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INDUCED ASH TOXICITY EFFECTS ON THE HEMATOLOGICAL AND BIOCHEMICAL CHANGES OF *CYPRINUS CARPIO* L. 1785

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ABSTRACT: This study was the first of its kind in Iraq, which aims to assess the risk of Durah power plant ash concentrations in hematological and biochemical changes of common carp. The result shows that the mean RBC value was from 1.3±0.015 to 0.93±1.54 was recorded in a group of 0.5and3ppt after two-six weeks of exposure, respectively. In contrast, the mean Hb value were from 10+0.26 to 6+0.11g/dl was recorded in blood sample exposed to 0.5and3ppt after two-six weeks of exposure, respectively. Regarding to PCV was decreased from 30%±0.54 to 18%±0.74 at the end of 45th days of exposure in treated group as compared to control group. Moving to WBCs increased from 10.87±0.31mm^3 in 0.5ppt to 20.4±0.14mm^3 at the 3ppt concentration after six weeks of exposure period. Turning now to activity of liver enzymes the results of SGPT value were from72±1.665 to126±0.48U/L was recorded in blood sample exposed to 0.5and3ppt after two-six weeks, respectively. All these data were higher than control group. However the mean SGOT values were from 274±13.52 to397±18.42 U/L was recorded in blood fish exposed to 0.5and3ppt after two-six weeks, respectively.Our study concluded that presence of toxic ash in aquatic environment may produce systemic toxic effects and reveal the changes in blood cells count of fish also pose hazardous alternations in biochemical parameters.

Key words : Fly ash, Durah Power Plant, common carp, hematological, SGOT, SGPT.

INTRODUCTION

Blood parameters are considered as the pathophysiological indicators of the whole body and are important in diagnosing the structural and functional process in fish.

Hematological studies are important to know the impact of pollutions on aquatic organisms which along with indicating the physiological disturbances also provides vital information on general fish health (Barton and Iwama, 1991).

In recent years the energy demands have been increased so rapidly which is beanies largely met by using fossil fuel, this increasing is considered one of the challenges that faces the development of the country (Bashar, 2010).

The main source of energy is thermal power plants were produced the energy by using the coal as the fossil fuel. The process of coal combustion caused in health and environmental effects. Especially, coal is containing a largely mounts of organic and inorganic compound such as heavy metals, Poly Aromatic Hydrocarbons (PAHs) and Poly Chlorinated Biphenyls (PCBs) which are transformed to surrounding environment by various pathways. Heavy metals pollution has been indicated in aquatic animals (Adham *et al*, 2002; Olojo *et al*, 2005). This contamination's impact on the potential toxicity for the humans and their environment (Lee *et al*, 2007; Gueu *et al*, 2007).

Fly ash is a waste material resulting from coal based on thermal power plant which continuously increasing source of pollution chemically ash components consist a large amount of heavy metals, cations, anions, PAHs, PCBs, and quantity of various potentially toxic metals.

Fish are located at the end level of the aquatic food chain and can't escape from the impact of these pollutants, which may bioaccumulation and pass through the food to humans with causing acute and chronic diseases (Farombi *et al*, 2007). Therefore, the current study efforts have been made to indicate the toxic effect of fly ash contamination on hematological and biochemical parameters of *C. carpio* as a test organism under the control and treated conditions

which consider the first of its kind study in Iraq.

MATERIAL AND METHODS

Ethical approval

Ethical approval is not applicable to this study as fish samples used for the exposed to multiple ash concentrations which were collected from local hatchery incubators in Iraq.

Study Description

Durah power plant (DPP) which is located in south-west of Baghdad Governorate on the right bank of Tigris River and discharged their toxicants effluents directly with simple and old treatment to this river (Figure 1). The plant is considered one of the important industrial facilities in Iraq. It is located by 5.5 Km to the west of the Durah refinery (Nashaat, 2010).

Sampling and sample preparation

Common carp (*Cyprinus carpio* L. 1785) was obtained from the local hatchery incubators in Al-Musayyib City with average length of 10-15 cm and weight ranged from 31(±3) gm. The health fish was selected; the photoperiod was 16/8.light/dark. Water temperature was examined by mercury thermometer, water temperature of aquaria was ranged from 22-26C (Shamaaon and Al-Habeb, 1987). Regarding to hydrogen ion concentration (pH), Total dissolved solids (TDS) and Electrical conductivity (EC), data were measured by using a portable pH, EC meter. The experimental pH ranged from 7±0.5, dissolved oxygen 6.5-7. While the value of EC was ranged from 900-1220 µs/cm (Nebeker and Miller, 1988).

