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Effect of Copper and Lead as Water Pollutants on Ectoparasitic Infested *Oreochromis niloticus*

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Abstract

In this study, the effects of 1/10 and 1/20 96 h LC_{50} of copper and lead on ectoparasitic infested *Oreochromis niloticus* (*O. niloticus*) were evaluated by measuring its impact on the intensity and vitality of Cichlidogyrus and Trichodina species. In addition, copper and lead effects on hematological, biochemical parameters and histopathological findings of infested *O. niloticus* gills were evaluated. Three hundred naturally infested *O. niloticus* with Cichlidogyrus and Trichodina species were divided into five equal groups, each with three replicates (20 fish/ replicate). The infested fish of the first group were kept as control. While, infested fish of the second and third groups were exposed to 1/10 (0.43 mg/L) and 1/20 (0.21 mg/L) of 96 h LC_{50} of copper sulphate, respectively. Fish of fourth and fifth group were exposed to 1/10 (20.2 mg/L) and 1/20 (10.1 mg/L) of 96 h LC_{50} of lead acetate, respectively. The results revealed an inverse relationship between the different concentrations of copper and lead (1/10 and 1/20 96 h LC_{50}) and the intensity of external parasites in the gills of fish where the increase of copper and lead concentrations resulted in decrease in the intensity and vitality of ectoparasites during the experimental period (30 days). Red blood cell count, platelets, mean corpuscular volume, mean corpuscular hemoglobin, lymphocyte, neutrophil, esinophil, Aspertate Transminase; Alanine Transferase, urea and creatinine showed the lowest significant value in groups 2 and 4 in comparison to group 1, besides, histopathological alterations, such as; congestion of blood vessels and fusion of secondary lamellae and complete absence of secondary lamellae in gills of the experimental groups. It could be concluded that, fish ectoparasites (Cichlidogyrus and Trichodina species) are considered as a biomarker for environmental pollution (copper and lead pollution).

Keywords: Lead, Copper, Ectoparasites Intensity, Mortality, *Oreochromis niloticus*.

Introduction

The increasing pollution in the aquatic environment is related to the increase in human activity especially the industrial and agricultural wastes, which had negative impact on ecosystems, fish and organisms [1]**.** Pollution with heavy metals especially copper and lead has adverse effect on the Egyptian water bodies and their aquatic organisms. Copper is used as catalytic in several industries and is used as antifouling agent for painting floating cages and boat hull [2]. Lead pollution sources are sewage discharge, waste discharge of some industries as pipeline, cables, pesticides and paints [3].

Parasites are considered one of the biological indicators of pollutant effects on aquatic organisms. Any minor environmental change will affect on the short-lived free-living parasitic stages, in terms of, effect on their life cycle or effect on their intermediate host or effect on ectoparasites on the host [4]. The effect of environmental changes on parasites differs from one to another, where some parasites are very sensitive to environmental changes, while others are more resistant than their host and their prevalence and intensity could be increased in pollution [4, 5].

Therefore, the aim of the present study was to investigate the effect of 1/10 and 1/20 96 h LC_{50} of copper and lead on ectoparasitic infested *Oreochromis niloticus.* The pollutant

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effects included evaluation of the ectoparasite intensity and vitality, hematological and biochemical parameters and gill histopathological changes.

Material and methods

Fish and experimental design

Three hundred naturally infested *O. niloticus* (with average body weight 30±5 g) by *Cichlidogyrus* and *Trichodina* species were collected from EL-Abassa fish hatchery, Sharkia Governorate. The fish were kept in fifteen glass aquaria (80 x40 x30 cm) filled with 50 L of dechlorinated tap water. Fish were kept for two weeks for acclimation. The random samples were parasitological examined before the beginning of the experiment for ectoparasites (*Cichlidogyrus* and *Trichodina* sp.) as described below to determine the average of the parasite intensity (*Cichlidogyrus* = 4-8 parasites/field and *Trichodina* = 6-9 parasites/field). Fish were divided into five groups, each with three replicates (20 fish/ replicate). The infested fish of first group were kept as control. Fish of second and third groups were exposed to 1/10 (0.43 mg/L) and 1/20 (0.21 mg/L) of 96 h LC_{50} of copper sulphate, respectively [6]. Fish of the fourth and fifth groups (G4 and G5) were exposed to 1/10 (20.2 mg/L) and 1/20 (10.1 mg/L) of 96 h LC_{50} of lead acetate, respectively [7]. Water parameters were adjusted during the experimental period (30 days) according to American Public Health Association (APHA) [8], where, pH was adjusted to 7 to 8, dissolved oxygen 5 - 7 mg/L and temperature 24± 2ºC. The experimental fish were fed on a basal diet contained 39.9% crude protein twice daily at a rate of 3% of their body weight. The aquaria were left without water change for the first week then were changed every three days till the end of the experiment with keeping the heavy metals concentrations in water. The clinical signs, postmortem findings and mortality rate were recorded [9-11].

