Efficiency of the aquatic macrophyte salvinia auriculata in purification of urban effluents, validated by allium test (*Allium Cepa* L.)

Eficiência da macrófita aquática Salvinia auriculata na purificação de efluentes urbanos, validado pelo teste da cebola (*Allium cepa* L.)

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Abstract

This study aimed to evaluate the efficiency of the aquatic macrophytes Salvinia auriculata Aublet in purification of polluted effluents, and evaluate macroscopically the efficiency of macrophytes using the Allium cepa Linnaeus (onion test). Three collections were performed in September 2011, the first analysis was performed with water collected directly from River Santa Catarina, the second was performed seven days after the contact of the effluent with the macrophytes, and the third, fifteen days after the first analyses. The data were analyzed using Student's t-test ($p<0.05$). To verify the normality of the data was used the Kolmogorov-Smirnov test ($p<0.05$), for each sample (treatment). According to the results of the Allium test, the aquatic macrophyte S. auriculata was efficient in the removing of pollutants agents after fifteen days in contact with the effluent.

Keywords: Bioindicator. Effluent. Phytoremediation. Aquatic plants. Urban rivers.

Resumo

O presente estudo teve como objetivo avaliar a eficiência da espécie de macrófita aquática Salvinia auriculata Aublet na purificação de efluentes poluídos, além de avaliar macroscopicamente a eficiência das macrófitas utilizando o teste da cebola (*Allium cepa Linnaeus*). Foram realizadas três coletas no mês de setembro de 2011, a primeira análise foi realizada com a água diretamente coletada do Rio Santa Catarina, a segunda foi feita sete dias após o contato do efluente com as macrófitas, e a terceira, quinze dias após a primeira a análise. Os dados foram analisados através do teste t-student ($p<0,05$). Para verificar a normalidade dos dados utilizou-se o teste de Kolmogorov-Smirnov ($p<0,05$), para cada amostra (tratamento). De acordo com os resultados do teste da cebola a macrófita aquática S. auriculata se mostrou eficiente na remoção de agentes poluentes após 15 dias em contato com efluente.

Palavras-Chave: Bioindicador. Efluente. Fitorremediação. Plantas aquáticas. Rios urbanos.

Introduction

Water is vital resource for living beings, being used by human for several purposes, such as drinking water supply, irrigation, navigation, energy generation, and others. However, for much time, this valuable natural resource has been degraded by indiscriminate and inconsequent anthropogenic actions (Barbério 2008).

In the last decades, the population growth and the consequent increasing of industrial activities have contributing to the worsening of environmental problems, mainly regarding to the preservation of superficial and underground waters. Because of this fact, the legislation has become more restrictive and the oversight more present. However, reports of effluents discharges into streams, rivers, lakes and seas still are often worldwide. In Brazil, the reality is not different. According to the report presented in 2011 by Agência Nacional de Águas, about 70% of rivers that belongs to drainage basins ranging from Sergipe to Rio Grande do Sul present high levels of contamination, mainly by urban effluents (Triburtius, Zamora, Leal, 2004).

When effluents are discharged, the typical pollutants cause quality changes and consequent pollution (degradation) of the receiving rivers. Historically, the urban and industrial development occurred along the rivers due to the availability of water for supply and the possibility to use the river as receptor of wastewater. The worrying fact is the increasing of both population and industrial activities and the number of times that the same river receives urban and industrial wastewaters

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(Giordano, 2004).

The principal changes observed in the water resources are: reduction in dissolved oxygen levels caused by biodegradable organic matter; excessive growth of some aquatic organism due to excess of nutrients; and transmission of diseases by pathogenic organism (Braga *et al.*, 2002).

An alternative for the environmental decontamination is the phytoremediation that is defined as the use of vegetal systems and microorganisms to remove degrade or isolate toxic substances from the environment. The concentration of pollutants and presence of other toxins should be within the tolerance boundaries of the plant (Esteves, 1998).

The aquatic macrophytes are originally terrestrial plants that suffered adaptive changes to colonize aquatic environments, being classified in submersed, emergent, with floating leafs and free-floating. These plants present adaptations that allow their growth in a gradient ranging from satured soils to submerged in the water column (Esteves 1998; Bianchini Jr., Pacobahyba & Cunha-Santino, 2002; Camargo, Pezzato & Henry-Silva, 2003).

