ISSN 1819-1878

Asian Journal of Animal Sciences

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Asian Journal of Animal Sciences 6 (4): 154-163, 2012
ISSN 1819-1878 / DOI: 10.3923/ajas.2012.154.163

The Alterations in the Hematological Parameters of Endangered Caspian Brown Trout, *Salmo trutta caspius*, Exposed to Waterborne Mercuric Chloride

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ABSTRACT

In the present study, Caspian brown trout (*Salmo trutta caspius*) were exposed to environmentally subchronic concentrations of mercury chloride. The effect of this contaminant on blood parameters was investigated. *Salmo trutta caspius* was exposed to 3.5, 4.8 and 10 μg L^{-1} of $HgCl_2$ waterborne for 35 days. After 35 days of exposure, Red Blood Cell (RBC) count in three treated groups (1.3±0.02, 1.2±0.02 and 1.1±0.01, 10^6 mm^{-3}, respectively), Hemoglobin content (9.2±0.3, 8.02±0.3 and 7.1±0.4, g dL^{-1}, respectively), Hematocrit percentage (38.02±2.1, 39.3±2.6 and 34.1±1.8, respectively), Mean Corpuscular Volume (MCV; 257.4±48.7, 172.5±17.6 and 160.9±15.7 μm^3, respectively) and Mean corpuscular Hemoglobin (MCH; 72.6±3.2, 61.7±2.5 and 59.6±2.4, pg, respectively) were decreased when compared to the control (RBC; 1.3±0.01, 10^6 mm^{-3}, Hb; 9.6±0.2 g dL^{-1} Hematocrit percentage; 38.2±2.2, MCV; 268.7±41.4 μm^3 and MCH; 73.5±2.8 pg). The number of white blood cells (WBC; 0.73±0.06, 0.86±0.06 and 0.93±0.09, 10^4 mm^{-3}) and the Erythrocyte-Sedimentation Rate (ESR; 2.7±1.8, mm h^{-1}) increased in mercuric chloride treated fish. Mean Corpuscular Hemoglobin Concentration (MCHC) values in treated fish (30.7±4.4, 29.6±3.4 and 29.9±5.03%, respectively) were not different compared to control group (31.6±5.6%).

Key words: *Salmo trutta caspius*, toxicity, mercuric chloride, blood parameters

INTRODUCTION

Expansion in agricultural and industrial activity have a results in increasing heavy metals deposition in natural water. The presence and loading heavy metals in water is more serious than in land and regard as pollutants of environmental. The toxicity effect of heavy metal is not the same and its effect changes according environmental condition. Beckvar *et al*. (1996) noted that when the temperature elevated, oxygen content is low, salinities in marine environments reduced and in the presence of metals such as zinc and lead, toxicity was found to be greater. Among heavy metals, Mercury (Hg) is one of the most hazardous environmental pollutants, due to its toxicity and its accumulation in aquatic organisms. There are several form of mercury and methyl mercury is toxic that are used in aquaculture activity such as using fungicides, bactericides. Some fish such as salmonids are more sensitive to mercury.

Caspian brown trout, *Salmo trutta caspius*, is the one and the vulnerable species of Caspian Sea (Yousefian, 2010). The adults live in the Caspian Sea (10-13 ppt) but spawning occurs in freshwater rivers (such as 'Tonekabon-rood', Iran); fry live in rivers for a couple of months and then they return back to the Caspian Sea. The Caspian brown trout (*Salmo trutta caspius*) is

distributed in southern basin of Caspian Sea and recently their natural stocks are endangered (Yousefian, 2010). Therefore, Caspian brown trout were used as a model to study the impact of mercurial products contamination as no data are available on this species and also because they represents important species for recreational fishing in Caspian Sea. The migration of fish is reduced to the river due to several causes such as pollution, therefore artificial reproduction is practice for many of Caspian Sea fishes (Yousefian and Mousavi, 2008; Yousefian *et al*., 2008). The Caspian Sea is a lake and an enclosed water body that is fed from several freshwater rivers. Till now several studies have been reported in different aspect related to this lake e.g., Biology (Maal *et al*., 2012; Safary *et al*., 2010; Ghaemi *et al*., 2006), Oceanography (Jamshidi and Abu Bakar, 2010, 2011), plant sciences (Abkenar and Safarpour, 2007) and fishes study (Sadeghi *et al*., 2009).