All the range of water environmental parameters which mentioned during the present study was within the acceptable limits of common carp. The fish were exposed to multiple ash concentrations 0.5, 1.5 and 3ppt as well as to the control group. Four groups were used in chronic toxicity test and left for 2, 4 and 6 weeks. Water was renewed every 48hr. to prevent depletion of dissolved oxygen concentration and avoid any wastes or food residues as well as growth the microorganisms (FAO, 1987). Fish were fed twice daily at a fixed feeding rate of 3% body weight per day for 45 days. Fish samples were divided into two groups (Each group contains ten fish), first group was control and the other was toxicity treatment groups with two replicates for each concentration in aquarium.

Fish samples were divided into two groups (Each group contains ten fish), first group was control and the other was toxicity treatment groups with two replicates for each concentration in aquarium. To prepared stock solution with

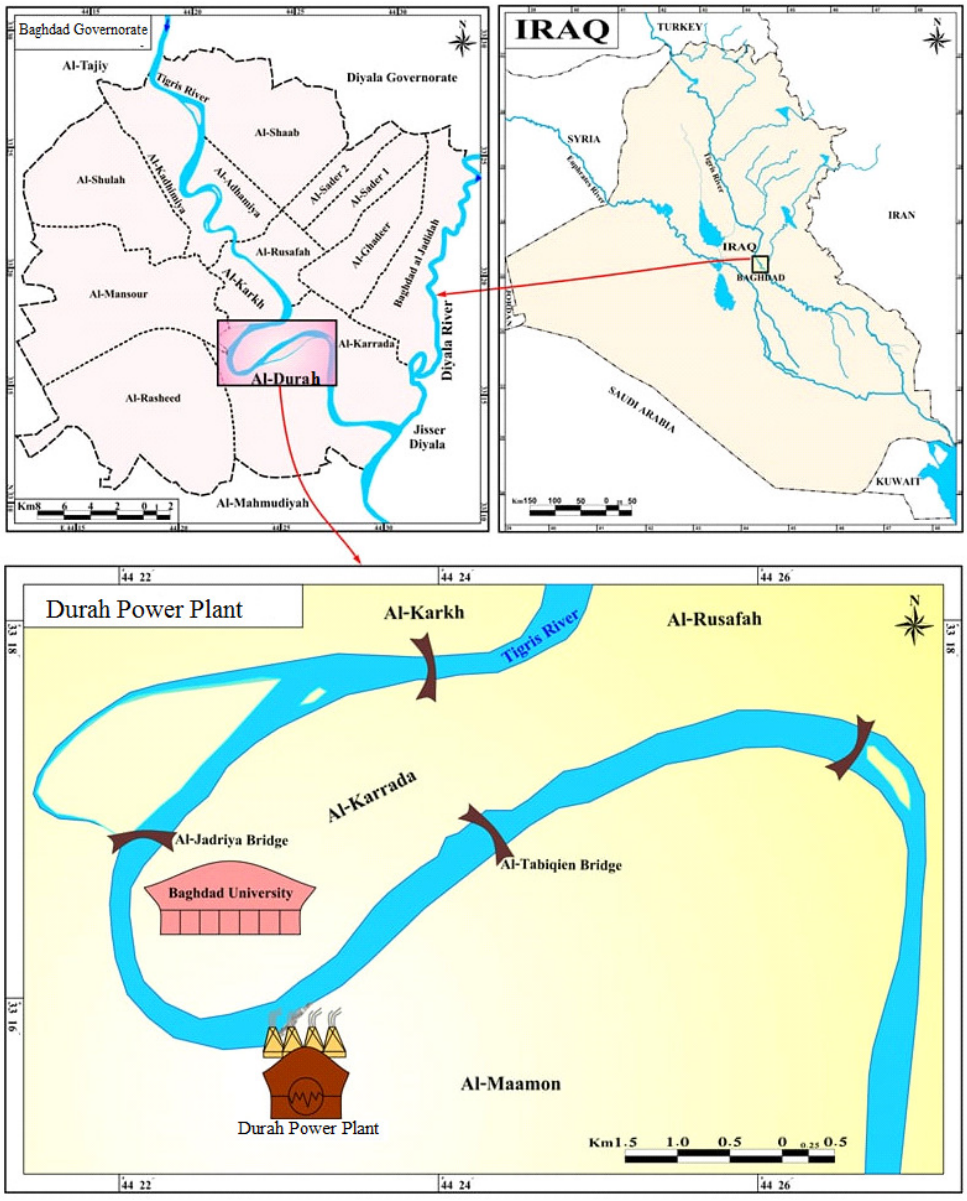

Figure 1 : A map shows Durah power plant (DPP) location

3ppt of ash concentration used in this test, weighting 3 gm of ash and dissolved in every 1L of aged tap water in aquaria. The minimum ash concentrations prepared depending on the following equation bellow:

$C_1 \times V_1 = C_2 \times V_2$

C_1: Concentration one of pollutant (fly ash)

V_1: Unknown

C_2: Concentration was requiring to obtain

V_2: Volume of water in aquaria (50 Liter)

Blood sample collection was done via a caudal vein by using a sterile disposable syringe, then transferred in EDTA tubes to calculate the hematological values after removing the needle from the syringe. Whereas using the heparin by wetting inside of the syringe with heparin before blood collected to prevent blood clots and to determine the hematological test (Mgbenka and Oulah, 2003).

The blood sample from each fish was collected separately and put into two containers containing EDTA: one for hematological tests and the other for plasma separation (Habiballah, 2001).

White blood cells count (WBCs) and Red Blood Cells (RBCs) were measured by using hemocytometer chamber and according to Mgbenka and Oulah (2003). In contrast, Packed cell Volume (PCV) was calculated by using microhematocrit capillary tube after filled with blood to 3/4 up to their length, then plugged the end of the tubes by using a clay then set in a microhematocrit centrifuge for five minutes at 1500 rpm, the result was read according to the number of the sample and the number of the rack. On the other hand, Hemoglobin Concentration was