Journal of Aquaculture Research & Development

Ali et al., J Aquac Res Development 2015, 6:1
DOI: 10.4172/2155-9546.1000294

Research Article Open Access

Effect of El-Sail Drain Wastewater on Nile Tilapia (*Oreochromis niloticus*) from River Nile at Aswan, Egypt

Ali SM[1], Yones EM[2], Kenawy AM[3], Ibrahim TB[3] and Abbas WT[3]*

[1]*Microbiology Department, National Institute of Oceanography and Fisheries, Aswan Research Station, Egypt*
[2]*Fish Diseases Lab, National Institute of Oceanography and Fisheries, Alexandria, Egypt*
[3]*Hydrobiology Department, Veterinary Research Division, National Research Center, Dokki, Giza, Egypt*

Abstract

This study demonstrates the impact of wastewater of El-Sail Drain on the health of *Oreochromis niloticus* collected from two sites of River Nile at Aswan Governorate. One of these sites is before (I) and the other is after (II) the disposal point of El-Sail drain. The physicochemical parameters of water (pH, electric conductivity, total dissolved solids, dissolved oxygen, biological and chemical oxygen demands, nitrite, nitrate and ammonia) were determined. Heavy metals (Cu, Pb, Cd and Ni) concentrations in water and fish tissues (gills, muscles, liver and gonads) were detected. The microbiological, parasitological and pathological conditions of fish were also investigated. Higher values of pH, EC, BOD and COD were detected in site II than from site I. In contrast to DO, nitrite, nitrate and ammonia which were lower in site II. Heavy metals concentrations in water of both sites, especially Ni, Pb and Cd exceeded the permissible limits and its abundance followed the order: Pb>Ni>Cd>Cu. Total bacterial count, total coliform, *Salmonella* sp., *Shigella* sp. and *E. coli* were detected in higher numbers in water samples from site II. Moreover, the fish caught from that site revealed higher bacterial and parasitic infection. The bioaccumulation of Ni and Pb exceeded the maximum permissible limit; however, Cu and Cd concentrations were below the permissible limit in different tissues. The bioaccumulation factor of Cu showed its highest value in liver. The histopathological lesions were more prominent in fish collected from site II. So, consuming fish caught from the studied sites around El-sail drain disposal point represents serious hazard on human health.

Keywords: El-Sail drain; Waste water; *Oreochromis niloticus*; River Nile; Aswan

Introduction

River Nile is the main source of fresh water in Egypt. It receives a lot of pollutants. Nevertheless, the river is still able to recover in virtually all the locations, with very little exceptions [1].

El Sail drain (Kima drain) is considered as one of the major sources of pollution of the River Nile at Aswan governorate. It is used for the disposal of either treated or not sewage wastewater, household solid waste, as well as industrial wastewater (Kima factory). These pollutants can elevate some water parameters, increase levels of BOD and COD in River Nile water and also increase the incidence of pathogenic bacteria, toxic organic compounds and heavy metals; El-Sail drain discharges high amount of organic matter estimated by 10.1 tons/day COD, 3.2 tons/day BOD, 0.03 tons/day heavy metals and 3.25 E+04 MPN/100 ml faecal Coliform bacteria [2]. Kima drain wastewaters exhibit high concentrations of dissolved salts, particularly close to where the waste of the Kima factory enters and decrease substantially near the end of the Kima drain [3].

Heavy metals are the most-active polluting substances; they affect the quality of the environment, with its long-term impact on living organisms. Determination of trace metals concentration in natural water system has received increasing attention for monitoring the environmental pollution, due to the fact that some metals are not biodegradable and accumulated in different organs of animals and human [4]. They can cause serious impairment to circulatory, metabolic, physiological and even structural systems when high concentrations are present in aquatic ecosystems [5].

Unfavorable environmental conditions are the main contributors to stress phenomenon that languish fish immunity and opens the pathway to pathogens and parasites [6]. Many previous studies have stated the presence of *Salmonella, Shigella* and *Escherchia coli* in fish harvested from water polluted with human and animal wastes. Also, representatives of family Enterobacteriaceae are usually found among the most prevalent bacteria on the fresh water fish. These microorganisms adsorbed on the surfaces of fish and may be found also in their intestinal contents [7].

Fish frequently serve as intermediate or transport host for larval parasites of many animals, including humans. Most of fish parasites are believed to cause little or no harm to their host under the natural environmental conditions. However, their mere presence often renders fish undesirable by consumers [8]. While; severe parasitic infection is becoming a threat for fish health management and production throughout the world. It cause decrease in growth rate, weight loss, spread human and animal diseases, postpone sexual maturity of fish and increase fish mortalities [9]. Moreover, the parasitic infection in fish may be detrimental to the fish industry because it lowers the quality, quantity and the economic value of fish [10].

Fish can be used as a monitoring tool for the quality of the aquatic environment and fish histopathology, with a broad range of causes, is increasingly being used as indicator of environmental stress since it provides a definitive biological end-point of historical exposure [11].

***Corresponding author:** Wafaa T Abbas, Department of Hydrobiology, National Research Centre, El-Bohooth Street, Dokki 12622, Giza, Egypt, Tel: 989163128034; E-mail: wtabbas2005@yahoo.com

Received November 10, 2014; **Accepted** November 17, 2014; **Published** January 20, 2015

Citation: Ali SM, Yones EM, Kenawy AM, Ibrahim TB, Abbas WT (2015) Effect of El-Sail Drain Wastewater on Nile Tilapia (*Oreochromis niloticus*) from River Nile at Aswan, Egypt. J Aquac Res Development 6: 294. doi:10.4172/2155-9546.1000294

As well as histopathology can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem [12].

