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THE FRESHWATER MUSSELS ARE A GOOD BIOINDICATOR FOR MONITORING THE WATER POLLUTION BY HEAVY METAL (THE SAINT–VICTOR–SUR–LOIRE LAKE, FRANCE)

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Abstract. The study has focused freshwater mussels to determine the risk of water pollution. We have shown that freshwater mussels are a good bioindicator for monitoring over time this type of water pollution due to their ability to filter water from the river. The entire food chain (freshwater mussels, mollusc, shrimp, fish, birds and humans) is affected by the pollution.

The Saint Etienne (France) has a large industrial and mining history. The end of these activities has given way to industrial wasteland and mine dumps. Leaching by rain industrial and mining waste may generate a very large urban pollution. Rivers (Ondaine and Loire) and lakes (Saint–Victor–sur–Loire) downstream of this zone are highly polluted by heavy metals.

Key words: Heavy metals, freshwater mussels; Bioaccumulation; Bioindicator.

Introduction

Due to their industrial and mining history, several agglomerations in the Rhône–Alpes region have mining and industrial wastes containing several metals pollution sources of groundwater, public health and urban management problem of some sites.

The study of pollution of the Saint–Victor–sur–Loire Lake lacks.

Indeed, considering the difficulty of the collection of valid samples for trace metal analysis in water as well as the low spatial and temporal representativeness of these, use of quantitative bioindicator of the chemical contamination attracts more and more attention in ecotoxicology.

We have elaborated this work to estimate the degree of metal contamination at the level of the Saint–Victor–sur–Loire Lake and Ondaine river mouth (figure 1) this by dosage of seven trace metals (Cu, Mn, Fe, Co, Cr, Pb and Cd) in fresh water Anodontes mussels.

In fact, freshwater mussels have been found to be suitable indicator species for trace metals [GOLDBERG, 1975; PHILLIPS, 1976; PHILLIPS, 1977; BROWN & LUOMA, 1995; JUMSHAMN & GRAHL–NIELSEN, 1996].

It is capable to accumulate trace metals such as cadmium, mercury and lead largely than for example fish and algae [JUMSHAMN & GRAHL–NIELSEN, 1996; RIGET *et al.*, 1996; AIRAS *et al.*, 2004, BUTNARIU *et al.*, 2015].

Context

Saint Victor Lake is bordered by two castles; it is a reservoir of 66 million m^3 of volume, 3.65 km^2 surface, built in 1957.

Decennial drains in 1977, 1987 and 2000 were not performed.

They were conducted between 2011 and 2012.

Saint Victor Lake is located downstream of Aurec–sur–Loire (Haute–

Loire) and upstream of Saint–Just–Saint–Rambert (Loire).

The density of carp is low but includes catfish, crayfish and mussels.

In March 1995, the volume of sludge deposited at Ondaine mouth was estimated at 40 000 m^3.

The cost of sludge removal was evaluated then at € 2 million.

The total volume of sludge deposited across the Grangent dam was between 1.5 and 2 million m^3, which represents 1.3 Mt.

Two–thirds of the sediment would have been deposited by the Loire River and a third by tributaries (primarily Ondaine).

Among the sediments, there were found metal oxides in significant quantities.

The thickness of sediment was about 5 meters at the confluence of Ondaine–Loire.

Water quality is still a major problem for the Saint Victor Lake.

Indeed, during the heart of the great industrial period, Ondaine brought 20,000 tonnes of materials such as iron, manganese, zinc and nickel.

Ondaine is an affluent of the Loire River, crossing several small towns.

The Ondaine River itself has several tributaries:

The Cotatay has long time been renowned for the quality of its waters.

Twenty–two ancient factories located on its shores, ended their industrial activity.

The Egotay's Ondaine tributary was also renowned for the quality of its waters.

Many laundresses and Unieux washerwomen were washing their line basins using its waters.

