Effects of greywater irrigation on germination, growth and photosynthetic characteristics in selected African leafy vegetables

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ABSTRACT

The reuse of greywater, wastewater from sources other than toilets, could enable low-income households to save potable water for drinking and cooking. Greywater irrigation of food crops is widely practised but its effects on African leafy vegetables (ALVs), which hold potential for cultivation to improve food security, are unknown. This study investigated the effects of synthetic greywater irrigation on germination in three ALVs, viz., *Amaranthus dubius, Cleome gynandra* and *Solanum nigrum*, and subsequent seedling growth in *A. dubius* and *S. nigrum*. Seeds and seedlings were treated with chlorinated and dechlorinated greywater and tap water, supplemented with nutrients. Greywater application decreased germination capacity (by 23–25%) when assessed in Petri dishes in *A. dubius* only. However, greywater application was less harmful to *A. dubius* seeds sown in soil. Vigour was compromised in greywater-treated seeds of all three species but greywater can be used to irrigate freshly-sown seeds of *A. dubius* without reducing percentage seedling production. However, greywater irrigation reduced capacity (by 21–23%) and rate of shoot emergence in *S. nigrum*, and growth and chlorophyll content in both species. These negative effects were accompanied by increased soil electrical conductivity (after 21 d) and pH (after 14 d). The reduced growth under greywater irrigation was most likely based on a reduction in light-harvesting capacity and/or nutrient availability. Overall, *S. nigrum* seedlings were significantly more sensitive to the negative effects of greywater, possibly due to increased transpirational water loss under greywater irrigation. The effects of greywater were largely independent of chlorine content. Applying greywater in excess of plant requirements and/or alternating greywater irrigation events with freshwater watering events could promote leaching of salts found in greywater. The effects of greywater irrigation on soil water and nutrient availability demand further investigation for ALVs.

Keywords: African leafy vegetables, greywater irrigation, germination, seedling growth

INTRODUCTION

The number of people living in either water-stressed or water-scarce countries is expected to reach 3 billion by 2025 (Hanjra and Qureshi, 2010). The increased pressure on water resources can be attributed to population growth, climate change, and the rising demand for water in industrial sectors. The abstraction of water for irrigation in the agricultural sector amounts to 80% of the global water consumption, and is the limiting factor in food production in many countries (Hanjra and Qureshi, 2010). Innovative approaches are therefore needed to attain both water and food security, particularly in sub-Saharan Africa (Finley et al., 2009; Hanjra and Qureshi, 2010; Rodda et al., 2011).

Innovative approaches to reduce the pressure on urban water supplies include the reuse of domestic greywater (wastewater from bath tubs, showers, hand-wash basins, laundry and kitchen sinks (Finley et al., 2009; Pinto et al., 2010) for irrigation (Pinto et al., 2010). Greywater is already used for irrigation of crops, more widely so in arid regions where its reuse reduces potable water use by up to 50% (Al-Hamaiedeh and Bino, 2010). In South Africa, parts of the country receive less than the required 500 mm annual rainfall for rain-fed cropping (Schulze, 1997). Recycled greywater could therefore be used in these regions for irrigation of crops, especially for small-scale implementation such as household and community food gardens. Greywater could be particularly useful for irrigation of subsistence crops such as African leafy vegetables (ALVs), which are consumed across Africa (Van Rensburg et al., 2007); however, its effects on germination and seedling growth in these species are presently unknown. Historically, in South Africa, the setting for this study, the collection of ALVs from the wild or from cultivated fields is still commonly practised among poor people in rural areas but there is evidence of an overall decline in their use (Van Rensburg et al., 2007). Among the leafy vegetables collected in the wild are *Amaranthus dubius* Mart. ex Thellung., *Cleome gynandra* L., and *Solanum nigrum* L., which have the potential to become cultivated species (Van Rensburg et al., 2007). This will greatly increase their availability in the face of impending water and food insecurity.

The reuse of untreated greywater does, however, hold risks to crops, soil and human consumers, which include the potential accumulation of pathogens, metals, and organic chemicals in the soil and/or in plants (Finley et al., 2009). The elevated salinity, pH and boron content of greywater can also adversely impact soil in the long term (Finley et al., 2009; Al-Hamaiedeh and Bino, 2010; Misra et al., 2010; Rodda et al., 2011). Previous studies have investigated the effects of greywater irrigation on several conventional crops such as tomato (Misra et al., 2010), carrot, lettuce, red pepper (Finley et al., 2010), green pepper and Swiss chard (Rodda et al., 2011). The effects of greywater on soil properties such as pH and electrical conductivity (EC) have also been investigated (Pinto et al., 2010; Misra et al., 2010; Rodda et al., 2011); however, information on the direct and indirect effects of greywater on seed germination, seedling growth and physiology of ALVs is scarce. The present study investigated the effects of greywater irrigation on seed germination capacity and velocity in *A. dubius, C. gynandra, and S. nigrum*. The germination data were used to select two of these species for investigating the effects of greywater irrigation on seedling growth and physiology.

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The selection of seedling growth and physiological parameters to be measured in this study was guided by the fact that photosynthesis contributes to plant growth and development, and determines biomass production (Parida and Das, 2005; Ashraf and Harris, 2013). The efficiency of photosynthesis is dependent on the integrity and efficiency of the photosynthetic apparatus (Ashraf and Harris, 2013; Parida and Das, 2005). This can be gauged from a measure of the photochemical efficiency of photosystem II (Fv/Fm), which was used here as an indication of plant health (Moradi and Ismail, 2007; Murchie and Lawson, 2013). Any environmental stress that affects photosynthesis in crops is likely to have an effect on yield (Cabot et al., 2014). One such stress is salinity which can arise from the accumulation of salts in soil irrigated with greywater (D'Odorico et al., 2013; Rengasamy, 2006). Abiotic stresses such as salinity can compromise both seed germinability (Hasanuzzaman et al., 2013) and seedling growth (Munns, 2002). Other stresses include increasing hydrophobicity of soils as a result of adsorption of surfactants to soil particles (Travis et al., 2010), and reduced water and nutrient availability (Al-Hamaiedeh and Bino, 2010). Soil pH, moisture content and electrical conductivity were therefore also measured in this study.

