Water and fish quality during the reuse in aquaculture of sewage treated in stabilization ponds: experience of Ceará state, Brazil

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Abstract The purpose of this study was to evaluate the water and fish quality (Nile tilapia, *Oreochromis niloticus*), during the reuse in aquaculture of sewage treated in waste stabilization ponds (WSP): anaerobic, facultative and two maturation ponds. The experiment was conducted at the Centre of Wastewater Treatment and Reuse, based in Aquiraz, Ceará, Brazil. Three tanks were constructed in concrete and the treated sewage was used as the water and food source. The treated sewage presented good physical-chemical and microbiological quality for aquaculture, in which the thermotolerant coliforms and Helminth eggs concentrations were below the limits suggested by the WHO. The microbiological results in the fish meat indicated that the pathogens concentrations were below the limits suggested by Anvisa (Agência de Vigilância Sanitária, Brazilian sanitary control agency). The zootechnical parameters indicated that it is possible the use in aquaculture of treated sewage coming from stabilization ponds as the only source of water and food.

Keywords aquaculture reuse; *Oreochromis niloticus*; stabilization ponds.

Introduction

The reuse of treated sewage in aquaculture is one of the many possibilities for water reuse, in which effluents coming from waste stabilization ponds (WSP) systems have been successfully used to cultivate fishes such as the specie Nile tilapia (*Oreochromis niloticus*).

According to Felizatto (2000), the reuse in aquaculture can be classified as direct, when aimed to produce fishes for human consumption, or indirect, when the goal is to produce microalgae or aquatic plants to be subsequently used as food.

Many authors are in favor of utilizing effluents from stabilization ponds to cultivate fishes such as tilapia and carp (Pescod, 1977; Silva; Mara; 1979 *apud* Kellner; Pires, 1998). According to Jana (1998), sewage-fed aquaculture is a unique system and has many advantages in developing tropical countries, acting as a major nutrients source for crop farming and aquaculture, helping to combat environmental pollution, and therefore pushing towards a sustainable production.

Regarding the fish productivity, the primary and secondary treated sewage were successfully applied to cultivate the Nile tilapia *Oreochromis niloticus* (L.). The fish growth rate in the tank with treated sewage was significantly higher compared to the growth in the natural habitat (Khalil; Hussein, 2008).

Several studies agree that wastewater-fed aquacultures, especially the ones consisting of several ponds, have fairly good wastewater hygiene properties. A series of 2 to 3 ponds should probably be sufficient to reduce the numbers of faecal bacteria to acceptable levels (Junke-Bierberovic, 2008).

Fish and plants passively accumulate microbial contaminant on their surface. Fish can concentrate bacteria, viruses and protozoa in their guts. Rarely pathogens (excluding trematoides) penetrate into the edible fish flesh (muscle). However, if fish is stressed – for example, by overcrowding, poor water quality or other conditions – the bacteria and viruses (there are no data of protozoa) may be able to penetrate into the edible fish (WHO, 2006). They may also be recovered from the fish skin surface (Edwards, 1992).

In 1989, the World Health Organization (WHO) proposed the following limits for treated sewage use in aquaculture: $\leq 10^3$ Faecal coliforms per 100 mL in the aquaculture tank; or $\leq 10^4$ thermotolerant coliforms in the aquaculture tank affluent; and absence of Helminth eggs (trematoides) (WHO, 1989).

In 2006, the WHO conducted a detailed revision on the sanitary limits to use treated sewage in aquaculture, which are in the book "Guidelines for the safe use of wastewater, excretas and greywater. Volume 3: Wastewater use in aquaculture" (WHO, 2006). Table 1 contains the WHO microbial quality targets for waste-fed aquaculture.

Table 1. Microbial quality targets for waste-fed aquaculture (WHO, 2006)

Media	Viable trematodes eggs (including Schistosome eggs where relevant) – number per 100 mL or per g total solids[a]	*E. coli* (geometric mean number per 100 mL or per g total solids[a,b])	Helminth eggs[c] (arithmetic mean number per litre or per g total solids[a,d])
Product consumers			
Pond water	Not detectable	$\leq 10^4$	≤ 1
Wastewater	Not detectable	$\leq 10^5$	≤ 1
Treated excreta	Not detectable	$\leq 10^6$	≤ 1
Edible fish flesh or plant parts	Infective metacercariae (presence or absence per fish or plant) not detectable or non-infective	Codex Alimentarius Commission specifications[e]	Not detectable
Aquacultural workers and local communities			
Pond water	Not detectable[f]	$\leq 10^3$	≤ 1
Wastewater	Not detectable[f]	$\leq 10^4$	≤ 1
Treated excreta	Not detectable[f]	$\leq 10^5$	≤ 1

