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COMPARATIVE STUDY BETWEEN DESERT CULTIVATED AND NATURAL FISHERIES OF MULLET FISH IN EGYPT, II- MICROBIOLOGICAL CONCERN

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ABSTRACT

Cultivated mullet fish were compared with the wild mullet fish from four locations during summer and winter 2005/2006. This work was evaluated via examination of three pathogenic microbial genera (*Salmonella, Shigella & E. colli*) in samples of rearing water, aquafeed, sediments and fish. From the results, it could be concluded that there is a pollution with pathogenic bacteria (specially *E. coli*) in all fish feeds, rearing water and sediments of the tested locations (mainly in summer season). Also, there is no difference between fish of natural resources and those of aquaculture concerning bacterial contamination. So, it is a legal must to take considerations from the responsible authorities for treating all kinds of waste waters before reaching water bodies to protect aquatic life and consumers.
Keywords: Mullet –*Salmonella – Shigella, E. colli.*

INTRODUCTION

Fish had long been regarded as a nutritious and highly desirable food due to their contribution of high quality animal protein, richness in calcium and phosphorus and generous supply of vitamins (Abdelhamid, 2003). Microorganisms are present everywhere on earth, water and atmosphere. Fish and shellfish harvested from water polluted with human and animal wastes can contain *Salmonella*, *Shigella* and *Escherchia coli*. It may be assumed that aquatic animals, including fish, are continually bathed in an aqueous suspension of microorganisms and that, consequently, external surfaces will be in frequent contact with these microbes. The initial microbial flora on the caught fish is dependent upon the contamination of the water and bottom sediment from the area of catch. Representatives of family *Enterobacteriaceae* are usually found among the most prevalent bacteria on the fresh water fish (Austin and Austin, 1987). Most of the microorganisms in tissue are thought to result from surface, gills, or intestinal contamination (Austin, 1982). Microorganisms, adsorbed on the surfaces of the fish and that found in their intestinal contents, do not affect the fish during life; but after death, saprophytic and commensally residents invade the flesh and bring about its decomposition, as well as it can induce disease of human consuming such flesh (Ayres *et al.*, 1980). The objective of the present work was to study the bacteriological status of the examined fish muscles, water, sediments and feed stuffs collected during summer and winter seasons from four locations in Egypt (Marsa Matroh – Alexendria – El-Bardaweel and Port Said).

MATERIALS AND METHODS

Sampling Locations:

As mentioned in Abdelhamid *et al.* (2006).

Bacteriological Techniques:

Under complete aseptic condition, 10 grams of the back muscles was aseptically transferred into a sterile homogenizer containing 90 ml of sterile 0.1% peptone water. The contents were homogenized for 2.5 minutes at 14000 r.p.m to provide a homogenate of 1/10 dilution and then allowed stand for about five minutes. The homogenate was transferred into a sterile test tube and 1 ml was transferred into a sterile test tube containing 9 ml of 0.1 peptone water from which ten-fold serial dilutions up to 10^6 were prepared (ICMSF, 1978). Prepared samples were examined for detection and enumeration microorganisms in fish as total bacterial count, colony forming unit (CFU)/g of sample. The pour plate technique recommended by AOAC (1990) was applied. 12 to 15 ml of melted standard plate count agar (cooled to 44 – 46°C) were added to each inoculated plate. Then, thoroughly and uniformly mixed with the sample and left to solidify. After solidification, the plates were incubated in inverted position at 35°C for 48 hours. The number of colonies in selected duplicate plates of the same dilution was enumerated, then the mesophilic count per gram of sample was calculated. Isolation of *Escherichia coli* using Mac- Conkey broth. Typical pink coloured colonies were picked up and purified (Cruickshank *et al.*, 1975). Isolation of *Salmonella* according to Fricker (1987) and Vassiliadis (1983). *Salmonella* produce colonies with black center (Biolife, 1996). Isolation of *Shigella* using selenite – cystine broth (Twedt, 1978). *Shigella* appear as colorless colonies (Biolife, 1996). For microscopical examination (staining), a film was prepared from the pure culture of isolated organisms (*E. coli* – *Salmonella* – *Shigella*), all were stained with Gram stain then examined microscopically to appear the gram negative rods (red rods). Under complete aseptic conditions, 10 ml of water samples were transferred into a sterile glass bottle containing 90 ml of 10% peptone water, from which ten-fold serial dilutions up to 10^6 were prepared. For enumeration of mesophiles (total bacterial count), one ml of the sample as well as of the dilutions were placed in separate petri dishes and 15 ml of liquefied agar medium at a temperature of 43 to 45°C were added to each dish. The numbers of colonies in selected dishes and the mesophilic count per ml of sample was calculated according to APHA (1992). Isolation of *Escherichia coli*, *Salmonella* and *Shigella* followed the same procedures as mentioned with fish samples. 10 grams of sediment and fish feed samples were aseptically transferred into a sterile homogenizer containing 90 ml of sterile 0.1% peptone water. The contents were homogenized for 2.5 minutes at 14000 r.p.m. to provide a homogenate of 1/10 dilution and then allowed stand for about five minutes. The homogenate was transferred into a sterile test tube and 1 ml was transferred into a sterile test tube containing 9 ml of 0.1 peptone water from which ten-fold serial dilution up to 10^6 were prepared (ICMSF, 1978). Prepared samples were

