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Research Article

Effect of Pozzolanic Cement Exposure in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

Cement used in structures such as bridges, dams, and retaining walls built on the aquatic ecosystem harms different organisms. The current study reveals the effect of pozzolanic cement (PC) on Nile Tilapia (*Oreochromis niloticus)*. The LC_{50} value for *O. niloticus* exposed to 6 different PC concentrations for 24 hours was calculated as 306.66 mg L^{-1}. Increasing PC concentration significantly increased the pH of water (<10) at the end of the study ($p<0.05$). The increase in pH level showed a positive correlation with the increase in fish mortality ($p<0.05$). In addition, PC negatively affected the hematological parameters of fish. Thus, the current study reveals the negative effects of PC on *O. niloticus* in the acute period. Future studies should focus on developing cement with environmentally friendly materials that will not affect the pH level of water.

Keywords: Aquatic toxicology, hematology, LC_{50}, pozzolanic cement

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INTRODUCTION

The construction industry is indispensable in that it meets the many visible and essential needs of humanity, such as new constructions, restorations, and post-earthquake reconstruction. Cement, as the basic material of the construction industry, is the second most produced material after steel, with a production of around 4.1 billion tons worldwide (Farfan et al., 2019). Since cement is the main material in the construction of many structures such as bridges, tunnels, dams, and retaining walls, the negative effects of possible effluents on natural water resources are inevitable (Kurtoglu et al., 2016). Even the cement wastes from construction sites that are far from the aquatic system eventually contaminate the aquatic ecosystems through rainwater and sewage systems. However, there are limited studies on the effect of cement in natural waters and habitats (Er & Kayış, 2022).

Natural water resources, which are generally the final destination of all pollutants, are the habitats of aquatic organisms. In order to ensure the continuity of biodiversity, it is necessary to protect organisms in water resources against anthropogenic pollutants and to investigate their resistance (Bashir et al., 2020; Gadzała-Kopciuch et al., 2004). Possible pollutants in the aquatic environment affecting water quality parameters such as pH, turbidity and dissolved oxygen are the primary factors that affect the living standards of organisms (Austin, 1998). Cement, which is often used in the construction industry, poses a danger to aquatic organisms because it has the potential to increase the pH of water. In a study where cement increased the pH level above 10.5 it was reported that the mortality rate of rainbow trout (*Oncorhynchus mykiss*) individuals was around 60% (Er et al., 2019). In general, a pH value between 9 and 10 can be harmful to fish, while pH values above 10 can be lethal to the rest (European Inland Fisheries Advisory, 1969).

Physical and chemical changes in the aquatic environment cause measurable physiological changes in fish (Fazio et al., 2013). This is because fish are in contact with the aquatic environment, which significantly affects blood homeostasis. Biomarkers are defined as a change in biological response, ranging from molecular to cellular and from physiological responses to behavioral changes (Depledge et al., 1995). As an indicator of water quality, the hematological profile in fish has been recently evaluated as a rapid analysis (Akram et al., 2021; Minaz et al., 2022a). In particular, automatic hematological assay is a significant diagnostic alternative to conventional methods today (Fazio, 2019). Blood parameters provide useful information about various body processes and are of great importance in assessing the harmful effects of anthropogenic pollution on aquatic environments (Guerrera et al., 2021).

The effect of cement as an anthropogenic pollutant on aquatic organisms has been discussed in several studies (Adamu et al., 2008; Er et al., 2019; Er & Kayış, 2022). Unpublished case studies of cement-related mortalities in aquaculture facilities are known. In this context, the impact of cement on Nile Tilapia (*Oreochromis niloticus*), one of the most cultured species in the world, is very significant. The current study focuses on the toxic effects of pozzolanic cement (PC), which is frequently used due to its qualities of permanent durability, easy workability and high impermeability. In our study the hematological response of *O. niloticus* against the potential sub-lethal concentration at the end of the acute period (24 hours) was also investigated.

MATERIALS AND METHODS

The toxic material (Pozzolanic Cement)

In the present study, the toxic effect of a commercial pozzolanic cement (PC) was evaluated (CEM IV/B (P) 32,5 R, Oyak Çimento, Türkiye). Pozzolan materials do not have individual binding properties. However, they have high binding abilities in aqueous ambient with calcium hydroxide. The PC in the current study includes 65-89% natural clinker (30% clay and 70% limestone) and 11-35% pozzolan materials. Therefore, the potential toxic material in the available study has a content of Silicon dioxide (SiO_2), calcium oxide (CaO), aluminum oxide (Al_2O_3), and iron oxide (Fe_2O_3).

