

Field Study on Cadmium pollution in relation to internal parasitic diseases in cultured Nile Tilapia at Kafr El-Sheikh Governorate

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Abstract: The aim of this study is to explain the relation ship between cadmium pollution and internal parasitic infestation in tilapia fish. The present study was carried out on 400 specimens of Tilapia fish (*Oreochromis niloticus* (*O.niloticus*) ranged from 20 - 30 cm. While as their average body weights were ranged from 180 ± 10 g. The clinical signs revealed no pathognomonic abnormalities on the external body surface except in heavily naturally infested fish, represented as respiratory manifestation. The postmortem findings of investigated fish revealed the presence of black spots in different parts of the body in some infested fishes. While, internal organs were appeared anemic with enlargement and congestion. As well as, haemorrhage and ulceration of intestine and stomach mucous membrane, white nodules in posterior kidney. The isolated parasites from examined tilapia were 6 types namely: *Enterogyrus cichlidarum*, *Orientocreadium batrochoides*, *Heterophidae*, *Polyonchobothrium sp*, *Paracamallanas cyathopharynx* and *Acanthocentis tilapiae*. Helminth infestations of *O. niloticus* in Sidi Salem district fish farms in autumn season were 11 , 8, 1 and 4 % trematodes , nematode , cestode and *Acanthocentis tilapiae* respectively. Also, in Alirad district fish farms were 9 , 4, 1 and 2 % respectively. While, in Meutobeus fish farms were 6 , 3 , 1 and 2 % respectively. The residues of cadmium in water and *O. niloticus* tissues naturally exposed to cadmium were determined and discussed. The correlation between naturally exposed to cadmium *O. niloticus* tissues and internal parasitic diseases was studied. Also, cadmium displayed a significant decrease in PCV%, RBCs and Hb while elevation in the level of WBCs , blood glucose , serum AST, ALT, urea and creatinine at Sidi Salem district fish farms decreased in Alirad district fish farms and Metobus District fish farms throughout the periods of study. Besides, the histopathological alterations in different organs of *O. niloticus* were recorded.

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Key words: cadmium, *O. niloticus*, internal parasites, histopathology, clinicopathology

1. Introduction

Today, the contamination of freshwater with a wide range of pollutants has become a matter of great concern over the last few decades. Heavy metal levels have increased due to domestic, industrial, mining and agricultural activities (Kalay and Canli, 2000). Aquatic organisms such as fish and shellfish accumulate metals to concentrations many times higher than present in water (Olaifa *et al.*, 2004). They can take up metals concentrated at different levels in their different body organs. Cadmium concentration is higher in gills and viscera than other organs (Khaled, 2004). Studies carried out on fish have shown that cadmium heavy metal may have toxic effects, altering physiological activities in tissue and in blood of fish (Larsson *et al.*, 1985). Therefore, it is important to monitor heavy metal in aquatic environments (water and fish). Now, there is more awareness of the importance of studying fish parasites as one of the major obstacles in fish production about 80% of fish diseases are parasitic especially for warm water fish

(Eissa *et al.*, 1996). The relationship of parasitism and pollution is not simple and essence involves a double edged phenomenon which parasitism may decrease host susceptibility to toxic pollutions may result in an increase or decrease in the prevalence of certain parasites. Pollutants may affect on intermediate or alternate hosts in parasite life cycle, on free-living life cycle stages of parasite invasion (Sindermann, 1990). This study was undertaken to investigate the levels of Cadmium heavy metal in water and *O. niloticus* fish in relation to internal parasitic diseases among Kafr El Sheikh fish farms

2. Materials and methods

2.1. Fish:

A total number of 400 adult cultured *O. niloticus* were randomly collected from Kafr El- Sheikh Governorate fish farms. The collected fish were obtained in autumn season 2010 from three districts areas (Sidi-Salem, Alriad and Metobus fish farms). The length of adult specimens was 20 - 30 cm. While

as their average body weights were ranged from 180 ± 10 g. The collected fish were transferred alive to lab of Hydrobiology Dept. in National Research Center in large plastic tanks filled with two thirds with their natural water from the same source and aerated with air battery pumps.

2.2. Water samples:

A total number of 36 water sample of 3 districts of cultured fish. They were collected from the same fish farms at the same time (midday). The collected water sample bottles were labeled with the locality, date, time and type of fish pond. The flasks, one litre volume were equipped with a cork stopper and open hand prides under water surface then equipped again. The water samples were collected as replicates from various distances along each location and the averages of their analysis were taken.

2.3. Aquaria:

Fourteen fully prepared glass aquaria, $40 \times 50 \times 100$ cm were used for holding the collected fish throughout the period of study. They were supplied with a chlorine free tap water with continuous aeration using electric air pumping compressors (Rena, France) according to Innes (1966); without water filtration or water heater.

