ORIGINAL RESEARCH

Evaluation of the sensitivity and production of flowers in *Tagetes erecta L*. exposed to high doses of sodium from irrigation with landfill leachates

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Abstract

Purpose Reuse of landfill leachate is an effective alternative for their nutrients to mitigate decrease in freshwater. On the other hand, the growth of vegetation in the final disposal areas provides many benefits such as improving the visual impact, controlling of hydric erosion, etc. The purpose of this work was to evaluate landfill leachate as irrigation water and source of nutrients for growth of *Tagetes erecta* L., an ornamental plant with phytoremediation capacities.

Method Vegetal growth, physiological responses and mineral elements uptake of the ornamental plant *Tagetes erecta* L. were studied with different levels of landfill leachate irrigation. The landfill leachate was provided by the municipal waste treatment. Experimental period covered 34 days of daily watering between the beginning and end of the flowering stage. Three different irrigation treatments were used: T1: 10% leachate; T2: 25% leachate; T3: 50% leachate. Hoagland's solution served as the control treatment.

Results The irrigation treatment with a dose of 50% leachate causes a clear deterioration in the plant and its flowers. In lower doses, the plant responds favorably to both the production of flowers and the main features of them. Also, the water-use efficiency (WUE) is diminished in those plants irrigated with the major dose of leachate.

Conclusion Maintaining controlled doses, landfill leachates can be used as an alternative source of water and nutrients. Irrigation with leachates of these characteristics should be done in dosages not higher than 25% to avoid possible damage of *Tagetes erecta* L. growth.

Keywords Alternative water sources, Water use efficiency, Plant growth, Anthocyanins, Landfill effluents, Nutrient recycling

Introduction

FAO (2015) and United Nations (2015) published that around 70% of water consumption is used for agriculture, estimating an increase of 19% by 2050. The use of unusual water resources such as raw or treated urban or industrial wastewater, landfill leachates may represent an optimal compromise between the need to produce renewable energy and conservation of water supply (Zema et al. 2012). Landfill leachate is defined as the liquid effluent generated from rainwater percolation through solid waste disposed in a landfill, as well as from the moisture present in the waste and the degradation products of residues (Salem et al. 2008; Costa et al. 2019). Landfill waste degradation occurs through various biological and chemical processes following four pathways: (1) initial aerobic phase; (2) anaerobic acidogenic phase; (3) methanogenic phase; and (4) stabilization phase (Mandal et al. 2017). Leachate produced during the decomposition of organic fraction of municipal solid waste is a complex mixture of various pollutants such as inorganic salts, organic compounds, nutrients and heavy metals. Consequently, the generated leachate is considered as a major pollution threat to the surface and groundwater, soil, environment and human health. In many of the developing countries, the con-

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tamination issue is more serious since most of the municipal solid waste (MSW) management facilities and landfills do not have leachate collection and treatment systems (Arunbabu et al. 2017). The quality of leachate depends on various factors such as the composition of waste, biological and chemical processes that occur during the degradation of waste, moisture content, rainfall, local climate, etc.; thus, it is site-specific (Kumar and Alappat 2005; Jones et al. 2006; Miao et al. 2019). Municipal waste leachate can be used as a liquid fertilizer mainly in calcareous soils due to the high amount of organic matter and plant nutrients. Other authors have evaluated the application of leachate as irrigation water in different crops and types of soil, generally obtaining positive results under applications of moderate concentrations of the leachate (Zalesny et al. 2008; Justin and Zupančič 2009; Singh et al. 2017; Yang et al. 2017; Miao et al. 2019). Since the leachate is highly saline in nature, its frequent high-dose application is not recommended for crops sensitive to salinity (Khoshgoftarmanesh and Kalbasi 2002). The application of MSW leachate on soil affects the physical and chemical properties of soil. Leachate application on plants imposes both positive and negative impacts on the vegetal growth (Cheng and Chu 2007) since the liquid can contain macro and micronutrients and trace elements. The reuse of this type of effluent for irrigation would reduce the use of freshwater and also could reduce the volume of effluent discharge in the watercourses (Pedrero et al. 2010; Bedbabis et al. 2015; Libutti et al. 2018). Furthermore, features of landfill leachates vary depending on the age of the landfill. For example, from the analysis of 64 landfills, Jones et al. (2006) found that COD varied between 2,740 and 152,000 mg L⁻¹ in acidogenic landfills, and between 622 and 8,000 mg L-1 in those in methanogenic phase. Furthermore, parameters such as trace metal content and nutrients tended to decrease in the older landfills (methanogenic phase). In general, the leachate from the methanogenic stage differs from the previous one, with a lower content of COD, BOD, NH⁺ and a higher pH. Considering the high pH, low COD and content of heavy metals in the leachate studied in this investigation, it could be said that the landfill was in a methanogenic stage. The electrical conductivity of the fillings surveyed in this stage varied between 5.9-19.3 mS cm⁻¹ (Jones et al. 2006). These values are a sample of the salinity problem, frequent in these effluents.

