Clinicopathological Studies in African Catfish (*Clarias gariepinus*) Affected By Ammonia Toxicity

Abdullah, O.A.M.[1], Mona M. Abdel-Wahab[2], Amina A. Dessouki[3], Haidy G. Abdel-Rahman[1], Asmaa F. Ibrahim[4].

[1]Dept. of Clinical Pathology, Faculty of Veterinary Medicine, Suez Canal University.
[2]Animal Health Research Institute, Ismailia.
[3]Dept. of Pathology, Faculty of Veterinary Medicine, Suez Canal University.
4-Directorate of Veterinary Medicine.

Abstract:
A total number of 60 *Clarias gariepinus* fish obtained from Ismailia governorate and its tributaries were collected from three locations. The fish were divided into three main groups, (group A) from El-Teraa, (group B) from El-Berkaa, (group C) from El-Rashah. These locations derived from Mohamed Ali channel which derived from River Nile. The fish and water of control group were obtained from central laboratory for Aquaculture Research, El-Abbassa, Abo-Hamad, Sharqia, Egypt. Water analysis of the examined polluted locations revealed high level of ammonia. Serum biochemical examinations revealed hypoproteinemia, hypoalbuminemia and hypoglobulinemia with increase in serum ALT, AST, total bilirubin, direct bilirubin, indirect bilirubin, glucose, urea, creatinine and serum ammonia level in the three groups compared with control one.

Key words: ammonia, biochemistry, glucose, protein, ALT, AST, *Clarias gariepinus*.

Introduction:

Fish and other aquatic organisms are exposed to great varieties of pollution that have found their way into water in the form of sewage, industrial and agricultural wastes. Many authors had studied the effect of different types of pollutants on fish. Fish production should be increased in Egypt to meet the demand of the increasing population. Several problems face fish production in Egypt. Among these problems are the most tropical species die via low water quality because of pollution with ammonia ***(Harris et al, 1998).***

Ammonia is the principal nitrogenous waste product of fish that represents 60% to 80% of nitrogenous excretion of fish ***(Salin and Williot, 1991).*** It is also, the main nitrogenous waste material excreted by gills in addition to urea and amines and an end product of

the protein catabolism ***(De Croux et al, 2004).*** Ammonia is toxic, not only to fish but also to all aquatic animals ***(Harris et al, 1998),*** especially in pond aquaculture at low concentrations of dissolved oxygen ***(Alabaster et al, 1983).***
Ammonia accumulates to toxic levels; fish cannot extract energy from feed and will fall into a coma and die ***(Hargreaves and Tucker, 2004).*** Ammonia tends to block oxygen transfer from the gills to the blood and can cause both immediate and long term gill damage ***(Joel and Amajuoyi, 2010).*** Also, it can cause impairment of cerebral energy metabolism, damage to gills, liver, kidneys, spleen and thyroid tissue in fish, crustaceans and mollusks ***(Smart, 1978).***
This work was conducted to study the harmful effects of ammonia toxicity on the African Catfish *Clarias gariepinus* in three different locations by evaluating: The biochemical analysis, water analysis study**,** the histopathological alterations induced by ammonia toxicity.

Materials and Methods:-

Fish

This study was carried out on Catfish *Clarias gariepinus* belonged to Ismailia Governorate & its tributaries over three months period from first of May 2014 till end of July 2014. A total number of 60 *C.gariepinus* with an average body weight 400 ± 50 g were collected from three locations {El-Teraa- El-Berka- El-Rashah} derived from Mohamed Ali channel which derived from River Nile. The fish were devided into three main groups according to the site they obtained from. The first group (group A) collected from El-Teraa including 15 fish. The second group (group B) collected from El-Berka including 15 fish. The third group (group C) collected from El-Rashah including 15 fish. , each group was subdivided into three subgroups each contain 5 fish according to time they obtained. The fish and water of control group were collected from Central Laboratory for Aquaculture Research, El-Abbassa, Abo-Hamad, Sharqia, Egypt including 15 fish. The fish were immediately transported alive in sterile bags to the lab of Clinical Pathology Dept., Faculty of Vet. Medicine, Suez Canal University.

