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POSSIBLE EFFECTS OF FEEDING FISH THE DRIED-TREATED SEWAGE:
II- CONCERNING BLOOD PROFILE AND HISTOLOGICAL STRUCTURE OF THE LIVER

Abdelhamid A. M.*, A. I. Mehrim*; A. A. M. Abdel Hamid and M. A. Alkatan***

***Department of Animal Production, Faculty of Agriculture, Al-Mansoura University, and**

****Department of Histology and Cell Biology, Faculty of Medicine, Al-Mansoura University, Egypt**

ABSTRACT

In a field study conducted for 102 days at feeding period, using a polyculture system (Nile tilapia, silver carp, common carp, and African catfish at a rate 1: 1: 1: 1) in two Hapas, one for control fish fed a commercial diet and the second one fish were fed a dried sewage sludge (DSS, product of treating sanitary and agricultural drainage of Al-Reiad, Kafr El-Sheikh, Egypt). At the end of the experiment, blood samples were withdrawn and liver samples were taken from each fish species for hematological, biochemical and histological examinations. From the obtained results, it was clear that DSS feeding did not negatively affect the blood picture in general, although the significantly elevated values of transaminases activity and triglycerides concentration. DSS feeding led to some drastic histological alterations in the liver structure. Conclusively, and because of the presence of some pollutants from agricultural and urban drainages whether in the rearing water or in the sewage sludge that can negatively affect fish health, production, and quality as well as could be inter the food chain and threat human health; so, it is recommended to give more concern on food and water quality (environmental friendly) used in aquaculture to offer safe products for human consumption.

Keywords: Fish,Sewage sludge, Blood parameters, Histology.

INTRODUCTION

Industrial effluents are major source of water pollution besides sewage, agricultural discharges and other household residues. The chief source of contaminants are the industrial waste discharge, mining, agriculture, household waste disposal and fuel combustion (Swarup*et al.,* 2006; Saxsena and Garg, 2010), where aquaculture is totally based on aquatic ecosystem. In addition, organic sewage, including fecal debris, which may contain large populations of bacteria (Dudley *et al.,* 1980).Most of the Egyptian wild (marine, brackish, and fresh) water bodiesis contaminated with agricultural, industrial, and urban drainage, which is responsible for their water and fish pollution with different heavy metals (Abdelhamid*et al*., 2006 and 2013a&b) at levels exceeding the Egyptians' tolerance limits (ES, 1993 and Abdelhakeem *et al.,* 2002). Heavy metals polluted water is causative for ca. thirty-two danger disease (Abdelhamid, 2006). So, many intoxications could be occurred in humans consume fish reared under polluted water conditions (Abdelhamid and El-Ayouty, 1991; Shata,1996 and Abdelhamid*et al*., 1999)

or fed contaminated diets (Abdelhamid, 1983 and Abdelhamid*et al*., 1996).This year (2014) will be a milestone year where the per capita consumption of farmed fish will be greater than wild fish consumption (Koeleman, 2014).

Different wastes are frequently used in fish feeding (Abdelhamid, 2014a) from plant, animal and/or variable sources (Abdelhamid*et al*., 2012a&b; Ibrahim *et al*., 2012; Khalafalla*et al*., 2012; Abd El-Hakim *et al*., 2013; Abdelhamid and Soliman, 2013 and Abou-Zied*et al*., 2013). Plant and animal wastes are used too as organic fertilizers for fish ponds (Agouz and Gomha, 2011 and Abdelhamid, 2014b). In this respect, sewage sludge may be used in aquaculture. However, the use of untreated night soil as a fertilizer as a source of nutrients in fish farming presents a considerable health hazard in the form of pathogens and parasites. Moreover, fish ponds receiving nutrients derived from treated night soil were less contaminated than ones to which untreated night soil was applied, and the fish reared in them were of superior quality (Ling *et al*., 1993). In addition,fish are used as excellent indicator of aquatic pollution due to their high sensitivity to environmental contaminants which may damage certain physiological and biochemical processes when contact with the organs of fishes (Saravanan*et al*., 2011).So, the aim of the present study was to investigate the effects of feeding dried sewage sludge (DSS) on hematological, blood biochemical parameters and histological structure of the liver of four fish species (Nile tilapia, common carp, silver carp, African catfish), for 102 days.

MATERIALS AND METHODS

The experimental management:

This study was conducted during the summer season in a private fish farm at Tolompat 7, Alriad, Kafr El-Sheikh governorate, Egypt. Nile tilapia, silver carp and African catfish were purchased from a private fish farm, Kafr El-Sheikh governorate, Egypt. While, common carp were purchased from Integrated Fish Farm at Al-Manzala (General Authority for Fish Resources Development, Ministry of Agriculture) Al-Dakahlia governorate, Egypt. Fish were stocked into a net Hapa for two weeks as an adaptation period, during which they were fed a basal diet.

Fish were distributed into two experimental treatments (in 2 net Hapas); in the first treatment (the control group), fish were fed the basal diet, whereas in the second treatment, fish were fed DSS. Each Hapa (8 × 3 × 1 m) were constructed and implanted in an earthen pond (irrigated from agricultural drainage). Four fish species were distributed with an average initial body weight of Nile tilapia,*Oreochromisniloticus* 178.0 ± 3.5 g, common carp,*Cyprinuscarpio*232.0 ± 2.7 g, silver carp,*Hypophthalmichthysmolitrix*344.0 ± 4.3 g and African catfish,*Clariasgariepinus*408.0 ± 3.2 g). Stocking density was 100 fish / Hapa at a rate of 1: 1: 1: 1 for each fish species. Throughout the experimental period,water quality parameters in each Hapa were measured weekly, including temperature (via a thermometer), pH-value (using Jenway Ltd., Model 350-pH-meter, Staffordshire ST15 0SA, UK) and dissolved oxygen (using Jenway Ltd., Model 970-dissolved oxygen meter, Staffordshire ST15

0SA, UK). Average values of water temperature were 25.0 ± 3.0 ºC, pH 8.80 and dissolved oxygen 2.66ppm.

The basal diet was purchased from the local market. This commercial diet contained yellow corn, soybean meal (44%), wheat bran, fish meal (65%), corn gluten (60%), lime stone, common salt, dicalcium phosphate, and molasses and had not less than 25% crude protein, 3% crude lipids, 3935 Kcal gross energy / Kg diet, and not more than 5.30% crude fiber, according to the manufacture's formula.

The treated DSS was obtained from the duple stage treatment project (Sanitary Drainage Station Al-Riad city, Kafr El-Sheikh governorate, Egypt). The tested diet and DSS were offered once daily (10:00 am) at 5% of the fish biomass at each Hapa. The feed quantity was adjusted each 21 days according to the actual body weight changes. The chemical analysis for the basal diet (control) and DSS was illustrated in Table 1. DSS seems to be CP-richer than the control diet; yet, DSS contains very high percentage of ash and very low percentages of EE and total carbohydrate percentages comparing to the control diet.

Table 1: Chemical composition of the tested commercial diet and dried sewage sludge

Composition	% on dry matter basis	
	Control (C) diet	Dried sewage sludge (DSS)
Dry matter, DM	92.75	92.12
Crude protein, CP	25.75	30.21
Ether extract, EE	3.56	0.89
Ash	6.42	41.54
Total carbohydrate	64.27	27.36

Blood analyses:

At the end of the experiment**,** blood samples were collected (in heparinized-evacuated test tubes) from the caudal vein of five fish / species / treatment and immediately sent to a private clinical chemistry lab. at Al-Riad city, Kafr El-Sheikh governorate, Egypt for hematological (using Swelab®Alfa Auto counter, USA) and blood biochemical parameters (using commercial kits).

Histological examination:

At the end of the experiment, samples of livers were taken from three fish / species / treatment, trimmed and fixed (preserved) in 10% phosphate buffered formalin (saline solution) for 4 days. Washed with tap water for 24 hours, and then dehydrated in ascending grade of ethyl alcohol, cleared in xylol, embedded for 2 hours in 2 changes of paraffin wax (melting point 56 °C) and sectioned at 5 microns thickness, stained with enriches acid haematoxylin, and eosin stains (H&E), mounted in balsam, then examined microscopically (Roberts, 2001).