Due to close condition of Caspian Sea, the fish are exposed to mercury and different heavy metal by the surrounding water or directly from food. To test of mercury effect in this fish several techniques and methods are suggested. Among Them hematological analyzing is a fast and cheap techniques are used to illustrate the status of fish. Hematological changes in some fish exposed to various toxicants have been studied in *Oncorhynchus mykiss* (Atamanalp *et al*., 2002), *Salmo trutta fario* (Atamanalp *et al*., 2010), rainbow trout, *Salmo gairdneri* (Niimi and Lowe-Jinde, 1984), *Oreochromis aureus* Allen (1994), Persian Sturgeon, *Acipenser persicus* (Khoshnood *et al*., 2011) and *Clarias batrachus* (Maheswaran *et al*., 2008).

The present study was undertaken to analyze the impact of sub-chronic concentrations of mercuric chloride on haematological parameters of Caspian brown trout (*Salmo trutta caspius*).

MATERIAL AND METHODS

Fish stock: The experiment was carried out at the Kalardasht Salmonids Reproduction Center (KSRC), Iran, after the spawning season of Caspian brown trout. Adult fish with 2-years-old (n = 44), female or male Caspian brown trout (*S. trutta caspius*) with a mean (±SD) weight of 267±19.68 g (total length: 35-45 cm) were randomly selected and transferred to one 6 m^3 concrete raceways supplied with running water, approximately 4 weeks prior to onset the experiment during March, 2011. Fish were maintained under natural water temperature (10±0.5°C) and photoperiod. Fish were acclimated for 2 weeks before they were used for the experiments.

Experimental design: In order to expose fish subjects to Mercury chloride, a flow through system was used which has been described previously (Lahnsteiner *et al*., 2005a, b) (Fig. 1). Briefly, the system consisted of four 0.5 m^3 tanks. The tanks were supplied with well water of 8°C, an oxygen content of >90% saturation (pH levels and total water hardness were 7.6 and 105 mg in $CaCO_3$, respectively). Mercuric chloride was added by means of an injection pump. Consequently, the Mercuric chloride concentrations were adjusted by changing the injection rates. Well water was supplied via a storage reservoir in a height of 1.5 m above the tanks where after the water flow was regulated by reduction pieces (diameter reduction from 30 to 6 mm) (Fig. 1).

This set up reduced variations in well water flow rates to <2.0%. The injection pumps were precise, having variations of <0.5%. Final mercuric chloride concentrations were calculated based on the flow rate of uncontaminated well water and on the injection rate of mercuric chloride.

The stock of mercuric chloride, containing 3 g $HgCl_2$ (Merck) was diluted to 580 μg L^{-1} with well water. In the experiment estimated final mercuric chloride concentrations of 3.5, 4.8 and 10 μg L^{-1} were used. To obtain these concentrations the well water flow through rate and the mercuric

