Canna x generalis irrigated with greywater in a nature-based solution

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Abstract

To achieve Goal 6 of the 2030 Agenda for Sustainable Development and provide water for all, water reuse is essential. We assessed the potential of treating greywater (LGw) while reusing it via irrigation of *Canna x generalis*, commonly used in nature-based solutions (NBS), with a view on plant development. The study was conducted at a mesocosm scale with factorial designs to test two substrates and four types of irrigation water. Stage 1 tested LGw, LGw with nutrients (LGw + N), and tap water with nutrients (TW + N). Stage 2 tested indoor and outdoor applications of TW (TWi and TWo, respectively) and LGw (LGwi and LGwo, respectively). All treatments resulted in reductions in total nitrogen and phosphate concentrations. The effluent turbidity, chemical oxygen demand, and surfactant contents of LGw + N and LGw decreased considerably after passing through the substrate. The results showed no statistical differences among the measured variables when LGw and TW received artificial fertilizers, which provided advantageous conditions for complete plant development, as evidenced by flowering. Conversely, compared with all other treatments, LGw treatment alone resulted in inferior development and exhibited some symptoms of nutritional deficiency, observed as reductions in dry biomass in the aboveground parts (range: 1.87–6.52 g) and belowground parts (10.03–36.19 g). Overall, *Canna x generalis* did not exhibit symptoms of toxicity and became fully developed, except for flowering, proving to be a robust species that is resistant to adverse environmental conditions, and is therefore recommended for cultivation in nature-based systems for landscaping integrated with LGw treatment and reuse.

1. Introduction

To be able achieve Goal 6 of the 2030 Agenda for Sustainable Development (United Nations, 2015) sustainable sanitation solutions and water reuse are mandatory. Within this context, nature-based solutions play an important role on the decentralized treatment and reuse of greywater (Marques et al 2021; Boano et al 2020).

Greywater (Gw) includes all domestic wastewater (WW) except for toilet water (Eriksson et al 2002) and contains chemical compounds and/or organic products resulting from cleaning and hygiene activities (Ziemba et al 2018). Such activities produce an estimated 75% of the total domestic wastewater volume, presenting a lower pathogen concentration than conventional domestic sewage (Shi et al 2018).

Constructed wetlands, a biological treatment system inspired by natural ecosystems such as swamps and marshes, comprise a notable nature-based solution (NBS) usually used for Gw treatment or as a secondary treatment for the effluent from wastewater treatment systems (Paulo et al 2009), offering several advantages over other methods, including ease of operation, low construction and maintenance costs, and low environmental impact (Kadlec and Wallace 2009).

According to Paulo et al (2013), constructed wetlands could be integrated into gardens and landscaping projects, which would increase the area of green spaces in urban zones. More recently, living (green) walls and green roofs have also been proposed as NBSs, as the outer surfaces of buildings can provide unused spaces in densely inhabited areas (Boano et al 2020). The natural cooling effect of evapotranspiration, occurring in NBSs, is one of the most cost-effective approaches for mitigating the urban heat island effect (Pradhan et al 2019).

Therefore, the use of NBSs to treat Gw has become an attractive option for (i) converting a significant fraction of WW to a valuable water resource and (ii) mitigating the impact of urban heat islands, flooding, and food supply, offering various ecosystem services beneficial to the environment (Arden and Ma 2018; Boano et al 2020; Pradhan et al 2019).

To make such systems feasible, studies must identify the optimal substrate types and adaptable plant species that can treat Gw while also providing aesthetic gains. In particular, vegetation used in constructed wetlands must be tolerant to constantly saturated or submerged areas and to continuous exposure to different types and concentrations of pollutants. According to Vymazal (2013), plants used in constructed wetlands designed for wastewater treatment should (i) be tolerant of high organic and nutrient loadings, (ii) have rich roots and rhizomes, and (iii) have high aboveground biomass. Several studies have focused on the adaptation of plants in different media to the removal efficiency of microbiological and physicochemical parameters, using ornamental plants in constructed wetlands (post)treating domestic sewage or Gw (Calheiros et al 2015; Leiva et al 2018; Konnerup et al 2009; Naik et al 2020), In a review paper, Vymazal (2011) presented a considerable list of ornamental species used for horizontal subsurface flow constructed wetlands, particularly in the tropics and subtropics.

Our research group has been working with ornamental plants in NBSs for treating LGw since 2006. Several species were examined, including *Heliconia psittacorum*, *Cyperus isocladus* (the dwarf type), *Bromelia* sp., *Canna* sp., *Arundina bambusifolia*, *Alpinia purpurata*, *Hedychium coronarium*, *Caladium hortulanum*, *Canna x generalis*, and *Equisetum giganteum* (Caputo et al 2019; Magalhães Filho et al 2019; Paulo et al 2007; Paulo et al 2009; Paulo et al 2013; Silva et al 2017). Among all species tested in the different experiments, *Canna x generalis* showed more resilience and adaptability to different conditions (e.g., operational, climate, and substrates). However, most studies have not focused on the plant itself, and the conclusions regarding the plants are usually field observations rather than experimental trials. Therefore, we assessed the effects of irrigation with various greywater formulations and two substrate types on the development of *Canna x generalis* in terms of its adaptability, nutrient status, and growth rate.

