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Chemical and Microbiological Examinations of Water and Fish Takenfrom Brackish Water of Damietta City, Egypt

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Abstract

Brackish water and fish samples of River Nile in Damietta city were examined. Morphological examination of fish obtained from brackish water of River Nile was examined. The highest value of BOD_5^{20} was found during summer being 17.4 mgO_2/L while the lowest value was found during winter being 9.9 mgO_2/L. Stannum and arsines did not present in all examined seasons of all samples (water and fish). Lead did not detected in all seasons samples except in spring being 0.003 and 0.003 ppm in water and fish, respectively. The highest value of total bacterial count in brackish water was found during summer being 4300cfu/ml x 10^3, while the lowest value was in winter being 2 cfu/ml x 10^3. The highest value of total fungal count was in summer being 18 while lowest value was in the winter being 0.8cfu/ml x 10^3. There was no bacterial growth on SS agar medium. The highest value of Staphylococci count was in summer being 30cfu/ml x 10^3. The highest value of *Aeromonas* count in summer is 50cfu/ml x 10^3. The highest value of coliform count was in spring being 150cfu/ml x 10^3. The total bacterial count of fish muscles were 9, 1600, 0.0 and 0.0cfu/ml x 10^3 in spring, summer, autumn and winter, respectively. On the other hand, the highest value of total bacterial count of fish intestine was 3000 cfu/ml x 10^3 in spring, but the lowest value was in the autumn being 0.1cfu/ml x 10^3. Total bacterial count of fish surface was 390, 1000, 0.1 and 1cfu/ml x 10^3 in spring, summer, autumn and winter, respectively. The highest values of fungal count in muscles, intestine and fish surface were during summer, spring, being 1600, 3000 and 1000 cfu/g x 10^3, respectively. Ahigh correlation coefficient value (r = 0.747) between the log of total bacterial count (LTBC) and BOD in the brackish water of River Nile was indicated, which means there are a highly pollution in river ecosystem by industrial pollutants. Among 18 bacterial isolates, only 4 were coccoid shape, 5 isolates were short rods and 9 isolates were long rods. 5 isolates were Gram negative and 13 isolates were Gram positive. 9 isolates were spore formers and 9 isolates were non spore formers. All isolates gave negative results in acid fast stain. *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Esherichia* sp. *Aspergillusglaucus*, *A. niger* and *A. flavus* were isolated and identified from water and fish.

Keywords

Brackish Water, Microbiological Examination, BOD, Correlation Coefficient, Isolation and Identification

1. Introduction

Brackish water (less commonly brack water) is salt water and fresh water mixed together. It is saltier than fresh water, but not as salty as seawater. It may result from mixing of seawater with fresh water or it may occur in brackish fossilaquifers. Technically, brackish water contains between 0.5 and 30 grams of salt per liter, more often expressed as 0.5 to 30 parts per thousand. Thus, brackish covers a range of salinity regimes and is not considered a precisely defined condition. It is characteristic of many brackish surface waters that their salinity can vary considerably over space and/or time [1].

According to FAO [2], Egypt has 2400 km of coastline in the Mediterranean and the Red seas. The total freshwater area

in Egypt is estimated as 6000Km^2, distributed mainly in the River Nile, its major two tributaries and many irrigation canals that flow through the country. Furthermore, several brackish and salty lakes are present, mainly Mariut, Edku, Manzala and Bardawil in the North; Qaroun in the Middle; and Timsah and Bitter Lakes in the North East. Another principal water body in Egypt is the greatest African artificial reservoir behind the Aswan High Dam, the Lake Nasser, which is a completely freshwater lake. In 2009, marine capture fisheries accounted for 127,821 tons, inland capture fisheries were 259,577 tons, and both were far less than aquaculture production that accounted alone for 705,490 tons [3].

Microbiological food safety is centered on the production of safer foods and mainly ensured by preventive approaches. Its primary goals are to minimize the risks of food borne pathogens and their toxins, reduce the incidence of human disease as well as facilitating domestic and international trade [4]. *Aspergillus*spp. and *Penicillium* spp. are the common genera of fungi generally isolated from the bakery products. These fungi have been known to produce toxins, which are both acutely and chronically toxic for animal and humans [5]. *A. flavus* and *A. parasiticus* producing aflatoxins were isolated from different Egyptian foods. Fungi can produce their mycotoxins naturally in various agricultural products [6].

