DOI: 10.2478/cjf-2020-0012

CODEN RIBAEG ISSN 1330-061X (print)
1848-0586 (online)

sciendo

USE OF NATURAL ZEOLITES AS A DETOXIFIER OF HEAVY METALS IN WATER AND THE FLESH OF REARED EUROPEAN SEABASS *Dicentrarchus labrax*

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ARTICLE INFO

Received: 11 September 2019
Accepted: 19 May 2020

ABSTRACT

A study was conducted to investigate the effects of natural zeolites as a water clarifier on the heavy metal removal efficiency from the underground saltwater used for rearing *Dicentrarchus labrax* fry. Five concentrations of zeolites were tested: 0 (Z0), 2.5‰ (Z2.5), 5‰ (Z5), 7.5‰ (Z7.5) and 10‰ (Z10). Fry with an initial body weight of 1.53±0.018 g/fish were stocked in 15 aquaria at a density of 10 fry/aquarium. The fish were fed a commercial diet (42% protein and 12.34% lipid) twice daily (09:30 and 14:00) at 5% of their body weight per day for 42 days. Growth, feed utilization, survival and heavy metal removal efficiency were evaluated. The growth performance and feed utilization indices gradually improved with increasing zeolite concentration, with the most significant ($P \leq 0.05$) values detected at Z10. The survival rate decreased significantly at Z10 compared with the control (Z0). Increasing the zeolite concentration significantly ($P \leq 0.05$) improved the removal efficiency of heavy metals in the rearing water with adsorption selectivity of Pb>Cd>Fe>Cu>Zn. Furthermore, an increase in the detoxification rate of heavy metals in fish flesh with increasing zeolite level was detected with the removal selectivity of Fe>Cu>Zn>Pb>Cd. In conclusion, it can be stated that natural zeolites can be used effectively to reduce heavy metals in polluted waters and subsequently in fish flesh in addition to improving fish performance.

Keywords:
Dicentrarchus labrax
Zeolites
Underground saltwater
Water clarification
Fish performance
Feed utilization

How to Cite

Aly, H. A., Abdel-Rahim, M. M., Sallam, G. R., Lotfy, A. M., Abdelaty, B. S. (2020): Use of natural zeolites as a detoxifier of heavy metals in water and the flesh of reared European seabass *Dicentrarchus labrax*. Croatian Journal of Fisheries, 78, 121-132. DOI: 10.2478/cjf-2020-0012.

INTRODUCTION

Marine aquaculture has been developed and globally expanded in many countries over the last three decades. That expansion refers to either technology development and/or the fulfilment of the increasing demand for seafood by customers worldwide. However, there are some obstacles to the expansion of coastal, inland marine aquaculture worldwide. One of these limitations is laws that prevent or limit the use of coastal areas in the aquaculture industry, especially in regions that are suitable for other investment sectors, such as tourism, agriculture, industry and building or development of coastal cities (Essa, 2013; Sadek, 2013). The procedures for obtaining a license to establish a coastal aquaculture allocated zone are globally very complicated. One of the most promising alternatives is the expansion of mariculture in the desert, depending on the utilization of underground brackish or saltwater. Desert mariculture could be a promising long-lasting production sector, especially in locations where it is challenging to achieve any degree of success for the plant production sector (Sadek, 2013). However, underground saltwater users have many difficulties, such as low dissolved oxygen content, high ammonia content, water hardness and high heavy metal content.

To achieve a durable desert mariculture development industry, many studies have discussed various techniques to enhance the quality of underground saltwater using many technologies, such as activated carbon (Nootong et al., 2011; Gaikwad, 2012; Aly et al., 2016), probiotic and biofloc (Mehrim, 2009; Brito et al., 2014; Emerenciano et al., 2014; Barman et al., 2017), biological and sand filters (Menasveta et al., 2001; Premalatha and Lipton, 2007; Saoud et al., 2007; Zhang et al., 2010; Trang and Brix, 2014) and zeolites (James and Sampath, 1999; Singh et al., 2004; Asgharimoghadam et al., 2012; Ghiasi and Jasour, 2012; Kanyılmaz et al., 2014; Aly et al., 2016; Abdel-Rahim, 2017; Fayed et al., 2019).

Zeolites have a highly porous structure that captures contaminants as small as 4 microns in size. Zeolites have a natural negative charge that enables them to adsorb cations and other organic pollutants in addition to undesirable odors. Therefore, zeolites are among the most recommended applications for use as a water quality enhancer by removing ammonia or heavy metals in fish ponds. Besides, zeolites are inexpensive and safe natural products compared with other materials, such as activated carbon (Abdel-Rahim, 2017) and biological filters (Trang and Brix, 2014). In previous studies, the effects of zeolites on both the ammonia removal efficiency and growth performance of *Dicentrarchus labrax* fry (Aly et al., 2016) and *D. labrax* juveniles (Fayed et al., 2019) were evaluated. Therefore, to complement the studies on improving the quality of well water, this study aims to assess the heavy metal removal efficiency of zeolites in both rearing water and fish flesh of *D. labrax*. The primary purpose of this study is to eliminate or reduce the drawbacks facing desert mariculture, especially in vast areas of Egypt that have no freshwater resources but only saline or brackish waters available.

MATERIALS AND METHODS

Experimental site

The experiment was carried out in the Fish Rearing Lab., El-Max Station for Applied Research, National Institute of Oceanography and Fisheries (NIOF), Alexandria, Egypt during 2017.

Experimental fish

European seabass *D. labrax* fry was purchased from the Marine Finfish Hatchery, K21, General Authority for Fish Resources Development (GAFRD), Ministry of Agriculture and Land Reclamation, located in West Alexandria, Egypt. After three days of acclimation, 150 seabass fry (average weight of 1.53±0.018 gm) were transferred to 15 experimental aquaria (50 liters each) at a stocking density of 10 fry/aquarium.