Water

Water samples were collected from the three locations at the same time of collection of fish. Water (2 Litre) was collected 50-80 cm below the water surface in bottles. Water samples were kept in an ice box and immediately transported to the lab of Animal Hygiene, Zoonoses and Animal Behaviour Dept., Faculty of Vet. Medicine, Suez Canal University to examine the physicochemical characteristics.

Blood sampling:-

The blood was collected from the caudal blood vessels. The blood was left in a plain centrifuge tube without anticoagulant in order to

clot and centrifuged at 5000 rpm for 5 min at room temperature, the supernatant serum collected and stored at -20 ^{o}C in screw epindorph tubes until used for serum biochemical analysis.

Serum biochemical examinations:-

ALT and AST were determined according to ***Reitman and Frankel (1957),*** bilirubin was determined according to ***Kaplan (1984),*** total protein was determined according to ***Henry (1964),*** albumin was determined according to ***Drupt (1974),*** globulin was determined according to ***Coles (1974),*** glucose was estimated according to ***Trinder (1969),*** urea was determined according to ***Reiss et al., (1965),*** creatinine was estimated according to ***Henry et al., (1974),*** serum ammonia was estimated by turbidmetry using ***Coppas 8000.*** All kits used in this study were obtained from ***BIO-Merieux (Brains / France)*** and ***TichoDiagnostic (Sees, France).***

Water analysis:-

Ammonium was determined by using UV screening spectrophotometric method according to ***APHA (1998),*** toxic (unionized) ammonia was calculated using ***Emerson et al., (1975).***

Histopathological examination:-

Tissue specimens from the different organs (gills, liver, kidneys and spleen) of fish were collected and immediately fixed in 10% formalin solution for 48-72 h. according to ***Drury and Willington (1980).***

Table 1 : Experimental design

Groups / Time of Collection	Control	Group A (El-Teraa)	Group B (El-Berka)	Group C (El-Rashah)	Total
1st month	5 fish	5 fish	5 fish	5 fish	20 fish
2nd month	5fish	5 fish	5 fish	5fish	20fish
3rd month	5 fish	5 fish	5 fish	5 fish	20 fish
Total	15 fish	15 fish	15 fish	15 fish	60 fish

Results and Discussion:

The presence of any substance in the water produces changes in their quality which are not always favorable for development and survival of aquatic organisms. When the water quality is affected by toxicant, any physiological changes will be reflected in the values of one or more of the hematological, biochemical and histopathological parameters

(Adham 2002 and Ishikawa et al, 2007).

Of all the water quality parameters that affect fish, ammonia is the most important after oxygen, especially in semi intensive systems. Ammonia is toxic not only to fish but also to all aquatic animals. Ammonia causes stress and damage to gills and other tissues, even in small amounts ***(de Oliveira et al., 2012)***.

In our study, results showed that ammonia level is increased in the three treatments where the highest level was obtained at the third month of collection in El-Berka. Our results are considered higher than the acceptable limits as recommended by ***Bhatnagar and Singh (2010)*** who reported that the maximum tolerance level of ammonia for most fish was about 0.1 mg/L of unionized ammonia (NH_3). Also, ***Buttner (1993)*** reported that ammonia must be limited between 0.2-2.0 mg/L. ***Yang (1999)*** concluded that the tolerable level of ammonia for fish culture is 1.2 mg/L. ***EPA (1998)*** reported that water with concentrations of less than 0.020 mg/L unionized ammonia is considered safe for fish reproduction. While ***Muir et al (2000)*** recommended that ideal NH_3 level for tilapia should be below 0.2 mg/L.