The most studied species for the phytoremediation of final disposal sites are those considered native (Song 2018; Vaverková et al. 2018). The physicochemical properties and organic matter concentration of landfill soils are generally poor for plant growth (Kim and Owens 2011). The plant species used in this type of soils must be characterized by their tolerance to heavy metals, infertile soils, low drainage and compact soil. On the other hand, the possibility of using leachate as a source of nutrients makes it necessary for the plant to be tolerant to its frequent usage and exposition to its particular features, such as high salinity (Nagendran et al. 2006). Another important feature of the plants used for phytoremediation is their ability to generate large amounts of biomass (Ali et al. 2013; Kumar Yadav et al. 2018). Tagetes erecta L. is an ornamental species of the family Asteraceae (Compositae) (Lorenzi and Souza 2001). Tagetes sp. are also of interest as sources of biologically active compounds (Vasudevan et al. 1997) and natural food colorants (Barzana et al. 2002). In this context, the pigment extracted from T. erecta L. is used in the preparation of vegetable oils, pasta, bread, juices, mustard and milk derivatives (Delgado-Vargas and Paredes-Lopez 2003), and is added to chicken feed in order to intensify the yellow coloration of broiler flesh and egg yolks (Martínez et al. 2004). It is a stout, erect and branching annual ornamental plant, 60-90 cm tall with the flower color ranging from bright yellow, brownish yellow, orange to brown (Pratheesh et al. 2009). Moreover, it is a plant capable of producing flowers of commercial importance and has been reported as a species with the possibility of use in phytoremediation technology. Thus, it can be grown on contaminated soils for phytoremediation as well as floriculture (Chitraprabha and Sathyavathi 2018; Kumar Yadav et al. 2018). Its high biomass production, its well-developed root system, and its ability to tolerate and accumulate a wide range of heavy metals in its aerial and harvestable parts make it favorable for use in phytoremediation (Pal et al. 2013; Coelho et al. 2017; Chitraprabha and Sathyavathi 2018). Furthermore, it has been reported that it can tolerate poor and saline soils, features that may be present in contaminated soils (Vasudevan et al. 1997; Coelho et al. 2017). On the other hand, being an ornamental crop due to the interest of its flowers reduces the risk of contamination of the food chain (Pal et al. 2013; Chitraprabha and Sathyavathi 2018).

By incorporating shrubs, floral species, etc., the visual impact is diminished and the social acceptance of final disposal sites is promoted. Thus, *Tagetes erecta* L. could be used as an ornamental plant and in turn decontaminate urban areas due to its ability to perform phytoremediation by phytoextraction. The main purposes of the present study include: (1) to study the growth status of *Tagetes erecta* L. in response to landfill leachate irrigation; (2) to determine the impact of different doses of landfill leachate on the physiological response of *T. erecta* L., including photosynthetic pigments, soluble protein and soluble sugar; (3) to examine the influence of landfill leachate on the uptake of the main mineral elements; and (4) to determine the influence of landfill leachate irrigation on the flowering stage, particularly on flower quality and yield.

Materials and methods

Leachate characterization and analytical determination

Landfill leachate was collected at a landfill site of Santa Fe Province, Argentina. The treatment plant facilities include a stage of equalization and separation of solids, two anaerobic sequential reactors, an aerobic reactor, one final equalization tank and a chlorination treatment prior to its overturning in the body of water. Samples were taken downstream of the equalization tank before chlorination. Collected samples were stored at 4 °C and then brought to room temperature before use. Nitrogen content was determined using the Kjeldahl method (Kjeldahl 1883). Metals were extracted and then analyzed by atomic absorption spectrometry (AAS) following EPA Method 200.2 (Martin et al. 1994). The COD analyses were performed according to Eaton et al. (1995) using a HACH DR900 spectrophotometer. Phytotoxicity test on different doses of landfill leachate (100%; 50%; 25%; 10%) consists in a modified germination test (Fiasconaro et al. 2015) utilizing lettuce (Lattuca sativa L.) seeds. The use of lettuce seeds for the germination test (phytotoxicity) is based on their sensitivity to different factors that are toxic to the plant, thus determining the presence of phytotoxic substances (Tam and Tiquia 1994; Ling et al. 2010; Visioli et al. 2013).

