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EFFECTS OF HEAVY METALS INTOXICATION ON SOME FRESH WATER FISH

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ABSTRACT

A total of 780 Fingerlings fish (390 Tilapia nilotica and 390 Cyprinus carpiol (common carp) were utilized for studying the toxicological effect of mercury, cadmium and lead as water pollutant heavy metals. The LC_{50} clinical signs, P.M. lesions, pathological changes and residual concentration were determined.

The results showed that the LC_{50} of mercuric chloride and cadmium chloride were 5.525 mg/L and 8.075 mg/L, respectively for Tilapia nilotica and 4.425 mg/L and 5.925 mg/L, respectively for Common carp. There are no mortalities among both fish species subjected to lead acetate within 96 hrs. The intoxicated fish by the three heavy metals showed the same signs of nervous and respiratory manifestation as well as hepatic and renal disorders. The residual concentration of mercuric chloride; cadmium chloride, and lead acetate were 1. 64 ± 0.04, 1.47 ± 0.02 and 3.70 ± 0.08, respectively in tissues of Tilapia nilotica and 2.85 ± 0.29, 2.18 ± 0.12 and 4.95 ± 0.17, respectively in tissues of Common carp.

INTRODUCTION

Various substances released from modern human societies and entering matters produce pollution of the aquatic environment. Industrial discharges, sewage and seepage from mining wastes are the most common sources for heavy metals intoxication to fish in addition to natural sources by geological and biological cycles[1].

Cadmium is present in some phosphate fertilizers which are used to fertilize fish ponds in fish farms. It is also present in low concentration in soils, sandstones and surface water[6,3]. The use of lead pipes or pipes with soldered joints for water supplies of fish is considered as a source of lead

pollution to aquatic environments. Also, lead shots that accumulate arround water fowl hunting sites are also source of lead poisoning for cultured fishes[17,11]. Also, the transformation of the vapor of metalic mercury to inorganic mercury is the major source of mercury in aquatic environment[12].

These heavy metals are highly toxic to fish and may lead to direct mortality, pathological changes and biological accumulation which directly affect the life of individuals or the population[7].

The toxic effect of heavy metals varies according to the concentration, period of exposure, species and age of exposed fish as well as water quality[18].

The present work was carried out to determine the toxicity of different concentrations of cadmium (Cd), lead (Pb) and mercury (Hg) on fingerlings of Tilapia nilotica and common carp (Cyprinus carpiol) by recording the mortalities and pathological lesions, in addition to determining the residual amounts of heavy metals in tissues of fish subjected to sublethal concentration.

MATERIAL AND METHODS

The LC_{50} of cadmium chloride and mercuric chloride was determined for Tilapia nilotica and Cyprinus carpiol (common carp) by adopting a reported method[5]. The fish used were of fingerlings with an average weight of 15 gm. and 11 cm length. They were obtained from Abbasah fish hatchery at Sharkia provience and left for two weeks in glass aquaria containing dechlorinated tap water with hardness 104 mg/L as $CaCO_3$. The water temperature was kept at 23 ± 1°C and the maitained pH was 7.4. Continous directions and observations were carried out before starting experimental work. During the observation periods bacteriological and parasitological tests were carried out to exclude any disease interference[4].

For chronic toxicity study of the LC_{50} either cadmium or mercury salts were used for tewelve weeks. For each salt and fish species twenty fish were used under the same previously described conditions. Clinical signs, post-mortem and histopathological examinations of the liver and kidney [10] were carried out during chronicity study.

For lead acetate intoxication several trials were carried out to determine the lethal dose, and the results showed that up to fourteen PPM in water, no mortalities were recorded. Accordingly, two doses of twelve and fourteen PPM were used for the study of its long-term effect for twelve weeks. Twenty fingerlings fish were used for each dose for both Tilapia nilotica and Common carp. During the twelve weeks intoxication clinical signs, post-mortem and histopathological changes in the liver and kidney occurred.

Residual analysis for each toxin was carried out by Atomic Absorption Spectorphotometry technique as previously described [20,22]. All chemical used were of a pure grade.

Statistical analysis of the data was carried out according to reported methods[24].

RESULTS

Determination of the LC_{50}:

The results of LC_{50} determination of the chloride of cadmium and mercury for Tialpia nilotica and Common carp are showen in Tables 1,2,3, and 4.

Although no mortalities occurred at dose levels of 12 and 14 PPM lead acetate for 96 hours, deaths started to be recorded after one week of intoxication for both Tilapia nilotica and Common carp.

For Tilapia nilotica it was 60,90 and 100% mortality after 1,6 and 12 week of exposure (12 PPM), respectively, and 40,70,80,90 and 100% after 1,3,6,9 and 12 weeks for Common carp, respectively.

At dose level 14 PPM the percentages of mortalities were 40,70,80,90 and 100 for Tilapia nilotica and 30,50,70,80,90 and 100 for Common carp after 1,3,6,9 and 12 weeks of exposure, respectively.

