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Original research

Bioaccumulation of some heavy metals in some organs of *Coptodon zilli* fish in Old Aswan Reservoirs and Aswan River Nile

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Abstract:

This study was carried out in Aswan's old Reservoirs between the old Aswan Dam at the north and the High Dam at the south and the River Nile at the area of entrance of Aswan town. Eighty (80) live (*Coptodon zilli*) fish species were caught and randomly collected. Water samples were collected at a depth of 30 cm. There were not any abnormal clinical signs between examined fish. The concentration of cadmium (Cd) and lead (Pb) in the liver and kidney exceeded the maximum permissible limit (MPL) at all seasons with significant differences during different seasons. On the other hand, the results of Cd and lead (Pb) in the muscles were within the maximum permissible limit (MPL) during the different seasons except in autumn without significant differences between results. The concentrations of Cd and Pb in the River Nile and Old Reservoir exceeded permissible limits in winter and in summer. While in Autumn were within the permissible limits and less than permissible limits in Spring. All Bioaccumulation factor (BAF) values within the examined organs liver, kidney and muscles are of low concentration according to the WMPT.

Keywords: precipitation, cadmium, lead, bioaccumulation

1- Introduction

Heavy metal pollution that occurs naturally has limited concentrations, but the intense and diverse human activities have caused an increase in the concentration of incident accumulations (Yona et al. 2018, Yona et al. 2021). These backlogs create numerous and growing community health concerns (Ananthan, et al. 2006, Gupta et al. 2009, Arikibe and Prasad 2020) Toxic concentrations have been detected at common levels in aquatic environmental systems, which poses a grave threat to the public health of both humans and aquatic animals (Yona et al., 2021).

The main sources of dangerous on the aquatic environment including fishes is contamination by inorganic chemicals, that affecting state of health of fish (ECDG, 2002). Heavy metals such as cadmium consider the sources of pond pollution in the ponds, lakes and river systems resulting from industries, sewage and agricultural drains which mainly affected fish (Basha and Rani, 2003).

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Fishes considered one of the most common bio indicators for environmental pollutants (Al-Busaidi et al., 2011), Entrance of heavy metals into fish occur through food, water, and sediments (Castro-González and Méndez-Armenta, 2008). There many factors play an important role in accumulation of heavy metals in fish as season, habitat and physiological conditions of fish (Kargin, 1996). Literatures by FAO/WHO (1989) indicate that geographical location play role in bioaccumulation of metal into fish.

Tilapia (Family Cichlid) is a freshwater fish and is considered the most commercially important, and the family Cichlid includes the most widespread fish species in Lake Nasser, Egypt (GAFRD, 2018).

The structural form of heavy metals is the main determinant of their toxicity. Their presence in the environment of fish, along with their solubility in water, increases the likelihood of fish absorbing them and the risk of their association with proteins and enzymes (Wayne and Ho, 1999). The waste resulting from the industry is the main source of lead metal, as the phosphate fertilizers that are used in the cultivation of various crops are the main source of cadmium (Mason, 2002). The River Nile in Aswan receives a lot of amounts of wastes as runoff due to presences of tourist's boats and industry wastes products while old Aswan Reservoirs appear as row area except presences of boats to Filla templates. The importance of this study comes from the real awareness of the health risks posed by heavy metals. This study aimed to determine the level of spoilage and accumulation (cadmium and lead) as samples of heavy metals in some fish organs. (kidney, liver, and muscles of *Coptodon zilli* fish in Old Aswan Reservoir and Aswan Nile at the north of Aswan town during the different four seasons. make a comparison of their concentration between two different localities throughout the four seasons.

2- Materials and Methods

Ethical approval

This study was carried out in the Aswan city and obtained the approval of the Animal Ethics Committee of the Faculty of Fish and Fisheries Technology on the protocol for conducting the research.

2.1. The Study Area

Aswan old Reservoirs which is the locality of Nile between the old Aswan Dam at north and the High Dam at south and **Aswan River Nile** at the area of entrance of Aswan town.

