PHYSIOLOGICAL EFFECTS OF LEAD ,CADMIUM AND THEIR MIXTURE ON SOME ORGANS OF *Alburnus Mossulensis* FISH

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ABSTRACT

***Alburnus mossulensis* has been exposed to lead and cadmium for (96)h in order to determination lethal concentration 50 (LC_{50}) value. A total of 140 fish used in this experiment divided into two groups each group contain 70 fishes .The first group exposure to $pbcl_2$ while the second group exposure to $cdcl_2$. Method of Finney Probit Analysis used to analysis data. LC_{50} value for $PbCl_2$ and $CdCl_2$ was (57.8 and 29.6) mg/l respectively. Then used about 200 fishes divided into 4 groups, the first group exposure to $1/2LC_{50}$ of $PbCl_2$,the second group exposure to $1/2LC_{50}$ $CdCl_2$ while the third groups contained ($1/2LC_{50}$ $PbCl_2$+$1/2LC_{50}$ of $CdCl_2$) as so as control group for peroids (1,4,7 and 14) days to determination level of antioxidants Glutathione (GSH) and the level of lipid peroxidation (Malondialdehyde MDA) . The testes were carried out as three replications, lead ,cadmium and their mixture induced oxidative stress . The results of this study explained significant decrease of the level Glutathione and significant increase (Tukey test, $P<0.05$) of the level of Malondialdehye compared with control in all treatments . It is possible to conclude that lead and Cadmium have cytotoxic effects, Fish mortality rates increase with increasing concentrations used and increasing exposure periods.**

Keywords: LC_{50} , toxicity, antioxidants, oxidative stress ,heavy metal.

التاثيرات الفسلجية للرصاص والكادميوم ومزيجهما في بعض اعضاء اسماك *Alburnus mossulensis*

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المستخلص

عرضت اسماك *Alburnus mossulensis* الى الرصاص والى الكادميوم لمدة 96 ساعة وذلك من اجل تحديد التركيز القاتل لنصف العدد من الاسماك. استخدمت 140 سمكة وقسمت الى مجموعتين، كل مجموعة احتوت على 70 سمكة. عوملت اسماك المجموعة الاولى بالرصاص ،بينما عوملت اسماك المجموعة الثانية بالكادميوم. استخدمت طريقة Finney Probit Analysis من اجل تحليل البيانات احصائيا. اوضحت النتائج ان قيمة التركيز المميت الوسطي لكل من الرصاص والكادميوم (57.8 و29.6) ملغم/لتر على التوالي. كما استخدمت 200 سمكة مقسمة الى 4 مجاميع: عوملت اسماك المجموعة الاولى بتركيز يعادل نصف قيمة التركز المميت الوسطي للرصاص، في حين عوملت اسماك المجموعة الثانية بتركيز يعادل نصف قيمة التركيز المميت الوسطي للكادميوم، اما اسماك المجموعة الثالثة فقد عرضت الى مزيج من كلا من (نصف التركيز المميت الوسطي للرصاص مضاف اليه نصف التركيز المميت الوسطي للكادميوم) فضلا عن مجموعة السيطرة وللفترات(1و4 و7و14) يوما، وبواقع 3 مكررات، لتحديد مستوى مضادات الاكسدة وتزنخ الدهن(المالون ثنائي الالديهايد). اوضحت النتائج وجود انخفاضا معنويا في مستوى الكلوتاثايون وارتفاعا في مستوى المالون ثنائي الالديهايد بالمقارنة مع مجموعة السيطرة وعند مستوى احتمالية ($P<0.05$)في جميع المعاملات.نستنتج ان للرصاص والكادميوم تأثيرات سمية خلوية، وان معدلات نفوق الاسماك تزداد بزيادة التراكيز المستخدمة وزيادة فترات التعريض.

الكلمات المفتاحية: التركيز القاتل لنصف العدد من الاسماك، السمية، مضادات الاكسدة، الكرب التاكسدي، المعادن الثقيلة.
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INTRODUCTION