Clinical signs, post-mortem and histopathological studies:

The behaviour of fish previously intoxicated with the three examined metals is the same. At low concentrations the fish were excited, showed hyperactivity and increased their locomotor in all aquarium directions. Fish swam while fines were held beside the body. The activity of fish was reduced, manifested by loss of any reflex action and they quite lie down on the bottom of the aquarium. Before death there was hurried respiratory movement and finally fish died.

In case of cadmium intoxication, beside nervous signs there was periodical attack of neuromuscular spasms followed by continous tension of opposing muscles. Fish remained lying down on the bottom of the aquorium with tension in their muscles for long periods that reached a few weeks before dying.

The post mortem findings of the two examined fish species were nearly similar despite the heavy metals used. The intoxicated fish appeared pale with yellow patches on the head operculum and loss of scales specially at the peduncle region. Abrasion on the skin of the head, destruction of the fins (specially caudal and pectoral) and accumulation of asitic fluid in the abdominal cavity were seen. The gills were congested in case of cadmium and mercuric intoxication while pale in colour in case of lead.

The pathologic findings in the two examined fish species were nearly similar.

Histopathological findings in lead acetate treated groups:

Liver:

The majority of hepatic cells had single or multiple clear large vacuoles of sharp border replacing cytoplasm and sometimes ruptures forming large cysts with flattened nuclei remaining situated on periphery (Fig. 2). The remaining hepatic cells showed granular and easinophilic cytoplasm with centrally located nuclei. The portal area revealed mild inflamatory reactions mainly lymphocytes besides a few fibroblast proliferations. The epithelial lining of the biliary system was completely distended with secretion or completely destructed with necrosis of the hepatic cells around portal area (Fig. 3). Focal haemarrhages among hepatic cells could be seen.

Kidneys:

Focal coagulative necrosis of renal epithelium with mild lymphocytic inflammations among renal tubules were seen (Fig. 4).

The blood vessels were congested. Some glomeruli revealed thickening of Bowman's capsule with shrinkage of glomerular tufts which appeared deeply basophilic.

Histopathological findings in cadmium cloride treated group:

Liver:

All the hepatic cells of cadmium treated fish showed degenerative changes mainly hydropic degeneration (Fig. 5). Lymp-hocytic infiltration among hepatic cells and portal areas, beside congestion of blood vessels were seen in some fish. Focal haemorrhages were evident in the biliary system.

Kidenys:

Some glomeruli had contracted glomerular tufts, with few red blood cells, and dilated glomeular areas. In addition to severe coagulative necrois

of renal epithelium which was represented by homogenous eosinophilic cytoplasm with absence of their nuclei and was infilterated with numerous golden yellow pigmented hemosiderosis (Fig. 5). Depletion of hemopiotic elements from intersitial tissues with focal haemorrhage among renal tubules could be seen.

Histopathological findings in Mercuric chloride treated group:

Liver:

The hepatic cells in some intoxicated fish contained one or more numerous vacuoles (Vacular degeneration) with hyperplasia of reticuloendothelial cells. Other hepatocytes suffered from coagulative necrosis with congestion of blood vessels and the portal areas were infiltrated with lymphocytes and granulocytes. Some blood vessels showed hyalinized media with endostheliosis of their intima (Fig. 7).

Kidneys:

The renal tubules showed necrotic epithelium which appeared dissociated from their basement membranes with more eosinophilic cytoplasm (Fig. 8). The renal tissue had numerous golden yellow pigment or granules (hemosiderosis). The lamina propia of ureters was midly inflitarted with lymphocytes and oedematous.

Means within the same column, with different superscripts are significantly different.

Residual analysis:

The residual study revealed that the concentration of the three studied heavy metals were significantly higher than that recommended by the Food and Drug Administration (F.D.A. 1976). The result is shown in Table (5).

Table (1): Determination of LC_{50} of Cadmium chloride in Tilapia nilotica after 96 hours exposure

Group	No. group	Dose (mg/L)	No. of dead	a	b	ε (axb)
1	10	1.5	0	--	--	--
2	10	2	1	0.5	0.5	0.25
3	10	4	3	2	2	4.00
4	10	6	4	2	3.5	7.00
5	10	8	4	2	4	8.00
6	10	10	5	2	4.5	9.00
7	10	12	8	2	6.5	13.00
8	10	14	10	2	9	18.00

LC_{50} = The lowest dose killing all fishes = $\frac{\varepsilon\ (axb)}{No./group}$ $\varepsilon\ (59.25)$

Where:

a = Difference between two successive doses.

b = Mean of death between two successive doses.

$$LC_{50} = 14 - \frac{59.25}{10} = 14 - 5.925 = 8.075 \text{ mg/L.}$$

Table (2): Determination of LC_{50} of Cadmium chloride in Common carp after 96 hours exposure.

