QUANTITATIVE AND QUALITATIVE STUDIES ON THE BACTERIAL MICROFLORA OF SOME FISH FARMS IN EL-FAYOUM GOVERNORATE, EGYPT

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ABSTRACT

Microbial assessment was carried out monthly during 2003 at four fish farms, in addition to Dayer El-Berka Drain (the source of water for the farms) in El-Fayoum. This study investigated the bacterial indicators of pollution, the bacterial biomass and the bacteria associated with nitrogen transformations in the aquatic ecosystem of the fish farms.

The data showed that the temperature ranged between 16.6 "C and 33.6 °C and the pH values were at the alkaline side (7.43- 8.91). On the other hand, the concentrations of ammonia, nitrite and nitrate were in the range of 0.2- 2.26 mg/l, 2.7- 96 μ g/l and 26.3- 300.3 μ g/l, respectively.

The bacteriological analyses revealed that the variation in the total viable bacterial counts in the farms was directly related to the type of diet used in fish nutrition. The bacterial counts, in the fish farms, varied from 1.8×10^{12} to 40.8×10^{12} cfu /ml and from 1.4×10^{12} to 1510^{12} cfu /ml at 22 °C and 37 °C, respectively. On the other hand, their counts in Dayer El-Berka Drain fluctuated between 2.8×10^{12} and 29×10^{12} cfu /ml and between 4×10^{12} and 30×10^{12} cfu /ml at 22 °C and 37°C, respectively. Whereas the bacterio-plankton biomass ranged between 496×10^3 and 1160×10^3 mg C m⁻³. The present data showed that the bacterial flora was markedly affected by water temperature. Bacteriological examination showed that the highest counts of nitrogen cycle bacteria recorded in Dayer El-Berka Drain was high compared with the fish ponds.

With regard to the bacterial pollution, the results revealed that the numbers of faecal indicator bacteria, in Dayer El-Berka Drain exceeded the Egyptian standards (1996) and the European Commission (EC) guide standards (1998). Thus, for public health concern, the drainage water should be treated before being used in aquaculture.

INTRODUCTION

Attention has been turned in recent years towards fish farming to increase fish yield. Public perception of the impact of aquaculture on the environment is increasingly placing pressure on the producers. Environmental protection policies are seeking assurances. as regards not only the quality of fish as a product but also the quality of the aquaculture ecosystem.

Exploitation of fisheries resources in Egypt. as well as elsewhere in Africa, has been carried out in absence of adequate ecological knowledge of the fish feed organisms (Mavuti, 1990).

Heterotrophic bacterio-plankton are not only responsible for the degradation of organic matter and recycling of nutrients but are also important producers of particulate organic matter (Azam *et al.*, 1983). Presently, the ultimate role of bacteria in the food chain is an issue at the forefront of aquatic ecology (Azam *et al.*, 1983; Sherr and Albright, 1987). Sometimes bacterial bimass attains higher annual value than the production of phytoplankton (Simon *et al.*, 1992). Data on the multiplication of bacteria and bacterial biomass production in fertilizer- treated fish ponds indicate a more or less significant growth of phytoplankton biomass and a manifold increase in bacterial biomass which shows a high fish production (Shroeder, 1978). However, the significance of bacteria as feed source for metazoans depends on the rate of transfer of bacterial biomass in the heterotrophic food web and the number of steps involved (Winker *et al.*, 1990).

There is appreciation now for the role of bacteria in the microbial loop (Kirchman, 2000) which emphasizes the role of bacteria in the trophic transfer through a micrograzer (protozoa) food chain resulting in transfer of carbon, nitrogen and other nutrients into the food web (Zehr and Word, 2002).

The planktonic heterotrophic bacteria utilize more than half the organic carbon produced in aquatic system. Because they are the only population control the movement of the major part of organic matter and capable of significantly altering both dissolved organic matter (DOM) and particulate organic matter (POM), their recycling processes affect composition and interactions in planktonic system (Overbeck and Chrost, 1990).

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Faecal indicator bacteria, coliforms and faecal streptococci, were enumerated for health reasons. If these indicator bacteria are present, there is a probability that pathogenic microorganisms (bacteria, viruses and protozoa) excreted in the faeces are also present and that water can transmit waterborne infectious diseases. Faecal bacteria are considered to be a good indicator of faecal contamination as they are present in the faeces of human beings and warm-blooded animals (Noble *et al.* 2004). Thereby, indicator bacteria have traditionally been monitored to control the spread of these diseases (Haile *et al.*, 1999).

