



# Revista Engenharia na Agricultura

ISSN 2175-6813

Viçosa, MG, DEA/UFV - DOI: 10.13083/reveng.v29i1.12465

v.29, p.347-354, 2021

#### REUSE OF AQUACULTURE WASTEWATER TREATED IN CONSTRUCTED WETLANDS

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#### Keywords:

Dissolved oxygen Nile tilapia Water quality Water recirculation

### **ABSTRACT**

Water reuse in fish farming is a practice that has been spreading significantly, therefore requiring more efficient and vigorous treatment systems. The objective of this study was to evaluate the potential reuse of aquaculture wastewater after being treated in horizontal subsurface flow constructed wetlands (HSSF-CW). Three HSSF-CWs with dimensions of 1.0 m in width, 3.0 m in length, and 0.30 m in depth were evaluated. One HSSF-CW had stargrass (*Cynodon nlemfuensis*) cultivated; the other had cattail (*Typha latifolia*), and the third was used as a control, where there was no cultivation. The wastewater was provided from three Nile tilapia breeding tanks, which recirculated the entire system after going through the HSSF-CW. The following variables were determined every five days at the HSSF-CW influent and effluent: temperature, pH, turbidity, electrical conductivity, redox potential, dissolved oxygen, ammonium, and nitrate. The treatment in the HSSF-CW allowed the reuse of aquaculture wastewater as it provides water quality levels according to the recommendation for Nile tilapia breeding. Furthermore, the water quality parameters monitored in HSSF-CW effluent remained within limits established by CONAMA Resolution No. 357/2005 for aquaculture and fishing activity.

# Palavras-chave:

Oxigênio dissolvido Tilápia do Nilo Qualidade de água Recirculação de água

# REÚSO DE ÁGUA RESIDUÁRIA DA PISCICULTURA TRATADA EM SISTEMAS ALAGADOS CONSTRUÍDOS

# RESUMO

Received 03 May, 2021 • Accepted 03 Aug, 2021 • Published 22 Nov, 2021

O reúso de água na piscicultura é uma prática que tem se difundido consideravelmente, demandando sistemas de tratamento cada vez mais eficientes e robustos. Neste trabalho objetivou-se avaliar o potencial reúso de água residuária da piscicultura, após ser tratada em sistemas alagados construídos de escoamento horizontal subsuperficial (SAC-EHSS). Foram avaliados três SAC-EHSS com dimensões de 1,0 m de largura, 3,0 m de comprimento e 0,30 m de profundidade. Em um dos SAC-EHSS foi cultivado a grama-estrela (Cynodon nlemfuensis), em outro a taboa (Thypha latifolia) e o terceiro empregado como controle, no qual não houve cultivo. A água residuária era proveniente de três tanques de cultivo de tilápia do Nilo, que após passar pelos SAC-EHSS, recirculava todo o sistema. A cada cinco dias, determinava-se, na entrada e na saída dos SAC-EHSS, as seguintes variáveis: temperatura, pH, turbidez, condutividade elétrica, potencial de oxirredução, oxigênio dissolvido, amônio e nitrato. O tratamento nos SAC-EHSS possibilitou o reúso de água residuária da piscicultura, por proporcionar níveis de qualidade de água em conformidade com o recomendado para a criação de tilápia do Nilo. Além disso, os parâmetros de qualidade de água monitorados no efluente dos SAC-EHSS atenderam aos limites estabelecidos pela Resolução CONAMA nº 357/2005 para aquicultura e atividade de pesca.

#### INTRODUCTION

The demand for the use of water resources has increased significantly in the last years, resulting in the scarcity of this resource in several regions. The reuse of wastewater can contribute to reducing the demand for water in agriculture and livestock, especially in developing countries where the treatment and use of effluents are still very low (OMOTADE *et al.*, 2019). According to Santos and Roston (2016), the reuse of wastewater contributes to the preservation and conservation of water sources, as it avoids the release of polluting loads, and at the same time reduces the water uptake in water bodies.