2.2. Fish and water samples.

Eighty (80) live (*Coptodon zilli*) fish species were caught and randomly collected) by trammel nets and bottom gill nets throughout the fourth seasons from November 2018 to August 2019 Tilapia fish (*Coptodon zilli*) the samples collected were from commercial catches in two regions (Old Aswan Reservoir and Aswan River Nile). Water samples in the two experimental areas were collected at a depth of 30 cm below the surface simultaneously using a PVC vertical water sampler. The water samples were then transferred to the Fish Health and Diseases Laboratory in the Faculty. Samples weighing about 1 g were taken from muscles, kidneys and liver (wet weight) and the samples were washed with distilled water and packed in bags after weighing and then stored until chemical analysis of heavy metals at -18°C.

2.3. Clinical and postmortem examinations

All experimental fish were clinically examined following the euthanasia protocol by percussion stunning the brain to destroy it (AVMA (American Veterinary Medical Association), 2013). Internal and external pests were identified in aggregate (Issa, 2016, Nougat 2010).

2.4. Determination of heavy metals concentrations

2.4. 1. Heavy metals analysis

Fish samples

Heavy metals cadmium and lead (Cd and Pb) concentrations in samples of *Coptodon zilli* fish organs (liver, kidney and muscles) were determined using Pye Unicum Atomic Absorption Spectrophotometer and Cd and Pb hollow cathode lamps and air-acetylene flame. Calibration was performed by analyzing five standard solutions and two reagent blank samples (Olaifa et al., 2004).

Water samples

Total metal nitric acid digestion method as well as atomic absorption spectrophotometer were used to estimate heavy metal concentrations of water (cadmium and lead) samples (PerkinElmer, A 800 analyzer) according to APHA (American Public Health Association) (2005).

2.4. 2. Bioaccumulation factor

According to Ezzat et al. (2012) The bioaccumulation factor (BAF) of a specific pollutant was calculated by calculating the ratio between its concentration in a fish organ and its concentration as a water soluble substance.

$$BAF = \frac{\text{Pollutant concentration in fish musculature (mg/kg)}}{\text{Pollutant concentration in water (mg/l)}}$$

The obtained values were compared with those of the Waste Minimization Prioritization Tool (WMPT), Where :

score of 1 = low concern (BAF <250),
score of 2 = medium concern (BAF from 250 to 1,000), and
score of 3 = high concern (BAF exceeding 1,000) (McGeer et al., 2004).

2.5. Statistical analysis

With regard to the two different experimental sites, to assess the significant difference in the concentration of the different metals studied, one-way ANOVA was used, and a probability level of 0.05 or less was considered significant (Bailey, 1981). Standard errors were also estimated.

3- Results and Discussions

3-1 Results of clinical signs examination:

Results of clinical signs examination reviles that there are not any abnormal clinical signs between examined fish.

3-1-1 Seasonal variations of heavy metals precipitation:

Heavy metals accumulate in water bodies (rivers and lakes) from natural sources such as atmospheric sedimentation, erosion factors, hydrodynamic processes, leaching, and rock carving, as well as from intensive human activities, which are more dangerous and more deadly to the environment than natural polluted sources (Paul, 2017). Through the skin and gills or through feeding, heavy metals are transmitted to aquatic animals (Pulatsü and Topçu 2015) causing a state of nervous poisoning, decreased reproduction, severe imbalances in bio-blood chemistry and changes in tissues. Exposure of fish to heavy metal pollution is also considered a cause of immune system dysfunction and high blood sugar, also depletion in enzymatic activities (Kumari et al., 2017, Ali and Khan, 2019). In the current study there were significant differences in the concentration of lead and cadmium (Pb, and Cd) in the organs examined (liver, kidneys and muscles) of *Coptodon zilli* fish which were collected from Aswan River Nile and Old Aswan Reservoirs, We found that the concentration of cadmium (Cd) in liver and kidney exceeded the maximum permissible limit (MPL) according to international standards as shown in table (1) at all seasons, and the results of analysis of variance showed significant differences in metal during different seasons, autumn > summer > winter > Spring, except results in fish muscles which within maximum permissible limit (MPL) which agree with results of the muscles of *T. nilotica* from the High Dam Lake in Egypt (Rashed, 2001). Kucuksegin et al. (2006) determined that the accumulation of heavy metals in the tissues of aquatic organisms is an indirect measure of the presence and availability of heavy metals in the water surface. According to this observation, it can be asserted that muscle tissue cannot be considered an organ characterized by the accumulation of cadmium.