Experimental design and plant determinations

The research was designed to assess the effect of different doses of leachate as irrigation water on ornamentals plants compared with a nutritive solution. The ornamental species selected were *Tagetes erecta* L. The experiment was performed during the flowering stage and lasted for 34 days. Dilutions of landfill leachate were made with ultrapure water. Three different irrigation treatments were used: T1: 10% leachate + 90% ultrapure water; T2: 25% leachate + 75% ultrapure water; T3: 50% leachate + 50% ultrapure water. Hoagland's solution (Hoagland and Arnon 1950) was used as the control treatment. Plants were cultivated in pots with soil and were watered daily with leachate dilutions or Hoagland's solution depending on the corresponding treatment (Table 1). The ornamentals plants were grown up in a glasshouse at 29 °C / 18 °C and 50% / 70% relative humidity (RH) (day/night). The photoperiod was 14 h under natural daylight. The principal cations were extracted following EPA Method 200.2 and then analyzed by atomic absorption spectrometry (AAS) (Martin et al. 1994). The total volume of irrigation leachate applied during the experiment was calculated according to the treatments in which the ornamental plants were developed.

Plants were harvested after 34 days of irrigation with landfill leachate for the determination of growth and yield parameters. Leaves and flowers were carefully separated, weighed and stored at -80 °C until analysis. Leaf chlorophylls were extracted in 95% (v/v) ethanol at 80 °C for 10 min and their concentration was quantified by measuring absorbance in a spectrophotometer (Perkin Elmer. Lambda 35. UV/VIS). Estimation of total chlorophylls was done by using the extinction coefficients and equations described by Lichtenthaler (1987). Leaf total soluble proteins (TSP) were measured by the protein dye-binding method (Bradford 1976) using bovine serum albumin as a standard. Leaf total soluble sugars (TSS) were analyzed using anthrone, according to Yemm and Willis (1954).

Roots, stems, and leaves dry matter (DM) were determined by drying samples at 85 °C to constant mass. The dry matters of the different plant organs were used to calculate several parameters as described by Ryser and Lambers (1995) and Correia et al. (2010): relative leaf weight ratio (LWR; leaf DM per unit plant DM), stem weight ratio (SWR; stem DM per unit plant DM), and root weight ratio (RWR; root DM per unit plant DM). The total plant dry matter (DM) was calculated as the DM sum of the leaves, stems, and roots.

Flower quality and yield

The measurements of flower quality included β -carotene and anthocyanin content. Fresh samples of flow-

Parameters		Initial Leachate	T1	T2	Т3
pН		8.62	7.68	7.57	7.50
EC	mS cm ⁻¹	8.65	1.27	2.34	4.64
COD	$\mathrm{mg}\mathrm{O}_{2}\mathrm{L}^{\text{-1}}$	458.37	45.84	114.59	229.18
GI	%	14.66	102.37	98.58	47.09
$\mathbf{N}_{kjeldhal}$	%	nd	nd	nd	nd
Κ	mg L ⁻¹	397.50	45.62	99.59	195.78
Na	mg L ⁻¹	626.50	63.00	157.36	314.50
Mg	mg L ⁻¹	104.50	11.00	27.10	51.75
Ca	mg L ⁻¹	52.50	4.95	14.50	27.52
Fe	mg L ⁻¹	0.07	0.01	0.02	0.04
Cu	μg L-1	0.14	0.01	0.08	0.10
Ni	μg L-1	2.11	0.22	0.66	1.11
Pb	μg L-1	0.71	0.07	0.18	0.42
Cr	μg L ⁻¹	3.25	0.30	0.90	1.75
Cd	μg L ⁻¹	0.10	0.01	0.03	0.05
As	μg L-1	0.17	0.02	0.05	0.08

Table 1 Main properties of initial leachate (100%) and different leachate dilutions used as irrigation water

T1, 10% leachate+90% ultrapure water; T2, 25% leachate+75% ultrapure water; T3, 50% leachate+50% ultrapure water. EC: electric conductivity; GI: germination index; COD: chemical oxygen demand. nd: not detected.

ers were homogenized using a pestle and mortar in the presence of liquid N2. β-carotene contents were calculated according to the Nagata and Yamashita (1992) equations. β -carotene were finally expressed as mg kg⁻¹ DM (Fiasconaro et al. 2015). Anthocyanins were determined by the pH-differential method (Giusti and Wrolstad 2001) according to Sutharut and Sudarat (2012) with some modifications. The buffers utilized were (a) pH 1.0 buffer (potassium chloride, 0.025 M) and (b) pH 4.5 buffer (sodium acetate, 0.4 M) as described by Lee et al. (2005). The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and rapid measurement of the total anthocyanins, even in the presence of polymerized degraded pigments and other interfering compounds (Sutharut and Sudarat 2012).

