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Microbiological Profiles of El-Qanater El-Khairia Fish Farm

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Abstract: Earthen ponds water and sediment in El-Qanater Fish Farm were assessed using microbiological and physico-chemical parameters as indices. The value of Total Coilforms (TC), Faecal Coliforms (FC), Faecal Streptococci (FS) in predicting the presence of *Salmonella/Shigella* (*S/S*) sp. and *Staphylococcus aures* were assessed. Total viable bacterial count (log_{10}) in pond water ranged from 2.15±0.316 to 8.53±0.18 cfu/ml and in pond sediment from 4.113±0.035 to 10.53±0.399 cfu/g. Also the total counts of yeast and mould were 1.259±0.241 to 5.2±0.1 cfu/ml and 00 (not detectable, ND) to 7.29±0.25 cfu/g in water and sediment, respectively. Value of TC, FC and FS in water were ranged from 3.24±0.06 to 5.153±0.418,00(ND) to 4.0±0.15 and 00(ND) to 4.1±0.19cfu/100ml, respectively. While the respective counts in sediment ranged from 3.29±0.16 to 5.78±0.132, 00(ND) to 4.45±0.15 and 00(ND) to 4.83±0.058 cfu/100g. *S/S* in the water ranged from 1.93±0.058 to 3.1±0.00 cfu/ml and 2.0±0.18 to 4.53±0.31 cfu/g in the sediment.

Key wards: Microbial flora · coliforms · faecal streptococci · pond water · sediment

INTRODUCTION

Aquaculture is the world's fastest growing food production sector. It was once considered as an environmentally sound practice because of it's traditional polyculture and integrated system of farming based on optimum utilization of farm resources [1]. As fish take a large number of bacteria into their gut from water, sediment and food, so microbial pollution of aquatic environment induces an increased public health risk where it is used as a source of potable water for fish and shell fish farming [2]. It is well know that freshwater fish and their aquatic environment can harbour human pathogenic bacteria, particularly members of the coliform group, which indicates the sewage pollution [3, 4].

E.coli is regarded as the most sensitive indicator of faecal pollution and it is use as the most sensitive indicator for faecal pollution [5].

If indicator bacteria (faecal indicator) are present, there is a probability that pathogenic organisms excreted in feces are also present and that the water can transmit water borne infectious diseases. Indicator bacteria have traditionally been monitored in water pollution contract [6]. Most Probable Number (MPN) test was done to detect the colifrom in water samples [7] and the TC is sufficient to predict the presence of *Salmonella* sp. or *Staphylococcus aureus* in water moderately affected by sewage pollution [8]. Aquatic animals take up various kinds of bacteria from food, water and sediments which may became constituents of bacterial flora of the digestive tract causing serious disease problems [9]. No sufficient data about the microbiological aspect of El-Qanater El-Khairia Fish Farm, so, the present work was designed to investigate some quantitative and qualitative aspects of the bacterial flora in cultured ponds and to determine the microbial population of water and sediment samples and to clarifying the relation between the bacterial load of pond water and that of pond sediment.

MATERIALS AND METHODS

Studying area: El-Qanater El-Kharia, fresh water farm, Inland waters and Aquaculture Branch, National Institute of Oceanography and Fisheries, Cairo, Egypt. Farm area about 1.3 Feedn, contains seven earthen ponds (˜867 m^2/pond). The ponds completely supplied with River Nile water over the year. Tilapia fish is the most common fish held in these ponds and feed an artificial diet.

Physical parameters: Temperature, pH and dissolved oxygen were measured on every sampling day at mid day time [10].

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Microbiological sampling and analyses: Three samples of each pond (water and sediment) were performed monthly during June 2006 until May 2007. Samples were collected in sterilized clean glass bottles (250 ml) and subjected to dilutions at rate of 10^{-1}-10^{-6}. Also, 10 g wet sediment were completed to 100 ml sterile saline and serially diluted (10^{-1}-10^{-8}). 100 µl of each dilution was spread on each agar plate surface [10].

Microbiological analyses: Spread plate technique was used for total viable bacterial count (TVBC) of water and sediment on nutrient agar (NA), (1-140, Scharlau Chemie, S.A.) and potato dextrose agar (PDA) (1-483, Scharlau Chemie,S.A.) for yeast and mould [10], S/S agar (1-171, Scharlau Chemie,S.A.) for detect *Salmonla /Shiggeilla*. All the inoculated plates (triplicates) were incubated at 37±2°C for 48h. and 30±2°C for 7 days for bacteria and yeast and mold, respectively.