Experimental design

Five zeolite concentrations were tested: 0 (Z0), 2.5‰ (Z2.5), 5‰ (Z5), 7.5‰ (Z7.5) and 10‰ (Z10). Zeolite was placed in fiberglass net sacs with a 1-mm mesh size. Each sac was filled with 125 g zeolite. The aquaria used for the Z2.5, Z5, Z7.5 and Z10 treatments were supplied with 1, 2, 3 and 4 sacs, respectively. The zeolite sacs were removed every week, washed with freshwater, sun-dried, and reused again for up to four weeks before being replaced with new ones. The natural zeolite (Z) clinoptilolite used in this experiment was purchased from Yemen (http://alixzeolite.com/en/), and the chemical composition and physical properties of this Yemen natural zeolite have been reported by Aly et al. (2016) and Abdel-Rahim (2017), respectively.

Feed and feeding

Fish were fed a commercial diet (42.63% crude protein, 12.34% ether extract, 7.8% ash, 2.35% fibre, 33.65% nitrogen-free extract (NFE), 495.22 Kcal/100 g gross energy and N:C ratio of 86.09 mg CP kcal) with a daily feeding rate of 5% of fish biomass through three feeding meals per day six days per week for a six-week experimental period. Uneaten feed and feces were removed every day, and only the water lost by siphoning was replaced daily.

Sample collection and analytical procedures

Feed and fish proximate chemical analyses

At the beginning of the experiment, a specimen of the tested fish (approximately 40 fish) was randomly collected and preserved for initial body chemical composition. At the end of this experiment, 15 fish from each treatment were sampled for the determination of the proximate chemical composition. Samples of the experimental diet and fish samples were subjected to proximate chemical analyses to measure the moisture, crude protein, crude lipid, crude fiber and ash, according to AOAC (2000).

Heavy metals determination

Copper (Cu), lead (Pb), cadmium (Cd), iron (Fe) and zinc (Zn) were determined in both water and fish muscles. Water samples were collected three times weekly from each aquarium to measure the content of heavy metals. Samples were analysed in an atomic absorption spectrophotometer (AAS), and data were recorded as weekly average concentrations for each aquarium. After fish samples (three replicates) were collected, 2 g of fish muscles were digested by 70% nitric acid and then filtered by filter papers, and the volume was completed in which de-ionized water was added to 25 ml. Then, the digested muscles were transferred to an atomic absorption flame spectrophotometer (AAFS) for heavy metal determination. Water analysis of heavy metals was performed based on the procedure of Shkinev et al. (1989), while the determination of heavy metals in fish samples was performed according to Bat et al. (2012).

Measurements/Calculations

Growth performance and feed utilization parameters

Every week, a sample of fish fry from each aquarium was weighed to verify the actual weight gain and to justify the amount of diet. At the end of the experiment, the fish fry in each aquarium was sampled and counted, and the average final weight was calculated. The growth performance and feed utilization parameters were calculated according to the following equations:

Weight gain (WG, g/fish) = Fw – Iw

where Iw is the initial mean weight of fish in grams, and Fw is the final mean weight of the fish in grams.

Average daily gain (ADG, g/fish/day) = Fw – Iw / n

where n is the duration period.

Specific growth rate (SGR, %/fish/day) = 100 * (ln Fw – ln Iw) / days

where ln is the natural logarithm.

Survival rate (%) = 100 * (final number of fish / initial number of fish)

Feed intake (g/fish): This is the amount of feed given or supplied during the experimental period for each fish per gram.

Feed conversion ratio (FCR) = dry matter feed intake (g)/weight gain (g)

Protein efficiency ratio (PER) = weight gain (g)/ protein intake (g)

Protein productive value (PPV, %) = 100 * (protein gain (g)/protein intake (g))

Energy utilization (EU, %) = 100 * (retained energy / energy fed)

Statistical analysis

Data of the investigated traits (survival, growth performance, feed utilization, heavy metal content in both rearing water and fish flesh) were analyzed with one-way analysis of variance (ANOVA) using SPSS version 22 (SPSS Company Inc., Chicago, IL, USA) to evaluate the differences between the tested treatments. The differences within each experimental treatment were assessed using Duncan's Multiple Range Test at a significance level of $P \leq 0.05$.

RESULTS

Growth performance and survival rate

Growth performance indices (final weight, FW; WG, ADG and SGR) and the survival rate are shown in Table 1. Increasing the zeolite concentration increased the growth performance of seabass. The highest final weight was obtained for the Z10 treatment (4.33 gm), while the lowest value was obtained for Z0 (2.85 gm), with highly significant differences ($P \leq 0.05$) among the treatments. The same trend was detected for WG, ADG and SGR, where the Z10 treatment had the highest values for each parameter. Concerning survival rate, the highest value (100%) was achieved for the control treatment (Z0), without any significant differences ($P > 0.05$) from the other zeolite treatments, except Z10.

Feed utilization

The feed intake, FCR; PER; PPV; energy gain, EG and EU data are shown in Table 1. The feed utilization indices improved significantly ($P \leq 0.05$) with increasing zeolite concentration. The highest value of FCR was achieved for the Z10 treatment (1.60), while the lowest value was achieved for Z0 (3.41). FCR decreased significantly by 53% at Z10 compared with Z0, whereas PER and PPV improved by 113 and 41% at Z10 compared with Z0, respectively. Additionally, improvements in the EG and EU values of 37 and 42% were recorded at Z10 compared with Z0, respectively.