determined by the UV spectrophotometer method. The blood sample was mixed with Drabkin's reagent (Potassium cyanide and potassium ferricyanide). According to Ramakrishnan (2012) Hb was react with ferricyanide to form methemoglobin. The final solution was then transferred to form stable called cyanmethmoglobin by the cyanide. Then intensity was measured at 540 nm. Biochemicals parameters also estimated in the present study. SGOT and SGPT activities were measured in fish *C.carpio* by using colorimetric methods which were described by Reitman and Frankel (1957) with utilizing a ready-made kit for determination of SGOT and SGPT levels.

RESULTS AND DISCUSSION

Table (1) and Figure (2) shows that the highest mean RBC value 1.3± 0.015 was recorded in a group of 0.5 ppt after two weeks' of exposure and almost similar to that of control group 1.52± 0.21, while the lowest mean value 0.93± 1.54 was found in the group of 3ppt after six week's exposure.

It was seemed clearly that an increased ash concentration and period of exposure had significantly impacted on the mean values of RBC for examining *C. carpio*. Statistically, there were significant differences $p<0.05$ between all the treated of *C. carpio*.

Our finding agrees with Satish and Singh (2017) when fly ash causes decreased significantly in RBC mean values of *Channa punctatus* (BLOCH) which was reaches 1.58± 0.21 under short term exposure to ash application as compared with 3.10± 0.36 was recorded at control group. Similarly, there was a reduction in RBC values for long term exposure to fly ash and coal combustion residue from 3.58± 0.25 at control group to 1.78± 0.33 at exposure group. The result of the present study is very near or close to the results mentioned above. The reduction in mean values of RBC in the present study may be related to the effect of chemical substances in the gills injury which was caused a bleeding and hemolysis, then affected on destroyed the osmoregulation system. Therefore, it had effect in response to oxygen transport capacity, or whether the erythrocyte production by the hematopoietic organs decreases due to the destruction of circulating cells. (Saravanon, *et al* 2011; Thangam *et al* 2014). On the other hand, an increase occurred in the mean value of WBC during the increasing the level of ash concentrations. The mean value of WBC showed that the lower mean value 10.87×10^3 mm^3 was recorded in 0.5ppt after two weeks, while the higher mean value 20.4×10^3 mm^3 was recorded for the groups treated with 3ppt after six weeks of exposure as compared with control group as in (Table1; Figure 3).