Several studies were carried out on aquatic environments to monitor the microbial pollution using coliform bacteria and faecal streptococci as indicators for faecal pollution (El-Hawwary *et al.*, 1992; Sabae ,1999 & 2000; Rabeh, 2000; Noble *et al.*, 2004).

The primary role of bacteria in the nitrogen cycle is probably the release of inorganic nitrogen (NH_4) during the decomposition of organic matter, thereby recycling N and other nutrients to phytoplankton.

Biological N₂- fixation is catalysed by nitrogen-fixing bacteria (*Azotobacter* and *Clostridium*). Several authors have studied the ecology of *Azotobacter* and *Clostridium* in aquatic environment. El-Samra (1983) studied their distribution in different fish ponds. Moreover. Sabae and Abdel- Satar (2001) studied the ecology of *Azotobacter* and *Clostridium* in El-Salam Canal and Rabeh (2001) in Lake Manzalah.

Nitrogen removal is partially achieved by aerobic nitrification, which is performed by two groups of gram- negative, aerobic bacteria and anaerobic denitrification, in which ammonium is oxidised directly to N_2 (Kuai and Verstraete, 1998).

The primary concern of fish culture system for high density fish culture operation is the toxic effect of ammonia on fish culture. To control and maintain safe ammonia level in this fish culture systems, biological filters have been designed to promote the growth of ammonia- and nitrite- oxidising bacteria (nitrifying bacteria).

Although considerable studies were carried out on the nitrogen cycle bacteria in aquatic environment (Abu-Sedra. 1990;Sabae.1996; Sabae and Abdel Satar, 2001; Rabeh, 2001). little is known about their role in aquaculture.

Some studies have been done on the food chain in the fish farms. Toulaibah (1992) evaluated the impacts of inorganic fertilization on phytoplankton community and fish production in El-Serw fish farm. Shehata *et al.* (1994) performed two experiments in El-Qanater fish farm to determine the optimum fertilization doses (urea and superphosphate) which increase the phytoplankton and zooplankton populations. Moreover, Mageed and Konsowa (2002) studied the relation between phytoplankton . zooplankton and fish production in freshwater fish farm.

The aim of the present investigation was to study the microbial quality of some fish farms in El- Fayoum and to evaluate the bacterial biomass and its role in productivity of the farm and to determine the variations of nitrogen cycle bacteria in their ecosystem.

The field of study:

Several fish farms are distributed around Lake Qarun in El-Faiyum Governorate which depend on agricultural drainage water. Most of them are simple and primitive with wide variation of management. Some of these fish ponds were used for rearing tilapias, while others were used to produce mullets. Four fish ponds were included in the present study:

- El-Shoura fish pond with an area about 4200 m², with 750 kg mullet and 1000 kg tilapias fish production.
- Gouda fish pond 1 with an area about 4200 m², with unknown fish production.
- Gouda fish pond 2 has an area 29400 m², with unknown fish production.
- Shalakany fish pond has an area about 8400 m², with 2000 kg of tilapias production.

MATERIAL AND METHODS

Sample collection:

Water samples were collected monthly from the four fish ponds. in addition to Dayer El-Berka Drain (the source of water) in El-Faiyum, during 2003. These ponds are located at the eastern bank of Lake Qarun. The water samples were taken in 500 ml sterilized glass bottles and transferred in an ice-box to the laboratory to be analysed.

1-Physicochemical parameters:

Water temperature $({}^{O}C)$

The temperature values of water were measured with standard thermometer immediately after collection.

Hydrogen ion concentration (pH)

The pH values of water were measured using pH-meter model Jenway 3150 UK.

Other chemical parameters including dissolved oxygen, ammonia, nitrite and nitrate were determined (by Dr. Mohammed H. Ali) at the same time of sampling, according to the method described in APHA (1992).