2. Material And Methods

2.1. Experimental design

The experiment was conducted in a greenhouse (6.00 m × 6.00 m) located at the Federal University of Mato Grosso do Sul, Campo Grande City, Mato Grosso do Sul, Brazil (20° 30¢S, 54° 36¢W; elevation: 546 m). The irrigation experiments were performed in mesocosms using buckets to simulate natural plant systems.

The experiment was divided into two 80-day stages. Two substrates were tested: substrate 1 (S1) combined a layer of nº 2 fine gravel topped with a layer of soil; substrate 2 (S2) was fine gravel. Ten treatments were carried out for four irrigation water types: tap water (TW), TW + nutrients (TW+N), LGw, and LGw + nutrients (LGw+N).

In stage 1, a 2 × 3 factorial experimental design was applied, with six replicates per treatment (online resource, Figure 1A). The factors tested were the two substrate types (S1 and S2) and three water types (TW+N, LGw+N, and LGw). Stage 1 was performed during a hot-dry period (September to November). Stage 2 used a 2 × 2 factorial design testing of two substrate types (S1 and S2) and two water types (TW and LGw). Stage 2 was performed during a rainy period (January to March). Each of these treatments comprised six replicates, of which three were placed outdoors (outdoor LGw (LGwo)) and three were placed inside the greenhouse (indoor LGw (LGwi)).

All treatments in stage 1, and TWi, TWo, and LGwo in stage 2, were performed using new plant propagules; however, the LGwi treatments with both substrates in stage 2 were performed using three out of the initial six replicates of LGw from stage 1. Therefore, during the final analyses, most plants had grown for only 80 days, whereas those in the LGwi treatments had grown for 208 days (duration of the entire experimental period).

2.2. Experimental plants and treatment

The present study was performed using the ornamental species *Canna x generalis*. Propagules collected in a pilot system (Paulo et al 2009) were planted in pots filled with sand and cultivated for 65 days with daily irrigation with Tw to support sprouting, growth, and acclimation. For the experiment, seedlings of a uniform size and in good health were selected, removed from the pots, and cleaned to remove all excess sand. Then, the seedlings were transplanted into translucent 13.5 L (26.3-cm diameter and 30-cm height) buckets (one seedling per bucket), the bottoms of which contained an opening that was connected to a hose that was used to drain the water when necessary. The buckets containing S1 were filled first with a 11.0 cm layer of nº. 2 fine gravel and then with a 14.0 cm of soil (stage 1 soil: 610, 230, and 160 $g{\cdot}Kg^{-1}$ of sand, silt, and loam; stage 2 soil: 810, 80, and 110 $g{\cdot}Kg^{-1}$ of sand, silt, and loam (Solos, Campo Grande City, MS, Brazil). The buckets containing S2 were filled with a 25.0 cm layer of fine gravel. All experimental buckets were placed on the countertops inside the greenhouse under non-controlled environmental conditions.

The mesocosms were irrigated via 20.0 cm diameter (3/4") polyvinyl chloride hoses placed at a 20.0-cm depth below the surface substrate. Thus, irrigation water was able to reach the roots via capillarity action. Buckets were irrigated every 5 days in stage 1 and every 7 days in stage 2. Before each irrigation, water was drained from the bottom of the buckets and collected to determine the water consumption rate. Then, new irrigation water was manually poured into the hose via a volumetric beaker. The volume was calculated based on the useful volume for each substrate: 2 L for S1 and 4 L for S2.

The Gw treatments received LGw simulating that produced during daily household activities except for dishwashers and kitchen sinks. The water underwent no treatment or removal of the rough materials before use. Every batch of LGw produced by the research group members was qualitatively characterized. The following parameters were analyzed: pH, electrical conductivity (EC), total dissolved salts (TDS), turbidity, chemical oxygen demand (COD), total nitrogen (TN), phosphate (PO_4^{-2}), and surfactants. Sampling and analyses were performed according to the recommendations of the Standard Methods for the Examination of Water and Wastewater (APHA 2012).

In stage 1, treatments receiving nutrients (i.e., TW+N and LGw+N) were supplied with Hidrogood Fert (Hidrogood, São Paulo, Brazil), a product used in hydroponic processes. The chemical composition (water %) of the fertilizer solution was as follows: macronutrients (10% nitrogen (N), 9% phosphorus pentoxide (P_2O_5), 28% potassium oxide (K_2O), 3.3% magnesium (Mg), and 4.3% sulfur (S)), and micronutrients (0.06% boron (B), 0.01% copper (Cu), 0.07% molybdenum (Mo), manganese (Mn), and 0.02% zinc (Zn)). In addition, 45.19% calcium nitrate ($Ca(NO_3)_2$) macronutrient was separately dissolved because it forms insoluble compounds with phosphates and sulphates.

2.3. Assessment of plant growth

Plants were assessed once every week using non-destructive measurements of total height, number of leaves, leaf width and length, and stem diameter. At the end of each stage, plants were removed for destructive measurements of aboveground and belowground initial and final dry mass, and foliar macronutrient and micronutrient contents. The plant matter was divided into aboveground parts (i.e., stem and leaves) and belowground parts (i.e., roots) and dried in an oven at 60 °C until a constant weight was attained to determine the dry biomass. Leaf samples were sent to the Solos lab (Campo Grande, MS, Brazil) to analyze the macronutrient and micronutrient contents using the Mehlich-1 (phosphorus, potassium, iron, manganese, zinc, and copper) and KCl 1M (calcium and magnesium) methods.

