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COMPARATIVE ANALYSIS OF HEAVY METALS AND ELEMENTAL PROFILES IN TROUT SPECIES FROM TWO DIFFERENT AREAS

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Abstract

An important concern of man has become to have high-quality food for a healthy life. Fish such as rainbow trout (Oncorhynchus mykiss) and brook trout (Salvelinus fontinalis) are an important bioindicator of pollution. The accumulation of heavy metals represents a potential danger to public health. Trout is preferred and consumed by many people so any possible risk of ingesting metals through food chains should be assessed. The main organs studied according to specialized literature regarding the accumulation of heavy metals are the liver an the kidney, these organs play an important role in preventing the transfer of heavy metals to other organs, for example muscle tissue. As is known, the pollution of aquatic ecosystems has a direct impact on the entire aquaculture activity. Therefore, the purpose of this study was to monitor metals present in the two trout species in different areas.

Key words*: biomonitoring, brook trout, heavy metals, human health risk, rainbow trout.*

INTRODUCTION

The process of human development in relation to the environment has gradually turned into an instrument of destruction with negative effects on the ecosystem, through the participation of "brutal" humanity in the irrational exploitation of nature (Benciu, 2007). Metals in very small quantities are useful to all forms of life, metals enter the cell in a controlled manner in the form of cations. A large amount of any metal causes toxicity to the human and animal body (Elumalai et al., 2023).

According to Kumar et al. (2024), the term heavy metal is commonly used for metals with a density greater than water, so heavy metals are described as elements with densities greater than 5.0 g.cm^{-3} and atomic masses greater than 20, of example: mercury (Hg), copper (Cu), nickel (Ni), cadmium (Cd), chromium (Cr), arsenic (As), zinc (Zn) and lead (Pb).

The term "heavy" also refers to a variety of metals, including certain metalloids, which can be dangerous to both plants and living organisms, even at very low concentrations.

Heavy metals are natural compounds of the earth's crust, they reach the body of animals and humans through various ways: food, water, air. Their effects are bioaccumulation, the concentration of heavy metals increases per mass unit, they block intracellular biochemical processes due to accumulation (Matta et al., 1999).

Heavy metals have mutagenic and carcinogenic properties in the body (Kumar-Sharma et al., 2024).

According to many studies, the deterioration of the ecosystem is primarily due to industrialization.

The Environmental Protection Agency and the World Agency for Cancer Research confirm the damage to the body even at low levels of exposure to heavy metals, the source of contamination being feed, water and air (Abida et al., 2009).

Heavy metals: iron (Fe), manganese (Mn), coblat (Co), nickel (Ni), copper (Cu), chromium (Cr) and zinc (Zn) are necessary for metabolic activities, since they form the cofactor for many enzymes, but their amount is very important, because if the limit is exceeded, they cause harm to the human body and animals (Javed & Usmani, 2017).

According to Zeynali et al. (2009), and other studies in the specialized literature, the factors that influence the accumulation of heavy metals in fish tissue can be the following: nourishment sources, seasonal changes, environmental conditions (temperature, salinity, pH) and biological variations (species, sex, age, size) etc.

Water being essential for life, but its quality has decreased more and more due anthropogenic activities (Koca et al., 2005).

Heavy metals in fish in high concentrations or by accumulation over time, cause acute and chronic diseases, slow metabolism, damage to the epithelial layer, gill disease, damage to liver tissue and ulcers (Market, 2007).

Useful tools in research are: monitoring or evaluation of heavy metals; evaluation of health indices, bioaccumulation, blood biochemistry, enzymes of tissue damage markers, genotoxicity, histopathology, water pollution (Pandey, 2006; Falllah et al., 2011).

Fish is consumed by a large mass of the population, including children, due to the content of high-quality protein and polyunsaturated fatty acids (Malik et al., 2010).

People are more and more cautious about the food they consume, paying attention to the quality of fish and seafood because they represent an important category for a balanced diet (Sneddon et al., 2007).

It is very important to implement remedial approaches to mitigate the negative effects of heavy metals, due to the bioaccumulative power in the fish body, there are some fish species that accumulate higher concentrations of heavy metals, usually fish species at levels higher trophic (Gleick, 1996; Wittmann, 1979).