All flowers from each plant were detached to calculate yield. The number of flowers was recorded and flower DM was obtained after drying at 60 °C for 10 days.

Water-use efficiency and volume of leachate used

Water-use efficiency (WUE) for flower yield was calculated as the ratio between flower DM and the volume of irrigation water applied during the experimental period. WUE was expressed by grams of flower DM per dm³ (Baigorri et al. 1999).

Statistical analysis

The experiment had a completely randomized block design, and the values obtained for each plant and each variable were considered as independent replicates. Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA). Data were submitted to an analysis of variance (ANOVA). Means \pm standard errors (S.E.) were calculated and, when the F ratio was significant, the least significant differences were evaluated by a Tukey's t-test (p < 0.05).

Results and discussion

Leachate properties and their use as irrigation water

It is known that the use of wastewater as an irrigation alternative is a practice that is increasingly used due to water scarcity worldwide. Particularly, Fornes et al. (2007) stated that the main constraint to ornamental plant production is water consumption. To produce 1 kg of ornamental plant dry matter, 100-350 kg of water is needed depending on the species and grown conditions. The use of landfill leachates has been studied for their use as irrigation water (Li et al. 2017; Singh et al. 2017). As a result of this practice, an increase in plant growth has been documented due to the presence of beneficial macro and micronutrients for plants growth (Singh and Agrawal 2010).

Results presented in Table 1 show the characterization of leachate and the dilutions used in the experiment. Initial leachate (100%) exhibited a high concentration of potassium (K) and sodium (Na) and a low concentration of trace elements. The germination index (GI) indicated a high phytotoxicity on 100% leachate. When the percentage of water increases for the leachate dilutions used at the different treatments, the concentration of chemical elements decreased while the GI augmented.

GI performed with lettuce seeds provided an estimate of the phytotoxicity of landfill leachate. Plants can be protected from growth inhibition when the leachate irrigation plan is designed with reference to phytotoxicity data (Cheng and Chu 2007, 2011). The phytotoxicity (lower GI) could be primarily attributed to the excessive amount of salts present on the undiluted leachate. As the leachate dose was increased within the irrigation treatments, GI decreased (GI_{T1}>GI_{T2}>GI_{T3}). Electrical conductivity (EC) of 4 mS cm⁻¹ would inhibit seed growth by impeding water uptake in salt sensitive plants (Bewley and Black 1994). According to Cheng and Chu (2007), the GI decreases with the increase of the EC in leachate causing damage to the growth of the root length of the species selected to perform the phytotoxicity test. The most widely used phytotoxicity test involving terrestrial plants is the seed germination test, which measures germination rate and/or root elongation as the end points (Pascual et al. 1997; Cheng and Chu 2007). Like Cheng and Chu (2007), an attempt was made to establish the most appropriate leachate irrigation doses based on the obtained phytotoxicity data. From the analyses carried out, it was determined that our leachate presented a high electrical conductivity mainly due to its high concentration of Na (Table 1) and an alkaline pH indicating the mature to old stage of the dumping site (Jorstad et al. 2004). Addition of Na⁺, another prevalent ion in landfill leachate, without the concomitant increase in Ca²⁺ has been shown to cause specific damage to cell membranes (Cramer et al. 1985).

Likewise, Table 2 shows the volume used as irrigation water and their dilutions. The volume of applied irrigation water (leachate dilutions and Hoagland's solution) during 34 days of experiment was the same in all treatments (1.25 L per plant). Finally, the water use efficiency calculated on flower yield basis (WUE) was lower in *T. erecta* L. grown at treatment T3.

	Irrigation water	Total Leachate	Leachate daily	WUE	
	applied (L plant ⁻¹)	(L plant ⁻¹)	(cm ³ plant ⁻¹)	(g of flower DM/ dm ³ of water applied)	
Treatments					
Control	1.25	0	0.0	1.24±0.07a	
T1	1.25	0.125	3.7	1.13±0.1a	
T2	1.25	0.312	9.2	1.4±0.13a	
Т3	1.25	0.625	18.4	0.69±0.14b	

Table 2 Volume of irrigation water applied, concentration of leachate utilized and water use efficiency in each treatment of *Tagetes erecta* L.

