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ORIGINAL ARTICLE

Hematotoxic effects of direct infusion of crude diesel oil on juvenile great sturgeon *Huso huso*

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Abstract Changes in the hematological and immunological parameters of fish due to anthropological pollutants, may lead to hematotoxic and immunotoxic effects. The objectives of current study were to determine the experimental effects of direct infusion of crude diesel oil on hematological and immunological features of juvenile great sturgeon *Huso huso*. During this toxicity test, juveniles of beluga were exposed to the acute doses (5, 10, 20, 40, 100, 500, and 1,000 ppm) of direct infusion crude diesel oil for 0, 48 h and 7 days. WBC, MCH, MCHC, and neutrophil in fish exposed to crude diesel oil for 48 h were significantly greater compared to the respective control groups and RBC, Hb, Ht, MCV, and lymphocyte were significantly lower than control groups ($P \leq 0.05$). Eosinophils did not vary significantly in the groups exposed to 48 h crude diesel oil compared to the respective control groups ($P > 0.05$). Furthermore, 7-day exposures showed that WBC and Neutrophil were significantly greater compared to the respective control groups ($P \leq 0.05$) and MCV was depleted within the crude diesel oil adjacency.

Keywords Biomarker · Diesel oil · Hematology · Immunology · Pollution · Sturgeon

Introduction

Hematological indices and blood parameters could be useful to evaluate the effects of contaminants such as oil pollutions in fish (Oliveira Ribeiro et al. 2006). Hematological indices are more often used when clinical diagnoses of fish physiology are used to determine sub-lethal exposure of pollutants (Kim et al. 2008). Blood indices are considered as health indices of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to xenobiotics (Adhikari et al. 2004).

Fish blood is sensitive to pollution-induced stress and its alternation due to the hematological and immunological parameters can be used as toxicity indices of xenobiotics (Sancho et al. 2000). Although fish blood indices have been increasingly examined in marine environmental monitoring as valuable parameters of physiological changes in the presence of pollutants, the lack of basic knowledge about the blood response to stressors mainly from industrial stressors is the most important leakage to using these indices in environmental monitoring programs (Affonso et al. 2002).

A little experimental and epidemiological report has confirmed the immunotoxicity and hematotoxicity of oil in a variety of marine biota; however, several studies on different condition of in vitro and in vivo experiment have confirmed that the immune system as a whole is affected by exposure to xenobiotics (Zelikoff and Thomas 1998). Though, the extent of immune dysfunction is dependent on the type of pollutant, duration of exposure, species, and even the strain of animal used (Sancho et al. 2000).

The Caspian Sea, one of the largest lakes in the world, is a terminal sink for water draining from many bordering countries like Russia and Iran (Kosarev and Yablonskaya 1994). This area belonged 85 % of the world's sturgeon population (Kim et al. 2008). In addition to over exploitation of the fisheries, this enclosed ecosystem has undergone an increase in chemical contamination over the past decades. This contamination stems from industrial and domestic effluent and petrochemical contamination (Kaplin 1995). The oil pollutants is a result of inundation of low-lying oil fields along the northeastern coast of the Caspian Sea and offshore drilling activities during which oil can be spilled during collection, storage, and transport.

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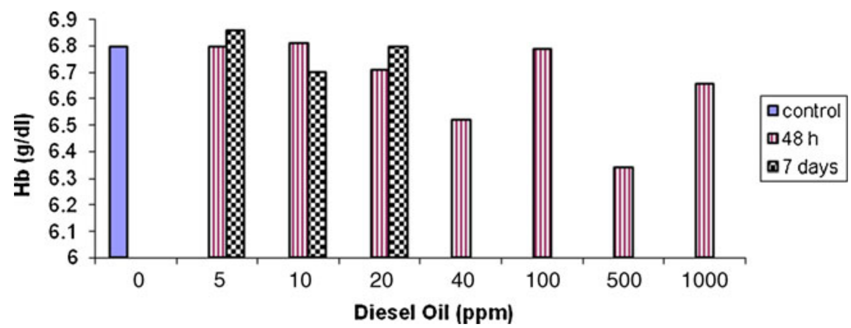

Fig. 1 Hemoglobin change of *H. huso* during acute and sub-acute exposure to crude diesel oil

Because the study on hematotoxicity and immunotoxicity of diesel is not well established, evaluation of their potential toxicity in valuable fish species is very important, so the objectives of current study were to determine the sub-lethal effects of direct diesel oil on hematological and immunological features of great sturgeon to aim toxicity of oil pollution in this valuable fish.

