EGYPTIAN JOURNAL OF AQUATIC RESEARCH ISSN 1110-0354
VOL. 31., 1. 2005

MICROBIAL ASSESSMENT OF EL- MAX FISH FARM

GEHAN M. ABO-ELELA, NERMEEN A. ELSERSY*, ELSHENAWY M., ABDEL- MAWLA, E.

National Institute of Oceanography and Fisheries, Microbiology laboratory, Kyed Bay, Anfoushy, Alexandria, Egypt.

Keywords: Vibriosis- Fish Farm - E.coli- Streptococcus faecalis

ABSTRACT

El-Max fish farm is one of the most important natural fish farm in Alexandria which is suffering from die-off of large amounts of fish. Facing this problem we must deal with the estimation of the quality of the farm raring unit environment (ponds). Analysis of samples using the Bray - Curtis similarity coefficient test yielded that, the input and El-Khandac were similar at (97%), while the eight ponds were classified at different level of similarity as follows: pond1 (B1) and pond7 (B7) were similar at (86%), B2 and B3 were similar at (90%), B14, B10 and B9 were similar at (93%), where as B11 is singly separated at (83%). Moreover, results indicate that distribution of the different bacterial groups was affected with one or more chemical parameters such as ammonia, nitrates, phosphates and alkalinity. Since the similarity between ponds was high, we can consider them all as a unique environment from the physicochemical and microbiological point of view.

INTRODUCTION

The demand for fish is expanding rapidly through out the world because of increasing population and income. Fish is also an important component of human food and animal feed too (Abdel-latif, 1996). In Egypt the need for rapid development and proper management of fishery is becoming a necessity in view of the high demand for fish as a relatively cheap source of animal protein. Fish culture in Egypt, embodies inland and coastal water. Inland fish culture embodies fresh and brackish water fish farming (Hamza, 1996).

The distribution and size of fish populations are largely determined by their interaction with other organisms and their environment (Krebs, 1994; Krebs, 2001). A population can grow by increasing its immigration or birth rate and can decrease by increasing its emigration or death rate (Scott and Smith, 1994).

El-Max fish farm is a highly economic raring farm. Many investigators have studied the effect of inorganic and organic fertilizers on the water quality and growth rates of some fishes (Wahby, 1974; Bishara, 1978 and1979). In addition (El-Banna, 1993) studied the phytoplankton and primary productivity, while (Abdel-latif, 1996) studied the distribution of heterotrophs in the different ponds and identified the isolated species from some fish tissues. The bacterium that causes vibriosis has been shown to survive in sea water for more than a year (Croze, 1981).

Lately, El-Max fish farm has been suffered from die-off of large amounts of fish. In order to face the problem two points must be considered, the first is to estimate the quality of the farm raring unit environment and the second is to treat or control the diseases and faecal pollution spreading via changing the feeding source features.

* Corresponding author

Therefore, the present work aimed to evaluate the water quality of the farm in order to define the first stage of the problem.

MATERIALS AND METHODS

Study area

El- Max natural occurring fish farm (Fig. 1) is located at 15 km west of Alexandria. It is adjacent to the pumping station belonging to the Ministry of water resources. It lies in the west southern part of Lake Maruit. Its total area is about 37 Fadans, divided into 14 ponds (coded B1-B14). The largest is no.14 with an area of about 14 Fadans while areas of the remaining ponds range from 0.75 to 1.25 Fadans. The water depth in the ponds varies from 0.5 to 1.8 m. This farm receives its feeding water from El-Nobaria fresh water mixed with the water drained through different waste production such as the irrigation water, industrial products and others which discharge into El-Umoum drain. Eight ponds were chosen for the work study (1, 2, 3, 7, 9, 10, 11 and 14) in addition to the input of the farm and El-khandac area. Nine research field trips during 2003, have been done to El-Max fish farm to study the microbial population.

Samples collection

The temperature and pH value of the water samples were measured in the field using graduated thermometer and portable digital pH meter respectively.

The salinity, alkalinity and nutrient salts were detected according to the methods described by (Grasshoff,1976). All chemical parameters were collected and analyzed in chemical laboratory of National Institute of Oceanography and Fisheries (NIOF). Samples for the physico-chemical analysis were fixed in position immediately after taking them.

The bacteriological sampling techniques of the International Organization For Standardization ISO5667/1(1980) and ISO 5667/2 (1990 a) were used for water sample collection. Sterile glass sampling bottles with wide-mouth and screw cap-with capacity of 500ml were used for collecting the water samples. Special stainless steel sampling rod was used. The bottles were kept unopened until the moment of collection. After collection the samples were sent to the laboratory, and examined within 2 or 3 hrs of sampling. Two separate samples were collected from the same source at the same time.

Sample analysis

Microbiological parameters

Bacterial analyses were performed by membrane filtration technique according to ISO 9308/1(1990 b) and 7899/2 (1984). Quantities of 0.1,1 and10 ml of each sample were filtered through 0.45µm pore size 47 mm diameter, grid sterile cellulose membrane (Gelman Laboratory) using a sterile glass filtration unit (Millipore, Befrid, UK) and a vacuum pump at a pressure of 65k Pa.