The trout is a biomonitor in the case of heavy metals confirmed by Barrientos et al., 2019; Market, 2007 and other researchers.

Certain aquatic plants, called macrophytes, are used for remedial purposes to remove heavy metals from aquatic sources as an environmentally friendly technology to reduce heavy metal pollution (Kumar et al., 2024).

Most aquatic organisms, including trout, can form a resistance against heavy metal pollution, and according to Klerks & Weis (1987), we can identify two main mechanisms: acclimatization and adaptation.

According to studies, acclimatization is described as an increased tolerance through exposure to metals, which is acquired at the individual level, through repeated and prolonged exposure to sublethal concentrations of heavy metals.

It should be noted that increased tolerance is not inherited, but acquired through exposure (Wirgin & Waldman, 2004; Hansen et al., 2006).

The most common heavy metals that contaminate the fish are: mercury (Hg), cadmium (Cd) and lead (Pb), and the degree of pollution and the feed mixture used in farms play a major role in heavy metal contamination (Majid et al., 2019).

Regarding the global seafood production, it registers a continuous increase, so that the Food and Agriculture Organization of the United Nations estimates a quantity of 195 million tons around the year 2025, (FAO, 2018).

As for the purpose of this study, it was to monitor metals in brook trout (*Salvelinus fontinalis*) and rainbow trout (*Oncorhynchus mykiss*) harvested from two different areas. These elements can have a major impact on health, so it is very important to know them, to establish their level, because fish meat is fully recommended for the proper functioning of the human body.

MATERIALS AND METHODS

In this study we used trout samples from the Cluj area, Romania. Brook trout (*Salvelinus fontinalis*) was harvested from Marisel, and rainbow trout (*Oncorhynchus mykiss*) was harvested from Gilau. In Transylvania (Romania), there are numerous trout breeding and development systems, and thus the importance of this fish can be seen due to the population's desire to own a farm to support their own trout consumption or for marketing purposes. Obtaining favorable results regarding the level of contaminants-heavy metals in the case of this study, helps us to confirm the quality of the trout and also of the surrounding environment. The impact will be favorable to consumers if the contaminant levels fall within the legal limits.

The trout samples were stored in laboratory conditions in a minifreezer used for research purposes only.

Metals, macro- and microelements from trout samples were analyzed: sodium (Na), magnesium (Mg), aluminium (Al), potassium (K), calcium (Ca), titanium (Ti), chromium (Cr), mangandese (Mn), iron (Fe), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), barium (Br), rubidium (Rb), strontium (Sr), molybdenum (Mo), staniu (Sn), iodine (I), barium (Ba) and phosphorus (P).

The first stage was that of mineralization with microwaves, 0.5 g of trout sample was taken and subjected to microwave mineralization with nitric acid (HNO3)-65% and hydrogen peroxide (H2O2)-30%. In the next stage, the digestive program was carried out (Table 1), followed by the cooling stage.

After this step, the samples were diluted to 20 ml, with ultrapure water for dilution, then the filtration step follows, using a cellulose filter of 0.45 µm.

Table 1. Operating conditions of the microwave digestion system

Specification	Stage				
	1	2	3	4	5
Temperature (°C)	145	170	190	100	100
Pressure (bar).	30	30	30	0	0
Ramp time (min)	5	1	1	1	1
Maitenance time (min)	25	10	15	10	10
Power (%*)	80	80	80	0	0

*100% power corresponds to 1400 W

ICP-MS (Inductively Coupled Plasma Mass spectrometry) / ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) was used to analyze the concentrations of the elements in the solutions that were initially mineralized. A Perkin-Elmer Elan DRC II inductively coupled plasma mass spectrometer ICP-MS was used for liquid sample analysis as well as a quantitative method with single-point calibration and response factor-based exploration, following a double calibration of the detector, to have information about as many elements as possible. All results obtained are expressed in ug/L (micrograms/liter) and the limit of quantification is 1 ug/L.

The normality of all datasets was assessed by the Shapiro-Wilk assay, using GraphPad Prism for statistical analysis. The results are presented as a mean ± standard error of the mean (SEM). The t-test was used to determine statistical significance, and the resulting P-values were annotated on accompanying graphs.