Group	No. group	Dose (mg/L)	No. of dead	a	b	ε (axb)
1	10	1.5	1	--	--	--
2	10	2	2	0.5	1.5	0.75
3	10	4	4	2	3	6.00
4	10	6	5	2	4.5	9.00
5	10	8	6	2	5.5	11.00
6	10	10	9	2	7.5	15.00
7	10	12	10	2	9.5	19.00
8	10	14	10	2	10	--

LC_{50} = The lowest dose killing all fishes = $\frac{\varepsilon\ (axb)}{No./group}$ $\varepsilon\ (60.75)$

Where:

a = Difference between two successive doses.

b = Mean of death between two successive doses.

$$LC_{50} = 14 - \frac{60.75}{10} = 14 - 6.075 = 7.925 \text{ mg/L.}$$

Table (3): Determination of LC_{50} of mercuric chloride in Tilapia nilotica after 96 hours exposure.

Group	No. group	Dose (mg/L)	No. of dead	a	b	ε (axb)
1	10	1.5	1	--	--	--
2	10	2	2	0.5	1.5	0.75
3	10	4	5	2	3.5	7.00
4	10	6	6	2	5.5	11.00
5	10	8	7	2	6.5	13.00
6	10	10	9	2	8.0	16.00
7	10	12	9	2	9.0	18.00
8	10	14	10	2	9.5	19.00
						ε (84.75)

LC_{50} = The lowest dose killing all fishes = $\frac{\varepsilon\ (axb)}{No./group}$

Where:

a = Difference between two successive doses.

b = Mean of death between two successive doses.

$LC_{50} = 14 - \frac{84.75}{10} = 14 - 8.475 = 5.525$ mg/L.

Table (4): Determination of LC_{50} of mercuric chloride in Common carp after 96 hours exposure.

Group	No. group	Dose (mg/L)	No. of dead	a	b	ε (axb)
1	10	1.5	3	--	--	--
2	10	2	4	0.5	3.5	1.75
3	10	4	6	2	5.0	10.0
4	10	6	8	2	7.0	14.0
5	10	8	8	2	8.0	16.0
6	10	10	9	2	8.5	17.0
7	10	12	9	2	9.0	18.0
8	10	14	10	2	9.5	19.0
						ε (95.75

LC_{50} = The lowest dose killing all fishes = $\frac{\varepsilon\ (axb)}{No./group}$

Where:

a = Difference between two successive doses.

b = Mean of death between two successive doses.

$LC_{50} = 14 - \frac{95.75}{10} = 14 - 9.575 = 4.425$ mg/L.