11-Bacteriological parameters:

Enumeration of bacterial populations:

Bacteriological analyses' were carried out monthly to determine the total viable bacterial counts (TVBCs) at 22^oC and 37^oC as well as the most probable number (MPN) of faecal indicative bacteria (total and faecal coliform bacteria and faecal streptococci) (APHA, 1992). Also, the MPN of nitrogen cycle bacteria were determined (aerobic nitrogen- fixing *Azotobacter*. anaerobic nitrogen-fixing *Clostridium*. nitrifying and denitrifying bacteria) (Sabae, 1993). *Media and temperatures:*

- I. Plate count agar medium, was used for detecting the total viable bacterial counts at 22°C and 37°C for 48 hr and 24 hr, respectively.
- II. MacConkey broth, was used to determine the total (TC) and faecal coliforms (FC) at 37^oC for 48 hr and 44.5^oC for 24 hr, respectively.
- III. Azide dextrose broth, was used for estimation of MPN of faecal streptococci at 37°C for 48 hr.
- IV. Modified Ashbys' medium, was used for determination of Azotobacter at 25-30 °C for 2 weeks.
- V. Modified Winogradskey's medium, was used to determine the MPN of *Clostridia* at 25- 30 °C for 3 weeks (the samples were pasteurised at 80 °C for 15 min. before inoculation).
- VI. Stephenson ammonium sulphate with CaCO₃, was used to determine the MPN of nitrifying bacteria at 25-30 0C for 3 weeks.
- VII. Nitrate-peptone medium. was used to determine the MPN of denitrifying bacteria at 25-30 °C for 2 weeks.

RESULTS AND DISCUSSION

The ranges and means of physico-chemical parameters of the water are shown in Table (1). The data showed that the maximum temperature value $(35.1^{\circ}C)$ was recorded during July, while the minimum one $(14.2^{\circ}C)$ was detected in January.

As shown in Table (1), the pH values of the water in the fish ponds ranged between 7.43 and 8.91, whereas its values in Dayer El-Berka Drain varied from 7.28 to 8.66. As a whole, the pH values were at the alkaline side, which represents favourable conditions for bacterial growth.

The present data showed that pH values in the Drain waters were lower than that in the fish ponds. This could be explained by its high turbidity which suppresses the photosynthetic activity of phytoplankton.

On the other hand, flourishing of phytoplankton in the fish ponds led to consumption of CO_2 by the photosynthetic process and consequently high pH values (Ueda *et al.*,2000; Sabae and Abdel-Satar, 2001).

The concentration of ammonia in the fish ponds ranged between 0.2 and 2.26 mg/l, while its values in Dayer El-Berka Drain varied from 0.16 to 2.54 mg/l. The nitrite content in the ponds fluctuated between 2.7 and 96 μ g/l, whereas the nitrate content ranged between 24.9 and 300.3 μ g/l water of the ponds and between 99.7 and 2285.1 μ g/l water in Dayer El-Berka Drain. On the other hand, the oxygen content of the water in the ponds were in the range of 3.2-12 mg/l and 4-11.2 mg/l in the Drain waters.

A complementary challenge in microbial ecology is to understand the functional role of defined bacterial populations in natural ecosystems (Ducklow, 2000).

Figure (1) shows variations in the total viable bacterial counts (TVBCs) at 22^oC and 37^oC. Their numbers in El-Shoura fish farm ranged between 2.2×10^{12} and 40.8×10^{12} cfu/ ml water at 22^oC and between 1.9×10^{12} and 18×10^{12} cfu/ ml water at 37^oC.On the other hand, the bacterial numbers in Gouda fish farms varied from 1.8×10^{12} to 17.8×10^{12} cfu /ml water and from 1.7×10^{12} to 15×10^{12} cfu /ml water at 22° C and 37^oC. respectively.

The bacterial counts in shalaqany fish farm were in the range of $2 \times 10^{12} - 19 \times 10^{12}$ cfu /ml water at 22° C and $1.6 \times 10^{12} - 15 \times 10^{12}$ cfu /ml water at 37° C.

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The bacterial counts in EI- Shoura farm recorded higher numbers than in other farms $(2.2 \times 10^{42} - 40.8 \times 10^{42} \text{ efu})$ ml at 22°C and $1.9 \times 10^{42} - 18 \times 10^{42}$ efu (ml water 37°C). This might be attributed to the effect of animal manure used for fish nutrition, which create favourable condition for the growth of bacteria (Gast and Gocke, 1988).

On the other hand, the bacterial count in Dayer El-Berka Drain were in the range of $2.8 \times 10^{12} - 29 \times 10^{12}$ cfu/ml water at 22° C and $4 \times 10^{12} - 30 \times 10^{12}$ cfu/ml water at 37° C.