In relation to fish farming, the renewal of water in the breeding ponds results in the disposal of an effluent enriched with nitrogen, phosphorus, organic matter, suspended particulate matter, in addition to antibiotics and other chemicals that can be used to control fish diseases (KONNERUP et al., 2011). Currently, more efficient cultivation methodologies, which employ partial or total water reuse, are being developed. However, this practice requires the use of a treatment system to remove pollutants from the water to be recirculated, so as not to compromise productivity in the fish ponds (ASSUNÇÃO et al., 2017).

Treatment systems that use conventional technologies are expensive and present complexity to be used in the treatment of fish farming wastewater (KONNERUP *et al.*, 2011). Thus, solutions that have low cost, high efficiency, convenient construction and operation, such as Constructed Wetlands (CWs), become a promising alternative for the treatment and reuse of these effluents (SILVA *et al.*, 2017).

Several studies demonstrate the high potential of CWs for the treatment of fish farming wastewater, as they allow a satisfactory removal of organic matter, suspended solids, nutrients, and indicator organisms for effluent contamination (KONNERUP *et al.*, 2011; AMORIM, 2014; ASSUNÇÃO *et al.*, 2017; OMOTADE *et al.*, 2019). According to Chakravartty *et al.* (2017),

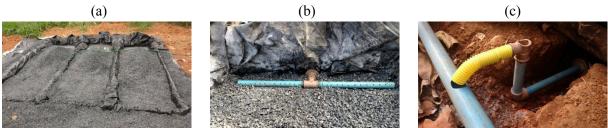
in addition to the high efficiency in removing pollutants, the CWs enable the cultivation of crops, contributing to food production. According to the authors, in systems with total water recirculation, for each kilogram of fish produced, a sufficient amount of nutrients to produce approximately 7 kg of aerial plant biomass is generated.

Plants grown in CWs act mainly in the removal of nutrients, enhancing the efficiency of the treatment in addition to providing it a pleasant appearance (FIA et al., 2017). Several crops have been studied for this purpose, including species of the genus *Thypha*, for promoting oxygenation in the root zone (MATOS et al., 2010; FIA et al., 2017). Regarding the stargrass, no reports were found in the literature on the use of this species in CWs, however, another genus of the same family, the Bermuda grass (*Cynodon* spp.) has a high potential for removing nutrients when cultivated in these systems (TEIXEIRA et al., 2021).

Considering the lack of technical information regarding the reuse of fish farm effluents treated in constructed wetland systems, the objective of this study was to evaluate the quality and potential reuse of fish farm wastewater, after being subjected to treatment in horizontal subsurface flow constructed wetlands (HSSF-CW), cultivated with stargrass (*Cynodon nlemfuensis*) and cattail (*Thypha latifolia*).

# MATERIAL AND METHODS

The experiment was installed and conducted in the experimental area of the Institute of Agricultural Sciences (ICA) of the Federal University of Vales do Jequitinhonha and Mucuri, Unaí *campus*, Unaí, State of Minas Gerais. For the execution of this experiment, three HSSF-CW were used, built in parallel in ditches dug in the ground, with dimensions of 0.30 m in depth, 1.0 m in width and 3.0 m in length, sealed with polyvinyl chloride geomembrane (PVC), and filled with a 0.25 m layer of zero gravel (total height) (diameter  $D_{60} = 7$  mm, uniformity coefficient (UC)  $D_{60}/D_{10} = 1.6$ , and initial void volume of 0.493 m<sup>3</sup> m<sup>-3</sup>) (Figure 1).



**Figure 1.** Construction of the horizontal subsurface flow constructed wetlands (a), drainage system (b), and water outlet from the HSSF-CW with level control (c)

The drainage system (Figure 1b) was built using 32-mm PVC pipes, installed opposite to the entrance of the HSSF-CW in order to allow a saturated layer of 0.20 m (useful height), across the full range of systems. In this way, the water level in the beds was kept at 0.05 m below the surface (Figure 1c).

