Biochemical analysis of liver and muscles of *Synodontis schall* fish species as a tool for pollution assessment on the White Nile River at south of Khartoum City, Sudan

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Abstract

Synodontis schall is important fish species that consumed popularly in Khartoum City restaurants. This study aimed to determinate the levels of heavy metals and enzyme activities in the liver and muscles of this fish species. Ethanol extract was taken for the determination of cholesterol, total lipid contents in fish muscles. While saline extract was used for the estimation of glucose, total protein, albumin, globulin and enzyme e.g., GOT, GPT, LDH, CPK and amylase. The concentrations of heavy metals were carried out by the flame atomic absorption spectrophotometer (UNICAM 929). Result indicated that there were no significant differences between enzyme activities in the liver extract of the tested samples and control. The lead (Pb) level in the liver was found to be higher than that in the muscle, whereas the copper (Cu) level in the muscle was found to be higher than that in the liver. It may be concluded that the maximum cadmium (Cd) concentration in the liver were remarkably higher than the maximum level set by the Commission Regulation while its concentration in the muscle tissues were slightly violated the recommended maximum level. Regular monitoring of biological analysis for fish species in the study area is of paramount importance.

Keywords: *Heavy metals, hematology, biochemical, Synodontis schall, pollution*

Introduction

It was well documented that there are important precedents on the serious consequences of heavy metals contaminated fish consumption on human health, being worth mentioning: Minamata, Japan tragedy, where over 900 people lost their lives and two million suffered health problems as a consequence of having eaten Hg contaminated fish. Minamata disease (McCurry, 2006) and the event at Jinzu River basin, at Toyama prefecture, where one of the most serious Cd prolonged intake intoxication cases takes place following the mining extractions that contaminated those waters (Inaba *et al*. 2005). However, fish are exposed to heavy metals in polluted waters. Heavy metals come into the water from the anthropogenic activities, and sources that continually released into the aquatic environment create serious health risks due to their toxicity, long persistence, bioaccumulation and bio-magnifications in the food chain (Bat *et al.*, 2013). It is vital to determine heavy metal levels in seafood, because heavy metal ions can accumulate more easily in fish as compared with other foodstuffs (Bat *et al.*, 2014). Aquatic systems especially lakes are more sensitive to heavy metal pollutants and are contaminated by chemical substances, industrial, domestic and agriculture wastes in the form of particles, metal ions, organic and inorganic compounds (Tarra-Wahlberg *et al*., 2001). River Nile and its main tributaries White Nile and Blue Nile Rivers are the most important rivers as a source of water supply and catfish area that supplies most of Khartoum City residents. The aquatic life of the White Nile River is very rich in fish species diversity. A study of hematological analysis of some fish species from the affected polluted site of White Nile River conducted by Nogod *et al*. (2020) concluded that in spite of being insignificantly different comparing to control, most of these hematological analysis found to be not compliance to the suggested hematology level of healthy fish. The area under the study is located south of Khartoum City nearby Jabal Awlia Dam. This area was selected for the study purpose because the river over there receives sewage effluent from Soba Treatment Plant and from the Military Factory. Therefore, this study aimed to evaluate the effect of the accumulation of heavy metals on the biological activity of *Synodontis schall* fish species.

Materials and methods

Collection of fish samples

A total of 120 Synodontis *schall* fish species, that commonly known as Gargor, (Figure 1) were collected, of which, a 60 tested fish were collected from the targeted polluted area; the first 30 fish of them were collected from the discharge zone of the White Nile River site (1), whereas the second 30 fish were collected from the main ponds of sewage site (2). The rest (120 fish) were collected from the upstream non-polluted area of Jebel Awlia Dam (site 3), as control (Figure 2). The collected *Synodontis schall* fish species were well washed with clean water and kept in polyethylene packages containing ice, then transported to the Central Veterinary Research Laboratory of the Omdurman University bending identification and analysis

Figure 1: *Synodontis schall* fish species, popular known as Gargor.