MATERIALS AND METHODS

Plant material and experimental design

The seeds of the three AVL species investigated, *A. dubius*, *C. gynandra* and *S. nigrum*, were collected from wild populations growing in the eThekwini municipal area, in KwaZulu-Natal, South Africa. The irrigation treatments included both chlorinated and dechlorinated versions of greywater and tap water, to investigate the potential interactive and/or cumulative effects of chlorine and conventional greywater constituents. The study commenced with a 2-week germination trial, conducted under controlled growth room conditions (see 'Germination studies' below), and involved watering the seeds with 4 irrigation treatments: chlorinated greywater (CGW), chlorinated tap water (CTW), dechlorinated tap water (DTW) and dechlorinated greywater (DGW). Chlorine levels were measured with a test kit for free and total chlorine (Hanna Instruments (Pty) Ltd, South Africa). Two of these species, *A. dubius* and *S. nigrum,* were then selected for subsequent seedling growth studies based on their relatively high germination capacity within the greywater treatments. Seedlings were subjected to the same four irrigation treatments for a 55-day growth period under greenhouse conditions.

Preparation of irrigation treatments

The four irrigation treatments (CGW, CTW, DTW and DGW) were prepared in 10 L containers. The composition of synthetic greywater is shown in Table 1. Tap water was used in the preparation of all four irrigation treatments and, in the case of the dechlorinated treatments, 20 L of tap water was aerated with an aquarium jet filter pump (MAGI-700, RESUN, Shenzhen, China) for 24 h prior to use. Greywater used for irrigation in the eThekwini area is generated from household water supplies used for bathing, laundry, and washing food, dishes and kitchen utensils. Synthetic greywater was used in the study to avoid the variability in quality associated with greywater collected from households (Finley et al., 2009). The synthetic greywater was prepared with ingredients representing the various components found in greywater, such as soap, oil and grease, carbohydrates and proteins (Table 1). The components included were based on Dalahmeh et al. (2013), with concentrations adjusted to yield chemical measures (chemical oxygen demand, biological oxygen demand, total nitrogen and total phosphorus) comparable to mixed domestic greywater from a household in an informal settlement (P. Naicker, unpublished data). Additionally, 1 g/L of essential nutrients (Dr Fishers Multifeed Classic, Plaaskem, Grovida, Durban) was added to each treatment container at every watering event to avoid nutrient deficiency acting as a confounding factor, since crop cultivation conventionally involves the addition of fertiliser(s).

Germination studies

Germination capacity and vigour were measured in CGW, CTW, DTW and DGW treated seeds of *A. dubius*, *C. gynandra*, and *S. nigrum* using a between-paper method (Rao et al. 2006), conducted under growth-room conditions (25°C, 40% RH, and 16 h day-8 h night cycle). For each species, 3 replicates of 25 seeds (per Petri dish) were exposed to each irrigation treatment. The seeds were placed between Whatman's No. 1 filter paper moistened with 4.5 mL of the selected irrigation treatment and received a further 1.5 mL every second day for a total of 14 days. During this period germination, defined as emergence of a radicle greater than 1 mm, was monitored daily. The time (in days) taken to achieve 50% of final germination capacity was used to compare germination velocity (Sershen et al., 2014) across the different irrigation treatments. Both shoot and root lengths were measured 14 days after the first radicle emerged. These data were used to calculate Seed Vigour Index (SVI) which is an important trait for crop establishment and uniformity (Singh and Srivastava, 2012). Two species (*A. dubius* and *S. nigrum*) were then selected for the seedling growth trials because they exhibited a germination percentage >50% within both the chlorinated and dechlorinated greywater treatments.

Seedling growth studies

A. dubius and *S. nigrum* seedlings were grown from seed under polycarbonate greenhouse conditions for 55 days at the University of KwaZulu-Natal (Durban, South Africa). Pots

TABLE 1
Ingredients used to prepare synthetic greywater

Ingredients	Amount
Tap water	9.99
Soap (g)	
Multi-purpose green bar	7.2
Body-care soap bar	2.0
Hand-washing laundry powder	6.4
Fats and greases (g)	
Cooking oil	0.15
Food product (carbohydrate and protein) (g)	
Cake flour	1
Nutrient broth	1
Final pH	9.9

(1 L polyethylene potting bags) were prepared using a 1:1 (v/v) mixture of air-dried river sand and pine bark–based potting mix (Grovida, Durban, South Africa) and seeds (one per pot) were sown to a depth of 1 cm. The same four irrigation treatments used in the germination studies were used in the seedling growth trial. Each treatment consisted of 3 replicates of 25 seeds each. Each pot was watered evenly with 100 mL of the respective treatment water (CGW, CTW, DTW or DGW). Shoot emergence was monitored every 3 days and weekly non-destructive growth measurements of shoot height were carried out from the root collar to the petiole of the youngest leaf for 10 seedlings per replicate trial, for each treatment (n = 30).

Biomass accumulation and leaf area

Plants were harvested 55 days after the seeds were sown. Root and shoot lengths of harvested plants were measured before each seedling (n = 30 for *A. dubius* and n = 25 for *S. nigrum*) was then separated into roots, shoots and leaves. Leaf area was measured using a leaf area meter (CID, Inc., Germany, CI-202 Area Meter) before all the plant material (roots, shoots and leaves) was dried in an oven at 55°C for 7 days. After 7 days the plant material was weighed and the biomass data generated were used to calculate root:shoot ratio, leaf area ratio (measure of the efficiency with which a plant deploys its photosynthetic resources) and specific leaf area ratio (fraction of total plant weight allocated to the leaves) (after Hunt et al., 2003).

Photosynthesis

Leaf photosynthesis, stomatal conductance and transpiration rates were measured using a portable photosynthesis system (Li-6400, LI-COR, Lincoln, NE, USA) between 10:30 and 15:00 during the final (8^{th}) week of seedling growth. Ten seedlings from each of the three replicates (n = 30) were selected randomly for these measurements in each treatment. One measurement per plant was taken for the second-youngest leaf at saturated light intensity of 1 000 µmol $m^{-2}\cdot s^{-1}$, with an air flow rate of 500 mol$\cdot s^{-1}$ and at a CO_2 concentration of 400 ppm (Moradi and Ismail, 2007; Naidoo, 2009).

Chlorophyll fluorescence

The maximum quantum efficiency of PSII (Fv/Fm) was measured using a portable pulse amplitude modulated fluorometer (Li-6400XT, LI-COR, Lincoln, NE, USA) during the final (8^{th}) week of seedling growth. Plants were dark adapted for 40 min to allow all electrons to drain from the photosystems (Moradi and Ismail, 2007; Sayed, 2003). Ten seedlings from each of the three replicates (n = 30) were selected randomly for these measurements in each treatment. One measurement per plant was taken on the lamina, midway between the base and the tip of the second youngest leaf (after Moradi and Ismail, 2007; Naidoo, 2009).

Chlorophyll content

Leaf chlorophyll content measurements were carried out for 10 randomly-selected seedlings from each of the three replicate trials for each treatment (n = 30) during the final (8^{th}) week of seedling growth. One measurement per plant was taken on the lamina, midway between the base and the tip of the second-youngest leaf using a hand-held chlorophyll absorbance meter (Minolta SPAD-502, Minolta Camera Co. Ltd.).

