Scientific Papers. Series E. Land Reclamation, Earth Observation & Surveying, Environmental Engineering. Vol. XIV, 2025
Print ISSN 2285-6064, CD-ROM ISSN 2285-6072, Online ISSN 2393-5138, ISSN-L 2285-6064

ASSESSMENT OF IRON, COPPER, AND ZINC IN THE MUSCLE OF PONTIC SHAD: INFLUENCE OF YEAR, WEIGHT, AND LENGTH

Nina-Nicoleta LAZĂR[1], Mădălina CĂLMUC[1], Ira-Adeline SIMIONOV[1, 3], Maxim ARSENI[1, 2], Mihaela TIMOFTI[1, 2], Puiu-Lucian GEORGESCU[1, 2], Cătălina ITICESCU[1, 2], Violeta DOMNITEANU[4]

[1]"Dunărea de Jos" University of Galați, REXDAN Research Centre, 98 George Coșbuc Street, 800223, Galați, Romania
[2]"Dunărea de Jos" University of Galați, Faculty of Sciences and Environment, 111 Domnească Street, 800201, Galați, Romania
[3]"Dunărea de Jos" University of Galați, Faculty of Food Science and Engineering, 111 Domnească Street, 800201, Galați, Romania
[4]Software Republic S.R.L., Șivita Village, Tulucești Commune, 4 Trandafirilor Street, 800204, Galați, Romania

Corresponding author email: nina.condurache@ugal.ro

Abstract

This study aimed to analyze the concentrations of iron (Fe), copper (Cu), and zinc (Zn) in pontic shad (Alosa immaculata) and assess the influence of factors such as capture year, length, and weight on these concentrations. Fish samples were collected during the 2023 and 2024 fishing seasons. After recording the length and weight of each specimen, the muscle underwent acidic digestion. The concentrations of Fe, Cu, and Zn were then determined using a Total Reflection X-ray Fluorescence (TXRF) spectrometer. The results revealed an overall increase in chemical elements concentrations from 2023 to 2024. While Cu levels showed no correlation with length or weight, Fe and Zn exhibited strong correlations with these parameters.

***Key words**: Alosa immaculata, chemical elements, correlation, Total Reflection X-ray Fluorescence (TXRF).*

INTRODUCTION

Fish size may influence the concentration of some important metals in its tissues. But does this association apply to all chemical elements?
Copper (Cu), zinc (Zn) and iron (Fe) are chemical elements that are vital to all living species, including fish, and play important roles in cellular metabolic processes. However, high concentrations of these substances may produce more harm than benefits (Simionov et al., 2023; Dinu (Iacob) et al., 2024). For instance, redox activity of Cu and Fe can produce harmful free radicals that can react with biomolecules including lipids, proteins, and DNA. The oxidation of these compounds can cause cellular damage, damage normal biological functioning, and contribute to the development of a variety of disorders (Bury, 2003; Kozlowski et al., 2009; Wang et al., 2024). In fish, these compounds can enter the body in two main ways: through the intestines from food or through the gills from the water (Bury, 2003). According to Kumar et al. (2024), many factors impact the concentrations of Fe, Cu, and Zn in fish, including age, size, sex, swimming behavior, the environment, and eating behaviors. Since larger fish generally have more body mass and muscle tissue, they tend to have higher concentrations of certain chemical elements compared to smaller fish, although the concentration per unit of muscle may be lower than in smaller fish. Additionally, larger fish are often older and may have accumulated more chemical elements due to longer exposure to their environment, particularly if those metals are present in the water, sediment, or food (Jezierska & Witeska, 2006; Łuczyńska & Tonska, 2006; Has-Schön et al., 2015; Balzani et al., 2022).
Alosa immaculata (Bennett, 1835), commonly referred to as the pontic shad, is a marine teleost fish native to the Black Sea. It belongs to the *Clupeidae* family, along with the herrings, sardines and sprats. As an anadromous

migratory species, it inhabits the sea but migrates to coastal lakes and lower sections of rivers (such as the Danube, Dniester, and Prut) to spawn in the springtime. It is predominantly a predator and a secondary macrophage that feeds solely in saltwater habitats, consuming small fish and crustaceans. It does not feed in freshwater (https://fish-commercial-names.ec.europa.eu/; https://fishbase.se/).

