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Accumulation of lead in the tissues and effects on growth rate of freshwater *Cyprinus carpio*

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Abstract
The present study was undertaken to determine the accumulation of heavy metal Lead (Pb) in different tissues of *Cyprinus carpio* as well as description the growth rate of *C. carpio*. The experimental fish were exposed to lead at sub lethal concentration (0.4 mg/l) and (0.2mg/l) for 60 days by added Pb in water aquarium. The element of lead was assayed by using flame atomic absorption spectrophotometer (FAAS) and the results were given as µg/gm. dry wt. The high level lead accumulation was found to be in liver in T1 (98.77±41.46) µg/gm and T3 (94.9568.20) µg/gm. The order of lead accumulation in organs was liver > gill > kidney > spleen. As well as showed a decrease in the growth rate of the fish because of the addition of lead element in a water aquarium.

Keywords: Lead, *Cyprinus carpio*, growth, accumulation

1. Introduction
Cyprinus carpio (Common carp) belong to the class actinopterygii, the order Cypriniformes, and the family Cyprinidae which is considered the largest family of freshwater fish. *Cyprinus carpio* generally lived in freshwater environments, especially aquarium, river and lakes, and also rarely lived brackish-water environments[1]. It is widely distributed in almost all countries of the world but is very high popular in Asia and some European countries [2]. Lead is from serious elements, because they serve no known useful purpose in fish, which have great and varied adverse effects [3]. As a result, lead may accumulate in particular tissues of the fish body. The common sources of lead is different in nature including natural and anthropogenic processes such as smelters, combustion of coal and mineral oil, paint industries, mining and alloy processing units [4]. Environmental pollution by lead is a worldwide public health problem, exemplified by elevated blood lead levels among people living in the polluted areas [5]. Atomic absorption spectrometry is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest [6]. Flame atomic absorption spectrophotometer is referred to as direct aspiration determinations. They are normally completed as single element analyses and are relatively free of inter element spectral interferences [7]. The present study was undertaken to determine the accumulation of heavy metal Lead (Pb) in different tissues of *Cyprinus carpio* as well as description the growth rate of *C. carpio*.

2. Materials and Methods
The experimental fish were obtained from Al-Swerra hatchery. A total of 100 fingerlings of carp fish *C. carpio* with an average weight ranging between 36-41gm. in body weight and 13-16 cm in total length were considered in this study. Fish were acclimated to laboratory condition for two weeks before beginning of the experiment and bathed in sodium chloride for five mints to remove external parasite if present. Fish weight was measured before the experiment and after the experiment ended for observation body weight changes during the experiment period. The experimental fish were distributed randomly upon four treatments in addition to control treatment. First treatment (T1) contained Pb (0.4 mg/l) with replacement water aquarium entirely per three days and added Pb continuously, the second treatment (T2) contained pb (0.4mg/l) with replacement water aquarium entirely per three days without adding Pb, third treatment (T3) contained Pb (0.2mg/l) with replacement water aquarium entirely per three days and adding Pb continuously, fourth treatment (T4) contained Pb (0.2mg/l) with replacement water aquarium entirely per three days without adding Pb. Fish samples were dissected (gills, liver, kidney and spleen), about 50gm. of organs were taken into

crucibles and washed with distil water and placed on clean slides and dried to constant weight in oven at 70 °C for 24 h. 1 ml of concentrated HNO_3 added to each piece of dried tissue and placed in clean tube for digestion, Samples were placed in shaker water bath at 70 °C for 4 h. After digestion was completed, the tubes were allowed to cool, then diluted with 4 ml of distil water, a tissue digests aliquot was stored at room temperature. After digestion was completed, Lead detection, particularly flame atomic absorption spectrophotometer (FAAS). Concentrations of lead in fish were measured in the central laboratories of Science College, University of Baghdad, according to [8]. Standards of lead were prepared in order to calibrate the instrument before analysis using 1000 µg/l lead stock solution (de ionized water), diluted by 2% nitric acid, The lead standards were used 2.5, 4.5, 6.5, and 8.5 µg/l. The wavelength chosen for lead measurements was 217.0 nm, with slit width -0.2 nm. Lead concentrations in the tissues were expressed as µg/gm. dry weight. The calculation to determine lead concentrations in the tissues was carried out as per the following equation [9, 10].

Lead C$\boldsymbol{oncentration}$ (µ$\boldsymbol{g}$/$\boldsymbol{g}$) =V$\boldsymbol{ol.}$ $\boldsymbol{ml}$ / S$\boldsymbol{ample\ wt.}$ $\boldsymbol{g}$ × d$\boldsymbol{igest\ conc.}$ ($\boldsymbol{mg}$/$\boldsymbol{l}$)

2.1 Statistical analysis

Results are expressed as M± S.E. One - way (ANOVA) was used to compare lead accumulation between treatments in such organ (significant values, $p \leq 0.05$) [11].