Soil characteristics

A separate set of plants, 3 replicates of 5 pots each per treatment, were grown under greenhouse conditions for 30 days and received the same irrigation treatments as in the seedling growth trial. Soil moisture measurements (n = 3) for each treatment were taken weekly for 4 weeks using a soil moisture meter (Delta T Devices, HH2 moisture meter, London, United Kingdom). Soil from 3 replicate pots per treatment was also collected weekly to determine soil pH and electrical conductivity (EC) using methods described by Gavlak et al. (2003). Both pH and EC samples were prepared at a soil:water ratio of 1:2. Soil $pH_{1:2}$ was measured using methodologies adapted from Gavlak et al. (2003). To determine soil $pH_{1:2}$, 20 mL of deionised water was added to 10 g of air-dried soil passed through a 2.0 mm mesh for each of 3 pots. The suspended solids were allowed to settle for 15 min before pH of the liquid fraction was measured for each replicate (n = 3) using a pocket pH meter (Checker HI 98103, Durban, South Africa).

To determine soil $EC_{1:2}$, 40 mL of deionised water was added to 20 cm^3 of air-dried soil passed through a 2.0 mm mesh for each of 3 pots. The suspended solids were allowed to settle for 15 min before the liquid fraction was pipetted into five 1.5 mL wells of a multi-welled conductivity meter (CM 100-2, Reid & Associates, Durban, South Africa), i.e., yielding 5 pseudo-replicate measurements for each of 3 pots (n = 15).

Statistical analysis

Inter-treatment differences in SVI, germination capacity and velocity, percentage shoot emergence, shoot height, root:shoot ratio, leaf area index, photosynthetic rate, stomatal conductance, transpiration, Fv/Fm, chlorophyll content, and biomass accumulation and partitioning were tested by analysis of variance (ANOVA SPSS version 22). Soil pH, EC, and soil moisture content data were also tested for inter-treatment differences within time intervals across treatments by ANOVA. Multiple comparisons were made using a Tukey's mean separation test. Percentage data were arcsine transformed, and non-parametric data ($p < 0.05$ following Kolmogorov-Smirnov test) were either log or square root transformed in order to conform data to parametric test assumptions. Where data remained non-parametric, inter-treatment differences were tested for using a Kruskall-Wallis test. All differences were considered significant at the 0.05 level.

RESULTS

Seed germination capacity and velocity

The three species, *A. dubius*, *C. gynandra* and *S. nigrum,* all exhibited germination capacities above 50% across all treatments (Fig. 1A). *A. dubius* had a significantly ($p < 0.05$) lower germination capacity in simulated greywater as compared with the tap-water treatments, while germination capacity remained unaffected by greywater in both *C. gynandra* and *S. nigrum* (Fig. 1A). There was a significant ($p < 0.05$) delay in the time taken by greywater-treated seeds of *A. dubius* to reach 50% of their final germination capacity (i.e. reduction in germination velocity), compared with tap-water treatments (Fig. 1B). Germination velocity remained unaffected by greywater in both *C. gynandra* ($p > 0.05$) and *S. nigrum* ($p > 0.05$) when compared with tap-water treatments (Figs 1C and 1D). Seed vigour (measured in terms of SVI) was significantly lower for

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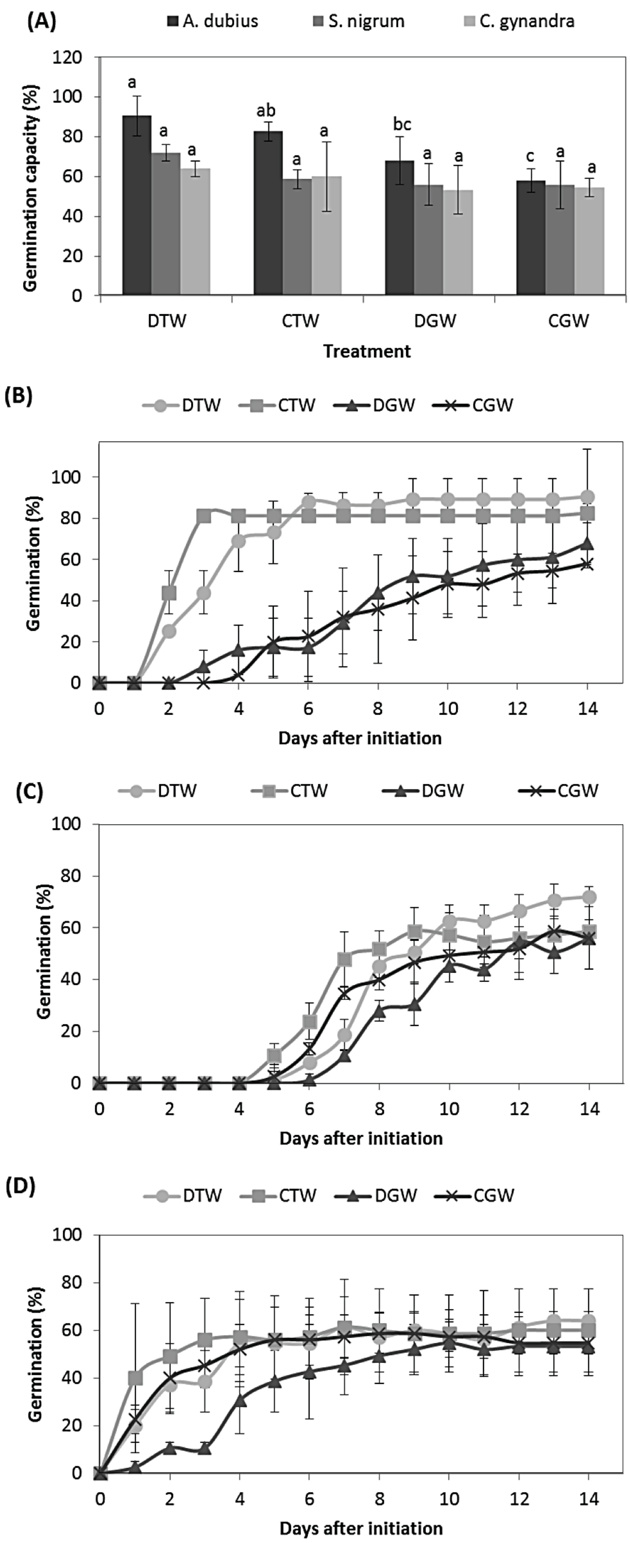

Figure 1

(A) Germination capacity at 14 days after initiation and percentage germination over time of (B) A. dubius, *(C)* C. gynandra *and (D)* S. nigrum, *seeds exposed to de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW), for 14 days in Petri dishes. Values represent mean±SD (3 replicates of 25 seeds each). Columns labelled with different letters are significantly different when compared within species (p < 0.05; ANOVA).*

greywater treatments compared with tap-water treatments in *A. dubius* ($p < 0.01$) and *C. gynandra* ($p < 0.05$) (Fig. 2). In *S. nigrum,* the SVI for greywater treatments was also relatively lower but these differences were only significant between dechlorinated greywater (DGW) and the two tap-water treatments ($p < 0.01$). There were no indications that germination capacity and velocity (Figs 1A–D) and seed vigour (Fig. 2) were significantly influenced by the presence of chlorine in the irrigation medium.

