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THE IMPACT OF ZEOLITE AGAINST CADMIUM CHLORIDE TOXICITY IN NILE TILAPIA *(Oreochromis niloticus)*

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Abstract

The present study aims to evaluat the possible ameliorative effect of Zeolite against cadmium toxicity in *Tilapia Nilotica*. fishes of Nile tilapia were divided into nine groups. The 1st group (lefet as control group without any addition. The 2nd, 3rd, 4th groups contains to 0.17, 0.35, 0.7 and 1.4 gm of Cdcl2/L, respectivelyy. Also the 5th, 6th, 7th, 8 th and 9 th contains (0.17, 0.35, 0.7 and 1.4Cd+ 8g of Zolite, respectively). After 90 days of exposurefish to Cd a reduction significantly ($P<0.05$) in haemoglobin content (Hb), haematocrit value (Hct). These parameters were improved when zolite was applied with Cd. The values of Hb and Hct were increased significantly to be as in the control fish group. There were significant decreased in total protein (TP) and albumen levels in plasma of fish exposed to Cd alone. While, these values were improved in fish after addition of zolite with low doses of cadmium. Also plasma glucose concentration, cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT) alkaline phosphatase (ALP), creatinine and Urea concentrations were increased significantly in fish exposed to Cd alone. Addition of zolite to Cd contaminated medium enhanced these biochemical parameters in fish and the enzyme activities returned to be as control group. Fish exposed to Cd alone accumulates 2.01, 2.38, 2.73 and 5.48 mg Cd/g dry weight in gills after exposure to all doses of Cd toxicity respectively. Addition of zolite to Cd contaminated medium considerably reduced cadmium absorption and accumulation in gills.

Keywords: Zeolite, cadmium chloride, *Nile tilapia*.

INTRODUCTION

Environmental pollution is a universal problem and most impotent pollutants are the heavy metals in aquatic network because of their toxicity, accumulation and bio-magnification by marine creature. Domestics industrial and anthropogenic activities may broadly become the source of natural aquatic systems contamination of heavy metal (Velez and Montero, 1998 and Conacher and Mes, 1993).

The pollution of water resources due to the indiscriminate disposal of heavy metals has been causing worldwide concern for last few decades. It is well known that some metals have toxic or harmful effect on many forms of life. Metals, which are significantly toxic to human beings and ecological environments, include chromium, copper, lead, mercury, manganese, cadmium, nickel, zinc and iron, etc. This problem has received considerable amount of attention in recent years. One primarily concern is that marine animals which can readily absorb those heavy metals is wastewater and directly enter the human food chains present a high health risk to consumers(Meena *et al*., 2005).

As a human food, fish are consider as an excellent source of poly unsaturated fully acid (predominantly omega – 3 fatly acids), protein, Zn, iron and calcium (Tucker, 1997 and Tooth & Brown, 1997).

Due to increased human activities and interests, cadmium has become one of the most hazardous heavy metals in aquatic environments and could threaten aquatic organisms including tilapia fish. Cadmium is a naturally occurring toxic heavy metal no known biological function in humans and animals and therefore considered non-essential, Food Drug Administration (FDA, 1993).

Cadmium is used in electroplating and galvanizing due to its non-corrosive and cumulative nature. It is used as color pigment for paints, plastics and as a cathode material for nickel-cadmium batteries Kalaiselvi *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 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 Zahra *et al*. (2016).

Most of the world's capture fisheries are considered fully exploited or overexploited. Aquaculture plays the main role in meeting food fish demand and remains one of the fastest-growing food producing sectors. Aquaculture provides half of all fish for human food. This share is forecasted to rise to 62% by 2030 as catches from wild capture fisheries will level off (FAO, 2014). With population growth, aquaculture will continue to expand, which means technologies with higher efficiency should be adopted to increase the production. On the other hand, due to concerns regarding detrimental impacts of aquaculture production on the environment and increased regulations on aquaculture effluents, the aquaculture industry is focused on the development of new methods to minimize the toxic contaminants in aquaculture waters in recycling systems and wastewaters of aquaculture ponds. Using eco-friendly adsorbents such as natural zeolitic minerals is considered as one of the promising approaches for treating contaminated waters and wastewaters Zahra *et al*. (2016). The aim of this study was to evaluate the possible ameliorative role of zeolite in reducing the toxicity of cadmium chloride and improvement physiological parameters in Nile tilapia *(Oreochromis niloticu*s)

MATERIALS AND METHODS

Cadmium chloride: The cadmium chloride was obtained from El- Nasr Chemical Company (Cairo, Egypt).

