

Health Safety of the Fish from the Luapula River in Democratic Republic of Congo

Koya Mawazo Kaya^{1*}, Ndibualonji Badibanga Bualufu²

¹Department of Public Health, University of Kalemie, Kalemie, Democratic Republic of the Congo

²Faculty of Veterinary Medicine, Department of Biochemistry, University of Lubumbashi, Lubumbashi, Democratic Republic of the Congo

Email: *koyamawazo@gmail.com

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Abstract

The Luapula River has received mining discharges from Lubumbashi and Kipushi Gecamines for several decades and from the CHEMAF company since 2005. It also received mining waste from SODIMICO. The Luapula River has Lake Banguelo as its source in Zambia and flows into Lake Moero, which is located on the border between Zambia and the Democratic Republic of Congo. The objective of this study is to assess the health safety of the fish from the Luapula River. Fish samples were collected in August 2015 at the site of the locality of Kasenga located downstream of the mining activities (n = 14) and presumed to be polluted. On the other hand, reference fish samples were taken upstream of any mining activity of the Panda, Kasungwe and Congo Rivers at the sections located near their sources and presumed not to be polluted by mining wastes. (n = 11). Ten Metal Trace Elements (MTE) were assayed at the laboratory of the Congolese Control Office (OCC) of Lubumbashi and at the laboratory of the Catholic University of Leuven in Belgium using ICP-OES and ICP-MS. To assess and evaluate the health safety of fish from the Luapula River, the concentrations of fish samples from the Luapula River were compared with the concentrations of the reference fish samples from the Panda Kasungwe and Congo rivers by the Wilcoxon test. The concentrations of fish samples from the Congo River were also compared with the maximum acceptable metal trace element concentrations established by the FAO, WHO, EU (European Union) and other regulatory bodies. as reported by Akoto et al. The results of this study showed that the fish from the Luapula River is contaminated in Cd, Cu, Ni and Pb, and are not fit for human consumption. The results obtained will be brought to attention of the decision-makers of the Province of Haut-Katanga, so that measures can be taken to ban metallurgical factories to drump their

mining wastes into waterways.

Keywords

Fish Health Safety, Luapula River, Metal Trace Elements, South-East of Democratic Republic of Congo

1. Introduction

The south-east of the Democratic Republic of Congo is a region currently characterized by a proliferation of several ore processing plants of all sizes: artisanal, semi-industrial and industrial (Kalenga et al., 2006). Unfortunately, it has been found that almost all mineral processing plants discharge their tailings into the environment without any treatment and discharge their liquid effluents directly into a watercourse (SNC-Lavalin, 2003; Kalenga et al. 2006). However, chemical analysis has shown that many of these wastes contain Metal Trace Elements such as Cu, Co, Cd, Ge, Zn, Pb and As (SNC-Lavalin, 2003; Kalenga et al., 2006; Koya, 2017).

Metal Trace Elements accumulate in living organisms. and have short- and long-term toxic effects. (Miquel, 2001; Bliefert & Perraud, 2011). Food remains a major source of human contamination with many pollutants. (Gérin et al., 2003) Indeed, the toxins in a food are carried by the food itself. (Dérache, 1986). This is why monitoring the quality of fish and its aquatic environment is of great importance for the protection and preservation of the health of the entire population of the Kasenga and Lubumbashi region, who regularly consumes fish from the Luapula River. We hope that the results of this study will tell us what impact the discharge of untreated mining waste into the Luapula River can have on the chemical quality of fish and what possible risk this can have on the health of the population.

Despite the fact that the congolese population of the South-East of the Democratic Republic of Congo consumes fish from rivers polluted with mining wastes, which are otherwise untreated, studies on the quality control of the fish are rare and almost non-existent. A part from our work on the quality of fish in Lake Tshangalele in 2012 and 2017, Katemo, 2010, studied the impact of mining effluents on the Shituru hydrometallurgical complex on the Lufira basin. The results showed that there was a high contamination of heavy metals, in the Upper Lufira bassin.

When the fish of the Luapula River bathe in an aquatic environment polluted with mining waste, themselves loaded with multiple pollutants, including Metal Trace Elements (SNC-Lavalin, 2003; Kalenga, et al., 2006), can we really believe that these fish of the Luapula River are not contaminated with various pollutants? For our part, we believe that the fish of the Luapula River are contaminated, particularly with Metal Trace Elements, which should be determined in this study.