Plants watered with different leachate dilutions, T1, 10% leachate+90% ultrapure water; T2, 25% leachate+75% ultrapure water; T3, 50% leachate+50% ultrapure water. Control: Hoagland's solution.

Values represent means (n = 9). Within each column, means followed by different letters are significantly different ($p \le 0.05$) according to a Tukey's test. DM: dry matter.

Plant features and flower quality

Growth features of *Tagetes erecta* L. are shown in Table 3. Results showed that the plant dry matter (DM) production of ornamental plants fertilized with 50% leachate (T3) was significantly reduced, but the leaf weight ratio (LWR) was increased in comparison to the other treatments. However, the root/aerial part, stem and root dry weight ratios (SWR, RWR) remained unchanged under the different fertigation treatments (Table 3). Biochemical analysis performed in leaves showed that the total chlorophyll, carotenoid and leaf TSS concentrations were decreased in T3 compared to the other treatments. By contrast, the TSP concentrations on leaves increased in ornamental plants grown at T1 (Table 3).

Table 3 Main features of *Tagetes erecta* L. plants watered with different leachate dilutions

Parameters		Control	T1	T2	Т3
Plant DM	g plant -1	4.36±028a	4.11±0.36ab	4.22±0.27ab	3.16±0.29b
Root/ aerial part		3.76±0.31a	3.53±0.48a	4.12±0.43a	4.28±0.72a
LWR		0.29±0.01b	0.29±0.02b	0.28±0.01b	0.38±0.03a
SWR		0.21±0.01a	0.19±0.01a	0.19±0.01a	0.17±0.02a
RWR		0.14±0.01a	0.17±0.03a	0.12±0.01a	0.18±0.03a
Leaf chlorophylls	mg $g^{-1}DM$	16.29±1.30a	15.89±1.82a	14.94±2.25a	4.70±0.95b
Leaf Carotenoids	mg $g^{-1}DM$	2.46±0.18a	2.48±0.24a	2.30±0.30a	0.86±0.17b
Leaf TSP	mg g ⁻¹ DM	6.51±1.00b	14.08±2.00a	6.97±1.08b	3.30±0.38b
LeafTSS	mg g ⁻¹ DM	30.11±2.52a	22.98±1.88ab	25.54±2.97ab	18.26±1.47b

T1, 10% leachate+90% ultrapure water; T2, 25% leachate+75% ultrapure water; T3, 50% leachate+50% ultrapure water. Control: Hoagland's solution.

Values represent means $(n = 9) \pm$ standard errors (SE). Within each file, means followed by different letters are significantly different $(p \le 0.05)$ according to a Tukey's test. DM: dry matter. LWR: leaf weight ratio; SWR: stem weight ratio; RWR: root weight ratio; TSS: total soluble sugars. TSP: total soluble proteins.

Likewise, yield, flower number and flower size (expressed as g FM flower⁻¹) were significantly decreased in treatment fertigation with 50% of leachate (T3) (Table 4). Fig. 1 shows the physical appearance of the plants at the end of the experiment for the different treatments. Regarding flower features as the pigmentation, results show that the β -carotene concentration was slightly improved in the T2 treatment (25% leachate) (Fig. 2). Nevertheless, the differences between treatments were more evident for anthocyanins, which were significantly affected by the presence of leachate in irrigation water in all cases.

In our case, the high EC seems to have affected not only the dry matter generation of *T. erecta*, but particularly its flower production (Table 3 and 4). Contrariwise, Singh et al. (2017) showed that the use of leachate as irrigation water on wheat favorably affected the plant growth by increasing the shoot and root length, the number of leaves and the harvest index. Land application of landfill leachate increases the quantity of macro and micronutrients in the soil, improving the soil productivity and crop yield (Khoshgoftarmanesh and Kalbasi 2002). It should be clarified that, in our study, the concentration of heavy metals in the leachate used was, in all cases, lesser than the limits required by the local dump legislation.