Although, Aswan is fortunate in having good quality fresh water from River Nile comparing to the upward Cairo Province, but it receives domestic, agriculture and industrial wastewaters from different drains. Therefore, this work was planned to determine the effect of El-Sail drain discharge on River Nile water at Aswan and its impact on heavy metals concentrations, microbial and parasitological infection and also the histopathological alterations in different tissues of *Oreochromis niloticus* fish.

Material and Methods

The experimental area

The studied area is selected to be under the influence of El-Sail drain effluent at Aswan city during autumn 2013. Water and fish samples were collected from two sites of the River Nile which were 141 m before the disposal point of El-Sail drain (site I, N:24° 06` 54.94"; E: 32° 53` 54.74") and 245 m after the disposal point (site II, N:24° 07` 05.14"; E: 32° 53` 50.33") as shown in Figure 1. Water samples were collected from the surface water, (ca. <1 m ashore), in sterile brown bottles (200 ml capacity) for microbiological analysis, and in one liter polyethylene bottle for chemical analysis. Samples of Nile Tilapia (*Oreochromis niloticus*) were collected from each site (80 fish/site). Fish were transposed a live back after catching to the laboratory for subsequent analysis. Means of fish total lengths and total weights were 16.98 ± 1.94 cm and 80.15 ± 16.05 g respectively.

Physicochemical analysis of water

Water samples have been subjected to various analyses including pH value, electric conductivity (EC) and total dissolved solids (TDS) by using portable devices (pH meter model HI 8314 and digital conductivity meter HI2300 Hanna Ins. Romania). Dissolved oxygen (DO) was measured using the modified Winkler method and biological oxygen demand (BOD) with the five-day incubation method [13]. Chemical oxygen demand (COD) was carried out using the potassium permanganate method [14]. Colorimetric methods were used to determine ammonia and nitrite [13] and nitrate [15].

Figure 1: Satellite image (A) and photos (B,C) locating El-Sail drainage effluent into River Nile at Aswan, Egypt. X: output of El-Sail drain into the River Nile.

Heavy metals analysis

Heavy metals (Cd, Pb, Cu and Ni) in water samples and Nile tilapia organs (gills, muscles, liver and gonads) were determined using atomic absorption spectrometry (Perkin-Elmer 3110, USA) with graphite atomizer HGA-600, after using the digestion technique by nitric acid according to the standard methods for examination of water and wastewater [16]. The bioaccumulation factor was estimated according to Authman and Abbas [17] as the following equation: Bioaccumulation factor (BAF)=(Pollutant concentration in fish organ (mg/kg)/Pollutant in water (mg/l)).

Bacteriological analysis

Ten ml of each water sample was subjected to serially dilutions (10^{-1} to10^{-5}) with sterile physiological saline (0.85% wt/vol. NaCl) in deionized water. The pour plate technique and the nutrient agar were used for the enumeration of total bacterial counts at both 22°C and 37°C incubation temperatures [13]. For total spore-forming bacteria, water samples and its successive dilutions were pasteurized for 15 min at 80°C, prior to plating in nutrient medium and incubating at 30°C. MPN technique was used for enumerated total and faecal coliforms (using MacConky broth medium) and faecal streptococci (using Azide dextrose broth medium), then incubated at 37°C for 24-48 h [13]. Eosin Methylene Blue (EMB) was used for enumerated Escherichia coli and Violet Red Bile agar (VRB) for enumerated Enterobacterease. As well as Salmonella Shigella agar (SS agar) was used for enumerated *Salmonella* sp. and *Shigella* sp. [16].

Also the fish body surface was wiped with 70% ethanol, and parts of gills, skin, dorsal muscles, liver, gut and gonads were taken from each fish. 10 g of each organ was aseptically transferred in to 90 ml of sterilized 0.85% normal saline, homogenized and centrifuged for 2.5 min at 14000 rpm and then allowed to stand for about five min. Ten-fold serial dilutions up to 10^{7} were done. Nutrient agar was used for total plate count at 22°C & 37°C, EMB for Escherichia coli, VRB for Enterobacterease spp., and SS agar for *Salmonella* sp., and *Shigella* sp. [16].

Parasitological examination

Examination of gills and branchial cavity: Branchial cavity was dissected and examined by naked eye for the presence of large cysts. The gill arches were isolated and put in a saline solution; monogenean worms and crustacean parasites were collected and permanently mounted un-stained in glycerol jelly [6].

Examination of muscles: Small snips of muscles (about one gram) were taken from fish samples, compressed between 2 large glass slides (compressorium) and examined under the binocular dissecting microscope for the presence of metacercariae. Isolated metacercariae were withdrawn by fine long tipped pipette into 0.5% saline solution, stained by acetic acid alum carmine stain, mounted and examined under the microscope [18].

Examination of gastrointestinal tract: The alimentary canal of each fish was separated, dissected and divided into small pieces, washed with physiological saline for several times to get rid of mucus and coarse particles that may be adherent to the parasites, then each part was opened and examined in a Petri dish under binocular dissecting

microscope, the helminthes were collected by Pasteur pipette, stained with carmine stain, mounted and examined under the microscope [6].

Histopathological examination

After dissecting the fish, gills, skin, liver, spleen and gonads were carefully removed and small pieces were fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax and sectioned at 5 microns. Sections were stained according to Harris Hematoxylin and Eosin method [19], examined microscopically and photographed by using a microscopic camera.

Statistical analysis

Data were statistically analyzed using analysis of variance [20], using the STATISTICA (6.0) computer programs.

