



Toxic Effects of Mercuric Chloride (HgCl₂) on the Common Carp (*Cyprinus carpio*) Larvae and Recovery Using Selenium and Vitamins

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ABSTRACT

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The harmful effect of waterborne mercury (Hg) as a common biotic stressor to aquatic environment has gained much more interest nowadays. The current study aimed to investigate the impact of lethal and sub-lethal doses of mercuric chloride (HgCl₂) on the embryonic development and dietary supplementation with selenium, vitamin C and vitamin E to mitigate its toxicity on the common carp (*Cyprinus carpio*) fish. Five hundred of newly hatched larvae were assigned into five groups for 60 days. The control (C) group and the other four groups were exposed to HgCl₂ at doses of 0.02, 0.04, 0.06, and 0.08mg/l, respectively. Only the group exposed to HgCl₂ at doses of 0.08mg/l was fed on basal diet treated with selenium(2mg/kg), vitamin C (3000IU/kg), and vitamin E (100mg/kg) for 20 days at the end of the experiment. The results revealed that the HgCl₂ exposed embryo experienced abnormal lesions such as lack of tail and yolk sac edema. After exposure to HgCl₂, the small larvae lost equilibrium, and their growth indices were retarded, resulting in an increased mortality rate. The morphological observation of the larvae under effects of HgCl₂ were classified into axial lordosis, lateral scoliosis and curvature of the spine. Since the larvae ranged from 10 to 25 days in age, the heavy metal became more dangerous, and the mortalities rate increased. Histopathological alterations revealed that the gills in HgCl₂ exposed group were more affected than the gills of the control and treated group. The gill lamellae revealed a decrease in heights and an increase in the thickness of the primary lamellae. Overall, selenium, vitamin C and vitamin E could be added to the diet of *Cyprinus carpio* larvae to alleviate stress condition caused by mercury.

INTRODUCTION

Heavy metals produce effects on the tissues of various terrestrial and aquatic animals (Sastry & Agrawal, 1979). Among the aquatic fauna, fish are the most sensitive group for heavy metal (El-Gamal 2002; El-Mattary *et al.*, 2019). Moreover, mercuric chloride (HgCl₂) is considered one of the most dangerous heavy metals in the aquatic environment (Asefi & Zamani, 2015; El Mattary *et al.*, 2019). Exposure to a high level of inorganic mercuric chloride causes a permanent damage to the kidney and brain of fish

(ATSDR, 2003). However, most researchers didn't give enough information on the nutritional factors that might affect the organism's response to heavy metal intoxication (Chapman & Chan, 2000).

Mercury as an industrial pollutant and immune toxicant has the potential to adversely affect the human and animal health. It induces a broad range of physiological, biochemical, and neurological dysfunction in the human health (Beheary & El-Mattary, 2018). The common carp *Cyprinus carpio* was chosen as an experimental fish in the current research for its importance and economic value in the fish sector. Nowadays in Egypt, the aquaculture of the common carp is increased for its importance (GAFRD, 2001). Fish are exposed to countless elements of heavy metals, among these metals is mercuric chloride. This heavy metal causes problems from its toxicity, resulting in a high mortality rate of the fish.

The input of heavy metals into the environment, whether from terrestrial or aquatic sources, is an important aspect of environmental pollution. Both liver, kidney, brain, and gills are the most susceptible organs to intoxication by mercury compounds (Rani *et al.*, 2022). Very slight information is available to reduce the troubles that result from intoxication with mercuric chloride. Few studies were carried out on the fertilized eggs and newly hatched larvae.

Therefore, some methods must be found to reduce the problems resulting from intoxication with HgCl_2 on the vital organs such as gills (El-Greisy & El-Gamal, 2015). Moreover, other methods should be initiated to reduce the high mortality rate in newly hatched eggs and small larvae.

Among the material that protects the animal from heavy metal is vitamin C since it has a beneficial impact on decreasing the harmful effects of heavy metal. Moreover, selenium mitigates the harmful effect of heavy metal and protects the animals by helping eliminate heavy metal. The role of vitamin E was not neglected in raising the immunity of the fish (Saul, 2005). Little attention has been paid on the effect of mercuric chloride on the early life stage larvae (El-Greisy & El-Gamal, 2015). Therefore, the current research was designed to show the impact of lethal and sub-lethal dose levels of HgCl_2 on the embryonic growth inside the eggs and the larval growth rate to improve their capability to survive during the first days of life.

Therefore, treatment with selenium as well as vitamins E and C was used to protect and retrieve these larvae from HgCl_2 intoxication, considering nutritional perspectives as stated by Clarkson (1998) and Moniruzzaman *et al.* (2017).

MATERIALS AND METHODS

The experimental study started on the 25th of May 2023 and continued for 60 days later after post hatching at El-Serw Fish Farm. Eggs and the larvae were obtained at a fixed time from the hatchery of the common carp *Cyprinus carpio* under semi-natural spawning as described by El-Gamal (2000) and El-Greisy and El-Gamal (2015).

1. Preparations of brood stock fish for spawning

Approximately, 50 healthy males and females were chosen with a sex ratio of 1:1, then segregated and put into two small earthen ponds, each with approximately 400m³ in size. The weight of mature females ranged from 2 to 3kg, while the weight of mature males ranged from 1.5 to 2kg. The fish were fed twice daily with food containing 30% protein for three weeks before spawning.

2. Collection of fertilized and small hatched eggs

After mating about 1200 fertilized eggs were collected. The fertilized eggs were dispersed at a rate of 200 eggs in each small clay pots containing 3L of dechlorinated water. The water parameters were adjusted to become pH 7.0- 7.5, water temperature between 25- 28°C, and dissolved oxygen between 6- 6.5mg/ L, as reported by **El-Gamal and El-Greisy (2008)**.

3. LC₅₀ for 96 hours

The standard of stock solution of mercuric chloride (HgCl₂) that has molecular weight of 325.29 was 1000mg/ L (**El-Mattary et al., 2019**). The working solutions were freshly prepared by a diluting stock solution according to the level of the required dose. The doses were divided into four level groups, and the control group without treatment was also used, and the number of the fertilized eggs in each treatment was 100.

1. The first container: the lethal dose was adjusted to be 0.2mg/ L of HgCl₂.
2. The second container: the lethal dose was adjusted to be 0.4mg/ L of HgCl₂.
3. The third container: the lethal dose was adjusted to be 0.6mg/ L of HgCl₂.
4. The fourth container: the lethal dose was adjusted to be 0.8mg/ L of HgCl₂.

Finally, the fifth container was used as a control. Water was changed daily, and then lethal dose was adjusted according to the design of the experiment in each container.

The white and deformed eggs were counted and removed daily from the surface of water. The fertilized eggs were fixed in a clearing solution for a short time to clarify the effect of the lethal doses of HgCl₂ on the embryonic development inside the eggs and the early hatched larvae. This technique was designed according to **Szczerbik et al. (2008)** and **Barakat et al. (2022)**.