Frequently these plants proliferate undesirably and affect the multiple uses of lakes, dams and rivers, requiring the application of control techniques. On the other hand, these plants have been used in wastewater treatment systems, in the recovery of degraded environments and also as ornamental plants (Camargo *et al.* 2003).

According to Joyce (1990), the aquatic plant *Salvinia auriculata* Aublet serves as biofertilizer and mulch cover in vegetable garden and orchard, and is able to remove and accumulate heavy metals. Being contaminated cannot be used as feed or fertilizer (Amaral & Bittrich, 2002).

The use of *Allium cepa* Linnaeus as test organism was introduced by Levan (1938). The genus Allium has been used for the study of basic mechanisms as well as for scoring the effect of chemicals. Among the species Allium, *A. cepa* has proved the most useful, and has repeatedly been suggested as a standard test material (Fiskesjö, 1985). The onion has the advantage that may be used without proceed any condensation, purification or sterilization of the polluted water, allowing an analysis of the total sample (Fiskesjö, 1985; Rank & Nielsen, 1994).

The onion (*A. cepa*) has been suggested for bioassays due to the characteristics such as cell proliferation kinetics, fast root growth, large number of dividing cells, high tolerance to different cultivation conditions and easy handling, and also confers even greater degree of closeness with species of biota exposed to toxic substances (Fiskesjö, 1985; Barbério, 2008).

This study aimed to evaluate the efficiency of the system composed of a species of aquatic macrophyte free-floating S. auriculata in purification of polluted effluents, and to evaluate macroscopically the efficiency of the macrophytes using the Allium test.

Material and Methods

Macrophytes

Among several aquatic plants used as biological filter, the eared watermoss – *S. auriculata* - was chosen. This plant belongs to the family Salviniaceae, division Pterydophyta.

The aquatic plants were collected at Instituto Federal do Rio de Janeiro, located in the municipality of Engenheiro Paulo de Frontin-RJ, and immediately taken to the Hospital Universitário Sul Fluminense, located in the municipality of Vassouras-RJ. These plants were placed in a water tank measuring 57 cm height, 62 cm length and width, with a total volume of 219.11 L (0.219 m^3). The tank was filled with water coming from the River Santa Catarina that passes across the hospital.

Samples

The samples were collected with two 2.5 L plastic bottles previously sanitized and taken to the laboratory of Environmental Science, Universidade Severino Sombra, where was performed the Allium test.

Collections

The analyses were performed in September 2011, in a total of three collections. The first analysis was performed with water collected directly from River Santa Catarina. The second collection was performed seven days after the contact of the effluent with the macrophyte, and the third, fifteen after the first analysis (Table 1).

Table 1. Steps of analysis of the effluents, containing the intervals of evolution

First step
Analysis of River Santa Catarina effluent (09/01/2011)
Analysis of River Santa Catarina effluent filtered by macrophytes (7 days)
Analysis of River Santa Catarina effluent filtered by macrophytes (15 days)

Allium test (*Allium cepa*)

To test the efficiency of the aquatic macrophyte *S. auriculata* was used the Allium test. In the local trade were obtained 30 equal size bulbs of common onion. The primordial roots were removed before start the assay. The bulbs were placed in trays containing distilled water during 24 hours for previous germination of root tips, to verify the feasibility of the onions. For analysis, were selected only the bulbs that pointed the growth of roots.

For experiment, were used 200 mL of effluent poured into 250 mL glass jars. Twelve repetitions were used for the effluent River Santa Catarina and normal tap water, obtained in the laboratory.

During the assembly process were applied four wood sticks in each onion, inclined slightly to stimulate the roots growth. For seven days the bulbs were submitted to tap water and crude/treated effluent protected against direct sunlight and in ambient temperature. On the seventh day the roots were cut, being counted, measured by a digital caliper (Mitutoyo-precision of 0.01 mm) and weighed by a digital scale (precision of 0.0001 g).

Figure 1. Onions distributed among the treatments, during the evaluation.

Statistical Analysis

The data were submitted to statistical analysis where were compared the data in pairs. Thus, for each collection were compared the data between water and effluent and then comparing the data of effluents among the collections. For analysis of comparison in pairs were used the Student's t-test ($p < 0.05$). To check the normality of data was used the Kolmogorov-Smirnov test ($p < 0.05$) for each sample (treatment).

