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Chemical and Microbiological Examinations of Water and Fish Taken from Mediterranean Sea of Ras El-Bar City, Damietta Governorate, Egypt

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Abstract

Mediterranean sea water and fish samples of Ras El-Bar city in Damietta governorate, Egypt were examined. Morphological examination of fish obtained from sea water was examined. The highest value of BOD_5^{20} was found during summer being 2.4 mgO_2/L while the lowest value was found during spring being 0.8 mgO_2/L. Lead, Arsines and Stannum did not presented in all seasons of all samples (water and fish). Cadmium and cupper did not presented in all fish samples, but presented in all water samples. The lowest value of cadmium in fish was in the spring being 0.014 ppm while the highest value was in winter being 0.028 ppm. The highest value of total bacterial count in sea water was found during summer being 20cfu/ml×10^3, while the lowest value was in spring and winter being 0.01cfu/ml×10^3. The highest value of total fungal count was in summer being 4cfu/ml×10^3 while lowest value was in the winter being 0.0cfu/ml×10^3. There were no bacterial growth on SS agar medium. The highest value of Staphylococci count was in summer being 20cfu/ml×10^3. The highest value of *Aeromonas* count in summer being 50cfu/ml×10^3. The highest value of coliform count was in autumn being 30cfu/ml×10^3. The highest value of total bacterial count of fish muscles, fish intestine and fish surface were 1600, 3000 and 1000cfu/g×10^3 in summer, spring and summer, respectively. The highest values of fungal count in muscles, intestine and fish surface were during summer, spring, being 0.4, 900 and 430cfu/g ×10^3, respectively. The highest values of Staphylococcal counts of fish muscles, intestine and fish surface were in summer, being 20, 80 and 200cfu/g×10^3, respectively. The highest count of *Aeromonas* sp. was in fish muscles, intestine and surface during summer, being 40, 1700 and 10cfu/g×10^3, respectively. Coliform was found in the highest values in summer being 36, 9.2 and 2400cfu/g×10^3 in muscles, intestine and surface, respectively. A strong correlation coefficient value (r = 0.79) between the log of total bacterial count (LTBC) and BOD in the sea water was indicated, which means there are a highly pollution by industrial pollutants. Among 10 bacterial isolates, only one was coccoid shape, 4 isolates were short rods and 5 isolates were long rods. Four isolates were Gram negative and 6 isolates were Gram positive. Five isolates were spore formers and 5 isolates were non spore formers. All isolates gave negative results with acid fast stain. *Micrococcus* sp., *Bacillus* sp., *Esherichia* sp. *Pseudomonas* sp., *Aspergillus niger*, and *A. flavus* were isolated and identified from water and fish.

Keywords

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

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1. Introduction

Over the years there have been very few publications summarizing available information in the microbiological properties of sea water of Ras El-Bar city in Damietta Governorate, Egypt. So that, the information about the seawater of New Damietta city on Mediterranean Sea take

