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Full Length Article

Soil Properties, Growth, Mineral Content and Ultra-structural Leaf Morphology of Swiss Chard in Response to Landfill Leachates Used as Irrigation Water

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Abstract

Swiss chard cultivar was raised in greenhouse and irrigated with different concentrations of leachate obtained from the municipal landfill in Bellville, Cape Town, South Africa. The greenhouse experiment was laid out in a completely randomised design (CRD) replicated three times. Soil and crop samples were taken weekly after transplant and analysed to assess soil properties, growth and mineral contents of Swiss chard, post-irrigation. The leachate samples had a high electrical conductivity (mean = 383 mS cm^{-1}) and high soluble salts content (mean values, Na: 714.5 mg/L, K: 56.8 mg/L, Ca: 133.7 mg/L, Mg: 68.8 mg/L, Cl: 983 mg/L); while the composition of heavy metals in the wastewater leachates were of low concentrations. The application of different concentrations of leachates as source of irrigation resulted in increased soil cation concentrations, particularly Na ions (increased sodicity). Similarly, an increase in electrical conductivity and pH were recorded in the soils after irrigation with leachates. The soil metal concentrations were low and there was no significant ($p<0.05$) difference in soil heavy metal concentrations between the soils irrigated with leachate and the controls. The result also shows significant ($p<0.05$) reduction (up to 50%) in Swiss chard growth (plant height) with application of all the three concentrations (100, 50 and 25%) of leachate as sources of irrigation water compared with the growth observed in leachate-free (control) irrigation systems. This reduction in growth was best attributed to the high cations content in plant tissue, picked up from the soil, which was high in these cations as a result of leachate irrigation. A study of the ultra-structural leaf morphology of the species using SEM revealed possible closure of the stomata opening induced by osmotic pressure, deposition of elements and reduced photosynthesis which would have limited carbon dioxide uptake and ultimately yield. The implication of this study on the possible agronomic improvement of Zn content in Swiss chard species tested is discussed.

Keywords: Landfill leachate; Potting soil; Swiss chard; Salinity; Electrical conductivity (EC)

Introduction

Landfill leachate has been for a long time recognized as a potential source of pollution to ground and surface waters (McDougall *et al.*, 1980). Toxic substances, which are commonly present in leachate, include major ions, heavy metals, a wide range of organic compounds and microorganisms. It is essential to collect and treat these leachates before they are discharged in main or municipal water in order to safeguard aquatic ecosystems. Utilization of municipal wastewaters on lands has been a low-cost treatment and disposal alternative. Municipal wastewater which contains considerable amounts of salts may serve as a source of nutrients for plant growth (Berry *et al.*, 1980). Similarly, it may be possible to recycle landfill leachate by land application; this decontamination is achieved by allowing the soil to act as a filter. Bennett *et al.* (1975) reported significant water quality improvement and no serious effect on a mature hardwood forest after using landfill leachate as irrigation water. It has also been demonstrated that forages contained higher concentrations of macronutrients after irrigation with leachate (Menser *et al.*, 1979). However, unlike sewage effluent, landfill leachate has not been considered as a potential resource for plant growth, mainly on account of excessive Fe and Mn. Apart from Fe and Mn, other metals may be present in toxic concentrations. Landfill leachate may also contain salts and organic compounds such as organic acids and phenols in quantities that may restrict biological activity in the root zone (Artiolar-Fortuny *et al.*, 1982). In a country like South Africa which is considered a water scarce country, the prudent use of water for agriculture purposes is always welcomed. Relatively few studies had been undertaken in recent past to evaluate toxicity and deleterious effects of leachate on soil and crop yields in the Western Cape Province of South Africa. Hence, this study evaluates the use of landfill leachate as a potential source of water for irrigation purposes in the Western Cape Province, South Africa. This study also determines the degree of uptake of essential and trace elements by Swiss chard vegetable irrigated with various concentrations of landfill leachate.