Microbiological examinations of Damietta Branch of River Nile from spring 2008 to winter 2009 was studied by Khalifa and Sabae [7]. Total Coliforms (TC), were 2400, 1900, 45 and 80 cfu/ml in spring, summer, autumn and winter, respectively. Faecal Coliforms (FC) were 930, 240, 35 and 40 cfu/ml in spring, summer, autumn and winter, respectively. Faecal Streptococci (FS) were 2900, 280, 460 and 150 cfu/ml in spring, summer, autumn and winter, respectively.

There are not any published date about the brackish water in the part of Damietta branch of River Nile from El-Shoara dam to the Mediterranean sea in El-Lesan area (Figure 1). This dame separate the fresh water of River Nile and salt water of Mediterranean sea. The space between El-Shoara dam and the Mediterranean sea is about 21 Km. Moreover, excess of salt water was added in this region from the navigational channel between the Damietta port and the brackish water of River Nile. From all of those conditions this region has a special satiation. So that, the purpose of this work was to examine the microbiological and chemical samples of brackish water and fish obtained from the region between El-Shoara dam and the Mediterranean sea in Damietta, Egypt.

Figure 1. *a) Delta region of River Nile. b) Region of Brackish water of River Nile. c) El-Lesan area d) Navigational channel e) El-Shoara dam.*

2. Material and Methods

2.1. Physical and Chemical Examinations

2.1.1. 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) [8].

2.1.2. Biological Oxygen Demand (BOD_5^{20})

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 materials using clean and dried 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 [9] using the equation of BOD^{20}_5 (mg/L) = (Initial DO - Final DO) x dilution factor

2.1.3. 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*. [10] using Perkin – Elmer atomic absorption spectrophotometer (A.A.S 2) with hydride generation system Perkin – Elmer model PinAAcle 900T, serial No. PTCS12032601 made in Germany.

2.2. Microbiological Examinations

2.2.1. Samples Collection and Preparation

Water and fish samples were collected in three replicates from the same site of brackish water of River Nile in Damietta city during spring, summer, autumn and winter of 2014 (Figure 2). Water samples were collected in 100 ml sterile glass bottles and then transferred to the microbiological laboratory of department of Agricultural Microbiology, Faculty of Agriculture, Damietta University, 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 done and one ml of each last three dilutions was used for microbiological examinations [11].

2.2.2. 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 [12].

2.2.3. 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 medium for maintenance and identification [12].

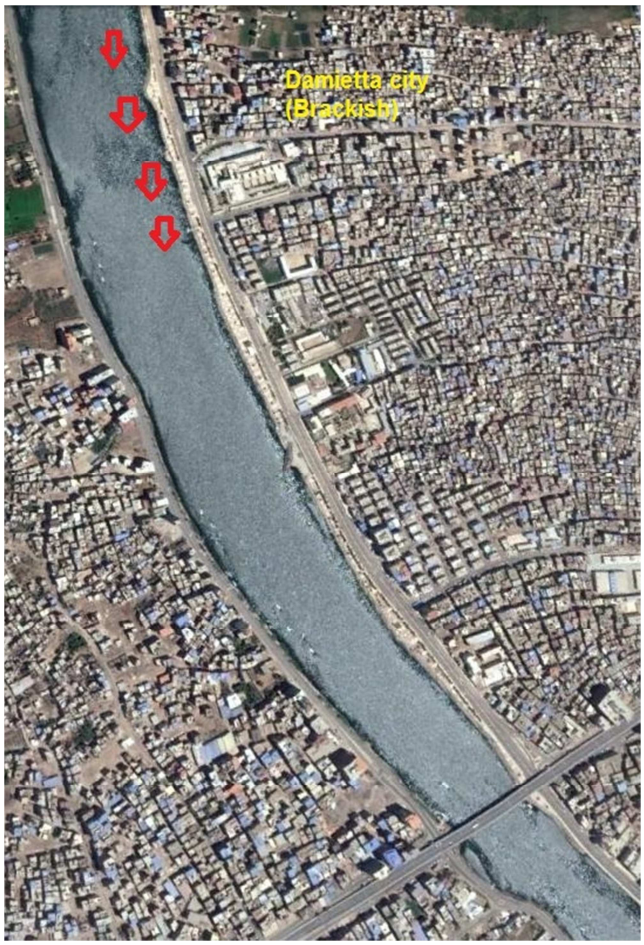

Figure 2. *Site of water and fish samples obtained from brackish water of River Nile.*

The following microbiological methods were carried out to identify the obtained bacterial isolates according to Holt et al. [13]. 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 [14]. 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 [15].