Results

Determination of water quality

The physicochemical, heavy metal and microbiological analysis of the water samples collected from two sites in River Nile; after and before El-Sail drain were showed in Table 1.

The physicochemical analysis revealed that, pH value, EC, COD and BOD were significantly higher in site II (8.01, 259 µm/l 2.4 mg/l and 1.06 mg/l) than site I (7.82, 253 µm/l, 1.2 mg/l and 0.83 mg/l), respectively. On the other hand, the lower DO value was recorded at site II (1.92 mg/l) and also the soluble forms of nitrogen; nitrite, nitrate and ammonia showed significant reduction in site II (0.009, 0.097 and 0.043 mg/l) comparing to site I (0.012, 0.128 and 0.091 mg/l), respectively.

In respect to the heavy metal analysis, in general, the abundance of tested heavy metals in water followed the order: Pb>Ni>Cd>Cu. Site II had lower Cd concentration and higher Cu concentration comparing to site I, while, Ni and Pb had convergent concentrations in both studied sites. Ni, Pb and Cd concentrations in water of both sites were exceeded the allowable limits.

Parameter	Site I	Site II
Microbiological analysis		
Total Bacterial Count at 22°C (cfu ml^{-1})	2.3×10^{4b}	13.9×10^{4a}
Total Bacterial Count at 37°C (cfu ml^{-1})	3.1×10^{4b}	21.9×10^{4a}
Total Spore-Forming bacteria (cfu ml^{-1})	9^{a}	7^{a}
Total Coliform (MPN/100 ml)	350^{b}	1600^{a}
Fecal Coliform (MPN/100 ml)	50^{b}	275^{a}
Fecal Streptococcus (MPN/100 ml)	110^{a}	110^{a}
Salmonella sp (cfu ml^{-1})	2^{a}	4^{a}
Shigella sp (cfu ml^{-1})	30^{b}	57^{a}
E coli (cfu ml^{-1})	16^{b}	35^{a}
Physico-chemical analysis		
pH	8.01^{a}	7.82^{b}
EC (µm^{-1})	253^{b}	259^{a}
Total dissolved solids (mg l^{-1})	162.2^{b}	165.7^{a}
DO (mg l^{-1})	2.04^{a}	1.92^{b}
BOD (mg l^{-1})	0.83^{b}	1.06^{a}
COD (mg l^{-1})	1.2^{b}	2.4^{a}
NO_2-N (mg l^{-1})	0.012^{a}	0.009^{b}
NO_3-N (mg l^{-1})	0.128^{a}	0.097^{b}
NH_3-N (mg l^{-1})	0.091^{a}	0.043^{b}
Heavy metals (ppm)		
Cu	0.26^{b}	0.41^{a}
Ni	1.81^{a}	1.9^{a}
Pb	2.63^{a}	2.59^{a}
Cd	1.04^{a}	0.13^{b}

Site I: before El-sail drain disposal point. Site II: after El-sail drain disposal point. Means followed by the same letter are not significantly different ($p \geq 0.05$).

Table 1: Water quality of the both studied sites.

The microbiological analysis of water samples showed increase in total bacterial counts in site II than Site I, while there was no difference between the two sites in the spore-forming bacterial count. Coliform bacteria were detected in the two sites with four fold increases in site II than site I, similarly the faecal coliform increased to about five folds in site II than site I. On the other hand, there was no significant difference in fecal Streptococcus count in both sites. The FC: FS ratio was 0.5 in site I and 2.5 for site II. Pathogenic bacteria (*Salmonella* sp., *Shigella* sp., and *E. coli*) were detected in water samples of both sites with higher values in site II than site I.

Concentration of heavy metals in fish tissues

The bioaccumulation of different heavy metals in fish tissues were in the following order, Cu: liver>gonads>gills>muscles; Ni: gills>liver>gonads>muscles; Pb: gills>liver>muscles>gonads and Cd: gonads>muscles>gills>liver. Generally, the highest metal concentrations were recorded in liver and muscles of fish from site II whereas, site I recorded the highest values of metals in gills and gonads. Moreover, the highest bioaccumulation factor (BAF) was that of Cu in liver, followed by Ni and Pb in gills and then Cd in gonads. However, the lowest values of BAF were Cu and Ni in muscles, followed by Pb in gonads and Cd in liver (Table 2).

Microbial load for various organs of Nile tilapia

In general, the highest bacterial load of various fish organs was recorded at site II, followed the order: gut>gills>skin>muscles. The highest TBC recorded in site II (5-2570 cfu$\times10^4$/g) compared with that of site I (1-124 cfu$\times10^4$/g). As well as, the highest count recorded in skin and gut and the lowest recorded in gonads. Similarly, the total spore-forming bacteria (TSF) counts showed increase in site II compared to site I; Gonads and gills of fish from site II recorded the highest TSF count comparing with other organs. Enterobacteriaceae spp. ranged 1–2309 cfu$\times10^3$/g and its number was the highest in gut organs at site II than other organs. *E. coli* detected in all studied organs in site II and detected only in 33% from the examined organs in site I. *E. coli* load in site II was $>10^3$, the highest count recorded in gills (3218 cfu/g) and skin (2356 cfu/g). *Salmonella* sp detected in 50% of tissue samples collected from site I while, it detected in all tissue samples collected from site II at a count of (17-215 cfu/g) in the following order: gills >gut>gonads>skin>liver>muscles. *Shigella* sp. ranged from 1-131$\times10^2$ cfu/g, high count showed in skin of fish collected from site II after El-Sail drain disposal point (Table 3).

