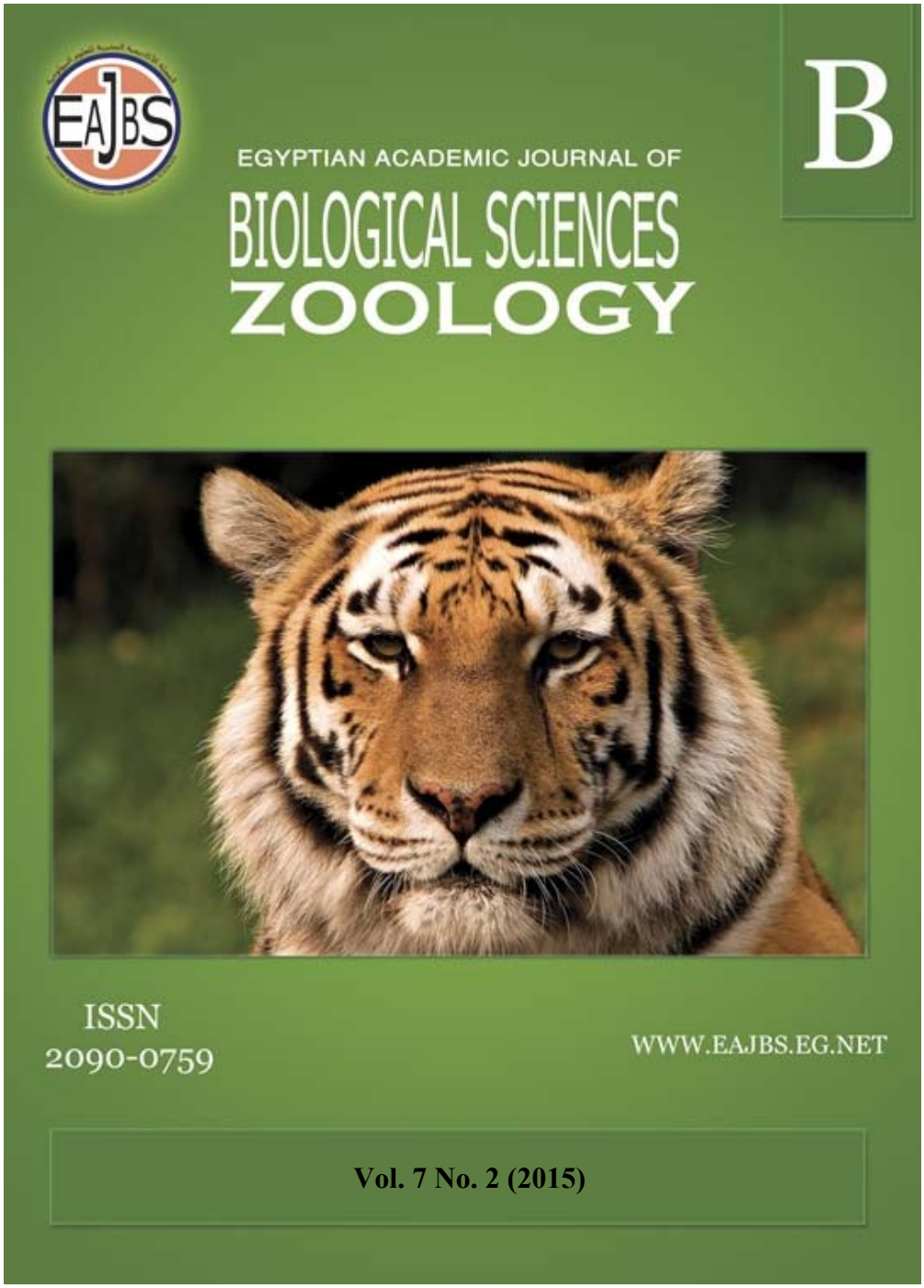


Egyptian Academic Journal of Biological Sciences is the official English language journal of the Egyptian Society of Biological Sciences, Department of Entomology , Faculty of Sciences Ain Shams University .