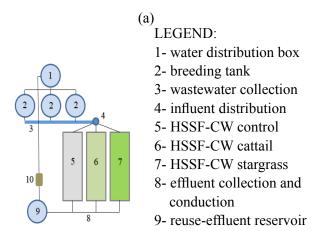
Cattail (*Thypha latifolia*) seedlings were collected in a flooded area on the Experimental Farm at ICA, and the aerial part was cut before planting. Stargrass (*Cynodon nlemfuensis*) was acquired in the forage sector. Both species were transplanted at a density of 8 propagules per m<sup>2</sup>, with a HSSF-CW used as a control, in which there was no cultivation.

After planting the seedlings, the HSSF-CW bed was saturated with a nutrient solution by Hoagland and Arnon (1950) to adapt the plants to the support medium. Thirty days after planting, the systems were completely drained and saturated using clean water. This procedure was repeated three times before starting the system operation, to ensure the

elimination of nutrients in the porous medium, so as not to interfere with the quality of the water used in the fish farming.

The wastewater treated in the HSSF-CW was provided by three Nile tilapia growing tanks, built with 500-L polyethylene boxes, which contained 40 fingerlings each. The fingerlings, with an average initial weight of 3.8 g, were fed ad libitum three times a day with commercial diets containing 32% crude protein throughout the experimental period.

The system was designed to carry out total water recirculation, thus, the wastewater from the breeding tanks was collected, mixed, and equally distributed among the three HSSF-CW, and after going through the treatment, the effluent was collected in a 250-L box and pumped to an upper box to be again distributed through gravity between the breeding tanks (Figure 2). The recirculation flow (2,160 L d<sup>-1</sup>) was kept constant using a tap, which was subjected to direct flow measurements twice a day.



10- pump



(b)

**Figure 2.** Schematic overview of the fish rearing and wastewater treatment system (a), an image of the system in operation (b)

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The water lost by evapotranspiration in the treatment systems was replaced daily in the water distribution box for the cultivation tanks, thus, there was no monitoring of individual evapotranspiration values for each HSSF-CW.

The HSSF-CW was fed continuously, with 720 L of fish farm wastewater being applied per day in each system, obtaining a hydraulic retention time (HRT) of approximately 9.86 hours, and a surface hydraulic application rate (TAS) of 0.24 m³ m²² d¹¹. The HRT was calculated based on the average influent flow data to the HSSF-CW and their useful volume. The low HRT is justified by the need for water recirculation in the culture tanks so that the adoption of higher values could make the treatment alternative unfeasible because it requires a volume of water greater than that necessary for the fish farming itself (SILVA *et al.*, 2017).

The HSSF-CW was controlled for a period of 60 days, starting in July and ending on August 31, 2019, totaling 12 sampling campaigns. Samples were collected every five days, at the entrance (influent) and the exit (effluent) of the treatment systems. The collection of samples was carried out at 8:00 am, with the analyzes being carried out shortly thereafter, at the Agricultural and Environmental Engineering Laboratory of the Institute of Agricultural Sciences.

The parameters monitored over the experiment were: water temperature, pH, turbidity, electrical conductivity (EC), redox potential (Eh) dissolved oxygen (DO), concentration of ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>). All analyses were performed according to the recommendations of the Standard Methods for the Examination of Water and

Wastewater (APHA, 2012).

The data obtained from the influent and effluent of the three HSSF-CW were submitted to the Bartlett and Lillierfors tests, to verify the conditions of homogeneity and normality, respectively. The 12 collections carried out over the experimental period were considered as repetitions. Then, the analysis of variance (ANOVA) was performed, followed by the test of Tukey at a 5% significance level, for comparison between the treatment means. All statistical analyses were performed using the STATISTICA® software.

#### RESULTS AND DISCUSSION

The quality of recirculated water remained within acceptable levels for rearing Nile tilapia throughout the experimental period (KONNERUP et al., 2011; SILVA et al., 2017). Regarding the limits established by CONAMA Resolution No. 357/05, which provides for the classification of water bodies and environmental guidelines for their classification (BRASIL, 2005), aquaculture and fishing activities require a compatible water quality level with Class 2. Thus, these pre-established limits will be used as a reference for comparison with the results obtained.

