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Research Article

Germination and initial growth of sunflower irrigated with untreated and treated (by distillation and electro-Fenton) landfill leachate

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Abstract

Currently, water scarcity, especially in irrigated agriculture, has led researchers to study reuse to study industrial and sewage reuse. Landfill leachates deserve special attention due to its difficulty of tratment they are effluents that are difficult to treat and because it contains essential susbstances for plant nutrition, such as nitrogen, phosphorus and potassium and its application in agriculture can be considered a noble use if it is properly handled and does not cause phytotoxicity. This study investigated the effects of different dilutions of treated and non-treated landfill leachate on germination and vitality of sunflower seed (cv. BRS 321), its effects in growth, and vitality and soil atributes under sunflower culture in greenhouse. The leachate was treated by two electro-Fenton process or distillation. The results showed that sunflower cv. BRS 321 seeds, germinated vigorously up to 25% effluent dilution, showing resistance to salinity and phytotoxicity at 50% raw leachate dilution. 15% dillution showed satisfactory results in sunflower growth and nutrition. However, the leachate uses of higher concentrations showed some changes in soil characteristics. This study suggests that landfill leachate can be used in seed germination under proper use and application, in their lowest leachate concentrations and always based on the chemical analyzes of the leachate.

Keywords: Landfill leachate - Agricultural irrigation – Sunflower – Soil - Phytotoxicity

1. Introduction

Currently water scarcity, especially in irrigated agriculture, has led researchers to study industrial and urban effluents reuse. In this context, landfill leachate deserves special attention because it contains high nutrient amount but are nevertheless difficult to treat [1, 2]. It is important to consider the correct disposal of solid urban waste (SUW) as one of the main environmental problems in the world and sanitary landfills are the current accepted alternative for waste destination. However, the by-products generated, such as leachate, also cause environmental damage. In this context, plants cultivation was studied as a possible alternative and solution for effluents destination [3-5].

Landfill leachate is a liquid that has been seeped through the solid waste disposed in a landfill and contains extracted, dissolved, or suspended materials. It is characterized by high levels of chemical oxygen demand (COD) (due to presence of non-biodegradable and high toxic organic compounds), salinity and sodium [6-8].

Since landfill leachate also contains nutrients that are essential to plants (such as nitrogen, phosphorus, potassium, as well as some micronutrients) researchers were encouraged to evaluate its Viability use in agriculture [9-12].

Using this effluent in agricultural irrigation requires treatment technologies to reduce the number of pollutants into acceptable levels. Furthermore, sodium concentrations in treated landfill leachate must be assessed to prevent soil salinization that inhibits the development of some forage seeds [7, 8].

Among the used technologies to treat landfill leachate, advanced oxidation processes (AOP), such as Electro-Fenton (E-Fenton) and evaporation, seems to be very promising since they have low costs and operational facilities [6, 13]. Leachate evaporation is a technique used to reduce the volume, concentrating solids, and promoting the volatilization of ammonia and organic compounds and according to Sprovieri, Souza and Contrera the recovery of ammonia can be reached by evaporation and vacuum distillation under controlled temperature and pressure [14].

Fenton processes are widely used to treat effluents with high COD levels (such as landfill leachates), since these technologies are very efficient to promote the mineralization of non-biodegradable organic material which is oxidized by hydrogen peroxide degradation catalyzed by Fe^{2}+ salts [6, 15, 16]. In Electro-Fenton (E-Fenton) direct reaction, ferrous ions and hydrogen peroxide are electrochemically generated in a reactor (in the anode and cathode, respectively). On the other hand, in E-Fenton indirect reaction, ferrous ions are generated "in situ" from oxidation of an iron sacrificial anode, while hydrogen peroxide is added to the electrochemical cell. Then, Fe2+ and H2O2 react like the Fenton process generating hydroxyl radicals (OH•) which are highly reactive [17]. Comparing to classical Fenton process, E-Fenton offers significant advantages like lower ferrous ions generation in cathode and sludge production [6, 18, 19].

Sunflower (Helianthus annuus L.) is one of the few plants that all its parts can be used, in addition to is an important option for farmers who apply crop rotation or sequence due to its short cycle and easy adaptation to different kinds of soil and edaphoclimatic conditions [20, 21]. According to Silva et al. Helianthus annuus L. has phytoremediation ability due to its tolerance to irrigation with brackish and saline waters containing a wide variety of cations and anions [22]. This plant is also economically important due to its large use both in food and inedible crops such as that destined for biodiesel production [20, 23, 24]. This fact is mainly due to the high oil content in its se creating a new market offer, that of biodiesel [25].

This study aims to evaluate the seeds germination in the laboratory and the initial growth of sunflower (Heliantus annus L.) in a greenhouse, irrigated with treated (electro-Fenton and distillation) and non-treated landfill leachate, respectively. Thus, the main objective was to carry out a preliminary assessment of these special wastewater reuse in sunflower cultivation, intended for biodiesel production.

2. Materials and Methods

2.1. Sampling

The raw leachate samples (RL) were collected in Jardim Gramacho Metropolitan Landfill (Rio de Janeiro State, Brazil). This landfill was considered the largest landfill in Latin America and received solid waste from metropolitan region of Rio de Janeiro Along 1976 and 2012.

1976–2012.The leachate samples were collected in February 2013 from the equalization lagoon, in polyethylene flasks (5 L capacity) whose external walls were previously painted in black and washed internally with alkaline solution Extran® 5% and then rinsed with deionized water [13].

2.2. Leachate treatment by Electro-Fenton process

RL was submitted to E-Fenton treatment (EFTL) in a 1L batch electrochemical reactor, according to the methodology described by Oliveira et al. as summarized below: pH of RL was adjusted to 4.0 using concentrated H2SO4 (Merck) under constant magnetic stirring, then 5 ml of 30% v/v H2O2 (Merck) added and the iron electrode inserted [13]. Then a 2A alternating current applied for 30 minutes. After this period, the supernatant was filtered, and the filtrate collected and stored in polyethylene bottles under refrigeration (4oC) for later physical-chemical characterization.

2.3. Leachate treatment by distillation

RL was distilled according to Couto et al. that is, in a fractional distillation unit 1 L capacity equipped with a spherical glass reactor with anti-bumping granules, electrical heating mantle, packed column (100 mm×10 mm), thermometer, cold finger, manual reflux divider product cooler, and a glass receiver [13]. The heating through was controlled so that the reflux ratio does not exceed 5 mL min−1. Samples collected in the bottles immersed in a nice bath. The first aliquot (DLT1), gotten when 10% of the leachate was distilled was evaluated. The distillates were collected, stored in polyethylene bottles under refrigeration (4oC) for later physical-chemical characterization.

2.4. Effluent sample characterization

All effluent sample (RL and treated leachates) were analyzed for physical and chemical characterization according to Standard Methods for the Examination of Water and Wastewater [26]. The following parameters were evaluated, total dissolved solids (TDS), pH, salinity, and electrical conductivity (EC) measured with a multi-parameter analyzer (PCS Test 35, OAKTON). Determination of chemical organic demand (COD) was obtained using a COD reactor (DRB 200, HACH) and a spectrophotometer (DR 5000, HACH). The concentrations for Na, K, Mg, Ca, NH4+, NO3−, PO43−, Cl- and SO4 2− determined by ion chromatograph (DIONEX ICS 3000) equipped with an IonPac® CS16 analytical (3×250mm), cation column preceded by two pre-columns and a cation suppressor CSRS 300 (2 mm) and an AS23 analytical (2×250 mm) anion column preceded by a pre-column and a 300ASR-S anion suppressor (2mm). The metals - Al, Cr, Cu, Fe, Mn, Ni, Pb and Zn - were determined by atomic absorption spectroscopy at VARIAN AAS 240 spectrometer, after digestion according to EPA-3051A adapted procedure by using 20 mL of crude leached sample and 8mL of concentrated HNO3 in a Teflon jar, which was closed and heated in a microwave oven 600 W power (for 20 minutes heating to 170 oC for 10 minutes and, in addition, holding at 170 °C for 10 minutes) [27]. The volume obtained was filtered on paper filter and swelled with 100 mL Milli-Q water. The concentration results for each parameter were the average of a three times measurement and summarized in Table 1.

