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Impact of the Carbamate Pesticide Sevin on Hematology and Histology of Teleost Fish (*Oreochromis niloticus*)

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Abstract: A total number of 48 *Oreochromis niloticus* the most economic and commercial fish in Egypt was dosed with 0.5 and 1.0 mg/l the Carbamate Pesticide Sevin for 5, 10 and 15 days. The alteration in some blood parameter and histology of gills and spleen were studied in relation to different doses. Results showed increased number of white blood cells (WBcs) and decreased number of red blood cells (RBcs) with decreased hemoglobin percentage (Hb%), packed cell volume (PCV) and glucose concentration by increasing the dose of Sevin and the period of exposure as compared to the control group. Histopathological alterations of gills include: hyperplasia in epithelial cells of secondary lamellae, severe damage and necrosis in fishes treated with 0.5 mg/l Sevin for 5, 10 and 15 days, respectively. Also fish exposed to 1 mg/l Sevin for 10 days suffer from severe destruction and necrosis in epithelial cells of lamellae. However, exposure to Sevin for 15 days induced severe necrosis in epithelial cells. In addition to some lesions in spleen; haemosidrinoses, increase in white pulp, decrease in red pulp and severe necrosis.It was concluded that the Sevin has a negative impact on the funa of the River Nile including *Oreochromis niloticus*.

Key words: Sevin · Gills · Spleen · Histopathology · Hematology · *Oreochromis niloticus*

INTRODUCTION

Pesticides are extensively used all over the world for several decades to control various pests, but their residues often reach either directly or indirectly to the aquatic environment. So pesticides are one of the most potentially harmful chemicals liberated into the environment in an unplanned manner, especially in the developing countries. In such way, the chemical characteristics of the aquatic ecosystem were changed and pollution of such habitat has been generated. The major sources of environmental contamination by these chemicals are in proper agricultural practices usage in public health programmers and industrial discharges [1,2].Therefore, it is necessary to study in detail the possible impacts of there hazardous chemicals on the aquatic organisms with special emphasis on the most economic ones, viz, the fish. Such pesticides are also known to have affinity for residing in animal tissues, especially in the fatty ones [3]. Toxic effects of pesticides on organ of fish have been studied by many workers in world [2, 4-13]. In general compounds like organophosphates alter the integrity of the respiratory epithelium and pillar cells decreasing ionic regulation and compromising the structure of the gills [14]. Sevin pollution in the aquatic environment has been recognized in many areas of the world and high concentrations of it have been detected in many species of fish [15]. Sevin active ingredient is 1-naphthyl-N-methyl carbamate. It is widely used in Egypt against cotton leaf worm on vegetables (tomato, potato, onion ct.), agricultural crops (Cotton, maije, Soybean ct.) and beetles on cucumber, cucurbit etc. It persists in the environment from few hours up to one month. Consequently it contaminate the food of man and his farm animals [16- 19].

The spleen is the important organ of the body's immune and lymphatic system as well as it is a haematopoeitic organ in which blood cells synthesized and stored [20]. Some authors studied the effect of water pollution by heavy metals and pesticides on some blood parameters and histology of spleen on *Oreochromis nitoticus* and *Clarias gariepinus* fish and some alterations in spleen histology and blood cells and chemistry were reported [2, 20-23]

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The present study was undertaken to understand the changes in the blood parameters and histopathology of gills and spleen of *Oreochromis nitoticus* fish after chronic exposure to Sevin pesticides.

MATERIALS AND METHODS

The synthetic Carbaryl insecticide, Sevin was kindly donated by Ministry of Agriculture in El-Qualyobia Province. The purity of the formulated Sevin is about 28%. The LD_{50} was determined according to Awwad [24].

Specimens of *O. nitoticus* of the same age and weight were collected from El-Kanater El-Khyria Research Station and acclimated under laboratory conditions for about two weeks in glass aquaria (50X40X85cm)with continuously aerated and dechlorinated tap water at room temperature (26.8°C).Fish were fed mainly on artificial feed of the following formula: 53% premix, 30% fish meal, 15% Soy-bean and 20% sorghum.

