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USED OF ZELOITE TO REDUCE THE IRON TOXICITY FOR ENHANCEMENT THE GROWTH PERFORMANCE AND PHYSIOLOGICAL STATUS IN NILE TILAPIA *(Orechromius nloticus)*.

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ABSTRACT

Iron is an essential element involved in many vital physiological functions in fish. Excess of iron concentration caused some toxic effects. The present study aims to evaluate the possible ameliorative effect of Zeolite against iron toxicity in Nile tilapia . Tilapia fry were divided into five groups. The 1st group (lefet as control group without any addition. The 2nd , exposed to 7٥mg/L of ferrous oxide. While 3rd , 4th and 5 th groups exposed to same concentration of ferrous oxide (Fe_2O_3) with 2, 4 and 8g of Zeolite/L, respectivelyy .The growth performance of *O. niloticus*, fry improved with the high concentration of zeolite compared to Fe group alone and control group. The best feed conversion ratio was recorded in the fish fingerlings exposed to 8g of zeolite/L compared with the control After exposure fish to Fe a reduction significantly ($P<0.05$) in erythrocyte count (RBC_s) haemoglobin content (Hb), haematocrit value (Hct). These parameters were improved when zolite was applied with Fe. The values of (RBC_s), Hb and Hct were increased significantly to be as in the control fish group. Similarly, there were significant decreased in total protein (TP) in plasma of fish exposed to iron alone and iron with low doses of zeolite. While, these values were improved in fish after addition of 8 g of zeolite/ L. Whereas, plasma glucose concentration, total lipid, cholesterol, uric acid and creatinine were increased significantly in fish exposed to Fe alone and mixture of same dose of Fe with low of zeolite. Similarly, alanine aminotransferase (ALT) and alkaline phosphatase (ALP), were increased significantly in fish exposed to Fe alone.

Addition of zeolite to Fe contaminated medium enhanced these biochemical parameters in fish and the enzyme activities returned to be as control group. Addition of zeolite to Fe contaminated medium considerably reduced Fe absorption and accumulation in muscle and gills.

Key wards: Nile tilapia, Fe, Zeolite, growth performance, Physiological parameter.

INTRODUCTION

Water born metals may alter the physiological and biochemical parameters in fish blood and tissues. The reaction and survival of aquatic animals depend not only on the biological state of the animals but also on the toxicity, and type and time of exposure to the toxicant (Brungs, 1977).

Iron (Fe) is an essential element for body functions such as oxygen transport and lipid oxidation and its presence constitutes a vital defense line to protect fish against infectious diseases (Hilty *et al*., 2011). However, iron excess can result in fish toxicity (Andersen *et al*., 1997 and Chu *et al*., 2007). The concentration of iron in body tissues must be tightly regulated because in excessive amounts it can lead to tissue damage, where it can form free radicals (Abbaspour *et al*., 2014). Besides, iron is a coenzyme and is necessary for the synthesis of hemoglobin (Yacoub, 2007) but very high intake of these elements can cause adverse health problems. Disturbances in AST and ALT activities are indicators of toxic conditions when these enzymes penetrate to the blood due to cytolyses of the liver (Kaya *et al*., 2014).

Iron is considered a heavy metal being found in high concentrations in aquatic environments near the miners that exploit this ore. In Brazil, the dam located in the municipality of Mariana - MG was built to serve as a deposit of the wastes generated during the mining process of iron, and ruptured, causing an unprecedented environmental disaster in Brazil's history (Barba, 2015). Iron is also regarded as an important chemical element for many living organisms (as a bio metal) and is vital for survival at low concentrations as it is essential for multiple metabolic processes such as oxygen transport, DNA synthesis, electron transport, and cofactor for many proteins (Bury and Grosell, 2003). Iron present in water can be absorbed by the fish via gills, skin or food (Cottet *et al*., 2015)

Excess iron dissolved in the water can cause the formation of flakes of this metal in the gills of the fish resulting in its obstruction, causing respiratory disorders Bury *et al*. (2003). Animals that consume diets with high levels of iron may have reduced growth, worse feed conversion, diet rejection, mortality, and histopathological damage in liver cells, where excess of iron in the body is stored in organ and tissutes (Bury and Grosell, 2003). Fish can be used as environmental indicators, reacting immediately to any changes in the aquatic ecosystem, through physiological changes such as changes in opercular beating, unusual swimming, gill changes and feeding changes or difficulties (Hundley and Navarro, 2018).

Zeolites are used in industry, agriculture, environment protection and even in medicine. Zeolites have a relatively high Si/Al compositional ratio which gives it is special ion-exchange selectivity for large monovalent cations. Natural or synthetic zeolites (sodium aluminum silicates) are known to easy adsorb metal ions by exchange reactions Jain (1999). Zeolites are microporous crystalline hydrated aluminosilicates, which have found various applications because of their very unique physicochemical characteristics such as ion exchange and adsorption–desorption properties. Significant progress has been made in recent years on applications of these inorganic adsorbents in different industries including agriculture, aquaculture, water and wastewater treatment, air purification and petrochemicals (Ghasemi *et al*. 2016). Natural zeolites are porous and hydrated aluminum silicates that have ion-exchange and adsorption properties that due to their a large surface area (Xia *et al.*, 2009). However, the findings of many other studies that zeolite (Clinoptilolites) are shown to be highly effective regarding the metabolic utilization of nitrogen in animals in addition to its ability to improvement of fish meat quality and growth (Mumpton & Fishman, 1977 and Stetca & Morea, 2013).