Figure 2: Fish sampling points Sites(1,2 and 3).Site(1) at river Nile, Site (2) at Soba Sewage plant (test samples) .Site(3) Jebel Awlia Fesheries (control samples) Modified from www.Googleearth.com, (2016).

Processing of liver and muscle tissues

Liver and muscle were cut with razor, washed with distilled water and blotted with blotting paper. A weighted portion (about 3 grams) of both liver and muscle was homogenized in 3 ml ice-cold saline (0.89% NaCl) solution for saline extract and 3ml ethanol for ethanol extract in a motor driven Teflon glass homogenizer. The homogenate centrifuged rpm at 4000 rpm (3.500×g) for 45 minutes at 5°C in a refrigerated centrifuge to get a clear saline supernatant and for 15 minutes at 5°C at the same speed for ethanol supernatant. Digestion methods described by Van Loon (1980) and Du Preez and Steyn (1992) were used for the estimation of heavy metals, e.g., Cr^{3+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Fe and Pb^{2+} (Ali, 2004).

Biochemical analysis of fish liver and muscle

Saline and ethanol extracts were analyzed for the following biochemical parameters, including some enzymes to evaluate the possible effect of water pollution on them. Ethanol extract was taken for the determination of cholesterol, total lipid contents in fish muscles. While saline extract was used for the estimation of glucose, total protein, albumin, globulin and enzyme like GOT, GPT, LDH, CPK and amylase. Detailed procedures for estimation of all above parameters were the same as given under the biochemical analysis of liver and blood (Ali, 2004).

Determination of heavy metals in fish tissues

Frozen tissue samples of liver and muscle were thawed, rinsed in distilled water and blotted in blotting paper. A known weight of liver and muscle of the fish was shifted to 250 ml volumetric flasks for digestion. Samples digestion done according to methods described by Van Loon (1980) and Du Preez and Steryn (1992). By this method digestion was completed in about 20 minutes instead of 3 to 4 hours as stated by Van Loon (1980). Sample after digestion were cooled and diluted to 10 ml with distilled water by proper rinsing of digestion flasks. Atomic absorption spectrophotometer was used to determine the concentration of Cr^{3+},Cu^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+} and Pb^{2+} in the tissues sample of the fish. A range of analytical standards for each metal was prepared from Merck Stock solution (Van Loon, 1980 and Du Preez and Steyn, 1992).

Statistical analysis

Student t-test analysis of variance, mean differences, correlation and chart were done by using SPSS (Statistical Package for Social Sciences) program version.

Result and discussion

Biochemical evaluation of liver and muscle of *Synodontis schall* fish species with respect to GOT, GPT, LDH, ALP, CPK and amylase

