Bull. Nat. Inst. Oceanogr. & Fish., A.R.E. 1994. 20 (1): 59 - 68

ACCUMULATION AND ELIMINATION OF PATHOGENIC BACTERIA BY TILAPIA FISH SPECIES.

By

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Key Words : Pathogenic bacteria, Fecal coliforms, Tilapia Fish Sp.

ABSTRACT

Laboratory experiments were done to investigate the rate of bacterial contamination in Tilapia sp. This include total aerobic bacteria, total coliforms and fecal coliforms. The fish was exposed to sewage water for 12 hours (contamination period) and subsequent elimination period in clean water for the next 180 hours.

During the contamination period, the numbers of total aerobic bacteria, total coliforms and fecal coliforms in skin, gills and alimentary canal were increased by 4.5, 41.6 and 4.1, and 105, 65 and 38, and 20,42 and 13 folds over the initial numbers respectively.

After 132 hours of elimination period, bacterial counts approximated the initial numbers or less, except in gills where slightly higher numbers of total coliforms were recorded. No coliform bacteria was detected in fish muscles during the accumulation and elimination periods.

INTRODUCTION

The increase of pollution in natural waters has intensified the detection frequency and persistence of pathogenic micro-organisms in areas affected by sewage discharge, which is causative agents to several human diseases. Consumption of contaminated fish is one of the main ways of human expose to pathogenic micro-organisms.

Fish accumulate micro-organisms from their environment so that their microbial safety as a human food is directly related to the quality of water-bodies from which they are caught (Brown and Dorn, 1979). their safety as a food is also affected by bacterial multiplication to infective levels during marketing and retailing periods.

The indicator micro-organisms mainly bacteria such as total coliforms and fecal coliforms or Escherichia coli, which established for water quality (WHO, 1971, Geldreich, 1976 and Jazrawy et al., 1988) have generally been used to evaluate the potential health hazards associated with the contaminated food and seafood (West et al., 1985, west and Colman, 1986, and Daw et al., 1990). Number of countries have adopted microbiological standards for seafood tests, for example a widely accepted standard is <2.3 E. coli cells per gram of oyster are considered safe for human consumption (Wood, 1976).

The objective of this study was to determine the possibility of elimination of pathogenic bacteria from fish Tilapia sp. exposed to sewage water. Thus, contaminated fish may be rendered safe for human consumption.

MATERIALS AND METHODS

Alive fish, Tilapia sp. (15-20 g weight) were collected from fish farm and acclimatized to indoor condition for one week. After then, the experiments were started by putting 2 groups of active and healthy fish (each group consists of 15 fish) in 2 glass tanks (100 liter) containing aerated sewage water. The initial total aerobic bacteria (TAB), total coliforms (TC) and fecal coliforms (FC) were counted in sewage water as well as in muscles, skin, gills and alimentary canal of fish. Contamination of fish by bacteria was measured after 12 hours of exposure to the sewage water. The rest fish (alive, active) were then transferred into 2 tanks containing clean water (dechlorinated, no ammonia and aerated). The bacterial numbers were counted at different times during the experimental period. Static renewal of clean water was done every 48 hours. At each sampling time, two fish were taken from each tank, measured and weighted. The organs were weighted, transferred to sterile blender, and blended for 60 seconds with sterile saline solution (8 g NaCl/liter). Then, serial dilutions of homogenates were done to determine the bacterial counts.

Total plate counts was done using spread plating method onto nutrient agar (Difco), and plates were incubated at 37°C for 48 hours. Total and fecal coliforms were counted by the five-tubes most probable number (MPN) technique. Tubes of Mac-Conkey broth (oxide) were inoculated with homogenate dilutions. The tubes

were incubated at 37°C for 24 hours to detect total coliforms. Tubes displaying positive (acid and gas production) were subcultured into the same media and incubated at 44.5°C. Positive acid and gas production tubes were counted to record fecal colifroms. The density of coliforms was determined by using a standard tables (APHA, 1989). The bacterial numbers were performed per cm^2 for external skin surface and per gram for muscles, gills and alimentary canal.