Parasitological examination

Three fish/replicate (9 fish/group) were parasitologically examined once on the middle of the first week of the experiment and then were examined daily at other three weeks of experiment. Scraping of the slime and outer layer of the skin and fins with cover slide was carried out. The scraping material was spread with a drop of normal saline on the slide, covered with clean cover slide and then examined at lower power magnification (4x). In addition, part of the gill arch was mounted between two slides with a drop of normal saline and then examined at lower power magnification (4x) for detecting microscopic ectoparasites [10].

The intensity of ectoparasites was determined according to Bush *et al.* [12]. The vitality of the parasite was based on a score system where score 1: parasite was inactivated and dead, Score 2: parasite was sluggish in movement and score 3: parasite was moderate in their vitality and movement. Finally, score 4: the parasite was with high vitality, where, *Cichlidogyrus* sp. appeared bobbing or stretching and compressing their body fast, while, *Trichodina* sp. appeared hyperactive, rotating and scooting movement [11, 13].

Hematological and biochemical examination

Blood samples were collected at the end of the experimental period from the caudal vein of nine fish from each treated group (three from each replicate) using sterile syringes with EDTA. The blood samples were used for determining red blood cell count (RBCs; 10^6/uL), hematocrit (HCT; %), hemoglobin concentration (Hb; g/dl), white blood cell count (WBCs; 10^3/uL), platelet count (10^3/uL), mean corpuscular volume (MCV, fl), mean corpuscular hemoglobin (MCH; pg) and mean corpuscular hemoglobin concentration (MCHC, g/dl) [14, 15]. In addition, differential leukocyte count (namely, lymphocytes, neutrophils, monocytes and eosinophils) were performed in Giemsa stained blood smear [14]. Another blood samples were withdrawn into Eppendorf tubes and centrifuged at 3000 rpm for 15 min for serum separation. The serum samples were used for the estimation of Aspertate Transminase (AST, IU/L); Alanine Transferase (ALT, IU/L); serum urea (mg/L) and creatinine levels (mg/L) [16-18].