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the same trend. Copper, iron and zinc concentrations in all water samples of New Damietta city on Mediterranean Sea were matching with the Egyptian law No. 4 / 1994 [1]. Sea water samples in New Damietta city showed minimum concentrations of dissolved Oxygen and maximum concentrations of biological oxygen demand, chemical Oxygen demand, ammonia and total nitrogen. In the same direction, another author evaluate the quality of the seawater of Alexandria, Egypt on Mediterranean Sea, in six different sites. One sample was positive for *Salmonella*. Neither *Shigella* or *Yersinia* were isolated from any sites. *E. coli* was identified in ten samples. Three samples were positive for the presence of enteric viruses. The analysis of phages showed a variable pollution values [2]. Moreover, another author evaluate the microbial diversity of bacteria, fungi and other pathogenic microorganisms present in seawater and sediments from Chennai shoreline (southeast coast of India). Chennai beaches are heavily polluted with untreated sewage effluents; municipal sewage disposal and recreational activities. Seawater was heavily contaminated with coliforms, *Vibrio* and *Pseudomonas* compare to sediment microbial contamination. Isolated bacteria are mostly pathogenic microorganisms including *Vibrio*, *Pseudomonas*, *Coliforms*, *Salmonella* and *Shigella* [3]. Another potential environmental threat noticed was heavy metal resistance of these pathogenic strains against 50mM of Ni, Cr, Cu, Co, Pb and Hg. The Chennai coast may cause health risk to the recreational users and fisher folk, ultimately warrants environmental quality management to control microbial contamination. Recently, the seawater collected from a selected bathing site in the West coast of Sri Lanka was examined, for pollution indicating bacterial parameters. Sand samples were subjected to similar evaluation. Two sites were selected from this location to collect samples [4]. The following microbial parameters were analyzed in the collected samples: enterococci, total coliforms, thermotolerant coliforms, *E. coli*, *Pseudomonas* sp. and *Staphylococcus* sp. In addition, physical parameters such as temperature, pH and conductivity were also measured in collected samples. Enterococci counts generally varied from 09 - <1600 (MPN/100 ml). The average enterococci and total coliform counts in beach sand were higher than that of seawater. However, *E. coli* showed a higher average count as 228 (MPN/100ml) in seawater than 72 (MPN/100 ml) in beach sand. Bacteria belonging to species of *Vibrio*, *Aeromonas*, *Pseudomonas*, *Bacillus*, *Staphylococcus* and *Listeria* were identified from both seawater and sand. The sampled seawater was characterized by a salinity of 20-33 (ppt) and varying pH of 6.7 – 8.20. Based on the obtained results for enterococci, the selected location was provisionally classified in the D category as per WHO guidelines. However microbiological quality of the seawater as per the water quality guidelines depends upon which indicator microbe is chosen. A brief outline is given of marine microbiology, relating to both natural microorganisms and those resulting from pollution. Microbial indicators of pollution and their methods of detection are discussed and recent epidemiological surveys of health effects associated with seawater recreational activities are reviewed. Reference is made to microbiological standards for bathing waters. It is evident that more work is required to elucidate the complex ecological interactions of marine microorganisms; that current microbial pollution indicators are in need of refinement; and that although recreational exposure to coastal waters has some association with the acquisition of minor illness, attempts to relate this to the microbiological quality of the water are confounding [5].

The presence of many types of microorganisms, including potential pathogens has been reported as an integral part of coastal management programs in many countries. Therefore the purpose of this study was to examine the chemical and microbiological properties of seawater and fish collected from Mediterranean sea water of Ras El-Bar city, Damietta Governorate, Egypt, during spring, summer, autumn and winter.

2. Materials 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) [6].

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 mg/l. BOD_5 was calculated by the method described using the equation of BOD_5^{20} (mg/L) = (Initial DO - Final DO) x dilution factor [7].

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 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 [8].

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 Mediterranean sea water of Ras El-Bar city, 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 into icebox to the microbiological laboratory of Department of Agricultural Microbiology, Faculty of Agriculture, Damietta University. One ml of each water sample (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 [9].

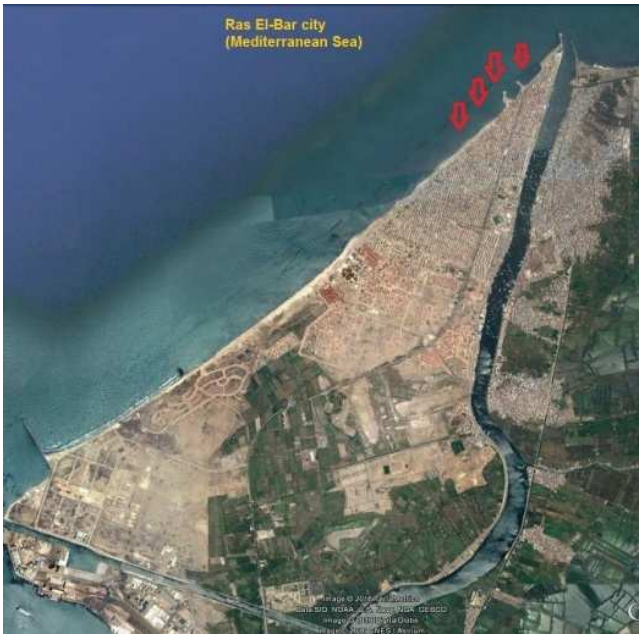

Figure 1. Site of water and fish samples taken from Mediterranean sea water of Ras El-Bar city, Damietta Governorate, Egypt.