Heavy metals assay in water

Table 2 presents the weekly recorded mean values of some heavy metals (copper, cadmium, iron, zinc and lead) in water. There were significant ($P \leq 0.05$) differences between the Zn concentrations among the tested weeks; the lowest Zn concentration (7.53 µg/l) was reported in week five among the measured weeks, followed by week three (17.605 µg/l). The highest Zn concentration (32.528 µg/l) was recorded in the sixth week. For the different treatments, the results showed that there were significant differences between the different levels of zeolite used. The Z10 treatment resulted in the lowest level of Zn

Table 1. The effects of different concentrations of natural zeolites on the growth performance and feed utilization of *Dicentrarchus labrax* fingerlings

Tested Parameters	Zeolite concentration (‰)^{1*}				
	Z0	Z2.5	Z5	Z7.5	Z10
Initial weight (IW), gm/fish	1.5±0.023	1.54±0.012	1.52±0.012	1.53±0.013	1.55±0.03
Final weight (FW), gm/fish	2.85±0.06^{e}	3.08±0.06^{d}	3.42±0.02^{c}	3.66±0.04^{b}	4.33±0.06^{a}
Weight gain (WG), gm/fish	1.35±0.08^{d}	1.54±0.07^{d}	1.90±0.02^{c}	2.13±0.04^{b}	2.78±0.09^{a}
Average daily gain (ADG), mg/fish/day	32.0±2.0^{d}	37.0±2.0^{d}	45.0±1.0^{c}	51.0±1.0^{b}	66.0±2.0^{a}
Specific growth rate (SGR), %/day	1.53±0.08^{c}	1.65±0.06^{c}	1.93±0.03^{b}	2.07±0.03^{b}	2.44±0.07^{a}
Survival rate (SR), %	100.0±0.0^{a}	93.3±3.33^{ab}	93.3±3.33^{ab}	90.0±0.00^{ab}	86.7±6.67^{b}
Feed intake (FI), gm/fish	4.58±0.11	4.52±0.17	4.67±0.18	4.55±0.07	4.45±0.10
Feed conversion ratio (FCR)	3.41±0.13^{a}	2.93±0.05^{b}	2.46±0.12^{c}	2.14±0.07^{d}	1.60±0.07^{e}
Protein efficiency ratio (PER)	0.69±0.03^{d}	0.80±0.01^{d}	0.96±0.05^{c}	1.10±0.03^{b}	1.47±0.06^{a}
Protein productive value (PPV), %	26.71±0.07^{c}	28.02±0.85^{bc}	29.24±0.72^{bc}	33.58±2.77^{ab}	37.91±2.82^{a}
Energy gain (EG)	4.50±0.26^{c}	4.52±0.16^{c}	5.16±0.16^{bc}	5.64±0.20^{ab}	6.18±0.24^{a}
Energy utilization (EU), %	19.84±0.97^{c}	20.23±0.73^{c}	22.34±0.70^{bc}	25.22±1.99^{ab}	28.14±1.65^{a}

[1] The five zeolite concentrations were 0 (Z0), 2.5‰ (Z2.5), 5‰ (Z5), 7.5‰ (Z7.5) and 10‰ (Z10)

*Means in the same row sharing a letter in their superscript are not significantly different at the 0.05 level

(13.474 µg/l), with a removal percentage of 46.23% of the control content of Zn, followed by the Z7.5 treatment (15.206 µg/l), which represented 39.32% removal of the control value. The lowest treatment was Z2.5, which resulted in only 20.26% removal of Zn.

The highest value in the Z10 treatment was obtained on day 20, with the removal of approximately 58.56% of Zn compared with the control treatment. Otherwise, the lowest percentage was collected on day 42 (the last day of the experiment), where only approximately 22.94% of Zn was removed compared with the control treatment. As the water was changed entirely every week, it was noticeable that on the days immediately before the change of water, the zeolite was more effective than on the days after the water had been changed, where the average percentage was 52.52% and 35.93%, respectively; these results indicated that changing the water results in an overload of zeolite, as it takes more time to recover and absorb more Zn. The lead (Pb) content in the water showed significant differences within the experimental period as well as among the different treatments. Although the lead contents were very high in all treatments, the lowest amount was obtained at week 5 (56.82 µg/l), followed by week 4 (58.94 µg/l). When the source value was 298 µg/l, the highest amount was detected at week 3 (221.27 µg/l). The amounts of heavy metals in the rearing water of seabass are shown in Table 3, while the removal efficiency (%/control) is shown in Table 4 and Figure 1.

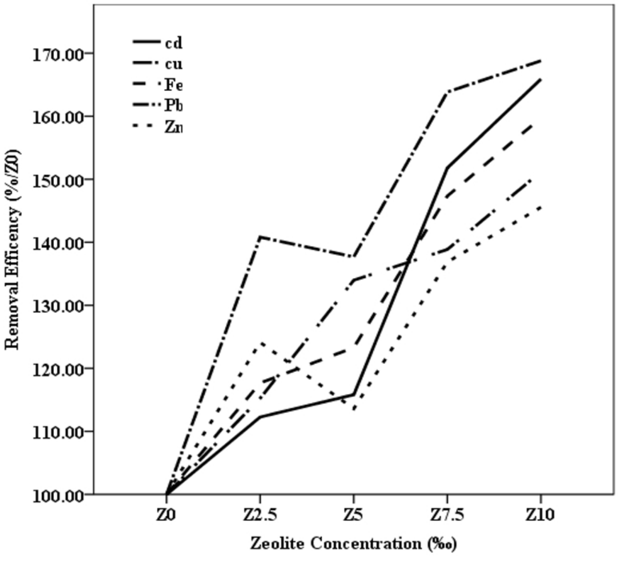

Fig 1. Removal efficiencies (%/control) of some heavy metals (copper, cadmium, iron, zinc, and lead) in the rearing water of *Dicentrarchus labrax* using different concentrations of zeolite as water additives

Table 2. Effect of different concentrations of zeolite on the weekly mean values of some heavy metals in ground saline water used in the rearing of *Dicentrarchus labrax* fingerlings