Parasitic infection

Different types of external and internal fish parasites were recorded in both studied sites with higher prevalence in site II than site I. The monogenean and crustaceans parasites were recorded in gills with percentages of 70% and 30% in site II and I respectively (Figures 2a and 2b). Regarding the internal parasites, *Clinostomum* sp. trematodes were detected in gills and branchial cavity of fish in lower infestation, 10% in both sites (Figure 2c). While, nematodes and *Acanthocephala* sp. were detected in the intestine of fishes with high percentage (40%) in site II and with low rate (10%) in site I (Figures 2d and 2e). Also, *Diplostomum* sp. encysted metacercareae were recorded in higher number in fish from

Organ	Site	Heavy metals concentrations (ppm)							
		Cu	BAF	Ni	BAF	Pb	BAF	Cd	BAF
Gills	SI SII	5.2 3.05	20 7.44	8.65 7.45	4.78 3.92	11.85 10.45	4.51 4.03	0.35 0.35	0.34 2.69
Muscles	SI SII	1.85 3.15	7.12 7.68	5.2 6.4	2.87 3.37	7.7 8	2.93 3.09	1 0.35	0.96 2.69
Liver	SI SII	46.1 68.05	177.31 165.98	8.4 7.7	4.64 4.05	7.2 11.3	2.74 4.36	0.45 0.05	0.43 0.38
Gonads	SI SII	17.4 0	66.92 0	7.7 5	4.25 2.63	7.25 6.35	2.76 2.45	0.8 0.95	0.77 7.31
Permissible level		30 mg/kg		0.4 mg/kg		2 mg/kg		2 mg/kg	

Site I: before El-sail drain disposal point. Site II: after El-sail drain disposal point. BAF: bioaccumulation factor.

Table 2: Heavy metals concentrations and bioaccumulation factor in different organs of *Oreochromis niloticus* from the both studied sites.

Organs	Sites	TBC at 37°C (cfu x10⁴/g)	SFB (cfu/g)	*Enterobacteriaceae* (cfu x 10³/g)	*E. coli* (cfu/g)	*Salmonella sp.* (cfu/g)	*Shigella sp.* (cfux10²/g)
Muscles	SI SII	17^{d} 80^{cd}	83^{c} 338^{bc}	3^{b} 4^{b}	35^{f} 419^{de}	0^{e} 17^{de}	1^{e} 5^{e}
Skin	SI SII	124^{cd} 2570^{a}	256^{c} 758^{bc}	127^{b} 128^{b}	0^{f} 2356^{b}	12^{de} 43^{c}	46^{cd} 131^{a}
Gills	SI SII	1^{d} 240^{c}	487^{bc} 2159^{ab}	2^{b} 19^{b}	0^{f} 3218^{a}	36^{cd} 215^{a}	19^{de} 91^{b}
Liver	SI SII	2^{d} 92^{cd}	58^{c} $399b^{c}$	1^{b} 7^{b}	0^{f} 759^{c}	0^{e} 29^{cd}	11^{e} 22^{de}
Gut	SI SII	32^{d} 2258^{b}	139^{c} 263^{c}	162^{b} 2309^{a}	0^{f} 471^{d}	115^{b} 122^{b}	61^{bc} 77^{bc}
Gonads	SI SII	1^{d} 5^{d}	190^{c} 3401^{a}	3^{b} 4^{b}	193^{ef} 794^{c}	0^{e} 96^{b}	9^{e} 18^{de}

Site I: before El-sail drain disposal point. Site II: after El-sail drain disposal point. TBC: Total bacteria counts. SFB: Spore-forming bacteria. Means followed by the same letter within the same columns are not significantly different ($p \geq 0.05$).

Table 3: Microbial load in various organs of *Oreochromis niloticus* collected from both studied sites.

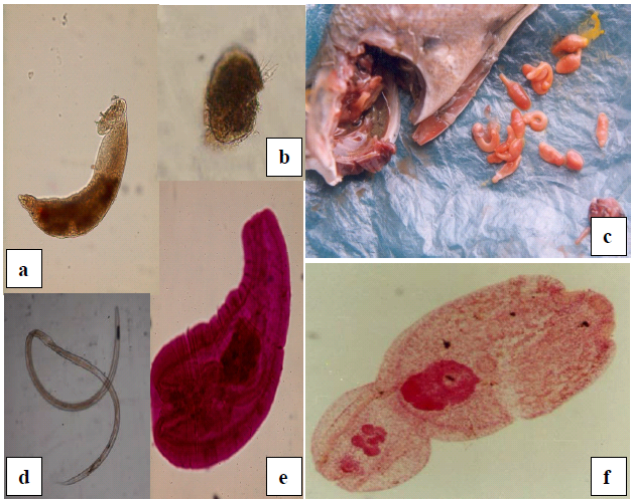

Figure 2: Parasitic infection in different fish organs. **a:** monogenea from gills, **b:** crustacean from gills, **c:** Clinostomatid excysted metacercariaefrom gills and branchial cavity, **d:** nematode larva from intestine, **e:** *Acanthothephala* sp. from intestine and **f:** Diplostomatidexcysted metacercarea from muscles.

site II (10/gm) than that in site I (3/gm) (Figure 2f).

