

Effect of irrigation with anaerobic baffled reactor effluent on Swiss chard (*Beta vulgaris* cicla.) yield, nutrient uptake and leaching

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ABSTRACT

The disposal of treated wastewater from an anaerobic baffled reactor (ABR) effluent into water bodies can cause pollution. Treated wastewater management through irrigation of crops has the potential of increasing crop production through nutrient uptake while reducing the risks of environmental pollution. However, this study aimed to investigate the effect of irrigation with ABR effluent on Swiss chard yield, nutrient (N and P) uptake and leaching. Field experiments were done over three seasons at Newlands, Durban, South Africa. The experiments were laid out in a randomised complete block design (RCBD) with three treatments: ABR effluent irrigation (ABR), tap water irrigation with fertiliser (TWF) and rain-fed with fertiliser (RFF). Data were collected on

nutrient (N and P) leaching at 30 and 50 cm depths, crop growth, soil chemical properties and nutrient uptake. Effects of irrigation with ABR effluent on soil chemical properties, Swiss chard growth, plant nutrient uptake and leaching were comparable to TWF and RFF treatments. This implies that irrigating crops with ABR effluent is a potential method for wastewater management in a manner that will not cause environmental pollution while benefiting peri-urban farmers.

Key words | ABR effluent, environmental pollution, eutrophication, nutrient leaching, soil, Swiss chard

INTRODUCTION

Decentralised wastewater treatment systems (DEWATS) have been developed as a low cost sanitation solution especially in densely populated informal settlements in developing countries such as South Africa (Foxon *et al.* 2004). Informal settlements are expanding beyond the ability of many municipalities to connect them to current centralised sewerage infrastructures (Foxon *et al.* 2004; Hudson 2010). The DEWATS involves the use of an ABR (anaerobic baffled reactor) where human waste is passed through a series of hanging and standing baffles. Human waste is degraded anaerobically leading to the production of biogas, low sludge yields and ABR effluent (Foxon *et al.* 2004; Nasr *et al.* 2009). The ABR effluent contains mineral elements (nitrogen and phosphorus) which are important for crop growth, which when disposed into water bodies can cause eutrophication and death of aquatic life. The use of treated wastewater in agriculture for irrigating crops can provide a sustainable method for wastewater disposal in a manner that will improve crop productivity, alleviate water scarcity and help recycle nutrients (Pedrero *et al.* 2010; Kalavrouziotis

2011; Mateo-Sagasta *et al.* 2013; Drechsel & Keraita 2014). Due to issues associated with water scarcity and climate change, reuse of treated wastewater in agriculture is of significant concern (Pedrero *et al.* 2010). When treated wastewater is used for irrigation adverse effects of nutrients on crops and the environment should be considered. Crops have different nutrient requirements, which might reduce crop yields when applied excessively, and also depending on the irrigation management practices might leach below the ground leading to ground water contamination (Pedrero *et al.* 2010; Kalavrouziotis *et al.* 2013; Tesfamariam *et al.* 2013).

Several studies have been done on the use of treated wastewater in agriculture with special reference to effects of heavy metals, salinity and sodicity on soils (Jiménez 2005; Kalavrouziotis *et al.* 2013). Some other miscellaneous effects, such as specific ion toxicities and damage on irrigation infrastructure, are well documented (Pescod 1992). Other studies have shown that treated wastewater can significantly affect soil pH which contributes to bioavailability of nutrients (N and P) (Rousan *et al.* 2007; Leal *et al.* 2009). Soil biotic and abiotic processes leading to the mineralisation or immobilisation of mineral elements (N and P) are affected by factors such as soil pH, temperature, weather conditions and soil physical properties (Tefamariam *et al.* 2013). Furthermore, subsequent processes leading to the depletion of these nutrients in the soil through nutrient uptake can be affected by plant vigour, root morphology and density (Somma *et al.* 1998). Root growth can be affected by factors such as weather conditions, soil physical and chemical properties such as pH (Somma *et al.* 1998). Moreover, the rate of leaching can be driven by soil physical properties such as texture, organic matter, hydraulic conductivity and rainfall intensity (Tefamariam *et al.* 2013).

Nitrates are very dangerous to human health when they are leached into the soil leading to contamination of groundwater resources (Scheierling *et al.* 2010). However, agricultural systems can provide crops which might act as soil purifiers that remove nutrient pollutants through crop uptake, leaving less available for leaching (García-Peréz *et al.* 2011). The ability of plants to take up mineral nutrients (N and P) from the soil is affected by the existence of forms which can be taken up by plant roots (Tisdale *et al.* 1966). In ABR effluent nitrogen predominantly exists in the form of ammonium (NH_4^+) and other organic forms such as proteins and uric acid (Foxon *et al.* 2004; Hudson 2010; Bame *et al.* 2014). Inorganic forms of P found in treated effluents include orthophosphates and pyrophosphate, which are readily available for plant uptake (Scharchtman *et al.* 1998; Lusk *et al.* 2013). Organic matter can adsorb mineral P within the soil and immobilise it for either plant uptake or leaching (Kim *et al.* 2011). Column studies by Bame *et al.* (2014) have shown that irrigation with ABR effluent can increase the retention of nutrients (nitrates and phosphorus) which can potentially be taken up by plant roots. They further investigated the ability of maize to uptake these nutrients under a pot trial, which are controlled conditions. Studies on different crops to take up nutrients from soils irrigated with ABR effluent are non-existent. Thus, the ability of different crops, such as Swiss chard, to take up nutrients from soils irrigated with ABR effluent and subsequent effects on groundwater pollution through leaching have never been done. Moreover, there is scarce information on the effects of irrigation with ABR effluent on crop growth, nutrient uptake and environmental pollution, especially under field conditions (Pedrero *et al.* 2010). Therefore, this study aimed to investigate the effect of ABR effluent on Swiss chard yield, nutrient uptake and leaching. The information was used to generate nutrient mass balances that would account for losses through leaching

and plant uptake within a Swiss chard field. The specific objectives were, however, to:

- 1) Investigate changes in soil chemical properties after irrigation with ABR effluent.
- 2) Determine the effect of ABR effluent irrigation on Swiss chard growth during different seasons.
- 3) Monitor nutrient leaching beyond the root zone after irrigating with ABR effluent during the summer season.
- 4) Determine N and P mass budgets after irrigating Swiss chard with ABR effluent.

METHODS

Experimental site

Field experiments were carried out at the Newlands Mashu Permaculture Centre in Durban (Figure 1). The site is located at a longitude of 30°57'E and latitude of 29°58'S. It has a mean annual rainfall of 1,000 mm and daily average temperatures of 20.5 °C (www.durban.climatemps.com). Table 1 shows the soil chemical properties of the experimental site before planting. The nitrogen and phosphorus content of the site is 0.22% and 74.6 mg kg⁻¹, respectively.

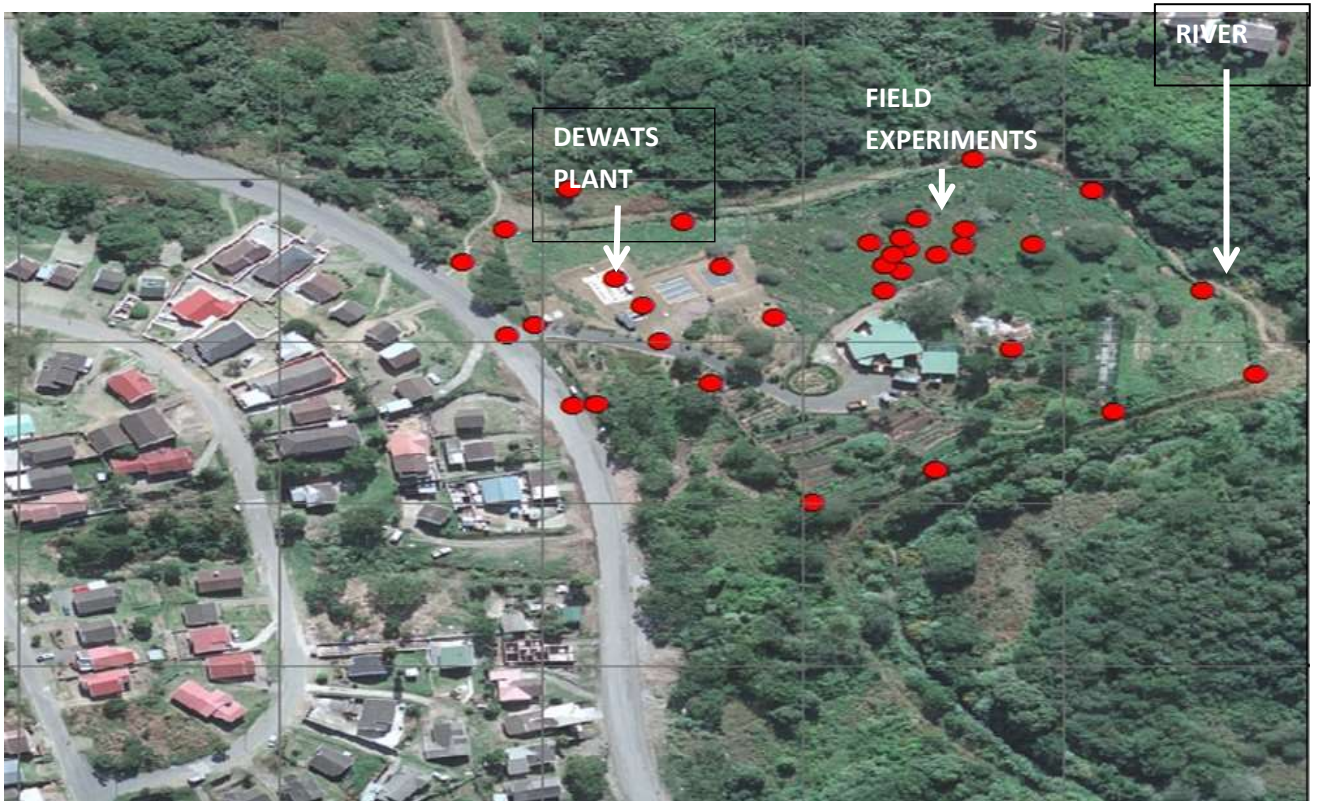


Figure 1| A map showing an aerial view of the experimental site, physical features (River) and the surrounding households

Table 1 | Soil chemical properties of Newlands Mashu sampled before planting, within the top 30 cm soil profile.