4. Experimental design

Based on the LC₅₀ (96 hours), the small hatching larvae were exposed to five sub lethal doses of HgCl₂, according to **El- Mattary et al. (2019)**, and five glass ponds were used. There were 100 small larvae in each aquarium, and the experiment lasted for 60 days post-hatching. Each glass aquarium contained 60 liters of dechlorinated water and aerated gently. The doses used in this experiment were applied as follows:

1. The first glass aquarium: the dose used was 0.02mg/ L of HgCl₂.

2. The second glass aquarium: the dose used was 0.04mg/ L of HgCl₂.
- 3- The third glass aquarium: the dose used was 0.06mg/ L of HgCl₂.
- 4- The fourth glass aquarium: the dose used was 0.08mg/ L of HgCl₂.
- 5- The fifth glass aquarium was used as a control without treatment.

The water in each glass pond was totally changed every three days, then the ponds were refilled with a new fresh water. The doses' level in each pond was adjusted to the selected dose. The small larvae were fed diet containing 30% protein during the period of experiment.

In the treatment group, sodium selenite (Na₂SeO₃) was added at a concentration of 2mg/ kg, along with vitamin C at 3000mg/ kg and vitamin E at a dose of 100mg/ kg of food. This treatment was used on the larvae that was subjected to a dose level of 0.08mg/ L of HgCl₂ and continued for about 20 days on the last post hatching larvae.

5. Collection of samples

Fish samples were collected three times at 40, 50, and 60 days post hatching, and the mortality rates were computed during the period of study. The lengths of larvae were scaled to the nearest mm, and the weights were weighed to the nearest mg.

6. Calculation formula

The data were calculated in detail in accordance with **Ye *et al.* (2013)** and **EL-Greisy and El-Gamal (2015)**.

Survival rate % = number of fish survival / total number of fish X100

Average of final weight (AFW) = Tw/n

Weight gain (WG) = W2-W1

Weight gain rate = W2 – W1/W1

Where, W1 and W2 are the preliminary weight and final weight in mg, respectively, and n is the number of fish.

Other parameters were also calculated according to **El-Greisy *et al.* (2016)** and used as follows:

Hatching rate = Number of hatching eggs / numbers of fertilized eggs × 100

Fertilization rate = Number of fertilized eggs / total number of eggs × 100

7. Histopathological observations

Ten newly hatched larvae were collected during the period of 15, 35, and 60 days. In case of small larva, the specimen was fixed as a whole. However, in a large fish at 35 and 60 days post hatching, specimens were dissected, and the parts containing the gill tissues were fixed in Bouin's solution for about 24 hours. The tissues of these organs were dehydrated in an ascending ethanol and cleared in xylene. After that, the tissue was embedded in wax with a melting point of 56-60°C and stained with Harris haematoxylin and counterstained with eosin (**Mousa, 1994; El-Gamal *et al.*, 2021**).

8. Statistical analysis

The statistical analysis between the control group and exposed groups were calculated in accordance with **Sokal and Rohlf (1969)** and **El-Greisy *et al.* (2016)**. The t-test in each group was applied to find out a significance difference in each treatment and compare it with that of the control group. A significance was recorded when $P < 0.05$; however, when $P > 0.05$, no significance difference was recorded as stated by **El-Greisy *et al.* (2016)**, **Barakat *et al.* (2022)** and **El-Gamal *et al.* (2023)**.

RESULTS

1. Effect of LC₅₀ (96 hours) of mercuric chloride on the mortality rate

The mortality percentages of fertilized eggs and newly hatched larvae exposed to the lethal doses (LC₅₀) of HgCl₂ were identified in Table (1). In the current study, it was found that the mortality rate increased upon increasing the doses' level of HgCl₂. This appeared upon comparing the percentage of the mortality rate in the control group with groups exposed to various levels of HgCl₂. Significance differences ($P < 0.05$) were observed while comparing the influence of high doses of HgCl₂, ranging from 0.4–0.8mg/ L, and the control group. However, no significance difference was observed ($P > 0.05$) when comparing the influence of HgCl₂ in a low dose level (0.2mg/ L) and control group. In this context, the rate of mortality in the newly hatched larvae increased with increasing the concentration of the HgCl₂. The maximum effect of the lethal dose reached a value of 0.8mg/ L, as shown in Table (1).

Table 1. Influence of lethal doses (LC₅₀) of mercuric chloride (HgCl₂) on mortality rates of fertilized eggs during the period of 96 hours of incubation

Parameter	Control	Lethal doses levels of HgCl ₂ (mg/ L)			
		0.2	0.4	0.6	0.8
Time /(hr)					
25	12	8	15	20	75
40	12	12	20	25	15
70	8	10	7	20	7
96 hrs	5	3	10	15	-
Mean of mortality rate ±SD	37±1.63 ^a	33±0.081	52±0.942 ^b	80±1.63 ^c	97±2.16 ^d

Values with the different superscript letters of control group (a) and the different letters in the exposed groups b, c and d letters were significantly different ($P < 0.05$).

The trial was replicated, and the number of eggs in each treatment was 100.

2. Effect of LC₅₀ (96 hours) of mercuric chloride on hatching rate and abnormality of eggs

The effect of different doses of mercuric chloride, ranging from 0.2- 0.8mg/ L of HgCl₂, on the hatching rate and abnormality of eggs is shown in Table (2). The results showed that the control group was less affected than the exposed group. Upon comparing the results of the control group and exposed groups, there were significant differences in the percentage of hatching and abnormality rates ($P < 0.05$).

The influence of the lethal doses on the incubation time revealed that a short time of hatching was recorded in the control group. However, a long time for hatching was observed in the fertilized eggs subjected to a high level of HgCl₂. In comparing these results, significance differences were recorded between the incubation time in the eggs of the control group and those groups exposed to different lethal doses ($P < 0.05$), as shown in Table (2).

Table 2. Effect of lethal doses (LC₅₀) of mercuric chloride on hatching rate and abnormalities rate of fertilized eggs after 40 hours of incubation

Parameter	Control	Lethal doses levels of HgCl ₂ (mg/ L) on hatched eggs			
Doses level		0.2	0.4	0.6	0.8
Hatching rate%	70 ^a	37 ^b	35 ^c	30 ^d	25 ^e
Abnormalities rate %	30	63	65	70	75
Incubation time in hrs	23	31	33	35	40

Values with different superscripts letters were significantly different in comparison with control group ($P < 0.05$).