Table 1: Seasonal variations in cadmium mean concentrations (mg/kg) within the liver, kidney and muscles of *Coptodon zilli* fish in Aswan River Nile.

	Winter_	Spring_	Summer_	Autumn_	IMP	REF
Cd in Liver	1.08±0.13[b]	0.74±0.10[d]	1.38±0.17[a]	1.42±0.17[a]	0.05	E.O.S.Q.C. (2005)
Cd in Kidney	0.73±0.11[b]	0.54±0.06[d]	0.96±0.09[a]	0.90±0.14[a]	0.1	FAO/WHO(1999)
Cd in Muscle	0.02±0.01[a]	0.02±0.02[a]	0.03±0.01[a]	0.03±0.01[a]	0.5	EOS (1993) and WHO (1989)

All results are expressed as Mean± Standard Error (SE) of Mean.
Data shown with different letters are statistically significant at the $P < 0.05$ level.

Also the concentration of cadmium in Aswan Old Reservoir which shown in table (2) in liver and kidney exceed the maximum permissible limit (MPL) at all seasons, with significant differences between results in liver at summer > autumn > spring > winter and in kidney results at summer > autumn, winter > spring. On the other hand the results of Cd in the muscles within the maximum permissible limit (MPL) during the different seasons except in autumn which is (0.06±0.08**)** according to E.O.S.Q.C. (2005) without significant differences between results.

Also we found that the concentration of lead (Pb) of *Coptodon zilli* in in Aswan River Nile which shown in table (3) in liver and kidney exceed the maximum permissible limit (MPL) at all seasons, with significant differences between results in liver at autumn > summer > winter > spring with significant differences and in kidney only exceed maximum permissible limit (MPL) according to FAO/WHO (1999) and EOS (1993) with variation during different seasons autumn > summer > spring > winter without significant differences between results and within maximum permissible limit according to EOS (1993), On the other hand the results of muscles samples within the maximum permissible limit (MPL) during the different seasons, without significant differences between results.

Table 2: (M+SE) of Seasonal variations in cadmium (Cd) (mg/kg) within the liver, kidney and muscles of *Coptodon zilli* in Aswan Old Reservoir.

Aswan old Reservoir	Winter	Spring	Summer	Autumn	IMP	REF
Cd in liver	0.72±0.06 b	0.99±0.14 a	1.18±0.19 a	1.09±0.15 a	0.05	E.O.S.Q.C. (2005)
Cd in kidney	0.59±0.13[b]	0.58±0.09[b]	0.74±0.05[a]	0.59±0.03[b]	0.1	FAO/WHO(1999)
Cd in muscle	0.02±0.01[a]	0.02±0.02[a]	0.03±0.02[a]	0.06±0.08[a]	0.5	EOS (1993) and WHO (1989)

Table 3: (M+SE) of Seasonal variations in lead (Pb) (mg/kg) within the liver, kidney and muscles of *Coptodon zilli* in Aswan River Nile.

Aswan River Nile.	Winter_	Spring	Summer	Autumn	IMP	REF
Lead (Pb) in liver	3.03±0.22[b]	2.85±0.35[b]	3.93±0.60[a]	3.98±0.39a	0.5	E.O.S.Q.C. 2005) and WHO (1989)
Lead (Pb) in kidney	1.55±0.24[a]	1.60±0.16[a]	1.68±0.31[a]	1.69±0.19[a]	0.214	FAO/WHO (1999)
Lead (Pb) in muscles	0.09±0.01[b]	0.08±0.01[b]	0.12±0.02[a]	0.12±0.02[a]	2.0	EOS (1993)

The concentration of lead (Pb) of *Coptodon zilli* in Aswan Old Reservoir. Which shown in table (4) in liver exceed the maximum permissible limit (MPL) according to international standards as at all seasons, with significant differences between results in liver at autumn > summer > spring> winter and in kidney mean results exceed permissible limit in FAO/WHO(1999) during all seasons except in winter while it consider within permissible limit according to EOS (1993) only exceed permissible limit according to E.O.S.Q.C.(2005) and WHO (1989) in summer , While the concentration within permissible limits in other seasons and in the results of muscles samples, without significant differences between results.