Biochemical profiles of blood can provide important information about the internal environment of the organism. The role of blood enzymes in monitoring and detecting stress or disease has led to a growing concern in using them as biochemical indicators to trace environmental pollutants ***(Adham et al, 1999).*** Data of *C. gariepinus* in our study revealed that the activities of serum enzymes (ALT and AST) were significantly elevated in response to exposure to high level of ammonia concentrations, with a positive correlation between ammonia concentration and enzyme level elevation, as AST increased than normal value**.** ***Krajnovic-Ozretic and Krajnovic-Ozertic (1992)*** recorded elevated activities of ALT in the plasma of adult gray mullets *Mugilavratus Risso* exposed to acute concentrations of phenol and cyanide. Increased level of ALT and AST in common carp after exposure to ammonia toxicity may be due to the loss of Kreb's cycle with the result that these enzymes compensate by providing alpha ketoglutarate ***(Chatty et al, 1980 and Salah El-Deen 1999).*** The observed changes could be also due to generalized organ system failure due to the effect of ammonia toxicity**.**

Bilirubin is a metabolic waste product which formed from the breakdown of erythrocytes. In our study, there was increase in total and direct bilirubin which are indicator for cholestasis and pathological alterations of the biliary flow ***(Lalitsingh et al, 2010).*** There was increase in direct and indirect bilirubin in the serum which is indicators for

hepatocellular jaundice caused by ammonia toxicity ***(Coles, 1986).*** Another possible reason may be a metabolic disturbance in liver involving defective conjugation and/or excretion of bilirubin. The bilirubin route of elimination is perhaps most important contributing source to the excretion of xenobiotics, but is of primary importance for the excretion of the animal's metabolites. Since the liver encounters nutrients, environmental toxicants and waste products, within this framework, it extracts the environmental toxicants and waste products to prevent their circulation to other parts of the body ***(Cheesborough, 1992).***

One of the important functions of plasma/serum protein is the maintainance of osmotic balance between the circulating blood and tissue fluids ***(Harper et al, 1979).*** The influence of toxicants on the total protein concentration of fish has also been taken into consideration in evaluating the response to stressors and consequently the increasing demand for energy. Concerning serum protein level in our study, the results showed that there was decrease in total protein, albumin and globulin level. These results may be attributed to the severity of the stressor, which causes osmotic imbalance. This result is in agreement with ***Elbealy (2012)*** and ***Alkahem et al, (1998)*** who attributed the reduction in the proteins to its conversion to fulfilling an increased energy demand by fish to cope with detrimental conditions imposed by a toxicant. This result was in contrary with ***Seham (2013)*** who attributed the increase in total protein, albumin and globulin to the changes taking place in serum globulin metabolism or to the input of different pollutants.

The blood glucose was the most sensitive parameter in detecting the sublethal stress response. The serum glucose level was elevated in our study. This result may be due to increase in plasma concentration of catecholamines and corticosteroids as stress response of fish subjected to environmental alterations ***(Tayel et al, 2008).*** Glucose increased to cope with stress and maintain homeostasis ***(Ackerman et al, 2006)***. Under stress conditions, hypothalamo-pituitaty interregnal axis elevated blood cortisol which in turn leads to glycogenolysis, lypolysis and gluconeogenesis to provide energy. The reported hyperglycemia may be due to withdrawn of water from blood to muscles to overcome the pollution present in water ***(Massoud et al, 1973)*** and/or due to the breakdown of glycogen in liver as a result of water pollution ***(Haggag et al, 1993).*** Also, this hyperglycemia may be caused by enhanced glycogen breakdown in liver, probably because of anaerobic stress and/or the discharges of various types of wastes. This result is in contrary with ***Buckley et al,***

(1979) who observed that blood glucose diminished whereas liver glycogen stores increased in Coho salmon exposed for 91 days to 3, 16, 47 mg N/L NH_4CL.