The important environmental heavy metal contaminants are lead and cadmium that has widespread distribution. Human and animal exposure were due to both natural and anthropogenic causes, such mining, smelting, and other chemical products. Most times, these contaminants are co pollutants, resulting in mutual damage to living creatures and complementary harmful effects on health (30). Lead has been one of the nutrient-free metals that causes contamination in the aquatic environment (4). Lead induces oxidative stress through a direct effect on membranes (28). The increased ROS level coincides with inhibition of Na +, K + -ATPase, which disrupts the regulation of osmosis as well as causes tissue damage (20).Cadmium becomes considered to be a significant industrial source of pollution in the aqueous medium and a serious threat to aquatic species, mainly fish (3). Ecological exposure to cadmium can increase the occurrence of the element being ingested, causing damage to the organ (25). Fish play a role as a physiological indicator of the quality of water (2) . So with their affinity to the accumulation of materials in their muscle tissue (32), they cause change in physical, biochemical as well as hereditary parameters in their body(16). Penetrating fish through direct water absorption via their gills and skin, or via ingestion of contaminated food (6). Cadmium been regarded to be among the greatest harmful pollutants of contaminated water which cause toxicity to every ecological component (27). Cadmium doesn't really produce additional Reactive oxygen species (ROS), but also can change GSH levels. Alterations in Glutathion (GSH) may give rise to lipid peroxidation (LPO) of the cell membrane. Except for cadmium, lead can cause LPO and this is demonstrated by the outcome of several scientists (10). The aim of study determination of LC_{50} for Pb and Cd and then Studying effects of cadmium, lead and their mixture on level of effectiveness of GSH and MDA.

MATERIALS AND METHODS

Collecting fish samples: The fish used during this study were collected from the waters of the Shalalat area in north of Mosul during the period from mid-October . A manual fishing net was used in the process of collecting fish, after which the fish were transported to the laboratory by a plastic basin in which water from the same aquatic environment was placed in it.

Specimens Preparation

Fish were placed in a glass tank containing chlorine-free water in addition to the water of the natural fish environment. The aquarium were provided with devices. Aeration and left for about two weeks for acclimatization, use Chinese-made commercial food.

Preparation of stock solution

The standard stock solution for lead was prepared at a concentration of 100 mg / l by dissolving 0.671 g of $PbCl_2$ with a quantity of distilled water and then the volume was completed to 5l , from which the concentrations (0,10, 30, 50, 70 and 90) mg/l used in the concentration determination experiment were prepared. As for cadmium, (0.812) g of $CdCl_2$ was dissolved in a quantity of distilled water and then the volume was completed to 5000 ml, from which the concentrations (0.10, 20, 40, 60 and 80) mg/l used in the concentration determination experiment were prepared.

Determination of LC_{50} of lead and cadmium

The lethal concentration was determined for half the number of fish during the acute exposure period 4 days, by exposing the fish to lead concentrations (0, 10, 30, 50, 70, 90 and 100) mg / l. By placing 10 fish in each aquarium, the number of deaths was recorded within 4 days. The same applies to cadmium, as the concentrations (0, 10, 20, 40, 60, 80 and100) mg / liter were used during the acute exposure period (4 days). In the current experiment, 1/2 LC_{50} for lead , 1/2LC_{50} for cadmium and their mixtures were used.

Statical Analysis

Concentration was determined for LC_{50} using a probit analysis software system, version 1.5 (12) The program used Graph padprism 5 to analyze the data statistically. The arithmetic mean was compared using the ANOVA One way program by means of a linear analysis of variance of the variables in the criteria under study for each group separately, in addition to the control group, using (Tukey's test) at a significant level ($p < 0.05$) To compare the effect of lead on the members under study within different exposure periods, and letters have been placed in the tables to denote the

significant differences (29). Similar letters demonstrate statistically significant differences in treatment and regulation. The values with different letters in the same row differ significantly.

Experimental design

The fish were divided into 4 groups: The first group: Control is represented by fish in aquarium containing dechlorinated water. The second group: is represented by fish in aquarium containing an aqueous solution of lead at a concentration of 28 mg /l (1 /2L C_{50}) for a period of 96 hours. The third group: is represented by fish in aquarium containing an aqueous solution of $CdCl_2$ at a concentration of 15 mg /l (1/2LC_{50}) for a period of 96 hours. Fourth group: is represented by fish in aquarium containing an aqueous solution of a mixture of lead (28 mg / l) and cadmium (15 mg / l).

RESULTS AND DISSCUSTION

Serious $PbCl_2$ toxicity demonstrates that the mortality rate dependent on the concentration of heavy metal lead, whereas the number of deaths is much absent from of the control (Tab. 1 and 2).

Table 1. Relationship between lead concentration and mortality rate of *A. mossulensis* fish exposed for a period of (96) hours.

