American Journal of Food Science and Health
Vol. 5, No. 2, 2019, pp. 50-60
http://www.aiscience.org/journal/ajfsh
ISSN: 2381-7216 (Print); ISSN: 2381-7224 (Online)

Chemical and Microbiological Examinations of Water and Fish Taken from Manzala Lake of Damietta Gavernorate, Egypt

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Abstract

Water and fish samples were collected in three replicates from the same site of Manzala lake water of El-Roda city in Damietta Governorate, Egypt were examined. Morphological examination of fish obtained from Manzala lake water was examined. The highest value of BOD_5^{20} was found during summer being 14.8 mgO_2/L while the lowest value was found during winter being 7.6 mgO_2/L. Arsines and Stannum 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. Also, cadmium and cupper did not presented in all fish samples. Lead did not detected in spring and summer samples while the highest value was during winter being 0.007 ppm in water and 0.032 ppm during autumn in fish. The highest value of total bacterial count in Manzala lake water was found during spring being 4400 cfu/ml $\times 10^3$, while the lowest value was in autumn. The highest value of total fungal count was in summer being 22500 cfu/ml $\times 10^3$ while lowest value was in the winter being 0.06 cfu/ml $\times 10^3$. There were no bacterial growth on SS agar medium. The highest value of Staphylococci count was in spring being 1760 cfu/ml $\times 10^3$. The highest value of *Aeromonas* count in spring being 66 cfu/ml$\times 10^3$. The highest value of coliform count was in spring being 1210 cfu/ml$\times 10^3$. The total bacterial count of fish muscles were 17.6, 6000, 0.06 and 45 cfu/g $\times 10^3$ in spring, summer, autumn and winter, respectively. On the other hand, the highest value of total bacterial count of fish intestine was 46200 cfu/g $\times 10^3$ in spring, but the lowest value was in the autumn. Total bacterial count of fish surface were 1980, 500, 0.2 and 10 cfu/g $\times 10^3$ in spring, summer, autumn and winter, respectively. The highest values of fungal count in muscles, intestine and fish surface being 1.87, 13200 and 46.2 cfu/g $\times 10^3$ during spring, respectively. The highest values of Staphylococcal counts of fish muscles, intestine and fish surface were in spring being 0.198, 33 and 77 cfu/g $\times 10^3$. The highest count of *Aeromonas* sp. was in fish intestine during spring, being 2530 cfu/g $\times 10^3$. Coliform was found in the highest values in spring being 1210000 cfu/g $\times 10^3$ in intestine, and the lowest values in the autumn and winter. There was no correlation coefficient (r = 0.00) between the log of total bacterial count and BOD_5^{20} in the Manzala lake water. Among 19 bacterial isolates, only one was coccoid shape, 13 isolates were short rods and 5 isolates were long rods. Thirteen isolates were Gram negative and 6 isolates were Gram positive. Six isolates were spore formers and 13 isolates were non spore formers. All isolates gave negative results with acid fast stain. *Micrococcus* sp., *Aeromonas* sp., *Esherichia* sp., *Pseudomonas* sp., *Aspergillus alliaceus*, *Aspergillus niger, Aspergillus flavus* and *Penicillium* sp. were isolated and identified from water and fish.

Keywords

Manzala Lake, Microbiological Examination, BOD, Correlation Coefficient, Isolation and Identification

Received: March 25, 2019 / Accepted: May 13, 2019 / Published online: May 27, 2019

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1. Introduction

Manzala lake is in grave danger of suffering pollution from the drainage of industries, agriculture and urban sewage that affects the physio-chemical and biological parameters in the lake. A geographical information system (GIS)-based method of lake trophic status assessment was undertaken to study the spatial distribution of eutrophic conditions of Manzala Lake. The lake changed to eutrophic freshwater. This change is due to the increase of freshwater inputs and nutrient loading associated with agricultural land reclamation and urban waste disposal [1].