For detection of total coliforms, the membranes were placed onto the surface of endo -Les agar and incubated at 37 C for 24h.The dark red color with golden green metallic sheen colonies on the used selective media were counted. Representative ten colonies were sub cultured onto nutrient agar (37 C for 24h) and the confirmatory tests including gas production (using lactose broth medium), oxidase test and Gram-stain were done.

For detection and counting the thermotolerant coliforms (*E.coli*), the membranes were placed onto the surface of m-FC agar and incubated at 44.5 C+0.5 for 24h .The blue colonies developed were counted and ten colonies were sub cultured on NA (Nutrient Agar) at 37 C for 24h.The confirmatory tests for ten sub cultured isolates including gas production, indole production, oxidase test and Gram stain were performed.

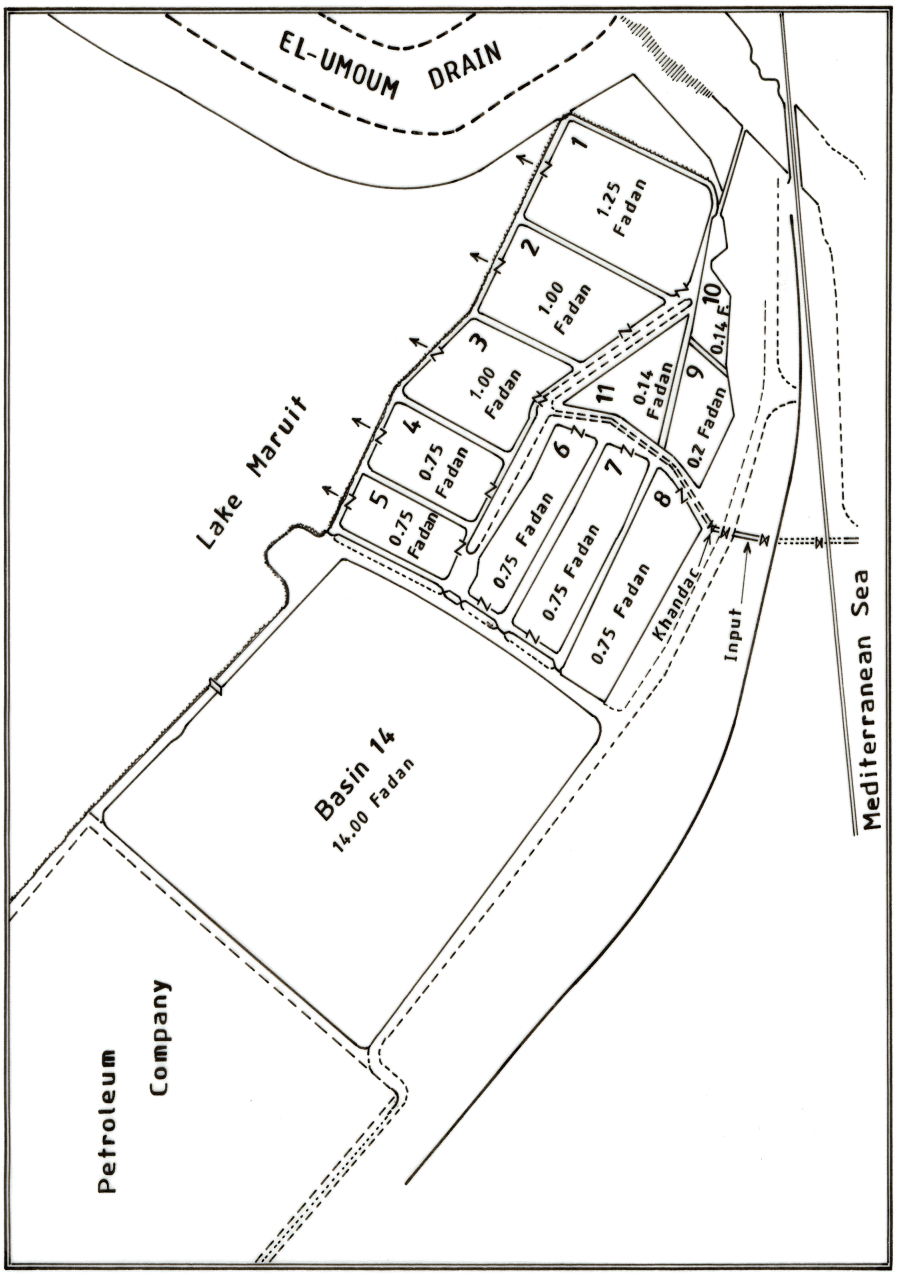

Fig. 1. Map showing the different ponds in the study area (El-Max fish farm).

For investigation and counting of members of *Streptococcus faecalis*, the membranes were placed onto the surface of m-enterococcus agar and incubated at 37 C for 48h. Red, maroon or pink colonies were counted, then sub cultured on Nutrient Agar at 37ºC for 24h. For the ten sub cultured colonies, confirmatory tests including Esculin hydrolysis, catalase test and Gram stain were done. Final counts of all the three faecal pollution indicators were calculated as Colony Forming Unit (CFU/gm).

