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ASSESSMENT RATING OF FE, CU AND ZN IN WATER AND IN TISSUES OF *LUCIOBARBUS CALLENSIS* (VALENCIENNES, 1842) AND *CYPRINUS CARPIO CARPIO* (LINNAEUS, 1785) AT K'SOB RESERVOIR (M'SILA - ALGERIA)

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Abstract

The aim of this study was to determine Fe, Cu and Zn in K'sob reservoir water (Msila - Algeria) and to estimate their concentration in the tissues of Cyprinus carpio carpio and Luciobarbus callensis, as an indicator of the quality of this aquatic ecosystem. The choice of these species is mainly based on endemism criteria; the Carp fish is representative species of the invasive fish communities but the Barbel is an endemic species inhabiting the course of a Wadi and the K'sob reservoir. Our study conducted from January to May 2014 with a monthly monitoring in order to analysis the concentration of the heavy metals in water and fish tissues. The reservoir water show different values of heavy metals between high concentration of Cu and a medium for Fe and Zn. While the higher concentrations of Copper were obtained from carp and important values of Iron and Zinc are shown in both species. These values appear compatible with our fish's habitat and its diet.

Keywords

Heavy Metal, Freshwater Fish, Luciobarbus Callensis, Cyprinus Carpio Carpio, Water, Reservoir, Algeria

1. Introduction

Over most of the Algerian territory is arid, this aridity combined with the fluctuation of the Mediterranean climate makes water a resource that is both rare and unequally distributed over time and space. Subsequently this variability has necessitated the mobilization of all water resources as well as the construction of dam reservoirs. These artificial aquatic ecosystems are often subject to physical and chemical pollution because they are exposed to many contaminations.

Many of pollutants are released every day into the environment. Among them, heavy metals are considered serious pollutants of the aquatic environment (Lu et al., 2015; Pandey & Madhuri, 2014) because of their persistence and their tendency to bioaccumulate in aquatic organisms (Harte et al., 1991; Schuurmann & Markert, 1998; Zenker et al 2014).

Heavy metals are natural trace components of the aquatic environment, but background levels in the environment have increased, especially in areas where industrial, agricultural and mining activities are widespread (Langston, 1990; Bryan & Langston, 1992).

Metals transferred through aquatic food webs to fish, humans, and other piscivorous animals are of environmental and human health concern (Chen et al., 2000). Heavy metals accumulate in the tissues of aquatic animals and may become toxic when accumulation reaches a substantially high level. Accumulation levels vary considerably among metals and species (Heath, 1987). Fish are at the higher levels of the food chain (Bosch et al., 2016; Eimers, 2001) and may concentrate large amounts of some heavy metals from the water (Eimers, 2001)

The aim of this study was to determine three essential heavy metals (Cu, Zn and Fe) from water and fish tissues of two Cyprinidae fish in the K'sob reservoir in arid region of Algeria.

2. Materials and Methods

2.1 Study Area

The watershed of Wadi K'sob (Fig. 1) is located in the southern slopes of the Hodna Mountains and its waters flow to the Chott Hodna which is an endorheic lake, outlet. The surface area of the basin is 1494.5 km².

The K'sob Wadi has an average annual abundance of 60 million m3 before entering the reservoir with a specific flow rate of 1.51 / s / km² or an average flow factor of 11%.
The average density of this Wadi is extremely high (5.45 km / km²), mostly near the dam (Mimeche, 2014). Benkadja et al. (2012) noted an average annual water discharge of 0.89 m^3 / s recorded for the period 1973-2010.

The K'sob reservoir (Fig 1) is an older man-made lake located in an arid region: North the Hodna Plaine (north-east Algeria). It was constructed with an agricultural purpose on the K'sob Wadi and it has an important temporal variation in water temperature and volume (Mimeche et al., 2013; Mimeche et al., 2015), nowadays its water storage is less than 07 hm^3.µ

2.2 Fish Sample

Two species of fish, the barbel (*Luciobarbus callensis*) and the common carp (*Cyprinus carpio carpio*), were selected as sentinel species. Carp are representative species of the invasive fish communities. The Barbel endemic species inhabiting the course of stream and the reservoir. The specimens were collected during monthly experimental samplings taken between January 2014 and May 2014. The fishing gears used by the fishermen were trawls (each with a length of approximately 100 m).