Property	Value
Organic C (%)	2.24
N (%)	0.22
P (mg kg ⁻¹)	74.6
K (cmol _c kg ⁻¹)	0.3
Ca (cmol _c kg ⁻¹)	10.2
Mg (cmol _c kg ⁻¹)	6.5
Exch. acidity (cmol _c kg ⁻¹)	0.05
Total cation (cmol _c kg ⁻¹)	17.1
Acid sat. (%)	0
pH (KCl)	5.23
Zn (mg kg ⁻¹)	43.5
Mn (mg kg ⁻¹)	4.81
Cu (mg kg ⁻¹)	14.6

Table 2 shows the site physical properties collected on each and at four different depths (10 cm, 30 cm, 50 cm and 100 cm). Block 1 and 2 are similar with regards to particle size distribution; however, block 3 is slightly different. Clay content tends to increase lower down the profile but the clay is higher in block 1 and 2 than in block 3. Organic C also decreases down the profile but the decrease is greater in block 1 and 2 than in block 3.

Table 2 | Soil physical properties for the experimental site collected at four depths (10 cm, 30 cm, 50 cm and 100 cm) in three different blocks.

Profile position	Soil depth (cm)	Particle size distribution (%)			Org C (%)	Water content at -33 kPa (cm ³ cm ⁻³)	Water content at -1500 kPa (cm ³ cm ⁻³)	K _{sat} (mm h ⁻¹)
		Sand	Silt	Clay				
Top (Block 1)	10	30	46	24	3.8	0.247	0.013	4.68
	30	22	43	35	2.5	0.251	0.045	0.10
	50	26	31	43	0.6	0.268	0.048	-
	100	26	29	45	0.3	0.292	0.018	0.03
Middle (Block 2)	10	32	39	29	3.7	0.261	0.139	1.60
	30	28	41	31	1.6	0.287	0.142	-
	50	31	26	43	0.4	0.262	0.118	-
	100	26	24	50	0.1	0.410	0.039	-
Bottom (Block 3)	10	33	46	21	2.4	0.233	0.162	0.17
	30	28	48	24	2.1	0.235	0.182	1.76
	50	28	44	28	2.0	0.446	0.278	-
	100	16	42	42	1.3	0.294	0.102	1.01

Experimental design

The experiments were carried out in a randomised complete block design (RCBD) with three blocks. The experiments consisted of the following three treatments: ABR effluent irrigation (ABR), tap water with fertiliser irrigation (TWF) and rain-fed conditions with fertiliser application (RFF). However, in summer 2012, ABR plots were irrigated with tap water and no fertiliser was applied.

ABR effluent characterisation for irrigation purposes

The ABR effluent was analysed to determine the mineral element content. Aliquots of 500 mL ABR effluent were collected after irrigation and analysed on site various elements (K, Fe, Cu, Na, Al, Ca, Mn, Mg, Pb, Cd and Ar) using the inductively coupled plasma atomic emission spectrophotometer (ICP-AES) as described by He (2009). Total N was done using the Kjeldahl method as described in Spellman (2000). The nitrates and phosphates were measured using the Merk® Reflectoquant.

Soil chemical properties

Soil samples representative of the experimental site were collected from five randomly chosen points on nine different plots measuring 25 m². Five samples were collected within each plot using an auger to a depth of 20–30 cm and bulked. Composite samples were sent to the Fertiliser Advisory Service, KZN Department of Agriculture and Environmental Affairs; Soil Fertility and Analytical Service, CEDARA for analysis. Changes in soil chemical properties were monitored from baseline studies and after the irrigation with ABR effluent. Soil sampling was done in April 2012 (baseline studies), August 2012 (winter

experiment) and April 2013 (summer experiment). Fertiliser application to the inorganic fertiliser treatments of follow-up crops was conducted based on soil analysis results after harvesting the previous crop.

Installation of access tubes and the calibration of the neutron probe for the determination of soil water content

Soil water content was measured using a neutron probe, which was calibrated for the study site according to Greacen (1981). Two representative spots were selected at the study site for wet and dry spot calibration. The wet spot calibration was determined by making a 4 m² pond. An access tube was installed to a 1 m depth at the centre of the dam. The access tube was inserted in the soil using an auger and a hammer and in such a way that 10 cm was left above the ground surface. The dam was then filled continuously with water until it reached steady state and was covered immediately with polythene plastic for 3 days to prevent evaporation. After 3 days the plastic cover was removed and neutron probe readings taken every 20 cm depth. A soil profile was dug immediately close to the access tubes and undisturbed core samples collected every 20 cm for gravimetric water content determination. The soil samples were weighed immediately after collection (wet mass) and dried in the oven at 105 °C until they reached steady mass (48 hours). Dry spot calibration was done as following the wet spot calibration procedures except that ponding was not done.

Determination of gravimetric water content

$$\theta_g = \frac{M_w}{M_s} \quad (1)$$

where θ_g is gravimetric water content (%), M_w is mass of water (kg), and M_s dry mass of soil (kg).

Determination of volumetric water content

This is the ratio of the volume of water to the volume of soil and is calculated by multiplying the gravimetric water content by the bulk density.

$$\theta_v = \theta_g \left(\frac{\rho_b}{\rho_w} \right) \quad (2)$$

where θ_g is gravimetric water content and ρ_b is the oven-dry bulk density (kg m^{-3}) and ρ_w density of water (kg m^{-3}).

A linear regression equation was fitted to the neutron probe reading ratios of the dry and wet spots against the corresponding volumetric water contents per each layer down to 60 cm (Figure 2). Reading ratios were calculated according to the formula below:

Neutron probe reading in soil / Average standard reading in air

Standard readings in air for that particular day were calculated by averaging ten neutron probe counts in air.

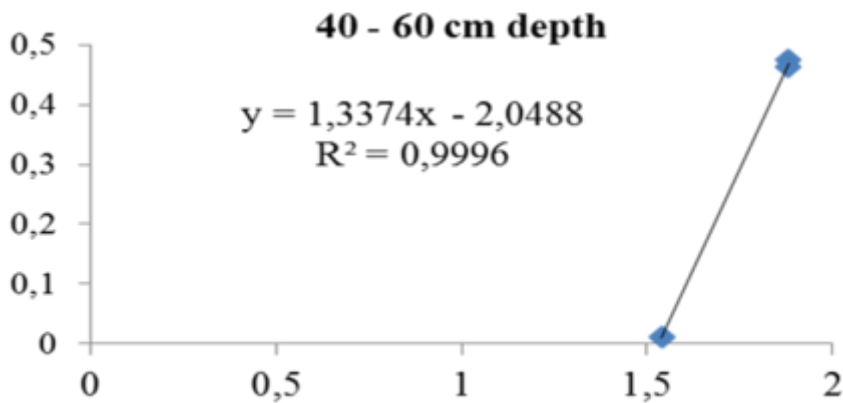
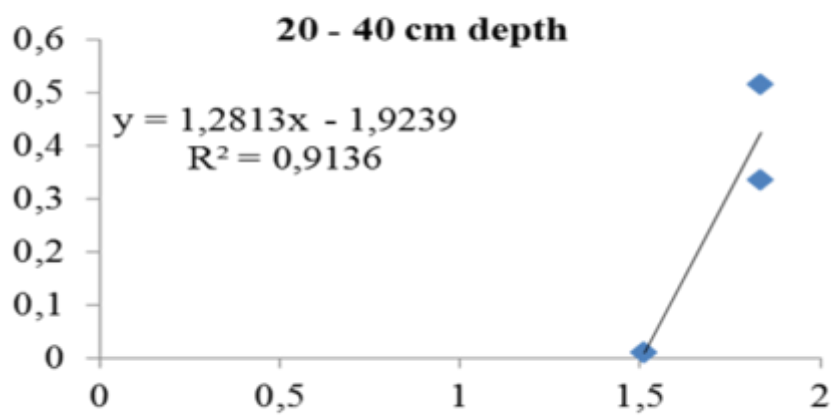
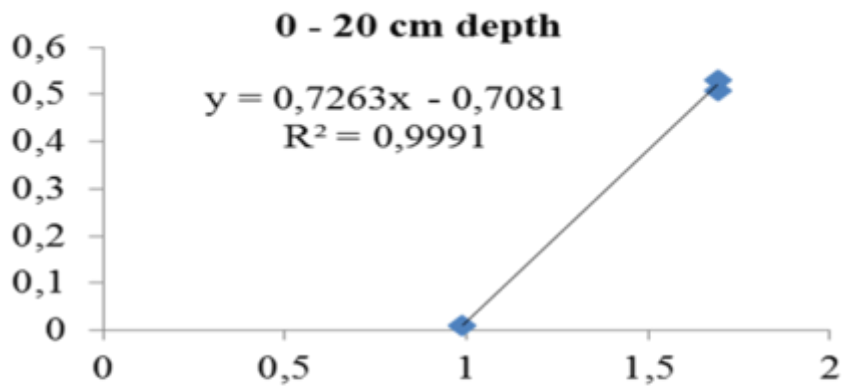


Figure 2 | Neutron probe calibration equations at different soil layers (0–20 cm, 20–40 cm and 40–60 cm) showing the relationship between dry spot count ratios (Y axis) and wet spot count ratios (X axis).