[a] Excreta is measured in grams of total solid (i.e. dry weight); 100 mL of wastewater/excreta contains approximately 1-4 g of total solids.
[b] An geometric mean should be determined throughout the irrigation season. For pond water and product consumers, for example, the mean value of $\leq 10^4$ *E. coli* per 100 mL should be obtained for at least 90% of samples in order to allow for the occasional high-value sample (i.e. with or 10^5 or 10^6 *E. coli* per 100 mL).
[c] Applicable when emergent aquatic plants are grown and when there is high contact with wastewater, excreta, contaminated water or contaminated soils.
[d] An arithmetic mean should be determined throughout the irrigation season. The mean value of ≤ 1 egg per litre should be obtained for at least 90% of samples in order to allow for the occasional high-value sample (i.e with > 10 eggs per litre).
[e] The Codex Alimentarius Commission does not specify microbial qualities for fish flesh or aquatic plants. Rather, it recommends the adoption of hazard analysis and critical control point (HACCP) principles as applied from production to consumption.
[e] Viable Schistosome eggs where relevant.

The purpose of this study was to evaluate the water and fish quality (Nile tilapia, *Oreochromis niloticus*), during the reuse in aquaculture of sewage treated in WSP: anaerobic, facultative and two maturation ponds

Material and methods

The experiment was conducted at the Center of Wastewater Treatment and Reuse, attached to the Wastewater Treatment Plant of Aquiraz, Ceará, Brazil, and owned by the Company of Water and Wastewater of Ceará - Cagece. About 25000 persons are served by WWTP.

Three tanks (ET-1, ET-2, ET-3) of 50 m^3 constructed in concrete and covered with a net against predators were used (Figure 1). They were fed with treated sewage as the only water and food source, in which the water was coming from the WSP system: anaerobic, facultative and two maturation ponds. The three tanks have the same operational characteristics.

Figure 1. Aquaculture tanks used in the experiment. Aquiraz, Ceará, Brazil, 2007.

As a fish model, the specie Nile tilapia, *Orechromis niloticus*, was selected, with an initial weight average of 0.45g, sexually reverted for male, and tested in an experimental density of 3 alevin/m² (150 alevins/tank). As mentioned above, no supplementary food was added to the tanks.

The physical-chemical and microbiological analyses of the treated sewage were done weekly in the Laboratory of Sanitation of the Federal University of Ceará, according to Standard Methods recommendations (APHA, 2005). The main zootechnical parameters used to evaluate the fish development were: length, weight gain, initial and final biomass, and productivity.

In the period that the fishes reached the expected size and weight, fish samples were collected to investigate the microbiological aspects with regard to the presence of Thermotolerant Coliforms, Staphylococcus Coagulase Positive and Salmonella sp. in three different parts: gill, skin and muscle. The analyses were done in the Laboratory of Sea Sciences of the Federal University of Ceará.

Results and discussion

Quality of the treated sewage used as water and food source in the tanks

The treated sewage quality with regard to the physical-chemical and microbiological parameters can be found in Table 2. The water showed good physical-chemical and microbiological quality for aquaculture, in which the thermotolerant coliforms and Helminth eggs concentrations were below the limits suggested by the WHO (2006).

Table 2. Water quality in the treated sewage-fed fish tanks. Aquiraz, Ceará, Brazil. 2007.

Parameter	N	average	st.dev	min	max	var.coef.
pH	13	7.8	1.2	5.8	9.4	0.15
Turbidity	11	29.6	24.2	10.2	99.5	0.82
Conductivity	13	727	137	420	870	0.19
Sodium	3	53.7	21.9	32.2	75.9	0.41
Calcium	3	45.4	12.9	36.0	60.1	0.28
Magnesium	3	28.0	3.6	24.3	31.6	0.13
Potassium	6	26.2	10.3	15.6	36.1	0.39
Alkalinity	11	148.1	19.8	105.8	167.2	0.13
Chlorine	4	92.5	7.3	85.4	101.7	0.08
BOD	5	37	21	23	74	0.58
Total COD	10	112	88	25	325	0.79
Filtered COD	6	51	27	30	105	0.53
TS	5	521	265	307	962	0.51
TSS	4	15	23	0	42	1.51
TDS	4	506	242	307	920	0.48
Ammonium	3	7.7	1.6	6.2	9.3	0.20
Phosphorus	7	12.8	12.6	0.0	27.2	0.99
Total Coliforms	6	6.5E+02	3.0E+03	2.0E+01	7.8E+03	1.40
E. coli	5	7.7E+01	5.0E+02	1.5E+01	1.2E+03	1.61
Helminth eggs	5	0.4	1.0	0.0	3.3	2.28

n – number of samples; st.dev – standard deviation; min - minimal; max – maximum; var.coef. – variation coefficient.

