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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effect of natural adsorbent clinoptilolite on some hematological parameters of *Cyprinus carpio*

Doğal bir adsorban olan klinoptilolitin, *Cyprinus carpio* 'nun bazı hematolojik parametreleri üzerine etkileri

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Abstract: Effect of natural chelating agent clinoptilolite on hematocrit level, erythrocyte numbers, mean corpuscular volume (MCV), erythrocyte and erythrocyte nucleus area of *Cyprinus carpio* were determined after exposing the animals to 2, 4, 8, 16, 32 gL-1 clinoptilolite (0.40 µ) (3, 7, 21 and 30 days). Hematocrit levels were determined using microhematocrit methods and erythrocyte numbers, erythrocyte and its nucleus areas and MCV were determined using microscopic methods.

Erythrocyte number and hematocrit levels decreased at 16 and 32 g L-1 of clinoptilolite with compared to control group in except 3 and 7 days of exposure ($p<0.05$). Clinoptilolite increased MCV, erythrocyte and its nucleus areas compared with the control. Erythrocyte numbers, erythrocyte and its nucleus area increased at 2, 4 and 8 g L-1 concentrations of clinoptilolite during 7, 21 and 30 days exposure compared to 3 days exposure while hematocrit and MCV decreased. Erythrocyte numbers, erythrocyte and its nucleus area decreased at 32 g L-1 concentrations of clinoptilolite during 7, 21 and 30 days exposure compared to 3 days exposure while hematocrit and MCV increased ($p<0.05$).

Changes observed in hematocrit level and erythrocyte morphology might be due to the effect of studied concentrations of clinoptilolite on both gill and blood cell permeabilities.

Key Words: *Cyprinus carpio,* clinoptilolite, adsorbent, blood

Öz: Araştırmada, doğal bir şelatlayıcı ajan olan klinoptilolitin (0,40 µ) 2, 4, 8, 16, 32 g/l'lik ortam derişimlerinin 3, 7, 21 ve 30 gün sürelerde, *Cyprinus carpio*'nun hematokrit düzeyi, eritrosit sayısı, ortalama eritrosit hacmi (MCV), eritrosit ve eritrosit nukleus alanı üzerine etkilerinin belirlenmesi amaçlanmıştır. İncelenen parametrelerden hematokrit düzeyinin analizinde mikrohematokrit yöntemi, eritrosit sayısı, MCV, eritrosit ve eritrosit nukleus alanının analizinde mikroskobik yöntemler kullanılmıştır.

Eritrosit sayısı ve hematokrit düzeyinin klinoptilolitin 16 ve 32 g/l ortam derişimlerinin 3 ve 7 günlük etki süreleri dışında, belirlenen süre ve derişimlerinin etkisinde kontrole göre düştüğü belirlenmiştir ($p<0,05$). MCV, eritrosit ve eritrosit nukleus alanının klinoptilolitin etkisinde kontrole oranla artış gösterdiği saptanmıştır. Klinoptilolitin 2, 4 ve 8 g/l derişimleri etkisinde eritrosit sayısı, eritrosit ve eritrosit nükleus alanı 3. güne oranla 7, 21 ve 30 gün etkisinde artarken, hematokrit düzeyi ile MCV düşmüştür. Klinoptilolitin 32 g/l derişimi etkisinde ise eritrosit sayısı, eritrosit ve eritrosit nükleus alanı 3. güne oranla 7, 21 ve 30 gün sürelerde düşmüş, hematokrit düzeyi ile MCV artmıştır ($p<0,05$).

Hematokrit düzeyi ile eritrosit morfolojisinde meydana gelen değişimlerin klinoptilolitin uygulanan derişim ve etki süresine bağlı olarak gerek solungaç gerekse kan hücrelerinin membran permeabilitesindeki bozukluktan kaynaklanabileceği olasıdır.

Anahtar Kelimeler: *Cyprinus carpio*, klinoptilolit, adsorban, kan

INTRODUCTION

Although industrial, agricultural and urban applications of modern designs, developed within the last 50 years, made social life easier, discarded waste materials become more dangerous to natural environments (Agarwal, 2009). Discharging these mainly anthropogenic materials to environment without reasonable treatment, negatively affect the life of organisms by changing the physical and chemical characteristics of these environments. Aquatic environments are the main sinks for terrestrial wastes due to hydrological cycle. Wastes entering aquatic environments affect water quality and while causing habitat change or mortality in sensitive species, they accumulate in species with high tolerance and cause metabolic, physiologic and biochemical changes.

