Pakistan J. Zool., vol. 48(6), pp. 1857-1863, 2016.

Effect of Zeolite and Copper Sulfate, Administered Alone and in Combination on the Biochemical Components of Blood Serum of Common Carp, *Cyprinus carpio*

Ekrem Mutlu,[1,]* Seyit Aydın,[1] Tuğba Demir[2] and Telat Yanık[3]
[1]*Faculty of Fisheries, Kastamonu University, 37150- Kastamonu, Turkey*
[2]*Kamer Örnek Vocational High School, Cumhuriyet University*, Sivas, Turkey
[3]*Faculty of Fisheries, Atatürk University, 25100- Erzurum, Turkey*

ABSTRACT

Effects of zeolite have been described on the biochemical parameters of blood of *Cyprinus carpio* exposed to copper sulphate. The control fish showed 57.67% increase in body weight over a period of 90 days. This increase was 26.45% after exposure to copper sulphate (2mg/L) and 32.01% after Zeolite treatment (60 mg/L). When Zeolite was administered along with copper sulfate the fish showed 46.24% increase in body weight. Blood sampling were made for the determination of hematological values (glucose (GLU), blood urea nitrogen (BUN), total protein (TP), albumin (ALB), globulin (GLB), creatinine (CR), total bilirubin (TBIL), uric acid (UA), and direct bilirubin (DBIL)) at 30, 60, 90 days. Fish weight increased 26.45%, 46.29%, 32.01% and 57.67% respectively in groups and differences between groups were significant ($p<0.05$). GLU, BUN, TP, ALB, GLB, CR, TBIL and UA values differed significantly among the groups ($P<0.05$). There were significant differences GLU, BUN, TP, ALB, GLB, CR, TBIL and UA values among the groups ($P<0.05$). TP value decreased, but GL, TBIL, UA and DBIL values of $CuSO_4$ group increased significantly compared to control group ($p<0.05$). Therefore, it was suggested to use of clinoptilolite in rearing waters when there is a problem related to metals.

Article Information
Received 20 March 2015
Revised 5 May 2016
Accepted 21 May 2016
Available online 25 September 2016

Authors' Contribution
EM conceived and designed the study. SA and TD collected and analyzed the data. TD and TY statistically analyzed the data. All authors contributed to writing of the article.

Key words
Carp, copper sulfate, zeolite, hematology, heavy metal, toxicity

INTRODUCTION

Arsenic, cadmium, chromium, copper, nickel, lead and mercury are the most common heavy metal pollutants in water sources (Kidwai and Ahmed, 1999; Yousafzai and Shakoori, 2006). Uptake of heavy metals occurred through absorption across the gill surface or through the gut wall in fish (Yousafzai, and Shakoori, 2008; Nwani *et al.*, 2009; Güllü *et al.*, 2014; Gündoğdu *et al., 2016*). Copper contamination affects fish adversely and high concentrations can cause toxicity in fish (Woodward *et al.*, 1994). Copper showed varied effects on different species (Mazon *et al.*, 2002). It enters to fish body mainly via gill followed by liver, stomach and intestine and becomes much more toxic by combining with other contaminants such as ammonia, mercury and zinc in fish (Rompala *et al.*, 1984; Vinodhini and Narayanan, 2008). Exposures to copper toxicity caused to negative effects on body weight of fish (Schjolden *et al.*, 2007; Hamilton *et al.*, 1977), and on digestive enzymes and lipase (Sastry and Gupta, 1978). It is also reported that higher concentrations of $CuSO_4$ damaged gill epithelium, hematopoietic tissues, kidney, spleen and liver of fish (Nussey *et al.*, 1995; Mazon *et al.*, 2002; Figueiredo –Fernandes *et al.*, 2007). It is well known that hematologic parameters have been used in many studies for the description of fish health (Nussey *et al.*, 1995a; Mazon *et al.*, 2002; Carvalho and Fernandes, 2006; Singh *et al.*, 2008).