Variables		Heavy Metals (µg/l)*				
Zeolite[1]	Weeks	Cd	Cu	Fe	Pb	Zn
Z0	1st	23.2 ± 0.202^{f}	15.2 ± 0.938^{mn}	18.2 ± 0.606^{ij}	266 ± 26.1^{ik}	31.4 ± 0.606^{hj}
	2nd	20.1 ± 0.0577^{cde}	9.77 ± 0.884^{pq}	14.3 ± 0.664^{kl}	244 ± 16.1^{ef}	46.4 ± 1.53^{ghj}
	3rd	19.8 ± 1.24^{gh}	18.3 ± 1.5^{egh}	10.6 ± 0.0289^{kl}	397 ± 31^{ghij}	23.4 ± 1.36^{l}
	4th	9.92 ± 1.43^{kl}	17.6 ± 0.635^{hij}	19.9 ± 0.00289^{jk}	104 ± 5.33^{n}	30.6 ± 1.01^{ghj}
	5th	19.9 ± 0.462^{i}	15.8 ± 1.01^{op}	20 ± 0.0115^{g}	127 ± 2.4^{n}	30.1 ± 1.34^{l}
	6th	5.15 ± 0.453^{l}	40 ± 0.578^{c}	55.4 ± 0.837^{c}	146 ± 3.26^{mn}	35.6 ± 0.924^{ce}
Z2.5	1st	20 ± 0.144^{g}	14.2 ± 1.33^{no}	14.1 ± 1.06^{jk}	164 ± 2.47^{jk}	21.8 ± 0.404^{l}
	2nd	19 ± 0.0866^{de}	5.55 ± 0.491^{q}	10.4 ± 0.636^{m}	213 ± 0.346^{fi}	36.8 ± 1.99^{fhi}
	3rd	17.3 ± 1.05^{kl}	16.5 ± 0.866^{jkl}	8.9 ± 0.924^{kl}	315 ± 18.8^{hij}	19.1 ± 1.15^{l}
	4th	8 ± 0.462^{l}	15.8 ± 0.563^{km}	19.1 ± 1.02^{l}	55.4 ± 3.06^{n}	16.4 ± 0.463^{l}
	5th	18.7 ± 0.296^{jkl}	14.4 ± 1.18^{oq}	14.1 ± 1.17^{hi}	54.4 ± 6.5^{n}	21.8 ± 0.551^{l}
	6th	4.31 ± 0.352^{l}	34.9 ± 1.77^{d}	51.7 ± 0.926^{d}	44.9 ± 1.27^{n}	35.2 ± 1.07^{f}
Z5	1st	18.3 ± 0.498b	12.6 ± 0.231^{gi}	13.6 ± 0.202^{fg}	149 ± 3.64^{e}	22.6 ± 1.1^{ghj}
	2nd	20 ± 1.36^{bd}	3.15 ± 0.433^{op}	8.2 ± 0.0^{h}	169 ± 10.9^{d}	35.3 ± 1.36b
	3rd	14.6 ± 1.4^{de}	14.1 ± 1.08^{eg}	9.48 ± 0.741^{hi}	164 ± 6.73^{b}	18.8 ± 1.1ijk
	4th	7.93 ± 0.845^{h}	13.8 ± 0.635^{egh}	15.4 ± 0.672^{e}	81.2 ± 7.69^{ln}	36.3 ± 1.03^{kl}
	5th	16.2 ± 0.693^{bd}	6.4 ± 1.21^{gi}	13.2 ± 0.625^{fg}	106 ± 2.22^{ln}	22.5 ± 0.909^{ghj}
	6th	4.72 ± 0.338^{ik}	34 ± 0.437^{b}	52.8 ± 0.808^{bc}	66.8 ± 5.74^{mn}	34.4 ± 1.62^{bc}
Z7.5	1st	14.6 ± 1.23^{be}	8.2 ± 0.866^{ik}	7.8 ± 0.231^{fg}	111 ± 1.52^{efg}	19.6 ± 1.23^{fh}
	2nd	17.7 ± 0.644^{b}	3.43 ± 0.437^{pq}	4.8 ± 0.404^{ij}	163 ± 8.66^{e}	21.4 ± 0.779^{bc}
	3rd	8.85 ± 0.26^{f}	15.8 ± 0.606^{gi}	5.62 ± 0.678^{hi}	126 ± 5.85^{ef}	13.9 ± 0.751^{jk}
	4th	2.35 ± 0.00577^{h}	13.3 ± 0.173^{hi}	6.5 ± 0.635^{f}	28.8 ± 4.47^{kl}	21.6 ± 0.871^{b}
	5th	5.6 ± 0.577^{ef}	5.55 ± 0.779^{no}	12.6 ± 0.318^{g}	29.6 ± 2.63^{ik}	14.9 ± 0.814^{fh}
	6th	2.15 ± 0.433^{ij}	32.3 ± 0.533^{bc}	50.3 ± 0.441^{b}	37.4 ± 0.491^{lm}	32.3 ± 1.04^{bcd}
Z10	1st	10.2 ± 0.404^{a}	7.2 ± 0.635^{fgi}	6.6 ± 0.462^{e}	98.4 ± 1.88^{c}	15.6 ± 0.26^{de}
	2nd	17 ± 0.52^{b}	2.45 ± 0.548^{lm}	2.1 ± 0.231^{fg}	134 ± 5.47^{c}	22.2 ± 0.779^{a}
	3rd	2.4 ± 0.0577^{bc}	11.2 ± 1.11^{e}	4.75 ± 0.491^{h}	118 ± 0.769^{a}	13.9 ± 0.651^{fg}
	4th	1.8 ± 0.346^{gh}	10.4 ± 0.0289^{ef}	4 ± 0.231^{e}	27.4 ± 1.41^{ik}	15.4 ± 0.411^{e}
	5th	2.75 ± 0.202^{b}	4.75 ± 0.202^{egh}	9 ± 0.0577^{e}	28.1 ± 0.884^{ghij}	14.7 ± 0.399^{e}
	6th	1.65 ± 0.202^{i}	28 ± 0.577^{a}	44.8 ± 0.0866^{a}	25 ± 0.462^{efh}	25.1 ± 0.635^{b}
P-value	Col1	<0.001	<0.001	<0.001	<0.001	<0.001
	Groups	<0.001	<0.001	<0.001	<0.001	<0.001
	C×G[1]	<0.001	<0.001	<0.001	<0.001	<0.001

