

**ROLE OF NATURAL ZEOLITE IN IMPROVING WATER QUALITY,
PERFORMANCE AND HEALTH STATUS OF NILE TILAPIA
(*OREOCHROMIS NILOTICUS* L.) IN EARTHEN PONDS**

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Abstract

This experiment was designed to determine the impact of natural zeolite on water quality, growth performance and health status of Nile tilapia freshwater fish, (*Oreochromis niloticus*) in earthen ponds. Three treatments included six earthen ponds (two replicates for each treatment) of one feddan each were stocked by *Oreochromis niloticus*. The first treatment (control) without zeolite, the second treatment (T1) in which natural zeolite was scattered on the bottom of the pond (about 500kg/feddan), while in the third treatment (T2) natural zeolite was used as a mechanical filter where zeolite (500kg) was placed in a concrete pond with dimensions (1.5 m length, 2.30 m width and 90cm height) in which the irrigation water passed through it and then distributed to the ponds by water inlet pipes. The results revealed that application of zeolite in fish ponds improves water quality parameters; decrease heavy metals accumulation in water, sediment and fish organs as well as it increases fish growth parameters. However, proximate chemical composition of the whole fish body of *O. niloticus* not significantly varied among different treatments. In conclusion, application of zeolite in fish ponds helps to get good fish production and improves water quality and fish health especially, when scattered on the pond bottom than used as filter. Moreover, using zeolite was a truly effective preventive measure for facing fish diseases.

Keywords: Natural zeolite, Water quality, Earthen ponds, Nile tilapia, Performance, Health status

INTRODUCTION

Water quality is determined by some variables like temperature, transparency, turbidity, water colour, carbon dioxide, pH, alkalinity, hardness, unionized ammonia (NH_3), nitrite and nitrate (Saeed, 2013; Saeed and Abdel-Mageed, 2011; Chandra Das *et al.*, 2005). For improving water quality and decreasing ammonia toxicity, there are several possible methods including zeolite addition (U.S.EPA, 2007; Shalaby, 2007). Zeolite addition has been used successfully in freshwater systems to decrease ammonia concentrations and toxicity for several decades. However, zeolite in marine systems has been used less because ions in the seawater interfere with zeolite's ability to adsorb ammonia (Burgess *et al.*, 2004).

Constantly ongoing research show promising opportunities for using natural and modified zeolites to purify wastewaters and environment protection, because of their unique molecular sieve, sorption and ion exchange properties (Kędziora *et al.*, 2014).

Natural zeolites represent a large and very diverse group of minerals such as water-silicates that are characterized by three-dimensional structure and belong to the class of aluminum silicates from a chemical point of view (Strakova *et al.*, 2008; Vaughan, 1988). Additionally, natural zeolites are porous and contain hydrated aluminum silicates that have ion-exchange and adsorption properties and have a large surface area that helps in the adsorption properties (Xia *et al.*, 2009). 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 (Wen *et al.*, 2010). Zeolites are used in industry, agriculture, environment protection and even in medicine. Zeolites have a relatively high Si/Al compositional ratio which gives it the special ion-exchange selectivity for large monovalent cations. Natural or synthetic zeolites (sodium aluminum silicates) are known to adsorb metal ions by exchange reactions (Jain, 1999). So, the natural zeolites, clinoptilolite may be useful as metal scavengers

in metal-rich sludges to its ability to take up heavy metals; Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn (Zorpas *et al.*, 2000).

As good water quality of fish culture ponds and aquatic environment play a critical role for successful fish production, water quality management in fish ponds is a deterrent step (Saeed, 2013). Thus, natural zeolites can apply successfully in aquaculture to remove ammonium from hatchery, transport, aquarium waters, generate oxygen for aeration systems in aquaria, transport and additives fish feed. Zeolite cation exchange removes NH_4^+ from recirculating fish production systems and hatcheries waters produced by the decomposition of excrement and/or unused food, much as NH_4^+ is removed from municipal sewage effluent and wastewaters (Bruin *et al.*, 1980; Piper and Smith, 1984). Therefore, the present study was conducted to evaluate the impact of adding natural zeolite for improving water quality of fish culture, growth performance and health status of Nile tilapia (*Oreochromis niloticus*) fish in earthen ponds.

MATERIALS AND METHODS

Experimental Design.

The study had been done in a private farm at Tollumbat No. 7 (agriculture drainage water) in Riyadh City, Kafr El-Sheikh governorate, Egypt. Water, sediment and fish samples were collected from 6 shallow (1feddan each and 1.25 m deep) earthen ponds represent three treatments (two ponds each). The first treatment (control) was under normal fish culture conditions without zeolite addition, and the second treatment (T1) in which natural zeolite was scattered on the pond bottom (about 500kg/feddan), while in the third treatment (T2) natural zeolite was used as a mechanical filter where zeolite (500kg) was placed in a concrete pond with dimensions (1.5m length, 2.30m width and 90cm height) in which the irrigation water passed through it and then distributed to the ponds by water inlet pipes. Natural zeolite as shown in Figure 1 are porous granules of sedimentary rocks which have the ability to gain and lose water with great ability for ions exchange.

