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Microbiology quality in continuous water flow fish ponds

Qualidade microbiológica em viveiros de criação de peixes com disposição seqüencial

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Resumo

The study verified sanitary aspects in fish ponds with sequential disposal and the effect of the tanks effluent in parallel in the system. Six fish ponds were studied in the dry and the rainy periods and were analyzed microbiological aspects (thermo tolerant coliforms, total coliforms and heterotrophic bacteria), DBO_5 and DQO. It had contamination in the water of supplying for thermo tolerant coliforms, either of human or animals, compromising all the fish ponds studied with sanitary indices undesirable and representing risk in the cultivation of fish. It was found a great contamination of thermo tolerant coliforms, total coliforms, and heterotrophic bacteria in the period of rains with significant differences for DBO5 and total coliforms variables in relation to the showed periods. The high temperatures and the waters of draining had favored the high level of coliforms and heterotrophic bacteria in the period of rains. Through the results was concluded that the parallel tanks and the rainy period had had greater influence that the proper sequential disposal of the fisheries.

Key words: Heterotrophic bacteria. Coliforms. Fish ponds.

Abstract

O estudo verificou aspectos sanitários em viveiros de criação de peixes com disposição seqüencial e o efeito dos efluentes de tanques em paralelo no sistema. Foram estudados seis viveiros de criação de peixes nos períodos de chuva e seca e analisados aspectos microbiológicos (coliformes termotolerantes, totais e bactérias heterotróficas), DBO_5 e DQO. Houve contaminação na água de abastecimento por coliformes termotolerantes, seja de origem humana ou animal, comprometendo todos os viveiros estudados com índices sanitários indesejáveis e representando risco na criação de peixes. Foi encontrado um maior aporte de coliformes totais e bactérias heterotróficas no período de chuvas com diferenças significativas nos períodos amostrados em relação à DBO5 e coliformes totais. As altas temperaturas e as águas de escoamento favoreceram as concentrações elevadas de coliformes e bactérias heterotróficas no período de chuvas. Através dos resultados obtidos concluiu-se que os tanques paralelos e o período chuvoso tiveram maior influência que a própria disposição seqüencial dos viveiros.

Palavras-chave: Bactérias Heterotróficas. Coliformes. Viveiros de Peixes.

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Introduction

Bacteria are naturally present in fish pond water and sediment, being regarded as integral to the biological structure, acting upon the metabolism of the aquatic ecosystem and bearing a basic role to water quality. A number of specific functions which can be pointed out are as follows: contribution to mineralization, utilization as probiotic, and employment of certain species as food supplement to several aquatic organisms in fish breeding systems (NAYAK; SAVAN, 1999; SONNENHOLZNER; BOYD, 2000; SIPAÚBA-TAVARES, 2006).

Heterotrophic bacteria presence is of great importance for determining water bacteriological quality. Heterotrophic, aerobic mesophyle, or viable facultative bacteria may point to the existence of different groups of pathogenic and opportunist microorganisms comprising a public health hazard, affecting the fish immunological system in conformity with bacteria concentration in water (MORITA; SIPAUBA-TAVARES; AMARAL, 2006).

The most employed indicators for thermo tolerant pollution are total coliforms, thermo tolerant coliforms and thermo tolerant streptococcus. Thermo tolerant pathogenic microorganisms are hard to detect, but may appear intermittently and be found in low numbers in water samples. However, *Escherichia coli* is the best thermo tolerant pollution indicator for its shorter survival in the environment, being the most precise indicator for water contamination by thermo tolerant material.

Contamination of the water used for fish ponds is not desirable as it brings pollution and sanitary risk for human consumption. Few studies have evaluated the sanitary aspects of fish ponds systems (NIEWOLAK; TUCHOLSKI, 2000; JANA et al., 2001; EL-SHAFAI et al., 2004; PILARSKI et al., 2004; MORITA; SIPAUBA-TAVARES; AMARAL, 2006; LAN; DAC CAM; MARA, 2007; MACEDO; SIPAÚBA-TAVARES; AMARAL, 2009; SIPAÚBA-TAVARES; IBARRA; FIORESI, 2009), especially management practices using animal manure, especially from swine, a widespread practice in the south of Brazil. Thus, fish pond systems may have their sanitary conditions deteriorate along time, when food wastes, feces, and swine manure, among others are constantly added to the ponds. The adoption of preventive measures, coupled to limnological monitoring are necessary tools for the obtaining of a high-quality and commercially-feasible product for the national and international markets. Thus, the present study has evaluated the degree of water contamination in fish ponds, aiming at as certaining the effect of their continuous water flow layout.

