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Microbiological and Chemical Examinations of Water and Fish Obtained From River Nile of Damietta Governorate, Egypt

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Abstract This research was carried out at Microbiol. Lab., Faculty of Agric., Damietta Univ. to evaluate the water and fish of River Nile. Water and fish samples were collected from the same site of River Nile in Damietta governorate namely Farskour city during spring, summer, autumn and winter at 2014. All fishes obtained from Rive Nile were belonging to one genus of fish namely, *Oreochromis niloticus* (Nile tilapia). Long, weight and color of examined fish were recorded. Temperature, electrolyte conductivity (EC) and dissolved oxygen (DO) of River Nile water were determined. Obtained results proved that the highest value of Biochemical Oxygen Demand (BOD_5) was found during winter being 7.0 mgO_2/L and the lowest value was during summer being 4.7 mgO_2/L. Cadmium was detected in summer, autumn and winter 0.001, 0.003 and 0.007, respectively. In case of spring these minerals were not detected. The highest value of total bacteria, total fungi, staphylococci, *Aeromonas* and coliform count in the water were 6.121, 4.231, 4.342, 3.301 and 5.997 log cfu/ml, respectively. The highest values of bacterial groups of muscles, intestine and surface of fish were in spring and the lowest values were in autumn. Twenty bacterial isolates were identified; 2 colonies were considered as *Staphylococcus* sp., other 2 were considered as *Micrococcus* sp. Six colonies were considered as *Bacillus* sp. Another five colonies were considered as *Aeromonas* sp. Three fungal isolates were identified as *Aspergillus oryzae*.

Keywords Heavy metals, BOD and Microbial populations.

Introduction

River Nile represents more than 90% of the Nile basin's water resources. Industrial waste waters are considered among the major sources of environmental pollution, endangering public health through direct use as well as feeding fish that live in the polluted streams. It is estimated that more than 400 factories continue to discharge more than 2.5 million m^3 per day of untreated effluent into Egypt's waters [1].

Nowadays, the demand of fish food is increasing throughout the world due to the recognition of its nutritional value. *Oreochromis niloticus* (Nile tilapia) dominates in fresh water due to its superior performance in this environment. Tilapias are considered as the best species for culture because of their high tolerance to both adverse environmental conditions and relatively poor water quality[2].

BOD is the total amount of dissolved oxygen required (milligrams per liter or parts per million, ppm) by microorganisms for biodegradation of organic matters. It is a common indicator used to measure organic water pollutants [3]. Fish and other aquatic animals depend on DO (the oxygen present in the water) to live. The amount of DO in streams is dependent on the water temperature, the quantity of sediment in the stream, the amount of oxygen taken out of the system by respiring and decaying organisms, and the amount of oxygen put back into the system by photosynthesis and aeration [4]. The optimum value of good water quality is 4 to 6 mg/l of DO, which ensures healthy aquatic life in a water body. The seasonal BOD values were slightly higher in summer low during winter and rainy season. Higher values of BOD in summer season due to higher microbial activity and elevated temperature [5].

Bacterial populations of fish skin ranged from 10^2 to10^4 (cfu/cm^2). Gill tissue has been found to harbour high bacterial populations, e.g., up to 10^6 cfu/g. Muscle was considered to be sterile [6]. Bacterial populations in the digestive tract can be up to 10^8 cfu/g. For aerobic heterotrophs and 10^5 cfu/g. For anaerobic bacteria Fish eggs may be populated by high numbers of bacteria 10^3–10^6 cfu/g. Incidentally, the digestive tract of newly hatched larvae contains scant bacterial populations, but are quickly colonized [7]. Fish of a good quality should have counts of total bacteria less than 10/g. Faecal coliforms and total coliforms should not exceed 10/g and 100/g, respectively. Total coliform count in water is not higher than of World Health Organization standard (WHO) ($1.0x10^3$ cfu/100 ml) [8].