Materials and methods

Preparing experimental condition and fish rearing

Juvenile specimens of Beluga, average weight 200 g, were supplied by fisheries research laboratory. Prior to the toxicity tests, fish were acclimated to laboratory conditions for a minimum of 7 days in a 300-L tank with dechlorinated tap water. During acclimitation, fish were fed with commercial pellet twice a day. To obtain the crude diesel oil, according to the concentration, a part of commercial diesel oil was directly injected to the tanks.

Fish were submitted to acute doses (5, 10, 20, 40, 100, 500, and 1,000 ppm) for 0, 48 h, and 7 days static toxicity tests, performed in tank of 200 L, each containing four fish. One control group, consisting of seven fish exposed only to water (the same as that used for acclimation), was sampled at each experimental interval along with the experimental groups exposed to water plus crude diesel oil. Three replicates were carried out for each experimental time. During the tests, water was continuously monitored for temperature, dissolved oxygen, pH, and conductivity (Hedayati et al. 2010).

Fish sampling and blood evaluation

Immediately after removing the fish from the tank, they were anesthetized with clove powder (200 ppm), and blood samples were taken from the caudal vein by means of heparinized plastic syringes (Hedayati et al. 2011). Subsequently, fish were killed by struck on head.

Determinations of the blood indices were performed immediately on fresh blood. Numbers of blood leukocytes and erythrocytes were determined by diluting heparinized blood with Giemsa stain at 1:30 dilution and cells were counted using a hemocytometer Neubauer under the light microscope (Stevens 1997).

The leukocyte differential count was made in peripheral blood smears stained by Merck Giemsa (Beutler et al. 2001), giving the neutrophils value of differential neutrophils and the mononuclear value of differential lymphocytes plus monocyte and eosinophile.

Hematocrit values (Ht, percent) were immediately determined after sampling by placing fresh blood in glass capillary tubes and centrifuged for 5 min at 10,000 rpm in a microhematocrit centrifuge (Hettich, Germany) then measuring the packed cell volume (Goldenfarb et al. 1971); hematocrit readings were performed with the aid of a microhematocrit reader. Hemoglobin levels (Hb, milligrams per liter) were determined colorimetrically by measuring the formation of cyanomethemoglobin according to Lee et al. (1998). Erythrocytes indices (MCHC or mean cell hemoglobin concentration, MCV or mean corpuscular volume, and MCH or mean corpuscular hemoglobin) were calculated from RBC, Ht, and Hb according to Lee et al. (1998).

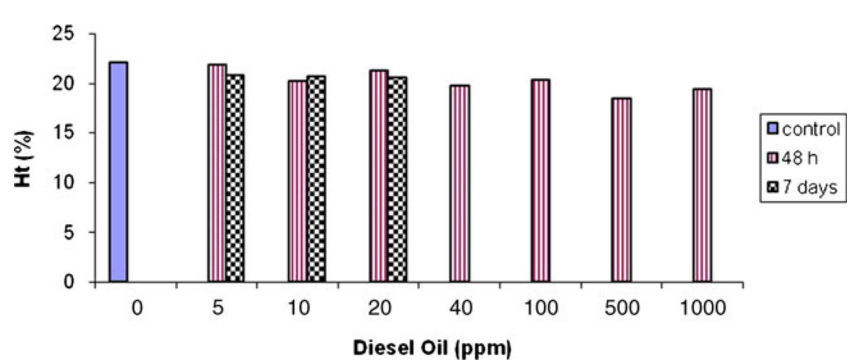

Fig. 2 Hematocite change of *H. huso* during acute and sub-acute exposure to crude diesel oil