Our plants were exposed to different concentrations of sodium according to the leachate dose received as irrigation water. At the end of the experiment, and in disagreement to the publication of Bañón et al. (2012), there was no detected inhibitory effect on the root growth of *Tagetes erecta* (Table 3). It is well document-

	8	1		
	Yield	Flowers	Flowers size	
	(g flowers DM plant ⁻¹)	(n° plant ⁻¹)	(g FM flower ⁻¹)	
Treatments				
Control	1.55±0.09a	6.33±0.44a	9.95±0.46a	
T1	1.41±0.12a	6.00±0.73a	8.67±0.47a	
T2	1.76±0.16a	5.44±0.37a	10.04±0.51a	
Т3	0.86±0.17b	2.22±0.28b	1.66±0.37b	

Table 4 Yield and some flower features of <i>Tagetes erecto</i>	a L. plants watered with different leachate dilutions
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T1, 10% leachate+90% ultrapure water; T2, 25% leachate+75% ultrapure water; T3, 50% leachate+50% ultrapure water. Control: Hoagland's solution.

Values represent means (n = 9) \pm standard errors (SE). Within each column, means followed by different letters are significantly different (p ≤ 0.05) according to a Tukey's test. FM: fresh matter.



Fig. 1 Plants of *Tagetes erecta* L. irrigated with increasing doses of landfill leachate during 34 days

T1, 10% leachate+90% ultrapure water; T2, 25% leachate+75% ultrapure water; T3, 50% leachate+50% ultrapure water. Control: Hoagland's solution.

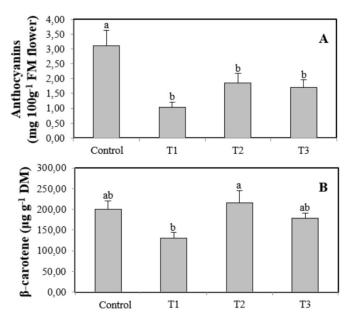


Fig. 2 Anthocyanin (mg $100g^{-1}$ FM flower) and β -carotene (µg g^{-1} DM) concentrations of *Tagetes erecta* L.

Plants irrigated with different leachate dilutions, T1, 10% leachate+90% ultrapure water; T2, 25% leachate+75% ultrapure water; T3, 50% leachate+50% ultrapure water. Control: Hoagland's solution. Values represent means (n = 9) \pm standard errors (S.E.). Histograms with different letter indicate that values differed significantly (p ≤0.05) according to a Tukey's test.

ed that, due to its direct exposure to salinity, the root is the most vulnerable organ of the plant subjected to salt stress, affecting the plant water absorption capacity, water use efficiency, etc. (Sánchez-Blanco et al. 2014). Although in our study any deleterious effect on root growth was not found, it can be observed a decrease in the DM of those plants exposed to the highest concentration of Na (treatment T3) as also indicated by Villarino and Mattson (2011). Our results show that plants watered with 50% leachate (T3) diminished WUE (Table 2). Several reports have shown that the exposure to saline stress in ornamental plants causes a reduction in plant height (Valdez-Aguilar et al. 2009; Zhang and Shi 2013). In our case, there were no significant differences between those plants irrigated with Hoagland's solution and with the different doses of landfill leachate (data not shown). In agreement with García-Caparrós et al. (2017), the proportion of biomass allocated in leaves (LWR) in *T. erecta* increased at higher Na levels (T3).

Regarding the flowers, some reports detailed that exposing ornamental plants to salinity stress can cause a decrease in the general growth of the plant and also a reduction of number, quality and fresh matter of flowers (Quist et al. 1999; Küçükahmetler 2002; Fornes et al. 2007; Trivellini et al. 2014). Also, under these conditions, ornamental plants may reduce the flowering intensity, bringing forward, delaying or shortening the flowering stage (Fornes et al. 2007; Álvarez et al. 2012). Correspondingly, our results show that plants irrigated with the T3 treatment diminished the number, fresh weight and yield of flowers (Table 4).

