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Evaluation of Activated Charcoal as a Protective Agent Against Toxicity of Cadmium or Pestban in African Catfish (*Clarias Gariepinus*)

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ABSTRACT

The polluted water constitutes one of the most important stressors leading to adverse effects on fish health. The objective of this study was to examine the effects of cadmium or pestban, alone and in combination with charcoal, on health of African catfish (*Clarias gariepinus*). The 96 hr LC50 of cadmium as a cadmium chloride and Pestban 48% (o.o.diethyl-o-3,5,6-trichloro-2pyridal) was found to be 40 and 5 ppm, respectively. During determination of the 96-hr LC50, fish intoxicated with pollutants exhibited loss of equilibrium, nervous manifestation with erected pectoral fins and decreased in feed intake. Also, increased in mucus secretion and the fish exhibited a respiratory distress. The postmortem alterations revealed congestion of the internal organs and reddish ascetic fluid. One hundred and forty four fish were divided into 6 groups. Gp 1 served as a control. Gp 2 was exposed to activated charcoal (2.5 mg/l). Gp 3 was exposed to the cadmium (20 ppm). Gp 4 was exposed to both cadmium (20 ppm) and charcoal (2.5 mg/l). Gp 5 was exposed to the Pestban (2.5 ppm). Gp 6 was treated with Pestban (2.5 ppm) and 2.5 mg/l charcoal. All fish groups were hold for 45 days to evaluate the effects of sublethal concentrations of cadmium or pestban with and without charcoal on physiological and biochemical assays. The addition of charcoal alone to water of reared fish had no side effect on fish health. RBCs counts, Hb and Ht were decreased in cadmium or pestban treated groups. An improvement in RBCs counts, Hb and Ht were noticed in cadmium metal and pestban groups treated with charcoal. Glucose levels, total protein, total lipids and serum enzymes as well as uric acid and creatinine were significantly affected by both cadmium and Pestban exposure. On the other hand, the addition of absorbance substance to the contaminated water improved significantly the biochemical parameters as compared to fish groups exposed to pollutants. In conclusion, the treatment of polluted water by activated charcoal during the experimental trials significantly reduced adverse effect of pollution and render water nonhazardous to environment.

Keywords:

INTRODUCTION

African catfish, *Clarias gariepinus*, is an economically important food fish species, cultured under different systems, a part from being a modal organism in research. The conflict between environment and development is becoming ever more prominent. Egyptian authority has prohibited the use of freshwater from Nile River for different aquaculture purposes due to the shortage of water resources. Therefore, fish farms utilize untreated agriculture drainage water.

The exposure to heavy metals even in a low concentration affects metabolism, growth, reproduction and/or survival of aquatic organisms. Cadmium is an element from a group known as "heavy metals". They are pollutants produced by industries and mining which can be dangerous to humans and animals even in very small quantities (WHO, 1992). Cadmium is very toxic to a variety of species of fish and wildlife (Hinck *et al.*, 2006). Cadmium causes behavior, growth, immunosuppressive and physiological problems in aquatic life at sub lethal concentrations (Baldisserotto *et al.*, 2005; Hewitt *et al.*, 2006 and Szczerbik *et al*., 2006). However, in freshwater ecosystems, contaminated food may sometimes be a more important source of toxic metals than the water itself. Chronic discharges of low levels of waterborne metals over long periods of time may lead to metal accumulation in sediments (Baldisserotto *et al.*, 2005).

The application of chemical pesticides in the agricultural, industrial drainage and domestic sewage and their deposition in the aquatic system is known to exhibited notable alterations in the aquatic organisms and finally death. Now, there is a growing concern worldwide over the indiscriminate use of such chemicals, which result in environmental pollution and toxicity risk to non-target organisms (Rao, $2006_{a\&b}$).

Most aquatic organisms have the capability of bioconcentrating pollutants in different tissues by feeding and metabolic processes. Therefore, there is an urgent need to develop more effective, cheap and target specific method to reduce the toxic elements to be disposed of without danger to human health. Adsorption is the process of removing soluble contaminants by attachment to a solid. Activated carbon adsorption has been recommended by the US Environmental Protection Agency (USEPA) as one of the best available technologies in removal of organic pollutants (Adams and Watson, 1996).