RESULTS AND DISCUSSIONS

The concentration of heavy metals for the two species of trout taken into account from diffe-rent places of the Transylvanian area, therefore the values of the metals can be seen from the graphs presented below (Figures 1, 2, 3).

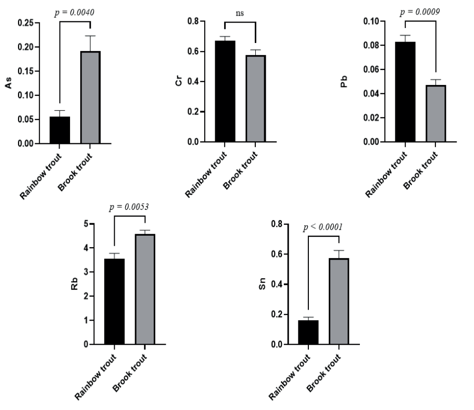

Figure 1. The levels of heavy metals (As, Cr, Pb, Rb and Sn) from Rainbow and Brook trout. Data represents the mean ± SEM (n = 5) expressed in ug/L

According to Figure 1, in the presence of heavy metals, rainbow trout from Marișel commune exhibited significantly reduced concentrations of arsenic (As), rubidium (Rb), and staniu (Sn). Conversely, lead (Pb) levels were substantially elevated in rainbow trout compared to brook trout obtained from the Gilău commune. Additionally, chromium (Cr) concentrations were slightly higher in Rainbow trout but not statistically different to the Brook trout group. Among the five elements considered in Figure 1, the lowest values for rainbow trout were represented for the following two elements: arsenic (As) and staniu (Sn).

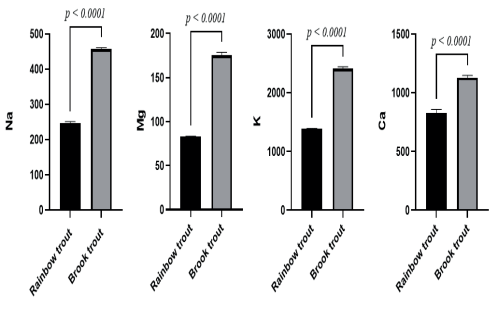

Figure 2. Concentrations of macroelements from Rainbow and Brook trout (Na, Mg, K and Ca) from Rainbow and Brook trout. Data represents the mean ± SEM (n = 5) expressed in ug/L

Differences were also observed in the macroelement profiles of the two fish species (Figure 2). Notably, concentrations of sodium (Na), magnesium (Mg), potassium (K), and calcium (Ca) displayed statistically significant increased trends in Brook trout indicating more nutritional value compared to Rainbow trout groups.

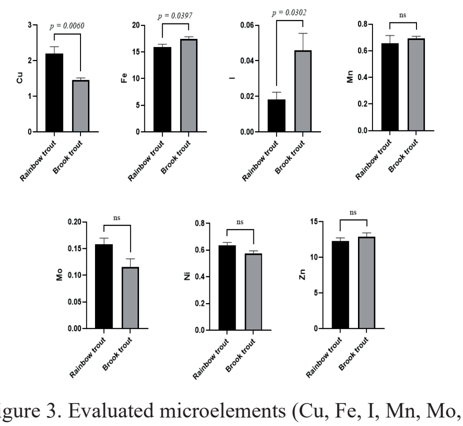

Figure 3. Evaluated microelements (Cu, Fe, I, Mn, Mo, Ni and Zn) from both trout species. Data represents the mean ± SEM (n = 5) expressed in ug/L

Differences in microelement profiles copper (Cu), iron (Fe), and iodine (I) between the two fish species from various locations were also notable (Figure 3). Specifically, copper (Cu) levels were substantially higher in Rainbow trout samples, whereas iron (Fe) and iodine (I) concentrations were significantly elevated in Brook trout samples. Furthermore, the levels of manganese (Mn), molybdenum (Mo), nickel (Ni), and zinc (Zn) exhibited minor variations between the experimental groups, although these differences were not statistically significant.