Material and Methods

The study was developed in semi-intensive fish ponds at the São Paulo State University Aquaculture Center. The six fish ponds studied displayed a mean surface area of 4301.18m^2 and a mean depth of 1.31m. The effluent from the entire set of fish ponds outflows into the Jaboticabal stream, a tributary of the Mogi-Guaçu River.

Sampling was performed, at the inlet of each of the six fish ponds, for fifteen days, in two seasons of the year, the rainy (November-December/2000) and the dry (May-June/2001) periods. Nine points were set, as follows: W = well source; F_1 - F_6 = fish ponds studied, in which fish ponds F_3 and F_5 displayed two inflow points, F_{3a} and F_{3b}, F_{5a} and F_{5b}, respectively (Fig. 1).

Water samples were collected in sterilized 500 mL flasks and taken to the laboratory in an isothermal material box containing ice cubes. Time between collection and seeding in culture medium was at most 8 hours. All material used in microbiological analyses had been previously sterilized, and samples were submitted to Most Probable Number (MPN) determination of total coliforms and thermo tolerant coliforms (GREENBERG; CLESCERI; EATON, 1992).

Bacteria counting was performed following bacterial growth in a colony manual counter, plates presenting between 25 and 250 colonies being used. The mean of the colony number counted on Petri plates, multiplied by the corresponding dilution factor expressed the number of heterotrophic colonies units and density was given in colonies formation units (CFU/mL) (GREENBERG; CLESCERI; EATON, 1992).

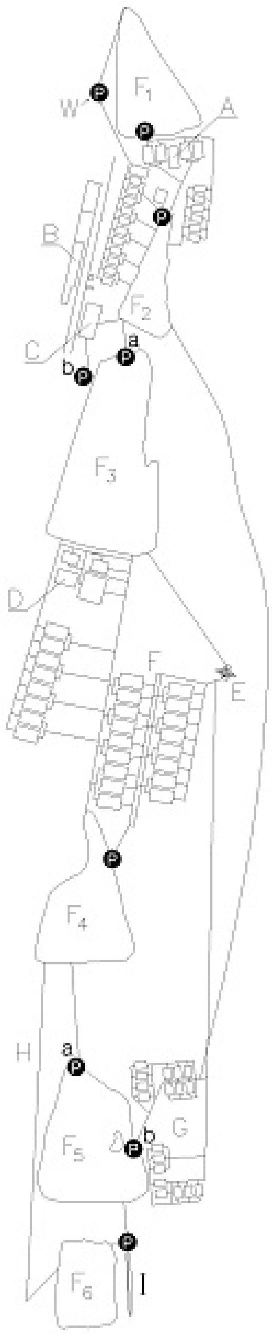

Figure. 1. Sequential layout plan of the fish ponds, in which: P=Sampling points, F_1 - F_6=Fish ponds, W=Well, A=Ornamental Fish Laboratory, B=Limnology and Plankton Production Laboratory, C=Aquatic Organism Nutrition Laboratory, D=Aquatic Organism Pathology Laboratory, E=Frog breeding Sector, F=Fish breeding Sector, G=Crustacean breeding Sector, H=Water out let, I= Macrophyte Biofilter.

BOD was determined by the initial and final differences of dissolved oxygen in the five day incubation period, at a temperature of 20 ± 1ºC. Chemical Oxygen Demand (COD) was ascertained by the colorimetric method, using a digestion block and a HACH DR-2000 digital spectrophotometer (ADAMS, 1990; GREENBERG; CLESCERI; EATON, 1992).

The periods were compared by Mann-Whitney test and fish ponds by Friedman test for each variable at the 5% level of probability (SIEGEL, 1975).

Results

Thermo tolerant coliforms were found in all collection points, with mean densities in supply water being lower than 10 coliforms MPN/100mL in the rainy period. The first fish pond, in both periods, showed the lowest mean concentrations of thermo tolerant coliforms whereas the highest were obtained in F_4 and F_{5a} fish ponds, with $4x10^6$ and $10x10^5$MPN/100mL in the rainy period and $8x10^2$ and $2.6x10^2$MPN/100mL in the dry period. Coliforms were found in all points studied, with total coliforms found in lower mean concentrations in the dry periods, with a minimum of 74 coliforms/100mL at the well source and maximum of 170 coliforms in the F_2, F_4 and F_6 fish ponds. Total coliforms were found in higher concentrations in all points in the rainy period, where it varied from $9.4x10^3$ to $3x10^8$ coliforms MPN/100mL in F_{3b} and F_4 fish ponds (Fig. 2).