2.5. Germination, seedling development and sunflower (Heliantus annus L.) growth

The Brazilian Agricultural Research Corporation (EMBRAPA) kindly provided Helianthus annuus L. cv. BRS 321 seeds.

Seed germination and vitality tests were performed in 4x6+1 completely randomized factorial design in four repetitions with 25 seeds per repetition, totaling 2,800 seeds. The seeds were disinfected by washing in 1% sodium hypochlorite and distilled water. This procedure repeated three times. After disinfection, the seeds were dried at room temperature [28, 29].

Germination tests were conducted with six dosages of each one of the three effluent samples: sanitary landfill leachate (RL), leachate treated by electro-Fenton (EFLT), leachate treated by distillation (1st aliquot - DTL1). Each effluent quality was diluted in distilled water in the concentrations (% v/v) of 0 (control), 5, 15, 25, 50, 75 and 100.

The germination test was performed using Germitest® paper rolls wetted with each dilution sample at a ratio 2.5 times the dry paper weight, rolled carefully and wrapped in polyethylene bags to reduce evaporation placed in an incubator for COD at ± 25 ºC under 12 h photoperiod. Germinated seeds were counted every day for seven days [30].

Germination rate and normal seedlings were evaluated according to International Seeds Testing Associations-ISTA rules [30]. Germinated seeds daily counted for seven days were used to calculate germination speed index (GVI). Seeds with a protruding primary root were considered germinated. The GVI was calculated using the number of germinated seeds counted daily in each repetition, according to equation 1 [31].

$$GVI = \sum \left(\frac{n}{t}\right) \qquad (1)$$

Where GVI is germination speed index, n is the number of seeds newly germinated at time t and t is the day from sowing.

The emergency speed index (IVE) was calculated using the number of germinated seeds with only primary root counted daily in each repletion, according to equation 2 [32]:

$$IVE = \sum e_n / \sum N_n \qquad (2)$$

Where IVE is emergency speedy index, en seedling emergency in the first count, n is the last count at time, Nn is the number of days of seeds at time t, and t is the day from sowing.

Seedling aerial part and primary root length were measured using a 0.1cm graduated ruler. Ten normal seedlings were randomly chosen from each repetition of each treatment on the 7th day, for evaluation.

Seedling development was observed throughout the experiment; however, on the last counting day (7th day), both normal and abnormal plants were observed following the ISTA rules .

2.6. Studies of raw leachate in leaf nutrition and chemical attributes of soil with sunflower cultivation in a greenhouse

To evaluate the use of raw leachate (RL) in initial sunflower cultivation (nutrition and growth) and its effect on soil chemical attributes, an experiment was conducted in a protected environment. Pots with 3.5 L capacity filled with characteristic soil of the State of Rio de Janeiro where sunflower seeds sowed (Helianthus annus L.) BRS 321. The greenhouse is located at the State University of Rio de Janeiro (Maracanã campus, Rio de Janeiro, Brazil latitude 22º 54 'South, longitude 43º 14 'West).

The soil used to fill the pots was collected in a natural profile of a Red - Yellow Argisol, in a superficial soil layer from the municipality of Resende - RJ. After collected, a sample was taken for physical and chemical characterization, following the methodology described by Embrapa [33]. After dried in open air, soil was removed, homogenized, and passed through a 3.35 mm mesh sieve and weighed for homogeneous pots filling. All pots received zero gravel 0.01 cm layer and the same mass of soil, being weighed on a semi-analytical balance, totaling 2,200 g of soil per pot.

The experimental design used was a completely randomized (DIC), using the factorial scheme 5x4 + 1 with 5 concentrations of mixture with leachate: 0% with drinking water, 5%, 15%, 25%, 50% RL with drinking water and one treatment with mineral fertilization based on Novais, Neves and Barros methodology that considered the soil volume in each pot [34]. The seeds (BRS 321 variety) used both in germination test and in greenhouse experiment were provided by Brazilian Agricultural Research Corporation (EMBRAPA).

In each pot, 10 seeds were planted. On the 15th day, the surplus seedlings removed, leaving only one seedling per pot. Irrigation with different concentrations of RL mixture and with clean water started from the first day of planting. In the experimental units with mineral fertilizer and the control (0%) the soil moisture maintenance was done by irrigating the pots only with clean water, the other units were irrigated with the leachate mixture concentrations being 5%, 15%, 25% and 50%. Both, the leachate, and the clear water were applied manually with the aid of a 1.0 L graduated cylinder, obeying an irrigation frequency every two days until the end of the BRS 321 seed cycle, which was at 45º day. Crop treatments, invasive plant and pest control done manually when needed.

To calculate the leachate and clean water applications, the soil water balance method adapted from Gonçalves et al (2014) was used. This method consists in weighing the vessels where the difference in mass will correspond to the volume of water to be applied to raise the soil to field capacity (saturation with water in the soil, at the level of 100%) (equation 3) [35].

$$V = P_{cc} - P \qquad (3)$$

Where,
V= Water volume (L);
P_{cc}= Pot mass pot saturated with water (kg); P= Pot mass before receiving water (kg)

2.7. Sunflower seedlings growth analysis

To evaluate the effects of leachate doses application on sunflower seedlings growth after 45 days, the following parameters was considered, height of the aerial part (cm), length of the stem (cm) and root system (cm) with the help of a ruler graduated in millimeters, from the neck of the plant to the apex. The fresh matter weighed immediately after each cut and packed in paper bags. The aerial part (stem and leaves) and the root put to dry in an oven with forced air circulation at a 65ºC temperature for 72 hours, until reaching constant masses. At the end, the material was weighed, having the fresh weight and the dry weight of the aerial part and the root. With these data, the plant water loss was assessed [36].

2.8. Sunflower seedings leaf analysis for macro and micronutrients

To obtain the macro and micronutrients leaf levels, after cutting the aerial part on the 45th day, counted from the treatment's application beginning, the cut material was weighed and submitted to drying in a forced ventilation oven at 65 ° C, for 72 h. Then, it was ground in an analytical mill (IKA A11 Basic ®) with a 30-mesh sieve, packed in paper bags and sent to the laboratory for macro and micronutrients (nitrogen, phosphorus, potassium, calcium, magnesium, zinc, iron, copper, chromium, manganese) determination, according to Carmo et al. methodology [37].

The concentrations of phosphorus, potassium, calcium, magnesium, zinc, iron, copper, chromium, and manganese were obtained from the dried and ground samples after digestion with nitric acid and perchloric acid. After digestion, phosphorus determined by colorimetry, potassium by flame photometry, calcium and magnesium by atomic absorption and the other nutrients determined by plasma induction atomic emission spectrometry (ICP). To determine the N content, the samples were submitted to digestion via moist sulfuric and the Nitrogen determination obtained by Kjeldahl method.

2.9. Soil physical and chemical characterization at the end of experiment

At the experiment end, soil samples from each experimental unit were air dried, ground and sent for physical and chemical analysis according to the procedure of the Brazilian Agricultural Research Corporation-Embrapa.

2.10. Statistical analysis

Seed germination and seeding vitality results were submitted to variance analysis (ANOVA) as well as linear and polynomial (second order) regression tests using the software SAS (version 9.1).The data related to the greenhouse experiment assayed by using ANOVA and when significant, submitted to Tukey test, adopting a 5% probability level, using the SAS software (version 9.1).

3. Results and Discussion

3.1. Landfill leachate treatment

Physical and chemical analysis of RL revealed high electrical conductivity, chemical organic demand, as well as ammonia-nitrogen (Table 1). Heavy metal contents were low due to immobilization by adsorption and precipitation, according to Teta et al. [38]. The high COD content is related to recalcitrant organic material in the leachate, as well as the high amount of ammonia-nitrogen, resulting in an increasing raw leachate toxicity [39]. The high ammonia–nitrogen concentrations in leachate occurs throughout the landfill lifetime, even after its inactivity [13]. Ammonia-nitrogen can be used as fertilizer in agriculture however COD and conductivity contents found in RL (Table 1) were above the recommended levels for irrigation water settled down by World Health Organization [40, 41]. Hence, RL requires to be treated before reuse.