According to Zeynali et al. (2009), the liver is the organ with higher concentrations of heavy metals (except for Ba and Sr), compared to the muscle tissue of farmed or wild fish. Levels of barium (Ba), chromium (Cr), iron (Fe), manganese (Mn), zinc (Zn) are higher in wild rainbow trout tissues compared to farmed trout, and mercury (Hg) levels in all samples examined were lower than limits accepted by the European Commission, according to the study by Zeynali et al., 2009.

In the study from Chile, higher concentrations were found in trout that were near areas with agricultural and exotic plantations, and this study confirms also the existence of a higher concentration of heavy metals in the liver compared to muscle tissue (Barrientos et al., 2019).

By comparison with other studies, where the concentration of heavy metals in pork from 7 different areas of Romania was analyzed, using the spectrophotometric method, for example the amount of aluminium (Al) varied in the range of 1.68±0.34 µg/L (Cluj) at 9.42±0.21 µg/L (Mureș). Aluminum being considered very toxic for the central nervous system, hematopoietic organs, bone system, etc. (Puia et al., 2019).

Also, in a study for the same locality (Sălaj) Romania, the high degree of concentration of aluminium (Al) and lead (Pb) was found in a pork meat. Al showed a variation of 5.03 mg/kg in the samples from Bistrița-Năsăud and 9.64 mg/kg from Sălaj (Coroian et al., 2017).

Hg (mercury), was found in higher concentrations in liver than in muscle for several fish species, according to several existing studies (Linde et al., 2004).

In liver tissue, heavy metal levels were significantly higher in brown trout than in European eels, and metals levels in the tissue were similar, in both brown trout (*Salmo trutta*) and European eel (*Anguilla anguilla*) (Linde et al., 2004).

According to Jaffal et al. (2011), at the end of winter the levels of copper (Cu) and cadmium (Cd) in the muscle tissue were higher compared to the levels of copper (Cu) and cadmium (Cd) from the end of summer in the liver tissue. Trout farms are a major source of water pollution. The study was carried out in April, July and October.

A control locality and 3 downstream localities were established. According to the working hypothesis, the load with heavy metals was statistically significantly increased especially in sediments but also in E. danica larvae, which proved to be a good indicator for pollution systems (Jaffal et al., 2011).

In a study carried out in Colorado, Brown Trout was exposed for about 2 years to heavy metals: cadmium (Cd), zinc (Zn) and then samples were taken to evaluate the liver and the growth-promoting enzyme (ornithine decarboxylase)-ODC. The results were compared with samples collected from trout that were raised in uncontaminated places.

The activity of the ODC enzyme in both males and females was lower in the trout from the contaminated site (Norris et al., 2000).

CrPic (C18H12CrN3O6) is used in nutritional supplements, which represents a form of organic chromium - Cr (III), because it has a better absorption than other forms of dietary chromium- Cr (III). Also in some studies, CrPic confirms the advantageous role in growth, the improvement of serum protein and the reduction of stress, and in other studies, negative effects such as affecting the function of certain organs are highlighted (Yu Li et al., 2022).

According to Yu Li et al., 2022, in one experiment, the chromium (Cr) content of chromium (Cr) VI in tissues was significantly higher than chromium (Cr) III at 250 mg/kg from 7 to 66 days for most samples, with except muscle tissue, while Cr bioaccumulation of Cr (VI) and Cr (III) in whole fish were similar at 250 mg/kg. Cr (VI) had greater tissue aggressiveness than Cr (III) at a similar level of inorganic Cr bioaccumulation in whole fish, according to the study.

The bioaccumulation of heavy metals in fish according to Kumar et al., 2024, can induce histopathological changes in the essential organs, can also produce oxidative stress at the cellular level and inflammation, respectively the induction of various cellular apoptosis and autophagy pathways.

The most ubiquitous heavy metals are considered by some authors from the specialized literature: chromium, arsenic, mercury, cadmium, lead, copper and nickel, which can lead to pollution of the aquatic environment and also affect the physiology of fish (Kumar et al., 2024).

During an experiment, the authors found that exposure to chromium (Cr) caused major deformations in the liver (apoptosis, vacuolization, necrosis and hypertrophy), (Awasthi & Trivedi, 2019).

