



REMOVAL OF AMMONIACAL NITROGEN FROM MUNICIPAL LANDFILL LEACHATE WITH FLOATING *Typha domingensis* (TYPHACEAE)

Eliminación del nitrógeno amoniacal del lixiviado de vertedero municipal con *Typha domingensis* (TYPHACEAE) en flotación

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ABSTRACT

A promising method for the treatment of effluents is the use of floating macrophytes. Ammoniacal nitrogen is a typical compound present in urban landfill leachates and its removal is important due its toxicity to several organisms. Therefore, the study evaluated *Typha domingensis* survival and nitrification potential artificially floating in domestic solid waste leachate. Plants were exposed for 35 days to leachate (100, 75 and 50 %) and to rainwater with N:P:K (control). Dissolved oxygen (DO) of the treatments was periodically measured, and ammoniacal nitrogen, nitrite and nitrate were analyzed before and after exposure. At the end of the experiment, plant survival rate was calculated. After two weeks, DO increased twice in the control, three times in 50 % leachate, four times in 75 % leachate, and eight times in 100 % leachate. At the end of the experiment, ammoniacal nitrogen was no longer detected, there was a significant reduction of nitrite, and a significant increase of nitrate in the treatments containing leachate. Plant survival was higher in those individuals exposed to 100 % leachate and decreased at lower leachate concentrations: 98 %, 94 %, 92 %, and 86 %. The study demonstrated that the ammoniacal nitrogen concentration of the leachate was not toxic to *T. domingensis*, and that it was efficient in the removal of this compound from the effluent, indicating that the species may be used artificially floating for the removal of this contaminant from domestic solid waste landfill leachate when in low concentrations.

Keywords: Cattail, macrophyte, nitrification, phytoremediation.

RESUMEN

Un método prometedor para el tratamiento de efluentes es el uso de macrófitas flotantes. El nitrógeno amoniacal es un componente típico de lixiviados de vertederos urbanos y su remoción es importante debido a su toxicidad para diversos organismos. Por eso, el estudio evaluó la supervivencia y el potencial de nitrificación de *Typha domingensis* flotando artificialmente en lixiviados de residuos sólidos domésticos. Las plantas fueron expuestas por 35 días a lixiviado (100, 75 y 50 %) y al agua de lluvia con N:P:K (control). El oxígeno disuelto (OD) fue medido periódicamente; el nitrógeno amoniacal, el nitrito y el nitrato fueron analizados antes y después de la exposición; al final del experimento, la tasa de supervivencia de las plantas fue calculada. Después de dos semanas, el OD aumentó dos veces en el control, tres veces en el lixiviado 50 %, cuatro veces en el lixiviado 75 % y ocho veces en el lixiviado 100 %. Al final del experimento, el nitrógeno amoniacal no fue más detectado, hubo una reducción significativa de nitrito y un aumento significativo de el nitrato en los tratamientos que contenían lixiviado. La supervivencia de las plantas fue mayor en aquellos individuos expuestos al lixiviado 100 % y se redujo en las menores concentraciones: 98 %, 94 %, 92 % y 86 %. El estudio demostró que la concentración de nitrógeno amoniacal del lixiviado no fue tóxica para *T. domingensis* y que ésta fue eficiente en la remoción del compuesto del efluente, indicando que la especie puede ser utilizada flotando artificialmente para la remoción del contaminante de lixiviados de residuos sólidos domésticos en bajas concentraciones.

Palabras clave: Enea, fitorremediación, macrófita, nitrificación.

