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EFFECT OF EDTA ON SOME HEAVY METALS IN SEWAGE WASTE WATER USED IN AQUACULTURE OF *OREOCHROMIS NILOTICUS*

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Abstract

The effect of EDTA " Ethylendiaminetetraacetic acid" in reducing the soluble heavy metals (Fe, Mn, Zn, Cu and Pb)contents in sewage waste water and muscles of Nile tilapia (*Oreochromis niloticus*) was studied in an investigation for 90 days period. The work was carried out at Central Laboratory For Aquaculture Research (CLAR) Abbassa, Abu-Hammad, Sharkia Governorate). A total of 315 Nile tilapia fingerlings (20±2g weight) were divided randomly into 21 aquaria, 15 fish for each representing 7 treatment groups in triplicates. Results showed that, all fish in treatment containing the highest EDTA levels 0.750 g/l died after 3 days of the experimental start. The heavy metal contents sewage wastewater, and in fish muscles declined with each increase in EDTA concentration. The chelating agent of EDTA increased with decreasing of heavy metals in water.

INTRODUCTION

The indiscriminate discharge of industrial effluents, raw sewage and other waste pollute most environments and affect the survival and physiological activities of target organisms. Metals and pesticides, in particular, have a tendency to accumulate and undergo food chain magnification (Vinikour *et al.,* 1980). They cloud also cause catastrophic diseases like minamata and itai-itai, (James, 2000). Some of the probably affected organisms, like fish, are consumed by human beings. Hence, reduction of toxic elements in aquatic environments is one of the primary challenges in waste water treatment. Unfortunately in some regions of Egypt, where there is a shortage in fresh water, fish breeders in some private farms use untreated sewage wastewater for fish culture. This water may pose a potential health risk to handlers and consumers of such fish (Lawton and Morse, 1980). For this reason, the using of effluent for aquaculture has not yet been approved by health authorities. According to Sandbank and Nupen

(1984), the largest problem regarding aquaculture in wastewater effluent is the accumulation of heavy metals, pathogens and pesticides in the fish and as a result, the possible transmission of diseases to man.

Synthetic compounds like ethylenediaminetetraacetic acid (EDTA) are known to be effective chelating agents of heavy metals. Shaker *et al.* (2000) found that copper in fish livers started declining in this organ with each increase in EDTA concentration up to 0.750 g/L water. Also, they found that EDTA up to 0.250 g/L decreased the copper content in the muscles to its permissible level. The most widely used techniques for removal of heavy metals involve the process of neutralization and metalhydroxide precipitation (Himesh and Mahadevaswamy, 1994).

The present work was designed to study the effect of the chelating agent EDTA on the reduction of some heavy metals (Fe, Mn, Zn, Cu and Pb) in sewage wastewater and fish muscles as well as improvement of growth rate in *Oreochromis niloticus*.

MATERIALS AND METHODS

Experimental fish, *Oreochromis niloticus* fingerlings were transported from Abbassa fish hatchery to the Central Laboratory For Aquaculture Research (CLAR). Fish were stocked in fiber glass tank and acclimatized for the new water at the experimental site for three weeks. Thereafter, *O. niloticus* fingerlings were divided randomly into 21 glass aquaria (60 x 35 x 45 Cm), 15 fish each, to represent 7 groups with three replicates each. The average fish weight at the experimental start was 20±2g. The sewage wastewater was located in a private drain at Bahr El-Bakar drain Shader Azzam, Port Saaed Governorate, and transported to the Central Laboratory for Aquaculture Research (CLAR). The treatments applied were wastewater without EDTA, at levels of 0.00, 0.125, 0.250, 0.375, 0.500, 0.625 and 0.750 g/l (Table, 1).

Table 1. Experimental groups and their notation.

Group No.	Notation
1	Sewage wastewater without EDTA control
2	Wastewater + (0.125 g/l EDTA)
3	Wastewater + (0.250 g/l EDTA)
4	Wastewater + (0.375 g/l EDTA)
5	Wastewater + (0.500 g/l EDTA)
6	Wastewater + (0.625 g/l EDTA)
7	Wastewater + (0.750 g/l EDTA)

Water analysis

pH value was determined using pH meter model Corrning, 345.

Temperature and dissolved oxygen (DO) were measured using oxygen meter model YSI 57.