Fish were divided into three groups, each group comprised 24 fish. They were all healthy, nearly of the same age and weight (120-150g).Fishes of the first group were kept as a control in aquarium containing well aerated chlorine free water. The second and third groups of fishes were kept in an aquarium of aerated water containing 0.5 and 1.0 mg/l Sevin which amounted 1/20 and 1/10 LD_{50}, respectively. Fish samples were captured after 5, 10 and 15 days, ANOVA test was used to compare between the effects of the two sets of treatment within the different time intervals (SPSS, 16)

HEMATOLOGICAL STUDY

Fish from the above three groups were killed at the end of the experiment to provide blood from their cut caudal vein. The blood was collected in a dry glass vial containing the anticoagulant ethylene diamine tetra-acetic acid (EDTA). The red blood corpuscle (RBC) and white blood corpuscle (WBC) were counted using a Spencer Double Neubauer Haemocytometer as described by Baixhall and Diasley [25].Hemoglobin content was estimated in blood by using the cyanmethemoglobin method described by VanKampen and Zyistra[26].The microhaematocrit method was used to measure the packed cell volume (P.C.V.)according to McKnight [27].

For the determination of glucose concentration, blood was collected in a clean and dry test tube which kept for 15 min. at room temperature. It was then centrifuged at 2000 r.p.m. for 10 min. The decanted clear serum was used for determine glucose by the method of Folin and Wu [28].

Histological Studies: Gills and spleen samples from the same collected fish were carefully removed and fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned into five micrometers thick and then a half of these sections were stained with hematoxylin and eosin method according to Harris [29]. Then sections were examined by light microscopy and photographed by using a microscopic camera (Model No. Ts-242).

RESULTS

The main alterations observed in the studied hematological parameters after exposure to Sevin pesticides are elucidated in Table 1.

Red blood cells, packed cell volume, hemoglobin content and glucose concentration decreased. While white blood cells showed marked increase by increasing the dose and the period of exposures. From Table 1 it is

Table 1: Hematological parameters of *Oreochromis niloticus* after exposure to 0.5 and 1 mg/l Sevin in 5, 10 and 15 days (mean ±SD)

Days	Parameter	Control	Treatment (0.5 mg/l)	p-value	Treatment (1.0 mg/l)	p-value
5	R.B.Cs (10^6/mm3)	2.35±0.04	1.57±0.07	3.41E-10	1.67±0.03	1.29E-11
	Hb (g/dl)	9.64±0.14	8.42±0.18	1.54E-07	8.17±0.18	2.45E-08
	P.C.V %	22.07±1.13	16.42±0.70	1.00E-06	17.23±0.92	1.02E-05
	W.B.Cs (10^3/mm^3)	3.87±0.30	5.97±0.30	2.61E-07	6.78±0.48	1.95E-07
	Glucose (mg/100 ml serum)	87.17±0.92	88.73±0.88	0.01295	91.15±2.12	0.001749
10	R.B.Cs (10^6/mm^3)	2.35±0.03	1.68±0.03	2.88E-12	1.82±0.05	1.29E-09
	Hb (g/dl)	9.63±0.62	8.61±0.32	0.004845	8.46±0.34	0.002284
	P.C.V %	22.07±1.26	17.03±0.99	2.00E-05	15.60±0.88	1.22E-06
	W.B.Cs (10^3/mm^3)	4.18±1.26	6.04±0.17	0.004944	6.67±0.41	0.000989
	Glucose (mg/100 ml serum)	87.22±0.44	89.15±0.89	0.000754	92.27±1.36	6.00E-06
15	R.B.Cs (10^6/mm^3)	2.35±0.05	1.39±0.03	2.12E-12	1.93±0.04	1.7E-08
	Hb (g/dl)	9.63±0.57	8.23±0.50	0.001126	8.41±0.78	0.011303
	P.C.V %	22.08±1.30	18.32±0.92	0.0002	16.48±0.90	5.58E-06
	W.B.Cs (10^3/mm^3)	3.87±0.19	6.43±0.45	1.47E-07	7.63±0.62	6.45E-08
	Glucose (mg/100 ml serum)	87.15±0.86	90.35±1.21	0.000364	88.92±2.33	0.112263

Red blood cells (R.B.Cs), white blood cells (W.B.Cs), packed cell volume (P.C.V), hemoglobin content (Hb)