Results and Discussion

The values obtained in the results follow in Table 2 and Figure 2. When were compared the water and the effluent (river) of the first collection, there was statistical difference, according to the Student's t-test ($p<0.05$), among the treatments averages (Figure 3), the same occurred in the second collection (Figure 4), however, in the third, there was not statistical difference to $p<0.05$ (Figure 5). Studies prove that several aquatic plants have high capacity to purify wastewater and sewage (Salati Filho *et al.,* 1998; Skillicorn, Spira & Journey, 1993; Pedralli, 1999), including the species S. auriculata (Amaral & Bittrich, 2002).

These species require high concentrations of nutrients. For this reason have been used successfully in the recovery of polluted rivers and lakes, because its roots form a dense web able to retain even the finest particles in suspension and absorb toxic substances from industrial and domestic wastewater (Camargo *et al.* 2003).

It was observed that the longer period in which the effluent was in contact with the aquatic plant, higher capacity of absorption and reduction of pollutants.

Therefore, the length of onion roots did not differ in length in the first week showing that the aquatic plants allowed the reduction of pollutants present only after fifteen days (Pott & Pott, 2002).

Table 2. Length of onion roots (*Allium cepa* L.) in three samples divided in water and River Santa Catarina effluent. First collection: crude effluent; Second collection: effluent submitted for eight days to aquatic plants; Third collection: effluent submitted for fifteen days to aquatic plants, in the period from 09/01/2011 to 09/15/2011

Length of roots (mm)						
Repetitions	First Collection		Second Collection		Third Collection	
	Water	River	Water	River	Water	River
Mean	46.78	17.83	33.72	18.99	44.25	41.15
S. Deviation	15.6457	10.0068	13.5770	6.2264	11.9705	8.2738
Variance	244.7885	100.1351	184.3360	38.7677	143.2918	68.4565
T Calculated	4.991**		2.858**		0.739^{ns}	

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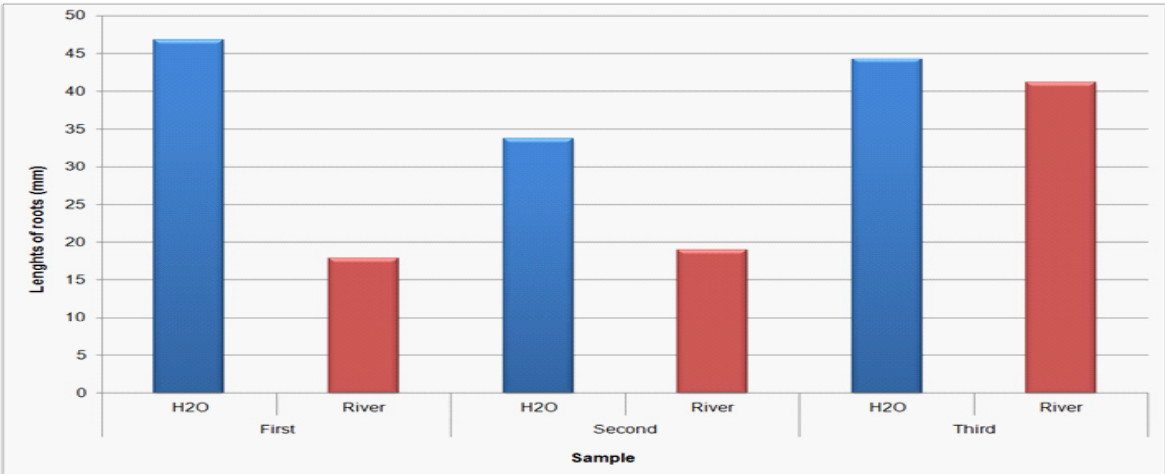

Figure 2. Mean length of onion roots (*Allium cepa* L.) submitted to the River Santa Catarina effluent and the water (control) in three situations. First collection: crude effluent; second collection: effluent submitted for eight days to aquatic plants; Third collection: effluent submitted for fifteen days to aquatic plants, in the period from 09/01/2011 to 09/15/2011.

