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## ROLE OF PALM-DATE PITS IN ENVIRONMENTAL PROTECTION OF *OREOCHROMIS NILOTICUS* FROM HEAVY METAL POLLUTANTS

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#### Abstract

Oreochromis niloticus exposed to a mixture of copper, lead and cadmium alone or in combination with either crushed raw palm-date pits or its chemically activated carbon for one month. The obtained results revealed that, heavy metals had adverse effects on health of exposed Oreochromis niloticus. Significant removal copper, lead of and cadmium concentrations onto the crushed raw palm-date pits and its chemically activated carbon from both water and exposed Oreochromis niloticus concomitant with amelioration of their adverse effects. The crushed raw palm-date pits was an inexpensive solid adsorbent compared to chemically activated one.

**Key words:** Palm-date pits - environmental protection – *Oreochromis niloticus* - heavy metal pollutants.

#### INTRODUCTION

Nile tilapia "*Oreochromis niloticus*" belongs to one of the most important groups of fish being recognized as good biological models due to its easy handling, culture and maintenance in the laboratory and for studying possible adaptations to pollutants in toxicological studies (Almeida *et al.*, 2002).

Heavy metals from man-made pollution sources are continually released into aquatic ecosystems. The contamination of heavy metals is a serious threat because of their toxicity, long persistence, bioaccumulation and biomagnification in the food chain (Yilmaz *et al.*, 2007). The impact

of heavy metals on aquatic environment affects directly or indirectly human health (NWQCU, 1995).

Industrial activities (e.g. mining, painting car manufacturing, metal plating and tanneries) and agricultural activities (intensively used fertilizers and fungicidal sprays) are the main sources of wastes containing heavy metals. Heavy metals are considered to be one of the most hazardous water contaminants. According to the WHO (2004 and 2006), among the most toxic metals are cadmium, chromium, copper, lead, mercury and nickel.

Date pits are a huge solid waste in Egypt and are of little or no economic value and in fact present a disposal problem. The quantity of date pits has been estimated at a million tons per year. Date pits have been used for the preparation of physically and chemically activated carbon(Awwad *et al.*, 2008).

Activated carbons represent a group of versatile adsorbents due to their extensive surface area, high degree of surface reactivity, and favorable pore size distribution. Chemical activation has become widely applied because of its lower activation temperature and high carbon product yield as compared to physical processing. The most commonly used chemical activation agent is  $H_3PO_4$ , but  $ZnCl_2$  is also used on a relatively small scale (Baker et al., 1992). From both economic and environmental perspectives, phosphoric acid is the preferred processing method (Hsisiheng *et al.*, 1998 and Diao *et al.*, 2002). In addition, activated carbon adsorption has proven to be a highly effective method to remove low concentration heavy metal ions from aqueous phase (Dastgheib and Rockstraw, 2001).

Therefore the aim of this study is directed to the use of crushed palm-date pits (raw and chemically activated) as adsorbent for minimizing some heavy metals pollutants (copper "Cu", lead "Pb" and cadmium "Cd") in water and exposed *Oreochromis niloticus* "*O. niloticus*".

#### **MATERIAL AND METHODS**

## Fish:

A total of 120 *O. niloticus* with an average body weight  $33\pm0.6g$  were obtained from Abbassa Fish Hatchery at Sharkia province. Fish were apparently healthy and free from any skin lesions or external parasites. They were maintained in glass aquaria (each, 80x40x30 cm capacity) with 80 liters of dechlorinated tap water in each aquarium. Fish were acclimatized to water environment for two weeks.

## Preparation of chemically activated carbon:

Palm-date pits were chemically prepared according to Al-Attas (2003). Characterization of crushed raw and chemically activated palm - date pits performance was carried out by measurement of iodine number according to ASTM D4607 (2006).

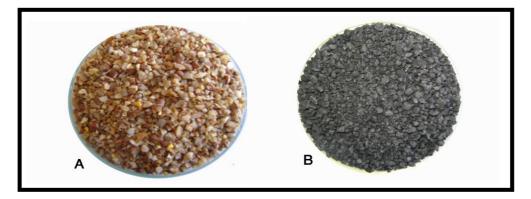


Fig. 1. Showed crushed raw palm-date pits (A) and crushed chemically activated palm-date pits (B).