2.4. Statistical analysis

The data were tested for normality of distribution using the Kolmogorov-Smirnov test. The plant growth data were subjected to analysis of variance (ANOVA), and significantly different means were identified using Tukey's test (alpha level: 5%). All statistical analyses were performed using the Sisvar 5.3 software (Ferreira 2011).

3. Results And Discussion

3.1 Irrigation water

Water inlet and outlet samples were collected and analyzed during the experiment to qualitatively characterize the irrigation water before application and after passing through the substrate. The results are shown in Table 1.

Table 1 Physicochemical parameters of the irrigation water types used in the experimental treatment groups

Sample			Parameter							
Stage	Irrig. water	Point	pH	Turbid. (NTU)	EC ($dS \cdot m^{-1}$)	TDS ($mg \cdot L^{-1}$)	COD	TN	PO_4^{2-}	Surfact.
1 (n = 16)	TW+N	I	6.6	1.1	1.6	1039.1	49.8	141.9	67.8	9.4
		SO1	6.9	1.6	1.4	922.2	49.6	94.3	28.1	7.8
		SO2	7.2	1.5	2.6	1689.7	47.5	134.0	12.9	10.5
	LGw+N	I	7.3	213.5	2.2	1420.0	590.2	132.2	80.5	43.9
		SO1	8.0	5.7	1.7	1074.7	94.5	22.7	12.0	7.6
		SO2	7.8	6.1	2.2	1409.4	129.5	27.6	16.9	15.4
	LGw	I	9.0	177.5	0.9	591.5	639.1	7.1	4.5	61.6
		SO1	7.7	27.0	0.9	566.5	146.5	2.3	2.0	28.2
		SO2	7.4	34.5	0.8	522.3	208.4	2.3	3.4	39.3
2 (n = 11)	TW	I	6.8	0.6	0.1	95.8	22.6	1.9	3.6	7.3
	LGw	I	9.0	222.7	1.7	1087.5	828.5	13.9	4.2	112.8
	LGwi	SO1	7.9	52.1	1.6	1032.4	433.7	3.6	1.5	62.6
		SO2	7.8	61.4	1.3	849.7	494.9	5.8	1.6	79.6
	LGwo	SO1	7.2	40.0	1.2	758.8	227.5	1.6	1.1	54.4
		SO2	7.6	35.8	1.0	623.8	292.9	2.8	1.1	61.6

Abbreviations: TW+N: tap water + nutrients; LGw+N: greywater + nutrients; LGw: greywater; TW: tap water; LGwi: greywater, indoor; LGwo: greywater, outdoor; I: inlet; SO1: substrate 1 outlet; SO2: substrate 2 outlet; Irrig. water: irrigation water; Turbid.: turbidity; EC: electrical conductivity; TDS: total dissolved salts; COD: chemical oxygen demand; TN: total nitrogen; Surfact.: surfactants.

The turbidity, COD, and surfactant contents of LGw+N and LGw decreased considerably after passing through the substrate, highlighting the filtration effect of these systems (Wu et al 2015). Furthermore, all treatments showed reductions in TN and PO_4^{-2} concentrations, indicating absorption by the plants. Substrate 1 performed better in the removal of COD and surfactants for both stages and was more representative for surfactants that achieved about 18% higher removal compared to S2. Average COD removal ranged from 84% (496 $mg.L^{-1}$) for LGw+N, stage 1 to a minimum of 48% (395 $mg.L^{-1}$) for LGw, stage 2, indoors.

In addition, TW+N showed increased pH, EC, and TDS values after passing through S2. Thus, the plants in this group may have had more salt accumulation at the roots. Such retained salts could have been eliminated with additional irrigation and drainage (Feng et al 2017). In contrast, salts might have been withheld in the soil layer in S1.

According to Paganini (2003), irrigation water can be divided into five salinity classes. Most of the irrigation water in this study matched the high salinity class (TDS: 500-1500 mg·L^{-1}), except TW (stage 2), which presented low salinity (TDS: 0-175 mg·L^{-1}). High salinity can damage vegetation via direct contact between salts and plants. If the salts accumulated in the soil exceed the tolerance limits of plant roots, this can cause inhibition of germination and growth. Despite the increased salinity, this inhibition was not observed in the present study.

The EC varied between 1.6 and 2.2 dS·m^{-1} due to the nutrient solution applied. These values were within the ideal conductivity range) of 1.5-4.0 dS·m^{-1}, as suggested by Furlani et al (1999); values above this range can cause toxicity, and values below indicate a deficiency in some elements. Ding et al (2018) assessed the influence of EC increase on the fresh and dry weights of *Brassica campestris* L. ssp. *chinensis* in a hydroponic system, finding that the highest EC (9.6 dS·m-1) had toxic effects on plant growth, causing lower biomass production. For another species, chrysanthemum cv. Miramar, Beckmann-Cavalcante et al (2010) assessed an EC of 2.1 dS·m^{-1}, which supported chrysanthemum production that met commercial quality standards, although it influenced plant height. For comparison, in the present study, increments in EC and TDS showed greater ranges in S2 irrigated with TW+N, although the levels remained within the recommended range; therefore, EC did not impair plant development.