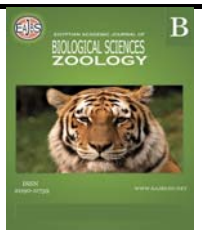
The Journal publishes original research papers and reviews from any zoological discipline or from directly allied fields in ecology, behavioral biology, physiology, biochemistry,

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 7(2)pp51-56 (2015)*

Egypt. Acad. J. Biolog. Sci., 7(1): 51- 56 (2015)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
www.eajbs.eg.net



Effect of Copper Pollution on the Biochemical Composition, Growth and Gill Structre of *Oreochromis niloticus*

Abdel-Salam Mohamed Ibraik Ohaida
Zoology Department. Faculty of Science. University of Misurata Libya.

ARTICLE INFO
Article History
Received: 15/10/2015
Accepted: 20/11/2015

***Keywords*:**
Copper pollution
Biochemical composition
Oreochromis niloticus

ABSTRACT

Alterations in the biochemical composition, growth and histological structure of the gills of fish *Oreochromis niloticus* following prolonged exposure (112 days) to a sub lethal concentration of copper (0.043 ppm), were investigated.

The tissue levels of total proteins, total lipids and total carbohydrates were decreased while muscle glycogen level was increased after was decreased significantly during copper exposure indicating that the organic matter (biochemical content) might be involved in the energy expenditure. The exposed to copper. Muscle glycogen change may give a good tissue total values of proteins, lipids and carbohydrates seem to be of limited value as potential stress in indicators in fish estimate of a stress condition.

Copper had resulted in an extracted appearance in gill lamellae; adjacent lamellae fused together and the epithelial lining of lamellae became disintegrated.

Growth, behavioral and histological responses were integrative to the biochemical changes. To sum up, the consistent changes in the biochemical composition, growth rate, behavior and histological structure noted in *O. niloticus* after 112 days exposure to the sublethal concentration of copper indicate that these fish have not acclimated to copper.

INTRODUCTION

Though copper is an essential micro-nutrient, yet it is highly toxic at high concentrations (El-Domiaty, 1987 and Tort *et. al.,* 1987). Numerous studies have shown that the addition of copper salts to natural water can seriously threaten aquatic life (Arthur and Leonard, 2010). The introduction of small amounts of many of the relatively toxic heavy metal cation, into an aquatic environment, dynamics of aquatic organisms event at nonlethal levels.

The problems of pollution in relation to fish and their environment have received increasing attention during the last decades.

For better understanding of the mode of action of toxicants, more knowledge about the biochemical and physiological changes is necessary.

A particularly significant attribute of sublethal physiological response is that it is amenable to both laboratory and field measurements unlike traditional toxicant testing. Such methods could be employed to develop environmental quality models to predict the biological effects of potential pollutants well as to directly monitor the effects in the environment (Widdows, 1985).

Clinical chemical analyses used in mammalian studies, are highly developed and reliable, but only modest application of the principles and methods have been extended to the realm of aquatic organisms. However, there is accumulating evidence that valid and useful analytical relations can be drawn between biochemical physiological factors and pathology of an aquatic species.

The present study examines the chronic influences of sublethal exposure of copper on *Oreochromis niloticus*, a fresh water fish species inhabiting Taif area. The analyses have been focused on the variables of total proteins, total lipids, total carbohydrates and glycogen representing tissue metabolism . Additional observations are reported on the growth rate, histopathological and behavioral responses.

MATERIALS AND METHODS

Adult specimens of the fresh water fish *Oreochromis niloticus* were used in this study. They were collected from the Tawarga pond at Misurata.

Initially, the 960-hr TL_m (0.214 ppm) was determined to establish the sublethal concentration of copper for ensuring chronic exposure (112 days). All acute lethality tests were conducted according to the methods of APHA(1965).