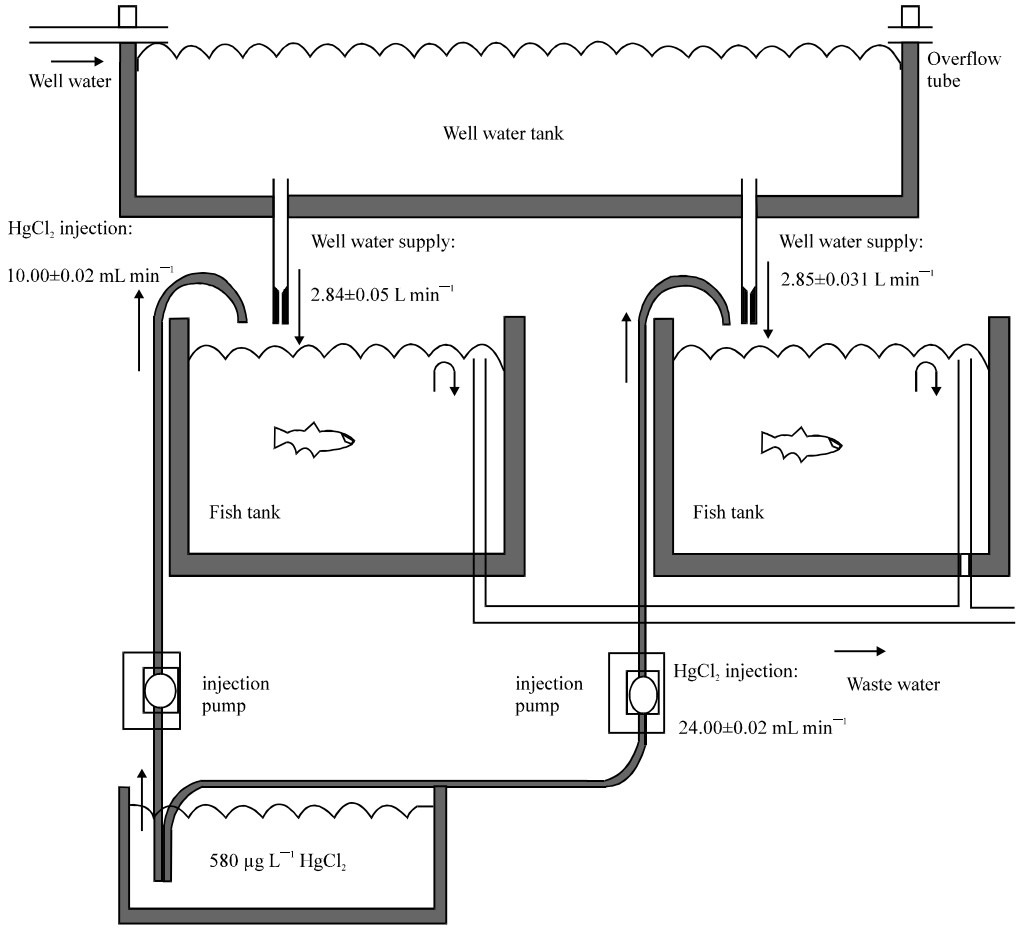

Fig. 1: Scheme of the flow through system used for exposure of Caspian brown trout to mercuric chloride ($HgCl_2$). Tanks had a volume of 0.5 m^3 and well water supply was adjusted by reduction pieces which reduced the tube diameter from 30 to 6 mm. The legend is relevant for tanks with final mercuric chloride concentrations of 3.5 and 4.80 µg L^{-1} (Lahnsteiner *et al*., 2005a, b)

chloride injection rate were adjusted in the following way; tank 1: well water flow through rate: 2.84±0.05 L min^{-1}, Mercuric chloride injection rate: 10.00±0.02 mL min^{-1}, resulting estimated Mercuric chloride exposure level 3.5 µg L^{-1}, tank 2: well water flow through rate: 2.85±0.03 L min^{-1}, Mercuric chloride injection rate: 24.00±0.02 mL min^{-1}, estimated mercuric chloride exposure level: 4.8 µg L^{-1}, tank 3: well water flow through rate: 2.72±0.05 L min^{-1}, mercuric chloride injection rate: 52.00±0.01 mL min^{-1}, estimated mercuric chloride exposure level: 10.0 µg L^{-1} and tank 4 served as control.

Before the onset of the experiment the system was calibrated for 1 week to reach equilibrium between potential mercuric chloride adsorption on equipment and concentrations in water. In the beginning of the experiment the system was controlled daily on well water flow rates and injection rates.

Experiment: To determine the influence of sub-chronic concentrations of mercuric chloride on blood parameters, 2-years Caspian brown trout were exposed to mercuric chloride during the

experiment. Four experimental groups were formed each consisting of 11 fish. Also the fish density (approximately 3 kg m^3) was approximately similar in the four tanks. Fish groups 1-3 were exposed to the described mercuric chloride concentrations for 35 days, group 4 served as control. Fish were fed two times per week with commercial pellet and had a natural photoperiod. At the end of 35 days exposure, fish were taken out and their blood was subjected to hematological analysis (Aziz *et al.*, 1993; Santhakumar *et al.*, 1999).

The collected blood from the control and mercuric chloride treated fishes was obtained by severance of caudal peduncle and collected in Eppendorf tubes containing EDTA anticoagulant (Mgbenka *et al.*, 2003). These treated and control blood samples were used to estimate the haematological parameters. Approximately, 2 mL venous blood was drawn from each group using heparin as an anticoagulant and for estimation of Red Blood Cell (RBC) count and the total White Blood Cell (WBC) (Blaxhall and Daisley, 1973). Hemoglobin (Hb) concentration (Soivio and Oikari, 1976) and Erythrocyte Sedimentation Rate (ESR) whereas the Mean Corpuscular Volume (MCV), the Mean Corpuscular Hemoglobin (MCH) and the Mean Corpuscular Hemoglobin Concentration (MCHC) were calculated according to Reddy and Bashamohideen (1989).