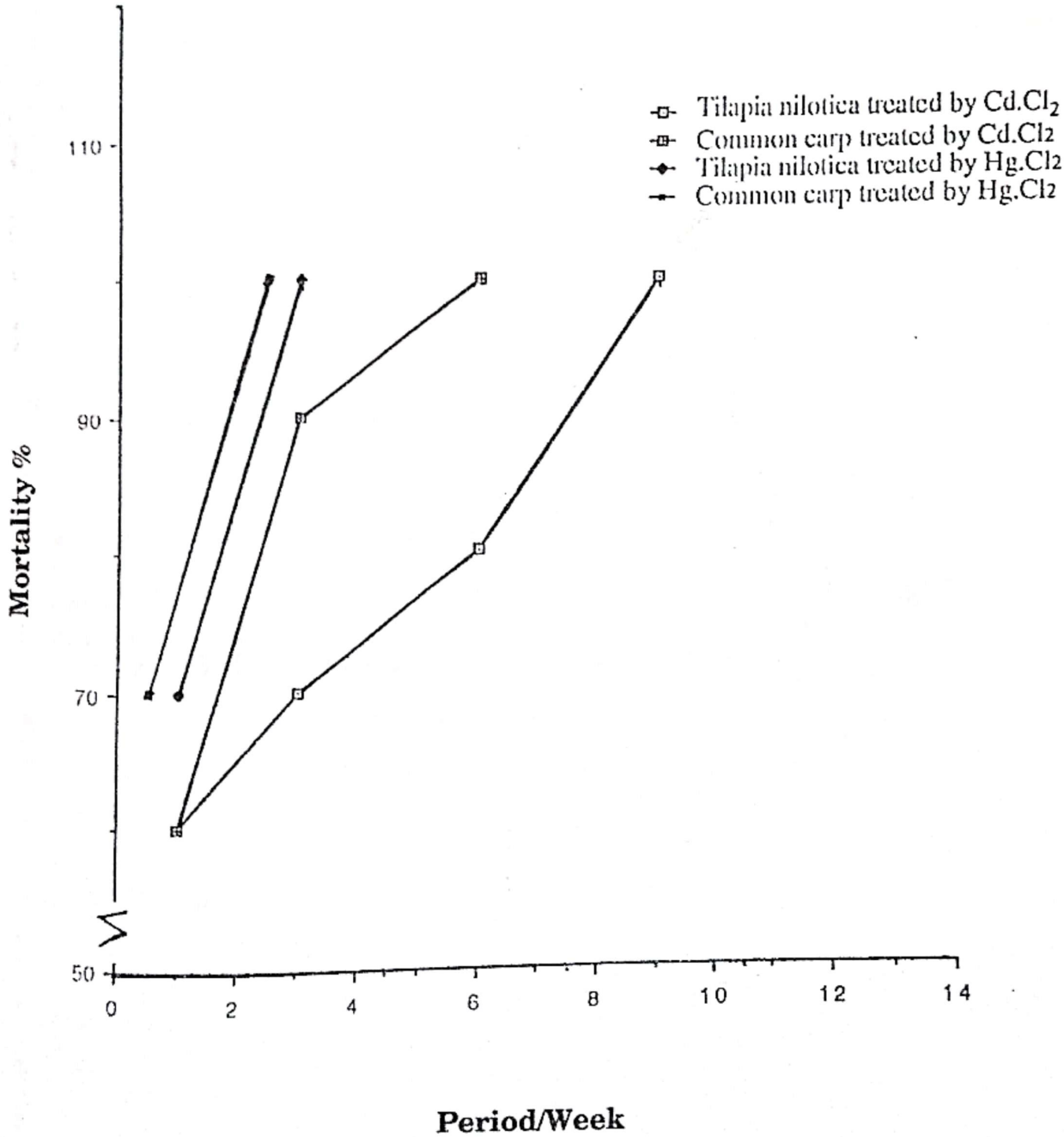

Fig. (1): Mortality rate in Tilapia nilotica and Common carp exposed to cadmium and mereuric chloride.

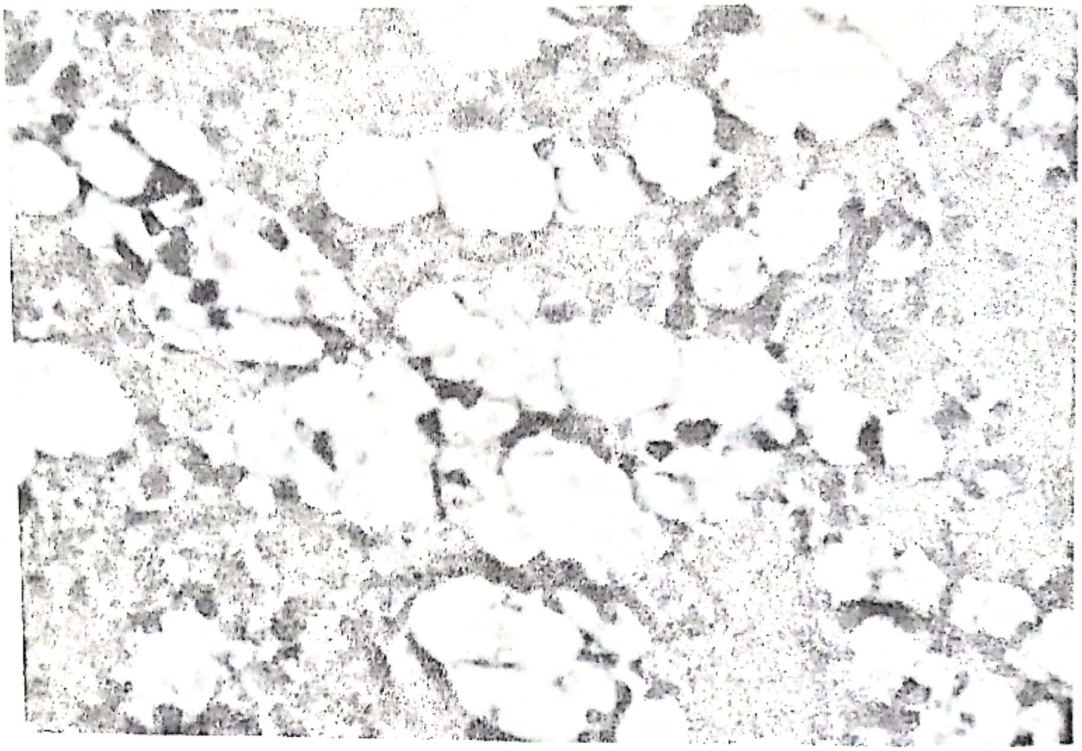

Fig. (2): Liver of fish treated with 14 PPM lead acetate showed granular and eosinophilic cystoplasm with centrally located nuclei. Fibroblast and lymphocytic proliferation in portal area. H & E X 600.