Statistical analysis:

Numerical data collected were statistically analyzed using SAS (2001) software package (version 9.2) to detect the overall effects of treatments (T_1-T_2). All percentages were arcsine-transformed prior to statistical analyses. The differences between mean of treatments were compared using Duncan's post hoc significant test (Duncan, 1955), and differences were considered statistically significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Blood analysis:

Regardless of fish species, only RBCs, WBC and PLT reflected significant differences between both dietary treatments (Table 2) with higher values for DSS fed fish. However, Cf gave significantly higher values for most of the hematological parameters referring to best tolerance among the studied fish species (Table 2). Except MCV, all other hematological parameters reflected significant interaction effects (Table 3), where the highest Hb and MCV values were of Cf fed the control diet, but DSS fed Cf gave the highest MCHC and WBCs, Cf fed DSS had also the highest RBCs and PCV, and control fed tilapia only had the highest MCH value. Regardless of fish species, blood creatinine concentration, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity were significantly increased with feeding fish DSS (Table 4) comparing with those fed the control diet. Regardless of dietary treatments, Cc gave significantly the highest values of urea, creatinine. AST and ALT; whereas, Sc gave significantly the lowest values for urea, AST and ALT (Table 4). That means that both of Cc and Sc were more influenced with feeding DSS (which affected both liver and kidney functions) than T and Cf. The interaction (dietary treatment × fish species) effect on urea, creatinine, AST and ALT was significant ($P \leq 0.0001$), and except urea, all other parameter's levels (creatinine, AST and ALT) were significantly at highest for Cc fed DSS; yet, control Cc gave the significantly highest urea concentration (Table 5).

Table 2: Effect of dietary treatment and fish species on some hematological parameters (hemoglobin, Hb; red blood cells, RBCs; packed cell volume, , PCV; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC; white blood cells, WBCs and platelet, PLT)

	Hb g/dl	RBCs $\times 10^6$/mm^3	PCV%	Blood indices			WBCs $\times 10^3$/mm^3	PLT $\times 10^3$/mm^3
				MCV (μ^3)	MCH (pg)	MCHC (%)		
Treatments								
Control diet	12.39	1.84^{b}	33.35	171.84	50.96	34.22	45.06^{b}	41.95^{b}
Dried sewage sludge	12.19	2.01^{a}	34.46	170.22	51.87	36.57	57.64^{a}	76.91^{a}
± SE	0.395	0.031	0.557	3.183	0.904	1.447	1.379	1.923
P- value	0.726	0.0005	0.174	0.722	0.484	0.261	0.0001	0.0001
Species								
Tilapia (T)	11.76^{b}	1.87^{c}	33.35^{c}	179.0^{ab}	61.93^{a}	33.63^{b}	47.33^{c}	47.66^{c}
Silver carp (Sc)	11.66^{b}	2.18^{b}	37.86^{b}	168.2^{b}	48.60^{c}	32.22^{b}	28.85^{d}	36.20^{d}
Common carp (Cc)	12.16^{ab}	1.03^{d}	21.29^{d}	182.4^{a}	41.50^{d}	43.20^{a}	58.70^{b}	69.41^{b}
Catfish (Cf)	13.57^{a}	2.62^{a}	43.12^{a}	154.5^{c}	53.65^{b}	32.53^{b}	70.52^{a}	84.45^{a}
± SE	0.559	0.044	0.788	4.501	1.278	2.046	1.950	2.720
P- value	0.035	0.0001	0.0001	0.0008	0.0001	0.002	0.0001	0.0001

Mean values in the same column for each category superscripted with different letters differ significantly.

Table 3: Effect of interaction between dietary treatment and fish species on some hematological parameters (hemoglobin, Hb; red blood cells, RBCs; packed cell volume, PCV; mean corpuscular volume, MCV; mean corpuscular hemoglobin, MCH; mean corpuscular hemoglobin concentration, MCHC; white blood cells, WBCs and platelet, PLT)

Treatment	Hb g/dl	RBCs $\times10^6$/mm^3	PCV %	Blood indices			WBCs $\times10^3$/mm^3	PLT $\times10^3$/mm^3
				MCV (μ^3)	MCH (pg)	MCHC (%)		
C* T	10.96^{ab}	1.70^{b}	31.20^{c}	182.5^{ab}	63.86^{a}	35.23	42.10^{b}	41.00^{b}
C * Sc	10.30^{b}	1.82^{b}	35.10^{b}	167.0^{bc}	45.80^{bc}	33.10	28.30^{c}	34.66^{b}
C * Cc	14.52^{a}	1.20^{c}	26.32^{d}	186.2^{a}	43.00^{c}	36.50	40.33^{b}	38.50^{b}
C * Cf	13.76^{ab}	2.62^{a}	40.80^{a}	151.6^{c}	51.20^{b}	32.06	69.53^{a}	53.66^{a}
S* T	12.56^{b}	2.03^{b}	35.50^{c}	175.4	60.00^{a}	32.03^{b}	52.56^{b}	54.33^{c}
S* Sc	13.02^{a}	2.55^{a}	40.62^{b}	169.4	51.40^{b}	31.35^{b}	29.40^{c}	7.75^{d}
S* Cc	9.80^{c}	0.85^{c}	16.26^{d}	178.6	40.00^{c}	49.90^{a}	77.06^{a}	100.3^{b}
S* Cf	13.37^{a}	2.62^{a}	45.45^{a}	157.4	56.10^{ab}	33.00^{b}	71.52^{a}	15.2^{a}
± SE	0.791	0.062	1.115	6.366	1.808	2.894	2.758	3.847
P- value	0.0005	0.0001	0.0001	0.644	0.021	0.033	0.0001	0.0001

Mean values in the same column superscripted with different letters differ significantly.

Table 4: Effect of dietary treatments and fish species on kidney and liver functions parameters (blood urea concentration, mg/dl; creatinine concentration, mg/dl; aspartate aminotransferase activity, AST, u/l; and alanine aminotransferase activity, ALT, u/l)

	Urea	Creatinine	AST	ALT
Treatments				
Control diet	13.48	0.51^{b}	50.02^{b}	49.43^{b}
Dried sewage sludge	12.83	0.66^{a}	98.72^{a}	102.2^{a}
± SE	0.354	0.034	2.572	5.566
P- value	0.203	0.003	0.0001	0.0001
Species				
Tilapia (T)	11.00^{bc}	0.60^{ab}	62.52^{b}	62.38^{b}
Silver carp (Sc)	9.76^{c}	0.60^{ab}	42.23^{c}	44.60^{b}
Common carp (Cc)	20.36^{a}	0.65^{a}	141.9^{a}	141.7^{a}
Catfish (Cf)	11.50^{b}	0.49^{b}	50.82^{c}	54.58^{b}
± SE	0.500	0.048	3.638	7.872
P- value	0.0001	0.137	0.0001	0.0001

Mean values in the same column for each category superscripted with different letters differ significantly.

Table 5: Effect of interaction between dietary treatments and fish species on kidney and liver functions parameters (blood urea concentration, mg/dl; creatinine concentration, mg/dl; aspartate aminotransferase activity, AST, u/l; and alanine aminotransferase activity, ALT, u/l)

Treatment	Urea	Creatinine	AST	ALT
C* T	11.20b	0.68a	37.52b	53.72ab
C * Sc	8.60b	0.48b	32.80b	30.56b
C * Cc	22.92a	0.44b	80.09a	70.12a
C * Cf	11.20b	0.44b	49.68b	43.32b
S* T	10.80b	0.52b	87.52b	71.05b
S* Sc	10.92b	0.72ab	51.66c	58.64b
S* Cc	17.80a	0.86a	203.7a	213.4a
S* Cf	11.80b	0.54b	51.96c	65.84b
± SE	0.708	0.068	5.145	11.13
P- value	0.0001	0.001	0.0001	0.0001

Mean values in the same column superscripted with different letters differ significantly.

Regardless of fish species, fish feeding DSS caused significantly higher globulin, but lower albumin and albumin / globulin ratio values. Regardless of dietary treatment, T blood contained the highest total protein, globulin and albumin (Table 6). The interaction effect confirmed that the significantly highest total protein and globulin concentrations were realized with T fish fed DSS, but the significantly highest albumin and albumin / globulin ratio values were found in T fish fed the control diet (Table 7).

