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Rhizofiltration system consisting of *Phragmites australis* and *Kyllinga nemoralis*: evaluation of efficient removal of metals and pathogenic microorganisms

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ABSTRACT

A rhizofiltration system planted with *Phragmites australis* L. and *Kyllinga nemoralis* L. was constructed at a wastewater treatment plant in KwaZulu-Natal, South Africa and evaluated for its efficiency in removing heavy metals and enteric pathogens from municipal wastewater. The rhizofiltration system that was kept unplanted acted as the reference section. Influent, effluent, plant tissue and sediment from the system were sampled bi-monthly for a period of six months and assessed for physicochemical parameters, removal of *Escherichia coli*, faecal coliforms and trace heavy metals. The biochemical oxygen demand and chemical oxygen demand values reduced as compared with the influent sample. Suspended solids, turbidity and alkalinity were reduced in the planted and reference sections. Considering the entire rhizofilter, heavy metals were accumulated at varying concentrations in the planted and reference section of the rhizofilter. Planted section showed a greater potential to remove heavy metals from wastewater than the reference section. The entire rhizofiltration system was found to have an average pathogen removal ratio between 45% and 98% for the various pathogens detected in the influent wastewater. *Ascaris lumbricoides* was reduced by 77% in the planted section and 53% in the reference section. The results obtained from this study suggest that macrophytes have the potential to remove heavy metals and pathogens from wastewater.

Keywords: Wastewater treatment; *Phragmites australis* L.; *Kyllinga nemoralis* L.; Heavy metals; Pathogens; Rhizofiltration

1. Introduction

Many industrial processes use substantial quantities of water and equally, release enormous wastewater into the environment [1]. Various forms of wastewater have been treated using conventional methods such as activated sludge processes and trickling filters [2] and stabilization ponds within municipalities and industries [3]. Such systems are less efficient in pathogen removal and require additional treatment by using disinfectants such as chlorine, ozone and ultraviolet radiation to achieve acceptable discharge limits [2]. Stabilization ponds which are cost-effective in installation and management have been successfully used for wastewater treatment in developing countries [4]. However, stabilization ponds cannot eliminate pollutants such as heavy metals and pathogens [5], which is the goal of efficient wastewater treatment.

Biological treatment systems that can achieve maximum wastewater treatment are the constructed wetlands [1]. Wetlands are known to achieve better treatment of wastewater including the removal of heavy metals, suspended solids and wastewater pathogens [4]. The use of rhizofilters in constructed wetlands may be appropriate for application in small communities and institutions because of cost-effectiveness in management and can remove significant

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amounts of pollutants from wastewater [1]. Rhizofiltration processes involve the mutual interaction of plant roots and microorganisms to remove contaminants from wastewater [6]. More specifically, it involves the filtration, adsorption, concentration and assimilation of pollutants such as heavy metal ions from the wastewater into the root systems [6].

Constructed wetland systems have been designed to use various species of plants with the potential to accumulate pollutants [7,8]. Some of the plant species used include *Phragmites australis*, *Typha latifolia* and *Eichhornia crassipes* [9]. However, the potential of *Kyllinga nemoralis* L. as a wastewater treatment plant species in constructed wetland has not been investigated. The macrophytes, *Kyllinga nemoralis* L. and *Phragmites australis* L., proved to be potential biosorbents and were also able to remove some specific pathogens such as *E. coli* from the wastewater. Selection of the most appropriate macrophyte for systems efficiency remained a challenge but considering the study by Vu et al. [10]; the antimicrobial characteristics of the two plants contributed to the decision to choose these plants in this study.

Heavy metals such as lead (Pb), zinc (Zn), cadmium (Cd), chromium (Cr), nickel (Ni) and copper (Cu) occur naturally and are potentially hazardous to both terrestrial and aquatic environments when present above permissible limits [6,11]. Traces of heavy metals usually occur in the water where some get immobilized in the sediment and form complexes with oxides and hydroxides of iron (Fe) and other particulate matter in the sediment [11]. The common methods to remove heavy metals from wastewater include removal of metal ions by chemical precipitation, ion exchange, coagulation, solvent extraction, hydroxide precipitation, electrodialysis and reverse osmosis but proved to have limitations including the formation of residual metallic compounds that require extra treatment of the effluents [12]. Hydroxide precipitation based on metals precipitation such as ions of Cu, As, Zn, Pb and Cd may be used to co-precipitate heavy metals with pyrite from wastewater [13]. New methods such as the application of rhizofiltration systems which are based on the application of aquatic plants (normally roots) to remove metals through adsorption and absorption processes have been adapted [14].

Municipal wastewater contaminated with several pathogenic microorganisms is another concern for human health [15]. Some pathogenic microorganism may survive the treatment system, whereas others have adaptations that compromise their removal depending on the type of wastewater treatment applied [16]. Some pathogens are very resilient and change their forms (form cysts e.g., *Ascaris lumbricoides*) to survive harsh environmental conditions [1,17]. The facultative bacteria *Escherichia coli* are considered harmless or may cause mild diarrhoea to humans. However, some serotypes of *E. coli* can cause food poisoning with symptoms of chronic diarrhoea, stomach crumbs and severe vomiting [9]. The higher-class organisms such as *Ascaris lumbricoides* form one of the most important groups of nematodes because of their parasitic nature in humans. The eggs are mainly found in wastewater and in the soil and can remain viable for a very long time (up to a period of 10 years or more) under unfavourable conditions. It causes the condition ascariasis in man through the ingestion of mature ova. The major route of removal from wastewater is filtration through the matrix, the length of the wetland, hydraulic loading and retention period of the wetland system [9].