Experimental design, fish, and water quality

This study took place in the toxicology laboratory, Recep Tayyip Erdoğan University, Rize, Türkiye. Nile Tilapia fish (50.4±4.4 g) were purchased from the Aquaculture Application and Research Center. The system design was triplicate configured with a 50 L aquarium and ten fish in each aquarium. For adaptation, the fish were placed in the aquariums ten days before the study. The study was carried out using rested tap water and in accordance with a 12-hour dark/light photoperiod. Aeration was distributed to the aquariums with air stones from a central pump system. Since PC is not a water-soluble material, it tends to aggregate at the bottom of the aquarium. In order to prevent this accumulation and keep the water homogeneous, a mixing system that provides water circulation was placed at the bottom of the aquarium. The fish were not fed in the acute period in order to provide stable water quality. All experimental studies were checked and approved by the Ethical Local Committee of the Recep Tayyip Erdogan University (Decision No: 2021/14).

Water quality parameters were measured at the beginning of the study to ensure their suitability for the living standards of the fish. Temperature (°C), pH and dissolved oxygen (mg L^{-1}) were measured with a portable multi-parameter (Hach, HQ40D 58258-00). Accordingly, water quality parameters were recorded as 17.1±0.6 °C, 6.51±0.2 and 7.65±1.4 mg L^{-1} for temperature, pH and dissolved oxygen, respectively.

Acute toxicity

Within the scope of the current study, the lethal concentration that killed 50% of the target population was determined. In this context, the fish were exposed to six different PC concentrations (100 mg L^{-1}, 200 mg L^{-1}, 300 mg L^{-1}, 400 mg L^{-1}, 500 mg L^{-1} and 600 mg L^{-1}) for 24 hours (Tablo 1). No PC was added to the control group but all other manipulative conditions were the same as for the PC groups. LC_{10}, LC_{50} and LC_{90} for 24 h values were determined as 255 mg L^{-1}, 306 mg L^{-1}, and 339 mg L^{-1} respectively.

Hematological analysis

Fish blood indicators were analyzed according to the automatic hematological assay method (Minaz et al., 2022a). Before the hematological analysis, five fish were randomly chosen from each group and transferred to an anesthesia tank that contained 60 mg L^{-1} clove oil. Afterward, 2.5 ml syringes were used to take blood samples from the caudal vein of the fish. Blood samples (0.3-0.5 mL) were fastish transferred to EDTA K3 tubes for the prevention of congelation. Finally, the following indicators were investigated using automatic hematological assay (Prokan-6800VET): leukocyte (WBC), erythrocyte (RBC), lymphocyte (LYM), monocyte (MID), granulocyte (GRAN), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV) mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The blood counter device was calibrated with blood from healthy fish prior to the study.

Statistical analyses

In the current study, all data were shown in mean ± standard deviation. The Kolmogorov-Smirnov test was used to determine normality distribution in the data set. According to the normality analysis, it was observed that all data sets showed normal distribution. Therefore, parametric tests were applied to determine statistical differences between the groups. Significant differences among groups were evaluated by a one-way ANOVA test. Also, the Tukey test was used to detect the differences in the groups. The Pearson Correlation test was performed to examine the statistical relationship between PC concentration, mortality rate and pH values. At the end of acute period, the Probit test was used to detect the LC_{50} value. P-values of less than 0.05 were considered statistically significant. All datasets were analyzed by SPSS 25 software package for Windows.

RESULTS AND DISCUSSION

Cement is the basic building block of many reinforced concrete structures in aquatic environments. When construction plans using cement are made to increase human welfare in an aquatic environment, most researchers typically focus on the fact that construction areas restrict the vital needs of organisms in the ecosystem, such as nutrition, reproduction and migration (Kocabaş et

Table 1. Lethal concentration of PC in *O. niloticus.*

Concentration (mg L^{-1})	Total fish (n)	Number of dead fish (mortality%)
100	30	0 (0%)
200	30	0 (0%)
300	30	12 (40%)
400	30	27 (90%)
500	30	30 (100%)
600	30	30 (100%)
LC_{10} (mg L^{-1})		255.08
LC_{50} (mg L^{-1})		306.66
LC_{90} (mg L^{-1})		339.40

al., 2013). Although many studies have been conducted on the effects of reinforced concrete structures on aquatic environments, the effect of cement on organisms has been ignored. Therefore, the current study focused on the effect of possible cement leakage from reinforced concrete structures on *O. niloticus* at the end of acute period.

Relationship between mortality rate and pH

The relationship between the mortality rate and pH are shown in Figure 1. Accordingly, while no death was observed up to 200 mg L^{-1} PC concentration at the end of 24 hours, death was observed in 40% at 300 mg L^{-1} PC, 90% at 400 mg L^{-1} PC, and in all individuals at 500 and 600 mg L^{-1}. Additionally, higher pH levels (pH level of up to almost 11) were observed with increasing PC concentration, while the pH was around 6.5 in the control group. At the LC_{50} level (306 mg L^{-1}), the pH level was estimated to be around 10.18. There was a significantly positive relationship between PC concentration, mortality rate and pH (Table 2, $p<0.05$).