The ponds were stocked with monosex males of Nile tilapia, *Oreochromis niloticus* fingerlings at an average initial length of about 11.40, 11.50 and 12.10 cm and an average initial weight of 22.90, 23.50 and 25.70 g for control, T1 and T2, respectively. Each pond was stocked with 12000 fish/feddan (3 fish/m²). Nile tilapia fingerlings were fed on a commercial diet containing 25% crude protein six days/week at a daily feeding rate of 3% of an average fish-body weight twice at 9.0 am and 3.0pm during the experimental period in each pond. The trial lasted for 22 weeks and at the end of the experiment, ponds were drained and fish were harvested, counted, and the individual weight and length were measured and also water and sediment were analyzed.



Figure 1. Commercial natural zeolite (Clinoptilolite) granules.

Sampling:

Water, sediment were collected monthly and fish samples biweekly from each pond. Water samples were taken from different places at each site by a PVC tube column sampler at depth of half meter from the water surface. The samples at each site were mixed in a plastic bucket and a sample of 1 liter was placed in a polyethylene bottle, kept refrigerated and transferred cold to the laboratory for analysis. Sampling of bottom sediments (from the upper 10 cm surface layer) using Peterson grab as described by Boyd and Tucker (1992) and kept in cleaned plastic bags. The wet sediment samples were air dried,

pulverized, and then mixed to provide a homogeneous mixture. Three sediment replicate samples from each site were combined. Nile tilapia freshwater fish were collected and transported to the laboratory in an ice box, where tissue samples were taken.

Laboratory Analysis.

a. Water:

Hydrogen ion concentration (pH) was measured with pH meter (Model 25, Fisher Scientific). Total dissolved solids (TDS as g/l) were determined using a salinity-conductivity meter (model, YSI EC 300). Temperature and dissolved oxygen were measured by using a digital oxygen meter (Model YSI 55). The concentration of total hardness (mg/l as CaCO₃), total ammonia (NH₄-N+NH₃-N), unionized ammonia (NH₃-N), nitrite (NO₂-N) and nitrate (NO₃-N) were measured by methods described in Boyd and Tucker (1992). Transparency (cm) was measured by using a Secchi Disc of 20cm diameter. Heavy metals were extracted with conc. HNO₃ and HCl and preserved in a refrigerator till analysis for Fe, Zn, Mn, Cu, Cd and Pb concentration as µg/l (EPA. 1992).

b. Metals residues in fish and sediments:

Fish, musculature and organs (gills and liver) were collected separately and metals were extracted by HNO₃ and HCl according to the methods described in AOAC (1990). Sediment samples were extracted with HNO₃, HCl acid and H₂O₂ according to EPA (1996). Atomic Absorption Spectrophotometer (Model Thermo Electron Corporation) instrument was used to detect metals concentrations which were expressed as µg/g dry wt. in both sediments and fish samples.

The degree of metal removal (%) (Removal efficiency or Purification rate %) from the investigated treatments was calculated by the following general mathematical equation;

$$\text{Purification rate \%} = \frac{C_I - C_T}{C_I} \times 100$$

Where, C_I and C_T are concentrations (mg/L) in control and zeolite treatments, respectively.

C. Growth parameters:

Random samples (30 fish from each pond-60 fish for each treatment) were taken biweekly during the experimental period. Body measurements (body weight in g and body length in cm) were conducted at biweekly intervals throughout the whole experimental period growth parameters were calculated as follows:

i. Fish condition factor (K) was calculated according to Schreck and Moyle (1990) as:

$$K = (W_t/L^3) \times 100$$

Where, W_t is the total gutted weight of the fish (g), and L is the total length (cm)

ii. Specific growth rate (SGR) was calculated according to Jauncey and Rose (1982) as:

$$SGR = (\ln W_2 - \ln W_1 \times 100) / t$$

Where, w_1 = first fish weight in grams, w_2 = final fish weight in grams, t = period in day.

iii. Daily weight gain (DWG) was calculated using the formula:

$$DWG = [\text{Average } W_2 \text{ (g)} - \text{Average } W_1 \text{ (g)}] / t$$

Where W_1 and W_2 = the initial and final fish weight, while t is the experimental period in days.

d. Chemical composition:

At the end of the experiment, six fish from each pond exposed to the chemical composition (moisture, protein, fat and ash content) of the whole fish body according to AOAC (2000).

e. Clinical examinations:

Clinical examinations of infested or infected fish was done according to Stoskopf (1993); for skin darkening, discoloration or paleness, skin congestion, ragged or torn fins, raised scales, haemorrhage and erosions. The abdomen was examined for enlargement and or distention.

Statistical Analysis:

One-way ANOVA and Duncan's multiple range tests were used to evaluate the significant difference of the data which were computed by applying the computer program (SAS, 1996). Significant differences are stated at $P < 0.05$. Data were statistically analyzed according to Bailey (1981).

RESULTS AND DISCUSSION

Physico-Chemical Parameters.

Water quality parameters are presented in Table 1. Water temperature not significantly varied among treatments throughout the experimental period and ranged from 26.02 C° in T2 to 27.15 C° in control. Application of zeolite caused a significant increase ($P < 0.05$) of water pH (8.22 in T1 and 7.89 in T2) compared with control ponds (7.54). This may be related to that zeolites, in general, are weakly acidic in nature and sodium-form exchangers are selective for hydrogen, which leads to high pH values when the exchanger is equilibrated with relatively dilute electrolyte solutions (Leinonen and Lehto, 2001). Both carbonate and nitrate ions are attracted by the negative charge within zeolites (Mumpton, 1999). The desirable pH range for most fish species is 7-9 (Boyd, 1998).