Manzala lake is a very important lake in Egypt due to its dimensions and economic activity. Manzala lake is highly contaminated with Mn, Cd, Zn, Pb and Cu due to the continuous discharge of different pollutants into it. Metal contamination in water, sediment, and fish organs followed the order of Zn > Mn > Pb > Cu > Cd. The highest metal concentrations were found in fish tissues from the most contaminated site, showing that metal accumulation in *Oreochromis niloticus* reflects the degree of water pollution. All the five sites were contaminated with high count of bacteria and fecal chloroform in water which is an indicator of untreated waste water which spilled directly or indirectly to the lake. The most alarming result was found when analyzing fish; all the fish samples were contaminated on surface and internally with very high amounts of bacteria at gill and intestine. This confirms that lake fish is highly polluted and dangerous for human health. The results of this study supplied valuable information on the level of metal contamination in Manzala lake. Great efforts and cooperation between different authorities are needed to protect the lake from pollution and reduce environmental risk. This can be achieved by treatment of the agricultural, industrial, and sewage discharge. Regular evaluation of pollutants in the lake is also very important [2].

The Egyptian Lakes represent about 15% of the total commercial fishing areas in Egypt. These Lakes receive inputs of sewage, industrial and agriculture effluent. Lake Manzala occupies the northeastern corner of the Nile delta between the Mediterranean Sea and Suez Canal. This lake receives untreated and/or primary treated wastewater through Bahr El-Baqar Drain. This drain collects effluents from two secondary drains (Bilbeis Drain and Qalubeya Drain). Bahr El Baqar (100 km length) is considered as one of the most polluted drains in Egypt. On the other hand, Lake Qarun is enclosed saline lake among other inland lakes of Egypt. This lake receives also agricultural drainage water and sewage through two main drains, El-Batts (50 km length) and El-Wadi (48.5 km length). Moreover, the lake receives sewage drainage water and fish farms' drainage from El-Fayyum governorate through a system of twelve drains. It has been estimated that the Delta and Fayyum drains receive about 13.5 billion m^3 of wastewaters per year. Almost 90% of which is contributed from agricultural diffuse source, 6.2% of domestic point sources, 3.5% from domestic diffuse sources and the rest (3.5%) from industrial point sources [3].

Water of Lake Manzala is well oxygenated during different time intervals except the inlet of Moheeb and Bahr El-Baqr station region which suffered from complete depletion of dissolved oxygen around the year especially during hot months. The maximum value of DO (16 mg/l) was recorded at El-Kanater El-Khayria during December due to decreasing of temperature and to the prevailing winds which permit to increase the solubility of atmospheric oxygen. DO level > 5 ppm is essential to support good fish production. Fish can die if exposed to less than 0.3 mg /l of DO for a long period of time, minimum concentration of 1.0 mg /l DO is essential to sustain fish for long period and 5.0 mg /l are adequate in fish ponds [4].

Aeromonas hydrophila was recovered from fish living in lake Vrana on the Croatian island of Cres. The occurrence of the bacterium in the fish was assessed and related to gross signs of disease and findings at necropsy as a potential health hazard for fish [5].

The maximum isolation ratio of 4310 (22.3%) of the total bacteria isolates from fish of Oguta Lake (South-Eastern Nigeria), while scale had the maximum isolation ratio of 2530 (27.1%) of the total isolates from fish of Agulu Lake (South-Eastern Nigeria). The common isolates namely *Escherichia coli, Salmonella typhi* and Coliforms of the family Enterobacteriaceae formed 79.9% of the total bacterial isolates from Oguta Lake fish and 74.1% from Agulu Lake fish. *Staphylococcus aureus* was in significant in Oguta Lake fish (19, 0.1%) but was prominent in Agulu Lake fish where it contributed 150 (1.6%) of the total isolates. Of all the bacteria species identified, only *Escherichia coli* was isolated from the body parts of fish of both Lakes. Aerobic mesophilic bacteria and *Staphylococcus aureus* were encountered only in the scales and gut of fish of both Lakes. *Salmonella typhi* occurred only in the scale of Oguta fish. *Vibrio parahaemolyticus* was recorded only in the gonads [6].

Various bacterial species were isolated and identified from skin of freshwater fish of Punja. Total 210 cases were examined during the period July 2009 to March 2010. Bacterial isolation was done from forty seven cases of skin affections. The isolation and identification of bacteria was

done depending upon morphology, staining characteristics and biochemical testing using conventional methods as per the standard protocol. The bacterial species isolated were *Aeromonas spp*., *Bacillus spp., Citrobacter spp., E. coli, Enterobacter spp., Flavobacter spp., Klebsiella spp., Lactobacillus spp., Micrococcus spp., Proteus spp., Pseudomonas spp., Salmonella spp., Staphylococcus spp., Streptococcus spp*. and *Vibrio spp*. [7].