Consumption of aquatic organisms effected by pollutants along the food web not only result in the extinction of sensitive species and therefore habitat destructions but also endanger public health. Polluted aquatic environments also limit the consumption aquatic organisms, an important and valuable edible protein source for human (İslam and Tanaka, 2004).

Hence, removing any pollutant that disturbs environment is crucial for a sustainable ecosystem. Serious precautions were begun to be taken by developed countries and a number of regulations were established in this context. One of the most important of these is the "Water Environment Directive" dated 23 October 2000 and numbered 2000/60/EC (Akkaya et al., 2006).

Concepts such as clean production in industry and green development began to took place in world agenda (Tütüncü, 2012). Environmental policies developed on one hand and regaining valuable minerals from waste and process waters to provide economic profit on the other, helped to increase environmental awareness to prevent pollution.

Adsorption, absorption, chemical precipitation, ion exchange, neutralization, reverse osmosis, vaporization and membrane methods are used in prevention of pollution in aquatic systems (Patterson, 1985; Marani et al., 1995; Smith, 1996; James and Sampath, 1999; Papini et al., 1999). Phytoremediation techniques were added to these application in recent years. (Terzi and Yıldız, 2011). These methods are generally used for removing heavy metals from industrial waste water.

Not only inorganic but also organic pollutants cause important health problems in aquatic ecosystems. Raw material selection become important to be used in the method selected. Most effective and practical methods are absorption and ion exchange in this regard (Gisi et al., 2016). Clay, active carbon, resin, gel and zeolite are used as raw materials in these methods.

It is expected that the adsorbents to be used should be economical, environment friendly and easy obtainable since large quantities of these materials are used depending on the purpose of use. Zeolite is one of the chelators having these specifications which are aluminum silicates ($Na_{12}[(AlO_2)_{12}(SiO_2)_{12}]27H_2O$) and clays formed by chemical reactions between lake or sea waters and ashes and lava surfaced the after volcanic eruptions. It has natural and synthetic forms. Clinoptilolite is a natural form of zeolite which has reserves in Turkey. It is produced by crashing and drying process without using and chemical in its processing which makes clinoptilolite an environment friendly while its low cost production makes it economic. Clinoptilolite, beside its use in pollution control studies is also used in energy, agriculture and animal husbandry, mining and metallurgy, paper, construction, detergent and health sectors (Gülen et al., 2012).

Some materials in nature although known as non-toxic may have harmful effects on living organisms above known concentrations. Hence it is important to determine maximum concentrations of this raw material to be used as chelator. Most pollutants discharged ends in aquatic environments which effect on organisms living here which in turn has an upmost importance on both environment and on aquatic products that are significant protein source for human.

Blood parameters are one of the first indicators that reflect changes in metabolic and physiologic events in animals. Effects of clinoptilolite on hematocrit levels, erythrocyte numbers, mean corpuscular volume (MCV), erythrocytes and their nucleus areas were determined after exposing *C. carpio* to 2, 4, 8, 16 and 32 gL^{-1} clinoptilolite over 3, 7, 21 and 30 days.

MATERIAL AND METHODS

C. carpio, 130±31 g in weight and 20,5±2 cm in length were used as the experimental material. Fish were obtained from the experimental unit of MERSIN University, Faculty of Aquaculture and the experiments were carried out in the faculty laboratories under constant temperature of 24±1°C and 12 hour light/dark regime.

Fish were placed in in glass aquaria, 40x100x40 cm in height, for 15 days for them to adapt laboratory conditions. Fish were fed with fish feed (Pınar, Pellet No: 2) in daily amounts 2 % of the total biomass during adaptation and experimental periods. Aquaria were aerated by central aerating system.

Six glass aquaria were used in the experiments taking the clinoptilolite concentrations tested into

account. 120 liters of stabilized tap water was added to the first aquarium and was used as control. The same amount of 2, 4, 8, 16 and 32 gL^{-1} clinoptilolite solutions were added in the following 5 aquaria respectively and used as experimental aquaria. Three fish were removed from each aquarium at the end of 3, 7, 21 and 30 days of exposure periods hence 12 fish were placed in each aquarium, which made 72 fish in total. Experimental solutions were changed once in two days with fresh solutions prepared from a new stock solution by serial dilutions for possible changes in clinoptilolite concentrations due to evaporation, precipitation and adsorption.