INTRODUCTION

Solid waste landfill leachates are produced when moisture and rainwater infiltrating and percolating through landfill waste mix with the products of its decomposition (Souto, 2009; Barros, 2013). Leachate composition depends on several factors, such as the diversity and characteristics of the dump, the availability of water and oxygen, the way the landfill is operated, and its age (Muzaini *et al.*, 1995; Aziz *et al.*, 2004). Leachate from younger landfills generally exhibit higher concentrations of organic matter, presenting, for instance, high chemical oxygen demand (COD). Metals are often mentioned as part of the leachate composition; however, the metal content in leachates from old landfills (over 10 years old) is generally low, due to the methanogenic fermentation process in the landfill (Bozkurt *et al.*, 1999; Kulikowska and Klimiuk, 2008). Other common compounds are nitrogen (N) and phosphorus (P), nutrients used for biomass growth. Leachates from old landfills are rich in ammoniacal nitrogen derived from the hydrolysis and fermentation of nitrogenous fractions of biodegradable substrates and, thus, have a low concentration of dissolved oxygen (Kulikowska and Klimiuk, 2008; Wojciechowska *et al.*, 2010). It is therefore important to remove ammoniacal nitrogen from this type of effluent, considering its toxicity to many organisms, like plants (Bernard *et al.*, 1996; Clarke *et al.*, 2002; Arunbabu *et al.*, 2017), microalgae (Bernard *et al.*, 1996; Tam and Wong, 1996), crustaceans (Bernard *et al.*, 1996; Valencia-Castañeda *et al.*, 2018), and fish (Randall and Tsui, 2002; Zhang *et al.*, 2018). In addition, the release of this compound into natural water bodies may cause eutrophication, making it also toxic to the aquatic environment (Aziz *et al.*, 2004; Wojciechowska *et al.*, 2010). Clarke *et al.* (2002) observed that the growth rate of *Typha latifolia* was reduced when exposed to 200 mg L⁻¹ of ammonia; and Valencia-Castañeda *et al.* (2018) estimated that the safe levels of ammonia for the shrimp *Litopenaeus vannamei* at salinities of 1 and 3 g L⁻¹ were 0.54 mg L⁻¹ and 0.81 mg L⁻¹, respectively.

Due to the wide variety of landfill leachate composition, it is difficult to determine effective treatment techniques, and not necessarily the method adopted for a particular landfill will be applicable to others (Wojciechowska *et al.*, 2010). Among the methods used to treat urban landfill leachate, it is included physical, chemical and biological treatments. The most common biological treatment methods are the use of microbes (Mehmood *et al.*, 2009; Zhang *et al.*, 2016), and by constructed wetlands (Bulc, 2006; Yalcuk and Ugurlu, 2009; Ogata *et al.*, 2018; Silvestrini *et al.*, 2019). However, when the concentration of ammoniacal nitrogen is very high, direct biological treatment is not recommended, since, as already mentioned, in high concentrations this compound is toxic and inhibits the nitrification process (Aziz *et al.*, 2004).

Conventional physical and chemical methods of effluent treatments usually have a high cost considering the

treatment itself, as well as the construction and maintenance of the stations (Mojiri *et al.*, 2013; Pandey *et al.*, 2016). As an alternative, the use of aquatic macrophytes acts as a non-invasive and low cost method for the treatment of effluents. These plants are known for their ability to remove nutrients and for creating favorable conditions in the environment for organic matter degradation (Goldoni *et al.*, 2014). A promising method is the use of floating emergent macrophytes, where plants grow in a hydroponic environment, artificially floating in rafts rather than being rooted in the sediment (Headly and Tanner, 2012; Martelo and Borrero, 2012). This method offers the advantage of composing a system of simple operation and low maintenance to the treatment of effluents. Other advantages include the non-generation of sludge, the reduction of energy consumption by the improvement of solar energy, the disappearance of bad odors by their oxygenation, and also, it is not necessary to trim the plants, because the floating system naturally regenerates and the compounds incorporated to the plant return to water by the processes of decomposition of the vegetal tissue (Arcadia Ingeniería del Agua, Aire y Suelo, 2019). Besides, it has been understood that the assimilation of nutrients and other elements, such as metals, may be greater in this type of system when compared to systems in which the plants are rooted in the sediment (Headley and Tanner, 2012). This happens because the roots of the plants are not in contact with the benthic sediment or soil, and thus, they are in contact only with the nutrients limited in the water column (Kadlec and Wallace, 2008). In fact, Zhang *et al.* (2014) obtained better results for BOD₅, COD, nitrate, total nitrogen and total phosphorus in floating macrophyte systems compared to other constructed wetland systems.

Typha species are efficient plants for the removal of ions from effluents compared to other macrophytes due to their aerated internal structure formed by tissues with open spaces, allowing better absorption of pollutants, optimum evapotranspiration, and the transport of oxygen from the atmosphere to the leaves and from the leaves to roots and rhizome, besides its great tolerance to toxic agents (Mannarino *et al.*, 2006; Hegazy *et al.*, 2011; Barros, 2013; Mojiri *et al.*, 2013). Song *et al.* (2018) observed that *Typha angustifolia* stood out with the highest potential for landfill leachate remediation compared to other species (*Phragmites australis* and *Scirpus tabernaemontani*): in the end of six weeks of treatment, *T. angustifolia* showed great biomass and high accumulation of sodium, magnesium, phosphorus, manganese, iron and zinc. Therefore, the authors conclude that the species should be preferentially used in phytoremediation of landfill leachate.