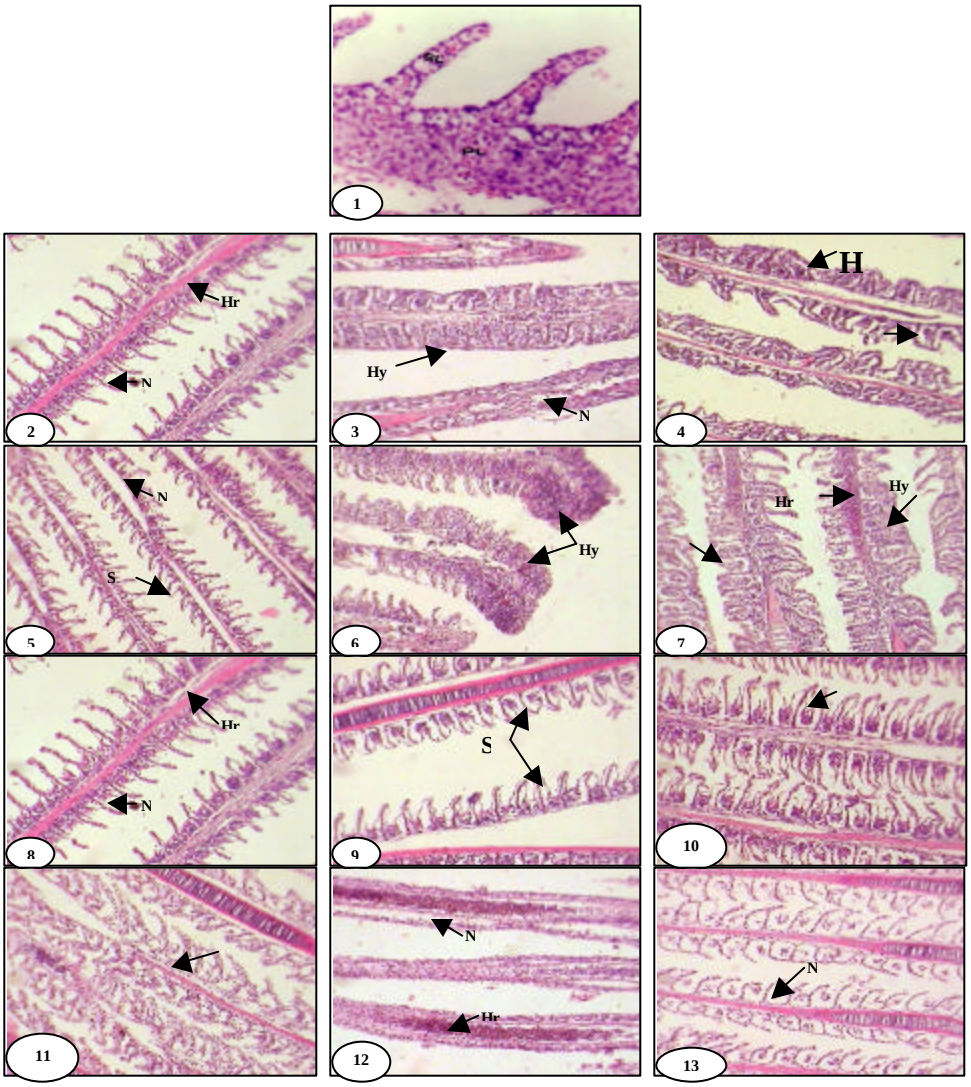

Fig. (1-13): Histological section in gills of *O. niloticus* stained with H&E; (1)control fish showing normal structure (X400),(2) fish exposed 0.5mg/l treated sevin at 5 days showing necrosis (N) of primary and secondary lamellar and hemorrhages (Hr) in primary lamellae(X250),(3&4) at 10 days showing hyperplasia (Hy)and separation (S) in secondary lamellar(X250),(5-7) at 15 days showing necroses(N) and hemorrhages(Hr) in primary lamellae(X250) ,(8&9) fish exposed 1 mg/l treated sevin at 5 days showing severe separation (S) in secondary lamellar(X250),(10&12) at 10 days showing severe necroses(N) and hemorrhages(Hr) (X250), (11&13) at 15 days showing severe necroses(X100)

obvious that there are highly significant differences ($P<0.05$) between the Control group and the other two sets of treatment within the desired intervals.

