

Few Heavy Metals and their Impact on Crab *Sesarma* and their Activities in River

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ABSTRACT

The present study investigated the effect of crude oil on bioaccumulation in tissues of carp fish exposed to concentrations of crude for two periods of acute exposure for 96 hours and for 15 days for chronic exposure to calculated the rate of accumulation in tissues of ovaries, muscles and liver, where the values showed differences in the levels of bioaccumulation depending on the concentration to which the fish was exposed and the type of tissue shows the bioaccumulation mean in the ovary tissues of carpio during exposure to crude oil concentration (0.12,0.25,0.33 and 0.36) mg/L . The bioaccumulation mean increased with the concentrations. Therefore, the (0.36 mg/l) recorded the highest value of bioaccumulation in the ovary tissues was (232.81 microgram/mg) and also recorded Significant differences, as the higher the concentration, the greater bioaccumulation mean lead to the hematological changes after exposed to lethal period and concentration. The values of blood cells, hemoglobin, hematocrit and platelets were significantly reduced during 48 hours period of exposure. The values of HB and some blood indicators were found to decrease, respectively as shown in the all period of exposure While the PCV blood volume recorded a decrease, rates reached as follows, (37.6, 34.48, 24.2, 19.12 mg/l), compared the control (39.72 mg/l)

Keywords: Oil Concentrations; Pollution; Blood; Hemoglobin; Bioaccumulation

INTRODUCTION

The pollution of the water environment is a very important topics at various levels and it constitute the biggest dilemma facing human in this area .The phenomenon of the spread of environment pollution with oil was after its discovery, as it became at the global level at the present time, as well as its entry in it many industries as petrochemicals, in addition the oil derivatives, which are considered one of the most important pollutants of the water ecology, also the marine exploration operations, therefore, the increase in all these activities led to the many oil pollutants which effective on the live of the environment in terms of quantity and systematic effects under these impacts of different oil concentrations [1,2]. The damage caused by oil pollution to the aquatic system also varies, as it depends on the chemical composition and released quantity from it. Where it forms a floating layer on the surface of the water [3]. The components of thin layer that has a negative impact on the process of gaseous exchange, including oxygen,

and this layer works to reduce the amount of evaporation in the environment, Which effects the amount of the rain as well as, the surface layer of the dark sleeping oil works to raise the temperature of the water beneath, so the amount of dissolved gases decrease with increase of physiological processes and the accompanying lack of dissolved oxygen, which leads to the death of some organisms [4,5]. The damage caused by oil pollution to the water environment also various m as it depends on the chemical composition and quantity, where it forms a floating layer on the surface of the water components of a thin layer that has negative impact on the process of oxygen exchange, and the layer works to reduce the amount of evaporation in the environment [6]. Water beneath it, so the amount dissolved gases decreases with increase of physiological process and the accompanying of dissolved oxygen which leads to the death of organisms. When crude oil reaches the aquatic environment from different sources, the harm of hydrocarbon compounds that the a susceptibility to water increase such as aromatic compounds with medium and high molecular weight, on aquatic

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organisms because of their ability to enter the bodies of organisms and accumulate in their tissues [7]. When these pollutants enter their bodies aquatic organisms either dispose of them or store them in concentrations greater than what is present in the environment and it's called bioaccumulation which takes place by adsorbing ions on the membrane in contact with water through the passage of water by diffusion through semi-permeable membranes to the fluid of the body, indicated that the direct entry of substance chemical coating across the respiratory surface of fish is mostly by diffusion [8]. In young and old fish, the respiratory coating is a large and very thin barrier between water and blood where the entry of toxic pollutants unto the fish by the gills is more convenient and faster than taking them from other sources such as feed [9,10]. Therefore, many studies indicated the effects of these hydrocarbons and crude oil on physiological processes, respiration, reproduction and growth [11]. And many researches have referred to toxic effects on histological changes in addition to well sub lethal effects and inhibition in growth. Behavioral and low density of population of fishes, where the current study indicated changes in the level of bioaccumulation between the different tissues of the body of the fish, in addition some of the blood changes of the common carp fish that face these variables in freshwater rivers [12-14].

MATERIALS AND METHODS

Fish samples were collected from the bank of Shatt al Arab in the north of the Basra city. The samples were transferred to the Tank for 10 days to acclimation under laboratory conditions at a temperature $24 \pm 2^{\circ}\text{C}$ and also provided with continuous ventilation and feed with dry protein diet. After that, they measured the average weight as 7 ± 2 gm. and 11 ± 3 cm length, after acclimation period samples are divided with three replicate in with 5 samples of fish in each replicate for each oil concentration.