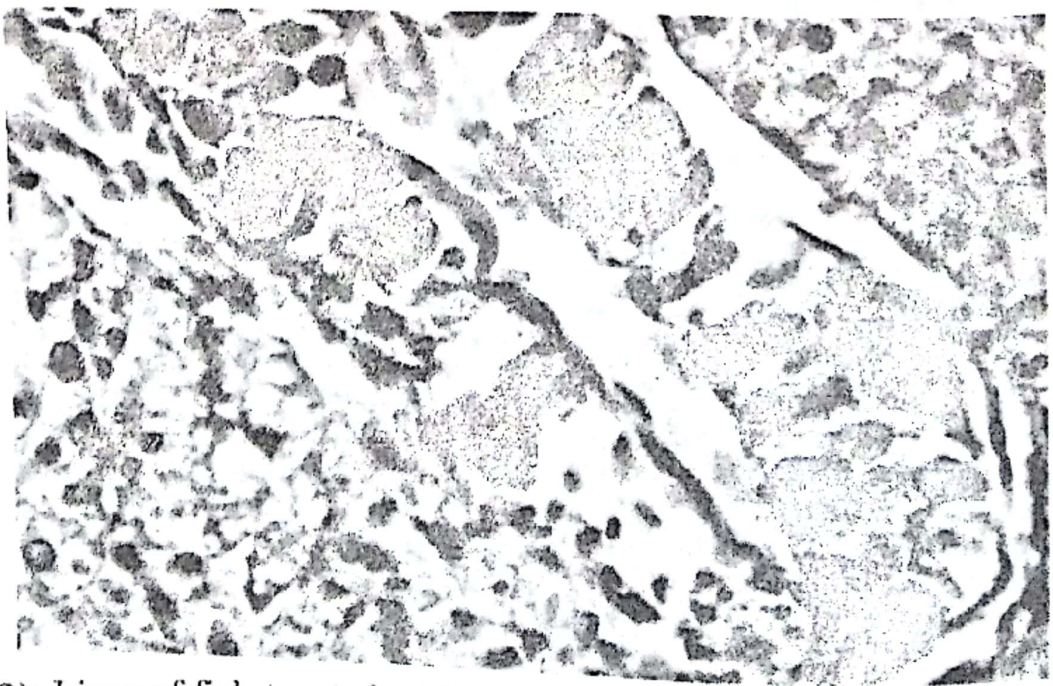

Fig. (3): Liver of fish treated with 14 PPM lead acetate showed necrosis of the hepatic cells around portal area. Focal haemorrhage among hepatic cells. H & E X 600.

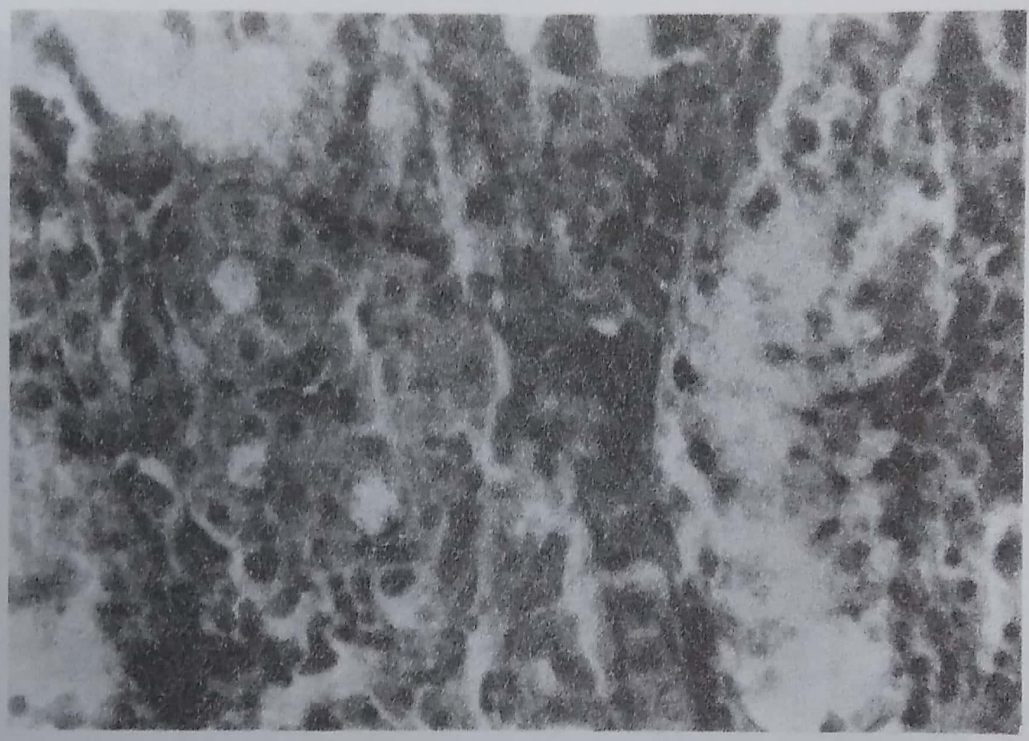

Fig. (4): Kidneys of fish treated with 12 PPM lead acetate showed focal coagulative necrosis of renal epithelium with mild lymphocytic inflammation among renal tubules. H & E X 600.

Fig. (5): Liver of fish treated with LC_{50} cadmium chloride showed hydropic degeneration, lymphocytic infiltration among hepatic cells and portal areas, focal haemorrhage were evident with congestion of blood vessels. H & E X 150.