[1] The five zeolite concentrations were 0 (Z0), 2.5‰ (Z2.5), 5‰ (Z5), 7.5‰ (Z7.5) and 10‰ (Z10)

* Means in the same column sharing a letter in their superscript are not significantly different at the 0.05 level

Table 3. Effect of different concentrations of zeolite on the content of some heavy metals in the rearing water of *Dicentrarchus labrax* fingerlings reared in underground saline water

Variable (µg/l)	Treatments^{1*}				
	Z0 (E)	Z2.5 (A)	Z5 (B)	Z7.5 (C)	Z10 (D)
Pb	214.0±45.2^{a}	141.1±44.7^{ab}	122.7±17.9^{ab}	82.6±23.7^{b}	71.8±20.6^{b}
Cd	16.35±2.90^{a}	14.55±2.72^{a}	13.63±2.46^{ab}	8.54±2.64^{ab}	5.97±2.57^{b}
Fe	23.07±6.64	19.72±6.56	18.78±6.89	14.60±7.23	11.88±6.65
Cu	19.45±4.29	16.89±3.95	14.01±4.39	13.10±4.28	10.67±3.72
Zn	32.92±3.14^{a}	25.18±3.52^{abc}	28.32±3.20^{ab}	20.62±2.69^{abc}	17.82±1.90^{c}

[1] The five zeolite concentrations were 0 (Z0), 2.5‰ (Z2.5), 5‰ (Z5), 7.5‰ (Z7.5) and 10‰ (Z10)
* Means in the same row sharing a letter in their superscript are not significantly different at the 0.05 level

Table 4. Removal efficiencies (%/control) of some heavy metals in the rearing water of *Dicentrarchus labrax* fingerlings using different concentrations of zeolite as water additives

Heavy Metals	Removal Efficiency (%/control)^{1*}				
	Z0	Z2.5	Z5	Z7.5	Z10
Pb	100.0±0.0^{c}	140.8±8.8^{b}	137.7±7.1^{b}	163.9±6.7^{a}	168.8±5.5^{a}
Cd	100.0±0.0^{b}	112.3±2.3^{b}	115.8±3.9^{b}	151.8±9.8^{a}	165.9±11.3^{a}
Fe	100.0±0.0^{c}	117.7±4.3^{bc}	123.3±5.8^{b}	147.4±9.0^{a}	159.7±9.6^{a}
Cu	100.0±0.0^{c}	115.2±5.6^{bc}	134.0±9.5^{ab}	138.9±9.4^{a}	151.2±7.4^{a}
Zn	100.0±0.0^{d}	124.1±6.1^{bc}	113.6±7.4^{cd}	136.9±6.6^{ab}	145.6±3.6^{a}

[1] The five zeolite concentrations were 0 (Z0), 2.5‰ (Z2.5), 5‰ (Z5), 7.5‰ (Z7.5) and 10‰ (Z10)
* Means in the same row sharing a letter in their superscript are not significantly different at the 0.05 level

Concerning the different treatments, the highest removal efficiency (%/control) was obtained for Z10, with a value of 168.8% removal compared with Z0, and 176.93% removal compared with the original water source. According to the lead contents after Z10 treatment, the highest removal of lead was obtained on day 38, with a value of 182.96% of the content at Z0. However, the lowest removal value was obtained on day 15, with a value of only 137.91% of the Z0 value. In contrast, for Zn, it was noticeable that the zeolite was more active after the water had been changed, where the average removal percentage was 174.60% of the control treatment two days after the water had been changed, while this average percentage was only 165% the day immediately before the water was changed; these results indicate that, concerning Pb removal, the zeolite was more effective when the lead concentration in the water was very high.

Additionally, these data reflect the high level of lead in the well water used in this experiment. Cadmium was the second most efficiently removed element after Z10 treatment of rearing water, with a 165.9% removal rate compared with the control (Table 4).

The zeolite detoxifier performance at Z10 for the other analysed elements (Fe, Cu and Zn) was very similar, with ratios varying between 159.7 and 145.6% as an average of all readings during the experimental weeks. The selectivity of natural zeolites for Pb and Cd is noticeably superior to that for Fe, Cu and Zn.

Heavy metal assay in fish flesh

The amounts of heavy metals in fish flesh are shown in Table 5, while the removal efficiencies (%/control) in the fish flesh of seabass fingerlings are shown in Table 6 and Figure 2. The data showed that the tested levels of zeolite significantly decreased the heavy metal content in fish muscle. The heavy metal values in fish muscle naturally reflect the results in the water. The copper content in fish muscle was the lowest value among all the tested elements (Cu, Pb, Zn, Cd and Fe), while the Zn content was the highest value. The copper content after the Z7.5 treatment was the lowest and was significantly lower than that of the control but was not significantly different from the contents observed after the other zeolite-added treatments (Z2.5, Z5 and Z10).