In 1960s, the first usage experiments of zeolites have been started with soil experiments in Japan. In Japan, the zeolite rocks have been used for increasing the productivity of volcanic soils by controlling the water content, and increasing the pH level. Clinoptilolite is one of the zeolites used for this purpose. Zeolites such as Bentonite and Mordenite -natural or synthetic crystalline aluminosilicates with ion exchanging properties have been used in animal production as supplemental feed additives to diets. Some of them such as Clinoptilolite have been used for preventing the toxic effects of minerals. By means of these important advantages of zeolites, they have attributed increasing attention of researchers. There are dozens of researches and experiments aiming to reveal the role and importance of zeolite use in agricultural production. The low concentration of clinoptilolite addition to diets has been shown to increase the body weight of animals and to improve the general health status (Mumpton, 1985; Tepe *et al.*, 2004). There are some controversial reports about

* Corresponding author; ekrem-mutlu@hotmail.com
0030-9923/2016/0006-1857 $ 8.00/0

the usage of zeolites in aquaculture. Tepe *et al.* (2004) advised using zeolite to remove excess ammonia from the waste waters. On the other hand, it was suggested not to use it for ammonia removal from pond waters (Silapajarn *et al.,* 2006). Toxic Cu concentrations of 0.036 ppm (max) were determined in sampling waters in spring time. Therefore, the present study was aimed to show effects of zeolite administered alone or in combination with copper sulphate on biochemical components of blood serum of carp, *Cyprinus carpio.*

MATERIALS AND METHODS

This study has been carried out Toxicology Institute of Hafik Vocational School of Cumhuriyet University in Turkey. Prior to the experiment, fish were acclimatized to laboratory conditions for 15 days. In river water, at spring season, copper value was recorded as 0.036 mg/L (max) which was higher than acceptable level of 0.02 ppm. The dose of $CuSO_4$ ($5H_2O$) was decided as 2ppm which was lower than the 96 h LC_{50}. At the present study, considering alkalinity value of water, zeolite dose was used as 60 mg/l (Tepe *et al.,* 2004).

Three groups, each of three fishes, were administered with $CuSO_4$ ($5H_2O$) at 2 mg/L, Zeolite at 60 mg/L and $CuSO_4$ ($5H_2O$) at 2 mg/L + zeolite at 60 mg/L. The fourth group was maintained as control and was without any treatment. Fish were kept in 100 L glass aquaria including 10 fish with 37.8 ± 0.01 g. $CuSO_4$ and Zeolite were added to aquariums in aqueous solutions. The fish were fed twice daily with a commercial carp pellet. Aquariums were cleaned daily. The mean temperature of the water during experiment was 22±1°C. pH values of water in aquariums were between 7.8 and 8.1 during the experiment. Hardness, alkalinity, dissolved oxygen and pH vales were between 234 - 240 mg/L $CaCO_3$; 238 - 240 mg/L $CaCO_3$; 5.9 mg/L - 6.2 mg/L and 7.8 - 8.1 respectively.

The study lasted for 90 days. Blood samples were taken at 30, 60, and 90 days from the end of caudal fin by injection needle and were centrifuged at 4000 rpm for 10 min prior to biochemical analysis by using Vitros System Chemistry 350 auto analyzer (Blaxhall and Daisley, 1973; Aziz *et al.,* 1993; Atamanalp *et al.,* 2002).

Statistical analysis

The effects of $CuSO_4$ concentrations on blood parameters obtained from the blood of fish in the in vivo experiment were determined using one-way ANOVA, and means were subjected to a multiple comparison test (Duncan) at the a= 0.05 level (Winer *et al.,* 1991).

RESULTS

Table I shows change in the body weight, while Table II shows effect of zeolite alone or in combination with $CuSO_4$ on the blood serum biochemical components of the fish.

Fish behaviour

Although there was no significant change in proportion to beginning of the experiment, the fish behaviour after $CuSO_4$ but they seemed to move slower than control and $CuSO_4$ + Zeolite group. The fish have started gathering generally at the bottom edges of the aquarium containing $CuSO_4$. The carp fish in Zeolite group have not shown distinguishing behaviors significantly different from the control and $CuSO_4$ + Zeolite groups. Although the pellet intake of fish in $CuSO_4$ group did not show any difference from the control, $CuSO_4$ + Zeolite and only Zeolite groups, their willingness and behavior of feeding decreased after 72nd day of the experimental duration.