Histological Studies

I-Gills: The normal gill histological picture is shown in Fig. 1. The filament consists of primary and secondary lamellae. The secondary lamellae include bronchial epithelium consisting of a layer of one or two cells of inter digitating squamous epithelial cells. Also mucous secreting cells and chloride cells are found scattered between the lamellae. The gills of *O. niloticus* which exposed to 0.5 mg/l and 1 mg/l Sevin for 5, 10 and 15 days show several histopathological alterations. gills obtained from fish exposed to 0.5 mg/l sevin at 5 days, show eosinophilic tendency in secondary gill lamellae and necrosis in epithelial cells of those lamellae (Fig. 2).

After 10 days of exposure to 0.5 mg/l Sevin, fish presented severe damage in gill lamellae.The damage including: vacuolated primary filament,eosinophilic material in gill filament, swellings accompanied by cytoplasmic vacillations and the lamellae were limed by spaces filled with hyperplastic epithelium (Fig. 3 and 4). Fish which exposed to 0.5 mg/l of Sevin for 15 days, show hyperplastic necrosis of epithelium around the gill ray of that fish (Fig. 5-7).

While, gills of fish exposed to 1 mg/l Sevin for 5 days showed tissue destruction and deformed gill lamellae tissues (Fig. 8 and 9).The exposed fish gills to 1 mg/l sevin for 10 days revealed severe destruction and deformation such as separation in epithelial cells of secondary lamella and hemorrhage (Fig. 10 and 11). Finally, gill filaments exposed to 1 mg/l sevin for 15 days showed separation in primary lamellae. Also some lamellae of these gills appeared distracted and necrotized tissues were aggregated in between their filaments (Fig. 12 and 13).

II-Spleen: In the present work, the normal histological picture of the spleen of *O. nitoticus* is shown in Fig.14.The spleen is enclosed with a capsule and consists of lymphatic laden area, white pulp. The surrounding pink areas are the red pulp which consists of large numbers of free cells and sinusoids

Histopathological changes in the spleen of the studied *O. nitoticus* (Fig. 15-26) were summarized in the following: degeneration in spleenic tissue leads to necrosis, fibrosis, haemosidrinoses, increasing in white pulp and decreasing in red pulp.

DISCUSSION

Blood offers an important profile to study the toxicological impact on animal tissue. Different blood parameters are often subjected to changes depending upon the stress, condition and various other environmental factors [30].The present results establish a condition of erythropenia and haemolysis in *O. nitoticus* [2,20,23,31]

Increased white blood cells count establishes leucocytosis, which is considered to be of an adaptive value for the tissue under chemical stress. This also helps in the removal of cellular debris of necroses tissue at a faster rate [32]. In the presence of foreign substances or under pathological conditions leucocytosis in fish may be the consequence of direct stimulation of immunological defense [33]. Also these observations were in agreement with the findings of John [2] and Tayel *et al.* [20, 23] after the exposure to Metasystox, Sevin pesticides and heavy metals.

The present elevated blood glucose concentration may be due to severe spleenic disorder. Omkar *et al.* [34] reported similar results in *S. fossilis* after Chloe dance intoxication. Also, it was reported that alteration in glucose concentration is directly related to pesticide intoxication in fish[35,36]. John [2] revealed similaralterations in fish exposed to Metasystox and Sevin pesticides. According to Tayel [20, 23], the elevated blood glucose level (hyperglycaemia) is indicative of disrupted carbohydrate metabolism due to water pollution.

Histology has been used as a test for evaluating toxic effects of water pollutants in fish [36]. Results from the histological studies are useful in establishing water quality criteria [37].

The fish gill is a multifunctional organ involved in respiration and homeostatic activities such as osmorgulation, metabolism, circulation of hormones, nitrogen excretion and acid base balance [38]. They are among the most delicate structures of the teleost body and they have an external location so they are subjected to damage by irritant whether dissolved or suspended in the water. External irritant are the most frequent causes of significant gill pathological changes [39].

Sevin (carbamate) was used in the present study with less than 10% of the LD_{50}. Due to gills are in contact with water and are exposed to dissolve contaminates and trophic contamination, fusion of lamellae and gill damage suggests an acute exposure to contaminate [13].

