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EFFECT OF EXPOSURE OF FRESH WATER CATFISH TO SOME HEAVY METALS

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ABSTRACT

Two hundreds and thirty catfish (Clarias lazera) weighing 200-250 gm each were obtained from abbasa fish farm were used in this investigation. LC_{50} of Copper Sulphate, lead acetate and Zinc sulphate was determined by exposure of the fish to different concentrations of the tested metals for 96 hours. It was 0.2, 0.1 and 0.1 ppm respectively. Determination of the residues of these heavy metals in the fish muscles and organs was studied after exposure of the fish to 0.02, 0.01 and 0.01 ppm (1/10 LC_{50}) of the tested metals respectively. Blood picture, liver and kidney functions and some biochemical parameters were also studied after exposure of the fish to the tested salts of heavy metals. It was found that liver contains the highest amount of copper (Cu) and lead (Pb) 20.1 & 12.7 ppm respectively while zinc (Zn) was concentrated in the kidney (7.1 ppm). The muscles contained Cu, Pb & Zn (3.6, 0.3 and 0.4 ppm) respectively lower amounts than the permissible limits.

RBCs count decreased by exposure of the fish to Cu, Pb and Zn (1.9, 1.95 and 1.5 million/ µl versus 2.4 for the control) respectively. Hemoglobin concentration and Packed Cell Volume (PCV) were also decreased. On the other hand total leucocytic count increased by the exposure of the fish to Cu and Pb (32.77 and 39.7 versus 27.3 10^3/µl), for the control. Exposure to Cu increased serum AST activity from 150 to 170 µ/L whereas serum ALT, AP, total proteins, albumin, total bilirubin, cholesterol did not significantly altered by exposure to tested metals. Exposure to Cu and Pb increased serum glucose level from 90 to 115 and 100 mg/dl respectively. Cu decreased the sodium, chloride, potassium and calcium (123, 112, 2.4 m.mol / L and 6.9 mg/dl versus 140, 123, 2.7 m. mol/L and 9.6 mg/dL versus 140, 123, 2.7 m.mol/L and 9.6 mg /dL) of the control respectively. On the contrary it increased blood urea nitrogen from 2.5 to 3.5 mg/dL. Exposure to lead and zinc did not significantly alter these parameters.

INTRODUCTION

Copper is an essential element and a part of about thirty enzymes and glycoproteins as catalase, cytochrome oxidase, peroxidase, dopamine, dopamine hydroxylase and others. Since oxidative enzymes requires copper to promotes iron absorption from gastrointestinal tract, thus it is necessary for hemoglobin synthesis. Because copper is essential for many functions in the vertebrate body, therefore, regulation of its level is necessary [(1)].

Also lead is a bone-seaking element, it is processed along with Ca^{2+} because of their chemical resemblance. However, tissues other than bone are storage sites for lead in fish. Lead salts are deposited as a layer in scales, fin rays, vertebrae, and opercula which are areas of calcification. More calcium is retained than lead because lead is processed as a trace contaminant of Ca[(2)].

Zinc is found in the vertebrate body second to iron in its quantity. It is an important element for many functions. It is a cofactor for a number of enzymes, including carbonic anhydrase, carboxy peptidase, phosphatases and glutamate dehydrogenase [(3)].

Particularly with heavy metals, pollution of aquatic environment, is one of the serious problems that create deleterious effects for fish and its consumers. Some heavy metals, such as lead, mercury and cadmium, are not essential for organisms, but induce toxic effects to fish and mammals, including man. The toxic effect of non-essential heavy metals have been recorded[(4)]. Other heavy metals such as copper (Cu), Zinc (Zn), iron (Fe) manganese (Mn), are essential for metabolic activities of living organisms. However, less is known about toxic effects of essential heavy metals on fish, although fish show toxic effects when exposed to higher levels than normal [(4)].

The purpose of the present work was thus initiated to throw a light on Cu, Zn and Pb residues in fish tissues and the determination of LC_{50} due to exposure for 96 hours of these elements. Effects of Cu, Zn and Pb exposure on haematological picture and serum biochemical parameters of fish were also evaluated.

MATERIAL AND METHODS

Drugs :
Copper sulphate (Sigma Chemical Co USA).

Lead acetate and zinc sulphate (Merck Sharp and Dolme, Germany).

Fish:- 230 catfish, *(Clarias lazera)*, weighing 250g each, were obtained from El-Abbasa fish farm, Sharkia governorate, Egypt.

LC_{50} of copper sulphate, zinc sulphate and lead acetate was determined by exposing fish to 0.03, 0.06, 0.1, 0.2 and 0.5 mg /L of the tested metals for 96 hours. Ten catfish were used for each concentration of each chemical. Dead fish were removed daily and mortality% was calculated [(5)].