Freshwater fish, *Alburnusalburnus* (bleak), were captured from Lake Mogan, situated in Ankara, during spring. The surface mucus of the fish was collected and associated bacteria were cultured and isolated. Eleven different genera were as following: *Acinetobacter, Aeromonas, Bacillus, Brevundimonas, Gordonia, Kocuria, Microbacterium, Mycobacterium, Pseudomonas, Rhodococcus and Staphylococcus* [8].

According to FAO [9], 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 [10].

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 [11]. *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 [12]. *A. flavus* and *A. parasiticus* producing aflatoxins were isolated from different Egyptian foods. Fungi can produce their mycotoxins naturally in various agricultural products [13-15].

The purpose of this work was to examine the microbiological and chemical samples of Manzala lake water and fish obtained from the region of El-Roda city in Damietta Gavernorate, Egypt.

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) [16].

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 using the equation of BOD_5^{20} (mg/L) = (Initial DO - Final DO) x dilution factor [17].

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 Perkin – Elmer atomic absorption spectrophotometer (A.A.S 2) with hydride generation system Perkin – Elmer model PinAAcle 900T, serial No. PTCS12032601 made in Germany [18].

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 Manzala lake water of El-Roda city in Damietta Governorate, Egypt during spring, summer, autumn and winter of 2014 (Figure 1). Water samples were collected in 100 ml sterile glass bottles and then transferred to the microbiological laboratory of Agricultural Microbiology department, 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 manually shaken 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 [19].

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 [20].

2.2.3. The Relationship Between the Log TBC and the BOD

The correlation coefficient value (r) was calculated according the following equation [21]. Where x means the values of log of total bacterial count, means $\bar{x}$ arithmetic mean of log total bacterial count, Y the values of BOD_5^{20}, $\bar{Y}$ arithmetic mean of BOD_5^{20} values.

$$r = \frac{\sum(X-\bar{X})(Y-\bar{Y})}{\sqrt{\sum(X-\bar{X})^2}\sqrt{\sum(Y-\bar{Y})^2}} \quad (1)$$

If the value of the correlation coefficient (r) =+1, perfect positive correlation, r=-1, perfect negative correlation or r=0, no correlation. If the values of correlation coefficient occur between 0.2-.0.4, 0.4-.0.6, 0.6-.0.8 or 0.8-.1.0 that means weak correlation, moderate correlation, strong correlation or very strong correlation [21].

2.2.4. Counting, Isolation and Maintenance of Some Pathogenic Bacteria

One ml of the last three dilutions of all samples (water and fish) were transferred to 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. 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 slant for maintenance and identification [20].

Figure 1. Site of water and fish samples obtained from Manzala Lake water of El-Roda city in Damietta Gavernorate, Egypt.

The following microbiological methods were carried out to identify the obtained bacterial isolates according to [23]. 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 [24]. 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 [24].

Coliform counts were detected using the most probable number (MPN) technique [20]. 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 [25].

2.2.5. 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 [20]. Approximately fifteen ml of potato dextrose agar (PDA) medium at 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 [26]. 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 following taxonomic keys were used [27-29].

3. Results and Discussion

3.1. Morphological Characteristics of Examined Fish

All fishes obtained from Manzala lake water of El-Roda city were belonged to one genus of fish namely, *Oreochromis niloticus* (Nile tilapia). The mean value of fish long of three individuals were 15, 14, 15 and 14 cm in spring, summer, autumn and winter, respectively (Table 1). The highest weight of fishes were obtained during spring being 116.1 gm, while the lowest weights were in case of summer being 100 gm. It was observed that, the color of fishes were differed according to seasons. Fishes obtained from Manzala lake water in the spring were white and black parts (Figure 2a). Most of fish during summer black and yellow parts (Figure 2b). Also, during autumn most of fish was black and yellow parts black with yellow parts (Figure 2c). In winter, most of fish was black and yellow and red parts (Figure 2d).

Table 1. Morphological examination of Nile tilapia (Oreochromis niloticus) obtained from Manzala lake water of Damietta Gavernorate, Egypt.