In this study, a rhizofiltration system was established with the aim of treating municipal wastewater. The system was tested to assess its capability and efficiency in the reduction of physicochemical parameters such as pH, salinity, temperature, turbidity, chemical oxygen demand (COD) and biochemical oxygen demand (COD). The system was first constructed and divided into the various matrix layers and then the macrophytes (*Phragmites australis* L. and *Kyllinga nemoralis* L.) were planted according to a study by Vymazal [18]. A unique feature of the rhizofiltration system was the matrix composition of large boulders, fine and coarse aggregate and graded sand, the height, length and width. Detailed mechanisms that are involved in pollutants removal and systems functionality of rhizofiltration treatment systems such as the one used in this study are remote. A system with these design characteristics is envisaged to require minimal management challenges and with a preference for use in small communities and learning institutions. The system was tested to assess its capability and efficiency in the reduction and removal of heavy metals and pathogens from wastewater. It also aimed to investigate the potential of macrophytes *P. australis* and *K. nemoralis* in the treatment of wastewater through the removal of metals and pathogens. *P. australis* has been investigated as a constructed wetlands treatment plant, while there is no documented study of the potential of *K. nemoralis* as a treatment wetlands plant. This forms part of the novelty of this study. This study, therefore, seeks to add knowledge on the efficiency of the constructed rhizofilter in wastewater purification. The study was conducted at the Kingsburgh wastewater treatment works located in Amanzimtoti, South – East of Durban, in the province of KwaZulu-Natal (South Africa) from the period of March 2012 to November 2012. Additionally, this study is aimed at adding more information on the performance of a rhizofiltration system in Durban, South Africa, which experiences a subtropical climate.

2. Materials and methods

2.1. Establishment of the rhizofiltration unit

A combined vertical and horizontal flow rhizofiltration system (VHFS) was constructed at the Kingsburgh wastewater treatment works located in Amanzimtoti, South – East of Durban, in the province of KwaZulu-Natal (South Africa). The rhizofilter was divided into two sections of planted and reference. The rhizofiltration system measured approximately 9 m (L) × 2 m (W) × 1 m (D) and was packed with filter material arranged in three zones of 1 m each according to the study design. The first zone was composed of coarse material of half-broken bricks and blocks (100–120 mm Ø and 250 mm deep) while the second zone was composed of crushed stone of between 63 and 150 mm sizes and the third zone was a mixture of small tones (19–25 mm) gravel and coarse river sand (Fig. 1). The sand layer at the top was overlaid with fine gravel to shield the sand from being blown away by the wind. The rhizofiltration system was divided into two sections (planted and reference). One section was planted with macrophytes *Phragmites australis* and *Kyllinga*

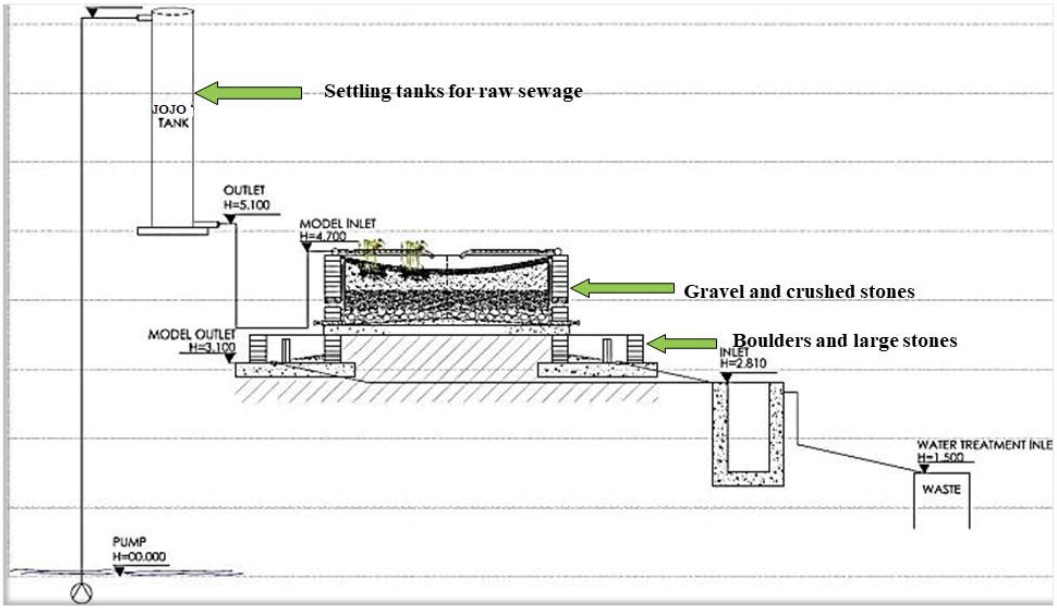

Fig. 1. Schematic representation (not to scale) of the rhizofiltration system in Kingsburgh showing gradient flow of pre-settled wastewater.