examined for detection and enumeration of microorganisms in samples as mentioned before with fish and water samples.

Statistical Analysis:

Using S.A.S. (2001) and Duncan (1955), numerical data collected were statistically analyzed for analysis of variance and least significant difference. Chi-square, t-test and correlation were calculated too when required.

RESULTS AND DISCUSSION

Total bacterial count (TBC) in fish diets ranged between 6.6 – 8 x 10^4 CFU/g in summer and 8 – 9.5 x 10^4 CFU/g in winter. *E. coli, Shigella* and *Salmonella* were presented in both locations and seasons, but no *Shigella* was found in diet of Port Saied in summer. In rearing water, TBC reached 1 – 3 x 10^5 CFU/ml in summer and 2.8 – 9.5 x 10^4 CFU/ml in winter. The three bacterial genera were found in location No. 2 in summer and locations No. 3 and 4 in winter. *Shigella* presented in all sampling locations, except in Marsa Matroh water during summer and in Alexandria water during winter. *E. coli* was presented in all locations in winter water, but only in Alexandria water in summer. The incidence of these bacteria in water was related to their presence in the diets. The sediments contained TBC as 1.2 – 2.5 x 10^5 CFU/g and 1.5 – 3 x 10^5 CFU/g in summer and winter, respectively. All tested bacterial genera were found in all sediment samples collected from different locations. From Table (1), Marsa Matroh fish contained TBC significantly ($P \leq 0.05$) higher than those of the other locations. The summer TBC was significantly ($P \leq 0.05$) higher than that of winter fish samples. Yet, fish sex had no significant ($P \geq 0.05$) effect on TBC of fish. However, the high TBC of summer fish is related to the high TBC in summer water. From Chi-square test data for distributing the negative and positive samples of fish for different genera of tested bacteria, it is clear that 115 (71.9%) samples of fish were positive for *E. coli*, 54 (33.8%) for *Shigella* and 21 (13.1%) for *Salmonella*. Marsa Matroh fish were the most contaminated (55%) with *Shigella*. Summer fish presented more *E. coli* (73.8%) and *Shigella* (38.8%) positive samples than those of winter fish samples (70.0 & 28.8%, respectively) , whereas *Salmonella* positive fish samples were more in winter (13.8%) than in summer (12.5%). Male fish samples were more positive for *E. coli* (73.3%) and *Salmonella* (16.0%) than the other fish sex (70.6 & 10.6%, respectively). However, these data confirmed the previous mentioned results of the significantly ($P \leq 0.05$) higher TBC in summer than in winter fish samples, although the non significant ($P \geq 0.05$) effect of fish sex on the TBC of fish (Table 1). Yet, female fish contained more (36.5%) *Shigella* positive samples than the males (30.7%). Generally, Chi-sqare test was not significant ($P \geq 0.05$), except for fish *Shigella* in different locations.