Zeolite has been recommended and used effectively in reducing toxic effects of feed materials infected with aflatoxins (Jand *et al*., 2005). Moreover, zeolite can be used as an antimicrobial agent (Haile and Nakhla, 2010). Forms of zeolite have been used to remove ammonium from aqueous solutions by cation exchange capacity (Wen *et al*., 2010) and help in reducing ammonia production by pellets (Wu-Haan *et al*., 2007) because the zeolites have many important characters such as ion exchange, filtering, odor removal, chemical sieve, water softener, stabilization of soil and gas absorption (Kocakusak *et al*., 2001). Shalaby *et al*. (2018) Showed that the addition of zeolite to Cd contaminated medium enhanced these biochemical parameters in fish and the enzyme activities returned to be as control group., also addition of zolite to Cd contaminated medium considerably reduced cadmium absorption and accumulation in gills. Simillary, these result are agreement with those of Shalaby *et al*. (2021) in Same of trend after exposure of tilapia to iron toxicity.

MATERIALS AND METHODS

Ferric oxide: It was obtained from El-Nasr Chemical Company (Cairo, Egypt).

Zeolite: Samples of zeolite were taken from the Enli Mining Company open pit mine in Manisa-Gördes in Western Anatolia.

The crushed original zeolite was ground and passed through 300 × 600-µm sieves and was dried in an oven at 100 ± 5 ºC for 24 h. It was characterized by X-ray diffraction (XRD) and chemical analysis. Chemical composition of zeolite samples was determined by the usual analytical methods for silicate materials. Standard wet chemical analysis was adopted.

Table 1. Chemical composition and physical properties of natural zeolite.

Chemical composition	Wt.	Physical properties
SiO_2	69.31	Appearance porosity (%), 41.5
Al_2O_3	13.11	Appearance density (g/cm3), 2.27
Fe_2O^3	1.31	Weight of per unit volume (g/cm3), 1.32
CaO	2.07	Water absorption (original) (%), 31.3
MgO	1.13	Water absorption (grinding) (%), 103.7
Na_2O	0.52	Oil absorption (g oil/100 g sample), 51
K_2O	2.83	Whiteness (%), 68
SO_3	0.10	Original bleaching (g sample/g tonsil), 1.95
H_2O	6.88	Active bleaching (g sample/g tonsil), 1.92
Si/Al	4.66	pH 7.5

Experimental fish:

Healthy Nile tilapia "*Oreochromis niloticus*" specimens with an average body weight of 9.5 ± 0.3 g obtained from Abbassa fish farm, Abbassa, Abou-Hammad, Sharkia, Egypt were acclimated in laboratory conditions for two weeks prior the experiment.

The experiment consist of five groups of the studied fish were holded for 90 days to evaluate the effects of different sublethal concentrations of ferrous oxide with or without Zeolite on the growth performance , physiological and biochemical assays. These groups were divided to the first group was kept as control. Second group was exposed to the 7٥mg ferrous oxide /L only . The third ,fourth and fifth groups exposed to same dose of Fe with 2, 4 and 8 g of zeolite /L respectively Each group consisted of 2o fish was divided into two replicates of tenth fish each replicate .Then maintained in glass aquaria supplied with dechlorinated aerated tap water at a temperature of 26±2 ^{o}C, pH 7.2±0.2 and dissolved oxygen 5.5 ± 0.5 mg/l.. The feeding rate was holded at the percent of 3% of the body weight daily.

Table 2. Experimental Design Outline.

Treatment	Ferrous oxides (Mg/L)	Zeolite (G)
T1	-	-
T2	75	-
T3	75	2
T4	75	4
T5	75	8

Water quality measurements:

Water samples were collected weekly at 15 cm depth from each aquarium. Dissolved oxygen (DO) and water temperature were measured in situ with an oxygen meter (YSI model 58, Yellow Spring Instrument Co., Yellow Springs, OH, USA). Unionized ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, CO, USA) and pH with a pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). All treatments, DO concentrations ranged from 4.1 to 4.6 mg/L, and water temperature average was 26.5±0.8 ^{o}C, pH value ranged from 7.2 to 7.6. Unionized ammonia were measurement according to (APHA, 2000). Water quality parameters were within acceptable ranges for fish growth (Boyd, 1984).

Fish growth measurement:

Growth performance was determined and feed utilization was calculated as follows equation: Wg= W2-W1 was Weight gain

Specific growth rate (SGR) was calculated according to Jauncey and Rose, (1982). SGR = 100 (ln W2 – ln W1) / T

Relative body weight gain (RBWG) was calculated according to the following equation RBWG= (wt2-wt1) x100/wt1

Where W1 and W2 are the initial and final weights, respectively, and T is the number of days of the experimental period.