Table 1 showed that all of the parameters of GOT, GPT, LDH, ALP, CPK and amylase of the tested liver extract were found to be not significantly different comparing to the control. With regard to muscle extract, GOT level was found to be significantly lower (79.7) in the fish sample from contaminated site 1 comparing to the control site 3 (87.8). In muscles, both LDH (81.8) and CPK (35.5) in contaminated site 1 were found to be lower comparing to that of the control site 3 (89.6 and 38.4, respectively). Whereas only GPT was found to be not significant in the contaminated sample site 1 comparing to the control. Noteworthy that, LDH level in the liver extract of the control sample (90.2) was found to be higher than that of the muscle extract (81.8) for the same control fish sample. The activity of serum enzyme in this study found to be varied according to the organ from which the extract is extracted. This observation confirmed the result that was observed by a previous study conducted by Krajnovic-Ozretic and Ozretic (1987) which stated that the LDH activity was found to be varied and it was primarily related to the organ from which the blood sampled. As well, similar result was observed by an earlier study conducted by Panteghini *et al.* (1984), which reported that the determination of the basic enzymes provides the opportunity to identify their origin and also permits estimation of the severity of injury at the tissue/cellular level. The findings of this study were in accordance with the results obtained by the study conducted by Dorcas and Solomon (2014), which reported both significant and insignificant differences between the levels of ALT, ALP and AST of fishes that studied and the levels of the standards. Ali (2004) reported that the concentration levels of blood serum enzyme of GOT, GPT and CPK was found to be much higher in blood tissues of *Tur-putitora* fish species from polluted waters River Kabul, Pakistan, when compared with that in control dams waters. Sagar *et al.* (2015) reported that in liver cell injury, a considerable increase in the serum level of both GOT and GPT enzymes was observed, whereas serum CPK activity is raised in all varieties of muscular dystrophy. While raised LDH values are found in renal disease, liver disease, disseminated malignancy and certain hematological disorders. The increased serum levels of ALP are found in both skeletal and hepatic disorders, and amylase enzyme raised in acute abdominal conditions, e.g. perforated peptic ulcer, cholecystitis, common bile duct and intestinal obstruction, but the higher levels are found in acute pancreatitis. Moreover, Sagar *et al.* (2015) stated reference range values of 5 - 50 U/L for both GOT and GPT, and for LDH to be 230-400U/L, ALP to be 70-230U/L, CPK to be 24-105 U/L, and for amylase is to be 60-160U/L. Previous studies conducted by Lemarie *et al.* (1991); De La Torre *et al.* (2000); Panigrahi *et al.*, 2010 and Tahmasebi-Kohyani *et al.* (2012) stated that the alterations of blood enzymes' activities were associated with liver damage, induced by pathological or stress situations or with feeding nutrients with hepatic protective effects. Perez-Jimenez *et al*. (2013) and Harikrishnan *et al*. (2011) reported that the increase of serum LHD activity may indicate hypoxia conditions, limiting water temperature or toxins, whereas the increase of serum CPK activity which is particularly active in heart and skeletal muscle, may indicate damage to these tissues. As well, Almeida *et al.* (2002) and Rehulka and Minarı´k, (2007) declared that pollutants and stress conditions were reported to increase CPK activity, probably due to muscle injury.

Biochemical evaluation of liver and muscle of *Synodontis schall* fish species with respect to total protein, albumin, globulin, glucose, cholesterol and total lipids

Table 2 revealed that all of the parameters of total protein, albumin, globulin, glucose, cholesterol and total lipids of both the tested liver and muscle extracts were found to be not significantly different comparing to the control. But some of the concentration levels of these parameters were found to be varied between liver and muscle. Albumin level in the liver was found to be 4.08 and 4.14 in control site 3 and contaminated site 1, respectively, these levels were higher than in muscles, which in muscle were found to be 3.52 and 3.45 in both control site 3 and contaminated site 1, respectively. Globulin level in the liver was found to be 2.34 and 2.24 in control site 3 and contaminated site 1, respectively, these levels were much lower than in muscles, which in muscle were found to be 3.24 and 3.18 in both control site 3 and contaminated site 1, respectively. Moreover, cholesterol level in the liver was found to be 70.8 and 71.7 in both control site 3 and contaminated site 1, respectively, in muscle these levels were found to be 54.5 and 55.4 in both control site 3 and contaminated site 1, respectively. In spite of being insignificant different to control, the levels of glucose and cholesterol in both liver and muscle of the fish were found to be slightly higher in the contaminated site 1 comparing to the control site 3. Sandnes and Waagb (1988) considered the range for total protein of normal healthy fish was to be 41.6–56.6 gl^{-1}, for albumin: 18.3–24.3 gl^{-1}, for cholesterol: 9.3 - 12.8 μmol, for triglycerides: 2.53–4.98 μmol, and for creatinine: 26–46 μmol**.** It was reported that plasma protein levels are often associated with fish nutritional and physiological status (Rehulka *et al*. 2005; Maita 2007). Basal plasma protein values averaged was stated by Polakof *et al*. (2012) to be 4.9 g dl-1. Whereas basal blood glucose levels are known to differ considerably among fish species. Basal plasma cholesterol levels reported by this study are lower comparing to the previously reported values by Peres *et al*. (2014). It also confirmed by Peres *et al*, 2014 that, in seabass, liver has a pivotal role in the maintenance of glucose homeostasis.