nemoralis, while the other section remained unplanted acted as the reference section [19]. The system was designed to function as a vertically integrated rhizofiltration wetland with 10 inlet zones that opened directly onto the sand media. The designed outlets (top overflows and bottom outlets for low flows) were considered for channelling different flow regimes. The design favoured an inlet feed into the rhizofilter and drainage back to wastewater treatment works by gravity. The combined vertical (VFCW) and horizontal flow (HFCW) rhizofiltration system had two zones to compare pollutant removal efficiencies (planted and reference). On the planted section of the rhizofilter 20 rhizome stalks each of two species of macrophytes (*P. australis* L. and *K. nemoralis* L.) were initially planted (well-established rhizomes of about 25 cm in length were used). *P. australis* was the pioneer species (Fig. 2a), which was planted in two rows and *K. nemoralis* was added after a period of 2 months to increase the plant cover and as a second experimental macrophyte which has not been investigated elsewhere and forms part of the novelty of this study. Acclimatization and stimulation of plant growth in the system were performed according to the methodology adopted by Di Luca et al. [20]. This was followed by application of settled pre-treated wastewater from the municipal treatment system in the mix ratio of 10%, 20%, 30%, 50%, 80% and finally 100% settled wastewater for a period of 3 months to establish the treatment process (microorganisms and biofilm establishment). Adjacent to the rhizofiltration system was two settling tanks of 10,000 m^3 (large) and 5,000 m^3 (small) capacity. Raw sewage was pumped into the 5,000 m^3 tank where it could settle before flowing over to the large tank as settled sewage through the overflow outlet which was fitted at the top of both tanks. From the large tank, the settled sewage was channelled by gravity into the vertical feed system of the rhizofilter in comparison with the previous designs [21].

A rhizofiltration system is said to achieve a steady state of operation when there is a significant reduction in organic loading and suspended matter according to wastewater discharge standards [18]. The biomass is also at an optimum and steady state when about 80% of the soil is covered by the plants (Fig. 2b). The hydraulic loading of the rhizofiltration system was tested by performing flow rate test based on influent and effluent discharges and was adjusted using valves. The design of this system accommodated a flow rate of 25–50 m^3 d^{-1} from the pre-settling tank to the inlet of the rhizofilter. The system was fitted with 10 sampling points (A–E on planted and F–J on the reference) on the opposite sides where water samples were taken periodically for various tests to assess the efficiency of the system in pollutant removal (Fig. 3). Raw wastewater from the conventional municipal treatment system was channelled to the 5,000 m^3 and allowed to settle before being released into the 10,000 m^3 tank which finally channelled the wastewater to the rhizofiltration unit by gravity flow.

2.2. Measurement of flow rates in the rhizofilter

The flow rate of raw wastewater from the municipal treatment inlet into the 5,000 m^3 tank was measured and time taken for the tank to fill up was noted and regulated from time to time. The water level in both tanks was checked using a transparent graduated tubing with 10 cm interval markings, which was fitted at the side of the tank. The flow of the water entering the rhizofilter was measured by observing the decrease and increase in the markings and regulated using valves as was required from time to time. Flow rate from the various outlet sampling points was measured using a graduated bucket which could fill up and time is taken to fill it up was also noted and used to calculate the flow rate from each sampling point against time. Water

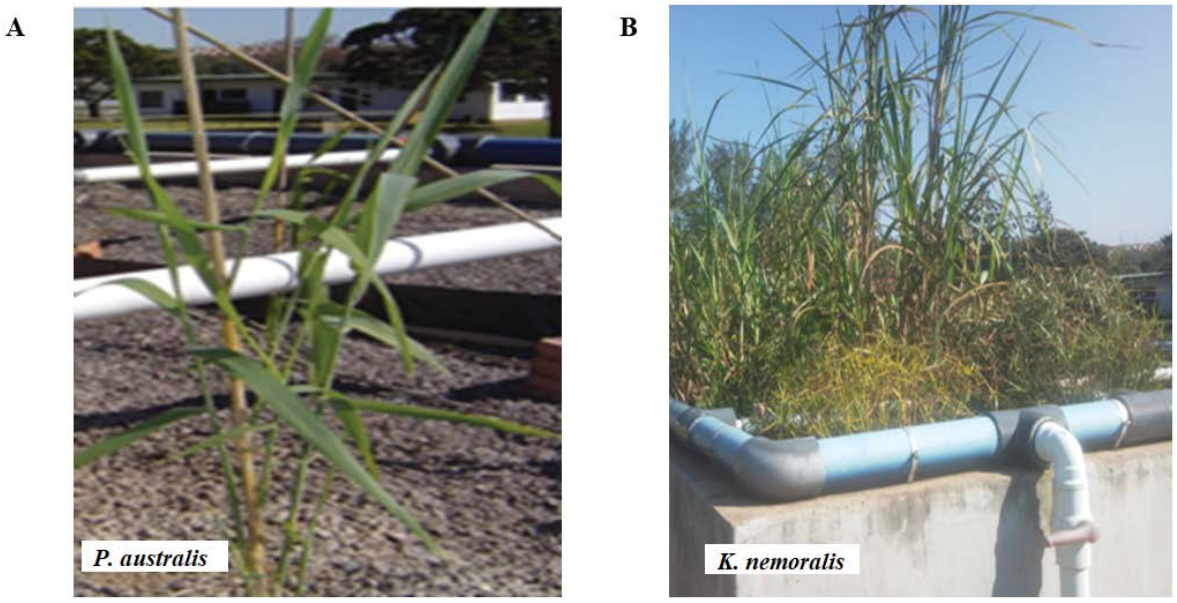

Fig. 2. Healthy growth of *P. australis* at the initial stages of the rhizofilter establishment (A) and a section of optimum macrophyte growth showing the macrophytes *P. australis* and *K. nemoralis* (B).