Zeolite: Samples of zeolite were taken from the Enli Mining Companyopen 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, along with instrumental methods, 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 animal:

Healthy *Oreochromis niloticu*s of 35-45, grams were collected from the ponds of the Central Laboratory for Aquaculture Research at Abbassa, Abo-Hammad, Sharkia, Egypt. Fish stay in large plastic aquaria for 15 days in the laboratory for acclimation. Fish were distributed in twenty seventh glass aquaria of about 100-litre capacity each and stocked at a rate of 10-fish/ aquarium. The glass aquaria were supplied with dechlorinated tap water and air stones average water temperature was maintained at 27±2 ºC. The aquaria were divided into 9 groups with three replicates per group. The 1st group was left as control, 2nd, 3rd, 4th, and 5th groups received 1/200 and 1/100, 1/50 and 1/25 LC_{50} of Cd (0.17, 0.35,0.7 and 1.4mg Cd/l) respectively according to Shalaby

(1997) other groups (received same doses of Cd ,respectively in addition to 8 g/l of zolite (groups 6 th,7 th,8 th and 9 th). The treatment groups are in illustrated in Table (2)

Table 2. Design of experimental.

	Cadmium		Zeolite
Groups	LC_{50}	Dose(mg/l)	Dose(g/l)
1	-	-	-
2	1/200	0.17	-
3	1/100	0.35	-
4	1/50	0.7	-
5	1/25	1.4	-
6	1/200	0.17	8
7	1/100	0.35	8
8	1/50	0.7	8
9	1/25	1.4	8

Fish of the experimental groups were fed on a pelleted fish diet containing 32%C, and fish was fed at a rate of 3% of live body weight twice daily for 90 days. Semi-dynamic method for removal of excreta was used every day by siphoning a portion of water from the aquarium and replacing it by an equal volume of water sampling.

Physiological Analyses:

After 90 days of the experiment. Samples of blood, were taken from five Nile tilapia from each aquarium.

Fish were not fed for 24 h before sampling and anaesthetized with buffered MS 222 (50 mg /L). Blood samples were taken from caudal vein of an anaesthetized fish by sterile syringe using EDTA solution as anticoagulant. These blood samples were used for determining hemoglobin content (Vankampen, 1961) and heamatocrit value (Hct) were calculated according to the formulae mentioned by Britton (1963).

Plasma was obtained by centrifugation of blood at 3000 rpm for 15 min and nonhaemolyzed plasma was stored in deep freezer for further biochemical

analyses. Total protein content and creatinine concentration were determined colorimetrically according to Henry (1964). Also the albumin concentration was determined according to young (2000) Plasma glucose was determined, using glucose kits supplied by Boehring Mannheium kit, according to Trinder (1969). Triglycerides were determined using Biocon Kits according to Fossati and Principe (1982). Cholesterol was determined as CHOD-PAP method according to Allain *et al* (1974). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957), while alkaline phosphatase (ALP) was measured by using Diamond diagnostics kits according to the method of Bergmeyer (1972). Also urea concentration was determined according Patton and Crouch (1977).

Cd residue: Cadmium was measured in 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

Hematological parameters:

Heamoglobin content:

The heamoglobin content in the blood in control fish group was 10.90± 1.15 (g/100 ml), these values were highly significant decrease in fish groups exposed to all doses of cadmium alone as compared with control group, Meanwhile Zeolite plus Cd toxicity in 6th, 7th and 8th treated groups elucidate non significant decrease in hemoglobin level 10.25± 1.27, 10.07± 0.84 and 9.500± 1.00 (g/100 ml, respectively) comparing to control group (10.90 g/100ml).

However, addition of zeolite for low dose of cadmium toxicity were improved level of heamoglobin concentration, While, Zeolite + 1.4 mg $CdCl_2$

/L treated group showed a significant decrease in hemoglobin level as compared with control groups (Table 3).

Table (3) showed that 1.4 ppm of Cd toxicated groups afforded highly significant decrease in Hct level to 17.50 ± 0.57 % when compared with control group (23.50 ± 1.29). While, the fish exposed to 0.17, 0.35 and 0.70 Cd mg/L showed a non significant decrease in Hct levels when compared with control group. However, Zeolite + 0.17, 0.35 mg $CdCl_2$/L in treated groups elucidate a significant increase in Hct level compared with control group. While, the fish exposed to Zeolite with 0.70 and 1.4 mg Cd /L showed non significant change in Hct level compared with control group.

Table 3. The heamoglobin content (g/100ml) and haematocrit value (%) in the blood of Nile tilapia after exposed to cadmium toxicity alone or in addition of zeolite.