MPN of TC: The MPN was estimated by detecting the number of tubes in each group showed gas following the incubation period [10]. Plates of Eosin methylene Blue (EMB) agar (1-068, Scharlau Chemie, S.A.) were inoculated from each positive tube and incubated at 37±2°C for 24h. to detect the coliform growth.

MPN-index of FC: Transfer from all positive tubes to fresh single strength McConkey broth (2-118, Scharlau Chemie,S.A.) tube using 4 mm diameter loop. Incubate at 44.5 °C for 24h. in water bath. MPN index /100ml water or 100g sediment was determined from the tables of [11].

MPN of FS: Faecal streptococci was determined using Azide dextrose broth (2-343, Scharlau Chemie, S.A.) the corresponding MPN index was computed from the tables of [11].

Characterization of the obtained bacterial isolates: Micro and macro-morphological characteristics: All the bacterial isolates were purified on NA plates to study cell shape, color, motility and /or pigment production. Also, the stained smear of each bacterial isolate was applied for studying the size, cell shape, spore forming and Gram stain.

Biochemical characteristics: The most of biochemical testes as oxidase, catalase, VP test, nitrate reduction, hydrogen sulfide production, hydrolysis of starch, gelatin, esculin, arginine and casein, oxidation/fermentation (OF) of glucose and the bacterial growth relationships to pH, temperature and some chemical inhibitors are based on [12] to identify the genus or species level.

Statistical analysis: Mean, standard deviation (SD), correlation coefficient and the degree of significance of the obtained results were calculated using SPSS a computer program of biostatistics.

RESULTS

Physical parameters: The temperature of water ranged from 25.0±0.0 to 42.6±0.79°C and dissolved oxygen of water ranged from 2.01±0.005 to 6.21±0.84 ppm and water ranged from pH 6.53±0.237 to 8.9±0.1 during the period of the study (Table 1).

Microbiological parameters of water: Table 2 shows the bacterial load and yeast and mould (log_{10}) distribution in water. It was evident that the highest value of TVBC was recorded during July (8.53±0.18) and the lowest value was recorded in November (2.151±0.136). Also the yeast and mould counts recorded the highest values during July (5.2±0.1) and the lowest values during December (1.259±0.241). Water TC and FC were significantly correlated ($P<0.05$) with both temperature and water TVBC. The highest numbers of TC and FC were recorded

Table 1: Mean values±SE of temperature, Dissolved Oxygen (DO) and pH of the water samples in earthen ponds, from June 2006 to May 2007

Parameters Month	Temperature (C°)	D.O (ppm)	pH
June	42.6±0.79	2.53±0.46	6.89±0.06
July	35.6±0.100	5.88±0.171	8.23±0.25
August	38.3±0.153	5.7±0.33	8.61±0.20
September	35.5±0.500	5.82±0.136	7.65±0.15
October	32.66±0.580	4.7±0.67	6.53±0.237
November	30.0±0.0	5.6±0.70	6.57±0.266
December	28.0±0.0	5.81±0.96	6.73±0.226
January	25.0±0.0	2.01±0.005	7.56±0.11
February	32.0±0.0	5.035±1.005	8.25±0.45
March	32.3±0.58	3.02±0.34	8.8±0.2
April	33.0±0.0	6.04±0.67	8.25±0.35
May	35.0±0.0	6.21±0.84	8.9±0.1

Mean value±standard deviation of three replicates SE: Standard error

Table 2: Mean values±SE of total viable bacterial count (TVBC) and yeast and mould (cfu/ml) in pond water, from June 2006 to May 2007

Parameters Month	TVBC	Yeast & mould
June	6.155±0.122	4.03±0.208
July	8.53±0.18	5.2±0.1
August	6.215±0.59	4.18±0.137
September	6.092±0.77	5.14±0.15
October	5.213±0.038	4.00±0.0
November	2.151±0.316	4.54±1.22
December	3.906±0.43	1.259±0.241
January	3.62±0.325	2.63±0.208
February	3.85±0.136	2.63±0.015
March	4.58±0.434	3.0±0.0
April	4.88±0.458	3.4±0.36
May	4.58±0.055	5.1±0.1