Detection of residues: Concentration of copper, lead and zinc in fish tissues was determined using atomic absorption spectrophotometer (Pye Unicum Sp.9)[6].

EXPERIMENTAL

Four groups of catfish each of ten were used. The first group was left as a control. The 2nd, 3rd and 4th groups were exposed for 96 hours to 0.02 ppm of copper sulphate, 0.01 ppm lead acetate and 0.01 ppm zinc sulphate (1/10 LC50) respectively. Blood samples were collected by cutting the tail vein. A small amount was used as a whole blood for haematological picture using EDTA as an anticoagulant. The remaining amount of blood was placed in clean centrifuge tubes and left to clot, then centrifugated at 3000 rpm for 15 min. Serum was separated for estimation of some biochemical parameters. Muscle, liver and kidneys were obtained from each fish for estimation of copper, lead and zinc residues. The samples were kept in plastic marked containers at –18 °C in freezer for assaying residues.

Haematological studies:

Total red and white blood cells, blood haemoglobin, packed cell volume (PCV) were estimated [7-9].

The values of MCV, MCH and MCHC were calculated [10].

Serum biochemical parameters:

The following parameters were estimated using commercial kits supplied by Sigma. The activities of serum Aspartate aminotransferase (AST), alanine aminotransferase (ALT)[11]. Alkaline phosphatase (AP) [12], total protein (TP) [13], albumin (Alb) [14], were estimated.

Globulin and total bilirubin (TB)[15], glucose,[16] cholesterol[17] blood urea nitrogen (BUN),[18], creatinine (Creat)[19], uric acid [20] sodium (Na) potassium (K) [21] and phosphorus[22]. Chloride (Cl)[23]. Calcium (Ca)[24] and magnesium (Mg)[25] were analysed.

Statistical analysis: Data were statistically analysed using Student "t" test [26].

RESULTS AND DISCUSSION

The heavy metals pollution should be regarded carefully as it forms a great public health hazard for both human and animal. Pollution with heavy metals is a problem of magnitude of ecological significance and they accumulate in aquatic living beings [27].

Copper, lead and zinc were highly cummulative in liver and kidney, while the muscle tissue took up the smallest quantities. Liver and kidney are considered reliable bioindicators of metals exposure. Lead and zinc are toxic metals for their high affinity to the tissues and their cummulative posisoning effect, while copper is an essential element for all living beings [28].

Copper was more cummulative in the liver than kidney and muscle since the liver is the main organ of storage and metabolism for copper[29]. The permissible limit of copper and lead in sea foods fish intended by Egyptian General Authority of Standerdization and Quality Control[30], FAO & WHO [31] is 20 and 0.5 ppm. The muscle is the most commonly consumed protein of fish. The international permissible limit of zinc ranged from 5o-150 ppm as mentioned by National Health Medical Research Council NHMRC[32]. Our results showed that the concentrations of copper, lead and zinc in the muscle tissues were 3.6, 0.4 and 0.3 ppm.

The concentrations did not exceed the permissible limits (20, 0.5 & 50-150 ppm respectively). Our results on fish also coordinated with those reported before [33], who exposed juvenile catfish, (Ictalarus punctatus) to copper, lead and zinc at a concentration of 0.2, 0.1 and 0.1 ppm respectively.

Fish haematology is used for diagnosis of abnormal functioning of physiological mechanisms in fish. In the present study, copper, lead and zinc exposure resulted in anaemia. White blood cells in channel catfish, play a major role in the defense mechanism. Therefore, increase of total leucocytic count due to lead, copper and zinc exposure might be possibly attributed to stimulation of leucopoiosis resulting from tissue destruction[34]. Elevated levels of AST, ALT and AP activities could be interpreted to minimal hepatic injury. Elevations of these enzymes after exposure to Cu, Pb and Zn in raibow trout were previously recorded [35].

The decline in total serum proteins concentration may be due to its renal excretion, impaired protein synthesis and /or liver disorder caused by copper[36]. The hypoglobulinaemia observed in our study may be the result of liver dysfunction[37]. The total bilirubin level did not show any significant difference among the different concentrations used of Cu, Pb and Zn exposure.

This result is not compatible with that obtained before[38], who reported an elevated level of total bilirubin in Indian catfish. He attributed this elevation to liver dysfunction and or destruction of erythrocytes. The pathological condition of fish resulting from exposure to these levels of heavy metals did not reach to the level of haemolytic anaemia and total bilirubin level was not elevated.