Water deficit was estimated as the sum of the difference between the field capacity and neutron probe readings of each layer until 60 cm depth.

Trial establishment and management of field experiments

Three experiments were carried out, with two experiments carried out in summer and winter of 2012 and the other one during the summer of 2013 (February to April). The winter experiment focused on investigating the effect of ABR effluent on soil chemical properties and Swiss chard biomass production; whereas the summer experiment included the determination of the effect of ABR effluent nutrient (N and P) leaching and Swiss chard yield.

Swiss chard seeds purchased from McDonalds store in Pietermaritzburg were planted in the plots (16 m²) on 15 May 2012 (winter planting) and 31 January 2013 (summer planting) at a spacing of 30 cm × 30 cm. Three seeds were planted per planting station at 25 mm depth and all plots were irrigated using tap water for 2 weeks until seedling establishment. Swiss chard from ABR effluent (ABR) and tap water + fertiliser treatment (TWF) were irrigated using drip irrigation while the rain-fed + fertiliser treatment (RFF) was not irrigated further after seedling establishment. Thinning was done to leave one plant per planting station 3 weeks after planting and the final emergence was recorded. No fertiliser was applied to the ABR plots; however, fertilisers urea (46%: N), DAP (46%: P₂O₅) and KCl (52%: K) were applied to the TWF and RFF treatments according to the soil fertility analysis and Swiss chard crop nutrient requirements (Table 3).

Table 3 | N (Urea), P (DAP) and K (KCl) fertilisers applied to the Swiss chard during the experiment showing the mineral nutrients (N, P and K) applied to the crop.

Treatment	Block	N (kg ha⁻¹)	P (kg ha⁻¹)	K (kg ha⁻¹)
Winter 2012				
TWF	1	100	40	145
TWF	2	100	40	180
TWF	3	100	0	120
RFF	1	100	40	190
RFF	2	100	40	260
RFF	3	100	40	160
Summer 2013				
TWF	1		40	180
TWF	2	100	40	280
TWF	3	100	40	180
RFF	1	100	40	185
RFF	2	100	40	305
RFF	3	100	40	190

Plastic coke bottles (2 litres) perforated at the bottom using a 15.24 cm nail were submerged 5 cm below the ground level to provide a modified drip irrigation system according to Rodda *et al.* (2011). Crop management operations (weeding, pest and disease control) were carried out when necessary to keep the field weed free and maintain healthy plants. Irrigation was done at a 4–5 day interval to maintain water at field capacity within the 30 cm soil depth and the soil moisture content was determined using a neutron probe (CPN 503DR Hydroprobe, Campbell Pacific Nuclear, CA, USA).

Data collection

Plant growth and chlorophyll data during the winter experiment

Data on chlorophyll content, fresh and dry biomass were collected from plants which were randomly sampled from nine quadrants measuring 1 m². From each quadrant, 18% of the plants were selected for data collection. Chlorophyll content was measured using a CCM200 chlorophyll meter (Optisciences Inc., USA) 4 weeks after crop emergence and at harvesting (8 weeks after crop emergence) (Figure 3). Fresh biomass was measured on site immediately after harvesting using a CPA22025 precision balance (Sartorius CPA Precision balances, LA). Dry mass was determined from the plants measured fresh mass by oven drying at 70 °C for 72 hours.

Determination of plant biomass, chlorophyll content and plant tissue nutrients during the summer experiment

Chlorophyll content, dry mass and fresh mass were measured as done in the winter season and results were compared among the three seasons (summer 2012, winter 2012 and summer 2013). Swiss chard plants were cut 1 cm from

the ground and dried at 70 °C for 72 hours, crushed and sieved through a 1 mm sieve before being taken for macro and micro nutrients analysis at the KZN Department of Agriculture and Environmental Affairs; Soil Fertility and Analytical Service, CEDARA. The plant tissue was ground and sieved through a 0.84 mm sieve before being analysed using an ICP-AES for macronutrients and micronutrients (Rodda *et al.* 2011). The plant tissue analysis results obtained after irrigation with ABR effluent were compared to the baseline studies results.

Collection and analysis of leachates from the soil

Wetting front detectors (WFD) installed in the plots at 30 cm and 50 cm depth were used to collect leachates at the respective depths. Due to low rainfall in winter leachates were only collected during the summer 2013. The WFDs were sampled immediately after a heavy rain event and emptied after sample collection. The collected leachates were analysed immediately at the site for nitrates and phosphates using the Merck® Reflectoquant (Figure 3). Leachate samples showing values outside the range for the nitrate test were diluted $\times 5$, $\times 10$ and $\times 15$ and the results obtained multiplied by the dilution factor to get the actual nitrate concentration within the sample.

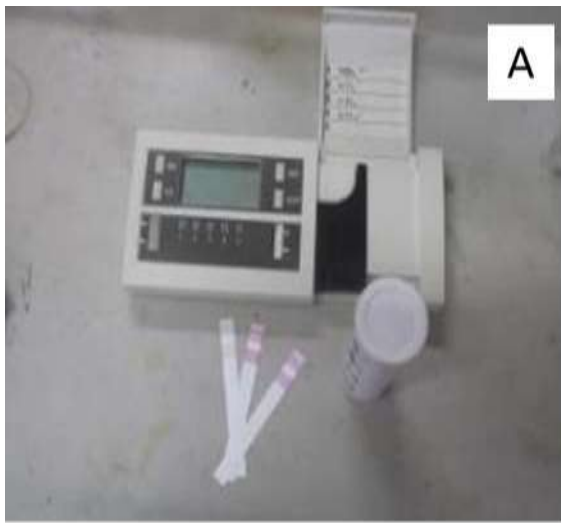


Figure 3 | Merck Reflectoquant test kit (A) and leachates collected from the field (B) and the CCM200 used for measuring chlorophyll content (C).

Determination of N and P mass balances

Nutrient mass balances were calculated according to Tesfamariam *et al.* (2013). The amounts of N and P entering the soil system (irrigation), currently available (initial and final soil nutrients) and losses (leaching and crop uptake) were accounted for.

Data analysis

All statistical analyses were performed using GenStat® 14th Edition (VSN International, Hemel Hempstead, UK). Means were compared using SEDs at the 5% level of significance.

RESULTS

Field experiments on irrigation with ABR effluent were carried out during winter 2012 and summer 2013. Results on plant growth and soil chemical properties obtained after irrigation with ABR effluent were compared to the baseline studies carried out in summer 2012.

Characterisation of the effluent

Table 4 shows the chemical characteristics of the effluent used to irrigate the Swiss chard. The ABR effluent did not contain significant amounts of heavy metals (Al, Pb, Ni, Hg, Cd and Cr). The recommended crop nutrient requirements for Swiss chard were N (100 kg ha⁻¹), P (40 kg ha⁻¹) and K (123 kg ha⁻¹). The N supplied to the Swiss chard crop by the effluent amounted to 91 kg ha⁻¹ which was close to the N requirements. Phosphorus content supplied amounted to 7.6 kg ha⁻¹ which was considerably lower than the crop needs. The potassium concentration in the effluent amounting to 21.2 kg ha⁻¹ was similarly much lower than the crop requirements. The average sodium (Na) concentration in the ABR effluent applied to the Swiss chard crop is 52.8 kg ha⁻¹.

Table 4 | Elemental composition of the ABR effluent used for the irrigation and estimates of the major nutrients supplied during the irrigation of the Swiss chard plants

Element	ABR effluent sampling				Average	Nutrient content (kg ha ⁻¹)			Average
	ABR1	ABR2	ABR3	ABR4		Block 1	Block 2	Block 3	
Element	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹	kg ha ⁻¹
N	61	-	-	-	61	99	77	97.6	91.2
Ca	22.1	31.2	48.2	14.2	28.9	46.9	36.5	46.3	43.2
K	15.7	19.4	8.34	13.3	14.2	23.1	17.9	22.7	21.2
Mg	14.9	12.5	27.1	4.2	14.7	23.9	18.6	23.5	22.0
P	4.46	9.34	3.48	3.01	5.1	8.3	6.4	8.2	7.6
Al	0.36	0.02	0	0	0.1	0.2	0.1	0.2	0.1
Na	nd	35.9	36.8	33.3	35.3	57.3	44.6	56.5	52.8
Fe	0.12	0.09	0.08	0.09	0.1	0.2	0.1	0.2	0.1
Mo	0.11	0.07	0.04	0.05	0.1	0.2	0.1	0.2	0.1
Zn	0.06	0	0	0.01	0	0	0	0	0
B	0	0.1	0.07	0.03	0.1	0.2	0.1	0.2	0.2
Cu	0	0	0	0	0	0	0	0	0
Mn	0	0	0	0	0	0	0	0	0
Co	0	0	0	0	0	0	0	0	0
Cr	0	0	0	0	0	0	0	0	0
Cd	nd	0	0	0	0	0	0	0	0
Hg	0	nd	nd	nd	0	0	0	0	0
Ni	0	0	0	0	0	0	0	0	0
Se	nd	0	0	0	0	0	0	0	0
V	nd	0.01	0	0	0	0	0	0	0
S	nd	13.8	24.8	16	18.3	29.7	23.1	29.3	27.4
Pb	nd	0.09	0.12	0.07	0.1	0.2	0.1	0.2	0.1

*nd- not detected

Comparing the effect of ABR effluent irrigation on soil chemical properties over three seasons and between treatments

Table 5 describes the mean square values for the soil chemical analysis over three seasons (summer 2012, winter 2012 and summer 2013) comparing the effect of ABR effluent on soil chemical properties. Contrasts were done to compare the chemical properties at different seasons (Table 5). Generally, no significant differences were observed across all seasons with respect to changes in soil chemical properties except for N, P and Mn. Significant differences were observed for N ($P < 0.001$), P ($P < 0.01$) and Mn ($P < 0.001$) (Figure 4). N, P and Mn content decreased generally over the seasons for all the plots irrespective of ABR irrigation.