According to some authors, to estimate the concentration of heavy metals in various vital organs of fish, the following methods can be used: AAS (Atomic Absorption Spectroscopy), ICP-AES (Inductively Coupled Plasma Atomic Emission Spectroscopy), ICP-MS (Inductively Coupled Plasma Mass spectrometry) and ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) (Sibal &Espino, 2018).

Following a study carried out in Brazil, the authors highlighted the value of heavy metals in water, sediment and fish, during four seasons. The largest concentration of heavy metals was found in sediment, followed by water and the least in fish. According to other studies, suspended sediments adsorb pollutants from water, thus reducing their concentration in the water column (Weber et al., 2013).

In another study carried out in Hungary, where fish were taken from Lake Balaton, metal concentrations in samples taken from different organs (muscle tissue, liver, gills) were between the following ranges: (Cd) cadmium: 0.098; 0.20; 0.106; (Cu) copper: 0.358; 0.327; 1,536; (Pb) lead: 0.3; 0.257; 0.196; (Zn) zinc: 3.00; 7,390; 4.99 μg/g dry weight. The highest concentrations of Cd, Cu, Pb and Zn were detected in the gills and liver of the fish, and the highest concentrations of Hg were measured in the muscle tissue (Farcas et al., 2003).

The results of a study of fish samples taken from the Mexican Pacific coast from two different localities are as follows: in liver tissue were higher concentrations of Cu (Sinaloa 28.3, Guerrero 16.3 $\mu g\ g^{-1}$), Fe (Sinaloa 1098, Guerrero 636 $\mu g\ g^{-1}$) and Zn (Sinaloa 226, Guerrero 186 $\mu g\ g^{-1}$) compared to muscle of fish from both study areas, according to Spanopoulos-Zarco et al. (2017).

In a study conducted on fish harvested in the Black Sea, Turkey, in different seasons, the samples were analyzed by graphite atomic absorption spectrometry and flame furnace after microwave digestion. According to Mendil et al. (2009), the following metal concentrations were obtained: 25.5-41.4 microg/g iron (Fe), 17.8-25.7 microg/g zinc (Zn), 0.28-0.64 microg/g lead (Pb), 0.64-0.99 microg/g chromium (Cr), 1.3-3.6 microg/g manganese (Mn), 1.4-1.9 microg/g copper (Cu), 0.18-0.35 microg/g cadmium (Cd) and 0.25-0.42 microg/g cobalt (Co) for fish species. In this study, it was observed that the levels of trace elements in the fish species analysed were acceptable for human consumption, while the levels of lead and cadmium in the fish samples were higher than the recommended legal limits.

According to a study carried out in France, certain species of fish were caught in different periods of years (1987-2007), and the results show the decrease of heavy metal concentrations in fish tissue between 1987 and 2007, which reflects the decrease of heavy metal concentrations in environment. According to this study, the results found in 2007 are comparable to those published by another study carried out in the 1990s. It should be specified that the values of the average concentrations of cadmium in fish muscle in 2007 were above the maximum safe level for human consumption defined by the European Commission (Shinn et al., 2009).

CONCLUSIONS

Nowadays, due to various industrial or agricultural activities, heavy metal pollution has become a priority.

The aquatic ecosystem is considered as a final reservoir for the accumulation of heavy metals from various activities.

According to studies, the concentration and distribution of heavy metals is essential for the proper functioning of the aquatic ecosystem, because heavy metals disrupt the aquatic balance, being non-biodegradable and bioaccumulative in nature.

The bioaccumulation of heavy metals leads to various anomalies, structural injuries, functional disorders, cellular dysfunctions, etc. and disrupting the global ecological balance.

To protect the global ecosystem, more regulations must be implemented to conserve water sources, because most of the methods used to treat heavy metals are expensive and also difficult to use.

REFERENCES

Abida, B., Harikrishna, S., & Irfanulla, K. (2009). Analysis of heavy metals in water, sediments and fish samples of Madivala Lakes of Bangalore, Karnataka. *Int. J. Chem. Technol. Res.*, 1, 245–249.

Awasthi, Y., & Trivedi, S. P. (2019). Evaluation of glutathione Reductase (GR) Activity in liver of fish, Channa Punctatus (Bloch, 1793) Exposed to Hexavalent Chromium. *Journal of Advanced Zoology*, 90.