Some physical and chemical characteristics of water in experimental and control aquaria are given in Table 1.

Table 1. Some physical and chemical characteristics of water in experimental tanks

Temperature	22 ± 1 °C
pH	7.4 ± 0.7
Dissolved Oxygen	6.6 ± 0.51 mgL^{-1}
Total Hardness	225 ± 0.45 ppm $CaCO_3$
Alkalinity	331 ± 0.49 ppm $CaCO_3$

Due prevent to stress induced changes that may occur in the parameters studied during sampling, fish were anaesthetized using ethylene mono phenyl ester (= Phenoxyethanol, $C_8H_{10}O_2$; Merck) and dried using blotting paper before taking measurements and blood samples. Blood samples were taken by cutting the caudal peduncle vertically after measuring the weight and length of the fishes.

Spread blood preparations were made by dropping a drop of blood onto a microscope slide for determining erythrocyte and erythrocyte nucleus areas, blood samples were taken into citrated tubes to determine erythrocyte numbers and blood samples for the determination of hematocrit were directly taken into heparinized capillary pipettes.

Erythrocyte and erythrocyte nucleus areas were determined by morphometric measurements of microscopic inspection of the dyed spread slides. Giemsa method was adopted in the preparation of dyed spread slides (Grimstone, 1972). The length and width of a minimum of 150 erythrocyte and 150 erythrocyte nucleus in dyed spread slide belonging to each specimen were measured under a Nikon, H550-L research microscope and the areas were calculated using following formulas (Gregory, 2009).

$$\text{Erythrocyte Area} = \pi \frac{\text{Erythrocyte Width}}{2} \times \frac{\text{Erythrocyte Length}}{2}$$

$$\text{Erythrocyte Nucleus Area} = \pi \frac{\text{Erythrocyte Nucleus Width}}{2} \times \frac{\text{Erythrocyte Nucleus Length}}{2}$$

Hematocrit levels were determined according to microhematocrit method (Wedemeyer, 1990).

Erythrocyte numbers of blood numbers were determined under a Leica, CME microscope using Thoma slides (Wedemeyer and Yasutake, 1977). Erythrocyte numbers and MCV were calculated using the formulas given below (Konuk, 1981; Gürgün and Halkman, 1988).

$$\text{Erythrocyte Numbers} = \frac{\text{Erythrocyte Cell Numbers x Dilution Rate}}{\text{Numbers of Small a Squares Counted}} \text{x100}$$

$$\text{MCV} = \frac{\text{Hematocrite level}}{\text{Erythrocyte Numbers}} \text{x10}$$

Data obtained were analyzed statistically by a series of ANOVA and SNK using SPSS 16 pocket program. Arcsine transformation was applied to hematocrit data before statistical analysis since they are expressed as percentages.

RESULTS

No mortality was observed in *C. carpio* exposed to 2, 4, 8, 16 and 32 gL^{-1} clinoptilolite over 3, 7, 21 and 30 days. Although no behavioral changes was observed in fish exposed to low concentrations of clinoptilolite, moving towards the surface and color change after 25 days of exposure was observed at higher concentrations. Gill degenerations and hemorrhage was observed in fish dissected after exposing to high concentrations.

Erythrocyte numbers decreased compared to control under the effect of 32 gL^{-1} clinoptilolite at all exposure periods except 3 days. Number of erythrocytes increased in fish exposed to 2 and 4 gL^{-1} concentrations of clinoptilolite for 7, 21 and 30 days compared to day 3 where as the numbers decreased when exposed to 16 and 32 gL^{-1} concentrations ($p<0.05$; Table 2).