Table 5. Effect of different concentrations of zeolite on the content of some heavy metals in the flesh of *Dicentrarchus labrax* fingerlings reared in underground saline water

Heavy Metals	Initial	Treatments[1*]				
		Z0	Z2.5	Z5	Z7.5	Z10
Pb	2506.67±17.64	2274.17±2.20^{a}	2094.17±37.15^{b}	2041. 67±41.06^{bc}	1974.17±2.20^{c}	1360.83±52.76^{d}
Cd	681.67±1.67	657.83±28.00^{a}	519.17±10.24^{b}	485.83±9.61^{b}	468.33±16.67^{b}	478.337±8.82^{b}
Fe	17525.83±391.47	23560.0±167.43^{a}	23201.67±98.21^{a}	20075.00±188.61^{b}	19259.17±814.07^{b}	17503.33±455.13^{c}
Cu	1400.00±10.41	1508.33±66.73^{a}	1448.33±47.81^{ab}	1376.67±9.39^{ab}	1378.33±62.07^{ab}	1332.50±30.31^{b}
Zn	1783.33±1.67	1746. 67±8.33^{a}	1728.33±1.67^{a}	1715.00±17.56^{a}	1723.33±8.82^{a}	1681.67±1.67^{b}

[1] The five zeolite concentrations were 0 (Z0), 2.5‰ (Z2.5), 5‰ (Z5), 7.5‰ (Z7.5) and 10‰ (Z10)
* Means in the same row sharing a letter in their superscript are not significantly different at the 0.05 level

Table 6. Removal efficiencies (%/control) of some heavy metals in the fish flesh of *Dicentrarchus labrax* fingerlings using different concentrations of zeolite as water additives

Heavy Metals	Removal Efficiency (%/control)[1*]				
	Z0	Z2.5	Z5	Z7.5	Z10
Pb	100.0±0.0^{d}	107.9±1.7^{c}	110.2±1.7^{bc}	113.2±0.0^{b}	140.2±2.3^{a}
Cd	100.0±0.0^{b}	120.9±2.0^{a}	125.9±3.6^{a}	128.7±2.3^{a}	127.0±3.8^{a}
Fe	100.0±0.0^{c}	101.5±1.1^{c}	114.8±1.4^{b}	118.3±2.9^{b}	125.7±2.3^{a}
Cu	100.0±0.0^{b}	103.9±1.1^{ab}	108.3±4.6^{ab}	108.6±0.5^{ab}	111.4±3.4^{a}
Zn	100.0±0.0^{b}	101.0±0.4^{b}	101.8±1.5^{ab}	101.3±0.6^{ab}	103.7±0.3^{a}

1 The five zeolite concentrations were 0 (Z0), 2.5‰ (Z2.5), 5‰ (Z5), 7.5‰ (Z7.5) and 10‰ (Z10)
* Means in the same row sharing a letter in their superscript are not significantly different at the 0.05 level

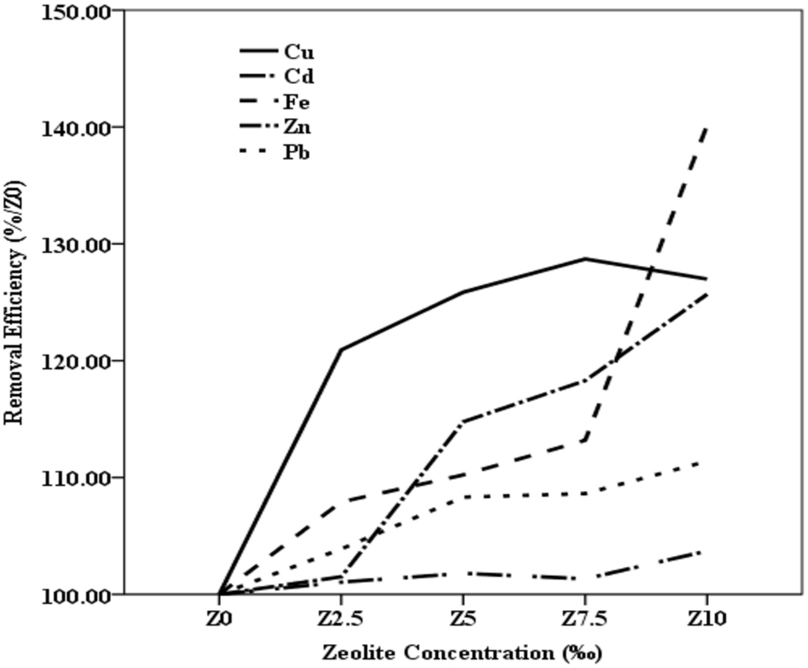

Fig 2. Removal efficiencies (%/control) of some heavy metals (copper, cadmium, iron, zinc, and lead) in the fish flesh of *Dicentrarchus labrax* fingerlings using different concentrations of zeolite as water additives

A highly significant reduction in the content of Fe in seabass fish flesh was recorded at Z10, compared with the other treatments, with a removal efficiency of 140.2% compared with the control. The other tested heavy metals (Pb, Zn, Cd and Fe) exhibited the lowest content at Z10 in comparison with the other treatments and the control. The zinc content after Z10 treatment (17503.33 µg/g) was significantly lower than those after the other zeolite-added treatments, as well as the control treatment (23560.0 µg/g), with a 125.7% removal efficiency relative to the control treatment (Table 6). Additionally, the Z10 treatment resulted in the lowest Pb content (1332.50 µg/g) among all tested treatments, which was significantly different from the control treatment value (1508.33 µg/g), indicating a 111.4% removal efficiency. The cadmium content after Z10 treatment (1681.67 µg/g) was significantly lower than the contents after the other zeolite-added treatments and the control (1746.67 µg/g), achieving 103.72% removal efficiency relative to the control.