Salinity was measured by conductivity meter model YSI 33 S.C.T. meter.

Ammonia was measured by Hack Comparison Apparatus No. 1954 (A.P.H.A., 1993).

Total alkalinity was measured according to A.P.H.A. (1993).

Total hardness was measured by titration with standard solution of EDTA according to A.P.H.A. 1993.

The heavy metals (Fe, Mn, Zn, Cu and Pb) concentrations in water were estimated following the method of A.P.H.A. (1993).

Fish analysis

Heavy metals (Fe, Mn, Zn, Cu and Pb) content in muscle of fish were estimated at 0, 7, 15, 30, 60 and 90 days. Three replicates of samples were digested with a mixture of concentrated nitric acid and perchloric acid at the ratio of 1:2 until the formation of white residue at 100 ºC in a water bath. The cooled residue was dissolved completely by adding 1N HCl and made up to 25 ml with distilled water (FAO 1975).

Statistical Analysis

Data were analyzed using ANOVA and Duncan's test at a probability level of <0.05 according to SAS (1987).

RESULTS AND DISCUSSION

The present study revealed that the addition of EDTA to the sewage wastewater significantly ($p<0.05$) reduced the heavy metals in water and their uptake in fish tissues as compared to tilapia exposed to sewage water without EDTA. In Table 2, at the end of the experiment, the concentrations of iron were 4.22, 3.75, 3.21, 2.63, 2.25,1.77 and 1.5 ppm for sewage water $group_1$, $group_2$, $group_3$, $group_4$, $group_5$, $group_6$ and $group_7$, respectively. These results revealed that iron contents in sewage water gradually decreased as the concentration of EDTA increased which reflects the efficiency of this agent in chelating the iron in water. These results supported those obtained by Shaker *et al.* (2000), who found that EDTA reduced the copper in water and fish.

The highest reduction of iron was in the 7th group. The percentages of reduction were 11.3, 24.05, 37.8, 46.8, 58.0 and 64.45% for groups 2, 3, 4, 5, 6 and 7, respectively.

On the other hand, the concentration of manganese were 0.55, 0.43, 0.34, 0.26, 0.17, 0.08 and 0.06 ppm for the sewage water for groups 1, 2 , 3, 4, 5, 6 and 7, respectively. The reduction percentage were 22.5, 37.8, 53.6, 69.5, 85.8 and 89.1% for groups 2, 3, 4, 5, 6 and 7, respectively.

It was clear that the reduction percentage in manganese was comparatively higher than in iron. These results agreed with those obtained by James and Sampath (1997), who reported that the reduction of heavy metals by chelating agent EDTA was highest in lower heavy metal concentration than in higher concentration.

The concentration of Zn in sewage wastewater was 0.6 ppm, while, after adding EDTA, it was 0.5, 0.39, 0.27, 0.18, 0.09 and 0.08 ppm for groups 2, 3, 4, 5, 6 and 7, respectively. The reduction of Zn, Cu and Pb in water followed the same pattern with the results obtained by Sorvari and Sillanpää (1996) who reported that the complexion by either EDTA or DTPA resulted in significant toxicity decreases with most of studied metals "Fe, Cu, Mn, Zn, Cd and Hg".

The collected data for water quality during the study are summarized in Table 2. Water temperature was almost stable at 29.9 to 30.1°C in all groups during the study.

Also, dissolved oxygen, total hardness and salinity did not differ significantly between all groups during this study. pH and Total alkalinity decreased as EDTA levels increased. These results may be due to the acidic effect for EDTA on water. The death of all fish in treatment "EDTA7" referred to the increased acidity in these treatments " pH 5.1". These results are in agreement with those obtained by Shaker *et al.* (2000). Also, similar trend was observed in NH_3; the concentration of NH_3 gradually decreased with increased EDTA levels.

Results presented in Tables (3 - 7) showed that, Fe, Mn, Zn, Cu and pb contents in fish muscles at all periods tested after experimental start, decreased significantly ($P<0.05$) with each increase in the level of EDTA.