After passing through both substrates, pH of LGw+N increased, in contrast with LGw, whose pH decreased. This could be explained by the better COD removal (von Sperling 2005) in the LGw+N treatment (Table 1). The ideal pH, according to Furlani et al 1999, is 5.5-6.5, within which plants can achieve their maximum development potential. Although the pH remained above the recommended range, the use of artificial fertilizer did not impair plant growth.

3.2. Growth parameters

Table 2 presents the effects of all substrate and irrigation treatments on plant growth parameters, as indicators of plant health and nutritional status.

Table 2 Effects of irrigation water type and substrate on plant growth parameters after 80 days of treatment in stages 1 and 2. The indoor greywater treatment in stage 2 was omitted due to the different age of these plants, which prevented comparison

Sample			Parameter						
Substrate	Stage	Irrig. Water	No. leaves	Height	Stem diam.	Length 2nd leaf	Width 2nd leaf	Length 3rd leaf	Width 3rd leaf
				(cm)					
S1	1	TW+N	8.0^{a}	109.8^{ab}	3.9^{b}	16.3^{c}	5.8^{cd}	22.6^{d}	6.7^{e}
		LGw+N	8.0^{a}	98.9^{b}	3.6^{bc}	16.3^{c}	6.1^{bcd}	23.1^{d}	6.3^{e}
		LGw	3.8^{def}	39.2^{de}	2.3^{de}	19.8^{bc}	6.3^{bcd}	25.5^{cd}	7.4^{cde}
	2	TWi	5.3^{cd}	58.4^{c}	3.0^{bcd}	32.2^{a}	8.2^{a}	26.2^{cd}	8.8^{abcd}
		TWo	5.0^{cde}	48.4^{cd}	3.7^{b}	23.2^{bc}	7.3^{abc}	38.8^{a}	10.5^{a}
		LGwo	3.0^{f}	28.8^{e}	2.5^{cde}	23.0^{bc}	7.6^{abc}	36.2^{ab}	7.6^{bcde}
S2	1	TW+N	7.7^{ab}	114.7^{a}	4.1^{ab}	17.8^{c}	6.4^{bcd}	22.3^{d}	6.4^{e}
		LGw+N	8.7^{a}	104.8^{ab}	5.1^{a}	17.7^{c}	6.8^{abc}	21.9^{d}	7.3^{de}
		LGw	2.2^{fg}	27.4^{e}	1.4^{ef}	20.0^{bc}	7.3^{abc}	25.0^{cd}	9.0^{abcd}
	2	TWi	3.3^{ef}	37.0^{de}	2.3^{de}	27.5^{ab}	7.6^{ab}	30.0^{bcd}	7.9^{bcde}
		TWo	6.0^{bc}	46.6^{cd}	3.9^{b}	17.2^{c}	4.9^{d}	37.8^{ab}	9.3^{abc}
		LGwo	1.0^{g}	36.5^{de}	0.6^{f}	-	-	32.3^{abc}	9.5^{ab}
		CV (%)	17.61	10.86	19.77	20.28	14.13	15.02	12.68

Abbreviations: TW+N: tap water + nutrients; LGw+N: greywater + nutrients; LGw: greywater; TWi: tap water, indoor; TWo: tap water, outdoor; LGwo: greywater, outdoor; CV: coefficient of variation; No. leaves: number of leaves per plant; Stem diam.: stem diameter. Values are reported as the mean; values within the same column that are not connected by the same letter differ significantly (Tukey's test, $P < 0.05$).

Average plant growth differed among the treatment groups at the end of each stage. In stage 1, plants in S1 and S2 irrigated with TW+N and LGw+N had larger stems, greater heights, and higher number of leaves.

In the natural environment, the average height of *Canna x generalis* is 0.5–1.5 m (Zsiláné et al, 2017). In this study, plant height was significantly higher in treatments receiving artificial fertilizer, underscoring the advantages of such treatments.

In particular, all plants irrigated with TW in stage 2 (grown indoors and outdoors) had heights less than half of those from the TW+N treatment in stage 1. Regardless, these values were within the typical range found in the literature. In contrast, irrigation with LGw, in both stages 1 and 2, resulted in plant heights below the typical range.

Irrigation with TW+N and LGw+N presented no statistical differences among the measured variables, likely because the same concentrations of added nutrients (especially nitrogen and phosphorus) were provided in both treatments, which provided advantageous conditions for plant development. Thus, artificially fertilized LGw did not inhibit plant growth; indeed, LGw treatment alone resulted in inferior growth in three parameters (number of leaves, plant height, and stem diameter) and exhibited some symptoms of nutritional deficiency, which may have limited plant development according to Liebig's law.

Plants of the same age irrigated with TW in stage 2 (TWo and TWi) had more leaves, greater heights, and larger stem diameters than those irrigated with LGw (stage 1) and LGwo (stage 2). It should be noted that LGwi plants were older, and thus were not included in this comparison.

For all plants in all treatments, the number of leaves increased throughout the duration of the experiments. However, irrigation with LGw resulted in the emergence of fewer leaves, in addition to the loss of senescent leaves observed for all treatments. The first leaves of the plants, for all treatments, did not last the complete duration of each stage (80 days).