Table 2. Effect of clinoptilolite on erythrocyte numbers (x10^6) of *C. carpio*

Clinoptilolite Concentration (gL^{-1})	Exposure Period (Days)			
	3	7	21	30
	*	*	*	*
Control	1.08 ± 0.01 as	1.08 ± 0.03 as	1.17 ± 0.08 as	1.14 ± 0.05 as
2	0.77 ± 0.05 at	1.09 ± 0.03 bs	1.11 ± 0.11 bs	1.00 ± 0.01 bst
4	0.51 ± 0.04 ax	0.96 ± 0.08 bt	0.85 ± 0.01 bt	1.00 ± 0.08 bst
8	0.81 ± 0.03 at	0.85 ± 0.00 ax	0.84 ± 0.02 at	0.86 ± 0.04 at
16	0.94 ± 0.02 acy	0.71 ± 0.03 by	0.99 ± 0.01 cst	0.86 ± 0.01 at
32	1.34 ± 0.06 az	0.68 ± 0.03 by	1.00 ± 0.03 cst	1.08 ± 0.03 cst

*= SNK; Letters a, b, c and s, t, x, y and z show differences among the exposure periods and concentrations respectively. Data shown with different letters are significant at the p < 0.05 level.

$\bar{X} \pm S\bar{x}$ = Mean ± Standard error

Hematocrit levels increased compared to control on the 3rd day of exposure at 2 gL^{-1}, on 7th day at 8, 16 and 32 gL^{-1} and on 30thday at 16 gL^{-1} concentrations of clinoptilolite. There was a decrease in hematocrit levels in other concentrations and exposure periods studied. Clinoptilolite concentrations tested, with the exception of 2 gL^{-1}, increased hematocrit levels compared to day 3 at the exposure periods studied ($p<0.05$; Table 3).

Table 3. Effect of clinoptilolite on hematocrit levels (%) of *C. carpio*

Clinoptilolite Concentration (gL^{-1})	Exposure Period (Days)			
	3	7	21	30
	*	*	*	*
Control	42.5 ± 2.50 as	44.0 ± 4.00 as	41.0 ± 1.00 as	43.0 ± 1.00 as
2	55.5 ± 2.50 at	41.0 ± 2.00 bs	42.5 ± 1.50 bs	43.0 ± 1.00 bs
4	35.0 ±1.00 as	37.0 ± 1.00 as	45.0 ± 1.00 bs	37.5 ± 0.50 ast
8	35.5 ± 0.50 as	57.0 ± 1.00 bt	34.0 ± 2.00 at	35.0 ± 1.00 at
16	34.0 ± 2.00 as	61.0 ± 3.00bt	41.0 ± 1.00 as	53.5 ± 2.50 bx
32	32.5 ± 2.50 as	47.0 ± 1.00 bs	40.0 ± 1.00 as	33.0 ± 1.00 at

*= SNK; Letters a, b and s, t, x show differences among the exposure periods and concentrations respectively. Data shown with different letters are significant at the p < 0.05 level.

$\bar{X} \pm S\bar{x}$ = Mean ± Standard error

MCV increased compared to control in fish exposed to the tested concentrations of clinoptilolite except in fish exposed to 32 gL^{-1} concentration for 3 days. MCV value increased in fish exposed to 32 gL^{-1} concentration increased in exposure periods tested and decreased at all the remaining concentrations ($p<0.05$; Tablo 4).

Tablo 4. Effect of clinoptilolite on MCV (fl) of *C. carpio*

Clinoptilolite Concentration (gL^{-1})	Exposure Period (Days)			
	3	7	21	30
	*	*	*	*
Control	356.0 ± 8.0 as	379.5 ± 10.5 as	351.5 ± 5.5 as	363.0 ± 5.0 as
2	743.0 ± 2.0 at	498.0 ± 4.0 bt	372.0± 8.0 as	512.0 ± 3.0 bt
4	649.0 ± 5.0 ax	386.5 ± 7.5 bs	529.5 ± 8.5 ct	355.5 ± 12.5 bs
8	417.0 ± 2.0 ay	664.5 ± 1.5 bx	402.0 ± 9.0 ax	406.0 ± 6.0 ax
16	882.5 ± 4.5 az	850.5 ± 1.5 ay	416.0 ± 6.0 bx	574.0 ± 8.5 cy
32	316.5 ± 8.5 aw	663.5 ± 13.5 bx	427.0 ± 2.0 cx	439.0 ± 4.0 cz

*= SNK; Letters a, b, c and s, t, x, y, zshow differences among the exposure periods and concentrations respectively. Data shown with different letters are significant at the p < 0.05 level.