Histological alterations

Fish collected from site II showed much greater damage in their histopathological examination compared to site I which revealed mild changes. The skin of fish collected from site I revealed slight accumulation of melanophores in the epidermal layer (Figure 3a), while severe hyperplasia, hypertrophy and excessive accumulation of melanophores were observed in the epidermal layer of fish collected from site II (Figure 4a). Gills exhibited slight congestion in the lamellar and branchial blood vessels in site I fish (Figure 3b), where gills of site II fish showed edema with epithelial lifting and telangiectasis (Figure 4b) and severe lamellar fusion and epithelial hyperplasia with external parasites were shown in between the gill tissues (Figures 4c and d). Liver in site I fish showed moderate vacuolar degeneration with mononuclear inflammatory cells infiltration in between hepatic parenchyma (Figure 3c) accompanied with slight congestion and widening of the hepatic blood (Figure 3d), on the other hand severe degenerative and necrotic changes in the hepatocytes and pancreatic tissues with aggregation of mononuclear inflammatory cells were observed in site II fish (Figure 4e). Spleen of site II fish had hyper activation of melanomacrophage cells (Figure 4f) accompanied with depletion of the lymphocytic tissues and blood vessels hyperplasia (Figure 4g). Ovary of site I fish revealed normal oocytes with different stages of maturation, stromal edema, (Figure 3e), however site II fish ovary seen in ripe stage with histological changes of oocytes as liquefaction of cytoplasm, nucleus loses and degeneration in oocyte wall with liquefaction of the yolk sphere (Figure 4h). Testis of site I fish exhibited degeneration and necrosis leading to decrease in the number of spermatogenic cells in the numerous siminiferous tubules (++) (Figure 3f), but site II fish testis showed severe degeneration in semniferous tubules, associated with decrease in the number of spermatocytes or spermatids (+++) (Figure 4i).

Discussion

River Nile is the donor of life to Egypt; it represents the principle freshwater source that meets nearly all the demands for drinking water

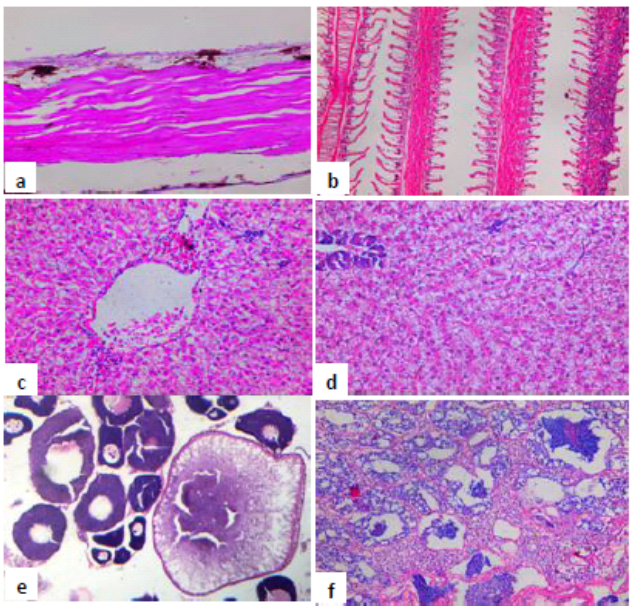

Figure 3: Histopathological changes in *Oreochromis niloticus* collected from site I showed: **a.** Skin with slight accumulation of melanophores in the epidermal layer. **b.** Gills showing congestion in the lamellar and branchial blood vessels associated with hyperplasia at the base of secondary lamellae. **c.** Liver showing moderate vacuolar degeneration with mononuclear inflammatory cells infiltration and slight congestion. **d.** Liver showing widening of hepatic blood vessels and slight congestion. **e.** Ovary showing stromal edema, degenerated and necrotic follicles and oocytes with different stages of maturation. **f.** Testis showing degeneration and necrosis leading to decrease in the number of spermatogenic cells in the numerous siminiferous tubules, and vacuolar degeneration in the germinal epithelium. (H&E, X400).

and irrigation. In recent years, the problems of pollution in water bodies have become a point of local concern. The water quality released from the High Dam in Aswan shows little degradation and it remains remarkably clean from chemical pollution until it reaches to the Delta [21]. Water quality of the River Nile in Aswan is generally meeting the water quality standards stipulated by the Egyptian governmental law 48/1982 [22]. While there are some polluted drains in Aswan which have very bad effect on the water quality; El Sail (KIMA), El Ganayen and El Berba drains. Many health problems were determined previously especially with the high bacterial counts in that water [2].

The present study indicated that, El Sail drain wastewater caused many changes in water quality of River Nile and consequently affects the fish health. Although physicochemical valuesare within the allowable values stated in the Egyptian law 48/1982 [22] but, there is more or less differences comparing the two studied sites after and before the drain disposal point. In general, El-Sail drain waste water causes oxygen content reduction in site II compared with site I, this may be due to the presence of high load of organic pollutants that consumes the dissolved oxygen during oxidation processes, this result was agree with that revealed by other researchers [23,24]. As well as, the BOD values of water from site II was lower than COD which may reflect the hardly biodegradable of discharge compounds [25]. Also, this study reported a decrease in the soluble nitrogen in water of site II comparing to site I, this may be attributed to the decrease in biological activities of aquatic organisms and nitrification in the water column due to the presence of pollution [26].

Regarding the heavy metal concentration, the present study indicated that the concentration of Cu in the water of two sites (0.26-0.41 mg/l) were lower than the permissible levels (1 mg/l) permitted by the Egyptian Organization for Standardization [27]. On the other hand, a high Pb concentration (2.59–2.63 mg/l) which exceed the Egyptian Standards of the Environmental Laws no. 48/1982 [22] (the maximum Pb concentration in water is 0.05 mg/l), Pb can find its way to the water of the River Nile through the leaching of gasoline from the fishery boats and the tour ships travels from Aswan to Sudan. Ni concentrations of water in the two sites were also exceeded the permissible level of EOS (0.07 mg/l) [27]. The Cd concentrations in the water of tested site (0.13–1.04 mg/l) are higher than the permissible level (0.01 mg/l) recommended by the Egyptian Organization for Standardization. Generally, the increase in heavy metals concentrations at the two studied sites around the drain can be attributed to the huge quantities of sewage and industrial wastes via El-Sail drain. Since metals are regarded as serious pollutants of the aquatic environment because of their environmental persistence and tendency to be concentrated in