Table 6: Effect of dietary treatments and fish species on plasma protein concentrations

	Total protein (g / dl)	Globulin (g / dl)	Albumin (g / dl)	Albumin / Globulin ratio
Treatments				
Control diet	4.36	1.43^{b}	2.93^{a}	2.08^{a}
Dried sewage sludge	4.25	1.82^{a}	2.43^{b}	1.33^{b}
± SE	0.123	0.043	0.108	0.069
P- value	0.550	0.0001	0.002	0.0001
Species				
Tilapia (T)	5.33^{a}	2.00^{a}	3.33^{a}	1.86
Silver carp (Sc)	3.35^{b}	1.32^{b}	2.03^{b}	1.57
Common carp (Cc)	3.57^{b}	1.32^{b}	2.25^{b}	1.73
Catfish (Cf)	4.98^{a}	1.87^{a}	3.11^{a}	1.66
± SE	0.174	0.061	0.153	0.097
P- value	0.0001	0.0001	0.0001	0.205

Mean values in the same column superscripted with different letters differ significantly.

Table 7: Effect of the interaction between dietary treatments and fish species on plasma protein concentrations

Treatment	Total protein (g / dl)	Globulin (g / dl)	Albumin (g / dl)	Albumin / Globulin ratio
C* T	4.94^{a}	1.44^{b}	3.50^{a}	2.46^{a}
C * Sc	3.36^{b}	1.20^{c}	2.16^{c}	1.82^{bc}
C * Cc	3.98^{b}	1.20^{c}	2.78^{b}	2.29^{ab}
C * Cf	5.16^{a}	1.88^{a}	3.28^{ab}	1.74^{c}
S* T	5.72^{a}	2.56^{a}	3.16^{a}	1.26^{ab}
S* Sc	3.34^{c}	1.44^{c}	1.90^{b}	1.31^{ab}
S* Cc	3.16^{c}	1.44^{c}	1.72^{b}	1.17^{b}
S* Cf	4.80^{b}	1.86^{b}	2.94^{a}	1.58^{a}
± SE	0.246	0.087	0.217	0.138
P- value	0.020	0.0001	0.234	0.001

Mean values in the same column superscripted with different letters differ significantly.

Data of liver function criteria as total cholesterol, triglyceride, HDL and LDL are given in Tables 8 and 9. Regardless of fish species, feeding fish DSS resulted in significantly higher values of triglyceride, HDL and LDL comparing with those fed the control diet. Regardless of the dietary treatment, T fish represented the significantly highest concentration of both triglyceride and HDL but Cf contained the significantly highest levels of total cholesterol, HDL and LDL (Table 8). The significant interaction effect (Table 9) revealed that the highest total cholesterol and LDL concentrations were found with control Cf, but T and Cf fed DSS gave the highest triglyceride and HDL levels, respectively.

Table 8: Effect of dietary treatments and fish species on concentrations of some blood biochemical parameters (mg/dl) (HDL: high density lipoprotein; LDL: low density lipoprotein)

	Total cholesterol	Triglyceride	HDL	LDL
Treatments				
Control diet	168.9	108.1^{b}	86.05^{b}	62.10^{b}
Dried sewage sludge	172.4	158.0^{a}	96.10^{a}	38.95^{a}
± SE	7.927	6.327	6.325	6.155
P- value	0.756	0.0001	0.269	0.012
Species				
Tilapia (T)	177.0^{ab}	206.4^{a}	104.1^{a}	34.30^{b}
Silver carp (Sc)	165.2^{bc}	107.8^{c}	88.1^{ab}	50.70^{b}
Common carp (Cc)	134.0^{c}	138.3^{b}	68.2^{b}	36.90^{b}
Catfish (Cf)	206.6^{a}	79.70^{d}	103.9^{a}	80.20^{a}
± SE	11.21	8.948	8.945	8.705
P- value	0.0008	0.0001	0.023	0.002

Mean values in the same column superscripted with different letters differ significantly.

Table 9: Effect of the interaction between dietary treatments and fish species on concentrations of some blood biochemical parameters (mg/dl) (HDL: high density lipoprotein; LDL: low density lipoprotein)

Treatment	Total cholesterol	Triglyceride	HDL	LDL
C* T	144.8^{b}	126.0^{b}	102.4^{a}	22.20^{c}
C * Sc	170.0^{b}	70.8^{c}	72.00^{b}	68.60^{b}
C * Cc	140.2^{b}	177.0^{a}	76.80^{ab}	32.20^{bc}
C * Cf	220.8^{a}	58.6^{c}	93.00^{ab}	125.4^{a}
S* T	209.2^{a}	286.8^{a}	105.8^{ab}	46.40
S* Sc	160.4^{ab}	144.8^{b}	104.2^{ab}	32.80
S* Cc	127.8^{b}	99.6^{b}	59.60^{b}	41.60
S* Cf	192.4^{a}	100.8^{b}	114.8^{a}	35.00
± SE	15.85	12.65	12.65	12.31
P- value	0.029	0.0001	0.240	0.0002

Mean values in the same column superscripted with different letters differ significantly.

Hematological changes were detected in fish exposed to pollutants in laboratory studies. The present findings revealed that DSS feeding did not negatively affect the hematological parameters. Inversely with our results, the thrombocyte fraction significantly reduced in dabs (*Limandalimanda*) exposed to sewage sludge (Secombes*et al.,* 1991 & 1992). Also,goldfish (*Carassiusauratus*) exposed to 50% raw and 100% treated sewages on day 30, showed lower values of red blood cells, granulocytes and lymphocytes counts, activity of phagocytic cells, Hb, and plasma protein concentrations than those of the control group. Similar changes in blood parameters were observed in gold fish exposed to 5% raw and 10% treated sewages for 30 days (Kakuta, 1997).

The measurement of suitable biomarkers in liver becomes useful and can give an idea about the health state of fish. Toxicological studies have shown that the impact of contaminants on aquatic ecosystems can be evaluated by measuring biochemical parameters in the liver of the fish that respond specifically to the degree and type of contamination (Barhoumi*et al*., 2012). The transaminases, AST and ALT are two key enzymes considered as

a sensitive measure to evaluate hepatocellular damage and some hepatic diseases (Ibrahim and Mahmoud, 2005). In different fish species including *C. gariepinus*, AST and ALT enzymes activity were found to increase in response to heavy metals (Mekkawy*et al*., 2011). An increase in plasma AST and ALT activities due to metals (Zn, Cu and Cd) was also found in experimental conditions (Zikic*et al*., 2001), as well as in fish chronically exposed to metals (Levesque *et al*., 2002). In addition, the present findings coincide with the reported histopathoiogical lesions, which revealed a marked degeneration and necrosis of hepatocytes as the elevation in transaminases activities may be attributed to liver injury (Aly*et al*., 2003).

In this context, a study investigated the levels of PCBs (polychlorinated biphenols) in the sludge cake and in tilapia, *O. mossambicus* fed on sludge supplemented diet, and the biochemical responses in the liver of tilapia fed the diet. A significant increase in the PCB concentrations was found in the flesh of fish fed on sludge (30%) supplemented diet. Total PCBs was detected in the fish and higher chlorinated PCB isomers were much more common than lower chlorinated PCB isomers. The sludge was found to be contaminated with PCBs. The sludge - supplemented diets did not impose any effect on both ALT and AST activities up to 30 days. However, the liver AST activity was significantly increased ($P < 0.05$) in fish receiving 10 and 30% of sludge-supplemented diets while ALT activity was significantly decreased ($P < 0.05$) in fish receiving 30% of sludge- supplemented diet only, when compared with the control group, at the end of the experiment. The level of triglyceride of the treated fish was not significantly different ($P > 0.05$) from fish fed the control diet. The changes in liver metabolism in fish fed with sludge diets indicated the existence of xenobiotics (Yang *et al*., 1993).