Zootechnical parameters

The fish growth curves in terms of length and weight, for the three tanks, can be observed in Figures 2 (length) and 3 (weight).

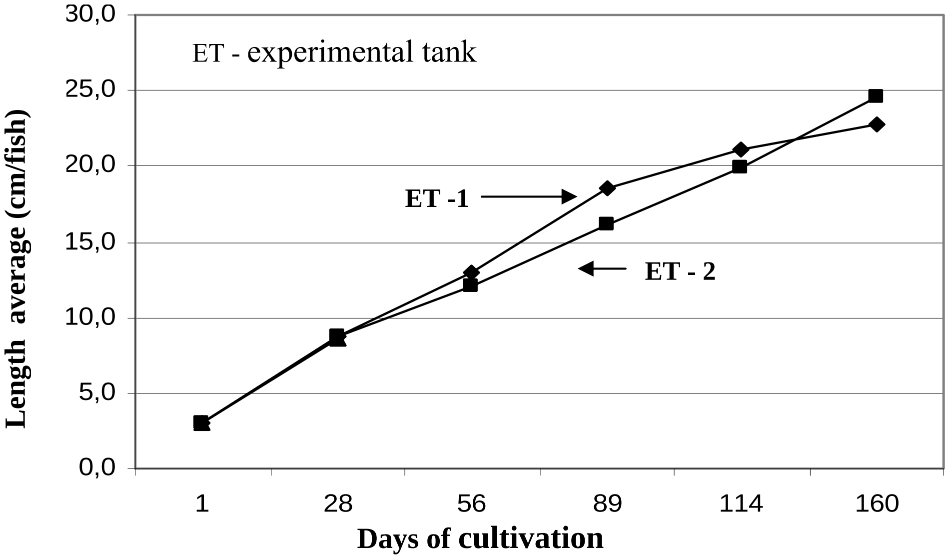

Figure 2. Growth curves in terms of length average (cm/fish), for the sewage-fed tanks without food supplementation. Aquiraz, Ceará, Brazil. 2007.

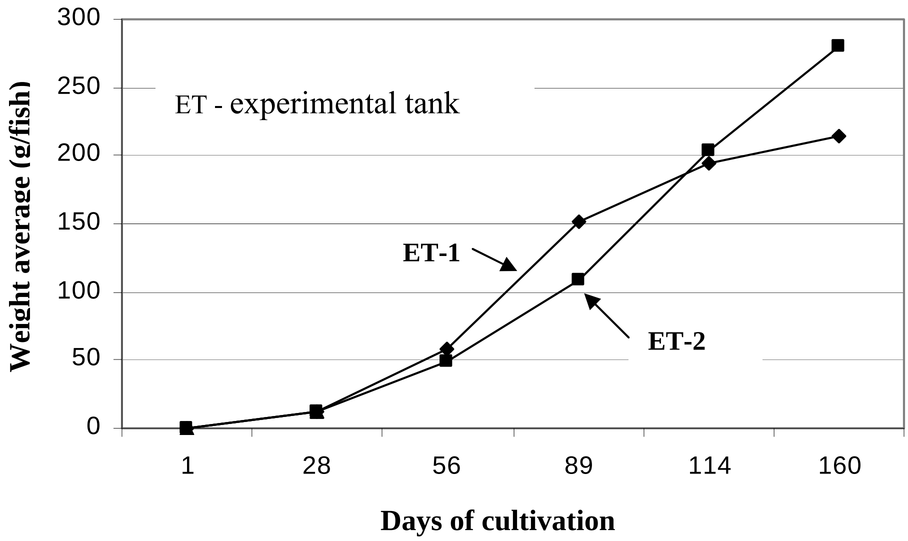

Figure 3. Growth curves in terms of weight average (g/fish), for the sewage-fed tanks without food supplementation. Aquiraz, Ceará, Brazil. 2007.

In the Experimental Tank 3 (ET-3), 100% mortality of the initial fish population was found at the end of the experiment. It is worth to mention that outside the Research Centre, in the same region, also occurred for unknown reasons mortality of fishes cultivated in conventional tanks fed with water.