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 [10].

2.2.3. The Relationship Between the Log TBC and the BOD

The correlation coefficient value (r) was calculated according the following equation [11]. 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.

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 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. All typical obtained colonies were isolated on the same specific cultivation medium for maintenance and identification [10].

The following microbiological methods were carried out to identify the obtained bacterial isolates [12]. 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 [13]. The colonies count per ml or gram of samples was calculated as follows: The bacterial or fungal count (cfu/ml orcfu/g) = average number of triplicates of the same dilution x reciprocal of the dilution used [14].

Coliform counts were detected using the most probable number (MPN) technique [10]. 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 [15].

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 [10]. 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 [16]. 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 [17] was used.

3. Results and Discussion

3.1. Morphological Characteristics of Fish

All fishes obtained from Mediterranean sea of Ras El-Bar city, Damietta Governorate, Egypt were belonged to tow genera of fish namely, *Oreochromis niloticus* (Nile tilapia) and *Liza carinata*. The mean value of fish long of three individuals were 18, 17, 12 and 11 cm in spring, summer, autumn and winter, respectively (Table 1). The highest weight of fishes were obtained during spring and summer being 187 and 180 gm, while the lowest weights were in case of autumn and winter being 90 and 24gm, respectively. It was observed that, the color of fishes were differed according to seasons. Fishes obtained during the spring and summer were white and gray region (Figure 2a and 2b), while, during autumn most of fish was white and black parts (Figure 2c). In winter, most of fish was white, yellow and black part (Figure 2d).

Figure 2. Fishtaken from Mediterranean sea water of Ras El-Bar city, Damietta Governorate, Egypt. a) Oreochromis niloticus during spring, b) Oreochromis niloticus during summer, c) Liza carinata during autumn and d) Liza carinata during winter.

Table 1. Morphological examination of fish taken from Mediterranean sea of Ras El-Bar city, Damietta Governorate, Egypt.

Seasons	Scientific name	Long (cm)	Weight (g)	Fish color
Spring	*Oreochromis niloticus*	18	187	Most of fish wear white and gray parts
Summer	*Oreochromis niloticus*	17	180	Most of fish wear white and gray parts
Autumn	*Liza carinata*	12	90	Most of fish was white and black parts
Winter	*Liza carinata*	11	24	Most of fish was white, yellow and black parts

3.2. BOD and EC Values of Sea Water

Data in Table 2. showing that, temperature varied between 15 and 27°C. Initial DO were 7.0, 6.0, 7.4 and 8.0 mgO_2/L in spring, summer, autumn and winter, respectively. Final DO were 6.2, 4.8, 6.8 and 8.0 mgO_2/L in spring, summer, autumn and winter, respectively. The highest value of BOD_5^{20} was during summer being 2.4 mgO_2/L while the lowest value was during spring being 0.8 mgO_2/L. This phenomena owing to the increase in the biological activities in summer than that occurred in winter. The lowest value of EC was in winter being 167.9 mhos/cm and the highest value was in summer being 248 mhos/cm.

The DO of this study was higher than that another article who measured 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 [18]. Also, the BOD_5^{20} values of El-Rahawy drain and found that, BOD_5^{20} values were 3.5, 2.0 and 16.5 mg/l during summer, autumn and winter, respectively [19]. Obtained results were lower than that another article [20] 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). On the other hand, Mediterranean Sea water samples in New Damietta city showed minimum concentrations of dissolved Oxygen and maximum concentrations of biological oxygen demand, chemical Oxygen demand, ammonia and total nitrogen [1].