The iron content in fish muscles (Table 3) at all periods tested after experiments started to decline with the increase in EDTA concentration up to 0.625 g/l water. The statistical analysis revealed, also, that the decrease in iron uptake found in most all periods was significant ($P<0.05$). These results are in agreement with results obtained by

James *et al.* (1998), who showed that addition of chelating agent to sub lethal levels of cadmium significantly reduced the retention of cadmium in body tissues, and this indirectly improved the growth of catfish. Also, similar manners were observed in Mn, Zn, Cu and pb (Tables 4, 5, 6 and 7). The uptake of Mn in muscles of *O. niloticus* (Table 4) exposed to sewage wastewater without EDTA was 0.198 mg/g wet tissues, and it significantly ($P<0.05$) declined to 0.114, 0.092, 0.072, 0.056 and 0.044 mg/g wet tissues for $EDTA_1$, $EDTA_2$, $EDTA_3$, $EDTA_4$ and $EDTA_5$, respectively.

Concerning zinc, it was clear that a more or less similar pattern of the previous metals was followed. In sewage wastewater without EDTA treatment, zinc concentration in muscles was 0.265 mg/g wet tissues, while, in fish muscles after experiment in other treatments it was 0.144, 0.114, 0.102, 0.084 and 0.076 mg/g for the same groups, respectively.

Also, copper in the first treatment after study was 0.545mg/g, but in other treatments, its concentration was reduced by EDTA agent to 0.382, 0.314, 0.224, 0.176 and 0.149 mg/g wet tissues. Lead concentration in sewage wastewater without EDTA was 0.084mg/g, and in other treatments it was 0.063, 0.049, 0.033, 0.009 and 0.007mg/g wet tissues for $EDTA_1$, $EDTA_2$, $EDTA_3$, $EDTA_4$ and $EDTA_5$, respectively. These results are in agreement with those obtained by James (2000).

Overall, heavy metals contents in fish muscles decreased significantly ($p<0.05$) with each increase in the level of EDTA. These results revealed that chelating agent of EDTA increased with decreasing of heavy metals in water. The accumulation rates of metals in fish in this study were in the order Fe>Pb > Cu> Zn> Mn. These results are in agreement with those obtained by Srinivas (1993) who found that chelated metal was less toxic than that of their ionic forms. Also, he found that the chelating agent of EDTA increased with decrease of heavy metals concentration in water.

Generally, results of Tables 3 to 7 showed that EDTA up to 0.375 g/l decreased the heavy metal contents in the muscles to the saved permissible level of these metals in fish muscles according to World Halth Organization (WHO, 1984) and United States Environmental Protection Agency (USEPA, 1986). They reported that the permissible levels of Fe, Mn, Zn, Cu and Pb in fish muscles are 30.0, 6.5, 50.0, 20.0 and 2.0 ppm, respectively.

Table 2. Effect of EDTA on Heavy metals concentrations (ppm) in water and water quality.

Items \ Treatments	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Temp. °C	30.0±1.5a	30.1±2.0a	30.0±1.0a	29.9±1.0a	30.0±1.0a	30.0±1.5a	30.0±1.0a
D.O. mg/l	7.4±0.3a	7.5±0.2a	7.6±0.3a	7.7±0.2a	7.7±0.2a	7.7±0.1a	7.4±0.2a
PH	8.9±0.1a	8.3±0.2a	7.9±0.2a	7.5±0.2b	7.0±0.2b	6.6±0.1b	5.1±0.1c
NH3 mg/l	0.94±0.02a	0.76±0.04a	0.55±0.01b	0.45±0.02b	0.42±0.06b	0.38±0.01b	0.33±0.01b
Total alk.mg/l	324.0±16.0a	291.0±11.0a	270.0±8.0a	206.0±6.0b	182.0±7.0b	166.0±5.0b	96±3.6c
T.H. mg/l	674.0±21.0a	694.0±18.0a	714.0±14.0a	735.0±19.0a	750.0±16.0a	750.0±12.0a	766±8.0a
Sal. mg/l	2.42±0.01a	2.45±0.0a	2.5±0.02a	2.55±0.04a	2.6±0.04a	2.6±0.04a	2.65±0.05a
Fe. ppm	4.22±0.14a	3.75±0.076b	3.21±0.088c	2.63±0.024d	2.25±0.02e	1.77±0.03f	1.5±0.06f
Mn ppm	0.55±0.06a	0.43±0.009b	0.34±0.02c	0.26±0.01d	0.17±0.004e	0.08±0.002f	0.06±0.002f
Zn ppm	0.6±0.024a	0.5±0.018b	0.39±0.034c	0.27±0.004d	0.18±0.024e	0.09±0.016f	0.08±0.001f
Cu ppm	0.86±0.028a	0.73±0.038b	0.56±0.021c	0.32±0.008d	0.27±0.004e	0.16±0.014f	0.11±0.01f
Pb ppm	1.28±0.056a	1.09±0.072b	0.91±0.04c	0.71±0.004d	0.52±0.008e	0.36±0.012f	0.28±0.01f

a-f Means within a raw with the same superscript are significantly different ($P<0.05$).