Plants irrigated with LGw in stage 2 were divided into replicates grown inside and outside the greenhouse. Notably, the three indoor LGw replicates (LGwi) grown in S1 and S2 were derived from the LGw system used in stage 1; therefore, three replicates from both LGw treatments in S1 and S2 from stage 1 were maintained throughout the entire experimental period (208 days). This impaired the comparison between these and the other treatments since the plants did not present the same age during the same period. Table 3 lists the average plant growth results for all LGw treatments. Over time, plants treated with LGw lost their leaves and showed reductions in stem diameter (LGwi treatment, Table 3). At the end of stage 2, LGwo treatment did not show statistical differences in leaf number compared with the LGw treatment at the end of stage 1. Interestingly, there were significant differences among treatments for plant height in S1 and stem diameter in S2.

Table 3 Effects of irrigation water type and substrate on plant growth parameters in plants irrigated with greywater. Plants in LGw and LGwo were 80 days old, and plants in LGwi were 208 days old

Sample			Parameter						
Substrate	Stage	Irrigation water	No. leaves	Height	Stem diam.	Length 2nd leaf	Width 2nd leaf	Length 3rd leaf	Width 3rd leaf
				(cm)					
S1	1	LGw	3.8^{a}	39.2^{a}	2.3^{a}	19.8^{b}	6.3^{b}	25.5^{c}	7.4^{c}
	2	LGwi	1.3^{bc}	41.1^{a}	1.1^{bc}	-	-	-	-
		LGwo	3.0^{a}	28.8^{b}	2.5^{a}	23.0^{a}	7.6^{a}	36.2^{a}	7.6^{bc}
S2	1	LGw	2.2^{ab}	27.4^{b}	1.4^{b}	20.0^{ab}	7.3^{a}	$2.,0^{c}$	9.0^{ab}
	2	LGwi	0.3^{c}	32.3^{ab}	0.8^{bc}	-	-	-	-
		LGwo	1.0^{bc}	36.5^{ab}	0.6^{c}	-	-	32.3^{b}	9.5^{a}
		CV (%)	45.52	15.30	29.53	17.47	12.29	7.22	14.43

Abbreviations: LGw: greywater; LGwi: greywater, indoor; LGwo: greywater, outdoor; CV: coefficient of variation. No. leaves: number of leaves per plant; Stem diam.: stem diameter. Values are reported as the mean; values within the same column that are not connected by the same letter differ significantly (Tukey's test, $P < 0.05$).

Cerqueira et al (2008) assessed the development of *Heliconia psittacorum* and *Gladiolus hortulanus* irrigated with treated residual water (i.e., domestic wastewater) or stream water, and found no significant differences among water types; however, plants irrigated with domestic wastewater were taller than those irrigated with stream water. These findings contradict those of the present study, probably due to the characteristics of treated domestic wastewater, which might be more diluted and homogenous than LGw. Furthermore, the LGw physicochemical composition presented low concentrations of nutrients, which could impede plant development.

3.3. Water consumption

Figure 1 presents the plant water consumption rates during the course of the experiments. The control buckets contained only substrate, with no plants, and were irrigated with TW.

Water consumption differed greatly under different irrigation water types over time and among treatments. In the TW+N and LGw+N treatments, water consumption increased over the 80-day experiment, especially in S2. This may have been due to the emergence of plants in these systems since plant water consumption changes with plant development stage and atmospheric conditions. Concurring with this, the plants in these treatments showed better growth due to fertilization. In contrast, water consumption was relatively low in the LGw treatment, possibly due to poorer plant growth. It should be noted that the issue related to the increase in TDS and EC in TW+N (see Table 1) may have resulted from relatively high evaporation, as evidenced by the high water consumption. With decreasing water volume, the salt concentration increases. Interestingly, water consumption was greater in S1 than in S2 for all greenhouse LGw treatments (LGw+N, LGw, and LGwi), In addition, outdoor treatments (TWo and LGwo) exhibited negative water consumption over time since they were subjected to rainfall events throughout the study period.

3.4. Analysis of leaf nutrient status

The results of the macronutrient and micronutrient analyses of leaf samples (Table A1, online resource) could not be compared with optimal ranges, since no relevant literature on macronutrient and micronutrient levels for *Canna x generalis* were found.

At the end of stage 1, all treatments showed increases in calcium (352–615%), magnesium (103–267%), iron (113–245%), and boron (290–982%) compared with the plant seedlings before the experiment; however, there were varying reductions in nitrogen (38–98%), copper (13–56%), and sulfur (50–63%). Plants cultivated in S2 had slightly higher nitrogen and phosphorus concentrations than those in S1, regardless of irrigation water type.

Irrigation with LGw resulted in increased macronutrient (calcium) and micronutrients (iron, manganese, and boron) in plants when compared with that in the seedlings. For comparison, Santos et al (2012) studied the development and nutritional status of *Heliconia* plants irrigated with treated wastewater from domestic use associated with chemical fertilizer and found that the application of this wastewater increased micronutrient levels in leaves, especially iron (final concentration: 127.97 mg·Kg^{-1}) and manganese (561.58 mg·Kg^{-1}).

The nitrate found in calcium nitrate, which was used as a component of the nutrient solution in this study, freely moves within irrigation water because it is not absorbed by soil colloids. Thus, nitrogen level maintenance in plants irrigated with nutritional solutions could be explained by the fact that these fertilizers contain additional available nitrogen for absorption by plants (Parewa et al 2014; Vieira and Ramos 1999).