Seedling growth and physiology

Percentage shoot emergence, which is an indication of percentage seedling production, was comparable across treatments in *A. dubius* (Fig. 3A), but was significantly ($p < 0.05$) lower in greywater, as opposed to tap water, treatments in *S. nigrum* (Fig. 3B). Similarly, whilst shoot emergence velocity was relatively unaffected by greywater irrigation in *A. dubius*, this parameter was significantly ($p < 0.05$) reduced in the greywater treatments for *S. nigrum* (Figs 3A and 3B, respectively).

Shoot height in *A. dubius* was significantly ($p < 0.01$) lower in greywater compared with tap-water treatments from 35 days after initiation (DAI) up until the final harvest (55 DAI) (Fig. 4A). *S. nigrum* also displayed a significantly ($p < 0.01$) lower shoot height in greywater treatments compared with the DTW treatment from 35–55 DAI (Fig. 4B). Thereafter, *S. nigrum* shoot height in the greywater treatments remained significantly lower ($p < 0.01$) than both tap-water treatments up until 49 DAI. Though the trends described above (i.e. lower shoot height in greywater-treated seedlings) were also true at the final harvest, these differences were not significant for both species.

At the final harvest, both *A. dubius* and *S. nigrum* had a significantly lower total biomass in greywater compared with tap-water treatments ($p < 0.01$), with no significant differences between de-chlorinated and chlorinated treatments (Fig. 5). More specifically, there was a ±57.3% reduction in total dry biomass in *A. dubius* seedlings treated with greywater as opposed to tap water, ±10.3% of which was attributed to a reduction in

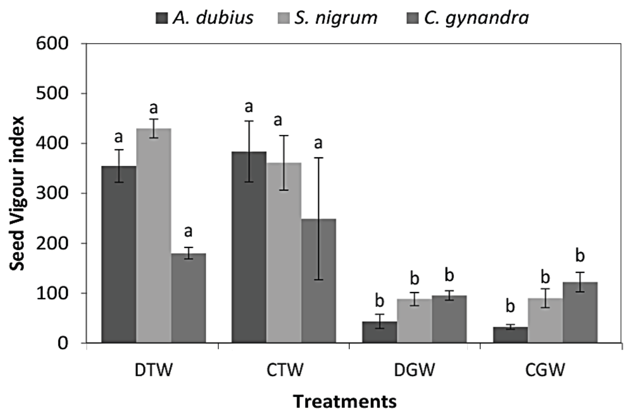

Figure 2

Seed vigour index for A. dubius, S. nigrum *and* C. gynandra, *exposed to the following irrigation treatments within Petri dishes: de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW). Values represent mean ± SD (3 replicates of 25 seeds each). Columns labelled with different letters are significantly different when compared within species (p < 0.01; ANOVA).*

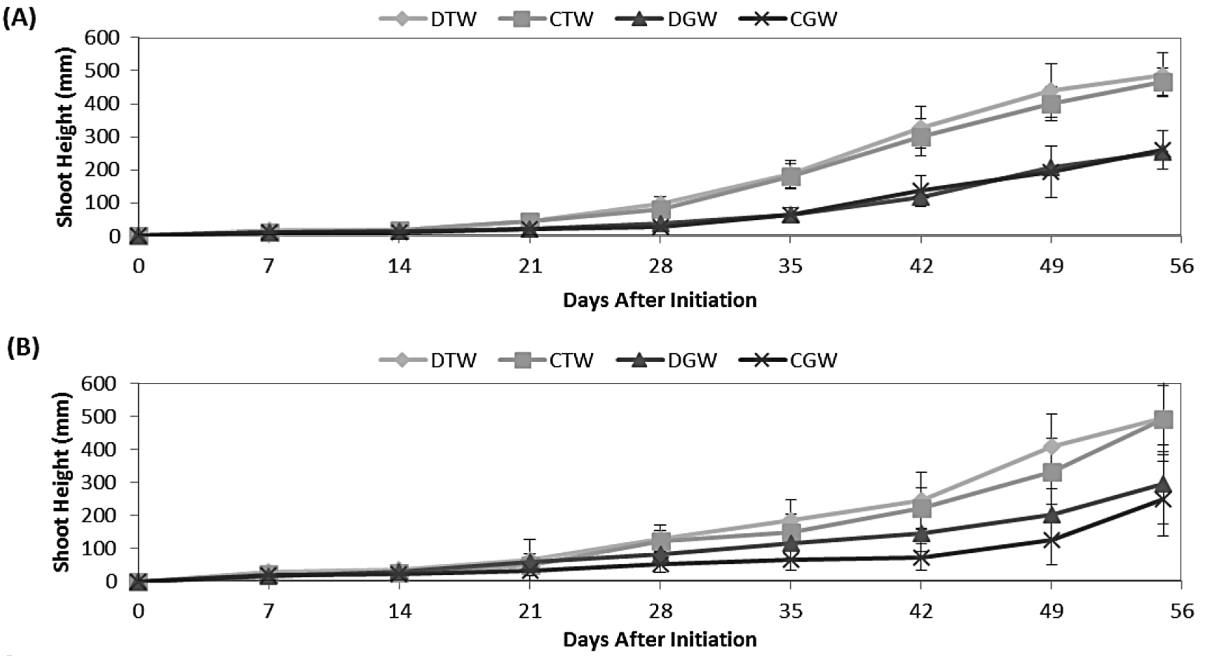

Figure 3

Percentage shoot emergence over the first 18 of 55 days exposure of (A) A. dubius *and (B)* S. nigrum *seeds and subsequent seedlings to the following irrigation treatments: de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW). Seed and seedlings were irrigated within soil-filled pots. Values represent mean±SD (3 replicates of 25 seeds each). Final percentage shoot emergence was significantly different when compared within species, across treatments, for* S. nigrum *($p < 0.05$; ANOVA) but not for* A. dubius *($p > 0.05$; ANOVA).*