Dissolved oxygen (DO) was not affected significantly by treatments ($P > 0.05$) and it was higher (4.16 mg/l) in T1 followed by T2 (3.99 mg/l) than that of control (3.89 mg/l). DO attained lower value in control. The higher values of DO in T1 and T2 may be attributed to good water quality conditions caused by zeolite addition, where uneaten food and nutrients were decreased. Ferdous *et al.* (2013) found that DO content increased significantly after zeolite

treatment. However, DO was still lower than that recommended by Boyd (1998), who advised 5.0 mg/l to satisfies the needs of fish welfare.

Regarding, total dissolved salts (TDS), it was obviously higher in control ponds (2.31 g/l) than those in zeolite treated ponds T1 (1.87 g/l) and T2 (2.07 g/l). There was a significant ($P < 0.05$) variations in water TDS among treatments. The reduction in TDS values in T1 and T2 ponds revealed that zeolite highly absorbed various salts from pond water (Mumpton, 1999). Water hardness increased in control with a total mean of (1879.0 mg/l). On the other hand, it was decrease in T1 (834.0 mg/l) and T2 (1216.0 mg/l). This possibly could be from the adsorption activity of zeolite for removing divalent cations (Ca^{++} , Mg^{++} and/or Fe^{++}) from water (Mumpton, 1999). In addition to iron removal, zeolites can be used for water softening, through Ca^{2+} ion removal and replaced by ions of Na^+ (Nazarenko and Zarubina, 2013).

Table 1. Some water quality parameters in different treatments during the experimental period based on means \pm SE.

Parameters	No.	Control	T1	T2
Temp (C°)	3	27.15 \pm 0.10 ^a	27.09 \pm 0.11 ^a	26.02 \pm 0.10 ^a
pH	3	7.54 \pm 0.012 ^b	8.22 \pm 0.011 ^a	7.89 \pm 0.014 ^{ab}
DO oxygen	3	3.89 \pm 0.011 ^a	4.16 \pm 0.013 ^a	3.99 \pm 0.011 ^a
Total dissolved salts (mg/l)	3	2.31 \pm 0.010 ^a	1.87 \pm 0.008 ^b	2.07 \pm 0.008 ^{ab}
Total hardness (mg/l)	3	1879.0 \pm 5.62 ^a	834.0 \pm 5.29 ^c	1216.0 \pm 4.89 ^b
Secchi disck (cm)	3	19.88 \pm 0.48 ^b	31.20 \pm 0.48 ^a	22.86 \pm 0.49 ^b
Total ammonia-N (mg/l)	3	0.46 \pm 0.005 ^a	0.16 \pm 0.004 ^c	0.29 \pm 0.004 ^b
NO ₂ -N mg/l	3	0.79 \pm 0.003 ^a	0.34 \pm 0.003 ^b	0.46 \pm 0.003 ^b
NO ₃ -N mg/l	3	0.82 \pm 0.005 ^a	0.55 \pm 0.005 ^b	0.67 \pm 0.006 ^b

^{a, b, c} \pm Means with the same letter in each column are not significantly different ($P \geq 0.05$).

Secchi disc readings was positively affected by treatments ($P < 0.05$). The average values of transparency were generally higher in T1 (31.20 cm), whereas T2 showed Secchi disc readings (22.86 cm) compared with lower secchi disc

reading of control ponds (19.88 cm). This resulted from the purification role of zeolite which was consistent with the results of Ferdous *et al.* (2013) who observed that zeolite increase water transparency. Application of zeolite significantly decreased all the inorganic dissolved nitrogen, namely total ammonia ($\text{NH}_3\text{-N} + \text{NH}_4^+\text{-N}$), nitrite ($\text{NO}_2\text{-N}$) and nitrate ($\text{NO}_3\text{-N}$) content in the water. The levels of these nutrients in the three treatments were significantly ($P < 0.05$) different with higher values in control ponds (0.46, 0.79 and 0.82 mg/l, respectively).

Significantly lower concentrations of nitrogen nutrients in T1 (0.16, 0.34 and 0.55 mg/l, respectively) and T2 (0.29, 0.46 and 0.67 mg/l, respectively) might have been principally due to the adsorption capacity of zeolite as it attracted nitrate ions and improve nutrient retention from soil and water (Mumpton, 1999). Also, Polat *et al.* (2004) mentioned that zeolite clinoptilolite retains valuable nitrogen. The negative charge on zeolite allows for the adsorption of certain positively charged ions as NH_4^+ which is preferentially adsorbed to the zeolite matrix. Durhan *et al.* (1993) and Besser *et al.* (1998) mentioned that zeolite has been used to decrease the concentrations of ammonium in freshwater. A direct relationship between the mass of zeolite and total ammonia removal was evident (Burgess *et al.*, 2004). The desirable ranges are 0.2-2.0 mg/l for total $\text{NH}_4\text{-N}$, < 0.3 mg/l for $\text{NO}_2\text{-N}$ and 0.2-10 mg/l for $\text{NO}_3\text{-N}$ in fish ponds (Boyd, 1998).

Heavy Metals.

a. Water:

Metals concentration values showed significant differences ($P < 0.05$) for the three treatments (Table 2), in which, control ponds had the highest total mean concentrations of all metals (1822.78 $\mu\text{g/l}$) compared to T1 (563.83 $\mu\text{g/l}$) and T2 (613.14 $\mu\text{g/l}$). Hamed *et al.* (2013) attributed the increase of metals content in water to the decomposition of organic matter in sediments and release of metals to the overlying water. Also, Fe showed the highest accumulated metal in different sites, while Pb was the lowest one.