In Romania, the Pontic shad is an economically valuable fish species, with its population in decline (Milea et al., 2023). For this reason, the Pontic shad has been added to the IUCN Red List of Threatened Species (Lazăr et al., 2024).

In the scientific literature there are several studies on the presence of heavy metals in the transition zone of the Pontic shad, the presence of these heavy metals being characteristic of the Lower Danube area (Burada et al., 2015).

Considering that the Pontic shad is not a fish species as extensively studied as others, yet it holds significant importance for Romanian traditions, customs, gastronomy, and economy, the aim of this study was:

- The use of the TXRF method for precise determination of the Fe, Cu, and Zn.
- The analysis of Fe, Cu, and Zn content in *Alosa immaculata* captured in 2023 and 2024.
- The preliminary investigation of the correlations between fish length, weight, and the concentrations of these metals.

MATERIALS AND METHODS

Chemicals and reagents

The following reagents were used: Gallium (Ga) standard solution purchased from SCP Science (Canada); polyvinyl alcohol (PVA), Suprapur nitric acid 65% (HNO_3), and perhydrol 30% (H_2O_2) purchased from Sigma-Aldrich (Germany), and silicone solution in isopropanol purchased from Serva Electrophoresis GmbH (Germany).

Sample preparation

Three Pontic shad specimens were collected during each fishing season (April-May) in 2023 and 2024, utilizing traditional methods associated with commercial fishing in the Lower Danube Basin. Following capture, they were transported to the REXDAN Research Infrastructure, at the "Dunărea de Jos" University of Galați, Romania. After having measured the total length and weight, the fish were washed with ultrapure water, dissected, and muscle tissue samples were taken for analysis. Each muscle sample was subsequently digested using a microwave digestion procedure, as described by Simionov et al. (2023), with a mixture of 65% HNO_3 and 30% H_2O_2 in a 9:1 ratio. Following digestion, the samples were diluted to 50 mL with ultrapure type I water and stored in Falcon tubes for further analysis using TXRF (Figure 1).

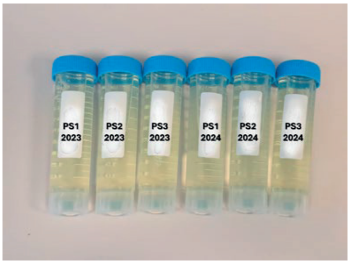

Figure 1. Digested samples of pontic shad

TXRF analysis

The sample preparation process included mixing the digested samples with a 0.3 g/L PVA solution at a 10:1 ratio and incorporating an internal standard. In our study, gallium (Ga) was used as the internal standard at concentrations ranging from 50 to 100 µg/L, depending on the sample matrix. Subsequently, 10 µL of each prepared sample was transferred onto a siliconized quartz carrier and dried on a hot plate at 50°C for 5 min. Measurements were conducted in triplicate for 1000 sec per sample, using a Bruker S4 T-Star spectrometer (Bruker AXS Microanalysis GmbH, Billerica, MA, USA) illustrated in Figure 2. The results were reported as the mean ± standard deviation of the triplicates. The elemental concentrations in the samples were calculated using the following equation:

$$Ci = CIS \cdot Ni \cdot SIS/NIS \cdot Si$$

where:

- Ci represents the element concentration;
- CIS is the Ga concentration;
- Ni is the element net count rate;
- NIS is the Ga net count rate,
- Si is the element sensitivity factor;
- SIS is the Ga sensitivity factor.

Before measurements, the equipment was calibrated as described by Lazăr et al. (2025).

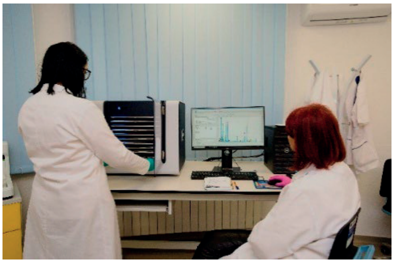

Figure 2. Sample analyzis using Bruker S4 T-Star spectrometer at REXDAN Research Infrastructure

Statistical Analysis

The data were analyzed using descriptive statistics (mean and standard deviation), inferential statistics (ANOVA), and exploratory and relational analysis (correlation matrices), employing Minitab 17 software and Microsoft Excel.