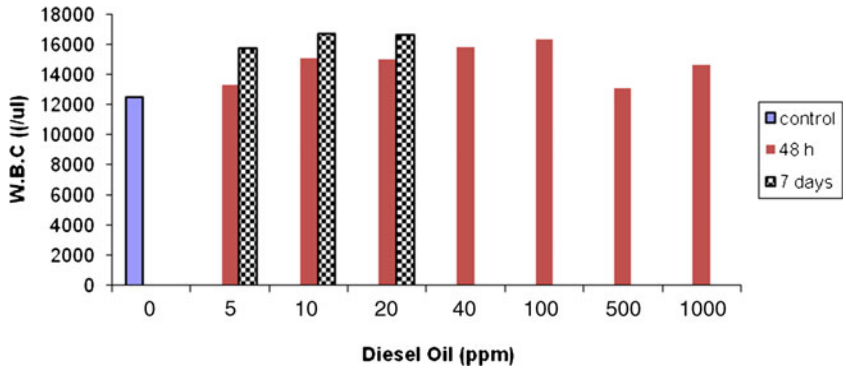

Fig. 3 White Blood Cell change of *H. huso* during acute and sub-acute exposure to crude diesel oil

Statistical analysis

One-way analysis of variance ANOVA with Duncan post hoc was used to determine significant differences to evaluate the effect of diesel oil on blood parameters. To investigate associations between bioaccumulation and its effects, Pearson correlation coefficients (*r*) were calculated between diesel oil concentrations and blood parameters. Multiple regressions were used to determine the relationship between diesel oil concentration and blood parameters. The differences between means were analyzed at the 5 % probability level (*P* value of less than 0.05 was considered as statistically significant). Data are reported as means±standard deviation ($X \pm SD$).

Results

Hematological indices

With respect to in vitro raw data, the Kolmogrov–Smirnov normality test was significant at a $P<0.05$, for all parameters. Results of crude diesel oil hemoglobin and hematocrit activities analysis are presented in Figs. 1 and 2.

Fish exposed to crude diesel oil for 48 h showed a significant elevation in WBC, Hb, Ht, MCV, MCH, MCHC, neutrophil, and lymphocyte concentration, in relation to the respective control ($P \leq 0.05$), whereas among significant indices WBC, MCH, MCHC, and neutrophil in fish exposed to crude diesel oil for 48 h were significantly greater compared to the respective control groups and RBC, Hb, Ht MCV, and lymphocyte were significantly lower than control groups ($P \leq 0.05$). Eosinophils did not vary significantly in the groups exposed to 48-h crude diesel oil compared to the respective control groups ($P>0.05$) (Figs. 2, 3, 4, 5, 6, 7 and 8).

During the 48-h exposure, the correlation between crude diesel oil with all parameters was statistically tested by analyzing the data obtained during the crude diesel oil exposed. Only the RBC, Ht, and MCV levels showed significant correlation ($P<0.05$) with crude diesel oil exposure, all correlations were negative.

Curve estimation regressions data were used to determine the relationship between crude diesel oil concentration with hematological and immunological activity. Only the RBC, Ht, and MCV levels showed significant linear regression ($P<0.05$) $Y=a \pm bX$ with crude diesel oil.

Furthermore, 7-day exposures showed a significant change in WBC, MCV, and neutrophil levels, in relation to the control treatment ($P \leq 0.05$).

Whereas among significant indices, WBC and neutrophil were significantly greater compared to the respective control groups ($P \leq 0.05$), and MCV was depleted within the crude diesel oil adjacency. Because of high value of experiment doses (more than LC_{50}), after 7-days exposure, there were 100 % mortality in 40, 100, 500, and 1,000 ppm, and we could not have serum samples.

