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Snails and Fish as Pollution Biomarkers in Lake Manzala and Laboratory A: Lake Manzala Snails

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Abstract

Physiological, hematological and biochemical parameters have been used as biomarkers for water quality in snail samples collected from Lake Manzala.

The results showed significant increase in AST, ALT, and ALP in *Planorbis* and *Physa* snail samples collected from Dakahlya site in Lake Manzala. Most of snails are collected from of Port-Said and Dakahlya sites showed significant increase in urea. On the other hand, alteration in creatinine values in samples from different lake sites was recorded. Significant increase of total protein level and total bilirubin was obtained in all samples. Most of snail samples showed significant decrease in hemocytes count. The oxidative enzymes (CAT, GGT and GST) recorded alteration in their activity.

Regarding Histopathological observations, in the foot region of *Biomphalaria* snails collected from Port Said and Dakahlya governorates are the most affected. The head foot showed splitting in the longitudinal and oblique muscle fibers and increased empty spaces within muscle. Shrinkage, focal areas of necrosis, large fat vacuoles and enlargement were observed in the salivary gland. Snail's ganglia showed enlargement of neurosecretory neurons, degeneration with large vacuoles and fibrosis. Hepatopancreas became much more distorted with necrosis, atrophy, degeneration and fat vaculation especially in Port Said and Damietta samples. Also, hepatopancreatic acini filled with different developmental stages of *S. mansonai* cercariae were observed in *Biomphalaria* snails collected from Port Said. Severe degenerative changes were observed in most of gonad's cells including ova and sperms especially in snails collected from Damietta. Also, *Biomphalaria* snails collected from Lake Mazala showed accumulation of heavy metals in the head foot tissues. In conclusion, the severe alteration and degeneration recorded in the physiological and hematological parameters and also histopathological observations are clear evidence for the pollution of the water from which these snails were collected.

Keywords: Lake manzala; Aspartate aminotransferasel; Alanine aminotransferase; Alkaline phosphatase; Total protein; Bilirubin; Hemocytes; Oxidative enzymes and histopathology

Abbrevations: AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase; ALB: Albumin; CAT: Catalase; GST: Glutathione-S-Transferase; GGT: Gamma Glutamyltransferase; TBRI: Theodor Bilhaz Institute; HE: Hematoxylin and Eosin Stain; A/G ratio: Albumin/Glubuline ratio

Introduction

Lake Manzala is considered one of the most important lakes in Egypt. It is exposed to high levels of pollutants from industrial, domestic and agricultural resources [1-3]. Ali reported that Lake Manzala receives about 4000 million cubic meters of untreated industrial, domestic and agricultural waste water annually [4].

The use of physiological and biochemical parameters as indicators of water quality has been developed to detect sublethal impacts of pollutants. Prominent among these biomarkers are physiological variables, such as plasma levels of metabolites [5], haematological data [6,7], levels of hormones [8-11] and biochemical variables such as detoxifying enzyme activities [12,13]. Interesting reports concerning the mechanisms of metal uptake, accumulation, transport, and elimination of metals in molluscs are usually focused on chemical, biochemical, molecular, and physiological aspects [14-21]. El-Khayat assessed genetic variation and genetic pattern of *Lymnaea* snails collected from irrigation canals in four different Governorates using ISSR markers, with the characterization of environmental parameters of the collecting *Lymnaea* sites. The authors showed high polymorphism by using for the first time the ISSR PCR technique for studying genetic variations of *L. natalensis* snails in Egypt and concluded that *L. natalensis* snails can survive associated with other snails, plants, and insects and can be tolerate the heavy metals in water [22].

Similarly, histopathological changes have been widely used as biomarkers in the health evaluation of animal organisms. The discharge of toxic elements into the rivers, estuaries and coastal waters poses serious pollution and consequently affects the fish, flora and fauna as snail.

Moreover, freshwater molluscs play an important role in aquatic ecosystems, providing food for many fish species and vertebrates [23].

This work aims to record the alterations of the Physiological, hematological and histopathological parameters in snails collected from Lake Manzala as a bio-indicator for water pollution.

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Materials and Methods

Snail samples were collected from 8 sites in Lake Manzalafrom 3 governorates; Port-Said (Kobry El-Lansh, Kaar El-Bahr and El-Khankak), Dakahlya (Gammalya, Matarya and Nasayma) and Damietta (Ananyya and Sayala). The snails collected were kept in water from their habitat and examined for natural infection. The negative (uninfected) *Biomphalaria* snails and other collected species (*Physa* and *Planorbis*) were contributed in the physiological studies. On the other hand, both negative and positive *Biomphalaria* samples were examined histologically.

Biochemical studies

Determination of liver and kidney functions: The assessment of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea, creatinine, total and direct bilirubin, albumin (ALB) and total protein were examined in snail tissue extracts. They were assayed biochemically using biosystem autoanalyzer, Backmann at Theodor Bilhaz Institute (TBRI) hospital laboratories. Snail tissues were dissected out, homogenized in bi-distilled water (1:1 w/v) using motor homogenizer and centrifuged at 5000 rpm for 20 min at 4°C and the supernatants were taken and kept at -20°C till used as described by [24].

Creatinine was determined according to [25]. In this method, creatinine reacts with picrate to form a coloured complex and the rate of formation of the complex is measured photometrically at 492 nm.

Urea was determined by using the coupled urease/glutamate dehydrogenase (GLDH) enzyme system according to [26].