2.4. Clinical picture:

Alive fish were clinically examined and postmortem examination according to the methods described by Noga (1996).

2.5. Parasitological examination:

Musculature, gastrointestinal tract and internal organs were examined according to Paperna (1980).

2.6. Blood sampling:

It was collected from the caudal vein of the examined fish using a plastic syringe and divided into two portions. The first portion was kept as a whole blood in heparinized tubes for hematological examination. Serum was separated from the second portion for biochemical analysis according to Dacie & Lewis (1991).

2.7. Cadmium residues:

In liver, kidneys, and musculature were estimated according to Combs *et al.* (1987).

2.8. Histopathological examination:

Autopsy specimens were taken from liver, intestine, spleen, gills and musculature of fish in different groups and fixed in 10% formalin solution for 24 hrs. Washing was done in tap water then serial dilutions of alcohol (70, 90% and absolute ethyl) were

used for dehydration. Specimens were cleared in xylene and embedded in paraffin and sectioned 4 microns thickness by slide microtome. The obtained sections were collected on glass slides, deparaffinized, stained by hematoxylin and eosin and examined microscopically (Banchroft and Stevens, 1996).

3. Results

3.1. Clinical picture:

O. niloticus exposed to cadmium showed slimy body with pale skin, signs of restlessness, some fish suffered from emaciation. Also, abnormal movement and shape (**scoliosis**) were shown with loss of appetite and escape reflex. Postmortem lesions revealed inflamed, enlarged pale spleen and liver spotted with inflammatory patches, while the intestines were darker in colour (**Plate.(A):(1 to 4)**).



Plate.(A): Showing *O. niloticus* naturally exposed to Cadmium suffering from emaciation (1), **scoliosis** (2), degeneration in ovaries (3) enlarged pale spleen and spotted liver with inflammatory patches (4).

3.2. Parasitological examination:

Adult worms were isolated from the stomach of infested fish. Such adult worms are trematodes belonged to class Trematoda, order Monogenea, and genus *Enterogyrus* and identified as *Enterogyrus cichlidarum*. They were isolated from midgut of infested fish adult worms are related to class Trematoda, order Digenea and genus *Orientocreadium* and identified as *Orientocreadium batrochoides*. Cysts were embedded in musculature as black colour, oval in shape with thin double wall cyst. They were related to Heterophid metacercariae. Adult worms were isolated from the intestine of infested *O. niloticus* as small whitish in colour, segmented and flat. Such adult worms belonged to subclass Eucestoda order Pseudophyllidea, family Ptychobothriidae, genus *Polyonchobothrium*. Concerning the

parasitological examination it was revealed funnel shape and armed with large tridents with sclerotized posterior ends, the worm is yellowish in colour when fresh, the buccal capsule was chitinous. Such adult worms belonged to order Spiruridea, family Camallanidae, genus Paracamallanus and identified as *P. cyathopharynx*. Thorny-head worms were isolated from hindgut. Such adult worms were belonged to phylum Acanthocephala, class Eoacanthocephala, order Gyraacanthocephala, family Quadrogyridae, genus Acanthosentis and identified as *Acanthosentis tilapiae* (PLB,1-9).

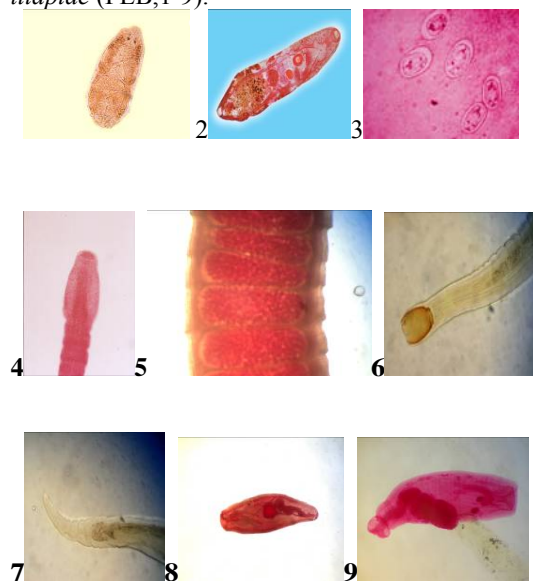


Plate. (B): Showing *Enterogyrus cichlidarum* wet mount (1), Adult fluke, *Orientocreadium batrochoides*. (2), heavy infestation of *Heterophidae* encysted metacercariae in musculature (3), *Polyonchobothrium* sp. (Anterior end). Wet mount (4), *Polyonchobothrium* sp. (gravid segment) (5), *Paracamallanus cyathopharynx*. (Anterior end). Wet mount (6), *Paracamallanus cyathopharynx*. (Posterior end). Wet mount X 10. (7): *Acanthosentis tilapiae* ♂. (8): *Acanthosentis tilapiae* ♀. (9) All stained with acetic acid alum Carmine X 40 except wet mount one.