To correlate TBC with the other tested parameters, whether of body measurements, heavy metals concentration in fish flesh, or their bioaccumulation factors (BAFs), correlation coefficients were calculated. Significant positive correlations were found between TBC, on one side, and Pb level in fish and fish length, depth and weight, on the other side. T-test data cleared that the *E. coli, Shigella* and *Salmonella* positive samples were

115, 54 and 21, respectively from total of 160 samples. T-values between each of these bacterial genera and the other tested parameters (heavy metals and their BAF as well as body measurements, Abdelhamid *et al.*, 2006) of the tested *M. cephalus* were not significant ($P \geq 0.05$), except between *Shigella*, from one side, and Pb content and TBC, on the other side. Yet, Tate (1995) cited that the diversity of the soil microbial genome suggests that a variety of organisms must exist in soil with augmented resistance to heavy metal pollutants. For example, a variety of soil bacteria have been isolated with augmented resistance to metal contaminants. He added that microbial populations may be inhibited by release of organic-matter-associated cautions through microbial catabolism of the soil's organic matter. The availability of the toxic caution may become the controlling factor in bacterial population development with the impact of those environmental properties normally considered to determine community diversity being minimized.

Table (1): Total bacterial count (means* ± SE) of the tested *M. cephalus* (CFU/g).

Variables	T.B.C.
Locations	
Marsa Matroh	204975^{a} ± 22430.59
Alexandria	132125^{b} ± 8128.39
Port Saied	135900^{b} ± 11132.00
El-Bardaweel Lake	135400^{b} ± 10378.46
Seasons	
Summer season	171075^{a} ± 12320.91
Winter season	133125^{b} ± 7771.79
Sex	
Male	151160^{a} ± 12048.15
Female	152929.412^{a} ± 9113.33

*Means within the same variable superscripted with different letters differ significantly ($P \leq 0.05$).

Nile tilapia fish grew in treated-waste effluents reflected very low total aerobic bacterial count (9.3×10^2 CFU/g) in the edible muscles and complied with WHO guidelines (< 10^5 CFU/g). *Salmonella, Shigella* and *Staphylococcus* were absent (Khalil and Hussein, 1997). Although the general microbial quality differed significantly among the production systems (source of rearing water); yet, human bacterial pathogens like *E. coli* and *Schigella spp.* were isolated from Egyptian naturally infected fishes of freshwater, mainly *Oreochromis spp., Clarias lazera* and common carp (Enany *et al.*, 2004). Egyptian General Authority of Standardization and Quality Control (2000); recommended 10^6 total bacterial count per gram as a maximum permitted limit for fish, and added that fish must be free from *Salmonella* and *Shigellia*. Food fish, whether under cooked, fecally contaminated, or inadequate refrigerated, may be contaminated by bacteria, e.g. *Salmonella, Shigella* and *Escherichia coli* causing foodborne illness, such as salmonellosis, shigellosis and Hamburger disease, respectively. Their toxicity symptoms including abdominal pains, vomiting, fever, diarrhea, chills, and cramps (Work book, 2005).

Eating fish at least once a week is good for the brain, may help slow age-related mental decline by the equivalent of three to four years (10% slower annual decline in thinking), and helps keep the mind sharp. It lowers the risk of Alzheimer's disease and stroke. Fish which are rich in omega-3 fatty acids also have been shown to prevent heart disease (Morris, 2001). Yet, pathogens are parasites which cause diseases (WRC, 2005). Particularly, sea foods may cause acute diarrhea due to *Salmonella* contamination (Harrison's, 2005). So, *Salmonella, Shigella*, and *E. coli*, all capable of inducing gut-wrenching gastroenteritis. But, the incidence of diagnosed infections for pathogens was increased for *E. coli* and *Shigella* but decreased for *Salmonella* in 2000 than before (Colorado, 2001). A recent report (Anon., 2005) showed important declines in food borne infections due to common bacterial pathogens in 2004. From 1996 – 2004, the incidence of *E. coli* infections decreased 42%. *Salmonella* infections dropped 8%. The incidence of *Shigella* did not change significantly. However, 76 million food borne illness, or food poisoning, cases occur in the USA every year (30% are caused by bacteria, e.g. *E. coli*, *Salmonella* and *Shigella*).