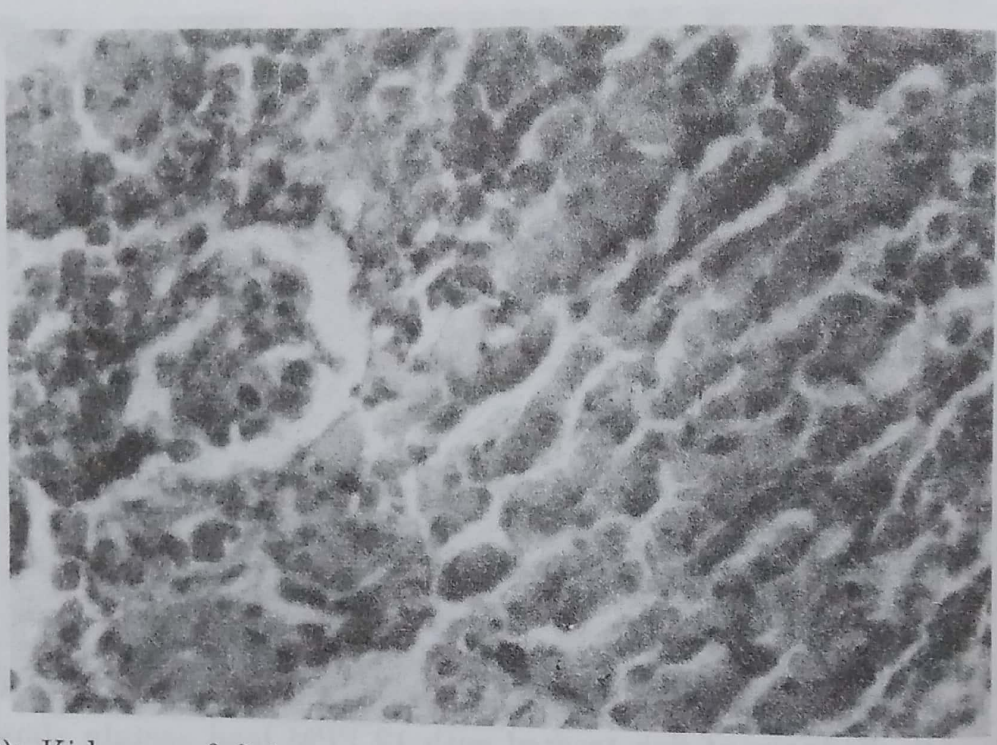

Fig. (6): Kidneys of fish treated with LC_{50} cadmium chloride showed coagulative necrosis of renal epithelium and infiltration of yellow pigment (hemosiderosis). H & E X 600.

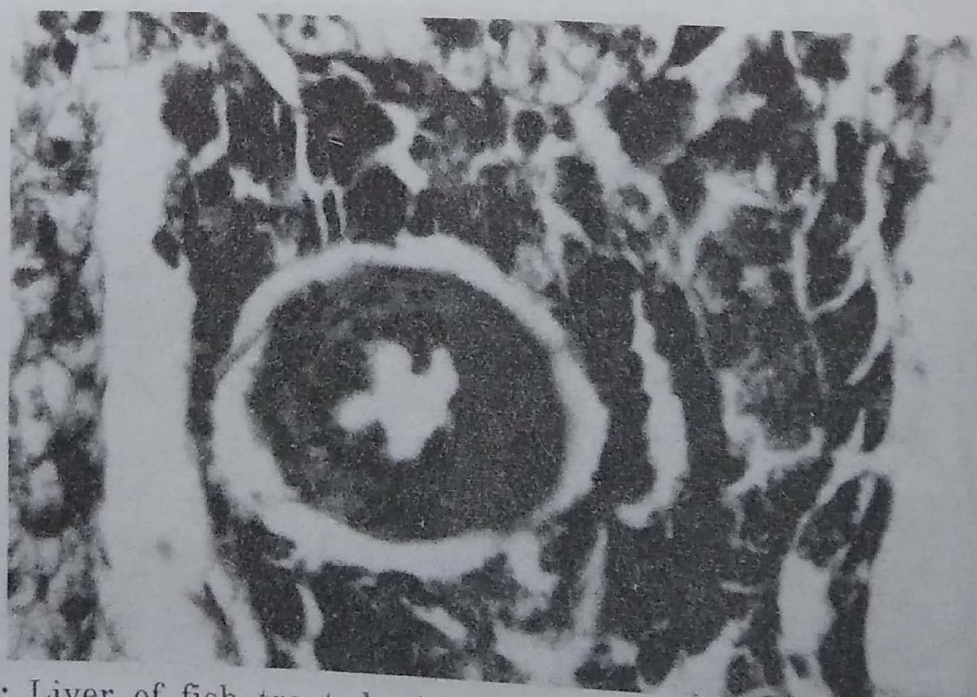

Fig. (7): Liver of fish treated with LC_{50} Mercuric chloride vacular degeneration with hyperplasia of reticuloendothelil cells. Some blood vessels showed hyalinized media with endotheliosis of their intima. H & E X 600.