Table 3. Effect of EDTA on Iron (Fe) content in muscles of *O. niloticus* as mg/g wet tissue.

Treatments		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Days	0	0.077±0.002a	0.077±0.002a	0.077±0.002a	0.077±0.002a	0.077±0.002a	0.077±0.002a
	7	0.545±0.019a	0.455±0.024b	0.409±0.022b	0.370±0.016c	0.272±0.012d	0.266±0.001d
	15	0.946±0.054a	0.776±0.036b	0.701±0.024c	0.612±0.012d	0.473±0.012e	0.465±0.012e
	30	1.256±0.046a	1.017±0.03b	0.942±0.03b	0.754±0.02c	0.628±0.016d	0.618±0.012d
	60	1.595±0.038a	1.276±0.028b	1.148±0.024b	0.957±0.01c	0.76±0.008d	0.74±0.008d
	90	1.975±0.026a	1.521±0.022b	1.383±0.018c	1.145±0.021d	0.908±0.014e	0.880±0.008e

a-e Means within a raw with the same superscript are significantly different ($P<0.05$).

Table 4. Effect of EDTA on Manganese (Mn) content in muscles of *O. niloticus* as mg/g wet tissue.

Treatments		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Days	0	0.015±0.001a	0.015±0.001a	0.015±0.001a	0.015±0.001a	0.015±0.001a	0.015±0.001a
	7	0.052±0.001a	0.034±0.002b	0.028±0.002b	0.02±0.001c	0.018±0.001c	0.016±0.001c
	15	0.084±0.005a	0.045±0.004b	0.037±0.002b	0.029±0.002c	0.024±0.001c	0.021±0.001c
	30	0.114±0.003a	0.068±0.003b	0.055±0.003c	0.038±0.001d	0.031±0.002d	0.028±0.002d
	60	0.159±0.007a	0.099±0.008b	0.074±0.007c	0.056±0.004c	0.042±0.001d	0.035±0.003d
	90	0.198±0.005a	0.114±0.007b	0.092±0.005c	0.072±0.001d	0.056±0.001e	0.044±0.002e

[a-e] Means within a raw with the same superscript are significantly different ($P<0.05$).

Table 5. Effect of EDTA on Zinc (Zn) content in muscles of *O. niloticus* as mg/g wet tissue.

Treatments		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Days	0	0.023±0.001a	0.023±0.001a	0.023±0.001a	0.023±0.001a	0.023±0.001a	0.023±0.001a
	7	0.051±0.006a	0.042±0.005b	0.039±0.001b	0.031±0.001b	0.027±0.002c	0.024±0.002c
	15	0.084±0.004a	0.064±0.009b	0.056±0.007c	0.046±0.002d	0.035±0.002de	0.0.3±0.002e
	30	.126±0.009a	0.086±0.004b	0.074±0.003c	0.065±0.002cd	0.052±0.002e	0.049±0.004e
	60	0.095±0.01a	0.112±0.01b	0.098±0.009c	0.082±0.007d	0.070±0.007e	0.064±0.002e
	90	0.265±0.015a	0.144±0.008b	0.114±0.012c	0.102±0.009d	0.084±0.005e	0.076±0.006e

[a-e] Means within a raw with the same superscript are significantly different ($P<0.05$).

Table 6. Effect of EDTA on Copper (Cu) content in muscles of *O. niloticus* as mg/g wet tissue.