3. Effect of mercuric chloride on embryonic development

The influence of HgCl₂ on the embryo development of *Cyprinus carpio* includes body malformation of the embryos. In this respect, our results showed that high doses ranging from 0.04 to 0.08mg/ L of HgCl₂, were more effective causing various degrees of deformation. The eggs wall can't completely protect the eggs from the hazardous of heavy metal. The most malformations on the embryos inside the eggs included: A: the eyes were not completely formed and yolk sac edema; B: the tail was short; C: the head was deformed; and D: the yolk sac appeared in a deformed shape inside the egg, as displayed in Fig. (1A- D).

In Fig. (2A), the embryo in the control eggs exhibits a normal head, yolk sac, and complete tail. In Fig. (2B), the embryo shows a small yolk sac and a short tail. Fig. (2C) depicts the newly hatched larvae in the control group, displaying a normal head and complete tail. Fig. (2D) illustrates an egg containing yolk sac edema and a lack of tail.

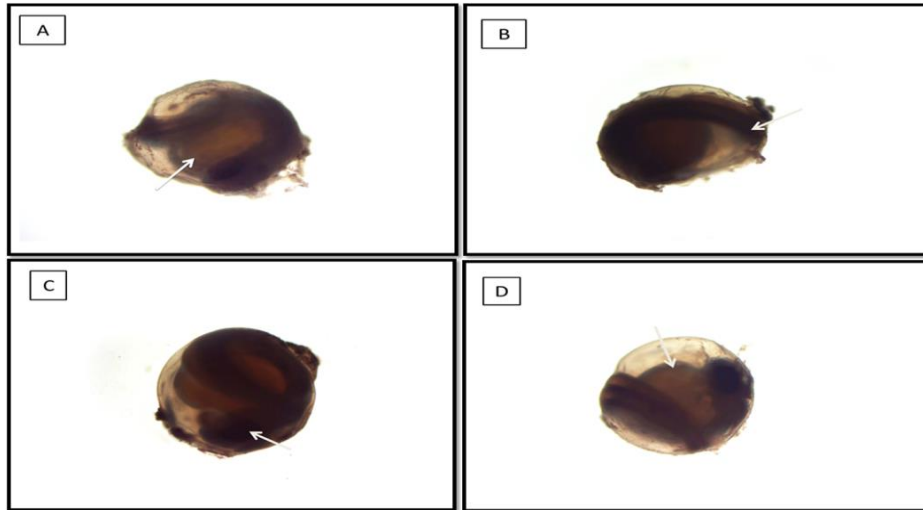


Fig. 1. Photomicrograph pictures (A -D) ($\times 40$) taken by dissecting microscope and clearing solution was used to describe the embryos inside the eggs (96 hours of incubation) showing: **A.** Yolk sac edema (arrow); **B.** Short tail (arrow); **C.** Deformed head (arrow), and **D.** Deformed yolk sac (arrow)

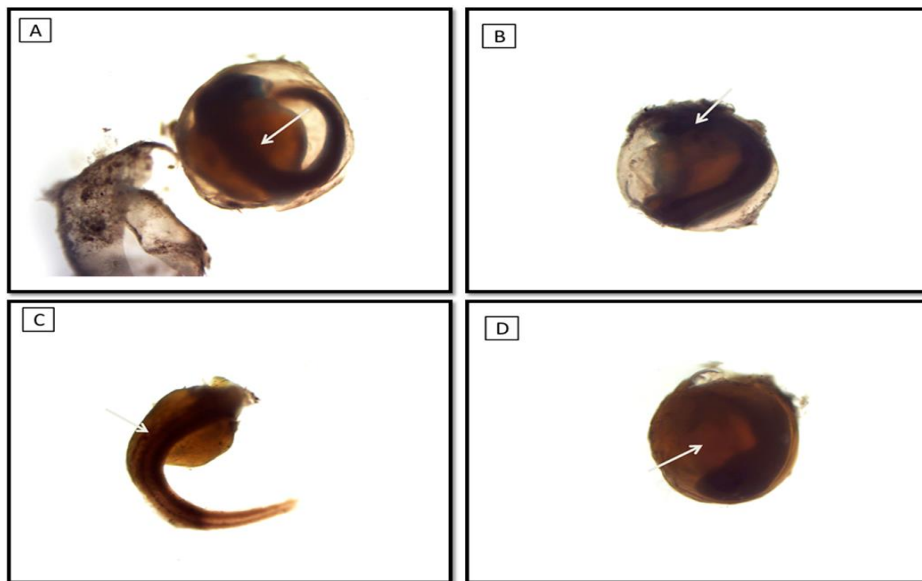


Fig. 2. Photomicrograph picture (A- D) ($\times 40$) taken by using dissecting microscope and clearing solution was taken to describe the embryos inside the eggs (96hrs. of incubation) showing: **A.** Embryo in control egg having normal structure (arrow); **B.** Embryo with a short tail and small yolk sac (arrow); **C.** Newly hatched larvae from the control group (3 day in age) (arrow), and **D.** Embryo with a yolk sac edema and short tail (arrow)

4. Effect of mercuric chloride on growth rate after 40 days post hatching

Our results revealed no significance differences ($P > 0.05$) in both the growth rate in length and growth rate in weight between the control group and other groups exposed to the sub-lethal doses of HgCl_2 . However, there was a significance difference ($P < 0.05$) in the survival rate between the control group (47.66 ± 2.07) and exposed groups (25.5 ± 4.402). Moreover, there was a significance difference ($P < 0.05$) in mortality rate between the control group and other exposed groups to HgCl_2 , as exhibited in Table (3).

Table 3. Effects of sub-lethal doses of mercuric chloride on growth rate of the common carp *Cyprinus carpio* larvae for a period of 40 days post hatching

Variable parameter	Control group	Sub-lethal doses levels in (mg/ L)			
		0.02	0.04	0.06	00.8
Mean of growth in length (mm) \pm SD	1.23 \pm 0.25	1.07 \pm 0.09	1.03 \pm 0.16	0.98 \pm 1.23	0.65 \pm 0.020
Mean of growth in weight (mg) \pm SD	0.04 \pm 0.08	0.02 \pm 0.008	0.02 \pm 0.004	0.01 \pm 0.006	0.009 \pm 0.008
Mean of Survival rate \pm SD	47.66 \pm 2.07 ^a	35.5 \pm 3.08	29.17 \pm 2.13 ^b	30.5 \pm 5.14 ^c	25.5 \pm 4.408 ^d
Mean of Mortality rate \pm SD	52.33 \pm 3.07 ^a	64.17 \pm 2.85 ^b	70.33 \pm 2.48 ^c	71.17 \pm 4.16 ^d	75.5 \pm 4.408 ^e

The value with different superscripts letters with different treatments in comparison with control group was significantly different ($P < 0.05$).

5. Effect of mercuric chloride on growth rate after 50 days post hatching

There were no significant differences ($P > 0.05$) in both weight and length between the control group and groups exposed to the sub-lethal doses of HgCl_2 . However, more significant differences ($P < 0.05$) were observed in the survival rate between the control group and exposed groups. Additionally, the mortality rate of the larvae increased by increasing the level of doses, reaching a percentage of 75 ± 4.08 after the larvae was exposed to 0.08mg/ L of HgCl_2 .