Metals are a major category of globally-distributed pollutants and are natural elements that have been extracted from earth and harnessed for human industry and products for millennia. Heavy metal is a term used to define metallic elements with atomic weight higher than 40.0. Metals are notable for their wide environmental dispersion from such activity; their tendency to accumulate in selected tissues of human body; and their overall potential to be toxic even at relatively minor levels of exposure. Some metals, such as copper and iron, are essential to life and play an irreplaceable role in the function of critical enzyme systems. Other metals have no useful role in human physiology (and most other living organisms) and, even worse, as in the case of lead and mercury may be toxic even at trace level of exposure. Metals that are essential have the potential to be toxic at very high level of exposure. One reflection of the importance of metals relative to other potential hazard is their ranking by the United State Agency for Toxic Substances and Disease Registry (ATDSR), which lists all hazards present in toxic waste site according to their prevalence and the severity of their toxicity The first, second, third, and sixth hazards on the list are heavy metals: lead, mercury, arsenic and cadmium, respectively [9]**.**

Therefore, the present study was aimed to determine the physical and chemical state of water and fish obtained from River Nile, Egypt. Also, the microbiological examination including the identifying of bacterial and fungal isolates obtained.

Materials and Methods

Physical and Chemical Examination

Electrolyte Conductivity and Temperature

Electrolyte conductivity (EC) and Temperature were determined using a conductivity meter (CM) (Model: CD-4301, Power: 9 V battery, Lutron Electronic Enterprise Co., LTD Made in Taiwan) [10]**.**

Biological Oxygen Demand (BOD_5)

Dissolving oxygen was determined using a dissolved oxygen meter (Model: YK-22DO, Power: 9 V battery, Lutron Electronic Enterprise Co., LTD Made in Taiwan). The initial dissolving oxygen (initial DO) was determined using a dissolved oxygen meter directly in the site. Water samples (125 ml) were collected from 20 cm below the water surface to avoid floating material using brown glass bottles. These samples were firmly covered and placed in an incubator in the dark for 5 days at 20°C. At the end of this time, the dissolved oxygen level was determined and considered as final DO. BOD_5 was calculated by the method described by Stirling [11]. (BOD^{20}_5) mg/L = (Initial DO - Final DO) x dilution factor

Heavy Metals

These analyses were carried out at Central Laboratory of Damietta. To determine Lead (Pb), Cadmium (Cd), Stannum (St), Arsines (As) and Copper(Co) concentrations, collected water samples were conducted according to the methods of Gloterman *et al*. [12] using Perkin – Elmer atomic absorption spectrophotometer (A.A.S 2) with hydride generation system Perkin – Elmer model PinAAcle 900T, serial No. PTCS12032601.

Microbiological Examinations

Samples Collection and Preparation

Water and fish samples were collected in three replicates from the same site of River Nile in Damietta governorate namely Farskour city during spring, summer, autumn and winter of 2014. Water samples were collected in 100 ml sterile glass bottles and then transferred to the microbiological laboratory into the icebox. One ml of water samples (each is mixed one of the three bottles) or one gram of each fish intestine or fish muscles sample were aseptically transferred to 9 ml of sterile buffer phosphate PH7. For the microbiological examination of fish surface, 10 ml of sterile water were aseptically transferred to a plastic bag containing the tested fish and samples were shaken manually for 2 min, the suspension was collected aseptically in sterilized test tube. The suspension of all samples were shaken for 10 min using a vortex (VM-300 power: 220 VAC, 50Hz, 0.16A/Made in Taiwan-Associated with Cannic, in the U.S.A.) to homogenate the obtained solution. Serial dilutions were performed and one ml of each last three dilutions was used for microbiological examinations [13].

Total Bacterial Count

For total bacterial count of all samples (water and fish), poured plate method was used After preparing suitable serial dilutions of water samples, 1 ml was transferred into sterile glass Petri dish in triplicates. Approximate 15 ml of melted nutrient agar medium at 45-50 °C was poured in each plate, then thoroughly mixed and left for solidification. The plates were incubated at 37 °C for 72 hours in a digital incubator (Switc, MPM Instruments S.R.L., Bernareggio/Made in Italy). After the incubation period, developed separated colonies were counted per each plate of the same dilution and the mean value was calculated [14].