Table 1: Physical chemical mean parameters in raw and treated leachates.

Evaluated parameters	RL	EFTL	DTL1
pH	9.00	6.30	9.77
Conductivity (mS Cm^{-1})	10.48	18.52	8.23
Chemical oxygen demand (mg L^{-1})	3,494	1,350	n.d.
Total dissolved solids (mg L^{-1})	6,721	19,400	5,815
Salinity (mg L^{-1})	5,585	14,250	4,420
Ammonia-nitrogen (mg L^{-1}) **	2,165	2,000	3,047
Nitrite (mg L^{-1}) **	277.10	n.d.	282.35
Nitrate (mg L^{-1}) **	119.2 1	35.40	211.37
Phosphorus (mg L-^{1}) **	17.10	n.d.	143.01
Sulfate (mg L-^{1}) **	391	8,741	655
Chloride (mg L-^{1})	5,319	5,015	221
Potassium (mg L-^{1}) **	2,182	1,770	2,237
Sodium (mg L-^{1}) **	2,667	2,686	1,635
Magnesium (mg L-^{1}) **	65.49	14.80	n.d.

Zinc (mg L^{-1}) *	n.d.	0.171	n.d.
Chromium (mg L^{-1}) *	0.233	0.099	n.d.
Nickel (mg L^{-1}) *	0.194	0.284	0.005
Copper (mg L^{-1}) *	0.346	0.237	0.004
Aluminum (mg L^{-1}) *	0.056	0.810	n.d.
Cadmium (mg L^{-1}) *	1.080	0.029	0.001
Iron (mg L^{-1}) *	0.013	4.310	0.022
Plumbum (mg L^{-1}) *	3.224	0.360	0.010

*Determined by Atomic absorption; ** Determined by ion chromatography; RL: Raw Leachate, EFTL: leachate treated by Electro-Fenton process; DTL1: Distillation-treated leachate - 1st aliquot; nd = not determined.

In this study, two different treatment processes were employed aiming RL agricultural use for irrigation: Electro-Fenton (EFTL) and distillation (DTL1).

In the EFTL treatment, organic matter is oxidized by OH• radicals produced from the reaction between hydrogen peroxide and Fe2+ generated in situ [6]. The leachate was acidified until pH 3-4, since the Fenton reactions occur better at low pH values [42, 6]. The electro-Fenton lasted for 30 minutes in a current of 2A. In the final solution the pH was slightly acid (6.0) and the COD removal was 61 %. EFTL was not able to remove ammonia-nitrogen, which favors its use as fertilizer in the crops. However, salinity and total dissolved solids increased, which may be an impediment to its reuse in irrigation [43]. Moreover, DLT1 treated leachate has higher ammonia-nitrogen content and low salinity than EFTL, which can be good features for its reuse in agriculture.

As excessive salinity and mainly elevate sodium contents are inadequate for agriculture and can cause adverse effects on plant growth and pollute the soil and groundwater germination and seedling development tests with sunflower seeds were performed in order to determine the appropriate dilution rates for each leachate treatment (EFTL and DTL1) [43].

3.2. Germination, seedling development and growth

The analysis of variance for sunflower germination tests irrigated with three types of effluents (RL, EFTL and DTL1) with seven dilutions grades (from 0 % v/v -control with distilled water- to 100 % v/v), showed a significant effect of the isolated factors and their interactions - degree of dilution and effluent quality (LB, LTEF and LTD1) - for all germination and vigor processes analyzed. In this sense, linear and quadratic regression were used to evaluate the influence of the dilution grade of each effluent quality on germination.

The germination results for sunflower seeds treated with the three different effluent qualities (RL, EFTL and DTL1) in seven dilution degrees (% v/v) from 0% (control with distilled water) to 100%, are presented in Fig. 1. Quadratic effect was observed in all effluent's quality: RL (Fig. 1A), EFTL (Fig. 1B) and DTL1 (Fig. 1C), with adjusted coefficients (R2) observed around 0.90.

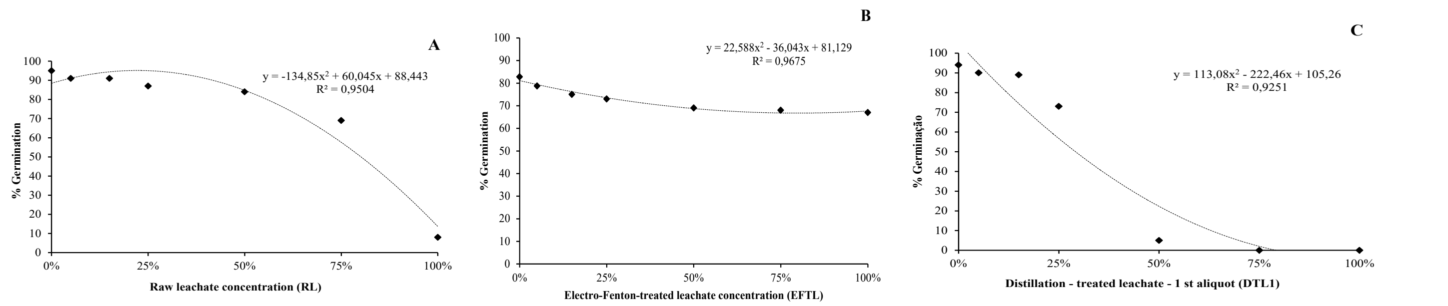

Figure 1: Effects of untreated and treated sanitary landfill leachate dilution degrees (% v/v) on sunflower seed germination percentage. A. Raw leachate; B. Raw leachate treated by electro-Fenton (EFTL); C. Distilled raw leachate - first aliquot (DTL1).

Concerning to RL and DTL1 effluents, the use of higher concentrations significantly decreased sunflower seed germination (Fig. 1A and 1C, respectively). This effect may be due to high salinity. High salt concentration induces a reduction in internal hydric potential interfering in water availability for the seeds, which directly influences on germination and mainly in plant vigo [44, 45]. Ammonia-nitrogen (NH4+) concentration in wastewater may also be influencing reduction in germination percentage. According to Pan et al. plants are particularly sensitive to NH4+ during germination and early seedling development. Mechanisms of toxicity include salt toxicity and specific NH4+ toxicity [46]. In DTL1 solution which has the higher ammonia-nitrogen concentration (3,470 mg L-1), the germination was totally inhibited at the 50% dilution. According to Cheng and Chu high ammonia-nitrogen concentration is highly phytotoxic to seed nutrient storage tissues as well as alters germination sequence [7].

On the other hand, the high salinity of EFTL solution (Table 1) did not hinder significantly the germination potential of the sunflower seeds in all dilution degrees (Fig. 1B). It has been proposed that seed germination is influenced by total dissolved salts concentration (or osmotic pressure) as well as by the type of salts involved [47]. Regarding to EFTL solution, Table 1 also shows that this treated effluent has hight iron (Fe) concentration, which is generated in situ by oxidation of the anode in EFTL treatment [19, 18]. Fe ions promotes the formation of chlorophyll and acts as an oxygen carrier, necessary for cell division and growth [48-50]. Also participates in formation of some enzymes (catalase, peroxidase, cytochrome oxidase and xanthine oxidase), and is indispensable for respiration, photosynthesis, nitrogen (N2) and electron transfer through the cycling between Fe2+ and Fe3+ [51]. Thus, the high concentration of iron ions in EFTL effluent (Table 1) may be minimizing the negative effects of salinity and ammonia-nitrogen concentration, improving germination by facilitating the breaking seeds dormancy and exerting a stimulatory effect [44].

Germination speed index values (GVI) were higher in all treatments at dilution rates up to 25% (Fig. 2). When using higher effluent concentrations (75 and 100% v/v), GVI was probably influenced by salinity and ammonia-nitrogen concentrations, which slowed down the post-seminal seed development, promoting phytotoxic effects on germination [52, 53].