RESULTS AND DISCUSSIONS

Understanding fish weight, length, and metals' concentrations offers essential information for managing fish populations, protecting human health, and ensuring environmental sustainability. These factors contribute to the responsible use of fishery resources while protecting aquatic ecosystems and public health. Table 1 shows the length and weight ranges of the pontic shad specimens captured and analysed in our study. The lengths of the fish from 2023 ranged between 26.70 cm and 32.40 cm, while for those of 2024 varied between 27.90 cm and 31.60 cm. The weight of the fish captured in 2023 varied from 144.10 g to 206.17 g, while for the specimens from 2024 varied between 200.58 g and 273.21 g, with a mean weight of 226.76 ± 40.33 g. Stroe et al. (2024) reported a mean weight of 223.5 ± 44.95 g and a mean length of 29.53 ± 1.79 cm for the Pontic shad specimens captured during March-June 2023 between 169-197 river km along the Danube.

In our study, it can be noted that the Pontic shad specimens captured in 2024 were larger in terms of weight compared to the specimens captured in 2023, which may suggest a possible greater food availability or favorable environmental factors.

Table 1. Biometric data of the analyzed pontic shad specimens

Fish specimen	Length (cm)	Weight (g)
PS 1 (2023)	29.30	206.17
PS 2 (2023)	32.40	179.42
PS 3 (2023)	26.70	144.10
PS 1 (2024)	27.90	206.50
PS 2 (2024)	28.70	200.58
PS 3 (2024)	31.60	273.21

Figure 3 illustrates the average metal concentrations in the muscle tissue of pontic shad, along with their standard deviations. The highest Fe concentration was observed in PS1 captured in 2024. The highest Cu concentrations were identified in PS2 from 2024. The highest Zn concentration was observed in PS3 from 2024.

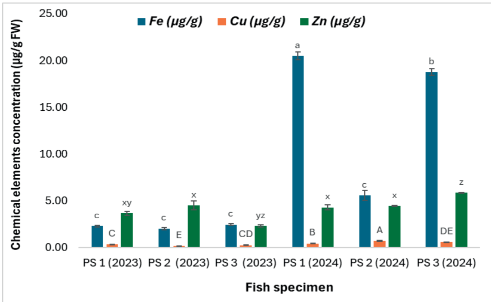

Figure 3. Concentration of Fe, Cu, and Zn in pontic shad. Statistical differences in Fe concentrations are indicated by lowercase letters (a-c), in Cu by uppercase letters (A-E), and in Zn concentrations by lowercase letters (x-z). Values that share the same letter are not significantly different ($p > 0.05$)

Numerous studies have analyzed heavy metal concentrations in fish from the Danube River, yet few have focused on the pontic shad. Stancheva et al. (2014) reported Fe concentrations of 9.00 ± 1.00 µg/g wet weight in pontic shad from the Bulgarian coast of the Black Sea, captured in 2010. Jitar et al. (2015) documented Cu concentrations ranging from 0.73 to 2.10 µg/g wet weight and Zn concentrations between 2.22 and 4.12 µg/g wet weight in specimens collected along the Romanian coastline of the Black Sea, between 2011 and 2012. Makedonski et al. (2017) identified Cu concentrations of 0.45 ± 0.03 µg/g wet weight and Zn concentrations of 9.00 ± 1.00 µg/g wet weight in Pontic shad from the Bulgarian coast, also from 2010. Bat et al.

(2018) reported a Cu concentration of 2.25 ± 0.81 µg/g wet weight in the muscle tissue of Pontic shad fished from Turkish waters of the Black Sea in 2016. More recently, Simionov et al. (2021) reported Cu concentrations of 0.40 ± 0.10 µg/g wet weight in Pontic shad from both the Black Sea and the Danube River. They also recorded Zn concentrations of 4.00 ± 0.40 µg/g wet weight in specimens from the Black Sea and 3.60 ± 0.30 µg/g wet weight from the Danube River. Additionally, Fe concentrations were reported at 10.50 ± 1.60 µg/g wet weight in Pontic shad from the Black Sea, and 6.00 ± 1.00 µg/g wet weight from the Danube River.

Comparing our results with previous studies on metals' accumulation in pontic shad highlights both similarities and differences across various geographic areas and years, providing insight into potential environmental and ecological influences.