The significance of differences was recorded ($P < 0.05$) in the mortality rate upon comparing the control group with groups exposed to the different sub-lethal doses of HgCl_2 , as shown in Table (4).

Table 4. Effects of sub-lethal doses of mercuric chloride on growth rate of the common carp *Cyprinus carpio* larvae for a period of 50 days post hatching

Variable parameter	Control group	Sub-lethal doses levels in (mg/ L)			
		0.02	0.04	0.06	0.08
Mean of growth in length (cm) \pm SD	1.78 \pm 0.46	1.62 \pm 0.47	1.48 \pm 0.35	1.48 \pm 0.31	1.30 \pm 0.23
Mean of growth in weight (gm) \pm SD	0.081 \pm 0.015	0.054 \pm 0.014	0.09 \pm 0.012	0.038 \pm 0.013	0.063 \pm 0.015
Mean of Survival rate \pm SD	42.83 \pm 1.48 ^a	30.33 \pm 2.32 ^b	27.66 \pm 3.6 ^c	20 \pm 3.15 ^d	17.33 \pm 2.05 ^e
Mean of Mortality rate \pm SD	57.16 \pm 3.38	61.78 \pm 3.57	65.33 \pm 3.61 ^a	69.6 \pm 3.38 ^b	75 \pm 4.08 ^c

There was no significant difference between the length in the control group and the other exposed groups ($P > 0.05$).

There was a significant difference between the survival rate in control group and the other exposed groups ($P < 0.05$).

The value with different superscripts letters with different sub lethal doses in comparison with control group was significantly different ($P < 0.05$).

6. Size and behavior of newly hatched larvae

The larvae subjected to a high level of mercuric chloride showed a clinical pathology in the form of the malformation of the yolk sac, body shortening, and reduced size.

The high doses of HgCl₂ (0.06, 0.08mg/ L) had greater effect on the behavior of the newly hatched larvae. The small larvae lost equilibrium in the water, and some of these larvae tried to swim toward the surface of water. After age 10 to 25 days, the effect became more dangerous, and the mortalities rate increased.

The control fish group at 25 days old had no lesions on their bodies, as shown in Fig. (3A). While larvae under the effect of HgCl₂ were classified according to the pronounced deformation into B: Lateral spine curvature scoliosis, C: Malformation of yolk sac, and D: Axial spine curvature lordosis (Fig. 3B- D). Moreover, Fig. (4) shows A: lost eye and lateral spine curvature, B: C- shaped body, C: body shortening and loss of caudal fin, and D: the treated larvae with selenium, vitamin E and vitamin C morphologically appeared to be similar to those of the control (Fig. 4A- D).



Fig. 3. Photomicrograph picture of control and exposed larvae to HgCl_2 , taken with dissecting microscope ($\times 40$) (A - D) showing: **A.** control larvae without treatment 25 days (arrow); **B.** Larvae with lateral spine curvature (arrow); **C.** Larvae having malformation of yolk sac (arrow), and **D.** Axial spine curvature with observed lordosis (arrow)

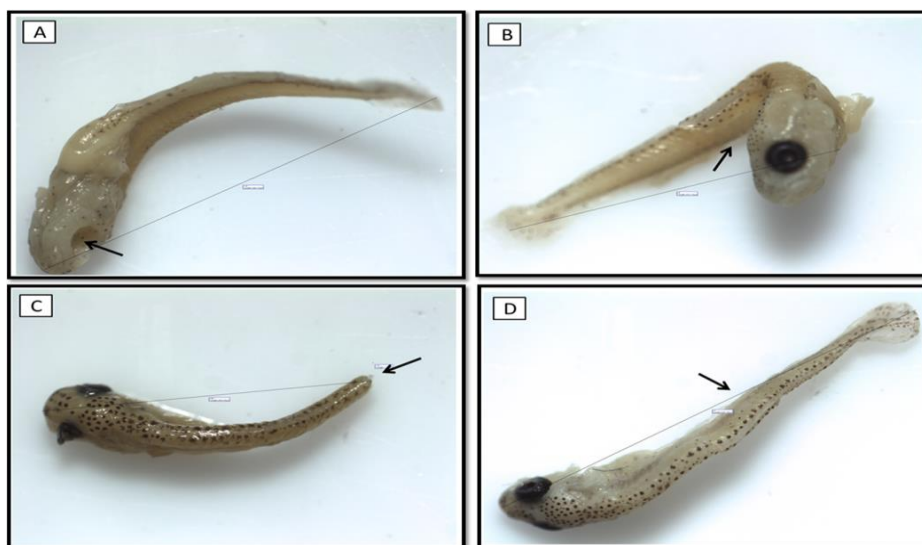


Fig. 4. Photomicrograph pictures of the exposed larvae to HgCl_2 and treated larvae with selenium and vitamins taken by dissecting microscope ($\times 40$) (A - D) showing: **A.** Larvae with no eyes (arrow), **B.** Larvae in a C- shaped body (arrow), **C.** body shortening and absence of caudal fin (arrow), and **D.** Treated larvae with selenium and vitamins (E, C) appearing to be similar to control larvae (arrow)

7. Remediation of toxic effects of HgCl₂ with diet treated with selenium, vitamins C and vitamin E

Our results revealed that the growth in length and weight was improved in the treated group after 40 days post hatching, as shown in Table (5).

There was no significant difference ($P > 0.05$) in the growth in length between the control group and treated group with mixed selenium, vitamin C, and vitamin E, as exhibited in Table (5). In addition, the survival rate in the control group was not significantly different from that of treated group with the mixed selenium and vitamins ($P > 0.05$), as presented in Table (6).

After 60 days of post hatching, the survival rate reached $65 \pm 1.15\%$ in the control group, and in the treated group, it reached 62 ± 1.15 . Upon comparing the mortality rate in the treated group with the control group, no significant difference was recorded ($P > 0.05$), as shown in Table (6).

Table 5. Toxicity effects of mercuric chloride (0.08mg/ L) on growth rate and treatment of this effect using selenium, vitamin C and vitamin E for periods of 40, 50 and 60 days of post hatching

Variable parameter	Days of post hatching	Control group (without treatment)		Treated group with selenium, vitamin C, and vitamin E	
		Mean of growth in length (cm) \pm SD	Mean of growth in weight (gm) \pm SD	Mean of growth in length (cm) \pm SD	Mean of growth in weight (gm) \pm SD
HgCl ₂ (0.08 mg), Selenium (2 mg), Vitamin C (3000mg), Vitamin E (100 mg)	40	1.7 \pm 0.188	0.095 \pm 0.008	1.5 \pm 0.39	0.085 \pm 0.011
	50	1.9 \pm 0.152	0.180 \pm 0.003	1.73 \pm 0.28	0.123 \pm 0.03
	60	2.1 \pm 0.170	0.19 \pm 0.024	1.9 \pm 0.23	0.17 \pm 0.02

Number of fish in each treatment was 100.