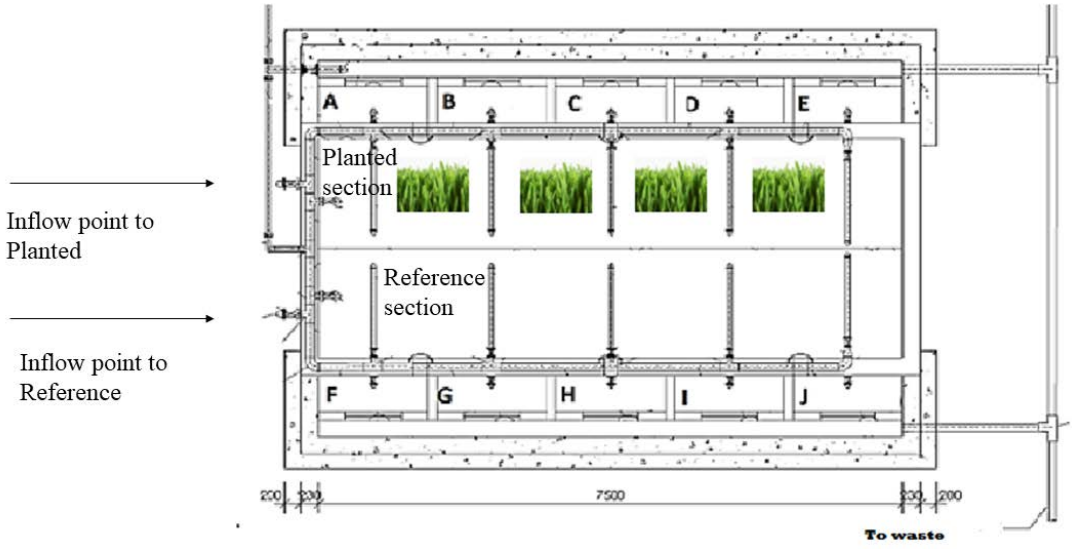

Fig. 3. Design of the rhizofilter showing outlet sampling points A–E (planted) and F–J (reference) sections.

and plant samples were collected monthly for analysis of the various parameters to establish system functionality and efficiency from March 2012 to November 2012, when the system was assumed to have reached a steady state of operation.

2.3. Sampling procedures for wastewater and plant material

Water samples were collected in 1 L sterile Schott bottles, placed in a polystyrene cooler box packed with ice and transported to the laboratory for the various analyses. Various physicochemical tests such as pH, electrical conductivity (EC), turbidity (nephelometric turbidity unit, NTU), total dissolved solids (TDS) and suspended solids (SS), BOD and COD were carried out using conventional procedures according to standard methods APHA [22]. Likewise, three cuttings (weighing approximately 200 g) of each specimen of *K. nemoralis* and *P. australis* were randomly collected from the rhizofiltration unit using a pair of secateurs according to standard methods for the examination of water and wastewater APHA [22]. The specimens were placed in transparent plastic bags, labelled and taken to the laboratory for further analysis, that is, measurement of heavy metals in different parts of plants such as leaf, stem and roots. The system achieved about 80% plant cover at the time of sampling, a situation that displayed negligible disturbance of the macrophytes density within the system.

2.4. Measurement of physicochemical parameters

All the physicochemical parameters were measured according to APHA [22]. A calibrated electronic meter (YSI 556 MPS-United Kingdom) was used in the field for the determination of EC (μs cm^{-1}), salinity (mg L^{-1}), TDS (mg L^{-1}), pH (pH units), dissolved oxygen (mg L^{-1}) and temperature (°C). The electrode was rinsed each time between sample measurements using distilled water to avoid contamination from the preceding sample. BOD was determined by the respirometric method using the OxiTop TS 606/2-i system. COD was determined by the closed reflux colorimetric method while alkalinity was determined using the titration method. Suspended solids were determined by using glass fibre filter disks (GF/A Cat No. 1820 047 with 47 mm∅). Metals analysis was done according to a method by Hou et al. [23] and concentration of the various elements was determined using the inductively coupled plasma-optical emission spectroscopy (ICP-OES) [20] (Supplementary information Method S1).

2.5. Preparation of plant material and determination of metal contents

The specimens were washed with distilled water to remove attached soil particles and placed in a sieve to drain out excess water. The plant specimens were further immersed in a solution of HCl (0.01 M) in order to remove any exterior metal deposits. The material was then rinsed with double distilled water for about 1 min. The material was then partitioned into leaves, stems and roots and then shredded using a pair of scissors and secateurs. The sample was air-dried to remove excess water and then dried at 80°C for 24 h. The material was blended to a fine powder using a Milestone start D Mellerware 50 g capacity blender. The homogenized dried plant material (0.3 g) was weighed in triplicate and placed in a microwave Teflon tube. The tube was introduced into the Teflon safety shield and 8 mL of HNO_3 (65%) followed by 2 mL of H_2O_2 (30%) were carefully added and mixed in a fume cupboard. The solution was gently swirled to ensure homogeneity. The Teflon tube was tightly closed and placed in the microwave (Milestone START D) cavity and digested for 35 min at 180°C. The residue was evaporated on a hot plate at 50°C and diluted using double distilled water to a volume of 50 mL. The prepared samples were used to measure the metal content using inductively coupled plasma optical emission spectrometer (ICP-OES) [24].