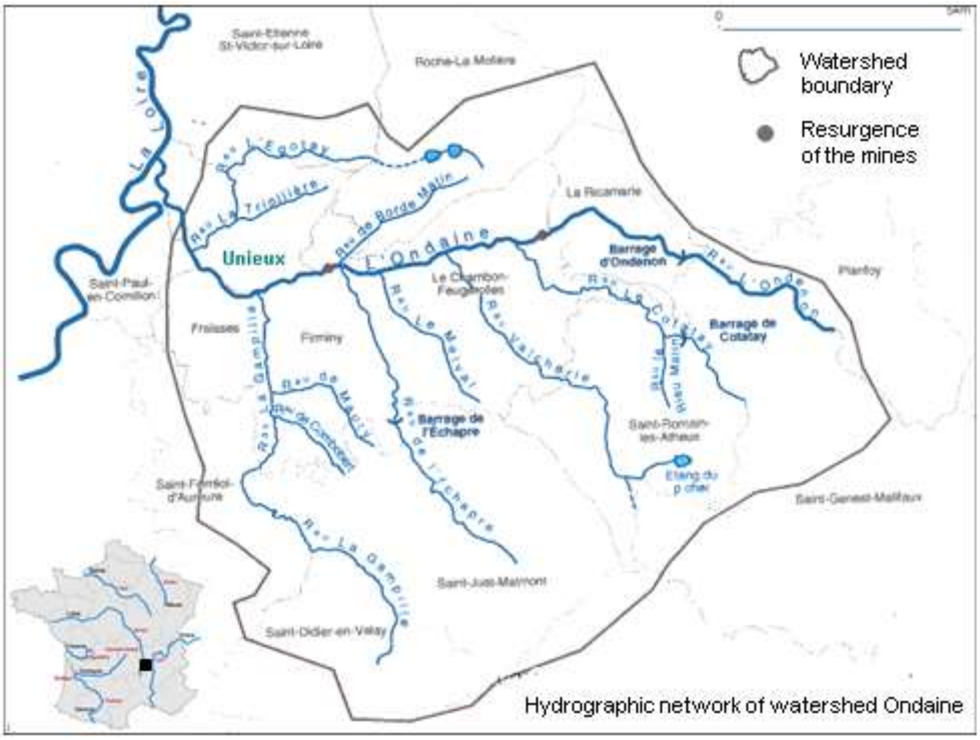

Figure 1. Localization of the Ondaine Valley

Since the late 1960, quality of rivers has deteriorated.

In November 2002, Egotay was still receiving Charles's area wastewater and ones of Beaulieu town, located in the town of Roche–la–Molière, and domestic discharges of Bécizieux, small town of Saint–Victor–sur–Loire via a collector of sewage and other agricultural and industrial untreated waste.

The first traces of pollution of the Gampille tributary of the Ondaine were

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identified in 1964. In 1989, the Agency of Loire–Brittany Basin is committed to Department of the Haute–Loire to stop pollution problems of the Gampille and its tributary Combobert, caused by the waste disposal of Saint–Just–Malmont and the malfunction of the treatment plant.

The causes of "historic" pollution of the Ondaine River have many origins:

- Industrialization of the valley from 1840, receives without any treatment wastewaters from many industrial plants.
- Mine tailing and industrial waste.
- Direct discharges of wastewater into the river.
- The small size of the watershed that provides the preponderance of urban discharges during low flow periods and the excessive and uncontrolled use of pesticides also contributes to the degradation of Ondaine and Loire water quality.

The mines are no longer operating in the Ondaine Valley since 1983.

The basement of valley contains 304 galleries.

Mine wastewater was pumped by Charbonnages de France Company but rejected in part on bed of the Ondaine water course.

The slag wastes are still important.

The management of these historical slag wastes still constitutes to be a major problem for the region.

For 150 years, many companies operated production of special steels but dairy has accumulated in many slag wastes.

The sources of pollution of river were still potentially important in 2003.

Industrial equipment is not waterproof. Wastes were sometimes stored near watercourses.