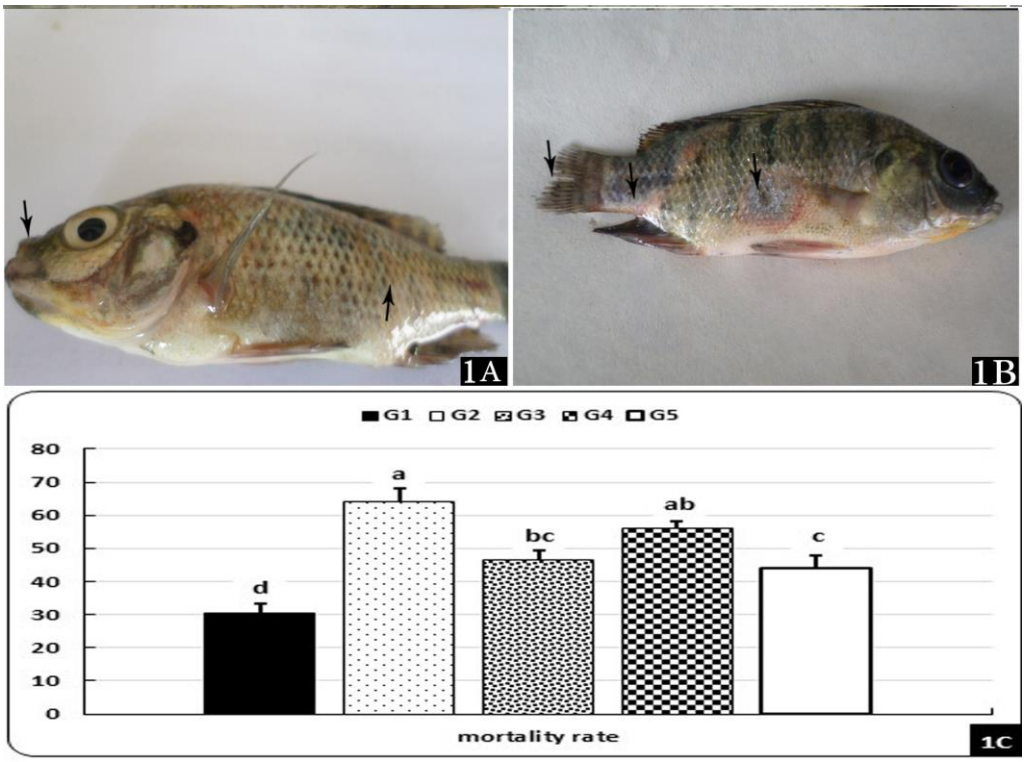

Figure (1): Effect of 1/10 and 1/20 96 h LC_{50} of copper sulphate and lead acetate on fish mortality during the experimental period (30 days). (1A) *Oreochromis niloticus* exposed to 1/10 and 96 h LC50 of copper sulphate showing open mouth, erected fin and slimy body. (1B) *Oreochromis niloticus* exposed to 1/10 and 96 h LC_{50} of lead acetate showing fin rot, loss of body scale, loss of skin shinny appearance and dark body color (1C) showing the mortality rate at the end of experimental period (30 days). Bars with different superscripts (a, b and c) are significantly different (P < 0.05, using a one-way ANOVA).

Histopathological examination

Gill samples from three fish in each replicate were taken from different experimental fish groups and preserved in 10% neutral buffered formalin. The gill specimens were dehydrated in ascending grades of ethyl alcohol, then clarified in xylol, embedded in paraffin wax and cut into thin sections at 3-5 um thickness. Finally, the sections were stained with Hematoxylin and Eosin stain (H & E) [19].