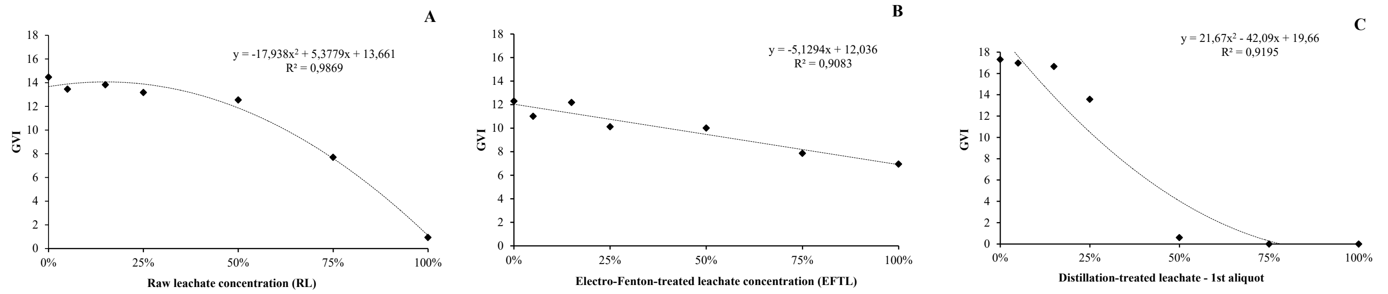

Figure 2: Effects of untreated and treated sanitary landfill leachate dilution degrees (% v/v) on Germination Velocity Index (GVI). A. Raw leachate; B. Raw leachate treated by electro-Fenton process (EFTL); C. Distilled raw leachate – 1st aliquot (DTL1).

Fig. 3 shows normal seedlings percentage as a function of effluent dilution. In all cases, the effluent application had a significate effect. DTL2 (Fig. 3D) had satisfactory and similar results for normal seedlings percentage in lower dilutions. At 75 and 100% v/v dilution, some abnormal seedlings were observed. For EFTL (Fig. 3B), despite the successful germination (Fig. 1B), the seedlings did not develop properly. We can infer that seed water potential was altered and water uptake did not occur, which consequently affected storage tissues responsible for initial seedling growth. For LTD1, concentrations above 50% v/v resulted in the death of all seeds (0.0 % of germination) and, consequently, there was no seedling development. Therefore, these dilutions were not included in Fig. 3C.

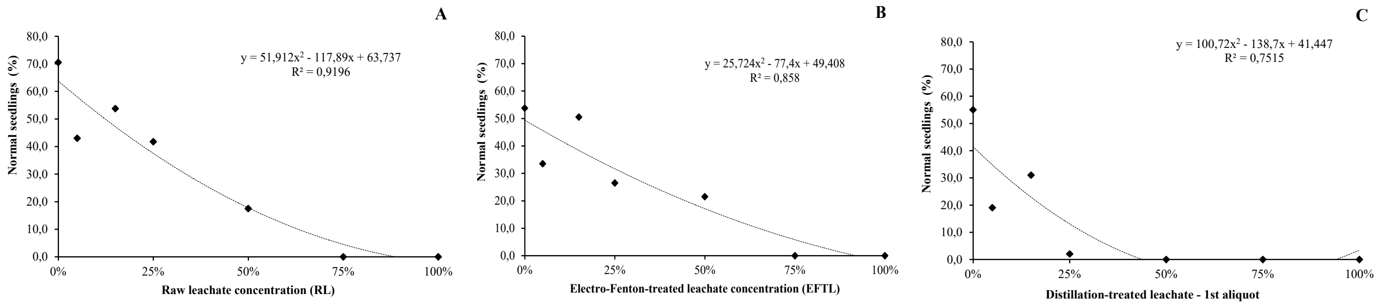

Figure 3: Effects of untreated and treated sanitary landfill leachate dilution degrees (% v/v) on normal seedlings percentage of sunflower. A. Raw leachate; B. Raw leachate treated by electro-Fenton (EFTL); C. Distilled raw leachate - first aliquot (DTL1).

Untreated and treated effluent effects on root and aerial part growth of normal seedlings are shown in Figs. 4 and 5. According Žaltauskaitė and Čypaitė root growth is a great sensitive parameter in plant phytotoxicity assessment and toxics effluents impact its growth [54]. RL dilutions up to 50% v/v has promoted acceptable root length and aerial part growth, but above this value, no seedling development occured. These data confirm that sunflower is moderately tolerant to salinity (3,310 mg L-1), but growth is progressively reduced by the increasing of salt concentration at the radicle medium, as demonstrated by Dickmann et al. [55].

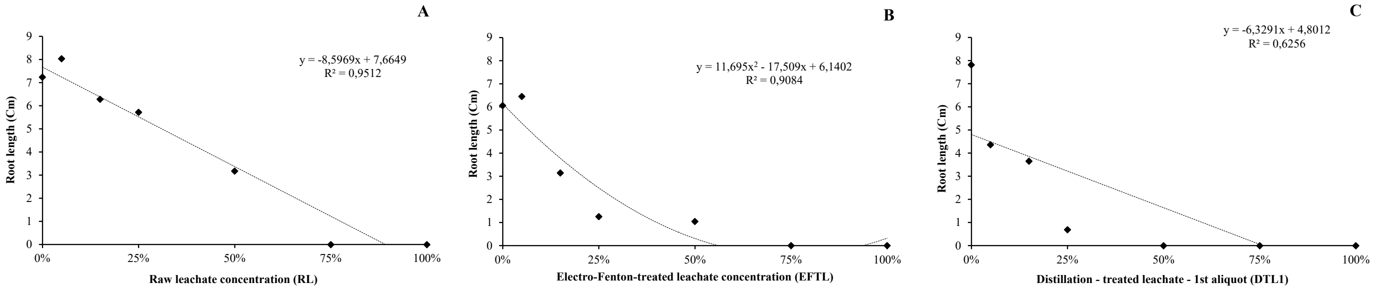

Figure 4: Effects of untreated and treated sanitary landfill leachate dilution degrees (% v/v) on seedling root length. A. Raw leachate; B. Raw leachate treated by electro-Fenton (EFTL); C. Distilled raw leachate - first aliquot (DTL1).

For EFTL, dilutions above 15% (Figs. 6 and 7) reduced or strongly inhibited seedling growth. The higher salinity attributed to high sulfate content did not influenced the germination velocity, but these parameters caused variations in their development processes. Thus, the results described above show that seedling growth sequence in relation to effluent quality was RL>EFTL>DTL1.

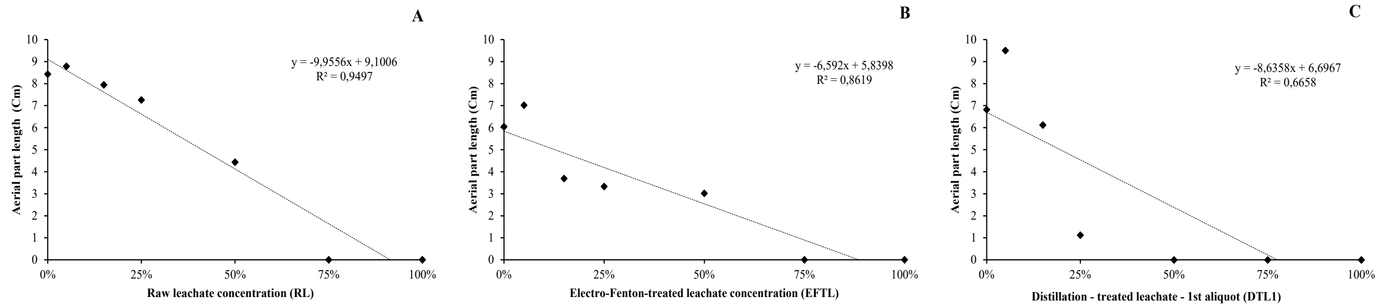

Figure 5: Effects of untreated and treated sanitary landfill leachate dilution degrees (% v/v) on seedling aerial part length. A. Raw leachate; B. Raw leachate treated by electro-Fenton (EFTL); C. Distilled raw leachate - first aliquot (DTL1).