3.3. Prevalence of internal parasitic diseases in *O. niloticus*

From **Table (1)** it was indicated that a great variation in the infestation % in adult *O. niloticus* naturally exposed to cadmium.

Helminth infestations of *O. niloticus* in Sidi Salem district fish farms, trematodes were 11 %, nematode was 8 %, cestode was 1 %, *Acanthosentis tilapiae* were 4 % in autumn season. Also, Helminthes infestations of *O. niloticus* in Alirad district fish farms, trematodes were 9 %, nematode

were 4 %, cestode were 1 %, *Acanthosentis tilapiae* were 2%. While, Helminthes infestations of *O. niloticus* in Meutobeus fish farms, trematodes were 6 %, nematode were 3%, cestode were 1%, *Acanthosentis tilapiae* were 2% in autumn season (Table,2).

Table(1): - Prevalence of parasitic infestation in adults *O. niloticus* naturally exposed to cadmium in relation to different localities.

Locality	No of Fish	Number of infested Fish		Total
		No	%	
Sidi Salem fish farms	200	48	24	12
Alirad fish farms	100	16	16	4
Meutobeus fish farms	100	12	12	3
Total	400	76		19

3.4. Cadmium residues in water :

Results are shown in **Tables 4&5** (Cadmium concentrations in water of Nile Tilapia farms).

3.5. Cadmium residues in *Oreochromis niloticus* tissues:

Results are shown in **Table 5** (Concentration of cadmium in fresh Nile tilapia tissues). Cadmium residues were significantly increased in internal organs of *O. niloticus* tissues comparing to musculature.

3.6. Hematological studies:

The present study demonstrated that *O. niloticus* naturally exposed to cadmium displayed a significant decrease in P.C.V, RBCs and Hb while elevation in the level of WBCs at Sidi Salem district fish farms these parameters were decreased in Alirad and Meutobeus Districted fish farms throughout the periods of study (Table 6 and 7) .

3.7. Biochemical studies:

The present study demonstrated that *O. niloticus* naturally exposed to cadmium displayed a significant elevation in the level of blood glucose, serum AST, ALT, UREA, Creatinine. at Sidi Salem district fish farms these parameters were decreased in Alirad and Meutobeus Districted fish farms throughout the periods of study (Table 8).

3.8. Histopathological examination:

The histopathological alterations in the affected liver was manifested as melanin pigmented cells with leucocyte inflammatory cells infiltration were observed in the portal vein associated with

congestion in the central vein.while in the affected kidney was manifested as focal haemorrhage in between the degenerated and necrosed tubules associated with dilatation and congestion in the blood vessels with perivascular deposition of melanin pigmented cells .also, in the affected musculature was manifested as hyalinization in some muscular bundles. Oedematus musculature and infested with encysted metacercariae were appear surrounded with serous fluid which contain a network of fibrin and in the affected intestine was manifested as hypertrophy, hyperplastic, proliferation and necrobiosis in the lining epithelium associated with inflammatory cells infiltration in the lamina propria. There was Variations were observed in the thickness of the villi , while the goblet cells were observed in diffuse manner all over the mucosal epithelium . The histopathological alteration in the affected spleen was manifested as hemosiderin was detected in the congested red pulps ,focal melanin pigment cells deposition was observed in the white pulps and in the perivascular tissue of the dilated and congested blood vessels(PL.C,1-5).

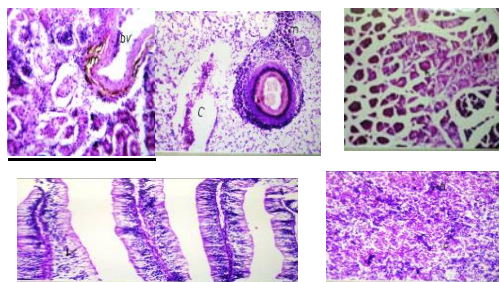


Plate.(C): Showing Liver of *O.niloticus* naturally exposed to Cadmium suffered from melanin pigmented cells and leucocyte inflammatory cells infiltration (m) were observed in the portal area associated with congested central vein © (1), kidney suffered from degeneration in the tubules with dilatation in the blood vessels (bv) (2), hyalinization in some muscular bundles (3), necrobiosis in the mucosal epithelium with inflammatory cells infiltration (v) of villi. (4) and hemosiderin (h) and congested red pulps © (5) . All stained by (H& E) X 40.

Table (2): The correlations between the average of some water parameters in different fish cultures with endoparasitic infestations.