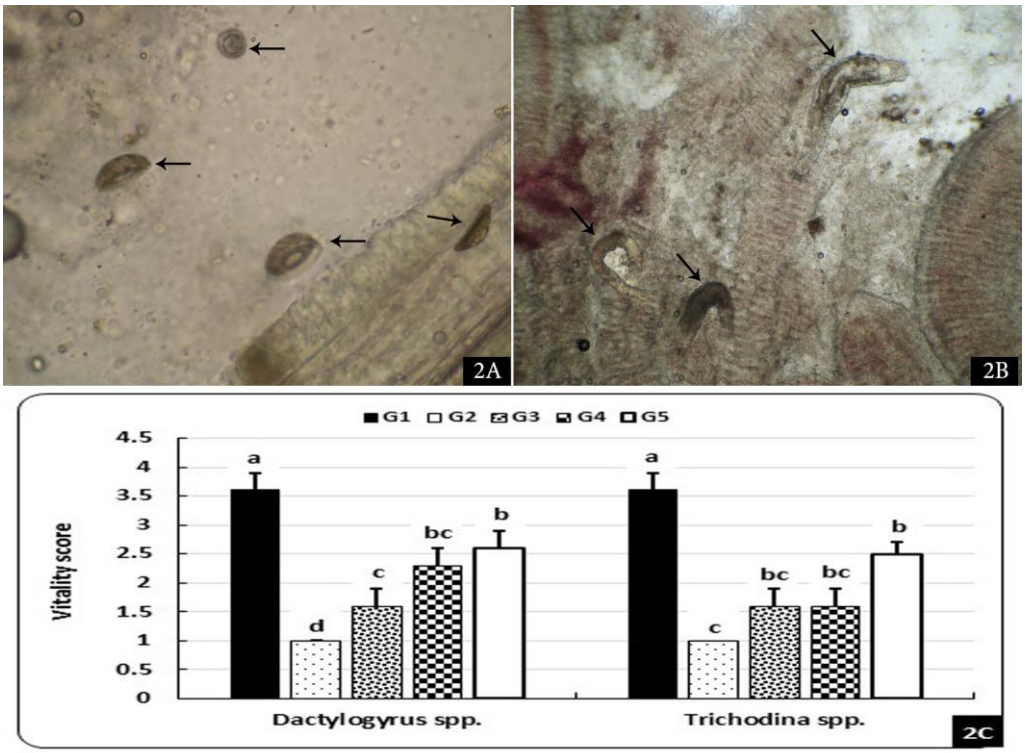

Figure (2): Effect of 1/10 and 1/20 96 h LC_{50} of copper sulphate and lead acetate on *Cichlidogyrus* and *Trichodina* species vitality during the experimental period (30 days). (2A) Showing *Trichodina* species (X500), in (G1) which appear dorsal ventrally flattened oval ciliated protozoan parasites (2B) Showing *Cichlidogyrus* species (X500), in (G1) which characterized by the presence of two pairs of anchors. (2C) Showing vitality scores at the end of experimental period (30 days). Bars with different superscripts (a, b and c) are significantly different (P < 0.05, using a one-way ANOVA).

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) (SPSS for Windows 21.0, Inc., Chicago, IL, USA). Results were presented as mean ± standard errors (SE). The P value (< 0.05) was used to indicate statistical significance.

Results and Discussion

Clinical signs and post mortem findings

Infested fish with ectoparasites (*Cichlidogyrus* and *Trichodina* species) and kept at 1/10 and 1/20 96 h LC_{50} of copper sulphate showed hyperactivity with unbalanced fast movement, difficult respiration, gasping with increase ventilation rate and speed operculum movement and swim near to the surface with loss of scale. Fish died with erected fins, open mouth and their body were covered with mucous (Figure 1A) and gills color were pale. While, those infested with ectoparasites (*Cichlidogyrus* and *Trichodina* species) and kept at 1/10 and 1/20 96 h LC_{50} lead acetate were dark in color with large amount of mucous all over the body surface, loss of skin shinny appearance, loss of scales, fin rot and black caudal peduncles (Figure 1B) with slow movement and loss of escape reflex. Gills were congested with congested liver, kidney and gall bladder. These results agreed with Mahmoud *et al*. [20] who reported asphyxia, other pathological changes, tissue damages and even mortality that occurred in *O. niloticus* as a result of synergism of parasitism and water pollution.

The significant high mortality rate at the end of the experiment was noticed in group 2 where infested fish were exposed to 1/10 96 h LC_{50} of copper sulphate followed by groups 3 and 4. The significant low mortality rate was recorded in G1 (Figure 1C). This could be attributed to the multi directional toxic effect of heavy metals on fish from where

physiological and chemical process of body systems were altered [21]. In addition, ion regulatory disruption effect of heavy metals toxicity [22]. These results are comparable to those of Taweel *et al.* [23] who recorded that fish mortality increased with increasing concentration and/or exposure time of *O. niloticus* to heavy metal. They also recorded that *O. niloticus* was more sensitive to copper than lead and copper is more toxic to fish.

Table (1): Effect of 1/10 and 1/20 96 h LC_{50} of copper sulphate and lead acetate on *Cichlidogyrus* and *Trichodina* species intensity during the experimental period (30 days

Groups No = 60*	Treatment	*Cichlidogyrus* species intensity during the experimental period					*Trichodina* species intensity during the experimental period				
		3 days	7 days	14 days	21 days	30 days	3 days	7 days	14 days	21 days	30 days
1	Control not exposed to 1/10 or 1/20 96 h LC_{50} lead or copper	7.3 ± 0.3 ^{a}	6.6 ± 0.3 ^{a}	6 ± 0.5 ^{a}	5.3 ± 0.3 ^{a}	4.6 ± 0.3 ^{a}	7.3 ± 0.6 ^{a}	6.6 ± 0.3 ^{a}	6.6 ± 0.3 ^{a}	6.3 ± 0.3 ^{a}	5.6 ± 0.3 ^{a}
2	exposed to 1/10 96 h LC_{50} of copper sulphate	5.3 ± 0.3 ^{c}	4.6 ± 0.3 ^{c}	3.6 ± 0.3 ^{c}	1.6 ± 0.3 ^{c}	0.6 ± 0.3 ^{c}	4.3 ± 0.3 ^{c}	3.6 ± 0.3 ^{c}	2 ± 0.5 ^{c}	1.3 ± 0.3 ^{c}	0.3 ± 0.3 ^{c}
3	exposed to 1/20 96 h LC_{50} of copper sulphate	7 ± 0.5 ^{ab}	6.3 ± 0.3 ^{ab}	5 ± 0.5 ^{bc}	3.3 ± 0.6 ^{b}	1.6 ± 0.3 ^{bc}	5.6 ± 0.3 ^{bc}	4.6 ± 0.3 ^{bc}	3.6 ± 0.3 ^{b}	3 ± 0.5 ^{b}	1.3 ± 0.3 ^{bc}
4	exposed to 1/10 96 h LC_{50} of lead acetate	5.6 ± 0.3 ^{bc}	4.6 ± 0.3 ^{c}	4.6 ± 0.3 ^{bc}	2.6 ± 0.3 ^{bc}	1.3 ± 0.3 ^{bc}	4.6 ± 0.3 ^{c}	4.3 ± 0.3 ^{bc}	3.3 ± 0.3 ^{b}	2.3 ± 0.3 ^{bc}	0.6 ± 0.3 ^{bc}
5	exposed to 1/20 96 h LC_{50} of lead acetate	7.3 ± 0.6 ^{a}	5.3 ± 0.3 ^{bc}	5.3 ± 0.3 ^{b}	3.6 ± 0.5 ^{b}	2.3 ±0.3 ^{b}	6.3 ± 0.3 ^{ab}	5 ± 0.5 ^{bc}	4.3 ± 0.3 ^{b}	3.3 ± 0.5 ^{b}	1.6 ± 0.3 ^{b}