A preliminary study by Geosciences & Environment department of Ecole Nationale Supérieur des Mines de Saint Etienne of a one hundred samples showed that the soils of the Ondaine valley are very rich in metal oxides, especially those growing on industrial waste.

Industrial waste and harvested soils are highly reactive (figure 2 and 3) and metal oxides are mobilized and easily pass into solution. Water filled metal oxides exceed safety standards.

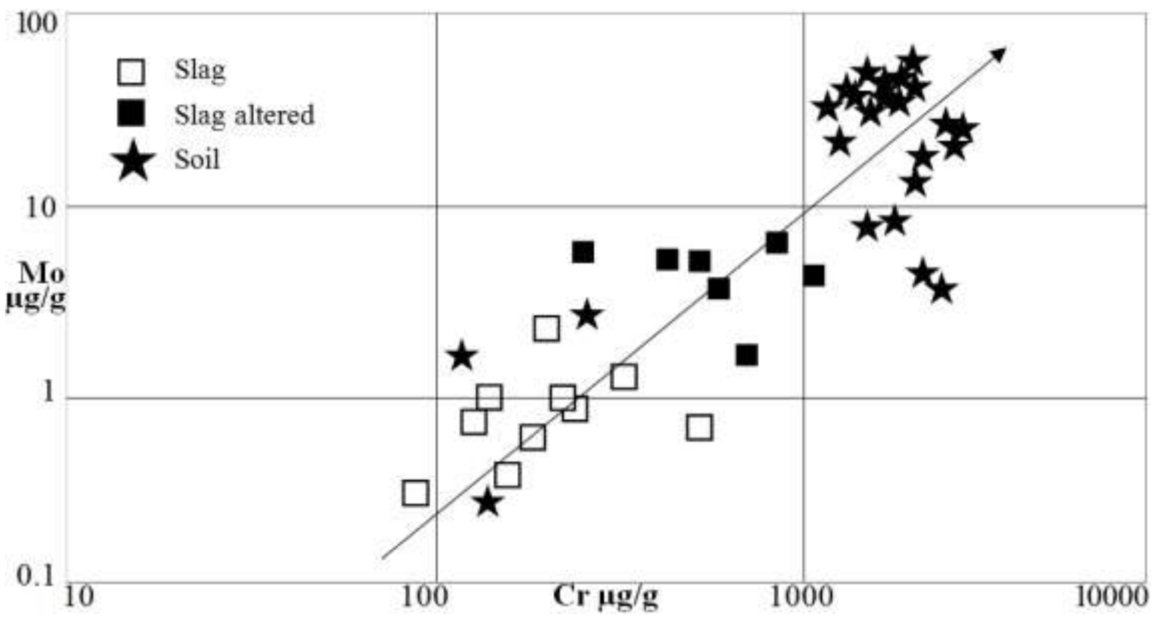

Figure 2. Mo versus Cr content from slag, altered slag and soil of Ondaine valley.

Leaching tests conducted in laboratory have shown that water charged resided 3–4 days, depending on location, before returning to streams and Ondaine River.