Consequently, this study highlighted to evaluate the ability of charcoal to reduce the adverse effects of water contamination with cadmium or pestban with regard to the physiological and biochemical aspects of African catfish; *Clarias garpiepinus.*

MATERIALS AND METHODS

Experimental Fish

An apparently healthy African catfish (*Clarias gariepinus*) weighing 100-125 gm were collected from earthen ponds of El-Abbassa fish farm, transferred to the laboratory, maintained in glass aquaria filled with dechlorinated tap water supplied with continuous aeration and acclimatized to laboratory conditions for 15 days before the start of experiment. The temperature was kept at 24±1^{o}C throughout the experiment. Photoperiod was maintained at 12 h light and 12 h dark. Fish were fed twice daily with standard commercially prepared pellets at 2% of their body mass/day throughout the experimental period. Uneaten feed and feces were siphoned daily. Dead fish were removed daily. The biomass of fish in each aquarium was measured at the beginning of experiment and after each sampling; thereby the daily ration was adjusted.

Determination of the 96 hour half lethal concentration (96 hr LC50)

The 96 hour half lethal concentrations (96 hr LC_{50}) of cadmium in the form of cadmium chloride (El-Nasr Company for drug chemicals, Egypt) and pestban pesticide 48% (o.o.diethyl-o-3,5,6-trichloro-2pyridal) from Agri-chem, Egypt were determined for *C. gariepinus* according to Behreus and Karbeur (1953). For determination of 96 hr LC_{50} cadmium, fish were divided into ten equal groups (each contains 10 fish) and subjected to 0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 ppm concentrations, respectively. On the other hand, for determination of 96 hr LC_{50} pestban pesticide 48%, fish were divided into ten equal groups (each contains 10 fish) and subjected to the following concentrations 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 ppm, respectively. Fish were starved during the experiments. Gross mortality in each group was recorded and removed every 8 hr for 96 hr. The treated fish was observed to clinical and postmortem alterations according to Amlacher (1970).

Experimental Design

One hundred and forty four fish were distributed in 6 groups. Each group consists of 3 replicates; each replicate consists of 8 fish in 100-L glass aquarium. The first group (Gp. 1) served as a control. The 2nd one (G_2) was exposed to activated charcoal from El-Nasr Company for drug chemicals (2.5 mg/l). The 3rd group (G_3) was exposed to the cadmium (20 ppm). The 4th group (G_4) was exposed to both cadmium (20 ppm) and charcoal (2.5 mg/l). The 5th group was exposed to the Pestban (2.5 ppm). The 6th group was exposed to the Pestban (2.5 ppm) and 2.5 mg/l charcoal. A three quarters of aquarium's water was removed with fish excreta by siphoning and replaced with an equal volume of water maintains the same pollutants or charcoal concentration per each treatment group. All fish groups were hold for 45 days to evaluate the effects of sublethal concentrations of cadmium or pestban with and without charcoal on physiological and biochemical assays.

Blood and serum analysis

Fish were anaesthetized using buffered tricaine methane sulfonate (20mg/L) and blood was collected from fish caudal vein by a sterile syringe. Each sample was divided in two sets of eppendorf tubes. One set contained heparin, used as anticoagulant for hematological investigations. The second set, without anticoagulant, was left to clot at 4^{o}C and centrifuged at 3000 rpm for 10 minutes at low temperature. The collected serum was stored at –20 ^{o}C for biochemical assays. Red blood cells (RBCs) were counted using a Neubauer hemocytometer after blood dilution with phosphate-buffered saline. Packed cell volume values (PCV %) were immediately determined after sampling by placing fresh blood in glass hematocrit-capillary tubes and centrifuging for 10 minutes. Hemoglobin levels (Hb, g/dl) were determined colorimetrically by measuring the formation of cyanomethemoglobin after using a specific kit. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined according to Reitman and Frankel (1957). Total serum protein was determined according to Henry (1964). Total serum lipid was determined according to Schmit (1964). Serum glucose was determined colorimetrically using glucose kit according to Trinder (1969). Serum uric acid was measured according to Barham and Trinder (1972) and serum creatinine was measured colorimetrically as described by Henry (1974). All used kits were produced by Egyptian American Co. for Laboratory Services, Egypt.