Data represents (mean ± SE) from three replicate per group (n = 9). Values with different superscripts (a, b and c) within the same column are significantly different (P<0.05, using a one-way ANOVA). * Naturally infested *O. niloticus* with *Cichlidogyrus* and *Trichodina* species.

There is an inverse relationship between the different concentrations of copper and lead (1/10 and 1/20 96 h LC_{50}), and the intensity of external parasites (*Cichlidogyrus* and *Trichodina* species) in the gills and skin of fish. The significant increase in the *Cichlidogyrus* and *Trichodina* sp. intensity mean was recorded in control group followed by group 5 then groups 3 and 4. While, the significant decrease in the *Cichlidogyrus* and *Trichodina* sp. intensity mean was reported in group 2 (Table 1). In similar manner, the significant high *Cichlidogyrus* and *Trichodina* sp. vitality score was noticed in group 1 where *Trichodina* species appeared rotating, scooting, erratic, whirling, and hyperactive (Figure 2A), while, *Cichlidogyrus* species appeared bobbing or stretching and compressing its body fast (Figure 2B) followed by G5. The *Cichlidogyrus* and *Trichodina* species vitality score was significantly low in group 2 in which the parasites were inactivated and died**.** No significant difference between groups 3 and 4 in *Trichodina* species vitality score was observed, while, G4 showed highly significant increase than G3 in *Cichlidogyrus* species vitality score (Figure 2C). These results agreed with El-Seify *et al.* [24] who found a negative relationship between heavy metal pollution and prevalence of monogenetic infection. This may be due to the toxic effect of the heavy metal on the parasite itself [25]. The parasite response score was different according to the contamination type and the parasite taxon, where, the digeneans and protozoans taxa were mostly respondent to contamination [5, 26].

The results of hematology, which was represented by different leukocyte counts and biochemical parameters are present in Table 2. After 30 days, RBCs, platelets, MCV, MCH, lymphocyte, neutrophil, esinophil, ALT, AST, urea and creatinine showed the lowest significant values in the second and fourth groups in comparison with the control group. The monocyte counts and MCHC showed significant decrease in all experimental groups in comparison to the control group but no significant difference was observed among experimental groups. The stressors, water quality, sources of fish samples, feeding of fish and parasitism may affect hematological and biochemical parameters of fish [27]. It was reported that anemia may be found in some species of Cu-exposed fish but not in others [28]. Also, Dawson [29] observed direct erythrocyte injury which was considered the first and most important sign in lead poisoning of catfish. Dawson [29] recorded a decrease in RBCs, Hb % and PCV% in Channa punctatus upon treatment with heavy metals. In contrast to our results, both Singh [30] and Mazon *et al.* [31] had record a significant increase in WBCs count in fish exposed to copper toxicity.

Table (2): Effect of 1/10 and 1/20 96 h LC_{50} of copper sulphate and lead acetate on hematological and biochemical parameters of infested *Oreochromis niloticus* at the end of experiment (30 days).