Glucose level was elevated from 90 to 100 and 115 mg/dl as a result of exposure to lead and copper respectively. This may be attributed to either reduction of hepatic glycolytic enzymes and insulin hormone or stimulation of glucogenesis. Similar results were reported before[39], who reported elevated levels of glucose in fish exposed to heavy metals.

Elevation of blood urea nitrogen (BUN) was reported in this study as a result of exposure to copper. Urea in fish is produced by liver, it is excreted primarily by the gills rather than the kidney[35]. The elevation of BUN may be attributed to gill dysfunction. Serum uric acid concentration did not show any significant difference in comparison with the control.

Uric acid is formed by fish from exogenous and endogenous purines. It is converted in the liver to urea for excretion by the gills[35]. Normal value of uric acid in this work may be attributed to liver dysfunction which was not severe enough to alter the uric acid level in the blood. Our present work showed a decrease in the serum concentration of Na, K, Cl and Ca due to copper exposure.

Hyponatraemia, hypokalemia, hypochloremia and hypocalcemia were recorded in rainbow trout and tilapia[37]. Whereas, phosphorus and magnesium levels were not significantly altered by the exposure of the fish to Cu, Pb and Zn. This result is not in accordance with those previously reported[38], they recorded an increased serum magnesium level in rainbow trout intoxicated by Cu, Pb and Zn. This might be due to the difference in the concentrations used or the period of exposure or species variations.

It could be concluded that the residues of Cu, Pb and Zn in the fish muscle were below the permissible limits after 96 hours of exposure but these heavy metals had some deleterious effects on fish.

On exposure for longer duration, the problem of residues will appear with severe economic loss due to the toxic effect of the heavy metals on fish farming.

Table (1) : Mortality rate of channel catfish *(Clarias lazera)* exposed to different concentrations of copper, lead and Zinc for 96 hours at 23 °C (water hardness 55 mg /L) n = 10

Concentration (ppm)	Copper (Cu)		Lead (Pb)		Zinc (Zn)	
	No. of deaths	Mortality %	No of deaths	Mortality %	No of deaths	Mortality %
0.03	1	10	1	10	2	20
0.06	2	20	2	20	2	20
0.1	3	30	6	60	4	40
0.2	6	60	8	80	4	40
0.5	8	80	9	90	6	60

Table (2) : Concentration of copper, lead and zinc in ppm in tissues of catfish *(Clarias lazera)* exposed to 0.02, 0.01 and 0.01 ppm respectively for 96 hours. (Mean ± S.E.) n = 10

Heavy Metals	Tissues		
	Liver	Kidney	Muscle
Copper	20.1 ± 2.9	7.6 ± 1.7	3.6 ± 0.7
Lead	12.7± 1.9	2.4 ± 0.9	0.4 ± 0.1
Zinc	5.9 ± 0.9	7.1 ± 1.0	0.3 ± 0.1

Table (3) : Haematological picture of channel catfish *(Clarias lazera)* exposed to copper, lead and zinc (0.02, 0.01 and 0.01 ppm) respectively. (Mean ± S.E.) n = 10

Concentration ppm	RBCs Mill/μl	Hb g/dL	PCV %	MCV DL	MCH Pg	MCHC %	TLC 10^3/μl
Control	2.4 ±0.2	8.7 ±0.4	25.4 ±1.3	102.01±1.7	30.9 ±1.7	33.4 ±1.3	27.3±2.4*
Copper (0.02)	1.9 ±0.2*	5.5 ±0.3*	18.1± 0.5*	99.1 ±1.8	30.9± 1.8	29.7 ±1.4	32.7±2.3*
Lead (0.01)	1.95±1.6*	4.9 ±0.2	16.5± 1.0*	100 ± 1.9	31.9 ±1.9	30.4 ±1.4	39.7±2.3*
Zinc (0.01)	1.5 ±0.2*	5.9 ±0.4	15.1± 1.2*	101.2±2.1	34.3± 2.4	33.9± 1.1	29.5 ±1.5

* Significant at $P < 0.05$

Table (4) : Liver function tests of channel catfish *(Clarias lazera)* exposed to Copper, Lead and Zinc (0.02, 0.01 and 0.01 ppm) respectively for 96 hours. (Mean ± S.E) n = 10