## **Experimental design:**

Fish were divided into four equal groups, each consisting of two replicates (15 fish replicate <sup>-1</sup>). The first group was kept as control. The second, third and fourth groups were exposed to a mixture of metals solution containing  $1/_{20}$  72 hrs. LC<sub>50</sub> for copper sulfate (21.12 mg/L) and lead acetate (2.03 mg/L) according to Mohammed et al. (2008) and  $1/_{20}$  96 hrs.LC<sub>50</sub> for cadmium chloride (2.65 mg/L) according to Lopez (1995-1996). The third and fourth groups were treated with crushed raw and chemically activated carbon of palm-date pits respectively (Fig.1).

The amount of crushed raw palm-date pits or its chemically activated carbon was 5g./L of water and the adsorption time was 72hrs. according to Lyubchik *et al.*, (2008). The amount of crushed raw palm-date pits and its chemically activated carbon, metals mixture and water were changed every 72 hrs along one month.

Water temperature, PH and dissolved oxygen were adjusted to be  $23\pm1$  °C, 7.2 and  $5\pm0.4$  mg/ L respectively.

## Clinical examination of tested O. niloticus:

The behavioural responses, clinical signs and post-mortem changes were recorded according to Lucky (1977).

#### Sampling and metal analysis:

Crushed raw palm-date pits , chemically activated carbon of palmdate pits and water were collected every 72 hrs. While Samples from exposed *O. niloticus* (gills, liver and muscle) were collected at the end of the experiment. Fish and palm-date pits samples were stored at -20 °C for digestion and analysis (AOAC, 1995). Water samples were filtered and 0.3ml of nitric acid was added to each 100 ml and kept in refrigerator till analysis (APHA, 1998). Samples were analyzed by using Buck Scientific Atomic Absorption Spectrophotometer 210 VGP at Central Laboratory, Faculty of Veterinary Medicine, Zagazig University, Egypt.

## Statistical analysis:

Analysis of variance (ANOVA) and Duncan's multiple range test (Duncan, 1955) was used to determine the differences between treatments. The mean values were significant at the level of ( $P \le 0.05$ ). Standard errors of treatment – means, were estimated. All the statistical analysis were carried out by using SAS program (2005).

## **RESULTS AND DISCUSSION**

## 1- Performance of palm-date pits:

The efficiency and quality of the produced activated carbon from palm-date pits were characterized by measuring the iodine number. The iodine number of crushed raw and chemically activated carbon of palm-date pits was 329 mg g<sup>-1</sup> and 480 mg g<sup>-1</sup> respectively which nearly similar to that obtained by Haimour and Emeish (2006) for the chemical activated one. The differences of iodine number between raw and chemically activated carbon of palm –date pits may be attributed to treatment with phosphoric acid and high temperature for chemically activated one.

## 2- Clinical findings of tested O. niolticus:

Behavioural response and mortality of *O. niloticus* exposed to a mixture of metals (copper, lead and cadmium) and the effect of addition of crushed palm-date pits (raw and chemically activated) are depicted in Table 1 and the survivability was showed in Fig. 2. *O. niloticus* (2<sup>nd</sup> group) exposed to a mixture of metals only were not respond well to escape reflex, anorexia and darkening of the skin and pale gills and liver. The mortality rate was highest (56.7%) compared to other treated groups. These may be attributed to the adverse effects of heavy metals on aquatic

organisms ranges from slight reduction in growth rate and poor health to death (Joel and Amajuoyi, 2009). Copper lethality in freshwater fish has been related to disruption of osmoregulation at fish gills. In particular copper reduce sodium uptake and increase sodium loss at fish gills with death occurring because of decreased blood sodium concentration (Wood, 1992). Lead exposure reduced feeding ability (Weber *et al.*, 1991). Lead is a potent neurotoxicant including vascular and neuroglia injury, oxidative stress and failure of neuronal migration (De Gennaro, 2002). Cadmium exert a wide range of pathological effects like damage in gill structure (Thophon et al., 2003), disturbances in respiration (Chowdhury *et al.*, 2004), changes in blood parameters such as cortisol and glucose which reveal the stress response in fish (Lacroix and Hontela, 2004) and plasma ion regulation especially calcium balance (Chowdhury *et al.*, 2004).

*O. niloticus* exposed to a mixture of metals and treated with crushed raw palm-date pits ( $3^{rd}$  group) and chemically activated form ( $4^{th}$  group) were respond well to escape reflex with survivability  $^{\circ}6.7\%$  and 93.3% respectively. This may be due to the adsorption of metallic ions, on crushed raw palm-date pits and chemically activated form, which have an electrostatic nature, small size and frequently charged in the aqueous solution (Lopez-Ramon et al., 2002, Awwad *et al.*, 2008 and Al-Ghothi *et al.*, 2010). Consequently, the toxic effects of heavy metals on health of tested *O. niloticus* were ameliorated.