Determination of antioxidant enzymes: The antioxidant enzymes catalase (CAT), Glutathione-S-Transferase (GST) and Gamma Glutamyltransferase (GGT) were assayed in snail tissue extract using spectrophotometer. Snail's tissues were dissected out. Each snail tissue from each treatment was homogenized in bi-distilled water (10:1 w/v) using motor homogenizer. Homogenates were centrifuged at 5000 rpm for 20 min at 4°C and the supernatants were taken and kept at -20°C till used.

Determination of snail hemolymph components: Snail hemolymph was collected in accordance to the technique of [27]. The hemolymph was obtained via small hole made in the shell into which capillary tube was inserted then it was drawn into tube by capillary suction. The hemocytes of the samples hemolymph were determined by haemocytometer. For total and differential counting, monolayer of hemocytes were stained with Giemsa stain for 20 minutes, according to the methods of [28] and counted by light microscopy.

Histopathological examinations: Snail specimens collected from Lake Manzala were dissected, removed from their shells gently and fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin sections were prepared, stained by hematoxylin and eosin (HE) and then examined microscopically and photographed for histopathology observations [29].

Statistical analysis: Data are expressed as means ± SD. The results were computed statistically (SPSS software package, version 20) using the T-test analysis**.** Values of $p<0.05$ were considered statistically significant.

Results

Biochemical parameters

The present results showed significant increase in AST, ALT, and ALP in *Planorbis* and *Physa*, respectively in most samples collected from Nasayma site in Lake Manzala. Most of snails collected from Port-Said and Dakahlya sites showed significant increase in urea. Results of creatinine in samples from different lake sites showed alteration, ranged between non-significant decrease and increase (Table 1). Significant increase of total protein level was obtained in all field samples while total bilirubin showed the highest levels in *physa* and *planorbis* samples collected from Nasayma, Dakahlya and in *Biomphalaria* samples collected from Matarya, Dakahlya. Also, results showed higher levels of indirect bilirubin than direct. Most snail samples showed approximately normal A/G ratio (Table 2).

Antioxidant enzymes

Significant alterations in catalase (CAT) level were noticed in all snail samples collected from Lake Manzala as compared with lab bread controls (except in *Planorbis* collected from Kobry El-Lansh and *Biomphalaria* from Gammalya and *Biomphalaria* from Nasayma). The recorded alterations in the snail samples was increased by 18 to185%, or decreased by -13 to -90% (Table 3).

Glutathione-S-transferase (GST) alteration was demonstrated in all samples includes decrease in activity ranging from -21% to -83% ($P<0.001$) and increase in activity ranging from 13% to 119%.

The same result was noticed in Gamma-glutamyl transpeptidase (GGT) in snail samples as compared with lab bread controls, some samples showed decrease change activity ranging from -1% to -35% and other samples showed increase change activity ranging from 6% to 666%, (Table 3).

Determination of hemolymph components

The majority of snail samples showed significant decrease in total and differential cell count as compared with lab bread controls (Table 4). The higher percent of decrease in the total cell count (-72%) was recorded in *Biomphalaria* collected from Nasayma, Dakahlya. Hemoglobin concentration showed alteration; increased to 2.6 g in *Planorbis* collected from Kobry El-Lansh and decreased to 0.8 g in *Physa* collected from Annanya, Damietta.

The histopathological observations

A knowledge of the normal histology and structure of snails is guided by [30].

Head foot: The normal foot region has an outer cuticular layer as a protective layer of the foot. Inner to this lining there is a tall columnar epithelium with basal nuclei in its cell. Amongst the columnar epithelium there are modified sacs like cells in the form of unicellular glands which open through the cuticular layer exterior to the foot surface. These unicellular glands are involved in mucous secretion. Embedded in between there are transversely muscle fibers, called as longitudinal muscle fibers. Major part of the foot muscles are made up of thickly arranged oblique muscle fibers.

Histopathological observations in foot region of *Biomphalaria* snail samples showed necrotic change (shrinkage) in the mucous secreting unicellular glands (Figure 1b) and hyaline substances are shown in samples collected from Port Said (Figure 1c) and splitting fiber tissues in Dakahlya and Damietta snails (Figure 1d,1e). Also, results showed oblique splitting muscle fibers, increased empty spaces and atrophy within muscles of snail head in Dakahlya samples (Figure 1f,1g,1h).

Salivary gland: The normal salivary gland of *B. alexandrina* snail composed of two lobs found in the buccal mass as shown in (Figure 2a).