Fish were then acclimated for two weeks in glass aquaria (50 × 50 × 40 cm) containing freshwater (pH 7.1 – 7.2; oxygen content 7.8 – 8.8 ppm; hardness 92–102.0 ppm; as C_aCo_3 ; temperature 22-24˚C) taken from the area they were collected. Dacie and Lewis (1984)

After the acclimation period, fish were the acclimation period, fish were subjected for 112 days to copper (as $CuSO_4.7H_2O$) at a sublethal concentration (0.043pmm) equivalent to 0.2 of the 96hr TL_m value for this species. For measuring growth rate, animals were weighed biweekly throughout the period of exposure. Behavioral responses were also recorded throughout this period. After the long-term period surviving fish both control and copper-treated aquaria were caught, sacrificed and the muscle and gill tissues were excised. Muscle tissue, as well as whole fish, were weighed and taken for determination of the biochemical content (total proteins, total lipids and total carbohydrates). Total protein level was determined in muscle by the method of Dacie *et al.* (1991).Total lipids were estimated in the whole fish using the method of Entenman (1957). The content of carbohydrate was determined in the whole fish as described by Hassid and Abraham (1963). The muscle content of glycogen was analyzed by the method of Barnes *et al*. (1963). For microscopic analysis, gill tissue was washed in physiological saline (0.9%), fixed in Bouins solution, dehydrated in graded series of alchol, cleared xylol and embedded in paraffin wax. Serial section of gill lamellae were cut at 8μ.

The biochemical data were analyzed for statistical differences between control and experimental means by Students t-test. Growth rate data were statistically evaluated using One-way Analysis of variance (ANOVA). $P<0.05$ was taken as the level of significance in both t-test and ANOVA.

RESULTS

The changes in the tissue levels of total proteins, total lipids, total carbohydrates and glycogen, produced in *Oreochromis niloticus* exposed to the sublethal copper concentration for 112 days are Summarized in Table (1).

Table (1) demonstrates that the biochemical content of the copper exposed *Oreochromis niloticus* showed marked variation in relation to their corresponding controls. Statistical analysis of the data indicated that total proteins, total lipids and total carbohydrates had decreased, whereas glycogen had increased after chronic copper exposure.

Table 1: Changes in the biochemical content of *Oreochromis niloticus* chronically (112 days) exposed to 0.043 pmm of copper.

	Unit	control	Copper - treated	Percent change
Total proteins	Mg/g wet tissue	121.18±8.40	85.36±5.91*	-29.56
Total lipids	Mg/g wet tissue	235.55±29.23	80.57±7.27*	-65.80
Total carbohydrates	Mg/g wet tissue	199.22±13.50	134.62±18.28Z*	-32.43
Glycogen	Mg/g wet tissue	8.11±0.82	17.12±1.58*	+111.10

- Or- indicates increase or decrease.

* Statistically significant at $P \leq 0.05$; student t-test

It is evident from Table (1) that the biochemical factors showing the maximal change were glycogen (+ 111.10%) and total lipids (-65.7%) and those showing the minimal change were total proteins (-29.75) and total carbohydrates (-32.43%).

The effect of sublethal exposure to copper on the growth of *Oreochromis niloticus* for 112 days is illustrated in table. The growth rate of copper treated fish is noticeably dropped, in relation to controls. The marked depression in growth rate started from the eighth week ($P<0.01$) and continued evidently until the end of the exposure period ($P<0.001$).

Light microscopic study showed that the sublethal copper exposure for 112 days caused an extracted appearance in gill lamellae. Adjacent instances. The epithelial cells lining of each lamellae were not as tall as in the control and often became so disintegrated that there was little observable cellular detail. The remains of the detached from the more central portion of each lamellae.

Behavioral responses during metal exposure showed great deviation from the normal behavior. After introducing the fish in the test aquaria excessive secretion of mucus was observed. At the first hours of exposure, hyperexcitability was exerted, as revealed by muscle spasms and opercular movements. This period was followed by modification of body pigmentation. Finally, fish were found to move to water surface in the test aquaria to gulp excessive ventilation.

DISCUSSION

Changes in the tissue levels of total proteins, total lipids, total carbohydrates and glycogen are quite good indicators of pollutant influence. After 112 days of copper exposure a significant increase in the muscle glycogen, and significant declines in the levels of total proteins, total lipids and total carbohydrates were observed in *Oreochromis niloticus*.

The observed elevation in the glycogen level of muscle in *O. niloticus* presumably be caused by the increase in the rate of glycogenesis or gluconeogenesis. The present results seem to contradict the general trend of muscle glycogenolysis noted in all previous studies (Shaffi, 1980 and chaudhry, 1984) on heavy metals and other toxicants. The stepped up muscle glycogen content (111.10%) was to build up the fuel reserves of the tissues because it is the muscle that drives the movements of the fish.