Weight gain

Significant differences were observed between weight gains of carp ($p<0.05$) (Table I). The mean weight of the fish at the beginning of the study was 37.8±0.01 g. At the end of 35 day trial period, the mean weights of the fish were found to be 44.2±0.01g in control group, 43.8±0.01 g in copper and zeolite group, 41.9±0.01 g in zeolite group, and 41.7±0.01 g in copper sulfate group. At the end of 65 day trial period, the mean weights of the fish were 51.3±0.01g in control group, 48.6±0.01 g in copper sulfate and zeolite group, 45.7±0.01 g in zeolite group, and 44.9±0.01 g in copper sulfate group. At the end of 90-day trial period, the mean weights of the fish were 59.6±0.01g in control group, 55.3±0.01 g in copper and zeolite group, 49.9±0.01 g in zeolite group, and 47.8±0.01 g in copper sulfate group.

Biochemical components of blood serum

There were significant differences GLU, BUN, TP, ALB, GLB, CR, TBIL and UA values among the groups ($P<0.05$) (Table II). TP value decreased, but GL, TBIL, UA and DBIL values of $CuSO_4$ group increased significantly compared to control group ($p<0.05$). As seen in Table II, GLU level in $CuSO_4$ group was 143.30 mmol/L at the end of 90-day trial group. In $CuSO_4$ + Zeolite group, the same value was found to be 140.50 mmol/L, whereas in Zeolite group, it was found to be 161.07 mmol/L. Glu values in Control group were found to be 135.77 mmol/L, 137.40 mmol/L, and 136.93 mmol/L in 35, 65, and 90 days of trial periods, respectively. The differences among the groups were

Table I.- Changes in weight (g) of carp with 37.8 ± 0.01 initial weight exposed to $CuSO_4$ ($CuSO_4$ ($5H_2O$) 2 mg/l), $CuSO_4$ ($CuSO_4$ ($5H_2O$) 2 mg/l) + Zeolite (60 mg/l), Zeolite (60 mg/l) for 90 days.

Treatment	Period (Day) 0	30	60	90	Weight increase (%)
Control (0 mg)	37.8±0.01	44.2 ± 0.01	51.3 ± 0.01	59.6 ± 0.01	57.67
$CuSO_4$ (2 mg/L)	37.8±0.01	41.7 ± 0.01*	44.9 ± 0.01	47.8 ± 0.01	26.45
Zeolite (60 mg/L)	37.8±0.01	41.9 ± 0.01	45.7 ± 0.01	49.9 ± 0.01	32.01
$CuSO_4$+Zeolite (2 mg/L+60 mg/L)	37.8±0.01	43.8 ± 0.01	48.6 ± 0.01	55.3 ± 0.01	46.29

* Values represent mean ±SEM.

statistically significant ($P<0.05$). While the minimum glucose value at the end of 35-day trial period was observed in $CuSO_4$ group, the minimum values in 65-day and 90-day trial periods were observed in $CuSO_4$ and Control groups, respectively. The maximum glucose value at the end of 90-day trial period was observed in Zeolite group.

The BUN in $CuSO_4$ group was 2.30 µmol/L in 35 days trial period, 2.60 µmol/L in 65-day trial group, and 2.80 µmol/L in 90 days trial group. The differences among the above groups were statistically significant ($P<0.05$). Minimum BUN values at the end of 35-day, 65-day and 90-day trial periods were observed in Zeolite, Zeolite, and control groups, respectively. The maximum BUN value was observed in $CuSO_4$ + Zeolite group at the end of 90-day trial period.

The total protein (TP) content in $CuSO_4$ group were 1.54 g/l at the end of 35 days, 1.26 g/l at the end of 65 days, and 1.10 g/l at the end of 90 days trial group. In analyses performed in Control group, the total protein values were found to be 4.21 g/l, 4.15 g/l, and 4.12 g/l in 35, 65 and 90 days of trial periods, respectively. The differences among the groups were statistically significant ($P<0.05$). The minimum TP values at the end of 35-day, 65-day and 90-day trial periods were observed in $CuSO_4$ group, whereas the maximum TP value was observed in Control group at the end of 90-day trial period. This result indicates that the $CuSO_4$ implementation decreases the total protein content of blood.