Parameter			AST (Unites/ml)		ALT (Unites/ml)		ALP (IU/L)		Glucose		Creatinine (mg/dl)		urea (mg/dl)	
Treatments			Mean ± SD	Change %	Mean ± SD	Change %	Mean ± SD	Change %	Mean ± SD	Change %	Mean ± SD	Change %	Mean ± SD	Change %
Control lab			21.9 ± 4		41.5 ± 10		38.4 ± 8		45.0 ± 8		0.55 ± 0.22		9.4 ± 1	
Bort-Said	Kaar El-Bahr	*Biomphalaria*	26.2 ± 2	20	64.3 ± 24	55	42.5 ± 4	11	76.1 ± 27	69	0.35 ± 0.0	-36	13.8 ± 1	47
		Planorbis	30.2 ± 4	38	50.6 ± 7	22	56.8 ± 11	48	72.4 ± 11	61	0 ± 0.0	-100	12.4 ± 0	32
	El-Khankak	*Biomphalaria*	11 ± 0**	-50	57.9 ± 0	40	106 ± 0.0	176	85.7 ± 0.0	90	1.46 ± 0.0	165	30 ± 0.0*	219
	Kobry El-Lansh	*Biomphalaria*	37.4 ± 5*	71	74.8 ± 12	80	94.0 ± 29	145	99.2 ± 6*	120	0 ± 0.0	-100	27.3 ± 1.2**	190
		Planorbis	40.5 ± 6*	85	65.7 ± 11	58	101.4 ± 11*	164	96.8 ± 13*	115	1.53 ± 1.25	178	25.9 ± 1.1**	176
Dakahlya	Gammalya	*Biomphalaria*	31.1 ± 0**	42	65 ± 15	57	65.5 ± 10	71	91.0 ± 23	102	0.40 ± 0.04	-27	22.9 ± 13**	144
	Nasayma	*Biomphalaria*	29.6 ± 3*	35	44.1 ± 10	6	52.0 ± 4	35	80.1 ± 19	78	1.31 ± 0.94	138	15.7 ± 3	67
		Planorbis	56.4 ± 2**	158	85.8 ± 9*	107	105.6 ± 10*	175	131.1 ± 6**	191	0.38 ± 0.0	-31	19.3 ± 2*	105
		Physa	53.8 ± 3**	146	66.4 ± 10	60	162.3 ± 4.7**	323	119.3 ± 9*	165	1.41 ± 0.0	156	20.4 ± 2*	117
	Matarya	*Biomphalaria*	32.1 ± 2*	47	44 ± 17	6	66.3 ± 3.3*	73	85.3 ± 3.1*	90	0.12 ± 0.0	-78	22.8 ± 8	143
Damietta	Annanya	*Physa*	34.2 ± 5	56	53.6 ± 7	29	112.7 ± 4**	193	66.4 ± 12	48	0.08 ± 0.0	-85	14.5 ± 3	54
	Sayala	*Planorbis*	30.6 ± 4	40	51.2 ± 8	23	57.9 ± 11	51	67.4 ± 16	50	0 ± 0.0	-100	12.3 ± 1	31

*, ** & *** significant compared to control value at p<0.05, p<0.01 & p<0.001, respectively.

Table 1: Aspartate amino transferase (AST), alanine amino transferase (ALT) alkaline phosphatase (ALP), glucose, creatinine and urea in tissue extract of snails collected from Lake Manzala.

Parameter			Total protein (g/dl)		Albumin (g/dl)		Globulin (g/dl)		A/G Ratio ◊	Total Bilirubin (Umol/l)		Direct Bilirubin (mg/dl)		In-Direct Bilirubin (U/mg)	
Treatments			Mean ± SD	Change %	Mean ± SD	Change %	Mean ± SD	Change %		Mean ± SD	Change %	Mean ± SD	Change %	Mean ± SD	Change %
Control lab			6.21 ± 0.1		3.27 ± 0.02		2.94 ± 0.11		1.13	2.8 ± 0.1		0.2 ± 0.04		2.5 ± 0.1	
Bort-Said	Kaar El-Bahr	*Biomphalaria*	10.35 ± 0.4**	67	6.84 ± 0.30**	109	3.54 ± 0.11*	20	1.93	4.4 ± 0.0*	1.6	0.6 ± 0.0	200	3.8 ± 0.0*	52
		Planorbis	11.97 ± 0.6**	93	5.52 ± 0.57*	69	6.45 ± 0.04***	119	0.85	4.8 ± 0.8	2	0.1 ± 0.0	-50	4.7 ± 0.8	88
	El-Khankak	*Biomphalaria*	12.42 ± 0.04**	100	7.53 ± 0.26**	130	4.89 ± 0.30*	66	1.63						
	Kobry El-Lansh	*Biomphalaria*	12.39 ± 0.8**	100	6.42 ± 0.08***	96	3.87 ± 0.74	32	1.22	6.3 ± 0.3**	3.5	0.3 ± 0.0	50	6.0 ± 0.3**	140
		Planorbis	10.86 ± 0.6**	75	6.24 ± 0.51*	91	4.62 ± 0.12**	57	1.34	6.5 ± 1.2*	3.7	1.1 ± 0.1**	450	5.4 ± 0.4**	116
Dakahlya	Gammalya	*Biomphalaria*	11.49 ± 0.3**	85	6.72 ± 0.40**	106	4.77 ± 0.12**	62	1.44	5.3 ± 0.1**	2.5	0.1 ± 0.4	-50	5.1 ± 0.2**	104
	Nasayma	*Biomphalaria*	23.04 ± 0.5***	271	13.95 ± 0.30***	327	9.12 ± 0.22***	210	1.53	4.5 ± 0.0*	1.7	2.5 ± 0.0**	1150	2.0 ± 0.0	-20
		Planorbis	24.27 ± 0.7***	291	12.66 ± 0.04***	287	11.61 ± 0.63**	295	1.12	10.4 ± 0.0**	7.6	0.4 ± 0.0	100	10.0 ± 0.0*	300
		Physa	8.94 ± 0.2**	44	6.24 ± 0.04***	91	2.7 ± 0.15	-8	2.35	14.7 ± 1.8*	11.9	1.8 ± 1.3	800	12.9 ± 0.6**	416
	Matarya	*Biomphalaria*	11.67 ± 0.59**	88	7.95 ± 0.35**	143	3.72 ± 0.25	27	2.16	5.0 ± 0.1**	2.2	0.4 ± 1.5	100	4.4 ± 0.2**	76
Damietta	Annanya	*Physa*	9.9 ± 0.6*	59	5.1 ± 0.41*	56	4.8 ± 0.16**	63	1.05	5.7 ± 0.9*	2.9	2.1 ± 0.4*	950	3.6 ± 1.3	44
	Sayala	*Planorbis*								4.8 ± 0.8	2	0.3 ± 0.2	50	4.5 ± 0.7	80