DISCUSSION

Adding zeolite to fish rearing water or feed has many benefits that directly or indirectly affect the vitality of farmed fish. In the present study, an improvement in seabass growth and feed utilization indices with increasing zeolite concentration was recorded. Similar to the results of the current study, the results of Ali et al. (2016) indicated that application of 10 ppt natural zeolite to rearing tanks as a water additive resulted in a higher ADG (+30.7%) and a higher survival rate (+22.7%) of European seabass (*Dicentrarchus labrax*), and a 96.4% ammonia removal efficiency compared with zeolite-free treatment. As in previous work, Rabiatul et al. (2018) found a significant positive correlation between the growth performance of red hybrid tilapia (*Oreochromis* sp.) with increasing zeolite levels from 0 to 20 ppt. The highest values of the growth parameters were obtained with 20 ppt zeolite, with 37.3% higher ADG. The 20 ppt zeolite treatment was also associated with a higher oxygen content (+23.5%), lower ammonia content (-77.8%), lower nitrite content (-63.9%) and lower turbidity (-11.7%) than the control. Additionally, Ghiasi and Jasour (2012) concluded that the growth performance of angelfish and *Pterophyllum scalare* was higher in the 10 and 15 ppt zeolite groups than in the control. The addition of zeolite to fish feed also improved the performance and health status of the fish. Obradovic et al. (2006) concluded that the addition of 1% zeolite to feed and 1.2 ppt zeolite to water had a positive, stimulating effect on all morphometrical and biochemical indices of rainbow trout (*Oncorhynchus mykiss*). The zeolite group exhibited a higher weight increase (+18.04%), higher length increase (+12.00%), lower mortality rate (-32.0%), higher daily feed consumption (+3.102%), lower feed conversion (-13.62%), lower protein conversion (g/g) (-14.78%) and a higher production index (+32.96%) than the zeolite-free treatment. This result was attributed to the substantial improvement in the haematological and blood biochemical analyses. The noticeable decrease in the survival rate recorded in the current study and other studies after treatment with high contents of zeolite in both water and feed may be attributed to the potential negative effects of zeolite as an ion exchange material that reduces some essential elements (e.g. activated carbon), which correlates with some vital biological activities of fish (Sigworth and Smith, 1972; Caglia, 2013; Ali at al., 2016; Jawahar et al., 2016). Therefore, further studies should be conducted on this issue.

Zeolites are highly selective cleaners of a variety of metal cations that can be removed from liquid effluents through the ion exchange process. Natural zeolites are excellent detoxifiers of heavy metal cations (Mn, Fe, Cd, Zn, Pb, Cu, Co, Cr, Cu and Pb) with efficiencies as high as 97% from waste and drinking waters (Margeta et al., 2013). Previous studies illustrated the capability of zeolite to adsorb heavy metals from industrial, municipal and agricultural wastewater (Hokkanen et al., 2013; Wen et al., 2016). The results of the current study revealed that the removal percentage of heavy metals increased gradually with increasing zeolite content.

The results showed the fluctuation of the Cu^{2+} concentration at the point source, with the highest Cu^{2+} concentration recorded in the final week. The zeolite adsorption ability decreased with the elevated levels of Cu^{2+} at the point source. Similarly, Erdem et al. (2004) indicated that natural zeolites have great potential to remove heavy metals such as Cu^{2+} from industrial wastewater. After the Z7.5 treatment, the highest removal percentage of Cu^{2+} was 87% when the Cu^{2+} concentration was 11.5 µg/l, but the removal efficiency decreased significantly to 6% when the Cu^{2+} concentration increased to 28.9 µg/l. Moreover, when the Cu^{2+} level increased from 8.6 to 11.5 µg/l at the point source, the removal efficiency (in %) after the Z7.5 treatment increased from 60.4 to 86.7%. Correspondingly, the results herein agree with those of Hegazi (2013) who reported that by increasing the amount of absorbent, the removal percentage of Cu^{2+} increases from 24.5% to 98.2%. Consequently, for the same point source level, the Z5 removal percentage of Cu^{2+} decreased from 53% to 11%, and the lowest Cu^{2+} removal percentage was detected for the Z0 treatment, with a decrease from 25% to 4.5%. On the other hand, when the Cu^{2+} level at the point source increased from 13.6 to 20.9 µg/l, the removal efficiency for adsorbing Cu^{2+} decreased from 38.7% to 16.7% for the Z7.5 treatment.

Accordingly, high Cd levels were detected in the point source during the experimental period, with values of 23.2 µg/l, and the lowest value was detected for the Z7.5 treatment, with a value of 1.65 µg/l during the last week of the experiment. Thus, an increased level of zeolite improves the potential ability of the zeolite to adsorb Cd from water and enhances the water quality. However, James and Sampath (1999) found that the presence of zeolite in water positively affected the removal of Cd (mg/g muscle) from fish bodies (*Oreochromis mossambicus*) when they exposed the fish to Cd alone or in a mixture with zeolite (0.5, 2 and 4 ppt zeolite). Their results indicated that there were significant differences between the control treatment (Cd only) and the zeolite treatments. Higher concentrations of zeolite resulted in lower levels of Cd (1.78, 1.36, 1.17 and 0.90 mg/g Cd). Nevertheless, they illustrated that 45 days (the experimental period) was not adequate for the complete removal of Cd from water and fish muscle. Cadmium and copper are considered the most toxic metals due to their ability to cause morphological changes (Thophon et al., 2003) and ecological damage (Al-Weher, 2008) in aquatic organisms. The harmful effect of cadmium was observed on fish gills where the metals accumulated to concentrations many times higher than those present in water (El Deen et al., 2011). However, the fluctuating salinity of water bodies causes damage to the chloride cells of *D. labrax*, which accelerates the rate of ion loss and impairs ion uptake (Laurent and Perry, 1991). Furthermore, in the present study, various concentrations of Cu and Cd were detected in *D. labrax* carcasses. The results illustrated in Table 4 revealed that the levels of Cu, Cd and Fe in the carcasses of *D. labrax* fingerlings decreased significantly ($P<0.05$) with increased levels of zeolite in the water. However, the fish carcasses in point source tanks had high levels of Cu, Cd and Fe, with values of 681.67, 1783.33 and 2506.67 µg/g, respectively. This finding indicates that treating water with zeolite has a positive effect on eliminating the harmful effects that heavy metals pose to the welfare of fish health during the grow-out stage.