Groups	Hct %	Hb %
Control	23.50 ± 1.29^{c}	10.90 ± 1.15^{a}
$CdCl_2$ 0.17 treated group	22.75 ± 1.50^{c}	8.875 ± 1.16^{bcd}
$CdCl_2$ 0.35 treated group	22.25 ± 2.06^{c}	8.175 ± 1.07^{cd}
$CdCl_2$ 0.7 treated group	19.00 ± 1.15^{cd}	7.950 ± 0.05^{d}
$CdCl_2$ 1.4 treated group	17.50 ± 0.57^{d}	5.650 ± 0.43^{e}
Zeolite+ $CdCl_2$ 0.17 treated group	31.50 ± 1.73^{a}	10.25 ± 1.27^{ab}
Zeolite + $CdCl_2$ 0.35 treated group	28.50 ± 4.43^{b}	10.07 ± 0.84^{ab}
Zeolite + $CdCl_2$ 0.7 treated group	21.50 ± 0.57^{c}	9.500 ± 1.00^{abc}
Zeolite + $CdCl_2$ 1.4 treated group	20.50 ± 0.57^{c}	8.500 ± 0.57^{cd}

Means within the same column in each category carrying different litters are significantly different at ($P \leq 0.05$).

Biochemical changes:

1- Total protein content:

Date Table (4) showed significant reduction in plasma total protein of fish exposed to Cd alone and high dose of Cd (0,7 and 1.4 mg Cd/L) with zeolite when compared to control group. Addition of zeolite improve the total protein in blood than that of Cd alone, but still lower than that of control group ($P<0.05$). This result indicated that the addition of zeolite failed to recover the total protein in blood fish at the high doses of cadmium.

2- **Albumin level**: The plasma albumin level in control group was 6.32± 2.07[a]g/100, this value was decreased significantly to 4.22± 0.37, 4.25± 0.4, 3.97± 0.53 and 3.77± 0.32 after exposed the fish to sublethal doses of cadmium (0.17, 0.35, 0.7 and 1.4 mg/L), respectively. At the same time, low doses of cadmium +8g /l zeolite treated groups elucidated non significant change in albumin level as compared with control group. On the other hand, this level was decreased significantly in fish exposed to high dose of Cd with zeolite as compared to control group as shown in (Table 4).

3- Glucose levels: The plasma glucose concentration showed higher significant values (P< 0.01) (181.75± 19.80, 233.0± 20.78, 244.0± 19.60 and 298.25± 16.16 mg%) in fish groups exposed to all doses of cadmium alone (0.17, 0.35, 0.7 and 1.4 mgCd/l, respectively) when compared to the control group value (156.50± 10.31 mg/dI). The glucose concentration in fish subjected to low doses of Cd plus zeolite (0.17, and 0.35 mg of Cd+8g of zeolite /l) did not significantly be affected. On the other hand, the plasma glucose concentration were significantly increased (P <0.05) in all treatments of high dose of Cd with zeolite as seen in Table (4).

Table 4. Total protein contain, albumin and glucose concentrations in the blood of *O. niloticus* exposed to sublethal dose of cadmium alone or combined with zeolite.

Groups	Protein (g/dI)	Albumin (g/dI)	Glucose (mg/dI)
Control	9.0± 0.93[a]	6.32 ± 2.07[a]	156.50 ± 10.31[de]
Cadmium 0.17 ppm alone	5.60 ± 0.27[d]	4.22 ± 0.37[bc]	181.75 ± 19.80[b]
Cadmium 0.35 ppm alone	5.25 ± 0.65[d]	4.25 ± 0.40[bc]	233.00 ± 20.78[abc]
Cadmium 0.7 ppm alone	5.15 ± 0.85[d]	3.97 ± 0.53[bc]	244.00 ± 19.60[ab]
Cadmium 1.4 ppm alone	4.92 ± 1.06[d]	3.77 ± 0.32[c]	298.25 ± 16.16[a]
Cadmium 0.17 ppm + zeolite (8g/L)	8.87 ± 0.21[a]	6.30 ± 1.19 [a]	141.750 ± 21.26[e]
Cadmium 0.35 ppm + zeolite (8g/L)	7.90 ± 0.14[a]	5.80 ± 0.23[abc]	137.75 ± 24.08[de]
Cadmium 0.7 ppm + zeolite (8g/L)	7.00 ± 0.81[c]	5.70 ± 0.08[bc]	171.50 ± 42.96[bce]
Cadmium 1.4 ppm + zeolite (8g/L)	4.50 ± 0.69[d]	4.12 ± 0.61[bc]	203.75 ± 38.57[bc]

Means within the same column in each category carrying different litters are significantly different at (P ≤ 0.05).