Natural zeolite, clinoptilolite has the ability to take up heavy metals; Cd, Cu, Fe, Mn, Pb and Zn (Barloková and Ilavsky, 2010). Thus, application of zeolite (clinoptilolite) decreased heavy metals in pond's water and was more effective in T1 which comply with studies of showed that natural zeolites was able to remove cationic heavy metal elements from industrial wastewater. Moreover, Kocakusak *et al.* (2001) mentioned that zeolites application in wastes increased both heavy metals and ammonium ions removal. Metal ions' removal efficiencies for tested pond's water treated with zeolite are shown in Table 2. The degree of metal removal (%) (Removal efficiency of ions or Purification rate %) from the investigated treatments using natural zeolite was calculated by the following mathematical formula;

$$\text{Purification rate \%} = \frac{C_I - C_T}{C_I} \times 100$$

In which, zeolites had selectivity chelated Cd, Pb, Mn and Zn. In the two treatments the selectivity of ions was: Cd > Pb > Mn > Zn > Fe > Cu. The difference in selectivity might be attributed to ion-exchange processes. Also, data demonstrated the preference of zeolite for Cd and Pb compared to other metals. Similar results were recorded by Shaheen *et al.* (2012) and Mieret *et al.* (2001) who describing more interactions of Pb²⁺ and Cd²⁺ competing for ion-exchange sites in natural zeolite; clinoptilolite.

Table 2. Means±SE of heavy metals concentration ($\mu\text{g/l}$) in water of different treatments.

Parameters	No.	Control	T1	Rate of purification %	T2	Rate of purification %
Fe	3	1460.12 ± 1.26 ^a	490.48 ±1.30 ^c	66.41	530.56 ± 1.29 ^b	63.66
Zn	3	81.23 ± 0.19 ^a	16.19 ±0.21 ^b	80.07	18.89 ± 0.21 ^b	76.75
Cu	3	59.45 ± 0.42 ^a	21.08 ± 0.39 ^b	64.54	20.19 ± 0.38 ^b	66.07
Mn	3	87.16 ± 0.37 ^a	15.22 ± 0.40 ^b	82.54	17.99 ± 0.38 ^b	79.36
Cd	3	71.71 ± 0.29 ^a	9.89 ± 0.26 ^b	86.21	11.77 ± 0.28 ^b	83.59
Pb	3	63.11 ± 0.30 ^a	10.97 ± 0.32 ^b	82.62	13.74 ±0.29 ^b	78.23
Total		1822.78	563.83	77.06	613.14	74.60

^{a, b, c} ± Means with the same letter in each column are not significantly different ($P \geq 0.05$).

Zeolite, clinoptilolite as a bulking material has the ability to increase the porosity of the substrate and as a result, to improve the composting process and the biodegradability of the organic matter (Wagner *et al.*, 1990). The net negative charge in zeolite is balanced by the positive cations (sodium, potassium, or calcium). These cations are exchangeable with certain cations in solutions such as lead, cadmium, zinc, and manganese (Barrer, 1978) and make zeolite suitable for removing undesirable heavy metal ions from industrial effluent waters.

b. Sediments:

Results of heavy metals in sediments are presented in Table 3. The mean concentrations of metals differed between sediments of the three treatments and were detected in the following order: Fe > Mn > Zn > Pb > Cu > Cd. These results showed that sediments of control ponds accumulate high levels of all studied metals. The distributions of metals are associated mainly with the organic matter fraction of sediments as found by Saeed and El-Gammal (2009), who

also observed a significant positive correlation between organic matter and each of Fe, Zn, Cu and Mn and no correlation with Pb.

The increases of Fe and Mn could be attributed to their increase in the earth's crust. Zinc and Cu accumulation could come from feed as recorded by Wudtisin and Boyd (2006). They also suggested that the accumulation of Fe, Mn, Cu, and Zn could have occurred by precipitation with hydroxyl ions and carbonates under aerobic conditions. The pH increase causes precipitation of hydrous ferric oxides and decreased dissolved metal concentrations as metals precipitate as hydroxides (Wingenfelder *et al.*, 2005). The ability to exchange cations and adsorb inorganic and organic molecules of certain sizes and catalytic properties are among the most significant features of zeolites. So, using the natural material zeolite is one of the methods to make iron and manganese removal more effective (Barloková, 2008).

Table 3. Means±SE of heavy metals concentrations ($\mu\text{g/g}$ dw except Fe mg/g dw) in sediments of different treatments.

Parameters	No.	Control	T1	T2
Fe	3	46.7 ±0.42 ^a	26.53 ±0.39 ^c	39.12 ±0.39 ^b
Zn	3	218.4 ±1.06 ^a	164.11 ±0.97 ^b	200.12 ±0.91 ^{ab}
Cu	3	82.7 ±0.11 ^a	48.69 ±0.11 ^b	79.94 ±0.10 ^{ab}
Mn	3	346.55 ±1.45 ^a	216.08 ±1.32 ^b	294.13 ±1.49 ^{ab}
Cd	3	13.15 ±0.03 ^a	10.80 ±0.03 ^b	11.12 ±0.03 ^b
Pb	3	80.35 ±0.89 ^a	73.16 ±0.90 ^b	76.61 ±0.92 ^{ab}

^{a, b, c} ± Means with the same letter in each column are not significantly different ($P \geq 0.05$).

c. Fish:

Metals accumulation in musculature and liver of *O. niloticus* in different treatments are shown in Table 4. All metals showed significant ($P < 0.05$) difference among different treatments. It was observed that, the concentrations of all studied metals in fish reared in T1 and T2 were lower than those reared in control ponds. This may be explained by the assumption that dissolved metals

are adsorbed on zeolite (Shaheen *et al.*, 2012), thus leads to decreasing metals bioaccumulation in fish.