Coliform counts were detected using the most probable

number (MPN) technique [12]. Three decimal dilutions for each sample in three replicated tubes were used. One ml of each suitable dilution was added to test tube containing MacConkey broth medium and Durham tubes, then incubated at 37°C 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 [16].

2.2.4. 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 [12]. Approximately fifteen ml of potato dextrose agar (PDA) mediumat about 45°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 PDA medium slant for identification tests. The fungal isolates were subcultured then maintained on PDA slants at 5°C till use [17]. Fungal isolates were identified by morphological characteristics of colonies in PDA medium as well as spore morphology, hyphae and conidiophores. 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 [18] and Yoshida *et al*. [19] were used.

3. Results and Discussion

3.1. Morphological Characteristics of Fish Obtained from Brackish Water of River Nile

All fishes obtained from brackish water of Rive Nile were belonged to one genus of fish namely, *Oreochromisniloticus* (Nile tilapia). The mean value of fish long of three individuals were 5.5, 8, 6 and 8 cm in spring, summer, autumn and winter, respectively (Table 1). The highest weight of fishes were obtained during spring being 32gm, while the lowest weights were in case of autumn, winter and summer being 20, 24 and 25 gm, respectively. It was observed that, the color of fishes were differed according to seasons. Fishes obtained from brackish water in the spring were green with red and black color in abdomen region (Figure 3a). Most of fish during summer was black and yellow with red parts (Figure 3b). Also, during autumn most of fish was black with yellow parts (Figure 3c). In winter, most of fish was black and yellow with red part (Figure 3d).

Table 1. *Morphological examination of Nile tilapia (Oreochromisniloticus) obtained from brackish water of River Nile.*

Seasons	Long (cm)	Weight (g)	Fish color
Spring	5.5	32	Green with red and black color in abdomen region
Summer	8	25	Most of fish was black and yellow with red parts
Autumn	6	20	Most of fish was black with yellow parts
Winter	8	24	Most of fish was black and yellow with red parts

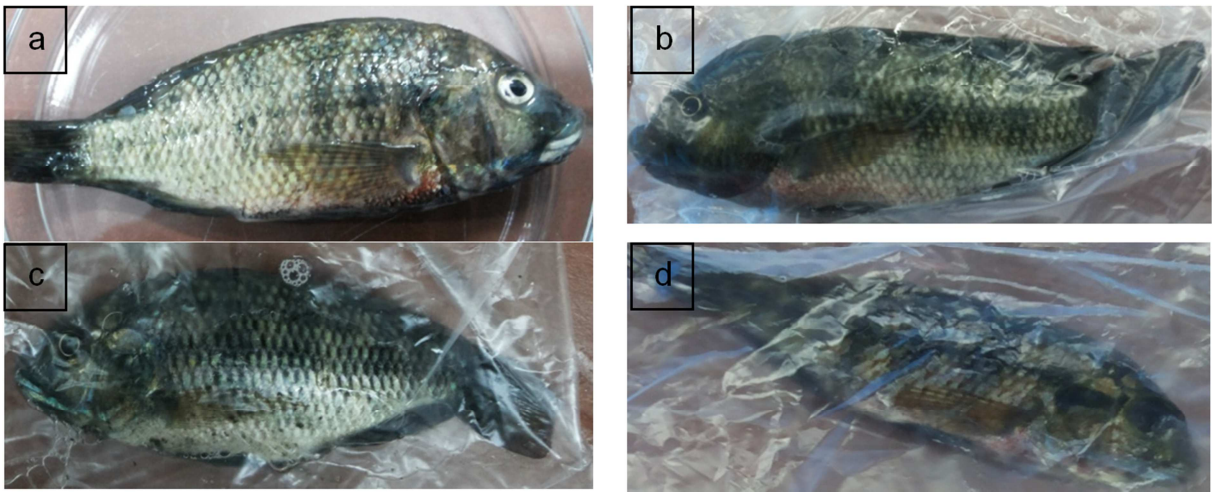

Figure 3. *Oreochromisniloticus obtained from brackish water of River Nileduring a) spring, b) summer, c) autumn and d) winter.*

3.2. BOD and EC Values of Brackish Water of River Nile

Data in Table 2. showing that, temperature varied between 15 and 28°C. Initial DO were 8.5, 8.0, 8.2 and 8.4 mgO_2/L in spring, summer, autumn and winter, respectively. Final DO were 3.2, 2.2, 3.3 and 5.1 mgO_2/L in spring, summer, autumn and winter, respectively. The highest value of BOD_5^{20} was during summer being 17.4 mgO_2/L while the lowest value was during winter being 9.9 mgO_2/L. The lowest value of EC was in winter being 145.9 mhos/cm and the highest value

was in summer being 179.8 mhos/cm.