Enzyme activities:

Aspartate amino transferease (AST) in the plasma of Nile tilapia as in (Table5) showed that CdCl2 treated groups afforded highly significant increase in AST level as compared with control group. Meanwhile, Zeolite with all concentration of Cd (0.17, 0.35 and 0.7ppm) treated groups elucidate significant decrease in AST level as compared to CdCl2 treated groups only while they also, afforded a non significant increase in AST level as compared with control group. At the same time, Zeolite + 1.4mg of Cd treated group elucidate significant increase in AST level as compared with control group.

ALT levels were markedly elevated after cadmium expoure to normal fish when compared control groups and 1.4% caused the highest toxic effect. Meanwhile, a non significant change in plasma ALT level compared with control groups was recorded in Zeolite+ $CdCl_2$ 0.17, 0.35, 0.70 and 1.4 % treated group as shown in Table (5).

Alkaline pohosphatase (ALP) level was decreased significant to 663.25 ± 10.7, 736.25 ± 41.5, 774.5 ± 9.0, and 2657.0± 36.8 IU/L in plasma of fish exposed to all dose of cadmium, respectively when compared to the control group (393.0 ± 17.5 IU/L) **.** Meanwhile, 8gm/l Zeolite + CdCl2 0.17, 0.35, 0.7 and 1.4 afforded non-significant change in ALP level as compared to control group, as shown in (Table 5).

Table 5. The AST, ALT and ALP levels (IU/L) in the blood of *O. niloticus* exposed to sublethal concentration of cadmium alone or combined with zeolite.

Groups	ALT	AST	ALP
Control	69.50 ± 2.88^{c}	17.75 ± 10.75^{e}	393.00 ± 17.5^{b}
Cadmium 0.17 ppm alone	71.50 ± 4.04^{b}	31.25 ± 3.685^{c}	663.25 ± 10.7^{b}
Cadmium 0.35 ppm alone	76.50 ± 4.04^{b}	33.50 ± 2.886^{c}	736.25 ± 41.5^{b}
Cadmium 0.7 ppm alone	80.00 ± 2.58^{a}	44.00 ± 3.464^{b}	774.50 ± 9.0^{a}
Cadmium 1.4 ppm alone	91.00 ± 3.46^{a}	55.50 ± 4.041^{a}	2657.0 ± 36.8^{a}
Cadmium 0.17 ppm + zeolite (8g/L)	69.00 ± 2.30^{c}	11.50 ± 3.872^{e}	317.50 ± 15.1^{b}
Cadmium 0.35 ppm + zeolite (8g/L)	71.00 ± 1.15^{c}	11.50 ± 3.872^{ed}	543.00 ± 2.30^{b}
Cadmium 0.7 ppm + zeolite (8g/L)	71.25 ± 4.34^{c}	18.50 ± 3.316^{ed}	380.50 ± 16.1^{b}
Cadmium 1.4 ppm + zeolite (8g/L)	72.00 ± 5.77^{c}	21.25 ± 4.787^{d}	394.00 ± 17.4^{b}

Means within the same column in each category carrying different litters are significantly different at ($P \leq 0.05$).

The cholestrerol level in the plasma of *O. niloticus* has shown highly significant increased in all toxicity groups of Cd only when compared to the control group while, these levels were non significantly change in all toxic group of Cd combined with zeolite when compared to control group (Table 6). Also The triglycerides concentration in fish plasma as shown in Table (6) was highly significant increased in all treated groups exposed to cadmium with or without zeolitecomared to control group.

Table 6. The cholesterol and triglycerides concentration in the plasma of *O. niloticus* exposed to sublethal dose of cadmium alone or combined with zeolite

Groups	Cholesterol	Triglycerides
Control	127.00 ± 15.42^{c}	87.25 ± 4.85^{b}
$CdCl_2$ 0.17 treated group	245.25 ± 50.39^{b}	173.70 ± 58.5^{a}
$CdCl_2$ 0.35 treated group	247.25 ± 44.95^{b}	182.00 ± 25.5^{a}
$CdCl_2$ 0.7 treated group	248.50 ± 92.85^{b}	169.50 ± 51.34^{a}
$CdCl_2$ 1.4 treated group	387.75 ± 172.0^{a}	188.50 ± 30.82^{a}
Zeolite+ $CdCl_2$ 0.17 treated group	125.50 ± 33.03^{c}	141.50 ± 35.70^{ab}
Zeolite + $CdCl_2$ 0.3 5 treated group	125.00 ± 1.410^{c}	102.75 ± 21.09^{b}
Zeolite + $CdCl_2$ 0.7 treated group	120.20 ± 24.28^{c}	133.75 ± 42.41^{ab}
Zeolite + $CdCl_2$ 1.4 treated group	155.00 ± 43.45^{bc}	134.50 ± 46.93^{ab}

Means within the same column in each category carrying different litters are significant at ($P \leq 0.05$).

