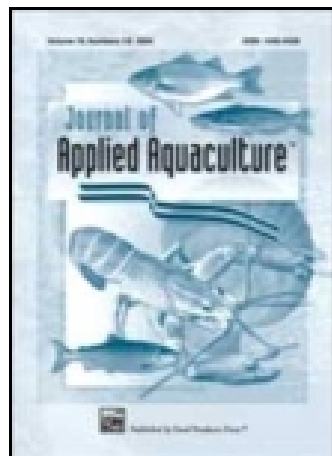




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Duckweed Bioconversion and Fish Production in Treated Domestic Wastewater

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Duckweed Bioconversion and Fish Production in Treated Domestic Wastewater

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We evaluated the bioconversion of domestic wastewater into protein biomass through duckweed based ponds and fish farming in a pilot recirculating system. The average duckweed wet biomass yield obtained during the experimental period was 4.96 kg/m² (1.3 kg dry matter; 63 g/m²/day), with crude protein and fiber content of 38.03% and 16.17%, respectively. Tilapia final average weight, feed conversion rate, and specific growth rate when fed pelletized duckweed were similar to values with commercial feed. Duckweed production removed 30.8% and 28.7% of ammonia and phosphate, respectively, from stabilized domestic wastewater.

KEYWORDS *Lemna, tilapia, effluent, reuse, alternative dietary source, production costs*

INTRODUCTION

Water quality deterioration is related to uncontrolled waste discharge into surface waters. Waste stabilization pond systems are inexpensive and known

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for their ability to achieve good removal of pathogens and organic pollutants (Zimmo et al. 2004). However, they may not achieve secondary effluent standards in terms of suspended solids and nutrient reduction due to algal growth in the ponds (Smith and Moelyowati 2001). In contrast, duckweed based wastewater systems are promising for use in effluent polishing through nutrient removal (Smith and Moelyowati 2001). Duckweeds also reduce suspended solids by blocking light penetration, causing algae to die off, which then settle or disintegrate. Duckweeds have high production rates, are easy manually harvest from the surface, and contain high protein and low fiber (Ran et al. 2004).

Bioconversion of domestic sewage in fish farming and agriculture can contribute to cost recovery and protein production (El-Shafai et al. 2007). Treated sewage is used in fish farming to fertilize ponds and enhance natural productivity (Mara and Cairncross 1989).

Many studies have explored alternative dietary protein sources for tilapia culture (Nguyen et al. 2009; Zhao et al. 2010). Studies have been conducted on the use of aquatic plants in tilapia feeds (El-Sayed 1999; Mandal et al. 2010). Duckweeds are good food for tilapia, as it contains about 35%–45% crude protein, good amino acid balance, and mineral profile (Mbagwu and Adeniji 1988). Mohedano et al. (2005) reported a reduction in feeding costs when replacing fishmeal with duckweed meal in tilapia diets. Tavares et al. (2008) showed that pelletized duckweed can represent up to 50% of a tilapia diet without affecting weight gain in tilapia fingerlings. The goal of this study was to evaluate the feasibility of duckweed-based-ponds as a tertiary sewage treatment for removal of pollutants and the use of duckweed as a feed source in tilapia production.

MATERIALS AND METHODS

Outdoor experiments were conducted at the Municipal Wastewater Treatment Plant administrated by CASAN (a Sanitation Company) located in the metropolitan region of Florianópolis, SC, Brazil. The plant is composed of one anaerobic (7.3 ha) and three facultative (10.5, 6.7, and 3.2 ha) stabilization ponds in series. Treated domestic wastewater from the last facultative pond (3.2 ha) was pumped to a duckweed pilot scale system (Figure 1).

This pilot system was composed of four duckweed tanks (first phase) and six red tilapia production tanks (second phase), as shown in Figure 1. In the first phase, the effluent went through four duckweed tanks in series to evaluate nutrient removal efficiency. Then, in the second phase, effluent was pumped to six fish tanks to evaluate growth parameters and environmental impacts caused by the administration of both treatments tested (commercial feed and pelletized duckweed).