Materials and Methods

Materials

All reagents used for this study: super-pure 65% nitric acid, 30% hydrogen peroxide, and 35% hydrochloric acid were of analytical grades and were obtained from Merck KGaA Company. Pots (size: 20 cm) were purchased from AgriMark (South Africa). Potting soil, Swiss chard seedling, pelleted organic fertilizers were purchased from Stodels Nurseries, Cape Town, South Africa.

Experimentation

Fifty pots (size: 20 cm each) were washed with tap water several times and rinsed. Each pot was filled with potting soil and placed on metal bench inside the greenhouse of the Horticulture Department, Cape Peninsula University of Technology, Bellville Campus. The pots were separated into five groups of ten pots according to the irrigation treatments used. One seedling was planted in each pot after measuring the soil pH using handheld multi-system meter and suitable fertilizer was added to each pot at commercial recommended rate to enhance growth; based on pre-soil analysis (Table 1). The Swiss chard seedlings used in the experiment were of the same cultivar and age. Treatments (leachate and controls) were applied daily at regular interval throughout the duration of the experiment to maintain good soil moisture. Three soil samples on 2, 5 and 8 weeks after transplant were collected for analysis to determine the influence of leachate irrigation on soil properties (McLaughlin *et al*., 2000).

Leaf samples of Swiss chard were collected from each treatment in appropriately labeled brown paper bags for tissue analysis. Samples were rinsed thoroughly with tap water and distilled water in order to remove all traces of soil and dust particles and to ensure there was no contamination. The leaves were later dried in the oven at 60°C. Swiss chard leaf samples were collected in triplicates every week over a period of two months to investigate trend in possible accumulation of nutrients over time.

Soil and Plant Tissue Analyses

Soil samples were initially sieved through a 2 mm sieve. The soil was thoroughly mixed to achieve homogeneity and then dried in the oven at 60°C till constant weight was achieved. Once the soil was cool, 1.0 g of the dried soil was accurately weighed and transferred to a 50 mL Phillips beaker. To this, 2 mL of nitric acid and 6 mL of hydrochloric acid was added. The solution was transferred to a round bottle flask and heated to approximately 85°C for approximately 30 min. After wards, the sample was cooled, filtered through Whatman GF/C filter papers, transferred to a 50 mL volumetric flask, and diluted to the mark with distilled water. The sample was then taken for analysis using MP-AES (EPA Method, 1994).

Swiss chard leaves were dried in the oven at 60°C till constant weight was achieved. Moisture content was determined by difference. The dried leaves were milled and 0.1 g of each sample were weighed out accurately and transferred to a microwave digestion reaction vessel. A 2 mL of 30% hydrogen peroxide and 5 mL of 65% nitric acid were added to the contents in the vessel. The vessels were placed in the carousel digestion proceeded by means of a temperature programmer. The digestion procedure was carried out in a Milestone-MLS 1200 Mega microwave oven capable of accommodating 6 digestion vessels. After digestion, the samples were cooled, filtered using Whatman GF/C filter paper and transferred to the volumetric flask and made up to the mark with ultra-pure water.

Scanning Electron Microscopy (SEM)

Scanning Electron Microscope was used to study the morphology of the fresh leaves samples of leachate treated Swiss chard to determine possible structural changes due to heavy metals accumulation and the result was compared with the controls. The SEM was used to generate images of specimens with magnifications of 50 KX to examine fractured surfaces and changes in structural integrity of leaves due to different application of leachate concentrations.

Results

Effect of Leachate, Tap and Distilled Water Irrigation on Soil pH

Data of different soil pH resulting from leachate tap and distilled water irrigations are presented in Fig. 1. The pH of the potting soil progressively increased along leachate concentration gradient. The potting soil itself had an initial pH above 7 which implies it is an alkaline soil. The analysis of the leachate also indicated the presence of alkaline forming salts or cations. Therefor pH steadily rose from week one to week eight reaching a pH peak of 9.3 for 100% leachate irrigation. In contrast, only a slight variation was observed in pH values of soils irrigated with tap and distilled waters, as the concentration of these ions were low in the control. The 100% leachate irrigation recorded a 15.8% rise in soil pH compared with the control due to continuous accumulation of base forming cations from the leachate.