For detection of *Vibrio spp.* the membranes were placed onto the surface of alkaline peptone agar for 6h at 25 C then carefully transferred onto TCBS (Thiosulphate citrate bile salt sucrose) agar and incubated at 37 C for 24h. Large green and /or yellow colonies were considered to be *Vibrio* spp.

Statistical analysis

The multiple regression analyses were carried out on data to find the relations between the count of the different bacterial groups in the different ponds and the measured environmental variables according to Steel and Torrie (1960)

to identify the most effective environmental variables related to the bacterial count.

For studying the similarity between the ponds depending on the count of the different bacterial groups, the Bray and Curtis similarity test (Bray and Curtis, 1957) was used. Moreover, the % of similarities were plotted into cluster analysis to identify the % of closeness of the different ponds from the biological point of view. This analysis was performed using Statistica -5 statistical package (StatSoft, Inc.2001).

RESULTS

Table 1 shows the annual average concentrations of different measured chemical parameters. The annual temperature range of all sampling sites was from 23.9 ºC to 26.6 ºC, while salinity ranged from 3.87 ‰ to 6.79 ‰. Moreover, pH ranged from 7.1 to 8.9.

The data From Fig. 2 to Fig.6 represent the seasonal average of the different bacterial groups in the different ponds. In general, El-Khandac and Input contained the highest counts of all bacterial groups compared to other tested sites with the except of pond 11. The count of total coliform was higher in summer than in autumn and spring exhibited the highest record for all tested sites compared to other bacterial groups. The data of pond 11 indicates a comparatively high numbers of tested bacteria especially in spring.

The highest numbers of bacteria were detected in the input and El-khandac, the annual averages were 257780 and 48384 CFU/100 ml for total coliforms, 9494 and 35339 CFU/100 ml for *E. coli*, 24170 and 43147 CFU/100 ml for *S. faecalis* and 71810 and 73500 CFU/100 ml for vibrios. While the lowest annual average numbers of total coliforms that was detected in pond 11 and pond 2 (62886 and 25540 CFU/100 ml), also pond 7 harbored the lowest annual average number of *E.coli* (489 CFU/100 ml) followed by pond 14 (5480 CFU/100 ml). pond 1 showed the less annual average number of *S. faecalis* (288 CFU/100 ml), then pond 10 and pond 11 (2650 and 38135 CFU/100 ml). Comparatively with the other ponds, vibrios annual average numbers were low at pond 2 and pond 10 (1177 and 4546).

Stepwise multiple regression models showed the dependence of bacterial counts on the correlative environmental factors as follows:

Total coliforms = 1.52-0.339 alkalinity + 0.061 nitrate (r2=0.2265).

E .coli = -1.36 + 0.107 NH_4 + 0.1013 NO3 - 0.295 PO_4 (r2=0.3260).

S. faecalis=1.89+ 0.27 NH_4 (r2=0.0825).

Vibrio sp. = 9.608-2.027 alkalinity + 0.214 NO3 (r2=0.2665).

While, bacterial counts showed no significant correlation with the physical parameters (Temperature, salinity and pH).

Analysis of the 10 ponds using the Bray-Curtis similarity coefficient test yielded the dendogram shown in Fig. 7. As clearly observed, at 55% similarity level, two main clusters were formed, the first cluster included the input and El-Khandac (similarity level was 97%), while the second cluster included all ponds and were grouped at 78% similarity level. Also B1 and B7 were similar at 86% and B2&B3 were similar at 90%, in addition to that B14, B10 and B9 were similar at 93%, but pond 11 is singly separated at 83%.

Table 1. Annual average concentrations of different chemical parameters (NIOF, 2004)

Station	Ammonia (µg at*./l)	Nitrate (µg at./l)	Phosphate (µg at./l)	Alkalinity (ml eq./l)
Input	91.34	27.69	14.48	5.53
El-Khandac	88.84	34.35	12.67	5.90
B7	4.09	14.40	4.44	4.98
B1	19.32	21.20	2.81	4.41
B11	31.20	20.05	4.04	4.98
B2	23.65	15.10	3.74	4.49
B3	53.16	25.34	10.70	4.97
B9	47.07	22.64	9.90	5.52
B10	49.41	19.58	8.95	5.85
B14	2.99	6.25	4.41	4.66