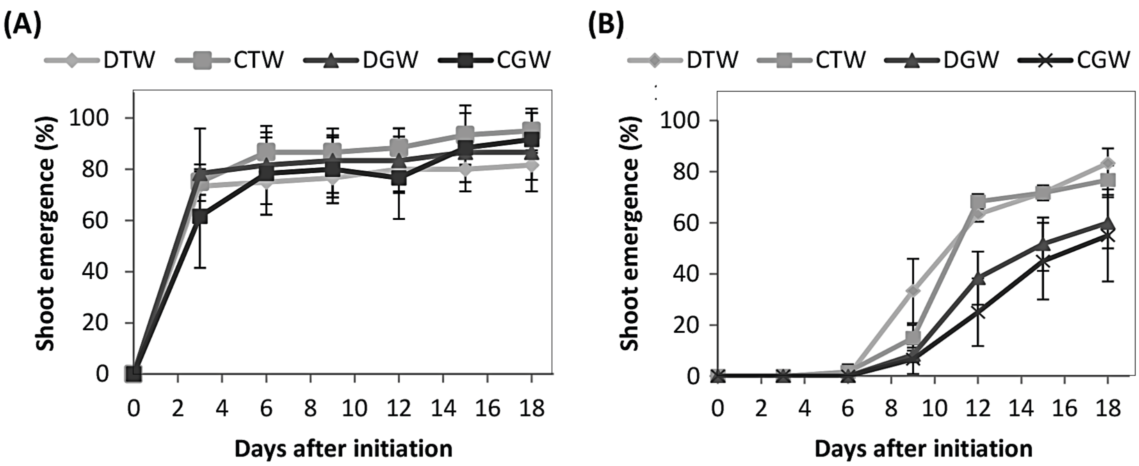

Figure 4

Shoot height of (A) A. dubius *and (B)* S. nigrum *seedlings exposed to the following irrigation treatments for 55 days: de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW). Values represent mean±SD (n = 30 for* A. dubius *and n = 25 for* S. nigrum*). For* A. dubius *$p < 0.01$ from 35–55 DAI (ANOVA) and $p < 0.01$ for* S. nigrum *from 42–55 DAI (ANOVA).*

roots, ±30.6% in stems, and ±16.6% in leaves (Fig. 5). There was also a ±57.7% reduction in total biomass in *S. nigrum* seedlings irrigated with greywater compared with those irrigated with tap water (Fig. 5), ±9.0% of which was attributed to a reduction in roots, ±29.3% in stems and ±19.3% in leaves (data not shown).

There was a significant decrease in biomass allocation to stems and an increase in biomass allocation to leaves in *A. dubius* under greywater irrigation relative to the tap-water treatments ($p < 0.05$; Fig. 6A). Biomass partitioning to roots in *A. dubius* was unaffected by greywater irrigation (Fig. 6A). Greywater irrigation did not alter biomass partitioning in *S. nigrum* (Fig. 6B). There were also no significant differences in biomass partitioning between chlorinated and de-chlorinated treatments for both species (Figs 6A and 6B).

For *A. dubius*, root:shoot ratio appeared to be slightly higher in the greywater treatments ($p < 0.05$ for DGW only) while this ratio was slightly lower in the chlorinated treatments in *S. nigrum* ($p < 0.05$, for CGW only) (Table 2). Specific leaf

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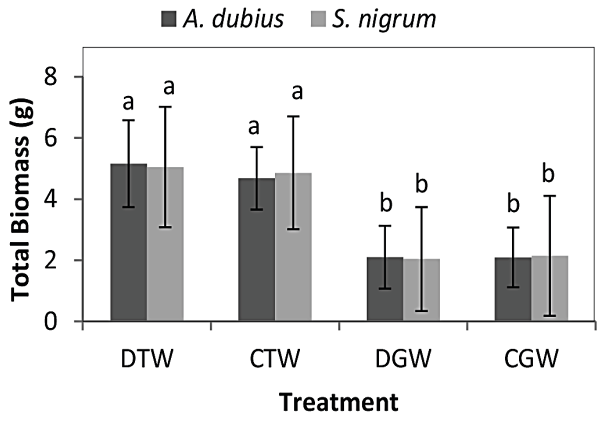

Figure 5

Total biomass of A. dubius *and* S. nigrum *seedlings exposed to the following irrigation treatments for 55 days: de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW). Values represent mean±SD (*n = 30 *for* A. dubius *and* n = 25 *for* S. nigrum*). Columns labelled with different letters are significantly different when compared within species (*p < 0.05*; ANOVA).*

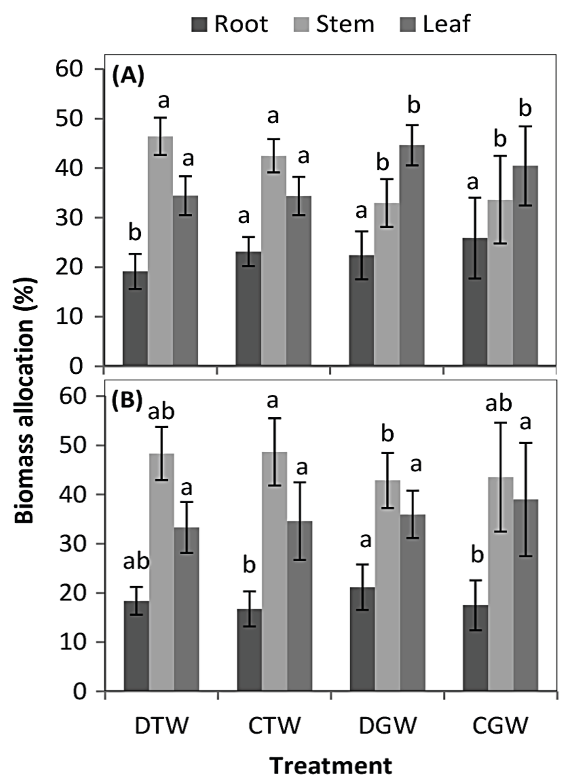

Figure 6

Root, stem and leaf percentage biomass allocation for (A) A. dubius *and (B)* S. nigrum *seedlings exposed to the following irrigation treatments for 55 days: de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW). Values represent mean ± SD (*n = 30 *for A. dubius and* n = 25 *for* S. nigrum*). Columns labelled with different letters are significantly different when compared within organs (*p < 0.05*; ANOVA).*

TABLE 2
Root:shoot ratio, specific leaf area and leaf area ratio of *A. dubius* and *S. nigrum* exposed to the following irrigation treatments for 55 days: de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW).