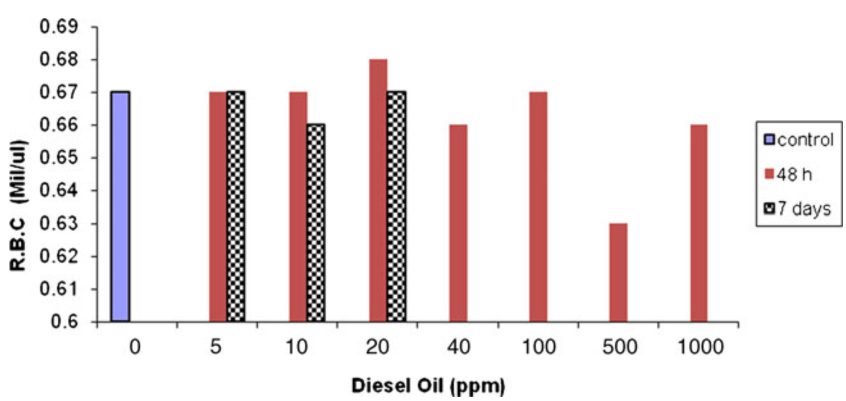

Fig. 4 Red Blood Cell change of *H. huso* during acute and sub-acute exposure to crude diesel oil

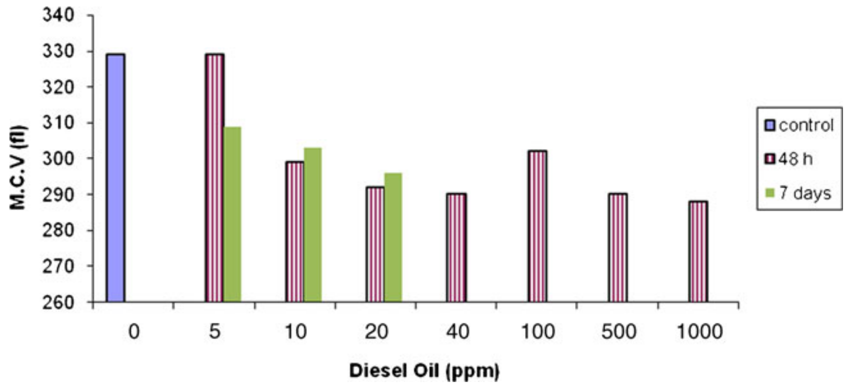

Fig. 5 MCV change of *H. huso* during acute and sub-acute exposure to crude diesel oil

During the 7-days exposure, the correlation between crude diesel oil with all parameters was statistically tested by analyzing the data obtained during the crude diesel oil exposed. Only the WBC, Ht, MCV, and MCHC levels showed significant correlation ($P<0.05$) with crude diesel oil exposure; among that correlation, WBC and MCHC were positive and the other ones were negative.

Curve estimation regressions data were used to determine the relationship between 7-days crude diesel oil concentration with hematological and immunological activity. Only the WBC, Ht, MCV, and MCHC levels showed significant linear regression ($P<0.05$) $Y=a\pm bX$ with crude diesel oil.

Discussion

There is little research related to hematological responses in fish-exposed oil pollutants (Sancho et al. 2000). In this work, a series of hematological and immunological parameters were examined in *Huso huso* after exposure to acute doses. Our result declared increase of WBC, MCHC, and neutrophil and decrease of RBC, MCV, MCH Hb, Ht, lymphocyte, and eosinophil within acute exposure.

The chemicals that stimulate blood cell/hemoglobin production, generally induce a hypoxic condition in fish that stimulated the spleen, which produces the blood cells in fish (Fange 1992), to contract and release stored erythrocytes into the circulation. The values observed for hematocrit and