Cement content tends to increase the pH level of water (Lee & Hur, 2005). However, studies have shown that this increase in pH level disappears within 24 hours in static water and returns to normal levels (Law et al., 2013). Therefore, a 24-hour LC_{50} value

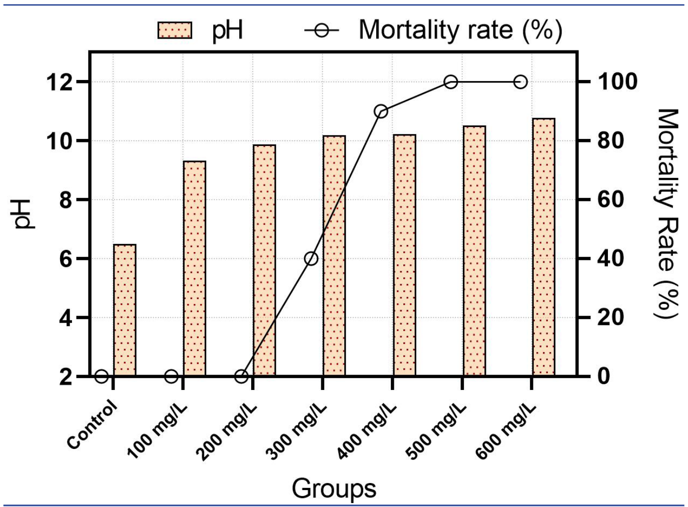

Figure 1. Mortality rate and pH depending on PC concentration.

Table 2. Correlation table between PC concentration, mortality rate, and pH.

		Concentration	Mortality Rate	pH
Concentration	Pearson Correlation	1		
	Sig. (2-tailed)			
Mortality Rate	Pearson Correlation	0.938**	1	
	Sig. (2-tailed)	0.002		
pH	Pearson Correlation	0.825*	0.759*	1
	Sig. (2-tailed)	0.022	0.045	

* Correlation is significant at the 0.05 level (2-tailed);
** Correlation is significant at the 0.01 level (2-tailed)

was determined in the current study. In a study conducted to determine the toxic effects of portland cement on tilapia (7.2 g), the LC_{50} value was found as 41.21 mg L^{-1} (Adamu et al., 2008). In the current study, this value was calculated as 307 mg L^{-1}. The difference between these two studies involving the same species may be due to the type of cement or the difference in fish size (34.8 g). In another study, the 96-hour LC_{50} value in rainbow trout exposed to different concentrations of PC was 440 mg L^{-1} (Kurtoglu et al., 2016). The reason for the high 96-hour LC_{50} value in the previous study may be related to the fact that the temperature was lower than in our study and more suitable for rainbow trout. It would be expected that the 24-hour LC_{50} value for rainbow trout, which is a more sensitive fish than tilapia, would be much higher.

In an aquatic ecosystem, pH is vital to organisms and can affect the solubility and toxicity of chemicals in the water. Although most aquatic organisms live in the pH range of 6.5-9, there may also be organisms living outside this range. However, high or low pH levels have negative effects on growth and reproduction. Cement tends to increase pH levels, resulting in toxicity to aquatic organisms. For instance, the increase in pH level caused a decrease in the EC_{50} (median-effective concentration) value for *Daphnia magna* (Rodrigues et al., 2020). In Atlantic salmon eggs, a low pH level (pH 2.78) caused the death of 98% of all individuals after 18 hours, while a high pH level (12.23) caused all individuals to die within 15 minutes (Foldvik et al., 2022). The toxicity ability of high pH level is much more effective. In the current study, the pH level showed a positive relationship with increasing concentration in the first 24 hours. However, pH levels in all groups returned to levels similar to the control group after 24 hours. In our previous study, a different group was designed whose pH level was reduced with HCL to reveal the toxic driving force of cement (Er & Kayış, 2022). At the end of the study, it was observed that there was no death in the group in which the pH level was manipulated. Therefore, it has been proven that the main driving force in cement toxicity studies is pH. The lethal effect of high pH level on organisms will contribute to the management of the contamination process both in nature and in aquaculture ambients. However, today's common belief is that in cases of cement-related contamination, losses should be determined by sampling fish rather than the characteristics of the water. Additionally, there is a deficiency regarding the time period for providing water samples.