Typha domingensis Pers., known commonly as southern cattail, is found throughout temperate and tropical regions worldwide, occurs in a wide variety of aquatic habitats from wetland to totally submerged environments, and has been used to treat the most varied types of effluents (Hegazy *et al.*, 2011;

Barros, 2013; Gomber *et al.*, 2013; Schierano *et al.*, 2016), but few studies used the species artificially floating (Mojiri *et al.*, 2013; Goldoni *et al.*, 2014; Luca *et al.*, 2019).

Considering that the reduction of ammoniacal nitrogen concentration is often the most important and the most difficult objective to be achieved in effluent treatments, the study aimed to evaluate the survival and the nitrification potential of *Typha domingensis* artificially floating in different concentrations of domestic solid waste leachate.

MATERIALS AND METHODS

Leachate was collected in plastic vials from the wastewater outfall of a deactivated domestic solid waste landfill of a southern city in Brazil, at a depth of 15 to 30 cm below the surface. All vials were stored in Styrofoam box with ice until analysis in the laboratory.

Collection, storage, preservation and transport of the samples were performed according to the criteria established by the Brazilian Association of Technical Norms (ABNT/NBR 9898/1987) and Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 2012). Leachate samples and the control (rainwater with N:P:K [1g L⁻¹]) were analyzed in triplicate for ammoniacal nitrogen by titration; nitrite and nitrate by visible UV spectroscopy, according to criteria established by the Standard Methods for the Examination of Water and Wastewater (APHA *et al.*, 2012). These analyzes were repeated after the plants were exposed to the treatments.

Typha domingensis individuals of one year of age were collected from a cultivation site where they were artificially floating in a nutrient solution (tap water with N:P:K [1 g L⁻¹]). The plants were transferred to plastic containers containing 3 L of each treatment: leachate 50 % (leachate in 50 % dilution with rainwater), 75 % (leachate in 75 % dilution with rainwater) and 100 % (raw leachate), and control. Ten containers were used for each treatment, with five plants in each container, according to the scheme presented in

figure 1. The plants remained in flotation for a period of 35 days and twice a week the solution in each container was stirred to avoid decantation of material. The experiment was developed in a greenhouse to avoid influence of rainfall. Immediately before plant exposure, at 14, 28 and 35 days, dissolved oxygen (DO) and pH were measured in each of the treatments using a multi-parameter meter AK88 (AKSO®). To verify the tolerance of the individuals of *Typha domingensis* exposed to the different treatments, their survival rate after the exposure was calculated.

Data normality was evaluated using the Shapiro-Wilk test. As the data met the assumptions of normality the analysis of variance (ANOVA) was performed and the mean values of nitrate, nitrite, ammoniacal nitrogen, DO and pH in the treatments and between treatments were compared by the Duncan test, at 5 % probability. For the analysis of nitrate, nitrite and ammoniacal nitrogen means in each treatment, before and after the experiment the Student's t-test of paired samples (5 % probability) was applied. All tests were performed using the SPSS software, version 20.

RESULTS

Before the exposure of the individuals of *Typha domingensis* to the treatments, the pH of the solutions was neutral, between 7.2 and 7.4. During the experiment, the pH of the leachate samples increased to alkaline (mean of 8.25), while the control's pH remained neutral. When comparing between dates, there was a great variation of pH in all treatments (Fig. 2). DO of the different leachate concentrations and control differed significantly among all treatments before plant exposure, when the control showed the highest value (10.5 mg L⁻¹) and the concentration 100 % the lowest value (3.2 mg L⁻¹). After only two weeks of exposure, DO of the treatments increased twice in the control, three times in 50 % leachate, four times in 75 % leachate, and eight times in 100 % leachate. At 28 and 35 days, there was no significant difference between the treatments, and comparing the