The albumin (ALB) values in the $CuSO_4$ group was found to be 0.78 mmol/L at the end of 90 days trial group. In the Zeolite group, these values were found to be 1.47 mmol/L, 1.48 mmol/L, and 1.42 mmol/L, respectively. The differences among the groups were statistically significant ($P<0.05$). The minimum albumin values at the end of 35-day, 65-day and 90-day trial periods were observed in $CuSO_4$ group, whereas the maximum albumin value was observed in Zeolite group at the end of 90-day trial period.

The globulin (GLB) values in the $CuSO_4$ group were found to be 3.34 mmol/L at the end of 35 days, 3.60 mmol/L at the end of 65 days and 4.07 mmol/L at the end of 90 days trial group. In Control group, the GLB values were found to be 1.68 mmol/L, 1.68 mmol/L, and 1.68 mmol/L in 35, 65 and 90 days of trial periods respectively. The differences among the groups were statistically significant ($P<0.05$). The minimum GLB values at the end of 35-day, 65-day and 90-day trial periods were observed in Control group. The maximum albumin value was observed in $CuSO_4$ group at the end of 90-day trial period.

The creatinine (CR) in $CuSO_4$ group indicated the results of 0.33 µmol/L at the end of 35 days trial period, 0.38 µmol/L at the end of 65 days trial group, and 0.40 µmol/L at the end of 90 days trial group. The CR in Control group, the CR values were found to be 0.22 µmol/L, 0.20 µmol/L, and 0.20 µmol/L in 35, 65 and 90 days of trial periods respectively. The differences among the groups were statistically significant ($P<0.05$). While the minimum CR values at the end of 35-day, 65-day and 90-day trial periods were observed in Control group, The maximum CR value was observed in $CuSO_4$ group at the end of 90-day trial period.

The total bilirubin (TBIL) in $CuSO_4$ group was 0.73 µmol/L at the end of 35 days, 0.74 µmol/L at the end of 65 days, and 0.12 µmol/L at the end of 90 days trial group. These values in $CuSO_4$ + Zeolite group were found to be 0.53 µmol/L, 0.53 µmol/L, and 0.65 µmol/L, respectively. In Zeolite group, these values were 0.57 µmol/L, 0.57 µmol/L, and 0.57 mmol/L, respectively. In Control group, the total bilirubin values were found to be 0.41 mmol/L, 0.43 mmol/L, and 0.43 mmol/L in 35, 65 and 90 days of trial periods, respectively. The differences among the groups were statistically significant ($P<0.05$). The minimum total bilirubin values at the end of 35-day, 65-day and 90-day trial periods were observed in control group, the maximum total bilirubin value was observed in $CuSO_4$ group at the end of 90-day trial period.

The uric acid (UA) concentration in $CuSO_4$ group was 3.89 mmol/L at the end of 35 days, 3.85 mmol/L at the end of 65 days, and 3.94 mmol/L at the end of 90

Table II.- Effect of Zeolite and copper sulphate administered alone and in combination for 90 days on the various biochemical components of *Cyprinus cario*.

	Control				$CuSO_4$				Zeolite				CuSO4 + Zeolite			
	30	60	90	Mean±SD	30	60	90	Mean±SD	30	60	90	Mean±SD	30	60	90	Mean±SD
Glucose (mmol/L)	135.78	137.4	136.93	136.70± 0.83	121.8	135.6	148.3	135.23± 13.25*	160.4	158.43	161.07	159.97± 1.37	108.3	128.57	140.5	125.8± 16.28
Total protein (g/L)	4.21	4.15	4.12	4.16± 0.05	1.54	1.26	1.1	1.30± 0.22	4.6	4.35	4.1	4.35± 0.25	3.7	3.2	3	3.3± 0.36
Albumin	1.37	1.35	1.32	1.35± 0.03	1.18	1.06	0.78	1.01± 0.21	1.47	1.48	1.42	1.46± 0.03	1.34	1.16	1.02	1.2± 0.16
Globulin	1.68	1.68	1.68	1.68± 0.00	3.43	3.6	4.07	3.70± 0.33	2.93	2.89	2.98	2.93± 0.05	2.05	2.08	2.15	2.1± 0.05
Total bilirubin (µmol/L)	0.41	0.43	0.43	0.42± 0.01	0.73	0.74	0.72	0.73± 0.01	0.57	0.57	0.57	0.57± 0.00	0.53	0.53	0.65	0.6± 0.07
Direct bilirubin (µmol/L)	0.21	0.23	0.23	0.22± 0.01	0.47	0.52	0.57	0.52± 0.05	0.38	0.28	0.27	0.31± 0.06	0.27	0.38	0.45	0.4± 0.09
Blod urea nitrogen (µmol/L)	2.12	2.1	2.1	2.11± 0.01	2.3	2.6	2.8	2.57± 0.25	1.87	2	2.19	2.02± 0.16	2.42	2.7	2.89	2.7± 0.24
Uric acid (µmol/L)	1.27	1.36	1.36	0.21± 0.01	0.33	0.38	0.4	0.37± 0.04	0.3	0.27	0.29	0.29± 0.02	0.28	0.29	0.31	0.3± 0.02