Most teleost fish is obligate ammonioteles excreting the bulk 75 - 90 % of their waste nitrogen as ammonia ***(Hamdy and Poxton, 1993)***, together with only small amounts (5 - 15 %) of urea produced by uricolysis ***(Wood, 1993).*** Urea occurs in natures as the major nitrogen containing end product of protein metabolism by vertebrates, which excrete urea in urine. Creatinine is a nitrogenous waste product, which is synthesized in the body at a fairly constant rate from creatine. The serum urea and creatinine levels in our study were increased in ammonia exposed fish. This may be attributed to renal damage which could be due to the toxicity lead to decrease the filtration rate of the kidneys and thus retention of the urea excretion and creatinine. These results are in agreement with ***Mcdonald and Milligan (1992)***. ***Harvey (1997)*** reported that the creatinine measurement was more indicative and of more diagnostic value in assessment of renal function activities than blood urea level.

African catfish successfully control plasma NH_4^+ concentrations within physiological concentrations over a wide range of water ammonia concentrations that would be lethal to many other fishes. In African catfish plasma total ammonia is predominantly present (84-98%) as NH_4^+ ***(Ip et al, 2004)***. In our study, results showed that there was increase in serum ammonia level. This may be due to that in African catfish, exposure to high water ammonia (NH_3) initially results in a plasma NH_4^+ peak due to an NH_3 influx followed by the onset of NH_3 defense mechanisms over time. Our results was in agreement with ***Knoph and Thorud (1996)*** who observed that plasma total ammonia level increased linearly with the water total ammonia level in Atlantic salmon. Also, ***Person et al (1997)*** observed that blood plasma TAN contents were positively correlated with ambient ammonia concentrations in three batches of turbot *Scophthalmus maximus* juveniles exposed for 4-6 weeks to constant ammonium chloride solutions.

From the present study, it was concluded that there is a real need to study the interrelationships between the pollution of surface waters by a wide range of chemicals and diseases in natural fish populations, and the processes involved. This represents an important but at present under-developed field of scientific research. It is very important that this water quality stressor (ammonia) be monitored regularly and level should be controlled through various management practices when necessary.

Table 2: *Ammonia Level alterations of water obtained from El-Teraa, El-Berka, El-Rashah:*

groups / Months	Control	El-Teraa	El-Berka	El-Rashah
1st month of collection				
1- TAN (mg/L)	0.52± 0.08 [d]	6.63± 0.01 [a]	4.89± 0.04 [c]	5.11± 0.01 [b]
2- UIA-N (mg/L)	0.012± 0.013 [d]	0.13± 0.001 [a]	0.08± 0.003 [c]	0.09± 0.001 [b]
2nd month of collection				
1-TAN (mg/L)	0.3± 0.06 [d]	8.04± 0.04 [a]	6.23± 0.11 [C]	7.29± 0.11 [b]
2- UIA-N (mg/L)	0.006± 0.002 [d]	0.39± 0.01 [a]	0.12± 0.01 [c]	0.31± 0.02 [b]
3rd month of collection				
1-TAN (mg/L)	0.49± 0.14 [d]	10.18± 0.07 [b]	13.27± 0.18 [a]	9.15± 0.18 [c]
2- UIA-N (mg/L)	0.009± 0.012 [d]	1.52± 0.01 [b]	2.23± 0.03 [a]	0.91± 0.02 [c]

Means in the same row having different letters are significantly different at ($p \leq 0.05$).

Table 3: *Serum biochemical findings of examined C. gariepinus fish at first month of collection from the three different locations (El-Teraa, El-Berka, El-Rashah).*

Parameters / groups	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Ammonia (mg/L)
Control	19.8 ±0.89 b	83 ±0.72 b	0.62 ±0.14 c	0.33 ±0.03 a	0.28 ±0.03 c	4.63 ±0.16 a	2.14 ±0.12 a	2.49 ±0.1 a	0.86 ±0.02 a	76.15 ±0.1 c	9.71 ±0.38 b	0.3 ±0.13 b	1.43 ±0.05 c
A (ElTeraa)	29.8 ±1.98 a	105.2 ±3.62 a	0.73 ±0.1 a	0.4 ±0.09 a	.33 ±0.09 a	3.73 ±0.08 b	1.35 ±0.02 c	2.38 ±0.09 a	0.56 ±0.02 b	83.6 ±1.5 b	12.4 ±1.16 a	0.38 ±0.02 a	1.74 ±0.02 a
B (El-Berka)	21.8 ±1.88 b	85.4 ±2.25 b	0.64 ±0.13 b	0.37 ±0.05 a	0.27 ±0.05 b c	3.95 ±0.02 b	1.53 ±0.05 b	2.43 ±0.06 a	0.64 ±0.03 b	79.6 ±0.51 b	11.4 ±0.67ab	0.33 ±0.01 b	1.51 ±0.08 b
C (ElRashah)	29.4 ±1.63 a	105 ±3.78 a	0.71 ±0.08 a	0.39 ±0.08 a	0.32 ±0.06ab	3.61 ±0.07 b	1.41 ±0.03 b c	2.21 ±0.04 a	0.63 ±0.01 b	80.2 ±0.96 a	12.8 ±0.66 a	0.38 ±0.02 a	1.52 ±0.06 b