*at: atom

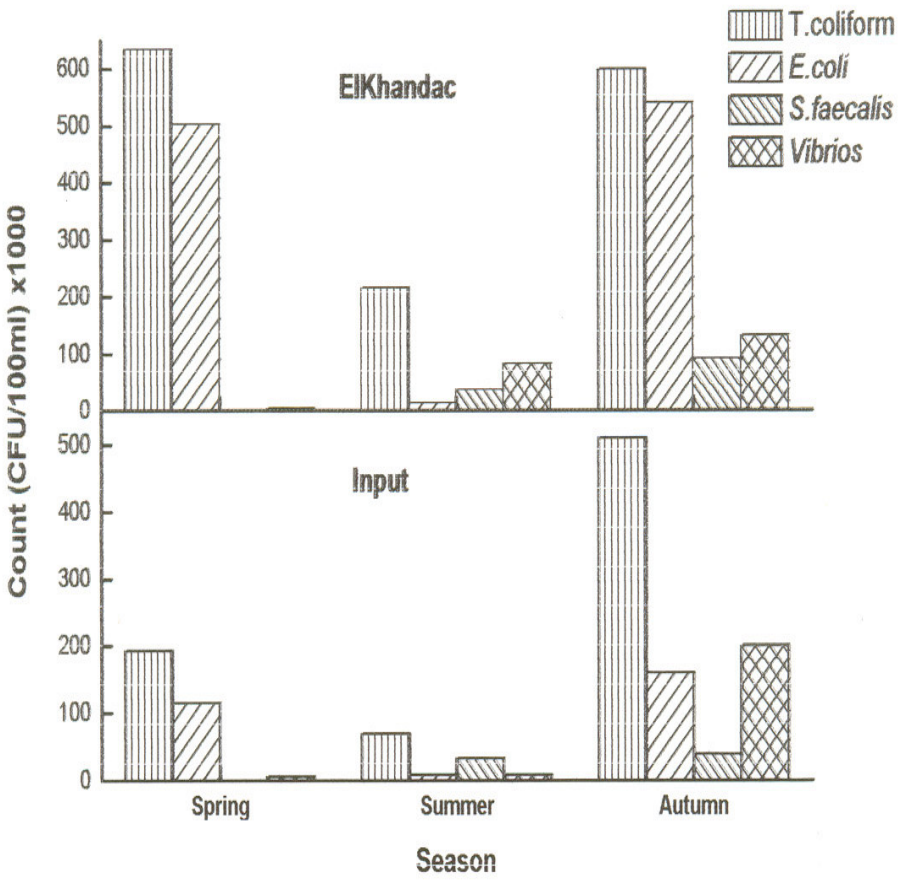

Fig. 2. Seasonal bacterial variation in El-khandac , and input

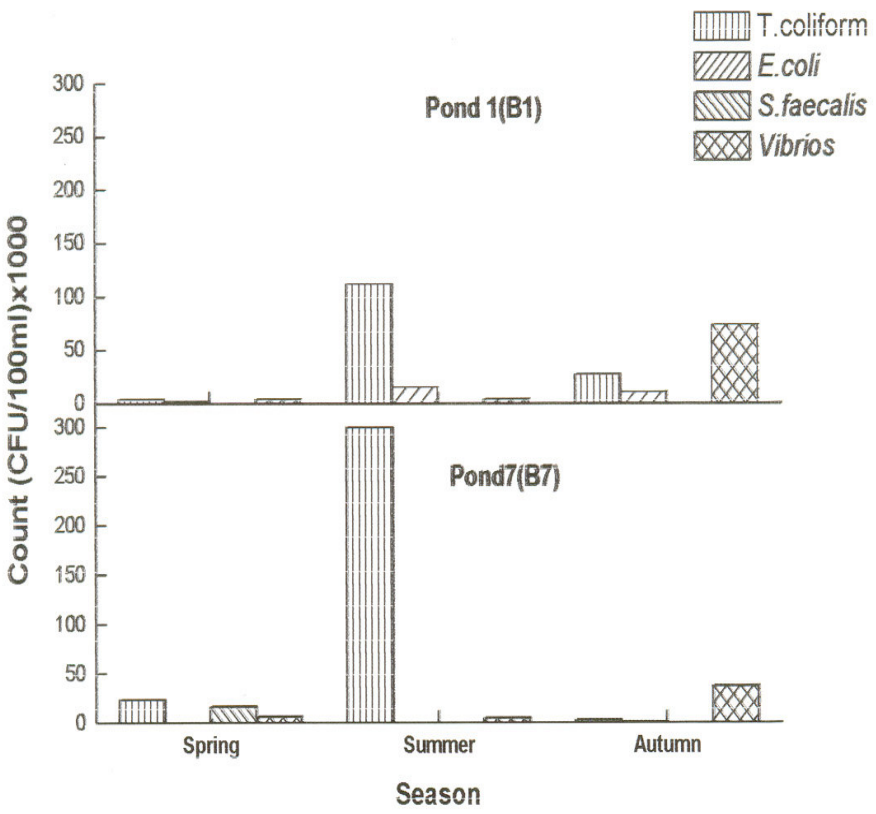

Fig.3. Seasonal variation of different bacterial groups in Pond 1 and Pond 7