Table 1: Characteristic of soil and leachate used in this study

Potting soil		Leachate (mg/l)	
pH	7.3	pH	7.8
EC (mS/m)	80	EC (mS/m)	383
Na (cmol/kg)	2.06	OP (kPa)	137.8
Ca (cmol/kg)	7.3	SAR	6.93
Mg (cmol/kg)	4.25	Na	714
K (cmol/kg)	1.87	Cl	983
B	3.41	K	56.8
Cu	13.83	Ca	133.7
Cr	4.2	B	0.42
Zn	26.0	Cu	0.80
Mn	43.3	Cr	0.30
Fe	169.4	Zn	0.62

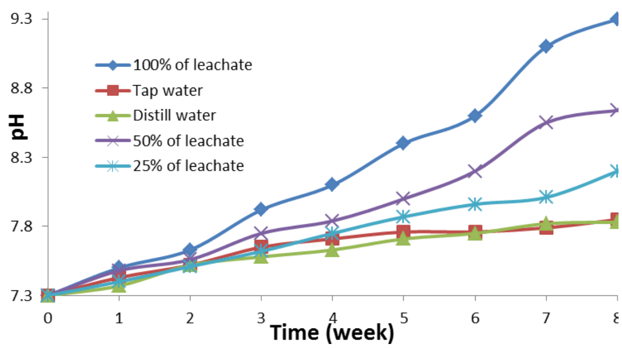

Fig. 1: Progressive increase in soil pH as affected by different concentrations of leachate irrigation

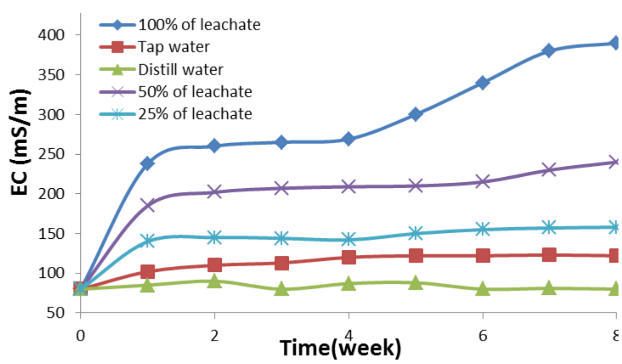

Fig. 2: The effect of irrigation waters on soil electrical conductivity

Effect of Irrigation Waters on Electrical Conductivity of the Soil (EC)

The influence of different leachate concentrations on electrical conductivity is presented in Fig. 2. As expected, the EC of the soil was higher due to treatment with different leachate concentrations compared with the control. The 100% leachate irrigated treatment showed an initial steep rise in the first week of the trial and flattened out up to week 4. After 4 weeks, soil EC recorded a progressive rise up to week 7 and increased slightly thereafter. The 50% leachate irrigation recorded up to 38.5% drop in soil EC compared with the 100% leachate treatment. On the other hand, the EC of soil irrigated with tap water increased slightly from 80 to 122, while EC for distilled water remained unchanged throughout the study.

Influence of Leachate Irrigation on the Growth of Swiss Chard

Fig. 3 shows the change in heights of Swiss chard species during the applications of different leachate concentrations and the controls. Although yield increased gradually from week one to eight across the whole treatments, the result indicates a significant ($p<0.05$) progressive drop in growth (height) in response to increasing concentration of leachate; reaching a steady peak at week five with a recorded maximum of 18.5 cm for 100% leachate treatment. The result generally showed about 50% drop in height of Swiss chard plant at 100% leachate irrigation compared with the distilled and tap water irrigated treatments which recorded 37.7 cm height at eight weeks after transplant.

Effect of Leachates Irrigations on the Level of Trace Elements in Swiss Chard

The accumulation of copper, zinc and chromium in Swiss chard irrigated with different leachate concentrations and the two control treatments during the study are presented in Table 3. After eight weeks of irrigation with concentrated leachate (100%), the results show accumulation of these elements in the leaf tissues of Swiss chard with the highest concentration of 2.00, 11.0 and 2.50 mg/kg for Cu, Zn and Cr respectively. In general, Zn accumulation in the tissue of Swiss chard recorded a significant ($p<0.05$) 300% increase between week two to week 5 for 100 and 50% leachate treatment with over five and half times accumulation of the same element (Zn) in 25% leachate treatment.