Statistics analysis: All haematological values of the *S. trutta caspius* are reported as means±SD. One way analysis of variance was used to determine significant variation between the treatments existed. Difference between means were determined and compared by multiple comparison test (Duncan). All tests used a significance level of $p<0.05$. The data were analysed by SPSS 18 software.

RESULTS

The erythrocyte count of healthy controls showed a mean value of 1.3±0.01, 10^6 mm^{-3}. The fishes exposed to environment relevant concentrations of mercuric chloride showed mean±SD values of RBC's as 1.3±0.02, 1.2±0.02 and 1.1±0.01, 10^6 mm^{-3} for 3.5, 4.8 and 10 μg L^{-1} treatments, respectively. The treatment with mercuric chloride was found to inflict a drastic reduction in the total count of RBC's. The reduction was dosage dependent; as concentration of mercuric chloride increased the RBC levels declined (Table 1). The values mentioned above showed a significant decrease when compared to the control ($p<0.05$). There was no significant change in RBC's count between treated group at 3 μg L^{-1} concentration of mercuric chloride and the control group ($p>0.05$).

The results of the total count of white blood cells revealed that the blood of the control fish showed a mean±SD value of 0.7±0.01, 10^4 mm^{-3}. The fishes exposed to environment sub-chronic concentrations showed the mean values of WBC as 0.73±0.06, 0.86±0.06 and 0.93±0.09, 10^4 mm^{-3} for 3.5, 4.8 and 10 μg L^{-1} of mercuric chloride treatments, respectively (Table 1). The values mentioned above showed a significant increase when compared to the control ($p<0.05$).

Hematocrit and MCHC percentages in treated group were not significantly change in compared to control group ($p>0.05$). The values of ESR was showed a significant increase only in the treated group at 10 μg L^{-1} mercuric chloride concentration ($p<0.05$). Hematocrit values showed a significant decrease in treated group at 10 μg L^{-1} concentration of waterborne mercury chloride (34.1±1.8%) in compared to control group (38.2±2.2%, $p<0.05$).

The control fishes showed mean±SD value of 9.6±0.2 g dL^{-1} for haemoglobin. The fishes were exposed to different concentrations of mercuric chloride showed the haemoglobin mean±SD values

Table 1: Changes in the hematological parameters of Caspian brown tout exposed to mercuric chloride for 35 days

Parameters	Control (n = 11)	3.5 μg L^{-1} (n = 11)	4.8 μg L^{-1} (n = 11)	10 μg L^{-1} (n = 11)
RBC (10^6 mm^{-3})	1.3±0.01	1.3±0.02^{NS}	1.2±0.02*	1.1±0.01**
WBC (10^4 mm^{-3})	0.7±0.01	0.73±0.06*	0.86±0.06*	0.93±0.09*
Hematocrit (%)	38.2±2.2	38.02±2.1^{NS}	39.3±2.6^{NS}	34.1±1.8*
ESR (mm h^{-1})	1.8±1.7	1.9±1.6^{NS}	1.9±1.6^{NS}	2.7±1.8*
Hemoglobin (g dL^{-1})	9.6±0.2	9.2±0.3^{NS}	8.02±0.3*	7.1±0.4*
MCV (μm^3)	268.7±41.4	257.4±48.7^{NS}	172.5±17.6*	160.9±15.7**
MCH (pg)	73.5±2.8	72.6±3.2^{NS}	61.7±2.5*	59.6±2.4*
MCHC (%)	31.6±5.6	30.7±4.4^{NS}	29.6±3.4^{NS}	29.9±5.03^{NS}

NS: Not Significant, *Significant ($p<0.05$), **Highly significant ($p<0.01$)

of 9.2±0.3, 8.02±0.3 and 7.1±0.4 g dL^{-1} haemoglobin at 3.5, 4.8 and 10 μg L^{-1} treatment, respectively (Table 1). The values for treatments showed a significant decrease when compared to the control ($p<0.05$). There was no significant change in the haemoglobin assessment between treated group at 3.5 μg L^{-1} concentration of mercuric chloride and the control group ($p>0.05$, Table 1).

RBC and MCV parameters in treated groups were found as very significant decrease compared to control group ($p<0.01$). The values of MCV and MCH parameters showed a significant decrease (at 4.8 and 10 μg L^{-1} concentration) when compared to the control ($p<0.05$).

