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HISTOPATHOLOGY OF RAINBOW TROUT (*Oncorhynchus mykiss*) AND STURGEON (*Acipenser baerii*) EXPOSED TO SUBLETHAL CONCENTRATIONS OF CEMENT

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ABSTRACT

In the present study, histopathologic effects of the cement mixing with water in aquatic environment on rainbow trout (*Oncorhynchus mykiss*) and Siberian sturgeon (*Acipenser baerii*) has been examined. Both of the two fish species were exposed to 125 and 500 mg/l of concentrated cement for 96 hours. LC_{50} values were calculated 0.44 g/l for trout (*O. mykiss*) and 0.62 g/l for Siberian sturgeon (*A. baerii*) at the end of the experiment for 96-hour test period. No mortality was observed in control groups. Multiple deformations became remarkable in the gills of both species. However, some hyperplasia was observed on secondary lamellae of gills of both fish species. Hyperplasia rates were found to be much higher in the sturgeon individuals than the trout individuals. Also, lamellar fusion was another important pathology in the gills of the sturgeon individuals. Few necroses have been encountered on the lamellas of the trout gills. Multiple fat granules were observed in the trout liver tissue, melanomacrophage centers and necrosis on sturgeon liver tissue treated with 500 mg/l cement. As a result, this study expressly shows that both of the two species, Siberian sturgeon and rainbow trout, cannot tolerate cement-sourced contamination.

KEYWORDS:
Aquatic Sustainability, Cement, Histology, LC_{50}, Pathology, Toxic Effect.

INTRODUCTION

Aquatic ecosystems in the inland waters, retaining walls constructed to avoid floats and overflows, concrete constructions made for hydroelectric power plant (HEPP) projects, the water mixed with concrete coming from manufacturing materials of concrete constructions, which are constructed into the stream beds like bridge constructions to aquatic ecosystem affect the life at aquatic ecosystem in a negative way. In order to evaluate these effects at macro and micro levels and take the necessary precautions, different studies are conducted, and preventive measures are applied by Turkish environmental authorities and organizations. Despite this, lots of negative cases are observed during the construction work around aquatic areas.

The amount of the issued reports which are related to negative effects on water sources like stream bed remediation studies, bridge constructions, tunnel constructions, discharge of ready mixed concrete manufacturing facilities into water sources are increasing day by day. The ready-mixed concrete is prepared by mixing proper amounts of aggregate compounds, cement, some kind of additional chemicals and water in the ready-mixed concrete plants.

It was stated that there should be a “Washing Barrel” and a “Settling Pool” in the facility, and the recycling process must be applied in order to regain the aggregates in the wastewater and the concrete mixer. It is stated that the types of waste materials give damage by leading to the prevention of the development of spawns and larvae of other organisms.

So far, the micro and macro effects of the construction work in inland waters of Turkey have not been studied in detail. Environmentalists and fish farms which are acting in these types of water sources cannot prove their damages which are related to the construction work. The chemicals which have been studied to determine the potential negative effects on fish are, generally pesticides [1, 2, 3, 4], fertilizer combinations [5], disinfectants [6], and nanoparticles [7] in recent years. These material groups have been in active interaction with the natural environment.

In this context, the aim of this study is to determine the potential negative effects of the cement, which is the unchanging element of concrete construction, in terms of toxicology and histopathology studies in rainbow trout (*Oncorhynchus mykiss*) and Siberian sturgeon (*Acipenser baerii*). These data will be helpful in solving the future problems in wetlands.

MATERIALS AND METHODS

Fish Material. In this study, rainbow trout (*Oncorhynchus mykiss*) and Siberian sturgeon (*Acipenser baerii*) were obtained from Recep Tayyip Erdoğan University Aquaculture Research and Application Center. Ninety trout individuals (40 g of average weight) and 30 sturgeons (120 g of average weight) were received for toxicological experiments.

Experimental Design. The experiments were performed in 70 lt tanks. The tanks were designed to include10 fish in each tank with 2 recurrences. Water temperature and pH values were measured before trials and after adding cement. 125 and 500 mg/L concentrated cements were added to the tanks in this study. At the same time, control groups were formed for each species [8].

Calculating the LC_{50} Value. The study was designed to calculate the concentrations that would kill 50% of the fish in the experiment in 96 hours. This process has been performed by Probit Analysis, which is a program within the SPSS 10.0 Statistical Software.

Histologic Studies. After the experiments, each group of the fish have been treated with benzocaine (25 mg/l) before mortality; and their livers, kidneys, spleens and gill tissues were fixed with 10% neutral buffer formalin (NBF). After 24 hours, the tissues were kept at 50% ethyl alcohol solution for 48 hours. Then the tissues went through alcohol series and they were incubated at 65°C water temperature in paraffin for overnight.
The tissue samples were embedded in paraffin for the tissue section process. In the tissue section process, the tissues were sliced into 0.5 µm thickness, deparaffinization process was applied and tissues were subjected to 30%, 50%, 70% and 100% alcohol series. Tissue sections were stained with hematoxylin and eosin, fixed with entellan and histopathologic changes were examined [3].