Preparation of solution

The concentrations of crude oil dissolved in water were prepared in glass tanks of 50 leach, with that were experimental jars used according to methods of Meador JP [15]. After that, each tank contains 45 l, to which 500 ml of crude oil is added. It is placed into magnetic stirrer to mix the storage solution, at the end floating oil layer id separated, the stock solution is preserved, and the concentrations for the experiment are prepared .The various concentrations used were in the following order concentrations of oil were (0.12, 0.25,0.33 and 0.36 mg/l), the lasts aquaria used as the control [16].

Bioaccumulation test

Extraction from tissues: Materials used to measure the bioaccumulation method in some tissues of carprio, Basra crude oil, benzene, methylhexan al alcohol, KOH, sodium sulfate and glass wool. Grimalt and Oliver method was used to Extract of HBC from samples of fish tissues then the hydrocarbons were extraction by thimble extraction for 24 hours after drying to temperature for 70°C after that, the HCB were measured in a spectroflurometer by wave length 360λ , where the

spectroflurometer method was adopted by (FAO1977) To measure the certain concentration of oil that calculates the bioaccumulation factor in the tissues of the ovaries, muscles liver. Of the fish C, Carprio under study using the method Doherty by used spectroflurometer instrument was calculated accumulation rates [17,18].

Statistical analyses: All data obtained from experimental groups were analyzed in Statistical Package for the Social Sciences (SPSS) 16.0. For the purpose of comparison between the accumulations rates by ($M \pm SE$) then compared these values of variance and the focus has been the correlation coefficient calculation.

Hematological test

Hematological parameters test: Blood Calculations are taken after the end of each period of exposure Blood samples are collected by cardiac puncture, hematological parameters for (R.B.C, Hb, HTC, PLT were determined Mean corpuscular (MCHC, MCH, MCV) were Calculations by using the method by Khoshbavar Rostam HA [19].

RESULTS

Bioaccumulation

The amount of bioaccumulation estimated by the, where the values showed differences in the levels of bioaccumulation depending on the concentration to which the fish was exposed and the type of tissue. The values indicated recorded in fish C. carprio ovaries higher than in liver and muscles during the exposure to concentration of crude oil for 15 days of exposure, The results illustrate that the level of accumulation in the ovary tissues was recorded according to the gradation of concentrations and respectively. The bioaccumulation mean in the ovary tissues of carprio during exposure to crude oil concentration (0.12, 0.25, 0.33, 0.36) mg/L in Figure 1. The bioaccumulation mean increased with the concentrations. Therefore, the (0.36 mg/l) recorded the highest value of bioaccumulation in the ovary tissues was (232.81 microgram/mg) and also recorded Significant differences ,as the higher the concentration , the greater bioaccumulation mean (Figures 2 and 3).

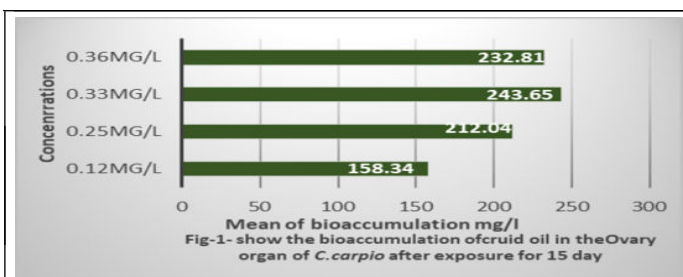


Figure 1: The bioaccumulation of cruid oil in the ovary organ of C.carpio after exposure for 15 day.

The bioaccumulation means in the carp muscle tissues. The concentration (0.36 mg/l) recorded higher mean of bioaccumulation compared to the Concentrations (0.33 mg/l), thus a significant differences was observed the concentrations

gradient and an increase in the bioaccumulation mean of hydrocarbons.

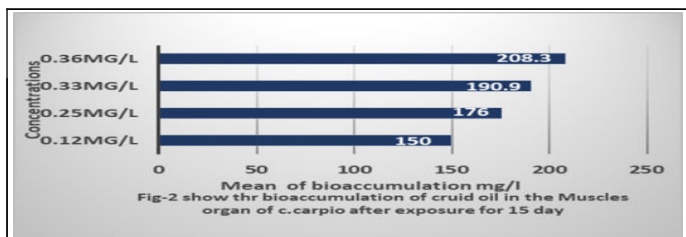


Figure 2: The bioaccumulation of crude oil in the muscles organ of C. carpio after exposure for 15 day.