DISCUSSION

Our findings showed that mercuric chloride had some effect on the hematological parameters of Caspian brown trout. It is shown that the 10 μg L^{-1} dose examined in this study also causes important changes in the hematological properties of Caspian brown trout. There is a lack of studies regarding to researches related to hematological responses in Caspian brown trout chronically and sub-chronically exposed to heavy metals. In the present study a series of hematological parameters were examined in *Salmo trutta caspius* after exposure to *in vitro* doses of mercuric chloride over a period of 35 days.

The use of immune system parameters to assess hormonal growth hormonal effect alterations in fishes experiencing heavy metal exposure and interest in defense mechanisms stem from the need to develop healthy management tools to support a rapidly growing aquaculture industry (Yousefian and Shirzad, 2011; Jones, 2001). However other techniques such as using supplementary feed or using inhibitory effects of some organism in preventing disease should not put out of our mind (Srinivasan *et al.*, 2007; Radfar and Farhoomand, 2008; Yesillik *et al.*, 2011).

The results of the present investigation show that the mercuric chloride treatment inflicted a drastic reduction in the total count of RBC's. The reduction was dosage dependent. Chloride mercury exposure, decreased RBC, hematocrit and haemoglobin values of *Salmo trutta caspius* in treated group. The difference was evaluated as highly significant ($p<0.01$) in the statistical analysis. Previously decline in RBC values and anemia were reported in fishes such as *Salvelinus fontinalis* (Maheswaran *et al.*, 2008), *Salmo gairdneri* (Johansson-Sjobeck and Larsson, 1979), *Anguilla anguilla* (Haux and Larsson, 1982) which were exposed to heavy metals.

The decrease in haemoglobin concentration may be is due to decrease in oxygen carrying capacity of the treated fish by the effect of the chloride mercury. Similarly result are stated by Atamanalp and Yanik (2003) that demonstrated a significant decrease in Hb content of *O. mykiss* and reported this situation with Hb destroying or to a decrease in the rate of Hb synthesis.

In the fish *Salmo trutta caspius* exposed to mercuric chloride, haemoglobin percentage decreased significantly. This indicates that mercury caused anaemia and this may be due to a decreased rate of production of red blood cells or an increased loss of these cells. Our finding is in agreement with those statement of Gill and Epple (1993). They have attributed anemia to impair erythropoiesis due to a direct effect of metal on hematopoietic centers and accelerate erythroclasis due to altered membrane permeability or increased mechanical fragility. Anyway the effect of mercury in decrease in the haemoglobin concentration can be due to increase in the rate of haemoglobin destroying or prevent its synthesis.

Mercury in blood have effect in hematology as we found in our experiment on Caspian sea trout and also may have biochemical changes in blood. According to Clarkson *et al.* (1961) about half of the mercury in blood is associated with red blood cells and the remaining half forms a complex with serum albumin by combination with sulfhydryl groups. Haematocrit decreased significantly in the mercuric chloride-treated fish when compared with the control fish. The disturbed haemoglobin synthesis due to an effect of lead on ALA-D may also result in anemia (Santos and Hall, 1990).

In the present investigation, leucocyte concentrations showed greater and quite different pattern of change with the effect of mercury when compared with the erythrocyte levels of the control group. White blood cells play a major role in the defense mechanism of the fish and consist of granulocytes, monocytes, lymphocytes and thrombocytes. Granulocytes and monocytes function as phagocytes to salvage debris from injured tissue and lymphocytes produce antibodies (Wedemeyer and Mcleay, 1981).

In present study, blood of all experimental groups contained higher concentrations of WBC (leucocytes) than those of controls. Allen (1994) observed increased WBC (leucocytes) counts in *Oreochromis aureus* after mercury exposure. An increase in WBC (lymphocyte) number may be the compensatory response of lymphoid tissues to the destruction of circulating lymphocytes (Shah and Altindag, 2005).

In the present study, white blood cell count was observed a significant increase in the treatment group in compared with control group and this is consistent with other previous studies (Santhakumar *et al.*, 1999).

The increase in WBC observed in the present study could be attributed to a stimulation of the immune system in response to tissue damage caused by mercuric chloride. Brandao *et al.* (2009) found a reduction in some immunological parameters (platelet, leukocyte and lymphocyte counts) and increase in neutrophil and monocyte percentages were demonstrated in $HgCl_2$ exposed.