**Table 1.** Behaviour and mortality of *O. niloticus* exposed to a mixture of metals (Cu, Pb and Cd) after treatment with crushed raw palm-date pits and its chemically activated carbon after one month.

Group n=30	Treatment	Escape	Mort	tality	Survivability	
		reflex	No.	%	%	
1	Control	+	0	0.0	100	
2	*Metals mixture	-	17	56.7	43.3	
3	*Metals mixture + **Crushed raw palm-date pits	+	٧	۲3.3	۷6.7	
4	*Metals mixture + **Crushed chemically activated carbon of palm-date pits	+	2	6.7	93.3	

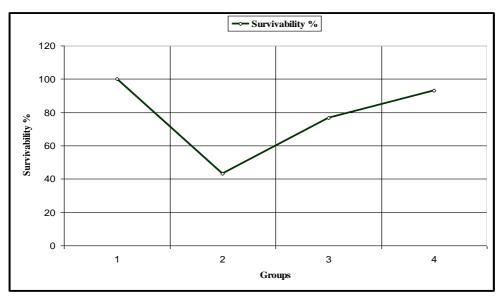
 $*^{1}/_{20}$  72 hrs. LC<sub>50</sub> for copper sulfate (21.12 mg/L) , lead acetate (2.03 mg/L) and

 $\frac{1}{20}$  96 hrs. LC<sub>50</sub> cadmium chloride (2.65 mg/L)

\*\* 5 g. crushed raw or chemically activated palm-date pits/ L of water.

+ The fish respond well to escape reflex.

-The fish not respond to escape reflex.



**Fig. 2**. Showed the survivability of *O. niloticus* exposed to a mixture of metals (Cu, Pb and Cd) after treatment with crushed raw palm-date pits and its chemically activated carbon after one month.

#### **3-** Metal concentrations:

Table 2 showed variation in the adsorption of each metal cations onto crushed raw palm-date pits and its chemically activated carbon, whereas the initial metal aqueous concentration was 21.12 mg/L  $\cdot$ 2.03mg/L and 2.65 mg/L for Cu, Pb and Cd respectively which significantly decreased after 72 hrs. post addition either crushed raw palm-date pits or its chemically activated carbon along the experimental period. This is concordant to that reported by Awwad et al.,(2008) for Pb<sup>2+</sup>, Cd<sup>2+</sup> and Fe<sup>3+</sup> and Al-Omair and El-Sharkawy (2007) for Pb<sup>2+</sup>, Cu<sup>2+</sup> and Cd<sup>2+</sup> using activated carbon from palm-date pits. Similarly a potential usefulness of raw palm-date pits adsorbent for Cu<sup>2+</sup> and Cd<sup>2+</sup> (Al-Ghothi *et al.*, .2010), Banat *et al.*, (2002) for Cu<sup>2+</sup> and Zn<sup>2+</sup> and Banat *et al.*, (2003a) for Cd<sup>2+</sup>.