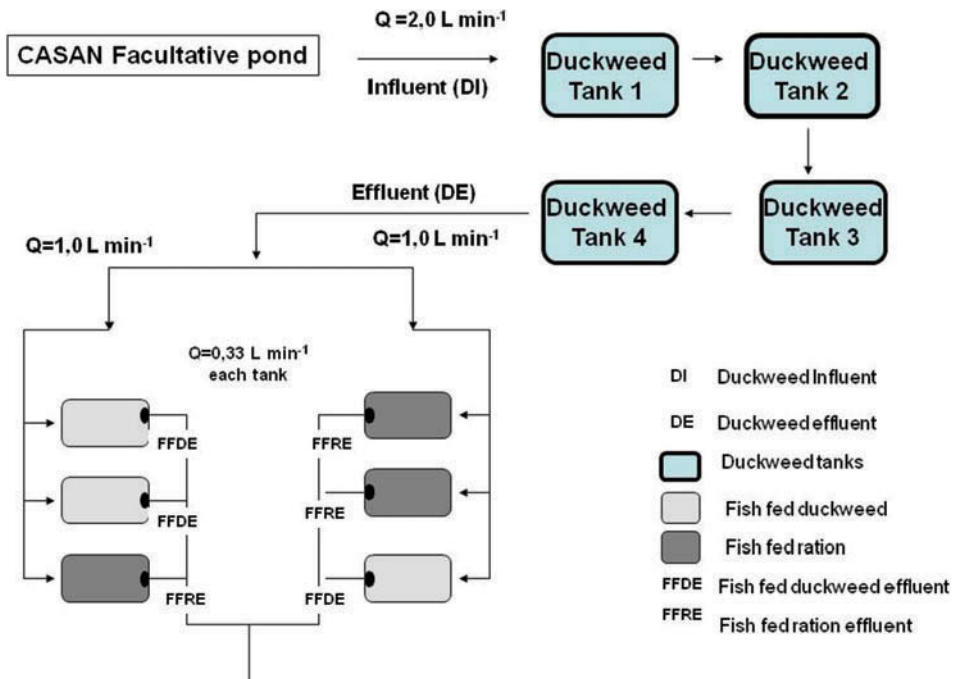


FIGURE 1 Duckweed-fish reuse pilot system.

The duckweed-based tank system (Figure 1) was composed of four fiberglass tanks with a depth of 0.90 m, surface area of 2.57 m², and volume of 2.3 m³ each. All tanks were connected and operated under a continuous water flow of 2 L min⁻¹. The retention time adopted was 1 day per tank, totaling 4 days.

Effluent sampling and analysis from duckweed tanks was conducted weekly. The following parameters were measured: total ammonia nitrogen (NH₃ + NH₄⁺) (Nessler method), nitrite (NO₂⁻), nitrate (NO₃⁻), total nitrogen (TKN), chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended, total and fixed solids, orthophosphate, turbidity, and total and fecal coliforms. Also, pH (digital YSI pH meter pH-100), conductivity, temperature, dissolved oxygen (YSI 55 probe, Yellow Spring Instruments, Ohio, USA), and transparency (secchi disk) were measured. All analyses followed APHA (1998) recommendations. For total and fecal coliforms analysis, we used a Colilert 18/Quanti-tray (IDEXX Laboratories, USA). Effluent samples were collected in sterile test tubes and transferred to the laboratory in less than five minutes. The equipment and solutions were sterilized in an autoclave before each use and all analyses were carried out in triplicate.

Duckweed growth rate was measured weekly through manual harvesting and weighing of biomass in a 20×20 cm square PVC sampling frame installed in the last duckweed tank. An initial duckweed amount (30 g) was

weighed and stocked into the square. After estimating the weekly production, the same initial amount was weighed again (30 g) and put back into the square. This procedure was adopted and repeated weekly during the experimental period in order to calculate and estimate duckweed biomass yield.

Duckweed nutritional value was determined monthly through proximate analysis (Table 1). Sampled material was sun dried for 24 h and in an oven at 50°C for another 24 h to reach constant weight. Thereafter, it was grinded and submitted to analysis, following AOAC (2000).

During the experimental period, duckweed biomass was collected weekly from the tank water surface with a screen so as to keep the plants growing at the maximum possible rate. Plants were placed in plastic nets for two days to drain and then transferred to a drying oven at 50°C for 24 h. After drying, the plant material was ground and water was added in a proportion of 40% to bind the mixture. Then, it was pelletized with a meat grinder at facilities of the Aquaculture Department, Universidade Federal de Santa Catarina, Brazil. Afterwards, pellets were oven dried for another six hours, packed and stored in a freezer at -20°C until utilization.

