

Results on Copper (Cu) Concentration in Wild Fish *Squalius Cephalus* & *Barbus Barbus* Tissues in Vardar River, North Macedonia

LULZIM SHAQIRI¹

PhD student, Department of Veterinary Public Health
Faculty of Veterinary Medicine,
Agricultural University of Tirana, Albania

JANI MAVROMATI

Department of Veterinary Public Health
Faculty of Veterinary Medicine
Agricultural University of Tirana, Albania

Abstract

*The concentrations of Copper (Cu) in fish from the Vardar river have been investigated in order to assess safety for consumers and the level of contamination. The selected tissues muscle, liver and skin of two fish species: European Chub (*Squalius cephalus*) and Common barbel (*Barbus barbus*) from Vardar river stream, Republic of North Macedonia. Samples are collected in nine different points (Hot Spots) in total distance of 301km and approximate 33 km distance between sampling sites. During the experiment it was investigated the effect of environmental conditions and urban discharges on Copper accumulation in muscles, liver and skin. The metal analyses were performed using Inductively coupled plasma mass spectrometry (ICP-MS). The average of metal concentrations (microgram per gram wet weight) in nine hot spots (HS) occurred in the following ranges: HS-1: muscles 1.35-liver 7.13-skin 0.74, HS-2: muscles 1.00-liver 3.31-skin 0.56, HS-3: muscles 0.60-liver 3.47-skin 0.27, HS-4: muscles 0.75-liver 3.92-skin 0.65, HS-5: muscles 0.83-liver 3.57-skin 0.68, HS-6: muscles, 0.57-liver 6.41-skin 1.03, HS-7: muscles 1.10-liver 3.42-skin 1.93, HS-8: muscles 1.67-liver 6.96-skin 2.19 and HS-9: muscles 2.05-liver 6.90-*

¹ Corresponding author: lulzimsh@yahoo.com

skin 4.05. The lowest levels of the lead were detected in the skin. The muscles and skin were found to accumulate the highest amounts of Cu. In case of organs, the highest levels were identified as follows: liver > skin > muscles. Further investigation of heavy metals is recommended, including a survey of fish consumption frequency among the local inhabitants.

Keywords: heavy metals, Copper, fish tissue, Republic of North Macedonia

1. INTRODUCTION

Fish is an important part of the human diet, but also a good indicator of trace metal pollution in the aquatic ecosystem. Fish samples are considered as one of the most indicative factors in fresh water systems, for the estimation of trace metals pollution potential (Rashed, 2001). Organisms retain Cu through gastrointestinal system to liver into the soft tissue muscle and skin. Pollution of water bodies is becoming a major cause of concern with respect to human health (Jarup, 2003). Metals in waters may be of natural origin from the rocks and soil or from human activities, including industry, domestic wastewater, agricultural discharge, mine runoff, solid waste disposal and atmospheric deposition. Metals generally enter the aquatic environment through atmospheric deposition, erosion of the geological matrix, or due to anthropogenic activities caused by industrial effluents, domestic sewage, and mining wastes. Increase in the human population has greatly contributed towards the conversion of these water bodies to impending contamination sinks (Tarvainen et al., 1997; Stephen et al., 2000).

Heavy metals are well known to be non-biodegradable and when present at high concentrations, they tend to bioaccumulate (De Forest et al, 2007). Being non-biodegradable,

metals can be concentrated along the food chain, producing their toxic effects at points often far away from the source of the pollution (Fernandez et al., 2000). Heavy metals can cause a variety of diseases in humans depending on the degree of exposure. These vary from minor skin irritation to severe damages of the liver, kidney, skeleton, nerve tissues and circulatory system. Copper belongs to the essential metal according to this in the lower concentrations is necessary for the living organisms, because Cu is a microelement for normal progress of the metabolic process in cell and it is a functional part with big influence on the activity of proteins and enzymes (Mumtaz,2002). In the nature we can find him as form of its minerals Chalcopyrite (CuFeS_2), Cuprite and Azurit.

River Vardar is the longest river in the Republic of North Macedonia and Republic of Greece with distance of over 388 km, with his source in the location Vrutok and stream at the Aegean Sea. The Vardar river, with its tributaries, makes up a great part of the total water resource of Macedonia. These tributaries and river Vardar are directly or indirectly connected with the mining areas and foundry for Copper (Cu) in the locations. The first reported information about ichthiofauna of river Vardar was from (Steindachner, 1892) and then he describes the *Leucos macedonicus* from the Vardar river. Some fish species in the river Vardar (*Squalius cephalus*, *Barbus Barbus*, *Vimba vimba*) are good examination samples for pollution because they are in the all stream of river. The ichthiofauna of the Vardar river is important part of the aquatic food chain for their omnivorous way of feeding. The river is widely used for fisheries (wild fish and fish farming in the upper part), sports and recreation. Very little recent information is available regarding the contamination with metals in the Vardar river fish species. The river Vardar passes near and across the biggest cities including Gostivar, Tetovo, Skopje, Veles, Negotino, Demir Kapija and Gevgelija with

possibility of environmental contamination from domestic and industrial sewage Hot point spots in Fig.1.



Figure.1 In the map of the Republic of North Macedonia, showing hot point spots

The aim of this study is to provide information and evaluate the level of Copper as a heavy metal in fish organs (muscle, liver and skin) from European Chub (*Squalius cephalus*) and Common barbell (*Barbus barbus*) fish species.

2. MATERIAL AND METHODS

Samples were collected in nine (9) places with distance between them in around 33km along the river Vardar. From each place fishes were collected from both species with support from the licensed fishermen for sport fishing in the North Macedonian fishing Federation.