Gills alteration such as epithelial hyperplasia and separation of the epithelial layer from supportive tissues

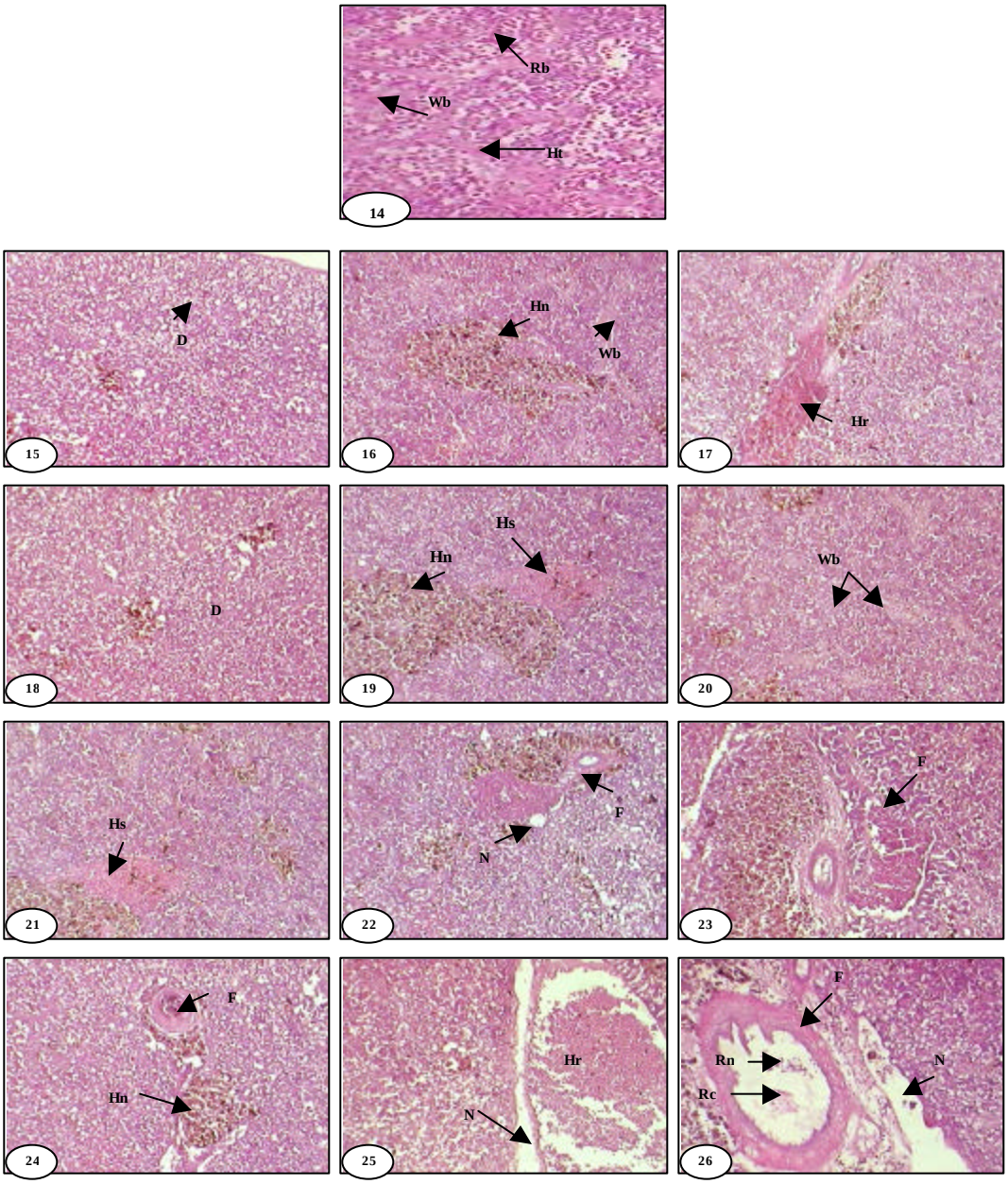

Fig.(14-26): Histological section in spleen stained with H &E;(14)control fish showing normal structure(X400),(15&16) fish exposed 0.5mg/l treated sevin at 5 days showing degeneration (D)and haemosidrinoses (Hn) (X250) , (17&18) at 10 days hemorrhages (Hr) (X250), (19&20) at 15 days hemolysis (Hs) and increase in white pulp (Wp) (X250), (21&22) fish exposed 1 mg/l treated sevin at 5 days showing hemolysis (Hs) and fibrosis (F) (X250) , (23&24) at 10 days haemosidrinoses (Hn) and fibrosis (F) (X250), (25&26) at 15 days necrosis (N), hemorrhages (Hr) and remaining of nucleus (Rn) (X250)

are usually leads directly to gill function disorders, which may cause the death of fish [40- 43]

The results in this work were more or less similar to results obtained by Thurston *et al* [44].