The changes observed in the level of total proteins in the tissues of the copper exposed *Oreochromis niloticus* refers to the higher toxicity of the test metal.

The influence of stress on lipid metabolism was not understood. Reports were given for an increase (Wedemeyer and McLeay, 1981)and for a decrease (*Sivaramakrishna et al.,* 2010) of lipid during stress. The present results suggest the mobilization of energy rich lipids for production of energy toxic stress.

With regards to the percentage decrease of total protein, total lipid and total carbohydrate levels, it is suggested that the test fish might mainly use the three organic matters for energy production. This explains why muscle glycogen did not decline as was expected.

A calculated copper concentration of 0.043 ppm affected growth of *O. niloticus* after 112 days. This result indicates that the test species was extremely sensitive to copper. It can be supported by the results obtained from the biochemical analysis.

The significant biochemical changes induced by chronic exposure may be more hazardous and could reduce the growth rate and fecundity, affect the ability to assimilate food, survival of eggs and embryos, and alter the behavior of fish and hence make the fish more susceptible to attack by predators. The chronic effects of such biochemical changes induced by aquatic pollutants are poorly known and need to be investigated more extensively.

It has been shown that the test fish, chronically exposed to the sublethal concentration of copper exhibit pathological conditions in gill tissue. Whether the changes in gill lamellae observed under light microscope are secondary or primary effects is difficult to tell. The sequence of damage during copper exposure in the present study may perhaps follow a similar pattern for other teleost (*Biagianti et al.,* 1986; Ruey Ping, 1987; Rajbanshi and Gupta, 1988). Schweiger (2006) showed that copper as well as mercury, cadmium, nickel, cobalt and manganese induced variable effects on the gill epithelium of several fish species.

Behavioral changes in response to copper began within a short time after exposure. Test fish displayed extreme sensitivity to the metal. The response included hyperecitability and mucus secretion. These observations are typical of copper poisoning of fish (Schweiger, 2006). The behavioral features provide useful measures of sublethal toxicity because they represent the integrated results of any biochemical and physiological processes (Anderson, 1971). Increase in the rate of opercula movement in *Cyprinion mhalensis* copper exposed cases clearly indicates the physiological stress to aquatic organisms. Thus copper stress apparently leads to higher metabolic rate which require an additional expenditure of energy. This is evident from the rapid movement of the test fish and gulping of air by them. This situation may be analogous to mammalian skeletal muscle in heavy exercise, with increased breathing rate (*Lal et al.,* 1984). Although respiratory stress imposed by copper may have a detrimental effect on fish, there are a very few studies in this direction in which a sublethal effect has been provided.

REFERENCES

Amerlcanpublic Health Assoiation (1965): Standard methods for the examination of water and wastewater. 12th ed. APHA, Ner York, 769.

Anderson, J. M., (1971): Assessment of the effects of pollutants on physiology and behavior. Proc. Soc. Lond. B., 177: 307-320.

Arthur, J. W. and Leonard, E. N. (2010): Effects of copper on *Gammarus pseudolimmaeus*, physa intgra and *Campeloma decisum* in soft water. J. Fish. Res. Bd. Can., 27: 1277-1283.

Barnes, H.; Margaet, B. and Finlayson, D. M. (1963): The seasonal changes in body weight, biochemical composition and oxygen uptake of two common bovearctic cirripedes, U. K., 43 : 185 – 211.

Blaglanti, S.; Gony, S. and Lecomte, R. (1986): Experimental effects of cadmium on glass-eels (*Anguilla Anguilla*). Vie Milleu, 36 : 317 – 322.

Chaudhry, H. S. (1984): Nickel toxicity on carbohydrate metabolism of a freshwater fish, *Colisa fasciatus*. Toxicol. Lett., 20: 115 – 121.

Dacie, O. H., Rosebrough, N. J.; Farr, A. L.; Randll, R. J. (1991): Protein measurement with the folin reagent. J. Biol. Chem. 93 : 265-275.

EL-Domiaty, N. A. (1987): Stress response juvenile *Clarias lazera* elicited by copper. Comp. Biochem. Physiol., 88: 259 – 262.