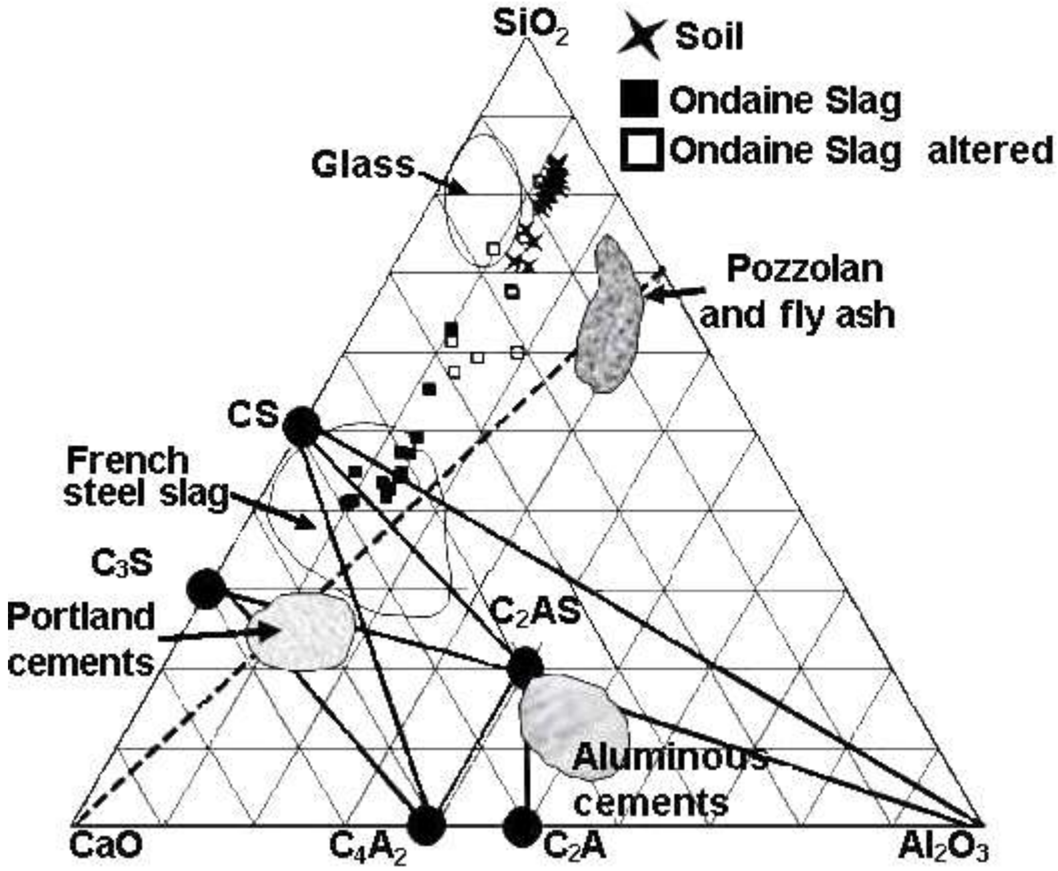

Figure 3. Distribution of slag, altered slag and soil of Ondaine valley in $CaO–SiO_2–Al_2O_3$ diagram. The SiO_2/Al_2O_3 ratio is same in slag, altered slag and soil and CaO/SiO_2 ratio decrease substantially. The composition of soil from slag waste is near glass raw material.

The SiO_2/Al_2O_3 ratio is same in slag, altered slag and soil and CaO/SiO_2 ratio decrease substantially to slag at soil.

The composition of soil and altered slag is near glass raw material.

Material and methods

The sampling of freshwater mussels was elaborated at four stations belonging to the mouth of the Ondaine estuary and Saint Victor Lake.

The shells were carefully opened so as not to damage tissues. The samples were conserved in sterilized petri dishes, weighed and stored at a temperature of–18°C. The adopted procedure for preparation of mussels was as follows: lyophilization during 48 hours under vacuum and a temperature of –50°C, and under a pressure of about 4000 bar.

Grinding of each sample in a porcelain mortar until obtaining of a fine powder. Addition of 3 mL of concentrated HNO_3 (69°C) in each bomb to solubilize metals and 1 mL of H_2O_2 to oxidize the organic matter and digestion at high pressure and high temperature.

Analyses of final solutions were done for all trace elements detected by ICP–AES and Atomic Absorption Spectroscopy in graphite furnace.

The results are expressed in µg/g of dry weight (ppm). Scanning electron microscopy (SEM) coupled with energy dispersive spectroscopy (EDS).

Observations and elemental analyzes were performed on a scanning electron microscope Jeol JSM 6400 coupled to an Oxford EDS analyzer (ENSM–SE). Observations were carried out on secondary electrons mode in case of granular raw unpolished samples and backscattered electron mode. To obtain comparable results, all analyzes were performed under identical conditions with an accelerating voltage of 20 kV.