The zootechnical parameters results are shown in Table 3. The highest productivity could be achieved in the experimental tank 1 (ET-1), i.e. about 6,204 kg/ha.

Table 3. Zootechnical parameters of the sewage-fed tanks without food supplementation. Aquiraz, Ceará, Brazil. 2007.

Zootechnical parameters	ET-1	ET-2	ET-3
Days of cultivation	160	160	160
Average of the initial length (cm/fish)	3.0	3.0	3.0
Average of the final length (cm/fish)	22.8	24.6	**
Growth (cm/fish)	19.8	21.6	**
Dairy growth (cm/fish/day)	0.12	0.14	**
Average of the initial weight (g/fish)	0.45	0.45	0.45
Average of the final weight (g/fish)	214.4	280.6	**
Weight gain (g/fish)	214.0	280.1	**
Dairy weight gain (g/fish/day)	1.34	1.75	**
Initial biomass (kg/tank*)	0.07	0.07	0.07
Final biomass (kg/tank*)	31.1	27.5	**
Biomass gain (kg/tank*)	31.0	27.4	**
Productivity (kg/ha)	6.204.1	5.485.7	**

*Tank with 50.0m³.

** Data not collected because of massive population death.

Sanitary fish conditions

The microbiological results of fishes cultivated in the tanks 1 and 2 can be found in Table 4. According to the Resolution RDC n° 12, ANVISA (Brazilian sanitary control agency), from January 2nd of 2001, the concentration limits for pathogens are:

Staphylococcus Coagulase Positive, 10^3 CFU/g, and Salmonella sp, absence in 25 g of skin. Therefore, no contamination was found in the three tissues analyzed, accomplishing the limits of the current legislation adopted in Brazil.

Table 4. Microbiological results of fishes cultivated in sewage-fed tanks 1 and 2, without food supplementation. Aquiraz, Ceará, Brazil. 2007.

Tank	Microorganism Analyzed	Faecal coliforms	*Salmonella* spp	Staphylococcus Coagulase Positive	Sanitary Conditions
	Microbiological limit	*	Absence/25g	10^3 CFU/g	
ET-1	Muscle	<1.8	Absence	<10	Satisfactory
	Skin	4.5	Absence	<10	Satisfactory
	Gill	24	Absence	<10	Satisfactory
ET-2	Muscle	7.8	Absence	<10	Satisfactory
	Skin	7.8	Absence	<10	Satisfactory
	Gill	14	Absence	<10	Satisfactory

* There is no reference value on the current Brazilian legislation

During treated sewage reuse in aquaculture must be considered a possible fish contamination by heavy metals. Although the levels of these compounds detected in sewage are usually low, there is still a possibility of industrial discharge in the pipes, therefore representing a risk.

In another experiment, the heavy metals levels in fishes cultivated with treated sewage coming from the same system used in the present investigation, were very low (Mota *et al.*, 2007). This indicated that they did not accumulate in the fishes, and the limits established by the Brazilian legislation and USFDA (United States Food and Drug Administration), the latter specifically for Chromium, were accomplished (Table 5).

Table 5. Heavy metals levels in the Nile Tilapia cultivated with effluents from WSP. Aquiraz, Ceará, Brazil. 2006.

Heavy metal	Limit (mg/kg)		Result (mg/kg)		
	Brazil	USFDA	ET-1	ET-2	ET-3
Lead (Pb)	2.00		1.59	1.43	0.46
Copper (Cu)	30.00		0.52	0.66	0.66
Chromium (Cr)		11.00	0.75	<0,49	<0.49
Zinc (Zn)	50.00		13.14	14.57	18.04

Pb, Cu and Zn = Limits specified by the Brazilian Ministry of Health
Cr = Limit specified by USFDA (United States Food and Drug Administration)
ET – Experimental tank

In an experiment conducted in Egypt, Khalil and Hussein (2008) concluded that the highest heavy metals concentrations were found in liver tissues, followed by intestine and gills, and then the muscles. Accumulation levels were within the acceptable limits when compared to the international legal standards for hazardous elements in fish and fishery products.

Conclusions

The main conclusions of the present research are:

- Systems of four stabilization ponds in series, produced effluents with good quality for use in aquaculture, which were below the limits suggested by WHO.
- The use of treated sewage in aquaculture as the only source of water and food is feasible, and offers a good prospect mainly for arid and semi-arid regions.
- The microbiological results of the fishes cultivated in the sewage-fed tanks indicated good sanitary conditions according to the Brazilian legislation.

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