$\bar{X} \pm S\bar{x}$ = Mean ± Standard error

Except the effects of 2 gL^{-1} clinoptilolite on 7th and 32 gL^{-1} concentration on 21st days, erythrocyte area increased compared to control (p<0.05; Table 5).

Tablo 5. Effect of clinoptilolite on erythrocyte area of *C. carpio* (μm^2)

Clinoptilolite Concentration (gL^{-1})	Exposure Period (Days)			
	3	7	21	30
	*	*	*	*
Control	0.78 ± 0.01 ast	0.74 ± 0.01 as	0.74 ± 0.02 as	0.74 ± 0.02 ast
2	0.70 ± 0.00 as	0.57 ± 0.02 bt	1.01 ± 0.05 ct	0.93 ± 0.01 cx
4	0.74 ± 0.02 ast	0.86 ± 0.01 ax	0.75 ± 0.03 as	0.85 ± 0.03 ay
8	0.77 ± 0.02 at	0.76 ± 0.00 as	0.85 ± 0.01 bs	0.74 ± 0.01 ast
16	0.83 ± 0.02 ax	0.81 ± 0.03 as	0.81 ± 0.01 as	0.68 ± 0.01 bs
32	0.74 ± 0.01 ast	0.76 ± 0.01 as	0.58 ± 0.02 bx	0.79 ± 0.00 at

*= SNK; Letters a, b and s, t, x show differences among the exposure periods and concentrations respectively. Data shown with different letters are significant at the p < 0.05 level.

$\bar{X} \pm S\bar{x}$ = Mean ± Standard error

Erythrocyte nucleus areas increased in fish exposed to the tested concentrations of clinoptilolite except under the effect of high concentrations on the 21st day. Compared to 3rd day erythrocyte nucleus areas increased with exposure period and decreased under high concentrations of clinoptilolite (p<0.05; Table 6).

Table 6. Effect of clinoptilolite on erythrocyte nucleus area of *C. carpio* (μm²)

Clinoptilolite Concentration (gL^{-1})	Süre (Gün)			
	3	7	21	30
	*	*	*	*
Kontrol	0.15 ± 0.002ast	0.14 ± 0.004ast	0.15 ± 0.002ast	0.14 ± 0.003 as
2	0.14 ± 0.003 as	0.13 ± 0.003 as	0.16 ± 0.011at	0.15 ± 0.006 as
4	0.14 ± 0.001 as	0.17 ± 0.001 bx	0.15 ± 0.001ast	0.15 ± 0.005 as
8	0.16 ± 0.00 at	0.15± 0.001 astx	0.13 ± 0.010 as	0.15 ± 0.004 as
16	0.18 ± 0.007ax	0.15 ± 0.008 astx	0.13 ± 0.002 bs	0.16 ± 0.003 as
32	0.19 ± 0,002ay	0.16 ± 0.006 btx	0.12 ± 0.001 cs	0.14 ± 0.004 ds

*= SNK; Letters a, b, c and s, t, x, y, z show differences among the exposure periods and concentrations respectively. Data shown with different letters are significant at the p < 0.05 level.

$\bar{X} \pm S\bar{x}$ = Mean ± Standard error

DISCUSSION

Chelators although have an important role in eliminating the toxic effects of pollutants, it was shown that the synthetic chelator EDTA causes mortality over a given dose James et al., 1998). A number of studies carried out with various fish species showed that clinoptilolite used in various concentrations to prevent ammonia (Asgharimoghadam et al., 2012; Farhangi and Rostami, 2012) and heavy metal (Tepe et al., 2004; Mishra and Jain, 2009; Ghiasi and Mirzargar, 2015) toxicities without causing mortality. No mortality was observed in *C. carpio* exposed to 2, 4, 8, 16 and 32 gL^{-1} of clinoptilolite over 3, 7, 21 and 30 days. Being a natural chelator and that no chemicals are used in its production might prevent mortality in *C. carpio* or else the concentrations tested were low and/or exposure periods were short for mortality in this species.

Various substances entering the aquatic environments change the physical and chemical composition of water. Aquatic organisms first react these changes by changing their behaviors. Swimming towards the surface, irregular or fast swimming movements, increase in operculum movements and reject feeding can mentioned among these behavioral changes. Clinoptilolite 2-5 mm in diameter was shown to increase turbidity in water and in high concentrations block fish gills and cause asphyxia (Rubec and Cruz, 2005). Adsorbent originated turbidity increase was reported to cause behavioral changes in salmonids (Bash et al., 2001). Low concentrations of clinoptilolite used in the present study did not cause any behavioral change in *C. carpio*, whereas fish started to swim near surface under the effect of high concentrations. This might be due to stress caused by intense turbidity as result of high concentrations of clinoptilolite which in turn increase mucus secretion and limit respiration.