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Chemical analyzes are semi–quantitative. Mappings XR heavy metals helped to highlight their distributions in mussel shells. Previously, samples were metallized with carbon or gold depending on type of observations and analyzes made.

Results

The organic portion of these freshwater mussels is richer in metals than the shell (Figure 4). The freshwater mussels filter the water of the river and therefore concentrate metals in the organic part. Metal concentrations are above the safety and health standards [BILAL, 2013]. Ondaine Valley drains the major part of the heavy metals that we find in freshwater mussels. The contents of heavy metals in both cases, Loire River and at the mouth of Ondaine are comparable. The same pattern is found in the freshwater mussels of the Ribeira de Iguape River in Brazil and the freshwater mussels from Sebu River in Morocco.

The processes of accumulation in tissues are extremely complex and depend on several factors, including properties of receiving tissue [MAURY & ENGRAND, 1986, FERENCZ et al., 2012]; nature of the contaminant and intra and extracellular environment [BOWEN, 1966].

Contrary to fishes, the organs of the freshwater mussels are all exposed to chemical pollutants suspended in water; direct contamination could have the same significance as trophic contamination.

Heavy metals are in relationship with different pollution incidents and continuous leaching of industrial and mining and industrial waste.

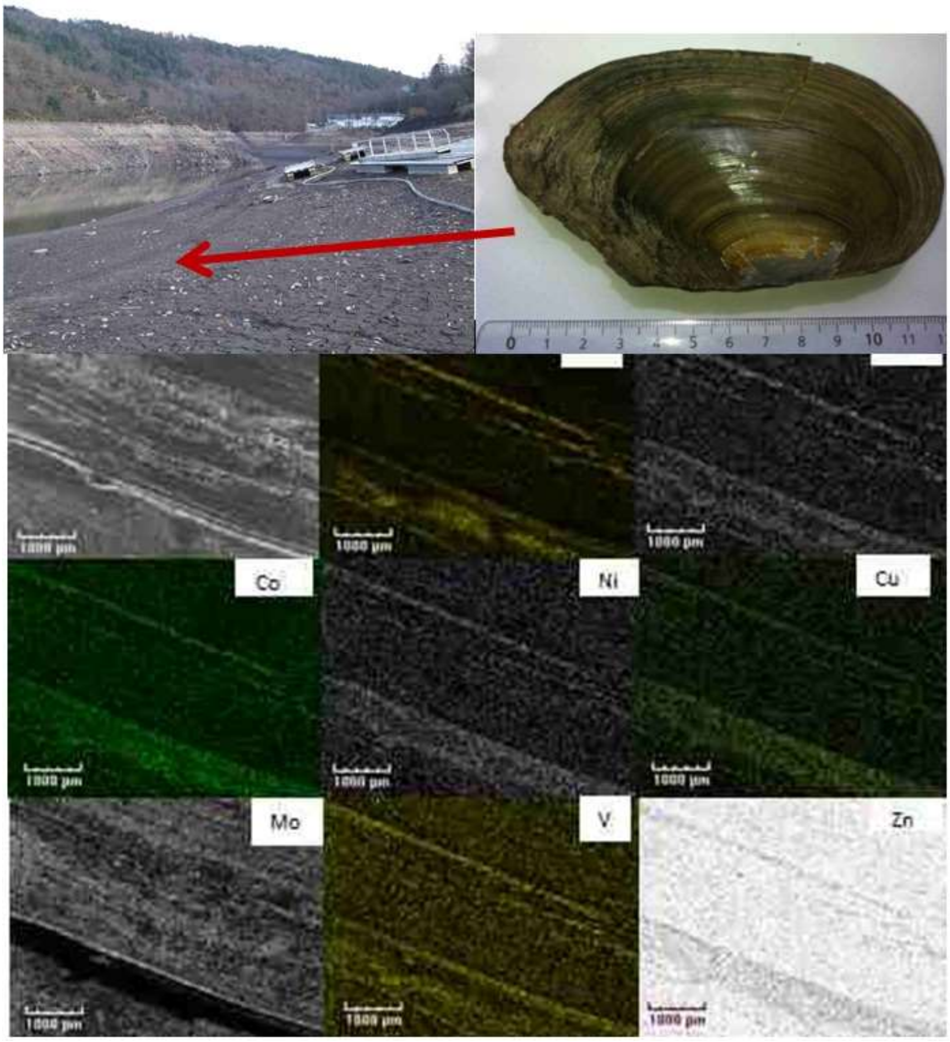

Figure 4. SEM image and distribution of heavy metals in shells of freshwater mussels by XR mapping [BILAL, 2013]. Fe, V: yellow, Mn, Mo, Ni: white; Co, Cu: green and Zn: grey.