District	No of Exam. fish	No of Infested. fish	Type of parasites	Infestation		Total	
				No	%	No	%
Sidi Salem fish farms	200	48	Trematodes	22	11	22	5.5
			Cestodes	2	1	2	0.5
			Nematodes	16	8	16	4
			Acanthocephala	8	4	8	2
Alirad fish farms	100	16	Trematodes	9	9	9	2.25
			Cestodes	1	1	1	0.25
			Nematodes	4	4	4	1
			Acanthocephala	2	2	2	0.5
Meutobeus fish farms	100	12	Trematodes	6	6	6	1.5
			Cestodes	1	1	1	0.25
			Nematodes	3	3	3	0.75
			Acanthocephala	2	2	2	0.5

Table (3) : Cadmium concentrations in water of Nile Tilapia farms (three localities).

Metal	District	In Autumn season water samples (ppm)		
		Min.	Max.	Mean ± SE
Cadmium	Sidi Salem fish farms	0.13	0.62	0.35± 0.009
	Alirad fish farms	0.062	0.082	0.07± 0.002
	Meutobeus fish farms	0.01	0.07	0.04± 0.015

Table (4): Residue of Cadmium in different *Oreochromis niloticus* tissues :

samples location	Musculature			Liver			Spleen			Kidney		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Sidi Salem fish farms	0.052	0.092	0.072±0.05	0.085	0.095	0.09±0.02	0.049	0.058	0.054±0.02	0.035	0.049	0.036±0.02
Alirad fish farms	0.026	0.046	0.036±0.05	0.053	0.073	0.063±0.02	0.007	0.0097	0.009±0.03	0.0086	0.0098	0.009±0.02
Meutobeus fish farms	0	0	0	0.008	0.01	0.009±0.02	0.007	0.0095	0.009±0.02	0.005	0.009	0.007±0.02

Table (5): Residue of Cadmium in different fish tissues correlated to internal parasitic infestation.

samples location	Musculatures	Liver	Spleen	Kidney	Infested fish %	
					NO	
Sidi Salem fish farms	0.072±0.05	0.09±0.02	0.054±0.02	0.036±0.02	48	24
Alirad fish farms	0.036±0.05	0.063±0.02	0.009±0.03	0.009±0.02	16	16
Meutobeus fish farms	0	0.009±0.02	0.009±0.02	0.007±0.02	12	12

Table (6): Erythrogram (RBCs (10⁶) , Hb and P.C.V.) in naturally exposed *O. niloticus* to cadmium pollution in three different locations in Kafr El Sheikh Governorate fish farms (10 samples from each location).

Districts	Parameters	Control fish		Naturally exposed fish	
		Range	mean	Range	Mean
Sidi Salem fish farms	R.B.Cs (10 ⁶)	1.7 -1.9	1.8 ±0.08Aa	1.6 -1.7	1.65±0.07Cb
	Hb(g%)	8.3-8.6	8.45±1.45Ba	6.6-7.4	7±2.75Aa
	P.C.V%	19.5-22.5	21±2.22Ab	16-18.5	17.25±2.75Ab
Alirad fish farms	R.B.Cs (10 ⁶)	1.7-1.8	1.75 ±0.07Bb	1.8 -1.9	1.85±0.08Aa
	Hb(g %)	8.1-8.4	8.22±1.22Ca	7.1-8.2	7.65±1.65Cb
	P.C.V%	19-21	20±2.10Bb	20.5-23	21.75±2.15Ca
Meutobeus fish farms	R.B.Cs (10 ⁶)	1.75-1.85	1.8 ±0.08Aa	1.7-1.75	1.725±0.05Bb
	Hb(g %)	8.3-8.8	8.55±1.55Aa	7.9-8.7	8.3±1.13Bb
	P.C.V%	20-22.5	21.5±2.15Ab	21-23.5	22.25±2.12Ba

For the same parameter of different locality under study:-

Capital letters: Means within the same column of different letters are significantly different at (P < 0.05).

Small letters: Means within the same row of different letters are significantly different at (P < 0.05).

Table (7): Leukogram parameter (total WBCs and differential count) in naturally exposed *O. niloticus* to cadmium pollution in three different locations in Kafr El Sheikh Governorate fish farms (10 samples from each location).