The acute toxic action of various concentrations of Sevin in this study caused severe necrosis, hyperplasia, degeneration and terminal desquamation of the epithelium. This observation agreed with those reported by Ibrahim *et al.*[15] after the exposure to same various concentrations of Sevin on gills of *Tilapia zillii.*

Spleen of fish is an important member of the body's immune and lymphatic system. It is a haematopoeitic tissue, which from the red blood cells. It is found as a small red mass. Its functions are filtration of blood, producing and storage of red blood calls, removing old and abnormal erythrocytes and producing antibody against blood born antigens.

Histopathological changes in the spleen of the studied *Oreochromis nitoticus* were summarized in the following: Fibrosis, necrosis, haemosidrinoses, increasing in white pulp and decreasing in red pulp. These changes may be due to trace metals pollution, pesticide lendane, organochlorine pesticides and heavy metals [20, 21, 23, 45- 47].

There was a direct relation ship between the reductions of red pulp that produces the red blood cells, which in turn showed the reduction in its number count. In addition the increase in white pulp produces large count of the white blood cells.

These results are in agreement with those of Lamas, *et al.* [48] and Tayel, *et al.* [20, 23.]. Morphological change were recorded in the spleen of *O. nitoticus* after exposure to the sewage water, heavy metals and pesticides [2, 20, 23,47]

it can be concluded that Sevin pesticide effect on gill and spleen histopathology and some blood parameters of *Oreochromis nitoticus.* These alterations depend on the dose and the time of exposure.

REFERENCES

1. Hazarika, R. and M. Das, 1998. Toxicological impact of BHC on the ovary of the air-breathing catfish Heterpneustes fossilis (Bloch). Bullet. Environ. Contamination Toxicol., 60: 16-21.
2. John, P.J., 2007. Alteration of certain blood parameters of freshwater teleost *Mystus vittatus* after chronic exposure to Metasystox and sevin. Fish Physiol. Biochem., 33: 15-20.
3. Sakr, S.A., S.M. Hanafy and N.I. El-Dosouky, 2002. Histopathological, histochemical and physiological studies on the effect of the insecticide (Hostathion) on the liver of the catfish *Claias gareipinus.* Egyptian J. Aquat. Biol. Fishries, 6: 103-123.
4. Sadhu, D.N., 1993. Toxicity of an organophosphorus insecticide monocil to air-breathing fish, channa punctatus. J. Ecotoxical Monitoring, 3 : 133-136.
5. Thakur, P.B. and V.S. Bais, 2000. Toxic effect of aldrin and fenvalerate on certain haematological oarameters of a freshwater Heteropneustes Fossilis (Bl.). J. Environ. Biol., 21: 161-163.
6. Kalavathy K.A., A. Sirva Kumar and R. Chandran, 2001. Toxic effects of pesticides dimethoate on the fish sarother odon mossambicus. J. Ecol. Res. Bio Conservation, 2: 27-32.
7. Prashanth, M.S., M. David and S.G. Mathed, 2005. Behavioural changes in freshwater fish Cirrhinus mrigala (Hamilton) exposed to Cypermethrin. J. Environ. Biol., 26: 141-144.
8. Guimaraes, A.T.B., H.C. Silvade Assis and W. Boeger, 2006. The effect of trichlorfon on acetylchotinesterase activity and histopathology of cultivated fish *Oreochromis niloticus.* Ecotoxicol. Environ. Safety, 4: 1-6.
9. Mohamed, F.A.S. and N.S. Gad, 2007. Impacts of pesticides residues in *Oreochromis niloticus* and Mugil cephalus from some fish farms in El-Fayoum governorate on some biochemical parameters and tissues structures. Egyptian J. Aquat. Res., 33: 247-275.
10. Mohamed, F.A.S., 2004. Histological Alterations in the intestine and Gonads of *Oreochromis niloticus* induced By reldan (organophosphorous insecticide) and Lannate (Carbamate insecticide). J. Egyptian Academic Soc. Environ. Develop., 5: 59-88.
11. Ayas, Z., G. Ekmekci, M. Ozmen and S.V. Yerli, 2006. Histopathological changes in the liver and kidney of fish in sariyar Ressrvoir, Turkey. Environ. Toxicol. Pharmacol., 23: 242-249.
12. Capkin, E., I. Allinok and S. Karahan, 2006. Water quality and fish size affect toxicity of endosulfan an organochlorine pesticide, to rainbow trout. Chemosphere, 64: 1793-1800.
13. Ribeiro, C.A., Y. Vollaire, A. Chardi and H. Roche, 2005. Bioaccumulation and the effects of organ chlorine pesticides, PAH and heavy metals in the Eel (Anguilla anguilla) at the Cam argue Nature Reserve, France. Aquatic Toxicol., 74: 53-69.