Table 2. *Temperature, BOD and EC values of brackish water of Damietta governorate.*

Seasons	Temperature (°C)	DO, mgO_2/L		BOD_5^{20} (mgO_2/L)	EC (mhos/cm)
		Initial	Final		
Spring	23	8.5	3.2	15.9	157.6
Summer	28	8.0	2.2	17.4	179.8
Autumn	21	8.2	3.3	14.7	149
Winter	15	8.4	5.1	9.9	145.9

The DO of this study was higher than that of Surendraraj *et al*. [20] who detected the DO of feeder canal water and they found that, the values were ranged between 0.89-3.53 ppm. BOD_5^{20} and salinity were lower which were ranged between 4.83-13.6 ppm and 0.07-0.23 mhos/cm, respectively. Also, Ahmed [21] studied the BOD_5^{20} values of El-Rahawy drain and he found that, BOD_5^{20} values were 3.5, 2.0 and 16.5 mg/l during summer, autumn and winter. Obtained results were lower than that obtained by EL-Shafei [22] since he found that the maximum value of DO (16 mg/l) was recorded at El-Kanater El-Khayria during December were (16 mg/l).

3.3. Heavy Metals Values of Examined Water and Fish Muscles

Results in Table 3 showing that, stannum and arsines did not presented in all seasons of all samples (water and fish). Also, cadmium and cupper did not presented in all fish samples. On the other hand, cadmium was presented in all water samples. The lowest value was in the spring being 0.011 ppm while the highest value was in autumn being 0.027 ppm. Lead did not detected in all seasons samples except spring samples being 0.003 and 0.003 ppm in water and fish, respectively. 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.

Table 3. *Heavy metals values of water and fish muscles.*

Seasons	Examined Heavy metal concentration (ppm)									
	Pb		Cd		Cu		As		Sn	
	Water	Fish	Water	Fish	Water	Fish	Water	Fish	Water	Fish
Spring	0.003	0.003	0.011	ND	ND	ND	ND	ND	ND	ND
Summer	ND	ND	0.026	ND	0.359	ND	ND	ND	ND	ND
Autumn	ND	ND	0.027	ND	0.038	ND	ND	ND	ND	ND
Winter	ND	ND	0.024	ND	ND	ND	ND	ND	ND	ND

3.4. Microbiological View of Brackish Water of River Nile

Results in Table 3 showing that, the highest value of total bacterial count was during summer being 4300cfu/ml x 10^3, while the lowest value was in winter being 2 cfu/ml x 10^3. The counts of total bacteria was related to the temperature, which was high when it was high. The high bacterial load may be enplaned by the observation of Ali *et al*. [25] 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 being 18 cfu/ml x 10^3 and lowest value was in the winter being 0.8cfu/ml x 10^3. It was observed that, there were no bacterial growth on SS agar medium. Obtained results were similar to that of Rokibul *et al*. [26] who found that, *Salmonella* and *Shigella* were not detected. The highest value of Staphylococci count was in summer being 30cfu/ml x 10^3, while it were in the lowest value in spring and autumn being 0.0 cfu/ml. The highest value of *Aeromonas* count in summer being 50cfu/ml x 10^3, but the lowest values was during winter to be 0.25cfu/ml x 10^3. The highest values of coliform count was in spring being 150cfu/ml x 10^3, while coliform count was in the lowest value in the autumn and winter being 0.0 and 0.0 cfu/ml, respectively.

The current results were higher than that obtained by Khalifa and Sabae [7] who reported that, total Coliforms of Damietta Branch of River Nile during spring was 930 cfu/100ml. Obtained results were lower than those obtained by Surendraraj *et al*. [20] who determined the total Coliform of farmed fish water and they found that, the counts were ranged between log 2.0 and 3.4 cfu/ml. According to the guideline criteria for faecal indicator organisms of WHO [27] which accept the guide values of the investigated bacteria up to 500/100ml for total Coliform and 100/100ml for both faecal Coliform and faecal Streptococci. So, these data revealed that the Nile water at the investigated sites is subjected to sewage pollution which considered to be very serious concept.