The increase in neutrophil and monocyte percentages, which represents the activity of the first and second lines of defense against the cellular damage after mercury exposure has been reported previously (Perlingerio and Queiroz, 1995). According to Wedemeyer *et al.* (1990), the suppression of the immune system increase the susceptibility to diseases in fish, a significant aspect considering the presence of heavy metal in natural ecosystems as a result of human activities.

Significant decrease were observed in MCH and MCV values are similar to other studies. MCH was significantly decreased in *C. idella* after 48 h exposure to fenvalerate (Mughal *et al.*, 1993). The decreased MCH and MCV levels may be a sign of hypochromic microcytic anemia (Shakoori *et al.*, 1996). In contrast to our findings, there were increases in Hb and MCH values in *T. mossambica* exposed to cadmium chloride (Aziz *et al.*, 1993) and *C. idella* exposed to sublethal doses of mercuric chloride (Shakoori *et al.*, 1996) and in *O. mykiss* exposed to cypermethrin (Atamanalp *et al.*, 2002).

RBC indices such as MCV and MCHC fell during prolonged exposure to Mercuric chloride. The decrease in other hematological parameters is attributable to reduced MCV (Ahmad *et al*., 1995) while Carvalho and Fernandes (2006) found no significant effects in MCHC fish, *Prochilodus scrofa* on copper toxicity, that are consistent with present study regarding no significant in MCHC values.

In addition, in present study, the Erythrocyte Sedimentation Rate (ESR) showed significant increase in treated fish. The increase in ESR shows that fish were intoxicated by Chloride mercury. This finding was similar to Atamanalp *et al*. (2002) and Kumar *et al*. (1999), these studies demonstrated that the cypermethrin exposure to *O. mykiss* and deltamethrin exposure to *H. fossilis*, causing significant increase in ESR values of fish.

The present study showed that under experimental conditions, blood parameters were sensitive to different aspects of heavy metals exposure. In conclusion, the major finding of present study were indicate that the sub-acute mercury concentrations tested, may cause several changes in the hematological parameters of treated fish, therefore, estimation of these indices, could provide a useful indicator regarding to ecosystem pollution.

In addition, the results of the present investigation show that mercuric chloride caused immunological impairments in *Salmo trutta caspius*, which suggests that the heavy metal may weaken the immune system and may result in severe physiological problems, ultimately leading to the death of fish.

ACKNOWLEDGMENT

The author would like to thank the staff of the Kelardasht Salmonids Reproduction Center (KSRC), Kelardasht, Iran, for providing experimental material and fish.

REFERENCES

Abkenar, K.T. and E. Safarpour, 2007. Performance of planted maple in Western Guilan province, Iran. Asian J. Plant Sci., 6: 1143-1146.

Ahmad, F., S.S. Ali and A. Shakoori, 1995. Sublethal effects of Danitol (Fenpropathrin), a synthetic pyrethroid, on freshwater Chinese grass carp, *Ctenopharyngodon idella*. Folia. Biol. (Krakow), 43: 151-159.

Allen, P., 1994. Changes in the hematological profile of the cichlid, *Oreochromis aureus* (Steindachner) during acute inorganic mercury intoxication. Comp. Biochem. Physiol. Part C: Pharmacol. Toxicol. Endocrinol., 108 C: 117-121.

Atamanalp, M., M.S. Keles, H.I. Haliloglu and M.S. Aras, 2002. The effects of cypermethrin (a synthetic pyrethroid) on some biochemical parameters (Ca, P, Na and TP) of rainbow trout (*Oncorhynchus mykiss*). Turk. J. Vet. Anim. Sci., 26: 1157-1160.

Atamanalp, M. and T. Yanik, 2003. Alterations in the hematological parameters of rainbow trout, *Oncorhynchus mykiss*, exposed to Mancozeb. Turk. J. Vet. Anim. Sci., 27: 1213-1217.

Atamanalp, M., E.M. Kocaman, A. Ucar and G. Alak, 2010. The alterations in the hematological parameters of brown trout *Salmo trutta fario*, exposed to cobalt chloride. J. Anim. Vet. Adv., 9: 2167-2170.

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

Beckvar, N., J. Field, S. Salazar and R. Hoff, 1996. Contaminants in aquatic habitats at hazardous waste sites: Mercury. NOAA Technical Memorandum NOS ORCA 100. Hazardous Materials Response and Assessment Division, National Oceanic and Atmospheric Admin.