2.6. Protozoa detection

An estimated amount of 1 L of the sample was collected from the inlet and 5 L from the designated effluent sampling points on the planted and reference sections, respectively, to detect the protozoa [25]. The further procedures were adapted from Vymazal [18] and total number (*N*) of the eggs per litre sample was determined from Eq. (1):

$$N = \frac{AX}{(1.5 \times P)} \quad (1)$$

where *N* is the number of eggs per litre of sample, *A* is number of eggs counted in the McMaster slide or the mean of counts from three slides, *X* is the volume of the final product (mL) and *P* is the volume of the McMaster slide (0.3 mL or 1.5 mL).

2.7. Coliform bacteria detection

Faecal coliforms and *Escherichia coli* were detected using the Colilert-18 method (based on the most probable number, MPN, USEPA protocol – 40CFR136) – IDEXX defined substrate technology [26].

2.8. Data analysis

Data were analysed using Microsoft Excel (Microsoft Corporation, USA) and GraphPad Prism (GraphPad Software, USA). The pollutant removal rates were estimated based on percentage removals and mass removals. Adsorption, absorption and desorption of metals tested by the macrophytes was statistically determined using ANOVA. The means were determined using Bonferroni tests. Percentage reductions and removals were calculated according to Eq. (2) while mass removal rate (*r*, in g mL^{-2} d^{-1}) was calculated according to Eq. (3) [27].

$$= \frac{(C_{in} - C_{out})}{C_{in} \times 100} \quad (2)$$

$$= q\,(C_{in} - C_{out}) \quad (3)$$

where C_{in}, C_{out} and q are inflow concentration (mg L^{-1}), effluent concentrations (mg L^{-1}) and hydraulic loading rate (m d^{-1}), respectively.

3. Results and discussion

3.1. Construction of rhizofilter

The macrophytes achieved optimum growth after a period of 1 month though the plant cover on the rhizofilter was not at 100%. However, initial tests were carried out on influent and effluent from both sections of the rhizofilter. *K. nemoralis* grass species was added as an experimental macrophyte. After a period of 3 months, the rhizofilter showed about 90% cover of the macrophytes and considered as optimum plant growth (Fig. 4). The rhizofiltration performance is affected by a number of factors including operating mode (batch or continuous, horizontal or vertical), loading rate and environmental conditions such as climate and season [6]. Overloading of such systems may result in clogging, decreased treatment efficiency and odour emissions, as well as large oxygen demand upon roots and resultant death of the plants. Higher organic loads resulted in hydraulic dysfunction due to internal clogging. However, the current rhizofiltration system did not experience any clogging during the entire study period. Instead, the system experienced channelling conditions and prominent channels were formed at some parts of the system. These channels allowed high flows of the water through them which gave some sampling taps higher flow rates than the others.

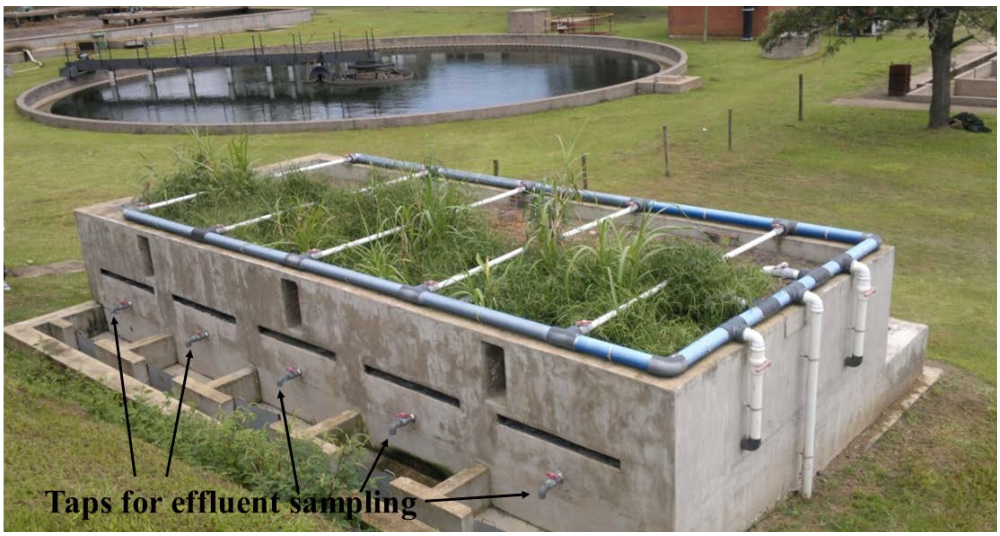

Fig. 4. Rhizofiltration unit at Kingsburgh showing about 80% plant cover on the planted section, 3 months after the initial planting of the macrophytes. Arrow indicates the sampling points.