Means in the same column having different letters are significantly different at ($p \leq 0.05$).

Table 4: *Serum biochemical findings of examined C. gariepinus fish at second month of collection from the three different locations (El-Teraa, El-Berka, El-Rashah).*

Parameters / Groups	ALT (U/L)	AST (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G ratio	Glucose (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Ammonia (mg/L)
Control	20 ±0.89 ^{c}	80 ±0.89^{d}	0.61 ±0.01 ^{d}	0.34 ±0.04^{b}	0.26 ±0.03 ^{c}	4.58 ±0.18^{a}	2.16 ±0.08^{a}	2.42 ±0.1 ^{a}	0.88 ±0.02^{a}	76.45 ±0.69^{c}	9.73 ±0.43^{c}	0.3 ±0.1^{c}	1.42 ±0.02^{d}
A (El-Teraa)	37 ±2.23^{a}	139.4 ±5.89^{a}	0.83 ±0.01^{a}	0.45 ±0.01^{a}	0.38 ±0.01^{a}	3.28 ±0.05^{c}	1.1 ±0.05 ^{c}	2.18 ±0.02^{a}	0.5 ±0.02 ^{c}	88.4 ±1.07^{a}	18.8 ±1.07^{a}	0.39 ±0.02^{a}	2.01 ±0.01^{a}
B (El-Berka)	29.4 ±1.91 ^{b}	99 ±3.05 ^{c}	0.73 ±0.01 ^{c}	0.41 ±0.02^{a}	0.32 ±0.02^{b}	3.67 ±0.05^{b}	1.34 ±0.01^{b}	2.33 ±0.05^{a}	0.57 ±0.01^{bc}	81.2 ±1.06^{b}	15 ±1.14 ^{b}	0.38 ±0.02^{b}	1.66 ±0.03 ^{c}
C (El-Rashah)	34.2 ±1.85^{ab}	115 ±6.94 ^{b}	0.78 ±0.04 ^{b}	0.43 ±0.05^{a}	0.35 ±0.05^{ab}	3.45 ±0.12^{bc}	1.3 ±0.06 ^{b}	2.15 ±0.16^{a}	0.62 ±0.06 ^{b}	86.4 ±1.16^{a}	19.2 ±1.68^{a}	0.44 ±0.11^{b}	1.91 ±0.06^{b}

Means in the same column having different letters are significantly different at (p≤ 0.05).

Table 5: *Serum biochemical findings of examined C. gariepinus fish at third month of collection from the three different locations (El-Teraa, El-Berka, El-Rashah).*