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	Metal concentrations (mg/L)											
Groups	(1) *Control			(2) ** Metals mixture		(3) ** Metals mixture +			(4) ** Metals mixture +			
Samples						***crushed raw palm-date pits			<b>***</b> chemically activated carbon of palm-date pits			
	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd
Water: P.L+	$0.015 \pm 0.001$ <sup>d</sup>	$0.005 \pm 0.001^{\ d}$	$0.011 \pm 0.001$ d	21.234± 0.252 <sup>a</sup>	2.048± 0.005 <sup>a</sup>	2.672± 0.006 <sup>a</sup>	13.744± 0.506 <sup>b</sup>	1.462± 0.046 <sup>b</sup>	1.286± 0.043 <sup>b</sup>	8.816± 0.293 °	0.892± 0.023 <sup>c</sup>	0.988± 0.041 <sup>c</sup>
<b>Palm-date pits:</b> Raw-	$0.015 \pm 0.001$ c	$0.005 \pm 0.001$ c	$0.011 \pm 0.001$ c	-	-	-	7.302± 0.337 <sup>b</sup>	0.628± 0.035 <sup>b</sup>	1.524± 0.032 <sup>b</sup>	-	-	-
Chemically activated-	0.009± 0.003 <sup>c</sup>	0.003± 0.005 <sup>c</sup>	0.008± 0.005 <sup>c</sup>	-	-	-	-	-	-	12.990± 1.089 <sup>a</sup>	1.202± 0.026 <sup>a</sup>	1.730± 0.025 <sup>a</sup>
Fish: Gills- P.L++	$0.050 \pm 0.001^{\ \ d}$	$0.013 \pm 0.001^{\text{ d}}$	$0.041 \pm 0.017^{\ d}$	45.222± 1.257 <sup>a</sup>	5.426± 0.421 <sup>a</sup>	3.370± 0.044 <sup>a</sup>	36.140± 1.213 <sup>b</sup>	2.186± 0.019 <sup>b</sup>	1.892± 0.176 <sup>b</sup>	27.230± 0.364 °	1.090± 0.015 <sup>c</sup>	1.284± 0.035 °
Liver-	$0.077 \pm 0.001^{\ d}$	0.020± 0.001 <sup>d</sup>	0.044± 0.002 <sup>d</sup>	514.598± 2.158 <sup>a</sup>	13.354± 0.717 <sup>d</sup>	8.048± 0.565 <sup>a</sup>	481.964± 2.214 <sup>b</sup>	8.712± 0.236 <sup>b</sup>	3.090± 0.194 <sup>b</sup>	232.628± 2.785 °	4.754± 0.237 <sup>℃</sup>	2.138± 0.135 °
Muscle-	${}^{0.027\pm}_{0.001}{}^{\rm d}$	$\begin{array}{c} 0.011 \pm \\ 0.001 \ ^{\textbf{d}} \end{array}$	$0.014 \pm 0.002$ <sup>c</sup>	38.456± 0.451 <sup>a</sup>	1.986± 0.065 <sup>a</sup>	2.418± 0.230 <sup>a</sup>	27.036± 0.937 <sup>b</sup>	1.222± 0.059 <sup>в</sup>	1.192± 0.025 <sup>b</sup>	20.506± 0.924 °	0.886± 0.029 °	0.962± 0.024 <sup>b</sup>

**Table 2.** Metal concentrations (Means ± S.E.) of tested water, palm-date pits and O. niloticus exposed to a mixture of metals (Cu, Pb and Cd) and after treatment with crushed raw palm-date pits and its chemically activated carbon after one month.

\* pretested analysis.

 $**^{1}/_{20}$  72 hrs.  $LC_{50}$  for copper sulfate (21.12 mg/L), lead acetate (2.03 mg/L) and  $^{1}/_{20}$  96 hrs.  $LC_{50}$  cadmium chloride (2.65 mg/L).

\*\*\* 5 g. crushed raw or chemically activated palm-date pits/ L of water.

P.L+ = Permissible Limit (WHO,1990 & 1993) for Cu, Pb and Cd were 2.00, 0.50 and 0.70 ppm respectively.

P.L++ = Permissible Limit (WHO,1990 & 1993) for Cu, Pb and Cd were 20.00, 2.00 and 0.50 ppm respectively.

Means within the same row with different letters are significantly different at  $P \le 0.05$ .

The mechanism of heavy metals adsorption on raw palm-date pits is by breaking up of the large particle to form smaller one probably serves to open the sealed channels in the adsorbent which then becomes available for adsorption (Al-Gothi *et al.*, 2010). Adsorption of metallic ions from aqueous solution on chemically activated carbon depends on metallic species which have small size and being frequently charged in solution; therefore, predominant electrostatic interactions between adsorbate and carbon surface occur (Lopez-Ramon *et al.*, 2002). Activated carbon has a great influence on both electrostatic and non electrostatic interaction. When the adsorbate is an electrolyte dissociate in aqueous solution , electrostatic interactions occur whereas their nature can be attractive or repulsive depends on the chemistry of chemical metal ion (Speciation) or metal ion complex , the solution PH and the point of zero charge of the surface , the surface porosity, the surface composition (oxygen functionality) and the size of adsorbing species (Dias *et al.*, 2007).

The present study revealed that the chemically activated carbon is most effective for adsorption of the positively charged metals. Exposure of the stones of palm-date pits to concentrated phosphoric acid and high temperature appears to be as an acid catalyst to promote bond cleavage reaction and formation of cross –links via processes such as cyclization and condensation and to combine with organic species to form phosphate and polyphosphate bridges that connect and crosslink biopolymer fragments (Jagtoyen and Derbyshire 1998).