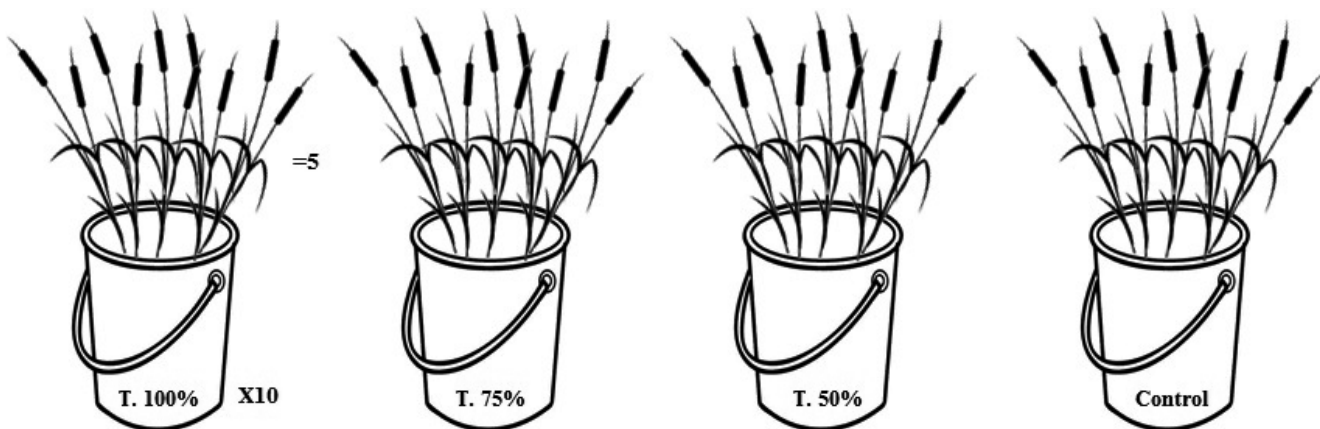


Figure 1. Scheme of the exposure of *Typha domingensis* individuals to different concentrations of leachate (treatments: 100 %, 75 %, and 50 %) and to control.