Table 6. Toxicity effects of mercuric chloride (0.08mg/ L) on mortality and survival rate and treatment of this effect using selenium, vitamin C, and vitamin E for periods of 40, 50, and 60 days post hatching

Variable parameter	Days of post hatching	Control group		Treated group with (0.08 mg / L) of HgCl ₂	
		Mean of Mortality rate \pm SD	Mean of Survival rate \pm SD	Mean of Mortality rate \pm SD	Mean of Survival rate \pm SD
HgCl ₂ (0.08 mg),	40	41.5 \pm 1.29	58.5 \pm 1.91	44.5 \pm 4.12	51.73 \pm 11.31
Selenium (2 mg),	50	25.3 \pm 1.20	70.20 \pm 1.53	34.25 \pm 4.34	58.73 \pm 3.37
VitaminC (3000mg)	60	30.3 \pm 1.63	65 \pm 1.15	27 \pm 2.87	62 \pm 1.15
Vitamin E (100 mg)					

After 60 days of post hatching there is no significance difference between the mortality rate of control group and treated group ($P > 0.05$). There is no significance difference between survival rate between control group and treated group ($P > 0.05$).

8. Pathological and histological studies in the gills

In the control groups at 25 days post hatching larvae (20- 23mm) in the total lengths, the histological characteristics of gills were composed of functional units of primary and moderate heights of secondary lamellae. There were no lesions in both primary and secondary lamellae. The secondary lamellae was composed of pillar cells that have vital role as an actual site for oxygen exchange from water (Fig. 5A).

Larvae exposed to a low dose (0.04mg/ L) of HgCl₂ for 30 days post hatching showed slight pathological lesions in both primary and secondary lamellar epithelium, as exhibited in Fig. (5B). After the post larval stage was 28mm in length (35 days of post hatching), the histopathological lesions increased in both secondary lamellae and some of the primary lamellae (Fig. 5C). Severe pathological modification were observed in the gills after the small young fish reached 35mm in length (40 days post hatching).

The mortality rate increased at this stage as result of deformation and necrosis of pillar cells that constitute the secondary lamellae (Fig. 6 A, B)

The most striking picture was observed in the gills of *Cyprinus carpio* after exposure to 0.08mg/ L HgCl₂ for a long period.

The treatment involved administering selenium at a dose of 2mg/ kg, vitamin C at a dose of 3000 IU/kg, and vitamin E at a dose of 100mg/ kg for 20 days after the end of the experiment. Some modifications appeared as an improvement in both secondary and primary lamellae. The gill lamellae and structure recovered to a state similar to the normal structure, as shown in Fig. (6C).

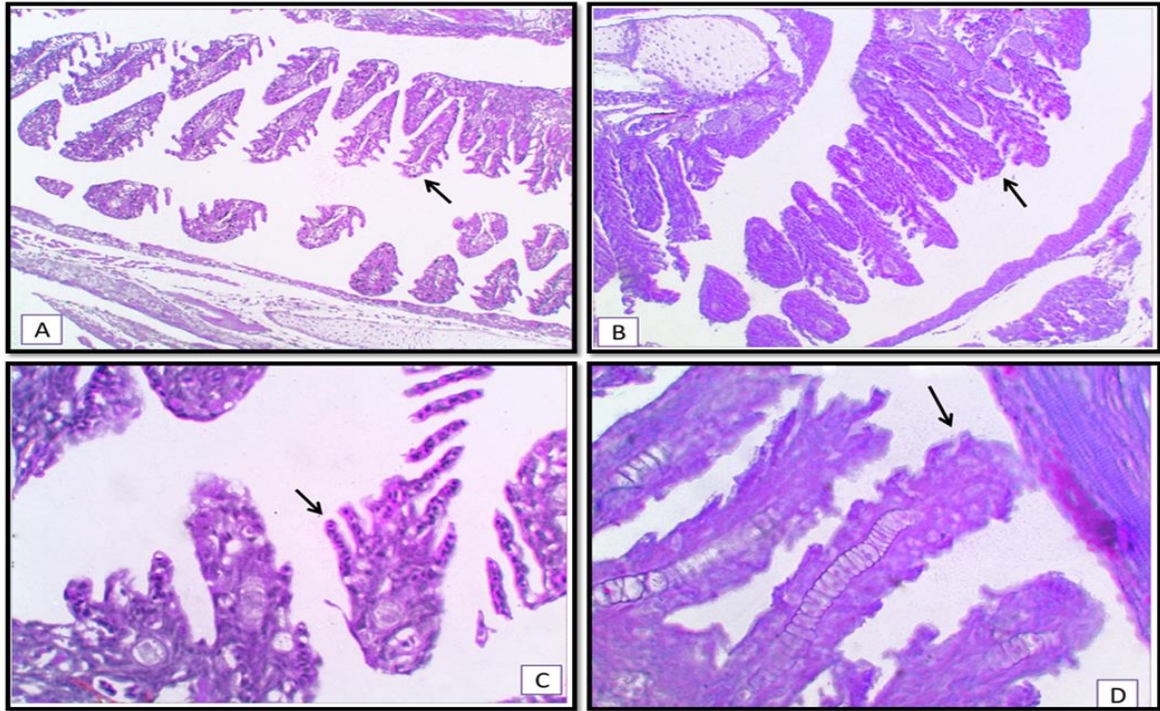


Fig. 5. Photomicrograph sections of gills (A- D) of control and exposed larvae (25– 35) days post hatching of *Cyprinus Carpio* showing: **A.** Normal structure in secondary lamellae (arrow) (H&E, $\times 100$); **B.** Gills exposed to 0.04mg/ L. HgCl₂ showed slight modification in secondary lamellae (Arrow) (H&E, $\times 100$); **C.** Gills exposed to 0.04mg/ L HgCl₂ (35 days) showed severe modifications and necrosis in secondary lamellae (arrow) (H&E, $\times 400$), and **D.** Gills exposed to 0.08mg/ L HgCl₂ showed that the lamellae were more effected (arrow) and necrosis in pillar cells (arrow) (H&E, $\times 400$)