Parameters	Group 1 (naturally infested with *Cichlidogyrus* and *Trichodina* species)	Group 2 (naturally infested and exposed to 1/10 96 h LC_{50} of copper sulphate)	Group 3 (naturally infested and exposed to 1/20 96 h LC_{50} of copper sulphate	Group 4 (naturally infested and exposed to 1/10 96 h LC_{50} of lead acetate)	Group 5 naturally infested and exposed to 1/20 96 h LC_{50} of lead acetate
RBCs (10^6/uL)	2.5±0.2^{a}	0.81±0.1c	1.18±0.08^{bc}	0.8±0.07^{c}	1.34±0.17^{b}
HCT %	19±0.28^{a}	13.8±1.09^{cd}	15.6±0.18^{c}	12.06±1.03^{d}	16.16±0.92^{b}
Hg (g/dl)	6.08±0.2^{a}	3.9±0.3^{c}	4.9±0.08^{b}	4.4±0.17^{bc}	4.3±0.16^{bc}
WBCs(10^3/uL)	18.3±1.7^{a}	11.2±0.92^{bc}	13.83±0.92^{b}	9.5±0.7^{c}	14.5±0.7^{b}
Platelets (10^3/uL)	163±13.5^{a}	130±2.6^{b}	145.3±6.8^{ab}	129.3±5.4^{b}	146.3±4.3^{ab}
MCV(fl)	158.6±7.4^{a}	95.6±2.3^{c}	124±2.6^{b}	94±0.5^{c}	112.3±8.08^{b}
MCH(pg)	55.36±2.6^{a}	32.68±1.32^{c}	47.8±3.8^{ab}	30.3±0.58^{c}	42.23±3.8^{b}
MCHC(g/dl)	36.53±2.31^{a}	26.13±2.08^{b}	31±0.62^{b}	28.6±1.1^{b}	30±0.62^{b}
Lymphcytes %	57.16±1.01^{a}	39.3±1.2^{c}	42.3±1.45^{bc}	40±0.57^{c}	44.3±1.45^{b}
Neutrophiles %	50.3±3.7^{a}	40.6±1.2^{c}	45±1.7^{ab}	40.16±1.1^{c}	47±3.7^{ab}
Monocytes %	3.6±0.3^{a}	1.3±0.3^{b}	2±0.5^{b}	1.3±0.3^{b}	1.6±0.3^{b}
Eosinophiles %	2.6±0.3^{a}	1.3±0.3^{b}	2.3±0.3^{ab}	1.3±0.3^{b}	2.3±0.3^{ab}
AST (IU/L)	68.6±0.8^{a}	43.6±10.91^{b}	53.6±4.6^{ab}	48.6±5.2^{b}	52.3±1.4^{ab}
ALT (IU/L)	62.6± 0.8^{a}	39.6±9.9^{b}	48.6±4.05^{ab}	44.3±4.9^{b}	47.3±1.4^{ab}
Urea (mg/L)	31.3±0.4^{a}	19.8±4.9^{b}	24.3±2.02^{ab}	22.1±2.4^{b}	23.6±0.7^{ab}
Creatinine (mg/L)	0.26±0.01^{a}	0.11±0.008^{d}	0.18±0.01^{c}	0.11±0.0008^{d}	0.22±0.005^{b}

Data represents (mean ± SE) from three replicate per group (n = 9). Values with different superscripts (a, b and c) within the same column are significantly different (P<0.05, using a one-way ANOVA).

The percentage of neutrophils, lymphocytes, monocytes and eosinophils generally decrease during acute exposure to copper [32]. Long-term exposure of catfish to 49 and 104 µg/L for 30 days lead to ALT and AST reduction [33]. Folmar [34] stated that the reduction in ALT and AST activities in fish exposed to metals could be attributed to the high accumulation of metals in fish tissues. Marie [35] noted that values of ALT and AST may increase or decrease which indicate damage in liver, kidney, muscle and gills. The transamination and oxidative deamination lead to lower values of ALT and AST enzymes due to exposure to pollution [36]. Nevertheless, these results are in disagreement with El-Seify *et al.* [24] who found ALT and AST as well as urea and creatinine values increased with exposure to both heavy metals and infestation with external parasites.