Effect of Different Concentrations of Leachate, on the Exchangeable Cation Concentrations in the Leaf of Swiss Chard

The results (Figs. 4–6) show different accumulations of the three cations in the leaf of Swiss chard during the study. Unlike Na where continuous application of all the concentrations of leachate encouraged the accumulation of the nutrient in plant tissue along concentration gradient from week two to week eight, Ca and Mg concentration recorded continuous significant ($p<0.05$) drop in the tissue of the species over the same period. However, Ca, Mg and Na, concentrations increased gradually in the leaf tissues of Swiss chard when tap and de-ionized water were used for irrigation (Figs. 4–6).

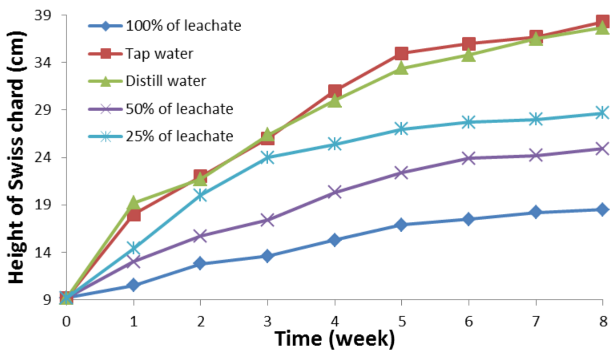

Fig. 3: Influence of leachate irrigation on the growth of Swiss chard

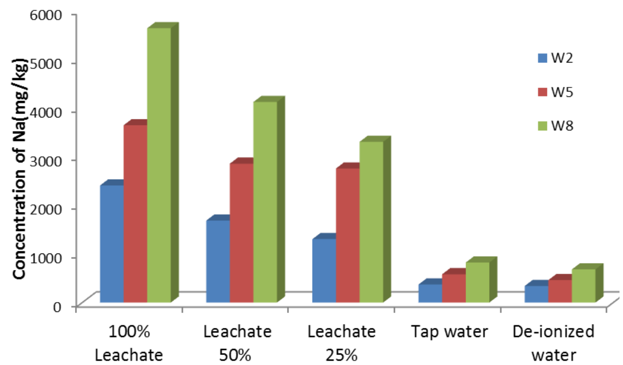

Fig. 4: Change in concentration of Na in Swiss chard leaves during second, fifth and eighth weeks of the experiment

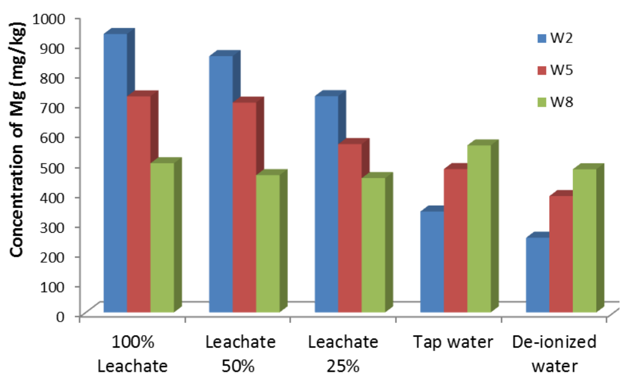

Fig. 5: Change in concentration of Mg in Swiss chard leaves during second, fifth and eighth weeks of the experiment

Effect of Different Irrigation Waters on Ultra-structural Leaf Morphology of Swiss Chard

The micrographs of the Scanning Electron Microscope (SEM) revealed non-uniform granules with rough surfaces of Swiss chard leaves with probable presence of stomata as shown in (Fig. 7a–7e; Fig. 8a–8e).