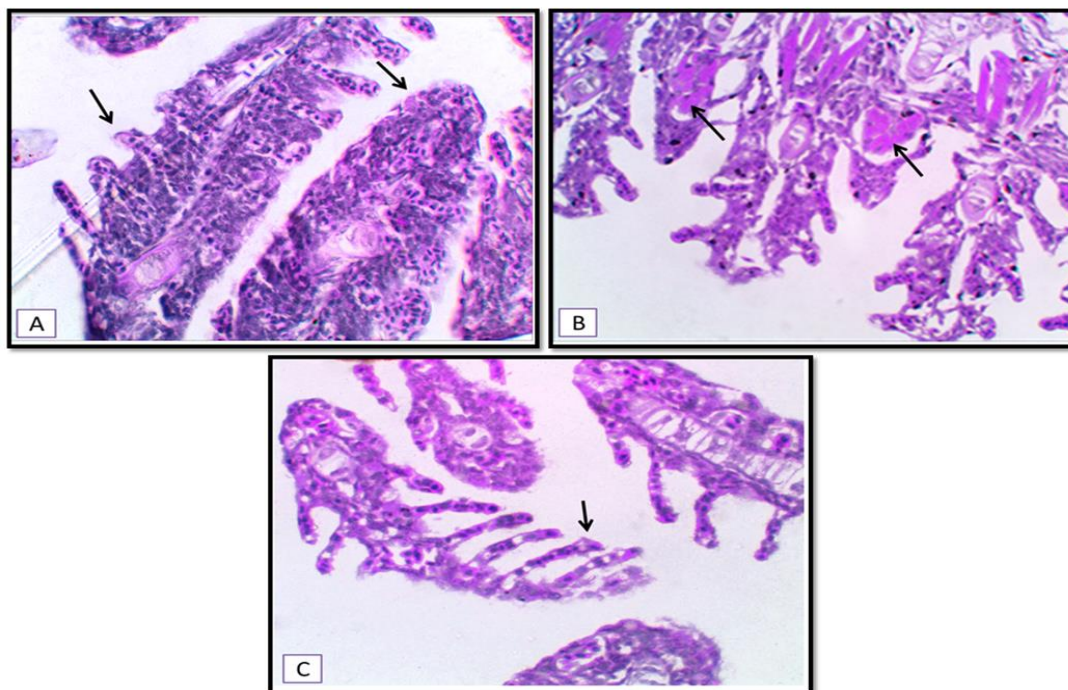


Fig. 6. Photo micrographic sections of gills (A- C) of fish exposed to 0.08mg of HgCl_2 , a period of exposure was 60 days and treated gills with mixture of selenium, vitamin E and vitamin C. **A.** Sections showing that the gill became more modified (arrow), and secondary lamellae became more necrotic (arrow) (H&E, $\times 400$). **B.** Severe modifications were observed in some gills after 60 days post hatching; blood clotting was observed in the base of secondary lamellae (arrow) (H&E, $\times 400$). **C.** Gill of fish exposed to 0.08mg/ L of HgCl_2 and fed diet treated with selenium, vitamin C and vitamin E for the last 20 days post hatching showed that the secondary lamellae improved and pillar cells appeared as in normal gills (arrow) (H&E, $\times 400$)

DISCUSSION

Mercury and its derivatives have been considered as important widespread contaminants in the aquatic environment. Mercury absorbed by fish and passed up the food chain in the carnivorous fish causing what is called bioaccumulation. It not only affects the aquatic environment but also bioaccumulates in humans, as stated by **Marcillo *et al.* (2017)**. The effect of various pollutants on fish has been studied, and the others are still under research. The most frequent pollutants are heavy metals and pesticides, although there are up to now researches on the effect of the nano-particles (**Khoshnood, 2016**). In this respect, the hatching rates of chronically exposed embryos were significantly decreased at a concentration greater than 10ppb Hg^{+2} .

Under all conditions of exposure, the survival rate was reduced at the concentration greater than 40ppb Hg^{+2} (**Sharpt & Neff, 1980**). In this context, waterborne heavy metals

have a large influence on different developmental stages in the embryonic period that leads to a decrease in the offspring quality and quantity (**Jeziarska et al, 2009; El-Greisy & El-Gamal, 2015**).

In the current study, the embryos inside the eggs of *Cyprinus carpio* were affected after exposure to HgCl₂ ranging from 0.06- 0.08mg/ L of water. Severe modifications were observed after the fertilized eggs were exposed to 0.08mg of HgCl₂ for a period of 96 hours of incubation. The most malformations of this heavy metal on the embryos inside the eggs were in the form of retardation in eye formation, yolk sac edema, and short tail. In this respect, **Witseka et al. (1995)**, **Slominska (1998)** and **Lugoweska (2005)** indicated that copper caused retardation in eyes pigmentation for the exposed *Cyprinus carpio*; this delay occurred during organogenesis. In the present study, the heavy metal in the form of HgCl₂ affected the rate of survival of the newly hatched larvae and led to an increase in the mortality rate. In this respect, **Jeziarska and Lugoweska (2002)** stated that the heavy metal affected the embryos of the *Cyprinus Carpio* fish and showed reduction in the offspring quality and quantity. Similar findings were observed in the current study where the mortalities increased after the eggs were exposed to high levels of HgCl₂.

In the current research, HgCl₂ caused various type of body deformation at the early life stage. These defects were classified according to the most malformation into axial lordosis or lateral scoliosis curvature, eyes deformation, and shortening of the body.

Similar results were obtained by several researchers. **Lugowska (2005)** studied the *Cyprinus Carpio* larvae and found vertebral column fractures, tail absence, and body malformations, rendering the larvae unable to swim and feed, ultimately resulting in death after yolk sac resorption. In this respect, some malformation such as vertebral curvature was observed by **Jeziarska and Lugowska (2002)** on *Cyprinus carpio* larvae.

In the present study, the effect of heavy metals depends mostly on the dosage and exposure time to the pollutant. Therefore, the present research was more concerned on the effect of heavy metal like HgCl₂ on the embryo growth and hatching rate of eggs. The lethal doses of HgCl₂ ranging from 0.2– 0.8mg/ L showed an increase in the mortality rate by increasing the doses' level, reaching to the maximum rate at 0.8mg/ L of HgCl₂.

Moreover, mercuric chloride induced a decrease in the hatching rate and an increase in the abnormalities rate during the early life stage. Similar findings were reported by **Lugowska and Jeziarska (2000)**, **Lugowska (2005)** and **El -Greisy and El-Gamal (2015)**.

In the present study, the incubation time for the hatching of eggs depended mainly on the level of the dose. In this context, **Weiteska et al. (1995)** recorded a delay in the hatching rate of the common carp embryos exposed to cadmium at concentrations more than 0.01mg/ dm³, and some eggs were deformed.

As mentioned before in the present study, the effect of the mercuric chloride depended on the level of doses used and the duration of exposure to the heavy metals.