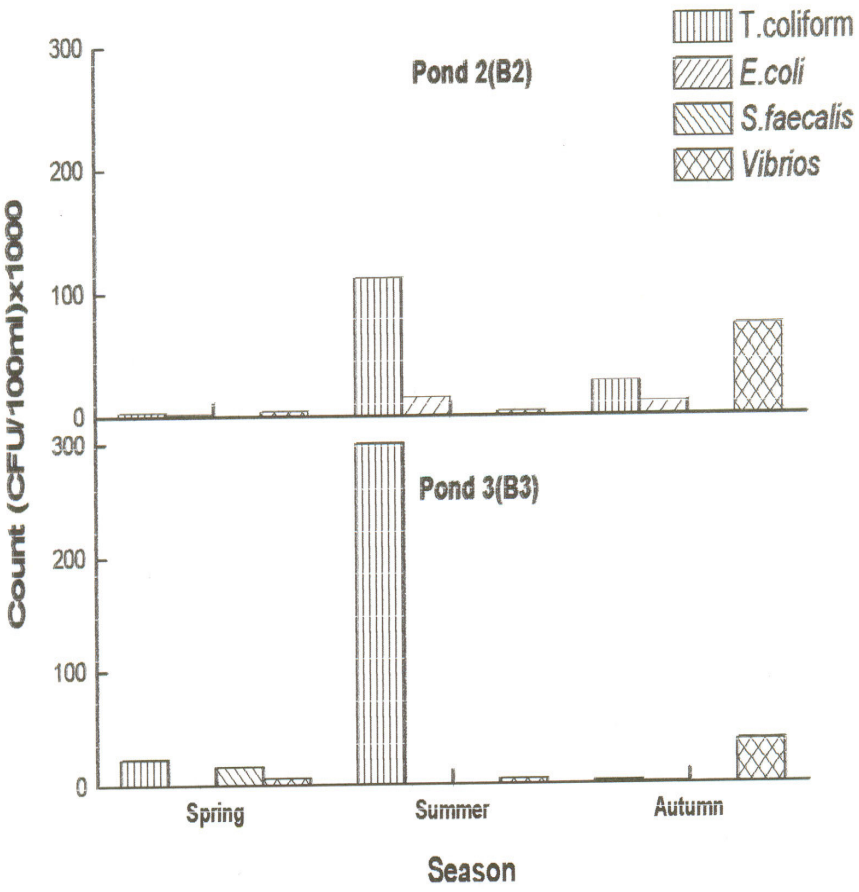

Fig. 4. Seasonal variation of different bacterial groups in Pond 2 and Pond 3

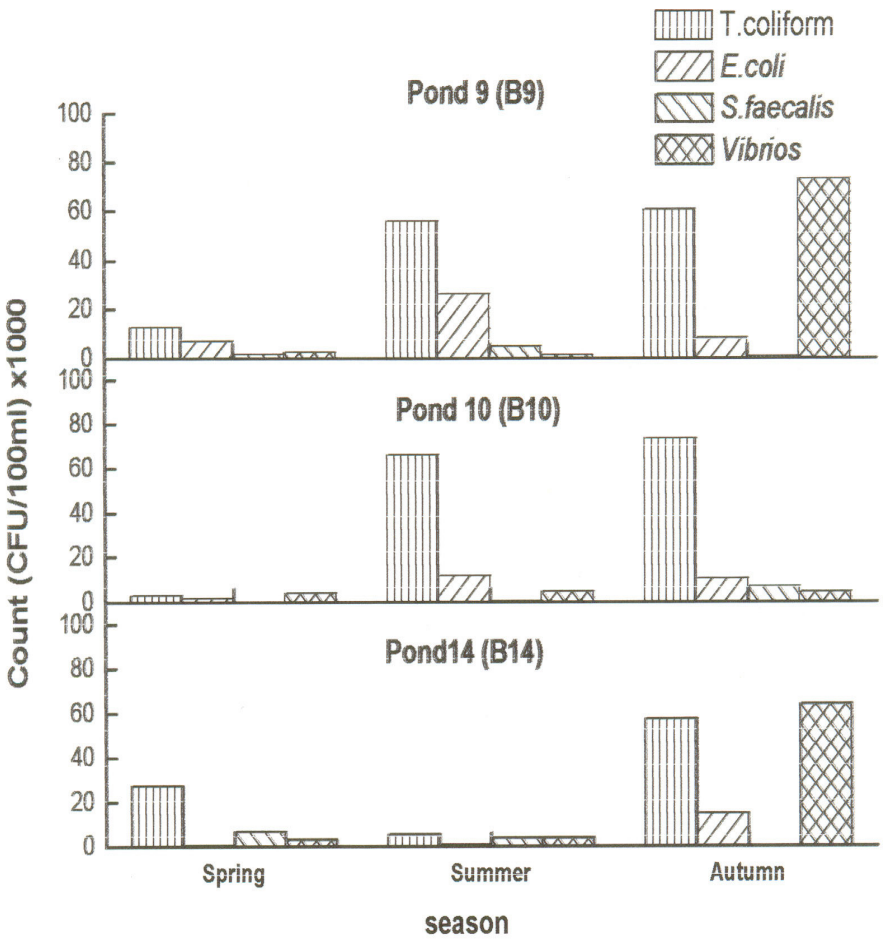

Fig. 5. Seasonal variation of different bacterial groups in Pond 9, Pond 10 and Pond 14