* Values in parentheses represents overall mean ±SD.

days trial group. In Control group, the uric acid values were found to be 1.27 mmol/L, 1.36 mmol/L, and 1.36 mmol/L in 35, 65 and 90 days of trial periods respectively. The differences among the groups were statistically significant ($P<0.05$). The minimum uric acid values were observed at the end of 35-day, 65-day and 90-day trial periods in the Control group. The maximum value was observed in $CuSO_4$ group at the end of 90-day trial period. These results indicate that the Zeolite is useful in controlling the uric acid increase due to $CuSO_4$.

The direct bilirubin (DBIL) concentration in $CuSO_4$ group 0.47 µmol/L at the end of 35 days, 0.52 µmol/L at the end of 65 days, and 0.57 µmol/L at the end of 90 days trial group. In Control group, the direct bilirubin values were found to be 0.21 µmol/L, 0.23 µmol/L, and 0.23 µmol/L in 35, 65 and 90 days of trial periods respectively. The differences among the groups were statistically significant ($P<0.05$). The minimum direct bilirubin values at the end of 35-day, 65-day and 90-day trial periods were observed in Control group. The maximum direct bilirubin value was observed in $CuSO_4$ group at the end of 90-day trial period. These results indicate that the Zeolite is useful in controlling the direct bilirubin increase in presence of $CuSO_4$.

Figure 1 depicts the general trend in biochemical changes in the fish after exposure to $CuSO_4$, Zeolite and their mixture. $CuSO_4$ administered at 2 mg/L drastically reduce the total protein (73%) and albumin (41%). Conversely increase in globulin (142%), total bilirubin (70%), direct bilirubin (104%), blood urea nitrogen (33%), creatinine (65%) and uric acid (186%) show liver and kidney damage. The treatment with Zeolite show the same trend as $CuSO_4$, though the extent of damage is much less.

The increase in globulin (77%), total bilirubin (33%), direct bilirubin (17%), creatinine (45%) and uric acid (131%) after zeolite treatment is significantly lesser than for $CuSO_4$ exposure. The mixture of CuSO4 and zeolite seem to accentuate the liver damage (total bilirubin, + 51%; direct bilirubin, + 96%, total protein, -27%) and kidney (blood urea nitrogen, (+38%; creatinine, +55%) damage. Uric acid level (+68%) and globulin (+28%) however show moderating effect of Zeolite on $CuSO_4$ toxicity.

DISCUSSION

It has clearly been shown that zeolite minerals addition into the feeds has contributed to the health status, feed efficiency, and growth rates. Moreover, when zeolites were part of the diet, it was observed that the intestinal disease incident throughout the young animals showed the tendency towards decrease (Mumpton, 1999).