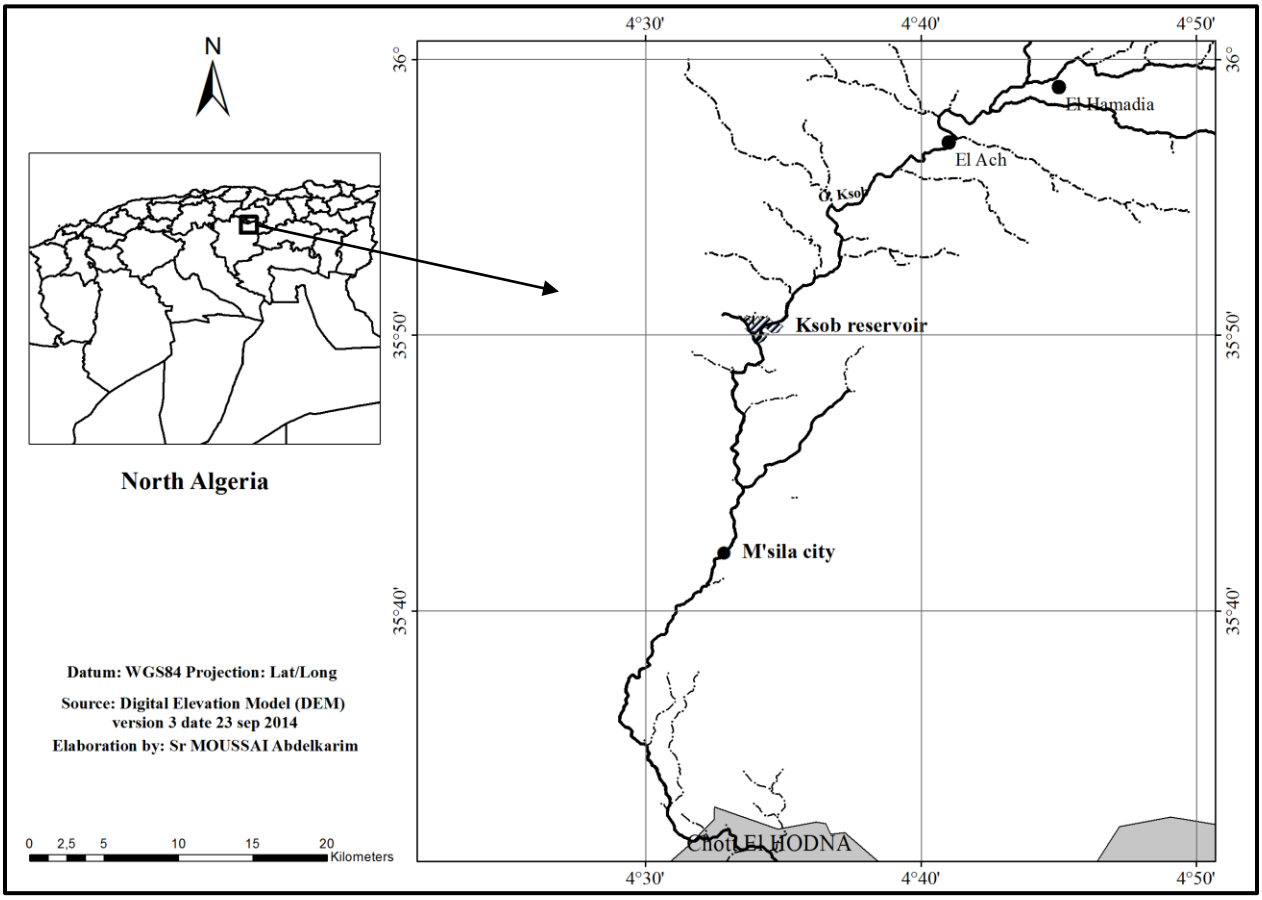

Figure 1: Map of study area

The specimens were preserved in neutralized formaldehyde solution (7%) and transported to the laboratory, where their total length (TL; ±0.1 mm) and total weight (TW; ±0.1 g) were recorded. All specimens were dissected to determine their sex by direct observation of the gonads.

2.3 Water Sample

Water samples were taken 50 cm below the water surface in 500 ml bottles, filtered through a Whatman 0.45 µm glassfiber filter, transferred to the polypropylene bottle of 500 ml, acidified with 5 ml of concentrated HNO 3 to less the pH than 2.0. Then water samples are stored at 4ºC and analyzed directly.

