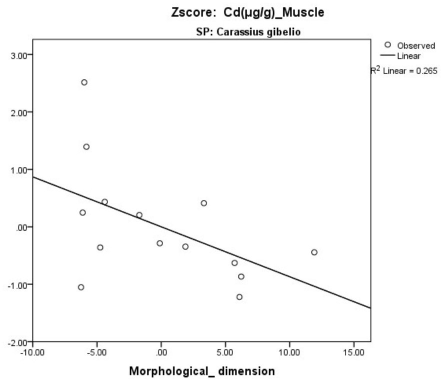


Figure 11. *The mean value of cadmium concentration in the muscles of the Carassius gibelio species*

Allometric parameters

In this study, there were estimated allometric coefficient (*a*) and allometric scaling (*b*) as well as the determination coefficient (R^2).

The statistical analysis of the LWR showed that parameters *a* and *b* obtained from length-weight relationships represents indicative of isometric or allometric growth differences between groups of categories [8, 13]. When *b* = 3, the fish grows isometrically resulting in an ideal shape of fish. A negative allometric growth corresponds to a value of $b < 3.0$ and a positive allometric growth corresponds to a value of $b > 3.0$ [15]. The growth differences could be due to different environmental and biological factors and also to the characteristics of the samples (size ranges, number of individuals sampled, etc) [10, 16, 17]. Also, an important factor in the relation length-weight is the collection area [18].

In our case, it can be observed that the sampling sites have a large influence on the allometric factor of the three species investigated. Thus, the exponent *b* of LWR, for the samples collected from the old riverbed, ranged between 2.560 for *Scardinius erythrophthalmus* and 3.064 for *Rhodeus sericeus amarus*. For the infiltration channel, the estimated value of allometry scaling (*b*) ranges between 3.088 for *Scardinius erythrophthalmus* and 3.486 for *Rhodeus sericeus amarus*.

CONCLUSIONS

In the present study, negative allometry registered in the case of *Scardinius erythrophthalmus* from the old riverbed samples as well as positive allometry recorded for all three species collected from the infiltration channel indicates the existence of disturbing factors in the environment that may affect the harmonious development of fish.

The correlation between morphological characteristics and heavy metals concentration in three species of freshwater shows that *Scardinius erythrophthalmus* can be considered an indicator of lead pollution of the fresh waters and *Carassius gibelio* can be used to monitor a freshwater aquatic ecosystem in terms of cadmium pollution.

These results are in accordance with the literature which argues that the metal accumulation in fish is closely related to the morphology of fish and the sampling area.

AUTHOR CONTRIBUTIONS

All authors have equal contribution to this research.

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