The results presented in Fig. 1A indicate that with the use of crude leachate (RL) at dilution rates from to 50%, germination rates greater than 80% were obtained, indicating the feasibility of using this effluent in irrigation of sunflower crop, thus avoiding the costs of distillation or electro Fenton treatment. Based on this analysis, seeking a broad assessment of the viability of agricultural reuse of this effluent without treatment, but with different dilution rates, the sunflower was grown in a greenhouse to verify the effects on its initial growth (root length, shoot length , dry root weight, green root weight, dry weight of the aerial part, green weight of the aerial part), in foliar absorption of macro (N, P, K, Ca and Mg) and micronutrients (Zn, Cu, Mn and Fe) and the soil chemical attributes.

3.3. Raw leachate concentrations effect on initial growth and leaf nutrition of sunflower

There was a significant statistical effect ($p < 0.05$) for the factor concentrations of leachate in all variables treated in ANOVA for growth and biomass, after 45 days planting (see supplementary Table 2).

Table 2: Summary of analysis of variance (ANOVA) for growth and biomass variables after 45 days of planting in a greenhouse

Variation source	GL	CR	CPA	MSR	MVR	MSPA	MVPA	BV/BS R	BV/BS PA
		Medium square							
Concentration	5	10,62*	627,63*	4,56*	106,91*	10,85*	437,70*	27,41ns	29,10ns
Repetitions	3								
Error	18	1,49	39,63	0,77	12,66	0,25	17,01	20,27	21,33
Total	23								
CV		31,30	29,60	84,27	56,20	37,30	45,84	59,64	61,43

Legend: Root length (CR); aerial part length (CPA); dry root mass (MSR); green root mass (MVR); aerial part dry mass (MSPA); aerial part green mass (MVPA); green biomass / dry root biomass (BV / BS-R); and Green biomass / dry shoot biomass (BV / BS-PA). n.s = not significant and * = significant at 5% probability. GL: degrees of freedom. CV: coefficient of variation

Fig. 6 shows the effects of different raw leachate (RL) concentrations on root length (CR) and aerial part (CPA) of the sunflower after 45 days of growth. At concentrations of 15%, 25% and 50%, the CPA values were statistically equal according to the Tukey test (average length of 30 cm). The highest CPA value (close to 40 cm) was obtained when mineral fertilizer was used. Similar behavior was observed for CR, except in 15% concentration that did not differ statistically from the mineral fertilization (Ad. Mineral), indicating that the use of this concentration of RL is able to allow satisfactory sunflower root and aerial part growth, minimizing soil mineral fertilization costs.

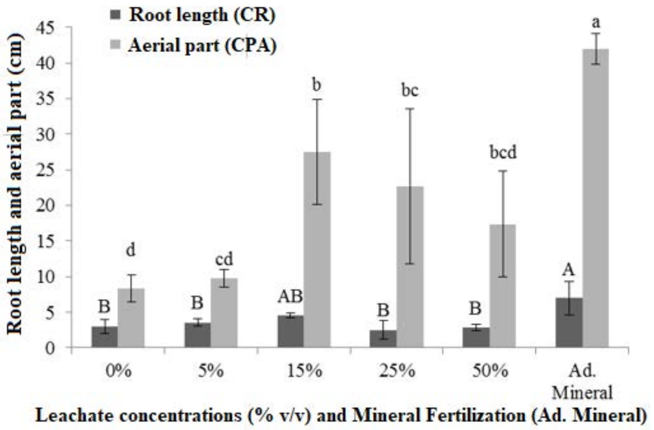

Figure 6: Root length and aerial part of BRS 321 sunflower grown in a greenhouse. The bar indicates the standard error; Average followed by the same lowercase or uppercase letter does not differ statistically from each other by Tukey's 5% test.

The low phosphate concentration (17.10 mg L^{-1}), high ammoniacal nitrogen (2.165 mg L-1) and chloride (5.319 mg L-1) concentrations in RL (Table 1) may have had a negative effect on sunflower growth, once the increasing in leachate concentrations, was noted a reduction in the averages obtained for CR and CPA (Fig. 6). These results are compatible with those obtained by Białowiec who observed a phytotoxic effect on the growth of five plants irrigated with two leachates from different landfills in Poland with lower NH4+, Cl- and P concentrations than the RL used in the present study [56].

The results referring to the green root mass (MVR) and dry root mass (MSR), were statistically equal both in 15% RL concentration and those obtained with mineral fertilization (where the highest averages were obtained), according to Tukey test ($p \leq 0.05$) (Fig. 7). In the other RL concentrations, the averages obtained for MVR and MSR were statistically equal according to Tukey test ($p \leq 0.05$), however, was observed a reduction in total sunflower biomass when compared to mineral fertilization.

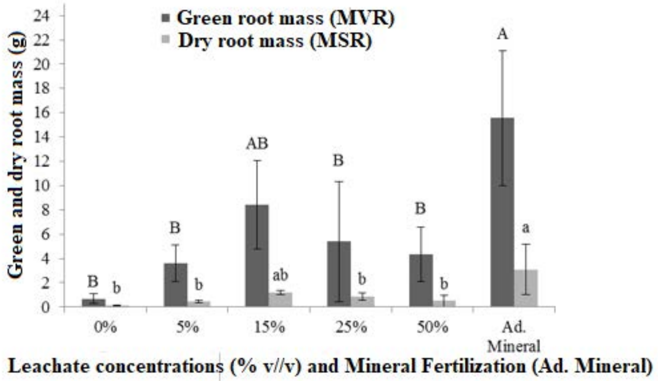

Figure 7: Green mass and dry mass of BRS 321 sunflower root grown in a greenhouse. The bar indicates standard error; Averages followed by the same lowercase or uppercase letter does not statistically differ from each other by Tukey's 5% test.

A Cheng and Chu study, with tree species using different concentrations of leachate from the Hong Kong landfill, did not show statistically significant differences for the species evaluated. However, the biomass values for aerial part / root of seedlings that were treated with the leachate were not higher than those obtained by the control with fertilizers. These results differ from those obtained by the present study, where the highest averages for these parameters were obtained with the use of 15% RL concentration and with the use of mineral fertilization and did not statistically differ (Fig. 7).

The Tukey test ($p \leq 0.05$) identified that averages of green mass (MVPA) and dry mass of aerial part (MSPA) did not differ from each other except for mineral fertilization (Fig. 8).

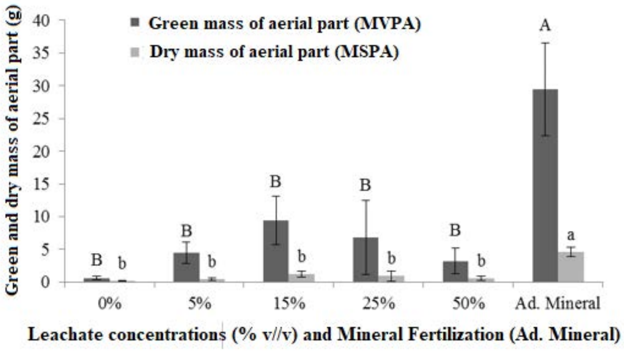

Figure 8: Green mass and dry mass of aerial part for BRS 321 sunflower grown in a greenhouse. The bar indicates the standard error; Averages followed by the same lowercase or uppercase letter does not differ statistically from each other by Tukey's 5% test.

Is important to note that with appliance of 25% and 50% RL concentrations, the plants at the end of the experiment showed stunted growth, with small yellowish leaves (Fig. 9 A and B), probably due to salt deposition on the leaves, since leachate has a high sodium concentration (2,667 mg L^{-1}). According to Ayers and Westcot the most common effect of salinity on plants, in general, is the cell expansion limitation due to increased osmotic pressure at the medium and the consequent cell plasmolysis, thus affecting cells division and elongation, impairing plant growth and mass volume [55]. Therefore, the results in Figure 8 indicate that the use of 15% RL concentration is able to promote the sunflower aerial part growth without damaging the leaves, demonstrating that the leachate was not toxic and / or statistically equal to water appliance.