Counting, Isolation and Maintenance of Some Pathogenic Bacteria

One ml of the last three dilutions of all samples (water and fish) were transferred into Petri dishes in three replicates and approximately 15.0 ml of a specific cultivation medium (Staph. 110 medium, *Aeromonas* selective agar medium or S. S. agar medium) was added and left to hardness. Petri dishes were placed upturned in incubator at 37°C for 72 h. The obtained colonies which were produced yellow-orange pigment on Staph. 110 medium was monitored as *Staphylococcus* sp. Also, The colonies which were a yellow color on *Aeromonas* selective agar medium were considered as *Aeromonas* sp. The colonies which were black-center colonies or pink to red colonies were monitored as *Salmonella* sp or *Shigella* sp. All typical colonies were isolated on the same specific cultivation for maintenance and identification [14].

The following microbiological methods were carried out to identify the obtained bacterial isolates according to Holt *et al*. [15]. Shape, arrangement of the cells, the Gram reaction, spore stain and acid fast stain were microscopically examined in stained preparations of 24-48 hrs old bacterial cultures. Presence of spores were recognized in stained smears using Schaeffer and Fulton's method after 2 days old cultures [16]. The colonies count per ml or gram of samples was calculated as follows: The bacterial or fungal count (cfu/ml or cfu/g) = average number of triplicates of the same dilution x reciprocal of the dilution used [17].

Coliform counts were detected using the most probable number (MPN) technique [14]. Three decimal dilutions for each sample in three replicated tubes were employed. One ml of each suitable dilution was added to test tubes containing MacConkey broth medium and Durham tubes, then incubated at 37ºC for 48 hours. The number of positive tubes showing acid and gas were recorded. The MPN of coliform bacteria per gram of sample was calculated from standard Table according to Sutton [18].

Total Fungal Count, Isolation, Maintenance and Identification

One ml of suitable serial dilutions of all water or fish samples were inoculated onto three plates using poured plate method [14]. Approximately fifteen ml of potato dextrose agar (PDA) medium at about 50°C was poured in each plate, then thoroughly mixed and left for solidification. The plates were incubated at 25°C for 5 days. After the incubation period, developed separated colonies were counted per each plate and the mean count of 3 plates was recorded to represent fungal count. Single different developed colonies were isolated on a PDA medium slant for identification tests. The fungal isolates were subcultured then maintained on PDA slants at 5°C till use [19]. Fungal isolates were identified by morphological characteristics of colonies in PDA medium. In addition, the vegetative and reproductive features observed using a light microscope (Olympus CX31 Binocular Halogen Microscope, Made in Japan) with a magnification power 400x, was also used. The taxonomic keys of Chung and Bennett [20] were used.

Statistical analysis

The differences between means were calculated by Duncan`s Multiple Range Test. Regression coefficient was analyzed with original data [21].

Results and Discussion

Morphological examination of fish obtained from River Nile during seasons of 2014

All fishes obtained from Rive Nile were belonging to one genus of fish namely, *Oreochromis niloticus* (Nile tilapia).

Table 1: Morphological examination of Nile tilapia (*Oreochromis niloticus*) obtained from River Nile during four seasons of 2014

Seasons	Long (cm)	Weight (g)	Fish color
Spring	7	37	Green with red color in abdomen region
Summer	10	33	Black with yellow and red parts in abdomen region
Autumn	7	22	Gray with white parts in abdomen region
Winter	4	15	Dark gray with yellow and red parts in abdomen region

BOD and EC values of water obtained from River Nile during seasons of 2014

The mean value of long of three individuals were 7, 10, 7 and 4 cm in spring, summer, autumn and winter, respectively **(Table 1)** Each value represents the mean of 3 individuals. The highest weight of fishes were obtained during spring and summer being 37 and 33 gm, respectively, and the lowest weight were in case of autumn and

winter being 22 and 15 gm, respectively, It was observed that, the color of fishes were differed according to seasons, where it was green with red color in abdomen region **(Fig. 1a)** during spring. On the other hand, fish color was black with yellow and red parts in abdomen region **(Fig. 1b)** during summer. It was grey with white parts in abdomen region **(Fig. 1c)** in the autumn, and dark grey with yellow and red parts in abdomen region **(Fig. 1d)** in the winter.