LGw applied as irrigation water had an average pH of 9.0 in both stages. According to Martinez (1999), pH values above 7.0 restrict micronutrient and phosphorus availability to plants. The results of the foliar analysis suggest that this phenomenon may have occurred in these treatments. The plants in S2 irrigated with LGw did not survive the entire duration of stage 2; therefore, it was not possible to obtain foliar samples for the macronutrient and micronutrient analyses. Furthermore, it was not possible to measure all nutrients in some samples due to the small amount of material available for analysis.

Zhang et al (2008) investigated the interactive effects of nitrogen and phosphorus on the growth of *Canna indica* and found that this species accumulated high nitrogen and phosphorus concentrations in leaves and stems under high-concentration conditions. This capacity for uptake, along with its high growth rate, are desirable features for plants used in constructed wetlands for wastewater treatment. Furthermore, among the tested samples, Zhang et al (2008) found higher nutrient concentrations in leaves (nitrogen: 30.8 g·Kg^{-1}; phosphorus 4.9 g·Kg^{-1}), which were similar to the concentrations found in leaf tissue in the present study (nitrogen: 30 g·Kg^{-1}; phosphorus: 3.6 g·Kg^{-1}).

Considering the irrigation water results in Table 1, LGw had relatively low nitrogen and phosphorus levels in stage 1. The LGw used in stage 2 had higher nutrient concentrations than TW (Table 1), albeit still below plant nutrient thresholds. However, the differences between LGw and TW in stage 2 were small in the foliar analysis (Table 4). Thus, plants irrigated with LGw appeared to show nutrient absorption limitations owing to the chemical composition of LGw.

Macronutrients and micronutrients perform specific functions necessary for plant development, and deficiencies or excesses in such nutrients cause characteristic symptoms. Some symptoms were observed in the treatments, as shown in online resource (Figure A2).

Amending the irrigation water with the nutrient solution did not cause nutrient deficiency or toxicity; throughout the experiment, leaves maintained an intense green color, indicating good growth. LGw irrigation caused nitrogen deficiency symptoms in both stages, as evidenced by pale yellowish-green leaves (Borges and Oliveira 2006), as well as phosphorus deficiency, as evidenced by chlorosis and, subsequently, necrosis of the leaf margin and center. These symptoms were initially observed in older leaves, and over time, spread to newer leaves. Both symptoms were also observed in stage 2 TW treatments.

3.5. Biomass production

The aboveground and belowground dry biomasses of plants subjected to different treatments after the completion of each stage (80 days) are shown in Figure A3 (online resource). Belowground biomass decreased with increasing nutrient availability (i.e., TW+N and LGw+N), but increased under low-nutrient conditions (i.e., TW and LGw). When facing nutrient deficiencies, plants often allocate more energy towards expanding the biomass of the root system. Furthermore, deficiencies in essential macronutrients may alter the aboveground-to-belowground biomass ratio (Hermans et al 2006). In fact, among all greenhouse experiments in plants 80 days old, only those irrigated with TW+N and LGw+N flowered on days 57 and 64, respectively, likely due to the satisfactory nutrient availability.

The greater aboveground biomass in plants irrigated with artificial fertilizer can be explained by the greater production of leaves, larger stem diameter, and greater height. Zhang et al (2008) and Jiang et al (2011), using *Canna indica*, showed similar changes in biomass allocation in response to nutrient availability compared with the present findings.

In the outdoor treatments in stage 2, TWo plants grown in S2 showed variations in aboveground and belowground dry biomass compared with TWi plants (Figure A3, online resource). Meanwhile, LGwo plants had a greater belowground dry biomass in both substrates compared to LGwi plants. This may have been the result of exposure to rain, resulting in decreased nutrient concentrations compared with indoor treatments and increased energy allocation toward root biomass growth. Thus, it was observed that plants irrigated with LGw presented differences in belowground biomass production when placed in an environment simulating real planting conditions. The other differences between the indoor and outdoor replicates (LGwi and LGwo) (e.g., water consumption and evaporation, reduced COD, turbidity, and surfactants in outdoor replicates) could also be explained by increased leaching and/or overflow due to rainwater in outdoor plants.

Plants irrigated with the nutrient solution showed higher water consumption (especially in S2), whereas LGw plants had lower water consumption, and thus had longer contact with soil water. However, constant high substrate moisture content due to frequent irrigation might inhibit root growth and increase fungal and bacterial growth.

Plants growing in the wild or cultivated artificially outdoors are exposed to various environmental stresses that cause negative effects, leading to changes and responses at all functional levels. Such changes and responses can be reversible or permanent (Osakabe et al 2014; Larcher 2006). In stressful environments, the survival strategy of plants does not rely on increasing productivity, but rather on balancing performance and survival (Salamoni 2008). In this study, all plants were susceptible to hydric, thermal, and saline stresses. Furthermore, LGw plants were susceptible to nutritional stress. Temperatures inside the greenhouse varied throughout the day, from a maximum of 46 °C at midday to 20 °C at night. Such exposure to heat might have caused some tissue damage over the course of the study. Regarding hydric stress, water consumption by plants and evaporation could have resulted in water deficits (TW+N and LGw+N) or excesses (LGw, LGwi, LGwo, TWi, and TWo), which could also affect vital processes. Finally, salinity is among the most important factors that can limit plant growth and nutrient status, since high salinity reduces ion activity within the solution, and alters nutrient absorption, transportation, assimilation, and distribution processes within the plant. Interactions between salinity and plant nutrient status become complex due to differences in concentrations and ionic compositions of the saline media (water and soil) to which plants are subjected. This can affect the efficiency of mineral acquisition from the soil (Lacerda 2005) and cause nutritional stress, which could impede plant growth. Nutritional stress can also be caused by imbalances in the concentration of a given nutrient; since different nutrients perform specific functions, growth and production may be altered (Deon, 2007). According to Lima (2009), some elements can influence the uptake and use of other elements, and such interactions can alter the lead mineral contents of plants.