From the chemical quality of the samples, we will be able to say whether the fish from the Luapula River is safe and therefore fit for human consumption. Food is safe when it can be consumed without risk of transmitting disease or causing poisoning. Thus, the objective of this study is to assess the health safety of fish from the Luapula River.

2. Materials and Methods

2.1. Study Environment

The Luapula River, in the Congolese locality of Kasenga, about 200 km from the city of Lubumbashi, as well as the Congo, Panda and Kasungwe Rivers, in the section located near their source and in the Haut-Katanga Province, in the south-east of the Democratic Republic of Congo, constituted our research environment.

The Luapula River has Lake Bangwelo as its source in Zambia and flows into Lake Moero, which is located on the border between Zambia and the Democratic Republic of Congo; it acts as the border between the two countries (Charlier, 1955). It provides the fishes to Riparian's population who is living to the right's river in Zambia and to the left's river in the Democratic Republic of the Congo.

The Luapula River has received mining waste for several decades, through the Kafubu, Lubumbashi and Musoshi rivers, mining waste from Lubumbashi and Kipushi Gecamines, as well as from SODIMICO (Congo's Industrial and Mining Development Company). Indeed, the Lubumbashi Electric Smelter (FEL) produces slag containing mainly Cu, Co, Cd and Ge. Unfortunately, this slag was stored next to the Lubumbashi River. And thanks to the rain, part of the slag poured into the Lubumbashi river each time. In addition, the cooling water from the molten furnace is directly discharged into the same river (Kalenga et al., 2006). SODIMICO had a floating copper ore concentrator with a capacity of 5000 tons per day. The floatation overflows and the overflow of the settling tank of the solutions are discharged into the Musoshi River which flows into the Kafubu river. The waters of the Musoshi River contained xathanes, sodium hydrosulfides, and foaming agents. Releases from the concentrator also contained 0.4% copper (Kalenga et al., 2006). Wastes from Kipushi's new copper and cobalt mill are discharged into the Kipushi tailings ponds and some of the discharges reach the Kafubu River. Analysis of a sample of these tailings collected in 2003 showed that these tailings contained As (740 mg/kg), Cd (110 mg/kg), Cu (360 mg/kg), Pb (200 mg/kg) and Zn with 15,000 mg/kg (SNC-Lavalin, 2003). The Musoshi and Lubumbashi rivers flow into the Kafubu River with all their untreated mining wastes, and the latter river, Kafubu, in turn discharges into the Luapula River.

2.2. Fishes Studied

The fishes studied belong to different families including Cyprinidae (Pseudocrenilabrus philander and Tilapia rendalli), Clariidae (Clarias buthupogon, C. dumerlii), Mochokidae (Synodontis sp, Chilogramis sp), Cyprinidae (Enteromius neefi), Cyprinodontidae (Lacustricola katangae), Anabantidae (Ctenopoma multispine).

2.3. Sampling and Samples Preparation

Fish samples were collected in August 2015 at the site of the locality of Kasenga, located downstream of the mining activities ($n = 14$) and presumed to be polluted. On the other hand, reference fish samples were taken upstream of any mining activity of the Panda, Kasungwe and Congo rivers at their sections presumed not to be polluted by mining waste and located near their sources ($n = 11$).

The fishes were caught either by net or by hook at the Kasenga site. After capture the fish samples were placed in small packing bags $28 \text{ cm} \times 17 \text{ cm}$ and transported in insulated boxes to the freezer for conservation. The preparation consisted of stripping the samples of their viscera and scales using a knife. After cleaning with distilled water, put in a bucket, the samples were dried in Binder and Thermosi SR2000 brand ovens at 70°C for 48 hours. After drying, the samples were crushed and powdered using porcelain mortars and pestles. The samples were then sent to the laboratory.

2.4. Chemical Analysis of Samples

Ten metal trace elements namely Al, As, Cd, Co, Cu, Mn, Ni, Pb, Se, and Zn were selected and assayed using ICP-MS at the laboratory of the Catholic University of Leuven in Belgium and ICP-OES at the laboratory of the Congolese Control Office (OCC) in Lubumbashi, DR Congo.

2.5. Statistical Analysis

To assess and evaluate the health safety of fish from the Luapula River, the concentrations of fish samples from this river were, by the Wilcoxon test as described by Ancelle (2002), compared with the concentrations of the reference fish samples from the Panda and Kasungwe rivers as well as from the Congo River at the level of their sections not polluted with mining wastes.