Morbidity and mortality cases of food borne pathogens (*Salmonella spp.* and *E. coli*) in the United States reduced between 1987 and 2000, and even estimated to be reduced sharply till 2010 (Wesley *et al.*, 2005). Yet, five commercial systems of freshwater fish culture contained *E. coli*. The presence of such organisms creates a potential for microbiological hazards in these systems. That could indicate the presence of human pathogens that may be hazardous to fish handlers and consumers. That indicates also that monitoring fish culture facilities for microbiological safety should be considered. In addition, workers should be aware of personal hygiene when entering, while working in, and when departing fish culture facilities (Mc Keon *et al.*, 2000). However, all strains of *Shigella* and *Salmonella* are considered pathogen, but only the pathogenic strains of *Escherichia* are associated with foodborne illness (Ray, 2001). Goosney *et al.* (2001) reported that bacterial pathogens can survive and persist to exploit their host's cellular processes to mediate their effects extracellularly or intracellularly. In either case, the pathogen hijacks the host's cytoskeleton, so the pathogen is involved in mediating numerous cellular functions, from cell shape and structure to programmed cell death. The incidence of salmonellosis has generally been declining since 1997 (Ethelberg *et al.*, 2005). Yet, Guerin *et al.* (2004) reported that in February 2001, an increased incidence of infection caused by *S. livingstone* was observed in Norway and Sweden. By July 2001, 44 cases wee notified in Norway and 16 in Sweden. There were three deaths, and 22 patients were hospitalized. *S. livingstone* was subsequently recovered from a processed fish product at the retail level. *Salmonella* enterica was responsible for the foodborne outbreaks in Portugal, 2002 due to inadequate processing, preparing or handling of foods (Correial *et al.*, 2004). There was no correlation between sediment metal content and the total hyporheic microbial biomass present within each site. However, microbial community structure showed a significant linear relationship with the sediment metal loads (Feris *et al.*, 2003).

REFERENCES

Abdelhamid, A.M. (2003). Scientific Fundamentals of Fish Production and Husbandry.2nd Rev.Ed.,Mansoura Univ. Press,Deposit No.1424/ 2003.

Abdelhamid, A. M.; Gawish, M.M. and Soryal, K.A. (2006). Comparative study between desert cultivated and natural fisheries of mullet fish in egypt, i- concerning heavy metals. J. Agric. Sci. Mansoura Univ., 31: 5665 - 5680

Anon. (2005). Foodborne Illness: Food Poisoning. Outbreak, Inc.

A.O.A.C. (1990). Association of Official Analytical Chemists. 14th Ed., Public A.O.A.C. P.O. Box 540. Benjamin Franklin Station, Washington, 4. D.C.

APHA (1992). Standard Health Methods for the Examination of Waste and Wastewater. American Public Health Association, 18th Ed., Washington.

Austin, B. (1982). Taxonomy of bacteria isolated from a coastal-marine fish – rearing unit. J. Appl. Bacteriol., 53: 253 – 268.

Austin, B. and Austin, D.A. (1987). Bacterial Fish Pathogens: disease in farmed and wild fish. Ellis Horwood, Chichester.

Ayres, J.C., Mundt, J.O. and Sandine, W.E. (1980). Microbiology of Foods. W.H. Freeman and Company, San Francisco.

Biolife (1996). The biolife manual. Supplement to the second edition. Biolife Italiana S.R.I.

Colorado (2001). Colorado State University Cooperative Extension Safefood Rapid Response Network. Safefood News, 5(4): 1 – 2.

Correial, A.M. Goncalves, G. and Saraiva, M.M. (2004). Foodborne outbreaks in Northern Portugal, 2002. Eurosurveillance Monthly, 9: 5 – 6.

Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. (1975). Medical Microbiology. 12th Ed., Vol. II, Churchill Lvingstone Ltd., Edinburgh, London and New York.

Duncan, D.B. (1955). Multiple range and multiple F-test. Biomet., 11: 1 – 42.

Egyptian General Authority of Standardization and Quality Controll (EGASQC) (2000), Cairo, Egypt (ES 344 – 2000).

Enany, M.E., El-Sayed, M.E., Diab, A.S., Hassan, S.M. and El-Gamal, R.M. (2004). Bacterial causes of fin rot in some fresh water fishes. Proc. 6th Int. Symp. on tilapia in Aquaculture. Manila, Philippines, Sept. 12 – 15, p: 229.

Ethelberg, S., Olsen, K.E. and Scheutz, F. (2005). Zoonotic intestinal infections 2004, Denmark. Epi-News, 9.