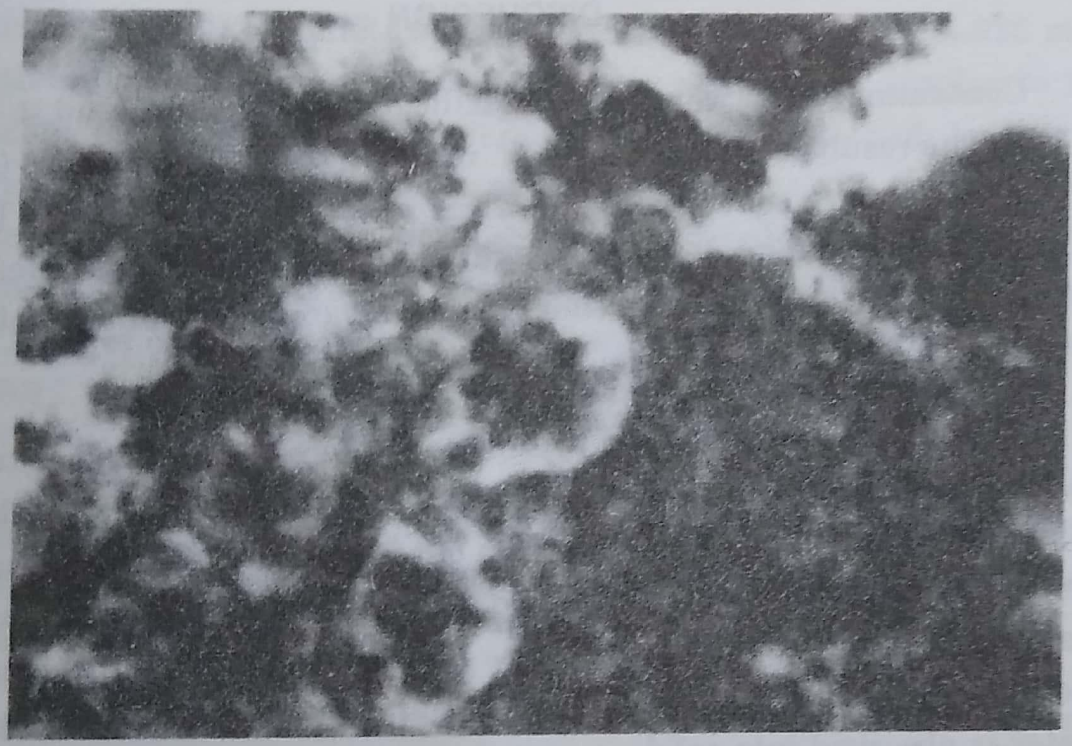

Fig. (8): Liver of fish treated with LC_{50} Mercuric chloride necrotic epithelium with more eosinophilic cytoplasm. H & E X 600.

Table (5): The analysis of variance of heavy metal residues (PPM) on Fish tissues compared with F.D.A. permissible limit.

Fish species	Heavy metal concentration (X ± S.E.)		
	Mercuric chloride	Cadmium chloride	Lead acetate
Tilapia nilotica	$1.46 \pm 0.04^{B*}$	$1.47 \pm 0.02^{B*}$	$3.70 \pm 0.08^{B*}$
Common carp	$2.85 \pm 0.29^{A*}$	$2.18 \pm 0.12^{A*}$	$4.95 \pm 0.17^{A*}$
F.D.A. (1976)	0.35^{C}	1.302^{C}	0.40^{C}
L.S.D.	0.26	0.46	0.44
F. value	78.09	19.40	91.52

* = Highly significant at $P \leq 0.01$. L.S.D. = Least significant difference.

Means within the same column, with different superscripts are significantly different.

DISCUSSION

Contamination of water with metallic compounds may lead to toxemia in fish. The results of direct mortality revealed that, common carp (Cyprinus carpio) was more sensitive to the three examined metals than Tilapias. This may be attributed to species susceptibility or to high tolerance of Tilapia to lower oxygen than Common carp. Meanwhile, metallic substances are able to block oxygen uptake in gills and liver mitochondria[15].

This result was supported by reports[2] which recorded that trout and salmon are more sensitive to metallic intoxication than other non salmonides with the possible exception of common carp which are sensitive. Mercury was more toxic to examined fish followed by cadmium, while the lead occupied the last situation. These may be attributed to the solubility of metallic compounds, where a substantial proportion of the cadmium in water is absorbed into solids or suspensions, but the soluble forms are not toxic to fish[26]. While lead is relatively insoluble, but its soluble forms such as lead acetate change into the insoluble lead sulfate in the gastrointestinal tract[8].

Also, the less toxic effect of cadmium and lead to fish may be attributed to the high protein content in the diet of examined fish where protein and Vit. C. reduce lead and cadmium absorption[16].

Although there was low mortality rate among fishs subjected to lead acetate, most fish appeared morbund and reduced activity till the end of the experiment. This toxicant is very dangerous in fish farms because it cannot be detected through the acute toxicity tests and may still have permanent adverse effects which directly interfere with the health or the ability to survive and reproduce.