Data in **Table 2**. showing that, temperature varied between 18 and 25°C. Initial DO were 6.6, 6.0, 7.0 and 7.5 mgO_2/L in spring, summer, autumn and winter, respectively. Final DO were 5.6, 4.7, 5.9 and 7.0 mgO_2/L in spring, summer, autumn and winter, respectively. The highest value of BOD_5^{20} was during winter being 7.0 mgO_2/L and the lowest value was during summer being 4.7 mgO_2/L. The highest value of EC was round in winter being 1.95 mhos/cm and the lowest value was in spring being 1.48 mhos/cm. The DO of **this study was higher than that of Surendraraj** *et al*. [22] who detected the DO of feeder canal water and they found that, the values ranged between 0.89-3.53 ppm. BOD and salinity were lower which were ranged between 4.83-13.6 ppm and 0.07-0.23 mhos/cm.

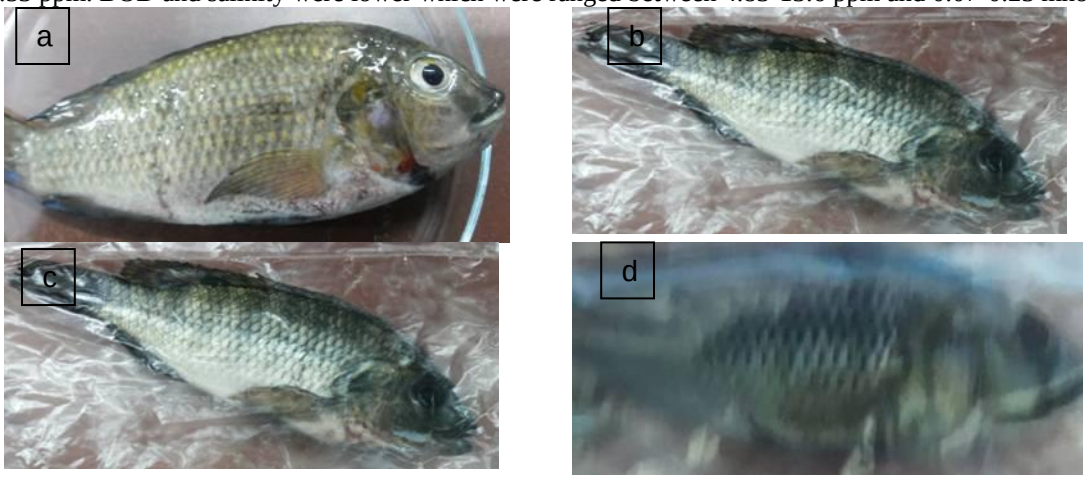

Figure 1: Oreochromis niloticus obtained from River Nile during
a) spring, b) summer, c) autumn and d) winter

Table 2: BOD and EC values of water obtained from Damietta governorate during four seasons of 2014

Seasons (2014)	Temperature (°C)	DO, mgO_2/L Initial	Final	BOD_5^{20} (mgO_2/L)	EC (mhos/cm)
Spring	20	6.6	5.6	2.6	1.48
Summer	25	6.0	4.7	3.1	1.58
Autumn	22	7.0	5.9	2.0	1.60
Winter	18	7.5	7.0	1.5	1.95

Heavy metals values of water and fish muscles during seasons of 2014.

Lead and cupper were not detected in all seasons samples except in spring being 0.006 and 0.106 ppm, respectively. On the other hand, Cadmium was detected in all seasons to be 0.001, 0.003 and 0.007 during summer, autumn and winter, respectively, while in spring it was not detected. Stannum and arsines did not present in all seasons. Also, heavy metals were determined in all fish muscles samples obtained from River Nile. Cadmium, Cupper, Arsines and Stannum did not found in all seasons. The values of lead were 0.088 and 0.040 ppm in spring and summer ,respectively, while they not detected in autumn and winter. These results are lower than the permissible levels (1 mg/L) permitted by the Egyptian Organization for Standardization [23]. Also, Pb concentration did not exceed the Egyptian Standards of the Environmental Laws No. 48/1982 [24]. which the maximum Pb concentration in water was 0.05 mg/L.