The results showed that the maximum bacterial counts were recorded during warm months (June and July). This might be attributed to the effect of high temperature which helps the bacterial growth and production. (Gocke *et al.*, 1990; Sabae, 1999).

The bacterial cell volume of individual varied between less than 0.005 to more than $5\mu m^3$. Therefore, the bacteria in the water sample might differ in their volumes by the ratio of 1: 1000 (Zimmerman, 1977).

Examination by transmission microscopy revealed that the bacteria were bacilli. The overall average volume of the examined bacterial cells in the studied fish ponds was 0.069 μ m³. Niewlak and Sinica (1981) adopted an average cell volume of 0.35 μ m³ in four fertilized polish lakes.

The bacterial biomass production much better characterises the trophic value of water bodies than does the primary production, since the production of bacterial biomass increases with their fertility (Nagata, 1988).

Figure (2) illustrates the bacterioplankton biomass in the studied fish ponds which ranged between 496 $\times 10^3$ mg C m⁻³ and 11260 $\times 10^3$ mg C m⁻³.

On the other hand, their biomass in Dayer El- Berka Drain ranged varied from 1214×10^3 mg C m⁻³ to 8004×10^3 mg C m⁻³. The minimum bacterial biomass were recorded during October and January, while the maximum one was detected in June and July. This pattern was in harmony with the count of bacteria. The high bacterial biomass in the fish ponds might be due to the high content of nitrogen and organic matter, added as diet for fish. This finding is in agreement with that of Verde (1996) and Rabeh (2003).

The interpretation of the results of the bacterial indicators of faecal pollution detected during this study was made according to European Commission (EC) guide standards (1998) and the Egyptian guide srandards (1996) Both accept the guide values of the investigated bacteria at 500 cfu /100 ml of water for total coliforms and 100 cfu /100 ml for each of faecal coliforms ($E. \ coli$) and faecal streptococci.

The most probable number (MPN) of total coliforms (TC), faecal coliforms (FC) and faecal streptococci (FS) are shown in Fig. (3). In El- Shoura fish farm, the MPN of faecal indicative bacteria were in the range of 29 - 1100, 10 - 290 and 150-1600 cells/ 100ml water for TC, FC and FS, respectively. whereas, their densities in Gouda fish farms (1&2) were in the range 0 - 1100, 0 - 290 and 0 - 1600 cells/ 100ml water.

However, in Shalakany fish pond, the MPN of total coliforms. faecal coliforms and faeacal streptococci were in the range of 0-240, 0-120 and 0 - 1100 cells/ 100ml water. respectively. moreover, coliform bacteria and faecal streptococci were not detected in Gouda and Shalakany fish farms during January. This might be due to the effect of low temperature in addition to the antagonistic effect of some microorganisms (Burlingame *et al.*, 1984; Kheiralla *et al.*, 1995).

The results showed that the most probable number of faecal indicative bacteria were higher in El-Shoura than Shalakany and Gouda fish ponds. This is explained by the effect of animal manures used in fertilization (personal communication). Animal manures may serve as direct source of feed for fish or decompose by microorganisms to inorganic nutrients (Boyd and Lichtkopper, 1979).

On the other hand, the MPN of total coliforms and faecal coliforms, in Dayer El-Berka Drain, ranged between 6×10^3 and 16×10^4 cells/100ml and between 3×10^3 and 9×10^4 cells/100ml, respectively., whereas faecal streptococci varied from 40×10^2 to 16×10^4 cells/100 ml.

The present results revealed that the number of faecal indicator bacteria exceeded the Egyptian and European guide standards in Dayer El-Berka Drain. Moreover, their numbers exceeded the guide limits during June in fish farms.

It is well known that coliform bacteria and faecal streptococci recorded their maximum numbers during warmer months. These results indicated that their growth was associated with the temperature, which agree with results reported by Lechevallier *et al.* (1991).

The data showed that the total bacterial counts and the numbers of faecal indicator bacteria were much higher in Dayer El- Berka Drain than in the fish farms. This might be explained by:

(i) the grazing effect of zooplankton on the bacteria, whereby the zooplankton populations recorded greater numbers in the farms (1054728 organism/ m^3 water of fish farm, 35091 organism/ m^3 water of Dayer El-Berka drain) (personal communication). This agrees with Fenchel (1989) and Tranvik (1989) who concluded that the major sink for bacteria is through grazing by zooplankton.