Kidney functions:

The creatinine and urea concentration in the plasma of *O niloticus* exposed to all doses of cadmium were increased significantly as comared to the control group. Whereas the addition of zeolite caused non significant changes compared to the control group, as shown in Table (7).

Table 7. The creatinine and urea concentration mg/L) in the blood of *O. niloticus* exposed to sublethal dose of cadmium alone or combined with zeolite.

Groups	Creatinine	Urea
Control	0.75 ± 0.25^{e}	43.50 ± 5.44^{e}
$CdCl_2$ 0.17 treated group	1.37 ± 0.20^{c}	71.50 ± 3.10^{b}
$CdCl_2$ 0.35 treated group	2.15 ± 0.64^{b}	74.25 ± 5.67^{b}
$CdCl_2$ 0.7 treated group	2.17 ± 0.37^{b}	76.50 ± 2.34^{ab}
$CdCl_2$ 1.4 treated group	2.67 ±0.29 ^{a}	84.25 ± 5.43^{a}
Zeolite+ $CdCl_2$ 0.17 treated group	0.60 ± 0.16^{e}	50.75 ± 6.70^{de}
Zeolite + $CdCl_2$ 0.35 treated group	0.91 ± .037^{cd}	58.00 ± 13.0^{cd}
Zeolite + $CdCl_2$ 0.7 treated group	0.72 ± 0.09^{e}	58.50 ± 2.38^{cd}
Zeolite + $CdCl_2$ 1.4 treated group	0.85 ± 0.12^{d}	60.75 ± 3.59^{c}

Means within the same column in each category carrying different litters are significantly different at ($P \leq 0.05$).

Cd bioaccumulation:

The Cd residue in gills of fish was increased significantly ia all treated groups of cadmium when comared to the control group. Addition of zeolite to the Cd polluted media reduced the Cd level in gills of Nile tilapia as compared to all concentration of Cd alone (Table 8).

Table 8. The cadmium residue in gills of Nile tilapia *(O. niloticus*) exposed to sublethal dose of cadmium alone or combined with zeolite

Groups	Residus (mg/g)
Control	0.095 ± .005^{e}
$CdCl_2$ 0.17 treated group	2.010 ± .011^{bcd}
$CdCl_2$ 0.35 treated group	2.380 ± .008^{bc}
$CdCl_2$ 0.7 treated group	2.735 ± .363^{b}
$CdCl_2$ 1.4 treated group	5.480 ± 1.35^{a}
Zeolite+ $CdCl_2$ 0.17 treated group	1.270 ± .008^{d}
Zeolite + $CdCl_2$ 0.3 5 treated group	1.830 ± .008^{cd}
Zeolite + $CdCl_2$ 0.7 treated group	1.850 ± .184^{cd}
Zeolite + $CdCl_2$ 1.4 treated group	2.710 ± .230^{b}

Means within the same column in each category carrying different litters are significantly different at ($P \leq 0.05$).

DISCUSSION

Cadmium is a highly toxic heavy metal, since it causes deleterious effects in organisms at low levels of exposure (Cope *et al.*, 1994). The present study demonstrates the effect of sub-lethal Cd exposure for 90 days on several pollution indicative biomarker enzymes and the possible protective effects of zeolite material in *O. niloticus*. Serum biomarkers such as ALT, AST, urea and creatinine have been used to detect cellular damage in liver and kidneys, and measure the responses to metals (Yang and Chen, 2003) It was emphasized that their measurement can be useful as a diagnostic tool in fish toxicology to identify their general health status and target organs affected by toxicants (McDonald and Grosell, 2006*)*.