A comparison of the irrigation treatments (Table 6) showed a significant increase in Ca content in plots irrigated using ABR effluent ($12.25 \text{ cmol}_c \text{ kg}^{-1}$) compared to tap water with fertiliser and rain-fed (11.84 and $9.63 \text{ cmol}_c \text{ kg}^{-1}$, respectively). Contrastingly, plots irrigated with ABR showed the lowest Mn content (7.45 mg kg^{-1}) compared to tap water with fertiliser and rain-fed (8.25 mg kg^{-1} and 10.79 mg kg^{-1}), respectively. ABR effluent seemed to have caused an increase in pH as observed; ABR had a pH of 5.4, TWF was 5.1 and RFF was 4.9 (Table 6).

Table 5 | Mean squares for soil chemical properties for samples collected from plots irrigated with tap water (TW), tap water with fertiliser (TWF) and rain-fed (RFF) after planting in 2012 and before and after ABR irrigation in 2013.

Source of Variation	D.f.	N	P	K	Ca	Mg	Exch. Acid	Total. Cat	pH	Zn	Mn	Org. C
Block	2	0.002505	707.9	0.000998	13.752	4.915	0.004378	14.15	1.4412	674.9	6.752	0.4583
Season	2	0.011183**	4931.8**	0.004227	1.342	1.156	0.002543	6.34	0.1261	3396.6	196.509***	0.8829
Treatment	2	0.000043	102.1	0.000854	17.886*	0.859	0.001591	6.23	0.7*	30.5	27.317*	0.1156
Season x Treatment	4	0.000521	237.2	0.011237	5.623	3.786	0.002411	33.96	0.0401	152.5	2.528	0.203
Residual	16	0.001238	711.4	0.00578	3.371	2.312	0.001549	26.94	0.1537	494.1	6.682	0.3541
Total	26											

*Significance at 5% level **Significance at 1% level ***Significance at <0.001 level

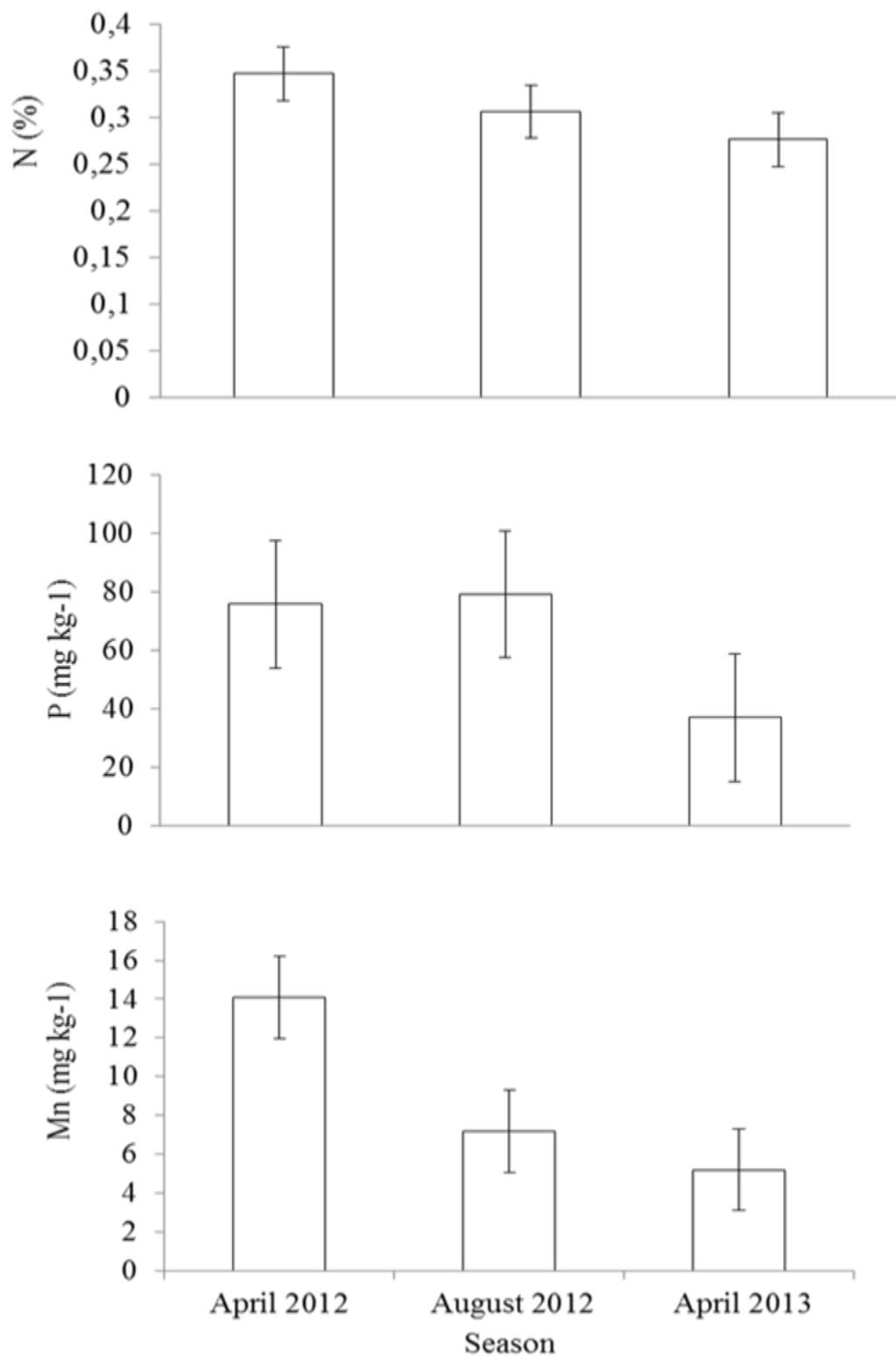


Figure 4 | Changes in soil N, P and Mn in the experimental plots over three planting seasons.

Table 6 | The effect of ABR effluent irrigation (ABR), tap water with fertiliser (TWF) and rain-fed on Ca and Mn content and soil pH over three seasons.

Treatments	Mineral elements		Soil pH
	Ca (cmol _c kg ⁻¹)	Mn (mg kg ⁻¹)	(KCl)
ABR	12.25 ^a	7.45 ^a	5.4 ^a
TWF	11.84 ^a	8.25 ^a	5.1 ^{ab}
RFF	9.63 ^b	10.79 ^b	4.9 ^b
SED	0.87	1.22	0.18

Superscripts ^{a, b and ab} denote means which are significantly different.

Comparison of plant tissue analysis results before (baseline studies) and after irrigation with ABR effluent

Table 7 shows the mean squares for the plant tissue analysis over two seasons (summer 2012 and summer 2013) and between irrigation treatments (ABR, TWF and RFF). There was a statistically significant difference for P ($P < 0.001$), Mg ($P < 0.01$) and Fe ($P < 0.05$) across seasons. Furthermore, significant differences were observed for the season and treatment interaction with regards to Na ($P < 0.01$) and K ($P < 0.05$).

Table 8 shows the concentrations of P, Mg, Fe and Mn (mg kg⁻¹) in Swiss chard plant tissue before (summer 2012) and after irrigation with ABR effluent (summer 2013). During summer 2013 there was a significant uptake of P (0.655 mg kg⁻¹), Mg (1.1 mg kg⁻¹) and Fe (2,036 mg kg⁻¹) compared to summer 2012 P (0.367 mg kg⁻¹), Mg (0.720 mg kg⁻¹) and Fe (625 mg kg⁻¹).

The interaction between seasons and treatments with respect to K and Na taken up are described in Table 9. High uptake of K was recorded in summer 2013 within the TWF treatment (3.46%) followed by ABR (2.73%) and

RFF (2.17%) as compared to summer 2012. In summer 2012, TWF treatment had high K uptake (1.66%) as compared to TW (1.32%) and RFF (1.41%). Na uptake was high in RFF treatment (4.6%) as compared to TW (3.8%) and TWF (3.1%) in summer 2012. On the contrary, in summer 2013, high Na was recorded in ABR treatment (4.19%) as compared to TWF (4.17%) and RFF (3.88%).