Table 4. *Microbiological values (cfu/ml x 10^3) of brackish water of River Nile.*

Seasons	Temperature of water samples (°C)	Count of					
		Total bacteria	Total fungi	*Salmonella* sp. and *Shigella* sp.	Staphylococci	*Aeromonas* sp.	Coliform
Spring	23	4000	3	0.0	0.0	1.25	150
Summer	28	4300	18	0.0	30	50	36
Autumn	21	60	1	0.0	0.0	0.5	0.0
Winter	15	2	0.8	0.0	0.001	0.25	0.0

Obtained results are in good agreement with published data of Osman [28] 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 average log number of each indicator was varied from site to another according to the environmental conditions. The highest average log number of total Coliform, faecal Coliform and faecal Streptococci were 4.38, 3.29 and 2.58, respectively. Moreover El-Kadi and El-Morsy [29] 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.

3.5. Microbiological View of Fish Taken from Brackish Water of River Nile.

The total bacterial count of fish muscles were 9, 1600, 0.0 and 0.0cfu/ml x 10^3 in spring, summer, autumn and winter, respectively (Table 5). On the other hand, the highest value of total bacterial count of fish intestine was 3000 cfu/ml x 10^3 in spring, but the lowest value was in the autumn being 0.1cfu/ml x 10^3. Total bacterial count of fish surface were 390, 1000, 0.1 and 1cfu/ml x 10^3 in spring, summer, autumn and winter, respectively. The highest values of fungal count in muscles, intestine and fish surface were 1600, 3000 and 1000 cfu/g x 10^3 during summer, spring, respectively. On the other hand, all lowest fungal counts of muscles, intestine and surface were during winter being 0.0, 0.1 and 1, respectively. 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 50, 1000 and 90cfu/g x 10^3, but the lowest values were in autumn. The highest count of *Aeromonas* sp. was in fish intestine during spring, being 5400cfu/g x 10^3 while the lowest value was during summer. Coliform was found in the highest values in spring being 11cfu/g x 10^8 in intestine, on the other hand, coliform count was in the lowest values in the autumn and winter.

Table 5. *Microbiological values (cfu/gm x 10^3) of fish taken from brackish water of River Nile.*

Seasons	Count of						
	Fish part	Total bacteria	Total fungi	*Salmonella*sp and *Shigella*sp	Staphylococci	*Aeromonas* sp.	Coliform
Springer	Muscles	9	0.0	0.0	50	0.0	2.3
	Intestine	3000	2000	0.0	1000	5400	1100000
	Surface	390	380	0.0	90	6.4	110
Summer	Muscles	1600	0.0	0.0	7.2	0.0	0.0
	Intestine	800	2500	0.0	30	0.0	0.0
	Surface	1000	490	0.0	30	0.0	36
Autumn	Muscles	0.2	0.02	0.0	0.0	0.05	0.0
	Intestine	0.4	1	0.0	0.0	30	0.0
	Surface	0.1	0.3	0.0	0.0	0.4	0.0
Winter	Muscles	0.0	0.01	0.0	0.01	0.02	0.0
	Intestine	0.1	0.7	0.0	0.01	0.02	0.0
	Surface	1	0.08	0.0	0.0	0.06	0.0

Obtained results are lower than those obtained by Surendraraj [20] who counted the total Coliform bacteria associated with mussels of fish feeder canal water ranged between log 1.68, 2.4 cfu/g. On the other hand, obtained results were higher than that obtained by Rokibul *et al*. [26] who determined the microorganisms among the fish samples collected from Dhaka city, Bangladesh. Fungal growth was observed in all samples within a range of 1.6×10^4 - 3.6×10^5cfu/g.

3.6. The Relationship Betweenthe Log of Total Bacterial Count and the Biological Oxygen Demand

Kagalou *et al*. [30] reported that, the correlation coefficient values occurred between 0.6 and 1.0 means the correlation was strong between the log of total bacterial count and BOD_5^{20}. The correlation coefficient between the log of total bacterial count (LTBC) in the brackish water of River Nile and the BOD_5^{20} was calculated. Statistical analysis indicated a high correlation (r = 0.747) between the log of total bacterial count and BOD_5^{20}. Kagalou *et al*. [30] reported that, a positive correlation between BOD_5^{20} and total bacterial count in a river ecosystem highly polluted by industrial pollutants.

Moreover, El-Kadiand El-Morsy [29] recorded a high values of correlation coefficient between the log of total bacterial count and BOD_5^{20} being 0.678, 0.869, 0.879 and 0.896. Similar results were obtained by El-Moghazy and El-Morsy [31]. In contrast, a low correlation (r = 0.552) between the log of total bacterial count and BOD_5^{20} was reported by El-Fadaly *et al*. [32]. Other author [33] found a negative correlation between total bacterial count and faecal coliforms with BOD_5^{20} in a slightly polluted water.