The present study reveals that the *O. niloticus* exposed to Cd exhibited a significant reduction in their Hb and Hct in comparison with control. These results are in agreement with Gill and Epple (1993). And with data obtained by Mekkawy *et al.* (2011), who reported that fish exposed to 4.64 mg Cd/l showed a significant decrease in their RBC, Hb and &Hct in *O. niloticus* for 15 and 30 day. Also (Shalaby, 2007) found that similar results for *O.niloticus* after exposed to 10 ppm Cd for 15 and 45 days .The reduction in these parameters at sub lethal levels of cadmium might be due to the destruction of mature RBC and the inhibition of erythrocytes production due to acute hemolytic crisis which intern results in severe anemia in most vertebrates exposed to various environmental contaminant (Khadre, 1988*)*

Metal exposure induces alternation in hematological parameter, generally because of change in blood water content. Both haemoconcentration and haemodilution have been described in different experiments. It is accepted that muscle is the water exchange tissue with blood (Waiwood, 1980). Anaemia caused by metal poisoning in fish are diagnosed by low haemoglobin content ,reduced haematocrite value and or decreased number of erythrocytes (Sorensen and Baure, 1983). Combination of 8 gm zeolite/l with sub lethal of cadmium (0.17, 0.35 and 0.7mg/l) treated group eludicate non significant decrease in

hemoglobin level when compared with control group. However, zeolite plus 1.4 mg/l Cd showed significant decreased in hemoglobin so, zeolite increased and improved the hemoglobin content to Nile tilapia but non significant as compared with control group. In groups treated with 0.17 and 0.35 mg/lCd eludicate significant increase in Hct level in blood of Nile tilapia compared with control group, but in high concentrations (0.7 and1.47mg/l Cd) shows non significant increase. The reduction of toxic elements like cadmium in aquatic environments is needed by any acceptable method and is primary challenge of waste water treatment. The most widely used technique for removal of toxic elements involves the process of neutralization and metal hydroxide precipitation (Himesh and Mahadevaswamy, 1994). According to (Mariora *et al*., 2010) Zeolite administration in cadmium contaminated reduces the hemoglobin and by addition the concentration 4gm/l of Z, Iron level in tissues return to value recorded in control group.

The present study shows that the total protein and albumin in the Nile tilapia was significantly decreased after exposure to sublethal dose of cadmium, these results agree with those of (Shalaby, 1997), who attributed these decreases in plasma total protein may be attributed to the great demands and cellular damage occurred in the tissues of fish exposed to Cd. It may be possible cause of increase protein breakdown. The inhibition of protein synthesis may caused lowering of protein content in fish tissues exposed to any heavy metal or may reflect the disturbances that occurred in the protein metabolism due to the presence of toxicant. (Mukherjee and Sinha, 1993), who reported that sublethal concentration of cadmium chloride caused decrease in muscle total protein of the major carp. Measurement of albumin , globulin , and total protein in plasma is of considerable diagnostic value in fish as it relates to general nutritional status (Schaperclaus and Kulow, 1992), or may be due to the disturbances in the liver protein metabolism due to cd toxicity , as was found to be the case with other contaminants (Abdel-Tawwab *et al*., 2007) Addition of zeolite to Cd toxitcity improved plasma total protein of Nile tilapia than that of cadmium alone, but still lower than that of control group ($p<0.05$). While,

the combination between Cd+zeolite eludicate non significant change in albumin levels as compared with control group. On the other hand, this level was decreased significantly in fish exposed to high dose of Cd with zeolite (1.4mg/l cd +8 gm/l of Z). Zeolite is a frame work of sodium aluminium silicate and the valuable property of the substance is to exchange its sodium for cations like Ca, Mg, Cu, Cd and other divalent in water. The complete removal or reduction of toxic elements in polluted environments is outmost importance (Boyd 1990 and James *et al*., 1998). According to (James and Sampath, 1999) shows the addition of zeolite to cadmium comtaminated media, significantly reduced ($p<0.05$) the metal level in the water and helped to eliminate metal from the fish body, which in turn improved the biochemical parameters as compared to fish exposed cadmium alone. The protein is an important component to stimulate growth in fish and the linear increase of protein content with rearing time in fish exposed to Cd plus Zeolite confirmed the increase of growth rate over those exposed to Cd alone and the results showed that all the tested biochemical parameters were improved.

Blood glucose is sensitive and reliable indicator of pollutant, causing environmental stress in fish. Our results agree with (Shalaby, 2007) In the present study, glucose and plasma lipids were significantly increased during cd exposure. Mukherjee and Sinha (1993) found that the glucose concentration was significantly increased in cadmium treated major carp. The increase of glucose and lactate are the usual responses after action of most stressors (Sivio and Oikari, 1976) .In addition, the expected increase of glucose as a secondary response of stress (Chapatwala *et al*., 1980). The present study shows that, the combination between cadmium and zeolite lead to decrease in glucose level as compared with treated groups with cadmium only. Zeolite afforded non significant change in glucose level as compared with control group. Zeolite is cheaper, no side effects, and is more suitable than EDTA, NTA and hence it may be considered as best chemical agent to remove toxic elements from polluted environment (James and Sampath, 1999).