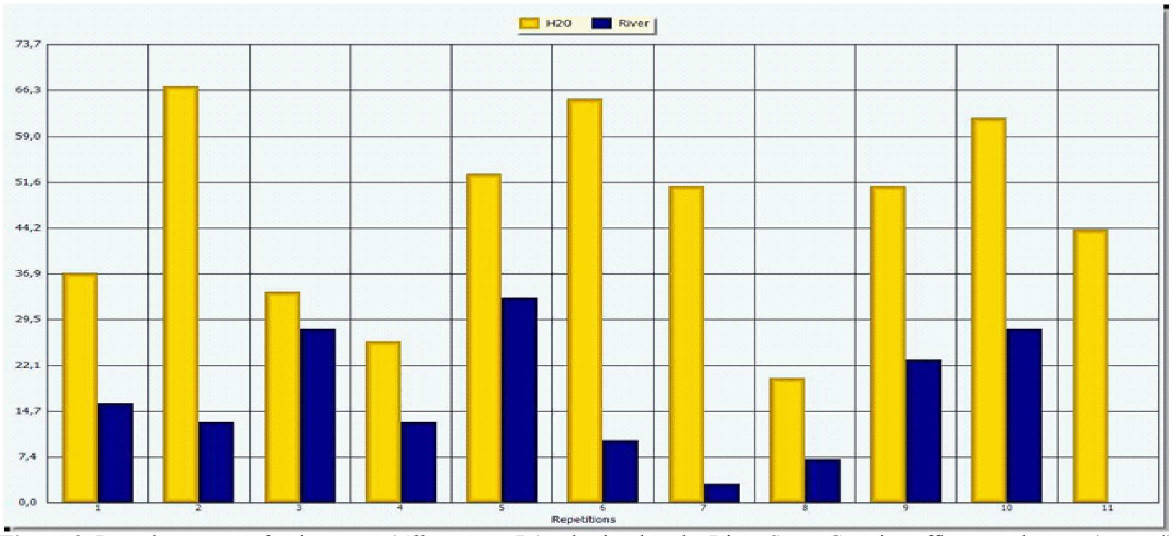

Figure 3. Length average of onion roots (*Allium cepa* L.) submitted to the River Santa Catarina effluent and water (control) in the first collection: crude effluent.

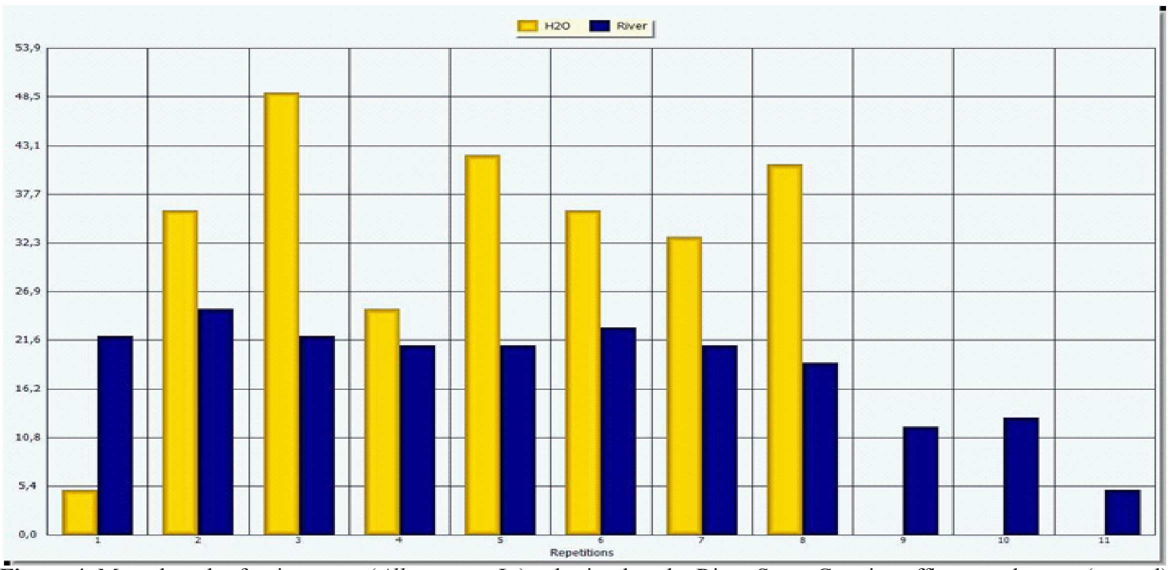

Figure 4. Mean length of onion roots (*Allium cepa* L.) submitted to the River Santa Catarina effluent and water (control) in the second collection: effluent submitted for eight days to aquatic plants.