Conc. \	Number	No. of mortality	Observed Proportion Responding	Proportion Responding Adjusted for Controls	Predicted Proportion Responding
10	10	0	0.0000	0.0000	0.0149
30	10	3	0.3000	0.3000	0.2083
50	10	4	0.4000	0.4000	0.4284
70	10	5	0.5000	0.5000	0.5932
90	10	6	0.6000	0.6000	0.7077
100	10	9	0.9000	0.9000	0.7508

Table 2. The upper and lower limits and the LC (LC1-99) of *A. mossulensis* fish exposed to lead for a period of (96) hours

Lethal Concentration (1-99)	Exposure concentration	Lower Limits	Upper Limits
LC_1	8.824	0.800	18.104
LC_5	15.306	2.703	26.212
LC_{10}	20.531	5.143	32.121
LC_{15}	25.032	7.901	37.020
LC_{50}	57.854	40.294	81.202
LC_{85}	133.714	91.654	399.350
LC_{90}	163.026	105.784	612.623
LC_{95}	218.675	129.784	1164.132
LC_{99}	379.328	188.095	3929.443

Severe $CdCl_2$ poisoning demonstrates that the death rate is dependent on the concentration of heavy metal lead whereas the mortality rate is virtually absent from the control (Tab. 3 and 4).

Table 3. Relationship between cadmium concentration and mortality rate of *A. mossulensis* exposed fish for a period of (96) hours

Conc.	Each number Detected	Number Resp.	Observed Proportion Responding	Proportion Responding Adjusted for Controls	Predicted Proportion Responding
10	10	0	0.0000	0.0000	0.0212
20	10	4	0.4000	0.4000	0.2692
40	10	6	0.6000	0.6000	0.7879
60	10	10	1.0000	1.0000	0.9481
80	10	10	1.0000	1.0000	0.9866
100	10	10	1.0000	1.0000	0.9962

Table 4. Upper and lower limits and LC (LC1-99) of *A. mossulensis* exposed to cadmium for a period of (96) hours

Lethal Concentration (1-99)	Exposure Concentration	Lower Limits	Upper Limits
LC_1	8.646	3.232	13.350
LC_5	12.074	5.648	17.175
LC_{10}	14.4 27	7.572	19.733
LC_{15}	16. 270	9.201	21.737
LC_{50}	29.638	19.780	34.672
LC_{85}	44.933	35.015	67.160
LC_{90}	50.671	39.012	80.683
LC_{95}	60.546	45.332	106.949
LC_{99}	84.551	58.973	184.832

The difference in the value of LC_{50} in the studied fish varies with species and this may be due to the difference in the age, size, sex, feeding quality of fish, and the conditions of the experiment (23). Acute toxicity also differs due to different water quality such as hardness (8) and different pH and water temperature (21). The toxicity of minerals to fish also varies; Some minerals may be highly toxic and at low concentrations for species of fish, but they may be less toxic or non-toxic to other species of fish and when they are present in the same or even higher concentrations than when present in the ecosystem (14).

Antioxidant and lipid peroxidation level

The activity of antioxidant(GSH) in the various organs of *A. mossulensis* has decreased significantly ($p<0.005$) in the gills , brain, liver, intestine and muscles Compared to the control in all periods ,Also, Significant differences were observed between the control and Cd ,Pb and mixture groups. The decrease in the level of GSH in the group exposed to lead was greater than in the group exposed to cadmium , There was no significant difference in muscle compared to the other groups and to the control group . The lowest level of GSH was found in muscles in all periods. As for the effect of treatments, the lowest level were found in the mixture group, which differed significantly from other of treatments Table(5,6,7and 8).

Table 5. Level of GSH(Mean ±SE nmol/ml) in some organs of fish in 24 h

Organs	Con.	$Pbcl_2$	$Cdcl_2$	Mix	Effect of organs
Gills	1430 ± 10 A	1288 ±2.0 b	1300±10 bc	1346 ±2.0 D	1341 A
Brain	2026 ±7.0 E	1924 ±3 ef	2003±4 g	1806 ±6 H	1940 B
Liver	3361±4 I	3241 ±5 j	3266 ±10 jk	3248 ±12 Ljk	2467 C
Intestine	2385±5 M	2241 ±8 n	2293 ±3 o	2218 ±2 Np	2284 D
Muscles	976 ±1 Q	958 ±9 qrt	962±12 qrst	960 ±9 qrt	964 E
effect of treatment	2036 A	1947 B	1948 BC	1916 D	