Salt stress affects photosynthesis resulting in stomatal limitations, causing a decrease in carbon assimilation. In the long term, the consequences of salt stress caused the accumulation of salt in the young leaves and the decrease in photosynthetic pigments concentrations (Acosta-Motos et al. 2017). It is recognized that chlorophyll and carotenoids concentrations in leaves directly correlate to the healthiness of plants (Barry 2009). A decrease in chlorophyll concentration under salt stress is a frequently reported phenomenon used as a sensitive indicator of the cellular metabolic state. This decrease may be related to membrane deterioration (Silveira and Carvalho 2016). Some studies have reported a reduction of the concentrations of chlorophylls and carotenoids in leaves of different plants subjected to saline conditions (Jaleel et al. 2008; Lee and Van Iersel 2008; Cantabella et al. 2017). Similarly, our data show that the T. erecta plants irrigated with the highest dose of leachate (T3) exhibited a decrease in chlorophylls and carotenoids in leaves (Table 3). Likewise, the plants grown with irrigation of 50% of leachate (T3) showed a lower concentration of TSS than those which were irrigated with 10% and 25% of leachate (Table 3). These results are in concordance with those published by Sami et al. (2016), who stated that sugars play a central role as osmoprotectants, taking part in the osmotic adjustment, carbon storage and radical scavenging under salt stress. The decrease in their concentration would indicate significant damage to the plant's metabolism. Therefore, the content of TSS can be used as a physiological indicator of salt tolerance evaluation (Liang et al. 2018). However, for the rest of the treatments, all these parameters behave similar to the control, which would indicate that at low doses, the leachate can be used as a source of water and nutrients.

Salt stress modifies the synthesis of secondary metabolites in plants (Zhu 2003). Specifically, anthocyanin content can be increased during the salt stress response or can be decreased in salt-sensitive plant species (Liang et al. 2018). Anthocyanins are primarily involved in a color-mediated attraction strategy in flowers (Stintzing and Carle 2004). Moreover, these pigmented flavonoids are considered a very important category of phytochemicals due to their strong antioxidant activity and other beneficial physicochemical and biological properties (De Pascual-Teresa and Sanchez-Ballesta 2008). Our study shows that plants irrigated with leachate reduced by approximately 50 percent the content of anthocyanins of flowers, whatever the dose applied respect to control (Fig. 2). Similarly, Trivellini et al. (2014) and Chrysargyris et al. (2018) reported that under saline stress, the content of anthocyanins was negatively affected on Hibiscus rosa-sinensis and Tagetes patula L., respectively.

The decrease in anthocyanin concentration would be related to the saline stress to which the plants that received leachate as irrigation water were subjected. Under conditions of saline stress, plants can show variations including different physiological disorders, among which the osmotic potential and the photosynthetic rate stand out. In addition, this stress can determine changes in the phenological features such as in flowers and in the accumulation of pigments in plant tissues (Borghesi et al. 2011; Ferrante et al. 2011). The decrease of anthocyanins in flowers subjected to salinity could be correlated with the decrease in plant growth and development due to the slowing down of metabolic processes. Likewise, anthocyanins could have already been involved in the antioxidant mechanisms of the plant in response to stress, causing a decrease in their content (Chrysargyris et al. 2018). However, Trivellini et al. (2014) stated that the positive or negative effects caused by saline stress will depend on the degree of tolerance of the plant and the saline concentrations to which it is subjected. Likewise, they affirmed that the mechanisms of action against saline stress are not entirely clear, especially the impact on the composition of the pigment and the expression of the color in flowers (Trivellini et al. 2014).

Respect to β -carotene concentration (Fig. 2), Fercha (2011) observed a decrease in chlorophyll and β -carotene content in salt-treated wheat. The decrease in chlorophyll and β -carotene was probably due to the inhibitory effect of salt on the accumulation of ions for the biosynthesis of the different chlorophyll pigments. A decrease in the chlorophyll level is only evident in the T3 treatment. It is not possible to say that there are real differences between the rest of the treatments, since the statistical analysis could not detect them. On the other hand, the decrease in chlorophylls in T3 could be related to the stress suffered by plants due to the high salinity of the irrigation water.

Plants exposed to T2 treatment showed an increase on β -carotene concentration, indicating that the inhibitory effect of salt on the biosynthesis of the different chlorophyll pigments was probably not very pronounced. This level of treatment results interesting because it maintains quality conditions for plants development while maximizing effluent reuse.

Plant elemental analysis

Table 5 presents the results obtained from the nutrients analysis of ornamental plants and soil at the beginning and at the end of the experimental period. The results showed that in general, all the elements increased their concentration at the end of the treatments in the plant and soil, although not always in a statistically significantly way. At the end of the experiment the results showed that, in the plants subjected to T3 treatment, the concentration of Na was significantly higher, but the plant Mg concentration decreased due to the presence of leachates. The Ca concentration in the plants was considerably reduced concomitantly with the increased doses of leachate applied. On the other hand, the concentrations of Na and Ca in soil were higher for the control treatment (Table 5).