Hematological and biochemical analysis:

At the end of the experiment, blood samples were taken from the caudal vein of non-anaesthetized fish by sterile syringe. 0.5 ml of the blood containing EDTA as an anticoagulant was used for erythrocyte count (Dacie and lewis, 1984), haemoglobin content (Van kampen, 1961) and haematocrit value (Britton, 1963).

Plasma was obtained by centrifugation of whole blood at 3000 rpm for 15 min and nonhaemolyzed plasma was stored in deep freezer for biochemical analyses. The muscle and gills of all fish specimens were collected and tpred for tissue analysis.

Alanine amino transferase (ALT) were determined according to the method of Reitman and Frankel (1957). Alkaline phosphatease was measured by using Diamond diagnostics kits according to the method of Bergmeyer (1972). The uric acid a determined according to Caraay (1955) and the glucose were estimated according to the method of Trinder (1969), while creatinine was determined according to (Kaplan, 1984). Total protein was determined according to Henry (1964) and total lipids according to Schmit (1964). Cholesterol and High-density lipoprotein (HDL) was determined as CHOD-PAP method according to Allain *et al*. (1974). All of these parameters were measured using specific reagent kits purchased from Diamond Diagnostic Company.

Fe residue: Iron was measured in muscles and gills according to method of Eaton and Stinson (1983).

Statistical Analysis:

The obtained data were subjected to analysis of variance according to Snedecor and Cochran (1982). Differences between means were done at the 5% probability level, using Duncan' s new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Water quality parameters :

Water quality parameters were analyzed as represented in Table (3). Water temperature was to (24.06 ^{o}C) consistent in all treatments. It was within the limits of optimal survival and growth of tilapia (Yıldırım *et al.*, 2009).. Similarly, salinity showed no variation among treatments. Throughout the experiment, dissolved oxygen level was decreased significantly to (3.02) in Fe group alone as compared to control and other treatments. Limits of dissolved oxygen for tilapia is ranged between 3.0-4.0 mg/L (Popma, 1999). In the present study, the DO values were below the limits except for treatment with 15 g/L zeolite. This was due to the high concentration of ammonia (Table 3). Mokhtari-Hosseini *et al*. (2016) stated that the existence of ammonium ion in water can cause the depletion of dissolved oxygen levels in water .

pH were measured at their lowest levels as 7.47± 0.15 and the highest was recorded as 9.34±0.16. In general, tilapia can survive in pH ranging from 5 to 10 but do best in a pH range of 6 to 9. In addition, pH is an important parameter affecting the sorption process at the water-dsorbent interfaces (Mazloomi and

Jalali, 2016). The total ammonia NH_3, total alkalinity and No_2 concentration was increased significantly in Fe alone group (T2) when compared to the control group. While, addition of zeolite to anther treatment caused reduction of these parameters similar the control group. EC were significantly different between the treatments when compared to the control group. In addition, Oz *et al*. (2016) found that addition of zeolite inside or outside net bag had reduced the concentration of ammonia in aquarium water. However, the use of zeolite in a net bag gave more advantages in terms of high adsorption levels of ammonia. Zeolite exchanges sodium ions for ammonium ions and shifts the ammonia equilibrium away from toxic un-ionized ammonia, thus preventing an increase in its level (Ghiasi and Jasour, 2012). The ranges of water quality parameters were within the acceptable ranges for fish culture (Boyd, 1984). On the other hand, the reduced ammonia concentrations at zeolite fish groups may be due to zeolite efficacy to adsorb ammonia. This adsorption efficacy depends on zeolite dose (Shalaby *et al*., 2021).

Table 3. The water quality in experimental of *O. niloticus* after exposed to iron alone and mixed with doses of zeolite.

Treatment	pH	DO	Total ammonia	NH3	Total alkalinity	Total hardness	NO2	Temp	E.C	Salinity
T1	7.51± 0.13e	5.23± 0.19ab	0.44± 0.02e	0.02± 0.01e	142.00± 3.74cd	141.60± 10.78	1.43± 0.51b	24.06± 1.24a	457.20± 9.28c	0.26± 0.01ab
T2	9.34± 0.16a	3.02± 0.11ab	2.55± 0.07a	1.76± 0.23a	149.60± 2.04bc	126.00± 2.97	2.31± 0.19a	24.06± 1.24a	515.00± 23.69ab	0.33 0.02ab
T3 L)	8.72± 0.19bc	5.44± 0.25a	1.48± 0.04c	0.46 0.12c	133.60± 3.08de	128.80± 2.42	1.19± 0.23b	24.06± 35.40a	480.20± 10.25bc	0.26± 0.003ab
T4	7.89± 0.16d	5.16± 0.22ab	1.49± 0.06c	0.10± 0.04de	145.60 2.32bc	115.60± 1.47	1.53± 0.34ab	24.06± 1.24a	478.00± 19.24bc	0.28± 0.08ab
T5	7.47± 0.15e	5.05± 0.09ab	0.46± 0.040e	0.012 0.003e	142.00± 3.74cd	111.20± 2.15	0.92 0.08b	24.06 1.24a	495.20± 7.94abc	0.31± 0.02ab

The same letter in the same column is not significantly different at $P<0.05$.