In the present study, performing blood chemistry analyses often provide vital information aiding the diagnosis for health assessment and management of cultured fish (Abdel- tawwab *et al.,* 2007).

In present study, there were significant changes in AST and ALT activities in plasma of *O. niloticus* exposed to Cd for 90 days. The increased levels of AST and ALT in blood plasma indicate impairment or hepatocellular damage and cellular degradation caused by Cd toxicity, probably in the liver, heart, muscle and plsma (Mukhopadhyay *et al*., 1982 and Yamawaki *et al.,* 1986).

In the addition of zeolite to cadmium chloride shows non significant change in ALT in all concentrations (0.17, 0.35, 0.7 and 1.4mg/L) as compared with control group. The combination of zeolite and cadmium chloride eludicate significant decrease in AST level as compared with Cd treated groups only while they also, afforded a non significant increase in AST level as compared with normal control group . According to (James and Sampath, 1999).

The addition of zeolite to cadmium contaminated media, significantly reduced metal level in the water and helped to eliminate metal from the fish body, which in turn improved the biochemical parameters as compared to fish exposed to cadmium alone. (Hikmet and Mehmet, 2013).

The present study also shows a significantly decreased in ALP, they agree with those of Ibrahim *et al*. (1991) who concluded that the decrease in enzyme activity in liver may be due to hepatocellular damage. On the other hand, alkaline phosphatase activity in liver and brains has been reported to decrease after treatment with Cu, Mn, Zn and other metals (Hegazy, 1991) .In this study, the combination between cadmium and zeolite shows non significant change in all concentrations (0.17, 0.35, 0.7 and 1.4mg/l) on ALP as compared with control group. James and Sampath (1999) shows that, the addition of zeolite to cadmium contaminated media, improved the biochemical parameters as compared to fish exposed to cadmium alone.

The cholesrol and triglycerides are significantly increased after exposure to cadmium. This increase in total lipids in plasma and liver may occurred as a result of oil droplets existed in the liver tissue with fragment of necrotic cell. This accumulation in fat may be either originated from destruction of liver cells increased mobilization and transportation of fat to the blood (Shalaby, 1997). The addition of zeolite to cadmium chloride to sub lethal concentrations (0.17, 0.35 and 0.7 mg/ L) and high concentration (1.4mg) elucidate non significant change in cholestrol level as compared with control group but less intense as compared with control group, and also in triglycerides the combination between cadmium and zeolite elucidate non significant change in all concentrations (0.17, 0.35, 0.7 and 1.4mg/l CdCl2) as compared with control group but triglycerides level in zeolite with cadmium is less than groups which treated with cadmium only .According to (James and Sampath, 1999), concluded that the addition of zeolite to cadmium contaminated media, improved the biochemical parameters as compared to fish exposed to cadmium only.

Creatinine and uric acid showed significant increase in fish exposed to Cd. These results may be due to the action of heavy metal on glomeruli filtration (Abbass *et al.*, 2002) and , or Cd may cause pathological changes to the kidney resulting in dysfunction. Addition of 8gm zeolite /l to cadmium afforded significant change in sub lethal concentrations (0.17, 0.35 and 0.7mg/l) as compared to control group. Also, in the combination of zeolite to cadmium chloride shows a non significant change in urea level as compared to control group but less intense as compared with cadmium only.

This study agree with Rashed (2001) who reported that the liver accumulate and concentrate high concentrations of cadmium. Velkova-Jordanoska and Kostoski (2005) recorded high levels of cadmium in the liver of fish accompanied by histological. Fish exposed to pollutants will have several pathological alternation can be used as biomarker to be able to detect cellular change in the affected organism. Norey *et al.*, (1990) examined the distribution .of cadmium within the three major cadmium sequestering organ

(liver, kidney and gills) in salmo gardner and stone loach .They found that the gills were the primary organ of accumulation of cadmium in rainbow trout.

Shalaby (1997) concluded that, the highest cadmium residue was detected in kidney, then liver, then gills and lowest in the muscle and plasma. In the present the addition of zeolite to cadmium chloride elucidate significant decrease in Cd residue in gills compared to all concentration of cadmium group. Also the effect is less intense compared with cadmium only. James and Sampath, (1999) reported that, Zeolite improving of feed conversion ratio and keeping animals healthy and reduce the cadmium bioaccumulation and concluded that, Zeolite addition in concentration of 0.5, 2.0 and 4.0 g/l respectively in water allowed reduction of metal bioaccumulation in gills when treated with 5gm/l z. According to James and Sampath (1999) Zeolite addition reduce Cd accumulation, this explained that: Cd and Z could interact in the aqueous medium as follows: Zeolite has extra frame work ions Na, and frame work ions (Al and Si), which are easily negligible. There is possible that Cd ions binding Na extra frame work ions from zeolite and this Cd wil be immobilized so Cd is reduced in this way. The Zeolite administration afforded significant ameliorative effects on nearly all studied parameters.