Districts	Parameters	Control fish		Naturally exposed fish	
		Range	mean	Range	Mean
Sidi Salem fish farms	W.B.Cs (10 ³)	40-44	42±2.40Ab	108-116	112±12.11Aa
	Heterophiles%	3.35-4	3.75±3.15Bb	25.1-27.3	26.5±2.65Aa
	Agranulocytes%	95-98	96.25±2.65Ba	70.3-75-1	73.5±7.33Cb
Alirad fish farms	W.B.Cs (10 ³)	38-40	39±2.39Cb	48-54	51±5.11Ca
	Heterophiles%	4.25-4.65	4.5±0.45Ab	8-8.40	8.25±5.22Ba
	Agranulocytes%	90-98	95.5±5.62Ca	90-92.15	91.25±9.25Bb
Meutobeus fish farms	W.B.Cs (10 ³)	40-42	41±4.11Bb	52.5-57.5	54±4.53Ba
	Heterophiles%	2.75- 3.25	3±3.14Cb	5.1-5.85	5.75±1.75Ca
	Agranulocytes%	95-99	97±7.98Aa	92.3-96.35	94.25±4.25Ab

For the same parameter of different locality under study:-

Capital letters: Means within the same column of different letters are significantly different at (P < 0.05).

Small letters: Means within the same row of different letters are significantly different at (P < 0.05).

Table, (8): Biochemical parameters in naturally exposed *O. niloticus* to cadmium pollution in three different locations in Kafr El Sheikh Governorate fish farms (10 samples from each location)

District	Parameters	Control fish		Naturally exposed fish	
		Range	Mean	Range	Mean
Sidi Salem fish farms	AST(u/l)	88-94	91±9.11Bb	126-146	136±11.36Aa
	ALT (u/l)	18-27	22.5±2.25Bb	37-43	40±4.11Aa
	Urea(mg/dl)	2.9-3.45	3.175±0.03Ab	3.8-4.5	4.15±0.04Aa
	Creatinine(mg/dl)	0.68-0.97	0.74±0.07Ab	0.8-1.3	1.05±0.05Aa
	Glucose(mg/dl)	58-63	60.5±6.50Bb	71-93	82±8.12Aa
Alirad fish farms	AST(u/l)	90-96	93±9.3Ab	120-136	128±12.18Ba
	ALT (u/l)	18-23	20.5±2.05Cb	33-42	37.5±3.75Ba
	Urea(mg/dl)	2.7-3.33	3.015±0.03Aa	2.8-3.7	3.25±0.03Ba
	Creatinine(mg/dl)	0.63-0.68	0.65±0.06Bb	0.63-1.1	0.85±0.08Ba
	Glucose(mg/dl)	60-62	61±6.11Ab	67-73	73±7.3Ba
Meutobeus fish farms	AST(u/l)	84-93	88.5±8.55Bb	96-107	103.5±10.37Ca
	ALT (u/l)	20-28	24±4.22Ab	19-33	26±2.66Ca
	Urea(mg/dl)	2.15-2.85	2.5±0.02Ba	2.3-3.1	2.7±0.02Ca
	Creatinine(mg/dl)	0.71-0.78	0.74±0.07Ab	0.54-1.1	0.82±0.08Ba
	Glucose(mg/dl)	57-64	60.5±6.05Bb	63-69	66±6.16Ca

For the same parameter of different locality under study:-

Capital letters: Means within the same column of different letters are significantly different at ($P < 0.05$).

Small letters: Means within the same row of different letters are significantly different at ($P < 0.05$).

4. Discussion

In Egypt, some areas especially those of Kafr El-Sheikh Governorate, fish farms are depending on agriculture drainage water mixed with industrial and the phosphate fertilizer which is considered the main source of Cd in the environment (Osman, 2009). Both types are considered an important source of cadmium pollution affecting the prevalence of internal parasitic diseases in cultured fishes similar to recorded by Dimari *et al.* (2008). The present study deals with most of different internal parasitic diseases among naturally infested cultured *O. niloticus* in relation to the cadmium concentration in water in Kafer El- Sheikh fish farms. In this work, the main clinical picture in naturally infested *O. niloticus* revealed that some aggregated on the water surface, accumulated at the water inlet of the pond. Also, some fish showed abnormal movement and shape (scoliosis). The results in this investigation showed that cadmium can primarily cause the backbone deformities in fish due to musculature spasms. This result is in agreement with that reported by Olsson (1998). Also, loss of appetite and escape reflex. These signs may be nearly similar to that recorded by Noga (1996) and Eissa *et al.* (2010). These results may be attributed to the prolonged exposure to heavy metals resulted in respiratory, osmoregulatory and circulatory impairment. The internal organs of naturally infested fish appeared pale, anemic with enlargement of liver and spleen and distended gallbladder. Signs of emaciation with petechial haemorrhage on the surface

of abdomen and slight bulging of stomach was observed, while intestinal wall was congested with the presence of ulcer and protruded from anus accompanied with large amount of catarrhal mucoid secretion. These may be nearly similar to that recorded by Ibtsam (2004) and Osman (2005). This may be explained due to the presence of trematodes, nematode, cestode or thorny headed worms which cause harmful effect as they embedded themselves between the villi of intestine causing local damage to the intestinal mucosa and possibly peritonitis. Proteolytic enzymes may be discharged from some adult worms degrading the intestinal tissues (Woo, 1995).