Table 2. Temperature, BOD and EC values of seawater of Ras El-Bar city, Damietta Governorate, Egypt.

Seasons	Temperature (°C)	DO, mgO_2/L		BOD_5^{20} (mgO_2/L)	EC (mhos/cm)
		Initial	Final		
Spring	22	7.0	6.2	0.8	193
Summer	27	6.0	4.8	2.4	248
Autumn	20	7.4	6.8	1.5	179
Winter	15	8.0	8.3	0.9	167.9

3.3. Heavy Metals Values of Examined Water and Fish Muscles

Results in Table 3 showing that, Lead, Arsines and Stannum did not presented in all seasons of all samples (water and fish). While, cadmium and cupper did not presented in all fish samples, but presented in all fish samples. The lowest value of cadmium in fish was in the spring being 0.014 ppm while the highest value was in winter being 0.028 ppm. Cupper was detected in all seasons samples in fish being 0.008, 0.032, 0.052 and 0.036 ppm in spring, summer, autumn and water, respectively. These results are lower than the permissible levels (1 mg/L) permitted by the Egyptian Organization for Standardization [21]. Also, all concentrations did not exceed the Egyptian Standards of the Environmental Laws No. 48/1982 [22]. The maximum concentration in water was 0.05 mg/L. Obtained result were lower than that obtained by another article who reported that, samples were collected along the Saudi Arabian coast of the Red Sea to assess the accumulation and ecological risks of heavy metals [23]. Results showed that the following mean concentrations of heavy metals: Cr (46.14 mg g^{-1} 18.48) > Cu (22.87 mg g^{-1} 13.60) > Ni (21.11 mg g^{-1} 3.2) > Pb (3.82 mgg^{-1} 2.46) > Cd (0.75 mg g^{-1} 0.87). The maximum concentrations of the studied metals were above the threshold effect level, indicating a limited impact on the respective ecosystems. The maximum concentration of Cd exceeded its toxic effect [23]. Similar results were obtained by [1] who reported that, copper, iron and zinc concentrations in all water samples of New Damietta city on Mediterranean Sea were matching with the Egyptian law No. 4 / 1994.

Table 3. Heavy metals values of water and fish muscles obtained from seawater of Ras El-Bar city, Damietta Governorate, 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	0.014	ND	0.008	ND	ND	ND	ND	ND
Summer	ND	ND	0.020	ND	0.032	ND	ND	ND	ND	ND
Autumn	ND	ND	0.026	ND	0.052	ND	ND	ND	ND	ND
Winter	ND	ND	0.028	ND	0.036	ND	ND	ND	ND	ND

3.4. Microbiological View of Sea Water

Results in Table 4 showing that, the highest value of total bacterial count was during summer being 20cfu/ml×10^3, while the lowest value was in spring and winter being only 0.01cfu/ml×10^3. These results were lower than that obtained by another article [24] where their total bacterial count was 120×10^{3cfu}/ml. The high bacterial load may be explained by the observation of another article [25] who reported that, the richness of the effluent in organic carbon exerted a specific enrichment effect on the microbial population.

Table 4 also showed that, the highest value of total fungal count was in summer being 4cfu/ml×10^3 and lowest value was in the winter being 0.0cfu/ml×10^3. It was observed that, there were no bacterial growth on SS agar medium. Obtained results were similar to that of another article [26] who found that, *Salmonella* and *Shigella* were not detected. Similar results were obtained by [24] who did not detected any of *Staphylococcus*, *Streptococcus*, *Salmonella*, *Vibrio* or *Pseudomonous* in their samples.

The highest value of Staphylococci count was in summer being 20cfu/ml×10^3, while it was in the lowest value in winter being 0.001cfu/ml. The highest value of *Aeromonas* count in summer being 50cfu/ml×10^3, while the lowest values was during winter to be 0.0cfu/ml×10^3. The highest values of coliform count was in autumn being 30cfu/ml×10^3, while coliform did not detected in other seasons. These results were higher than that obtained by another article [24] where their coliform count was 210cfu/100ml.