Table (1): Summary of the experimental design

Groups	Cadmium (20 ppm)	Pestban (2.5 ppm)	Charcoal (2.5 ppm)
G_1 (Control)	No	No	No
G_2	No	No	Yes
G_3	Yes	No	No
G_4	Yes	No	Yes
G_5	No	Yes	No
G_6	No	Yes	Yes

Statistical Analysis

The data were statistically analyzed using Duncan's multiple range test to determine differences in means (Duncan, 1955).

RESULTS

The 96 hr LC_{50} of cadmium and pestban was found to be 40 and 5 ppm, respectively. During determination of the 96-hr LC50, fish exhibited loss of equilibrium, dullness, erratic swimming movements, nervous manifestation with erected pectoral fins and decreased in feed intake. The skin had a mottled appearance and mucus secretion appeared to increase and accumulated on the gills and the fish exhibited a respiratory manifestation such as surfaced swimming, opening their mouth with rapid and frequent respiration. The postmortem alterations revealed congestion of the internal organs and reddish ascetic fluid.

The addition of chelating agent (charcoal) alone to water of reared fish (G2) had no side effect on fish health (Table 2,3&4). Table (2) showing the changes in red blood cells (RBCs), hemoglobin (Hb) and hematocrit of African catfish; *C. gariepinus* exposed to sublethal concentrations of cadmium element or pestban pesticide for 45 days without or with charcoal addition. RBCs count was significantly decreased in cadmium element and pestban treated groups (G3&G5) compared with control and charcoal treated groups(G1&G2). An improvement in RBCs count was noticed in cadmium element and pestban groups treated with charcoal (G4&G6). Also, a significant reduction was observed in both Hb and Ht due to cadmium element and pestban exposure (G3&G5). On the other hand, Hb and Ht levels in G4&G6 were significantly increased as a result of addition of charcoal to the polluted water.

Table 2: ***Changes in red blood cells (RBCs), hemoglobin (Hb) and hematocrit (mean ± SE) of African catfish; Clarias gariepinus exposed to sublethal concentrations of cadmium element or pestban pesticide for 45 days without or with charcoal addition.***

Parameters / Treatments	RBCs (Cellx10^6/mm^3)	Hb (g/dl)	Ht (%)
G_1 (Control)	$2.55±0.28^a$	$12.15±1.14^a$	$35.42±1.23^a$
G_2	$2.70±0.33^a$	$12.10±1.12^a$	$35.57±1.21^a$
G_3	$0.99±0.18^d$	$6.45±0.28^c$	$15.27±1.14^d$
G_4	$1.81±0.10^b$	$10.34±0.11^b$	$22.21±2.17^c$
G_5	$0.78±0.21^d$	$5.24±0.23^c$	$16.78±1.47^d$
G_6	$1.62±0.14^c$	$9.88±0.34^b$	$28.14±2.41^b$

Means with the same letter in the same column are not significantly different at P≤0.05.

Results of table (3) and table (4) show that the addition of absorbance substance to the contaminated water improved significantly the biochemical parameters as compared to fish groups exposed to pollutants. In Table 3, glucose levels, total protein and total lipids were significantly affected by both cadmium and pestban exposure ($P<0.05$). The plasma glucose concentrations showed lower significant values in fish exposed to pollutants as compared to the control

Table 3: ***Changes in plasma glucose, total plasma protein and total plasma lipid (mean ± SE) of African catfish; Clarias gariepinus exposed to sublethal concentrations of cadmium metal or pestban pesticide for 45 days without or with charcoal addition.***