Thus, the final results may be as damaging to the population as a concentration which kills many or all individuals quickly.

LC_{50} of mercuric and cadmium were 5.525 mg/L and 8.075 mg/L, respectively for Tilapia nilotica. While, it was 4.25 mg/L and 5.925 mg/L, respectively for common carp. These results were nearly similar to that previously recorded[21] and differed than that obtained by various authors[13,23]. These differences may be related to the environmental conditions under which the experiments were carried out. This finding was supported by previous work[9,19], which recorded a positive relation between 2 days LC_{50} of cadmium and water hardness, where the 2 days LC_{50} being about 0.12, 0.44 and 3.8 mg/L at water hardness 20,80 and 320 mg/L as $CaCO_3$, respectively.

The three examined metals have curmulative effect. The lower concentrations which do not kill fish after short periods were lethal after long periods of exposure.

The residual concentrations of mercuric chloride, cadmium chloride and lead acetate were 1.64 ± 0.04 PPM, 1.47 ± 0.02 PPM and 3.70 ± 0.010 PPM, respectively in tissues of Tialpia nilotica and 2,85 ± 0.29 PPM, 2.18 ± 0.12 PPM and 4.95 ± 0.17 PPM, respectively in tissues of common carp. These results exceeded the permissible limits indicated before[14] which considered fish tissues that contain more than 0.53 PPM, 1.30 PPM and 0.40 PPM of mercuric, cadmium and lead respectively as indication for contaminations.

The most common lesions observed were congestion and haemogenization of liver and kidneys in fish intoxicated with cadmium. While, haemorrhages in the intestinal tract were observed among fish intoxicated with mercury. Finally, fish intoxicated with lead showed paleness of gills and viscera. The microscopic changes of liver and kidneys of intoxicated fish by the three heavy metals revealed a hepato and renal toxic effect.

It could be concluded that, common carp is more sensitive to heavy metal pollutants.

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تأثير التسمم بالمعادن الثقيلة فى بعض أسماك المياه العذبه

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أجرى هذا البحث على ٧٨٠ سمكة نصفها من أصبوعيات البلطى النيلى والنصف الأخر من أصبعيات المبروك العادى . تم الحصول على تلك الأسماك من المفرخ السمكى بالعباسة بمحافظة الشرقية .

قسمت الأسماك إلى ثلاثة مجموعات رئيسية متساوية وكل مجموعة قسمت إلى ثلاثة عشرة مجموعة صغيرة كلا منها تحتوى على عشرة سمكات بلطى وعشرة سمكات مبروك . تم تعريض المجموعات الفرعية من ١ إلى ١٢ إلى تركيزات مختلفة من كلا من كلوريد الزئبق - كلورى الكادميوم وخلات الرصاص . تركت المجموعة الفرعية ١٣ فى كل مجموعة رئيسية كضوابط للتجارب إستمرت التجربة ١٢ أسبوع لدراسة التأثير السمى المزمن للمعادن المذكورة كملوثات لبيئة الأسماك وأوضحت نتائج البحث مايلى :

بتسجيل نسبة وتحديد التركيزات التى تؤدى الى نفوق ٥٠٪ من الأسماك فى ستة وتسعين ساعة LC_{50} وجد أن كلوريد الزئبق أكثر سمية لكل من النوعين من الأسماك يليه كلوريد الكادميوم أما خلات الرصاص فلم يحدث نفوق للأسماك خلال الفترة المذكورة . واظهرت أسماك المبروك حساسية للتلوث بالمعادن الثقلية أعلى منها فى البلطى حيث كانت التركيزات الجرعة المميتة للنصف لكل من كلوريد الزئبق وكلوريد الكادميوم على النحو التالى ٨ر٧٥ ملليجرام/لتر و ٥ر٥٢٥ ملليجرام/لتر على التوالى فى البلطى بينما ٥ر٩٢٥ ملليجرام/لتر ٤ر٤٢٥ ملليجرام/لتر على التوالى فى أسماك المبروك .

بدراسة الاعراض الإكلينيكية والصفة التشريحية والتغييرات الباثولوجية وجد أن المعادن الثلاثة تتشابه فى الأعراض العصبية والإضطرابات النفسية كما أوضحت الدراسة التأثير الكمى التراكمى لهذه المعادن .

بتحديد الكميات المختزنة من المعادن التقلية فى أنسجة الأسماك لكل من كلوريد الزئبق والكادميوم وخلات الرصاص وجد أنها تصل إلى ١ر٦٤ ± ٤ر. ملليجرام/كجم ، ١ر٤٧ ± ٢ر. ملليجرام/كجم ، ٣ر٧٠ ± ٨ر. ملليجرام/كجم على التوالى فى البلطى ، ٢ر٨٥ ± ٢٩ر. ، ٢ر١٨ ± ١٢ر ، ٤ر٩٥ ± ١٧ ملليجرام/كجم على التوالى فى المبروك . وهذه التركيزات أعلى من المسموح به فى هيئة .F.D.A.