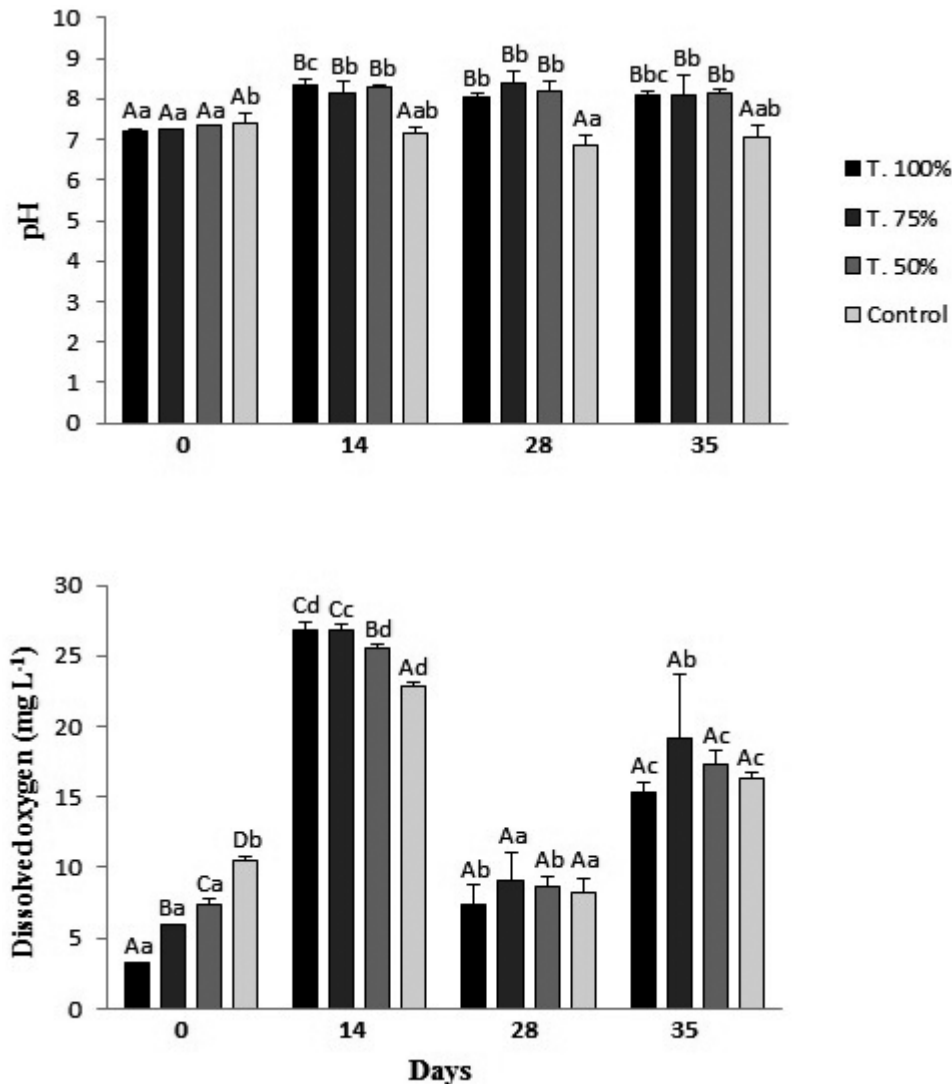


Figure 2. pH and dissolved oxygen mean values of the different treatments during the exposure period of *Typha domingensis*. Different upper-case letters indicate significant difference between treatments and different lower-case letters indicate significant difference between days for the same treatment, according to the Duncan test, at 5% probability. DO detection limit: 0 mg L⁻¹.

same treatment over time, DO concentration presented a significant difference in all the measurements in the control and in 50 and 100% leachates (Fig. 2).

Before the exposure of *Typha domingensis* to leachate concentrations and control, all treatments showed significant differences between each other on ammoniacal nitrogen concentrations, and these values were decreasing from the highest concentration of leachate to the lowest, and it was not detected in the control. At the end of the experiment, in all treatments this compound was no longer detected (Table 1). Nitrite concentration was significantly higher in the 100% leachate compared to the other treatments before plant exposure, and it was not detected by the analytical method in the control. In all treatments containing leachate, there was a significant decrease in nitrite concentration after plant

exposure. At 35 days, the compound remained undetected in the control, and in 100% leachate its concentration was again higher in relation to the other treatments (Table 1). At the beginning of the experiment, treatments 75 and 100% presented higher nitrate concentrations than the control and did not differ significantly from each other. After the exposure of *Typha domingensis*, as leachate concentration decreased, so did nitrate concentration, and all treatments differed significantly from each other. The treatments containing leachate presented concentrations significantly higher at the end of the experiment in relation to the beginning (Table 1).

Survival rate at the end of the experiment was higher in those individuals of *Typha domingensis* exposed to the 100% treatment and decreased as lower concentration of leachate: 98%, 94%, 92%, and 86% (control) (Fig. 3).

Table 1. Nitrate, nitrite and ammoniacal nitrogen concentrations in the different treatments before and after *Typha domingensis* exposure. Different letters in the line indicate a significant difference between treatments, according to the Duncan test, at 5 % probability. Asterisks in the column indicate a significant difference in each treatment at the end of the experiment in relation to the beginning, according to the paired Student's t-test, at 5 % probability.

		T. 100%	T. 75%	T. 50%	Control	Detection limit	
Nitrate	Before exposure	0.252 ± 0.0084 b	0.242 ± 0.0054 b	n.r.	0.078 ± 0.0002 a	0.078	mg L ⁻¹
	After exposure	2.325 ± 0.0880 d *	1.152 ± 0.2020 c *	0.829 ± 0.1050 b	0.407 ± 0.1510 a		
Nitrite	Before exposure	0.289 ± 0.031 c	0.206 ± 0.012 b	0.225 ± 0.037 b	n.d. a	0.002	mg L ⁻¹
	After exposure	0.079 ± 0.017 c *	0.046 ± 0.004 b *	0.042 ± 0.005 b *	n.d. a		
Ammoniacal nitrogen	Before exposure	36.30 ± 1.47 d	28.23 ± 3.26 c	18.10 ± 0.10 b	n.d. a	5.00	mg L ⁻¹
	After exposure	n.d. *	n.d. *	n.d. *	n.d.		

n.r.: not rated; n.d.: not detected by the analytical method.