The current results were higher than that obtained by another article [27] who reported that, total Coliforms of during spring was 930cfu/100ml. Obtained results were lower than those obtained by another article [18] who determined the total Coliform of farmed fish water and they found that, the counts were ranged between log 2.0 and 3.4cfu/ml. According to the guideline criteria for faecal indicator organisms of WHO [28] 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, respectively. So, these data of sea water of investigated sites were subjected to sewage pollution which considered to be very serious concept. Moreover, another article [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 7.3×10^2cfu/ml, respectively.

Table 4. Microbiological values (cfu/ml×10^3) of seawater of Ras El-Bar city, Damietta Governorate, Egypt.

Seasons	Count of					
	Total bacteria	Total fungi	*Salmonella* sp. and *Shigella* sp.	Staphylococci	*Aeromonas* sp.	Coliform
Spring	0.01	1.5	0.0	8.4	0.15	0.0
Summer	20	4	0.0	20	50	0.0
Autumn	0.01	0.1	0.0	0.01	0.013	30
Winter	0.1	0.0	0.0	0.001	0.0	0.0

3.5. Microbiological View of Fish

The total bacterial count of fish muscles were 9.6, 6700, 1.0 and 0.0cfu/ml×10^3 in spring, summer, autumn and winter (Table 5), respectively. On the other hand, the highest value of total bacterial count of fish intestine was 1000cfu/ml×10^3 in spring, but the lowest value was in the winter being 0.0cfu/ml×10^3. Total bacterial count of fish surface were 1500, 1000, 0.0 and 0.0cfu/ml×10^3 in spring, summer, autumn and winter, respectively. The highest values of fungal count in muscles, intestine and fish surface were 0.4, 900 and 430cfu/g×10^3 during spring, summer, spring, respectively. On the other hand, all lowest fungal counts of muscles, intestine and surface were during winter being 0.0, 0.07 and 0.5, 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 summer, being 20, 80 and 200cfu/g×10^3, while the lowest values were in autumn. The highest count of *Aeromonas* sp. was in fish muscles, intestine and surface during summer, being 40, 1700 and 10cfu/g×10^3, respectively, while the lowest value was during autumn. Coliform was found in the highest values in summer being 36, 9.2 and 2400cfu/g×10^3 in muscles, intestine and surface, respectively, on the other hand, coliform count was in the lowest values in the autumn and winter.

Obtained results are lower than those obtained by another article [18] who counted the total Coliform bacteria associated with muscles of fish feeder canal water ranged between log 1.68, 2.4cfu/g. On the other hand, obtained results were higher than that obtained by another article [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 Between the Log of TBC and the BOD

The correlation coefficient between the log of total bacterial count (TBC) in the Mediterranean sea waterand the BOD_5^{20}

was calculated (Table 6).

Table 5. Microbiological values (cfu/gm×10^3) of fish taken from seawater of Ras El-Bar city, Damietta Governorate, Egypt.

Seasons	Count of						
	Fish part	Total bacteria	Total fungi	*Salmonella*sp. and *Shigella*sp.	Staphylococci	*Aeromonas* sp.	Coliform
Springer	Muscles	9.6	0.4	0.0	0.3	0.015	1.5
	Intestine	1000	1000	0.0	0.0	20	0.0
	Surface	1500	430	0.0	60	1.4	0.0
Summer	Muscles	6700	0.0	0.0	20	40	36
	Intestine	600	900	0.0	80	1700	9.2
	Surface	10000	400	0.0	200	10	2400
Autumn	Muscles	1	0.05	0.0	0.0	0.0	0.0
	Intestine	0.01	0.3	0.0	0.0	0.0	0.0
	Surface	0.0	4	0.0	0.0	0.0	0.0
Winter	Muscles	0.0	0.0	0.0	0.02	0.1	0.0
	Intestine	0.0	0.07	0.0	0.01	0.21	0.0
	Surface	0.0	0.5	0.0	0.0	0.09	0.0