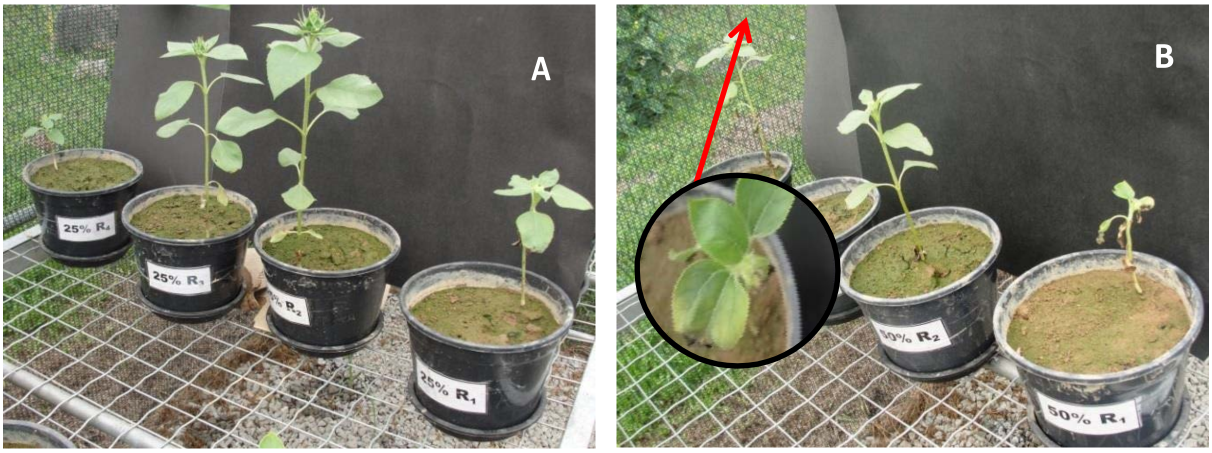

Figure 9: Demonstration of the problems found in the experiment using landfill leachate as irrigation. A. 25% v / v treatment; B. 50% v / v treatment.

The present study evaluated sunflower cultivation until the beginning of its flowering (45 days). The analysis of variance results (see supplementary Table 3) showed that there were statistically significant changes (p <0.05) in macro and micronutrients levels due to raw leachate different concentrations.

Table 3: Summary of analysis of variance for leaf macro and micronutrient variables after 45 days of planting in a greenhouse.

Variation source	GL	N	K	P	Ca	Mg
	Medium square					
Concentration	5	18,28ns	842,37*	7208218,9*	2934321,8ns	2760121,2*
Repetitions	3					
Error	18	28,25	19,78	864459	860462	49160
Total	23					
CV		14,09	11,90	37,72	19,05	9,31
Variation source	GL	Cu	Fe	Mn	Zn	
		Medium square				
Concentration	5	56,189 ns	388989,5ns	107866,3*	28935,2*	
Repetition	3					
Error	18	17,69	193037	15713	478,81	
Total	23					
CV		3679	62,19	22,68	17,08	

Legend: n.s = not significant and * = significant at 5% probability. GL: degrees of freedom. CV: coefficient of variation

When applying the Tukey test (p≤0.05) for macronutrients, was found that N and Ca did not show a statistically significant difference, therefore, there was no effect for applied treatments on these nutrient levels in the plant (Fig. 10 A and D). For nitrogen there were average variations between 35.57 g kg-1 and 41.29 g kg-1; for calcium, the averages were between 3.0g kg-1 and 5.0 g kg^{-1}.

Although the plant responded positively to the leachate concentrations when compared to mineral fertilization, the ideal absorption may have been affected by the leachate high salinity; however, the leachate concentration provided satisfactory growth in the initial concentrations. This may be due to the ionic competition that occurred in the soil adsorption sites during the experiment period, since aerial part and root development were compromised (Fig. 10 A). Bosco et al.stated that plants grown under high salinity tend to absorb less nitrogen and increase the absorption of Cl- accumulation [57].

Phosphorus showed high average levels in drinking water treatment (0%) and mineral fertilization, followed by highest averages for RL treatments with 5% and 15%, being statistically equal to mineral fertilization (Fig. 10 B).

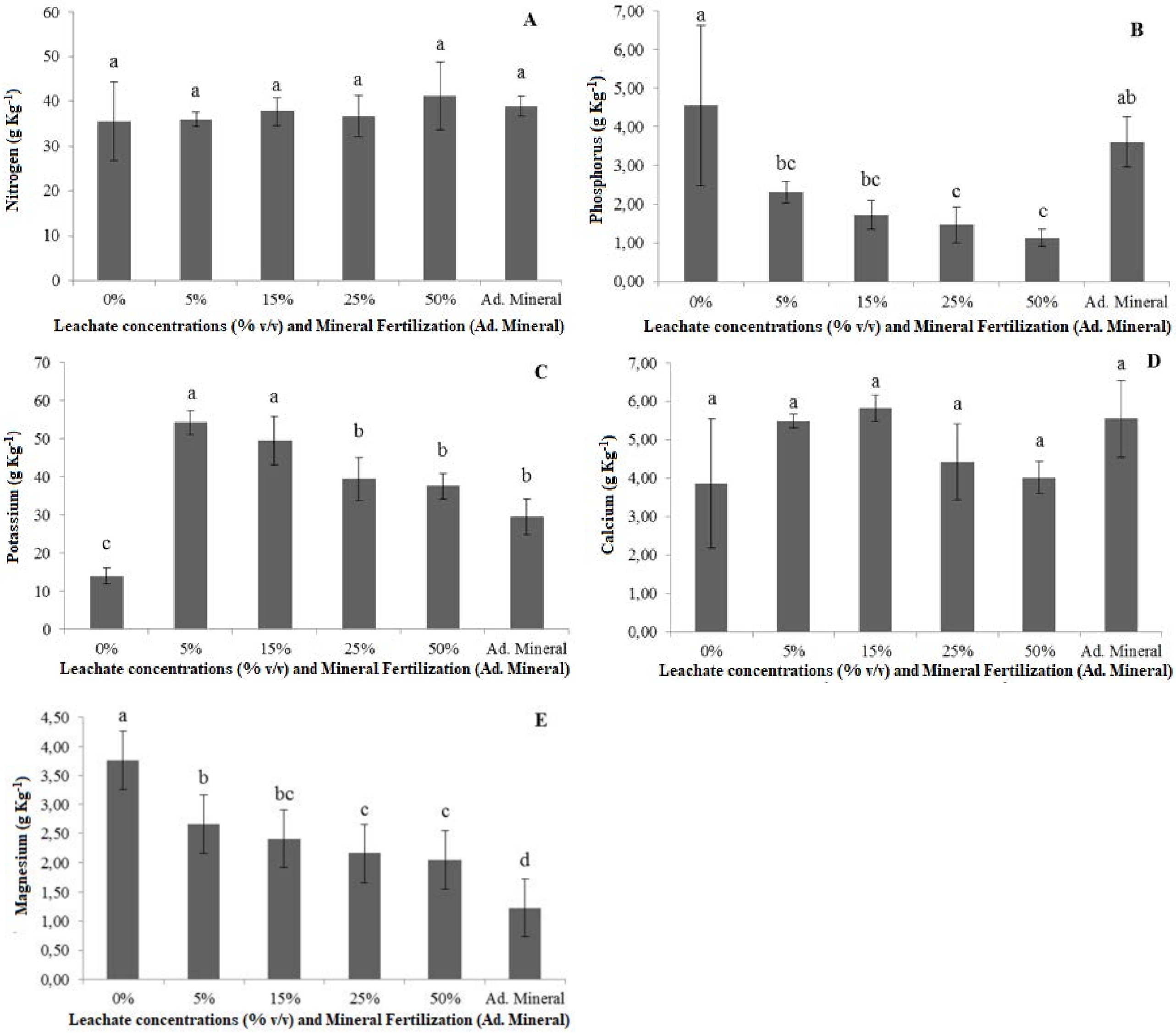

Figure 10: Macronutrients in sunflower aerial part grown in a greenhouse and irrigated with landfill leachate in different concentrations. A. Nitrogen; B. Phosphorus; C. Potassium; D. Calcium; E. Magnesium; The bar indicates the standard error; Averages followed by the same letter in columns do not differ statistically by the Tukey test ($p \leq 0.05$).