RESULTS

Experimental Water and pH values.
The water temperature was 10±0.5°C at the beginning of the study and it was fixed at this value till the end of the study. The pH value of the water was measured as 7.3 at the beginning for each fish species. It was measured as 9.5 after adding cement at 125 mg/l cement concentration trial; and as 10.5 after adding 500 mg/l cement concentration.

Macro Findings. It has been observed that trout (*Oncorhynchus mykiss*) gets stressed since the beginning of adding the cement. Also, it has been observed that they were much calmer and stress-free in comparison with the trout when changes in the fish was monitored during the experiment. No cataracts formation was observed inside the eyes of the fish during the experiment. Unbalanced floating and floating on water surface were observed in both species. Mortality have been reported since the first day in the trout groups which were treated with 500 mg/L dosage.

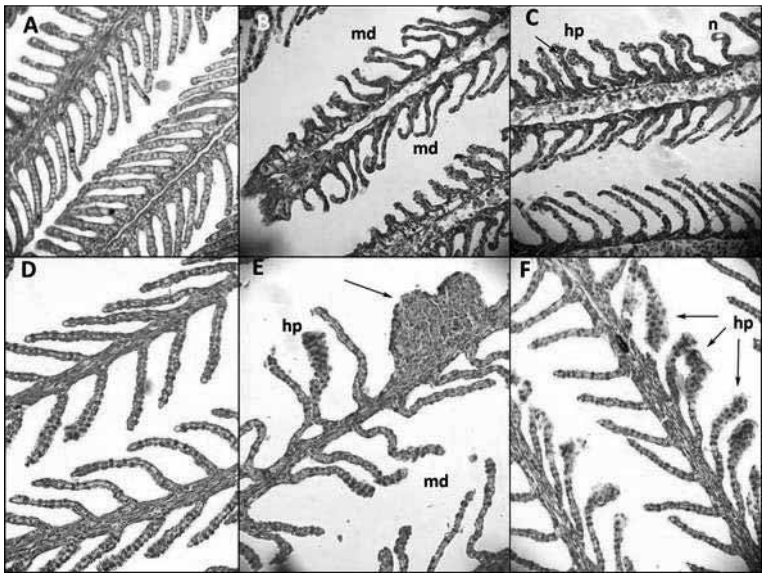

FIGURE 1
Histopathology in the gills of the trout and the sturgeon fish species, which were used in the experiments, A; Trout control group, B, C; gill tissue of trout applied 500mg/l dose, (md) multiple deformations, (hp) hyperplasia, (n) necrosis, D; sturgeon control group, E, F; gill tissue of sturgeon applied 500 mg/l dose, (md) multiple deformations, (hp) hyperplasia and lamellar fusion have been shown with black arrow.

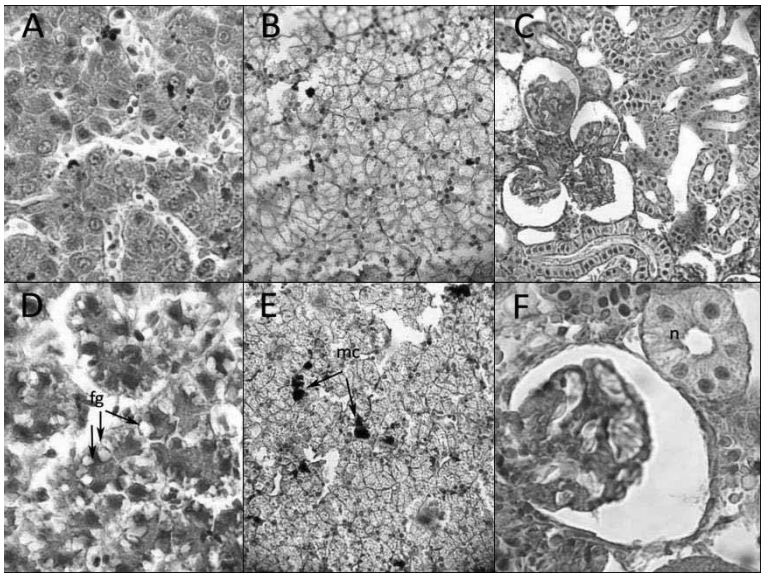

FIGURE 2
Histopathologies of the liver and the kidney tissues of the trout and the Siberian sturgeon in trials; A: Trout control group, D: Trout liver tissue treated with 500mg/l dosage, (fg) fat granules B; liver of sturgeon control group, E; Sturgeon liver treated with 500 mg/l dosage, (mc), melanomacrophage centers, C; sturgeon fish control group, F; sturgeon fish kidney tissue treated with 500 mg/l dosage, (n), necrosis.