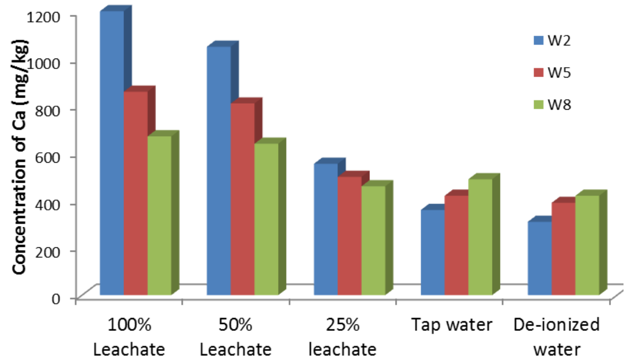

Fig. 6: Change in concentration of Ca in Swiss chard leaves during second, fifth and eighth weeks of the experiment

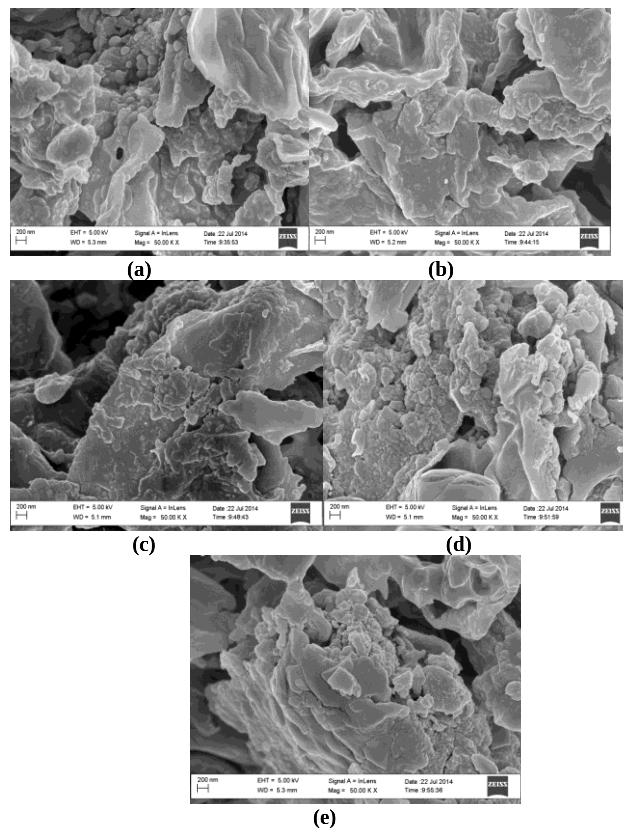

Fig. 7: 1 Scanning electron micrographs of (a) 25%LL, (b) 100% LL, (c) Tap water, (d) 50% LL (e) Distilled water, in the first week of the experiment

After irrigation with leachate, the leaves exhibited agglomerated smooth surfaces and the stomata may have been embedded with Na and Cl, which were confirmed by Energy Dispersive Spectroscopy (EDS). The accumulation of these elements cause increases in epidermal and mesophyll thickness, and elongation of the palisade cells.

Table 2: Irrigation water ratings based on electrical conductivity

EC (μS cm^{-1})	Water salinity rating	Plant suitablility (based on salt tolerance)
<650	Very low	Sensitive
650-1300	Low	Moderately sensitive
1300-2900	Medium	Moderately tolerant
2900-5200	High	Tolerant
5200-8100	Very high	Very tolerant
>8100	Extreme	Generally too saline