The mean value of WBC was increased when *C.carpio* exposed to 0.5, 1.5 and 3ppt of ash and this agrees with Javad and Usmani (2012) who found that heavy metals exposed to *Mastacembelus armatus* caused an increase in mean value of WBCs to 3.84± 0.18 in treated groups, as compared with 2.55± 0.16 which was recorded at control group. The same things were observed by Satish and Singh (2017) when *Channa punctatus* exposed to fly ash showed an increase in mean value of WBC to 8.96± 0.20 in treated groups, as compared 5.9± 0.45 in the control group. Similar results were shown by Amsath *et al*(2017) who reported an increase in mean value of WBC to 87.00×10^3 after 30th days of exposed *Channa punctatus* to arsenic, as compared with 56.67×10^3 which was recorded in the control group.

The results in the present study were explained by Ramesh *et al* (2009) and Satish and Snigh (2017) that

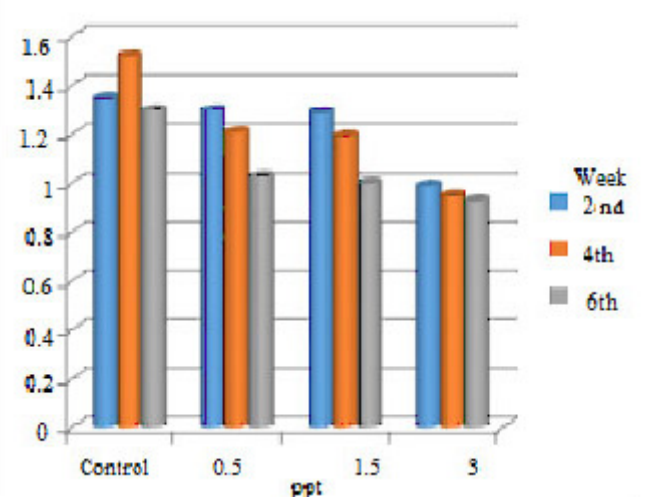

Figure 2 : Mean ± SE for RBCs in *C.carpio* after exposure to ash concentrations.

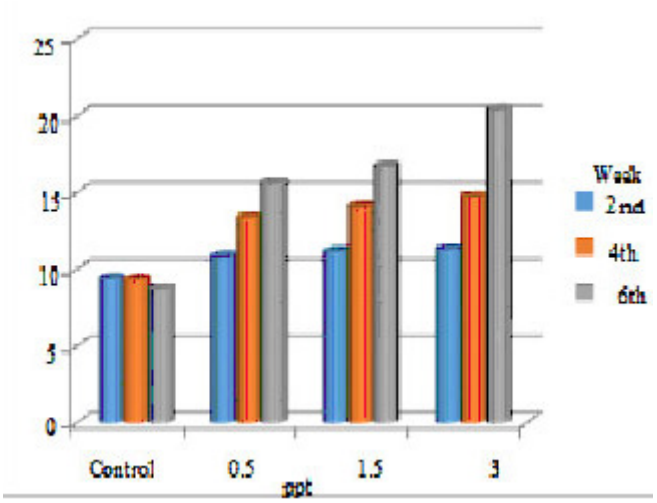

Figure 3 : Mean ± SE for WBCs in *C.carpio* after exposure to ash concentrations.

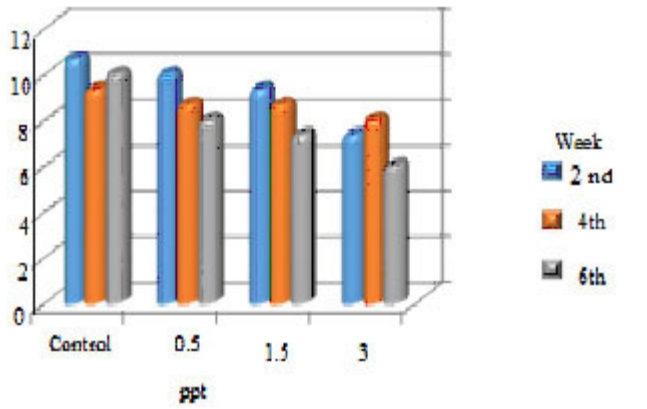

Figure 4 : Mean ± SE for Hb in *C. carpio* after exposure to ash concentrations.