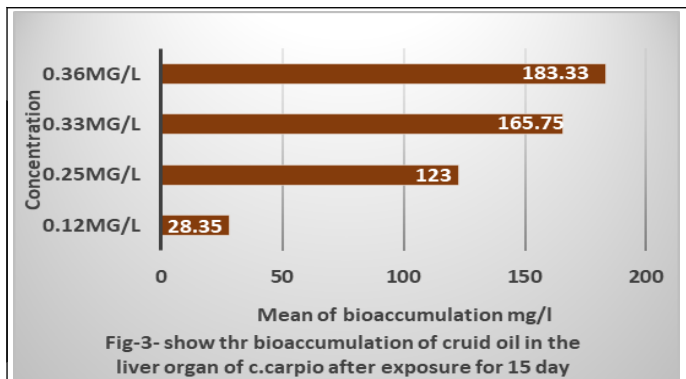


Figure 3: The bioaccumulation of crude oil in the liver organ of C. carpio after exposure for 15 day.

Bioaccumulation mean of hydrocarbons. Indicate low levels in the liver tissues compared to ovarian and muscles tissue, according to what found in Figures 1-3, where it was found

during exposure of the liver to the concentration of crude oil mg/L, that accumulation occurred at high concentration, where the 0.36 mg/l) recorded (183.33 ml/mg) value the bioaccumulation mean of hydrocarbons. Compared with the other conventions, it also recorded significant differences with concentrations.

Blood calculation: Blood indicators are used to diagnose and describe the health status of common carp fish *Cyprinus carpio* that are exposed to different concentrations of pollutants .including dissolved oil in the water column, which reflects this type of influence on the characteristics of blood parameters as shown in Tables 1-3, clear differences were noticed in the values of blood cells during exposure to concentrations of oil (0.12,0.25,0.33,0.36 mg/l), according to the time period of exposure and concentrations [20]. The values of blood cells ,hemoglobin, hematocrit and platelets were significantly reduced during 48 hours period of exposure, So the RBC recorded the higher decrease, which was in the 3.36 mg/l as 2.0 x RBC (10⁶/mm³ compared with control (3.65 RBC (10⁶/mm³) [21]. The values of HB and some blood indicators were found to decrease, respectively as shown in Table 1, in the all period of exposure while the PCV blood volume recorded a decrease, rates reached as follows (37.6, 34.48, 24.2, and 19.12%), and compared the control (39.72%). The tables also indicated the levels of some blood count [22]. As the MCV, MCH values showed a decrease in all time periods , and the decrease was 27,2 fl in 48 hours under concentrations of 0.36 mg, while increase significantly for values of MCHC as shown (%) significant increase was (37.06, 41.6, 45,52%) compared with control (35.72%) in Table 1.

Oil concentrations mg/l)	R.B.C (10 ⁶ /mm ³)	HB (gm. %)	PCV (%)	PLT (mm ³)	MCV (fl)	MCH (pg.)	MCHCH (%)	Confection coloration R
0	3.65 ± 1.03	11.3 ± 1.09	39.72 ± 3.03	362 ± 5.15	66 ± 3.2	48.5 ± 1.45	35.72 ± 3.61	0.531
0.12	3.74 ± 0.7	10.91 ± 1.13	37.6 ± 1.14	547 ± 5.02	57 ± 2.02	42 ± 2.05	37.06 ± 2.14	0.62
0.25	3.37.18 ± 0.2	2 ± 0.53	34.48 ± 1.21	592 ± 6.03	53 ± 2.15	37.14 ± 3.12	41.6 ± 2.03	0.784
0.33	3.5 ± 0.05	6.5 1 ± 0.13	24.2 ± 1.71	633 ± 6.08	48 ± 3.23	35.03 ± 4.08	45 ± 0.3	0.885
0.36	2.0 ± 0.04	4.02 ± 0.94	19.12 ± 0.14	681 ± 7.12	46 ± 1.9	27.12 ± 5.16	52 ± 3.01	0.91

Table 1: Showing the effect of Oil concentrations on the hematological parameters in exposure period 48 hours for fresh water fish *Cyprinus carpio* P<0.05.