Table 4. Means \pm SE of heavy metals concentration ($\mu\text{g/g}$ dry wt.) in musculature and liver of *O. niloticus* in different treatments.

Heavy Metals	NO.	Musculature			Liver		
		Control	T1	T2	Control	T1	T2
Fe	3	249.61 $\pm 0.46^a$	20.78 $\pm 0.48^c$	70.88 $\pm 0.48^b$	2042.13 $\pm 0.71^a$	470.71 $\pm 0.70^c$	618.9 $\pm 0.73^b$
Zn	3	206.26 $\pm 0.63^a$	17.06 $\pm 0.60^c$	25.36 $\pm 0.61^b$	1114.34 $\pm 0.80^a$	26.05 $\pm 0.79^c$	182.72 $\pm 0.85^b$
Cu	3	46.34 $\pm 0.10^a$	3.18 $\pm 0.11^c$	4.54 $\pm 0.11^b$	256.09 $\pm 0.58^a$	39.06 $\pm 0.49^c$	137.43 $\pm 0.52^b$
Mn	3	19.12 $\pm 0.14^a$	1.36 $\pm 0.09^b$	2.59 $\pm 0.10^b$	29.57 $\pm 0.16^a$	2.28 $\pm 0.12^c$	9.78 $\pm 0.21^b$
Cd	3	8.29 $\pm 0.02^a$	0.49 $\pm 0.02^b$	0.67 $\pm 0.02^b$	28.17 $\pm 0.04^a$	1.02 $\pm 0.04^c$	2.06 $\pm 0.04^b$
Pb	3	8.19 $\pm 0.03^a$	0.61 $\pm 0.03^b$	0.89 $\pm 0.03^b$	31.14 $\pm 0.07^a$	1.19 $\pm 0.06^b$	1.93 $\pm 0.07^b$

^{a, b, c} \pm Means with the same letter in each column are not significantly different ($P \geq 0.05$).

The abundance of heavy metals in different tissue/organs showed that liver had a high tendency to accumulate heavy metals than musculature. This may be explained as liver is the main target organ for detoxification and excretion of toxicants besides gills which being the key interface for the uptake of waterborne metal from water (Noegrohati, 2006).

Growth Parameters.

Growth in the fish can be simply monitored by measuring the increase of fish weight and length traits. Condition factor as an index of growth provides a measure of fatness "plumpness" or "robustness" of fish and food conversion efficiency. Condition factor is frequently assumed to reflect not only characteristics of fish such as health, reproductive state and growth, but also characteristics of the environment such as water quality (Schreck and Moyle, 1990).

Concerning the values of condition factor (k) of *O. niloticus* (Table 5) for different treatments; were 1.67, 1.67 and 1.56, respectively. Control ponds and T1 had the highest ($P<0.01$) K value compared to T2. Fish reared in T1 showed the highest ($P<0.01$) final body weight followed by T2 then control ponds (268.20, 248.30, and 199.80g), respectively. Average fish final length at the end of the experimental period were found to follow the same order ($P<0.01$) of final body weight and being 27.2, 26.0 and 24.8 cm, respectively.

Regarding the survival rate of Nile tilapia, it improved ($P<0.05$) with both types of application addition either in the pond bottom or used as a filter. The highest value of survival (96.4%) was obtained with addition of zeolite in the pond bottom (T1), followed by filter zeolite (T2), while the lowest (89.9%) was observed for control. These results are in accordance with those obtained by Osman *et al.* (2008), who found a strong correlation between body weight and body length for tilapia. The fluctuations in fish growth (length and weight) are affected by different factors such as feeding regime (Saeed and Abdel-Mageed, 2011) and environmental conditions (Saeed, 2013).

On the other hand, the highest averages daily gain and SGR were recorded at T1 (1.59 and 1.58%/day, respectively) followed by T2 (1.44 and 1.47%/day, respectively), while the lowest values (1.15 and 1.41%/day, respectively) were found in control ponds. The good conditions of water quality in T1 and T2 (treated with zeolite) may be a reason for higher harvest weights than those reared in control ponds. The data in the present study matches the conclusions of Xia *et al.* (2009) who reported that natural zeolite helps to prevent the occurrence of disease and enables to promote growth and survivability and higher profits.

Chemical Composition of Fish.

The results of proximate analysis of the whole fish body of *O. niloticus* for moisture, protein, fat and ash are shown in Table 6. Its averages were 61.85 % moisture, 60.52 % protein, 23.13 % lipid and 16.35 % ash in control ponds. The corresponding values of fish in T1 were 62.09% moisture, 62.89 % protein,

19.37 % lipid and 17.74 % ash. The values for fish in T2 were 62.12% moisture, 62.19 % protein, 19.14 % lipid and 18.67 % ash content. It is clear that, application of zeolite had no significant effect on the proximate composition of the fish. This may be that all fish in all ponds fed artificial feed. According to Hanley (1991), endogenous factors (size, sex and stage of life cycle) and exogenous factors (diet composition, feeding frequency, temperature etc.) affect the body composition of fish.

Table 5. Means \pm SE of some growth parameters of *O. niloticus* in different treatments.