The effect of the mercuric chloride in the lower doses' level is more dangerous than cadmium and zinc in a large doses' level (**El-Greisy & El-Gamal, 2015**). The present finding showed malformation in the yolk sac and abdominal curvature in the newly hatched larva after exposure to a high dose level of 0.8mg/ L of HgCl₂. Similar results were reported by **Lugowska (2005)** and **El-Greisy and El-Gamal (2015)**. Retardation in eggs hatching of the common carp probably resulted from the influence of the high doses' level of the mercuric chloride on the physiological condition of the small fry. **Jeziarska et al. (2009)** attributed the retardation in eggs hatching after exposure to the heavy metals to the impaired function of the hatching gland. The informed data showed delay in the hatching onset, which is possibly correlated to the function of the hatching gland, but the whole process may be accelerated or slowed down (**Cleveland et al., 1986; Dave & Xiu, 1991**).

In general, the yolk sac in the small larvae of various fish species is very important since it is the only source of nutrients for larvae before the mouth is completely formed. The alterations in the yolk sac absorption could directly affect the growth of larval stages (**Huang et al., 2010**).

In the present study, the effect of toxicity with mercuric chloride was recorded, no significant differences were detected in the growth rate (length and weight) between the exposed groups and control group. On the other hand, significant differences were detected in the survival rate between the exposed groups and control .

As observed in present study, the larvae in the control group hatched under the optimum condition and ranged in the total length from 3.5- 4mm long, while for those exposed to mercuric chloride, the larvae were smaller in size and the eggs were delayed in hatching. **Woodworth and Pascoe (1982)** recorded that larvae hatched in polluted environment with heavy metal were smaller in size than those from an unpolluted one. Generally, heavy metal could decrease the absorption of nutrient, increase the consumption of the further energy for detoxification, disable the growth hormone and inhibit the growth of the larvae (**Cao et al., 2012**).

The toxic effect of HgCl₂ can be treated by using a mixture of vitamin C, selenium and vitamin E. The present research showed that the growth rate (length and weight) was improved even after 60 days of post hatching. The survival rate enhanced in treated group, and no significant difference was recorded compared to the control group.

Our results are similar to those of **El -Greisy and El-Gamal (2015)** upon using vitamin C at a dose of 4000mg/ kg of food. The authors explained that vitamin C supplementation improved the larval quality and quantity and may prevent the dangerous effects of heavy metal (HgCl₂).

In addition, the simultaneous treatment of selenium (1- 2mg/ L) mixed with Hg exposure proved its protective effect against mercury toxicity (**El-Matary et al., 2019**). Moreover, vitamin E was used in a dose of 100mg/ kg of food. This treatment was extended for 20 days. The protective doses of vitamin E may be used for raising the

immunity against various diseases (**El-Demerdash et al., 2004; Ozeden et al., 2013**). Altogether, vitamins C and vitamin E mixed with the selenium play an important role in protection of larvae from the toxicity of mercuric chloride, and simultaneously rising the immunity against various diseases.

The present results showed that the degree of gill damage depended on the doses and the time of exposure to heavy metal. Similar results were observed by **El - Mattary et al. (2019)** upon using HgCl₂ on the gills of the elder *Oreochromis niloticus*.

The present study showed that more modifications were observed in gills by using the sub-lethal dose of HgCl₂ ranging from 0.06- 0.08mg/ L. Compared to the results of investigators, the present study examined the newly hatched larvae until 60 days of post hatching stages, while few studies addressed the early life stage.

CONCLUSION

It could be concluded that the heavy metals at high doses' level (0.06- 0.08mg/ L) had more effects on the embryos inside the eggs. The growth rate in lengths and weights were more retarded. The gills of the small fish were more deformed after 35 days of post hatching. The small larvae faced some problems in breathing oxygen from the water. To treat these effects resulting from the heavy metals, selenium and vitamin C were used. Additionally, vitamin E played an important role in raising the immunity and increasing the survival rate.

REFERENCES

- Asefi, M. and Zamani-Ahmad Mahmoodi, R.** (2015). Mercury concentrations and health risk assessment for two fish species, *Barbus grypus* and *Barbus luteus*, from the Maroon River, Khuzestan Province, Iran. *Environ. Monit Assess.*, 187: 1-10.
- ATSDR (Agency for Toxic Substances and Disease Registry)** (2003). Toxicological Profile for Mercury. Atlanta, GA, USA: U.S. Department of Health and Humans Services. Public Health Service, Centers for Diseases Control.
- Barakat, R.O.; El- Mezayen, M.M.; El- Gamal, A.E. and Soliman, A.A.** (2022). Effect of pH on Egg Hatching, Larval Growth Performance and Enzymatic Activity of the Nile tilapia *Oreochromis niloticus*. *Egypt. Aquat. Biol. Fish.*, 26(6): 1115-1131.
- Beheary, M.S. and El-Mattary, F.A.** (2018). Bioaccumulation of Heavy Metals and Implications Associated with Consumption of the Thinlip Mullet (*Liza ramada*) Collected from Sites of Varying Salinity. *Asian J. Fish. Aquat. Res.*, 2(2): 1-15.
- Cao, L.; Huang, W.; Shan, X.; Ye, Z. and Dou, S.** (2012). Tissue-specific accumulation of cadmium and its effects on antioxidative responses in Japanese flounder juveniles. *Environ. Toxicol. Pharmacol.*, 33(1): 16-25.