Table 7 | Mean squares for mineral nutrient concentrations in plant tissues sampled from Swiss chard plants irrigated with tap water (TW), tap water with fertiliser (TWF) rain-fed (RFF) in summer 2012 and after ABR irrigation in summer 2013

S.O.V.	D.f.	N	P	K	Ca	Na	Mg	Fe
Block	2	0.1263	0.013958	0.03183	0.01993	0.5934	0.0152	242606
Season	1	0.22311	0.373233***	7.843***	0.0005	0.2719	0.65098*	8955407*
Treatment	2	0.28165	0.01205	1.86921**	0.04301	0.5186*	0.02465	1029131
Season x Treatment	2	0.37609	0.001752	0.82129*	0.00396	1.2075**	0.06485	2092813
Residual	10	0.09318	0.005436	0.77573	0.01378	0.1152	0.08622	1240026
Total	17							

*Significance at 5% level **Significance at 1% level

***Significance at <0.001 level

Table 8 | P, Mg and Fe (mg kg⁻¹) concentration in plant tissue before (summer 2012) and after irrigation with ABR effluent (summer 2013)

Season	Mineral elements		
	P (%)	Mg (%)	Fe (mg kg ⁻¹)
Summer 2012	0.367 ^a	0.720 ^a	625 ^a
Summer 2013	0.655 ^b	1.100 ^b	2036 ^b
SED	0.0348	0.1384	524.6

Table 9 | Interaction between season and treatment with respect to K and Na uptake in Swiss chard plants irrigated with ABR effluent (ABR), tap water with fertiliser (TWF) and under rain-fed conditions (RFF).

Summer 2012		
	K	Na
	(%)	(%)
TW	1.32 ^a	3.8 ^b
TWF	1.66 ^a	3.1 ^c
RFF	1.41 ^a	4.6 ^a
Summer 2013		
ABR	2.73 ^c	4.19 ^d
TWF	3.46 ^e	4.17 ^d
RFF	2.17 ^d	3.88 ^d
SED	0.2274	0.2772

Superscripts ^{a, b, c, d} and ^e denotes means which are significantly different

*Note that in summer 2012 TW refers to tap water with no fertiliser which was the treatment assigned to the ABR plots in 2013.

Plant growth analysis among the three different seasons and treatments

Chlorophyll content determination

Table 10 shows chlorophyll content results at 4 and 8 weeks after plant emergence. Significant differences in chlorophyll content were observed among the three seasons and between different growth stages, i.e., 4 and 8 weeks of growth ($P < 0.001$). There were significant differences between summer 2012 and summer 2013 at week 4 ($P < 0.05$) and week 8 ($P < 0.01$) and this was similar to winter 2012 and summer 2013 at weeks 4 and 8, respectively (Figure 5). The difference between summer and winter 2012 was very significant ($P < 0.01$) at both week 4 and week 8. However, the differences between the irrigation treatments were not significant at both week 4 and week 8 across the three seasons.

Plant biomass results

Table 10 shows crop growth variables (fresh mass, dry mass and yield) among three treatments across the three seasons. There were significant differences in fresh mass ($P < 0.01$), dry mass ($P < 0.001$) and yield ($P < 0.01$) across the three seasons. A comparison between summer 2012 and summer 2013 showed significant differences in fresh mass ($P < 0.05$), dry mass ($P < 0.001$) and yield ($P < 0.05$). Significant differences ($P < 0.001$) were observed with respect to fresh mass, dry mass and yield among the three seasons (summer 2012, winter 2012 and summer 2013).

Mean squares for treatments showed highly significant differences for fresh mass and yield ($P < 0.001$) and dry mass ($P < 0.05$). Highly significant differences were observed in fresh ($P < 0.001$) and dry mass ($P < 0.05$) in response to ABR effluent irrigation and rain-fed irrigation (RFF). Similarly, the TWF treatment differed significantly with RFF ($P < 0.05$) with respect to fresh mass and yield. However, there were no significant differences observed between ABR irrigation and TWF irrigation with respect to all growth variables (fresh mass, dry mass and yield). Significant interactions were observed between season and treatment for fresh mass and yield ($P < 0.001$) and dry mass ($P < 0.05$). There were highly significant differences in Swiss chard growth between treatments especially in winter as shown in Figure 5 and 6.

The results shown in Figure 5 compares fresh and dry mass over three seasons and for the three treatments. During the summer 2012 planting no ABR effluent or fertiliser was applied to the experimental units assigned for the ABR irrigation. The reason for not irrigating with ABR was to allow a comparison of ABR effluent irrigation on the same plots so as to take into account the effect of the inherent soil fertility. The ABR plots showed lower mean values for fresh mass compared to TWF and RFF; however, this was not significantly different. ABR effluent was used to irrigate Swiss chard plants planted in these experimental units (plots that were assigned for ABR effluent irrigation in summer 2012) in winter 2012 and the results showed a significant difference with respect to fresh mass between the treatments. Plots irrigated with ABR effluent produced Swiss chard plants with the highest fresh mass than those irrigated using tap water with fertiliser and rain-fed (Figure 5). Mean values for fresh mass of Swiss chard plants within TWF treatment were significantly higher than those from RFF. The results on the effect of ABR effluent irrigation on dry mass showed a more or less similar pattern.

Table 10 | Mean squares for fresh and dry mass , yield and chlorophyll content for Swiss plants irrigated with tap water (TW), tap water with fertiliser (TWF) and rain-fed in summer 2012 and ABR irrigation in winter 2012 and summer 2013.

Source of Variation	D.f.	Fresh mass	Dry mass	Yield	Chlorophyll week 4	Chlorophyll week 8
Block	2	36743	348.45	453.79	10.03	4.474
Season	2	51480**	1568.02***	633.83**	480.2***	724.448***
Treatment	2	58904***	302.14*	728.16***	11.03	5.529
Season x treatment	1	50512***	172.2*	624.32***	14.11	0.686
Residual	16	6337	54.3	42.56	29.45	7.281
Total	26					

*Significance at 5% level **Significance at 1% level ***Significance at <0.001 level

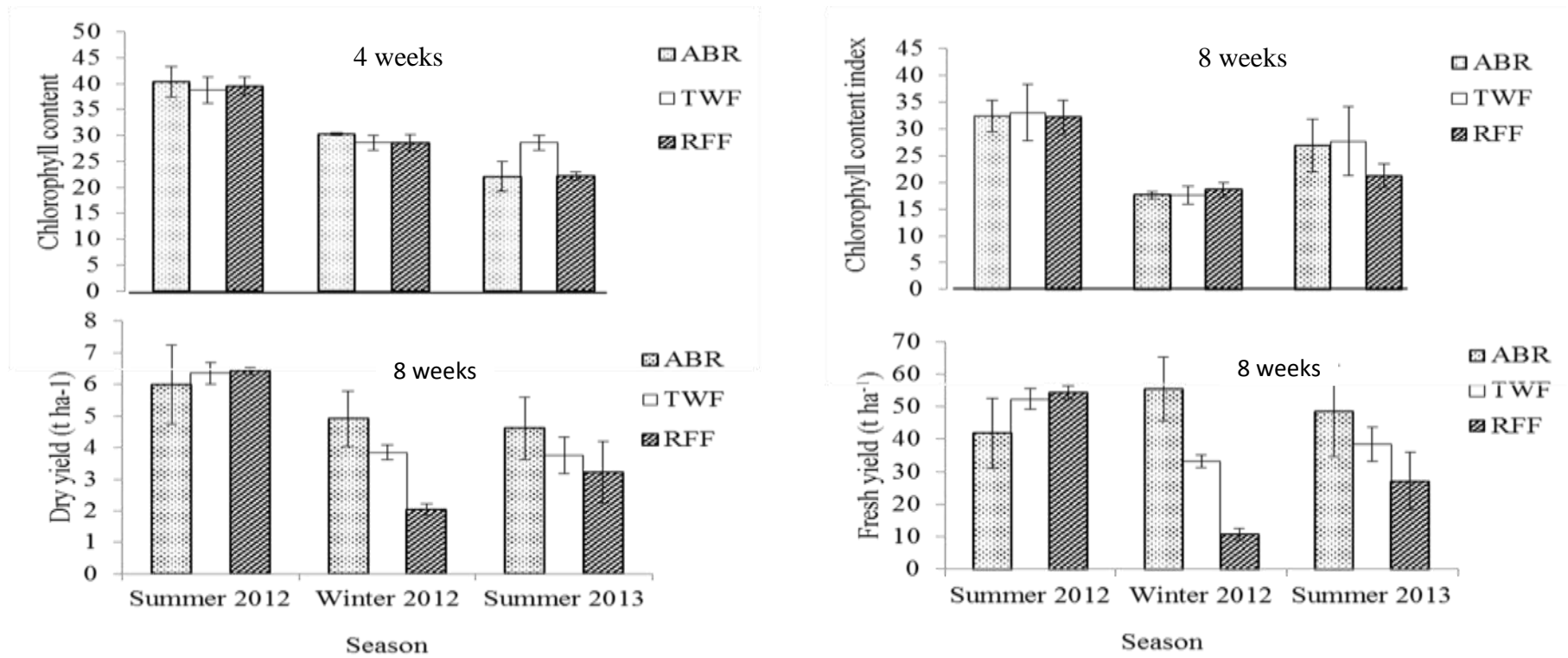


Figure 5 | Swiss chard growth (dry mass, fresh mass and chlorophyll content) within three seasons and different treatments (ABR, TWF and RFF). *In summer 2012 ABR plots were irrigated with tap water only and no fertiliser was applied

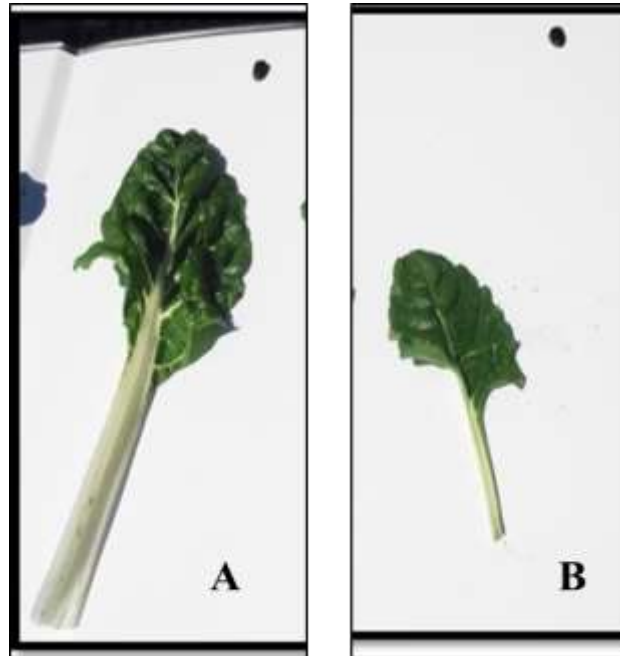


Figure 6 | Swiss chard leaves from the ABR treatment (a) and RFF treatment (b) showing differences in growth during the winter season.