Treatments	Leaf area ratio	Specific leaf area (mm^2 mg^{-1})	Root:shoot ratio
A. dubius			
DTW	9.81±2.26^{b}	28.96±8.09^{b}	0.24±0.05^{b}
CTW	8.67±2.00^{b}	25.15±4.92^{b}	0.30±0.05^{b}
DGW	10.75±2.99^{b}	23.82±5.12^{b}	0.30±0.08^{b}
CGW	9.26±1.77^{b}	23.78±7.72^{b}	0.36±0.15^{b}
S. nigrum			
DTW	9.62±3.58^{b}	30.33±13.88^{b}	0.23±0.04^{ab}
CTW	12.28±4.76^{bc}	35.69±11.05^{b}	0.20±0.05^{b}
DGW	16.97±4.39^{b}	46.76±8.66^{b}	0.27±0.07^{b}
CGW	14.24±5.23^{ab}	38.13±12.72^{b}	0.22±0.07^{b}

*Values represent mean ± SD (*n = 30 *for* A. dubius *and* n = 25 *for* S. nigrum*). Values followed by different letters are significantly different when compared across treatments, within species (*p < 0.05*; ANOVA).*

area (SLA) was comparable across treatments in *A. dubius* but for *S. nigrum* this parameter was significantly highest in DGW ($p < 0.05$; Table 2). For *A. dubius* leaf area ratio (LAR) was significantly ($p < 0.05$; Table 2) lowest in CTW, while for *S. nigrum* there was a trend for this ratio to be higher in the greywater treatments ($p < 0.05$ for DGW only; Table 2).

No significant differences in photosynthetic rates existed across the four treatments for both *A. dubius and S. nigrum* (Table 3). Except for CTW being significantly lower than DTW in *A. dubius*, Fv/Fm did not differ significantly across treatments (Table 3). There was a trend for leaf chlorophyll content in both *A. dubius* and *S. nigrum* irrigated with greywater to be lower than that in the tap-water treatments ($p < 0.05$ for all greywater treatments except *A. dubius*-CGW; Table 3).

Stomatal conductance was unaffected by greywater irrigation in *A. dubius* but was significantly higher in CGW compared with CTW in *S. nigrum* ($p < 0.01$; Table 3). Transpiration rates were unaffected by greywater in *A. dubius* but for *S. nigrum* transpiration rates in CGW and DGW were significantly ($p < 0.01$ and $p < 0.05$, respectively) higher than that in CTW (Table 3).

Soil characteristics

Soil electrical conductivity in greywater treatments was significantly higher than in tap-water treatments from 21 DAI onwards ($p < 0.01$; Fig. 7A). Soil pH was also significantly higher in greywater treatments from 14 DAI onwards ($p < 0.05$; Fig. 7B). Soil moisture content remained comparable across treatments throughout the experimental period (Fig. 7C).

DISCUSSION

Germination and shoot emergence

Greywater compromised germination capacity in only one of the three species investigated, viz., *A. dubius*, when assessed in Petri dishes (Fig. 1A). However, these effects on *A. dubius*

TABLE 3
Photosynthetic rate, maximum PSII efficiency (Fv/Fm), leaf chlorophyll content, stomatal conductance and transpiration rate of *A. dubius* and *S. nigrum* exposed to the following irrigation treatments for 55 days: de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW).

Treatments	Photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Flouresence (Fv/Fm)	Chlorophyll content (Spad Units)	Stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$)	Transpiration rate ($mmol \cdot m^{-2} \cdot s^{-1}$)
A. dubius					
DTW	26.02±4.55[a]	0.76±0.04[a]	44.06±4.18[a]	0.015±0.004[a]	0.49±0.14[a]
CTW	23.9±4.91[a]	0.73±0.03[a]	45.27±4.60[a]	0.015±0.003[a]	0.52±0.10[a]
DGW	25.73±3.75[a]	0.74±0.05[a]	39.55±5.84[a]	0.015±0.003[a]	0.51±0.09[a]
CGW	23.54±4.61[a]	0.75±0.04[a]	42.5±6.13[ab]	0.015±0.003[a]	0.53±0.10[a]
S. nigrum					
DTW	15.98±3.26[a]	0.85±0.06[a]	41.04±4.75[b]	0.023±0.007[ab]	0.79±0.23[a]
CTW	13.40±4.08[a]	0.83±0.04[a]	40.25±2.62[b]	0.017±0.008[a]	0.62±0.28[a]
DGW	15.06±4.93[a]	0.82±0.06[ab]	34.96±2.83[b]	0.022±0.009[ab]	0.84±0.30[ab]
CGW	15.12±4.61[a]	0.81±0.06[ab]	35.55±3.63[b]	0.027±0.011[a]	0.89±0.32[ab]

Values represent mean±SD (n = 30). Values followed by different letters are significantly different when compared across treatments, within species (p < 0.05; ANOVA)

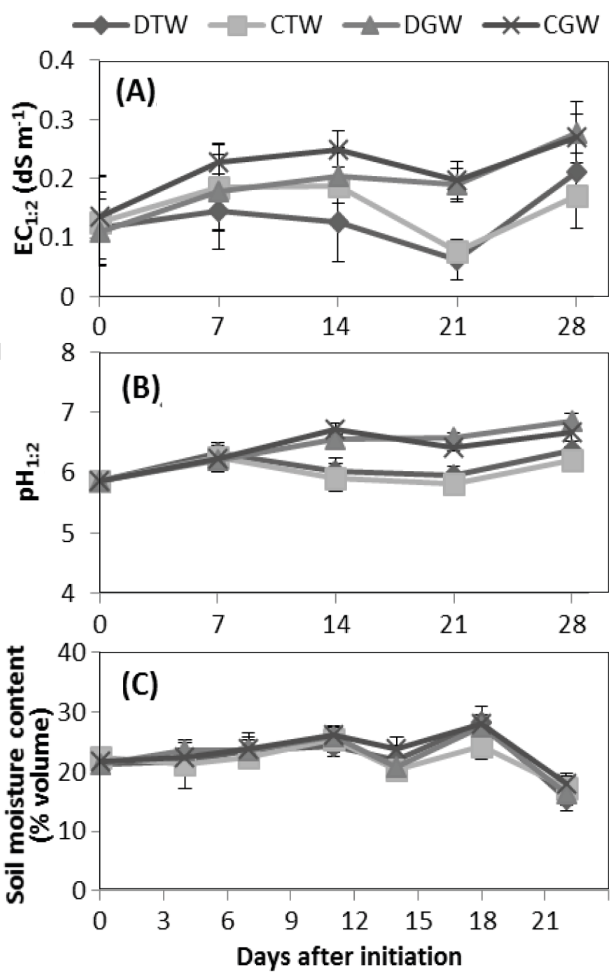

Figure 7
(A) Soil electrical conductivity, (B) pH and (C) moisture content within pots containing A. dubius *and* S. nigrum *seeds irrigated with de-chlorinated tap water (DTW), chlorinated tap water (CTW), de-chlorinated greywater (DGW), and chlorinated greywater (CGW), for 55 days. Values represent mean±SD* (n = 3). p *< 0.01 for soil electrical conductivity from 21 DAI onwards (ANOVA);* p *< 0.05 for soil pH from 14 DAI onwards (ANOVA);* p *> 0.05 for soil moisture throughout the experimental period (ANOVA).*

were negated by the fact that greywater irrigation did not compromise percentage shoot emergence relative to tap-water treatments when the seeds were sown in soil. Also, since germination capacity was not influenced by the presence/absence of chlorine (Fig. 1A), these data show promise for the use of greywater to irrigate freshly-sown seeds of these species for subsistence farming purposes. Other studies on the effects of greywater irrigation on seed germination generally show either no effect or reduced germination when compared with tap water (Garland et al., 2000; Misra et al., 2010; Himanen et al., 2012).