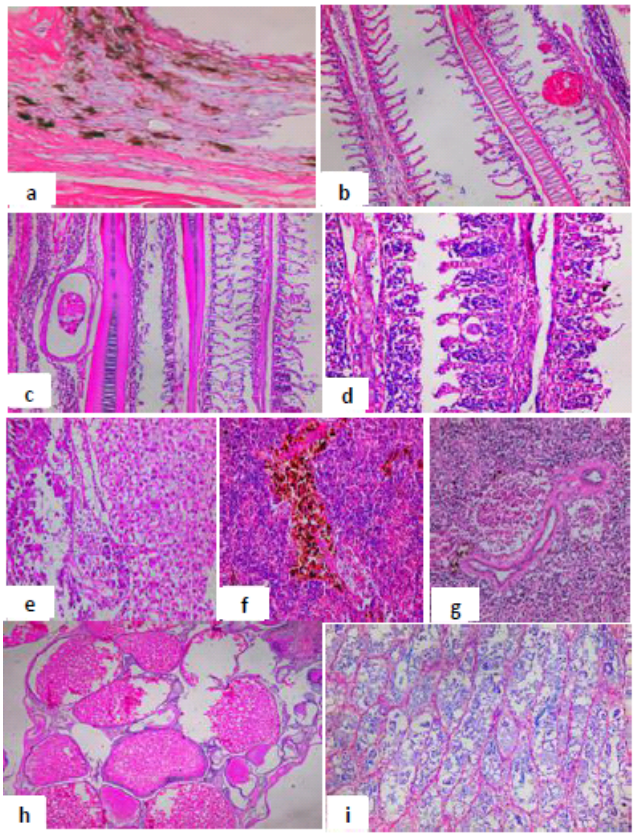

Figure 4: Histopathological changes in *Oreochromis niloticus* collected from site II showed: **a:** Skin with severe hyperplasia and hypertrophy in the epidermal layer and excessive accumulation of melanophores in the dermal layer. **b:** Gills showed edema with epithelial lifting, telangiectasia. **c&d:** Gills showed lamellar fusion, epithelial hyperplasia, proliferation of hypertrophic cells, external parasites in between the gill tissues surrounded with fibrous connective tissue sheet. **e:** Liver showed severe degenerative and necrotic changes in the hepatocytes and in the pancreatic tissues with aggregation of mononuclear inflammatory cells. **f:** Spleen showed congestion and hyperactivation of melanomacrophes cells. **g:** Spleen showed depletion of the lymphocytic tissues, congestion and hyperplasia in the wall of blood vessels. **h:** Ovary showed in ripe stage with liquefaction of cytoplasm of oocyte, nucleus loses and degeneration in wall of oocyte with liquefaction of the yolk sphere with large vacuoles of ripe stage and irregular wall of oocytes. **i:**Testis showing degeneration and necrosis in the primary spermatocytes in semniferous tubules and decreased number of spermatogenic cells. (H&E,X40).

aquatic organisms; heavy metals in fish tissues can reach concentrations up to 20000 fold higher than its concentrations in the surrounding water environment [28]. The difference in the tendency of different organs to accumulate different metals depends upon the target organ, fish species as well as the metal type [29]. The present results showed that the order of metal concentrations was Pb>Ni>Cu>Cd in the gills and muscles and Cu>Pb>Ni>Cd in the liver and gonads. The present data also showed that liver accumulated higher amounts of Cu and this may be due to its ability to retain and store Cu [30]. Similarly, some previous studies found that Cu exhibited its highest levels in the liver and the lowest values in the muscles [31,32]. The high accumulation of Cu in the liver could be attributed to the specific metabolic processes and enzyme catalyzed reaction involved Cu that taking place in the liver. The sulfur legends in the liver also have a great tendency to co-ordinate with Cu via oxygen carboxylate amino group nitrogen and/or sulfur of the mercapto group in the metalothionin protein which is in the highest concentration in the liver [33]. The concentrations of Cu in the muscles of the studied fish are still below the permissible level for Cu (30 mg/kg) recommended by the National Health and Medical Research Council [34]. On the other hand, Pb concentrations in all organs of fish are higher than US FDA maximum permissible level (2.0 mg/kg) and its accumulation in the tissues was in the following order: gills>liver>muscles>gonads. This high Pb accumulation was in agreement with its high concentration in water due to the gasoline pollution caused by boat traffic in this location. The concentrations of Cd in all fish organs are still below the WHO permissible level (2.0 mg/kg) [35].

Regarding the bacterial load in water, the present study indicated an increase in bacterial count in water from site II compared to site I. This may be attributed probably to the sewage disposal from El-Sail drain which spills its untreated waste water directly to river near this site. The ratio of TBC at 22°C to TBC at 37°C not exceeded 10 in both two sites, which explain the high pollution from El-Sail drain wastewater. The bacterial load in water increases by increasing the water temperature and the organic matter [36]. The total bacterial count of skin and gut of fish caught from site II was exceeded the permitted limit. Also, the total spore-forming bacteria, Enterobacteriacae, *E. coli, Salmonella* sp. and *Shigella* sp. was detected in all fish organs and it was the lowest in fish muscles from the two sites comparing with other organs. Microorganisms adsorbed on the surfaces of the fish and that found in their intestinal contents; They do not affect the fish during life but after death saprophytic and commensally residents invade the flesh and bring about its decomposition [37], as well as it can induce disease to humans when handling and consuming such flesh. The recommended total count of bacteria is106 per gram as a maximum permitted limit for fish, and the fish must be free from *Salmonella* sp. and *Shigella* sp. as documented by EGASQC [38]. Faecal coliform bacteria, a subgroup of the total coliform population, had a direct correlation with faecal contamination from warm-blooded animals. This results were emphasizes on a large amounts of sewage disposal directly in the River Nile without any treatments. Fish and fish products have long been considered a vehicle of food-borne bacterial and parasitic infections leading to human illness. Further research is needed to elucidate the behavior of bacterial contaminants in tilapia fish as well as in River Nile ecosystem.