Furthermore, beyond concentration levels, it is also crucial to explore how these metals relate to the biometric characteristics of the specimens. Figures 4 and 5 present the correlation between the concentrations of metals identified in the muscle of the pontic shad fished in 2023 and 2024, and their biometric data. In the case of the specimens from 2023, both negative and positive correlations can be observed. For instance, between Fe and Zn, a strong negative correlation can be observed with a value of -0.96. Fe also showed a strong negative correlation with a value of -0.99 with the length, and a moderate negative correlation with the weight of the fish. Zn has a strong positive correlation with the length (0.98) and moderate positive correlation with the weight (0.67). Cu has a moderate positive correlation with Fe (0.69), suggesting that these metals may be regulated similarly in the body. Cu also showed a negative correlation with length (-0.62), indicating a possible dilution of copper in larger fish. Weight and length are somewhat positively correlated, with a value of 0.52, suggesting allometric growth (not necessarily proportional between length and weight).

In the case of the pontic shad caught in 2024, a strong negative correlation between Fe and Cu (-0.85) was also observed. Fe showed a low correlation with the length and Zn concentration, but a high correlation with weight.

	Fe (µg/g)	Cu (µg/g)	Zn (µg/g)	L (cm)	G (g)
Fe (µg/g)	1				
Cu (µg/g)	0.691987871	1			
Zn (µg/g)	-0.963653642	-0.473975182	1		
L (cm)	-0.996288626	-0.62728103	0.983073	1	
G (g)	-0.449639507	0.33367145	0.671922	0.524854	1

Figure 4. Correlation matrix of Fe, Cu, Zn, and biometric data of the pontic shad from 2023. Green color - highest value; yellow color - average value; red color - lowest value

	Fe (µg/g)	Cu (µg/g)	Zn (µg/g)	L (cm)	G (g)
Fe (µg/g)	1				
Cu (µg/g)	-0.847525735	1			
Zn (µg/g)	0.333034566	0.218200625	1		
L (cm)	0.207227518	0.343602444	0.99146	1	
G (g)	0.469863086	0.070296626	0.988828	0.960946	1

Figure 5. Correlation matrix of Fe, Cu, Zn, and biometric data of the pontic shad from 2024. Green color - highest value; yellow color - average value; red color - lowest value

Zn had very strong correlations with the length (0.99) and weight (0.99). In addition, the length (L) and weight (G) had a very strong correlation (0.96). This fact confirms that as fish grow in length, they also increase in weight, but not necessarily in a linear manner (possible allometric growth as already stated by other authors).

In our study, Fe and Cu had an antagonistic relationship in both data sets, with strong negative correlations. This suggests a biological competition between the two metals, either at the level of absorption or in the tissue distribution of the fish. On the other hand, Zn was strongly correlated with length and weight in both cases indicating that Zn is an essential element for fish growth, as it is involved in the metabolic and structural processes of the fish. In correlation with weight and length, fish growth does not seem to be directly proportional to the accumulation of Fe, Zn, Cu. In a similar study, a positive correlation between Zn content and biometric data of pike and negative correlation between Zn and weight and length of perch was reported (Łuczyńska & Tonska, 2006). Positive correlations between the Cu and Zn concentrations and the length of fish were reported in various species of fish from Yangtze River in China (Yi & Zhangm, 2012). Contrary to our study, other authors observed a negative correlation between Cu concentrations and fish length and weight (Balzani et al., 2022; Kaçar, 2022). Furthermore, a positive correlation between Fe and the length of *S. glaris* captured in Arno river in Italy was reported (Balzani et al., 2022).

To address the initial question raised at the beginning of the manuscript, fish size and weight can affect the bioaccumulation of certain elements, while having no impact on others. As previously stated, the concentration of metals in fish is influenced by multiple factors, such as age, size, sex, swimming behaviour, the environment, and feeding habits. Therefore, a more in-depth study is necessary to fully understand the process of bioaccumulation in fish.

CONCLUSIONS

Our research highlighted variations in Fe, Cu, and Zn concentrations among Pontic shad specimens. Zn was positively associated with fish growth, while Fe and Cu showed an inverse relationship, suggesting that the metal metabolism and absorption in fish are complex and primarily influenced by interactions between metals rather than by size. These findings contribute to sustainable fisheries management, ensuring both environmental protection and public health. However, further research is needed to fully understand bioaccumulation mechanisms and their potential effects on fish health and human consumption. Studies on biota can be associated with studies on the quality of surface aquatic ecosystems that include the determination of the presence of heavy metals, studies that use statistical methods of integration of major pollutants with an impact on biodiversity (Popa et al., 2018).