3.7. Characterization and Identification of Bacterial Isolates

Table 6 showing bacterial isolates numbers, characterization and its sources. Seven different bacterial isolates were isolated from nutrient agar medium, 4 isolates were isolated on *Aeromonas* agar medium and 4 isolates were found on Staph 110. medium. Three isolates were obtained from McaConkey broth which gave acid and gas were picked up and streaked onto EMB medium. Six isolates were isolated from water, 5 from muscles, 3 from intestine and 4

from surface of fish. Among 18 bacterial isolates, only 4 were coccoid shape, 5 isolates were short rods and 9 isolates were long rods. Five isolates were Gram negative and 13 isolates were Gram positive. Nine isolates were spore formers and 9 isolates were non spore formers. All isolates gave negative results with acid fast stain.

Only one typical colony 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. 27 was considered as *Staphylococcus* sp. according to Bergey's Manual of Determinative Bacteriology [13]. Isolates Nos. 1, 2 and 20 were considered as *Micrococcus* sp. [13]. Nine 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 Isolates Nos. 10, 13, 19, 26, 29, 32, 43, 45 and 46 were considered as *Bacillus* sp. according to Bergey's Manual of Determinative Bacteriology [13]. Jimoh *et al*. [34] isolated *Bacillus alvei* and *Bacillus megaterium* from the microbial flora of the gastro-intestinal tract of *Clariasgariepinus* caught from river Dandaru Ibadan, Nigeria.

Table 6. *Bacterial isolates numbers, characterization and sources obtained from fish and brackish water of River Nile (Damietta governorate).*

Sources (2014)	Cultivation media	Isolates Nos.	Characterization of isolates				
			Shape	Arrangement	Gram stain	Spore stain	Acid fast stain
Water	Nutrient Agar	19	Long rods	single	+	+	-
		20	Coccoid	single	+	-	-
	Aeromonas	26	Long rods	chain	+	+	-
		43	Long rods	single	+	+	-
	Staph 110.	27	Coccoid	staph	+	-	-
		32	Long rods	single	+	+	-
Fish Muscles	Nutrient Agar	1	Coccoid	single	+	-	-
		2	Coccoid	single	+	-	-
		63	Short rods	single	-	-	-
	Aeromonas	13	Long rods	single	+	+	-
	McaConkey broth	82	Short rod	single	-	-	-
Fish Intestine	Nutrient Agar	10	Long rods	single	+	+	-
	Aeromonas	45	Long rods	single	+	+	-
	Staph 110.	36	Short rods	single	-	-	-
Fish Surface	*Aeromonas*	46	Long rods	single	+	+	-
	Staph 110.	29	Long rods	single	+	+	-
	McaConkey broth	80	Short rod	single	-	-	-
		83	Short rod	single	-	-	-

The cells were short rods, Gram negative, non sporeformer and non acid fast. Its arrangement was single. Isolates Nos. 80, 82 and 83 after growth onto EMB medium, the colonies were green and metallic sheen were considered as genus *Esherichia* according to Bergey's Manual of Determinative Bacteriology [13].

3.8. Characterization of Fungal Isolates

Seven fungal isolates were isolated from all examined samples. Characterization of the isolates showed that, isolate No. 24 grew on PDA medium at 25°C and the color was grayish turquoise to deep green. Reverse was pale yellow to pale brown hyphae are septets and hyaline with a cleistothecia. Conidial heads were radiate to loosely columnar. Conidiophores were smooth walled and uncolored to pale brown. Vesicles were globose to subglobose and uniseriate. Conidia are globose to subglobose. From these characteristics, isolate No. 24 was identified as *Aspergillusglaucus* following the protocol of Yoshida *et al*. [19].

Mycelium of colonies on PDA medium were white, conidial heads dark brown, greenish black, brownish black to black reverse colorless, conidial heads globose, radiate or splitting into several irregularor well-defined columns of conidial chains, conidiophores hyaline to brown and smooth-walled. Vesicles globose to subglobose hyaline to dark brown. From these characteristics, isolate Nos. 52, 58, 62 were identified and designated as *Aspergillusniger* [18].