For potassium there is a variation of averages between 13.85 g kg-1 up to a maximum of 54.28 g kg-1, with the highest average concentrations being obtained in concentrations of 5% and 15%. This effect may be related to the presence of potassium or to excess sodium in the RL (Table 1) that competes for the same active site as potassium, impairing this nutrient absorption by the plant. According to Feigin et al. even if there is an increase in available K content through fertigation of wastewater in the soil, this nutrient amount required by plants is so high that hardly only irrigation with effluent could adequately supply the plants [58]. This fact is proven by this present study with significant differences in this element content in the aerial part under RL irrigation in different concentrations (Fig. 10) and there were some visual deficiency symptoms in the leaves (such as necrosis) while conducting the experiment.

The variance analysis results by F test for micronutrients such as copper and iron were not significant, indicating that different leachate concentrations did not promote changes in these elements concentrations (see supplementary Table 3), which were statistically equal according to Tukey test ($p \leq 0.05$) (Figs. 11C and 11D). The control remained in the average, due drinking water that presented for Fe = 4.7g mL-1 - 7.7g mL-1 and Cu = 0.14g mL-1 - 0.23g mL-1. Unlike this work, FRIEDMAN et al. obtained low levels for iron in sunflower irrigated with treated effluent in relation to that irrigated with drinking water [59].

Figs. 11A and 11B show that after using drinking water, the average concentrations of zinc and manganese were higher than those obtained with using RL (with exception of Zn in RL 5% concentration), which possibly indicates that the content of these elements in the drinking water is higher than in the RL, contributing to the availability of these nutrients for the plants. This explains why the highest zinc average concentration was obtained at a 5% RL concentration (0.250 g kg-1), followed by the control (0.200 g kg-1). For manganese, the highest averages were obtained in treatments with mineral fertilization, control and in RL dosage of 5%. The cobalt, nickel, cadmium, and lead levels were below the detection limit of the method in analyzed samples.

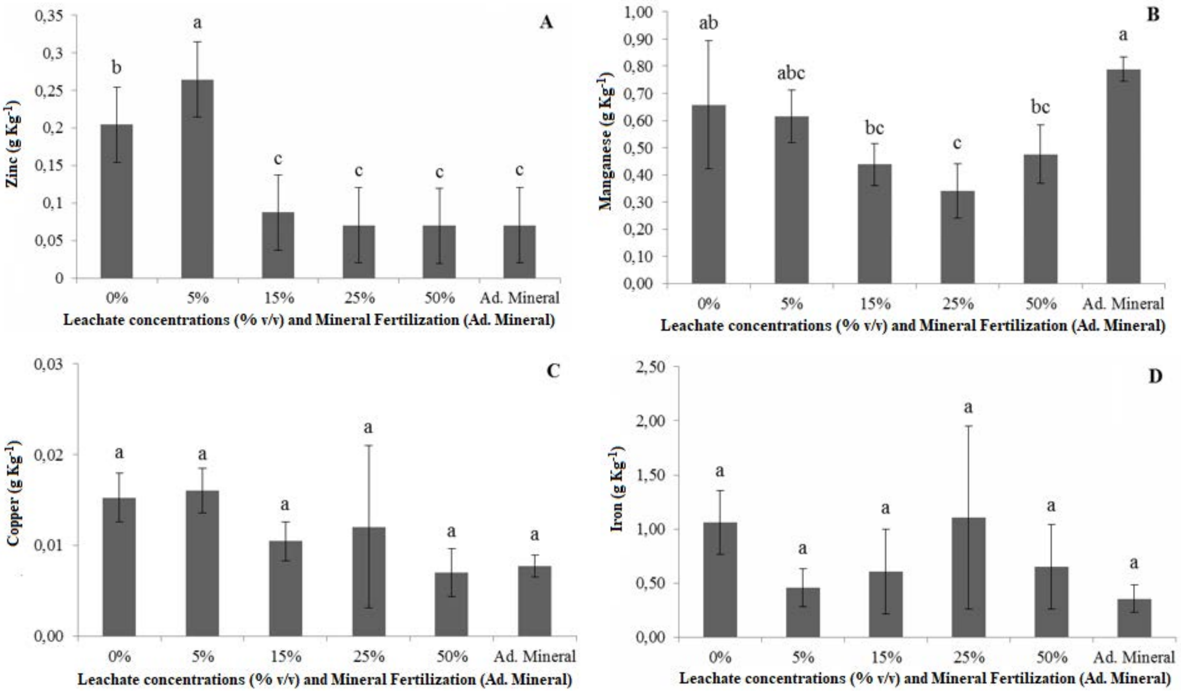

Figure 11: Micronutrients in aerial part of sunflower plants grown in a greenhouse and irrigated with landfill leachate in different concentrations. A. Zinc; B. Manganese; C. Copper; D. Iron; the bar indicates the standard error; Averages followed by the same letter in columns do not differ statistically by the Turkey test (p≤0.05).

3.4. Effect of raw leachate concentrations on chemical attributes of soil cultivated with sunflower in greenhouse.

From the variance analysis on studied factor (RL), there was a significant leachate concentrations effect on the soil used in the experiment (Argisol Red - yellow) (see supplementary Table 4).

Table 4 : Summary of analysis of soil variance after 45 days of cultivation with sunflower in a greenhouse

Variation source	GL	pH (H2O)	pH (KCl)	Ca^{2+}+Mg^{2+}	K^+	Na^+	$H^+ + Al^{3+}$
		Medium square					
Concentration	5	0,356*	0,199*	0,057*	0,828*	0,576*	3,03
Repetition	3						
Error	18	0,040	0,003	0,017	0,014	0,012	0,174
Total	23						
CV		4,38	1,47	15,56	23,25	22,51	7,04
Variation source	GL	Al^3+	P	C	N	C/N	
		Medium square					
Concentration	5	2,22*	164,77*	0,554 $^{n.s.}$	0,042*	0,366$^{n.s.}$	
Repetition	3						
Error	18	0,024	26,76	0,485	0,003	0,305	
Total	23						
CV		9,92	12,81	6,22	3,46	8,29	

Legend: n.s = not significant and * = significant at 5% probability. GL: degrees of freedom. CV: coefficient of variation

Soil tends to undergo changes in its chemical attributes with wastewater application. According to Jnad et al. addition of exchangeable cations and anions present in the effluents can raise or reduce the pH due to accelerated ammonia and ammonium nitrification, releasing hydrogen ions that remain adsorbed to the soil colloids [60]. Some works, such as Fonseca et al. and Leal even indicate that pH in soils irrigated with treated domestic sewage effluent increased by at least one unit [61]. In conducting this experiment, the RL pH average was 9.2 (Table 1) and in drinking water was 6.9. The soil did not have the pH corrected, starting the experiment with pH 4.1 in water and pH 3.6 in KCl (Table 5). The results indicate that the soil pH showed a higher average value in 50% RL concentration in both, water and KCl and was observed that an increase in pH occurs with the increase in RL concentrations (Table 5).

Table 5 also shows that the average values of K^+, Na^+, N and (Ca^{2+}+ Mg^{2+}) increased with landfill leachate concentrations elevation and Aluminum (Al3+) showed a slight reduction with the pH increase. A similar result was obtained by Krob et al. using urban waste compost, since it caused increases in pH values, CTC, sodium absorption ratio (RAS), total nitrogen (N), phosphorus (P), extractable sodium (Na), exchangeable calcium (Ca) and magnesium (Mg) and reduction in exchangeable aluminum (Al) in the soil [62].

Na+ and K+ concentrations in the soil increased with the rise in leachate concentration (Table 5) indicating that in long-term, the application of waste water rich in Na^+ and K^+ may modify the soil chemical and physical attributes. According to Santos et al. Na^+ and K^+ excess in the soil can cause clay fraction dispersion causing modification on the structure and altering fluids dynamics in the soil [11].

Carbon (C) concentrations in soil showed no statistically significant difference according to the Tukey test (≤0.05) after application of RL different concentrations (Table 5). On the other hand, total nitrogen concentrations showed great variation according to Tukey test (p≤0.05) where highest average values were obtained, respectively, in 50%, 25% and 15% RL concentrations (Table 6). Carbon is related to the organic matter of the soil that also acts as a protection on its surface, with the reduction of the contents of organic matter, the soil suffers great losses of carbon (C) and Nitrogen (N). Some authors have observed a reduction in carbon levels in soils irrigated with wastewater with high concentrations of nitrogen, as is the case with the RL used (Table 1), since it is subject to microbial decomposition of the soil, with N being later transformed into simple inorganic compounds available to plants, such as ammonium and nitrate [59, 63].