3.2. Flow rate analysis

The flow rate experienced between the various sampling points at the establishment of the rhizofilter was measured and recorded (Table S1). The settling tank which received the raw sewage first before the rhizofilter showed a flow rate of 0.62 L s^{-1} and increased to 1.12 L s^{-1}. The flow rate was varied within the various sampling points of the system. The lowest recorded flow rate was from sampling point "*E*" at 0.3942955 L min^{-1} on the planted section while highest was at sampling point "*J*" at 0.8710061 L min^{-1} on the reference section. The valves that released or channelled the flow into the rhizofilter further regulated the flow by reducing it to 0.02 L s^{-1}. The reduction was found to form ahead of about 100 mm on the filter surface of the unit. This phenomenon may have caused pressure and eventual channelling of the flow-through some specific areas within the rhizofilter. This may also be the reason for the difference in flow rates at the various sampling points of the system. The other factor that may have positively influenced the soil hydraulics in the rhizofilter was the distribution of the varied sizes of the grain in the matrix. The topmost layer which was a mixture of sand and fine gravel played one key role in contaminant removal. Similar observations were reported by Stottmeister et al. [28] on the effects of plants and microorganisms in constructed wetlands for wastewater treatment. The plant parts tested for metals uptake and deposition showed the deposition and uptake of metals, thereby reducing the level of metals in the wastewater. Similarly, the top layer of the rhizofilter may have played a role in the reduction of microorganisms such as bacteria, viruses, fungi as compared with the soil samples analysed.

Due to the variations in the wastewater contents, the change in the flow rate of the system (Fig. S1) was observed, as the pump was placed on the bottom of the sump (domestic wastewater receiver) where raw wastewater was collected. Flow rate variations were due to varying solidity and viscosity of the effluent rather than the pump itself. The flow rate of the water plays a key role in the pollutant reduction mechanisms in a constructed wetland for wastewater purification. The faster the flow, the less the pollutant trapping by the plant roots is achieved. Therefore, the flow needs to be regulated in such a way that the pollutant trapping is possible.

The system received wastewater from people around Kingsburgh, Durban, with an estimated population of about 200,000 people. All the pumps installed in the system were found to be in good working condition and produced an adequate flow rate for running the system. Measurements were taken for system calibration to ensure the repeatability and standardization of the procedures for results replication when necessary. These measurements were also important as enabled to track and monitor the changes in the system flow rates over time due to change with continual usage. Wastewater treatment in the constructed rhizofiltration system is known to be affected by the system's flow rate. Thus, it was first imperative to analyse the causes for the different flow rates in the rhizofilter unit before it could be operated. Monitoring and regulating water flow rate was a critical aspect of the success of the constructed rhizofiltration treatment process. The system consisted of three sections; inflow, settling basin (disposal section) and outflow. rhizofiltration systems are constructed with the reservoirs from which the inflow is controlled. The prevention of the clogging and short-circuiting of the rhizofiltration system flow, as well as initial filtration of wastewater, was of equal importance. This was prevented in the system by filling the inflow section with gravel, which consisted of different layers of varied sizes of gravel particles.

3.3. Physicochemical parameters

The physicochemical parameters studied showed variations according to the sampling points and season (Table 1). The pH increased on average from 6.9 ± 0.08 to 7.55 ± 0.01 pH units and 7.23 ± 0.07 on the planted and reference sections, respectively. However, the pH values were not statistically different between the planted and reference sections (p = 0.9925). High pH values were observed between the

Table 1
Summary of means of physicochemical parameters from influent and effluent from the planted and reference sections during 1 year of study

Parameter/unit	Influent (pre-chlorinated sewage) Mean* ± S.D.	Effluent (planted) Mean* ± S.D.	Effluent (reference) Mean* ± S.D.	Discharge limits (maximum)
pH (pH units)	6.90 ± 0.08	7.53 ± 0.01	7.23 ± 0.07	5.5–9.5
Temperature (°C)	21.9 ± 0.05	20.0 ± 0.15	21.5 ± 0.10	30/Not to alter ambient temperature
EC (μs/cm)	0.6 ± 0.05	0.5 ± 0.01	0.5 ± 0.05	70–250
Salinity (mg L^{-1})	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.05	1
Turbidity (NTU)	10.7 ± 0.10	9.9 ± 0.17	11.1 ± 0.29	5
TDS (mg L^{-1})	0.3 ± 0.05	0.4 ± 0.05	0.3 ± 0.05	250
TSS (mg L^{-1})	1.1 ± 0.05	0.8 ± 0.15	0.9 ± 0.05	25–30
DO (mg L^{-1})	3.8 ± 0.09	6.7 ± 0.08	5.7 ± 0.10	Objectionable
COD (mg L^{-1})	53.9 ± 1.71	30.9 ± 1.06	39.9 ± 1.43	75
BOD (mg L^{-1})	19.7 ± 0.83	7.3 ± 0.29	8.9 ± 0.36	20

*Mean value shown is the average of three replicates

months of February and April while comparatively low values were recorded in May and July. The flow rate was varied within the various sampling points on the planted and reference sections of the system. The lowest flow rate was from sampling point '*E*' at 0.3942955 L min^{-1} on the planted section while the highest was from sampling point '*J*' at 0.8710061 L min^{-1} in the reference section. The variations in flow rate within the sampling points could be attributed to establishing channelling caused by variations in flow rates as water flowed through the substrate, particle consistency of the wastewater and the location of the pump at the bottom area of the Jojo tank which may have hampered continuous flow [18].

The choice of physicochemical parameters studied was based on previous studies [21]. The investigation of temperature changes was because above a certain threshold, macrophytes cannot grow and below a threshold temperature, the nitrogen-converting bacteria would not efficiently work. Higher pH values were observed in the month of February and lower values were recorded in May and July [29]. In this study, the pH values were not significantly different for all sampling points as was an average of 6.5–7.2 pH units suggesting that the wastewater was majorly from domestic source [21]. Flow rate varied within the various sampling points of the system.