Growth performance:

Table (4) shows that there was no significant difference between the experimental groups in body weight at the start of the experiment. At the end of the experiment (after 12-week culture), it was observed that the exposed to iron alone (T2) significantly decreased (to 23.00±0.115g/fish and 13.81±0.106 g/fish $P<0.05$) in final body weight and weight gain of Nile tilapia respectively as compared to the to the control group (28.47±0.087 and 19.20±0.063, respectively). Similarly, the addition of zeolite by 2 and 4 g/L with same dose of iron toxicity was non significantly variation in same parameter. While addition of high dose of zeolite 8g/l with same dose of iron was improved significantly in final body weight and weight gain of Nile tilapia to 30.53 ±0.087 g and 21.36 ±0.121 (g/fish), respectively. when compared to Fe alone (T2) and similar the control group (28.47±0.087 and 19.20±0.063, respectively). In same table, the specific growth rate (SGR) and relative growth rate (REG) were decreased significantly to 1.53±0.008 and 1.504 ±0.012% in Nile tilapia exposure to iron toxicity when compared to the control group. On the other hand, addition of 8g/l of zeolite with same iron dose toxicity was improved significantly in SGR and REG respectively in Nile tilapia when compared to iron toxicity alone and similar the control groups (Table 3). Ghiasi and Jasour (2012) found that, the final weight gain of Angel fish (*Pterophyllumscalare*) with exposed to 10 & 15 g/L zeolite were higher compared to control group (without addition of zeolite). Related to the results of current study, addition of 20 g/L showed the highest weight gain and 15 g/L zeolite resulted in second highest in weight gain of Red Hybrid Tilapia compared to other treatments. According to these findings it can be concluded that addition of 15 g/L of zeolite in water not just the best for water quality improvement but was also the best dose for better growth performance of fish. Besides, the study performed by Yıldırım *et al*. (2009) showed that, the growth performance of tilapia zilli fed with fish feed diets containing 2% of zeolite showed an significant increase compared to control group.

Table 4. Growth performance in *O. niloticus* after exposed to iron alone and mixed with doses of zeolite**.**

Item Groups	Initial Weight	Final weight	Weight gain	DWG	SGR	RGR
T1	9.27[a] ±0.031	28.47[b] ±0.087	19.20[a] ±0.063	0.319[a] ±0.0011	1.87 [a] ±0.003	2/068[a] ±0.006
T2	9.17 [a] ±0.070	23.00[d] ±0.115	13.81[d] ±0.106	0.230[d] ±0.0018	1.53[d] ±0.008	1.504[d] ±0.012
T3	9. 23 [a] 0.070±	25.52[c] ±0.029	16.29[b] ±0.042	0.271[b] ±0.0007	1.69 [b] ±0.010	1.763[b] ±0.017
T4	9.17 [a] ±0.067	25.51[c] ±0.289	16.34[b] ±0.289	0.272[b] ±0.0048	1.71[b] ±0.018	1.785[b] ±0.031
T5	9.19 [a] ±0.034	30.53[a] ±0.087	21.36[c] ±0.121	0.355[c] ±0.002	2.00[c] ±0.011	2.236[c] ±0.227

The same letter in the same column is not significantly different at P<0.05.

Results in Table (5) show that FCR, FER and PER were decreased significantly to 1.097±0.012, 42.57±0.523 and 1.44±0.018, ($p<0.05$), respectively in Nile tilapia exposed to Fe alone as compared to the control group. Whereas the addition different dose of zeolite to iron toxicity was caused improved significantly ($p<0.05$) in this parameters when compared to the Fe alone and similar to the control group.

Table 5. Feed intake (FI), feed conversation ratio (FCR), feed E ratio(FER), protein E ratio (PER), protein valuation (PPV) and (E.U) in *O. niloticus* after exposed to iron alone and mixed with doses of zeolite**.**

Items treatment	FI	FCR	FER	PER	PPV	E.U
T1	35.418[c] ±0.555	1.85[d] ±0.023	54.07[a] ±0.675	1.82[a] ±0.023	26.21[a]b ±0.537	21.07[a] ±0.408
T2	27.263 [d] ±0.844	1.097[c] ±0.012	42.57[d] ±0.523	1.44[d] ±0.018	17.75[d] ±0.446	15.09[d] ±0.325
T3	35.288 [c] ±0246	2.00[c] ±0.046	46.31[c] ±0.495	1.56[c] ±0.017	24.91[b c] ±0.414	16.78 [c] ±0.262
T4	38.26b[c] ±0.5652	2.16[bc] ±0.0239	50.05[b] ±1.157	1.69[b] ±0.039	23.43[c] ±0.302	17.34[c] ±0.197
T5	42.023[a] ±0.428	2.355[a] ±0.089	50.76[b] ±0.298	1.71b[c] ±0.010	27.86[a] ±1.01	19.76[b] ±0.678

The same letter in the same column is not significantly different at P<0.05.