Two tilapia diet treatments were tested: (1) a commercial diet, where tanks received commercial feed (Guabi brand, 40% crude protein) and (2) pelletized duckweed, where tanks received pelletized duckweed as feed source. Diet composition of the commercial feed and pelletized duckweed are shown in Table 1. Crude protein and fiber content of the pelletized duckweed used in the present study (39.9% and 20.5%, respectively) were similar to crude protein (30%–40%), and crude fiber (5%–15%) reported by Gijzen et al. (1997). Once dried, duckweed can be stored in a cool environment (4°C) in plastic bags, and no other treatment or process is necessary to use it directly as feed for tilapia. Pelletizing duckweed improves water stability in water to up to three hours. The commercial diet had higher protein, fat, and energy, and lower fiber and ash than the duckweed diet.

TABLE 1 Composition of the commercial feed and the pelletized duckweed (*Lemna valdiviana*) experimental diets (dry matter).

Component (%)	Commercial feed	Pelletized duckweed
Moisture	5.5	4.50
Crude protein	53.3	39.9
Crude fat	9.93	4.63
Crude fiber	4.57	20.5
Ash	9.24	13.96
Gross energy (KJ g ⁻¹)	15.08	13.64

¹Commercial feed contained a vitamin and mineral premix with the following composition (kg/ product): folic acid: 250 mg; pantothenic acid: 5000 mg; biotin: 125 mg; copper: 2000 mg; choline: 25,000 mg; iron: 13,820 mg; iodine: 100 mg; magnesium: 3750 mg; niacin: 5000 mg; selenium: 75 mg; vitamin: 1,000,000 IU; vitamin B₁: 1250 mg; vitamin B₁₂: 3750 mg; vitamin B₂: 2500 mg; vitamin B₆: 1875 mg; vitamin C: 42,000 mg; vitamin D₃: 500,000 IU; vitamin E: 20,000 IU; vitamin K₃: 500 mg; zinc: 17,500 mg.

Six rectangular 500-L fiberglass tanks composed the experimental tilapia units. All tanks were independent from each other. Water exchange was 100% daily, with an average influent flow rate of 0.33 L min^{-1} (Figure 1). Tanks were aerated constantly with an air compressor. All six tanks were stocked with red tilapia (*Oreochromis mossambicus* × *Oreochromis niloticus*) fingerlings with an average initial weight of 0.8 g at a density of 10 fish per tank with three replicates per treatment.

The fish production tanks were installed and operated over 83 days. The system was set in an open field, exposed to weather conditions, with mean temperatures of 23.5°C , average humidity of 77%, and precipitation of 129 mm. All tanks received effluent treated by the duckweed-based ponds. Feed amount was 5% of body weight in the first week and thereafter on 1% until the end of the trial, divided into two feeding periods (0900 and 1600 h). Leftover feed was collected with a plastic screen to avoid water-quality deterioration. Effluent and water analysis in fish tanks was done as per the methods used in the duckweed ponds.

Fingerlings in each experimental unit were weighted every two weeks to calculate weight gain (final weight – initial weight), feed conversion (total dry feed fed /total wet weight gain), and specific growth rate ($100 \times \text{Ln final average weight} - \text{Ln initial average weight}/\text{days}$). Effluent polishing ability of the Duckweed Pilot system was estimated by nutrient removal efficiency. All data were analyzed by one-way ANOVA (Statistica 7.0).

RESULTS AND DISCUSSION

Duckweed Tanks

The average duckweed wet biomass yield obtained during the experimental period was 12.75 kg per tank (4.96 kg/m^2), which at 90% water is equivalent to 1.3 kg dry matter. Total dry matter production over 83 days was 5.2 kg ($63 \text{ g/m}^2/\text{day}$). Gijzen and Khondker (1997) demonstrated that duckweed productivity can reach from 2.7–8.2 kg dry matter $\text{m}^{-2}\text{year}^{-1}$, depending on the species, climatic conditions, available surface area, amount of nutrients, and management. Crude protein content averaged 38.08%. Nitrogen and phosphorus levels were 6.08% and 0.4%, respectively (dry matter).