2.4 Chemical Analysis

Fish muscle samples were obtained from the fish body in the dorsal part, without bones. For the analysis, two grams of each tissue sample were dissolved in a solution of nitric acid (HNO3: H2O = 2: 1) at 130 ° C for 2 hours. The water analysis and tissue of the fish were made using an Shimadzu AA 6200 flame atomic absorption spectrophotometer.

2.5 Statistical Procedures

All statistics were performed using the PAST program (Paleontological Statistics) Version 3.05 (1999-2015). Values of heavy metals in water and tissues are presented as means and standard errors. ANOVA and Pearson's correlation coefficient was used to test for significant associations between heavy metal levels in water and fish tissues, levels of probability are $p < 0.05$ was accepted as significant.

3. Results

3. 1 Biological Data of Samples

Table 1 shows the sex, age, total body length and total weight of fish used in this study.

Table 1: *Sex, age and Morphometric data (Minumum, maximum, mean and Std. error values of length and weight) of common carp (Cyprinus carpio carpio) and barbel (Luciobarbus callensis) from the K'sob reservoir*

Species	Sex	n	Age	Total Length (cm)	Total Weight (g)
Carp	Males	6	2-3	22.53±0.94 (19.9-25.5)	168.0± 26.31 (110-257)
	Females	4	3-4	33.95±1.80 (30.9-38.0)	480.9± 57.58 (371.0-594.5)
	Total	10	2-4	27.1±2.05 (19.9-38.0)	293.16± 57.30 (110.0-594.5)
Barbel	Males	14	2-5	17.73 ± 0.77 (13.1-21.9)	68.2±7.45 (28.5-109.1)

	Females	6	2-5	16.56±1,05 (14.1-19.9)	58.0±11.94 (32.1-96.1)
	Total	20	2-5	17.38±0.6 (13.1-21.9)	65.14± 6.24 (28.5-109.1)

Ten carp are collected in K'sob reservoir, with the size range of males (n= 6) was 19.9 cm and 25.5 cm, for the female (n= 4) between 30.9 cm and 38.0 cm. Age determination by scales showed 3 age-groups (2+ to 4+ years) in females and males.

The age determination of *L.callensis* showed 4 age-groups (2+ to 5+ years) in the both sex. The size range of males (n=14) was 13.1cm and 21.9 cm, for females (n=6) between 14.1 cm and 19.9 cm

3.2 Heavy Metal Content of Reservoir Water

Metal concentrations in water are presented in Table 2, which include minimum, maximum and mean concentrations with associated standard error values, results from other studies and guidelines.

The iron (Fe) has the highest concentrations among the studied metals and ranged between 0.084 mg l^{-1} and 0.67 mg l^{-1}, followed by Zinc with the values between 0.09 mg l^{-1}-and 0.47 mg l^{-1}. The value of copper ranged 0.025 mg l^{-1} and 0.10 mg l^{-1}. Three essential heavy metals (Cu, Zn and Fe) a significant difference was found between heavy metal levels in water of K'sob reservoir $F(14,2)= 3.91$ and $P < 0.05$.

Table 2: *The concentrations (minimum, maximum, mean and Std. error values) of heavy metals in the K'sob reservoir (mgl^{-1})*

	Min	**Max**	**Mean ± Std. Error**
Iron (Fe)	0.084	0.67	0.3008±0.112
Zinc (Zn)	0.09	0.47	0.328±0.072
Copper (Cu)	0.025	0.10	0.05 ± 0.015

3.3 Heavy Metal Uptake by the Fish

Heavy metal concentrations in the tissues of carp and barbel are shown in Table 3.

Iron and zinc concentrations content in carp tissues ranged respectively between 3.36 $mgkg^{-1}$ to 26.8 $mgkg^{-1}$ and 3.51 $mgkg^{-1}$ to 18.53 $mgkg^{-1}$. The Copper levels are 1.92 $mgkg^{-1}$ to 8.5 $mgkg^{-1}$.

The high levels of iron and copper shown to the females, and the zinc levels shown in the males. Both Fe and Cu concentrations between females and fish males were statistically significant ($P < 0.0001$, df =9)

In barbel the concentrations of iron and copper are lower than the carp tissues, ranged respectively 2.70 $mgkg^{-1}$ to 22.11$mgkg^{-1}$ and 0.94 $mgkg^{-1}$ to 7.80 $mgkg^{-1}$. Zinc values are high in barbel tissues with 2.51 $mgkg^{-1}$ to 21.60 $mgkg^{-1}$.