Exposure to sewage sludge also resulted in a decrease in total serum proteins (Secombes*et al.,* 1992) which agrees with the present findings on the experimental fish species exposed to dried sewage sludge. Serum albumin and globulin have been used as indicators of healthy status of fish and considered as important indicators for the effect of pollutants in fish (Tayel*et al*., 2007). Protein is also one of the important biochemical parameters which have been used to understand the general state of health and biological mechanism of metabolism under pollutant stress (Saravanan*et al*., 2011). During stress conditions fish need more energy to detoxify the toxicant and to overcome stress. So, due to this, proteins in liver degrade and the serum protein level increase. Reddy *et al.* (1995) and Singh and Sharma (1998) reported decline in protein constituent in liver and increase in serum in different fish under stress of pollutants. Results of the present study revealed increased values of protein, albumin, globulin and A/G ratio in blood of experimental fish species. In the present study, the increase of these parameters goes in parallel with the elevation in the levels of water parameters studied and heavy metals concentrations as a result of pollution stress (Tayel*et al*., 2007).

Fish exposure to chemical contaminants induces lesions in different target organs, especially in liver (Rabitto*et al*., 2005; Mela*et al*., 2007; Miranda *et al*. 2008). According to Aya*set al*. (2007) and Lemes and Braccini (2004), liver is an important target organ related to important metabolic and

detoxification mechanisms. Data of liver function criteria as total cholesterol, triglyceride, HDL and LDL is given in Tables 8 and 9. Regardless of fish species, feeding fish DSS resulted in significantly higher values of triglyceride, HDL and LDL comparing with those fed the control diet. Regardless of the dietary treatment, T fish represented the significantly highest concentration of both triglyceride and HDL but Cf contained the significantly highest levels of total cholesterol, HDL and LDL (Table 8). The significant interaction effect (Table 9) revealed that the highest total cholesterol and LDL concentrations were found with control Cf, but T and Cf fed DSS gave the highest triglyceride and HDL levels, respectively.

Exposure to sewage sludge has been implicated with effects on growth and protein synthesis in common dab (*Limandalimanda*) (Houlihan*et al.,* 1994). In addition, liver damage in fish has been associated with contamination by components of sewage sludge (Moore *et al*., 1996). Generally, increased Hb and RBC_s are found with dehydration as a consequence of pseudopolyglobulie; but increased WBC_s with bacterial infection and inflammatory reactions (Merck, 1976). Moreover, increased blood ammonia-N causes ataxia and death (Clarke and Clarke, 1978). Also, increased blood urea may occur in a number of diseases in addition to those in which the kidneys are primarily involved. Increased blood protein because of the hemoconcentration, so that the albumin-globulin ratio remains unaltered. Apart from these cases an increase in albumin occurs very rarely indeed, so that with very few exceptions an increase in total proteins arises only when there is an increase in one or more of the globulin components; the albumin either remains normal or is reduced to a smaller extent than the globulin is increased. As a result the albumin-globulin ratio falls. A decrease in total proteins is always due to a low albumin level or to edema. Low serum albumin may be due to decreased formation in the liver because of severe liver disease. Increases in cholesterol occur in nephritis (Varley, 1978).Also,Sweilum (2006) found that sub lethal doses of mercuric chloride negatively affected blood profile of tilapia species (*O. niloticus*, *O. aureus,* and *Sarotherodongalilaeus*). But, El-Saidy*et al*. (2012) working with *O. niloticus* registered higher total protein and globulin but lower albumin and albumin / globulin ratio than the values obtained herein. Additionally, Metwalli (2013) gave higher levels of total protein, albumin, creatinine and AST but lower values of urea and ALT for Nile tilapia than given in the present research.

Histological examination:

The present histological findings of *O. niloticus* fed the control diet showed that normal hepatocytes and enlargement, slight congestion and infiltration of the blood vessel (Bv)(Fig. 1a). However, the histological examination of liver of *O. niloticus* fed DSS showing necrotic hepatocytes and enlargement, severe congestion and infiltration of the portal blood vessel (PBV) (Fig. 1b).Figure 1c illustrated that *H.molitrix* fed the control diet showed normal hepatic lobules with normal hepatocytes and diffusion of melanomacrophage centers (MMCs). While, *H. molitrix* fed DSS showed

large areas of necrotic hepatocytes, infiltration of BV, areas of degenerated hepatocytes (Fig. 1d).

Microscopically, *C.carpio* fed the control diet showed normal hepatic lobules with normal hepatocyte arrangement around the central vein (CV) adjacent with blood sinusoids (Fig. 1e). Meanwhile, *C. carpio* fed dried sewage sludge showed severe necrotic hepatocytes and severe congestion, enlargement of PBV with monocytes, fibroblast infiltration and large area of degenerated hepatocytes (Fig. 1f).

Under present histological investigation, it has been observed that the liver of*C. gariepinus* fed the control diet showed normal hepatic lobules with normal hepatocyte arrangement around CV, adjacent with blood sinusoids, slight congestion of BV (Fig. 1g). However, *C. gariepinus* fed DSS showed large areas of degenerated hepatocytes around CV and diffusion of hemosiderin in the hepatic lobule (Fig. 1h). In addition, highly magnification (Fig. 1i) for the same treatment of catfish showed that large areas of degenerated hepatocytes and diffusion of hemosiderin in the hepatic lobule.

The slightly drastic effects on liver tissues in all the experimental fish species fed the control diet were detected, which were markedly increased in all fish species fed DSS. These severe alterations in the hepatocytes were confirmed by potential negative effects on hematological (Tables 2 and 3regarding to the dietary treatments, fish species and their interaction) or blood biochemical parameters(Tables 4, 5, 6, 7, 8 and 9 regarding to the dietary treatments, fish species and their interaction) in both fish fed the control diet or treated by DSS.

The fish liver plays an important role in vital functions in basic metabolism and it is the major organ of accumulation, biotransformation and excretion of contaminants in fish (Figueiredo-Fernandes*et al*., 2006). Also, the liver histology is used as biomarker for the environmental pollution (El-Serafy*et al*., 2009) and there have been numerous reports of histopathological changes in livers of fish exposed to a wide range of organic compounds and heavy metals (Au, 2004; Abdel-Moneim*et al*., 2012). So, it is not surprised that, the present harmful effects of all experimental fish species fed the control diet; concerning the hematological, blood biochemical parameters or histological structure of the liver may be related to fish rearing water quality in the experimental site, (Tolompat 7, Alriad, Kafr El-Sheikh governorate, Egypt). Where, the agriculture drained water was polluted with different types of pesticides, fertilizers and some heavy metal's residues not only in this fish farm, but also in all fish farms and fish hatcheries in Egypt according to the dishonorable Egyptian law No. 124/1983. Currently, this law prohibits aquaculture projects from drawing surface water, leaving more than 90% of the country's fish farms and fish hatcheries to operate on polluted agricultural drainage water. In addition, Vos*et al*. (2000) and Kolpin*et al*. (2002) reported that sewage effluents contain complex mixtures of chemicals such as natural and synthetic hormones, alkyl phenols, phthalates, bisphenolA, pharmaceuticals and some pesticides. So, these drastic effects of all experimental fish species fed the dried sewage sludge in the present study, may be due to the organic pollutants (PCBs, PBDEs and HBCDs) contamination in sludge, sediments and fish (Ilyas*et al*., 2013), as well as may be due to the long exposed time to the dried sewage sludge (102 days) in the present study. In addition, Moore *et al*. (1996) reported that exposure to sewage sludge has caused liver damage in fish.