Table 6. Correlation coefficient value between the log of total bacterial count and the BOD_5^{20} of sea 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	-2	0.8	-1.075	-0.6	1.155625	0.36	0.645
Summer	1.3	2.4	2.225	1	4.950625	1	2.225
Autumn	-2	1.5	-1.075	0.1	1.155625	0.01	-0.1075
Winter	-1	0.9	-0.075	-0.5	0.005625	0.25	0.0375
∑	-3.7	5.6	0	0	7.2675	1.62	2.7
	-0.925 ($\bar{x}$)	1.4 ($\bar{Y}$)	0	0	---	---	---

Statistical analysis indicated a strong correlation (r = 0.79) between the log of total bacterial count and BOD_5^{20}. [30] reported that, a positive correlation between BOD_5^{20} and total bacterial count in ecosystem highly polluted by industrial pollutants. Moreover, another article [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 [31]. In contrast, a low correlation (r = 0.552) between the log of total bacterial count and BOD_5^{20} was reported by another article [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 7 showing bacterial isolates numbers, characterization and its sources. Four different bacterial isolates were isolated from nutrient agar medium, 3 isolates were isolated on *Aeromonas* agar medium and 2 isolates were found on Staph 110. medium. One isolate was obtained from McaConkey broth which gave acid and gas were picked up and streaked onto EMB medium. Three isolates were obtained from water, 1 from muscles, 3 from intestine and 2 from surface of fish. Among 10 bacterial isolates, only one was coccoid shape, 4 isolates were short rods and 5 isolates were long rods. Four isolates were Gram negative and 6 isolates were Gram positive. Five isolates were spore formers and 5 isolates were non spore formers. All isolates gave negative results with acid fast stain.

Only one colony gave orange 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. 25 was considered as *Micrococcus* sp. according to Bergey's Manual of Determinative Bacteriology [12].

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. 28, 30, 35, 47 and 48 were considered as *Bacillus* sp. according to Bergey's Manual of Determinative Bacteriology [12]. The cells were short rods, Gram negative, non sporeformer and non acid fast. Its arrangement was single. Isolate No. 84 after growth on EMB medium, the colony was green and metallic sheen was considered as genus *Esherichia* according to Bergey's Manual of Determinative Bacteriology [12].

3.8. Characterization of Fungal Isolates

Five fungal isolates were isolated from all examined samples. Characterization of the isolates showed that, the mycelium of colonies of isolates Nos. 12, 53 and 69 which grew 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. 12, 53, 69 were identified and designated as *Aspergillus niger* [17].

Table 7. Bacterial isolates numbers, characterization and sources obtained from fish and water of Mediterranean sea of Ras El-Bar city.

Sources	Cultivation media	Isolates Nos.	Characterization of isolates				
			Shape	Arrangement	Gram stain	Spore stain	Acid fast stain
Water	Nutrient Agar	25	Coccoid	Pair	+	-	-
	Aeromonas	48	Long rods	Single	+	+	-
	Staph 110.	35	Long rods	Single	+	+	-
Fish Muscles	Nutrient Agar	14	Short rods	Single	-	-	-
	Aeromonas	47	Long rods	Single	+	+	-
Fish Intestine	Nutrient Agar	67	Short rods	Single	-	-	-
	Aeromonas	28	Long rods	Single	+	+	-
	Staph 110.	30	Long rods	Single	+	+	-
Fish Surface	Nutrient Agar	68	Short rods	Single	-	-	-
	McaConkey broth	84	Short rod	Single	-	-	-

Isolates Nos. 50, and 66 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 [17].

Another authors [34 and 35] isolated *Aspergillus niger, Aspergillus flavus,* from the gastro-intestinal tract of fish. In addition, Another authors [29 and 36] isolated five fungal isolates 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 spring. The lowest value of EC was in winter whilethe 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 summer while the lowest values were in winter and autumn. *Micrococcus* sp., *Bacillus* sp., *Esherichia* sp. *Pseudomonas* sp., *Aspergillus niger*, and *A. flavus* were isolated and identified from water and fish.

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