Regarding the internal monogenea (*Enterogyrus cichlidarum*) they were morphologically and parasitologically described and were nearly similar to the descriptions given by Osman (2005) and Noor El Deen (2007). Adult flukes isolated from midgut (*Orientocreadium batrochoides*) was identified depending on the morphological and parasitological characters and the encysted metacercariae, were identified as (Heterophidae). These findings are nearly similar that recorded by Yamaguti (1985). Concerning to the cestodes it was identified as (*Polyonchobothrium sp.*). Such identification is nearly similar that recorded by Ibtsam (2004).

Regarding to the isolated nematodes from naturally *O. niloticus*, isolation and identification of *Paracamallanus cyathopharynx* were undertaken that nearly similar to those of original descriptions by Woo (1995). Finally, the morphological and

parasitological examinations of Tilapia fish revealed isolation and identification of *Acanthosentis tilapiae* whose descriptions are nearly similar to those of original description by Yamaguti, (1985) and Ibtam, (2004).

In the present study a total prevalence of helminth infestations of *O. niloticus* in Sidi Salem district fish farms represented trematodes as 11% , nematode 8 % , cestode 1 % and *Acanthosentis tilapiae* 4 % in autumn season.

Such results are lower than recorded by Osman (2005) who found a prevalence of Enteroglyosis as 67.2%. These variations may be attributed to the water quality criteria and age of fish as such worms are stomach flukes need aged fish have well developed stomach and its wall was thicker for adaptation and fixation for such parasite. These results higher than that recorded with Hassan (1992) who found prevalence as 6% in *O. niloticus*. Such result disagree with that recorded by Tawfik (2005) who recorded a prevalence of digenia in autumn 22.7%. These results may be attributed to different types of fish, the presence of intermediate host (snails), the suitable temperature which consider the main survival factors for these intermediate hosts and aquatic birds (piscivorous birds) according to Noor El Deen (2007). Concerning a total prevalence of *P. cyathopharynx* was 8 % from *O. niloticus*. These findings disagree with that met by Abd El- Wahed (1992) who recorded a prevalence of *P. cyathopharynx* was 1.4% in *O. niloticus*. These results may be attributed to different types of fish, the presence of intermediate host copepods and the suitable temperature. In this study, *Polyonchobothrium* sp could be detected with an prevalence 1 % from *O. niloticus*. These findings are lower than that recorded by Hassan (1992) who found 7.5%. These results disagreed with that recorded by Nadia Mahfouz (1991) who recorded a prevalence of nematodes infestation in autumn were 0% . These differences may be due to variation in climatic and ecological factors which affect on intermediate host copepods (Cyclops) and aquatic birds.

Finally, the prevalence of *Acanthosentis tilapiae* in cultured *O. niloticus* was 4 %. These findings nearly higher than with that recorded by Rawia Adawy (2000) who recorded a prevalence of *A. tilapiae* in cultured two Tilapia sp as 2.4 and 3.7% and lower than that recorded by Eid (1997) who recorded 37.8% in tilapia sp. Also, disagree with Bassiony (2002) who mentioned that the highest infestation rate a prevalence of *A. tilapiae* in cultured Tilapia sp was in autumn 16.2 %. Also, Ibtam (2004) in cultured tilapia sp in autumn season as 10 %. This result may be attributed to different types of fish the presence of intermediate host (amphipod and isopod), the suitable

temperature which consider the main survival factors for these intermediate host.

Cadmium concentration in water of Sidi Salem farms was 0.35 ± 0.009 ppm which higher than the maximum permissible limits recommended by WHO (1984) 0.005 ppm, FAO/WHO (1992) [0.05 ppm] and Egyptian Organization for Standardization and Quality Control "E.O.S.Q.C". [0.1 mg kg⁻¹]. This result may be attributed to presence of industrial activity and agriculture drench branches were supply Sidi Salem fish farms .While in Alrad and Metobus areas were 0.07 ± 0.002 and 0.04 ± 0.015 ppm respectively within the permissible limits. This result may be attributed to absence of industrial activity.

It was observed that cadmium concentration in liver, kidney, intestine and spleen was significantly higher in fish exposed to cadmium. While cadmium concentration was in the permissible limits in musculature. The high contents of heavy metal found in viscera may be due to the fact that most of the heavy metal are accumulated in the liver, spleen, intestine and kidney after ingestion. Khaled, (2004) reported that Cadmium is stored in the body in various tissues but the main site of accumulation in aquatic organisms is in the kidney and liver. Fish musculature is important part to be used for human consumption. This result recorded with Yilmaz (2003) who found that concentrations of heavy metals were higher in all internal organ samples than in muscles.