Mo, V and Co gets to control the composition of mussel's shells. Fe, Mn, Ni appear sporadically (Figure 5) and indicate an occasional pollution.

The striations of these shells show growth and it represents a very good bioindicator to monitor the pollution of water.

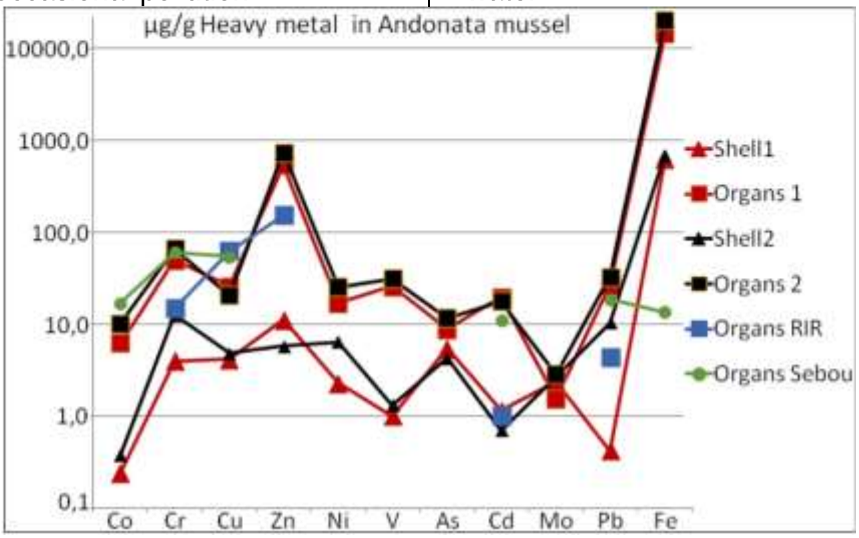

Figure 5. Distribution of metals in freshwater Andonata mussels at the Saint Victor Lake, Loire river (Shell 1, organs 1), at the mouth of Ondaine, Shell 2 and organs 2 [BILAL, 2013] and freshwater mussels at the Ribeira de Iguape River, organs RIR, Brazil [RODRIGUES *et al.*, 2013] and green: Organs from Sebou River Morocco [BOUNAKHLA *et al.*, 2011].

Conclusions

Understanding the phenomenon of bioaccumulation of heavy metals in living substance is of extremely complex.

This is due to the interpenetration of several parameters, which influence the bioaccumulation namely in general:

- the physico–chemical characteristics of the environment,
- the chemical properties of contaminant and
- the biologic factors of the organism.

The freshwater mussels are reliable as bioindicator and easy to use to track the evolution of water pollution.

This study showed that all measured heavy metals are higher than normally permitted levels and reveal a health hazard for the population.

Notably, Saint–Victor–sur–Loire Lake is a station where take place a lot of nautical activities and swimming from spring to late summer.

In conclusion, the results of this study show the persistent degradation of the Ondaine Valley and Loire River as a result of industrial, mining, agricultural and domestic discharges, which it would increase if no strategy of protection of this environment by concerned authorities, is developed and implemented.