Protein content in fish body was significantly lower in the group exposed to Fe alone than the control group and all other groups (mixture of same doses of Fe with different dose of zeolite) (Table 6). Contrarily, total lipids content in fish body high significantly (23.11%, $p<0.05$) in fish exposed to Ferric oxides when compared to the control group (19.15%); while it values were at same trend in all other groups (mixture of Fe with zeolite) when compared to the control group (Table 6). Ash content was significantly higher to (19.22, 19.10. 19.90 and 20.07 %; $p<0.05$) in fish exposed to Fe alone and mixture of different levels to zeolite respectively when compared to the control group (17.04 %) .Dry meter in fish body was significantly affected with treatments.

Table 6. Body composition 0f *O. niloticus* after exposed to iron alone and mixed with doses of zeolite (n=6).

Items treatment	DM	C.P	E.E	ASH
Start	23.54[e] ±0.0.23	57.85[f] ±0.026	19.15[f] ±0.046	23.13[a] ±0.035
T1	27.35[b] ±0.029	59.85[a] ±0.015	23.11[a] ±0.006	17.04[f] ±0.020
T2	26.75[d] ±0.029	58.43[f] ±0.015	22.35[b] ±0.009	19.22[d] ±0.015
T3	27.97[a] ±0.03	59.74[b] ±0.012	21.15d ±0.0.009	19.10[c] ±0.014
T4	27.40[bc] ±0.029	58.65[d] ±0.009	21.45[c] ±0.009	19.90[c] ±0.017
T5	26.98[c] ±0.020	58.91[c] ±0.006	21.02[e] ±0.012	20.07[b] ±0.009

The same letter in the same column is not significantly different at $P<0.05$.

Physiological parameter:

In vertebrates including fish, blood is the most frequently examined tissue in efforts to establish their health status or physiological status. Accordingly, health status such as oxygen carrying capacity has been directly determined by reference to main hematological indices including red blood cell (RBC), hemoglobin concentration (Hb) and Hematocrit values (Hct) by Houston (1990). Haematological parameters play an important role in the environmental monitoring of toxicants in aquatic ecosystem and also act as indicators of disease and stress (Li *et al.*, 2010 and Lavanya *et al.*, 2011). Parameters such as RBC, WBC, PCV, Hb and haematological indices (MCV, MCHC and MCHC) are widely used as strong bio indicators in aquatic toxicology (Singh & Srivastava, 2010 and Saravanan *et al.*, 2011). Similarly, biochemical parameters (glucose and protein) after due analysis are used as important indicators of physiological status of fish and the health of the aquatic environment (Pimpao *et al.*, 2007). Also, Saravanan *et al* (2015) Measured that the potential harmful effects of Fe_2O_3 (1 and 25 mg/L) on haematological, and biochemical, responses in an Indian major carp, *Labeo rohita* for a short-term period of 96 h. The results revealed significant (P\0.05) decreases in erythrocyte count, haemoglobin, haematocrit, and protein content in the blood of fish. In the present study, the erythrocyte count and haematocreat value in Nile tilapia were decrease significantly to (1.36±0.014 and 10.10±0.45 %, respectively) after exposed to iron alone when compared to the control group (1.43 ±0.017 and 29.37 ±0.43 %). On other hand, the hemoglobin content was high significantly in fish after exposed to Ferric oxide. Addition of zeolite improved health which increased hematological parameter (RBC_S, H and Hct) compared than control and treatment 2 which toxic by iron alone (Table 7).

The haemoglobin was more relevant, due to the toxicity of this contaminant being greater and causing damage to the organism in a shorter time. With the Fe2+ contaminated groups, there was also an increase, but smaller when compared to the Fe3+ treatments. Fish exposed to Fe ions have demonstrated a functional increase in blood hemoglobin levels, as Hb is converted to MeHb, causing intoxication in animals Aggergaard and jensnm (2001). Iron is involved in hepatic processes, and is also associated with oxygen transport through Hb, being considered one of the most important elements for fish homeostasis (Meynard *et al.*, 2014). However, excess iron dissolved in the water can cause iron flakes to form in the gills of the fish resulting in their obstruction, causing respiratory disorders Bury *et al*. (2003) and the increase of OB to overcome this provoked deficiency. In acute cases of iron poisoning there may be necrosis of the gill tissue and loss of ammonia excretion capacity that concentrated in the blood of fish. Slaninova *et al*. (2014). While the decrease in WBCs count in iron exposed group may be due to the increase in corticosteroids hormones that are secreted in fish exposed to any stress, such decrease in WBCs was modulated in pre curcumin exposed groups that may be explained by the chelating effect of curcumin and its ability to reduce free iron concentrations. Also, curcumin was found to be safe in Anabas testudineus at doses of 0.5 and 1% and did not produce any hematological changes Aggergaard and jensnm (2001). These results are in agreement with those of. Shalaby (2007) who found that a decrease in RBCs, Hb and packed cell volume (PCV) in the blood of Nile tilapia after cadmium intoxication. The obtained results revealed that toxicity fish were recovered when addition of zeolite as the RBCs, Hb and Hct in fish exposed to mixture of iron with zeolite became similar to those of the control fish. Similar results as Shalaby *et al*., 2018).