The Results of this study are lower than those obtained by Ali *et al*. [25] who tested heavy metals in Nile Tilapia (*Oreochromis niloticus*) from two sites of River Nile at Aswan (water and fish) and they found that, the concentrations of heavy metals (Cu, Ni, Pb and Cd) of water in the first site were 0.26, 1.81, 2.63 and 1.04 ppm, respectively. In the site II the values were 0.41, 1.9, 2.59 and 0.13 ppm, respectively. On the other hand, the concentrations of heavy metals of fish muscles in site I and II were 1.85, 5.2, 7.7, 1.0 and 3.15, 6.4, 8.0, 0.35 ppm, respectively.

Ali *et al*. [25] also explain that, 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. Moreover the increasing of heavy metals concentrations at River Nile can be attributed to the huge quantities of sewage and industrial wastes via drains.

Microbiological examination of water taken from River Nile (Damietta governorate) during seasons of 2014

Results in **Table 3** showing the highest value of total bacteria count was during spring being 6.121 log cfu/ml, while the lowest value was in winter being 2.477 log cfu/ml. The high bacterial load may be enplaned by the observation of Ali *et al*. [1] who reported that, the richness of the effluent in organic carbon exerted a specific enrichment effect on the microbial population**. Table 3** also showed that, the highest value of total fungal count was in summer and spring being 4.231 and 4.041 log cfu/ml, respectively. the total fungal count was in the lowest value in the autumn and winter being 1.0845 and 1.845 log cfu/ml, respectively. It was observed that, there were no bacterial growth on SS agar medium. The highest value of Staphylococci count was in spring being 4.342 log cfu/ml, while it was in the lowest value in autumn being 1 log cfu/ml. The highest value of *Aeromonas* count in summer being 3.301 log cfu/ml, but the lowest values were during spring, autumn and winter to be 2.797, 2.602 and 2.544 log cfu/ml, respectively. The highest values of coliform count were in spring and the summer being 5.997 and 4.964 log cfu/ml, respectively, while coliform count was in the lowest value in the autumn and winter being 0.477 and 0.301 log cfu/ml, respectively.

These results are in good agreement with those of Osman [26] who showed that, the microbiological quality of the River Nile was carried out from three different sites i.e. Helwan, El-Giza and Shoubra. The highest average log number of total coliform, faecal coliform and faecal streptococci were 4.38, 3.29 and 2.58, respectively. Those values are lower than that obtained by Ali *et al.* [25]. who reported that, the total bacterial count of the samples ranged from 2.3 $x10^4$-2.19 x 10^5 cfu/g. Also, these results did not similar to that obtained by Ali *et al.* [25] who reported that, the *Salmonella* and *Shigella* counts ranged from 2 and 57 cfu/g. This study took the same trend of Ali *et al*. [1] who found that, the total microbial load of various bacterial groups being highest in autumn and lowest in winter. They also found that, water load of bacterial indicators of pollution, in particular faecal coliforms, was higher in spring compared to other seasons. Moreover El-Kadi and El-Morsy [27] studied the microbiological populations of water of Nile tilapia and they found that, the maximum value of total bacterial count and total yeast and fungal in the water of fish were 2.88×10^4 and 7.3×10^2 CFU/ml, respectively.

Table 3: Microbiological values (Log cfu/ml) of water taken from River Nile (Damietta governorate) during seasons of 2014

Seasons (2014)	**°C**	**Count of Total bacteria**	**Total fungi**	**Staphylococci**	***Aeromonas***	**Coliform**
Spring	23	6.121	4.041	4.342	2.797	5.997
Summer	28	4.114	4.231	3.000	3.301	4.964
Autumn	20	3.301	1.845	1.000	2.602	0.477
Winter	14	2.477	1.840	1.041	2.544	0.301

Microbiological examination of fish obtained from River Nile (Damietta governorate) during seasons of 2014.