3-1-3 Heavy metals concentrations in water:-

Heavy metals are pollutants that are difficult to get rid of, as they remain in the environment for a long time, and they are not degradable, in addition to their impact extending to the next chain levels in the ecosystem (Maurya et al., 2016). Heavy metals are transferred from water to aquatic animals, benthic algae and plankton, and accumulate at varying levels in the internal organs. This accumulation depends on the osmosis of the organisms and animals. It can also be transmitted to humans and cause many diseases and the accumulation of toxins (Usmani et al., 2017).

Table 4: (M+SE) Seasonal variations in lead (Pb) (mg/kg) within the liver, kidney and muscles of *Coptodon zilli* in Aswan Old Reservoir.

Aswan Old Reservoir	Winter	Spring	Summer	Autumn	IMP	REF
Lead (Pb) in liver	2.09±0.27[b]	2.11±0.19[b]	2.67±0.19[a]	2.85±0.61[a]	0.5	E.O.S.Q.C. 2005) and WHO (1989)
Lead (Pb) in kidney	0.50±0.07[b]	0.44±0.06[b]	0.59±0.05[a]	0.34±0.05[d]	0.214	FAO/WHO(1999)
Lead (Pb) in muscles	0.05±0.02[a]	0.07±0.01[a]	0.08±0.08[a]	0.19±0.29[a]	2.0	EOS (1993)

The concentrations of heavy metals in water are presented in Table (5). Heavy metals we found increasing of concentrations Cd at locality of River Nile of Aswan in different seasons as the following: exceed permissible limits in winter and in summer (12±0.89, 15.6±0.25µg/l), in Autumn within the permissible limited and less than permissible limited in Spring also the results in Aswan Old Reservoir exceed the permissible limits in all with variation of differences seasons except Spring. As a result of the rapid growth of industrial development and human activities all over the world, the problem of pollution of water systems has emerged, which is one of the serious environmental problems that the world suffers from (Arifin et al., 2016).

The average concentration of Pb in the water samples of tow localities exceeded the permissible limits all seasons prescribed by EOS (1993), with variation of differences between different seasons.

Table (5) Seasonal variations heavy metal concentrations (µg/l) in Aswan River Nile and Old Reservoir.

	Winter	Spring	Summer	Autumn
Cd in water in River Nile	12±0.89[b]	8.4±0.25[b]	15.6±0.25[a]	10±0.45[b]
Cd in water in Old Reservoir	11±0.45[ab]	9.6±0.25[b]	13.4±0.25[ab]	16.8±1.36[a]
Pb in water in River Nile	191.2±14.45[ab]	258±1.45[ab]	142.6±7.89[b]	311.4±50.6[a]
Pb in water in Old Reservoir	93.8±6.83[b]	277.6±1.89[a]	162.6±19.58 [ab]	229.8±6.70[a]

The permissible limits of Cd and Pd concentration in Water are 10 (µg/l) according to **EOS (1993)**

3-2 Results of Bioaccumulation factor

Bioaccumulation factor (BAF) of fish organs showed that:

All Bioaccumulation factor (BAF) values within the examined organs liver, kidney and muscles are of low concentration according to the WMPT.

Table 6: Bioaccumulation factor of cadmium mean within the liver, kidney and muscles of *Coptodon zilli* fish in Aswan River Nile.