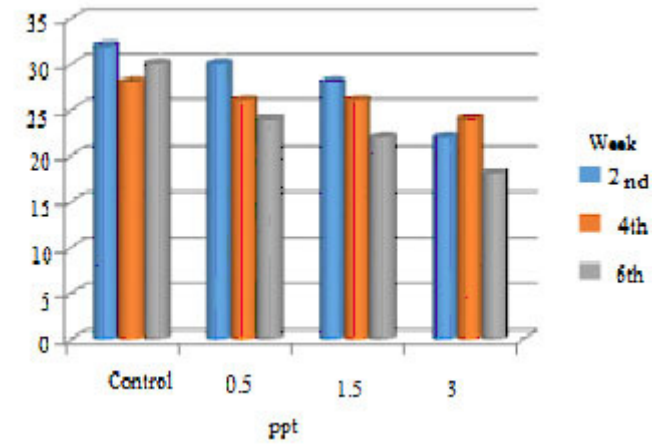

Figure 5 : Mean ± SE for PCV in *C.carpio* after exposure to ash concentrations.

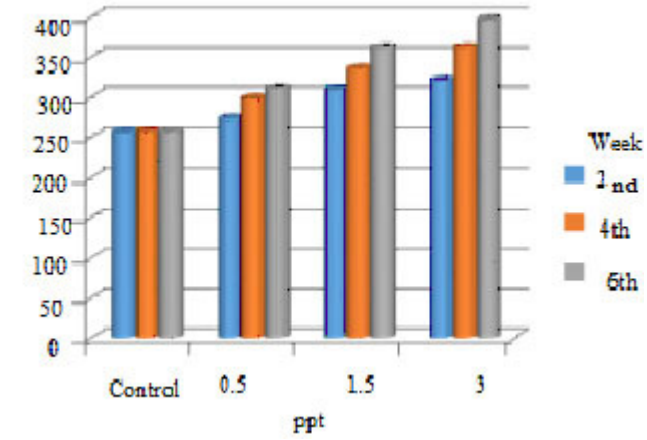

Figure 6 : Mean ± SE for SGOT in *C.carpio* after exposure to ash concentrations.

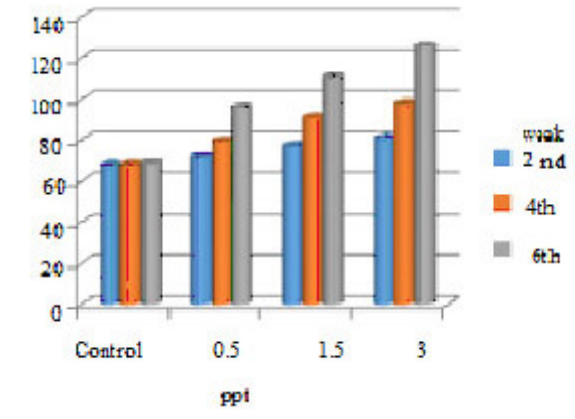

Figure 7 : Mean ± SE for SGPT in *C.carpio* after exposure to ash concentrations.

the increased in mean value of WBC may be due as a result of direct stimulation of its defense from the proportional to severity of stress conditions in fish during the presence of polluted substances, thus it was considered a result of direct stimulation of immunological defense and protection against the pollution as well as the anthropogenic impacts. Regarding to concentration of ash, the highest mean Hb value 10±0.26 was recorded in blood sample exposed to 0.5ppt after two weeks, while the lowest mean value 6±0.11 was recorded in blood sample exposed to 3ppt after six weeks (Table1; Figure 4).