RESULTS AND DISCUSSION

The detection of such bacteria in fish has been considered important as indicator of fecal pollution. The present technique depends on exposing fish to sewage water to simulate natural conditions. This technique showed effective contamination, in which high counts of bacteria was achieved over a short period of 12 hours. Zuaretz et al., (1993) reported that E. coli, reached maximum levels after 12 hours of inoculation in the digestive tract of Tilapia fish. Tilapia fish accumulated total aerobic bacteria (TAB) of 98 X 10^4 cells/cm^2 on skin surface, 488 X 10^4 cells/g in gills and 86 X 10^4 cells/g in alimentary canal (Table, 1). The concentration factor of TAP were 4.5, 41.6 and 4.1 in corresponding organs respectively, while it was 105,65 and 38 for total coliforms (TC) in the same respect organs. Present concentration factors were usually higher than those of Timony and Abston (1984) (6.5 and 8.5 for E. coli and Salmonella typhimurium respectively) on hard clams. The bacterial numbers in milliliter of sewage water in the exposure tanks were higher than the numbers accumulated in fish organs (Table, 1) and did not significantly affect the accumulation factor. Accordingly, the accumulation factor seems to be independent on the level of organisms in the sewage water. The same result was achieved by Cabelli and Heffernan (1970).

After removal of the fish to the depuration tanks in which the clean water renewed every 48 hours. TAB decreased gradually within the 84 hours on the skin surface to approximate the initial numbers, and reached less than the initial numbers after 132 hours (Fig. 1). Gills and alimentary canal, on the other hand, showed 5 folds decrease in numbers after 36 hours, and after 84 hours became less than initial ones. The same elimination pattern was found in TC and FC numbers (figs., 2 and 3). Haven et al., (1978) and Timony and Abston, (1984) have noted the same rhythm of elimination in E. coli numbers on oyster and hard clams. Small proportion of bacteria was found in the tanks of clean water, suggesting their association with fish fecal materials. It was found from the results that the gills needed more depuration time and shorter time

Table (1): Contamination and elimination by bacteria in organs of Tilapia sp.

	Time (hours)	Water (No./ml)			Skin (/cm²)			Gills (/ g)			Alimentary canal (/g)		
		TAB	TC	FC	TAB	TC	FC	TAB	TC	FC	TAB	TC	FC
Contamination period (Sewage water)	0	80×10^6	7500	210	28×10^4	0	0	12×10^4	10	2.5	24×10^4	63	5
	12	nd	nd	nd	126×10^4	105	20	500×10^4	657	105	110×10^4	2400	65
Elimination period (Clean water)	48	20×10^3	7	0	95×10^4	35	5	150×10^4	360	60	36×10^4	1600	40
	96	1×10^3	3	0	31×10^4	6	3	24×10^4	67	19	13×10^4	21	4
	144	1.5×10^3	0	0	17×10^4	3	1.5	3×10^4	80	0	30×10^4	32	1.5
	192	1.3×10^3	0	0	20×10^4	3.5	3	8×10^4	17	1.5	17×10^4	15	2

TAP : Total aerobic bacteria
TC : Total coliform
FC : Fecal coliform
nd : not determined