Heterotrophic bacteria were found from the well source to F_6 fish pond, with the lowest mean concentrations observed at W and F_1 in the rainy period, with $4.1x10^2$ and $6,9x10^2$CFU/100mL, respectively. Concentrations increased from fish pond F_1 to F_2 and later from F_3 to $F_{4.}$ During the dry period, concentrations varied from $1.5x10^3$ to $3.3x10^4$ CFU /100mL at the source and F_{3b} points, respectively (Fig. 2).

There was significant difference (P<0.05) between the rainy and the dry periods for total coliforms and between the fish ponds for thermo tolerant coliforms, total coliforms and heterotrophic bacteria (Tables 1 and 2).

COD displayed a different behavior between the two periods, with lower concentrations from W to F_{3b} and an increase from F_4 toward F_6 fish pond in the rainy period. Concentrations were lower in the dry period, with a variation in COD mean concentration from 2 to 11mg/L at W toward F_{3a} fish pond and from 10 to 12mg/L at F_4 to F_6 fish pond. BOD5 concentrations were very low from the well source to F_{3b}, varying from 0.5 to 3mg/L in the rainy period and concentrations varied from 5 to 7mg/L from fish pond F_4 toward F_6. Concentration varied 0.75 to 4mg/L, from W toward F_{3b} fish pond in the dry period, with very similar values between F_4 and F_6 fish ponds, around 5mg/L. In the same period, F_{5a} and F_{5b} showed the greatest concentrations (Fig. 3). A significant difference (P<0.05) was found between the rainy and the dry periods for BOD5 and between the six fish ponds for BOD5 and COD (Tables 1 and 2).

Table 1. Mean values of variables with non-parametric data distribution in the rainy (md_A) and dry (md_B), periods, reduced score values (Z) and Mann-Whitney's test (U).

Parameters	Md_A	Md_B	Z	U
BOD_5 (mg/L)	3.7	5.9	-2.015	P<0.05
COD (mg/L)	13.3	15.2	-0.411	P>0.05
Thermo tolerant coliforms (MPN)	154	140	-0.909	P>0.05
Total Coliforms (MPN)	479050	170	-6.653	P<0.05
Heterotrophic bacteria (CFU)	3265	3900	-0.961	P>0.05

Table 2. Mean values of variables with non-parametric data distribution in fish ponds (mdF_1, mdF_2, mdF_3, mdF_4, mdF_5, mdF_6), reduced score values (Z_r^2) and Friedman's ($Z^2_{0.05}$), in which CF= thermo tolerant coliforms and CT= total coliforms.

Parameters	mdF_1	MdF_2	mdF_3	MdF_4	MdF_5	mdF_6	Z_r^2	$Z^2_{0.05}$
BOD_5 (mg/)	0.55	2.05	2.25	7.48	6.83	7.96	45.05	P<0.05
COD (mg/L)	3.60	8.25	8.73	17.00	18.35	17.00	40.13	P<0.05
CF (MPN)	10.5	70	155	12181	10016	140	33.69	P<0.05
CT (MPN)	669	4689	38035	$1.2x10^6$	$1.2x10^6$	23685	32.98	P<0.05
Heterotrophic Bacteria (CFU)	550	16150	3265	23295	6425	2952	28.68	P<0.05

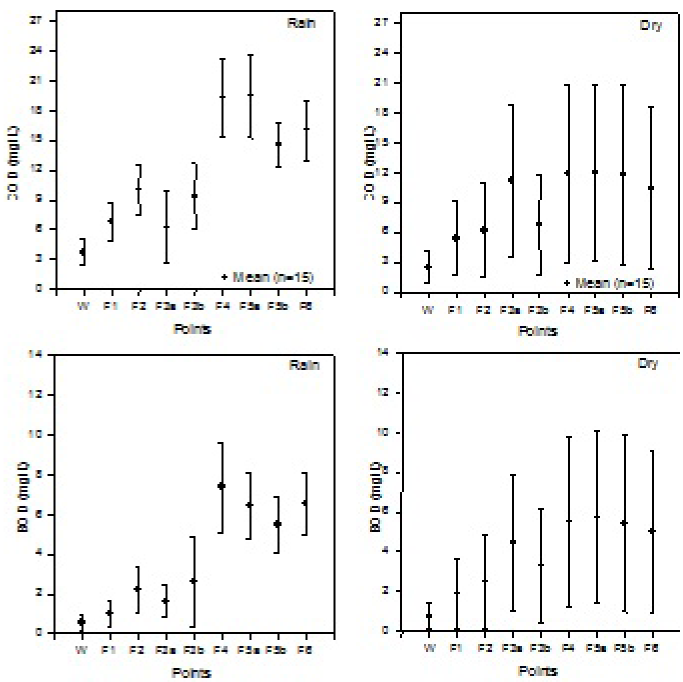

Figure 3. Mean BOD and COD (mg/L) concentrations in the rainy (A) and dry (B) periods, in which: W = well source; F_1 - F_6 = fish ponds.