Oil concretion	R.B.C (10 ⁶ /Mm)	HB (Gm. %)	PCV (%)	PLT (Mm ³)	MCV (Fl)	MCH (Pg.)	MCHC (%)	Coloration Factor R
0	3.53 ± 0.21	11.5 ± 25	35.2 ± 1.06	485.17 ± 9.65	68.17 ± 2.61	35.78 ± 2.12	34.78 ± 1.34	0.781
0.12	3.53 ± 0.45	9.13 ± 1.85	36 ± 1.3	230.22 ± 7.12	62.05 ± 2.98	33.53 ± 2.41	37.21 ± 1.6	0.844
0.25	2.76 ± 0.37	6.12 ± 2.0.9	29 ± 2.25	225.09 ± 7.99	59.18 ± 3.32	29.91 ± 2.08	42.2 ± 2.97	0.916
0.33	2.02 ± 0.41	5.37 ± 2.11	25 ± 0.17	185.13 ± 5.23	55.09 ± 2.71	25.86 ± 1.41	46.43 ± 3.17	0.937
0.36	1.14 ± 0.3	4.51 ± 1.0.2	20.22 ± 0.88	117.04 ± 4.91	47.21 ± 1.07	23.24 ± 2.02	56.18 ± 3.01	0.941

Table 2: The effect of crude oil thickness film on the hematological parameters in exposure period 72 hours for fresh water fish *Cyprinus carpio* P<0.05.

Oil concentrations mg/l	R.B.C (10 ⁶ /mm ³)	HB (gm. %)	PCV (%0	PLT (mm ³)	MCV (fl)	MCH (pg.)	MCHCH (%)	coloration factor R
0	3.22 ± 1.25	10.3 ± 0.63	39.1.4 ±	180 ± 3.09	66.41 ± 5.78	47.3 ± 2.04	34.27 ± 1.23	0.651
0.12	311 ± 1.09	7.63 ± 0.12	37 ± 1.34	136.6 ± 3.22	51.18 ± 5.09	39.02 ± 2.88	35.29 ± 1.76	0.792
0.25	2.43 ± 0.87	6.13 ± 0.22	34.7 ± 1.78	124.5 ± 2.91	47.33 ± 4.97	33.5 ± 3.91	47.37 ± 2.11	0.865
0.33	1.97 ± 0.05	4.6 ± 0.5	29 ± 1.2	119.8 ± 1.07	42.14 ± 3.15	29.03 ± 4.07	61.2 ± 3.15	0.901
0.36	1.03 ± 0.13	3.2 ± 0.07	11.3 ± 004	106 ± 1.02	27.12 ± 2.77	22.16 ± 4.33	74 ± 5.18	0.93

Table 3: The effect of crude oil thickness film on the hematological parameters in exposure period 96 hours for fresh water fish *Cyprinus carpio* P<0.05.

DISCUSSION

Bioaccumulation of petroleum hydrocarbons by fluorescence methods, This method is suitable for determining the amount of hydrocarbons in fish tissues, as fish are considered to have the ability to accumulate hydrocarbons in their tissues in higher concentrations than its media [23], Especially, within the tissues of fatty organs, and indicated by many studies that the most way pollutants enter through gills or skin, as it is the waterway for the entry all materials also the digestion of polluting materials [24]. This process leads to the accumulation of pollutants inside the bodies of living things in the aquatic system to levels higher than those found in the water column [25]. The oil can cover the gills of fish and prevent exchange of dissolved oxygen with aquatic environment; leading to obstruction of breathing through the formation of a thick mucus layer mixed with the compounds of oil is a source [26]. Fish exposure to some petroleum compounds of low molecular weight leads to numbness of fish due to its rapid dissolution in water even if the concentrations of these compounds is low because it is easily permeated through fatty tissues and cell membrane [27]. The present study found that ovarian tissues increased a significant (P<0.05) in the values of bioaccumulation, followed by muscles and liver, confirmed that the fat content varies from one tissue to another. While the concentrations of accumulation in ovarian tissues reached (232.81 mg/l) at concentration (0.36 mg/l) compared to author tissues under study [28]. The fish differed accumulation of hydrocarbons depending on Blood measurement are often used as a function of the health status of fish. Moreover to the level of stress to which these fish and living organisms in general exposed, as it is estimated that pollutants can cause damage to physiological functions [29]. The results of the present study showed a significant decreases in the average of the studied blood parameters for *C. carpio* compared with control groups, which are, the average RBC count, the percentage of Hb and percentage of combined blood volume PCV, for all concentrations in current experiment, where [30] they noticed a decrease in blood cells and times blood bleeding, also indicated during his study on blood variables of the fat content and metabolic speed [31]. Therefore, pollutants accumulate. The bioaccumulation of hydrocarbons is explained by the increase in the molecular dimension of hydrocarbons, where their ability affects the solubility in the lipid phase, changes in the direction of the structure of the molecules .This is due to the increase the extraction of the chemical fat molecules,