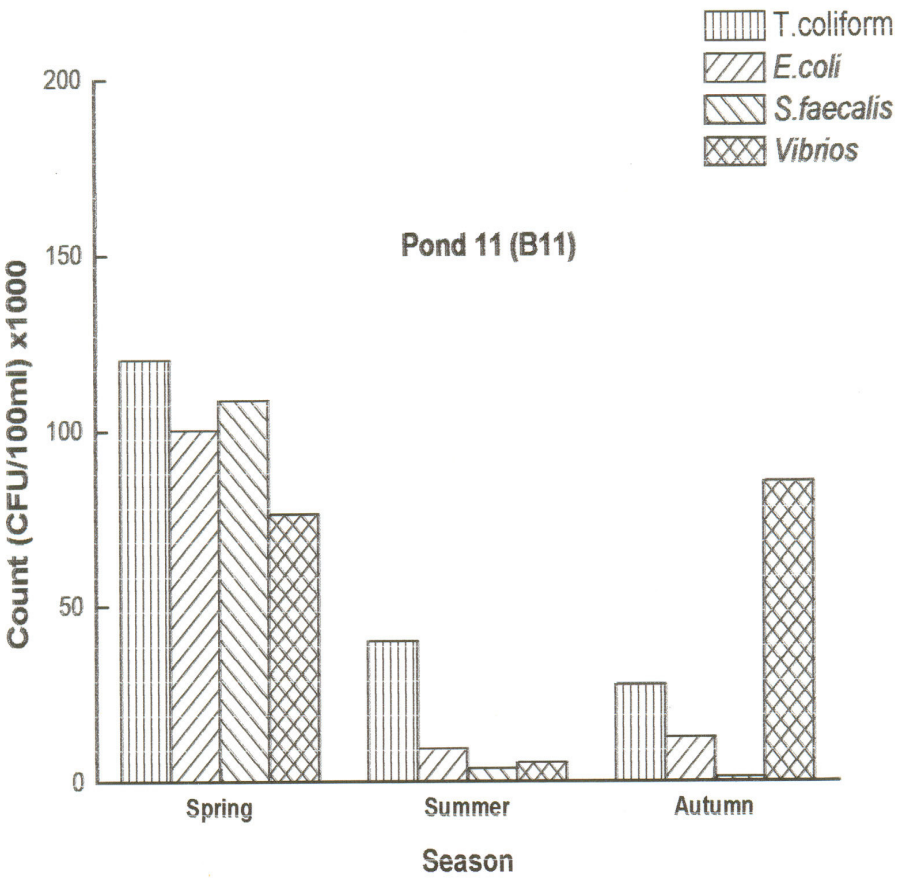

Fig. 6. Seasonal variation of different bacterial groups in pond 11.