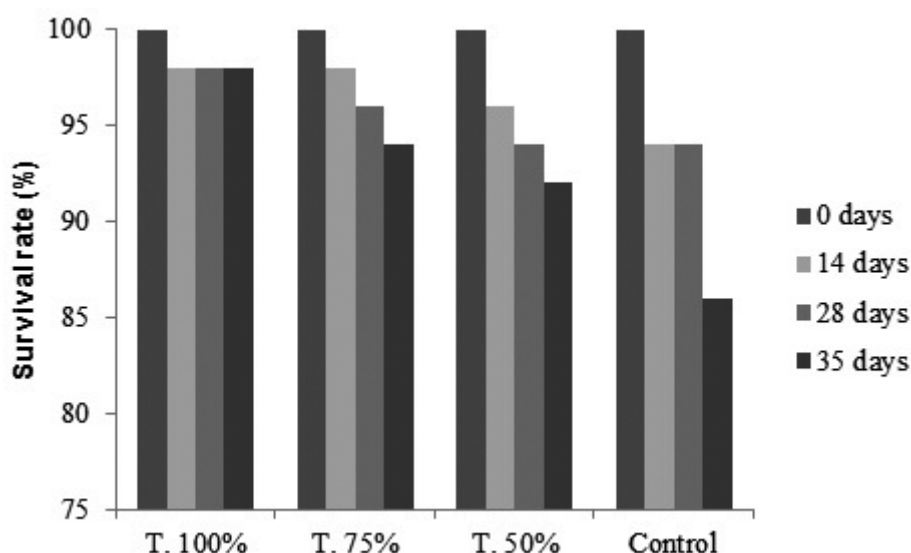


Figure 3. Survival rate of *Typha domingensis* individuals exposed to the different treatments throughout the experiment.

DISCUSSION

Several factors can lead to changes in a solution's pH, such as the chemical components present in the solution, temperature and gas exchange with the atmosphere (Oliveira, 2007). In addition, the biological activity stimulated by the action of living organisms, such as bacteria and plants, influences the maintenance of the organization of the solution constituents. Plant roots, for example, can change the pH of the environment in which they are inserted (Moreira and Siqueira, 2002). As observed, after plant exposure the pH of the treatments increased only in those containing leachate, evidencing that the rhizome of the plants, together with the components present in the effluent, created conditions for the development of biological activity and consequent pH alteration.

The DO results indicate that the species was effective in transporting oxygen to the environment by its root system, as stated by Mannarino *et al.* (2006), Andrade *et al.* (2007), and Sales Filho *et al.* (2015). *Typha* plants have their internal

structure formed by tissues with open spaces through which oxygen transport occurs: from the atmosphere to the leaves, from these to the root system, and then to the area around the rhizosphere, creating conditions for organic matter decomposition and for the growth of nitrifying bacteria (Mannarino *et al.*, 2006). Oxygen is consumed during the process of organic matter degradation per chemical and biochemical oxidation due to respiration of microorganisms (Valente, 1997; Bulc, 2006; Mannarino *et al.*, 2006), which may explain the drop in DO levels in all treatments observed at 28 days.

At the end of the experiment, ammoniacal nitrogen was no longer detected in all treatments. Thus, 35 days were sufficient for ammoniacal nitrogen removal on 30 L of raw leachate with 50 individuals of *Typha domingensis* in flotation. This result corroborates with several studies that have also demonstrated that *Typha* species are efficient in the removal of ammoniacal nitrogen from many effluents (Mannarino *et al.*, 2006; Escosteguy *et al.*, 2008; Yalcuk and Ugurlu, 2009;

Barros, 2013; Mojiri *et al.*, 2016; Mojiri *et al.*, 2017). Beltrão *et al.* (2005) also recorded higher nitrate concentrations at the exit than at the entrance of a biochemical system for the treatment of sanitary landfill leachate with the presence of *T. domingensis*, and Aluko and Sridhar (2014) also observed reduction of ammoniacal nitrogen and increase of nitrate in different chemical and biological combinations of solid waste landfill leachate treatment. Nitrogen is a constituent of proteins, chlorophyll and many biological components, and in organic matter from landfill leachate, it is broken down into amino acids and then reduced to ammoniacal nitrogen. In the presence of DO, ammoniacal nitrogen is oxidized to nitrite and then to nitrate, which explains the increase of nitrate in the effluents (Aluko and Sridhar, 2014).

The process of oxidation of ammonia to nitrite and then to nitrate is called nitrification and occurs by the action of nitrifying bacteria that grow in aerobic medium (Barbieri *et al.*, 2014). According to the Office of Water Programs (OWP, 2009), nitrification is the most efficient process in reducing ammonia and it is mainly dependent on the availability of dissolved oxygen. In addition, the optimum pH for nitrifying bacteria is alkaline (> 8.0) (Zoppas *et al.*, 2016).