Parameters	No.	Control	T1	T2
Condition factor (K)	60	1.67 \pm 0.11 ^a	1.67 \pm 0.07 ^a	1.56 \pm 0.08 ^b
Initial weight (gm)	60	22.90 \pm 0.25 ^a	23.50 \pm 0.31 ^a	25.70 \pm 0.33 ^a
Final weight(gm)	60	199.80 \pm 2.23 ^c	268.20 \pm 3.07 ^a	248.30 \pm 2.69 ^b
Initial length (cm)	60	11.40 \pm 0.25 ^a	11.50 \pm 0.26 ^a	12.10 \pm 0.21 ^a
Final length (cm)	60	24.80 \pm 1.11 ^b	27.20 \pm 1.03 ^a	26.00 \pm 1.07 ^a
DWG, g/fish	60	1.15 \pm 0.20 ^c	1.59 \pm 0.18 ^a	1.44 \pm 0.17 ^b
SGR, %/d	60	1.41 \pm 0.17 ^b	1.58 \pm 0.16 ^a	1.47 \pm 0.16 ^{ab}
Survival rate %		89.9 \pm 0.11 ^c	96.4 \pm 0.11 ^a	94.7 \pm 0.11 ^b

^{a, b, c} \pm Means with the same letter in each column are not significantly different ($P \geq 0.05$).

Table 6. Proximate chemical analyses (dry wt. basis) of *O. niloticus* reared in different treatments.

Parameter	No.	Control	T1	T2
Moisture%	6	61.85 \pm 0.48 ^a	62.09 \pm 0.63 ^a	62.12 \pm 0.35 ^a
Protein%	6	60.52 \pm 0.22 ^a	62.89 \pm 0.25 ^a	62.19 \pm 0.25 ^a
Fat%	6	23.13 \pm 0.18 ^a	19.37 \pm 0.13 ^a	19.14 \pm 0.14 ^a
Ash%	6	16.35 \pm 0.12 ^a	17.74 \pm 0.14 ^a	18.67 \pm 0.17 ^a

^{a, b, c} \pm Means with the same letter in each column are not significantly different ($P \geq 0.05$).

Clinical Examinations of Fish.

The clinical examination of some moribund and mortalities from all groups showed that some aggregated on the surface, accumulated at the water inlet and appeared dull, lethargic with loss of escape reflex. On the other side, the control group showed yellowish discoloration of the skin, dermatitis surrounded with petechial hemorrhage at the peduncle region indicating liver failure (jaundice like appearance) and less microbial resistance. This may be due to the pollution of the source of water supplying fish farms with some industrial wastes reaching the agriculture drainage waste water used for aquaculture in Egypt (Mohana, 1996; Fajardo, 2002). The improved production of treated groups could be as a result of the ability of zeolite to adsorb heavy metals and other pollutants like ammonia besides its antimicrobial effect (Haile and Nakhla, 2010).



Figure 2. Some fish of the control group suffering yellowish discoloration, dermatitis surrounded with petechial hemorrhages at the peduncle region and at the fin base.

In conclusion, application of zeolite in fish ponds improved fish health, disease resistance, water quality parameters and decreased heavy metals accumulation in water, sediment and in organs as well as improved growth parameters. However, proximate chemical composition of fish musculature did

not significantly vary. Application of zeolite scattered on the pond bottom increased the efficiency of improving pond quality than used as a filter.

REFERENCES

- AOAC, 1990. Association of Official Analytical Chemists, Official Methods of Analysis. International 15th edition. "Atomic Absorption Method for Fish", Washington, D.C.
- AOAC, 2000. Association of Official Analytical Chemists, Official Methods of Analysis. Chemists International 17th ed. Gaithersburg, Maryland, USA, AOAC International.
- Bailey, N.T., 1981. Statistical Methods in Biology, 2nd ed. (Biological Science Texts).
- Barloková, 2008. Natural zeolites in the water treatment process, (Slovak University of Technology), Slovak Journal of Civil Engineering, 2: 8-12.
- Barlokova, D. and J, Ilavsky, 2010. Removal of Iron and Manganese from Water Using Filtration by Natural Materials, Polish Journal of Environmental Studies, 19(6) :1117-1122.
- Barrer, R.M., 1978. Zeolites and clay minerals as sorbent and molecular sieves, Academic Press, London, 407–486.
- Besser, J. M.; C. G. Ingersoll; Leonard, E. N. and D. R. Mount 1998. Effect of zeolite on toxicity of ammonia in freshwater sediments: Implications for toxicity identification evaluation procedures, Environ Toxicol Chem, 17: 2310–2317.
- Boyd, C. E. and C. S. Tucker, 1992. Water quality and pond soil analysis for aquaculture, Alabama agric. Experim. Station. Auburn. Uni.,: 183 pp.
- Boyd, C. E., 1998. Water Quality for Pond Aquaculture. International Center for Aquaculture and Aquatic Environments, Alabama Agricultural Experiment Station, Auburn University, Alabama.