Parameters / Groups	Control	A (El-Teraa)	B (El-Berka)	C (El-Rashah)
ALT (U/L)	21.3 ±0.83 ^{b}	87 ±3.97^{a}	94.4 ±4.01^{a}	70.6 ±2.63^{a}
AST (U/L)	81 ±0.92 ^{c}	205.2 ±5.9^{ab}	211.4 ±6.58^{a}	191 ±3.98 ^{b}
Total bilirubin (mg/dl)	0.61 ±0.05^{c}	0.96 ±0.01^{a}	0.97 ±0.05^{a}	0.86 ±0.1 ^{b}
Direct bilirubin (mg/dl)	0.34 ±0.1^{c}	0.55 ±0.03^{a}	0.56 ±0.07^{a}	0.46 ±0.38^{b}
Indirect bilirubin (mg/dl)	0.26 ±0.07^{b}	0.41 ±0.02^{a}	0.42 ±0.05^{a}	0.39 ±0.12^{a}
Total protein (g/dl)	4.6 ±0.21^{a}	2.86 ±0.09^{b}	2.72 ±0.06^{b}	2.94 ±0.05^{b}
Albumin (g/dl)	2.2 ±0.1 ^{a}	1.03 ±0.04^{b}	0.75 ±0.06^{c}	1.04 ±0.03^{b}
Globulin (g/dl)	2.41 ±0.12^{a}	1.82 ±0.11^{b}	1.97 ±0.12^{b}	1.9 ±0.07 ^{b}
A/G ratio	0.9 ±0.03^{a}	0.58 ±0.05^{b}	0.39 ±0.05^{c}	0.55 ±0.03^{b}
Glucose (mg/dl)	78.13 ±0.69^{b}	93.6 ±1.28^{a}	94.4 ±1.32^{a}	91.6 ±1.07^{a}
Urea (mg/dl)	9.85 ±0.23^{d}	37 ±1.31^{b}	42.6 ±1.75^{a}	30.4 ±0.93^{c}
Creatinine (mg/dl)	0.29 ±0.09^{c}	0.47 ±0.02^{ab}	0.49 ±0.01^{a}	0.44 ± 0.02^{b}
Ammonia (mg/L)	1.5 ±0.07^{d}	2.36 ±0.02^{b}	2.54 ±0.03^{a}	2.18 ±0.01^{c}

Means in the same column having different letters are significantly different at (p≤ 0.05).

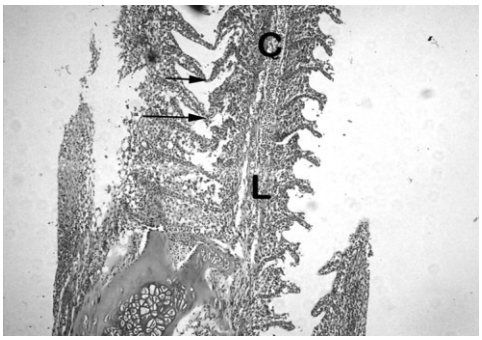
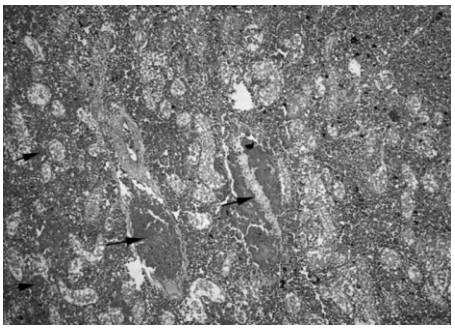


Figure 1: Gills, catfish exposed to 13.27 mg/L TAN at El-Berka showed epithelial hyperplasia, adhesion of secondary lamellae (arrows), congestion (C), mononuclear cells infiltration in primary and secondary lamellae (L). H&E. X 100.

Figure 2: Kidney, catfish exposed to 13.27 mg/L TAN at El-Berka showing diffuse congestion of blood vessel (arrows) necrotic change of melanomacrophages and degeneration of renal tubules. H&E. X 100.



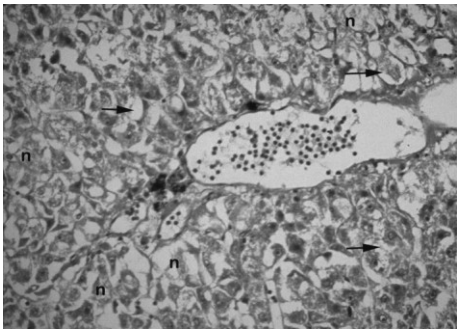
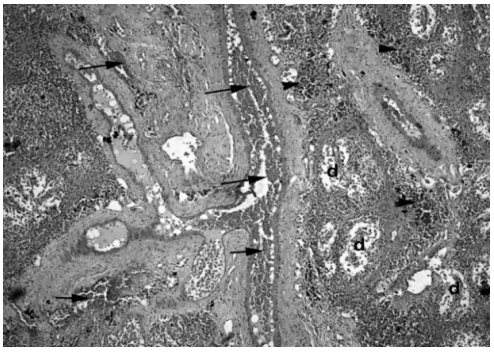