Biochemical evaluation of liver and muscle of *Synodontis schall* fish species with respect to Cd, Pb, Cu, Cr, Fe and Zn

Table 3 summarizes that all of the heavy metals levels of Cd, Pb, Cu, Cr, Fe and Zn of the tested liver extracts were found to be not significantly different comparing to the control except for Pb level, which was found to be much higher in the contaminated site 1 (0.439) comparing to the control site 3 (0.305). In muscle extract, the significant variation was observed for Cr, Fe and Zn, which were found to be higher in the contaminated site 1 comparing to the control site 3. The Pb level in the liver was found to be higher than that in the muscle. The findings of this study confirmed that the liver is the most organs accumulate pollutants of various kinds at higher levels their environment as reported earlier by Galindo *et al*. (1986) and recently by Bat *et al*. (2015). On contrast, the Cu level in the muscle was found to be higher in control site 3 than that in the liver, this result disagree with that reported by Dugo *et al*. (2006). Pb levels in the liver were found to be much higher than that found for other metals. However, other studies stated that gills uptake certain metals such as Pb (Coban *et al*., 2009). The accumulation of metals in the liver found to be followed the order: Fe, Pb, Cu, Cd, Cr and Zn, while in the muscle followed the order of Fe, Cu, Cd, Pb, Cr and Zn. Topçuoğlu *et al*. (2002) found that the accumulation of metals in muscle in wild Seabass taken from Perşembe, eastern Black sea showed the order of: Cd, Cr, Cu, Fe, Pb, Zn. Catsiki *et al*. (1999). Various studies suggest that metal uptake by fish performed via food that constitutes the major pathway for accumulation in liver and then muscle. However, when contamination occurs, concentration of metals in certain tissues increases in proportion with that of seawater (Coban *et al*., 2009). The results of this study showed that the Fe concentrations were the highest in both liver and muscle and Zn concentrations were found to be the lowest. This result may be comparable to that

Table 1: Biochemical parameters of liver and muscle of *Synodontis schall* fish species (commonly known as gargor)

sampled from Jebel Awlia Dam Site 3 (control) and White Nile River site 1 (contaminated).

parameter	liver				muscle			
	site 3 (control)	site 1 (test)	P-value	Sig-level	site 3	site 1	P-value	Sig-level
GOT (IU/g)	88.4	87.2	0.718	Ns	87.8	79.7	0.021	*
GPT (IU/g)	39.3	38.4	0.676	Ns	38.5	36.6	0.276	Ns
LDH (IU/g)	90.2	91.0	0.653	Ns	81.8	89.6	0.009	**
ALP (IU/g)	95.9	92.9	0.713	Ns	ND	ND	ND	ND
CPK(IU/g)	ND	ND	ND	ND	35.5	38.4	0.009	**
Amylase (IU/g)	62.6	63.6	0.104	Ns	ND	ND	ND	ND

Ns = No significant difference, ND – Not determined, * = Significant difference at 5%, **GOT**, glutamate oxaloacetate transaminase. **GPT** = glutamate pyruvate transaminase. **LDH** = lactate dehydrogenase, **ALP** = alkaline phosphatase, **Cd** = cadmium, **Pb** = lead, **Cu** = copper, **Cr** = chromium, **Fe** = iron, **Zn** = zinc.

