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EFFECTS OF CHRONIC EXPOSURES TO COPPER ON PRODUCTION AND TOTAL RESIDES AMONG TILAPIA SPECIES.

By

A.A. DRAZ*; I.A. SAMAHA; M.M. El-TELBANY AND A.I. EL-SARHA.

* Dept. of Microbiology and Animal hygiene.Faculty of Vet. Med.
Alexandria University.

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ABSTRACT

Two Oreochromis species and one tilapia named Oreochromis galilaeus, Oreochromis niloticus and Tilapia zillii respectively, were subjected to chronic sublethal dose of copper sulphate (0.25 ppm). Along three months, growth in body weights and body lengths were recorded, at the end the dorsal muscles of the fishes under research were examined for copper residues. The obtained results revealed that, there were a significant differences in the final body weight and body length between treated and non treated species, the highest final body weight and length were (90.3 ± 7.9, 18.5 ± 3.9) in case of non treated Oreochromis niloticus compared to (78.3 ± 7.9, 17.5 ± 3.9) in case of treated Oreochromis niloticus. On the other hand the lowest final body weight and body length were (44.2 ± 7.5, 13.3 ± 3.0) and (49.0 ± 6.7, 14.1 ± 2.9) in case of treated and non treated Tilapia zillii respectively. Data about estimated total residues revealed that there were a significant differences at ($P < 0.01$) among different species, treatment and the control as well as among species and treatment interaction. The highest LSM ± SE was 0.4390 ± 0.3626 in case of Tilapia zillii and the lowest was 0.2100 ± 0.1251 in case of Oreochromis niloticus. Significant differences was found between treated and non-treated replicates (0.4742 ± 0.2137) and (0.0357 ± 0.0183) respectively.

INTRODUCTION

Copper is a common pollutant in surface water and found as a trace metal in natural water i.e. usually at concentration of > 5 ug/ l, but can also be present at much higher concentration (Several mg/l), as a result of mining activities and other industrial processes, to the detrimenent of fish (Alabaster and Lioyd, 1982).

Copper compounds are used to treat a variety of fish diseases (Herwig, 1979). Numerous reports exist in the literature on the use of copper sulphate as an algicide in fishbearing water. However, the concentrations applied are higher than those which predicated to kill or harm fish. In addition to its role in hematopoiesis, it is also required for normal biological activity of many enzymes (Evans, 1973).

Concentrations from 0.02 to over 10 mg/liter of copper are reported to be lethal to fish. This wide range being attributed mainly to different water hardness, species of fish, duration of test and stage of life cycle. The mode of action of copper is not clear but toxicity is increased by reduction of water hardness, temperature and dissolved oxygen and decreased in the presence of chelating agents (Alabster and Lioyod, 1982). Nehring (1964) reported that an oral dose of 400 mg Cu/kg. was lethal to Common carp after three days. The toxicity of copper salts to fish was attributed to the precipitation of mucus on the gills thereby causing suffocation and also to direct damage of the gills (Ellis, 1937).

Lett, et al ., (1976) found that exposure of rainbow trout to sublethal of water - borne copper initially depressed feed intake and weight gain. In addition at a level of 0.035 mg/l was found to decrease ascorbic acid concentration in the liver of rainbow trout (Yamamoto et al ., 1981). Similarly carp exposed to water - borne copper (0.05 mg/l) showed decreased ascorbic acid concentration in the hepato-pancreas and a reduced hepatic L-gulano-lactene oxidase activity (Yamamoto et al., 1977) indicating that sublethal level of water-borne copper may reduce ascorbic acid levels in the hepato-pancreas by inhibiting ascorbic acid synthesis.

The minimum requirement and maximum tolerance for dietary copper (Cu) remain to be determined for most species of fish. Murai, et al. (1981) observed a toxic effect of supplemental copper at 32 mg Cu/kg diet in channel catfish. Toxicity was characterized by a reduction in growth and altered blood parameters. The effects of varying levels of dietary copper intake on several fish species have been studied (Ogino and Yang, 1980; Murai, et al., 1981; Knox, et al., 1982 and 1984; Satoh, et al., 1983 a,b,c). A basal diet containing 0.7 mg Cu/kg diet was reported to suppress growth in carp *Cyprinus carpio* as compared to control diet containing 0.3 mg Cu/kg diet (Ogino and Yang, 1980).