Gills are the main respiration organs of fish and are in direct contact with the environment. Hence chemicals entering water increase mucus secretion in gills and cause to develop defense mechanisms such as hyperplasia, hypertrophy, and proliferation (Wedemeyer et al., 1990) which increases oxygen diffusion distance and cause hypoxic conditions. Stress developed under hypoxic conditions have profound effects on hematologic parameters (Heath, 1995). Increase in the amount of suspended solid material (Becke et al., 2017) in *Oncorhynchus mykiss*, SiO_2 nanoparticles in *Labeo rohita* (Priya et al, 2015), heavy metals such as copper and lead (Çiftçi et al., 2015) result in stress and cause changes in hematologic parameters.

Studies carried out with a number of fish species showed that clinoptilolite had no histopathologic effect up to a certain concentration (15 gL^{-1}) (Asgharimoghadam et al., 2012; Farhangi and Rostami, 2012; Ghiasi and Jasour, 2012). External inspection of gill tissues revealed no deformation in *C. carpio* exposed to low concentrations of clinoptilolite. Whereas degenerations such as adherence of gill filaments, gill tissue dissolutions and hemorrhage were observed in fish exposed to 32 gL^{-1} clinoptilolite. This might result from friction of clinoptilolite particles with epithelial tissue during water passage through gills depending on particle size and concentration of clinoptilolite.

Deformations on gill tissue effect respiration negatively. Erythrocyte numbers, hematocrit levels, MCV, erythrocyte and erythrocyte nucleus areas are indicator parameters used in the determination of changes that occur in metabolic and physiologic

events. A decrease in hematocrit level and erythrocyte numbers and an increase in erythrocyte and nucleus areas was observed in *O. niloticus* exposed to Cd alone whereas when exposed to in mixture with the chelator chitosan (10 ppm) no change was detected in the parameters studied compared to control (Çiftçi et al., 2013).

Erythrocyte numbers of *C. carpio* decreased compared to control at all clinoptilolite concentrations and exposure periods tested except in 32 gL^{-1} concentration at 3 days of exposure. The numbers increased, however, compared to 3rd day at lower concentration sat the exposure periods tested. Since erythrocytes reflect the oxygen carrying capacity of blood, increase in their numbers show release of erythrocytes from hemopoietic tissues such as spleen and kidneys by feedback mechanisms to increase oxygen carrying capacity of blood. Whereas the decrease in numbers might reflect osmotic hemolysis due to impairments in membrane permeability.

Hematocrit level increased at 2 gL^{-1} clinoptilolite concentration at the beginning of exposure whereas exposures to 8, 16 and 32 gL^{-1} concentrations increased hematocrit after 7 days of exposure compared to control There was a decrease in its level at the other concentrations and exposure periods. Hematocrit is the ratio of shaped elements of blood to serum, it is directly related to erythrocyte numbers and area. Hence, the increase in hematocrit level under the effect of clinoptilolite at the concentrations and periods tested is directly related to erythrocyte area.

MCV increased compared to control in fish exposed to clinoptilolite at the concentrations periods tested except at 32 gL^{-1} exposure to 3 days where it decreased. Increase in MCV, can either be a result of swelling of erythrocytes by taking water due to failure in osmoregulation or numbers of primary erythrocytes compared to mature erythrocytes was higher.

Erythrocyte and erythrocyte nucleus areas increased compared to control except erythrocyte area in fish exposed to 2 gL^{-1} for 7 days and in fish exposed to 32 gL^{-1} for 21 days. Changes in erythrocyte and nucleus areas might be a result of swelling of erythrocytes by taking water due to failure in membrane permeability.

In conclusion, 32 gL^{-1} clinoptilolite concentration did not cause mortality in *C. carpio*, however due to deformations observed in gills using these concentrations in micronized sizes would not be suitable for fish health. Moreover high concentrations of clinoptilolite tested in this study caused changes in hematological parameters.

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