Parameters / Treatments	Plasma glucose (mg/dl)	Plasma protein (g/dl)	Plasma lipids (g/dl)
G_1(Control)	$65.02±1.01^a$	3.55±0.29a	3.31±0.24c
G_2	$66.22±1.33^a$	3.41±0.31a	3.21±0.12c
G_3	$28.14±2.32^c$	1.14±0.17c	5.27±0.75a
G_4	$64.17±1.32^a$	2.75±0.52b	4.11±0.04b
G_5	$20.71±1.65^d$	1.12±0.34c	5.13±.01a
G_6	$52.11±3.21^b$	2.55±0.33b	3.89±1.05b

Means with the same letter in the same column are not significantly different at P≤0.05.

Table 4: ***Changes in serum aspartate amninotransferase (SAST), serum alanine aminotransferase (SALT), uric acid and creatinine (mean±SE) of African catfish; C. gariepinus exposed to sublethal concentrations of cadmium metal or pestban pesticide for 45 days without or with charcoal addition.***

Parameters / Treatments	AST (µ/l)	ALT (µ/l)	Uric acid (mg/dl)	Creatinine (mg/dl)
G_1(Control)	28.73±0.05[a]	23.61±0.70[a]	1.82±0.02[c]	0.80±0.02[c]
G_2	28.41±0.72[a]	22.83±0.14[a]	1.34±0.03[c]	0.82±0.03[c]
G_3	10.16±0.25[c]	7.57±0.69[c]	3.47±0.15[a]	1.41±0.07[a]
G_4	20.41±0.70[b]	18.50±0.47[b]	2.51±0.26[b]	0.94±0.05[b]
G_5	9.98±0.22[c]	7.33±0.78[c]	3.58±0.70[a]	1.34±0.04[a]
G_6	19.53±0.62[b]	15.62±0.45[c]	2.67±0.18[b]	0.91±0.12[b]

Means with the same letter in the same column are not significantly different at P≤0.05.

group. These values were also increased significantly in fish exposed to mixture of pollutants and charcoal. The total protein values were significantly decreased in fish exposed to cadmium or pestban (G3&G5) versus to control group (G1). While an increasing in total protein values were reported by addition of charcoal to the polluted water. On the other hand, levels of total plasma lipids were significantly increased in G3 vs G1 and G5 vs G1. In table 4, the average values of SAST and SALT were significantly decreased by both cadmium and pestban (P<0.05). The values of uric acid and creatinine were increased significantly (P<0.05) in G3 and G5 vs G1.

DISCUSSION

Sustainable development of aquaculture activity as important strategy is gained considerable amount of attention. Potentially damaging chemicals are used in fish culture, such as disinfectants, therapeutics, feed additives, algicides, pesticides, and fertilizers threatening health of marine and freshwater fishes worldwide. Responses to pollutants by aquatic organisms are broad ranged depending on the compound, exposure time, water quality and the species (Richmonds and Dutta, 1992).

Biosorption is a term that describes the removal of heavy metals by the passive binding to non-living biomass from an aqueous solution. This implies that the removal mechanism is not metabolically controlled. Of the many types of biosorbents, the ability of charcoal to overcome the toxic activity of some pollutants having potential interest has been documented. Therefore, the objective of the current study was to evaluate the passive removal of toxic agents by inexpensive biomaterials, charcoal.

Fish intoxicated by cadmium and pestban showed erratic swimming, nervous manifestation, loss of appetite, increased mucus secretion and respiratory distress. Also, congestion of the internal organs was observed. Similar pictures were observed by El-Shaieb *et al.* (2001); Marzouk *et al.* (2006); Shalaby *et al.* (2006); Rao, ($2006_{a\&b}$); Mousa and El-Banna (2009); Mousa *et al.* (2009) and Mohammed (2010). Atallah *et al.* (1997) attributed such changes to the extraordinary need for the oxygen which could be attributed to coating of the gills with profuse mucus together with congestion and hyperplastic epithelium of the secondary lamellae.