Seasons	Long (cm)	Weight (g)	Fish color
Spring	15	116.1	Most of fish were white and black parts
Summer	14	100	Most of fish were black and yellow parts
Autumn	15	105	Most of fish were black and yellow parts
Winter	14	103	Most of fish were black and yellow and red parts

Figure 2. Oreochromisniloticus obtained from Manzala lake of Damietta Gavernorate, Egypt during a) spring, b) summer, c) autumn and d) winter.

3.2. BOD and EC Values of Manzala lake

Data in Table 2. showing that, temperature varied between 13.5 and 26°C. Initial DO were 6.0, 5.2, 6.2 and 7.0 mgO_2/L in spring, summer, autumn and winter, respectively. Final DO were 2.7, 0.6, 1.4 and 5.3 mgO_2/L in spring, summer, autumn and winter, respectively. The highest value of BOD_5^{20} was during summer being 14.8 mgO_2/L while the lowest value was during winter being 7.6 mgO_2/L. The lowest value of EC was in winter being 1.28 mhos/cm and the highest value was in spring being 23.3 mhos/cm.

Obtained results (Manzala lake) are higher than these of another author [30] who detected the BOD of pond water that ranged between 5.57, 11.13 ppm. Also, [30] detected the salinity and they found that, the salinity of pond water and feeder canal water were ranged between 0.03, 0.2, and 0.07, 0.23 mhos/cm, respectively. These results were lower than that obtained by another author [31] who studied the BOD values of El-Rahawy drain and he found that, BOD values 3.5 mg/l during summer

Table 2. Temperature, BOD and EC values of Manzala lake water of Damietta Gavernorate, Egypt.

Seasons	Temperature (°C)	DO, mgO_2/L		BOD_5^{20} (mgO_2/L)	EC (mhos/cm)
		Initial	Final		
Spring	21	6.0	2.7	10.4	23.3
Summer	26	5.2	0.6	14.8	22.2
Autumn	20	6.2	1.4	12.2	3.8
Winter	13.5	7.0	5.3	7.6	1.28

3.3. Heavy Metals Values of Examined Water and Fish Muscles

Results in Table 3 showing that, Arsines and Stannum did not presented in all seasons of all samples (water and fish). Also, cadmium and cupper did not presented in all fish samples. Lead did not detected in spring and summer samples while the highest value was found during winter being 0.007 ppm in water and 0.032 ppm during autumn in fish. On the other hand, the highest value of cadmium was found during winter being 0.036 ppm. Generally, cadmium presented in all water samples except during spring in water or all of samples of fish. Cupper presented in only two samples in water during spring and summer being 0.18 and 0.204 ppm and did not presented in all fish samples. These results are lower than the permissible levels (0.01 ppm) permitted by the Egyptian Organization for Standardization [32]. Also, Pb concentration did not exceed the Egyptian Standards of the Environmental Laws No. 48/1982 [33]. which the maximum Pb concentration in water was 0.01ppm.

Table 3. Heavy metals values of water and fish muscles taken from Manzala lake of Damietta Gavernorate, Egypt.

Seasons	Examined heavy metal concentration (ppm)									
	Pb		Cd		Cu		As		Sn	
	Water	Fish	Water	Fish	Water	Fish	Water	Fish	Water	Fish
Spring	ND	ND	ND	ND	0.18	ND	ND	ND	ND	ND
Summer	ND	ND	0.004	ND	0.204	ND	ND	ND	ND	ND
Autumn	0.001	0.032	0.005	ND	ND	ND	ND	ND	ND	ND
Winter	0.007	ND	0.036	ND	ND	ND	ND	ND	ND	ND

3.4. Microbiological View of Manzala Lake

Results in Table 4 showing that, the highest value of total bacterial count was during spring being 4400 cfu/ml $\times 10^3$, while the lowest value was found in autumn being 0.0 cfu/ml $\times 10^3$. Table 4 also showed that, the highest value of total fungal count was found in summer being 22500 cfu/ml $\times 10^3$ and lowest value was in the winter being 0.06 cfu/ml $\times 10^3$. It was observed that, there were no bacterial growth on SS agar medium. Obtained results were similar to [34] who found that, *Salmonella* and *Shigella* were not detected. The highest value of Staphylococci count was in spring being 1760 cfu/ml $\times 10^3$, while it was in the lowest value in autumn being 0.0 cfu/ml $\times 10^3$. The highest value of *Aeromonas* count in spring being 66 cfu/ml $\times 10^3$, but the lowest values was during autumn to be 0.1 cfu/ml $\times 10^3$. The highest values of coliform count was in spring being 1210 cfu/ml $\times 10^3$, while coliform count was in the lowest value in the autumn and winter being 0.0 and 0.0 cfu/ml, respectively.