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تأثير االزيوليت ضد سمية كلوريد الكادميوم فى اسماك البلطى النيلى (*O. niloticus*)

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الملخـص العربـى

اجرى هذا البحث لدراسة تأثير تركيزات مختلفة عنصر الكادميوم ومعالجته باضافة 8 جرام زوليت /لتر فى الوسط المائى على بعض التغيرات الفسيولوجية البيوكيميائية والمدى التراكمى لهذا العنصر فى انسجة أسماك البلطى النيلى وزن (35– 40جم). وتم توزيع الأسماك فى تسعة معاملات حيث تركت المجموعة الأولى (المجموعة الضابطة بدون اضافات) وتعرضت المجموعة الثانية والثالثة والرابعة والخامسة الى 0.17، 0.35، 0.7، 1.4 مجم الكادميوم/ لتر على التوالى وكذلك تعرضت المجموعات السادسة والسابعة الثامنة والتاسعة الى نفس التركيزات السابقة مع اضافة 8 جرام من الزوليت /لترعلى التوالى واستمرت التجربة 90 يوما. وأسفرت النتائج عن الأتى:

1–حدوث نقص معنوى فى معدلات الدم (والهيموجلوبين والهياتوكريت فى البلطى النيلى بعد تعرضة لتركيزات المختلفة من الكادميوم فقط. بينما اضافة 8 جرام من الوليت الى هذا الوسط الملوث بالكادميوم ادى الى تحسن معدلات الدم السابقة فى هذة المجموعات.

2– لوحظ نقص المحتوى البروتينى والليبيومين فى دم الأسماك التى تعرضت لجرعات التحت مميتة لعنصر الكاميوم منفردا بينما حدث تحسين فى محتوى البروتين والليبيومين بالمعاملات التى تم اضافة الزوليت للمجموعات الملوثة لعنصر الكادميوم.

3– كما لوحظ زيادة نسبة سكر والدهون والكوليسترول باللاسماك المعرضة الى جميع المعملات الملوثة بالكادميوم بينما أظهرت المعالجة بالزيوليت مع المجموعات الملوثة بالكادميوم الى نقصا فى مستوى السكر والدهون الثلاثية والكوليسترول بالأسماك.

4– وجد زيادة معنوية بانزيم (AST)، (ALT) بالأسماك المعرضة للكاميوم. ونقص معنوى فى الألكالين فوسفاتير (ALP) بالمقارنة بالمجموعة الضابطة. بينما حدث المجموعات المعالجة

بالزيوليت نقصا معنويا فى انزيم (AST)> (ALT) اما بالنسبه لانزيم (ALP) فقد اظهرت المجموعات المعالجه بالزيوليت الى زيادة .كما وجد زيادة معنوية نسبة الكرياتينين وبولينا الدم بالمجموعات المعرضة للكادميوم عند مقارنتها بالمجموعة الضابطة بينما أظهرت المجموعات المعالجة بالزيوليت. زيادة غير معنزية فى الكرياتنيين أوالبولينا وهى زيادة طبيعية وقد أدت أيضا الى تحسن فى وظائف الكلى.

5- لوحظ عند تلوث الأسماك بجرعات تحت مميتة من عنصر الكادميوم حدوث زيادة معنوية بالمدى التراكمى لعنصر الكاميوم بالخياشيم الأسماك الملوثة عند مقارنتها بالمجموعة الضابطة. بينما حدث انخافض معنوى للمدى التراكمى للكادميوم بخياشيم الأسماك التى تم معالجتها بالزيوليت.

أوضحت النتائج حدوث تحسن ملحوظ فى التغيرات الفسولوجية والبيوكيميائية التى حدثت فى مكونات الدم وانخفاض المدى التراكمى لكادميوم بالأنسجة عندما تم اضافة زوليت الى الوسط المائى الملوث بالكادميوم. مما سبق نستخلص ان اضافة 8 جرام/لتر من الزوليت الى الوسط المائى الملوث بالكادميوم يؤدى الى ازالة هذا الملوث من المياة وبذلك تكون هذة الأسماك امنة صحيا. ولذا نوصى باضفة 8جرام زيوليت /لترماء بالمياة الصرف الزراعى المستخدمة لتربية السمااك لحمايتها من التلوث.