In the present study, it was also observed a decrease in the concentration of ammoniacal nitrogen to the point of being no longer detected, and of nitrite, and an increase in nitrate concentration. Although it was not possible to perform a correlation test between ammonia and nitrate due to the undetected values of ammoniacal nitrogen at the end of the experiment, these results indicate the occurrence of the process of nitrification, since there was also a considerable increase in the DO concentration in the treatments after plant exposure, and also an increase in pH to alkaline, which may have contributed to the growth of nitrifying bacteria in the treatments. The results still corroborate with Yalcuk and Ugurlu (2009), who observed 62.3 % of ammonia nitrification of landfill leachate by *Typha latifolia* in a horizontal flow wetland (plants rooted in sediment composed of layers of sand, zeolite, and gravel); with Mojiri *et al.* (2016), that removed 99 % of the ammonia from a landfill leachate mixture with municipal wastewater using *Typha domingensis* in a constructed wetland (plants rooted in sediment composed of zeolite, activated carbon, limestone, and rice husk ash); and with Mojiri *et al.* (2017), that used two systems in association (one with *Eichhornia crassipes* rooted in zeolite and cockle shell, and the other with *T. domingensis* rooted in powdered bentonite, zeolite and cockle shell), and observed an improvement in ammonia elimination efficiency from 87.7 % to 99.9 %. In fact, these plants do not only extract ions from the environment, but also promote the activity of microbial decomposers of organic and nitrifying compounds, favoring effluents remediation processes since the initial moment of their cultivation (Chen *et al.*, 2015; Ashraf *et al.*, 2018). The greater the oxygenation in effluent treatment systems with plants, more efficient the process of

pollutants biodegradation, because the transfer of oxygen from the plants to the solution stimulates the density, diversity and consequent activity of the microbiological community (Zhang *et al.*, 2014).

Survival rate results indicate that the species is tolerant to the 100 % leachate conditions of this experiment, which is an indication of the possibility of its use in the treatment of this effluent without the necessity of its dilution, corroborating with Wojciechowska *et al.* (2010), who also demonstrated that it is possible to use macrophytes directly in raw domestic waste leachate. In a study to examine the effects of ammonia on emerging macrophytes, Clarke *et al.* (2002) observed that *Typha latifolia* reached maximum biomass at 84 mg L⁻¹ concentration of ammonia and had minimal biomass and lower growth rate at 0 mg L⁻¹ concentration. In addition, the growth rate of *T. latifolia* was also reduced when exposed to 200 mg L⁻¹ of ammonia.

As observed in the present study, *Typha domingensis* plants exposed to the control, where the concentration of ammoniacal nitrogen was not detected by the analytical method, presented the lowest survival rate, and the plants exposed to 100 % leachate had the highest survival rate, where the concentration of ammoniacal nitrogen was 36.3 mg L⁻¹. This indicates that, at this concentration, ammoniacal nitrogen was not toxic to the plants, but on the contrary, it was important for the survival of the individuals.

Arunbabu *et al.* (2017) studied the effects of domestic solid waste landfill leachate on *Vigna unguiculata*. The plants were irrigated with different concentrations of leachate (0, 0.5 %, 1 %, 2 %, 5 %, 10 %, 25 %, 50 %, and 100 % v/v). Above 5 % concentration, root and shoot growth of plants was significantly inhibited, and at 25 % concentration, plants did not survive up to the eighth week. According to the authors, these results suggest that diluted leachate may have produced a positive effect on plant growth, and that the high concentration of ammoniacal nitrogen (2.240 mg L⁻¹ in the raw leachate) showed toxic effects on plants. When ammoniacal nitrogen presents very high concentrations, it is recommended to dilute the leachate, or to perform a pretreatment before plant exposure, thus emphasizing that the method adopted for the treatment of the effluent of a particular landfill will not necessarily be applicable to others.

CONCLUSIONS

The present study demonstrated that *Typha domingensis* was responsible for the increase of pH in the different concentrations of leachate to the point of making it alkaline. Also, the use of the species artificially floating proved to be efficient in the removal of ammoniacal nitrogen, possibly by the transport of oxygen to the effluent through its roots, which consequently created conditions for the occurrence of the nitrification process.

The species is tolerant to the 100 % leachate conditions of this experiment, which was evidenced by the high survival of plants, and so, it is possible the use of *Typha domingensis* in the treatment of this effluent without the necessity of its dilution. However, at higher concentrations of ammoniacal nitrogen than the concentration existent in the effluent of the present study, it is recommended to dilute the leachate or to perform a pretreatment on plant exposure due to ammonia toxicity to living organisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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