Isolates Nos. 44, 64 and 65 showed conidial heads pale to intense yellow green when young, colonies not shifting to brown in age on PDA medium. Conidia definitely echinulate predominance; conidial heads radiate or very loosely columnar, colonies shifting to brownish in age; conidia smooth to roughened; conidiophores arising primarily from the substrate. From these characteristics, these isolates were identified as *Aspergillusflavus* as reported [18].

Jimoh *et al*. [34] isolated *Aspergillusniger, Aspergillusflavus,* from the gastro-intestinal tract of *Clariasgariepinus*. In addition, El-Kadi and El-Morsy [29] isolated five fungal isolates and identified it as *Aspergillusochraceus*, *A. oryzae*, *A. niger*, *Geotrichumcandidum* and *Penicillium* sp.

4. Conclusion

Obtained results proved that the highest value of BOD_5^{20} was found during summer while the lowest value was during winter. The lowest value of EC was in winter while the

highest value was in summer. Heavy metal 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 values of total bacteria, total fungi, *Aeromonas* and coliform count in the water were in the summer. The highest values of bacterial groups of intestine and surface of fish were in spring while the lowest values were in autumn. *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp., *Esherichia* sp. *Aspergillusglaucus*, *A. niger* and *A. flavus* were isolated and identified from water and fish.

References

[1] El-Kholy, S. E. (2016). Microbiological and Chemical Examination of Fish and Water Samples of Damietta Governorate (M. S. Thesis). Agricultural Microbiology Department, Faculty of Agriculture, Damietta University, Damietta, Egypt.

[2] FAO (2010). (Food and Agriculture Organization). The State of World Fisheries and Aquaculture. *The United Nations. Rome*.

[3] Khallaf, A. G.; K. M. Geba; A. G. M. Osman, K. Y. Abouel Fadl, Y. J. Borrell, E. G. Vazquez. (2017). SNP-based PCR-RFLP, T-RFLP and FINS methodologies for the identification of commercial fish species in Egypt. *Fisheries Research*. 185: 34–42.

[4] Igbinosa, E. O., Uyi, O. O., Odjadjare, E. E., Ajuzie, C. U., Orhue, P. O. & Adewole, E. M. (2012). Assessment of physicochemical qualities, heavy metal concentrations and bacterial pathogens in Shanomi Creek in the Niger Delta, Nigeria. *African J. Environ. Science and Technol*., 6 (11): 419-424.

[5] Basavaraja, D., Narayana, J., Kiran, B. R. & Puttaiah, E. T. (2014). Fish diversity and abundance in relation to water quality of Anjanapura reservoir, Karnataka, India. Int. *J. Curr. Microbiol. App. Sci.*, 3 (3): 747-757.

[6] Apun, K., Yusof, A. M. & Jugang, K. (1999). Distribution of bacteria in tropical freshwater fish and ponds. *Int. J. Environ. Health Res*, 9: 285–292.

[7] Khalifa, N. and S. Z. Sabae (2012). Investigation on mutual relations between bacteria and zooplankton in Damietta Branch, River Nile, *Egy. J. Applied Scie. Res*., 8 (5): 2679-2688.

[8] APHA, American Public Health Association (2005). *Standard Methods for the Examination of Water and Wastewater*, Amer. Publ. Heal. Assoc., Amer. Water Works Assoc. and Water Poll. Contr. Fed., Washington, DC.

[9] Stirling, H. P. (1985). *Chemical and Biological Methods of Water Analysis for Aquaculturists*. Institute of Aquaculture, University of Stirling, Scotland.

[10] Gloterman, H. L., Clymo, R. S. &Ohnstad, M. A. M. (1978). *Methods for Physical and Chemical Analysis of Fresh Water*. The 2nd ed., IBP Hand book No. 8. Blackwell Scientific Publications. Oxford.

[11] Ozogul, Y., Ozogul, F., Kuley, E., Ozkutuk, A. S., Gokbulut, C. & Kose, S. (2006). Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmusmaximus*), from the black sea, during chilled storage. *Food Chemistry*, 99: 752–758.

[12] Ronald, M. A. (2010). *Hand Book of Microbilogical Media*. CRC Taylor and Francis Group Boca Raton London New York, USA.

[13] Holt, J. G., Krieg, N. R., Sneath, P. H. A., Stanley, J. T. & Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology*. 9th ed., Williams and Wilkins, Baltimore.

[14] Benson, H. J. (2001). *Microbiological Applications Lab Manual*. 8th Ed. The McGraw−Hill Companies, UK.

[15] Anon, (1992). *Compendium of Methods for the Microbiological Examination of Foods*. The 3rd Ed., American Public Health Association. Washington, D. C., USA.