14. Mallate, J., 1985. Fish gill structural changes induced by toxicants and other irritants a statistical review.Can. J. Fish. Aquat. Sci., 42: 55-66.
15. Ibrahim, S.A., A.A. Al Gebaly and M.A. El-Kashif, 2005. Histopathological changes associated eith sevin (carbaryl) accumulation in fresh water fish (*Tilapia zillii*) at El-Kanater El-Khyria (Egypt). Botany and Zoology. Journal of Faculty of Science. Zagazig University, 27: 277-292.
16. Duggan, R.E. and J.R. Weatherwax, 1967. Dietary intake of pesticide chemicals. Science, 157: 1006-1010.
17. Webb, F.E., 1978. In "Proceedings of Canada us. Symps. On spruce Bdworm control with sevin -4- oil, Dec., 6-8-1978, Montreal, Que. Union carbide Agric. Products. Div., Journal of Acksonville, Fl, 88.
18. Schmitt, D.M., D.G. Grimble and J.L. Searcy, 1984. Eds. Managing the splice Budworn in Eastern North America. U.S.A. for Serve. Cooperative state Research Service. Agricutural. Hand Book, 6: 20-192.
19. Klaassem, G.D., M.O. Amdur and J. Doull, 1986. Toxicology. 3rd Edn. Collier Macmillan Publisher London.
20. Tayel, S.I., S.A. Ibrahim, M.M.N. Authman and M.A. El-Kashef, 2007. Assessment of sable drainage canal water quality and its effect on blood and spleen histology of *Oreochromis niloticus*. African J. Biol. Sci., 2: 97-107.
21. Naigaga, I., 2003. Bioaccumulation and histopathology of copper in *Oreochromis mossambicus*. M. Sc. (Ichthyology), Rhodes University, Library Theses, pp: 3-78.
22. Hoger, B., B. Kollner, M.R.Van den, B. Hitzfeld and D.R. Dielrich, 2004. Influence of chronic exposure to treated sewage effluent on the distribution of white blood cell populations in Rainbow trout (*Oncorhynchus mykiss*) spleen. Toxicol. Sci., 82: 97-105.
23. Tayel, S.I., A.M. Yacoub and S.A. Mahmoud, 2008. Histopathological and haematological responses to freshwater pollution in the Nile catfish *Clarias gariepinus*. J. Egyptian Academic Soc. Environ. Develop., 9: 43-60.
24. Awwad, M.H., 1991. Effect of pollution on haemopoietic cells of freshwater fishes in Qualyobia province. Ph.D. Thesis, Faculty of Science, Benha Univeristy, pp: 129.
25. Biaxhall, P.C. and W.K. Daisley, 1973. Routine haematological methods for use with fish blood. J. Fish Biol., 5: 771-781.
26. VanKampan, E.J. and C.N. Zijlstra, 1961. Determination of haemoglobin, Clinical and Chemical Actra., 5: 719-720.
27. Mcknight, I.M., 1966. A haematological study on the mountain white fish,Prosopium Williamsoni. Journal of the Fisheries Research Board of Canada, 23: 45-64.
28. Folin, O. and H. Wu, 1920. Journal of Biological and Chemistry, 41, 367. In: Span Diagnostic Kits, article, pp: 915.
29. Harris, H.F., 1990. Cited from Ph.D. Thesis titled: Impact of environmental pollution on structural and functional integrity of certain organs in freshwater fishes in Egypt. By S.M. Kadry (1985), Faculty of Girls, Ain Shams University, pp: 176.
30. Goel, K.A., A.K.A wasthi and S.K. Tyagi, 1981. Haematology of heteropneustes fossilis under chemico azo stress of Bismark brown. Currrent Sci., 50: 875-876.
31. Varadaraj, G., M.A. Subramaniam and B. Nagrajan, 1993. The effect of sublethal concentration of paper and pulp mill effluent on the hematological parameter of orechromis mossambicus. J. Environ. Biol., 14: 321-325.
32. Mcleay, D.J. and A.D. Brown, 1974. Growth stimulation in Juvenile salmon (*Oncorhynchus kistuch*) exposed to bleached kraft pulpmill effluent for 200 days. J. Fish. Res. Board of Canada, 31: 1043-1049.
33. Verma, S.R., S.K. Bansal, A.K. Gupta and R.C. Dalela, 1979. Pesticide induced haematological alteration in a freshwater fish, Saccobranchus fossilis. Bull. Environ. Contamination Toxicol., 22: 467-474.
34. Omkar, V.B., V. Padhyay and G.S. Shukla, 1984. Endosulfan induced changes in the carbohydrate metabolism of a freshwater prawn, Macrobranchium lemerrii. Currrent Sci., 535: 280-281.
35. Husain, K., R.A. Ansari and P.K. Gupla, 1987. Effect of sub chronic exposure of malathion on blood and tissue enzyme activities in female rats. J. Environ. Biol., 82: 137-142.
36. EIFAC, 1983. Working party on toxic testing procedures, revised report on fish toxicity testing procedures. EIFAC Technical paper, 24: 1-37.
37. FAO, 1981. Manual de methods de investigation del medio ambient a cuatico- part 4: Bases are la aleccion de encayos biologicos paraevaluar la contaninacion marina FAO, Doscume wntos Tccnicos de pesca, 164: 1-34.