3. Results

The concentration of lead in gills was 76.77µg/gm in T1, while it was 58.51µg/gm and 65.04µg/gm in T2 and T3 respectively. Results of statistical analysis showed no significant differences ($P > 0.05$) in Pb residue in gills at T1, T2 and T3 during studied period. T1 recorded highest values of Pb in gills compared with the different treatment (Table 1). Low values of Pb in gills appeared in T4 (29.45µg/gm). Concentration values of Pb in *C. carpio* were also differed in liver according to treatment, which was 98.77µg/gm in T1. 94.95µg/gm in T3 and 22.25µg/gm in T2. Table 1 showed that T1 recorded highest values of Pb in liver compared with the T2 and T4. While low value of Pb recorded in T4. The spleen of *C. carpio* was exposed to concentration of Pb was also differed according to treatments. Its values were 49.71µg/gm in T1 and 16.52µg/gm in T2, while T3 recorded highest values compared with other treatment which was 55.98µg/gm. (Table 1). Lead concentration in kidney of *C. carpio*, recorded lowest valve in T4 was 12.93 µg/gm. The highest value was found in T1 reached 66.51 µg/gm (Table 1). Results of statistical analysis showed no significant differences ($P > 0.05$) in Pb residue in gills, liver, kidney and spleen at T1, T2 and T3 during studied period. The highest concentration of lead recorded in different organs of *C. carpio* at T1 in the following sequence: liver > gills > kidney > spleen, while T2: gills> liver > spleen> kidney, T 3 characterized as following sequence: liver> gills > spleen > kidney, while T4: gills > kidney > spleen > liver (Figure 1).

The results showed after 60 days a significant decrease at ($P \leq 0.05$) in Treatment 1 (T1) body weight values compared with the control treatment value. The highest reduction was recorded in T1. The Treatment 2 (T2) and Treatment 3 (T3) gave lowest a significant increase at ($P > 0.05$) compared with control treatment. Whereas the control treatment and Treatment 4 (T4) showed significant increases at ($P > 0.05$) in most body weight values with time progression (Table 2). Results showed the highest reduction in body weight values of *C. carpio* as the following sequence: T1 > T3 > T2 > T4 (Figure 2).

Table 1: Concentrations of Lead µg/gm. (mean ± SE.) in different organs of *Cyprinus carpi*o.

Organs / Treats.	Gills	Liver	Kidney	Spleen
T1	76.77±13.06 A a	98.77±41.46 A a	66.51±37.99 A a	49.71±43.85 A a
T2	58.51±5.19 A	22.25±19.06 A	15.32±13.99 A	16.52±31.33 A
T3	65.04±46.12 A a	94.95±68.20 A a	42.78±32.12 A a	55.98±18.80 A a
T4	29.45±11.59 B x	9.32±2.93 B x	12.93±10.0 B x	12.79±9.21 B x

Different horizontally capital letters indicate significant variations at ($p \leq 0.05$) between treatments. Small letters mark highest concentrations in single organ. X mark lowest metal concentration in single organ.

Table 2: Body weight gain in gram (mean ± SE.) of *Cyprinus carpi*o which exposed to a different Pb concentration

Body weight (gm.) / Treatment mg/l	Average initial weight (gm.)	Average final weight (gm.)
Control	38.08 ± 4.12 A	43.55 ± 4.70 A a
T1	40.6 ± 1.17 A	37.77 ± 1.38 B a
T2	36.11 ± 1.09 A	37.94 ± 1.47 A a
T3	41.08 ± 4.62 A	42.47 ± 4.66 A a
T4	41.20 ± 6.29 A	43.75 ± 6.87 A a

Different horizontally capital letters represent significant variations at ($p \leq 0.05$).