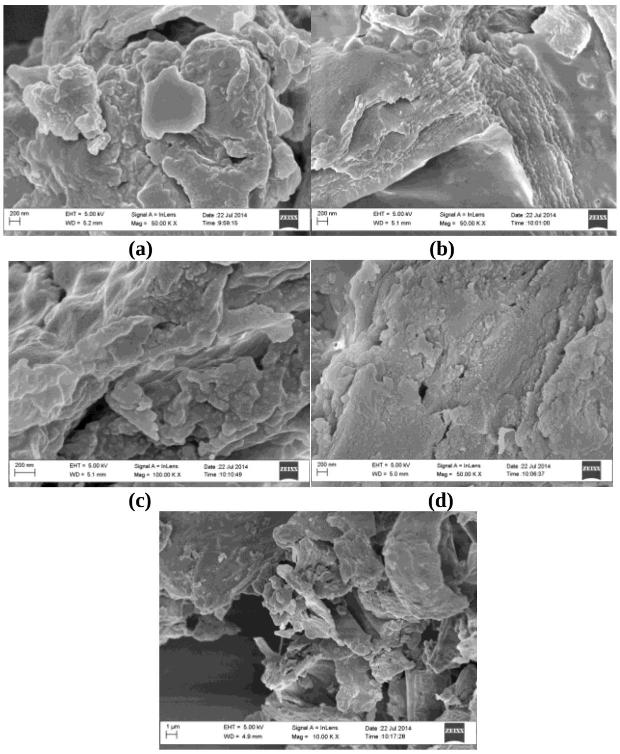

Fig. 8: Scanning electron micrographs of (a) 25% LL, (b) 100% LL, (c) Tap water, (d) 50% LL, (e) distilled water, in the eight week of the experiment

Discussion

The mineral constituents of the leachate and soil prior to the study (Table 1) indicated that the trial was conducted in alkaline soil and alkalinity increased over time. The soil pH result can be explained by the high concentration of monovalent and divalent cations contained in the leachate which accumulated over time during daily irrigation. As a result of negative charge developed by the soil particles, ions are absorbed on the soil lattice (Li *et al.*, 2010). Any process that encourages presence of high levels of exchangeable base in solution such as calcium, magnesium, potassium and sodium will reduce acidity and increase alkalinity. Hence there was a positive correlation between leachate concentration and increasing soil pH. In the current study, the result is indicative of a base saturated leachate solution.

The EC of control irrigation (water) was 95 μS cm^{-1} which indicates that the Salinity Rating was very low when compared with the standard values in Table 2. The Swiss chard species is moderately salt sensitive (Anonymous, 2000), therefore 100% leachate irrigation treatment affected growth rate by up to 50% compared with the controls. Plants are normally sensitive to soil properties such as electrical conductivity (EC). The result shows continuous increase in EC of the soil when irrigated with increasing concentration of (25%, 50% and 100%) leachate. The 100% leachate irrigation shows the highest EC ~ (400 mS/m) in the eight weeks of the experiment. The obvious reason for this increase is the availability of high concentration of cations such as Ca^{2+}, Mg^{2+}, K^{+}, Na^{+} and anions $SO4^{2-}$, Cl^{-}, HCO^{3-}, which are responsible for carrying electrical charges and therefore conduct electrical current (Garcia and Charbaji, 1993). Consequently, the high concentration of ions in the soil leachate increases the EC of the soil.

The decline in growth of Swiss chard leaves could be due to the high EC (salinity) value in the potted soil, which is resultant of high salinity of the leachate (Chiemchaisri *et al.*, 2005). As salinity increases each week due to application of irrigation waters, the amount of water available for plants decreases as the force with which the remaining water is held in the soil increases; making it progressively more difficult to withdraw water from the soil. The irrigation with tap and distilled water showed obvious growth increase from 9.2 cm in the first week to about 37.7 cm in the eighth week of the treatment. It could therefore be concluded that the application of the leachate has a deleterious effects on photosynthate production in Swiss chard species tested in this study due to accumulative toxic level of nutrients, high EC and osmotic limitations between the root and the soil solution.

High Sodicity concentration becomes a problem when enough salts accumulate in the root zone, which negatively affects crops growth. As the salt concentrations increase in the root zone, the water potential between the plant and soil increases, thereby reducing the plant-available water and making it harder for plants to take up water from surrounding soil. This condition lowers the amount of water available to the plant, regardless of the amount of actual water in the root zone (Hernández *et al.*, 1999). Nevertheless, the concentration of these elements in plant tissue does not exceed the globally expected safe value recommended for human health (Sayari *et al.*, 2005).