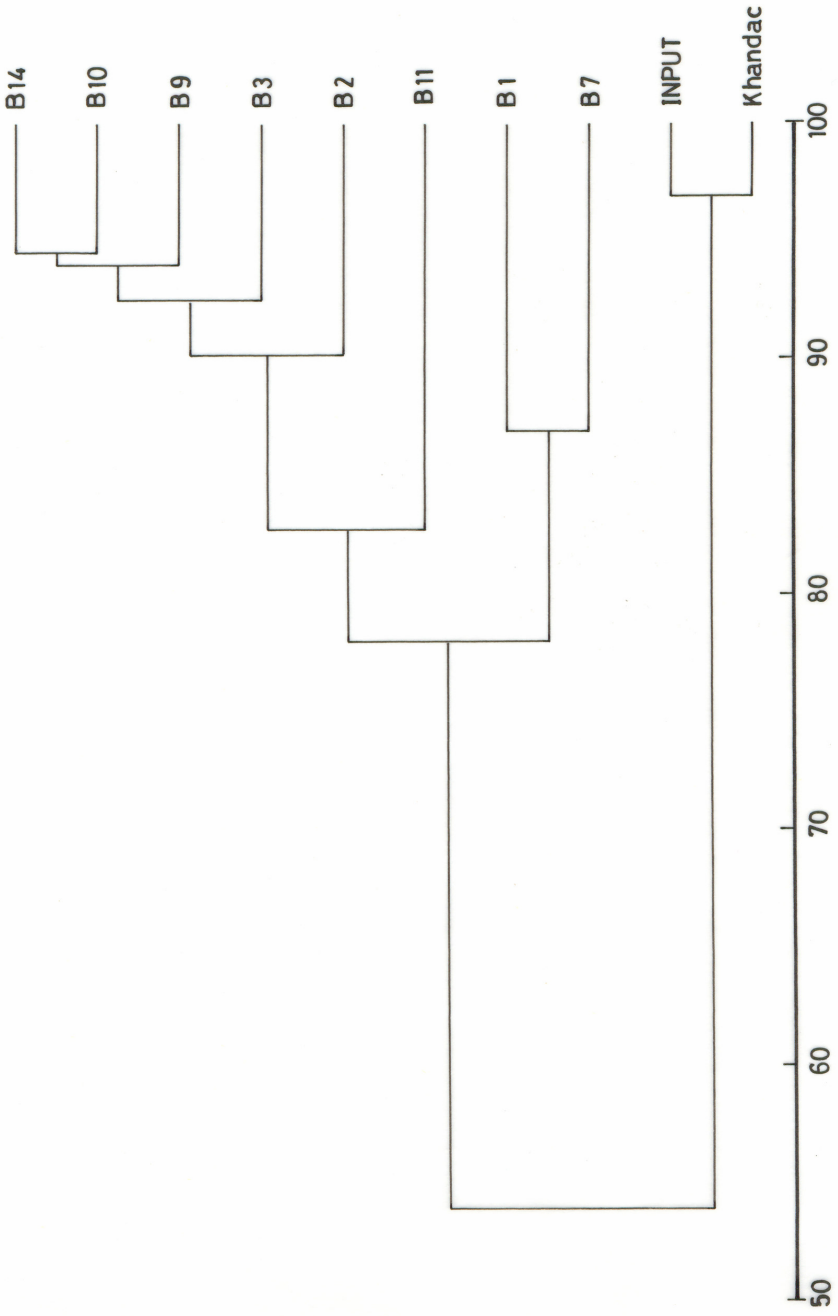

Fig. 7. Dendogram, similarities between ponds using Bray-Curtis similarity coefficient test.

DISCUSSION

Coliform bacteria, faecal coliforms and *E. coli* have, for almost a century, been used as indicators of the bacteriological safety of drinking water (Cliver and Newman, 1987; Marshall, *et al*.1997; Am. Public Health Assoc., 1998). *E.coli*, has been differentiated from total coliforms, as more specific indicators of faecal pollution (Leclerc *et al*, 2001). Generally, *E.coli* strains that colonize the human bowels are harmless commensals. However, within the species there are fully pathogenic strains that cause distinct syndromes of diarrheal disease because they possess virulence factors such as enteroadhession or enterotoxins. The main categories include enteropathogenic *E. coli*, enterotoxigenic *E. coli*, enteroinvasssive *E. coli*, enterohemorrhagic *E. coli*, enteroadherent *E.coli*, and enteroaggregative *E.coli* (Guerrant and Thielman 1995; Leclerc 1996 ;Chart 1998; Law and Chart 1998). In most well-studied waterborne outbreaks, described in Sweden (Bengstsson, *et al*. 1966) the casual organisms belong to the enteropathogenic *E.coli* class.

According to the European Commission (EC) guide standard (1998), which meet with the Egyptian guide standard (Ministry of Health, Egypt,1996), both accept the guide values of the investigated bacteria by 500 CFU/100 ml of water for coliforms, and 100 CFU/100 ml for *E.coli* and *S. faecalis*, so that all sites are considered to be faecally polluted, except pond 2 during spring. Also the presence of vibrios were highly condensed, especially the fish pathogen *V. damsela* which causes the intestinal infections in most fishes. This might be due to the fact that the source of feeding is highly polluted, where the annual averages were 257780, 56090, 24170 and 71810 CFU/100ml for total coliforms, *E.coli*, *S.faecalis* and vibrios respectively.

The highest numbers were detected in the input and El-khandac, where the highest concentrations of ammonia (91.34 and 88.84 NH_4–N µg at/l), nitrates (27.69 and 34.35 NO_3-N µg at./l) and phosphates (14.48 and 12.67 PO_4-p µg at./l). On the contrary ponds 1, 2 and 7 showed the lowest counts where the lowest concentrations of ammonia (19.32, 25.56 and 4.09 NH_4-N µg at./l), phosphate (2.81,3.74 and 4.44 PO_4-p µg at./l) and nitrate (21.20,15.10 and 14.4 NO_3-N µg at./l). Yusef, *et al*. (1995) reported that ammonia was a good indicator of sewage pollution into the flowing water. Another factor must be considered, pond 1 and pond 2 were the farest from Input so that they harbored the lowest counts, while El-khandac was the nearest, so that it contained higher percentages of faecal and pathogenic bacteria.

Moreover, pond 11 was singly separated as a single cluster at 83%, that may be due to its higher salinity (6.79‰).

Since the source of feeding was the same (El-Umoum drain) for all Ponds, the depth of water was also the same in all ponds, ranged from 1.0-1.5 meters, the presence of the same types of fishes (*Tilapia sp., Anguilla sp., Multets sp.*), the same type of sediment in the bottom, the presence of boughazes between the ponds, in addition to that, this farm is naturally occurring i.e. there is no determination to the initial time of cultivation, harvesting, size or age of fishes in it, all these factors led to the high similarity percentages between these ponds.

Abdel-latif, 1996, reported that the annual average of total heterotrophic bacteria in the different ponds did not differ significantly i.e. she agreed with our results, in that concern the similarity level between different ponds, was significantly high.

Since the similarity between ponds was high, we can consider all ponds as a unique environment from the microbiological and physicochemical point of view. Also we must treat the source of feeding chemically and micro-biologically in the second step of this work before entering to the different ponds.

REFERENCES

Abdel-latif, H.H. 1996. Studies on some microorganisms and their relationships with Multets and *Tilapia sp*. fishes reared in El-Max farm ponds. M.Sc. thesis Faculty of Science, Alexandria University, Egypt.

Am. Public health Assoc. 1998. Standard Methods for the Examination of Water and waste water. Washington, DC: American Public Health Association 20th ed.

Bengstsson, S., R.D. Berg, K. M. Danielsson, F. Lundmark, and O. Nordbring, 1966. Outbreak of gastrointestinal illness in Sweden Lakartidningen 63:4499.