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

Brandao, R., L. Pinto, C. Borges and W. Nogueira, 2009. Concomitant administration of Sodium 2, 3-dimercapto-1-propane sulphonate (DMPS) and diphenyl diselenide reduces effectiveness of DMPS in restoring damage induced by mercuric chloride in mice. Food Chem. Toxicol., 47: 1771-1778.

Carvalho, C.S. and M.N. Fernandes, 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.

Clarkson, T.W., J. Gatzy and C.H. Dalton, 1961. Studies of the equilibrations of mercury vapor with blood. University of Rochester. Atomic Energy Project, Report NR. UR-582.

Ghaemi, E.O., K. Ghazesaeed, F.F. Nasab, Z. Hashemzade, S. Vatani, M. Mohamedi and A.R. Mansourian, 2006. *Mycobacterium marinum* infection in caviar fishes and fisherman`s in a Caspian Sea province in North of Iran. J. Boil. Sci., 6: 1150-1152.

Gill, T.S. and A. Epple, 1993. Stress-related changes in the hematological profile of the American eel (*Anguilla rostrata*). Ecotoxicol. Environ. Saf., 25: 227-235.

Haux, C. and A. Larsson, 1982. Influence of inorganic lead on the biochemical blood composition in the rainbow trout *Salmo gairdneri*. Ecotoxicol. Environ. Safety, 6: 28-34.

Jamshidi, S. and M.N.B. Abu Bakar, 2010. An analysis on sound speed in seawater using CTD data. J. Applied Sci., 10: 132-138.

Jamshidi, S. and N.B. Abu Bakar, 2011. Oceanographic study in coastal waters of babolsar. Asian J. Earth Sci., 4: 1-8.

Johansson-Sjobeck, M.L. and A. Larsson, 1979. Effects of inorganic lead on delta-aminolevulinic acid dehydratase activity and haematological variables in the rainbow trout, *Salmo gairdneri*. Arch. Environ. Contam. Toxicol., 8: 419-431.

Jones, S.R.M., 2001. The occurrence and mechanisms of innate immunity against parasites in fish. Dev. Comp. Immunol., 25: 841-852.

Khoshnood, Z., S. Khodabandeh, M. Shahryari, S. Moghaddam and S.M. Khorjestan, 2011. Histopathological and pathomorphological effects of mercuric chloride on the gills of Persian sturgeon, *Acipenser persicus*, fry. Int. J. Nat. Resour. Marine Sci., 1: 23-32.

Kumar, S., S. Lata and K. Gopal, 1999. Deltamethrin induced physiological changes in freshwater catfish *Heteropneustes fossilis*. Bull. Environ. Contam. Toxicol., 62: 254-258.

Lahnsteiner, F., B. Berger, F. Grubinger and T. Weismann, 2005. The effect of 4-nonylphenol on semen quality, viability of gametes, fertilization success, and embryo and larvae survival in rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicol., 71: 297-306.

Lahnsteiner, F., B. Berger, M. Kletzl and T. Weismann, 2005. Effect of bisphenol A on maturation and quality of semen and eggs in the brown trout, *Salmo trutta f. fario*. Aquat Toxicol., 75: 213-224.

Maal, K.B., M. Bouzari and F.A. Zavareh, 2012. Characterization of two lytic bacteriophages of *Streptococcus sobrinus* isolated from Caspian sea. Asian J. Biol. Sci., (In press).

Maheswaran, R., A. Devapaul, S. Muralidharan, B. Velmurugan and S. Ignacimuthu, 2008. Haematological studies of fresh water fish, *Clarias batrachus* (L.) exposed to mercuric chloride. Int. J. Integ. Biol., 2: 49-54.

Mgbenka, B.O., N.S. Oluah and I. Umeike, 2003. Effect of gammalin 20 (Lindane) on differential white blood cell counts of the African catfish, *Clarias albopunctatus*. Bull. Envir. Contamination Toxicol., 71: 248-254.

Mughal, A.L., M.J. Iqbal and A.R. Shakoori, 1993. Toxicity of short term exposure of sublethal doses of a synthetic pyrethroid, fenvalerate, on the Chinese grass carp, ctenopharyngodon idella. Proc. Seminar Aquault. Develop. Pak., 5: 49-74.

Niimi, A.J. and L. Lowe-Jinde, 1984. Differential blood cell ratios of rainbow trout (*Salmo gairdner*) exposed to methylmercury and chlorobenzenes. Arch. Envir. Contamination Toxicol., 13: 303-311.