Fig. 1 (a-i):Cross section in liver of (a): *O. niloticus* fed the control diet showing normal hepatocytes (h) and enlargement, slight congestion and infiltration (arrow) of Bv; (b): *O. niloticus* fed DSS showing necrotic hepatocytes and enlargement, severe congestion (arrows heads) and infiltration (arrow) of PBV; (c): *H. molitrix* fed the control diet showing normal hepatic lobules with normal hepatocytes and diffusion of MMCs (arrows); (d): *H. molitrix* fed DSS showing large areas of necrotic (N) hepatocetes, infiltration (arrow) of BV and areas of degenerated hepatocytes (arrows heads); (e): *C. carpio* fed the control diet showing normal hepatic lobules with normal hepatocyte (h) arrangement around CV adjacent with blood sinusoids (S); (f): *C. carpio* fed DSS showing severe necrotic (N) hepatocytes and severe congestion, enlargement of PBV with monocytes, fibroblast infiltration (arrows heads) and large area of degenerated hepatocytes (arrows); (g): *C. gariepinus* fed the control diet showing normal hepatic lobules with normal hepatocyte arrangement around CV, adjacent with blood sinusoids (S) and slight congestion of BV (arrow); (h): *C. gariepinus* fed DSS showing large areas of degenerated hepatocytes (arrows) around CV and diffusion of hemosiderin in the hepatic lobule (arrows heads); (i): High magnification of (Fig. h) showing large areas of degenerated hepatocytes (arrows) and diffusion of hemosiderin in the hepatic lobule (arrows heads); a, b, d, e, f, g, h (× 100, H&E stains); c and i (× 400, H&E stains); Bv: blood vessel; PBV: portal blood vessel; MMCs: melanomacrophage centers; CV: central vein.

Egyptian drains receive large quantities of partially treated or untreated domestic and industrial wastewater and other human activities, which in turn ultimately discharge into River Nile, canals, lakes, or seas (El-Sheikh *et al*., 2010). The amount of waste discharged into receiving water bodies far exceeds the natural ability of these bodies to attenuate the pollutants (El-Sheikh *et al*., 2010) with dramatic consequences on water quality, sediments and biotic communities (Azzurro*et al*., 2010). The domestic wastewaters contain fairly high concentrations of metals (Authman*et al*., 2013), where these metals are derived from household products such as cleaning materials (detergents), toothpaste, cosmetic and human feces (Stephenson, 1987). Also, there are additional quantities introduced from industrial wastes (Alabaster and Lloyd, 1982) and washing of herbicides and pesticides of the agricultural lands (Khallaf*et al*., 1998).

The liver not only represents a central organ concerning basic metabolism (Gingerich, 1982), but is also a major site of the accumulation, biotransformation and excretion of toxic compounds (Meyers and Hendricks, 1985). It is the first organ to be exposed by the portal circulation to toxicants ingested by the body (Hibiya, 1982). Because of its unique position and proximity to the venous drainage of the digestive tract, the liver is susceptible to damage from absorbed toxic materials (Leeson and Leeson, 1976). The high degree of metabolic activity of hepatocytes renders them vulnerable and toxins can easily affect them. The harmful effects of ingested toxic substances are primarily exerted within the liver cells (Lloyd, 1992). Subsequently, hepatocytes respond to changes in the external and internal environments by alterations in both cellular structure and function (Wheater*et al.,* 1985). Since histopathological alterations are recognized and commonly used diagnostic tools in fish toxicological studies (Lloyd, 1992). Moreover, Gonzales *et al*. (1993) reported that necrosis found in the fish liver is usually related to contaminants found in water or sediment. Also, Khan *et al*. (1994) and Ayas*et al*. (2007) described that those lesions in liver of fish is one of the most important histopathological responses due to exposure to contaminants.

Moreover, brown trout *Salmotrutta*populations of numerous Swiss rivers are declining. Sewage plant effluents are discussed as a possible cause. To investigate the influence of sewage plant effluents, brown trout as well as rainbow trout *Oncorhynchusmy kiss* were exposed to 10% diluted wastewater. The effects were compared to those on trout kept in commercial tap water. Degenerative and inflammatory reactions in the liver of exposed animals were the most prominent findings. Several brown trout caught in the River Langete showed marked proliferative, degenerative and inflammatory lesions of gills, liver, and kidney (Schmidt *et al*., 1999).

Histopathological alteration on hepatocytes may be attributed to the direct toxic effects of pollutants, since the liver is the principal organ responsible for detoxification in vertebrates generally and in fish particularly (Freeman *et al*., 1983). The present study suggests a strong link between dietary dried sewage sludge and lesions in the liver. (Sorensen, 1991) cited that heavy metals were might cause liver damage. Aly*et al.* (2003) obtained similar results after exposure of *C. gariepinus*to lead pollution. Similar alterations in the liver of *Tilapia zillii*and*C. gariepinus*were observed in fishes

living in Nile water polluted with heavy metals (Ibrahim and Mahmoud, 2005 and Tayel*et al*., 2008). Yacoub and Abdel Sater (2003) observed histopathological effects of heavy metals on some fishes inhabiting Bardawil lagoon. Ptashynski*et al*. (2002) reported histopathological alteration after exposure of the lake whitefish (*Coregonusclupeaformis*) to nickel. Olojo*et al.* (2005) observed liver histopathological lesions after exposure of *C. gariepinus*to Pb for 9 days. Several histopathological changes in the liver were also observed in *O. niloticus*and *Tilapia zillii*collected from the southern region of Lake Manzalah contaminated with domestic, industrial and agricultural pollutants (Mohamed, 2001).

Histological biomarkers of toxicity in fish organs are a useful indicator of environmental pollution (Peebua*et al*., 2008). Several histopathological changes have been reported in the liver of experimental fish in response to agricultural, sewage and industrial pollutants (Mohamed, 2003). Similar results have been reported in *Channapunctatus*exposed to hexavalent chromium (Mishra and Mohanty, 2008), in *C. batrachus*exposed to 4 ppm and 8 ppm cadmium chloride for 90 days (Bilal, 2011), in *O. mossambicus*exposed to cadmium chloride at 5 and 50 ppm for 1, 7, 15 and 30 days (Rani and Ramamurthi, 1989), in *O. mossambicus*exposed to cadmium and zinc (Van Dyk*et al*., 2007), in *C. gariepinus*exposed to cadmium (Nuntiya*et al*., 2008), in liver of a freshwater catfish, *C. punctatus* exposed to 3 mg and 5mg cadmium chloride for 15 and 45 days (Amin *et al*., 2013) and in White Sea bass, *Latescalcarifer*exposed to 5 ppm cadmium chloride for 3 weeks (Thophon*et al*., 2003). These findings lend support to the observations of the present study. Additionally, several histopathological alterations, including vacuolar degeneration with focal areas of necrosis in liver were observed in the studied tissues of fish as a result of the accumulated metals (Mohamed, 2008).

The obtained results in the present study revealed that the feeding DSS led to slight effects on hematological parameters, but it had significantly drastic effects on blood biochemical parameters compared to the control group.Also, the harmful histological alterations in liver of all fish species fed DSS compared to the control group were detected.Consequently, no doubt these drastic effects related with the presence of some pollutants from agricultural and urban drainages whether in the rearing water or in the sewage sludge that can negatively affect fish health, production, and quality as well as could be inter the food chain and threat human health; so, it is recommended to give more concern on food and water quality (environmental friendly) used in aquaculture to offer safe products for human consumption.

REFERENCES

Abdelhakeem, N.F., Bakeer, M.N. and Soltan, M.A. (2002). Aquatic Environment for Fish Culture. Deposit No. 4774/2002.

Abdelhakeem, N.F.,Elgarhy, M.A. and Sayed, S.H. (2013).Studies of the use of fermented fish silsge in diets for all male Nile tilapia (*Oreochromisniloticus* Lin.). Egyptian J. Nutrition and Feeds, 16 (2): 501 (Summary).

Abdelhamid, A.M. (1983). Mykotoxin-Nachweis in Lebens-und Futtermitteln des subtropischenKlimas. In: Kurzfassungen der Vorträgezur 37. Tag.Ges.Ernährungsphysiol.Haustiere, Gőttingen, 5. Z. Tierphysiol., Tierernährug u Futtermittelkde., 50: 4-5.

Abdelhamid, A.M. (2006). Heavy metals pollution of the Egyptian aquatic media.The 2nd Inter. Sci. Con. For Environment "Recent Environmental Problems and Social Sharement", 28-30 March, South Valley University. Pp. 127 – 153.

Abdelhamid, A.M. (2014a). Fish Nutrition.An Arabic Textbook, Al-Mansoura Univ., 415p.

Abdelhamid, A.M. (2014b). The Blue Revolution (Pesciculture).An Arabic Textbook, Al-Mansoura Univ., 674p.

Abdelhamid, A.M. and El-Ayoty, S.A. (1991). Effect of catfish (*Clariaslazera*) composition of ingestion rearing water contaminated with lead or aluminum compounds. Arch. Anim. Nutr., Berlin, 41: 757 – 763.