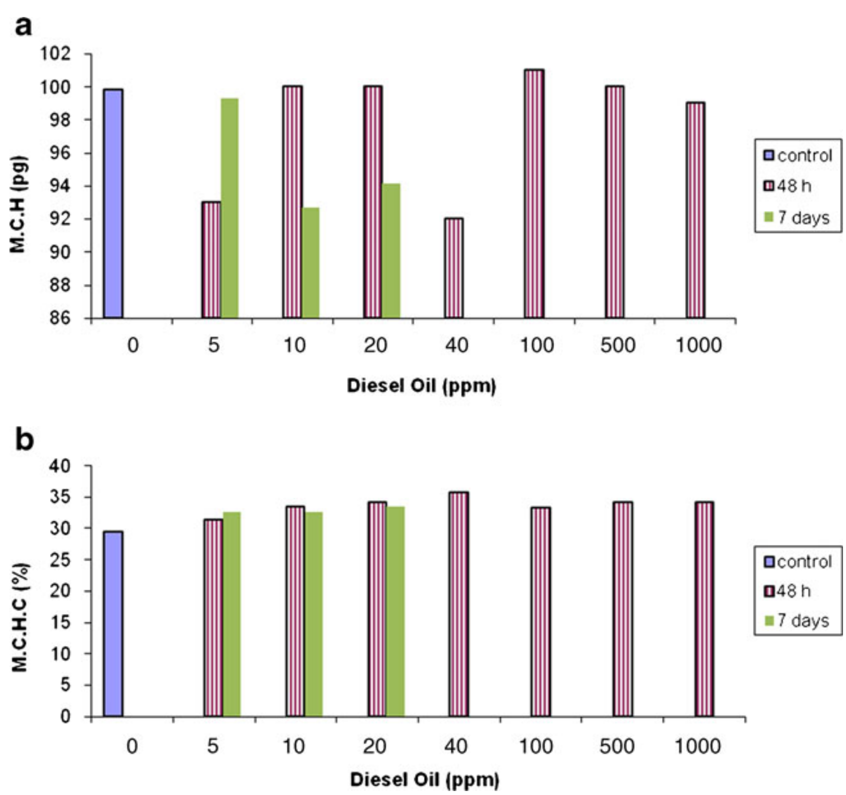

Fig. 6 MCH change of *H. huso* during acute and sub-acute exposure to crude diesel oil

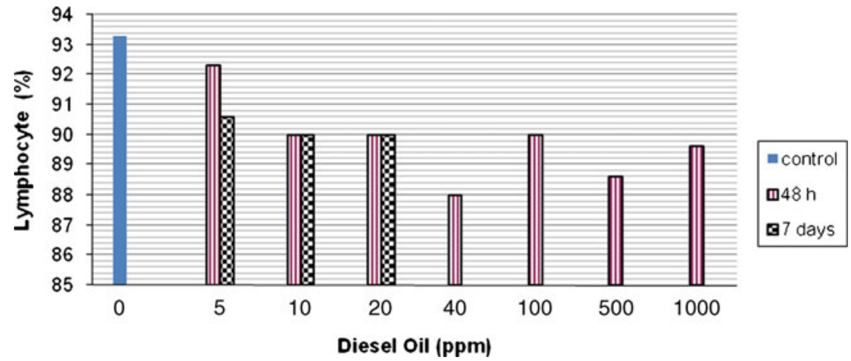

Fig. 7 Lymphocyte change of *H. huso* during acute and sub-acute exposure to crude diesel oil

hemoglobin are relatively close to those of other tropical and nontropical species of fish such as *Ictalurus punctatus* (23.9 %) (Lim et al. 2000) and *Colossoma macropomum* (20–23 %) and quite different from those of compared with other species (Affonso et al. 2002), *Oncorhynchus mykiss* (35.6 %) (Mattsson et al. 2001).

Elevation of hematocrit after acute exposure indicates the importance of the route of diesel oil contamination. Results observed accord with those of Chowdhury et al. (2004), who noted an increase of blood hematocrit and hemoglobin during environmental anoxia and acute exposure to pollutants will increase blood oxygen-carrying capacity when impairment of gas exchange occurs (Savari et al. 2011).