For future research, we aim to address Lower Danube areas located in the cross-border area of Romania, Ukraine and the Republic of Moldova, where, due to various anthropogenic factors, the presence of certain pollutants in surface waters has an important impact on the ichthyofauna (Iticescu et al., 2016).

ACKNOWLEDGEMENTS

This research is part of the project *"Innovative sediment management framework for a SUstainNable DANube black Sea system (SUNDANSE)"*, co-funded by the European Union. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union or the European Climate, Infrastructure and Environment Executive Agency. Neither the European Union nor the granting authority can be held responsible for them.

REFERENCES

Alosa immaculata (n.d.). Retrieved February 11, 2025, from https://fish-commercial-names.ec.europa.eu/fish-names/species/alosa-immaculata_ro

Alosa immaculata summary page (n.d.). *FishBase*. Retrieved February 11, 2025, from https://www.fishbase.us/summary/Alosa-immaculata.html

Balzani, P., Kouba, A., Tricarico, E., Kourantidou, M., & Haubrock, P. J. (2022). Metal accumulation in relation to size and body condition in an all-alien species community. *Environmental Science and Pollution Research, 29*(17), 25848–25857. https://doi.org/10.1007/s11356-021-17621-0

Bat, L., Öztekin, A., & Şahin, F. (2018). Trace metals amounts and health risk assessment of *Alosa immaculata* Bennett, 1835 in the Southern Black Sea. *Discovery Science, 14*, 109-116

Burada, A., Topa, C. M., Georgescu, L. P., Teodorof, L., Nastase, C., Seceleanu-Odor, D., & Iticescu, C. (2015). Heavy metals environment accumulation in Somova-Parcheş aquatic complex from the Danube Delta area. *Revista de Chimie, 1*.

Bury, N. (2003). Iron acquisition by teleost fish. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology, 135*(2), 97–105. https://doi.org/10.1016/S1532-0456(03)00021-8

Dinu (Iacob), A., Bounegru, A.V., Iticescu, C., Georgescu, L.P., & Apetrei, C. (2024). Electrochemical detection of Cd^{2+}, Pb^{2+}, Cu^{2+} and Hg^{2+} with sensors based on carbonaceous nanomaterials and Fe_3O_4 nanoparticles. *Nanomaterials, 14*(8), Article 8. https://doi.org/10.3390/nano14080702

Has-Schön, E., Bogut, I., Vuković, R., Galović, D., Bogut, A., & Horvatić, J. (2015). Distribution and age-related bioaccumulation of lead (Pb), mercury (Hg), cadmium (Cd), and arsenic (As) in tissues of common carp (*Cyprinus carpio*) and European catfish (*Silurus glanis*) from the Buško Blato reservoir (Bosnia and Herzegovina). *Chemosphere, 135*, 289–296. https://doi.org/10.1016/j.chemosphere.2015.04.015

Iticescu, C., Murariu, G., Georgescu, L. P., Burada, A., & Topa, C. M. (2016). Seasonal variation of the physico-chemical parameters and water quality index (WQI) of Danube water in the transborder Lower Danube area. *Revista de Chimie, 67*(9). https://revistadechimie.ro/Articles.asp?ID=5199.

Jezierska, B., & Witeska, M. (2006). The metal uptake and accumulation in fish living in polluted waters. In I. Twardowska, H. E. Allen, M. M. Häggblom, & S. Stefaniak (Eds.), *Soil and water pollution monitoring, protection and remediation* (pp. 107–114). Springer Netherlands. https://doi.org/10.1007/978-1-4020-4728-2_6

Jitar, O., Teodosiu, C., Oros, A., Plavan, G., & Nicoara, M. (2015). Bioaccumulation of heavy metals in marine organisms from the Romanian sector of the Black Sea. *New Biotechnology, 32*(3), 369–378. https://doi.org/10.1016/j.nbt.2014.11.004

Kaçar, E. (2022). Relationship of concentrations of some heavy metals with fish size in muscle tissue of *Carassius gibelio* (Bloch, 1782) from the Tigris River (Turkey). *Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 15*(2), 475–481. https://doi.org/10.18185/erzifbed.959413

Kozlowski, H., Janicka-Klos, A., Brasun, J., Gaggelli, E., Valensin, D., & Valensin, G. (2009). Copper, iron, and zinc ions homeostasis and their role in neurodegenerative disorders (metal uptake, transport, distribution and regulation). *Coordination Chemistry Reviews, 253*(21), 2665–2685. https://doi.org/10.1016/j.ccr.2009.05.011