2.6. Comparison with Thresholds

The concentrations of fish samples from the Luapula River were also compared with the maximum acceptable metal trace element concentrations established by the FAO, WHO, EU (European Union) and other regulatory bodies as reported by Akoto et al. (2014).

3. Results

It can be seen in **Table 1** above that the concentrations are quite low. Indeed, no concentration was found to be higher than the thresholds established by the WHO (30 mg/kg for Cu, 2 mg/kg for Cd and Pb and 1000 for Zn). (**Table 2**, **Table 3**)

It can be seen that, for all metal trace elements, all concentrations are below the WHO (30 mg/kg for Cu, 2 mg/kg for Cd and Pb, 1000 for Zn). However, the Pb has a sample with a concentration above the WHO threshold (2 mg/kg).

Table 1. Metal Trace Element concentrations of reference fish samples collected from the source stretch of the Kasungwe, Panda and Congo rivers (mg/kg).

N°	Code	Scientific name	Al	As	Cd	Co	Cu	Mn	Ni	Pb	Se	U	Zn
1	LUAS P22	<i>Enteromius neefi</i>	98.90	0.06	0.20	0.22	2.98	34.40	0.57	0.29	0.78	0.0	691.30
2	LUAS P23	<i>Unidentified</i>	292.70	0.14	0.43	0.62	5.40	24.72	0.43	0.59	0.85	0.02	205.80
3	LUAS P24	<i>Unidentified</i>	350.20	0.14	0.43	1.01	4.64	30.20	0.62	0.15	0.79	0.02	196.0
4	LUAS P25	<i>Unidentified</i>	307.70	0.11	0.24	0.45	3.84	22.41	0.62	0.17	0.82	0.02	184.50
										0.30	0.81	0.01	319.4
										0.2	0.0	0.0	248.1
5	KAS P27	<i>Clarias dumerlii</i>	166.8	0.17	0.02	0.11	1.91	8.42	0.30	0.14	2.1	0.01	29.2
6	KAS P28	<i>Clarias dumerlii</i>	130.8	0.205	0.02	0.11	1.53	8.88	0.20	0.22	.04	0.0	26.9
										0.18	2.07	0.0	28.05
										0.1	0.0	0.0	1.6
7	PAS P29	<i>Clarias dumerlii</i>	27.5	0.25	0.06	0.17	2.86	14.8	0.29	0.41	2.76	0.01	47.5
8	PAS P30	<i>Ctenopom multispine</i>	36.4	0.08	0.13	0.29	1.92	14.23	0.15	0.03	2.19	0.0	44
9	PAS P33	<i>Synodontis sp.</i>	28.3	0.07	0.3	0.34	1.72	16.15	0.11	0.05	1.83	0.0	58.1
10	PAS P34	<i>Synodontis sp.</i>	31.2	0.04	0.26	1.09	1.58	13	0.14	0.07	2.06	0.0	41.9
11	PAS P36	<i>Lacustricola katangae</i>	73.1	0.03	0.1	0.07	1.87	17.41	0.18	0.05	1.53	0.01	126.7
										0.12	2.07	0.0	63.64
										0.2	0.5	0.0	35.8
	average		162.8	0.2	0.18	0.41	2.75	18.6	0.33	0.2	1.6	0.01	150.2
	standard deviation		122.5	0.1	0.1	0.4	1.3	8.4	0.2	0.2	0.7	0.1	192.8

Table 2. Concentrations of Metal Trace Elements in fish samples collected from the Luapula River at the Kasenga locality site (mg/kg).