Table 5 Main properties of	of <i>Tagetes erecta</i> L. and s	soil at the beginning and th	e end of the ex	periment period (3	4 d)

		0				<u> </u>
Parameters		Initial	Control	T1	T2	Т3
Plants						
Κ	mg g ⁻¹ DM	22.21±1.39a	23.43±2.29a	25.74±2.22a	26.60±0.26a	29.99±0.32a
Na	mg g ⁻¹ DM	5.47±0.19b	5.84±0.06b	5.74±0.06b	6.57±1.08b	17.16±0.05a
Mg	mg g ⁻¹ DM	5.54±0.39b	9.31±0.64a	6.97±0.14b	7.21±0.47b	6.71±0.18b
Ca	mg g ⁻¹ DM	68.84±1.16c	114.66±2.91a	85.93±1.09b	90.57±0.92b	72.62±2.66c
Soils						
Κ	mg g ⁻¹ DM	2.32±0.47b	4.90±0.91a	2.88±0.14ab	2.55±0.46b	4.18±0.19ab
Na	mg g ⁻¹ DM	85±0.14b.0	4.95±0.84a	0.91±0.04b	1.36±0.32b	1.22±0.00b
Mg	mg g ⁻¹ DM	0.43±0.12a	0.80±0.07a	0.59±0.00a	0.88±0.37a	0.61±0.02a
Ca	mg g ⁻¹ DM	2.49±0.36b	5.59±1.21a	2.45±0.08b	2.75±0.06b	3.85±0.49ab

T1, 10% leachate+90% ultrapure water; T2, 25% leachate+75% ultrapure water; T3, 50% leachate+50% ultrapure water. Control: Hoagland's solution.

Values represent means $(n = 9) \pm$ standard errors (SE). Within each file, means followed by different letters are significantly different $(p \le 0.05)$ according to a Tukey's test. For the initial parameters, n=3.

Munns and Tester (2008) indicated that saline stress could affect the general nutritional status of plants showing a decrease in nutrient uptake. García-Caparrós et al. (2017) indicated that there is a decrease in the concentrations of N, P, K and Ca in leaves as the Na concentration increases. In our study, the Na content in the

plant was increased when the highest leachate dose was applied. These results coincide with different authors (Trejo-Téllez et al. 2013; Ashraf 2004). In turn, the influence of salinity on the concentration of K in the plant was not significant, again in concordance with Trejo-Téllez et al. (2013). However, the salinity negatively affected the Ca content in plants in accordance with different authors (Ashraf 2004; Niste et al. 2014; Shrivastava and Kumar 2015). The disproportionate presence of Na in both cellular and extracellular compartments, negatively impacts on the acquisition and homeostasis of essential nutrients such as Ca and this would be related to the antagonism that they present with respect to Na, whose content was increased (Niste et al. 2014; Shrivastava and Kumar 2015; García-Caparrós et al. 2017). In the case of Mg, a decrease in its content was observed for all plants irrigated with leachate, regardless of the dose, not expressing a clear antagonistic relationship with the accumulated sodium concentration in plant tissues.

Conclusion

The results obtained in this investigation show the sensitivity of Tagetes erecta L. to high doses of Na originated from the leachate-irrigation procedures, fundamentally affecting the color and the production of flowers. These facts are very important since this species is considered ornamental. However, our data indicate that is possible to use landfill leachate as irrigation water for T. erecta L. species at concentrations not higher than 25%. Irrigation with leachate in controlled doses would allow to recover the nutrients present in this effluent, decrease the consumption of water and chemical fertilizers in the revegetation of landfill areas and decrease the volume of discharge of effluents to rivers. Under these conditions, some important features of the vegetative (i.e., chlorophylls, carotenoids and soluble sugars) and the reproductive (number and size of flowers) development were clearly deteriorated. However, more research is essential to analyze in depth the reuse of leachate as irrigation water, to improve tolerance and use of nutrients, with special emphasis on dosages and the study of the application of different substrates that could improve stress tolerance. Also, it is necessary to establish criteria that allow establishing doses based on measurable characteristics in the leachate. The overall findings of this study show that vegetative and reproductive responses in the plants are directly related to

the germination index value and thus this parameter can be considered as a reliable indicator of the leachate phytotoxicity for further experimental designs of phytoremediation of leachate. However, the relationship between the phytotoxicity exhibited from leachates on seeds germination and the effects on subsequent physiological development should be studied further, since it may vary depending on the species under study and the temporal composition of the leachate.

Furthermore, greenhouse conditions imply a modification of the real features prevailing in landfills. As a future perspective, it is also expected to carry out vegetation implementation studies in conjunction with leachate irrigation at the sanitary landfill site.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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