Parasites are attracting increasing interest from parasite ecologists as potential bioindicators of environmental quality due to the variety of ways in which they respond to anthropogenic pollution [39]. Certain parasites can provide valuable information about the chemical state of their environment not only through their presence or absence but also through their ability to concentrate environmental toxins within their tissues in much more concentrations than found in their host. So the present study revealed that the higher percentage of parasitic infestation in site II may be attributed to the water pollution with heavy metals. This is in accordance with other studies which indicated that heavy metal pollution affecting the prevalence of internal parasitic diseases in cultured fishes [40]. In addition to the higher bacterial infection in that site which open the way to the second parasitic infection through weaken the fish and lower its immunity [6].

Concerning the histopathological examinations, fish collected from site II revealed much higher incidence of pathological alterations. The effect of sewage in the present study can be detected using an analysis of pathologies, where the pathological alterations in fish are the net result of adverse biochemical and physiological changes within the organism. Histopathologies are clear symptoms of in situ exposure to pollution in the form of structural alterations [41]. Also such pathological changes in histological structure can substantially impair the function of tissues and organs in fish [42]. A practical advantage of using fish histopathology in environmental assessments is that multiple organs can be examined; this increases the sensitivity at which pollution impacts can be detected. Fish exposed to sewage have more frequent and severe epitheliocystis [43]. Many toxicants (e.g. hydrocarbons, organochlorines and ammonia) cause a wide range of gill pathologies that include telangiectasis and lamellar fusion, epithelial hyperplasia, hypertrophy of chloride and mucus cells, and hyperplasia, as well as higher infestation rates with ectoparasites [44]. The liver is particularly susceptible to damage from a variety of toxicants; it is the major storage site of lipids in fish, liver metabolism is a potential target for the toxic action of chemicals [45]. Fish exposed to contaminated sediments are frequently affected by liver and kidney damage [44,46]. Structural abnormalities can result in the suppression or inhibition of physiological function, irrespective of whether the pathologies are caused by chemical, physical or secondary parasitic irritation. In this study, fish exhibited multifocal pathologies in the gills, liver, kidney, spleen and skeletal muscle tissue, indicating sublethal changes and potential reduction in the functional efficiency of these organs and hence affect the fish health.

Conclusion

This study provides evidence that of water quality and fish health is poorer in the area after disposal point (site II), affected by El-sail drain wastewater which spill the untreated sewage directly to the River Nile at Aswan. Thus, great efforts and cooperation between different authorities are needed to protect the River Nile from pollution and reduce environmental risk at this area which may achieved by treatment of industrial and sewage discharge and regular evaluation of pollutants.

References

1. Wahaab RA, Badawy MI (2004) Water quality assessment of the River Nile system: an overview. Biomed Environ Sci 17: 87-100.
2. Ezzat MN, Shehab H, Hassan AA, El-Sharkawy M, El Diasty A, et al. (2002) A Survey of Nile system pollution sources.
3. Soltan ME (1995) Effect of Kima drain wastewaters on Nile River waters. Environ Int 21: 459-464.
4. Saad SMM, El-Deeb AE, Tayel SI, Al-Shehri E, Ahmed NAM (2012) Effect of heavy metals pollution on histopathological alterations in muscles of *Clarias gariepinus* inhabiting the Rosetta branch, River Nile, Egypt.
5. Yang JL, Chen HC (2003) Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere 53: 877-882.

6. Eissa IAM (2002) Parasitic fish diseases in Egypt. Dar El-Nahda El-Arabia Publishing, 23 Abd El-KhalakTharwat St. Cairo, Egypt.

7. Austin B, Austin DA (1987) Bacterial Fish Pathogens: disease in farmed and wild fish.

8. Ryan L, Joseph E (2000) Biology, Prevention, and Effects of Common Grubs (Digenetic Trematodes) in Freshwater fish.

9. Chandra KJ (2006) Fish parasitological studies in Bangladesh: A Review. J Agric Rural Dev 4: 9-18.

10. Kaddumukasa M, Kaddu JB, Maranga B (2006) Occurrence of nematodes in the Nile Tilapia *Oreochromis niloticus* (Linne) in Lake Wamala, Uganda. Uganda J Agri Sci 12: 1-6.

11. Begum G (2004) Cabofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish *Clarias batrachus* (Linn.) and recovery response. Aquat Toxicol 66: 83-92.

12. Abd El-Aziz, Eman A, El- Habashi, Nagwan, Yones EM (2011) Histopathological alterations of Nile Tilapia (*Oreochromis niloticus*) induced by environmental Contamination in Lake Burullus, Egypt. Egypt. J Comp Path Clinic Path 24: 93-117.

13. APHA (American Public Health Association) (1995): Standard methods for the analysis of water and wastewater.

14. Golterman HL (1971) Methods for chemical analysis of freshwaters.

15. Mullin JB, Riley JP (1955) The spectrophotometric determination of nitrate in natural waters, with particular reference to sea-water. Ann Chim Acta 12: 464-480.