Hematological evaluation

For hematological studies, 100 mg L^{-1} PC (T100) and 200 mg L^{-1} PC (T200) concentration groups, which were 1/3 and 2/3 times the LC_{50} value and in which all fish survived, were evaluated (Figure 2). Accordingly, significant differences were observed between groups for all hematological parameters except MCV. Significant decreases were observed between all groups in WBC values depending on increasing concentration ($p<0.01$). PC groups showed significantly higher RBC, HGB, and HCT values compared to the control ($p<0.01$). For LYM value, the T200 group was significantly lower than the other groups ($p<0.05$). Finally, significantly lower values were observed in the PC groups for MID, GRAN, MCH, and MCHC values ($p<0.01$).

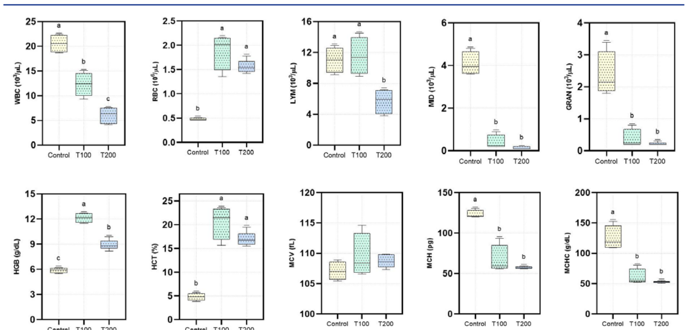

Figure 2. Hematological parameters of *O. niloticus* exposed to different PC concentrations.

One of the most important and rapid indicators for aquatic organisms encountering any toxic pollutant is hematological outputs (Ak et al., 2023; Fazio et al., 2017). This is because blood represents fish health depending on exposure to environmental stressors (Gabriel et al., 2011; Minaz et al., 2022a). Blood parameters may differ from normal levels in aquatic organisms during exposure to pollutants, transportation and anesthesia (Minaz et al., 2022b). In the current study, significantly decreased WBC values were noted in the increasing PC groups. WBCs are circulatory cells that assist in physiological responses and constitute different cell types such as lymphocytes and monocytes. Therefore, like WBC, lower MID and LYM in PC groups is an expected phenomenon. Although previous studies have rarely shown a tendency for WBC to increase in response to stressors, WBC tends to decrease in fish against many stressors (Esmaeili, 2021). Under stress conditions, the immune system is affected positively or negatively by many neuroendocrine elements (Khansari et al., 1990). The decrease in WBC, LYM and MID may be directly related to the decrease in cell production of the hematopoietic organs or in total leukocytes of the immune defense (Alam et al., 2021). It has previously been reported that exposure to herbicides as toxic substances reduces WBC values for the same fish species (Abdel-Warith et al., 2021). In general, exposure to toxic substances results in decreased leukocyte functions, subsequent inhibition of lymphocyte responses, apoptosis and cell death (Guzzi et al., 2012). RBC absorbs oxygen in the gills and carries it to the tissues. RBC in fish varies between 0.4×10^6 mm^{-3} and 5.2×10^6 mm^{-3} (Esmaeili, 2021). In the current study, this value remained within the general range. The immune system of fish may increase RBC under stress or due to infection. This is a defense mechanism to fight infection or toxic substances and it speeds up recovery. Like WBC, HGB is at higher levels in PC-exposed groups. The increase of HGB and HCT in fish may occur in order to increase the body's oxygen carrying capacity and respond to oxygen needs. However, this increase may vary under a particular fish species or in certain environmental conditions.

CONCLUSION

The release of cement, the basic building block of reinforced concrete structures, into the aquatic ecosystem has become an inevitable issue. Cement effluent entering the aquatic environment as a result of many developments such as agricultural activities and energy systems poses a danger to both the environmental environment and all organisms. In this context, the current study revealed the effects of pozzolanic cement exposure on Nile tilapia (*Oreochromis niloticus*). PC, which has a vital importance in the aquatic ecosystem due to its tendency to increase the pH level, had a toxic effect on *O. niloticus*. It also negatively affected the hematological system of the fish and created differences in blood parameters as a stress response. This study revealed the lethal and physiological effects of PC exposure during the acute period. Case studies are important to raise awareness by showing how the release of toxic PC material into natural waters affects the aquatic environment around the world. Future studies should focus on improving PC with supporting materials that do not increase the pH of water. In addition, using the current study as a reference, awareness should be raised for the construction industry and the use of environmentally friendly materials should be encouraged.

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Ethical Declaration: The current study was checked and approved by the Ethical Local Committee of the Recep Tayyip Erdogan University (Decision no:2021/14).

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Competing Interests: The authors have no relevant financial or non-financial interests to disclose.

Data Availability: All datasets used during the current study are available from the corresponding author on reasonable request.

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