In comparison to Cu and Cr, Swiss chard species tested in this study seems to have high affinity for Zn uptake than the elements above (Cu and Cr). Although total leaf yield was significantly compromised by all the leachate treatments, 25% leachate (or lower) could be considered as source of irrigation water to agronomically increase Zn accumulation in Swiss chard tissue; which could serve as a source of alleviating hidden hunger among marginal income population who rely mostly on this vegetable as source of nutrients.

Table 3: Trace elements content in Swiss chard leaves during irrigation with different concentrations of leachates, tap and de-ionized water

Element age at (mg/kg) harvest		100% Leachate	50% Leachate	25% Leachate	Tap water	De-ionized water
	*W2	0.00	0.00	0.00	0.00	0.00
Cu	W5	1.00	1.00	1.50	1.50	0.50
	W8	2.00	1.50	1.55	1.06	1.00
	W2	3.00	2.00	1.50	1.50	0.05
Zn	W5	9.50	7.00	8.50	4.00	3.50
	W8	11.0	6.50	6.00	4.50	4.50
	W2	0.00	0.00	0.00	0.00	0.00
Cr	W5	1.00	1.00	1.00	1.00	1.00
	W8	2.00	1.70	1.50	1.30	1.02

*W2: Week two, W5: Week five, W8: Week eight of the experiment

The increase in Na cation in the soils as a consequence of leachate irrigation was clearly reflected by the content of this element in the leaves of Swiss chard species. The uptake of Na by Swiss chard leaves significantly increased with time when leachate was used as irrigation water, which correlates with the concentration of this element in the leachate and ultimately in the soil. The results also shows decrease in Ca and Mg concentrations, which have also been reported in some grasses under leachate irrigations (Cureton *et al.*, 1991). Calcium deficiency was also reported by Grieve and Fujiyama (1987), in salt-stressed maize shoots. This could be due to the competition and replacement of Ca and Mg by Na (Grieve and Fujiyama, 1987). Such increase in the cation content (mainly Na) in the plants is one of the important aspects that must be taken into consideration when leachates are used as irrigation water, because high concentrations of cations in plant tissue may inhibit some biochemical processes, which are generally accompanied by a decline in growth (Greenway and Munns, 1980).

The authentication of microscopically important ultra-structures using the Scanning Electron Microscope (SEM) plays a vital role in the identification, location and distribution of some botanical features of plants (Vaishali *et al.*, 2008). While the tissue of Swiss chard leaves irrigated with control water showed no change in the surface structure between week one and eight, irregular surface with the presence of stomata, filled with deposition of elements was revealed for leachate treatment. Zooming in on Fig. 7a and 8a, (100% leachate irrigated treatment), the results of the SEM analysis revealed possible closure of the stomata opening induced by osmotic pressure, deposition of elements and reduced photosynthesis which would have limited carbon dioxide uptake (Zhu *et al.*, 2001). SEM results correlates well with the trend in plant height from week 2 to 8 and confirms early studies that the leaves of salt stressed plants are smaller with thick epidermal and mesophyll cells (Parida and Das, 2005).

Conclusion

The results of this study indicate that the concentrations of cations, electrical conductivity and pH in the potted soils increased with time when the leachate was used as irrigation system compared to utilization of tap and distilled water, where little or no change was observed. However the heavy metals concentration was within allowed threshold and shows no significant difference between leachate irrigated and water irrigated treatments, thus indicating the low concentration of these heavy metals in the leachate of the municipality.

The result indicated a 50% drop in growth as a result of leachate irrigation. This decline in growth could only be attributed to the higher Na content in the leachate used for the irrigation of the crop, which tends to replace Ca and Mg in solution thereby limiting their uptake and ultimately affecting structural growth in Swiss chard. However, because Swiss chard species used in the study survived, it could be concluded that the crop has high tolerance to Na. Furthermore, the results show the concentration of heavy metals in plant tissues over the period of this study falls within globally acceptable values which poses no harm for human consumption and health. Reducing the percentage concentration of the leachate could serve as an agronomic means of increasing Zn contents of Swiss chard without significantly compromising yield.

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