Table 7. The erythrocyte count (10^6 cells/ µl), haemoglobin content(g/ 100 ml) and haematocrit value (%) in blood of Nile tilapia after exposed iron alone and mixed with doses of zeolite.

Items treatment	Erythrocyte count	Haemoglobin content	Haematocrite value
T1	1.43 ^{a}±0.017	5.19 ^{b} ±1.4	29.37 ^{a}±0.43
T2	1.36 ^{d}±0.014	13.07 ^{a} ±2.66	10.10 ^{c}±0.45
T3	1.63 ^{c} ±0.025	14.59 ^{a} ±1.49	17.4 ^{b}±0.45
T4	1.57 ^{b}±0.038	8.56 ^{ab} ±0.272	24.1 ^{b}±2.26
T5	1.64 ^{b} 0.020	13.5 ^{a} 0 ±.29	30.13 ^{a}±1.04

The same letter in the same column is not significantly different at P<0.05**.**

Alteration of blood sugar level revealed a stress response of fish (Nemcsok *et al.*, 1987). In the present study, the blood glucose was increased significantly to (138.67±15.67 mg/L) in fish groups exposed to sublethal dose of iron alone, also it values was increased significantly to (135.84±2.255 & 134.40±5.807 mg/L, P<0.05) in Nile tilapia reared by iron combined with low doses of Zeolite (T3 &T4) respectively (Table 8). The increase in blood glucose might be resulted from an increase in plasma catecholamines and corticosteroid hormones (Pickering, 1981). Moreover, the hyperglycemia induced by any toxicant might be explained by the inhibition of the neuro-effector sites in the adrenal medulla leading to hyper secretion of adrenaline, which stimulates the breakdown of glycogen to glucose (Gupta, 1974).

The blood of common carp showed significant increase in glucose during 32 days of heavy metal intoxication. This might be due to the vulnerable stress induced by the heavy metals resulted in hyperglycemia. Previous investigation proved that, cadmium modulate the metabolism of carbohydrates, causing hyperglycemia by stimulating the glycogenolysis in some marine and fresh water fish species (Zikic *et al.*, 1997; Almeida *et al.*, 2001 and Levesque *et al.*, 2002). Similar trend with characteristic hyperglycemia was observed in Nile tilapia throughout our experiment. Heavy metals increase the glucose content in blood, because of intensive glycogenolysis and the synthesis of glucose from extra hepatic tissue proteins and amino acids. On other hand, the addition high levels of Zeolite to iron toxicity caused non-significant variation of blood glucose to (89.77±2.531mg/L) similar as control group (78.333 ±9.331 mg/L) (Table 8).

The quantitative determination of the total plasma protein reflects the liver capacity of protein synthesis and denoted the osmolality of the blood and the renal impairments. So it is of valuable effect in the diagnosis of the toxicity of the fish. In the present study, the total plasma protein was decreased significantly in Nile tilapia exposed to sub lethal doses of iron alone and the same dose combined with low level of Zeolite (2 g/L) (Table 8). The decrease was attributed to either a stage of hydration and change in water equilibrium and/or disturbances in the liver protein synthesis (Salah El-Deen and Rogers, 1996). While, the addition high to levels (4 or 8g of zeolite/L were improved synthase of protein in cells of Nile tilapia similar as control group (6.413 ± 0.420 mg/g) (Table 8).

Table 8. The changes of total protein and glucose concentration in plasma of blood Nile tilapia after exposed to iron alone and mixed with doses of zeolite.

Items treatment	Total protein (g/100ml)	Glucose (mg/L)
T1	6.413 [a] ± 0.420	78.333 [a] ±9.331
T2	4.710 [c] ± 0.253	138.67 [b] ±15.67
T3	5.543 [b] ±0.407	135.84 [b] ±2.255
T4	5.826 [ab] ± 0.135	134.40 [b] ±5.807
T5	6.010 [a] ± 0.263	89.77 [a] ±2.531

The same letter in the same column is not significantly different at $P<0.05$.

The uric acid and creatinine levels are indicators for kidney function and considered as important variables predicting to which limit the kidney is adversely affected. In the present study, creatinine and uric acid (Table 9) showed a significant increase in fish exposed to sublethal concentrations of iron alone and or combined with lower and medium doses of Zeolite (2 & 4 g zeolte/ L), respectively. These results may be due to the action of this herbicide on glomeruli filtration rate (Abbass *et al.*, 2002) and/or it may be cause pathological changes of resulting in kidney dysfunction. While, the addition of high level zeolite (8g zeolite/L) caused remove of iron toxicity and enhancement the health of fish to decreased its parameters similar as control.

Liver plays an important and vital role in iron homeostasis (Meynard *et al.*, 2014). The AST, ALT and ALP activities are associated with the tissues damages such as in the liver, gut and bile ducts (Peres *et al.*, 2013). The activity of liver enzymes; ALT and AST are considered as indicators for hepatotoxicity and histopathological changes (Hassaan *et al.*, 2018). High concentration of iron causes damage in the metabolically active liver organ. Fe caused increased release of the transaminase enzymes (ALT and ALP) into the blood circulation (Udotong, 2015 and Abbas *et al.*, 2019). The results confirmed the toxic effect of iron and elevation in plasma ALT and ALP enzymes in Fe group alone. Fortunately, zeolite modulated such toxic effect of iron and significantly decreased ALT and ALP values through chelating the excess free iron to not destroy cell (Table 9).