In the first 3 months of study, BOD removals were recorded at 38% for the planted section and 16% for reference, while COD was reduced by 16.5% and 9.6% for planted and references (Table 1), respectively. Table 1 displays result for various parameters including BOD at 79% and COD at 75% based on optimum removal efficiency by the system after a period of 6 months. The reductions of BOD and COD within the system are indicators of heavy removal of organic pollutants by the rhizofilter. The presence of macrophytes in the system may have played a key role in the reduction of organic matter and microbial activity, hence the reductions in COD and BOD were recorded. This was evidenced by the higher efficiency of reductions of COD and BOD from the planted section as compared with the reference section. On the contrary, another study suggested that increased microbial activity is attributed to the participation of macrophytes which allow for more microbial attachment and greater microbial activity associated with the rhizomes. The macrophytes also provided a pedestal (stems, roots and leaves) which supported the growth of microorganisms responsible for organic molecules breakdown. The reduction in COD may have also been dependent on temperature since organic matter removal and increased microbial activity tended to increase in the months of summer. The season between January and March has average temperatures of 28°C in Durban, South Africa. Further, there was an evident reduction in the levels of COD and BOD. This meant that the system was also able to reduce the organic load in the wastewater. This scenario was also observed in other studies of wetland treatment processes. The mass reduction rates for COD and BOD were similar to those reported by Abdelhakeem et al. [27]. *P. australis* reported to reduce up to 95% for BOD and 94% for COD [30]. The organic matter could possibly have been reduced through aerobic reactions and adsorption of solid particles within the rhizosphere which contributed to the breakdown of organic matters in conjunction with the macrophytes which provided oxygen to the root system. Major mechanisms for BOD and COD reduction were probably filtration by the filter matrix, sedimentation and microbial metabolism within the rhizofilter.

Total suspended solids were reduced by 86% on the planted section while the reference section showed a 59.8% reduction. The reduction of SS may have occurred due to sedimentation especially in the settling Jojo tanks and further onto the substrate of the rhizofilter [5]. Electrical conductivity (EC) was reduced on average by 7.7% on the planted section while the reference section had an average reduction of 0.83%; this may be due to the reduction of phosphorus and nitrogen salts by the microorganisms and plants interact within the porous substrate of the rhizosphere according to the study by Singh et al. [31]. Evapotranspiration and

temperature variations in summer have also been reported as one of the mechanisms involved in the reduction of EC in treatment wetlands and may be the reason for higher reductions of EC during the warm months observed in this study. The low reduction in conductivity levels during this initial system establishment could be attributed to an increase in nutrient and mineral levels occasioned by addition of compost manure which was required to enhance the growth of the macrophytes. TDS were reduced by 11.5% for the planted section and 3.5% on the reference section of the rhizofilter [3]. The temperature was reduced by 11.9% on the planted section and 1.2% in the reference section. The highest value of 35.3°C in the reference section was recorded in February while the lowest temperature reading of 17.1°C on the planted section was recorded during the month of June. Reductions in effluent temperature on the planted section were due to the plant cover and water retention, which created some macroclimate within the rhizosphere. Dissolved oxygen was raised by 10% on the planted section and 5% in the reference section. The recorded values were in the range of 3.2 mg L^{-1} at the inlet to an average of 6.5 and 6.0 mg L^{-1} on the planted and reference sections, respectively [32]. The increase in DO levels resulted from the physical and biological mechanisms of plant roots within the rhizosphere.

The alkalinity values remained constant between all the sampling points but were higher in the reference section at 0.34 mg L^{-1} as compared with 0.31 mg L^{-1} on the planted. Higher values were recorded in July while the lowest values were recorded in May. Alkalinity was varied between the different sampling points of the rhizofilter. Highest removal rates of 46.3% (sampling point *B*), 46.3% (sampling point *F*) and 45.5% (sampling point *I*) were recorded in April. The lowest reduction rate of alkalinity at 5.6% (sampling point A) was recorded in the month of May (Fig. S2). Low pH increased the alkalinities while high pH which was experienced at the sampling taps contributed to the reduced alkalinity at the effluents especially on the planted section in concurrence. This advanced that the calcium carbonate precipitation in the rhizosphere represented a trace governed by the inflow pH.

Turbidity varied between 8 and 11 NTU at the inflow and the various sampling points. February and May recorded the highest turbidity levels. The reductions were recorded at 11.97 NTU at the inflow to 9.7 and 9.1 NTU on planted and reference sections, respectively, and in agreement with the previous report [29]. The month of May showed the highest turbidity (up to 11.9 NTU) at the inflow during the study. This was reduced to an average of 8.3 NTU mostly on the reference section of the rhizofilter (Fig. S3). Turbidity was reduced at a steady rate on the reference section while the planted section had inconsistent reductions which can be attributed to the flow channelling caused by the root network of the macrophytes and the flow rate into the system [18]. The presence of macrophytes in the unit contributed to the reduction of turbidity and suspended solids in varying percentages. Suspended solids may have been removed by the filtration component and physical suspension offered by the extensive root network of the macrophytes. The process of pre-settling the raw sewage at the Jojo tanks before the gravity flow to the unit, also contributed to the reduction of both turbidity and suspended solids and prevented system clogging.

Salinity values remained constant between all the sampling points but were higher on the reference section at 0.34 mg L^{-1} as compared with 0.31 mg L^{-1} on the planted section. Higher values were recorded in July and the lowest values were recorded in May.