- Bruin, W. J.; J. W. Nightingale and L. Mumaw, 1980. Seattle Aquarium” Tech. Rep. 9: 1–17.
- Burgess. R.M.; M.M. Perron; M.G. Cantwell; K.T. Ho; J.R. Serbst and M.C. Pelletier, 2004. Use of Zeolite for Removing Ammonia and Ammonia-Caused Toxicity in Marine Toxicity Identification Evaluations. Arch. Environ. Contam. Toxicol., 47: 440–447.
- Chandra Das, P.; S. Ayyappan and J. Jena, 2005. Comparative changes in water quality and role of pond soil after application of different levels of organic and inorganic inputs”. Aquaculture Research, 36: 785-798.
- Durhan. E. J.; T. Norberg-King and L. P. Burkhard, 1993. Methods for aquatic toxicity identification evaluations, Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity, (EPA/600/R-92/080). Enviro. Res. Lab., Duluth, MN.
- EPA, 1992. Method 3005A, Test Methods for Evaluating Solid Waste, Laboratory manual physical/chemical methods, Washington, DC.
- EPA, 1996. Method 3050B”, Test Methods for Evaluating Soil, Sludges and Solid Waste, Laboratory Manual Physical/Chemical Methods, Washington, DC.
- Fajardo, L.J., 2002. Effect of zeolitic material on ammonia concentration during fish transport”, Undergraduate Thesis, College of Fisheries, Central Luzon State Uni., Sci. City of Muñoz, Nueva Ecija, 55 pp.
- Ferdous, J.; M. S. Reza; M. N. Khan; S. Saha; M. Alamgir; J. N. Akhter and M. K. Rahman, 2013. Effects of water treatment chemicals on plankton biomass in aquaculture”. J. Agrofor. Environ. 7 (1): 65-70.
- Haile, T. and G. Nakhla, 2010. The inhibitory effect of antimicrobial zeolite on the biofilm of *Acidithiobacillus thiooxidans*”. Biodegradation, 21:123-134.

- Hamed, Y. A.; T. S. Abdelmoneim; M. H. Elkiki; M. A. Hassan and R. Berndtsson, 2013. Assessment of heavy metals pollution and microbial contamination in water, sediments and fish of Lake Manzala, Egypt. *Life Science Journal*, 10 (1): 86-99.
- Hanley, F., 1991. Effect of feeding supplementary diets containing varying levels of lipid on growth, feed conversion and body composition of Nile Tilapia (*O. niloticus* L.), *Aquaculture*, 93: 323–334.
- Jain, S. K., 1999. Protective roles of zeolite on short and long term lead toxicity in teleost fish *Heteropneustes fossilis*, *Chemosphere*, 39 (2): 247-251.
- Kędziora, K.; J. Piasek; J. Szerement; A. Ambrożewicz-Nita, 2014. Use of modified zeolite in environmental engineering. a review, Institute of agrophysics PAS, Lublin, Poland. UDC 624. 04: 61-66.
- Khachatryan, S.V., 2014. Heavy Metal Adsorption By Armenian Natural Zeolite From Natural Aqueous Solutions, *Proc. of the Yerevan State Univ. Chemistry and Biology*, (2): 31–35.
- Kocakuşak, S.; Ö. Savaşç and T. Tve Ayok, 2001. Doğal Zeolitler ve Uygulama Alanları”, Tübitakmam, Malzeme ve Kimya Tek, Arş. Enst., Rapor No: KM 362, Proje No: 5015202, P.K.21, Gebze,.
- Leinonen, H. and J. Lehto, 2001. Purification of Metal Finishing Waste Waters with Zeolites and Activated Carbons, *Waste Management Research*, 19 (1): 45-57.
- Mieret, M.V. ; R.L. Callejas; R. Gehr; B.E. Cisneros; P. J. Alvarez, 2001. Heavy Metal Removal with Mexican Clinoptilolite: Multi-Component Ionic Exchange. *Water Res.*, 35 (2): 373–378.
- Mohana, E. E., 1996. Pathological studies on fish experimentally intoxicated by certain heavy metals. Ph.D. Thesis, Dept. of Path. and Par., Fac. of Vet. Med., Alex. Univ.

- Mumpton, F.A., 1999. Uses of Natural zeolites in agriculture and industry, National Academy of Sciences Colloquium, (Geology, Mineralogy and Human Welfare), Proc. Natl. Acad. Sci. USA, 96/7: pp. 3463-3470.
- Nazarenko, O. and R. Zarubina, 2013. Application of Sakhaptinsk Zeolite for Improving the Quality of Ground Water. Energy and Envir. Engin., 1: 68-73.
- Noegrohati, S., 2006. Bioaccumulation dynamic of heavy metals in *Oreochromis niloticus*. Berkala MIPA, 16 (2): 29-40.
- Osman, M. F.; H. M. Khattab; H. Mounes; and F. A. Hafez, 2008. Productive performance of *Oreochromis niloticus* under different nutritional and aquaculture systems. International Symposium on Tilapia in Aquaculture: 887-902.
- Piper, R. G. and C. E. Smith, 1984. Use of clinoptilolite for ammonia removal in fish culture systems. Pages 223–228 in W. G. Pond and F. A. Mumpton, editors. Zeo-agriculture. Westview Press, Boulder, Colorado, USA.
- Polat, E.; M. Karaca; H. Demir and A. N. Onus, 2004. Use of natural zeolite (clinoptilolite) in agriculture. J. Fruit and Ornamental Plant Research, Special ed., 12:183-189.
- Saeed, S. M. and M. A. EL-Gammal, 2009. Chemical characteristics of fish pond bottom sediment as a function of organic matter content. Egypt. J. Agric. Res., 87(3): 851-865.
- Saeed, S.M. and S.A. Abdel-Mageed, 2011. Impact of Poultry Slaughterhouse By-Product on the Aquatic Environment of Fish Ponds. J. The Arabian Aquaculture Society, 6 (1): 101-118.
- Saeed, S.M., 2013. Impact of environmental parameters on fish condition and quality in Lake Edku, Egypt. Egypt. J. Aquat. Biol. & Fish., 17, (1):101-112.