Abdelhamid, A.M. and Soliman, A.A.A. (2013).Comparative evaluation for dietary inclusion of some medical plants by common carp fish. Egyptian J. Nutrition and Feeds, 16 (2): 485-499.

Abdelhamid, A.M., El-Barbary, M.I. and Mabrouk, E.M.E. (2013a).Some heavy metals status in Ashtoum El-Gamil Protected Area. Journal of the Arabian Aquaculture Society, 8 (1): 69-86.

Abdelhamid, A.M., El-Mansoury, A.M., Osman, A.I. and El-Azab, S. M. (1999). Mycotoxins as causative for human food poisoning under Egyptian conditions. J. Agric. Sci., MansouraUniv., 24: 2751 – 2757.

Abdelhamid, A.M., Gawish, M.M.M. and Soryal, K.A. (2006). Comparative study between desert cultivated and natural fisheries of mullet fish in Egypt, I- concerning heavy metals. J. Agric. Sci. Mansoura Univ., 31: 5665 – 5680.

Abdelhamid, A.M., Gomaa, A.H. and El-Sayed, H.G.M. (2013b).Studies on some heavy metals in the River Nile water and fish at Helwan area, Egypt. Egypt. J. Aquat. Biol. & Fish., 17 (2): 105 – 126.

Abdelhamid, A.M., Khalil, F.F. andRagab, M.A. (1996). Survey of aflatoxin and ochratoxin occurrence in some local feeds and foods. Proceeding of conference on Food Borne Contamination and Egyptian's Health, MansouraUniv., Nov. 26-27, pp: 43-50.

Abdelhamid, A.M.,Maghraby, N.A, Mehrim, A.I. and Soliman, A.A.A. (2012a).Impact of non conventional feedstuffs on growth performance and nutrients utilization of Nile tilapia fish. Egyptian J. Nutrition and Feeds, 15 (1 Special Issue): 349-357.

Abdelhamid, A.M.,Maghraby, N.A, Mehrim, A.I. and Soliman, A.A.A. (2012b).Physiological, biochemical and microbiological assessment for non-conventional feedstuffs by Nile tilapia fish. Egyptian J. Nutrition and Feeds, 15 (1 Special Issue): 359-366.

Abdel-Moneim, A.M., M.A. Al-Kahtani and O.M. Elmenshawy, (2012). Histopathological biomarkers in gills and liver of *Oreochromisniloticus* from polluted wetland environments, Saudi Arabia. Chemosphere, 88: 1028-1035.

Abou-Zied, R.M.,Hassouna, M.M.E., Mohamed, R.A. and Abdl-Aziz, M.F.E. (2013).Effect of some environmental conditions and addition of palm pollen grains on the reproductive performance of red hybrid tilapia (*Oreochromis* sp.). Egyptian J. Nutrition and Feeds, 16 (2): 463-468.

Agouz, H.M. and Gomha, A.A. (2011).Evaluation study for the impact of poultry drops, silkworm wastes and fresh rumen contents in the Nile tilapia culture. Journal of the Arabian Aquaculture Society, 6 (1): 33-48.

Alabaster, J.S. and Lloyd,R. (1982).Water quality criteria for freshwater fish. London: FAO by Butterworth Scientific.

Aly, S.M., Zaki,M.S. and El-Genaidy,H.M. (2003). Pathological, biochemical, haematological and hormonal changes in catfish (*Clariasgariepinus*) exposed to lead pollution. Journal of the Egyptian Veterinary Medical Association, 63(1): 331-342.

Amin, N., Manohar, S., Qureshi, T.A. and Khan, S. (2013). Effect of Cadmium Chloride on the Histoarchitecture of Liver of a Freshwater Catfish, *Channapunctatus*. J. Chem. Bio. Phy. Sci. 3 (3):1906-1911.

Au, D.W.T. (2004). The application of histocytopathological biomarkers in marine pollution monitoring: a review. Marine Pollution Bulletin, 48: 817-834.

Authman, M.M.N., Abbas,H.H. and Abbas,W.T. (2013). Assessment of metal status in drainage canal water and their bioaccumulation in *Oreochromisniloticus* fish in relation to human health. Environmental Monitoring and Assessment, 185(1): 891-907.

Ayas, Z., Ekmekci, G., Ozmen, M. and Yerli, S.V. (2007).Histopathological changes in the livers and kidneys of fish in Sariyar Reservoir, Turkey. Environ. Toxicol.Pharmacol., 23 (2): 242-249.

Azzurro, E., Matiddi,M.,Fanelli,E.,Guidetti,P., La Mesa, G.,Scarpato,A. and Axiak,V. (2010).Sewage pollution impact on Mediterranean rocky-reef fish assemblages. Marine Environmental Research, 69: 390-397.

Barhoumi, S., Messaoudi, I., Gagné,F. and Kerkeni,A. (2012). Spatial and seasonal variability of some biomarkers in *Salariabasilisca* (Pisces: Blennidae): Implication for biomonitoring in Tunisian coasts. Ecological Indicators, 14(1): 222-228.

Bilal, A. (2011).Bioaccumulation and effect of cadmium chloride on certain organs of *Clariasbatrachus*.Ph.D Thesis, Barkatullah University Bhopal, India.

Clarke, E. G. C. and Clarke, M. L. (1978). Veterinary Toxocology.BaillièreTindall, Great Britain.

Dudley, D.J., Guentzel, M.N., Ibarra, M.J., Moore, B.E. and Sagik, B.P. (1980) Enumeration of potentially pathogenic bacteria from sewage sludges. Applied and Environmental Microbiology,39: 118-126.

Duncan, D.B. (1955). Multiple range and multiple F-test. Biometrics, 11: 1-42.

El-Saidy, D.M.S.D., El-Kashef, M.A., Wafa, M.A. and El-Hais, A.M. (2012). Growth performance, feed utilization, whole body composition and blood parameters of Nile tilapia *Oreochromisniloticus* (L.) fed faba bean meal (*Viciafaba*, L.) as a replacer for soybean meal. Egypt. J. Nutrition and Feeds, 16 (1).

El-Serafy, S.S., Abdel-Hameid,N.A.H. and El-Daly,A.A. (2009). Histological and histochemical alterations induced by phenol exposure in *Oreochromisaureus* (Steindachner, 1864) juveniles. Egyptian Journal of Aquatic Biology and Fisheries, 13(2): 151-172.

El-Sheikh, M.A., Saleh,H.I., El-Quosy,D.E. and Mahmoud,A.A. (2010). Improving water quality in polluted drains with free water surface constructed Wetlands. Ecological Engineering, 36: 1478-1484.

Figueiredo-Fernandes, A., Fontainhas-Fernandes,A., Rocha,E. and Reis-Henriques,M.A. (2006). The effect of paraquat on hepatic EROD activity, liver and gonadal histology in males and females of Nile Tilapia, *Oreochromisniloticus*, exposed at different temperatures. Archives of Environmental Contamination and Toxicology, 51(4): 626-632.

Freeman, H.C., Sangalang, G.B.,Uthe, J.F., Garside, E.T.and Daye,P.G. (1983).A histopathological examination of and analysis for polychlorinated hydrocarbons in, inshore Atlantic cod (*Gadusmorhua*). Archives of Environmental Contamination and Toxicology, 12(6): 627-632.

Gingerich, W.H. (1982). Hepatic toxicology of fishes. In: Aquatic Toxicology, Weber, L, Ed. Raven Press, New York, 55.

Gonzales, G., Crespo, S. and Brusle, J. (1993).Histo-cytological study of the liver of the cabrilla sea bass, *Serramuscabrilla* (Teleostei, Serramidae), an available model for marine fish experimental studies. J. Fish Biol., 43: 363-73.

Hibiya, J. (1982). An Atlas of Fish Histology: Normal and Pathological Conditions. Kodasha Ltd., Gustav-Fisher-Verlag, Stuttgard, New York, pp. 82-98.

Houlihan, D.F., Costello, M.J., Secombes, C.J., Stagg, R. and Brechin, J. (1994). Effects of sewage sludge exposure on growth, feeding and protein synthesis of dab (*Limandalimanda*L.). Marine Environmental Research, 37: 331–353.