The determination of nitrate and phosphate leaching

Table 11 shows mean squares for the analysis of nitrate and phosphate leachate collected to depths (30 cm to 50 cm) measured at four different time intervals: 7 days after crop emergence, 45 days after crop emergence, 53 days after crop emergence and 72 days after crop emergence in different treatments (ABR, TWF and RFF).

Significant differences ($P < 0.05$) were observed with respect to nitrate and phosphate concentrations between the 30 and 50 cm depths. Mean values for nitrate concentration at 30 and 50 cm were 64 and 47 mg L^{-1} , respectively, and clearly indicate that nitrate concentration was higher at the 30 cm depth (root zone) (Figure 7).

Significant differences ($P < 0.05$) were observed between the treatments and depth ($P < 0.001$) (30 and 50 cm) with respect to phosphate concentration (Table 11). There was a negligible concentration of phosphates within the 50 cm zone (Figure 7). The median value for phosphate concentrations at the two depths is highest for the ABR treatment, followed by TWF and RFF. However, the mean phosphate concentration for the ABR treatment was 1.76 mg L^{-1} and 1.98 mg L^{-1} for the TWF. The RFF treatment had the lowest value of 1.10 mg L^{-1} (Figure 8).

Table 11 | Mean squares for Nitrate-N and Phosphate-P concentrations (mg L^{-1}) at 30 and 50 cm depth for the three irrigation treatments (ABR, TWF and RFF) sampled over four time intervals.

S.O.V.	D.f.	N (mg L^{-1})	F prob.	P (mg L^{-1})	F prob.
Block stratum	2	6517		4.373	
Treatment	2	3400	0.081	5.046*	0.049
Time	3	2982	0.087	2.823	0.16
Depth	1	5250*	0.049	121.095****	<.001
Treatment x Time	6	2154	0.147	2.381	0.192
Treatment x Depth	2	1894	0.239	11.712**	0.002
Time x Depth	3	2778	0.104	6.32**	0.012
Treatment x Time x Depth	6	1718	0.258	1.448	0.486
Residual	46	1281		1.564	
Total	71				

*Significance at 5% level **Significance at 1% level ***Significance at <0.001 level

Table 11 shows that highly significant differences ($P < 0.001$), were observed with respect to phosphate concentrations at 30 cm and 50 cm. Figure 7 shows a box and whisker plot, comparing the variation in phosphate concentrations at 30 and 50 cm depths. The median phosphate value is clearly significant (above 2 mg L^{-1}) which is consistent with the observed mean values. The median value at 50 cm is very low and phosphates were hardly detectable at 50 cm. Mean phosphate values recorded at 30 and 50 cm were 2.91 mg L^{-1} and 0.31 mg L^{-1} , respectively. Figure 9 shows the interaction between depth and treatments. Results shows that P was not detected at 50 cm especially in the TWF treatment, although some was detected within ABR and RFF treatments.

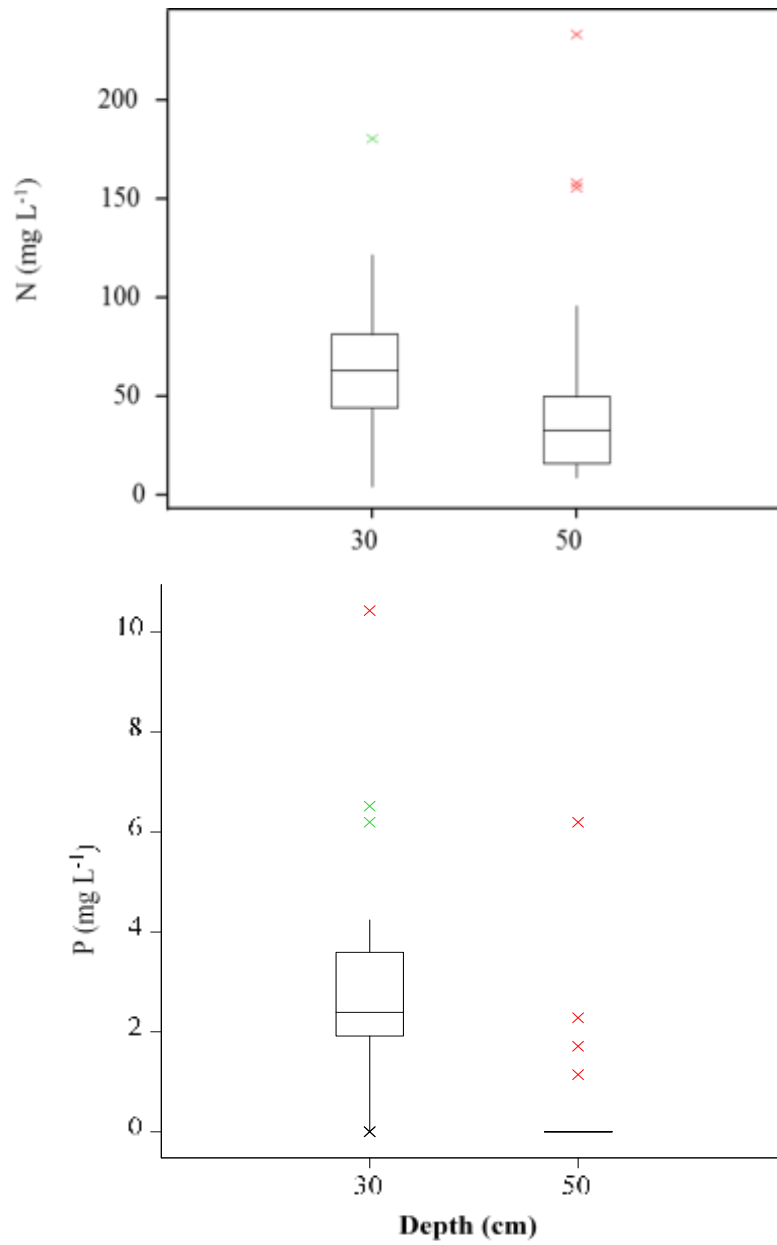


Figure 7 | Nitrate (N) and Phosphate (P) concentrations (mg L^{-1}) at two sampling depths (30 and 50 cm).

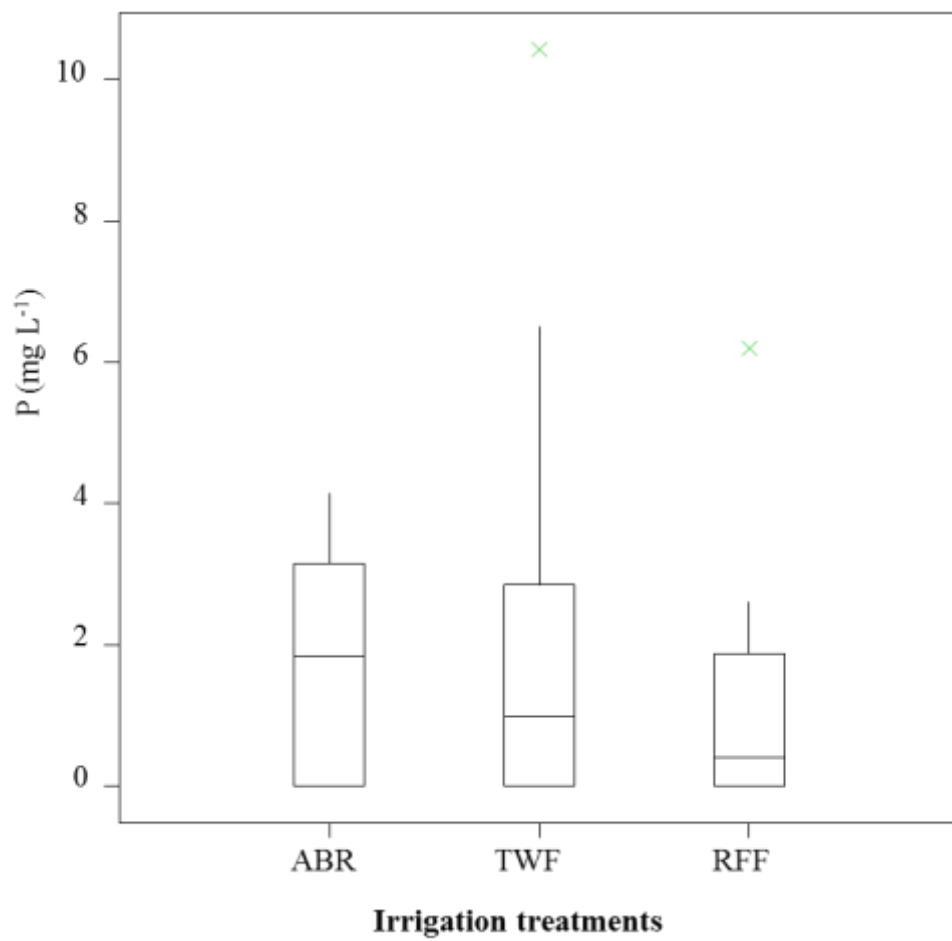


Figure 8 | Phosphate concentrations (mg L^{-1}) in plots irrigated with ABR effluent (ABR), tap water with fertiliser (TWF) and rain-fed (RFF).

