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MICROBIOLOGICAL ASPECTS OF FISH CULTURED IN WASTEWATERS—THE SOUTH AFRICAN EXPERIENCE

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ABSTRACT

Health risks associated with the utilization of wastewater for fish production were studied by investigating the possible incorporation of pathogenic microorganisms into fillets of fish. Fish were cultured in cages in stabilization ponds at two municipal wastewater treatment facilities, and in a flow through and recirculating system receiving humus tank effluent. Water and fish were analysed for total and faecal coliforms, *Salmonella* and coliphages. Even though high numbers of faecal coliforms (2.0×10^5/100 ml) and coliphages (1.2×10^6/100 ml) were detected in the wastewater, and in some instances *Salmonella* were isolated, none of these microorganisms were found in fish fillet. Results of this study indicate that fillets of fish grown in such wastewater pond systems are microbiologically safe for human consumption, provided that simple precautions are maintained in food handling and processing, and that ponds are restricted to domestic wastes.

KEYWORDS

Fish, aquaculture, wastewater, coliform, coliphage.

INTRODUCTION

The utilization of wastewaters for aquaculture is not a new concept. For centuries domestic and animal wastes have enriched fish ponds in Eastern countries (Edwards, 1980). Recent studies in South Africa have also demonstrated the high potential such schemes could have for the optimal utilization of limited water resources and associated food production (Gaigher and Krause, 1983; Bok and Jongbloed, 1984; Gaigher and Toerien, 1985).

Wastewater, although very satisfactory as a source of nutrients, also contains high numbers of pathogenic microorganisms. Because of the possibility of disease transfer, health authorities in many countries are still reluctant to approve the use of wastewaters for fish production (Bryan, 1977; Hejkal *et al.*, 1983).

Studies have been undertaken by the Division of Water Technology to evaluate the health aspects of fish culture in wastewater (Nupen, 1983; Turner *et al.*, 1986). As part of an ongoing investigation, fish were grown in cages in municipal stabilization ponds and in intensive aquaculture units. This paper discusses the associated health risks in relation to microbiological analyses.

MATERIALS AND METHODS

Field Studies

Two municipal stabilization pond systems, receiving activated sludge and humus tank effluent, were selected for the study. Fish were cultured in 2 m x 1 m x 1 m cages, manufactured from aluminium tubing covered in 30% shade cloth, resting on the bottom of the ponds. One cage each, stocked with one hundred fingerling carp (*Cyprinus carpio*), was placed in the first, second and fifth ponds at Site 1, and in the first and second ponds at

site 2. The microbiological quality of the fish and water was examined after two, three and four months of culturing, during a summer growth period.

Pilot Scale Studies

Fish were cultured in humus tank effluent supplemented with commercial trout pellets, in an intensive flow through, and recirculating system, at Daspoort, Pretoria. The flow through pond (8 000 l) received effluent at a flow rate of 10 l/min, and was stocked with one hundred fingerling tilapia (*Oreochromis mossambicus*). The recirculating system consisted of a 500 l pond, a pre-filter, a biofilter, and a sandfilter, through which 750 l of wastewater was continually circulated. The pond was stocked with fifty fingerling tilapia. Water of the two systems was microbiologically analysed every fortnight during a summer growth period of six months. Fish were analysed at the beginning and end of the growth period.

Preparation of Fish Fillet

For analyses fish were scaled, washed in tap water, and dried between sterile paper towels. The skin was then painted with a 0.5% gentian violet solution and, using sterile instruments, an incision was made along the base of the tail and the dorsal fin, and the skin detached from the flesh (Nupen, 1983). A fillet was dissected so that no contamination occurred from the exterior of the fish. Fillets of five fish were pooled. Weighed samples were macerated with 1:10 w/v sterile distilled water. Analyses were carried out directly on the prepared suspension.

Microbiological Analyses

Water and fish were analysed for total and faecal coliforms, *Salmonella* and coliphages. Total coliforms were enumerated on m-Endo agar by incubating at 35 °C for 18 h (Grabow and du Preez, 1979), and faecal coliforms on m-FC agar by incubating at 44 °C for 18 h (Grabow, *et al.*, 1981). For *Salmonella*, enrichment was carried out in Rappaport-Vassiliadis medium with incubation at 35 °C for 18 h and selection on bismuth sulphite agar, followed by picking off colonies and inoculation on triple sugar iron and urea agar, and serological testing. Coliphages were recovered using a modified double-layer-agar method (Adams, 1959) and *Escherichia coli* C as host. Plaques were counted after incubation at 35 °C for 18 h.

RESULTS

Table 1 shows that no faecal coliforms, *Salmonella* or coliphages were isolated from the fillets of fish grown in the stabilization pond systems or in the intensive aquaculture units. However, total coliforms were recovered from fish fillet, in most cases at the end of a growth period. Due to fish kills, fish from ponds 1 and 2 at Site 1 were not analysed at the end of the growth period.