Similar results were obtained from another author [35] who studied different kinds of fish collected from Damietta Governorate. These fish were in compatible with Egyptian standard specifications and results ensured that these fish were highly good for human consuming.

The results of another author [35] were higher than our results, where the range of total bacterial count was 0.6×10^6 to 0.2×10^8 /100 ml of water sample in winter and summer, respectively.

Obtained results were lower than those obtained by another author [30] 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 [37] 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 $\times 10^3$) of Manzala lake of Damietta Gavernorate, Egypt.

Seasons	Count of					
	Total bacteria	Total fungi	*Salmonella* sp. and *Shigella* sp.	Staphylococci	*Aeromonas* sp.	Coliform
Spring	4400	22000	0.0	1760	66	1210
Summer	39	22500	0.0	1	2	36
Autumn	0.0	0.3	0.0	0.0	0.1	0.0
Winter	3	0.06	0.0	0.015	0.2	0.0

Moreover, another author [38] 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 were 2.88×10^4 and 7.3×10^2 CFU/ml, respectively.

3.5. Microbiological View of Fish Taken from Manzala Lake

The total bacterial count of fish muscles were 17.6, 6000, 0.06 and 45 cfu/g $\times 10^3$ in spring, summer, autumn and winter,

respectively (Table 5). On the other hand, the highest value of total bacterial count of fish intestine was 46200 cfu/g $\times 10^3$ in spring, but the lowest value was in the autumn being 0.0 cfu/g $\times 10^3$. Total bacterial count of fish surface were 1980, 500, 0.2 and 10 cfu/g $\times 10^3$ in spring, summer, autumn and winter, respectively. The highest values of fungal count in muscles, intestine and fish surface being 1.87, 13200 and 46.2 cfu/g $\times 10^3$ during spring, respectively. On the other hand, all lowest fungal counts of muscles, intestine and surface were during winter being 0.6, 20 and 0.0, 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 0.198, 33 and 77 cfu/g $\times 10^3$, but the lowest values were in autumn and winter. The highest count of *Aeromonas* sp. was in fish intestine during spring, being 2530 cfu/g $\times 10^3$ while the lowest value was during summer and autumn. Coliform was found in the highest values in spring being 1210000 cfu/g $\times 10^3$ in intestine, on the other hand, coliform count was in the lowest values in the autumn and winter.

Table 5. Microbiological values (cfu/gm $\times 10^3$) of fish taken from Manzala lake of Damietta Gavernorate, Egypt.

Seasons	Count of						
	Fish part	Total bacteria	Total fungi	*Salmonella* sp. and *Shigella* sp.	Staphylococci	*Aeromonas* sp.	Coliform
Springer	Muscles	17.6	1.87	0.0	0.198	0.275	0.0
	Intestine	46200	13200	0.0	33	2530	1210000
	Surface	1980	46.2	0.0	77	1.01	12100
Summer	Muscles	6000	1.2	0.0	0.0	800	0.0
	Intestine	1000	12000	0.0	0.0	0.0	930
	Surface	500	68	0.0	0.0	0.0	160
Autumn	Muscles	0.06	0.7	0.0	0.01	0.07	110
	Intestine	0.0	40	0.0	0.0	0.0	0.0
	Surface	0.2	0.0	0.0	0.7	1	0.0
Winter	Muscles	45	0.6	0.0	0.01	0.2	0.0
	Intestine	10	20	0.0	1.5	30	1
	Surface	20	0.0	0.0	0.0	50	0.0

The highest pollution indicator was recorded in the water samples of Manzala, while the lowest pollution indicator detected in water sample of Mansoura city. The microbiological criteria and standards for drinking water supplies are based mainly on total and faecal Coliforms, faecal Streptococci and total bacterial counts [39]. On the other hand, the highest bacterial indicators were detected in warmer seasons which might be attributed to high temperature and the discharged waste water during this season [40].