The results recorded elevation of internal parasites with increase of Cd pollution comparing with that observed in control one. The relationship of parasitism and pollution is not simple and essence involves a double edged phenomenon which parasitism may decrease host susceptibility to toxic pollutions may result in an increase or decrease in the prevalence of certain parasites. Pollutants may affect on intermediate or alternate hosts in parasite life cycle, on free-living life cycle stages of parasite invasion (Sindermann, 1990).

Regarding hematological and biochemical parameters, a decrease in the concentration of haemoglobin in blood, which is usually caused by the effect of toxic metals on gills, as well as a decrease in oxygen also indicates anaemia that confirms negative changes occurring in fish. Glucose is one of the most sensitive indices of the stress state of an organism: its high concentrations in blood indicate that the fish is in stress and it is intensively using its energy reserves i.e. glycogen in liver and muscles. Meanwhile, a decreased concentration of glucose indicates the exhaustion of energy (glycogen) resources and, subsequently, the worsening of an organism status. Namely, a decrease in glucose in the blood of fish is observed during long-term exposure to heavy metals.

The present study demonstrated that *O. niloticus*

naturally exposed to cadmium revealed a decrease in RBCs count (erythropenia) ($1.65 \pm 0.07 \times 10^6$) while Hb (7 ± 2.75 g %) and P.C.V% (17.25 ± 2.75) in Sidi salim district area at Cd level (0.35 ppm). While RBCs count ($1.85 \pm 0.08 \times 10^6$), Hb (7.65 ± 1.65 g %) and P.C.V% (21.75 ± 2.15) in Alriad districted area at Cd level (0.07 ppm). In Metabus district area, RBCs count ($1.725 \pm 0.05 \times 10^6$), Hb (8.3 ± 1.13 g %) and P.C.V% (22.25 ± 2.12) at Cd level (0.04 ppm). The later 2 areas displayed nearly similar level with the finding net with control levels. This may be attributed to their water supply coming directly from Rasheed branch. Regarding Sidi salim district area its water supply coming directly from agriculture and industrials discharges. These results are similar with observations reported by Osman (2009) and Mona Zaki *et al* (2010).

However, an increase in WBCs count (leukocytosis) ($112 \pm 12.11 \times 10^3$) and heterophilis ($26.5 \pm 2.65\%$), while a decrease in agranulocytes ($73.5 \pm 7.33\%$) in comparing to control in Sidi salim district area at Cd level (0.35 ppm). The results shown in Alriad district area at Cd level (0.07 ppm) concerning WBCs count ($51 \pm 5.11 \times 10^3$), heterophilis (8.25 ± 5.22) and agranulocytes ($91.25 \pm 9.25\%$). In Metabus district area, concerning WBCs count ($54 \pm 4.53 \times 10^3$), heterophilis ($5.75 \pm 1.75\%$) and agranulocytes ($94.25 \pm 4.25\%$). This result is nearly similar to control. It may be attributed to the increase of cadmium level in Sidi Salim than Alriad and Metabus districts. These results are nearly similar to what recorded by Gill and Pant (1986) and Ahmed (1996) who recorded that the lymphocytosis condition may be occur due to the stimulatory effect of cadmium on haematopoietic tissues.

The present study showed that the exposure of cadmium was observed an elevation in the blood glucose (82 ± 8.12 mg/dl), serum AST and ALT (136 ± 11.36 and 40 ± 4.11 u/l) in Sidi salim district area respectively. While the glucose, AST and ALT (73 ± 7.3 mg/dl, 128 ± 12.18 and 37.5 ± 3.75 u/l) in Alriad district area respectively and in Metabus district area, the glucose, AST and ALT (66 ± 6.16 mg/dl, 103.5 ± 10.37 and 26 ± 2.66 u/l) respectively. These results in agreement with Mona Zaki *et al.*, (2010) who reported that experimental exposure of *Tilapia zillii* to cadmium sulphate at 0.25 ppm. Several investigations showed that these blood enzymes were highly increased in the fish treated with cadmium. In addition, Shakoori *et al.* (1990) who reported that the increase of blood enzymatic activity is either due to leakage of these enzymes from hepatic cells and thus raising levels in blood, increased synthesis and enzyme induction of these enzymes. Also, Campbell *et al.* (1984) who reported that these enzymes liberate to the blood stream when the hepatic parenchyma cells

are damaged Thophon *et al.* (2003) who found structural and ultrastructural damage in the liver of rainbow trout and white Sea bass following cadmium exposure. These results are nearly similar to what recorded by Attef, (2005) who reported that the fish *O. niloticus* exposed to sublethal concentration of cadmium displayed a significant elevation in the level of blood glucose after one day till the end of the experimental period.