Mean value±standard deviation of three replicates SE: Standard Error

Table 3: Mean values±SE MPN of TC, FC and FS (cfu /100ml) in pond water

Parameters Month	TC	FC	FS
June	3.243±0.06	3.0±0.0	3.17±0.231
July	5.153±0.418	4.0±0.15	3.72±0.332
August	1.643±1.43	1.0±0.0	4.03±0.058
September	3.07±0.029	2.0±0.18	ND
October	3.71±0.208	2.0±0.0	2.23±0.153
November	2.44±0.144	ND	ND
December	1.24±0.34	ND	1.49±0.12
January	0.87±1.5	ND	ND
February	1.96±0.42	ND	ND
March	2.6±0.0	ND	4.1±0.19
April	2.55±0.085	1.1±0.04	3.58±0.025
May	3.78±0.6	2.0±0.113	3.39±0.325

Mean value±standard deviation of three replicates, ND: Not Detected SE: Standard Error

Table 4: Mean values±SE of Total Viable Bacterial Count (TVBC) and yeast-mould (cfu/g) in pond sediment

Parameters Month	TVBC	Yeast and mould
June	7.295±1.153	4.9±0.10
July	10.53±0.339	7.29±0.25
August	7.36±0.256	6.09±0.09
September	8.518±0.256	5.37±0.30
October	6.438±0.112	5.14±0.15
November	4.113±0.035	4.30±0.300
December	5.58±0.21	3.392±0.089
January	5.85±0.3	ND
February	6.23±0.035	ND
March	6.47±0.035	2.83±0.05
April	6.58±0.04	3.0±0.0
May	6.58±0.04	4.4±0.361

Mean value±standard deviation of three replicates, ND: not detected SE: Standard Error

during June and July with the value ranged from 0.87±1.5 to 5.153±0.418 and 0.0(ND) to 4.0±0.15 cfu/100 ml, respectively, while FC wasn't detectable during November till March (Table 3).

FS group was not recorded during September, November, January and February, while the highest values were detected during March (4.1±0.19 cuf/100 ml) as seen in Table 3.

Microbiological parameters of sediment: Table 4 shows TVBC and yeast and mould in pond sediment. The minimum bacterial count was recorded during November (4.113±0.035 cfu/g), while the highest one was observed in July (10.53±0.339 cfu/g) with significant correlation (P<0.05) with water TVBC (P<0.05). The yeast and mould count in sediment were significantly correlated with temperature (P<0.05), their count were not detectable in

Table 5: Mean values±SE for MPN of TC,FC and FS(cfu/100g) in pond sediment

Parameters Month	TC	FC	FS
June	4.28±0.092	3.18±0.0	3.8±0.2
July	5.68±0.5	3.28±0.02	4.1±0.02
August	5.78±0.132	4.45±0.15	4.59±0.252
September	5.68±0.5	4.1±0.5	4.83±0.058
October	4.98±0.38	3.54±0.54	3.3±0.0
November	3.43±0.173	ND	3.08±0.072
December	4.46±0.1	ND	0.763±0.143
January	3.71±0.61	ND	ND
February	3.37±0.019	2.11±0.017	3.130±0.175
March	3.29±0.16	2.49±0.021	2.89±0.29
April	3.71±0.61	2.95±0.153	318±0.205
May	4.86±0.33	2.83±0.31	4.63±0.506

Mean value±standard deviation of three replicates, ND: Not Detected SE: Standard Error

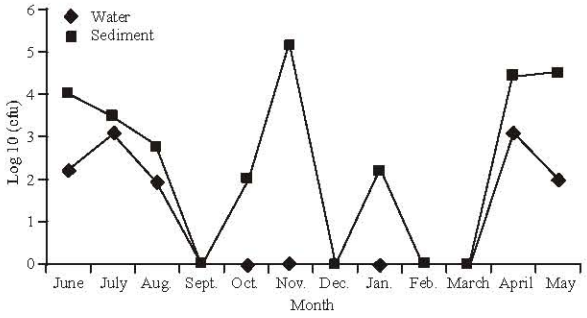

Fig. 1: Mean values±SE for *S/S* in the water and sediment

both January and February while maximum value and minimum value were recorded in July (7.29±0.25 cfu/g) and March (2.83±0.05 cfu/g), respectively.

MPN for TC, FC and FS are represented in Table 5. TC densities in sediment varied from 3.29±0.16 to 5.78±0.132 cfu/100 g with significant correlation with temperature (P<0.05).The highest counts of FC were recorded in August (4.45±0.15 cfu/100g), but it was not detectable during November, December and January. FS were not detectable during January and ranged from 0.763±0.143 cfu/100g to 4.83±0.058 cfu/100g, with significant correlation with temperature and water TVBC (P<0.05).