Chemical concentration ppm	AST μ/L	ALT μ/L	AP μ/L	TP g/dL	Alb g/dL	Globulin g/dL	T.B mg/dL	Glucose mg/dL	Cholesterol mg/dL
Control	150 ±5.2	79 ±2.9	17.1±1.7	2.0 ±0.1	0.6 ±0.08	2.01± 0.01	0.5 ±0.03	90 ±3.9	230 ± 5.9
Copper (0.02)	170±3.5*	90 ±4.5	20.1± 1.4	2.0 ±0.11	0.5 ±0.03	1.9 ±0.1*	0.6 ±0.04	115± 5.2*	240 ±6.7
Lead (0.01)	160 ±3.5	85 ±2.7	19.2 ±1.6	2.1± 0.01	0.4±0.03	1.9±0.08*	0.5 ±0.03	100± 4.0*	239 ±5.7
Zinc (0.01)	159±2.1*	80 ±3.9	17.0 ±1.4	2.4 ±0.07	0.7 ±0.05	2.0 ±0.07	0.7 ±0.04	95 ±2.6	230 ±4.9

* Significant at $P < 0.0.5$

Table (5) : Some kidney function tests and biochemical parameters of channel catfish *(Claries lazera)* exposed to copper, l and zinc (0.02, 0.01 & 0.01 ppm) respectively for 96 hours. (Mean ± S.E) n = 10

Chemical concentration ppm	Creatinine mg/dL	Uric acid Mg/dL	BUN mg/dL	Na mmol/L	CL mmol/L	K mmol/L	Ca mg/dL	P mg/dL	Mg mg/dl
Control	0.6 ± 0.1	1.5 ± 0.08	2.5 ± 0.18	140 ± 1.7	123 ± 3.1	2.7 ± 0.1	9.6 ± 0.5	12.5 ± 0.5	4.5 ± 0
Copper (0.02)	0.6 ± 0.10	1.4 ± 0.09	3.5 ± 0.2*	123± 2.9*	112± 1.5*	2.4 ± 0.1*	6.9 ± 0.3*	11.9 ± 0.5	4.0 ± 0
Lead (0.01)	0.6 ± 0.04	1.3 ± 0.1	3.1 ± 0.2	130 ± 2.6	120 ± 4.1	2.6 ± 0.2	8.6 ± 0.5	11.7 ± 0.4	4.0 ± 0
Zinc (0.01)	0.5 ± 0.03	1.1 ± 0.1	2.8 ± 0.6	136 ± 3.3	125 ± 3.2	2.5 ± 0.2	8.9 ± 0.4	11.8 ± 0.8	3.9 ± 0

• Significant at P < 0.0.5

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تأثير تعرض أسماك القرموط لبعض المعادن الثقيلة

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يعتبر النحاس والرصاص والزنك من العناصر الغذائية التى توجد فى الصخور والتربة والهواء والماء وأنسجة الحيوانات .

تم دراسة الجرعة السمية النصف مميتة لمدة ٩٦ ساعة لعنصر النحاس والرصاص والزنك لقرموط كلارياس لازيرا وتم دراسة التأثير السمى على صورة الدم ونسبة المركبات الكيميائية فى المصل .

كانت الجرعة هى ١/١٠ الجرعة السمية النصف مميتة لمدة ٩٦ ساعة للنحاس على هيئة كبريتات النحاس هى ٠٫٠٢ جزء فى المليون عند حرارة ٢٣°م وعسر الماء ٥٥ مجم /لتر . وللرصاص على هيئة كبريتات الرصاص هى ٠٫٠١ جزء فى المليون ، الزنك على هيئة سلفات الزنك هى ٠٫٠١ جزء فى المليون.

وخلاصة النتائج أن التسمم بالرصاص والنحاس والزنك نتج عنها أنيميا مع عدم تغيير الهيموجلوبين وزيادة فى معدل الاسبرتات أمينو ترانسفيراز وسكر الدم والبولينا بينما وجد نقص معنوى فى نسبة البروتين الكلى والجلوبيولين والصوديوم والكلوريد والبوتاسيوم . بينما معدلات الألانين أمينو ترانسفيراز والفوسفاتيز القاعدى والزلال والكوليستيرول وحمض البوليك والكرياتينين والفوسفور والماغنسيوم لم يتأثروا معنويا عند مقارنتهم بالمجموعات الضابطة .

وعند دراسة البقايا وجد تراكم النحاس والرصاص والزنك بمعدلات عالية جدا فى الكبد والكلى عنها فى العضلات .

من هذه الدراسة يتضح مدى السمية التى يحدثها التعرض للنحاس والرصاص والزنك بالنسبة لسمك القرموط ويمكن تشخيص التسمم فى الأسماك من خلال تغيير صورة الدم والتغيرات البيوكيميائية فى المعمل.

ولقد تم مناقشة الآثار السمية الضارة لمتبقيات النحاس والرصاص والزنك بأنسجتها وتأثيرها على صحة المستهلك .