Discussion

In the present study, the mean thermo tolerant coliform densities observed in fish pond 4 are related to the intense effluent flow from the several parallel tanks, in addition to the three first fish ponds flowing out into fish pond 4, with no previous treatment. This behavior reflects directly on the subsequent fish ponds, especially at the first point of fish pond 5, which receives water directly from fish pond 4. Conversely, the water out-flowing at the second point of fish pond 5 derives only from the tanks in the crustacean breeding sector, whose effluent contains a lower nutrient load than the one arriving in previous fish ponds.

Al-Harbi (2003) observed that *Escherichia coli* present in swine feces may be responsible for the contamination of the water, of the sediment and of the digestive system of tilapia in fish ponds. In the present study, the thermo tolerant coliforms reached fish ponds water and went through the intestinal excretions of mammals animals, as in the case of sheep, geese, herons and other birds found around the fish pond studied.

The bacterial community, together with the fungi, has been traditionally regarded as mineralizers, responsible for the conversion of organic matter into inorganic matter, thereby taking up a central role for nutrient return to the aquatic and terrestrial ecosystems. Organic decomposition occurs in conformity with microbial activity and density, which are regulated by the proportion of carbon, nitrogen and phosphorous, and, consequently, influenced by fertilizer introduction in the aquatic system.

The quantity of bacteria found in fish ponds was greater in the rainy period, corresponding to high level of fish production, when out flowing waters may carry large amounts of allochthonous materials to the fish ponds and also the high temperatures may favor the fast reproduction of total coliforms.

The water from livestock breeding farm wells may be a potential source of *Escherichia coli*. In the present study, the surroundings of the source to plantation areas and to the cattle and goat breeding farm sectors may have fostered contact with residual water and source contamination by coliforms, mainly during the rainy period. Until 14 years ago, swine breeding effluents flowed out directly into the fish pond 3, which has probably contributed to a greater coliform inflow and, consequently, contaminated subsequent fish ponds even further.

In the present study, microorganism concentrations have also demonstrated an increasing affluent trend toward the effluent in the fish ponds, considering as affluent the source water, and as effluent the water from the last fish pond, as these are continuous-flow systems. The E/A relationship of the whole system was raised in all parameters studied, with the relationship found for thermo tolerant coliforms in the rainy and dry periods being 29.18 and 4.00, respectively, demonstrating a high thermo tolerant coliform multiplication speed within the system, especially for the rainy period.

The fish ponds studied may be considered biodegradable (VALENTE; PADILHA; SILVA, 1997), as in all points, except for the source and the first fish pond in the rainy period, the COD/BOD mean ratio was lower than 5. The variation among fish ponds was greater than the variation between the periods, a fact which is related to the management employed and to the kind of continuous water flow system, with no previous treatment. In fish pond 4, the high BOD and COD values found indicate a high organic compound concentration. The higher BOD concentrations in the F_{3a}, F_{5a}, F5b and F_6 fish ponds may also be related to the organic solid availability in the above mentioned fish ponds, whereas the presence of coliforms determined a microbiological contamination potential.

Through the results obtained in the present study can be concluded that the supply water contaminated by coliforms has compromised the system of the fish ponds studied. According to public health fish in waters contaminated can not to be consumed. Although does not have certainty of the relation of water contaminated with eutrofication of fish ponds. The concentrations found had been above of the established standards of environment quality to effluent by Resolution 357 of CONAMA (CONAMA, 2005), influencing directly the quality of the water for the cultivation of fish. The microbiological parameters have determined an effluent with high concentrations of thermo tolerant coliforms, total coliforms, and heterotrophic bacteria. Undesirable sanitary indexes have indicated that the consumption of such fish may pose a risk to human health, mainly during the rainy period. Therefore can be recommended: treatments of the water of origin and of the effluent of the system before the launching in the receiving stream or disconection of the tanks in parallel for separate narrow channels.

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