The total bacterial count of fish muscles were 3.403, 5.602 , 0.954 and 2.000 log cfu/ml in spring, summer, autumn and winter **(Table 4)** ,respectively. On the other hand, the highest value of total bacterial count of fish intestine was 8.598 log cfu/ml in spring, but the lowest value was in the autumn being 1.398 log cfu/ml. Total bacterial count of fish surface were 6.297, 6.447, 3.114 and 3.114 log cfu/ml in spring, summer, autumn and winter, respectively. The highest values of fungal count in muscles, intestine and fish surface were during autumn, summer and spring being 1.477, 7.903 and 5.121 log cfu/g, respectively. On the other hand, all lowest fungal counts of muscles, intestine and surface were during winter being 0.699, 5.301 and 1.845, respectively.

Table 4: Microbiological examination (Log cfu/gm) of fish obtained from River Nile (Damietta governorate) during seasons of 2014

Seasons (2014)	**Count of Fish part**	**Total bacteria**	**Total fungi**	**Staphylococci**	***Aeromonas***	**Coliform**
	Muscles	3.403	1.041	2.083	2.422	4.218
Spring	Intestine	8.598	7.899	6.246	7.172	9.083
	Surface	6.297	5.121	4.917	3.513	5.083
	Muscles	5.602	0.954	0.778	0.778	1.230
Summer	Intestine	7.447	7.903	1.301	5.301	4.477
	Surface	6.447	5.086	1.114	0.602	1.041
	Muscles	0.954	1.477	0.945	0.477	0.301
Autumn	Intestine	1.398	6.114	1.477	2.301	1.322
	Surface	3.114	2.477	1.176	2.301	0.699
	Muscles	2.000	0.699	0.845	2.114	0.903
Winter	Intestine	3.000	5.301	2.301	3.398	1.361
	Surface	3.114	1.845	2.301	4.778	4.633

It was observed that, there were no bacterial growth on SS agar medium. The highest values of Staphylococcal counts of fish muscles, intestine and fish surface were in spring being 2.083, 6.246 and 4.917 log cfu/g, but the lowest values were in summer being 0.778, 1.301 and 1.114 log cfu/g, respectively.
The highest count of *Aeromonas* was in fish muscles during spring being 2.422 log cfu/g and the lowest value was during autumn being 0.477 log cfu/g. The highest count was in fish intestine during spring being 7.172 log cfu/gm and the lowest value (2.301 log cfu/gm) was during autumn. *Aeromonas* count on fish surface ranged between 4.778 and 0.602 during winter and summer, respectively. Coliform was found in the highest values in all fish parts in spring being 4.218, 9.083 and 5.083 log cfu/g in muscles, intestine and fish surface, on the other hand, coliform count was in the lowest values in the autumn.

These results are higher than those obtained by Surendraraj *et al.* [22]. who reported that, the total bacterial counts of muscles of carp farm fish ranged between log 4.19 – 4.85 cfu/g. Also, they found that, the total coliform of farmed fish ranged between log 2.0 – 3.4 cfu/ml. Mohammed and Hamid [28] studied the bacterial load of fresh fish (*Oreochromis niloticus* and *Clarias lazera)*, at the period from November 2009 to March 2010 in the Sudan University of Science and Technology, Department of Fisheries and Wildlife Science. The results were 8.4×10^5 and 1.7×10^5 cfu/g for fresh Tilapia and fresh catfish, respectively. These results are higher than obtained results. Jimoh *et al*. [29] studied the microbial flora of the gastro-intestinal tract of *Clarias gariepinus* caught from river Dandaru Ibadan, Nigeria. They found that, the total bacterial count, total fungal count and total coliform count were 6.5 x 10^5, 3.0 x 10^3 and 1.9 x10^4 cfu/g., respectively. In the same trend, El-Kadi and El-Morsy [27] reported that, the maximum value of total bacterial count and yeast and fungal count in fish intestine were 1.96×10^6 and 12.2 $\times10^2$ (CFU) /g, respectively.

The relationship between the log of total bacterial count and the biological oxygen demand
The correlation coefficient between the log of total bacterial count (LTBC) in the water of River Nile and the BOD was calculated **(Fig. 2)**. Statistical analysis indicated a low correlation (r = 0.552) between the log of total bacterial count and BOD. Kagalou *et al*. [30] reported that, a positive correlation between BOD and total bacterial count in a river ecosystem highly polluted by industrial pollutants. In contrast, other author [1] found a negative correlation between both total and faecal coliforms with BOD.