Excessive copper intake was toxic for human. These include renal malfunction, reduced plasma, neurologic disorder, cirrhosis and yellow atrophy of the liver, sever chronic diseases accompanied by anaemia and Wilson's disease (Cartwright, 1950; Churcb and Pond; 1974 and Blood, et al., 1979).

The purpose of the present work was to study the effect of chronic exposure of different species of Tilapia to sublethal concentration of copper on their growth and total muscle residues of copper (Cu).

MATERIAL AND METHODS

1- Experimental fish :

Three fish species belong to family Cichlidae were obtained from Fuwwa Freshwater Artificial Fish Hatchery, Fish weighed 30 ± 0.5 g/fish on the average were classified according to Holden and Reed 1972 into: Oreochromis galilaeus "Tilapia galilae", Oreochromis niloticus "Tilapia niloticus" and Tilapia zillii. The fish were raised in aquaria supplied with dechlorinated tap water for 2 weeks for acclimatization.

2- Experimental diet :

The formulated experimental diet and chemical composition were presented in table (1 a,b), a fixed feeding rate of 4 % from the biomass/day was offered for all replicates once daily at noon.

3- Treatment and experimental design :

Fishes were classified into 4 groups as following :

Group No.	Species involved	Replicate No.
(1)	O. galilaeus	A , b
(2)	O. niloticus	A , b
(3)	Tilapia zillii	A , b
(4)	(O. galilaeus, O.niloticus and Tilapia zillii)	A , b

Each group represented by two replicates 15 individual fish/replicate raised in separate aquarium. The first three groups (1,2 and 3) were subjected to sublethal concentration of copper (0.25 mg/l) adjusted every 24 hrs according to the amount of water of each aquarium by dissolving copper sulphate crystals

Table (1,a) Formula of the experimental diet.

Ingredients	%
Ground yellow corn	38.5
Soya bean meal	27.5
Fish meal	20.0
Rice bran	12.5
Sod. chloride*	0.5
Vitamin mix**	1.0
Total	100.0

* Sodium chloride analar.
** Vitamin mixture supplied by Pfeizer. Co. as Neomix, copper free.

Table (1,b): Chemical composition of the experimental diet.

Components	%
Moisture[a]	9.90
Crude protein[b]	26.67
Ether extract[c]	8.32
Total Coh[d]	47.17
Ash	7.94
ME/Mcal/Kg[e]	3549.69
Protein/energy ratio[f]	75.13

CoH = Carbohydrate.
ME = Metabolizable energy.

a & d : According to Association of Official Analytical Chemists. (A.O.A.C.) (1975) 12 th Ed. Washington D.C.

b : By kjeldahl method according to Randhir and Pradhan (1981).

c : According to Bligh and Dyer (1959). Technique as modified by Hanson and Olly (1963).

e : Metabolizable energy was calculated using a value of 4.5 k cal/g CoH according to Jancy and Ross (1982).

f : Calculated as mg of protein/Calories ME according to Jauncy and Ross (1982) and Alexis et al., (1985).

($CuSO_4$) directly in the aquaria. Group (4) raised together as a control test in dechlorinated non treated filtered tap water. Changing of the water occurred every 24 hrs and newly treated and non treated water were added.

Physico-chemical properties of water were recorded weekly during the experiment and are presented in Table (2).

4- Measurements :

In such studies, the condition of the fish growth could be recorded by determining the condition of the fish flesh K_f (Graham, 1923).

$$K_f = \frac{W \times 100}{L}$$

where W = Gutted weight of the fish
L = standard length of the fish

5- Determination of copper after 12 weeks "experimental period":

Copper analysis was performed by wet acid digestion procedure A weighed portion of dorsal muscle (1g) was put into a Kjeldahl flask containing 5 ml of nitric acid and 1 ml of perchloric acid. The mixture was heated to totally colourless solution and then diluted to 50 ml with bidistilled water.

Copper level was measured by Perkin Elemer 2380 Atomic absorption spectro-photometer at (324.7 nm) wave length.

Table 2: The most important measured physico-chemical parameters.

Item		Range	Mean
Water temperature	°C	23.0 -- 31.0	27.0
PH value		7.1 -- 8.9	8.0
Dissolved oxygen[a]	mg/l	4.7 -- 6.8	5.8
Organic matter[b]	mg/l	65.0 -- 174.0	119.5
Chloride content[c]	mg/l	12.0 -- 23.0	17.5
Salinity[c]	mg/l	21.7 -- 41.5	31.6
Hardness[a]	mg/l	75.0 -- 225.0	150.0

a : According to American Public Health Association (A.P.H.A.) (1985).
b : According to Attia (1964).
c : After Marriott (1974).