*, ** & *** significant compared to control value at p<0.05, p<0.01 & p<0.001, respectively.
◊ A/G = Ratio of albumin / globulin concentration

Table 2: Total protein, Albumin, globulin, A/G ratio, total Bilirubin, direct and indirect in tissue extract of snails collected from Lake Manzala.

Parameter			CAT (Unites/g)		GST (Unites/g)		GGT (Unites/g)	
Treatments			Mean ± SD	Change %	Mean ± SD	Change %	Mean ± SD	Change %
Field collected snails								
Control lab			9.06 ± 0.18		3.07 ± 0.5		999 ± 24	
Port-Said	Kaar El-Bahr	*Biomphalaria*	3.175 ± 0.37**	-65	1.52 ± 0.18	-50	653 ± 311	-35
		Planorbis	15.15 ± 1.9*	67	5.60 ± 0.13*	82	160 ± 19***	-84
	Kobry El-Lansh	*Biomphalaria*	3.96 ± 0.67**	-56	2.11 ± 0.26	-31	1241 ± 172	24
		Planorbis	7.59 ± 0.00	-16	4.66 ± 0.0	52	1227 ± 0*	23
Dakahlya	Gammalya	*Biomphalaria*	7.92 ± 1.59	-13	2.44 ± 0.33	-21	909 ± 147	-9
		Planorbis	16.7 ± 0.00*	84	5.31 ± 0.0	73	984 ± 0	-1
	Nasayma	*Biomphalaria*	10.69 ± 1.31	18	3.93 ± 0.49	28	716 ± 118	-28
		Planorbis	1.36 ± 0.25***	-85	0.83 ± 0.19*	-73	1152 ± 237	15
		Physa	0.89 ± 0.00**	-90	0.53 ± 0.0	-83	857 ± 0	-14
	Matarya	*Biomphalaria*	7.19 ± 0.05**	-21	3.63 ± 0.72	18	1274 ± 46*	28
		Physa	18.15 ± 0.00**	100	6.63 ± 0.0	116	915 ± 0	-8
		Planorbis	15.52 ± 0.00*	71	5.80 ± 0.0	89	1056 ± 0	6
Damietta	Annanya	*Physa*	7.195 ± 0.54*	-21	4.04 ± 0.93	32	7651 ± 651**	666
		Planorbis	25.785 ± 1.83**	185	6.71 ± 0.41*	119	3901 ± 731*	290
	Sayala	*Planorbis*	17.31 ± 0.00**	91	3.46 ± 2.7	13	1123 ± 266	12

*, ** & *** significant compared to control value at p< 0.05, p<0.01 & p<0.001, respectively.

Table 3: Catalase (CAT), glutathione-S-transferase (GST) and Gamma-glutamyl transpeptidase (GGT) in tissue extract of snails collected from Lake Manzala.

Parameters			Total cell count		Hyalinocytes			Round small hemocytes			Granuolocytes			Hemoglobin (g/100ml)	
Examined			Mean ± SD	Change %	%	Mean ± SD	Change %	%	Mean ± SD	Change %	%	Mean ± SD	Change %		
Control			3.2 ± 1.0		57	1.85 ± 0.5		27	0.8 ± 0.28		16	0.55 ± 0.2		1.8	
Port Said	Kaar El-Bahr	*Biomphalaria*	2.25 ± 0.7*	-30	61	1.4 ± 0.7	-24	24	0.5 ± 0.28	-38	15	0.35 ± 0.4	-36	2.3	28
		Planorbis	1.2 ± 0.3***	-63	57	0.7 ± 0.0***	-62	24	0.25 ± 0.21***	-69	16	0.25 ± 0.3*	-55	2.6	44
	Kobry El-Lansh	*Biomphalaria*	2.35 ± 0.6	-27	60	1.35 ± 0.4*	-27	24	0.55 ± 0.21	-31	16	0.45 ± 0.5	-18	1.8	0
		Planorbis	0.95 ± 0.1***	-70	45	0.4 ± 0.0***	-78	41	0.35 ± 0.07***	-56	14	0.20 ± 0.0***	-64	2.3	28
Dakahlya	Matarya	*Biomphalaria*	2.6 ± 0.1	-19	71	1.85 ± 0.2	0	18	0.45 ± 0.21*	-44	11	0.30 ± 0.3	-45	2.0	11
	Gammalya	*Biomphalaria*	1.4 ± 0.0***	-56	77	1.1 ± 0.0***	-41	16	0.2 ± 0.00***	-75	7	0.10 ± 0.0***	-82	1.7	-6
	Nasayma	*Biomphalaria*	0.9 ± 0.4***	-72											
Damietta	*Sayala*	*Planorbis*	1.95 ± 0.0**	-39	70	1.4 ± 0.0*	-24	15	0.3 ± 0.0***	-63	15	0.3 ± 0.0**	-45		
	Annanya	*Physa*	2.65 ± 0.5	-17	70	1.85 ± 0.6	0	23	0.55 ± 0.21	-31	7	0.25 ± 0.1**	-55	0.8	-56
		Planorbis	1.63 ± 0.04***	-49											

*, ** & *** significant compared to control value at p<0.05, p<0.01 & p<0.001, respectively.