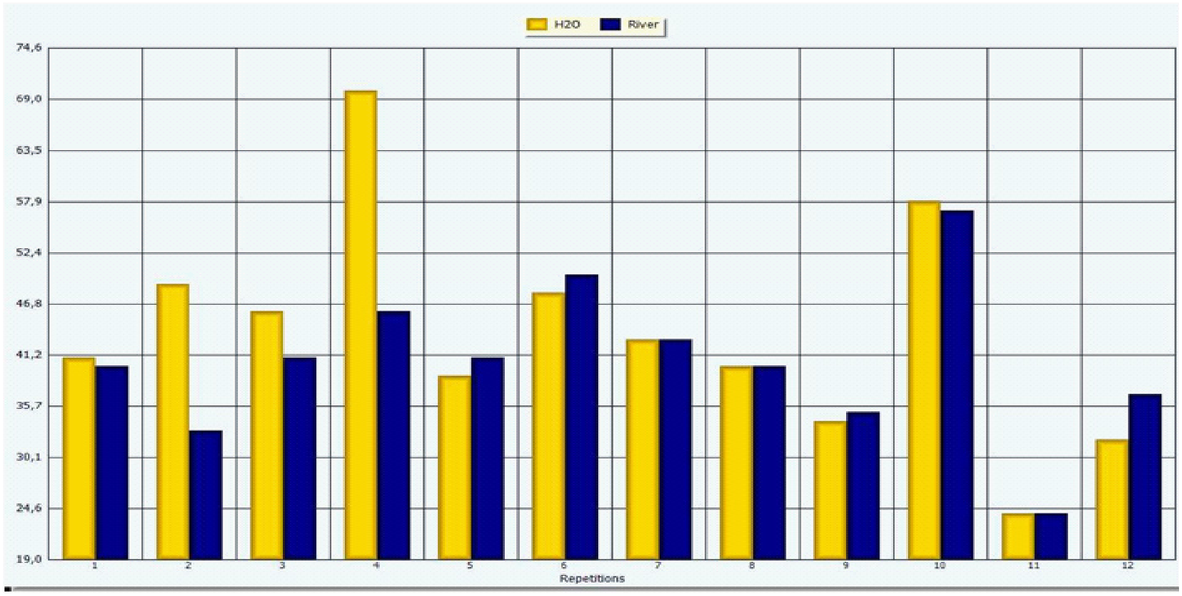

Figure 5. Mean length of onion roots (*Allium cepa* L.) submitted to the River Santa Catarina effluent and water (control) in the third collection: effluent submitted for fifteen days to aquatic plants.

The species *A. cepa* has been suggested due the high sensitivity, its roots are useful in biological assays because are the first to be exposed to chemical variations of water and soil (Fiskesjö, 1988; Grant, 1982).

As expected, in statistical and scientific hypothesis, there was no statistical difference among the length of roots submitted to tap water, according to the Student's t-test ($p < 0.05$).

When comparing only the data of River Santa Catarina effluent there are statistical difference ($p<0.05$ and $p<0.01$) according to the Student's t-test for the treatment T0 (Crude effluent) and T2 (Effluent submitted for fifteen days to aquatic plants) and T1 (Effluent submitted for eight days to aquatic plants) and T2, the statistical difference was not found between the treatment T0 and T1 ($p<0.05$) (Table 3 and Figure 6-9).

The statistical difference among the data suggests that the roots grew fifteen days after the effluent suffer filtering by the aquatic plants. The non-difference suggests that the roots still had similar average size, therefore, statistically non-differenced.

Table 3. Values of T-calculated ($p<0.05$ e $p<0.01$) for the River Santa Catarina effluent in three different moments: T0: Crude effluent; T1: Effluent submitted for eight days to aquatic plants; T2: Effluent submitted for fifteen days to aquatic plants, in the period from 09/01/2011 to 09/15/2011.