Feris, K., Ramsey, P., Frazar, C., Moore, J.N., Gannon, J.E. and Holben, W.E.(2003).Differences in hyporheic-zone microbial community structure along a heavy-metal contamination gradient. Appl. Environ. Microbiol., 69(9): 55623 – 5573.

Fricker, G.R. (1987). The isolation of Salmonellas and Campylobacters. J. Appl. Bact., 63: 99 – 116.

Goosney, D.L., Knoechel, D.G. and Finlay, B.B. (2001). Enteropathogenic E. coli, Salmonella, and Shigella: Masters of host cell cytoskeletal exploitation. File: //D:\DR_MAHA\goosney.htm.

Guerin, P.J., de Jong, B.H., Hasseltvedt, V., Kapperud, G., Styrmo, K., Gordrosen, B., Lassen, J., Andersson, Y. and Aavitsland, P. (2004). Outbreak of Salmonella livingstone infection in Norway and Sweden due to contaminated processed fish products.Epidemiol. Inf.,132:889 – 895.

Hamison's (2005). Acute Diarrhea. J.D. Harrison's.

ICMSF, International Commission on Microbiological Specification for Foods (1978). Microorganisms in foods. 1- Their significance and methods of enumeration. 2nd Ed. Toronto, Univ. of Toronto Press.

Khalil, M.T. and Hussein, H.A. (1997). Use of waste water for aquaculture: an experimental field study at a sewage-treatment plant, Egypt. Aquac. Res., 28(11): 859 – 865.

Mc Keon, C., Glenn, E., Gerba, C.P. and Fitzsinmons, K. (2000). Microbiological hazards of tilapia culture systems. Proc. Tilapia Aquaculture in the 21st Century, Sept. 3 – 7, Rio de Janeiro – RJ-Brazil, pp: 479 – 485.

Morris, M.C. (2001). Eating fish may help slow mental decline. http: //www.msnbe.msn.com/id/9651590/

Ray, B. (2001). Fundamental Food Microbiology, 2nd Ed. CRC Press. London. New York. Washington, D.C.

S.A.S. Institute, Inc. (2001). User's guide statistices. S.A.S. Institute, Inc. Cary, N.C.

Tate, R.L. (1995). Soil Microbiology. John Wiley & Sons, inc., New York.

Twedt, R.M. (1978). Shigellia. In: FDA Div Microbiology, Bacteriological Analytical Manual. Association of Official Analytical Chemists. Washington D.C.

Vassiliadis, P. (1983). The Rappaport – Vassiliadis (RV) enrichment for the isolation of Salmonellas: An overview. J. Appl. Bact., 54: 69 – 76.

Wesley, I.V., Hurd, H.S., Muraoka, W.T., Harbaugh, E. and Trampel, D. (2005). The effect of transport and holding on Salmonella and Campylobacter. World Poultry, 21(9): 28 – 30.

Work book (2005). Food Safe Work Book, Part Four. File: //H:\maha\Food Safe %204 %20 from %20 Hospitality %20 North,%20 Prince %20 Ge.

WRC (2005). Albany Waterways Resource Book: Water quality, Water quality Parameters. //H:\fish\WRC % 20 Albany % 20 Waterways % 20 Resource % 20 Book % 20 Water % 20.

دراسة مقارنة بين أسماك البورى المستزرعة والتى من المصايد الطبيعية فى مصر – من حيث الميكروبيولوجى

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تمت مقارنة أسماك البورى المستزرعة مع تلك من مصادرها الطبيعية فى أربعة مواقع خلال فصلى الصيف والشتاء ٢٠٠٥/٢٠٠٦م، وذلك من حيث محتواها من ٣ أجناس من البكتريا المرضية. ويستلخص من هذه الدراسة التأكيد على وجود تلوث بالبكتريا المرضية (خاصة الإيشريشيا كولى) فى كل من الأعلاف الصناعية للأسماك ومياه تربيتها ورسوبيات المواقع المدروسة (خاصة فى الصيف)، وأنه لا فرق بين أسماك المصايد الطبيعية والمزارع السمكية من حيث التلوث البكتيرى فكلاهما ملوث، مما يستوجب التنبيه على الجهات المسئولة بمعالجة كل أنواع الصرف قبل صبه فى الأجسام المائية حماية للكائنات المائية والمستهلك.