Table 4: Hematologic parameters of snails collected from Lake Manzala.

The histopathological effects of polluted water showed shrinkage and atrophy in the salivary gland of snails collected from Damietta (Figure 2b), focal areas of necrosis (Figure 2c,2d), large fat vacuoles (Figure 2e) and enlargement of the salivary gland (Figure 2f) in snails collected from Port said.

Central ganglia: The central nervous system ganglia are in the form of compact mass of ring surrounding the esophagus of the snail. (Figure 3) showed that all ganglia exhibit presence of enlarged neurosecretory neurons (Figure 3a). Fibrosis (Figure 3b,3c) and degeneration with large vacuoles (Figure 3d) were observed in snail samples collected from Damietta and Dakahlya (Figure 3).

Hepatopancreas: The normal histological structure of *Biomphalaria* hepatopancreas includes glandular tubules interspersed with connective tissues. The entire gland is enclosed within a thin walled sac called as tunica propria. The hepatopancreatic epithelium is rested on thin basement membrane; at least 3-4 types of cells can be recognized in the hepatopancreatic epithelium of the snail, digestive, calcium and excretory cells (Figure 4a). The histopathological changes showed cellular necrosis followed by loss of secretory activity of the epithelial cells in Port Said samples (Figure 3b). Also, atrophy, degeneration and fat vaculation were noticed in Port Said and Damietta samples (Figure 4c,4d). Dilated lumen and more than two hepatopancreatic tubules connected together with one larger lumen in Dakahlya samples (Figure 4e,4f).

Male organs (Prostate gland): The normal histological structures of the male organs of *B alexandrina* composed mainly of sperm duct and the prostate tubules (Figure 5a).

The histopathological observations of Port Said samples showed severe dilated sperm duct and prostate tubules, dilated lumen of prostate tubules which filled with hyaline and degeneration wall with necrotic change (Figure 5b,5c). While Dakahlya samples showed enlarged sperm duct, degenerated prostate tubules and clogged sperms.

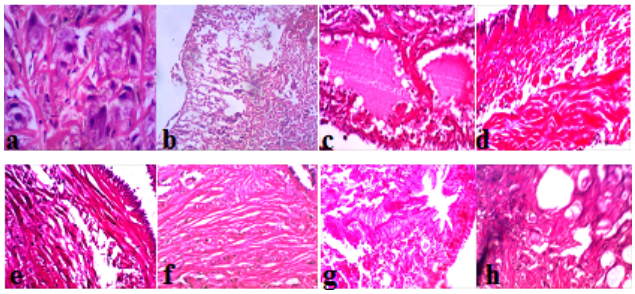

Figure 1: The normal histological structures of the head foot of snail *Biomphalria alexandrina* (a) (X400); shrinkage in the mucous secreting unicellular glands (b) (X100); hyaline degeneration (c) (X400); oblique muscle fiber got splitting and focal areas of necrosis (d &e) (X100); atrophy (f) (X100); empty spaces or vacuoles within muscle (g &h) (X400).

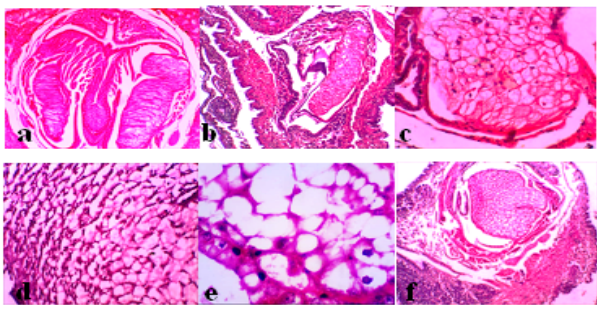

Figure 2: The normal salivary gland of *Biomphalria alexandrina* snail (a) (X40); shrinkage in and degeneration of one lobe (b) (X100); focal areas of necrosis 100x (c &d) (X400); large fat vacuoles (e) (X400); and enlargement of the salivary gland (f) (X400).

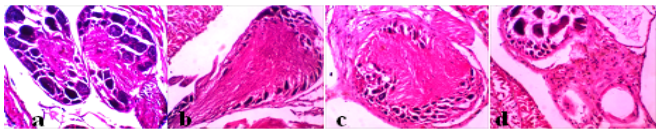

Figure 3: Enlargement of neurosecretory neurons (a) (X400); fibrosis (b, c) (X400); degeneration with large vacuoles (d) (X400).

The prostate gland in Damietta samples showed severe degeneration and atrophy (Figure 5b,5c).

The hermaphrodite gland: Histology of normal hermaphrodite gland of the adult *B. alexandrina* snails as that of any other pulmonate snail consists of number of vesicles known as acini separated from each other by thin vascular connective tissue (Figure 6a). Each acinus is enveloped in a sheath of squamous epithelium. In each acinus both male and female reproductive gametes are produced where mature ova are located at the periphery of the acini and bundles of male sperms are arranged in the center. Various stages of sperm and ovum development (simultaneous) are evident.