N°	Code	Scientific name	Al	As	Cd	Co	Cu	Mn	Ni	Pb	Se	Zn
1	LUKAS	<i>Clarias buthupogon</i>	13.06	<0.002	1.08	0.08	5.07	14.03	1.41	<0.001	<0.003	51.16
2	LUKAS	<i>Synodontis sp.</i>	79.13	<0.002	1.40	<0.0002	9.17	13.85	1.64	<0.001	0.87	109.1
3	LUKAS	<i>Coptodon rendalli</i>	157.5	<0.002	1.19	0.63	15.37	5.77	1.74	4.54	<0.003	58.18
4	LUKAS	<i>Coptodon rendalli</i>	34.31	<0.002	1.35	<0.0002	6.911	27.33	1.61	<0.001	<0.003	51.55
5	LUKAS	<i>Coptodon rendalli</i>	66.01	<0.002	0.92	<0.0002	3.14	8.31	0.86	<0.001	0.46	23.62
6	LUKAS	<i>Coptodon rendalli</i>	110.6	<0.002	1.52	0.35	14.17	33.06	1.98	<0.001	<0.003	96.35
7	LUKAS	<i>Clarias buthupogon</i>	15.78	<0.002	0.96	<0.0002	7.51	6.55	1.81	0.25	1.36	32.80
8	LUKAS	<i>Pseudocrenilabrus philander</i>	14.72	<0.002	1.55	0.22	7.84	12.96	2.15	<0.001	<0.003	113.3
9	LUKAS	<i>Synodontis sp.</i>	46.63	<0.002	1.31	0.07	14.20	20.99	1.78	<0.001	<0.003	58.95
10	LUKAS	<i>Synodontis sp.</i>	10.64	<0.002	1.44	0.24	6.99	11.90	1.77	0.25	3.11	49.82
11	LUKAS	<i>Synodontis sp.</i>	137.3	<0.002	1.45	<0.0002	8.98	13.66	2.14	<0.001	<0.003	63.10
12	LUKAS	<i>Clarias buthupogon</i>	108.5	<0.002	0.98	0.16	9.15	13.53	1.68	<0.001	0.30	38.66
13	LUKAS	<i>Synodontis sp.</i>	382.9	<0.002	1.31	0.23	10.83	39.54	1.39	<0.001	<0.003	48.06
14	LUKAS	<i>Chiloglanis sp.</i>	0.01	<0.002	<0.0001	<0.0002	<0.0004	0.01	<0.0005	<0.001	<0.003	0.01
	Average		84.1	-	1.3	0.2	9.2	15.8	1.6	1.7	1.2	56.7
	Standard deviation		100.0	-	0.2	0.2	3.6	10.9	0.3	2.5	1.1	31.6

Table 3. Results of comparison, by Wilcoxon test, of the Metal Trace Elements concentrations of fish samples from Luapula River to the Metal Trace Elements concentrations of reference fish samples (from Panda, Kasungwe and Congo Rivers).

	Luapula sample concentrations (mg/kg): Range	Reference fish samples Concentrations (mg/kg): Range	Wilcoxon test z value	Observations
Al	0.01 - 382.9	27.5 - 350.2	2.35 > 1.96	All the As concentrations of the Luapula River are below the detection limit.
Co	<0.0002 - 0.63	0.07 - 1.09	2.2 > 1.96	
Se	<0.003 - 3.11	0.78 - 2.76	3.17 > 1.96	
As	<0.002 mg/kg	0.03 - 0.25	-	
Cd	0.92 - 1.55	0.02 - 0.43	3.61 > 1.96	
Cu	0.07 - 0.63	1.53 - 5.4	3.39 > 1.96	
Ni	0.86 - 2.15	0.11 - 0.62	3.61 > 1.96	
Pb	0.25 - 4.54	0.03 - 0.59	2.73 > 1.96	
Mn	5.77 - 39.54	8.42 - 34.40	1.31 < 1.96	
Zn	0.01 - 113.3	26.9 - 691.3	0.93 < 1.96	

4. Discussion

4.1. Al, Co et Se

The comparison, by Wilcoxon test, of the Al, Co and Se concentrations of the Luapula River fish samples to the Al, Co and Se concentrations of the reference fish samples showed that the z values are as follows: $z = 2.35$ for Al, $z = 2.2$ for Co and $z = 3.17$ for Se, all greater than 1.96 ($p < 0.05$). So, the difference between the Al, Co and Se concentrations of the Luapula River and those of the reference fish Samples is significant ($p > 0.05$). But, the ranks of the AL (27.5 mg/kg - 350.2 mg/kg), Co (0.07 mg/kg - 1.09 mg/kg) and Se (0.78 mg/kg - 2.76 mg/kg) concentrations of the reference fish samples are higher than the ranks of the Al (0.01 mg/kg - 382.9 mg/kg), Co (<0.0002 mg/kg - 0.63 mg/kg) and Se (<0.003 mg/kg - 3.11 mg/kg) concentrations of the Luapula River fish samples (Table 2 and Table 3). Indeed, 6 concentrations out 14 for Co and 9 concentrations out 14 for Se are below the detection limit. It is concluded that, on average, the Al, Co and Se concentration values of the reference fish samples are higher than the Al, Co and Se concentration values of the Luapula River fish samples. It can be concluded that there was no Al, Co and Se contamination of fish from the Luapula River at the Kasenga site.