	Winter	Spring	Summer	Autumn
Cd Aswan River Nile				
LIVER	90.000	88.095	88.462	142.000
KIDNY	60.833	64.286	61.538	90.000
MUSCLE	1.667	2.381	1.923	3.000
Pb in Aswan River Nile				
LIVER	15.847	11.047	27.560	12.781
KIDNY	8.107	6.202	11.781	5.427
MUSCLE	0.471	0.310	0.842	0.385

Table 7 : Bioaccumulation factor of cadmium mean within the liver, kidney and muscles of *Coptodon zilli* fish in Aswan Old Reservoir**.**

	Winter	Spring	Summer	Autumn
Cd in Aswan Old Reservoir				
LIVER	65.4545	103.1250	88.0597	64.8810
KIDNY	53.6364	60.4167	55.2239	35.1190
Muscle	1.8182	2.0833	2.2388	3.5714
Pb in Aswan Old Reservoir				
LIVER	22.2814	7.6009	16.4207	12.3847
KIDNY	5.3518	1.5850	3.6039	1.4883
Muscle	0.5544	0.2522	0.4920	0.8268

4. Conclusions

The prevalence of two elements cadmium and lead was variable between the body organs liver, kidney and muscles during different 4 seasons liver is the target organs of accumulation of metals followed by kidney exceeding the permissible limit and muscles were less within two sampling sites **(Aswan River Nile and Aswan Old Reservoir).** Bioaccumulation factor in different examined organs in a score of 1 (low concern) (BAF values <250), according (WMPT) scoring system.

References

Al-Busaidi M., P. Yesudhason, S. Al-Mughairi WAK Al-Rahbi, KS Al-Harthy, NA Al-Mazrooei (2011). "Toxic metals in commercial marine fish in Oman with reference to national and international standards," Chemosphere, 85(1)67–73.

Ali, H. Khan K. (2019). Trophic transfer, bioaccumulation, and biomagnification of non-essential hazardous heavy metals and metalloids in food chains/ webs—Concepts and implications for wildlife and human health. Human Ecol. Risk Assessm. An Int. J., 25, 1353-1376, 10.1080/10807039.2018.1469398.

APHA (American Public Health Association) (1998): Standard methods for the examination of water and wastewater.

APHA (American Public Health Association) (2005). American Water Works Association: Standard methods for the examination of water and wastewater. New York.

Arefin, M.T., Rahman, M.M., Wahid-U-Zzaman, M., Kim, J.-E., 2016. Heavy metal contamination in surface water used for irrigation: functional assessment of the Turag River in Bangladesh. J. Appl. Biol. Chem. 59, 83–90. https://doi.org/10.3839/ jabc. 2016.015.

AVMA (American Veterinary Medical Association) (2013). Guidelines for the euthanasia of animals (2013rd ed.). American Veterinary Medical Association 1931 N. Meacham Road Schaumburg, IL 60173: American Veterinary Medical Association. https://www.avma.org/ sites/ defau lt/files/ resou rces/eutha nasia.pdf.

Bailey, N. T. (1981). Statistical Methods in Biology. 2nd ed. (Biological Science Texts).

Basha PS, Rani AU (2003). Cadmium-Induced Antioxidant Defense Mechanism in Freshwater Teleost Oreochromis Mossambicus (Tilapia). Ecotoxicol. Environ. Saf. 56(2): 218-221.

Castro-González M. I. and M. Méndez-Armenta (2008) Heavy metals: implications associated to fish consumption," Environmental Toxicology and Pharmacology, 26, 263–27.

E.O.S. (1993). Egyptian standard, maximum levels for heavy metal concentrations in food., 546-815 pp.

E.O.S.Q.C. (Egyptian Organization for Standardization and Quality Control), (2005). The permissible limits for fish., 1-889 .

Eissa, A. E. (2016). Clinical and Laboratory Manual of Fish diseases. Germany: LAP LAMBERT Academic Publishing. https://www. moreb ooks.shop/store/ gb/book/clini cal-and-laboratory -manual-of- fish-diseases/isbn/978-3-659-87612-7.

Ezzat, S. M., ElKorashey, R. M., & Sherif, M. M. (2012). The economical value of Nile Tilapia Fish "Oreochromis niloticus" in relation to water quality of lake Nasser, Egypt. Journal of American Science, 8(9), 234–247.

FAO, Food and Agriculture Organization (1983a). Manual of Methods in Aquatic Environmental Research, part 9.Analyses of metals and organochlorines in fish (p. 212). FAO FisheriesTechnical Paper.

FAO, Food and Agriculture Organization, (1983b). "Compilation of legal limits for hazardous substances in fish and fishery production," *FAO Fishery Circular*, vol. 464, pp. 5–100, 1983.