- SAS, 1996. SAS Procedure Guide, (Version 6.12 ed), SAS Institute Inc., Cray, NC, USA.
- Schreck, C.B. and P.B.A. Moyle, 1990. Methods for Fish Biology, Bethesda, Maryland: American Fisheries Society, USA, , ch.11.
- Shaheen, S.M.; A.S. Derbalah, and F.S. Moghanm, 2012. Removal of Heavy Metals from Aqueous Solution by Zeolite in Competitive Sorption System. Intern. J. of Environm. Sci. and Develop., 3(4): 362-367.
- Shalaby, A.M.E., 2007. Effect of EDTA on toxicity reduction of cadmium in relation to growth, some haematological and biochemical profiles of Nile tilapia (*O. niloticus*), J. Fisheries and Aquatic Science, 2 (2):100-109.
- Smith, C.F., 1981. Piper RG & Tisher HR, "U.S. Fish Wild l. Serv., Bozeman Inf. Leaflet, 20: 1-17.
- Stoskopf, M.K., 1993. Fish Medicine, (W.B. Saunders Co. Philadelphia, Pennsylvania, 19106, USA).
- Strakova, E.; R. Pospisil; P. Suchy; L. Steinhauser and I. Herzig, 2008. Administration of Clinoptilolite to broiler chickens during growth and its effect on the growth rate and bone metabolism indicators, Acta Vet Brno, 77: 199-207.
- USEPA, U.S., 2007. Environmental Protection Agency, Sediment Toxicity Identification Evaluation (TIE) Phases I, II, and III Guidance Document, (Edited by Kay T. Ho and Robert M. Burgess).
- Vaughan, D.E.W., 1988. The synthesis and manufacture of zeolites. Chem. Eng. Prog., 84: 25-31.
- Wagner, D.J.; G.D. Bacon; W.R. Knocke and M.S. Switzenbaum, 1990. Changes and variability in concentration of heavy metals in sewage sludge during composting". Environ. Technol., 11: 949-960.

- Wen, T., X. Zhang; H. Q. Zhang and J.D. Liu, 2010. Ammonium removal from aqueous solutions by zeolite absorption together with chemical precipitation”, *Water Sci. Technol.*, 61: 1941-1947.
- Wingenfelder, U; C. Hansen; G. furrer and R. Schulin, 2005. Removal of Heavy Metals from Mine Waters by Natural Zeolites, *Environ. Sci. Technol.*, 39: 4606-4613.
- Wudtisin, I. and C.E. Boyd, 2006. Physical and chemical characteristics of sediments in catfish, freshwater prawn, and carp ponds in Thailand, *Aquaculture Research*, 37: 1202-1214.
- Xia, Y.; G.S Walker; D.M. Grant and R. Mokaya, 2009. Hydrogen storage in high surface area carbons, Experimental demonstration of the effects of nitrogen doping, *J. Am. Chem. Soc.*, 131: 16493-16499.
- Zorpas, A.A.; T. Constantinides; A.G. Vlyssides; I. Haralambous; M. Loizidou. 2000. Heavy metal uptake by natural zeolite and metals partitioning in sewage sludge compost. *Bioresource Technology*, 72: 113-119.

دور الزيوليت الطبيعي في تحسين جودة المياه والأداء والحالة الصحية للبلطي النيلي في الأحواض الترابية

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الملخص العربي

الزيوليت معدن طبيعي من الثروات الطبيعية المفيدة والهامة، وقد أجريت هذه الدراسة في إحدى المزارع السمكية بكفر الشيخ لبحث مدى تأثيره على جودة المياه، وتراكم بعض العناصر مثل الحديد، الزنك، النحاس، المنجنيز، الكاديوم والرصاص في عضلات وكبد الأسماك المستزرعة وأيضا رسوبيات الأحواض، إلى جانب بعض قياسات النمو والتركيب الكيميائي (رطوبة، بروتين، دهن ورماد) للأسماك، حيث تم اختيار ثلاث مجموعات من الأحواض الترابية، المجموعة الأولى (control) خالية من الزيوليت، والمجموعة الثانية (T1) تستخدم الزيوليت موزع ومنتشر على قاع الأحواض، والمجموعة الثالثة (T2) وتغذيها مياه تم تمريرها على فلتر من الزيوليت. وقد أظهرت النتائج أن الزيوليت يؤدي الى تحسن خواص المياه وتركيز بعض العناصر الثقيلة في عضلات وكبد الأسماك وأيضا قياسات النمو في الأسماك، أما التركيب الكيميائي للأسماك فلم يظهر فروق ذات دلالة معنوية وأظهرت المجموعة الثانية تحسن أفضل من المجموعة الثالثة. وأيضا تم دراسة الحالة الصحية لأسماك التجربة، حيث أظهرت بعض أسماك المجموعة الضابطة الخالية من الزيوليت تغيرا في لون الجلد حيث ظهر اسوداد في جلد بعض العينات واصفرار في أخرى، كما لوحظ التهاب الجلد في بعض مناطق الجسم محاط بنزيف دموي بالقرب من الذيل وعند قاعدة الزعانف الصدرية ، في حين لم تظهر تلك العلامات المرضية على أسماك المعاملات الأخرى نتيجة لجودة خواص المياه بفعل الزيوليت. لذا توصى الدراسة باستخدام الزيوليت في المزارع السمكية بعد مزيد من الدراسة الاقتصادية.