Table 6. Level of GSH(Mean±SE) in some fish organs in 4 day

Organs	Con.	$Pbcl_2$	$Cdcl_2$	Mix	Effect of organs
Gills	1430 ± 10 A	1154 ±13 b	1178 ±18 c	1106 ±16 d	1217 A
Brain	2026± 23 E	1801 ±15 f	1895±10 g	1785 ±22 h	1877 B
Liver	3361 ±20 I	3029 ±15 j	3064±17 jk	3007 ±19 l	3115 C
Intestine	2385 ±9 M	2142 ±20 n	2159 ±27 no	2130 ±25 op	2204 D
Muscles	976 ±15 Q	930 ±13 qr	950 ±30 qrs	919 ±19 rst	948 E
effect of treatment	2036 A	1841 B	1819 BC	1789 D	

Table 7. Level of GSH (Mean±SE) in some organs of fish in 7 day

Organs	Con.	Pbcl2	Cdcl2	Mix	Effect of organs
Gills	1430 ±10 A	1123 ±20 b	1085 ±19 bc	1009 ±20 D	1161 A
Brain	2026±23 E	1740 ±18 f	1859±13 g	1644 ±12 H	1817 B
Liver	3361 ±20 I	2045 ±16 j	2195±11 k	1909 ±20 L	2378 C
Intestine	2385 ±9 M	2100 ±14 n	2134 ±19 no	2016 ±16 P	2158 D
Muscles	976 ±15 Q	898 ±17 r	900±12 rs	865 ±21 Rst	910 E
effect of treatment	2036 A	1635 B	1580 C	1489 D	

Table 8. Level of GSH(Mean ±SE) in organs of fish in14 days

Organs	Con.	Pbcl2	Cdcl2	Mix	Effect of organs
Gills	1430 ±10 A	996 ±18 b	1078±14 c	897 ±12 d	1100 A
Brain	2026 ±23 E	1530±20 f	1797 ±30 g	1004 ±12 h	1589 B
Liver	3361 ±20 I	1089 ±17 j	1216±16 k	984 ±11 l	1663 C
Intestine	2385 ±9 M	2078 ±14 n	2105 ±18 o	2006 ± 16 p	2144 D
Muscles	976±15 Q	814 ±16 r	824 ± 12 rs	816 ±13 rst	857 E
Effect of treatment	2036 A	1399 B	1307 C	1414 D	

The level of MDA significant increase in liver and intestine through(1 and 4) day Table(9 and 10), while it seen significant increase in all organs through in (7 and 14 days) Tables(11 and 12).The greatest higher level of MDA was found in brain and in mixture groups in all periods.

Table 9. Level of MDA(Mean ±SE) nmol/gm in some tissues of fish in 24h.

Organs	Con.	$Pbcl_2$	$Cdcl_2$	MIX	Effect of organs
Gills	127 ±25 A	131 ±15 ab	120 ±10 abc	145 ±5 Abd	130 A
Brain	221 ± 11 E	240 ±5 ef	225± 10 efg	249 ±16 Efh	234 B
Liver	195 ±16 I	228 ±8 j	210±7 jk	233 ±9 Jkl	217 C
Intestine	175 ±19 M	215 ±14 n	203 ± 12 on	225 ± 11 Np	205 D
Muscles	183 ±13 Q	198 ± 17 qr	180 ± 7 qrs	206 ± 12 St	192 E
Effect of treatment	180 A	202 B	188 AC	212 BD	

Table10. Level of MDA(Mean ±SE) nmol/gm in some tissues of fish in 4 days

Organs	Con.	Pbcl2	Cdcl2	MIX	Effect of organs
Gills	127 ±25 A	156± 14 b	132 ±11 abc	162 ±18 Bd	144 A
Brain	221 ±11 E	255±8 ef	238 ±12 efg	267 ± 11 Hf	245 B
Liver	195 ±16 I	248 ±24 j	225±16 kj	254 ± 13 Lkj	231 C
Intestine	175 ±19 M	235 ± 19 n	228 ±9 on	247 ±16 P	221 D
Muscles	183 ± 13 Q	225± 14 qr	200 ±20 qrs	229 ±23 qrst	209 E
Effect of treatment	180 A	224 B	204 C	232 D	

Table11. Level of MDA(Mean ±SE) nmol/gm in some tissues of fish in 7 days

Organs	Con.	Pbcl2	Cdcl2	MIX	Effect of organs
Gills	127±25 A	172 ±9 b	146 ±14 ac	180 ±16 Bd	156 A
Brain	221 ±11 E	284 ±8 f	249 ±14 eg	292 ±17 Hf	262 B
Liver	195 ±16 I	251 ±13 j	239 ± 19 k	270 ±25 L	239 C
Intestine	175 ±19 M	249 ± 24 n	240 ± 26 o	274 ± 23 P	234 D
Muscles	183 ± 13 Q	233 ± 18 rts±	227 ±16 s	240 ± 14 T	221 E
Effect of treatment	180 A	238 B	220 BC	251 BD	