Ibrahim, M.A,Khalafalla, M.M., Zaki, M.A. and AbdelHamid, H.M. (2012). Physiological and nutritional studies on improving growth of Nile tilapia (*Oreochromisniloticus*) fingerlings using ginger root meal as a feed additive. Egyptian J. Nutrition and Feeds, 15 (1 Special Issue): 377-389.

Ibrahim, S.A. and Mahmoud,S.A. (2005).Effect of heavy metals accumulation on enzyme activity and histology in liver of some Nile fish in Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 9(1): 203-219.

Ilyas, M.,Sudaryanto, A., Setiawan, I.E., Riyadi, A.S., Isobe, T. and Tanabe, S. (2013).Characterization of polychlorinated biphenyls and brominated flame retardants in sludge, sediment and fish from municipal dumpsite at Surabaya, Indonesia. Chemosphere, 93: 1500–1510.

Kakuta, I. (1997). Effect of sewage on blood parameters and the resistance against bacterial infection of goldfish, *Carassiusauratus*. Environ. Toxicol. Water Qual., 12:43-51.

Khalafalla, M.M., Mohsen, M.K, Eweeda, N.M., Belal, E.B. and Abozaid, S.A. (2012). Nutritional evaluation of some legumes by-products (biological treated) as a non-conventional feedstuffs in diets of Nile tilapia (*Oreochromisniloticus*) fingerlings. Egyptian J. Nutrition and Feeds, 15 (1 Special Issue): 337-347.

Khallaf, E.A., Galal,M. and Authman,M. (1998).Assessment of heavy metals pollution and their effects on *Oreochromisniloticus* in aquatic drainage water. Journal of the Egyptian German Society of Zoology, 26(B): 39-74.

Khan, R.A., Barker, D.E., Hooper, R., Lee, E.M., Ryan, K. and Nag, K. (1994).Histopathology in Winter Flounder (*Pleuronectesamericanus*) Living Adjacent to a Pulp and Paper Mill. Arch. Environ. Contamin.Toxicol., 26: 95-102.

Koeleman, E. (2014). Aquaculture takes over wild fisheries. AllAboutFeed, 22 (7): 18-20.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B. and Buxton, H.T. (2002). Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. streams 1999–2000: A national reconnaissance. Environmental Science and Technology, 36: 1202–1211.

Leeson, C.R. and Leeson, T.S. (1976).Histology (3rd edition). W. B. Saunders Company. Philadelphia, pp. 365-391.

Lemes, A.S. and Braccini, M.C. (2004).Descrição e análisehistológica das glândulasanexas do tratodigestório de *Hopliasmalabaricus* (Bloch, 1794) (Teleostei, Erythrinidae). Biodiv.Pamp., 2: 33-41.

Levesque, H.M., Moon, T.W., Campbell, P.G.C. and Hontela, A. (2002). Seasonal variation in carbohydrate and lipid metabolism of yellow perch (*Percaflavescens*) chronically exposed to metals in the field. Aquatic Toxicology, 60(3-4): 257-267.

Ling, B.,Den, T.X., Lu, Z.P., Min, L.W., Wang, Z.X.andYuan, A,X. (1993). Use of night soil in agriculture and fish farming.World Health Forum, 14 (1): 67-70.

Lloyd, R. (1992). Pollution and freshwater fish.The Buckland foundation. Fishing News Books, Oxford, pp 35-40, 77-81, 107-110, 122-124.

Mekkawy, I.A.A., Mahmoud,U.M.,Wassif,E.T. and Naguib,M. (2011). Effects of cadmium on some hematological and biochemical characteristics of *Oreochromisniloticus* (Linnaeus, 1758) dietary supplemented with tomato paste and vitamin E. Fish Physiology and Biochemistry, 37(1): 71-84.

Mela, M., Randi, M.A.F., Ventura, D.F., Carvalho, C.E.V., Pelletier, E. and Oliveira Ribeiro C.A. (2007). Effects of dietary methylmercury on liver and kidney histology in the neotropical fish *Hopliasmalabaricus*. Ecotoxicol. Environ. Saf., 68 (3): 426-435.

Merck, E. (1976). Labordiagnostik in der Tiermedizin.Diagnostica Merck, Deutschland.

Metwalli, A.A.A. (2013). Effect of partial and total substitution of fish meal with corn gluten meal on growth performance, nutrients utilization and some blood constituents of the Nile tilapia *Oreochromisniloticus*. Egypt. J. Aquat. Biol. & Fish., 17 (1): 91-100.

Meyers, T.R. and Hendricks, J.D. (1985).Histopatholgy. In: Fundamentals of Aquatic Toxicology. Methods and Applications (G.M.Rand and S.R. Petrocelli, eds.), Hemisphere Publishing Corp., Washington, DC, pp. 283-331.

Miranda, A.L., Roche, H., Randi, M.A.F., Menezes, M.L. and Oliveira Ribeiro, C.A. (2008). Bioaccumulation of chlorinated pesticides and PCBs in the tropical freshwater fish *Hopliasmalabaricus*: Histopathological, physiological, and immunological findings. Environ. Int., 34(7): 939-949.

Mishra, A.K. and Mohanty, B. (2008). Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channapunctatus* (Bloch). Environmental Toxicology and Pharmacology, 26(2):136-141.

Mohamed, F.A.S. (2001). Impacts of environmental pollution in the southern region of Lake Manzalah, Egypt, on the histological structures of the liver and intestine of *Oreochromisniloticus* and *Tilapia zillii*. Journal of Egyptian Academic Society for Environmental Development,2(2):25-42.

Mohamed, F.A.S. (2003). Histopathological studies on some organs of *Oreochromisniloticus, Tilapia zillii* and *Synodontisschall* from El-Salam canal, Egypt. Egyptian Journal of Aquatic Biology and Fisheries, 7(3): 99-138.

Mohamed, F.A.S. (2008). Bioaccumulation of selected metals and histopathological alterations in tissues of *Oreochromisniloticus* and *Latesniloticus* from Lake Nasser, Egypt. Global Veterinaria, 2 (4): 205-218.

Moore, M.J., Shea, D., Hilman, R.E. and Stegeman, J.J. (1996).Trends in hepatic tumors and hydropicvacuolation, fin erosion, organic chemicals and stable isotope ratios in winter flounder from Massachusetts, USA. Marine Pollution Bulletin,32: 458–470.

Nuntiya, G. Pantunga, K., Helander, H., Helander, F. and Voravit, C. (2008).Histopathological alterations of hybrid Walking catfish (*Clariasmacrocephalus* x *Clariasgariepinus*) in acute and subacute cadmium exposure. Environment Asia, 1: 22-27.

Olojo, E.A.A., Olurin,K.B.,Mbaka,G. and Oluwemimo,A.D. (2005). Histopathology of the gill and liver tissues of the African catfish, *Clariasgariepinus* exposed to lead. African Journal of Biotechnology, 4(1): 117-122.

Peebua, P., Kruatrachue,M.,Pokethitiyook, P. and Singhakaew,S. (2008).Histopathological alterations of Nile tilapia, *Oreochromisniloticus* in acute and subchronicalachlor exposure. Journal of Environmental Biology/Academy of Environmental Biology, India, 29(3): 325-331.

Ptashynski, M.D., Pedlar,R.M., Evans,R.E., Baron, C.L. and Klaverkamp,J.F. (2002).Toxicology of dietary nickel in Lake Whitefish (*Coregonusclupeaformis*). Aquatic Toxicology, 58(3-4): 229-247.

Rabitto, I.S., Alves Costa, J.R.M., Silva De Assis, H.C., Pelletier, F.M., Akaishi, F.M., Anjos, A., Randi, M.A.F. and Oliveira Ribeiro,C.A. (2005). Effects of dietary Pb(II) and tributyltin on neotropical fish, *Hopliasmalabaricus*: histopathological and biochemical findings. Ecotoxicol. Environ. Saf., 60: 147-156.

Rani, U.A. and Ramamurthi, R. (1989).Histopathological alterations in the liver of freshwater teleost Tilapia, (*Oreochromismossambicus*) in response to cadmium toxicity. Ecotoxicology and Environmental Safety, 17:221-226.