Kumar, M., Singh, S., Jain, A., Yadav, S., Dubey, A., & Trivedi, S. P. (2024). A review on heavy metal-induced toxicity in fishes: Bioaccumulation, antioxidant defense system, histopathological manifestations, and transcriptional profiling of genes. *Journal of Trace Elements in Medicine and Biology, 83*, 127377. https://doi.org/10.1016/ j.jtemb.2023. 127377

Lazăr, N.N., Simionov, I.A., Petrea, Șt.-M., Iticescu, C., Georgescu, P.L., Dima, F., & Antache, A. (2024). The influence of climate changes on heavy metals accumulation in *Alosa immaculata* from the Danube River Basin. *Marine Pollution Bulletin, 200*, 116145. https://doi.org/10.1016/j.marpolbul.2024.116145

Lazăr, N.N., Simionov, I.A., Călmuc, M., Călmuc, V.A., Iticescu, C., Georgescu, P.L., Timofti, M., & Drăgan, S. (2025). Cross-regional elemental comparison of mussels using total reflection X-ray fluorescence (TXRF). *Molecules, 30*(2), Article 2. https://doi.org/10.3390/molecules30020283

Łuczyńska, J., & Tonska, E. (2006). The effect of fish size on the content of zinc, iron, copper, and manganese in the muscles of perch (*Perca fluviatilis* L.) and pike (*Esox lucisus* L.). *Archives of Polish Fisheries, 14*, 5–13.

Makedonski, L., Peycheva, K., & Stancheva, M. (2017). Determination of heavy metals in selected Black Sea fish species. *Food Control, 72*, 313–318. https://doi.org/10.1016/j.foodcont.2015.08.024

Milea, Ștefania-A., Lazăr, N.N., Simionov, I.A., Petrea, Ștefan-M., Călmuc, M., Călmuc, V., Georgescu, P.L., & Iticescu, C. (2023). Effects of cooking methods and co-ingested foods on mercury bioaccessibility in pontic shad (*Alosa immaculata*). *Current Research in Food Science, 7*, 100599. https://doi.org/10.1016/j.crfs.2023.100599

Popa, P., Murariu, G., Timofti, M., & Georgescu, L.P. (2018). Multivariate statistical analyses of Danube River water quality at Galati, Romania. *Environmental Engineering and Management Journal, 17*(5), 1249–1266. https://doi.org/10.30638/eemj.2018.124

Simionov, I.A., Cristea, D.S., Petrea, S.M., Mogodan, A., Nicoara, M., Plavan, G., Baltag, E.S., Jijie, R., & Strungaru, S.A. (2021). Preliminary investigation of lower Danube pollution caused by potentially toxic metals. *Chemosphere, 264*, 128496. https://doi.org/10.1016/j.chemosphere.2020.128496

Simionov, I.-A., Călmuc, M., Iticescu, C., Călmuc, V., Georgescu, P.-L., Faggio, C., & Petrea, Șt.-M. (2023). Human health risk assessment of potentially toxic elements and microplastics accumulation in products from the Danube River Basin fish market. *Environmental Toxicology and Pharmacology, 104*, 104307. https://doi.org/10.1016/j.etap.2023.104307

Stancheva, M., Makedonski, L., & Peycheva, K. (2014). Determination of heavy metal concentrations of most consumed fish species from Bulgarian Black Sea coast.

Stroe, D.M., Cretu, M., Tenciu, M., Dima, F.M., Patriche, N., Tiganov, G., & Dediu, L. (2024). Age, growth, and mortality of pontic shad, *Alosa immaculata* Bennett, 1835, in the Danube River, Romania. *Fishes, 9*(4), Article 4. https://doi.org/10.3390/fishes9040128

Wang, L., Wang, H., Gao, C., Wang, C., Yan, Y., & Zhou, F. (2024). Dietary copper for fish: Homeostasis, nutritional functions, toxicity, and affecting factors. *Aquaculture, 587*, 740875. https://doi.org/10.1016/j.aquaculture.2024.740875

Yi, Y. J., & Zhang, S. H. (2012). The relationships between fish heavy metal concentrations and fish size in the upper and middle reach of Yangtze River. *Procedia Environmental Sciences, 13*, 1699–1707. https://doi.org/10.1016/j.proenv.2012.01.163