Table 1 shows the results obtained from the parameters monitored during the experimental period, in the influent and effluents of the HSSF-CW cultivated with stargrass and cattail, in addition to the HSSF-CW control.

It can be seen in Table 1 that some of the parameters did not show a significant difference between the HSSF-CW effluent and the influent.

**Table 1.** Average values of temperature (T), pH, turbidity (Turb.), electrical conductivity (EC), redox potential (Eh), dissolved oxygen (DO), ammonium (NH<sub>4</sub><sup>+</sup>), and nitrate (NO<sub>3</sub><sup>-</sup>) monitored in the HSSF-CW influent and effluent

Treatment	T	рН	Turb.	EC	Eh	DO	$NH_4^{+}$	$NO_3^-$
	(°C)		(NTU)	$(\mu S \text{ cm}^{-1})$	(mV)	(mg L <sup>-1</sup> )		
Influent	20.5 a	7.79 d	3.51 b	184.1 a	336.4 a	6.74 a	0.031 a	1.60 a
$HSSF\text{-CW}_{\scriptscriptstyle{\mathbf{C}}}$	20.3 a	8.12 a	3.28 b	183.0 a	314.8 c	6.59 a	0.020 b	5.20 a
$HSSF\text{-CW}_{S}$	20.5 a	7.90 c	3.32 b	186.3 a	328.6 b	6.18 b	0.021 b	2.38 a
$HSSF\text{-CW}_{T}$	20.4 a	7.99 b	4.43 a	188.8 a	322.8 b	6.21 b	0.022 b	3.89 a

Means followed by the same letter in the column do not differ significantly from each other, at 5% probability, by the test of Tukey HSSF-CW<sub>C</sub>: control; HSSF-CW<sub>S</sub>: cultivated with stargrass; HSSF-CW<sub>T</sub>: cultivated with cattail

These results may be related to the low hydraulic retention time (HRT) used in this work, which was approximately 9.86 hours. According to Kadlec and Wallace (2009), typical HRT values usually used in the design of constructed wetland systems vary between 4 and 15 days, depending on the type of wastewater to be treated.

In fish farming systems with water recirculation, it is recommended to use treatment systems with low HRT to reduce the amount of water needed initially (ASSUNÇAO *et al.*, 2017; SILVA *et al.*, 2017). However, the use of CWs may result in low treatment efficiency, as the shorter the contact time between the biofilm of the porous medium and the wastewater is treated, the greater the negative effect of biochemical transformations and absorption of nutrients by plants growing in these systems.

Data on the water temperature showed no significant difference in relation to the influent and effluents, which can be considered positive, as high variations in water temperature can affect the development of the fish. In this aspect, because the CWs are dug in the ground, they have a low thermal amplitude compared to air (KADLEC; WALLACE, 2009). According to Konnerup *et al.* (2011), the minimum temperature recommended for rearing tilapia is 20°C. In this work, the temperature of the effluents varied between 17.3 and 23.2 °C, relatively low values due to the season of the year and the time of measurements. Thus, it can be considered that these are minimum temperature values, reached throughout the day.

Regarding pH, all HSSF-CW contributed to increasing this parameter, but all values remained within the recommended range for raising tilapia (BRASIL, 2005; KONNERUP *et al.*, 2011). The HSSF-CW control was the one with the highest pH values, probably because of the absence of plants that tend to acidify the medium near the root zone (SILVA *et al.*, 2017). The presence of plants with slight acidification of the medium can be considered a positive factor, as significant rises in pH contribute to the formation of ammonia (NH<sub>3</sub>), from the ammonium ion (NH<sub>4</sub><sup>+</sup>) present in the wastewater. Because of its high toxicity, ammonia can lead to high mortality in fish.