The current results were higher than that obtained by another author [41] who reported that, total Coliforms during spring was 930 cfu/100ml.

The results of another author [26] were higher than our results, where the total bacterial counts was observed as a high bacterial load in both of gills and intestine comparing with the liver. the total bacterial counts were 0.8×10^7, 0.52×10^7 and 0.13×10^7 cfu / g in gills, intestine and liver, respectively.

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

The correlation coefficient between log of total bacterial count in Manzala lake water and BOD_5^{20} was calculated (Table 6). Statistical analysis indicated there was no correlation (r = 0.0) between log of total bacterial count and BOD_5^{20}.

Moreover, another author [42] reported that, a positive correlation between BOD_5^{20} and total bacterial count in ecosystem highly polluted by industrial wastes.

Table 6. Correlation coefficient value between the log of total bacterial count and the BOD_5^{20} of Manzala lake water.

Seasons	Log of Total bacterial count (X)	BOD_5^{20} (mgO_2/L) (Y)	$(X-\bar{x})$	$(Y-\bar{Y})$	$(X-\bar{x})^2$	$(Y-\bar{Y})^2$	$(X-\bar{x})(Y-\bar{Y})$
Springer	3.64	10.4	2.213	-0.85	4.897369	0.7225	-1.88105
Summer	1.59	14.8	-4.118	3.55	16.95792	12.6025	-14.6189
Autumn	0.001	12.2	-5.707	0.95	32.56985	0.9025	-5.42165
Winter	0.477	7.6	-5.231	-3.65	27.36336	13.3225	19.09315
∑	5.708	45			81.79	27.55	2.83
	1.427 ($\bar{x}$)	11.25 ($\bar{Y}$)			9.04	5.25	

Moreover, another author [38] 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 another author [43]. In contrast, a low correlation (r = 0.552) between the log of total bacterial count and BOD_5^{20} was reported by another author [44]. Other

author [45] 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 7 showing bacterial isolates numbers, characterization and its sources. Eight different bacterial isolates were isolated from nutrient agar medium, 5 isolates were isolated on *Aeromonas* agar medium and 3 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.

Five isolates were isolated from water, 5 from muscles, 3 from intestine and 6 from surface of fish. Among 19 bacterial isolates, only one was coccoid shape, 13 isolates were short rods and 5 isolates were long rods. Thirteen isolates were Gram negative and 6 isolates were Gram positive. Six isolates were spore formers and 13 isolates were non spore formers. All isolates gave negative results with acid fast stain.

Only one colony gave yellow color on nutrient agar medium, colony was 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 pair. Isolate No. 4 was considered as *Micrococcus* sp. according to Bergey's Manual of Determinative Bacteriology [22].

Five 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. 34, 51, 70, 71 and 72 were considered as *Bacillus* sp. according to Bergey's Manual of Determinative Bacteriology [22]. Another author [46] isolated *Bacillus alvei* and *Bacillus megaterium* from the microbial flora of the gastro-intestinal tract of *Clarias gariepinus* caught from river Dandaru Ibadan, Nigeria.

Table 7. Bacterial characterization and sources obtained from fish and brackish water of Manzala lake water of Damietta Gavernorate, Egypt.

Sources (2014)	Cultivation media	Isolates Nos.	Characterization of isolates				
			Shape	Arrangement	Gram stain	Spore stain	Acid fast stain
Water	Nutrient Agar	3	Short rods	Single	-	-	-
		4	Coccoid	Single	+	-	-
	Aeromonas	70	Long rods	Single	+	+	-
	Staph 110.	31	Short rods	Single	-	-	-
	McaConkey broth	86	Short rod	Single	-	-	-
Fish Muscles	Nutrient Agar	18	Short rods	Single	-	-	-
		51	Long rods	Single	+	+	-
		55	Short rods	Single	-	-	-
	Aeromonas	34	Long rods	Single	+	+	-
		71	Long rods	Single	+	+	-
Fish Intestine	*Aeromonas*	72	Long rods	Single	+	+	-
	Staph 110.	81	Short rod	Single	-	-	-
		85	Short rod	Single	-	-	-
Fish Surface	Nutrient Agar	5	Short rods	Single	-	-	-
		7	Short rods	Single	-	-	-
		56	Short rods	Single	-	-	-
	Aeromonas	73	Short rods	Single	-	-	-
	McaConkey broth	79	Short rod	Single	-	-	-
		87	Short rod	Single	-	-	-

Figure 3. Yellow color colony on Aeromonas medium.