In the present study SVI was compromised in greywater-treated material of all three species (significant for all except *S. nigrum* (Fig. 2)). *S. nigrum* appeared to be more sensitive to the negative effects of greywater irrigation than *A. dubius*: capacity and rate of shoot emergence were compromised relative to tap-water treatments in *S. nigrum* (Fig. 3B).

Soil moisture content, electrical conductivity and pH are among some of the most important abiotic factors influencing seed germination and seedling establishment (Mudgal et al., 2010; Rezvani and Fani Yazdi, 2013). In the present study, greywater irrigation increased soil EC from ±0.13 to ±0.27 dS m^{-1} (Fig. 7A), and pH from ±5.8 to ±6.7 (Fig. 7A), relative to tap-water irrigated soil. A greywater-induced increase in pH and EC has been reported in several other studies (Misra et al., 2010; Pandey et al., 2014; Pinto et al., 2010; Rodda et al., 2011). Interestingly, a study by Rezvani (2013) indicated that seeds of *S. nigrum* had high germination capacities in pH ranges between 4 and 10 but were sensitive to salinity in terms of germination capacity. Rezvani (2013) also showed that *S. nigrum* seeds are intolerant to short-term drought stress. Since pH in the greywater-treated soils was always <10, the reduction in the capacity and velocity of shoot emergence in greywater-treated *S. nigrum* seeds was possibly a consequence of the increase in soil EC, which can be likened to an increase in salinity (Corwin and Lesch, 2005). According to Hasanuzzaman et al. (2013), increased EC can lower the osmotic potential of soil and in this way compromise germination by altering the rate and/or amount of water imbibed by seeds, even in cases where soil moisture content is not reduced as in the present study (Fig. 7C). Other negative effects of increased salinity on seeds include altered activity of enzymes of nucleic acid metabolism, disruptions in hormonal balance, alterations in protein metabolism, and reduced utilisation of seed reserves (Hasanuzzaman et al., 2013).

Seedling growth

Shoot height in the greywater treatments was reduced by ±46% and ±55% for *A. dubius* and *S. nigrum,* respectively, when compared with tap-water treatments (Figs 4A & 4B). As alluded to earlier, the increase in EC in greywater treatments can be likened to a rise in salinity. An increase in EC/salinity has been associated with reduced crop yields (Corwin and Lesch, 2005). Parida and Das (2005), in reviewing the effects of salinity stress, also report reductions in shoot weight, plant height, number of leaves per plant, and root length. At the final harvest, *A. dubius* and *S. nigrum* plants treated with greywater had a significantly lower biomass compared with tap-water treated plants (Fig. 5). However, previous studies on the effects of household greywater report either an increase in yields of crop plants irrigated with greywater relative to tap water (Rodda et al., 2011; Reichman and Wightwick, 2013), or no differences in crop plant yield between tap water and greywater irrigation (Finley et al., 2009; Pinto et al., 2010; Travis et al., 2010). In the present study greywater irrigation did not have any significant effects on photosynthetic capacity (Table 3) but resulted in a reduction in yield, even though essential nutrients were added to both greywater and tap-water treatments. The reduced growth of plants treated with greywater could have therefore been (at least in part) due to the chemical constituents and/or characteristics of greywater, either directly or indirectly reducing the bioavailability of nutrients to plants.

Additionally, a study by Kang et al. (2011) showed that root dry biomass was maintained irrespective of nutrient concentrations and that improved water-use-efficiency to avoid water stress is usually achieved by reducing shoot biomass. Greywater irrigation resulted in an increase in EC relative to tap-water treatments and may have altered plant-water relations relative to tap-water irrigated pots by increasing the osmotic potential of the soil. Increasing the osmotic potential of soil can reduce soil water potential, which under water-limiting conditions can make it more difficult for plants to take up water from the surrounding soil. This reasoning may explain why, in the present study, the total reduction in biomass linked to greywater irrigation (±57.3% in *A. dubius* and ±57.7% in *S. nigrum* (Fig. 5)) was attributed largely to a reduction in stem biomass, followed by leaves and then roots.

Greywater irrigation led to an increase in biomass allocation to leaves in *A. dubius* (Fig. 6a); ALVs are primarily harvested for their edible leaves. There were indications of a slight increase in root:shoot ratio in the dechlorinated greywater treatments relative to tap-water treatments (significant for DGW-treated *A. dubius* only (Table 2)). Large root:shoot ratios are expected to be advantageous for seedling survival in water-stressed and nutrient-poor environments (Gedroc et al., 1996) which suggests that greywater led to a reduction in the availability of water and nutrients relative to tap water. Root:shoot ratio was slightly lower in the chlorinated treatments in *S. nigrum* (significant for CGW only) (Table 2); a reduction in root:shoot ratio is an indication of a decline in the overall health of a plant (Gedroc et al., 1996).

Specific leaf area (SLA) appeared to be unaffected by greywater in *A. dubius*, but greywater irrigation increased SLA in greywater-treated *S. nigrum* (significant for DGW only; Table 2). Leaves have a higher SLA if they are thinner (lower mass per volume). Increasing SLA is associated with increased allocation of biomass to metabolic components rather than structural components of the leaf and is associated with lower internal shading, and a reduction in potential diffusion limitations (Terashima and Hirosaka, 1995). An increase in SLA can therefore increase CO_2 assimilation rates (Epron et al., 1995). The LAR is the ratio of leaf area to total plant weight, indicating the fraction of total plant weight allocated to leaves. Both the species investigated here are 'leafy' vegetables and even though greywater irrigation reduced total plant biomass it did not alter/reduce LAR in both species relative to tap water (Table 2).