Reddy, A.N., Venugopal,N.B.R.K. and Reddy,S.L.N. (1995).Effect of endosulfan 35 EC on some biochemical changes in the tissues and haemolymph of a freshwater field crab, *Barytelphusaguerini*. Bulletin of Environmental Contamination and Toxicology, 55(1): 116-121.

Roberts, R.J. (2001). Fish Pathology, 3rd ed. WB.Saunders, Philadelphia, PA.

Saravanan, M., Kumar, K.P.and Ramesh, M. (2011).Haematological and biochemical responses of freshwater teleost fish *Cyprinuscarpio* (Actinopterygii: Cypriniformes) during acute and chronic sublethal exposure to lindane. Pesticide Biochemistry and Physiology, 100: 206-211.

SAS (2001). SAS/STAT Guide for personal computer. SAS Inst. Cary, N.C.

Saxsena, R. and Garg, P. (2010). Vitamin E provides protection against *In vitro* oxidative stress due to pesticide (Chlorphrifos and Endosulfan) in goat RBC. GERF Bull. Biosci., 1(1): 1-6.

Schmidt, H.,Bernet, D., Wahli, T., Meier, W. and Burkhardt-Holm, P. (1999).Active biomonitoring with brown trout and rainbow trout in diluted sewage plant effluents. Journal of Fish Biology, 54: 585–596.

Secombes, C.J., Fletcher, T.C., O'Flynn, J.A., Costello, M.J., Stagg, R. and Houlihan, D.F. (1991).Immunocompetence as a measure of the biological effects of sewage sludge pollution in fish. Comparative Biochemistry and Physiology, 100C: 133-136.

Secombes, C.J., Fletcher, T.C, White M.J., Costello M.J., Stagg R. and Houlihan, D.F. (1992). Effect of sewage sludge on immune responses in the dab *Limandalimanda* L. Aquatic Toxicology, 23: 217-230.

Shata, H.A. (1996). Pollution in El-Manzala Lake, possible contribution to incidence of chronic renal failure in Dakahlia. Proc. Foodborne Contamination & Egyptian's Health, Univ. of Mansoura, Nov. 26-27, pp: 197-200.

Singh, R.K. and Sharma, B. (1998).Carbofuran induced biochemical changes in *Clariasbatrachus*. Pesticide Science, 53(4): 285-290.

Sorensen, E.M.B. (1991). Metal poisoning in fish: Environmental and life sciences Associates. CRC Press Inc., Austin, Texas, Boston, pp: 374.

Stephenson, T. (1987).Sources of heavy metals in wastewaters. In: J.N. Lester, (Ed.), Heavy metals in wastewater and sludge treatment: Sources, Analysis and Legislation (1: 31-64). Cleveland, DH: CRC Press.

Swarup, D., Patra R.C., Naresh, Ram, Puneet K., Pallav, S., and Balagangatharathilagar, M. (2006). Deficiency of copper and cobalt in goats reared around lead zinc smelter. Small Ruminant Res.63(3): 309-313.

Sweilum, M.A. (2006). Effect of chronic exposure to sub lethal levels of mercury on total production, physiological functions and economic efficiency of tilapia fish, with regard to properties of ponds water. Egypt. J. Aquat. &Fish., 10 (3): 165-183.

Tayel, S.I., Yacoub, A.M. and Mahmoud, S.A. (2008).Histopathological and hematological responses to freshwater pollution in the Nile catfish *Clariasgariepinus*. Journal of Egyptian Academic Society for Environmental Development, 9(4): 43-60.

Tayel, S.I., Ibrahim,S.A.,Authman, M.M.N. and El-Kashef,M.A. (2007).Assessment of Sabal drainage canal water quality and its effect on blood and spleen histology of *Oreochromisniloticus*. African Journal of Biological Sciences, 3(1): 97-107.

Thophon, S., Kruatrachue, M., Upatham, E.S., Pokethitiyook, P., Sahaphong, S.andJaritkhuan, S. (2003).Histopathological alterations of white seabass, *Latescalcarifer*, in acute and sub chronic cadmium exposure. Environmental Pollution, 121(3): 307-320.

Van Dyk, J.C., Pieterse, G.M. and Van Vuren, J.H.J. (2007). Histological changes in the liver of *Oreochromismossambicus* after exposure to cadmium and zinc. Ecotoxicology and Environmental Safety, 66: 432–440.

Varley, H. (1978). Practical Clinical Biochemistry, 4th Ed., Arnold-Heinemann Publishers, India.

Vos, J.G., Dybing, E., Greim, H.A., Ladefoged, O., Lambre, C., Tarazona, J.V., Brandt, I. and Vethaaak, D.A. (2000). Health effects of endocrine-disrupting chemicals on wildlife, with special reference to the European situation. Critical Reviews in Toxicology, 30: 71-133.

Wheater, P.R., Burkitt, H.G., Stevens, A. and Lowe, J.S. (1985).Basic Histopathology.A Colour Atlas and Text. Churchill Livingstone, New York, pp. 1-4, 116.

Yacoub, A.M. andAbdel Satar, A.M. (2003). Heavy metals accumulation and macronutrients in the livers of some fish species of Bardawil Lagoon and their histological changes. Egyptian Journal of Aquatic Biology and Fisheries, 7(4): 403-422.

Yang, M.S., Fong, W.F., Chan, T.C., Chui, V.W.D. and Wong, M.H. (1993). Feasibility studies on the use of sewage sludge as supplementary feed for rearing tilapia II. PCBs of the treated fish, and biochemical response in the fish liver. Environmental Technology, 14 (12): 1163-1169.

Zikic, R.V., Stajn,A.S.,Pavlovic,S.Z.,Ognjanovic,B.I. and Saicic,Z.S. (2001). Activities of superoxide dismutase and catalase in erythrocytes and plasma transaminases of goldfish (*Carassiusauratusgibelio* Bloch.) exposed to cadmium. Physiological Research, 50(1): 105-111.

التأثيرات المحتملة لتغذية الأسماك على الحمأة الجافة المعالجة:٢- بالنسبة لصورة الدم والتركيب النسيجي للكبد

عبدالحميد محمد عبدالحميد*،أحمد إسماعيل محرم*،أحمدعبدالحميد محمدعبد الحميدو مصطفى السعيد القطان***

*** قسم إنتاج الحيوان، كلية الزراعة، جامعة المنصورة، مصر.**

****قسم الأنسجة وبيولوجيا الخلية، كلية الطب، جامعة المنصورة، مصر.**

أجريت تجربة حقلية لمدة 102 يوما على أربعة أنواع من أسماك المياه العذبة (بلطى نيلى، مبروك فضى، مبروك عادى، قرموط أفريقى، بمعدل تخزين من الأنواع الأربعة كنسبة ١: ١: ١: ١) فى نظام متعدد الأنواع فى هابتين موضوعتين فى حوض ترابى يُروى من ماء صرف زراعى، تم تغذية أسماك الهابة الأولى على عليقة تجارية والأخرى على حمأة (ناتج معالجة الصرف الصحى والزراعى لمنطقة الرياض بمحافظة كفر الشيخ)، وفى نهاية التجربة تم سحب عينات دموعينات من أكباد الأسماك للفحص. أظهرت النتائج المتحصل عليها أن التغذية على الحمأة لم تُسىء إلى صورة الدم رغم الارتفاع المعنوى فى نشاط إنزيمات نقل مجاميع الأمين وتركيزات الجليسريدات الثلاثية. ولقد أحدثت التغذية على الحمأة بعض التغيرات الضارة النسيجية فى أكباد الأسماك. ونخلص من ذلك وبسبب وجود ملوثات فى الصرف الزراعى والصحى والتى تنتقل إلىمياه رعاية الأسماك وفى الحمأة والتى يمكنها التأثير السيئ على صحة وإنتاج وجودة الأسماك ويمكن أن تدخل السلسلة الغذائية فتضر بصحة الإنسان، لذا يُوصى بتوجيه الاهتمام لجودة الأعلاف والمياه (صديقة للبيئة) المستخدمة فى الاستزراع المائى لإنتاج منتجات آمنة لاستهلاك الإنسان.