Variable amounts of heteroatoms can be found in activated carbon (e.g oxygen, hydrogen, nitrogen and sulphur) which might have origin in the raw material or could be introduced during preparation or further treatment deeply influence the charge , hydrophobicity and electronic density of the activated carbon surface (Radovic *et al.*, 2000). Moreover,

Dastgheib and Rockstraw (2001) proposed that various surface acidic functional groups (oxygen and /or phosphorous containing groups) are

developed through the surface oxidation as well as attachment of different oxygen/phosphorous groups to the surface while developing required porosity. Removal of metals is not efficiently complete which is concordant with Jha *et al.*, (2008) who found that the uptake of metal ions in multi metal- component system (Pb<sup>2+</sup>, Cu<sup>2+</sup>, Cd<sup>2+</sup> and Ni<sup>2+</sup>) is decreased than in single component system. This may be attributed to the interference or competition with metal ions on activated carbon / Zeolite which occurs in two steps the first is rapid quantitatively predominant sorption followed by second slower and quantitatively insignificant sorption which explained as the abundant availability of active sites on sorbent material become increasingly occupied so the sorption is to be less efficient and lower (Sag and Kutsal, 1996 and Nouri *et al.*, 2007).

The present study showed that the relationship between metals with respect to the levels of bioconcentration and among fish organs whereas, liver and gills accumulated the highest amount of three metals while the muscle recorded the lowest concentrations. This agree with that recorded by Allen (1995) who found that the highest bioaccumulation of cadmium and lead in kidney and liver of *O. niloticus* while the caudal muscle accumulated relatively low amount and the gills were intermediate after either single or mixture exposure. This may be due to the low binding rate for lead to sulfhydryl groups in the muscle (Moore and Ramamoorthy, 1984).

Concentrations of heavy metals in fish tissues were higher than that of water which confirmed by Chale (2002) whereas the entry of metal occurs either through the gill membrane or through ingestion. Bioaccumulation of heavy metals does not only depend on the structure of the organ but also on the interaction between metals and the target organs (Sorenen *et al.*, 1980). Copper accumulation in all tissue of fish whereas the highest amount recorded in the liver followed by the gills and lastly muscle which may be attributed to the liver and kidney may be the site of storage or metabolism (Kargm, 1998).

Gills of freshwater fish represent the largest fraction of the total body surface area which was in close contact with the water and only a few micrometers separate the blood from the external medium (Hughes, 1984). Their complexity and constant contact with the external environment make them the first target to water born pollutants (Perry and Laurent, 1993). Accumulations of heavy metals mostly depend on their concentration in the medium and the exposure period (Kargm and Erdem, 1991 and Authman, 2008).

Addition of crushed raw palm-date pits and its chemically activated carbon ,to the water where *O. niloticus* exposed to a mixture of metals at their toxic levels, reduced their concentrations in both water and fish organs as shown in Table 2. This may be attributed to variable adsorption capacities of crushed raw palm-date pits and its chemically activated carbon to these metals which consequently ameliorate their toxic effects.

## CONCLUSION

We concluded that heavy metals (Cu, Pb and Cd) had adverse effects on health of *O. niloticus*. The use of crushed raw palm-date pits and its chemically activated carbon in fish aquarium act as an available and an effective inorganic sorbent for the decrease of Cu, Pb and Cd concentrations from aqueous solutions. Therefore we recommended that the application of both in the fish farms where they are serving the double-fold aim of water treatment and solid waste disposal. The crushed raw palm-date pits are an inexpensive solid adsorbent compared to chemically activated one.

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دور نوى البلح فى حماية بيئة البلطى النيلى من ملوثات المعادن الثقيلة نبيلة امام الشرقاوى ، \*جمال النوبى أحمد قسم الطب الشرعى والسموم ، \* قسم امراض ورعاية الأسماك كلية الطب البيطرى – جامعة الزقازيق – مصر

تم تعريض البلطى النيلى لخليط من النحاس والرصاص والكادميوم وذلك بمفرده أوبعد اضافة نوى البلح المجروش الخام أو المنشط كيميائيا لمدة شهر . اوضحت النتائج ان هذه العناصر لها تاثيرات ضارة على صحة البلطى النيلى كما وجد ان اضافة نوى البلح المجروش الخام والمنشط كيميائيا له تأثير معنوى على خفض تركيز هذه العناصر فى كل من الماء وانسجة البلطى النيلى المعرض متزامنا مع تخفيف تلك الأثار الضارة . وقد خلصت هذه الدراسة الى أن استخدام نوى البلح المجروش الخام له تاثير فعال فى ادمصاص العناصر الثقيلة مع سهولة الحصول عليه وقلة تكلفتة مقارنة بالمعالج كيميائيا.