Seedling physiology

According to Hüner and Hopkins (2008), high growth rates are supported by a high shoot:root ratio, high stomatal conductance, and high photosynthesis rates. Environmental stresses can directly impact on the photosynthetic apparatus by disrupting the electron transport, stomatal control, and CO_2 assimilation, and could in effect lower growth rates (Anjum et al., 2011). The effects of increased EC/salinity on photosynthetic rates depend on the concentration of dissolved salts and the species (Hasanuzzaman et al., 2013). Whilst greywater irrigation led to an increase in EC in this study, the EC of greywater-irrigated soil remained within the recommended range of 0–1.5 dS/m for growth of most plant types (Pandey et al., 2014). This implies that the salinity stress (if any) associated with this increase in EC in greywater-treated pots may not have been severe enough to damage the photosynthetic apparatus as leaf photosynthetic rates and Fv/Fm were relatively unaffected by greywater in both species (Figs 8A and B). Salinity can either decrease or have no effect on Fv/Fm, depending on the species considered. Qin et al. (2013) for example, found that *Amaranthus tricolor* displayed high salt tolerance in terms of above-ground biomass output, leaf photosynthesis rate, Fv/Fm and photosynthetic pigment contents. However, Fv/Fm measures only the maximum photochemical efficiency of photosystem II, and therefore only gives an indication of the maximum quantum yield, which is the maximum efficiency at which light is absorbed by light-harvesting antennae chlorophyll of PSII and converted to chemical energy (Baker and Rosenqvist, 2004). Using rice, Moradi and Ismail (2007) showed that there was a significant difference in the actual quantum yield under saline conditions, even when Fv/Fm indicated no significance. Thus a reduction in photosynthate production can be brought about by a reduction in the efficiency with which light is harvested and/or a reduction in the quantity of light harvested, which is dependent on a number of factors including leaf chlorophyll content. In the present study, greywater irrigation reduced leaf chlorophyll content, possibly reducing the quantity of light harvested in both species relative to tap-water treatments (significant for all except *A. dubius*-CGW; Table 2). Parida and Das (2005) reported that saline conditions did not change thylakoid structure in *A. tricolor* (an ALV) and led to an increase in chlorophyll content. This suggests that *A. dubius* and *S. nigrum* may not be as tolerant of salinity as *A. tricolor*, and may explain the greywater-induced decline in total biomass accumulation observed in the two former species here. Additionally, leaf chlorophyll content is closely linked to leaf nitrogen in crops and can indicate soil nitrogen availability (Marenco et al., 2009). The decrease in chlorophyll content in *A. dubius* and *S. nigrum* leaves in the greywater treatments also suggests that greywater irrigation may have compromised nutrient availability relative to the tap water in this study.

In *S. nigrum* the decrease in the chlorophyll content was also accompanied by an increase in the transpiration rate compared with tap-water treatments (Table 3). This was possibly a consequence of the increase in SLA observed in greywater-treated *S. nigrum* plants and could have lowered the water use efficiency of the plants since transpirational losses generally increase as leaf thickness decreases and area increases (i.e. as SLA increases). An increase in the rate of transpiration is also indicative of salt stress (Parida and Das, 2005).

Soil characteristics

Many of the germination and plant growth and physiological responses discussed above have been discussed in the context of changes to the soil characteristics possibly induced by greywater irrigation, viz., increased electrical conductivity/salinity and pH, and reduced nutrient availability. Whilst the links between the increase in EC observed in greywater treatments (Fig. 7A) and the possible rise in salinity have been well-established, it is worth adding that previous studies have found irrigation of soils with high pH (i.e. >9) liquids to cause soil particles to separate, leading to soil structure decline (Anwar, 2011). The greywater used in this study possessed a pH of 9.9 and led to an eventual rise in soil pH (Fig. 7B); such increases can lead to micronutrient deficiencies (which are common under salt stress) and to macronutrient (e.g. phosphorous) deficiencies (Hasanuzzaman et al., 2013). The processes used by plants to optimize the use of P involve decreased growth rates, and an increased growth per unit of P uptake (Jenks and Hasegawa, 2008), which may also have contributed to the reduced growth observed in greywater-treated plants here. Soil moisture content remained unaffected by greywater (Fig.10C); however, this was measured for 4 weeks only and prolonged greywater irrigation has been shown to increase salinity, elevate pH (for example from 7.0 to 8.5 (Travis et al., 2010)) and boron concentration, and decrease capillary rise of water (Finley et al., 2009; Al-Hamaiedeh and Bino, 2010; Misra et al., 2010; Rodda et al., 2011).

Another possible explanation for the negative effects of greywater on the growth of the ALVs studied here is offered by investigations of the effect of greywater surfactants on soils, seed germination and plants. A few of these studies present evidence of reduced germination, reduced growth and phytotoxicity in plants irrigated with solutions containing surfactants or short-chain fatty acids (Garland et al., 2000; Misra et al., 2010, Hinamen et al., 2012; Taylor, 2014). Surfactants can also increase hydrophobicity and decrease wettability in greywater-irrigated soils, especially in sandy soils (Wiel-Shafran et al., 2006; Travis et al., 2010; Nowbakhat, 2011). In our own laboratory we have had mixed results, depending on the type of greywater used. Relative to tap water, irrigation with mixed greywater from an informal settlement increased plant growth (Rodda et al., 2011), but laundry greywater (both synthetic and community-sourced) decreased plant growth (Taylor, 2014). The decreased ability of surfactant-irrigated soil to hold water and, by implication, nutrients dissolved in soil water, could be expected to yield similar results in terms of symptoms of water and nutrient stress, as linked speculatively to increased EC, salinity and sodium concentrations in greywater in the preceding discussion.

CONCLUSION AND RECOMMENDATIONS

The mechanism via which growth was reduced by greywater irrigation in both species was most likely based on a reduction in light-harvesting capacity (i.e. reduced leaf chlorophyll content) and/or nutrient availability. This reduced growth in greywater-treated plants was accompanied by an increase in soil EC and pH (relative to tap-water treatments), both of which can bring about micronutrient deficiencies (Hasanuzzaman et al., 2013). Overall, *S. nigrum* seedlings were more sensitive to greywater irrigation than *A. dubius*, possibly due to increased transpirational water loss under greywater irrigation. There were indications that the presence of chlorine in the irrigation medium may compromise plant health (e.g. Fv/Fm in *A. dubius*-CTW was lower than *A. dubius*-DTW); however, the effects of greywater on both species appear to be largely independent of chlorine content.

When greywater is used for irrigation of *A. dubius* and *S. nigrum* plants, it should be applied in excess of plant requirements to promote leaching of salts found in greywater (Rodda et al., 2010). Alternating greywater irrigation events with freshwater watering events could also enhance leaching of accumulated salts and biodegradation of surfactants which can cause soil hydrophobicity. Future studies should incorporate measurements of plant nutrient uptake, leaf osmotic potential and soil water potential for a more robust assessment of the effects of greywater irrigation on plants. These studies should also be linked to the characterisation of surfactants in different types of greywater.

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