The females of *L.callensis* accumulate the iron and zinc more than the males, no statistical difference of heavy metal tissues was found between the sex.

Order accumulation of different traces metallic elements in carp and barbel are Fe> Zn> Cu.

Table 3: *Heavy metal concentrations (minimum, maximum, mean and standard error (SE) values) in tissues of Cyprinus carpio carpio and Luciobarbus callensis from the K'sob reservoir ($mgkg^{-1}$)*

Species		Carp			Barbel		
Sex		Males	Females	Total	Males	Females	Total
n		6	4	10	6	14	20
Iron (Fe)	Min	3.36	18.0	3.36	4.65	2.70	2.70
	Max	6.35	26.8	26.8	20.80	22.11	22.11
	Mean ± SE	4.99±0.51	23.21±1.88	12.28±3.07	13.33 ±2.89	8.79 ±1.83	10.15±1.58
Zinc (Zn)	Min	3.51	9.75	3.51	10.04	2.51	2.51
	Max	18.53	14.04	18.53	19.75	21.60	21.60
	Mean ± SE	12.60 ±2.88	12.32 ±0.93	12.49 ±1.70	13.67±1.67	12.71 ±1.85	13.00±1.37
Copper (Cu)	Min	1.92	5.95	1.92	0.94	1.09	0.94
	Max	2.88	8.50	8.50	7.8 0	5.90	7.80
	Mean ± SE	2.37 ±0.18	7.28 ±0.52	4.33 ±0.83	3.38 ±1.25	2.43 ±0.43	2.71 ±0.47

3.4 Heavy Metal Relation between Water and Tissues Fishes

The degree of correlation between the element pairs (Fe, Zn and Cu) is very high, so, the values are significant (Table 4).

Table 4: *Pearson correlation coefficient (R) and levels of significance (P) determined for the relationship between the content of heavy metals in water and t fish tissues*

	Carp		**Barbel**	
	R	**P**	**R**	**P**
Iron	0.999	< 0.05	0.986	< 0.0001
Zinc	0.996	< 0.05	0.841	< 0.05
Copper	0.996	< 0.05	0.729	< 0.05

4. Discussion

When a dam is constructed, the lotic system is replaced by an ecosystem with completely different characteristics (Mimeche et al., 2013). Given that the watershed is facing severe soil degradation (Benkadja et al., 2012), heavy silting has affected the K'sob dam, and suspended sediment also occurs commonly in the water during certain seasons (Mimeche et al., 2013). Consequently, environmental stress and the anthropogenic can be affected rapidly the quality of water and favorite the accumulation of heavy metals in the Wadi K'sob.

Based on the heavy metal level, the water of K'sob reservoir is classified as category II (water is of average quality) according to the standards of SEQ Littoral, (2003) (adopted by The National Agency for Water Resources -Algeria). The concentrations of Cu, Fe and Zn in the reservoir water are lower than WHO standarts (WHO, 2011). The metal levels were the highest of Fe in K'sob reservoir can be the natural leaching of the clay soil, or agricultural origin fertilization and pesticides (Cu) because the watershed of Wadi K'sob considered as a cereal area. Ghrefat & Yusuf (2006) confirmed this information in the Wadi Al-Arab Dam in Jordan. The origin of zinc are anthropic effect by evacuates some of used water in Wadi K'sob.

The heavy metal uptake occurs mainly from water, food and sediment (bottom feeders and burrowing animals) (Canli & Kalay, 1998). Heavy metals are of high ecological significance since they are not removed from water as a result of self-purification, but accumulate in reservoirs and enter the food chain (Loska & Wiechula, 2003).

The *C. carpio carpio* presents an important food source, mainly in proteins, for rural community situated far from the coast in Algeria (Mimeche et al., 2015). The first introduction of this species into the K'sob reservoir was in 1987.

The concentration values of iron found in *C.carpio carpio* in this study is near to the same reported in other regions such as Lake Isikli (5.52 -79.07 $mgkg^{-1}$) in Turkish by Tekin-Özan & Aktan (2012).