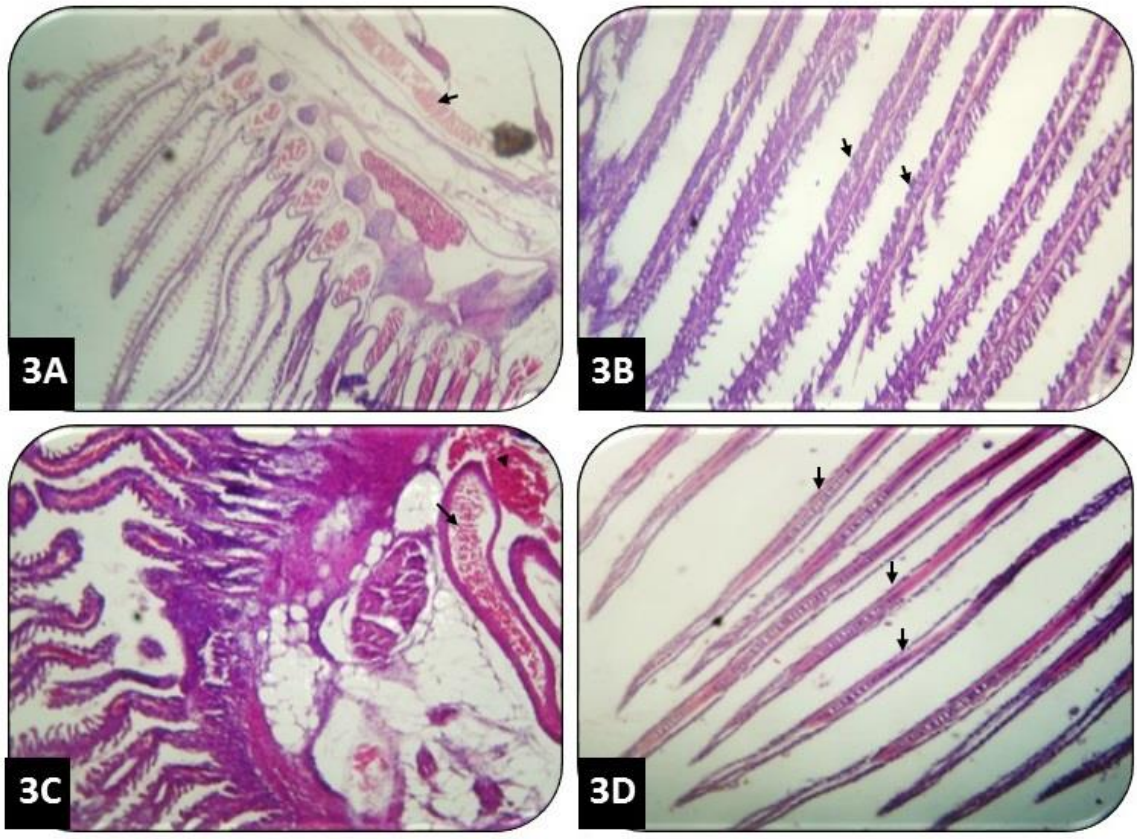

Figure (3): Histopathological changes in gills exposed to 1/10 and 1/20 96 h LC_{50} copper sulphate and lead acetate at the end of the experiment (30 days). (3A) Gills of control group, showing two *Trichodinia* spp. parasities embeded in the gills tissues (Arrowheads) with swollen and fusion of secondary gill lamellae tips (Arrows). H&E X100. (3B) Gills of control group, high power of the previous figure showing *Trichodinia* spp. parasite embedded in the gills tissues (Arrowheads). H&E X400. (3C) gills of *Oreochromis niloticus* exposed to 1/10 96-h LC_{50} of copper sulphate showing congestion of blood vessels (H & E x400). (3D) gills of *Oreochromis niloticus* exposed to 1/20 96 h LC_{50} of copper sulphate showing fusion of secondary lamellae (H & E x400). (3E) gills of *Oreochromis niloticus* exposed to 1/10 96 h LC_{50} of lead acetate showing congestion of blood vessels (arrow) and focal hemorrhage (head of arrow) (H & E x400). (3F) gills of *Oreochromis niloticus* exposed to 1/20 96 h LC_{50} of lead acetate showing complete absence of secondary lamellae (H & E x 1000).