The wastewater in the stabilization ponds at Site 1 was more contaminated than the water in the ponds at Site 2 and the humus tank effluent used in the intensive aquaculture units. Water from ponds 1 and 2 at Site 1 contained 10^6 total coliforms, 10^5 faecal coliforms, and 10^6 coliphages /100 ml. *Salmonella* was isolated from 100% of the water samples from pond 1, and 33% of the samples from pond 2. As the stabilization ponds were in series, the microbiological quality of the water was best in the final ponds. The microbiological quality of the water in the flow through and recirculating system was very much the same. Fluctuations occurred in the water quality of these systems, total coliforms ranging from 10^2 to 10^5, faecal coliforms from 10^1 to 10^4, and coliphages from 0 to 10^4 /100 ml water.

DISCUSSION

Findings that fillets of fish grown in municipal stabilization ponds and in intensive aquaculture units were free of pathogenic microorganisms were in agreement with results of previous studies using domestic wastewater for fish culture by the Division of Water Technology (Nupen, 1983; Turner *et al.*, 1986). Similar results were obtained by Cloete *et al.* (1984) studying the bacteriological quality of fish grown in cattle feedlot effluent. The presence of total coliforms in fish fillet was probably due to contamination by soil bacteria, growing on the skin of the fish in close contact with the flesh, and which might have been more resistant to disinfection than pathogenic microorganisms. These bacteria will be identified in future studies.

The fish in ponds 1 and 2 at Site 1 only survived for a period of 4 months. Fish kills might have been due to industrial waste discharged to the sewage works, or to a reduction in oxygen levels, or high ammonia levels.

TABLE 1 Microbiological quality of water and fish

	Total coliforms (number /100 ml water and 50 g fish)	Faecal coliforms (number /100 ml water and 50 g fish)	*Salmonella* (% samples positive /100 ml water and 50 g fish)	Coliphages (number /100 ml water and 50 g fish)
Site 1				
Pond 1				
Water	5.8×10^5 (1.4×10^5 - 1.1×10^6)	5.5×10^4 (2.1×10^4 - 1.0×10^5)	100	1.1×10^5 (8.7×10^2 - 2.0×10^5)
Fish - 3 months	0	0	0	0
Fish - 4 months	1.6×10^3	0	0	0
Pond 2				
Water	7.5×10^5 (3.0×10^4 - 2.0×10^6)	7.4×10^4 (8.2×10^3 - 2.0×10^5)	33	4.7×10^5 (1.3×10^4 - 1.2×10^6)
Fish - 3 months	0	0	0	0
Fish - 4 months	1.8×10^3	0	0	0
Pond 5				
Water	1.5×10^5 (1.0×10^5 - 2.0×10^5)	1.6×10^4 (5.0×10^3 - 3.5×10^4)	0 0	2.3×10^4 (1.0×10^4 - 4.7×10^4)
Fish - 3 months	0	0	0	0
Fish - 4 months	7.0×10^2	0	0	0
Fish - 5 months	0	0	0	0
Site 2				
Pond 1				
Water	1.4×10^5 (1.0×10^4 - 3.2×10^5)	1.1×10^4 (2.0×10^2 - 3.0×10^4)	67	2.7×10^2 (1.0×10^2 - 4.2×10^2)
Fish - beginning	0	0	0	0
Fish - 6 months	0	0	0	0
Pond 2				
Water	3.0×10^4 (2.0×10^4 - 5.0×10^4)	4.0×10^3 (4.0×10^2 - 9.0×10^3)	0	7.5×10^2 (5.0×10^1 - 2.1×10^3)
Fish - beginning	7.5×10^2	0	0	0
Fish - 6 months	4.5×10^3	0	0	0
Flow through system				
Water	8.0×10^4 (9.0×10^2 - 4.1×10^5)	9.0×10^3 (3.0×10^1 - 3.5×10^4)	17	1.3×10^4 (7.0×10^1 - 5.3×10^4
Fish - beginning	0	0	0	0
Fish - 6 months	9.2×10^3	0	0	0
Recirculating system				
Water	1.2×10^5 (1.6×10^2 - 5.9×10^5)	1.2×10^4 (1.0×10^1 - 6.3×10^4)	0	1.0×10^4 (0 - 8.4×10^4)
Fish - beginning	0	0	0	0
Fish 6 months	7.6×10^3	0	0	0

Note: Water quality given as an average, with the range in parenthesis.

CONCLUSIONS

Even though high numbers of faecal coliforms (2.0×10^5/100 ml) and coliphages (1.2×10^6/100 ml) were detected in the wastewater, and in some instances *Salmonella* were present, pathogenic microorganisms were never isolated from fish fillet. Results of analyses indicate that fillets of fish grown in stabilization pond systems and intensive aquaculture units receiving domestic wastewater are microbiologically safe for human consumption. Any danger of culinary work-surface contamination during preparation should be minimized, and subsequent cooking would provide the final safeguard (Nupen, 1983).

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