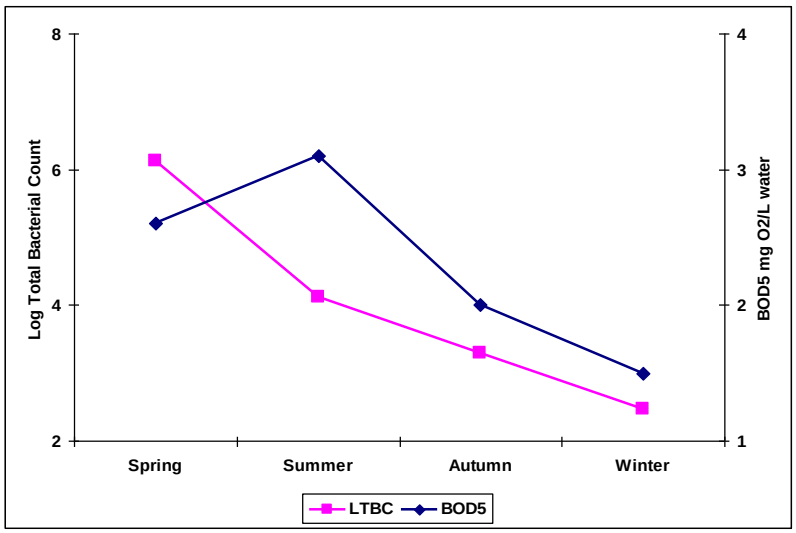

Figure 2: The relationship between the log of total bacterial count and the biological oxygen demand

Characterization and Identification of Bacterial Isolates
Table 5 showing bacterial isolates numbers, characterization and its sources. Seven different bacterial isolates were isolated from nutrient agar medium, 9 isolates were isolated on *Aeromonas* agar medium and 4 isolates were found on Staph 110. medium. Eleven isolates were isolated from water, 6 from muscles, 2 from intestine and 4 from surface of fish. Among 20 bacterial isolates, only 4 were coccoid shape, 10 isolates were short rods and 6 isolates were long rods. Ten isolates were Gram negative and 10 isolates were Gram positive. Six isolates were spore formers and 14 isolates were non spore formers. All isolates gave negative results in acid fast stain.

Table 5: Bacterial isolates numbers, characterization and sources obtained from fish and water of River Nile (Damietta governorate) during seasons of 2014

Sources (2014)	Cultivation media	Isolates Nos.	Characterization of isolates				
			Shape	Arrangement	Gram stain	Spore stain	Acid fast stain
Water	Nutrient Agar	9	Short rods	Single	-	-	-
		11	Short rods	Single	-	-	-
		22	Long rods	Pair	+	+	-
		23	Long rods	Chain	+	+	-
	Aeromonas	39	Short rods	Single	-	-	-
		6	Long rods	Single	+	+	-
		42	Long rods	Single	+	+	-
		77	Short rods	Single	-	-	-
		78	Short rods	Single	-	-	-
	Staph 110.	33	Coccoid	Staph	+	-	-
		37	Coccoid	Clusters	+	-	-
Fish Muscles	Nutrient Agar	40	Short rods	Single	-	-	-
		59	Short rods	Single	-	-	-
	Aeromonas	16	Long rods	Single	+	+	-
		76	Short rods	Single	-	-	-
	Staph 110.	15	Coccoid	Clusters	+	-	-
Fish Intestine	*Aeromonas*	75	Short rods	Single	-	-	-
Fish Surface	*Aeromonas*	41	Long rods	Single	+	+	-
		74	Short rods	Single	-	-	-
	Staph 110.	21	Coccoid	Pair	+	-	-

Two typical colonies representing Staphylococcal growth, orange color on Staph. 110 medium, colonies were picked up and streaked onto slant of the same medium. After growth, the morphological characteristics under light microscope were done. The cells were spherical, Gram positive, arranged in irregular clusters. Isolate No. 15 and 37 were considered as *Staphylococcus* sp. according to Bergey's Manual of Determinative Bacteriology [15]. Isolates Nos. 21 and 33 were considered as *Micrococcus* sp. This results are in harmony with those published by Ali [1] who isolated and identified of *Staphylococcus sp* from fish of fresh water in Mosul city. Five species of *Staphylococcus* sp, *S. saprophyticus*, *S. epidermidis*, *S. hyicus*, *S. aureus*, and *S. intermedius* were identified. The percentages of *Staphylococcus* isolates from skins was 35.5%, 17.7% in muscles, 25.8% in livers and 21% in intestines.