Regarding the naturally exposed fish to cadmium revealed an increase of glucose level may be attributed to stress. Also, De Smet and Bulst, (2002) who observed an increase in the activities of AST and ALT and they suggested that the observed proteolysis is intended to increase the role of protein in the energy production during cadmium stress. The results indicated that cadmium produces severe toxic effects in fish blood.

In the present study, in naturally exposed fish to cadmium was observed an elevation in the serum urea (4.15 ± 0.04 mg/dl) and Creatinine (1.05 ± 0.05 mg/dl) in Sidi Salim district area at Cd level (0.35 ppm) while the serum urea (3.25 ± 0.03 mg/dl) and Creatinine (0.85 ± 0.08 mg/dl) in Alriad district area at Cd level (0.07 ppm) and Metabus district area, the serum urea (2.7 ± 0.02 mg/dl) and Creatinine (0.82 ± 0.08 mg/dl) at Cd level (0.04 ppm). These results in agreement with Mona Zaki *et al.* (2010) who reported that experimental exposure of *Tilapia zillii* to cadmium sulphate at 0.25 ppm induced deleterious effects in fish such as damage of Kidney, liver, spleen and gills, which were reflected on the biochemical and hematological parameters. Also, this result agree with Abbas *et al.*, (2002) and Mona Zaki *et al.*, (2010) who recorded that a significant increase of urea and Creatinine of *Tilapia zillii* exposure to cadmium sulphate at 0.25 ppm.

Histopathologically, the liver showed degeneration of the hepatocytes, congestion of central vein and nuclear pyknosis in the majority of hepatic cells. These findings were apparent as the liver considered the organ of detoxification. Similar results were observed by Van Dyk (2007) who found that liver of fish is sensitive to environmental contaminants because many contaminants tend to accumulate in the liver and exposing it to a much higher levels than in the environment. In this study, the intestine of *O. niloticus* showed, hypertrophy and hyperplastic proliferation of intestinal villi, severe dilatation of blood vessels of the sub mucosa and desquamation of the epithelial lining of the interstitial villi attributed to mechanical injury of the infested parasites and the effect of toxic product. These findings are nearly similar to that recorded with Ibtisam (2004). Concerning histopathological alteration in the affected kidney was manifested as focal haemorrhage in

between the degenerated and necrosed tubules associated with dilatation and congestion in the blood vessels with perivascular deposition of melanin pigmented cells. The results of this investigation related to the kidneys of fish that were purposely poisoned. These results may be similar to that recorded with Tanimoto *et al.* (1999) who recorded that cadmium causing pathological changes of kidney tubules. Similar alterations in musculatures and kidney of Tilapia were observed in several species of fish exposed to heavy metals and these alterations were described by Gupta and Srivastava (2006). Regarding histopathological alteration in the affected musculature was manifested as hyalinization in some muscular bundles. Oedematus musculature and infested with encysted metacercariae were appear surrounded with serous fluid which contain a network of fibrin. These results may be attributed to cadmium pollution. This may be similar to that recorded with Kaoud and El-Dahshan (2010) who recorded that that several histopathological alterations were seen in the muscles of Tilapia which included degeneration in muscle bundles with aggregations of inflammatory cells between them and focal areas of necrosis. Also, atrophy and edema of muscle bundles as well as splitting of muscle fibers. The pathological findings in the intestine included atrophy in the muscularis, degenerative and necrotic changes in the intestinal mucosa and submucosa with necrotized cells aggregated in the intestinal lumen, edema and atrophy in the submucosa.

Finally, regarding histopathological alteration in the affected spleen was manifested as hemosiderin was detected in the congested red pulps and focal melanin pigment cells deposition was observed in the white pulps and in the per vascular tissue of the dilated and congested blood vessels. These observations in agreement with that recorded by Pirarat *et al.* (2008) who observed histopathological change in the spleen of *Oreochromis niloticus* fish exposed to different concentration of cadmium. Histopathological change including ellipsoidal tissue enlargement, melanomacrophage cell aggregation, vacuolar degeneration and edematous capillary and also with Suresh (2009) who observed a significant difference in the frequency and size of melano macrophage centres (MMC) and free macrophage found in, spleen of *Tilapia mossambica* exposed to 20.93 mg l⁻¹ of cadmium chloride and observed also two pigments hemosiderin and melanin.

From the present study, it was concluded that, scoliosis in *O. niloticus* may be an indicator to cadmium pollution and there was a positive correlation between cadmium pollution in water and the prevalence of internal parasitic infestation in *Oreochromis niloticus*. Finally, cadmium residues in

musculature was found in the permissible limits, while in internal organs were relatively high.

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