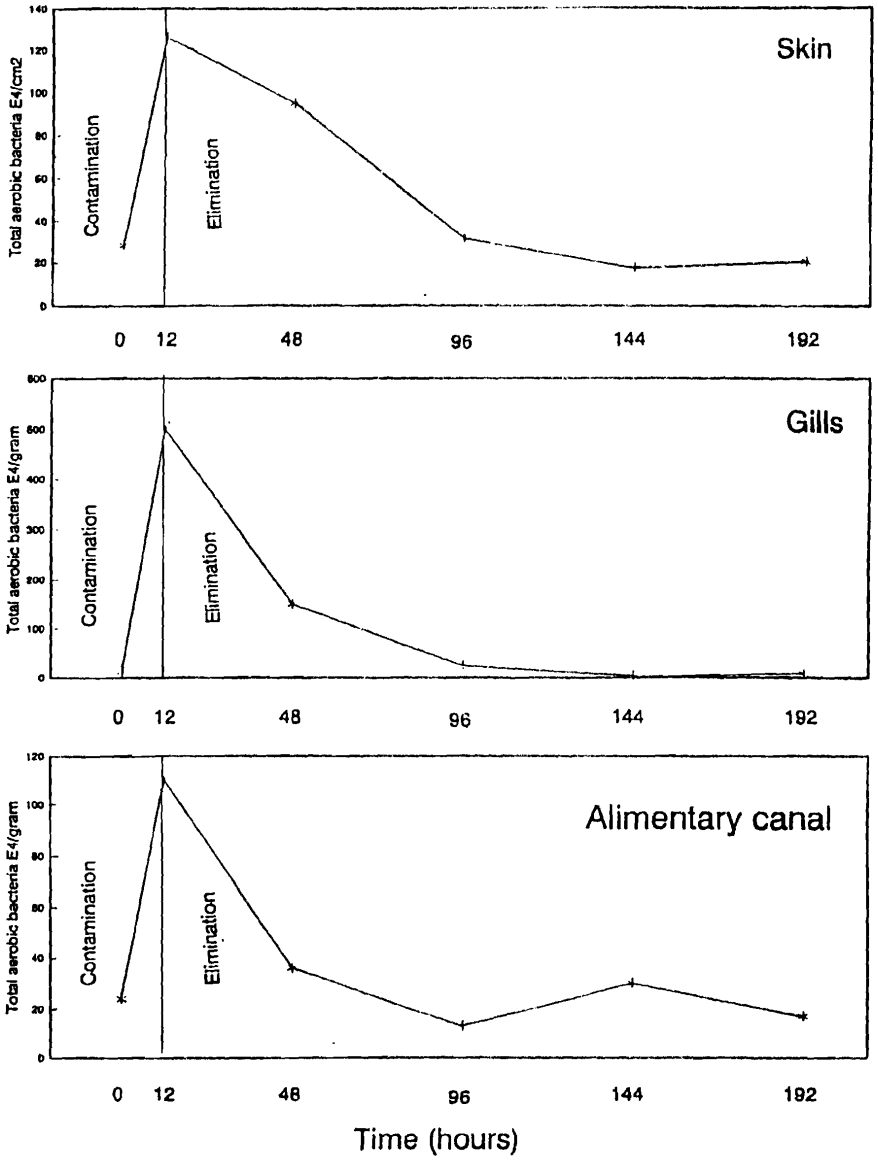

Figure 1: Contamination and elimination of total aerobic bacteria in organs of *Tilapia* sp.

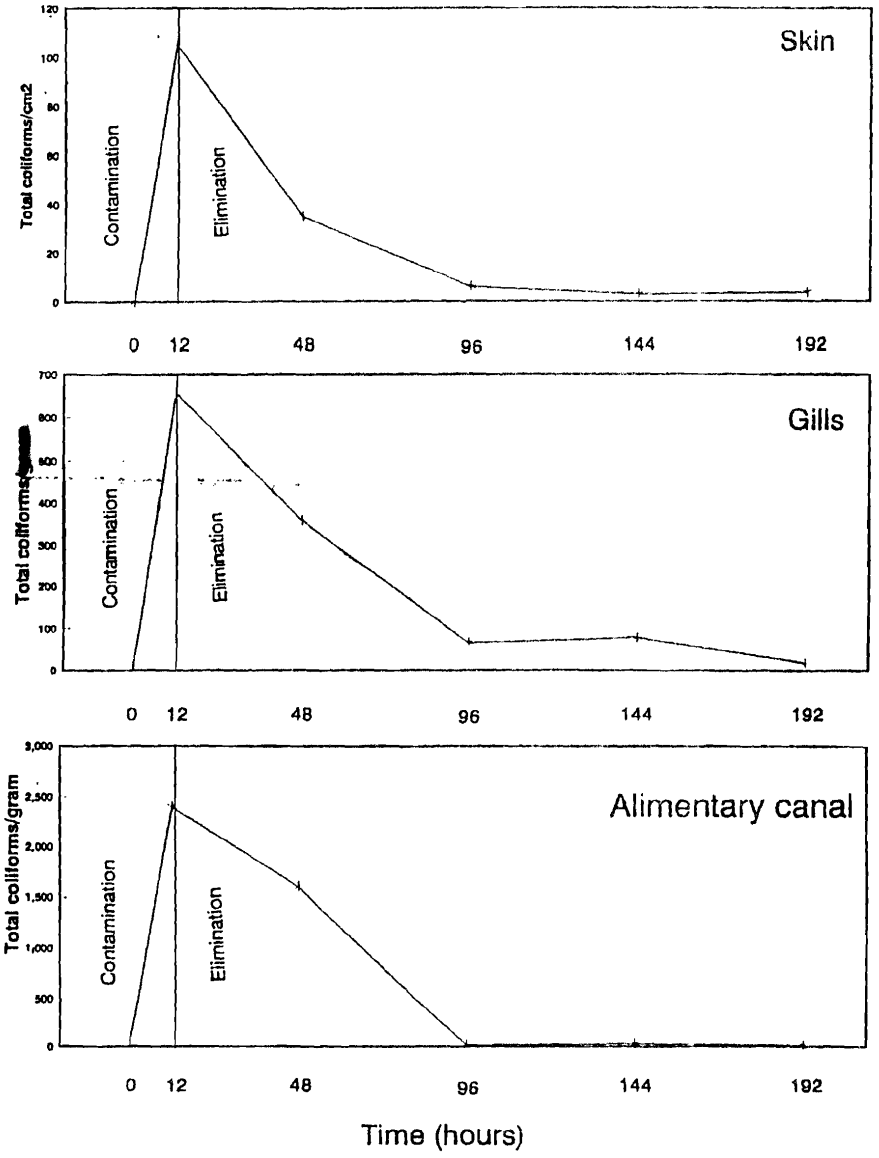

Figure 2: Contamination and elimination of total coliforms in organs of Tilapia sp.