Colony isolated on *Aeromonas* agar medium which were yellow colored (Figure 3), were picked up and streaked *Aeromonas* agar medium slant. After growth, the cells were short rods, Gram negative, non spore former and non acid fast. Its arrangement was single. Isolate No. 73 was considered as genus *Aeromonas* according to Bergey's Manual of Determinative Bacteriology [22].

The cells were short rods, Gram negative, non spore former and non acid fast. Its arrangement was single. Isolates Nos. 79, 86 and 87 after growth onto EMB medium, the colonies were green and metallic sheen (Figure 4) were considered as genus *Esherichia* according to Bergey's Manual of Determinative Bacteriology [22].

The cells were short rods, Gram negative, non spore former and non acid fast. Its arrangement was single. Isolates Nos. 3, 5, 7, 18, 31, 55, 56, 81 and 85 which grown on nutrient agar medium and the colony color was white and brown were considered as genus *Pseudomonas* according to Bergey's Manual of Determinative Bacteriology [22].

Figure 4. Green and metallic sheen colonies on EMB medium.

Similar results were obtained from [35] who studied total viable bacterial count, aerobic spore forming bacteria, *Staphylococcus aureus*, coliform group, *Clostridium* spp. and anaerobic spore formers producing H_2S. Their obtained results reported that all studied fish were compatible with their standard specifications from chemical and microbiological view.

Similar results were obtained by another author [47] who studied the microbiological load of mackerel (*Scomber scombrus*). Fourteen different bacterial isolates were isolated from all samples. Twelve isolates were isolated from staph 110 medium and two isolates were found on the SS agar medium. Twelve isolates were coccoid shaped bacteria and 2 isolates were spore forming long rods. All isolates were gram positive. Two isolates were spore forming and twelve isolates were non-spore forming. Six isolates were considered as *Staphylococcus* sp. and another six isolates were considered as *Micrococcus* sp. the last two isolates were considered as *Bacillus* sp.

3.8. Characterization of Fungal Isolates

Five fungal isolates were isolated from all examined samples. The isolate characteristics showed that, colony was green, on PDA medium. Conidiophores were smooth with globose vesicles that gave rise to radiating, biseriate conidial heads producing smooth-walled, globose conidia. From these characteristics, isolate No. 54 was identified as *Aspergillus alliaceus* (recently *Petromyces alliaceus*) [28].

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. 49 and 57 were identified and designated as *Aspergillus niger* [27].

Isolate No. 8 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 *Aspergillus flavus* as reported [27].

The colony of isolates No. 17 was rapid growing, flat, filamentous, and velvety, woolly, or cottony in texture on PDA medium (Photo 16.), the colonies are initially white and become blue green, gray green, olive gray, yellow or pinkish in time. visualized as globose to elongated sausage-shaped cells that multiply by fissio. From these characteristics, isolate 17 was identified as *Penicillium* sp. [29].

The following bacterial genera were isolated from water samples: *Aeromonas*, *Bacillus*, *Proteus*, *Pseudomonas*, *Streptococcus*, *S. epidermidis*, *S. aureus*, *Micrococcus*, *Vibrio* and *E. coli*. While the total bacterial counts from fish samples were higher in summer season in all tested organs than other seasons; also, the high bacterial load was observed in both of gills and intestine comparing with the liver. TBCs were 0.8×10^7, 0.52×10^7 and 0.13×10^7 cfu / g in gills, intestine and liver, respectively [36].

Aspergillus niger, Aspergillus flavus were isolated by many authors [45-48] from the gastro-intestinal tract of fish. In addition, another authors [38, 49 and 50] isolated five fungal isolates from the intestinal tract of fish. and identified it as *Aspergillus ochraceus*, *A. oryzae*, *A. niger*, *Geotrichum candidum* 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 spring. 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 spring and summer. The highest values of bacterial groups of intestine and surface of fish were in spring while the lowest values were in autumn. *Micrococcus* sp., *Aeromonas* sp., *Esherichia* sp., *Pseudomonas* sp., *Aspergillus alliaceus*, *Aspergillus niger, Aspergillus flavus* and *Penicillium* sp. were isolated and identified from water and fish.

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