Table 2: Biochemical parameters of liver and muscle of *Synodontis schall* fish species (commonly known as gargor) caught from Jebel Awlia Dam Site 3 (control) and White Nile River site 1 (contaminated).

parameter	liver				muscle			
	site 3 (control)	site 1 (test)	P-value	Sig-level	site 3 (control)	site 1 test	P-value	Sig-level
Total protein (mg/g)	6.34	6.39	0.730	Ns	6.72	6.63	0.393	Ns
Albumin (mg/g)	4.08	4.14	0.350	Ns	3.52	3.45	0.467	Ns
Globulin (mg/g)	2.34	2.24	0.517	Ns	3.24	3.18	0.343	Ns
Glucose mg/g)	65.0	67.9	0.252	Ns	64.4	66.8	0.148	Ns
Cholesterol (mg/g)	70.8	71.70	0.678	Ns	54.5	55.4	0.650	Ns
Total lipids (mg/g)	7.70	7.88	0.129	Ns	7.78	7.88	0.475	Ns

Ns = No significant different, * = Significant different at 5%, **GOT**, glutamate oxaloacetate transaminase.**GPT**, glutamate pyruvate transaminase. **LDH** = lactate dehydrogenase, **ALP** = alkaline phosphatase, **Cd** = cadmium, **Pb** = lead, **Cu** = coppor, **Cr** = chromium, **Fe** = iron, **Zn** = zinc.

Table 3: Biochemical parameters of liver and muscle of *Synodontis schall* fish species (commonly known as gargor) caught from Jebel Awlia Dam Site 3 (control) and White Nile River site 1 (contaminated).

parameter	liver				muscle			
	site 3 (control)	site 1 (test)	P-value	Sig-level	site 3 (control)	site 1 test	P-value	Sig-level
Cd (mg/g)	0.069	0.071	0.421	Ns	0.0572	0.0522	0.164	Ns
Pb(m/g)	0.305	0.439	0.030	*	0.0483	0.0417	0.149	Ns
Cu (mg/g)	0.158	0.163	0.339	Ns	0.4110	0.1723	0.147	Ns
Cr (mg/g)	0.0016	0.0018	0.336	Ns	0.00154	0.00198	0.036	*
Fe (mg/g)	1.75	1.65	0.684	Ns	1.50	1.66	0.01	**
Zn (mg/g)	0.00058	0.00080	0.590	Ns	0.00042	0.00035	0.001	**

Ns = No significant different, * = Significant different at 5%, **GOT**, glutamate oxaloacetate transaminase. **GPT**, glutamate pyruvate transaminase. **LDH** = lactate dehydrogenase, **ALP** = alkaline phosphatase, **Cd** = cadmium, **Pb** = lead, **Cu** = copper, **Cr** = chromium, **Fe** = iron, **Zn** = zinc.

reported by Bat *et al*. (2015). However, it was stated that fish contents of heavy metals varies depending on the zone, environmental conditions, the contamination level of the fishing site, and the characteristics of the fish (Roderiguez *et al.*, 2015). The maximum Cd concentrations (0.071) that detected in the liver tissues were remarkably higher than the maximum level (0.05 mg/g wet weights) set by the Commission Regulation (2006) whereas the Cd concentrations in the muscle tissues (0.0572) were slightly above that recommended maximum level. Similarly, the maximum Pb concentrations (0.439) found in the liver tissues were slightly higher than the maximum level (0.30 mg/ g wet weights) set by the Commission Regulation. This findings in accordance of that reported by Bat *et al*. (2015). Zuluaga *et al.* (2015) reported that Cd and Pb pose the highest risks for human health due to causing toxicity and possible carcinogenic effect.

Conclusion

It could conclude that the Pb level in the liver was found to be higher than that found in the muscle, and the Cu level in the muscle was found to be higher than that found in the liver. The results of this study showed that the Fe concentrations were the highest level of metals under the study, which was found to be higher in both liver and muscle, and Zn concentrations were found to be the lowest. The maximum Cd concentrations that detected in the liver tissues were remarkably higher than the maximum level set by the Commission Regulation whereas the Cd concentrations in the muscle tissues were slightly above the recommended maximum level. Similarly, the maximum Pb concentrations found in the liver tissues were slightly higher than the maximum level set by the Commission Regulation.

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