Treatment	River T0	River T2
River T0	-	
River T1	0.322^{ns}	7.202**

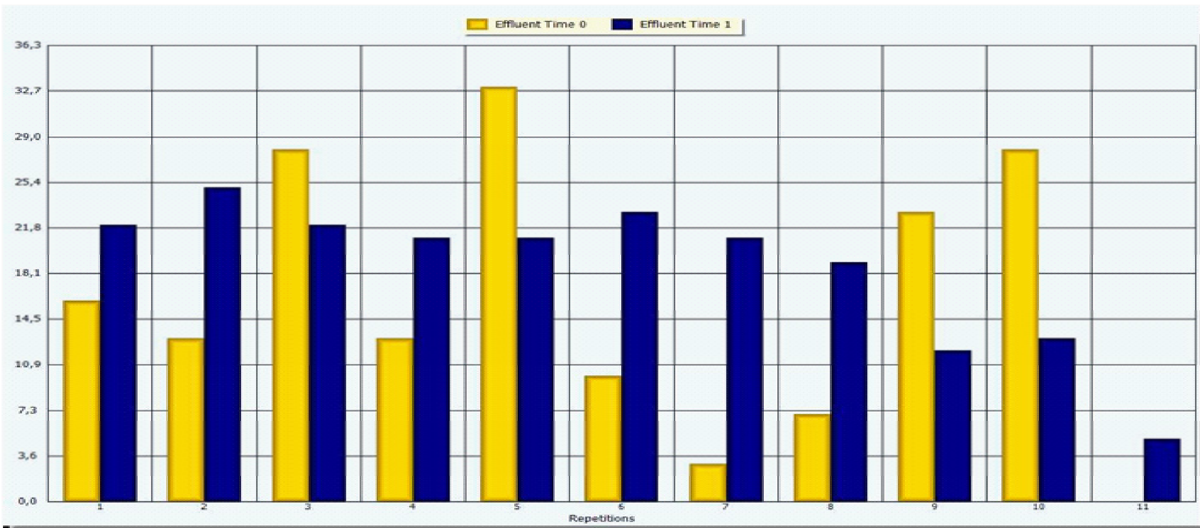

Figure 6. Mean length of onion roots (*Allium cepa* L.) submitted to the River Santa Catarina effluent in Time Zero (T0 = Crude effluent) and Time 1 (T1= Effluent submitted for eight days to aquatic plants).

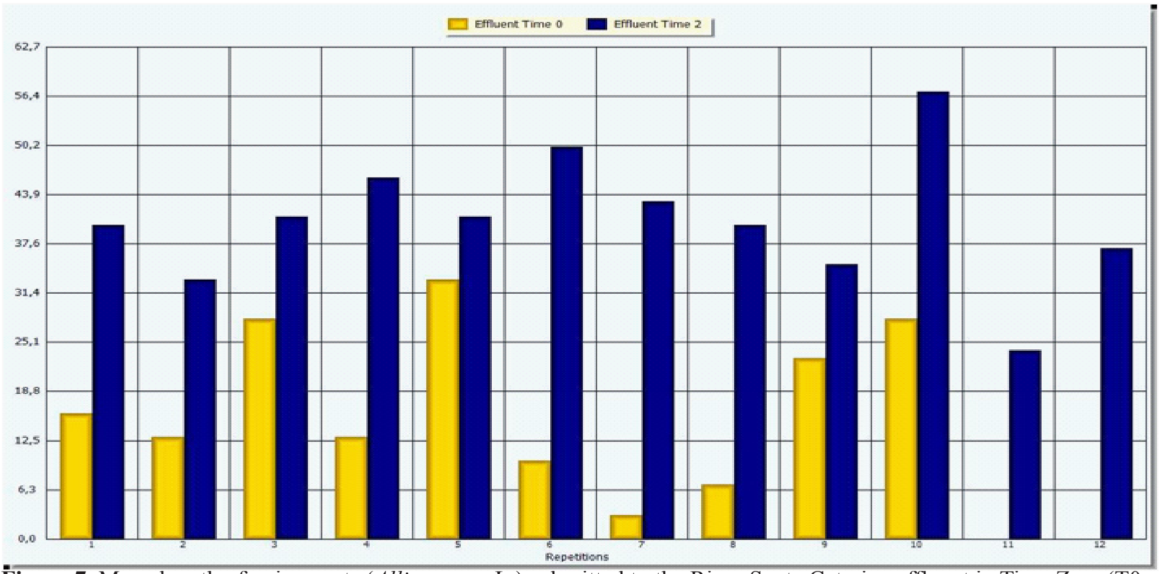

Figure 7. Mean length of onion roots (*Allium cepa* L.) submitted to the River Santa Catarina effluent in Time Zero (T0 = Crude effluent) and Time 2 (T2= Effluent submitted for fifteen days to aquatic plants).