Biochemical and physiological biomarkers are frequently used for detecting or diagnosing sublethal effects in fish exposed to different toxic substances. The decrease in RBCs, Hb and Ht was recorded in cadmium metal and pestban treated groups compared with control and charcoal treated groups. On the other hand, RBCs, Hb and Ht levels were significantly increased as a result of addition of charcoal to the polluted water. Similar results were reported by Mousa (2004); Shalaby *et al.* (2006); Shalaby *et al.* (2007) and Mousa and El-Banna (2009). The authors attributed the decrease in red blood cells and haemoglobin content to the disruptive action of the pollutants on the erythropoietic tissue as a result of which the viability of the cells might be affected.

The AST and ALT are liver specific enzymes and both are more sensitive measure of hepatotoxicity and histophathalogic changes and can be assessed within a shorter time (Balint *et al.*, 1997). The increase in AST and ALT values indicate the tissue damages in liver, kidney and gill (Oluah, 1999). In the present study, the average values of AST and ALT were significantly decreased by both cadmium and pestban exposure. These results were in agreement with Mousa (2004); Marzouk *et al.* (2006); Shalaby *et al.* (2007) and Mousa and El-Banna (2009).The inhibitory effects of insecticides are dependent on their binding capacity to the enzyme active site and by their rate of phosphorylation in relation to behavior and age (Dutta et al., 1995). The AST and ALT activities were increased in plasma and kidney, where in liver and gill decreased (Rao, 2006_a). Plasma activity concentrations of AST and ALT are the most commonly used biochemical markers of hepatocellular necrosis and time dependant (Friedman *et al.*, 1996; El-Shaieb *et al.*, 2001;

Zikic *et al.,* 2001; Shalaby *et al.,* 2006; Rao, 2006$_a$ and Rao, 2006$_b$).

The values of creatinine and uric acid were increased in fish exposed to intoxication. These could be suggested that both pollutants had hepatotoxic and nephrotoxic effects. This increase might be induced by glomerular insufficiency, increased muscle tissue catabolism or impairment of general metabolism as previously reported by Abd El-Meguid *et al*. (2002); Marzouk *et al.* (2006) and Mohamed (2010).

Liver is being considered the main site of metabolic activity in body and highly active in both toxifying and detoxifying insecticides (Jaroli and Sharma, 2005). The quantitative determination of total serum protein reflects the liver capacity of protein synthesis and denotes the osmolarity of the blood and the renal impairments. Total protein values were decreased in all intoxicated fish. These results agree with those of Jaroli and Sharma, (2005); Marzouk *et al.* (2006); Shalaby *et al.* (2007) and Mohamed (2010). They found that heavy metals caused decrease in total protein due to the great energy demands and the cellular damage that occur in the tissue of fish exposed to cadmium and/or inhibition of protein synthesis in fish tissue exposed to any heavy metal. Martinez *et al.* (2004) mentioned that fish under stress may mobilize protein to meet energy requirements needed to sustain increased physiological activity.

The increase of total serum lipids in this study may be due to the increase of lipids peroxides formation induced by the effect of pollutants as previously reported by Arias (1990). Otherwise, the destruction of the liver cells and other organs due to the effect of the toxicants increase the levels of total lipids in the serum (Mousa, 2004).

The present obtained data showed significant reduction in bad effect of pollutants by using a cheap and environmental friendly material. Activated charcoal is highly effective against both natural and synthetic toxins (Aiello, 1998; Shalaby *et al.,* 2006 and Mousa and El-Banna, 2009). Studies recorded the activated carbon to be effective in removing various mycotoxins, such as aflatoxin, fumonisins, ochratoxin A, trichothecene, and zearalenone (Huwig *et al.,* 2001). Natural toxins from plants are also removed or attenuated by activated charcoal treatment or supplementation (Bisson *et al.,* 2001).

In conclusion, the results of the current investigation indicated the directed toxic effects of cadmium and pestban on the health status of African catfish. Activated charcoal was proven to be the most effective, natural, promising and leading candidate in reduction the effects of pollutants.