The histopathological findings of the *O. niloticus* gills exposed to 1/10 96 h LC_{50} of copper sulphate (Figure 3A), showed congestion of blood vessels, while, gills of fish exposed to 1/20 96 h LC_{50} of copper sulphate (Figure 3D), showed fusion of secondary lamellae. These results agreed with Begum [37] who found that gills of *O. niloticus* exposed to 2 mg/L of copper sulphate for 5 and 10 days showed telangiectasis and focal hyperplasia in the secondary gill lamellae. This also agreed with Nouh and Selim [38] who found proliferative changes in gills of *O. niloticus* fish exposed to 600 µL of copper sulphate 3 times daily. Gills of fish exposed to 1/10 96 h LC_{50} of lead acetate (Figure 3C), showed congestion of blood vessels and focal hemorrhage, gills of fish exposed to 1/20 96 h LC_{50} of lead acetate, showed complete absence of secondary lamellae (Figure 3D). These results agreed with Chen *et al.* [39] who found gills of lead exposed *Cirrhinus mrigala* fish showed dilation and congestion in blood vessels of primary gill filament and hyperplasia of epithelial cells between secondary lamellae. These histological changes could be due to the direct effect of contaminants on gills since their direct and continuous contact with the external medium, which in turn, lead to disturbance in their functions such as respiratory gas exchange, osmoregulation, excretion of nitrogenous waste and acid-base regulation [40]. In addition to, the epithelial cells are mechanically injured by the

marginal hooklets of monogenia and cilia of *Trichodina* sp.

Conclusion

It could be concluded that, fish ectoparasites (*Cichlidogyrus* and *Trichodina* sp.) were sensitive to heavy metal pollution that consequently are consider as a biomarker for environmental pollution. There is an inverse relationship between the different concentrations of copper and lead on the intensity and vitality of *Cichlidogyrus* and *Trichodina* species in the gills of fish.

Acknowledgement

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Conflict of interest

The authors declare no conflict of interest.

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الملخص العربي

تأثير النحاس والرصاص كملوثات للمياه على اسماك البلطي النيلي المصابة بالطفيليات الخارجية

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في هذه الدراسة تم تقييم تأثير تركيز ١٠/١ و٢٠/١ لمدة ٩٦ساعة من الجرعة نصف المميتة لكل من النحاس والرصاص على اسماك البلطي النيلي المصابة بالطفيليات الخارجية عن طريق قياس تأثيرها على كثافة وحيوية طفيل الداكتيلوجيرس والتريكودينا إلى جانب تأثيرهم على قياسات الدم والقياسات البيو كيميائية والنتائج المرضية في خياشيم النيلي البلطي المصاب. تم تجميع ثلاثمائة من اسماك البلطي النيلي والمصابة طبيعيا بكل من الداكتيلوجيرس والتريكودينا وتم تقسيم الأسماك الى خمس مجموعات متساوية، ولكل منها ثلاث تكرارت (٢٠ سمكة لكل تكرار). المجموعة الاولى تمثل المجموعة الضابطه. فى حين تعرضت اسماك المجموعة الثانية والثالثة لتركيز ١٠/١(٠.٤٣ مجم/لتر) و٢٠/١ (٠.٢١مجم/لتر) من ٩٦ ساعة من الجرعة النصف مميته من كبريتات النحاس، على التوالي. كما تعرضت اسماك المجموعة الرابعة والخامسة لتركيز ١٠/١(٢٠.٠٢مجم/لتر) و٢٠/١(١٠.١ مجم/لتر) من ٩٦ ساعة من الجرعة النصف مميتة من خلات الرصاص، على التوالى. وقد كشفت الدراسة التجريبية وجود علاقة عكسية بين الجرعات المختلفة من النحاس والرصاص على كثافة وحيوية الطفيليات الخارجية الداكتيلوجيرس والتريكودينا في خياشيم الأسماك. حيث أدت الزيادة في جرعات النحاس والرصاص الي انخفاض كثافة وحيوية الطفيليات الخارجية خلال فترة التجربة. كما لوحظ انخفاض معنوي في معظم عناصر الدم وانزيمات الكبد والكلي بالمجموعات الثانية والرابعة بالمقارنة مع المجموعة الاولي. بجانب بعض التغيرات الهستوباثولوجية في أنسجة خياشيم الأسماك. وفي النهاية يمكن القول ان الطفيليات الخارجية الأسماك (التريكودينا و السيكليدوجيريس) انها واحده من اهم العلامات البيولوجية للتلوث البيئي (التلوث بالنحاس و الرصاص).