Histopathological alteration in Port Said samples included, acini lost their normal architechture and their separating connective tissues are almost degenerated (Figure 5b). The acinar epithilum showed necrotic changes in the form of decreasing cytoplasm of oocytes and partial destruction (Figure 6c). Atrophy and reduction in the number of sperms was also observed (Figure 6d). Degenerative changes were observed in most of the ova, where some of them have faint staining nuclei and others lost their nucleous (Figure 6e,6f). Some acini appear more or less evacuated and large fat vacuoles can be seen in Dakahlya samples (Figure 6g). Damietta showed the most degenerated features

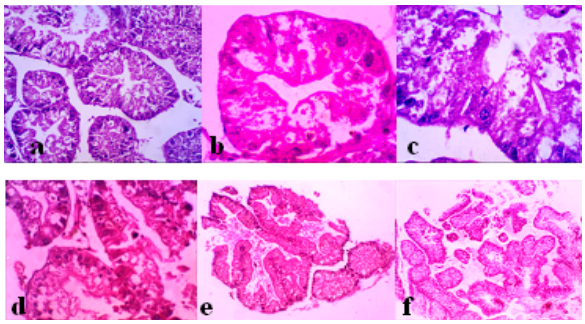

Figure 4: The normal histological structures of hepatopancrease of snail *Biomphalria alexandrina* (a) (X400); vacuolar degeneration of tubules cells (b)(X400); Atrophy, degeneration and fat vaculation (c & d) (x400); severe necrotic change of cells of tubules (b)(X400); Dilated lumen and more than two hepatopancreatic tubules connected together with one larger lumen (e, f)(X100).

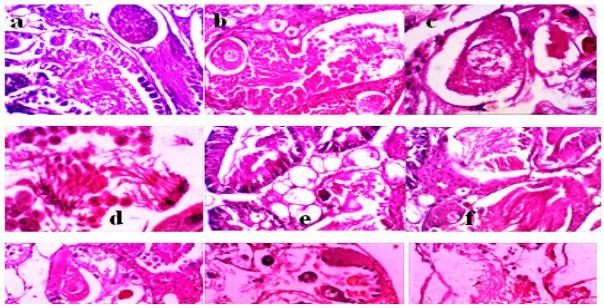

Figure 5: The normal histological structures of the male organs of *Biomphalaria alexandrina* (a) (X100). Severe dilated sperm duct and prostate tubule, dilated lumen of prostate tubules and filled with hyaline degeneration wall of prostate tubules with necrotic change (b & c, X100 & X400), enlarged sperm duct (d, X100), Clogged sperms (e, X100) and degenerated prostate gland (f, X100).

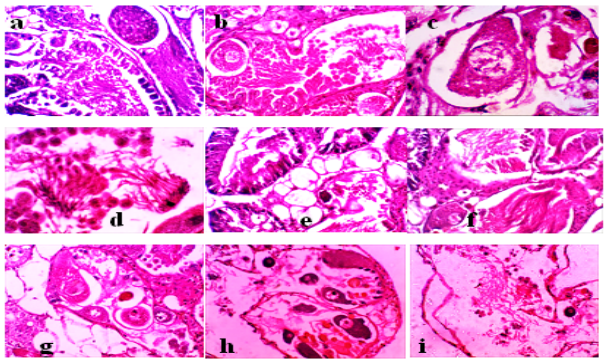

Figure 6: The normal histological structures of hermaphrodite of snail *Biomphalria alexabndrina* (a)(X400); vacuolation and atrophy of different stages of sperm (b)(X400); atretic and absorption of oocytes (c)(X400); atrophy and necrotic change of sperm stages(d)(X400); large fat vacuoles (e)(X400);severe necrotic change(f)(X100); severe fat vacuoles and degenerated hermaphrodite (g)(X100) and degenerated hermaphrodide , atretic oocytes, Atrophy of sperms (h & i, X100).

in the hermaphrodite gland as atretic oocytes and sperms and atrophy of most gland components (Figure 6h,6i).

The infected *Biomphalaria* samples collected from lake manzala

Some of *Biomphalaria* samples collected from Dakahlya and Port Said showed the presence of parasite sporocycts.

The oblique muscle fiber got damaged and mother sporocysts take place within foot muscles, thereby causing splitting, necrosis and increased empty spaces within muscle fibers (Figure 7a-7c).

The digestive gland was destructed while daughter sporocycts which contain many developing cercariae were noticed. The histopathological changes of digestive gland of *B. alexandrina* induced exudation in the lumen of tubules, expansion of hemolymphatic spaces between the tubules, loosing of connective tissue and increase of vaculation and necrotic changes in the digestive cells (Figure 7d,7e,7f).

Accumulation of heavy metals in snail tissues

(Figure 8) showed the accumulation of heavy metals in head foot tissues of *Biomphalaria* snails collected from Port Said, Damietta and Dakahlya samples.

Discussion

Under conditions of pollution mollusks are susceptible to the pathogenic effects of toxicants, which in turn may result in detrimental changes to their immunological and physiological processes [31].

The present results showed significant increase in AST and ALT, and ALP in *Planorbis* and *Physa* snail samples, collected from Nasayma site in Lake Manzala. Moreover, the results showed alterations in CAT, GST and GGT activity in snail samples collected from Lake Manzala.