38. Haaparanta, A., E.T. Valtonen and R.W. Haffman, 1997. Gill anomalies of perch and roach from lakes differing in water quality. J. Fish Biol., 50: 575-591.
39. Zaki, M.I. and S.G. Saad, 1987. Pathological changes in different species and embryological stages of fish induced by urea fertilizer waste water. Bulletin Institute of Oceanography and Fisheries. ARE, 131: 43-57.
40. Eller, L.L., 1975. Lesions in freshwater teleosts. In the pathology of fishes (Ribelin, W.E. and Magaki, G., Eds.). Madison, University of Wisconsin Press, pp: 305-330.
41. Gardner, G.R., 1974. Chemically induced lesions in esturine or marine teleosts. In: The pathology of fishes (eds. W.E. Riblein and G. Miggaki,). Univ. Wiscosin Press, Madison, pp: 657-693.
42. Smart, G., 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gair dness*). J. Fish Biol., 8: 471-475.
43. Cruz, E.R., M.C. De la Cruz and N.A. Sunaz, 1988. Hematological and histopathological changes in Oreochromis mossambius after exposure to the Molluscicides Aquatin and Brestan, In: R.S.V. Pullin; T. Bhukaswan; (eds. K. Tonguthai and J.L. MacLean), The second International Symposium on Tilapia in Aquaculture. ICLARM conference proceedings 15, 623 P. Department of Fisheries, Bangkok, Thailand and International center for living Aquatic Resources Management, Manila, Philippines, pp: 99-110.
44. Thurstone, R.V., R.C. Russo, R.J. Luedtk, R.C. Wang and C.J.D. Brown, 1983. Chronic toxicity of ammonia to rainbow trout. Transplantation and American Fifth Society, 113: 56-73.
45. Hart, L.J., S.A. Smith, B.J. Smith, J. Robertson and S.D. Holladay, 1997. Exposure of tilapia fish to the pesticide lindane results in hypocellularixy of the primary hematopoietic organ (pronephros) and the spleen without altering activity of phagocytic cells in these organs. Toxicology, 118: 211-221.
46. Haggag, A.M., M.A.S. Marie, K.H. Zaghloul and S.M. Eissa, 1993. Treatment of underground water for fish culture in Abbassa Farm, Sharkia. Bulletin of Faculty of Science, Cairo University, 61: 43-69.
47. Mazhar, F.M., M.A. Ashry and S.M. Kadry, 1986. Effects of environmental pollution on the histological features of the major organs of the Nile catfish. *Clarias lazera.* Proceed. Zool. Soc., Arab Republic Egypt, 12: 75-89.
48. Lamas, J., M. Noya, A. Figueras and A.E. Toranzo, 1995. Pathology associated with a viral erythrocytic infection in turbot, *scophthalmus maximus* (L.) J. Fish Diseases, 18: 425-433.

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