Six colonies which were white, yellow or orange color, were picked up and streaked onto nutrient agar slant. After growth, the morphological characteristics under light microscope were done. The cells were long rods, Gram positive, spore formers and non acid fast. Its arrangement were single, pair or chains. Isolates Nos. 6, 16, 22, 23, 41 and 42 were considered as *Bacillus* sp. according to Bergey's Manual of Determinative Bacteriology [15]. Jimoh *et al*. [29] isolated *Bacillus alvei* and *Bacillus megaterium* from the microbial flora of the gastro-intestinal tract of *Clarias gariepinus* caught from river Dandaru Ibadan, Nigeria.

Five colonies isolated on *Aeromonas* agar medium that gave yellow color were picked up and streaked onto the same medium slant. After growth, the morphological characteristics under light microscope were done. The cells were short rods, Gram negative, non spore formers and non acid fast. Its arrangement was single. Isolates Nos. 74, 75, 76, 77 and 78 were considered as *Aeromonas* sp. according to Bergey's Manual of Determinative Bacteriology [15]. Similar results were obtained by Rokibul *et al* [31] and Jimoh *et al.* [29] who isolated *Aeromonas* sp.

Colonies isolated on nutrient agar medium which were white or yellow color were picked up and streaked onto nutrient agar slant. After growth, the morphological characteristics under light microscope showed that. The cells of isolates Nos. were 9, 11, 39, 40 and 59. were short rods, Gram negative, non spore formers and non acid fast. Its arrangement was single.

Characterization of Fungal Isolates

Three fungal isolates were isolated from all samples. Characterization of the isolates showed that, colonies yellow green on PDA medium **(Fig. 3)**, conidiophores colorless, long, coarsely roughened. conidial heads typically radiate,

conidia globose to subglobose. From these characteristics, isolates Nos. 38, 60 and 61 were identified as *Aspergillus oryzae* [20]. Jimoh *et al*. [29] isolated *Aspergillus niger, Aspergillus flavus,* from the gastro-intestinal tract of *Clarias gariepinus*. In addition, El-Kadi and El-Morsy [27] isolated five fungal isolates and identified it as *Aspergillus ochraceus, A. oryzae, A. niger, Geotrichum candidum* and *Penicillium* sp.

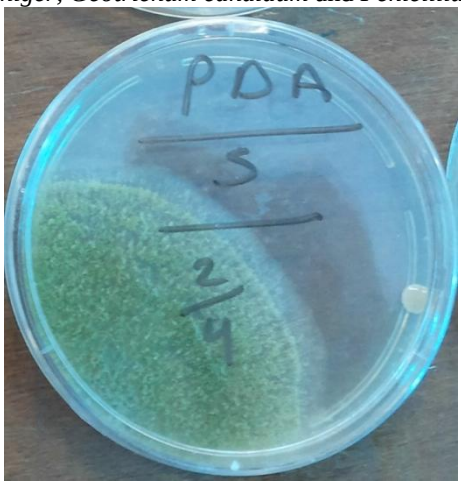

Figure 3: Yellow green fungal colony on PDA medium

Conclusion

Obtained results proved that the highest value of BOD was during winter and the lowest value was during summer. Heavy metals are lower than the permissible levels permitted by the Egyptian Organization for Standardization. Also, Pb concentration did not exceed about the Egyptian Standards of the Environmental Laws No. 48/1982. The highest value of total bacteria, total fungi, staphylococci, *Aeromonas* and coliform count in the water. The highest values of bacterial groups of muscles, intestine and surface of fish were in spring and the lowest values were in autumn. *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Aeromonas* sp. and *Aspergillus oryzae* were isolated and identified from water and fish.

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