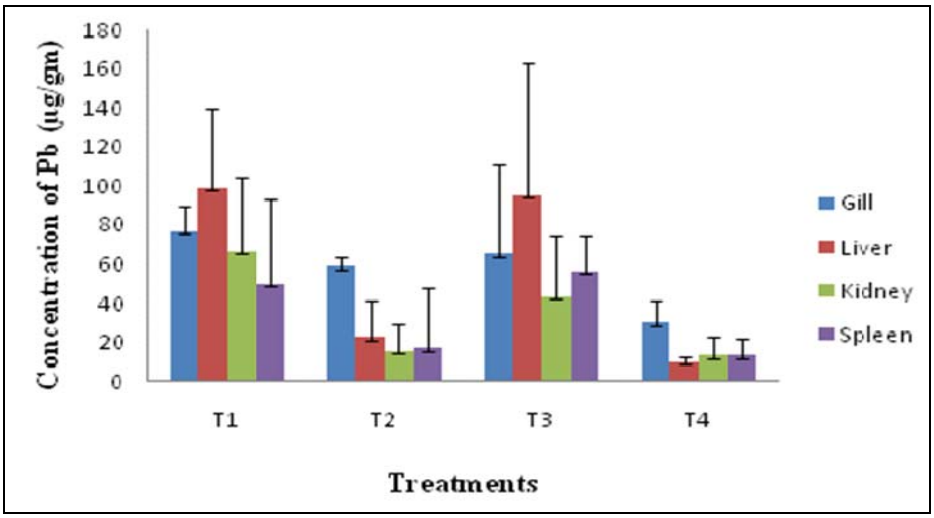

Fig 1: Concentrations of Pb (µg/gm.) (mean ± SE.) in all organs of *C. carpio* which exposed to a different Pb concentration (0.4 and 0.2) mg/l for 60 days.

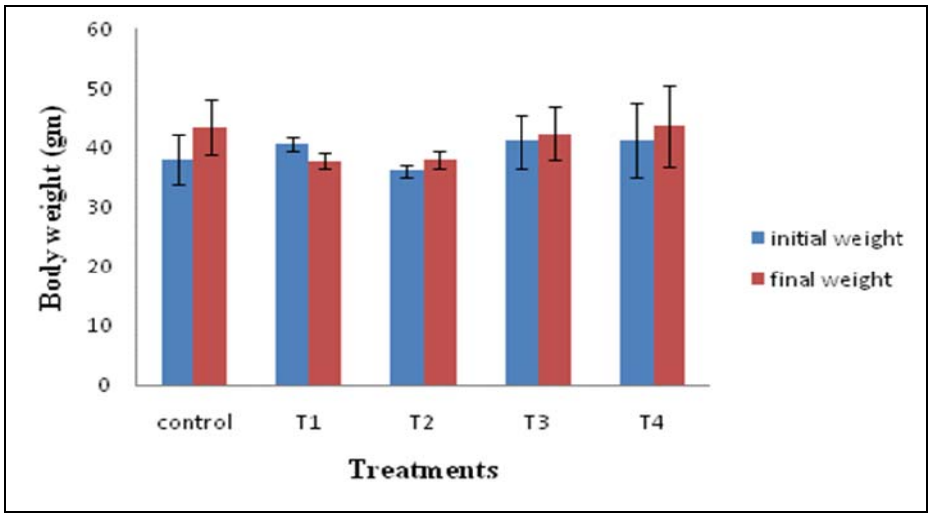

Fig 2: Body weight (gm) (mean ± SE.) of *C. carpi*o which exposed to a different Pb concentration (0.4 and 0.2)mg/l for 60 days.

4. Discussion

Lead is heavy metals which accumulate in the some tissues of fish and is extremely toxic in living organisms. Normal values recommended for lead in water is 0.05 mg/l according to [12, 13]. In present study, the highest concentration of Pb in studied organs of *C. carpio* at treatment1 as the following sequence: liver > gills > kidney > spleen, and treatment 3: gills > liver > spleen > kidney, while the lowest concentration of Pb in treatment 2 characterized as following sequence: gills > liver > spleen > kidney, and treatment 4: gills>spleen and kidney>liver. The high concentration of Pb in organs in T1 and T3 belong to add Pb continuously with changes water aquarium. While the lowest concentration of Pb in T2 and T4 in end experiment belong to changes water aquarium continuously without adds Pb matter.

Results appeared a marked decrease in total body weight (37.77 ± 1.38) gm. gain in T1 compared with control treatment (43.55 ± 4.70) gm. during the experimental period may, to some extent, support the observations reported by [14], who showed that lead administered may depress weight gain. The biological effects of sub lethal concentrations of Pb lead to delayed embryonic development, discouraged of growth, and weaken reproduction, neurological problems, increased mucous formation and kidney dysfunction [15]. T4 showed significant increasing ($p \leq 0.05$) in weight (43.75 ± 6.87) gm. after 60 day of the beginning of the experiment compared with initial weight, and that's nearly to results of control treatment due to changing water aquarium and adding of Pb only in the beginning of the experiment which led to improve health status of fish and remove stress of fish compared with T1 and T3. A substantial growth reduction caused by toxicant stress has important implications for survival in the natural situations. [16]. Fish growth is an indicator of populations' life conditions that could be used to detect stress due to contamination [17]. This study shows that Pb metal effects on the body weight of the fish when given at toxic doses. Also showed that continuous water change can reduce this effect and return fish to natural body weight.

5. Conclusion

The present study concluded that, the accumulation of Pb metal was generally the highest at the beginning of experiment where the lowest at the end of experiment. It is accepted that the concentration of pollutants in the tissues of aquatic animals results from both past and recent pollution of the environment [18]. As well as the results indicate that chronic exposure to Pb (0.2 and 0.4) mg/l for 60 day resulted in significant effects on the growth rates of *Cyprinus carpi*o in all treatments.

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