Perlingerio, R.C. and M.L. Queiroz, 1995. Measurement of the respiratory burst and chemotaxis in polymorphonuclear leukocytes from mercury exposed workers. Hum. Exp. Toxicol., 14: 281-286.

Radfar, M. and P. Farhoomand, 2008. The role of probiotic and source of lactose as feed additives on performance and gut improvement in broilers. Asian J. Anim. Vet. Adv., 3: 179-182.

Reddy, P.M. and M. Bashamohideen, 1989. Fenvalerate and cypermethrin induced changes in the haematological parameters of *Cyprinus carpio*. Acta. Hydrochim. Hydrobiol., 17: 101-107.

Sadeghi, S., M. Hedayati and S. Jamili, 2009. Determination of sperms` ATP content of golden grey mullet (*Mugil auratus*) at different conditions. Biotechnology, 8: 375-379.

Safary, A., M.R. Ardakani, A.A. Suraki, M.A. Khiavi and H. Motamedi, 2010. Isolation and characterization of biosurfactant producing bacteria from Caspian sea. Biotechnology, 9: 378-382.

Santhakumar, M., M. Balaji and K. Ramudu, 1999. Effect of sublethal concentrations of monocrotophos on erythropoietic activity and certain hematological parameters of fish *Unibus testudineus* (Bloch). Bull. Environ. Contam. Toxicol., 63: 379-384.

Santos, M.A. and A. Hall, 1990. Influence of inorganic lead on the biochemical blood composition of the eel, *Anguilla anguilla* L. Ecotoxicol. Environ. Saf., 20: 7-9.

Shah, S.L. and A. Altindag, 2005. Alterations in The Immunological Parameters of Tench (*Tinca tinca* L. 1758) after acute and chronic exposure to lethal and sublethal treatments with mercury, cadmium and lead. Turk. J. Vet. Anim. Sci., 29: 1163-1168.

Shakoori, A.R., A.L. Mughal and M.J. Iqbal, 1996. Effects of sublethal doses of fenvalerate (a synthetic pyrethroid) administered continuously for four weeks on the blood, liver and muscles of a freshwater fish, *Ctenopharyngodon idella*. Bull. Environ. Contam. Toxicol., 57: 487-494.

Soivio, A. and A. Oikari, 1976. Haematological effects of stress on a teleost *Esox lucius* L. J. Fish Biol., 8: 397-411.

Srinivasan, P., P. Ramasamy, G.P. Brennan and R.E.B. Hanna, 2007. Inhibitory effects of bacteriophages on the growth of *Vibrio* sp., pathogens of shrimp in the Indian aquaculture environment. Asian J. Anim. Vet. Adv., 2: 166-183.

Wedemeyer, G.R. and D.J. Mcleay, 1981. Methods of Determining the Tolerance of Fish to Environmental Stressors. In: Stress and Fish, Pickering, A.D. (Ed.). Academic Press, New York, USA., pp: 247-275.

Wedemeyer, G.A., B.A. Barton and D.J. McLeay, 1990. Stress and Acclimatation. In: Methods for Fish Biology, Schreck, C.B. and P.B. Moyle, (Eds.). American Fisheries Society, Bethesda, MD., pp: 451-489.

Yesillik, S., N. Yildirim, A. Dikici, A. Yildiz and S. Yesillik, 2011. Antibacterial effects of some fermented commercial and homemade dairy products and 0.9% lactic acid against selected foodborne pathogens. Asian J. Anim. Vet. Adv., 6: 189-195.

Yousefian, M. and H. Mosavi 2008. Spawning of South Caspian Kutum (*Rutilus frisii kutum*) in most migratory river of South Caspian Sea. Asian J. Anim. Vet. Adv., 3: 437-442.

Yousefian, M., H.G. Gezel and H.F. Masoud, 2008. Induction of ovulation in endemic *Chalcarburnus* (sic) chalcoides, living in the Caspian Sea, using LRH-Aa combined with metoclopramide. Afr. J. Biotechnol., 7: 4199-4201.

Yousefian, M., 2010. Stock identification and genetic variation at microsatellite loci of Caspian sea salmon (*Salmo trutta caspius*.). World J. Fish Mar. Sci., 2: 508-512.

Yousefian, M. and E. Shirzad, 2011. The review of the effect of growth hormone on immune system, metabolism and osmoregulation of fish. Aust. J. Basic Appl. Sci., 5: 467-475.