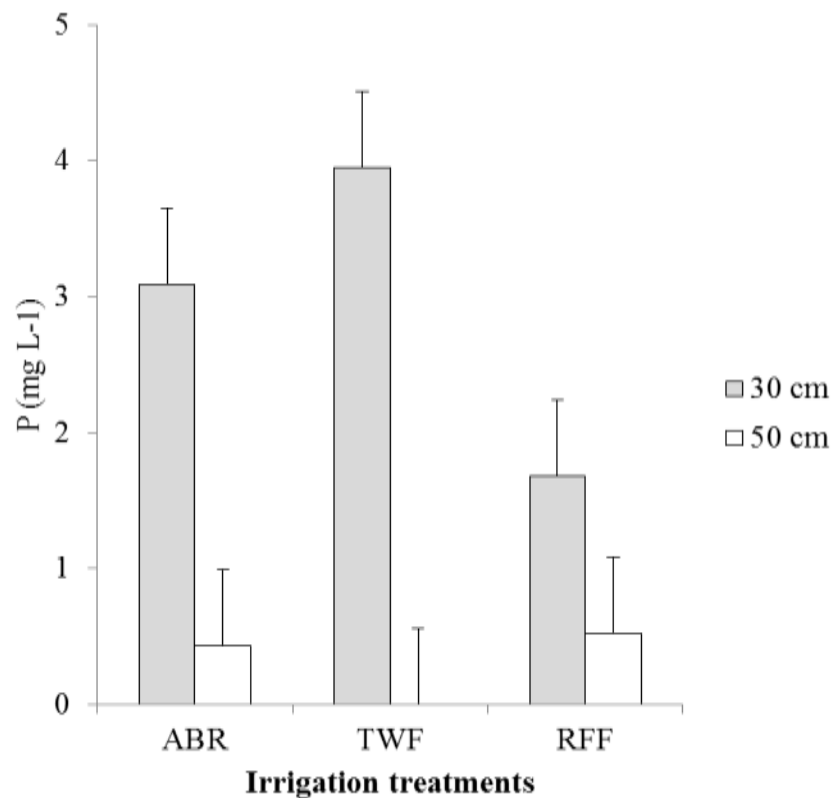


Figure 9 | The effect of depth and irrigation source treatments on phosphate concentrations (mg L^{-1}).

Highly significant ($P < 0.01$) interactions between depth and time were observed with respect to phosphate concentration ($P < 0.01$) (Table 11). Phosphates could only be detected at the 30 cm depth at early growth stages (Figure 10). There was a continuously higher concentration of phosphates within the 30 cm depth compared to 50 cm depth as the plants grew.

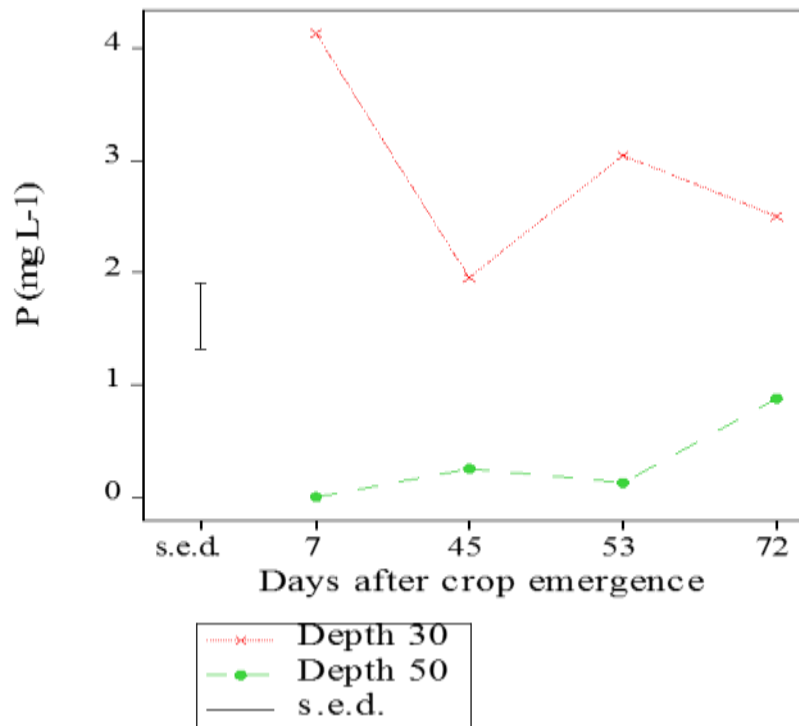


Figure 10 | Interaction between profile depth and time with respect to phosphate concentrations (mg L^{-1}).

N and P mass balances after irrigation with ABR effluent in summer

Table 12 shows mean squares for nutrient (N and P) mass balances in Swiss chard irrigated with ABR effluent during the summer season between three treatments (ABR, TWF and RFF). Insignificant differences in the amount of N added ($P > 0.05$) were observed while highly significant differences were observed with respect to P added to the soil ($P < 0.001$). No significant differences were observed with respect to N and P lost or gained within the soil system. Figure 11 shows the amounts of P supplied to the soil; ABR had the least amount of P ($0.00076 \text{ kg m}^{-2}$) supplied compared to TWF and RFF (0.004 kg m^{-2}).

Table 12 | Mean squares of Swiss chard nutrient (N and P) mass balance after irrigation with ABR effluent during 2013 summer season

Source of variation	D.f	Soil N					
		Soil N (before planting)	Soil N (after harvesting)	N (added to soil)	N (plant tissue)	N (leached)	N (Unaccounted loss)
Block	2	3.846	6.024	0.00013	0.023055	0.88664	3.217
Treatment	2	4.032	2.264	0.000198	0.010289	0.13969	2.391
Residual	8	1.219	3.362	0.00013	0.001523	0.06983	1.143
		Soil P					
		Soil P (Before planting)	Soil P (After planting)	P (added to soil)	P (plant tissue)	P (leached)	P (Unaccounted loss)
Block	2	0.02509	0.00544	9.76E-07	0.000567	8.43E-05	0.04327
Treatment	2	0.00571	0.00729	0.002682***	0.000147	8.43E-05	0.01016
Residual	8	0.01425	0.01086	9.76E-07	0.000143	0.000206	0.03121

*Significance at 5% level **Significance at 1% level ***Significance at <0.001 level

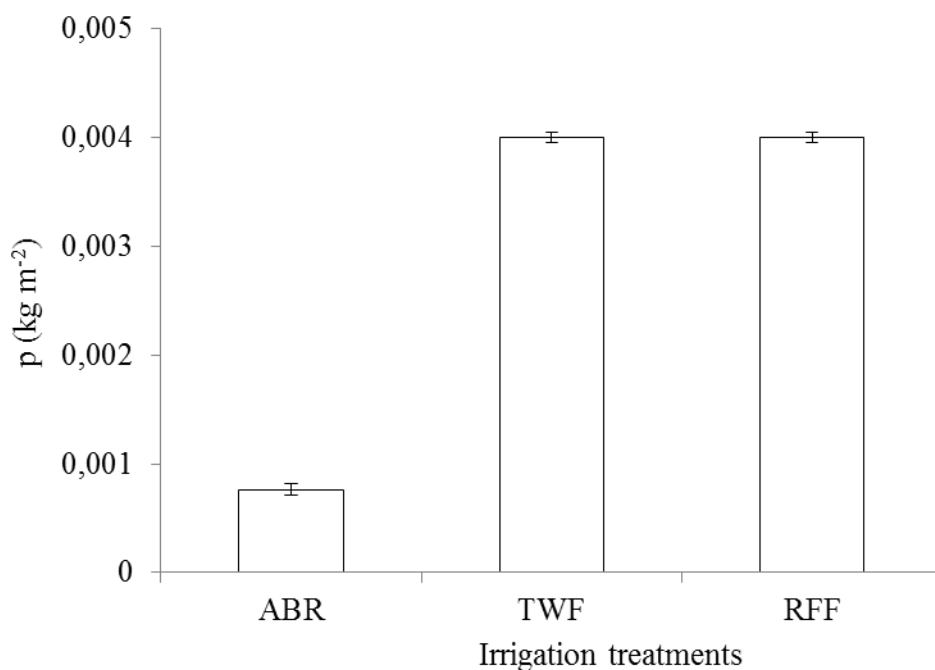


Figure 11 | Amounts of P (kg m⁻²) added to different treatments (ABR, TWF and RFF) during the Swiss chard growing season.