Another study (Paulo et al 2009) observed that *Canna x generalis* presented slow growth when irrigated with LGw. However, the studied system remained in operation for 5 years, and overall supported plant development. In fact, the seedlings used in the present study were obtained from these plants. Thus, this species is capable of being cultivated using LGw irrigation, with no signs of toxicity, albeit some signs of nutrient deficiencies. In a more recent study, the development of *Canna x generalis* was studied at both bench and pilot scales. The species was able to grow either in TW or LGw, with solar light input recommended if flowering is desired (Caputo et al 2019).

3.6. Soil chemical analysis

Samples of S1 soil were collected at the beginning and end of the experiments for chemical analysis (Table 4). Because the soils used in stages 1 and 2 were acquired at different times, they presented some distinct differences. In particular, the soil used in stage 2 had higher phosphorus concentrations. It should be noted that the LGwi treatment in stage 2 was a continuation of the LGw treatment in stage 1; therefore, this soil had relatively low phosphorus concentrations.

Table 4 Chemical analysis of the soils used in substrate 1 in stages 1 and 2

Sample		Parameter													
Stage	Irrig. water	pH	P	OM	K	Ca	Mg	Al+H	CEC	V	Fe	Mn	Zn	Cu	B
			(mg·dm^{-3})	(g·dm^{-3})	(cmol$_c$·dm^{-3})					(%)	(mg·dm^{-3})				
1	IS	6.46	4.06	13.38	0.24	2.80	0.95	1.70	5.69	70.12	39.49	67.22	1.32	5.79	0.12
	TW+N	6.61	4.63	9.91	0.05	3.65	1.10	1.55	6.35	75.59	61.28	56.04	1.14	4.84	0.45
	LGw+N	6.97	5.15	7.83	0.14	2.75	1.00	0.96	4.85	80.21	60.21	59.04	1.08	4.84	0.44
	LGw	7.40	3.90	5.96	0.03	1.55	0.95	0.44	2.97	85.19	102.77	51.01	1.29	3.09	0.15
2	IS	6.91	53.63	4.17	0.20	1.40	0.70	0.44	2.74	83.94	32.50	16.97	3.61	0.61	0.11
	TWi	7.80	50.69	7.09	0.04	1.75	1.00	0.00	2.79	100.0	44.80	28.10	4.33	0.75	0.33
	TWo	7.36	48.20	6.54	0.04	1.45	0.75	0.37	2.61	85.82	47.42	26.95	4.60	0.90	0.11
	LGwi	7.00	5.60	9.41	0.09	1.35	0.80	0.74	2.98	75.17	30.08	47.57	1.57	3.51	0.24
	LGwo	6.89	26.41	6.00	0.03	1.25	0.75	0.44	2.47	82.19	180.65	32.87	8.42	1.48	0.09

Abbreviations: IS: initial soil; TW+N: tap water + nutrients; LGw+N: greywater + nutrients; LGw: greywater; TWi: tap water, indoor; TWo: tap water, outdoor; LGwi: greywater, indoor; LGwo: greywater, outdoor; Irrig. water: irrigation water; OM: organic matter; Al+H: potential acidity; CEC: cation exchange capacity; V: saturation of bases.

In a study of soil fertility in the Minas Gerais state (Brazil), Alvarez et al (1999) proposed different classification levels based on lab analyses. According to the levels suggested in their work, the initial soil sample and soils irrigated with TW+N, LGw+N, and LGwo presented weak acidity, soils irrigated with LGw, TWo, and TWi presented weak alkalinity, and soils irrigated with LGw were neutral. All samples were considered inadequate by the agronomic standards mentioned above, having high or very high pH levels. According to the soil clay content, phosphorus availability was considered very low for all analyzed soils in stage 1 (as well as LGwi in stage 2). In stage 2, phosphorus availability was considered average for LGwo and very good for the other soils. Potassium availability was considered very low for soils irrigated with LGw, indicating that it may have been the limiting nutrient in this treatment.

Based on the organic matter content, the fertility of soils irrigated with LGw was very low (LGw and LGwo) or low (LGwi). Meanwhile, fertility was classified as average based on calcium, and average (LGwi and LGwo) or good (LGw) based on magnesium. The CEC was low for all solutions. Regarding base saturation, it was good (LGwi) or very good (LGw and LGwo).

After LGw irrigation in stage 1, the soil had high iron, manganese, and copper, good zinc, and very low boron levels. After LGwi irrigation in stage 2, the soil had average iron, high manganese and copper, good zinc, and low boron levels. In contrast, after LGwo irrigation in stage 2, the soil had high iron, manganese, and zinc, good copper, and very low boron levels. Thus, based on the results for LGwi, over time, soil iron concentrations decreased, and boron concentrations increased.