In relation to turbidity, low values are observed for both the influent and the effluents of the HSSF-

CW. Therefore, the evaluation of the efficiency of the system was partially affected as it is a robust system, recommended for the treatment of highturbidity wastewater (KADLEC; WALLACE, 2009). When they are used to treat effluents with a low concentration of solids, such as fish farming wastewater (KONNERUP et al., 2011; ASSUNÇÃO et al., 2017), the turbidity at the outlet of treatment systems can be greater than at the inlet, as a result of the detachment of solids from the support medium, which occur naturally in the beds of these systems, especially at the beginning of the operation. Osti et al. (2018) in evaluating the treatment of fish farming wastewater, observed that flooded systems built with horizontal subsurface flow tend to present greater turbidity in the effluent compared to surface flow CWs. These results are caused by carrying solids present in the HSSF-CW bed, due to the higher interstitial velocity in the porous medium, as previously mentioned.

One reason for greater turbidity in the HSSF-CW effluent cultivated with cattail (HSSF-CW<sub>T</sub>), is the transport of solids due to preferential paths in these systems. According to Lo Monaco *et al.* (2017), in CWs cultivated with white-ginger lily (*Hedychium coronarium*), greater turbidity was observed in the effluent in relation to the non-cultivated system, a consequence of the formation of preferential paths that facilitated the dragging of suspended solids over the CWs. Despite these results, all turbidity values obtained in this work are below 100 NTU, a value established by CONAMA Resolution No. 357/2005 as a threshold for the development of fish farming.

The EC of the HSSF-CW effluents did not show significant differences in relation to the influent, a positive factor for the treatment of this type of wastewater. In hot climates, there is a tendency for EC to increase due to evapotranspiration in the HSSF-CWs (OMOTADE et al., 2019; TEIXEIRA et al., 2020) and because of the decomposition of organic matter present in the systems, which can affect water reuse. On the other hand, the reduction in EC may occur due to the absorption and adsorption of ions present in wastewater, where plants greatly affect this process (FIA et al., 2017). Although the experiment was carried out in a hot and dry climate, the same occurred in

the winter period, with replenishment with water lost by evapotranspiration, therefore, further investigations should be carried out in the summer period, when higher values of evapotranspiration and rainfall are expected.

It can be seen in Table 1 the reduction of redox potential after the treatments, however, HSSF-CW beds remained as an oxidizing environment throughout the entire experimental period. This fact is related to the presence of oxygen in the medium, which showed a tendency to decrease when going through the treatment systems. Cultivated CWs had higher redox potential values compared to the control, however, in terms of dissolved oxygen, HSSF-CW<sub>c</sub> had a higher concentration. Even though cattail is considered a plant with high oxygenation potential in the root zone (MATOS et al., 2010), the HSSF-CW cultivated with this plant, presented redox potential values similar to the CW cultivated with stargrass, and lower concentration of DO than HSSF-CW<sub>c</sub>, probably due to the presence of organic material in the system bed, from the rhizomes used as seedlings during planting, corroborating the results obtained by Fia *et al.* (2017).

Positive values of redox potential are extremely beneficial for the treatment of wastewater reuse in fish farming, as the oxidative environment allows the oxidation of organic matter and the transformation of nitrogen, phosphorus, among other pollutants, present in the wastewater in treatment.

Regarding DO, a reduction is observed in the concentration after the treatments, however, at any moment did the concentration reach the critical level for tilapia production, which according to Konnerup *et al.* (2011), is 1 mg L<sup>-1</sup>. According to CONAMA Resolution No. 357/05, the limit concentration of DO for the fish farming activity is 5 mg L<sup>-1</sup>, therefore, all the evaluated treatments met this resolution.

Konnerup *et al.* (2011) found that the treatment of fish farming wastewater in CWs provided a reduction in the DO, especially for high values of hydraulic retention time. According to the authors, the reduction in DO concentration is related to the decomposition of organic matter in treatment systems (removal of biochemical oxygen demand).

Osti *et al.* (2018) found that DO concentration in subsurface flow systems remained lower when compared to surface flow systems due to the low incorporation of atmospheric oxygen into the water, and the presence of organic material retained in the HSSF-CW pore space.