Table 9. The changes of Creatinine, uric acid, Alkaline phosphatase and Alanine aminoreansferase (ALT) in plasma of blood Nile tilapia after exposed to iron alone and mixed with doses of zeolite.

Items treatment	Creatinine (mg/ dl)	Uric Acid (mg%)	ALP (Iu/L)	ALT (Iu/L)
T1	112.6 [c] ±0.210	1.87 [c] ±0.09	26.53 [c] ±1.37	13.57 [b] ±1.12
T2	129.33 [a] ± 1.83	2.72 [a] ±0.276	44.90 [a] ±2.89	47.43 [a] ±1.50
T3	121.33 [b] ±1.11	2.34 [b] ±0.912	41.97 [b] ±0.792	16.58 [b] ± 1.52
T4	109.67 [c] ± 0.422	2.29 b ±0.21	27/61 [c] ±1.99	14.26 [b] ±1.46
T5	106.67 [c] ± 2.23	1.78 [c] ±0.07	21.33 [d] ±1.37	14.24 [.b] ±0.78

The same letter in the same column is not significantly different at $P<0.05$.

On the other hand, total plasma lipids showed significant increase in all treated fish groups after the exposure time (Table 10). The intensity of hyper-lipemic state may reflect the degree of stress imposed on the animal under the influence of toxic reagents and environmental pollutants (Saeed, 1989). The increase of total plasma lipids may be due to the increase of lipids peroxides formation induced by the effect of iron toxicity as previously reported by Mousa (2004). Otherwise, the destruction of the liver cells and other organs due to the effect of the iron increase the levels of total lipids in the plasma (Inui 1968 and Mousa, 2004).

Cholesterol is the most important sterol occurring in animal fats. It is equally distributed between plasma and red blood cells, but in adrenal cortex, it occurs in the esterified form. The cholesterol occurs as white (or) faintly yellow almost odorless granules. In the present investigation, the blood cholesterol level and HDL concentration were significantly ($p<0.01$) increased in heavy metal exposed experimental groups. The increased levels of cholesterol develop weakness in the body and swimming ability of the fish was observed in our study. The level of cholesterol was found to be increased (Table 10) in the heavy metal toxicity that can generate free radicals through the oxidation of ferrous to ferric state by the Fenton's reaction. It is noticed that long-term effect of heavy metals stimulates lipid peroxidation (Faix *et al*., 2005). These results are agreement with those by (Vinodhini and Narayanan, 2009) who reported that the Concentrations of red blood cells, blood glucose and total cholesterol were significantly elevated after exposed the common carpe to 5mg/L of combined some heavy metals. Also, (Kanyılmaz and Tekellioğlu, 2016) showed that the cholesterol concentrations of gilthead sea bream were quadratic ally affected with dietary zeolite levels and varied between 240.70 and 384.70 mg/dL The increase in cholesterol and triglycerides with zeolite supplementation could be partly resulted from a linear increase in lipid retention by fish fed zeolite added diets (Kanyılmaz *et al*., 2015). Similarly, The High density lipoprotein (HDL) was increased significantly in fish exposed to all treatment when compared to the control group.

Table 10. The changes of lipids profiles in plasma of blood Nile tilapia after exposed to iron alone and mixed with doses of zeolite (n=6).

Items treatment	Total lipids (mg/dl)	Cholesterol (mg/dL)	HDL (Iu/L)
T1	$112.92^{a} \pm 1.65$	$75.600^{c} \pm 5.60$	$204.63^{c} \pm 5.14$
T2	$463.67^{b} \pm 30.577$	$139.98^{a} \pm 5.108$	$485.2^{a} \pm 9.08$
T3	$278.20^{c} \pm 23.040$	$113.56^{b} \pm 2.71$	$457.4^{b} \pm 10.65$
T4	$221.00^{d} \pm 18.166$	$108.43^{b} \pm 3.22$	$446.67^{b} \pm 7.24$
T5	$186.70^{e} \pm 5.369$	$67.41^{c} \pm 1.47$	$422.93^{b} \pm 9.25$

The same letter in the same column is not significantly different at P<0.05.

Fe accumulation:

Addition of zeolite to the iron polluted of the water reduced significantly of the iron level in aquariums water compared to that iron group only (Table 11). The data observed also a wide variation among the different organs of Nile tilapia subjected to Fe or Fe with different doses of zeolite. The highest concentration of Fe was found gills and the lowest amount of muscles. Table

(11) showed that the uptake of Fe in the gills and muscle of fish exposed to the Fe alone was 36.24±2.00 and 19.99±0.91 mg/ g dry weight respectively. It declined significantly to 10.29±0.74 and 5.19±0.39 in gills and muscle in fish exposure to Fe with 8 g zeolite / L (T5) respectively, similar as control groups. Similar result are obtained by (Mancy, 2017 and Shalaby *et al*., 2018) they showed that reduction of lead accumulated in the tissues of tilapia after addition of zeolite.