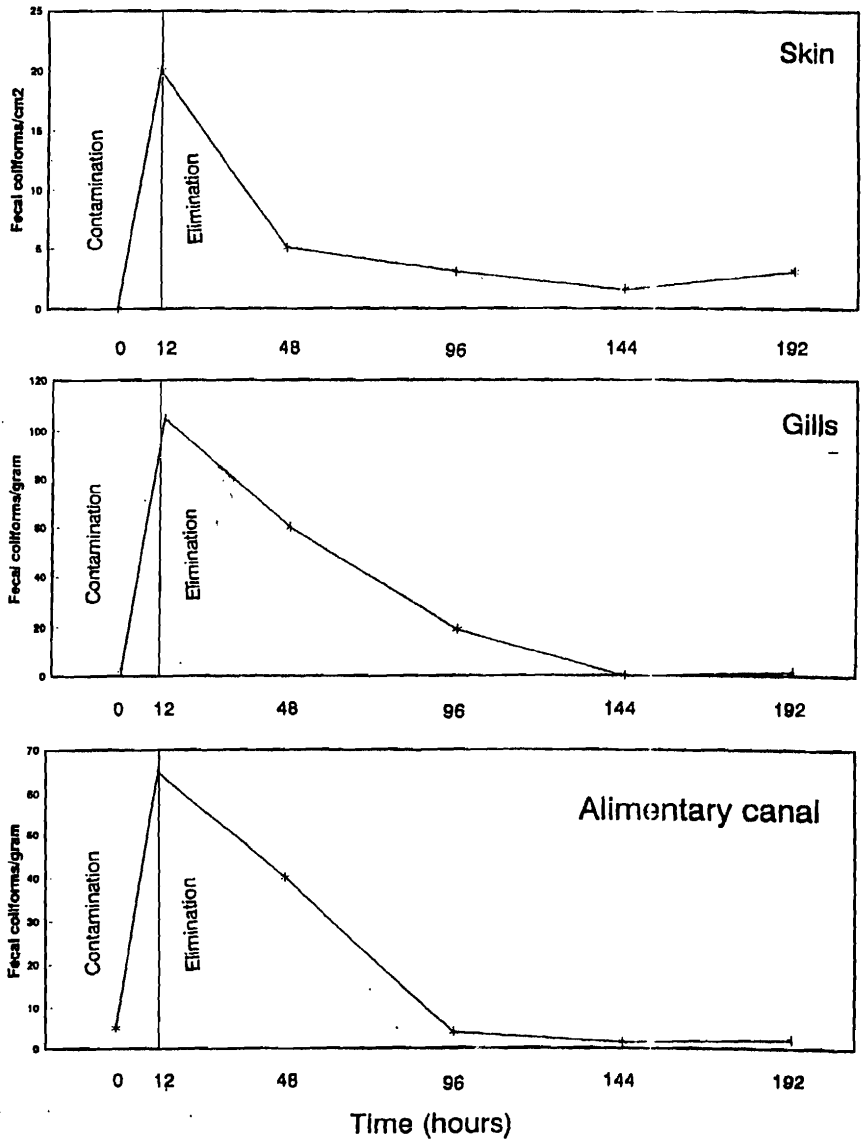

Figure 3: Contamination and elimination of fecal coliforms in organs of Tilapia sp.

for clean water renewal. No coliforms and fecal coliforms were detected in fish muscles in the contamination and elimination periods. The same results was found by Zuaretz et al., (1993) in Tilapia fish contaminated by E. coli.

The indicator organisms, although present in fish caught from polluted water are not generally considered part of the normal bacteria in fish or their environment. Matches and Abeyta (1983) reported that the freshly caught fish from temperate waters could carry bacterial populations of 10^2 to 10^3 per cm^2 of skin surface or per gram of gill tissue and often 10 to 100 fold higher, and the bacterial numbers in alimentary canal from very few to excess of 10^7 per gram of gut contents. However, because of handling or localized contamination on the fishing vessel, small numbers of coliforms were not uncommon.