Table 12. Level of MDA(Mean ±SE) nmol/gm in some tissues of fish in 14 days

Organs	Con.	$Pbcl_2$	$Cdcl_2$	MIX	Effect of organs
Gills	127±25 A	187 ±17 b	160 ± 21 bc	290±16 D	191 A
Brain	221 ± 11 E	298 ±22 f	268 ± 19 g	299 ± 15 H	272 B
Liver	195 ± 16 I	269 ± 27 j	246 ± 23 k	287± 26 L	249 C
Intestine	175 ± 19 M	278 ±14 n	257 ±11 o	288 ± 20 P	247 CD
Muscles	183 ± 13 Q	243 ±10 r	239 ± 19 s	256 ±17 Tr	230 E
Effect of treatment	180 A	255 B	234 BC	284 D	

This study agree with Thi et al.(31)who observed significant increase of the level of MDA in brain of fish exposure to lead . Changes in GSH, and MDA were used as indicators of cadmium and lead toxic effects in fish(18). The results of the present study agree with the findings of Elarabany and Bahnasawy (9) whom they studied the effect of lead and cadmium on *Clarias gariepinus* and observed a decrease in the level of GSH in fish gills. It also agrees with Alfakheri et al (1) finding of low GSH in the catfish serum *Clarias gariepinus*. It also agree with Jing et al. (19) who found, in terms of a decrease in the level of glutathione in the liver and gills of *Boleophthalmus pectinirostris* after exposured to Pb and Cd. Heavy metals deplete antioxidants (7). Heavy metals impedes the activity of antioxidant (13). Oxidative stress begins through a decrease in the defense system of the antioxidants, and the decrease in GSH in fish reflects the ability of heavy metals (lead and cadmium) to bind to a covalent bond with a group of sulfhydryl group of several antioxidant enzymes, which causes inactivation of these enzymes and renders them susceptible to oxidation by free radicals (24) The presence of Glutathione S-Transferase (GST) in the cytosol of most cells stimulates the binding of GSH to foreign substances in xenobiotic biology. By forming a pinhole Thio - ether between the thiol group of GSH glutathione and substance for detoxification purpose (11). Glutathione is an antioxidant rich in Sulfhydryl group, which gives it a distinctive electron-giving property and thus gives an electron to active oxygen species / free radicals which easily oxidizes GSSG. This rapid use of both GST and GSH leads to a decrease in their level (17). Glutathione binds to the metal lead. Thus, it works to protect cells from their negative effects(15). The results of the current study are in agreement by Thi et al. (31), as they found that when exposure to Zebra fish with the chronic effect of lead causes a significant increase in the level of MDA in the brain, the results of the current study also agree with what researchers Elarabany and Bahnasawy (9)whom found When exposed to $Pbcl_2$, *Clarias gariepinus* raised MDA in the liver and gills. The results of the current study are in agreement with the recent study conducted by Thi et al. (31), as they found that when exposure to Zebra fish with the chronic effect of lead causes a significant increase in the

level of maloonaldehyde in the brain. And Alfakheri (1)found an elevated level of MDA in the blood serum of the catfish *Clarias gariepinus* after exposure to lead. Also it was in agreement with that found by Zheng (33) who exposed fish to cadmium and found an increase in MDA level in the organs under study. The results of this study also agree with Ayoola (5) who exposed the fish *Hemichromis fasciatus* and *Chrysichthys nigrodigitus* they found high levels of malondaldehyde in the liver and gills. It is also inferred from the results of the current study that the effect of lead is greater than the effect of cadmium , This agrees with Mahjoub (22) and Rajeshkumar et al. (26)

CONCLUSIONS

Lead and cadmium has a harmful effect on the cells of living organisms, even at low concentrations. Fish mortality increases with increasing concentration and increasing duration of exposure. Exposure to lead and cadmium causes a change in the level of antioxidants. In addition, there was a significant increase in the level of MDA which indicates the occurrence of the lipid peroxidation process in the membranes and the increase of ROS.

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CONFLICTS OF INTEREST

There are no conflicts of interest declared by the authors in relation to the publication of this manuscript.

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