No physiological role of aluminum is known in a human or animal organism (Miquel, 2001). Several diseases have been associated with exposure to the Al (Wright & Melbourn, 2002). Squadrone et al. (2016) obtained values for the Lufira river at the Kapolowe-Gare (135.4 mg/Kg) and Koni (226 mg/Kg) sites that were much higher than those obtained in the present study (56.73 ± 53.5 mg/Kg)

Co is a constituent of vitamin B12 which is essential for the normal formation of red blood cells (Lauwerys, 1999). Koya (2017) obtained a Co average value of 1.5 mg/kg for the Lufira river, in Democratic Republic of Congo, at the Koni site

higher than that obtained in the present study on an average of 0.2 mg/kg for fish from Luapula River at the site of Kasenga.

Se is component of the enzyme glutathione peroxidase and numerous proteins including haemoglobin, myosin, cytochrome c and several ribonucleoproteins (Wright & Welbourn, 2002). Se can contribute to the proper functioning of the body, including immune system metabolism and thyroid function. However, consumption of high doses of Se can be toxic or even deadly. Signs of toxicity of the behaves hair loss, dizziness, nausea, vomiting, tremors, muscle aches (Show, 2023). The Se concentration value obtained in this study (1.2 mg/kg) is much higher than the Se concentration values obtained by Koya (2017) for the Lufira River at the Kapolowe-Gare (0.4 mg/Kg), Misisi (0.97 mg/kg) and Kapolowe-Mission (0.7 mg/kg) sites.

4.2. As

For arsenic, all the concentrations (<0.002 mg/kg) are below the detection limit (cfr (Table 2)). It can be concluded that there was no As contamination of fish from the Luapula River at the Kasenga site. In Egypt, El Nabawi et al. (1987) obtained for lakes Mariout and Edku an As concentration value of 0.03 mg/kg, but higher than that obtained in this study (<0.002 mg/kg).

4.3. Cd, Cu, Ni and Pb

The comparison of the Cd, Cu, Ni and Pb concentrations of the Luapula River fish samples to the Cd, Cu, Ni and Pb concentrations of the reference fish samples by Wilcoxon test showed that the z values are as follows: $z = 3.61$ for Cd, $z = 3.39$ for Cu, $z = 3.61$ for Ni and $z = 2.73$ for Pb. All these values are greater than 1.96 ($p < 0.05$). This means that the difference between the concentrations of Cd, Cu, Ni and Pb in the Luapula River samples and the concentrations of Cd, Cu, Ni and Pb in the reference fish samples is significant. This suggests that since the Luapula River has received water polluted with mining wastes from the Kafubu River, the fish in this river are indeed contaminated with Cd, Cu, Ni and Pb.

In this study, we can see that 13 Cd concentrations (0.92 to 1.55 mg/kg) out 14 in the fish samples from the Luapula River (Table 2 and Table 3) are all below the WHO threshold of 2.0 mg/kg, but all are above the EU threshold (0.02 mg/kg). For this reason, we prefer to consider the fish of the Luapula River as being of questionable safety in terms of cadmium. Prolonged exposure to cadmium in humans can lead to kidney damage, bone fragility, harmful effects on the respiratory system, and an increased risk of cancer and especially in the occurrence lung cancers (Lauwerys, 1999). Then, consumption of fish from Luapula River can lead to severe chronic Cd poisoning.

It should be noted that 13 Cu concentrations (3.14 mg/kg to 15.37 mg/kg) out 14 (Table 2 and Table 3) are below the WHO threshold of 30 mg/kg, but all these concentrations are above the EU threshold of 0.1 mg/kg. For this reason, we can consider the fish from the Luapula River to be of questionable health safety as far

as copper is concerned. In very low doses, Cu is a well-known trace element. It is essential for the synthesis of haemoglobin, normal bone formation, and maintenance of myelin in the nervous system (Wright & Welbourn, 2002). However, chronic exposure can cause irritation of the affected areas, including the mucous membranes, nasal cavities, eyes. It causes headaches, stomach aches, dizziness, digestive disorders such as vomiting and diarrhea (Lauwerys, 1999; ASEF, 2017). El Nabawi et al. (1987) obtained, for fish from Lake Idku and Mariout in Egypt, Cu concentration value (1.77 mg/kg) lower than that obtained in this study (2.5 mg/kg).