Our finding in the present study showed effects of ash concentrations on Hb mean value, which caused a significant decrease in the value of hemoglobin in the treated groups which containing the duration of exposure as compared with the control group. The Hb mean value of the present study was near to Hb mean which was obtained by Singh *et al*(2008) who determined that Hb mean value was decreased from 10.37% to 6.60% for *Channa punctatus* exposed to copper concentrations. Also agrees with the study of Satish and Singh (2017) who found that fly ash concentrations reduced the Hb mean value from 11.98± 0.59 to 9.34±0.45 and from 12.26±0.63 to 9.14±0.54 during the short and long term exposure of *Channa punctatus*.

The reduction in Hb mean value also reported by Amsath *et al* (2017) on *Channa punctatus* exposed to arsenic concentrations.

In contrast, this decreased in Hb mean values may be related to present pollutants in fly ash which can make a destructive action on erythrocytes, as a result of the cells ability of the cells may be affected or may be attributed to the damaged in gills and hemopoietic organs (Karuppasamy, 2005; Mazon *et al*, 2002).

The highest mean value of PCV 30% was found in the blood sample subjected to 0.5ppt after two weeks while, the lowest mean PCV value 18% was recorded in blood sample subjected to 3ppt after six weeks of exposure. However, we note that the mean PCV values are significantly decreased in the last weeks of exposure when compared to control 32% as in (Table1; Figure5).

Our finding result demonstrates that the mean PCV values decreases with the increasing in concentration of ash. This reduction in the mean value agrees with what was found by Yousafazi *et al*(2008) that aquatic pollution has an effect on decrease mean PCV value from 21.4% in control to 7% in treated group of *Tor putitora*. Singh *et al* (2008) also reported that the mean PCV values was reduced from 31% to 23.3% at the end of 45th day of exposed *Channa punctatus* to copper. Similar results were found by Obemeata *et al*(2012) who determined that the mean PCV value in fish species was observed to reduce with increasing concentrations of fly ash and exposure period.

Apart from the above cases improved that all these compressions of mean PCV values for different species

Table 1 : Effect of fly ash on haematological parameters in *C. carpio* L. during exposed to long- term (45th days or six weeks). (Values are expressed in Mean ± SE) of several blood parameter of *C. carpio* subjected to different fly ash concentrations for two, four and six weeks.

2nd week				
Concentration	**HB g/dl**	**PCV%**	**WBC*10³ mm³**	**RBC*10⁶ mm³**
Control	A10.67±0.33	A32±0.96	A9.37±0.26	A1.35±0.084
0.5	B10±0.26	B30±0.54	B10.87±0.31	B1.3±0.015
1.5	C9.333±0.18	C28±0.21	B11.125±0.35	B1.29±0.011
3	D7.333±0.47	D22±0.51	B11.25±0.16	C0.99±0.072
4th week				
Concentration	**HB g/dl**	**PCV%**	**WBC** *10³ mm³	**RBC** *10⁶ mm³
Control	A9.33±0.61	A28±0.63	A9.32±0.13	A1.52±0.21
0.5	AB8.667±0.52	B26±0.42	B13.37±0.45	AB1.21±0.13
1.5	AB8.667±0.14	B26±0.78	BC14.12±0.63	B1.195±0.07
3	B8±0.63	C24±0.82	C14.75±0.17	C0.95±0.041
6th week				
Concentration	**HB g/dl**	**PCV%**	**WBC** *10³ mm³	**RBC** *10⁶ mm³
Control	A10±0.13	A30±0.71	A8.75±0.65	A1.3±0.096
0.5	B8±0.21	B24±0.42	B15.67±0.34	B1.03±0.044
1.5	C7.33±0.31	C22±0.31	C16.75±0.09	B1.0±0.025
3	D6±0.11	D18±0.74	D20.4±0.14	B0.93±0.154

Table 2 : Biochemical changes in serum (SGOT and SGPT) of freshwater fish, *Cyprinus carpio* L. exposed to DPP effluent. (Values are expressed in Mean ± SE).

2nd week		
Concentration	**SGOT U/l**	**SGPT U/l**
Control	A257±11.63	A68±2.254
0.5	A274±13.523	B72±1.665
1.5	B309±8.306	C77±1.523
3	B321±11.975	D81±2.446
4th week		
Concentration	SGOT U/l	SGPT U/l
Control	A258±13.137	A68±1.589
0.5	B299±14.298	B79±4.746
1.5	C336±11.742	C91±2.831
3	D362±13.556	D98±3.790
6th week		
Concentration	SGOT U/l	SGPT U/l
Control	A258±12.633	A69±1.952
0.5	B311±19.671	B96±3.444
1.5	C362±21.305	C111±3.759
3	C397±18.427	D126±4.111

indicated that correct experimental results in present study.