16. APHA (American Public Health Association) (1998): Standard methods for the examination of water and wastewater.

17. Authman MM, Abbas HH (2007) Accumulation and distribution of copper and zinc in both water and some vital tissues of two fish species (*Tilapia zillii* and *Mugil cephalus*) of Lake Qarun, Fayoum Province, Egypt. Pak J Biol Sci 10: 2106-2122.

18. Pritchard MH, Kruse GOW (1982) The collection and preservation of animal parasites. University of Nebraska Press, Lincoln, Nebraska, United States of America.

19. Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T (1999) Histopathology in fish: Proposal for a protocol to assess aquatic pollution. J Fish Dis 22: 25-34.

20. Freed RE, Eisensmith SP, Goetz S, Reicosky D, Smail VW, Wolberg P (1990) STAT, a microcomputer program for the design, management and analysis of agronomic research experiments, version 4.0. Michigan State University, USA.

21. Saad MAH, Goma RH (1994) Effects of the High Dam and Aswan Cataract on the Chemical Composition of the Nile Waters. I. Major Anions. Verh. Internat. Verein. Limnol. 25: 1812-1815.

22. Egyptian Governmental Law No. 48 (1982) The implementer regulations for law 48/1982 regarding the protection of the River Nile and water ways from pollution.

23. Kakuta I, Murachi S (1997) Physiological response of carp, *Cyprinus carpio*, exposed to raw sewage containing fish processing wastewater. Environ Toxicol Water Qual 12: 1-9.

24. Ahmed NAM (2012) Biochemical studies on pollution of the River Nile at different stations of Delta barrage (Egypt).

25. Clark BG, Micheal AU (1972) Waste water engineering in the water Resources and Environmental Engineering, McGrow-Hill New York.

26. Saad SMM, El-Deeb AE, Tayel SI, Ahmed NAM (2011) Haematological and histopathological studies on *Clarias gariepinus* in relation to water quality along Rossetta branch, River Nile, Egypt. Egypt J Exp Biol (Zool.) 7: 223-233.

27. EOS (Egyptian Organization for Standardization) (1993) Egyptian standard, maximum levels for heavy metal concentrations in food.

28. Altindağ A, Yiğit S (2005) Assessment of heavy metal concentrations in the food web of lake Beyşehir, Turkey. Chemosphere 60: 552-556.

29. Mohamed FA, Aboul-Ezz AS (2006) Distribution pattern of some heavy metals in tissues of some fish species from the Mediterranean Sea. Afr J Biol Sci 2: 105-119.

30. Salanki I, Katalin V, Berta E (1982) Heavy metals in animals of Lake Balaton. Water Res 16: 1147-1152.

31. Soltan M, Moalla S, Rashed M, Fawzy E (2005) Physicochemical characteristics and distribution of some metals in the ecosystem of Lake Nasser, Egypt. Toxicol Environ Chem 87: 167-197.

32. Fernandes C, Fontainhas-Fernandes A, Cabral D, Salgado M (2008) Heavy metals in water, sediment and tissues of *Liza saliens* from Esmoriz-Paramos lagoon, Portugal. Environ. Monit Assess 136: 267-275.

33. Abdel-Baky TE (2001) Heavy metals concentrations in the catfish *Clarias gariepinus* (Burchell, 1822) from River Nile, El-Salam Canal and Lake Manzala and their impacts on cortisol and thyroid hormones. Egypt J Aquat Biol Fish 5: 79-98.

34. Marks PJ, Plaskett D, Potter I, Bradly J (1980) Relationship between concentration of heavy metals in muscle tissues and body weight of fish from the Swan-Avon estuary, Western Australia. Aust J Marine Freshwater Res 31: 783-793.

35. FAO (1992) Committee for inland fisheries of Africa: Working Party on Pollution and Fisheries.

36. Al-Harbi AH (2003) Faecal coliforms in pond water, sediments and hybrid tilapia *Orecochromis niloticus* and *Oreochromis aureus* in Saudi Arabia. Aquacult Res 34: 517-524.

37. Ayres JC, Mundt JO, Sandine WE (1980) Microbiology of Foods. W.H. Freeman and Company, San Francisco.

38. EGASQC (Egyptian General Authority of Standardization and Quality Control) (2000): Cairo, Egypt

39. Ogut H, Palm HW (2005) Seasonal dynamics of *Trichodina* spp. on whiting (*Merlangius merlangus*) in relation to organic pollution on the eastern Black Sea coast of Turkey. Parasitology Res 96: 149-153.

40. Overstreet RM (1993) Parasitic diseases of fishes and their relationship with toxicants and other environmental factors.

41. Hinton DE, Lauren DL (1990) Liver ultrastructural alterations accompanying chronic toxicity in fishes: potential biomarkers of exposure.

42. Couch JA, Fournie JWE (1993) Advances in Fisheries Science.

43. Nowak BF, LaPatra SE (2006) Epitheliocystis in fish. J Fish Dis 29: 573-588.

44. Mondon JA, Duda S, Nowak BF (2001) Histological, growth and 7-ethoxyresorufin O-deethylase (EROD) activity responses of greenback flounder *Rhombosolea tapirina* to contaminated marine sediment and diet. Aquatic Toxicol 54: 231-247.

45. Hinton DE, Segner H, Braunbeck T (2001) Toxic responses of the liver.

46. Hansen JA, Lipton J, Welsh PG, Calcela D, MacConnell B (2004) Reduced growth of rainbow trout *(Oncorhynchus mykiss)* fed a live invertebrate diet pre-exposed to metal-contaminated sediments. Environ Toxicol Chem 23: 1902-1911.