Comparing the Ni concentrations of fish samples from the Luapula River (0.86 mg/kg to 2.15 mg/kg) with the European Union (EU) threshold of 0.1 mg/kg, it can be seen that 13 Ni concentrations out of 14 of the Luapula River fish samples (Table 3) are above this threshold (0.1 mg/kg), with the exception of one sample whose concentration is below the detection limit. One can understand how extremely dangerous it is to eat such fish. In small quantities, nickel is essential, but if the absorption is too great, it can pose a health risk (Gates et al., 2023). Squadrone et al. (2016) obtained lower average values of Ni concentration for lake Tshangalele at the Kapolowe-Mission (0.9 mg/kg) and Mwadingusha (0.21 mg/kg) sites than those obtained in the present study (1.6 mg/kg).

Pb concentrations in fish samples from the Luapula River range from 0.25 mg to 4.54 mg/kg (Table 2 and Table 3), in addition to concentrations below the detection limit. At least one Pb concentration (4.54 mg/kg) is above the WHO (2 mg/kg), FAO (0.5 mg/kg), and EU (0.3 mg/kg) thresholds. This has just confirmed that the fish of the Luapula River do not present a reassuring health security. At high levels, lead poisoning is manifest by effects of the central nervous system, with stupor, coma and convulsions (Wright & Melbourn, 2002). The mean Pb concentration of fish samples from the Luapula River (1.7 mg/kg) is far higher than the values obtained by Koya (2017) for Lake Tshangalele at the Kapolowe-Mission locality site (0.35 mg/kg) and at the Kibangu locality site (0.64 mg/kg).

4.4. Mn et Zn

For Mn and Zn, the Wilcoxon test yielded $z = 1.31$ for Mn and $z = 0.93$ for Zn. Both z -values are less than 1.96 ($p < 0.05$). There is no significant difference in Mn and Zn concentrations between the fish from the Luapula River and the reference fish samples. It is concluded that the fish of the Luapula River at the Kasenga site are not contaminated with Mn and Zn.

Mn is essential in nucleic acid synthesis and the metabolism of carbohydrates, as well as in the maintenance of the nervous system (Wright & Welbourn, 2002). The mean Mn concentration of fish samples from Luapula (15.52 mg/kg) is higher than the values obtained by Koya (2017) for Lufira River (5.4 mg/kg), at Misisi site in the Democratic Republic of Congo.

Zn is an essential trace nutrient, a constituent of many enzymes, and a coenzyme for a number of systems (Wright & Welbourn, 2002). The mean Zn

concentration of fish samples taken from the Luapula River at the Kasenga site (56.7 mg/kg) is significantly higher than the mean values of Zn concentrations obtained by El Nabawi et al. (1987) for Lake Idku (7.4 mg/kg) in Egypt and by Kakulu et al. (1987) for the Niger Delta (4.8 mg/kg) in Nigeria.

5. Conclusion

We wanted to use this study to verify the health safety of the fish from the Luapula River, given that this river has been receiving mining waste from Gecamines Kipushi and Lubumbashi, SODIMICO and the CHEMAF plant for several years. The results of the chemical analysis as well as the comparison of the concentrations of Metal Trace Elements in the samples of fish from the Luapula River to the concentrations of Metal Trace Elements of the reference samples, by the Wilcoxon test have shown that the consumption of fish from the Luapula River presents a fairly serious risk to the health of the consumer. The content of Cd, Cu, Ni and Pb makes the fish of the Luapula River unsuitable and unfit for human consumption. In doing so, we wanted to give almost for each metal trace element treated in this study some examples of the pathologies that it can cause in order to show how dangerous it is to consume fish coming from an aquatic ecosystem polluted with mining wastes containing metal trace elements themselves. The results obtained will be brought to the attention of the decision-makers of the Haut-Katanga Province so that measures can be taken to ban metallurgical factories to dump their mining wastes into aquatic ecosystems, especially those which provide fish for the local population waterways and in particular the water was that supply fish to the local population.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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