[16] Sutton, S. (2010). The Most Probable number method and its uses in enumeration, qualification, and validation. *J. Validation Technology*, 35-38.

[17] APHA, American Public Health Association (1998). *Standard Methods for the Examination of Water and Wastewater*. The 20th Ed. APHA, Inc. New York.

[18] Chung, K. K. J. & Bennett, J. E. (1992). *Medical Mycology*. Lea & Febeiger. USA.

[19] Yoshida, K.; M. Ando; K. Ito; T. Sakata; K. Arima; S. Araki; and K. Uchida (1990). Hypersensitivity pneumonitis of a mushroom worker due to *Aspergillusglaucus*. Arch Environ. Health, 45: 245 – 251.

[20] Surendraraj, A., Farvin, K. H. S., Yathavam, R. &Thampuran, N. (2009). Enteric bacteriaassociated with formed fresh water fish and its culture environment in kerala, India. *Res. J. Microbiol*., 4 (9): 334-344.

[21] Ahmed, N. A. M. (2007). Effect of River Nile pollution on *Clariasgariepinus* located between El-Kanater El-Khayria and Helwan. M.Sc. Thesis, Faculty of Agriculture, Zagazig Univ, Egypt.

[22] EL-Shafei, H. M. (2016). Assessment of some water quality characteristics as guide lines for the management of pond fish culture in Lake Manzala, Egypt. *Inter. J. Fisheries and Aquatic Studies*. 4 (2): 416-420.

[23] ESO, Egyptian Organization for standardization (1993). *Egyptian Standard, maximum level for heavy metal concentrations in food*.

[24] EGL, 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*.

[25] Ali, S. M., Sabae, S. Z. Fayez, M. Monib, M. &Hegazi, N. A. (2011). The influence of agro-industrial effluents on River Nile Pollution. *J. of Advan. Res*., 2: 85–95.

[26] Rokibul, M. H., Mrityunjoy, A., Eshita, D., Kamal, K. D., Tasnia, A., Muhammad, A. A., Kazi, K. F. & Rashed, N. (2013). Microbiological study of sea fish samples collected from local marketsin Dhaka city. *Inte. Food Res. J.*, 20 (3): 1491-1495.

[27] WHO, World Health Organization (1996). Water Quality Monitoring - A Practical Guide to the Design and Implementation of Freshwater Quality Studies and Monitoring Programmes. WHO Technical Report Series, No 0419 223207. World Health Organization, Geneva.

[28] Osman, G. O. A. (2006). Studies on the microbial pollution indicators in water. Ph. D. Thesis, Agric. Microbiol. Dept., Fac. of Agric., Ain Shams Univ., Egypt.

[29] El-Kadi, S. M. & El-Morsy, A. M. (2016). The effect of water aquaria change on nutrient utilization and microbial activity of Nile tilapia *Oreochromisniloticus*. *International Journal of Fisheries and Aquatic Studies*, 4 (4): 196-205.

[30] Kagalou I, Tsimarakis G, Bezirtzoglou E. (2002). Interrelationships between bacteriological and chemical variations in lake Pamvotis-Greece. *Microbe. Ecol. Health Dis*. 14: 37-41.

[31] El-Moghazy, M. M. and A. M. El-Morsy (2017). Effect of water aquaria changes on growth performance of Nile tilapia *Oreochromisniloticus* and the relationship between bacterial load and biological oxygen demand. *International Journal of Fisheries and Aquatic Studies*. 5 (3): 341-349.

[32] El-Fadaly, H. A., S. M. El-Kadi and S. E. El-Kholy (2016). Microbiological and Chemical Examinations of Water and Fish Obtained From River Nile of Damietta Governorate, Egypt. *Chemistry Research Journal*. 1 (4): 132-140.

[33] Ali, S. M., Yones, E. M., Kenawy, A. M., Ibrahim, T. B. & Abbas, W. T. (2015). Effect of el-sail drain wastewater on Nile Tilapia (*Oreochromisniloticus*) from River Nile at Aswan, Egypt. *J. Aquac. Res. Development*, 6: 1-7.

[34] Jimoh, W. A., Bukola, M. O. O., Adebayo, M. D., Yusuff, A. A., Azeezand, F. A. & Salam, O. O (2014). Microbial flora of the gastro-intestinal tract of *Clariasgariepinus* caught from river Dandaru Ibadan, Nigeria. *Sokoto J. Veterinary Scieinces*, 12 (2): 19-24.