- Chapman, L. and Chan, H.M.** (2000). The influence of nutrition on methyl mercury intoxication. *Environ. Health Perspect.*, 108(1): 29-56.
- Clarkson, T.** (1998). Methyl mercury and fish consumption: weighing the risks. *Can. Med. Assoc. J.*, 158(11): 1465 -1466.
- Cleveland, L.; Little, E.E.; Hamilton, S.J.; Buckler, D.R. and Hunn, J.B.** (1986). Interactive toxicity of aluminum and acidity to early life stages of brook trout. *Trans. Am. Fish. Soc.*, 115(4), 610-620.
- Dave, G. and Xiu, R.** (1991). Toxicity of mercury, copper, nickel, lead, and cobalt to embryos and larvae of *zebrafish*, *Brachydanio rerio*. *Arch. Environ. Contam. Toxicol.*, 21: 126-134.
- El-Demerdash, F.M.; Yousef, M.I.; Kedwany, F.S. and Baghdadi, H.H.** (2004). Cadmium-induced changes in lipid peroxidation, blood hematology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β -carotene. *Food Chem. Toxicol.*, 42(10): 1563-1571.
- El-Gamal, A.E.** (2000). Induced spawning of cultivated common carp, *Cyprinus carpio* in fish farms. *Bull. Natl. Inst. Oceanogr. Fish.*, 26: 211-221.
- El-Gamal, A.E.** (2002). Experimental study on effect of polluted water with lead acetate on hatchability, growth and gonadal development of Nile tilapia, *Oreochromis niloticus*. *Bull. Natl. Inst. Oceanogr. Fish.*, 28: 217-233.
- El-Gamal, A.E. and El-Greisy, Z.A.** (2008). Effect of removal of egg adhesiveness on hatchability and effect of different levels of salinity on survival and larval development in common carp, *Cyprinus carpio*. *J. Appl. Sci. Res.*, 4(12): 1935-1945.
- El-Gamal, A.E.; El-Sayyad, H.; Sheha, M.A. and Barakat, R.O.** (2021). Histological and histochemical studies on the ovarian development of the grass carp, *Ctenopharyngodon idella* with special references to atretic phenomena. *Egypt. Aquat. Biol. Fish.*, 25(1): 477-492.
- El-Gamal, A.E.; El-Sayyad, H.E.; Sheha, M.A. and Barakat, R.O.** (2023). The Changes in the Biochemical Composition of Total Protein and Lipid in the Ovary, Liver Tissues and the Serum During the Process of the Ovarian Maturation in Female the Grass Carp, *Ctenopharyngodon idella*. *Egypt. Aquat. Biol. Fish.*, 27(2): 627-644.
- El-Greisy, Z.A. and El-Gamal, A.E.** (2015). Experimental studies on the effect of cadmium chloride, zinc acetate, their mixture and the mitigation with vitamin C supplementation on hatchability, size and quality of newly hatched larvae of common carp, *Cyprinus carpio*. *Egypt. J. Aquat. Res.*, 41(2): 219-226.
- El-Greisy, Z.A.; El-Gamal, A.E. and Ahmed, N.A.** (2016). Effect of prolonged ammonia toxicity on fertilized eggs, hatchability and size of newly hatched larvae of Nile tilapia, *Oreochromis niloticus*. *Egypt. J. Aquat. Res.*, 42(2): 215-222.
- El-Mattary, F.A.; Beheary, M.S.; Barakat, R.O. and El-Gamal, A.E.** (2019). Remediation of toxic effect of Mercuric Chloride on the Nile Tilapia, *Oreochromis niloticus* using Selenium. *Egypt. Aquat. Biol. Fish.*, 23(2): 109-121.

- GAFRD** (2001). General Authority for fish Resources Development Report on fish production, Cairo, Egypt.
- Huang, W.; Cao, L.; Ye, Z.; Yin, X.B. and Dou, S.** (2010). Antioxidative responses and bioaccumulation in Japanese flounder larvae and juveniles under chronic mercury exposure. *Comp. Biochem. Physiol. C: Toxicol. Pharmacol.*, 152(1): 99-106.
- Jeziarska, B. and Lugowska, K.** (2002). The quality of carp hatch subjected during embryonic development to copper and cadmium, proceeding of the 5th Czech conference of Ichthyology, PP. 246-252 .
- Jeziarska, B.; Lugowska, K. and Witeska, M.** (2009). The effects of heavy metals on embryonic development of fish (a review). *Fish Physiol. Biochem.*, 35: 625-640.
- Khoshnood, Z.** (2016). Using biomarkers in ecotoxicology: what and why. *Focus on Sciences*, 2(2):1-2.
- Lugowska, K.** (2005). The effects of copper and cadmium on embryonic development, and quality of newly hatched common carp (*Cyprinus carpio L.*) larvae. Doctoral dissertation, Ph. D Thesis, University of Podlasie (In Polish).
- Lugowska, K. and Jeziarska, B.** (2000). Effect of copper and lead on common carp embryos and larvae at two temperatures. *Folia Univ. Agric. Stetin. (Pisc.)*, 205(26): 29-38.
- Moniruzzaman, M.; Lee, J.H.; Lee, J.H.; Won, S.; Damusaru, J.H. and Bai, S.C.** (2017). Interactive effect of dietary vitamin E and inorganic mercury on growth performance and bioaccumulation of mercury in juvenile olive flounder, *Paralichthys olivaceus* treated with mercuric chloride. *Anim. Nutr.*, 3(3):276-283.
- Morcillo, P.; Esteban, M.A. and Cuesta, A.** (2017). Mercury and its toxic effects on fish. *Aims Environmental Science.*, 4(3): 386-402, doi: 10.3934/environsci.2017.3.386
- Mousa, M.A.** (1994). Biological studies on the reproduction of mullet (*Mugil cephalus L.*) in Egypt (PhD thesis). Cairo, Egypt: fact.Sci. Ain Shams Univ., pp. 278.
- Ozden, S.; Catalgol, B.; Gezginci-Oktayoglu, S.; Karatug, A.; Bolken, S. and Alpertunga, B.** (2013). Acute effects of methiocarb on oxidative damage and the protective effects of vitamin E and taurine in the liver and kidney of Wistar rats. *Toxicol. Ind Health*, 29(1): 60-71.
- Rani, R.; Sharma, P.; Kumar, R. and Hajam, Y.A.** (2022). Effects of heavy metals and pesticides on fish. In *Bacterial Fish Diseases*, Academic Press, pp. 59-86.
- Sastry, K.V. and Agrawal, M.K.** (1979). Effects of lead nitrate on the activities of a few enzymes in the kidney and ovary of *Heteropneustes fossilis*. *Bull. Environ. Contam. and Toxicol.*, 22: 55-59.
- Saul, A.W.** (2005). Vitamins and food supplements: safe and effective. Testimony before the Government of Canada, 38th Parliament. 1st Session, Standing Committee on Health, Ottawa, May 12, 2005 [http:// www.doctoryourself.com /testimony.htm](http://www.doctoryourself.com/testimony.htm).

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- Sharp, J.R. and Neff, J.M.** (1980). Effects of the duration of exposure to mercuric chloride on the embryogenesis of the estuarine teleost, *Fundulus heteroclitus*. Mar. Environ. Res., 3(3):195-213.
- Słomska, I.** (1998). Sensitivity of early developmental stages of common carp (*Cyprinus carpio L.*) to lead and copper toxicity .Doctoral dissertation, Ph. D Thesis, Institute of inland fisheries, Olsztyn (In Polish), pp. 104.
- Sokal, R.R. and Rohlf, F.J.** (1969). Biometry. Freeman and Company. San Francisco pp.776.
- Szczerbik, P.; Mikolajczyk, T.; Sokolowska-Mikolajczyk, M.; Socha, M.; Chyb, J. and Epler, P.** (2008). The influence of cadmium on *Prussian carp* oocyte maturation, development of eggs and hatching. Czech J. Anim. Sci., 53(1): 36-44.
- Witeska, M.; Jezierska, B. and Chaber, J.** (1995). The influence of cadmium on common carp embryos and larvae. Aquaculture, 129 (1-4): 129-132.
- Woodworth, J. and Pascoe, D.** (1982). Cadmium toxicity to rainbow trout, *Salmo gairdneri Richardson*: a study of eggs and alevins. J. Fish Biol., 21(1): 47-57.
- Ye, J.S.; Chen, X.J. and Zhu, Y.Y.** (2013). Influence of pH on survival, growth and activities of digestive enzymes of *Odontobutis obscurus*. Adv. J. Food Sci. Tech., 5(9): 1234-1237.