6- Statistical analysis :

The statistical analysis was carried out according to Statistical Analysis System (SAS) (1987)

Model

$$X_{ijk} = U + S_i + T_j + e_{ijk}$$

Where :

U = denoted the general means, S_i = effect of species

T_j = effect of copper treatment, e_{ijk} = error.

RESULTS

Gain in body weight and length were recorded in table (3). There were significant difference between weight gain and final body weight attributed to the effect of species difference. The highest final average body weight in treated species was 78.3 ± 7.9 in case of Oreochromis niloticus, while, the lowest final average body weight was 44.2 ± 7.5 in case of Tilapia zillii. Final average body weight of Oreochromis galilaeus being moderately affected by addition of 0.25 ppm (Cu SO_4). In the control groups there is no significant difference between Oreochromis niloticus and Oreochromis galilaeus. Moreover, there is no significant difference between treated and non treated species in final body lengths.

Copper residues estimated in the dorsal muscle from all fish species exposed to chronic sublethal level of copper sulphate 0.25 ppm including the control groups. Least square analysis and least square means and standard errors of estimated copper residue are presented in Tables (4,a) and (4,b) respectively. Least square analysis revealed significant differences at ($p < 0.01$) between species involved in the experiment, between treated and nontreated groups as well as between species treatment interaction (Table 4,a).

Concerning copper residues in the dorsal muscle of examined fish, the least square mean and standard errors showed a significant differences attributed to species difference where the highest least square mean and standard errors was (0.4390 ± 0.3626) estimated in the muscle of Tilapia zillii compared to the lowest (0.2100 ± 0.1251) in case of Oreopchromis niloticus. While, in case of Oreochromis galilaeus the least square mean and standard error being intermediate between Tilapia zillii and Oreochromis niloticus (Table 4,b). On the other hand, there were clear significant differences between treated and non treated groups. From Table (4,b) the most sensitive species was Tilapia zillii while the lowest interaction was associated with Oreochromis niloticus and Oreochromis galilaeus being intermediate, in addition there is significant difference between all species and the control interaction but some variation between the different species being observed.

Table (3): Average body weights and length recorded during the experiment.

Period	*Oreochromis galilaeus*				*Oreochromis niloticus*				*Tilapia zillii*			
	Treated		Non treated		Treated		Non treated		Treated		Non treated	
	B.W	B.L.	B.W	B.L.	B.W	B.L.	B.W	B.L.	B.W	B.L.	B.W	B.L.
	X±SE	X±SE	X±SE	X±SE	X±SE	X±SE	X±SE	X±SE	X±SE	X±SE	X±SE	X±SE
0 time	30.7±0.4	11.3±1.6	30.5±0.4	11.2±1.3	30.5±0.5	11.1±1.5	30.5±0.7	11.6±1.2	30.9±3.4	10.8±2.0	30.5±1.6	10.5±1.9
2nd week	33.7±0.4	11.5±2.0	34.7±2.1	11.9±2.1	33.5±0.5	11.5±1.9	33.8±0.9	11.5±2.0	32.1±3.7	11.6±2.1	31.5±3.0	11.3±2.5
4th week	42.3±1.1	12.5±2.1	46.5±3.0	13.2±3.2	44.5±2.5	13.0±2.5	46.7±3.2	13.5±2.0	35.4±5.1	11.9±2.0	36.0±4.1	12.1±2.0
6th week	48.0±5.1	14.0±1.9	52.1±3.9	14.0±2.0	51.1±6.2	14.4±2.0	56.3±7.1	14.7±3.1	38.7±6.2	12.2±2.3	40.2±3.9	12.7±2.6
8th week	50.7±6.2	15.4±2.3	55.0±5.1	14.9±3.3	57.2±7.1	15.7±2.0	61.2±7.2	16.2±3.9	39.0±7.3	12.5±2.5	43.0±4.5	13.1±3.4
10th week	61.8±7.1	16.5±3.3	64.9±7.0	15.1±3.1	68.6±7.0	17.5±3.7	72.5±5.6	17.7±3.6	40.5±6.5	12.9±2.5	47.5±5.1	14.0±3.0
12th week	66.3±6.9	16.9±3.4	76.2±6.4	16.9±4.0	78.3±7.9	17.5±3.9	79.3±7.9	17.2±3.9	44.2±7.5	13.3±3.0	49.0±6.7	14.1±2.9

NB: B.W. = Body weight (gram), B.L. = Body length (centimeter).
* Treated: exposed to 0.25 ppm ($CuSO_4$).
** Non treated : raised in copper free aquaria. (control).