Effluent nutrient removal efficiencies from the duckweed tanks are shown in Table 2. Notably, ammonia and phosphate removal efficiency was 30.8% and 28.7%, respectively. Al-Nozaily et al. (2000) reported that duckweed could readily absorb NH_4^+ and NO_3^- from wastewater. Organic nitrogen in colloidal or particulate form in the sewage needs to be decomposed first by bacteria into NH_4^+ , which can then be removed by plant uptake. Duckweed harvesting may also indirectly remove nitrogen compounds through harvest of bacteria and particulate matter attached to duckweed roots and leaves. Although biological nitrification-denitrification

TABLE 2 Average concentration of key water quality parameters influent and effluent, and removal efficiency by stabilized wastewater fed duckweed tanks (n = 20).

Variable	Duckweed Influent (DI)	Duckweed Effluent (DE)	Duckweeds removal efficiencies (%)
Turbidity (UNT)	195.8 ± 95.8	89.0 ± 79.7	54.5
Total COD (mgL ⁻¹)	195.3 ± 58.7	118.16 ± 47.8	39.4
Soluble COD (mgL ⁻¹)	147.7 ± 71.9	93.7 ± 27.3	36.5
NH ₄ ⁺ -N (mgL ⁻¹)	33.4 ± 6.0	23.1 ± 7.0	30.8
NO ₂ ⁻ -N (mgL ⁻¹)	0.07 ± 0.02	0.07 ± 0.11	8.39
NO ₃ ⁻ -N (mgL ⁻¹)	0.51 ± 0.16	0.53 ± 0.43	4.05
TKN (mgL ⁻¹)	34.6 ± 2.60	27.7 ± 9.28	19.9
P-PO ₄ ³⁺ (mgL ⁻¹)	11.07 ± 4.52	7.89 ± 3.60	28.7
Total solids (mgL ⁻¹)	50.4 ± 9.7	28.0 ± 5.9	44.4
Total Fixed Solids (mgL ⁻¹)	2518.9 ± 866.7	1718.1 ± 557.0	1.7
Total Volatile Solids (mgL ⁻¹)	2010.4 ± 705.1	1520.2 ± 572.1	24.3
Suspended Solids (mgL ⁻¹)	508.4 ± 257.6	274.2 ± 139.9	46.0
Total Coliforms (#/100 ml)	1.83E + 07	6.91E + 06	62.3
Log (10)	7.26	6.83	
<i>E. coli</i> (#/100 ml)	2.05E + 04	1.50E + 03	92.6
Log (10)	4.31	3.17	
Chlorophyll <i>a</i> (mgL ⁻¹)	0.221 ± 0.14	0.140 ± 0.15	36.4

processes have several advantages with respect to reliability and feasibility (Oron et al. 1987), removal of nitrogen by duckweeds is less energy consuming and easier for commercial implementation.

The duckweed pilot system also reduced total coliforms and *E. coli* by 62.3% and 92.6%, respectively. El-Shafai et al. (2007) showed that coliform removal in duckweeds systems occur due to the competition of duckweeds and bacteria for nutrients, as well as biomass harvesting. Suspended solid and chlorophyll *a* removal efficiencies in the duckweed tanks were around 46% and 36.4%, respectively, probably as a result of duckweed mat formation, which prevents sunlight penetration thus reducing phytoplankton growth. Turbidity removal was also significant, around 54.5%. Organic matter measured as total and soluble COD was less efficiently removed, 39.4% and 36.5%, respectively, while ammonia reduction was only around 30%. Oron et al. (1987) obtained an organic matter removal efficiency of 66.5% in 5 days and 73.4% within 10 days, using *Lemna gibba* in domestic wastewater treatment (COD from 500 to 750 mg.L⁻¹). In the present study, total and soluble COD removal efficiencies were 39.4% and 36.5%, respectively.

Overall, duckweed productivity and nutrient removal efficiency of our system was less than values reported in the literature. Either a higher retention time of the effluent in duckweed ponds to increase the removal efficiency of these nutrients, or higher rates of harvest of duckweed to provide more space for rapid growth should be tested as ways to improve the system.

Fish Tanks

Tilapia final average weight, feed conversion rate, and specific growth rate fed pelletized duckweed and commercial feed are shown in Table 3. Final average weight, feed conversion rate, and specific growth rate of red tilapia obtained in both treatments were 32.0 g, 1.59, and 5.56% and 25.9 g, 1.61, and 5.28%, respectively. Growth parameters obtained by fish fed commercial ration and pelletized duckweed were not statistically different ($p \geq 0.05$).