(ii) the point source of pollution to which the Drain is subjected.

Nitrogen-cycle bacteria:

Figure (4) shows the variations in the counts of non-symbiotic nitrogen-fixing bacteria (*Azotobacter* and *Clostridium*). In the fish farms, the maximum and minimum counts of aerobic nitrogen-fixing *Azotobacter* were recorded during October and January, respectively. Their numbers ranged between 4 and 18×10^2 cells/ml water. The highest numbers of *Azotobacter* were detected in Dayer El- Berka Drain, ranging between 35 and 46 × 10² cells/ml. This might be explained by:

(i) the availability of nutrients required for Azotobacter growth.

(ii)the drainage water may be the source of these bacteria. These obervations supported the idea that soil bacteria are dominant in agricultural drainage water (Rabeh, 2001).

The most probable number of *Clostridium* fluctuated between 0 and 19 cells/ ml. These results revealed that clostridial spores were not detected during December and January, whereas their maximum counts were recorded in August.

In accordance with Sabae (1996), the results show that the numbers of *Clostridia* were low compared to *Azotobacter*. This could be explained by the fact that *Clostridia* are strictly anaerobic.being unable to survive in the oxygenated water of the fish ponds ($3.5-16 \text{ mgO}_2/l$).

Monthly variations of nitrifying and denitrifying bacteria are shown in Fig. (5). The MPN of nitrifying bacteria fluctuated between 0 and 120 cells / ml. The present data show that the maximum number of nitrifying bacteria was recorded during October. which may be attributed to its mesophilic nature. Moreover, the monthly variations of nitrifying bacteria and zooplankton illustrated the inverse relationship as a result of grazing of zooplankton on bacteria (El-Sherif *et al.*, 1993). Similar observations were recorded by Gophen *et al.* (1979) and Laventyev *et al.* (1997).

The data showed that, the counts of nitrifying bacteria in Dayer El-Berka Drain were higher than their values in the fish ponds. This might be attributed to:

(i) the availability of ammonia which was used as substrate for nitrification.

(ii) the high number of nitrifying bacteria in the agricultural drainage water. These observations coincide with those reported by Sabae and Abdel-Satar (2001).

The tight coupling between nitrification and denitrification has been previously recorded (Jenkins and Kemp. 1984: Seitzinger. 1988 and Ahlgven *et al.* 1994).

The present data showed that the counts of denitrifying bacteria ranged between 0 and 39 cells/ml water of fish farm. Their numbers were much lower than that of nitrifying bacteria, which might be explained by the anaerobic nature of denitrifying bacteria that are unable to survive in oxygenated water (the oxygen content of the water was in the range of 3.5 - 12 mg/ l).

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Stall ar	W. temp ⁰ C	np ⁰ C	Нd	Ŧ	DO mg/l	mg/l	NH ₃ mg/l	mg/l	NO ₂ μg/l	µg/l	NO ₃ µg/l	۱g/I
"Tions Lables	Range	Mean	Rango	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
El-Shoura	16.6 - 32.6	25.08	7.78 - 8.77	8,52	4,8 - 9,6	7.38	0.31 - 1.42	0.71	2.7 - 53.3	18.40	28.3 - 206.3	22.41
Gouda_1	16.0 - 31.8	25.01	8.13 - 8.91	8.65	5.6 - 11.2	8.30	0.2 - 1.46	0.56	12,9 - 96.0	18,80	26.3 - 300.3	30.73
Gouda_2	17 1 - 32.7	24,53	8.02 - 8.78	8,54	3.20 - 11.2	7.62	0.2 - 2.26	0.69	4.2 - 66.3	20,00	31.1 - 267.4	29.10
Shalakani	17.5 - 34.7	25.93	7.43 - 8,87	8,40	6,4 - 12,0	8,72	0.21 - 1,12	0,42	4.2 - 49.5	22.20	24.9 - 244.9	25.50
Dayer El-Berka	14.2 - 35.1	24.96	7.28 - 8.66	8.13	4.0 - 11.2	8.15	0.16 - 2.54	1.04	13.3 - 257.2	18.60	99,7 - 2285,1	14.16

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