Figure 1 represented the total count of *S/S* in pond water and sediment. In water, it was absent during samples collection except April, May, June, July and August with value ranged from 1.93±0.058 to 3.1±0.00 cfu/ml. In the sediment, it was not different as comparing with that recorded in the water. In addition to the above months it was also recorded in October, November and January, where it ranged from 2.0±0.18 to 4.53±0.31 cfu/g.

Table 6: Bacterial composition and percentage distribution in the collected samples

Bacteria	Water	Sediment
Bacillus sp.	13	11
E. coli	18	22
Streptococcius faecils	17	20
Pseudomonas aerugenosa	6	6
P. flurescens	8	4
Staphylococcus aerus	10	11
Salmonella sp.	4	6
Shigella sp.	3	5

Bacterial distribution: Occurrence of some bacterial isolates from pond water and sediment were purified and identified up to species level. The data represented the identification of the bacterial flora in tested samples were recorded in Table 6. A wide distribution of bacterial communities and spices were obvious, while Gram negative bacteria represented the most distributed one. Seven genera were identified, where the most common bacteria were *E. coli* and *Streptococcus faecalis* and *Bacillus* sp., in all tested samples, while *Pseudomonas* sp., *Staphylococcus aerus*, *Salmonella* sp. and *Shigella* sp. were detected in a few numbers.

DISCUSSION

As far as it is known, this is the first record for monitoring the microbial flora in El-Qanater El-Khairia Fish Farm. Microbiological quality were controlled by certain parameters like density in terms of total viable bacterial count (TVBC) as well as coliforms in addition to the use of FS as a parameter for Judgment of water pollution[4,7].The results revealed that TVBC of pond water and sediment were varied according to both sample type and time of collection, whereas TVBC and yeast and mould counts in the samples showed variations with maximum number during July. This result may be due to the high water temperature and could be attributed to the load of organic matter in pond water resulted from the diet used for fish feeding. Water have temperature around 30°C for most of the year lead to directly proportional of bacterial action and growth to a temperature rise from 21°C to 30°C [13] and because the diet considered as a source of nutrients required for bacterial growth, the pond water becomes an ideal culture medium for the presence of pathogens. Bacterial infection may be important causes of food-born infection and other diseases in humans [14]. Water column alone gives a limited picture of aquatic microbial distribution. The bacterial and yeast and mould counts were higher in sediment than that in the pound water, that may proceed by the sorption of microorganisms to particles as organic matter suspended in the water, which may then sediment out [2]. Bacterial indicator testing is one of the main modes of water quality management in use today. Faecal bacteria are considered to be good indicators of faecal contamination. TC, FC and FS are bacterial indicators that have been used for decade to infer the presence of other potentially harmful pathogens in water [15]. In pond water faecal indicator values were varied with the temperature where the highest values recorded in July this due to the effect of high temperature and the effect of the water source in which the TC, FC and FS values are high during summer season [4]. Also sediment-bound bacteria create a potential for elevated bacterial concentrations in the overlying water, this likely explanation for the erratic variations in faecal indicator organism concentration frequently observed in water quality monitoring [16]. In general TC, FC and FS values in pond sediment were higher than that in water column, this due to that is known, sediment is prolong the survival of enteric bacteria, this may be because sediment may offer a more favorable chemical and biological environment [17,18,20]. Gram-negative bacteria were dominated the generic composition of isolated bacteria from pond culture system. However, Gram positive bacteria were still comprised a noticeable percentage. The most dominant bacteria was *E. coli* and *S. faecalis* that revealed the presence of sewage pollution that associated with *Pseudomonas* sp., *S/S* sp., this may be an indicator for the presence of other harmful pathogenic bacteria [15]. *P. areogenosa*, *S/S* sp. are the most important species in the present study as they are described as potential fish and shellfish pathogens [19].

In conclusion, the results obtained provided basic information for microbial flora in the El-Qanater El-Khairia El-Khairia Research Station Fish Farm.Faecal coliforms represented a potential problem in pond effluent management. Furthermore, attempts should be made to reduce as much as possible the pathogenic bacteria and further research is needed to elucidate the behavior of bacterial contaminants in tilapia fish as well as the pond ecosystem.

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