Table 11. The iron accumulation in muscles and gills of Nile tilapia (*O. niloticus*) after exposed to iron alone and mixed with different doses of zeolite.

Treatment / Organ	Fe (mg/g) Muscles	Fe (mg/g) Gills
T1	3.89 ± 0.52^{h}	8.02 ± 0.19^{fg}
T2	19.99 ± 0.91^{c}	$36.24 \pm ^{a}2.00$
T3	14.16 ± 0.88^{d}	27.21 ± 1.10^{b}
T4	12.04 ± 1.35^{de}	21.19 ± 1.14^{c}
T5	5.19 ± 0.39^{gh}	10.29 ± 0.74^{ef}

The same letter in the same column is not significantly different at $P<0.05$.

CONCLUSION

It could be concluded that water pollution with iron included significant negative impact in the growth performance, hematological and biochemical parameters together with marked high accumulation of iron in muscle and gills of Nile tilapia. Addition some chelating agents as zeolite could decrease the toxic effects of iron and improve fish condition

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استخدام الزوليت لاختزال سمية الحديد ولتحسين معدلات النمو والحالة الفسيولوجية فى اسماك البلطى النيلى (*Oreochromis niloticus*)

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الملخـــــــص العربــــــي

اجرى هذا البحث لدراسة تأثير عنصر الحديد ومعالجته باضافة مادة لها قدرة على امتصاص العناصر فى الوسط المائى (الزوليت) على بعض التغيرات الفسيولوجية والبيوكيميائية والمدى التراكمى لعنصر الحديد أنسجة العضلات والخياشيم فى اسماك البلطى النيلى.

تم توزيع الأسماك فى خمسة معاملات حيث تركت المجموعة الأولى (المجموعة الضابطة) للمقارنة بينما تعرضت المجموعة الثانية الى الجرعة العير مميتة لعنصر الحديد (٧٥ مجم حديد/ لتر فقط .وكذلك تعرضت المجموعات (الثالثة والرابعة والخامسة الى نفس الجرعة السابقة من الحديد وخلط ٢، ٤، ٨ جرام من الزوليت /لتر) على التوالى. وتم حساب معدلات النمو خلال فترة التجربة التى استمرت ٩٠ يوما وفى نهاية التجربة تم اخذ عينات لدراسة التغيرات الهيماتولوجية والبيوكميائية والمدى التراكمى لعنصر الحديد وأسفرت النتائج عن الأتى:

١- لوحظ نقص معنوى فى معدلات النمو (الوزن النهائى ومعدل النمو النسبى ومعمل التحول الغذائى بالمجموعه التى تعرضت الى جرعة الحديد فقط بينما لوحظ اضافة جرعات الزوليت المختلفة الى تحسين معدلات النمو.

٢-حدوث نقص معنوى فى دلالات الدم (كرات الدم الحمراء والهيموجلوبين والهياتوكريت فى البلطى النيلى بعد تعرضة للحديد فقط.بينما اضافة ٨جم /لتر من الزوليت الى هذا الوسط الملوث بالحديد ادى الى تحسن دلالات الدم.

٣- لوحظ زيادة معنوية فى جلوكوز الدم والأنزيمات الناقلة لمجموعات الأمينى ALT, والفوسفاتيز القاعدىِ ALP والدهون الكلية والكروستبرول والكرتنين ونسبة اليوريا فى بلازما دم الأسماك التى تعرضت جرعه للحديد فقط. وقد عادت هذة القياسات الى المستوى الطبيعى كما فى المجموعة الضابطة فى الأسماك التى تعرضت الى مخلوط من الحديد والزوليت.

٤- حدث انخفاض معنوى فى المحتوى البروتينى فى بلازما الأسماك التى تعرضت لعنصرالحديد منفرد. بينما حدث تحسين معنوى للمحتوى البروتينى فى الأسماك بعد اضافة الزوليت للوسط المائى.

٥- لوحظ زيادة المدى التراكمى لعنصر الخديد فى الخياشيم والعضلات بالاسماك المعرضة الى جرعة الحديد فقط بينما حدث انخفاض واضح بالدى التراكى لذلك العنصر بالمجموعات المعرضة لمحلوط من الجدسد والزوليت.

اوضحت النتائج حدوث تحسن ملحوظ فى التغيرات الفسولوجية والبيوكيميائية التى حدثت فى مكونات الدم ونسبة الجلوكوز والنشاط الأنزيمى والمحتوى البروتينى للأسماك وانخفاض المدى التراكمى للحديد بأنسجة الأسماك عندما تم اضافة جرعة ٨ جم من الزوليت الى الوسط المائى الملوث بالحديد مما سبق نستخلص ان اضافة الزوليت الى الوسط المائى الملوث باالحديد يؤدى الى ازالة هذا الملوث من المياة وتكون هذة الأسماك امنة صحيا. ولذا نوصى باضفة ٨ جرام زوليت /لترماء بمياة الصرف الزراعى المستخدمة لتربية الأسماك لحمايتها من التلوث.