DISCUSSION

Effects of ABR effluent irrigation on soil chemical properties

In this study, the effect of using ABR effluent on crop growth and the leaching of N and P from the soils was investigated. Initial tests included the characterisation of the ABR effluent used for irrigation. The general characteristics of the ABR effluent showed that the effluent was low in heavy metals. A number of studies have shown that household-treated sewage effluents rarely contain heavy metal unless contaminated with industrial effluent (Sharma *et al.* 2004). Treated wastewater is constituted by 20 to 85 mg L⁻¹ of total N and 6 to 20 mg L⁻¹ P depending on the strength (Pescod 1992). The

results observed suggest that ABR effluent can be classified as a major source of N. This implies that direct disposal of ABR effluent into rivers can cause water pollution. Its use in agriculture can be beneficial through nutrient recovery and leaching might lead to contamination of groundwater.

The study further determined the effect of the ABR effluent on soil chemical properties. A decrease in soil Mn and N content was observed during the winter season. This could perhaps be due to uptake by plants. During the preceding season (summer) there was high soil Ca content in the ABR treatment as compared to the RFF treatment (Table 6). This increase could perhaps be due to the concentration of Ca (28.9 mg L^{-1}) in the ABR effluent (Table 4). Although TWF was not irrigated with ABR effluent the soil Ca content was still statistically similar to the ABR treatment. The reason behind this was not well understood but might be related to other factors, such as variability in soil pH within the different plots. Ca losses and retention in the soil are affected by factors such as high rainfall, hydraulic conductivity and soil pH; basic cations such as Ca are easily leached at low pH (Levy 2011). Thus, the lower Ca concentration in the RFF treatment could perhaps be attributed to low pH (4.9) as compared to ABR (5.4). There was a decline in soil P, Mn and N over the three seasons independent of any treatment (Figure 4) probably due to uptake by plants. The lack of significant changes in the soil nutrients with respect to treatments (Table 5), suggests that using ABR effluent for irrigation did not significantly affect soil chemical properties in a short period of time.

Effects of irrigation with ABR effluent on nutrient uptake

The effect of using ABR effluent on Swiss chard nutrient uptake was investigated. Swiss chard has a nutrient sufficiency range of 4–6% (N) and 3–8% (K) (Campbell 2000); however, results obtained showed a higher uptake of N in RFF and ABR as compared to TWF. Although the difference was

statistically significant the values were within the optimum sufficiency range according to Campbell (2000). The reasons behind this observation could not be attributed to experimental treatments since there was adequate rainfall.

Furthermore, all the treatments received adequate N for Swiss chard growth. The leaching results showed insignificant differences among all the treatments, an indication a similar quantity of N was maintained in the soil regardless of treatments. However, further studies with more experiments will better explain the reason behind this observation. Except for K and Na, there was a significant increase in nutrient uptake during the second year independent of treatments (Table 9). According to Somma *et al.* (1998), dynamics in root growth due to soil and weather conditions can affect nutrient uptake in plants. High rainfall and temperature regimes increase root growth and nutrient uptake (Somma *et al.* 1998), however, this observation could be attributed to differences in rainfall distribution between the summer 2012 and summer 2013 seasons (Figure A1).

An antagonistic relationship with regards to K and Na uptake was observed among the treatments. ABR irrigated plants had a higher Na concentration compared to TWF and this was even higher than the RFF treatment during the second season (Table 9). High Na concentration within the ABR treatment could have been attributed to irrigation with ABR effluent since 52.8 kg ha^{-1} was supplied (Table 4). According to Pokluda & Kuben (2002), Swiss chard is categorised as a saline tolerant vegetable with a capacity to uptake large amounts of Na when it is present in the soil. This is an indication that salinity must be monitored when irrigating crops susceptible to salinity. Higher uptake of K within TWF as compared to ABR was due to the effects of KCl applied to the former. There was no K fertiliser applied to the ABR effluent irrigated plots and the effluent provided 21.2 kg ha^{-1} of K (Table 4), which was lesser than the amounts recommended for the crop based on soil analysis results (Table 3). Furthermore, Swiss chard requires about 225 kg ha^{-1} of K during its production (Schrader & Mayberry 2003).

Effect of irrigation with ABR effluent on Swiss chard growth

The interaction between seasons and treatments shows that the response of Swiss chard to irrigation was variable across seasons. The result shows that plant growth (dry yield, fresh yield and chlorophyll content) was high in summer 2012 compared to winter 2012 and summer 2013 (Figure 5). This was due to differences in weather conditions among the three seasons (Appendix 1). High fresh biomass values were observed in the ABR treatment compared to TWF and RFF treatment during the winter season. Furthermore, the ABR and TWF treatment had higher dry mass values than RFF treatment (Figure 5). The lower biomass observed in the RFF treatment during winter was due to low rainfall (Figure A1). During the summer 2013 season, similarly to summer 2012, no significant differences were observed in biomass (fresh and dry mass) among all the treatments (Figure 5). This implies that the use of ABR effluent for irrigation is very important in winter and a major concern for the management of excess effluent during summer.

Effect of irrigation with ABR effluent on N and P leaching

The analysis of leachate quality was conducted to monitor N and P leaching below the rooting zone. The higher concentrations of soil nitrates within the 30 cm depth indicate that there was a low rate of N leaching implying that most of the N was retained for plant uptake. This is expected since clay soils have a higher nutrient retention capacity due to texture and organic matter content compared to sandy soils (Tisdale 1966). However, the movement of nitrates within the soil could not be linked to irrigation with ABR effluent because the irrigation treatments did not differ with respect to nitrate concentration at the 30 cm depth (Table 11).

Phosphorus is a very immobile nutrient which can be adsorbed by organic matter in the soil rendering it less mobile especially in ferric clay soils and high

organic matter soils (Bame *et al.* 2014). The soil physical properties characterisation (Table 2) showed a significant decrease in hydraulic conductivity (Ksat) and organic C from 30 cm to 50 cm depth. This implies that lower movement of P from 30 cm to 50 cm was due to a decrease in organic matter and low hydraulic conductivity at these depths. A significant treatment \times depth interaction ($P < 0.01$) was observed with respect to phosphate concentrations (Table 11). The results show that there were higher concentrations of phosphates at the 30 cm depth compared to 50 cm depth for the ABR and RFF treatments (Figure 9). No phosphates were found at 50 cm depth for the TWF. The presence of phosphates at 50 cm depth for the RFF clearly suggests that phosphate movement in the soil cannot be conclusively attributed to ABR effluent irrigation only. The higher mean phosphate value for the TWF is inconsistent with the results shown in the box and whisker plots which show a higher median phosphate concentration for the ABR treatment. However, this can be easily explained by the large single value (outlier) observed in the TWF which most likely pushed the mean value upwards (Figure 7). Phosphate concentration within the 30 cm depth declined sharply at 45 days after crop emergence. It further increased from 53 days after crop emergence and the concentrations did not significantly change after 72 days. The lower phosphate concentration observed at 30 cm during the 45, 53 and 72 days after crop emergence (Figure 10) could probably be attributed to P uptake by the plants.

N and P mass balance budget following irrigation with ABR effluent

Insignificant differences in the N mass balances between all the treatments could be an indication that the N cycle system was in a balanced state regardless of treatments applied. The amount of N added to the soil through irrigation with ABR effluent did not differ significantly in comparison to the amount added through inorganic fertilisers (Table 3). Based on the actual Swiss chard water

requirement ABR effluent could provide sufficient amounts of N required by the plant. The amount of N taken up by plants between treatments did not differ significantly, implying that ABR irrigation did not affect N uptake. Insignificant N leaching values can further explain the fact that the N supplied by ABR effluent was taken up by plants. Ammonium nitrogen found in ABR effluent can be nitrified to nitrates, as reported by Bame *et al.* (2014), and subsequently taken up by plants. This may also explain the insignificant differences in amounts of soil N after harvesting. The ability of Swiss chard to take up mineral nutrients (N and P) implies that agricultural systems can be used sustainably to dispose treated wastewater (Abdel-Raouf *et al.* 2012).

Even though ABR effluent managed to provide about 16.7% of the total P requirements for Swiss chard production no significant differences were reported with respect to leached P, P taken up by plants, soil P after harvesting and before planting (Table 12). P is generally deficient in most soils, and exists mostly in organic forms unavailable for plant uptake (Schachtman *et al.* 1998). However, the mineralisation of organic P is affected by certain microorganisms which operate under certain conditions (pH, temperature and moisture) (Schachtman *et al.* 1998) but this process takes years. Moreover, Johns & McConchie (1994a) observed similar results after irrigating with sewage effluent from stabilisation ponds. The reasons behind this observation are not clear hence they need further study.

CONCLUSIONS

No significant changes were observed with respect to changes in soil physical and chemical properties over the three seasons following irrigation with ABR effluent due to short period of the experiment. Irrigation of Swiss chard with ABR effluent is comparable to other conventional agricultural practices mostly as a source of irrigation water. Movement of N and P from upper soil layers

cannot be attributed to ABR effluent irrigation. Irrigation with ABR effluent could not lead to the leaching of nutrients (N and P), implying that irrigation of Swiss chard with ABR effluent can have the potential to link urban sanitation with agriculture in a manner that is not harmful to the environment. Findings from this study cannot be conclusive in the long run; however, future research will be focused on modelling nutrient and water mass balances to predict the environmental sustainability of irrigating with ABR effluent, focusing on different soils, crops and environments.

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