Irrigation with greywater altered the soil pH and lowered the organic matter, potassium, calcium, and potential acidity, consequently reducing the CEC and increasing the saturation of bases. At the end of the experiments, higher values of some parameters, notably soil phosphorus and CEC, as well as irrigation water nitrogen, phosphorus, and potassium, might have influenced plant productivity. Azevedo and Oliveira (2005) observed that higher values of these parameters also influenced total plant production in cucumber. In sandy soils, CEC faces higher reductions, and nutrients (mainly nitrogen and potassium) are susceptible to leaching, which might have occurred in soils irrigated with TW and LGw, resulting in overall reductions in plant productivity.

4. Conclusions

We assessed the response of the ornamental plant *Canna x generalis* grown in different substrates to irrigation with domestic greywater compared to irrigation with TW, to determine the potential for simultaneous treatment and reuse of greywater through ornamental plant irrigation. Greywater irrigation caused no signs of toxicity to plants, but plants irrigated with greywater showed some visible symptoms of nutrient deficiencies and reduced growth, leaf number, plant height, and stem diameter in comparison with plants receiving artificial fertilizer. In terms of plant biomass, greywater irrigation resulted in increased belowground dry biomass due to poor nutrient availability. Regarding the tested substrates, there was no difference with respect to growth parameters in *Canna x generalis* irrigated with greywater. The combination of gravel and soil (S1) appeared to be more advantageous, especially for treatment comprising greywater without nutrient supplementation, with higher dry biomass, lower water consumption, and better removal of COD and surfactants.

The application of artificial fertilizer with greywater enabled the complete development of *Canna x generalis*, including flowering, which can be achieved if the system is constructed in a sunny area. Overall, *Canna x generalis* proved to be a robust species resistant to adverse environmental conditions and is therefore recommended for cultivation in NBSs receiving LGw.

Abbreviations

ANOVA, analysis of variance; COD, chemical oxygen demand; EC, electrical conductivity; LGw, greywater; LGWi, indoor greywater; LGwo, outdoor greywater; NBS, nature-based solution; TW, tap water; TDS, total dissolved salts; TN, total nitrogen; WW, wastewater

Declarations

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Figures

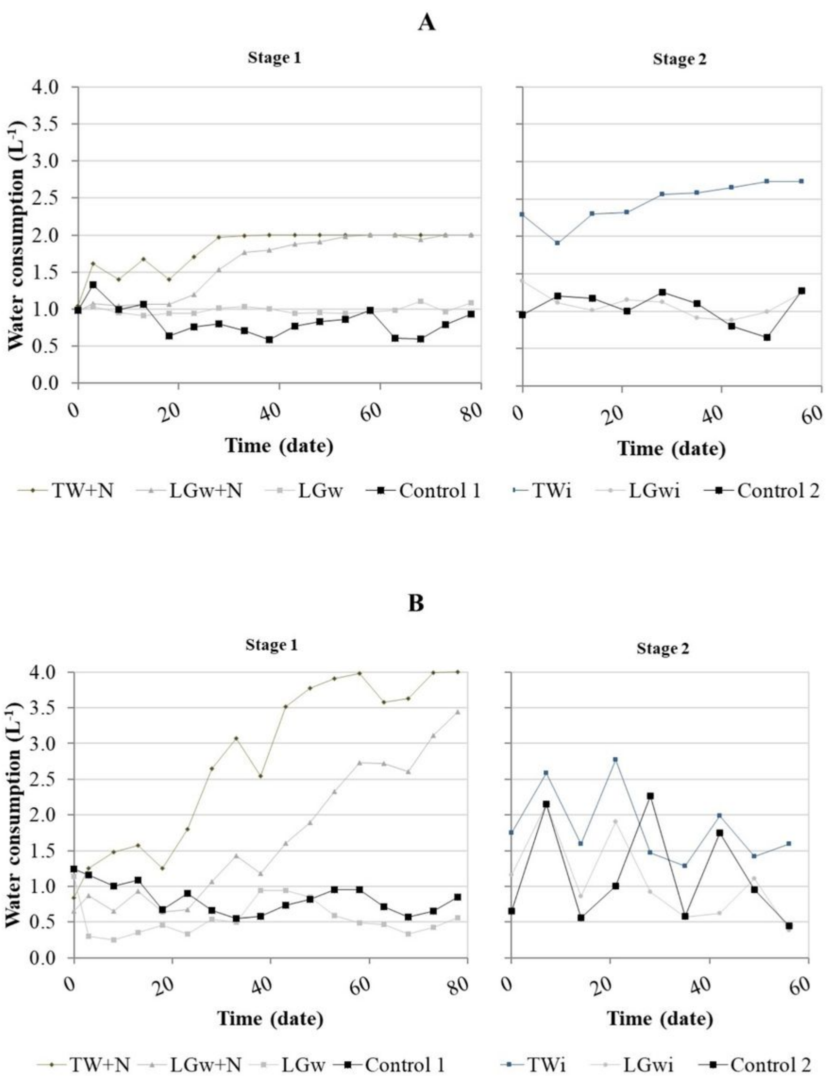

Figure 1

Mean water consumption in stages 1 and 2 in (A) substrate 1; (B) substrate 2

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