Figure 3: Liver, catfish exposed to 13.27 mg/L TAN at El-Berka showing vacuolated marked degeneration of hepatocyte (arrows), focal necrosis of some hepatic cells (n), and congestion of hepatic vessels. H&E. X 400.

Figure 4: Spleen, catfish exposed to 13.27 mg/L TAN at El-Berka showing congestion in splenic blood vessel (arrows), hyperactivation of the melanomacrophagecenters (arrow heads) with slight depletion of lymphoid follicles (d). H&E. X 100.



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الملخص العربى

دراسات باثولوجية اكلينيكية في أسماك القرموط الأفريقى المصابة بتسمم الأمونيا

أسامة علي محمد عبدالله - مني محمد عبدالوهاب- أمينة علي دسوقي- هايدي جلال عبدالرحمن- أسماء فؤاد ابراهيم فؤاد

-تتأثر الأسماك كأي كائن حي بالبيئة المحيطة بها فعندما يحدث خلل في أي من العوامل البيئية اللازمة لنموها فان ذلك ينعكس علي حياة وصحة هذه الأسماك ويسبب لها أضرارا وأمراضا يطلق عليها أسم الأمراض البيئية وهي عديدة ومتنوعة فمنها علي سبيل المثال التسمم بالأمونيا (مرض الخياشيم البيئي).
-تعد الأمونيا من الملوثات الشائعة في البيئة المائية و تدخل الي المجاري المائية من خلال المخلفات الصناعية والزراعية والمصارف الصحية. لذا فهي شائعة علي المستويين المحلي والعالمي ، لذا فان هذه الدراسة توضح الأثار الضارة المترتبة علي التلوث بالملوثات المائية في الأسماك.
-أجريت هذه الدراسة بمعمل الباثولوجيا الأكلينيكية بكلية الطب البيطري-جامعة قناة السويس.
-اشتملت الدراسة علي عدد ستون سمكة من أسماك القرموط الافريقي وتم تقسيمهم الي أربع مجموعات:
-المجموعة الضابطة (من العباسة بالشرقية)-المجموعة الأولي (من الترعة)- المجموعة الثانية (من البركة)-المجموعة الثالثة (من الرشاح). المجموعة الأولي والثانية و الثالثة متفرعين من ترعة محمد علي بالاسماعيلية المتفرعة من نهر النيل.
-الهدف من الرسالة دراسة الاختبارات الكيميائية وفحص أنسجة الأسماك المتعرضة لنسب عالية من الأمونيا وأسفرت النتائج عن الاتي:-
-بعد تسجيل التحاليل الفيزيوكيميائية للمياه التي تعيش فيها هذه الأسماك لوحظ وجود زيادة عالية في نسبة الأمونيا الموجودة في الماء مقارنة بالنسب الطبيعية المحددة للأسماك. كما أسفرت دراسة محتويات الدم الكيميائية الي نقص في نسب البروتين الكلي والزلال والجلوبيولين مع زيادة في نسبة انزيمات الكبد والبيليروبين والجلوكوز واليوريا والكرياتينين والأمونيا.
وقد أسفرت نتائج فحص الأنسجة (الخياشيم والكلي والكبد والطحال)عن كثير من التغيرات الباثولوجية نتيجة ارتفاع نسبة الأمونيا في البيئة المائية. هذا بالاضافة إلي نتائج الاختبارات الكيميائية في الدم أوضحت الكثير من التغيرات البيولوجية التي نجمت عن كثرة الملوثات في البيئة المائية الكائن بها تلك الأسماك محل البحث.