Table (4,a): Least square analysis of copper residues estimated in ug/g dry weight from the dorsal muscles among different species involved after 12 weeks experiment.

S.O.V.	Df	Ms	F	Pr > F
- Species	2	0.13090**	7.11	0.0017
- Treatment	1	2.7553**	149.63	0.0001
- Species X treatment interaction	2	0.1612**	8.76	0.0005
- Error	60	0.0184		

NB. ** significant at ($P < 0.01$).

Table (4,b): Least square means and standard errors of estimated copper residues (ug/g dry weight) in the dorsal muscles among different species involved in the experiment for 12 weeks.

S.O.V.	No.	LSM ± SE
1- Effect of species.		
Oreochromis galilaeus	22	0.3550 ± 0.2277[b]
Oreochromis niloticus	22	0.2100 ± 0.1251[c]
Tilapia zillii	22	0.4390 ± 0.3626[a]
2- Effect of treatment.		
- 0.25 ppm Cu so4	45	0.4742 + 0.213[a]
- Zero ppm Cu so4	21	0.0357 + 0.0183[b]
3- Effect of the interaction between species and treatment		
- Oreochromis galilaeus X treatment	15	0.4966 + 0.1006[b]
- Oreochromis galilaeus X control	7	0.0383 ± 0.0134[d]
- Oreochromis niloticus X treatment	15	0.2900 + 0.0430[c]
- Oreochromis niltoticus X control	7	0.0171 + 0.0075[d]
- Tilapia zillii X treatment	15	0.6360 + 0.2582[d]
- Tilapia zillii X control	7	0.0514 + 0.0143[d]

NB :- Values carry the same liters are non significant.
- Values carry different liters are significant.

DISCUSSION

The results of this study indicate that, species of fish differ in their ability to tolerate chronic sub-lethal level of copper, based upon the growth (Table 3, and Table 4,a and b), where there was a growth depression along with the exposure to chronic sub-lethal concentration of (Cu so4). Tilapia zillii being severely affected while Oreochromis niloticus affected to a lesser extent compared to Oreochromis galilaeus which was moderately affected. This may be due to species difference in physiological and biological activities. These results agree with those recorded by Lett et al., (1976); Mural et al., (1981); Yamamato et al., (1981); Knox et al., (1982) and (1984); Satoh et al., (1983 a,b,c); Lanno et al., (1985) and Gatlin and Wilson (1986). The herbivorous habit of Tilapia zillii may be a factor incriminated in rising its sensitivity to copper due to swallowing of all available macroscopical food particles of plant origin that containing higher Cu so_4 levels. Such phenomena was explained by Saleh et al., (1983). They proved that Tilapia zillii is less vulnerable to pollution of water environment compound with other fish species living in the polluted water localities of Lake Mariut. This was explained as due to the ability of Tilapia zillii to accumulate less amounts of heavy metals in its gills. i.e. the gills of Tilapia zillii permit the passage of heavy metals into flesh and so its flesh contains more heavy metals than the other ones. The mean copper level in the dorsal muscles in all the species under the experiment ranged from 0.21 - 0.43 ug/g dry weight in which the highest copper concentration was found in the samples obtained from dorsal muscles of Tilapia zillii in contrast the lowest (0.21 ug/g dry weight) estimated similarly for Oreochromis niloticus. This indicates species variation in capability to accumulate copper in their tissues and may also due to biological differences among the different species of Tilapia. Although all Tilapia species belongs to the same family they enjoyed a wide variety of feeding habits which allow swallowing a food particles containing higher copper residues. Generally this results similar to those obtained by Petirson et al., 1991 who studied copper levels in tissues of salmon species with different habits.

Generally, it is concluded that, there were a wide variation between the different species of Tilapia in tolerating chronic exposures to sub-lethal level, of copper as well as its accumulation in their muscles. It is clear that, Tilapia zillii was the mostly affected, in which depressed growth was clear and higher concentrations of copper accumulated in their muscles, whereas Oreochromis niloticus being the lowest in response to growth depression as well as residue estimated from their dorsal muscles, Oreochromis galilaeus being intermediate between Tilapia zillii and Oreochromis niloticus.

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