Histologic Studies. In the assessment of the fish tissue sections, first of all, alterations at gills were examined when the pathologies were checked. Multiple deformations were observed in the gills of both species. Apart from that, hyperplasia were observed in the gill lamellas of both species, too. In sturgeon, hyperplasia ration was found higher than in trout. Also in sturgeon gills, lamellar fusion was observed as another important pathology. In the trout, cellular necroses were seen rarely in the lamellae of gills (Figure 1). Multiple fat granules were observed in the trout liver tissue treated with 500mg/l dosage of cement. Melanomacrophage centers and necrosis were observed in the sturgeon liver treated with 500 mg/l dosage (Figure 2).

LC_{50} Value. LC_{50} value was calculated as 0.44 g/l for the trout (*Oncorhynchus mykiss*) and 0.62 g/l for the Siberian sturgeon (*Acipenser baerii*) at the end of the 96 hours experimental period. No mortality was observed in the control groups.

DISCUSSION AND CONCLUSIONS

The water pH level of 6-9 affects the normal physiological functions of the aquatic organisms, including the exchange of ions with the water and respiration. The acceptable range of pH in aquatic life, particularly in fish, depends on numerous other factors including prior pH acclimatization, water temperature, dissolved oxygen concentration, and the concentrations and ratios of various cautions and anions. Some aquatic organisms (e.g. certain species of algae) have been found to live at pH 2 and lower, and others at pH 10 and higher. Although fish had been found at pH values from 4-10, for maximum productivity, the pH level should be maintained between 6.5 and 8.5. The pH values between 9 and 10 can result in partial mortality for the rainbow trout (*Oncorhynchus mykiss*), the brown trout (*Salmo trutta*) and the salmon [9]. The pH range is not directly lethal to freshwater fish as 5.0-9.0. Chronic exposure to pH values above 10 can be harmful to all salmonids, and pH values higher than 9, can be harmful for some other species [10].

Domestic and industrial effluents discharges are considered one of the most significant threats of the aquatic environments worldwide [11]. High pH levels between 9 and 14 can be harmful for the living species by denaturing cellular membranes. Because alkalinity and pH are so closely related, changes in pH can also affect alkalinity, especially in poorly buffered waters [12].

Productivity and financial worries are the most important topics for developing countries in the case of utilizing natural sources efficiently. In this sense, aquatic resources are revised for especially obtaining energy, facilitating transportation and community safety by taking the reclamation works into account. Aquatic organisms can be affected after these activities usually at a later time period. Concrete and cement in the concrete are the main and fixed materials for nearly all activities in water. Many fish massacre have been reported at different times because of concrete activities in aquatic environments. Although there are various scientific studies which have examined the effects of cement on fish, the data in the literature are still limited.

[13] investigated the toxic effects of Portland cement in Salmonid fish in his study; and reported that 60% of the animals died in 24 hours after applying 100 mg/l dosage at 15°C. The researcher observed the pH value and stated that it exceeded 10.5 in his study, but he did not evaluate how toxic effects occur on tissues histologically. In the present study, the LC_{50} value was calculated for the trout as 0.44 gr/l at 10±0.5°C for 96 hours period and the pH value was 10.5 at 500 mg/L trial. There was no detailed information on the toxic effects of the cement in sturgeon, and this study enabled to compare responses of the trout and the sturgeon to cement-sourced toxic effects. In this context, it has been concluded that the sturgeon is more resistant to toxic effects than the trout under same environmental conditions.

Effects of different abiotic and biotic factors that affect fish have been examined extensively in histological studies [14, 15, 16]. Destructions made at tissues by manure, bacteria, virus and parasites have been reported [1, 2, 3, 4]. This study enables the histological comparison of the responses to toxic effect of two different species. Gills of fish are the preliminary impact area of contaminated toxicants in water. In this study, malformations and low level hyperplasia were observed at the trout gills, severe hyperplasia and low malformation were observed at sturgeon gills. Severe fat vacuoles at trout livers and only melanomacrophage formation at sturgeon livers were observed when the pathologies were considered in the liver and kidney tissues. Few necroses receive attention in the sturgeon when kidney tissues of each species are examined. According to these data, although the trout have fewer damages than the sturgeon in gill tissues during treatment with toxic material, they could enter stress conditions much earlier and they had high mortality levels than the sturgeon. This can be explained by the differences of weight rates and oxygen requirements of the two species. Also vacuolization was observed in liver tissue which can occur due to high mortality rate in the trout. It is a known fact that the aquatic ecosystem does not consist of macro living creatures. In future studies, which and how toxic materials affect the aquatic ecosystem, flora and fauna in a must be studied. This study is about how fish can be affected in the media where there are cement-added products, which is the most important part of the anthropogenic activities. This study has clearly demonstrated that fish species, the sturgeon and the trout, cannot tolerate cement-sourced toxicities. It is necessary that, in future studies that will be conducted in watery areas, the necessary precautions must be taken, and it must also be studied that how the other living creatures are affected by the toxic effects.

ACKNOWLEDGEMENTS

We offer our gratitude to RTEU BAP Coordinatorship and Fisheries Research & Application Center Directorate, because of study finance and live material supply.

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Received: **21.10.2015**
Accepted: **10.04.2016**

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