Lymphocytes are the most common circulating leukocyte found in fish (Campbell and Murru 1990). In fishes, the heterophil has been variably called heterophil or neutrophil depending on the size of cytoplasmic granules (Campbell and Murru 1990). Unlike avian and reptilian heterophils, fish heterophils contain large amounts of myeloperoxidase and their macrophages produce nitric oxide and reactive oxygen (Campbell and Murru 1990). There is a lack of knowledge regarding the full function of fish eosinophils, but they seem to function in a similar manner to mammalian mucosal mast cells. The eosinophils have been associated with antigenic stimulation and parasitic infestations (Ellis 2001).

Our results showed increase in differential neutrophil and decrease in lymphocyte. The monocytes and neutrophils increased and lymphocyte decrease during different stressors in cultured fish *Oreochromis aureus* has been confirmed (Silveira-Coffigny et al. 2004).

It is believed that neutrophils and monocytes have phagocytic activity which might explain their increased percentage during exposure time. Hlavek and Bulkley (1980) found a transient neutrophilia in rainbow trout 24 h after treatment with malachite green, but this decreased after 4 days. These authors stated that the white cells changes in the trout exposed to malachite green were the result of a stress syndrome not specific to vertebrates and not due to leucocytotoxic effects of this chemical compound in the fish. Darwish et al. (2001) also found an increase of neutrophil counts in channel catfish exposed to high doses of potassium permanganate.

Brandão et al. (2009) found a reduction in some immunological parameters (platelet, leukocyte and lymphocyte counts), and an increase in neutrophil and monocyte percentages were demonstrated in $HgCl_2$ exposed.

It is known that water pollutants can induce abnormal responses in the immune system, including leukocyte count, a marker of cellular defense (Friberg and Enestrom 1991). The increase in neutrophil and monocyte percentages, which represents the activity of the first and second lines of defense against the cellular damage, has been reported after mercury exposure (Perlingerio and Queiroz 1995).

Oliveira Ribeiro et al. (2006) confirmed significant decrease of mononuclear (differential lymphocytes plus monocytes) and significant increase of differential neutrophil to methyl mercury and inorganic lead. It is known that changes in leukocyte counts after exposure to pollutants may be

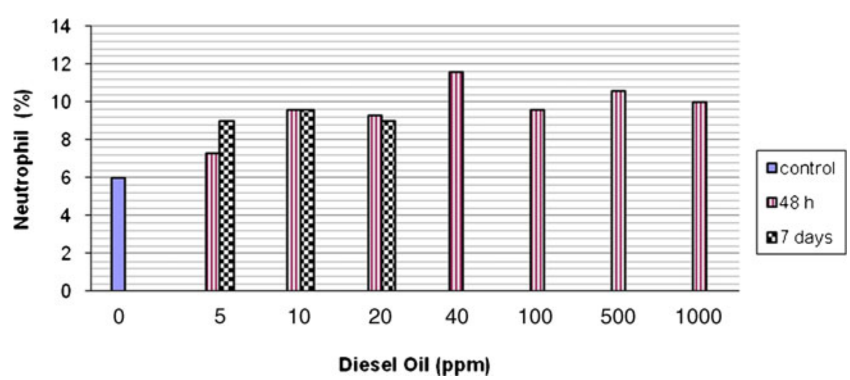

Fig. 8 Neutrophil change of *H. huso* during acute and sub-acute exposure to crude diesel oil

associated to a decrease in nonspecific immunity of the fish. The change of the leukocyte population could be related to the presence of tecidual damages such as necrosis (Oliveira Ribeiro et al. 2002).

According to Wedemeyer et al. (1990), the suppression of the immune system increases the susceptibility to diseases in fish, a very important aspect considering the presence of oil pollutants in natural ecosystems as a result of human activities. Although few studies have used dietary exposure to test the effects of contaminants in aquatic organisms (Rabitto et al. 2005), the present results showed that under experimental conditions blood parameters were sensitive to different aspects of oil exposure.

The major findings of this study are that acute diesel oil concentrations may cause several changes in the hematological and immunological parameters of the studied fish, so estimation of these indices, could provide a useful indicator of oil pollution of water bodies. It seems that WBC is a suitable biomarker of diesel oil in *H. huso*.

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