Barrientos, C., Tapia, J., Berton, C., Pena Cortes, F., Hauennstein, E., Fierro, P., & Vargas-Chacoff, L. (2019). Is eating wild rainbow trout safe? The effects of different land-uses on heavy metals content in Chile. *Environmental Pollution*, 254 (A), 112995.

Benciu, F. (2007). *Heavy metal pollution*. Bucharest, RO: Didactic and pedagogical Publishing House, 148.

Coroian, A., Miresan, V., Raducu, C., Longodor, A. L., Andronie, L., Odagiu, A., & Balta, I. (2017). The Influence of the Rearing Area upon Cd, Al and Pb Levels in Pork Meat. *Bioflux-ProEnvironment*, 10, 286 - 290.

Elumalai, S., Prabhu, K., Palani-Selvan, G., & Ramasamy, P. (2023). Review on heavy metal contaminants in freshwater fish in South India: current situation and future perspective. *Enviromental Science and Pollution Reseach*, 30, 119594-119611.

Falllah, A. A., Saei- Dehkordi, S. S., Nematollahi, A., & Jafari, T. (2011). Comparative study of heavy metal and trace element accumulation in edible tissues of farmed and wild rainbow trout (Oncorhynchus mykiss) using ICP-OES technique. *Microchemical Journal*, 98 (2), 275-279.

Farcas, A., Salanki, J., & Speczian, A. (2003). Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. *Water Research*, 37(5), 959-964.

Food and Agriculture Organization of the United Nations (FAO). (2018). The State of World Fisheries and Aquaculture -Meeting the Sustainable Development Goals.

Gleick, P. H. (1996). Water resources. In: Schneider SH (ed) Encyclopedia of climate and weather. *Oxford University Press, New York*, 2, 817–823.

Hansen, B. H., Romma, S., Garmo, A., Olsvik, P. A., & Andresen, R. A., (2006). Antioxidant stress proteins and their gene expression in brown trout (*Salmo trutta*) from three rivers with different levels of heavy metals. *Comparative Biochemistry and Physiology Part.C: Toxicology&Pharmacology*, 143 (3), 263-274.

Jaffal, A., Paris-Palacios, S., Jolly, S., Thailly, A. F., Delahaut, L., Beall, E., Roche, H., Biagianti-Risbourg, S., & Betoulle, S. (2011). Cadmium and copper contents in a freshwater fish species (brook trout, *Salvelinus fontinalis*) from the subantarctic Kerguelen Islands. *Polar Biology*, 34(3), 397- 409.

Javed, M., & Usmani, N. (2017). An Overview of the Adverse Effects of Heavy Metal Contamination on Fish Health. *Biological Sciences*, 89, 389-403.

Klerks, P. L., & Weis, J. S. (1987). Genetic adaptation to heavy metals in aquatic organisms: a review. *Environ. Pollut*., 45, 173-205.

Koca, Y. B., Koca, S., Yildiz, S., Gurcu, B., Osane, E., Tuncbas, O., & Aksoy, G. (2005). Investigation of histopathological and cytogenetic effects on *Lepomis gibbosus* (pisces: perciformes) in the Cine stream (Aydin/Turkey) with determination of water pollution. *Environ. Toxicol*., 20, 560–571.

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. *Jr. of Trace Elements in Medicine and Biology*, 83, 127377.

Kumar-Sharma, A., Sharma, M., Sharma, S., Singh-Malik, D., Sharma, M., Sharma, M., & Sharma, A. K. (2024). A systematic review on assessment of heavy metals toxicity in freshwater fish species: Current scenario and remedial approaches. *Journal of Geochemical Exploration*, 262, 107472.

Linde, A. R., Sanchez-Galan, A., & Vazquez, G. E. (2004). Heavy Metal Contamination of European Eel (Anguilla anguilla) and Brown Trout (*Salmo trutta*) Caught in Wild Ecosystems in Spain. *Journal of Food Protection*, 67, 2332–2336.

Majid, M., Janmohammad, M., Enayat, B., & Mehdi-Akbartabar, T. (2019). Heavy metal content in farmed rainbow trout in relation to aquaculture area and feed pellets*. Food and Raw materials*, 7 (2), 329-338.