Treatments		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Days	0	0.065±0.002a	0.067±0.002a	0.066±0.002a	0.065±0.002a	0.066±0.002a	0.066±0.002a
	7	0.195±0.025a	0.166±0.014b	0.142±0.01c	0.12±0.012d	0.088±0.008e	0.078±0.009e
	15	0.254±0.028a	0.203±0.022b	0.176±0.022c	0.148±0.022d	0.099±0.014e	0.089±0.016e
	30	0.396±0.018a	0.297±0.024b	0.204±0.026c	0.172±0.012d	0.124±0.007e	0.112±0.002e
	60	0.465±0.008a	0.326±0.022b	0.275±0.009c	0.196±0.008d	0.144±0.012e	0.128±0.002e
	90	0.545±0.006a	0.382±0.008b	0.314±0.004c	0.224±0.005d	0.176±0.002e	0.149±0.003±f

[a-f] Means within a raw with the same superscript are significantly different ($P<0.05$).

Table 7. Effect of EDTA on Lead (Pb) content in muscles of *O. niloticus* as mg/g wet tissue.

Treatments		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Days	0	0.001±0.0a	0.001±0.0a	0.001±0.0a	0.001±0.0a	0.001±0.0a	0.001±0.0a
	7	0.008±0.0a	0.006±0.0b	0.005±0.0c	0.004±0.0d	0.003±0.0e	0.001±0.0e
	15	0.019±0.001a	0.011±0.0b	0.009±0.001c	0.007±0.001d	0.004±0.001e	0.002±0.001e
	30	0.036±0.001a	0.021±0.002b	0.014±0.001c	0.009±0.0d	0.005±0.0e	0.003±0.0e
	60	0.065±0.002a	0.042±0.001b	0.032±0.0c	0.018±0.001d	0.007±0.0e	0.005±0.001e
	90	0.084±0.005a	0.063±0.002b	0.049±0.001c	0.33±0.002d	0.009±0.001e	0.007±0.0e

[a-e] Means within a raw with the same superscript are significantly different ($P<0.05$).

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تأثير الإديتا على بعض العناصر الثقيلة فى مياه الصرف الصحى المستخدمة فى إستزراع البلطى النيلى

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اجريت هذه الدراسه فى المعمل المركزى لبحوث الثروه السمكيه بالعباسه- محافظة الشرقيه. استخدم فيها عدد ٢١ حوضاً زجاجياً مقاس (٧٠ ×٤٥ ×٥٠سم) لكل منها تهويته الخاصه ووضع فى كل حوض زجاجى عدد ١٥ سمكه بلطى نيلى بمتوسط وزن ٢٠ ×٢ جم وقسمت الأحواض الى عدد ٧ معاملات لكل منها ثلاثة مكرراً وفيها استخدمت مياه صرف صحى منقوله من مصرف بحر البقر منطقة شادر عزام – محافظة بور سعيد. وكانت المعامله الاولى مياه صرف صحى بدون اضافة اديتا وباقى المعاملات كانت ١٢٥ . ٠، ٢٥٠ , ٠، ٣٧٥ , ٠، ٥٠٠ , ٠، ٦٢٥ , ٠، ٧٥٠ , ٠ جم/ لتر من ماء الصرف الصحى، وذلك بهدف دراسة تأثير استخدام الاديتا كمركب مخلبى على تقليل سمية العناصر الثقيلة الموجوده فى مياة الصرف الصحي وعلى سمكة البلطى النيلى. استمرت التجربه ٩٠ يوماً وتم أخذ عينات أسماك بصفه دوريه من كل معامله فى الأيام صفر، ٧، ١٥، ٣٠، ٦٠، ٩٠ يوماً لتقدير عناصر الحديد،المنجنيز، الزنك، النحاس والرصاص فى العضلات. وأشارت النتائج الى موت جميع الأسماك فى المعامله الأخيره (٧٥٠ . ٠ جم/لتر اديتا) بعد ثلاثة أيام. فى حين أن اضافة الاديتا أدى الى انخفاض تركيز العناصر الثقيلة المقدرة فى الماء وعضلات الأسماك. كما أن زيادة الاضافه من الاديتا أدت الى زيادة الانخفاض فى تركيز العناصر الثقيلة المقدرة فى الماء وعضلات الأسماك. بالاضافه الى ان نشاط الاديتا قد ظهر أكثر وضوحا فى التركيزات المنخفضه من العنصر عن المرتفعه والدليل على ذلك أن الانخفاض مع الحديد كان ٥٨٪ فقط بينما كان مع الرصاص ٧١٪ والنحاس ٨١,٩٪ والزنك ٨٥٪ والمنجنيز ٨٥,٨٪.