Entenman, G. (1957): In Methods in Enzymology. S. P. Colowick and N. O. Kaplan (eds), Academic press Inc., New York, 3: 299 – 304.

Hassid, W. Z. and Abraham, S. (1963): Chemical procedures for analysis of polysaccharides, in Colowick and Kaplan, eds Methods in Enzymology Academic press Inc., new York vol., 3 : 34-35.

Lal, H.; Misra, V.; Viswanathan, P. N. and Murti, C. R. K. (1984): Effect of synthetic detergents on some of the behavioral patterns of fish fingerlings (*Cirrhine mrigala*) and its relation to ecotoxicology. Bull. Environ. Contam. Toxicol., 32: 109-115.

Rajbanshi, V. K. and Gupta, A. K. (1988): Alterations in the architecture of the gill surface produced by water borne copper in *Heteropneustes fossilis* (Bloch). Acta Hydrochem. Hydrobiol., 16:355-331.

Ruey-Ping (1987) : The histological study of toxicity of mercury against freshwater fish. The Memoir of Virology and pharmacology in Fish Disease, 3:213-222.

Schweiger, V. G. (2006): Die Toxikologische Einwirkung von Schwermetallsalzen auf Fische und Fishahrtire. Arch. Fischereiwiss., 8:54-78.

Shaffi, S. A. (1980) : The acute industrial effluent toxicity to freshwater fish. Toxicol. Lett., 5: 183-190.

Sivaramakrishna, B.; Suresh, A.; Venkatamana, P. and Radhakishnalah, K. (2010): Copper influenced changes of lipid metabolism in the tissue of freshwater teleost *Labeo rohita* (Ham). Biochem. Int., 26 : 335-342.

Tort, L.; Torres, P. and Flos, R. (1987) : Effects on dogfish haematology and liver composition after copper exposure. Comp. Biochem. Physiol., 87(C): 349-353.

Wedemeyer, G. A. and Mcleay, D. J. (1981) : Methods for determining the tolerance of fishes to environmental stressors. In Stress and Fish (A. D. pickering ed.). Academic Press, New York 247-275.

Widdows, J. (1985): Physiological responses to pollution. Mar. Poll. Bull., 16: 129-134.

ARABIC SUMMERY

تأثير التلوث بالنحاس على التركيب الكيموحيوى والنمو وتركيب الخياشيم في سمكة البلطي (أوريوكروميس نيلوتيكاس)

عبد السلام محمد ابريك اوحيده
قسم علم الحيوان - كلية العلوم- جامعة مصراتة – ليبيا

تم دراسة التغيرات الحادثة في التركيب الكيموحيوي والنمو والتركيب الخيشومى في سمكة الماء العذب (أوريوكروميس نيلوتيكاس) أثناء تعرضها تعريضا مزمنا مدة 112 يوما لتركيز دون المميت (0.043 جزء في المليون) من النحاس. لوحظ بعد التعريض أن محتوى الأنسجة من كل من البروتين الكلي والليبيدات الكلية والكربوهيدرات الكلية قد تناقص بينما تزايد محتوى الجليكوجين فيها. كما ان معدل النمو قد تناقص أيضا خلال التعريض، مؤكداً أن المادة العضوية (المحتوى الكيموحيوى) قد استخدمت من قبل السمكة للحصول على الطاقة. هذا ويمكن القول بأن المعدلات الكلية للبروتين والليبيدات والكربوهيدرات تعد ذوات قيم محدودة كمؤشرات فعاله للمؤثر المجرب على هذه السمكة، لكن جليكوجين العضلات يمكن أن يعطي تقديراً جيداً لتأثير المؤثر عليها.

وقد أحدث النحاس مظهراً منكمشاً للصفائح الخيشومية. كما أن الصفائح الخيشومية المتجاورة قد التحمة مع بعضها البعض وتحللت البطانة الطلائية لها تحللا بالغاً.

هذا وقد كانت استجابات النمو والسلوك والتركيب النسيجى مكملة للتغيرات الكيموحيوية. ومن الخلاصة أن هذه التغيرات الحادثة جميعها في سمكة البلطي الأخضر بعد 112 يوما من التعريض المزمنة لتركيز دون مميت من النحاس تبين أن هذا النوع من الأسماك لم يتكيف مع التلوث بالنحاس.