Malik, N., Biswas, A. K., Qureshi, T. A., Borana K., & Virha, R. (2010). Bioaccumulation of heavy metals in fish tissues of a fresh water lake of Bhopal. *Environ. Monit. Assess*.,160, 267–276.

Market, B. (2007). Definitions and principles for bioindication and biomonitoring of trace metals in the environment. *J. Trace Elem. Med. Biol*., 21, 77-82.

Matta, J., Milad, M., Manger, R., & Tosteson, T. (1999). Heavy metals, lipid peroxidation, and cigateratoxicity in the liver of the Caribbean barracuda (*Sphyraena barracuda*). *Biol.Trace Elem. Res*., 70, 69-79.

Mendil, D., Demirci, Z., Tuzen, M., & Soylak, M., (2009). Seasonal investigation of trace element contents in commercially valuable fish species from the Black Sea, Turkey*. Food and Chemical Toxicology: an International Journal Published for the British Industrial Biological Research Association*, 48(3), 865-870.

Norris, D. O., Camp, J. M., Maldonado, T. A., & Woodling, J. D. (2000). Some aspects of liver function in wild brown trout, Salmo trutta, living in metal-contaminated water. *Comparative Biochemistry and Physiology- Toxicology & Pharmacology*, 127, 71-78.

Pandey, S.N. (2006). Accumulation of heavy metals (Cd, Cr, Ni, Cu, and Zn) in *Raphanus sativus* and *Spinacea oleracea* L. plants irrigated with industrial effluents. *J. Environ. Biol*., 27, 381–384.

Puia, M., Mihon, B., Ionescu, B. Butucel, E., Chicinas, A., Farcas, E., Puskas, H., Raducu, C., Longodor, A. L., & Coroian, A. (2019). Assessing the level of heavy metals in different geographical areas in Romania. *Banat s Journal of Biotechnology*, X (19).

Shinn, C., Dauba, F., Grenouillet, G., Lek, S. (2009). Temporal variation of heavy metal contamination in fish of the river lot in southern France. *Ecotoxicology and Environmental Safety*, 72 (7), 1957-1965.

Sibal, L. N., & Espino, M. P. B. (2018). Heavy metals in lake water: a review on occurrence and analytical determination. *Int. Jouranl Environ. Anal. Chem.*, 98, 536-554.

Sneddon, J., Rode, P. W., Hamilton, M. A., Pingeli, S., & Hagen, J. P. (2007). Determination of metals in seafood and fish in Southwest Louisiana. *Appl. Spectrosc. Rev.*, 42, 23-42.

Spanopoulos-Zarco, P., Ruelas - Inzunza, J., Aramburo-Moran, I. S., Bojórquez -Leyva, H., & Páez-Osuna, F. (2017). Differential Tissue Accumulation of Copper, Iron, and Zinc in Bycatch Fish from the Mexican Pacific. *Biological Trace Element Research*, 176, 201-206.

Weber, P., Behr, E. R., Lellis-Knorr, C., Vendruscolo, D. S., Flores, E. M. M., Dressler, V. L., & Baldisserotto, B. (2013). Metals in the water, sediment, and tissues of two fish species from different trophic levels in a subtropical Brazilian river*. Microchemical Jr.*,106, 61-66.

Wirgin, I., & Waldman, J. R. (2004). Resistance to contaminants in North American fish populations. *Mutat. Res.,* 552, 73-100.

Wittmann, G. T. W. (1979). Toxic metals. In: Förstner U, Wittmann G.T.W. (eds). *Metal pollution in the aquatic environment.* Berlin, GE: Springer Publishing House, 3–70.

Yu-Li, Lu-Wei, Zhang, P., Xiano, J., Zhiqiang-Guo, & Qiongyao-Fu. (2022) Bioaccumulation of dietary CrPic, Cr (III) and Cr (VI) in juvenile coral trout (Plectropomus leopardus. *Ecotoxicology and Enviromental Safty*, 240, 113692.

Zeynali, F., Tajik, H., Asri-Rezaei, S., Meshkini, S., Fallah, A. A., & Rahnama, M. (2009). Determination of copper, zinc and iron levels in edible muscle of three commercial fish species from Iranian coastal waters of the Caspian Sea. *J. Animal Vet. Adv*., 8, 1285-1288.