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References

1. Airas, S.; Duinker, A.; Julshamn, K. Copper, zinc, arsenic, cadmium, mercury, and lead in blue mussels (*Mytilus edulis*) in the Bergen harbor area, Western Norway. *Bulletin of environmental contamination and toxicology*, **2004**, 73(2):276–84.
2. Bilal, E. Environment Hazards: The recharge areas of the Guarani Aquifer in the state of São Paulo and comparison with the Loire and the Rhône basins in the Rhône–Alpes region. 1st International Research

Workshop, 13–15 November **2013**, Lyon, France.

3. Bounakhla, M.; Bounakhla, Z.; Tahri, M.; Zahry, F.; Zghaid, M.; Doukkali, A.; Benkirane, O.; Saidi, N.; Bilal, E. Evaluation of the Metallic Contamination Impact on *'Mytilus edulis*' Mussel at the Level of the Mouth of Sebou's Estuary, Marocco. *Carpathian Journal of Earth and Environmental Sciences*, **2011**, 6(2): 219–214.
4. Bowen, H.J.M. Trace elements in biochemistry. *Academic Press*, London. **1966**, pp. 241.
5. Brown, C.L.; Luoma, S.N. Use of the Euryhaline Bivalve Potamorcorbula Amurensis as a Biosentinel Species to assess Trace Metal Contamination in San Francisco Bay. *Marine Ecology Progress Series*, **1995**, 124:129–142.
6. Butnariu, M.; Goian, M.; Ianculov, I.; Gergen, I.; Negrea P.; Study regarding the influence of the Co (+2) on the development of soy plant and accumulation of some chemical elements (Fe, Mg, Ca, K and P). Rev Chim (Bucharest). **2005**, 56(8), p. 837–841.
7. Ferencz, Á.; Juhász, R.; Butnariu, M.; Deér, A.K; Varga, S.; Nemcsók J. Expression analysis of heat shock genes in the skin, spleen and blood of common carp (*Cyprinus carpio*) after cadmium exposure and hypothermia. *Acta Biologica Hungarica*, **2012**, 63(1), p. 15–25.
8. Goldberg, E.D. The Mussel Watch–A first Step in Global Marine Monitoring. *Marine Pollution Bulletin*, **1975**, 6(7), 111.
9. Julshamn K.; Grahl–Nielsen, O. Distribution of Trace Elements from Industrial Discharges in the Hardangerfjord, Norway: A Multivariate Data Analysis of Saithe, Flounder, and Blue Mussel as Sentinel Organisms. *Marine Pollution Bulletin*, **1996**; 32:564–571.
10. Maury, R.; Engrand, P. Recherches en écotoxicologie expérimentale sur la bioaccumulation et les transferts de mercure dans un système "sédiment–eau–macrophytes. M.S. *Thesis, University of Bordeaux 1*, **1986**, pp. 320.
11. Phillips, D.J.H. The Common mussel *Mytilus edulis* as an Indicator of Pollution by Zinc, Cadmium, Lead and Copper. II. Relationship of Metals in the Mussel to those Discharged by Industry. *Journal of Marine Biology*, **1976**, 38, 71–80.
12. Phillips, D.J.H. The Use of Biological Indicator Organisms to Monitor Trace Metal Pollution in Marine and Estuarine Environments–a Review. *Environmental Pollution Journal*, **1977**, 13, 281–311.
13. Riget, F.; Johansen, P.; Asmund, G. Influence of Length on Element Concentrations in Blue Mussels (*Mytilus edulis*). *Marine Pollution Bulletin*, **1996**, 32, 745–751.
14. Rodrigues, V.; Sigolo, J.; Guimarães, E.; Availability of metals in bottom sediments and freshwater bivalve (*Corbicula flumínea* and *Anondontites tenebricosus*)–Ribeira de Iguape River Brazil. *In Society for Environment geochemistry and health Symposium*, Paris 8–12, **2013**. p. 78.

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