An increased level of all the tested heavy metals during the last week of the experiment (week 6) was also observed for all zeolite treatments, even Z10. This finding indicates the possible release of adsorbed minerals from the zeolite particle and/or a full saturation of zeolite channels with adsorbed ions. In this regard, Erdem et al. (2004) stated that the adsorption efficiency decreased as the heavy metal concentration increased. Therefore, higher levels of zeolite treatment (more than 10 ppt) or the addition of new fresh zeolite material or recharged zeolite might be more suitable for groundwater with high levels of heavy metals. In accordance with this opinion, Rafiee and Saad (2010) discovered that the use of 0.011 ppt zeolite in fibreglass tanks as a bed medium in an aquaponic system (lettuce with red tilapia) significantly affected the Zn concentration in water, as the concentration was lower in the control treatment than in the zeolite-added treatment (0.13 and 0.14 mg/l, respectively) at the end of the experiment (7 weeks). Minceva et al. (2007) stated that Zn had the lowest adsorption selectivity when the efficiency of zeolite and granulated activated carbon in Zn, Pb and Cd removal from binary aqueous solutions was studied. Additionally, they specified that the adsorption capacity of Zn (as a single solution) by zeolite was 3.926 mg/g, while this value was reduced to 2.02 and 0.881 mg/g in the binary solutions containing Zn+Cd and Zn + Pb, respectively. However, the adsorption capacity of Pb (as a single solution) by zeolite was 27.174 mg/g, while the adsorption capacity was reduced to 24.51 and 18.622 in binary solutions containing mixtures of Pb+Zn and Pb+ Cd, respectively. The same authors also revealed that there was a positive relationship between the metal concentration and the adsorption capacity. In the same context, Oter and Akcay (2007) and Cincotti et al. (2006) reported that adsorption of Pb, Zn, Cu and Ni was reduced in mixed solutions compared with a single solution (containing only one heavy metal). Additionally, they stated that the zeolite selectivity in mixed and single solutions was Pb>Zn>Cu>Ni. Likewise, Erdem et al. (2004) found that Zn adsorption ranked third in the selectivity sequence of zeolite after Co^{2+} and Cu^{2+}. These findings show that the adsorption percentage depends on the charge density and hydrated ion diameter. Correspondingly, Ibrahim et al. (2010) stated that the selectivity sequence of zeolite (prepared from Egyptian kaolin) was Pb>Cd>Cu>Zn>Ni. Mihaly-Cozmuta et al. (2014), who investigated the influence of pH on zeolite efficiency, found that the solution pH significantly affected the zeolite adsorption selectivity, as the selectivity was Pb>Cd>Cu>Zn>Mn>Co>Ni at pH 4 and ~ Pb>Cd>Mn>Zn>Co>Cu>Ni at pH 1. Additionally, their results indicate that, regardless of the pH value, zeolite has the highest efficiency of Pb adsorption, followed by Cd. This result concurs with the results of the current study. The results obtained in both previous studies and this study explain the disparity in Zn removal efficiency and show that there was competition between the different metals tested in this study. In agreement with previous studies, the adsorption selectivity of zeolite in this study was Pb>Cd>Fe>Cu>Zn.

ACKNOWLEDGEMENTS

This research was financially supported by the National Institute of Oceanography and Fisheries (NIOF), Ministry of Scientific Research, Egypt. The authors hereby express their gratitude to the Head of NIOF, Aquaculture Division and Fish Rearing Lab. Especial thanks for Professor Ashraf Goda, Head of Aquaculture Division for his great help. We are grateful for Dr. Ahmed Sallam for helping. The authors also extend their gratitude to Prof. Dr. Mohamed Gomaa for his help and support during the experiment.

SAŽETAK

UPORABA PRIRODNIH ZEOLITA KAO PROČIŠĆIVAČA TEŠKIH METALA U VODI I MESU UZGAJANOG EUROPSKOG LUBINA *Dicentrarchus labrax*

Provedeno je istraživanje kojim su se ispitivali učinci prirodnih zeolita kao pročišćivača vode na učinkovitost uklanjanja teških metala iz podzemne slane vode koja se koristi za uzgoj mlađi *Dicentrarchus labrax*. Ispitano je pet koncentracija zeolita: 0 (Z0), 2,5 ‰ (Z2,5), 5 ‰ (Z5), 7,5 ‰ (Z7,5) i 10 ‰ (Z10). Mlađ s početnom tjelesnom masom od 1,53 ± 0,018 g su nasađene u 15 akvarija s gustoćom 10 jedinki/akvarij. Ribe su se hranile komercijalnom hranom (42% proteina i 12,34% lipida) dva puta dnevno (09:30 i 14:00), u udjelu od 5% tjelesne mase ribe, tijekom 42 dana. Evaluirani su rast, iskorištavanje hrane, preživljavanje i učinkovitost uklanjanja teških metala. Indeksi rasta i iskorištenja hrane postupno su se poboljšavali s povećanjem koncentracije zeolita, pri čemu su najznačajnije vrijednosti ($P \leq 0{,}05$) otkrivene sa skupinom Z10. Stopa preživljavanja značajno se smanjila u skupini Z10 pri usporedbi s kontrolom (Z0). Značajno povećanje koncentracije zeolita ($P \leq 0{,}05$) poboljšalo je učinkovitost uklanjanja teških metala iz uzgojne vode sa adsorpcijskom selektivnošću Pb>Cd>Fe>Cu>Zn. Nadalje, otkriveno je povećanje brzine detoksikacije teških metala u mesu ribe s povećanjem razine zeolita uz selektivnost uklanjanja Fe>Cu>Zn>Pb>Cd. Zaključno, može se reći da se prirodni zeoliti mogu učinkovito koristiti za smanjenje teških metala u onečišćenim vodama, a posljedično i u mesu ribe, uz poboljšanje performansi rasta ribe.

Ključne riječi: *Dicentrarchus labrax*, zeoliti, podzemna slana voda, pročišćavanje vode, performanse riba, iskorištavanje hrane

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