Results from present study occurs may be related to the changes in water balance which could cause a decrease in blood volume and an increase in WBCs values as a resulting in reduced PCV (Cameron, 1970).

In summary, the anemia might have led to a fall in RBC, HB and PCV volume, as well as, may be due to inactivity intestinal absorption of iron (Singh *et al*, 2008).

In contrast, an elevated value of liver enzymes compared to control was found. Moving to mean SGOT values in blood of common carp, the current study has found that the lowest mean SGOT value 274±13.52 U/L was recorded in blood fish exposed to 0.5 ppt after two weeks, while the highest mean SGOT value 397±18.42 U/L was found in blood fish exposed to 3ppt after six weeks, and the control blood fish sample has a mean of 257U/L (Table 2; Figure 6).

While the highest mean SGPT value 126±0.48 U/L was recorded in blood fish sample exposed to 3ppt after six weeks, while the lowest mean SGPT value 72±1.665 U/L was found in blood fish sample exposed to 0.5ppt after two weeks. All these data were higher than that of control fish sample 68±1.589 U/L(Table2; Figure7).

The present study shows significantly elevated on SGPT and SGOT enzymes of all exposed groups for ash concentrations when compared to control group. The results of the present study agree with the results obtained by Yousafzai *et al*(2008) to freshwater fish *Tor putitora* who reached a fact that the liver enzymes (SGOT and SGPT) showed 55.98% and 124.24% became increase in the fish caught from polluted water as compared with the control group. The same things were observed by Chavan and Muley (2014) when *Cirrhinus mirgala* exposed to sublethal concentrations of heavy metals. And these results in consistency with increase the liver enzymes of *C.carpio* which had been exposed to ash concentrations in the present study.

In sense, Anant Suresh (2015) found that the effluents of thermal power plant show a significantly greater increasing in the blood serum(SGOT and SGPT) in freshwater fish *Labeo rohita* exposed to power plant effluents , their results showed that the highest elevation in SGPT was 90.84 IU/L and the highest SGOT value was 421.388U/l . The increasing in the liver enzyme values in the studies which are mentioned previously are near to the values which were reported in this study for freshwater fish *C.carpio*.

The elevation in the values of liver enzymes could be due to increase in the expression of their proteins and signify pathology, or whether reflect to myocardial and hepatic toxicity leading to extensive liberation of the enzymes, this monitoring had proved a positive correlation between the elevates of these values and the toxicological liver when exposed to toxicants (Health, 1987; Chavan and Muley, 2014).

In addition, among other cell injuries caused by free radicals from heavy metals in hepatocytes is the lysosomal damages, fish hepatocytes often have a small number of lysosomes , and during heavy metal pollution the number and the size of lysosomes increase to store pollutants and lipids.

Although lysosome stability quickly monitors damage and the capacity of hepatocyte in detoxification, observation need to be confirmed with liver histopathologic and other physiologic biomarkers (Ardeshir *et al*, 2017).

CONCLUSION

Fly ash contamination alterations the aquatic ecosystem by changing in physical and chemical properties of water. Thus, some major differences in hematology and biochemistry was found by the present study which proved that fly ash is also impacting the total health of the food chain, which should be one of the major concerns in today's world. As *Cyprinus carpio* has been used as high nutritive value and it is used as the main source of protein rich food, Fly ashe of power plants are continually impacting the major fauna of water bodies.

Thus, by our work we were founded the change in various blood parameters of *Cyprinus carpio*. As no work was carried out on the effect of power plants effluent on the Iraqi rivers, so our model is considered as the first laboratory study for the assumption of the human health problems, thus ultimately benefiting the mankind.

Hence, the current investigation results confirm that stress due to fly ash present in the water does cause hematological disturbances, erythrocyte destruction (hemolysis), and leukocytosis in fishes, impacting the immune system and making the fish vulnerable to diseases. Therefore, the fish is provided as a bio indicator of deteriorating water quality and due care should be taken to monitor the environment of the waste water aquaculture pond.

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