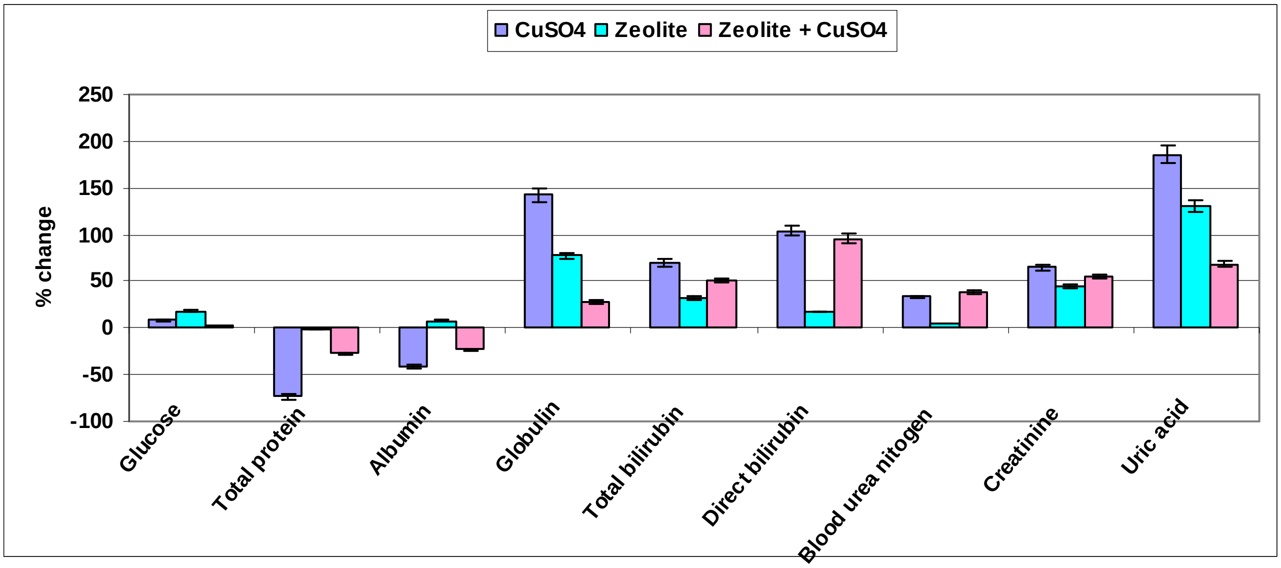

Fig. 1. Effect of $CuSO_4$, Zeolite and their combination on percent increase or decrease in the various biochemical components of blood sera of *Cyprinus carpio.*

It was reported that 2-5% of zeolite addition into the feeds of cattle has led to increase in body weight and decrease in diarrhea incidence throughout the animals. Zeolite minerals bound the free ammonia in gastrointestinal tract, and thereby prevent the buildup of ammonia to toxic levels throughout the system (Pond *et al.,* 1981). Determining significantly low growth percentages in all groups except for the control might be interpreted as that copper effected fish negatively in the present study.

The heavy metal exposure of fish leads to harmful effects on immunologic responses of fish (Mumpton, 1985). The serum globulin content was higher in the group exposed to $CuSO_4$ in this study. It is thought that this was the indication of copper toxicity.

Total glucose level was low in $CuSO_4$ compared to control group. It might be the sign of hypoglycemia occurring as a result of increases or decreases in liver enzymes' activity under the stress created by copper exposure (Mumpton, 1985).

There was a decrease in total blood protein of fish exposed to $CuSO_4$, which might be due to a nephritic syndrome (Fast *et al.,* 1975) or a liver disorder (Fukushima, 1980).

The total bilirubin and direct bilirubin levels in $CuSO_4$ group increased compared to control group. The creatinine, blood urea nitrogen and uric acid levels from the blood of $CuSO_4$ group were higher than that of Zeolite group. Similar results were reported in the presence of toxic materials such as exposure to copper (England, 1975), to pesticides (Fukushima, 1980), and to some other heavy metals and nitrate (Fujimori and Moriya, 1973). Obtaining these higher levels indicate the toxic effect of $CuSO_4$. The higher concentrations of blood urea nitrogen and creatinine have shown to be indicators of gill and kidney dysfunction (Fujimori and Moriya, 1973; Fugii, 1974).

Having higher or lower concentrations of hematological parameters of copper exposured group compared to control or zeolite group indicates the positive effects of zeolite used in this study. Similarly, clinoptilolite treatment protected the common carp against copper toxicity by decreasing the adverse effects of lead. It has been thought that high cation capacity of the clinoptilolite removed the copper load within the water, and therefore fish were subject to exposure low concentration copper (Tepe *et al.,* 2004).