Fish samples were transported with the plastic bags set in transport refrigerator in laboratory submitted for dissection of target organs were collected for study including skin, liver and muscle. Each sample of fish tissue has been measured with analytical scale then set in plastic bag, marked with number marking the catching location and tissue, then finally

refrigerated below the -18°C . Total numbers of samples were sixty (60) from European Chub and Common barbell.

Next step was the sample to set in the porcelain pots, heated in microwave in 105°C for 24h to drain. The next day samples were taken out from microwave and they passed to the stove for 24h in 550°C where they were burned. After 24h burning, samples were ready for digestion with HNO_3 65%. Prepared samples after digestion are ready for reading and analyze by Inductively coupled plasma mass spectrometry with the ICP-MS type Agilent 7500 series. All data are presented in the unit mg/kg wet weight of a sample tissue.

3. RESULTS AND DISCUSSION

Results of this study showed that the metal concentrations accumulated in the tissue samples were in descending order of liver > skin > muscles. In the study, we found that the concentration of Copper was different in the analyzed organs and differently in the sampling locations. The average of concentration of the Copper in the organs (muscle, liver and skin) and the locations (nine locations) is showed in Table 1.

Table1. Heavy Metal Content Copper (mg/kg wet weight) in Fish muscle, liver and skin

Sampling point	Muscle	Liver	Skin
HS-1	1.35	7.13	0.74
HS-2	1.00	3.31	0.56
HS-3	0.60	3.47	0.27
HS-4	0.75	3.92	0.65
HS-5	0.83	3.57	0.68
HS-6	0.57	6.41	1.03
HS-7	1.10	3.42	1.93
HS-8	1.67	6.96	2.19
HS-9	2.05	6.90	4.05

Higher Cu concentrations were found in liver tissue, while the lowest were detected in muscle tissues. This finding is in agreement with those of other studies regarding the differences between heavy-metal accumulation in fish tissues.

There are guidelines on acceptable levels of Cu in the edible parts of fish suggested in North Macedonia is according to international standards 20-100mg/kg shown in the Table 2.

Table 2 Guideline of International standards for heavy metal

Guidelines	Pb	Cd	Zn	Cu	Ni	Cr	Mn	Fe
FAO/WHO (1989)	0.50	0.50	40	30	-	-	-	-
Range of International Standards (Yamazaki <i>et al.</i> , 1996)	0.50-10	0.0-2	40-100	10-100	-	-	-	-
TFC (2002)	0.2	0.05	50	20	-	-	-	-
IAEA-407 (Wyse <i>et al.</i> , 2003)	0.12	0.19	67.10	3.28	0.60	0.73	3.52	146
EC (2006)	0.30	0.05	-	-	-	-	-	-
Chine (Cheung <i>et al.</i> , 2008)	0.50	-	-	50	-	2<	-	-

According to our results, there is metal contamination, but it is lower than the guidelines, in the edible part of the examined fish. The examined fish were not associated with enhanced Cu content in their muscle and were safe within the limits for human consumption.

From results we can see that we have a different Copper concentration, and the highest level is in the HS-9 sampling site, which is the location at the end near the border where the river Bregalnica is connected to the river Vardar, waste water and atmospheric water from the capital city of Skopje are loaded to the river Vardar at the upper part between city of Veles and Skopje, atmospheric water from the Copper mine Bucim so it is expected to have registered higher Copper presence to the fish from downstream of the river Vardar. In the most studies of similar analyzed samples, muscle accumulate the highest concentration of Copper, in our study this is shown in the samples of liver collected at sites from HS-4

until the HS-9, this shows that fishes in the part of river Vardar from Skopje till the border with Republic of Greece have accumulated higher concentration of Copper. In previous study, increased concentrations of these hazardous substances, especially Pb and Cd in water and sediment from the lower part of the Vardar River, influenced higher accumulations of metals in liver, gills and gonads of *Gobio gobio* L. (Nastova et al. 2017), concentration of Cu in muscle is not reported.

In our country the river pollution with heavy metal contaminations it is a concern because of potential thrived waste from domestic and industrial sewage, non-secured industrial landfill and mining fields. The Cu is calculated as potential hazards that can endanger both animal and human health. Knowledge of its concentration in fish is therefore important; both with respect to nature management and human consumption of fish as suggested in Amundsen et al., (1997).

In Vardar river, among all river/aquatic organisms, fish is the most interested for humans, in particular for sport activities, it also presents source of food mainly for fisherman families. Fishes are considered as indicators of the presence of the heavy metal contaminations in river ecosystem with light level risk as a potential for human food consumption, because they (fish) are at the top of aquatic food chain, this makes them easy transmitters of heavy metal accumulation to humans.

4. CONCLUSION

Our results provide information for the levels of Cu in common fish species of the Vardar river. Results will contribute to the effective monitoring of both environmental quality and the health of the organisms inhabiting the river ecosystem. According to the fish sample analyses, the range of concentration with Copper is close to the higher border with the

range of international standards 20-100 mg/kg wet weight, and it shows that the fishes from investigated Vardar river are safe for human consumption.

Aquatic organisms have been widely used in biological monitoring and assessment of safe environmental levels of heavy metals. In this study metal concentrations in the muscle of both fish species were used to investigate possible transfer of metals to human populations via fish consumption. Since accumulation of metals in the biological system is dangerous to human beings in our country, there is a need for regular or continuous monitoring of heavy metals concentrations in the aquatic environments.

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