Although slightly altered by the application of the RL, the soil pH remained acidic (Table 5), which allows the availability of toxic aluminum (Al^{3+}), responsible for impairing the root system development. In addition, acidic soils also allow phosphorus (P) fixation by iron (Fe) and aluminum (Al) forming insoluble compounds not available for plants [64].

Table 5: Soil fertility before and after landfill leachate application

Soil after treatments								
Parameters	Unit	Original Soil	0%	5%	15%	25%	50%	Mineral Fertilization
pH (H2O)	-	4,1	4,2b	4,5b	4,6b	4,7b	5,1a	4,4b
pH (KCl)	-	3,6	3,7c	3,8bc	4,0b	4,1b	4,3a	3,7c
Ca^2+ + Mg^2+	cmolc kg-1	0,6	0,80ab	0,75b	0,87ab	0,72b	1,05a	0,90ab
K+	cmolc kg-1	0,14	0,13d	0,27d	0,52bc	0,60b	1,36a	0,20d
Na+	cmolc kg-1	0,014	0,08c	0,48b	0,53b	0,65b	1,10a	0,07c
H+ + Al3+	cmolc kg-1	6,9	6,67ab	6,00bc	6,00bc	5,65c	4,50d	7,00a
Al^3+	cmolc kg-1	2,6	2,45a	1,87b	1,37c	1,30c	0,35d	2,12ab
P	mg kg-1	46,0	40,75b	36,25b	38,75b	37,25b	36,25b	53,00a
C	g kg-1	11,9	11,35a	10,52a	11,00a	11,55a	11,35a	11,37a
N	g kg-1	1,7	1,57cd	1,55d	1,70abc	1,72ab	1,82a	1,62bcd
C/N	-	7,0	7,00a	6,50a	6,75a	6,50a	6,25a	7,00a

Subtitle: Averages on the line followed by the same letter, do not differ significantly at 5% probability level by the Tukey test (p≤ 0.05).

The variance analysis showed that copper (Cu) and lead (Pb) levels in the soil did not show a statistically significant difference (p≤0.05) after different RL concentrations application, both, for Mehlich Extraction and for DTPA extraction (see supplementary Table 6).

Table 6 : Summary of analysis of variance for soil fertility after 45 days of sunflower cultivation

Variation source	GL	Mehlich Extraction				
		Cu	Fe	Mn	Zn	Pb
		Medium square				
Concentration	5	0,02$^{n.s.}$	60,17*	4,55*	0,527*	0,0167$^{n.s.}$
Repetition	3					
Error	18	0,008	3,76	1,62	0,091	0,023
Total	23					
CV		8,82	5,01	13,88	19,21	5,28
Fonte de variação	GL	DTPA Extraction				
		Cu	Fe	Mn	Zn	Pb
		Medium square				
Concentration	5	0,046*	157,6*	2,43$^{n.s.}$	0,38*	0,035$^{n.s.}$
Repetition	3					
Error	18	0,006	20,32	0,94	0,031	0,047
Total	23					
CV		9,88	6,34	16,17	18,68	7,039

Legend: n.s = not significant and * = significant at 5% probability. GL: degrees of freedom. CV: coefficient of variation

In comparison with the soil before the experiment, was observed that after treatments application there was no significant variation in these elements' concentrations (Table 7). Some elements such as cobalt (Co) and nickel (Ni) were not detected by the applied methodology (Table 7).

Iron presented higher average concentrations in soil treated by mineral fertilization, the control (0%) and 5% of RL in Mehlich extraction. By DTPA extraction, the averages showed greater variations, but the highest levels of this element were obtained with using mineral fertilizer, the control (0%) and 25% RL (Table 8). For others RL concentrations, the iron soil content was statistically equal to concentration of 25%. The results in Table 8 show that RL different concentrations application reduced iron content in the soil. Bearing in mind, that iron has great ability to form complexes with organic matter which facilitate its movement though soil profile and its absorption by plants, is inferred that this element when forming complexes with organic matter (expressed by the high COD) present in the RL (Table 1), may have been leaked during irrigation. The low carbon content in the soil used (Table 8), which is indicative of organic matter content, did not favor its incorporation into it.

Can be observed in Table 7, that soil zinc concentrations showed a slight increase with RL application in lower concentrations, by both types of extraction techniques used. According to Malavolta the lack of Zn decreases the size of new leaves, in addition to causing their narrowing and elongation [65]. These symptoms were not observed in the present study. However, because it is a micronutrient, would be necessary to have more sunflower cultivation time to observe such symptoms.

Manganese was the metal that showed the most striking variation after the treatments, and can be said that averages obtained, except for the control (0%), were statistically equal (Table 7).

Table 7: Soil metal content before and after landfill leachate treatment

		Soil after treatments					
Parameters	**Original Soil**	**0%**	**5%**	**15%**	**25%**	**50%**	**Mineral fertilization**
Mehlich extraction					mg		kg
	1						
Cu	1,17	1,13a	0,98a	0,98a	0,96a	0,93a	1,06a
Fe	55,1	43,25a	39,20ab	38,00b	36,85bc	32,67c	42,45a
Mg	15,9	8,00b	9,21ab	9,89ab	8,40ab	8,66ab	10,87a
Zn	1,40	2,14a	1,80ab	1,50ab	1,30b	1,13b	1,54ab
Co	0,091	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ni	0,131	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb	2,97	2,90a	2,86a	2,85a	2,93a	2,75a	2,92a
DTPA extraction	mg kg^{-1}						
Cu	1,08	1,00a	0,80b	0,70b	0,75b	0,71b	0,80b
Fe	96,5	77,72ab	68,00bc	67,02c	70,27abc	63,60c	80,00a
Mn	6,21	5,80a	6,54a	6,67a	5,30a	5,00a	6,84a
Zn	0,779	1,45a	1,14a	0,86bc	0,72c	0,60c	1,45bc
Co	-	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Ni	0,045	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Pb	0,071	3,13a	3,00a	3,11a	3,21a	3,07a	3,16a

Subtitle: Average on the line followed by the same letter, do not differ significantly at p ≤0.05 probability by the Tukey test.

The results analysis in tables 5 and 7, as well as Figures 8, 9, 10, 12 and 13, indicates that RL agricultural reuse for sunflower crops irrigation should be restricted to this effluent lowest concentration (less than 50%). As verified, the increase in RL concentration tends to generate soil-plant system negative impacts.

4. Conclusions

Raw leachate samples (RL) revealed high conductivity, chemical organic demand (COD) and ammonia-nitrogen but presented low heavy metal. The landfill leachate electro-Fenton treatment (EFLT) decreased COD in 61%, but did not decreased other parameters, especially ammonia-nitrogen and sodium. On the other hand, salinity and sulfate content increased strongly. Two aliquots of distillation treatment (DTL1 and DTL2) presented no detectable COD, but DTL1 had higher ammonia-concentration. In DTL2, all parameters were reduced, indicating that this treatment is the more efficient and may be an alternative for landfill disposal.

RL also presented good results. Germination and GVI percentage for sunflower BRS 321 seeds had an acceptable germination percentage in all effluent studied - raw and treated leachate. The best results were using a dilution between 15% and 25% relative to control (distilled water), although it was observed a reduction in the percentage in normal seedlings number with a normal cycle phenology.

The general satisfactory results obtained for dilutions below 50% for all the effluents investigated, lead to conclude that sunflower cv. BRS 321 is resistant, can germinate in extreme conditions and has good tolerance to toxic substances present in germination media.

The agricultural reuse of RL in sunflower crops irrigation must be restricted to this effluent concentration below 50%, to minimize the negative impacts on soil-plant system.

Considering that the RL used in this experiment presented essential nutrients for plants such as N and K, this effluent has a real possibility of being reused in sunflower crops irrigation, assuming that used at adequate dilution rates, promoting an environmentally appropriate destination for this by-product, in addition to reducing costs with commercial fertilizers.

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