These results differ to those reported by Tavares et al. (2010) when feeding unpelletized duckweed. In the present study, pelletizing increased duckweed consumption. According to Amaral (2002), the pelletizing process effects are: higher acceptability by the animals, higher consumption, and lower energy waste during the intake process, feed losses reduction and easy management. Pelletizing can also increase digestibility and nutrient absorption. In another study conducted by Tavares et al. (2008) with tilapia in net cages, commercial feed, pelletized duckweed, and 50% commercial feed/50% pelletized duckweed produced statistically similar weight gain, which could reduce feeding expenses up to 50%. On the downside, in this study, the tilapia nursery phase required 81 days compared to the typical 40 days in Brazilian aquaculture.

Influent and effluent water quality data from the fish production tanks are shown and compared in Tables 4–6. As with the duckweed tanks, fish production also modified effluent characteristics, increasing nitrite and nitrate concentrations in both treatments. Only fish tanks fed the commercial ration increased chlorophyll *a*, probably due to the higher nutrient density in the feed. Both feed treatments efficiently reduced total coliforms, turbidity and ammonia, by 100%, 80%, and 90%, respectively. Likewise, both treatments reduced total COD (50.5 and 42.7%), soluble COD (49.9 and 44.2%), and phosphate (60.9 and 67.9%). Removal efficiency of total solids (12.4 and 10.1%), total fixed solids (12.1 and 18.4), and total volatile solids (20.6 and 18.6%) were modest to low, especially in tanks fed the commercial diet probably due to the higher nutrient density of the commercial feed, which when excreted produced enhanced algal growth.

TABLE 3 Feed conversion rate and specific growth rate comparison between fish fed pelletized duckweed and commercial ration.

Variable	Diet		P value
	Pelletized duckweed	Commercial ration	
Final average weight	25.9 ^a	32.06 ^a	0.0805
Feed conversion rate	1.61 ^a	1.59 ^a	0.2515
Specific growth rate	5.28 ^a	5.56 ^a	0.1459

^aSignificance level of 5%.

TABLE 4 Average concentration of key water quality parameters influent and effluent, and removal efficiency by fish tanks fed pelletized duckweed (n = 20).

Variables	Duckweed Influent (DI)	Duckweed Effluent (DE)	Fish tanks Effluent fed Duckweed (FTE D)	FTE D Removal Efficiency (%)
Turbidity (UNT)	195.8 ± 95.8	89.0 ± 79.7	14.7 ± 8.8	89.8
Total COD (mgL ⁻¹)	195.3 ± 58.7	118.16 ± 47.8	93.13 ± 41.0	50.5
Soluble COD (mgL ⁻¹)	147.7 ± 71.9	93.7 ± 27.3	75.0 ± 59.0	49.9
NH ₄ ⁺ -N (mgL ⁻¹)	33.4 ± 6.0	23.1 ± 7.0	4.19 ± 5.0	88.0
NO ₂ ⁻ -N (mgL ⁻¹)	0.07 ± 0.02	0.07 ± 0.11	1.0 ± 1.4	—
NO ₃ ⁻ -N (mgL ⁻¹)	0.51 ± 0.16	0.53 ± 0.43	3.1 ± 1.4	—
TKN (mgL ⁻¹)	34.6 ± 2.60	27.7 ± 9.28	5.1 ± 1.6	85.2
P-PO ₄ ³⁺ (mgL ⁻¹)	11.07 ± 4.52	7.89 ± 3.60	4.14 ± 1.72	60.9
Total solids (mgL ⁻¹)	50.4 ± 9.7	28.0 ± 5.9	43.2 ± 12.5	12.4
Total Fixed Solids (mgL ⁻¹)	2518.9 ± 866.7	1718.1 ± 557.0	1937.7 ± 947.0	12.1
Total Volatile Solids (mgL ⁻¹)	2010.4 ± 705.1	1520.2 ± 572.1	1486.8 ± 648.3	20.6
Suspended Solids (mgL ⁻¹)	508.4 ± 257.6	274.2 ± 139.9	289.3 ± 112.8	22.5
Total Coliforms (#/100 ml)	1.83E + 07	6.91E + 06	3.51E + 05	98.5
Log (10)	7.26	6.83	5.54	
<i>E. coli</i> (#/100 ml)	2.05E + 04	1.50E + 03	1.00E + 03	69.8
Log (10)	4.31	3.17	3.0	
Chlorophyll <i>a</i> (mgL ⁻¹)	0.221 ± 0.14	0.095 ± 0.15	0.106 ± 0.09	59.2

TABLE 5 Average concentration of key water quality parameters influent and effluent, and removal efficiency by fish tanks fed a commercial tilapia diet (n = 20).