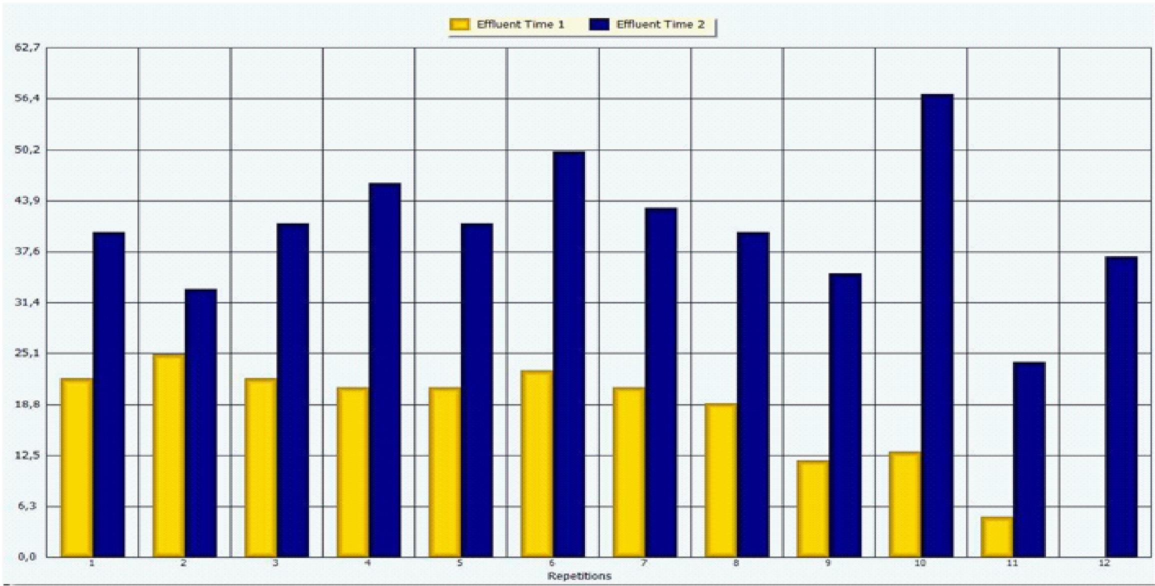

Figure 8. Mean length of onion roots (*Allium cepa* L.) submitted to the River Santa Catarina effluent in Time Zero e Time 1 (T1= Effluent submitted for eight days to aquatic plants) e Time 2 (T2= Effluent submitted for fifteen days to aquatic plants).

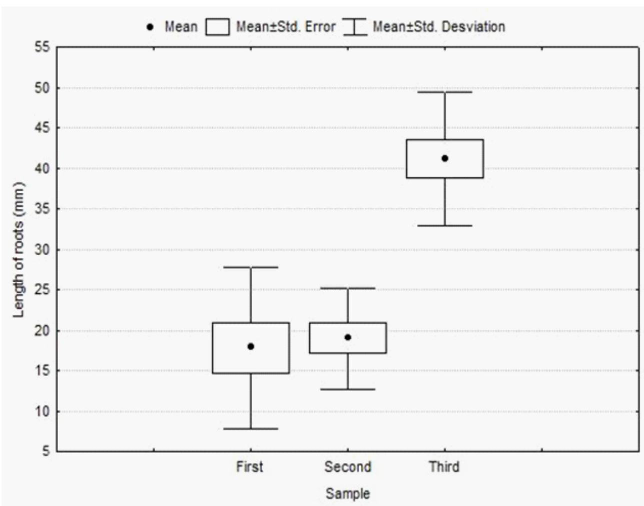

Figure 9. Mean, Standard Error and Standard Deviation of length of onion roots (*Allium cepa* L.) submitted to the River Santa Catarina effluent in Time Zero e Time 1 (T1= Effluent submitted for eight days to aquatic plants) e Time 2 (T2= Effluent submitted for fifteen days to aquatic plants).

Conclusion

Although there are many publications about the aquatic macrophytes and their importance in removing pollutants in Brazil, the research about the aquatic plant S. auriculata still is scarce, thus, this is one of the pioneering studies in this area.

This present study corroborate that S. auriculata is one of the promising species in removing pollutants and may be used in the decontamination of polluted urban effluents, being this one of the most important problems in these days, the water pollution.

However, it is necessary proper studies for the management of these aquatic plants, since they proliferate easily and may cause an even greater environmental impact.

The onions have eukaryote cells, therefore may confer higher degree of closeness to the aquatic biota exposed to pollutants, thus, being an effective assay for biomonitoring. The onion roots, being sensitive to several pollutants, show the efficiency of the aquatic plant *S. auriculata* after fifteen days, where showed equal growth to the tap water (control).

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