The concentration values of zinc found in *C.carpio carpio* in this study is lower than the reported by Tekin-Özan & Aktan (2012) in Lake Isikli in Turkish (1.19 - 390.17 $mgkg^{-1}$), and also low compared with values reported by Lavado et al. (2006) in the river Ebro Spain (33.17-329 $mgkg^{-1}$). The copper values concentration found in this species is similar to the results reported by Tekin-Özan & Aktan (2012) in Lake Isikli in Turkish (0.14 - 5.55 $mgkg^{-1}$), but is weak then findings notify by Lavado et al. (2006) The river Ebro in Spain (6.92- 40.69 $mgkg^{-1}$).

In the man-made K'sob lake, *L. callensis* is known to be one of the most abundant species and to show a certain degree of stability in its seasonal densities (Mimeche et al., 2013). The concentration of iron in the tissues of Barbel in this study (10.15±1.58 mg kg^{-1}) is lower than the declared values 16.50 ±5.50 $mgkg^{-1}$ by Maceda-Veiga et al. (2012) in Ripoll River in Spain.

The concentration of zinc in the tissues of *L.callensis* is low relative compared to reported values 69.00±18.00 $mgkg^{-1}$ by Maceda-Veiga et al. (2012) in Ripoll River and 28.35 - 48.34 $mgkg^{-1}$by Lavado et al. (2006) in the river Ebro in Spain.

The concentration of copper in the tissues of *L.callensis* is very high compared to reported values 2.290± 0.324 $mgkg^{-1}$ by Maceda-Veiga et al. (2012) in Ripoll River. And low values are compared the reported results 9.01 - 23.12 $mgkg^{-1}$ by Lavado et al. (2006) in the river Ebro in Spain.

According to the standards shown by UNESCO / WHO / UNEP (Chapman, 1992), the maximum zinc is 30 $mgkg^{-1}$and 2.0 $mgkg^{-1}$ of iron in Cyprinidae. The iron content in both species is high.

The concentration of metals in fish tissues may be directly related to their concentrations in water. These results indicate that the element pairs maybe have similar anthropogenic sources. Fe, Zn and Cu in water and fish tissues were found to have relatively higher positive correlation coefficients (Tab.4). However, the variations in the concentrations metals found in water from the K'sob reservoir were mainly attributed to changes in waste discharge rates. The levels of concentration of these metals in fish were much higher than in water. Fe, Zn and Cu are essential elements can be produce toxic effects when intake is excessively elevated (Pérez-Cid et al., 2001).

At the K'sob reservoir, *L. callensis* presents an omnivorous diet with a zoo-benthophage tendency (Mimeche, 2014) and Common carp are omnivorous, feeding on benthic invertebrates and vegetation (Brusle & Quignard, 2001). The relationships were found between Zn concentrations in zooplankton and fish (Spry et al., 1988; Papagiannis et al., 2004).

Chen et al., (2000) suggested that Zn may be biomagnified in a variety of aquatic food webs and lake types. The strong relationships were also found between Zn concentrations in zooplankton and fish (Spry et al., 1988). Sindayigaya et al., (1994) and Fisher & Reinfelder (1995) have confirmed that food is the important way of copper accumulation in aquatic animals, and diet choice influences body charge of copper. The greater part of benthic invertebrates (which are common prey items of benthic fish species) obtain nutrition through the digestion of sediment material (Campbell, 1994).

The difference in concentration metals between the sexes from the both species, can be explained by the competition increases between the two sexes to ensure food in K'sob reservoir, especially this study is coincident with the period of reproduction.

In the general order of bioaccumulation of metals analyzed in the muscles of fish species studied (*C.carpio carpio* and *L.callensis*) is as follows: Fe> Zn> Cu. The mechanisms absorption - absorption, metabolism and elimination détoxication- specific to each species studied could certainly explain the respective orders of bioaccumulation.

5. Conclusion

In this contribution of the study of heavy metals in water and tissues of Cyprinids fish, we obtained that the various metal levels for both species are rarely comparable, for the following reasons; the physiological behavior varies from one species to another and within the same ecosystem, variations in the concentrations of metals (Zinc, Copper and Iron) found in water from the reservoir were mainly attributed to changes in waste discharge rates. The levels of concentration of these metals in fish were much higher than in water;

The muscle is the most vital organ of toxicologically due to their key role in the phenomena of metabolism and accumulation; we estimate that the species of cyprinid fish could be seen as a good bio-indicator of the environment quality of the aquatic ecosystems (sentinel species).

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