CONCLUSION

Despite that the zeolites are widely used in industry, agriculture, animal husbandry and environmental protection, their effects in appropriate animal models and possible medical applications await detailed studies (Martin-Kleiner *et al.,* 2001). In the present study, it has been observed that the using zeolite in water had effects to prevent copper toxicity. Therefore, it may be suggested using zeolite in aquaculture to provide a safe environment for fish and to get higher production. On the other hand, the dietary and antibiotic effects of zeolites should also be determined in further experiments.

Statement of conflict of interest

Authors have declared no conflict of interest.

REFERENCES

Atamanalp, M., Yanık, T., Haliloglu, I. and Aras, M.S., 2002. Alterations in the hematological parameters of rainbow trout (*Oncorhynchus mykiss*) exposed to cypermethrin. *Israeli J. Aquacult.-Bamidgeh.*, **54**: 99-103.

Aziz, F., Amin, M. and Shakoori, A.R., 1993. Toxic effects of cadmium chloride on the haematology of fish, *Tilapia mossambica*. *Proc. Pakistan Congr. Zool.*, **13**: 141-154.

Blaxhall, P.C. and Daisley, K.W., 1973. Routine haematological methods for use with fish blood. *J. Fish Biol.*, **5**: 771-781.

Carvalho, C.S. and Fernandes, M.N., 2006. Effect of temperature on copper toxicity and hematological responses in the Neotropical fish *Prochilodus scrofa* at low and high pH. *Aquaculture*, **251**: 109-117.

England, D.C., 1975. Effect of zeolite on incidence and severity of scouring and level of performance of pigs during suckling and early post-weaning, *Rept. 17th Swine Day Spec. Rept. 447, Agricul. Exp. Sta., Oregon State University*, pp. 30-33.

Fast, A.W., Overholtz, W.J. and Tubb, R.A., 1975. Hypolimnetic oxygenation using liquid oxygen. *Water Resour. Res.*, **11**:294-299

Figueiredo-Fernandes, A., Ferreira-Cardoso, J.V., Garcia-Santos, S., Monteiro, S.M., Carrola, J., Matos, P. and Fontainhas-Fernandes, A., 2007. Histopathological Changes in liver and gill epithelium of Nile tilapia, *Oreochromis niloticus*, exposed to waterborne copper. *Pesquisa Vet. Brasil.*, Rio de Janeiro, **27**: 103-109.

Fugii, S., 1974. *Heavy metal adsorption by pulverized zeolites*, Japan, Kokai 74,079, 849, Aug. 1.

Fujimori, K. and Moriya, Y., 1973. Removal and treatment of heavy metals in industrial wastewater. 1. Neutralization method and solidification by zeolites. *Asashi Garasukogyo Gijutsu Shoreikai Kenkyu, Hokoku*, **23**:243-246

Fukushima, Y., 1980. Effect of zeolite addition in feed on the physiological condition of cows, *Chikusan no Kenkyu*, **33**: 1393-1395.

Güllü, K., Acar, Ü., Tezel, R., and Yozukmaz, A., 2014. Replacement of fish meal with fish processing by-product silage in diets for the rainbow trout, *Oncorhynchus mykiss*. *Pakistan J. Zool*., **46**:1697-1703,

Gündoğdu, A., Culha, S., Kocbas, F., and Culha, M. 2016. Heavy metal accummulation in muscles and total bodies of *Mullus barbatus*, *Trachurus trachurus* and *Engraulis encrasicolus* captured from the Coast of Sinop, Black Sea. *Pakistan J. Zool*., **48**: 25-34.

Hamilton, M.A., Russo, R.C. and Thurston, R.V., 1977. Trimmed Speakarman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Sci. Technol.*, **11**:417-719

Kidwai, S. and Ahmed, M., 1999. Heavy Metal Bioassays on Selected Fauna from the Karachi Coast (Northwest Arabian Sea), *Pakistan J. Zool.*, **31**: 147-157.