AST and ALT are vital enzymes in the metabolism and generation of energy from amino acids [32]. Therefore, the elevated transaminases may indicate the high energy demand of the snail under stressful conditions of intoxication. Also, the increase in ALT, AST and ALP enzymes were correlated with alteration in phospholipid metabolism [33] which indicated mainly to hepatocellular disorder [34]. Under physiological stress conditions in animals, the catalytic activity of the urea pathway enzymes is also accelerated [35].

These results are in agreement with [36] who recorded a significant increase of transaminases activity and catalase in the garden snail specimens (*Helix pomatia* L) which were collected from polluted area

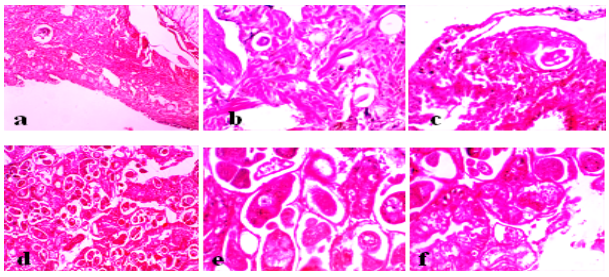

Figure 7: Head foot muscle of *Biomphalaria* snail containing mother sporocyct causing splitting, necrosis and increased empty spaces within muscle fibers (a, b, c X100); hepatopancrease acini filled with different stages of *S. mansonai* cercariae causing degeneration, loosing of connective tissue and increase of vaculation and necrotic changes in the digestive cells (d, X100 & e, f X400).

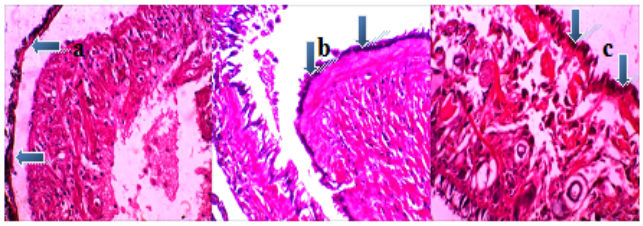

Figure 8: Port Said samples showed mantel layer more dark in color (arrows), separated from head foot (edema) and large number of pigment cells scattered in head foot with necrotic change in the middle of connective tissues (a, X400). Damietta samples showed darkened of mantel (arrows) closed to connective tissues of head foot (b, X400). Dakahlya samples showed atrophy of connective tissues with edema and darkened its outer layer (c, X400).

compared to control. Also, [37] indicated that there are significant elevations in the levels of acid phosphatase and alkaline phosphatase, after using of Profenophos against *B. alexandrina*, which can be explained by the destruction of internal snail cells. Mohamed revealed an elevation in the activities of AST, AlT and AkP enzymes in snails' tissues post treatment with LC_{10} and LC_{25} of Basudin, Selecron and Bayluscide in comparison with control groups [38]. Some other authors recorded increase of the activity of these enzymes, while others recorded decrease in intoxicated animals [36,39,40]. Abdel-Daim reported increased serum AST, ALT, ALP, cholesterol, urea, uric acid, creatinine and tissue MDA after application of deltamethrin subacute intoxication (1.46 µg/L for 28 days) against *Oreochromis niloticus* fish [41]. At the same time they found that tissue levels of GSH, GSH-Px, SOD and CAT were reduced. On the other hand, [42] recorded suppression of the antioxidant enzyme activity and alterations of serum biochemical parameters in freshwater fish Nile tilapia, *Oreochromis niloticus*.

Significant increase of total protein level was recorded also in all samples collected from Lake Manzala. This increase may be attributed to the changes in hepatic protein synthesis [43,44] due to the stress in the polluted habitat. These results go in the same direction as those of [45] who recorded an increase in the total protein concentration in *Helix* snails dependent in the presence of metal dust. Also, [46] highlighted a significant increase in the total protein rate under the effect of a chemical stress at different biological models. Mello observed significant changes in protein metabolism in response to exposure to different concentrations of *E. splendens* var. *hislopii* latex, with significant increases in snails exposed to 0.8 and 1.0 mg/l of the latex, indicating latex toxicity [47]. The same was observed by [48] using other plants and higher concentrations.

Snails collected from most Port-Said and Dakahlya sites showed significant increase in urea. Urea is only synthesized in liver from excess amino acids and excreted by kidney and major illness may increase urea levels [49]. The variation in the nitrogen degradation products showed that the increase of urea content occurred when the uric acid level declined. In accordance with this, the exposure of *Biomphalaria glabrata* to *Euphorbia splendens* var. *hislopii* latex caused the urea content increased which reflects a disturbance in the snail's regulation of their metabolism due to intoxication caused by the latex exposure [50].

Snail samples collected from most sites of Lake Manzala showed significant decrease in total hemolymph cell count, hyalinocytes, round small hemocytes and granuolocytes. The decrease in hemolymph

cells may be considered as a haemolysis response to the multiple pollution elements in Lake Manzala. This was mentioned by [51] that haemocytosis represents a response to external stress or certain stimuli and may originate from a variety of biotic or abiotic sources [52]. These results were in agreement with [53] who found that exposure to dyestuff and chemical effluent could result in decreases in RBC count and Hb content which are symptoms of anemia.

The histopathological changes produced by pollutants in organs and tissues can occur before they produce irreversible effects on the biota. So, histological methods can be used in conjunction with other parameters and/or ecotoxicological bioindicators as an early warning system for the survival of the species, as well as for environmental protection.