as it requires energy to keep the structure of the molecules and that is why the liver is considered as source of energy, so the fat amount decreased to consume it, the energy it needs to get rid dissolved and accumulated chemicals [32]. Because the main function of the liver is detoxification. Moreover, it was found that the physiological factors of fish are differing in their performance, according to the speed accumulation of hydrocarbons in their tissues [33]. Thus the fatty content of tissues is a key factor for the accumulation of pollutants of fish, where, it was found from this study that the value of accumulation higher in the ovary because it contents a high values of fat. Fish-*Clarias Gariepinus*, where he found successive decreases in the blood parameters during their exposure to hydrocarbons and other study confirmed in his study on fish [34]. During exposure to crude oil decrease in RBC and Hb. numerous studies have confirmed the effect of hydrocarbons on blood parameters [35], also (indicated a decrease in blood indicators is of lab fish exposed to a type of organic pesticides and increase MCHH. In another study, reduction in blood parameters of carp fish was found during exposure to WSF from crude oil, several studies also contributed to clarifying the importance of calculation and the study of blood composition as a confirming factor on the presences of environmental stresses resulting from the presence of dissolved petroleum toxic substance [36,37]. The present study also agrees with study by AL kaafaji, where he noticed a decline in the blood cells of carp and gold fish during their exposure to the residues of the refinery Dora, and also they found a decrease in the percentage level of Hb, which reaches low levels within 48 hours of exposure. As indicated by Turja reduction in PCV and Hb values in Tinca fish after 24 hours exposure to sub lethal mercury treatments in report by Mukbal found much variation between mean corpuscular hemoglobin after exposure *Labeo rohita* to pesticides [38,39]. That is study blood is one of the important connective tissues in the human and animal body at like, where its importance as physiology structure with a very important function. It is the liquid that determines the fate of the effectiveness of the circulation system with all its components, as the animal is impacted by pollutants surrounding it that have a negative hazard on the physiological e functions of the blood. Therefore, the reason for the values of the blood cells is due to exposing carp fish to the concentrations of crude oil, perhaps led to physiological stresses caused by toxicity of crude oil [39], impossible damage of the gill tissues, to obtain oxygen due to the decrease in RBC, as it causes, which are means for water

enter the body of fish. In addition to that compined by narcotic effects as result of toxins produce from crude oil. The decrease in the values of RBC, HB, PCV and some blood Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH)) and Mean Corpuscular Hemoglobin Concentration (MCHC) and during their exposure to crude oil pollutants, leads to the breakdown of blood cells and the occurrence coagulation. In addition, the percentage of Hb and PCV is related to the number of R.B.C cells, and therefore the values of these factors are affected by the number of red blood cells. Moreover, the changes are accompanied by pathological effects for fish, such as narcosis that effects the nervous system which leads to lethargy and weakness of muscles movement, as this effects swimming and lacked of resistance. The decline in the blood corpuscular Blood Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH)) due to the exposure of carp fish to acute concentrations of crude oil, especially after dissolved in the water, there is lack of oxygen to the respiratory plates, so structural damage occurs to the plates, and that is why crude oil used as evidence for fish exposed to many aquatic environment contaminated with crude oil, where reduction in blood indicators if reelected with accumulation of crude oil in the liver, ovaries, muscles, kidney and spleen of fish, where the kidney and spleen are responsible for reproduction of RBC and WBC as it is clear in Tables 1 and 3, the study also showed the decline in MCH values is an indicator of R.B.C swelling. In melted concentrations is the ratio between Hb/PCV, which is not affected by blood volume or number of RBC Also confirmed in his study, increase in the MCHCH values of fish (Silver Carp) exposed to a period of 96 hours of exposure to mercuric chloride.

CONCLUSION

Environmental pollutants are considered multiple, but the most dangerous are those toxins ,carried by chemicals and their repercussions on the components of the environment, including the aquatic system in which living organisms are taken as their place even if they are semi aquatic existent, but the spend their larval stages in the water medium, which is evidence of the vitality of the aquatic environment, hence this study on vital activity in light of these pollutants, and that is why heavy metals have a clear impact on vital activity of walking, and this reflects the extent of the toxic effect on aquatic organisms. Moreover the presences of pollutants in the water environment has negative effects and damage to the health of the environment, which in turn reflected on the health of humans and the living organisms that reside in this environment where the current study was conducted to test the activates of the organisms, which is explain the effect of heavy metals on movement as indicator the toxicity of the environment pollution.

COMPETING INTERESTS

There was no objection when completing the search

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