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تقييم الفحم النباتي في حماية سمكة القرموط الأفريقي ضد سمية عنصر الكادميوم ومبيد البستبان

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*قسم صحة الأسماك ** قسم بيولوجى وبيئة الأسماك ـ المعمل المركزى لبحوث الثروة السمكية بالعباسة- مركز البحوث الزراعية

تعتبر خاصية الإدمصاص في الكربون النشط من أهم العوامل التي جعلته من أهم وأفضل المواد التي يوصى بها لإزالة الملوثات من البيئة ولحماية الأسماك من خطرهم. تم اجراء هذه الدراسة لمعرفة تأثير عنصر الكادميوم ومبيد البستبان ٤٨% (كلوربيريفوس ٤٨%) ومعالجتهما باضافة الفحم النشط على بعض التغيرات الفسيولوجية والبيوكيميائية والتغيرات المرضية فى سمكة القرموط الأفريقي.

تم تعيين الجرعة المميتة للنصف لكل من الكادميوم ومبيد البستبان لسمكة القرموط كل على حدة ، فكانت للكادميوم هي ٤٠ اما مبيد البستبان ٥٠ جزء فى المليون. أظهرت النتائج أثناء تعيين الجرعة المميتة للنصف اضطرابا فى سلوك الأسماك المعاملة بالملوثات حيث لوحظت حركات غير متزنة ، زيادة في افراز المواد المخاطية وتراكمها على الخياشيم واضطرابات تنفسية والعوم على سطح الماء. أوضحت الصفة التشريحية عن وجود تغيرات مرضية فى صورة أحتقان للأعضاء الداخلية.

تم توزيع الأسماك فى ست معاملات حيث تركت المجموعة الأولى (المجموعة الضابطة) للمقارنة بينما تعرضت المجموعة الثانية للفحم المنشط (٢,٥ مجم/لتر) وكذلك تعرضت المجموعتين الثالثة والخامسة الى الكادميوم (٢٠ جزء فى المليون) ومبيد البستبان (٢,٥ جزء فى المليون) على التوالي. فى حين تعرضت المجموعتين والرابعة و السادسة لنفس تركيزي الكادميوم والفحم أو لنفس تركيزي البستبان والفحم على التوالي. وتم تخذ العينات بعد ٤٥ يوما من المعاملة.

أحدثت الجرعات تحت المميتة من عنصر الكادميوم أومبيد البستبان نقصا ذا دلالة فى عدد كرات الدم الحمراء ونسبة الهيموجلوبين والهيماتوكريت وسكر الدم والمحتوى البروتيني ونشاط الإنزيمات الناقلة لمجموعات الأمين AST, ALT ، بينما سجلت النتائج ارتفاعا ذا دلالة في محتوى الدهون وحمض اليوريك والكرياتينين ، غير أن الفحم النشط (٢,٥ مجم/لتر) قد أظهر تحسنا ملحوظا في هذه العوامل مقارنة بالمجموعة الضابطة. أحدثت الجرعات تحت المميتة من الكادميوم ومبيد البستبان نقصا ذا دلالة فى عدد كرات الدم الحمراء ونسبة الهيموجلوبين والهيماتوكريت وسكر الدم والمحتوى البروتيني ونشاط الإنزيمات الناقلة لمجموعات الأمين AST, ALT ، بينما سجلت النتائج ارتفاعا ذا دلالة في محتوى الدهون وحمض اليوريك والكرياتينين ، غير أن الفحم النشط (٢,٥ مجم/لتر) قد أظهر تحسنا ملحوظا في هذه القياسات الفسولوجية والبيوكيميائية.

نستخلص من هذه الدراسة أنه يجب استزراع الأسماك في مياه خالية من الملوثات حيث أنها تمثل خطرا شديدا على صحة وانتاج هذه الأسماك ، كما نوصي باستخدام الفحم النباتي النشط نثرا في المياه الملوثة للتخلص منها قبل استزراع الأسماك وبذلك تكون هذة الأسماك امنة صحيا.