The efficiency of fish purification by this way is monitored by the extent to which indicator bacteria such as fecal coliforms or E. coli have been cleansed to acceptable standards. It is considered that any pathogenic contaminants such as Salmonella will thus, have been equally cleansed. Son and Fleet (1980) established that in oyster, Salmonella typhimurium, S. Senftenberg, Bacillus cereus, Vibrio Parahaemolyticus and Clostridium perfringens could be ready cleansed under conditions which promoted decline of E. coli. This was demonstrated for oysters that were naturally contaminated with these pathogens and that had been contaminated in the laboratory. Such observation of considerable public health significance, challenges the long-accepted assumptions correlating the lowering of indicator bacteria with the cleansing of specific pathogenic bacteria (Hood et al., 1983 and Matched and Abeyta, 1983).

Commercial experience in a number of countries have shown that depuration cycle of 36 to 48 hours consistently yields oysters that meet E. coli standards (< 2.3 E. coli cells per gram) and have a good public health record (Souness et al., 1979). The same results were obtained by Son and Fleet (1980). They found that no E. coli was detected after 6 days of depuration.

In conclusion, present findings suggest that this system of depuration is efficient and rapidly eliminates large numbers of pathogenic bacterial indicators. This can be commercially applied by allowing the fish to actively feed in renewed clean water for a short time just prior to sale. Accordingly, we suggest to apply present technique of depuration on fish caught from Lake Mariut (Egypt), which is highly suffering from sewage contamination.

REFERENCES

APHA, 1989. Standard Methods for examination of Water and Waste water, American Public Health Association, Washington, DC 20036.

Brown, R.F. and C.R. Dorn, 1979. Fish, Shellfish and human health. J. Food Port. 40: 712-717.

Cabelli, V.J. and W.P. Heffernan, 1970. Accumulation of Esherichia coli by the northern quahaug. Appl. Environ. Microbiol., 19: 239-244.

Daw, Z.Y., G.M., Khalafalla, G.E. Mohamed, 1990. Effect of biological treatment on microbial flora of waste water. Annals Agric. Sci., Fac. Agric. Ain Shams Univ., Cairo, Egypt, 35: 71-79.

Geldreich, E.E., 1976. Fecal coliform and fecal Streptococci density relationships in waste discharges and receiving waters. CRC Crit. Rev. Environ. Control. 6: 349-369.

Haven, D.S., F.O. Perkins, R. Morales-Alamo, and M.W. Rhodes, 1978. Bacterial depuration by the American oyster (Crassostrea virginica) under controlled condition. Vol. 1, Biological and technical studies. Va. Inst. Mar. Sci. Spec. Sci. Rep. 88: 1-63.

Hood, M.A., G.E. Ness and N.J. Blake, 1983. Relationship among fecal coliforms, Escherichia coli and Salmonella spp. in Shellfish. Appl. Environ. Microbiol., 45: 122-126.

Jazrawy, S.F., Z.A. Al-Doori, T.A. Haddad, 1988. Antibiotic resistant coliform and fecal coliform bacteria in drinking water. Water, air and Soil pollution, 39: 337-382.

Matches, J.R. and C. Abeyta, 1983. Indicator organisms in fish and shellfish. Food Technology, June, 114-117.

Son, N.T. and G.H. Fleet, 1980. Behavior of Pathogenic bacteria in the oyster, Crassostrea commercialis, during depuration, re-laying and Storage. Appl. Environ. Microbiol., 40: 994-1002.

Souness, R.A., R.G. Bowrey and G.H. Fleet, 1979. Commercial depuration of the Sydney rock oyster, Crassostrea Commercialis. Food Technol. Aust., 31: 531-537.

Timoney, J.F. and A. Abston, 1984. Accumulation and elimination of Escherichia coli and Salmonella typhimurium by clams in an in-vitro system. Appl. Environ. Microbiol. 47: 986-988.

West, P.A., and M.R. Coleman, 1986. A tentative national reference procedure for isolation and enumeration of Escherichia coli from bivalve molluscan shellfish by most probable number method. J. Appl. bacteriol. 61: 505-516.

West, P.A., P.C. Wood and M. Jacob, 1985. Control of food poisoning risks associated with shellfish. J. Royal Soc. Health. 105: 15-21.

WHO, 1971. International Standards for Drinking water WHO. Geneva.

Wood, P.C., 1976. Guide to shellfish hygiene. WHO offset publication no. 31. World Health Organization, Geneva.

Zuaretz, S., A.M. Nasser, H. Shuval, and B. Fattal, 1993. Sea-food Safety Project, Sharm El-Sheikh Workshop. 20-23 December, 1993. Sharm El-Sheikh, Egypt.