Martin-Kleiner, I., Flegar-Mestric, Z., Zadro, R., Breljak, D., Janda, S.S., Stojkovic, R., Marusic, M., Radacic, M. and Boranic, M., 2001. The effect of the zeolite clinoptilolite on serum chemistry and hematopoiesis in mice. *Fd. Chem. Toxicol.*, **39**:717-727

Mazon, A.F., Monteiro, E.A.S., Pinhero, G.H.D. and Fernandes, M.N., 2002. Hematological and physiological changes induced by sort-term exposure to copper in the freshwater fish, *Prochilodus scrofa*. *Braz. J. Biol.*, **52**:621-631

Mumpton, F.A., 1985. Using Zeolites in Agriculture, Chapter VIII. In: *Innovative biological technologies for lesser developed countries*. Congress of the United States, Office of Technology Assessment. Washington D.C. pp. 127-158,

Mumpton, F.A., 1999. La roca: Uses of Natural zeolites in agriculture and industry. *Geol. Mineral. Hum. Welf.*, **96**:3463-3470

Nussey, G., Van Vuren J. H. J. and du Preez H. H., 1995. Effect of copper on the haematology and osmoregulation of the Mozambique tilapia, *Oreochromis mossambicus* (Cichlidae). *Comp. Biochem. Physiol.*, **11** IC: 369-380.

Nwani, C.D., Nwoye, V.C., Afiukwa, J.N. and Eyo, J.E., 2009. Assessment of heavy metal concentrations in the tissues (gills and muscles) of six commercially important freshwater fish species of Anambra River south-east Nigeria. *Asian J. Microbiol. Biotechnol. environ. Sci.*, **11**: 7–12.

Pond, W.G., Yen, J.T. and Hill, D.A., 1981. Decreased absorption of orally administered ammonia by clinoptilolite in rats. *Proc. Soc. exp. Biol. Med.*, **166**:369-373.

Rompala, J.M., Rutosky, F.W. and Putnam, D.J., 1984. *Concentrations of environmental contaminants from selected waters in Pennsylvania*. United States Fishery and Wildlife Service Report, Pennsylvania, USA.

Sastry, K.V. and Gupta, P.K., 1978. Alteration in the activity of some digestive enzymes by heavy metals and their reversal by a chelating agent. Lead nitrate intoxication, *Bull. environ. Contam. Toxicol*., **19**:549-555

Schjolden, J., Sorenson, J., Nilsson, G.E. and Poleo, A.B.S., 2007. Toxicity of the copper to Crucian carp (*Carassius carassius*) in soft water, *Sci. Total Environ.*, **384**:239-251

Silapajarn, O., Silapajarn, K. and Boyd, C.E., 2006. Evaluation of zeolite products used for aquaculture in Thailand. *J. World Aquacult. Soc.*, **37**:136-138.

Singh, D., Nath, K., Trivedi, S.P. and Sharma, Y.K., 2008. Impact of copper on haematological profile of freshwater fish, *Channa punctatus*. *J. environ. Biol. Lucknow*, **29**:

253-257.

Tepe, Y., Akyurt, I., Ciminli, C., Mutlu, E. and Çalışkan, M., 2004. Protective effect of clinoptilolite on lead toxicity in common carp (*Cyprinus carpio*). *Fresenius environ. Bull.*, **13**:639-642.

Vinodhini, R. and Narayanan, M., 2008. Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (common carp). *Int. J. environ. Sci. Technol.*, **5**:179–182

Winer, B.J., Brown, D.R. and Michels, K.M., 1991. *Statistical principles in experimental design*. 3rd ed. McGraw-Hill, New York

Woodward, D.E., Brumbaugh, W.G., Deloney, A.J., Little, E.E. and Smith, C.E., 1994. Effect of contaminant metals on fish in the Clark Fork River in Montana. *Trans. Am. Fish. Soc*., **123**: 51–62.

Yousafzai, A.M. and Shakoori, A.R., 2006. Bioaccumulation of chromium, nickel, lead, copper and zinc in the Tur putitora as an indicator of presence of heavy metals loads in River Kabul Pakistan. *Pakistan J. Zool.*, **8**: 341–347.

Yousafzai, A.M. and Shakoori, A.R., 2008. Heavy metal accumulation in the gills of an endangered south Asian freshwater fish as an indicator of aquatic pollution. *Pakistan J. Zool.*, **40**: 423-430.