Histopathological observations in head foot region of *Biomphalaria* snails showed shrinkage in the mucous secreting unicellular glands and hyaline substances in samples collected from Port Said sites, splitting fiber tissues, increased empty spaces and atrophy within muscles of snail head in Dakahlya and Damietta samples. The salivary gland of snails collected from Damietta showed shrinkage and atrophy while there were focal areas of necrosis, large fat vacuoles and enlargement of the salivary gland in snails collected from Port Said. All snails ganglia showed modified and enlargement of neurons, degeneration with large vacuoles and fibrosis in samples collected from Damietta and Dakahlya. The histopathological changes of hepatopancreas included cellular necrosis followed by loss of the epithelial cells were shown in Port Said samples. Also, atrophy, degeneration and fat vaculation were noticed in Port Said and Damietta samples. Dilated lumen and more than two hepatopancreatic tubules connected together with one larger lumen in Dakahlya samples. The prostate gland in snails of Port Said samples showed severe dilated sperm duct and prostate tubules, dilated lumen of prostate tubules which filled with hyaline and degeneration wall with necrotic change. While Dakahlya samples showed enlarged sperm duct, degenerated prostate tubules and clogged sperms. The prostate gland in Damietta samples showed severe degeneration and atrophy. Regarding the hermaphrodite gland in Dakahlya samples, decreasing cytoplasm of oocytes, partial destruction, lost nucleus, large fat vacuoles, atrophy and reduction in the number of sperms were observed. Damietta samples showed the most degeneration features in the hermaphrodite gland as atretic oocytes and sperms and atrophy of most gland components.

All these histopathological damages in snail organs may be due to the pollution of Lake Manzala water by heavy metals which recorded by [22]. Stress responses in invertebrates can occur following acute or chronic exposures to contaminated environments and as such, the overall health status of individuals within those environments, both in terms of histopathological lesions and the presence of infecting organisms, may ultimately reflect the general health status of these sites [54].

The digestive glands of molluscs have been known as target organs for contaminant effects because; this organ plays a major role in contaminant uptake, intracellular food digestion and metabolism of inorganic and organic chemicals in the organisms [55-57]. However, particulate metal uptake is mainly achieved via the digestive tract by endocytosis; further metals are transferred first to lysosomes and then to residual bodies, especially in the digestive cells of the digestive gland [58]. It could also be possible that in the damage in the snail's hepatopancreas including the alteration of liver and kidney enzymes is according to functionality analog with vertebrate's liver that accumulate mostly heavy metals compared to other organs, and which damage it also [59,60].

In agreement of these results, the exposure of the snails *Archachatina marginata* to sublethal concentrations of the metals resulted in a prevalence of hepatocellular foci of cellular alterations (FCA) in the hepatopancreas of snails. Basophilic adenoma and ovotesticular fibrillar inclusions were also observed in the ovotestes of snails exposed to the test metals [61]. Jonnalagadda have been reported histopathological alterations such as degeneration and the gathering of amebocytes in areas between the tubules in the digestive gland of snail *Bellamya dissimilis* exposed to endosulfan [62]. The histopathological examinations of *Lymnaea luteola* exposed to Paraquat (Gramoxone) revealed the following changes: amebocytes infiltrations, the lumen of digestive gland tubule was shrunken; degeneration of cells, secretory cells became irregular, necrosis of cells and atrophy in the connective tissue of digestive gland [63]. Moreover, it is worthy to mention that in the freshwater snails nervous system has been proved to be sensitive to many toxic materials and cytotoxicants that may induce injurious consequences [64-66].

Some of *Biomphalaria* samples collected from Dakahlya and Port Said showed the presence of parasite sporocycts. The most histopathological deleterious effects have been noticed within the tissues caused in the foot and hepatopancreas due to the invasion of larval trematode parasites to the host snail *B. alexandrina*. The oblique muscle fiber got damaged may be due to penetration of miracidia at the time of infection in the nature. Since earlier stages of larval development i.e. sporocyst and mother sporocyst, takes place within foot muscles, thereby causing increased empty spaces within muscle fibers after their entry in to the viscera of the snail. The digestive tubules epithelium got damaged to the extent of loss of normal tubular structure may be due to metabolic and other excretory materials in the form of granules found scattered in the connective tissue. The destruction of the digestive gland was even more severe may be due to the developing of daughter sporocycts which contains many of the developing cercariae.

Similar observations were recorded by [61] in the snail *Archachatina marginata* that the digestive gland tubule becomes compressed thereby resulting reduced tubular lumen of the gland as observed by that more cercaria and rediae were found in between the hepatic tubules and tunica propria causing extension of the space between tubules.

The histological observations of *Biomphalaria* snails collected from Lake Mazala showed accumulation of heavy metals in the head foot tissues. This was proved in the study of [22] who recorded that the metals concentrations were higher in snail tissues and water samples from Lake Manzala. The collected water samples from Damietta sites showed the highest significant Cu & Cd concentration while Port-Said samples showed the highest Pb concentration and Dakahlia showed the highest Zn concentration.

In conclusion, the severe alterations and degeneration recorded in the physiological and hematological parameters and also histopathological observations are clear evidence for the pollution of the water from which these snail samples were collected. This conclusion is confirmed by [67] who recorded highly significant concentrations of Cu, Cd, Pb and Zn in water samples from different Lake Manzala sites. Also, these metals were highly concentrated in snail and fish tissues and the higher metal bioaccumulation was determined in snails collected from sites showed higher water metals concentrations.

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