Variables	Duckweed Influent (DI)	Duckweed Effluent (DE)	Fish tanks Effluent fed Ration (FTE R)	FTE R Removal Efficiency (%)
Turbidity (UNT)	195.8 ± 95.8	89.0 ± 79.7	27.1 ± 22.7	82.9
Total COD (mgL ⁻¹)	195.3 ± 58.7	118.16 ± 47.8	109.1 ± 46.0	42.7
Soluble COD (mgL ⁻¹)	147.7 ± 71.9	93.7 ± 27.3	90.7 ± 62.6	44.2
NH ₄ ⁺ -N (mgL ⁻¹)	33.4 ± 6.0	23.1 ± 7.0	3.03 ± 3.6	91.0
NO ₂ ⁻ -N (mgL ⁻¹)	0.07 ± 0.02	0.07 ± 0.11	1.9 ± 2.2	—
NO ₃ ⁻ -N (mgL ⁻¹)	0.51 ± 0.16	0.53 ± 0.43	3.64 ± 1.3	—
TKN (mgL ⁻¹)	34.6 ± 2.60	27.7 ± 9.28	8.1 ± 4.6	71.4
P-PO ₄ ³⁺ (mgL ⁻¹)	11.07 ± 4.52	7.89 ± 3.60	3.2 ± 1.0	67.9
Total solids (mgL ⁻¹)	50.4 ± 9.7	28.0 ± 5.9	43.3 ± 14.3	10.1
Total Fixed Solids (mgL ⁻¹)	2518.9 ± 866.7	1840.1 ± 557	1747.8 ± 898.6	18.4
Total Volatile Solids (mgL ⁻¹)	2010.4 ± 705.1	1520.2 ± 572.1	1529.4 ± 696.8	18.6
Suspended Solids (mgL ⁻¹)	508.4 ± 257.6	274.2 ± 139.9	346.3 ± 201.4	2.22
Total Colifroms (#/100 ml)	1.83E + 07	6.91E + 06	2.11E + 05	99.1
Log (10)	7.26	6.83	5.32	
<i>E. coli</i> (#/100 ml)	2.05E + 04	1.50E + 03	1.00E + 03	69.8
Log (10)	4.31	3.17	3.0	
Chlorophyll <i>a</i> (mgL ⁻¹)	0.221 ± 0.14	0.09 ± 0.15	0.177 ± 0.15	—

The production of duckweed in wastewater can be an alternative that contributes to the sustainability of sewage treatment systems, mitigating environmental impacts. According to [Decamp and Warren \(2000\)](#) duckweed

TABLE 6 Statistical comparison of water quality improvement efficiency in fish tanks fed pelletized duckweed (FTED) or a commercial diet (FTE R) (n = 20).

Variables	FTE R Removal Efficiency (%)	FTE D Removal Efficiency (%)	p value*
Turbidity	89.8 ^a	82.9 ^a	0.2014
Total COD	50.5 ^a	42.7 ^a	0.3222
Soluble COD	49.9 ^a	44.2 ^a	0.5919
NH ₄ ⁺ -N	88.0 ^a	91.0 ^a	0.6030
NO ₂ ⁻ -N	-1673.3 ^a	-3803.8 ^a	0.2727
NO ₃ ⁻ -N	-540.4 ^a	-657.5 ^a	0.3246
TKN	85.2 ^a	71.4 ^a	0.0522
P-PO ₄ ³⁺	60.9 ^a	67.9 ^a	0.1702
Total solids	12.4 ^a	10.1 ^a	0.8692
Total Fixed Solids	12.1 ^a	18.4 ^a	0.8404
Total Volatile Solids	20.6 ^a	18.6 ^a	0.9064
Suspended Solids	22.5 ^a	2.22 ^a	0.6151
Total Coliforms	98.5 ^a	99.1 ^a	0.7040
<i>E. coli</i>	69.8 ^a	69.8 ^a	1.0000
Chlorophyll <i>a</i>	59.2 ^b	-7.3 ^a	0.0458

*Significance level of 5%.

can be used as a secondary treatment of effluents from activated sludge systems or stabilization ponds, since it contributes to nutrient uptake from the wastewater, improving effluent quality.

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