Bishara, N.F.1978. Fertilizing fish ponds. 2. Growth of *Mugil Cephalus* in Egypt by pond fertilization and feeding. Aquaculture, 13:361-367.

Bishara, N.F.1979. Fertilizing fish ponds. 3. Growth of *Mugil Capito* in Egypt by pond fertilization and feeding. Aquaculture, 16:47-55.

Bray, J.R. and J.T. Curtis, 1957. An ordination of the upland forest communities of Southern Wisconsin. Ecol. Monogr. 27: 325-349

Chart, H. 1998. Toxigenic. *E. coli*. Journal of Applied Microbiology. Suppl. 84: 775-865.

Cliver, D.O. and R.A. Newman 1987. Development of intestinal microbiota. In Gastrointestinal Microbiology,. Gastrointestinal Microbes and Host interaction Vol.2,. RI Mackie, BA White, RE Isaacson (eds.), New York: Champmam & Hall. 680 pp.

Croze, H. (1981). What ecologists think veterinarians should do. In Wildlife Disease Research and Economic Development. International Development and Research Centre. Ottawa.

Egypt, Guide standard, 1996. Ministry of health.

El-Bana, H.M. 1993. A. study on phytoplankton and primary productivity in El-Max fish farm. M.Sc. thesis, Faculty of Science, Alexandria University, Egypt.

European Commission Guide standard, 1998. Quality of bathing water, 1998: Document EUr 18166, Brussels.

Grasshof, K. 1976. Methods of seawater analysis .p. 317. with contribution by T. Almegreen, R. dawson, M. Ehrbodt, S.G. Fonselius, B. Josefrson, F. Korolef and K. Kremling. Aufl. Wrinheim. New York: Verlage Chemie.

Guerrant, R.L. and N.M. Thielman. 1995. Types of *E. Coli* enteropathogen. In: Infections of the gastrointestinal tract. Ed. MJ Blaser, PD Smith, JT Ravdin, HB Greenberg. 48: 687-691. New York: Raven. 1578 pp.

Hamza A.K. `1996. The aquaculture in Egypt. World aquaculture. 27: p.1

International Organization for Standardization (ISO 5667/1), 1980. Water quality sampling-Part 1: Guidance on the design of sampling programs, 13 pages, Geneva, Switzerland.

International Organization for Standardization (ISO 5667/2), 1990 a. Water quality sampling-Part 2: Guidance on the design of sampling techniques, 9 pages, Geneva, Switzerland.

International Organization for Standardization (ISO 7899/2), 1984. Water quality detection and enumeration of faecal streptococci, Part 2: Method by membrane filtration, 4 pages, Geneva, Switzerland.

International Organization for Standardization (ISO 9308/1), 1990 b. Water quality detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive *Escherichia coli*, Part 1: Membrane filtration method, 10 pages, Geneva, Switzerland.

Krebs, C.J. 1994. Ecology: The Experimental Analysis of Distribution and Abundance. Fourth Edition. Harper Collins College Publishers. New York

Krebs, C. J. 2001. Ecology: The experimental analysis of distribution and abundance 5th

ed. Bejamin Cumming, San Francisco, CA. 695 pp.

Law, D. and H. Chart, 1998. Enteroaggregative *E. Coli*. Journal of Applied Microbiology. 84:685-697.

Leclerc, H. 1996. Bacterial indicators of drinking water: concept and signification through epidermiological and bacteriological evaluation Proc. International Congress of Mineral waters, soft Drinks, 1st , pp. 52-77.

Leclerc, H., D.A.A. Mossel, S.C. Edberg, and C.B. Struijk. 2001. Advances in the bacteriology of the coliform group: Their suitability as markers of microbial water safety. Annual Review Microbiology. 55:201-234

Marshall, M.M., D. Naumovitz, Y. Ortega, and C.R. Sterling, 1997. Water borne protozoan pathogens. Clinical of Microbiological. Review. 10: 67-85

NIOF, 2004: Report on ecological and economic evaluation of El max fish farm. 64 p. National Institute of Oceanography and Fisheries (NIOF), Egypt , Alexandria (edit.).

Scott, M. E. and G. Smith 1994. Parasitic and Infectious Diseases. Academic Press. San Diego.

StatSoft, Inc. 2001. STATISTICA for Windows. Tulsa, OK: StatSoft, Inc., 2300 East 14th Street, Tulsa, OK, 74104-4442, (918) 749-1119, fax: (918) 749-2217, e-mail: info@statsoft.com, WEB: http://www.statsoft.com.

Steel, G. D. and J. H. Torrie. 1960. Principles and Producers of Statistics. Mc Graw-Hill. Book Company, Inc. New York. Toranto-London.

Wahby, S.D. 1974. Fertilizing fish ponds. 1-Chemistry of the waters. Aquaculture, 3: 245-259

Yusef, H. H., K. M., Ghanem, Z. M. El-Sherif and H.Y. El- Kasas. 1995. Coliforms as a measure of sewage pollution of Alexandria and coastal water. Bulletin of High, Institute of Public Healt, 25: 1019-1036.