In relation to nitrogen, specifically, the ammonium and nitrate ions, a reduction was observed in the ammonium concentration and no changes were observed in the nitrate concentration (Table 1). The HSSF-CW treatment contributed to a reduction in the NH<sub>4</sub><sup>+</sup> concentration and even though no significant difference was observed, there is a tendency for an increase in the concentration of NO<sub>3</sub><sup>-</sup> after treatment, as observed by Konnerup *et al.* (2011).

The reduction in NH<sub>4</sub><sup>+</sup> is related to the absorption of this nutrient by plants, and its transformation into NO<sub>3</sub><sup>-</sup> through the nitrification process as the evaluated HSSF-CW had favorable conditions, that is, an oxidative environment. Although no significant differences were found in the concentrations of NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> for the different treatments, the HSSF-CW<sub>C</sub> showed the greatest tendency to increase the concentration of NO<sub>3</sub><sup>-</sup>, possibly due to the higher DO concentrations.

At the beginning of the experiment, a low concentration of nitrate was observed, which may be related to the low development of nitrifying bacteria in the medium (KONNERUP et al., 2011). Nevertheless, this situation has been modified over time. In the nitrification cycle, the ammonium produced by fish is converted into nitrite by *Nitrosomonas* bacteria, and later into nitrate by *Nitrobacter*, an ion considered preferential in the nitrogen uptake plants grown in HSSF-CW (CHAKRAVARTTY et al., 2017).

Despite not having been monitored, the nitrite ion is extremely toxic to fish and should remain at concentrations lower than 0.5 mg L<sup>-1</sup> in tilapia breeding tanks (KONNERUP *et al.*, 2011). Concerning CONAMA Resolution No. 357/05, the limits are 1.0 and 10 mg L<sup>-1</sup> for ammonium and nitrate, respectively. Therefore, all HSSF-CW effluents evaluated in this work were in accordance with the limits established by the resolution. For tilapia rearing, it is recommended that the concentration of ammonium ion cannot be

greater than 2 mg L<sup>-1</sup>, associated with a pH of less than 7.0 to control the concentration of ammonia in the water, which is extremely toxic to fish (KONNERUP *et al.*, 2011).

According to Vilas Boas *et al.* (2018), despite the reduction of nitrogen in wastewater treated in HSSF-CW, it is essential to adopt a system that enables the transformation of nitrite and nitrate into gaseous nitrogen (N<sub>2</sub>), therefore promoting effective removal of N from the effluent. One of the possibilities is denitrification, a process that requires a low redox potential and, consequently, a low concentration of DO in the treatment system bed, conditions that were not observed in this work.

The results obtained in this experiment show that the use of HSSF-CW for the treatment and reuse of wastewater from intensive fish farming is an alternative to traditional treatment systems, due to the low cost of implementation, easy operation, and maintenance, in addition to contributing to the reduction of the amount of water needed for the activity.

# CONCLUSIONS

- The quality parameter of the water reuse remained within the recommended standards for tilapia rearing and below the limits established by CONAMA Resolution No. 357/05, demonstrating the feasibility of HSSF-CW for the fish farming wastewater treatment.
- The cultivation of cattail and stargrass in the HSSF-CW bed did not significantly contribute to increasing the efficiency of wastewater treatment from the fish farm. Even so, further works are recommended considering the entire production cycle of tilapia breeding.

# **AUTHORSHIP CONTRIBUTION STATEMENT**

**TEIXEIRA, L.D.:** Conceptualization, Formal Analysis, Project administration, Supervision, Writing—original draft; **SOUZA, A.:** Visualization, Writing—review & editing; **MOURA, G.S.:** 

Investigation, Methodology; **LEITE JÚNIOR**, **M.C.R.:** Validation, Writing – review & editing.

#### **DECLARATION OF INTERESTS**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## **ACKNOWLEDGEMENTS**

To Instituto de Ciências Agrárias (ICA) and Vales do Jequitinhonha e Mucuri University (UFVJM) for providing the facilities and granting undergraduate research scholarship.

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