FAO/WHO, (1989)"WHO technical report series No 505, Evaluation of certain food additives and the contaminants, mercury, lead and cadmium for environment monitory report No 52 center for environment," Tech. Rep., Fisheries And Aquaculture Science Lowest Tofit UK,.

GAFRD (2018). General Authority for Fish Resources Development. Fish statistics year book (28th ed.). Egypt: Ministry of Agriculture and LandReclamation.

Kargin, F. (1996). Seasonal changes in levels of heavy metals in tissues of Mullus barbatus and Sparus aurata collected from Iskenderum Gulf (Turkey). Water Air Soil Poll., 90: 557-562.

Kucuksezgin F, Kontas A, Altay O, Uluturhan E, Darılmaz E (2006) Assessment of marine pollution in Izmir Bay; Nutrient, heavy metal and total hydrocarbon concentrations. Environ Int 32(1):41–51. https://doi.org/10.1016/j.envint.2005.04.007.

Kumari B, Kumar V, Sinha AK, Ahsan J, Ghosh AK, Wang H, DeBoeck G 2017. Toxicology of arsenic in fish and aquatic systems. *Environ. Chem. Lett* 15, 43–64. 10.1007/s10311-016-0588-9.

Mason, C. F. (2002). Biology of freshwater pollution. 4rd ed. Essex Univ. England. 387 pp.

Maurya, P. K. and Malik, D. S. (2016). Bioaccumulation of xenobiotics compound of pesticides in riverine system and its control technique: A critical review. Jr Ind Pollut Control, 32, 580–94.

McGeer, J., Henningsen, G., Lanno, R., Fisher, N., Sappington, K., Drexler,J., & Beringer, M. (2004). Issue paper on the bioavailability and bioaccumulationof metals. U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/ produ ction/ files/ 2014-11/docum ents/bio_final.pdf.

Noga, E. J. (2010). Fish diseases, diagnosis and treatment (2nd ed.). Wiley. https://www.wiley.com/en-us/ Fish+Disea se%3A+Diagn osis+and+Treat ment%2C+2nd+Editi on-p-9780813806976.

Olaifa, F. G., Olaifa, A. K., & Onwude, T. E. (2004). Lethal and sublethal effects of copper to the African Catfish (Clarias gariepnus). African Journal of Biomedical Research, 7, 65–70.

Parker, R. C. (1972). Water analysis by atomic absorption spectroscopy. Varian techtron, Switzerland. In: E. I. Adeyeye (Editor), Determination of trace heavy metals in Illisha Africana fish and in associated water and sediment from some fish ponds. Int. J. Environ. Stud. 45: 231-238.

Paul, D. (2017). Research on heavy metal pollution of river Ganga: A Review. Annals of Agrarian Sci., (15): 278-286.

Pulatsü, S. Topçu S. A. (2015). Review of 15 Years of research on sediment heavy metal contents and sediment nutrient release in inland aquatic ecosystems, Turkey, J. Water Resour. Protect., 7 , 85-100, 10.4236/jwarp.2015.72007.

Rashed, M. (2001). Cadmium and lead levels in fish (Tilapia nilotica) tissues as a biological indicator for lake water pollution. Environ. Monitor. Assess, 68:75-89.

Rashed, M. N. (2001). Monitoring of environmental heavy metals in fish from Nasser Lake," Environment International, 27, (1), 27–33.

Usmani, Z. and Kumar, V. (2017). Characterization, partitioning and potential ecological risk quantification of trace element in coal fly ash. Environ. Sci. Pollut. Res., 24, 15547–15566

Wayne G, Ho Ya LM (1999). Introduction to Environmental Toxicology Impacts of Chemicals Upon Ecological Systems.

WHO World Health Organization, (1995).Heavy metals environmental aspects, Tech. Rep., Environmental Health criteria No. 85, Geneva, Switzerland, 1995. England, "Aquatic environment monitoring report no. 52," Tech. Rep., Center for environment, fisheries and aquaculture science Lowestoff UK.

WHO, World Health Organization (1989). Heavy metals-environmental aspects; Environment health criteria. No.85. Geneva, Switzerland.