3.4. Determination of metals concentration in planted and reference sections

There were statistically significant increases and reductions in the metals concentrations ($p < 0.05$) within the components of the rhizofiltration unit after a period of 3 months from the initial system set up (Table 2). Planted section showed lower concentrations of heavy metals than the reference section except for Ni (higher concentrations in planted section). Inlet and effluents from both planted and reference sections were shown to have reductions and increases in the metals concentrations for some metal ions (Table 2). Cd concentration (0.0311 mg L^{-1} in the inlet) was not significantly reduced to 0.03035 and 0.03085 mg L^{-1} in planted and reference sections, respectively. Cr (0.0671 mg L^{-1} in the inlet) was reduced to 0.046 and 0.047 mg L^{-1} in planted and reference sections, respectively. This could be attributed to uptake of metals by the plants in planted section and the amount in the reference section may have been absorbed into the sediment as reported in a study by Vymazal [18]. Similar observations were reported by Parmar and Thakur [11] during their study on heavy metal Cu, Ni and Zn toxicity, health hazards and their removal techniques by low-cost adsorbents. Co concentration was found to be 0.0614 mg L^{-1} in the inlet

Table 2
Metals concentration in the inlet, and effluent samples from the planted and reference sections of the system after 3 months of study

Metal	Inlet Mean# ± S.D.	Planted Mean# ± S.D.	Reference Mean# ± S.D.
Cadmium (mg L^{-1})	0.0311 ± 0.03030	0.03035 ± 0.00029	0.03085 ± 0.00006
Chromium (mg L^{-1})	0.0671 ± 0.05675*	0.04650 ± 0.00046*	0.04735 ± 0.00040*
Copper (mg L^{-1})	0.0614 ± 0.06145*	0.06835 ± 0.00029*	0.07965 ± 0.00017*
Nickel (mg L^{-1})	0.0481 ± 0.04755*	0.04560 ± 0.00023*	0.04050 ± 0.00011*
Lead (mg L^{-1})	0.5400 ± 0.14400*	0.58800 ± 0.00115*	0.62455 ± 0.00064*
Zinc (mg L^{-1})	0.4710 ± 0.16550*	0.43000 ± 0.00115*	0.54400 ± 0.00115*

*Statistically significant difference.
#Mean value shown is the average of three replicates.

and increased to 0.068 and 0.078 mg L^{-1} in planted and reference section, respectively. Ni was reduced to 0.046 mg L^{-1} in planted section and 0.041 mg L^{-1} in the reference section from initial concentration 0.0481 mg L^{-1}; while Zn was reduced to 0.43 mg L^{-1} in the planted section but increased in the reference section to 0.54 mg L^{-1} from an initial concentration of 0.47 mg L^{-1}. Zn is one of the micronutrients required by plants for growth [18]. This explains why there was a reduction in the planted section as more zinc may have been taken up by the plants for growth, while residual Cu from the plant tissues may have contributed to the increase witnessed in the planted section. The accumulation of metals in the stem and leaves of the macrophytes could be attributed to translocation [33]. The order of removal of metal ions by planted section was Cr > Zn > Ni > Cd while Co, Pb was found to be in higher concentrations as compared with the inlet section. The order of removal of metal ions by planted section was Cr > Ni > Cd while Co, Pb and Zn were found to be in higher concentrations as compared with the inlet section.

3.5. Determination of metals concentration within the plant tissue

After 3 months of system establishment, there were notable increases in the concentration of Cd (21%), Cu (60%) and Pb (50%) in the root of *K. nemoralis*. Cr decreased by 40% in the root of *K. nemoralis* and 70% in the root of *P. australis*. Ni decreased by 40% in the root of *P. australis* while Zn decreased by 20% in the leaf of *K. nemoralis*. There was an increased deposit of Zn in the root of *K. nemoralis* and in the stem tissue of *P. australis*. A significant amount of Ni was deposited in the root of *K. nemoralis* and stem tissue of *P. australis*. Results of sediment analysis also show that more metal deposits were recorded in the planted section as compared with the reference section of the rhizofilter. Notably, higher levels of Zn were deposited in the planted section (Fig. 5).

Considering the distribution of the metal ions in the components of the rhizofilter, sediment accumulated more of the ions as compared with the plant parts and the final effluent in 2012. The total amount of metal ions adsorbed by the sediment, absorbed by the plant tissues and concentration in wastewater were considered to create a mass balance. The highest accumulation was on sediment with Zn recording 56%. The effluents did not have many deposits except for chromium and Ni which recorded 17% and 9%, respectively (Fig. 6). The highest accumulation in the macrophytes was showed for Zn at 51%. This may be attributed to the fact that Zn is a micronutrient required by plants and that there may have been already some substantial amount of Zn deposits on the plant tissues before exposing to the wastewater [11]. However, some amount of metal ions could not be accounted for and was assumed to have been taken up by bacteria and fungi present in the rhizosphere. Metals may have been removed through various processes including precipitation and the ability of organics in the wastewater to bind to metal ions. This binding ability of metals onto organic matter may explain the reason for metals decrease in above-ground plant parts. Another metals removal process may have occurred through the accumulation of organic matter from shoots and leaves of the macrophytes in the rhizofilter. The metal ions present bound directly onto the organic matter which provided carbon and therefore energy for the microbial metabolism.

3.6. Ascaris lumbricoides and coliform bacteria

Effluent from the planted section recorded a lower (10 ova L^{-1}) concentration of the ova of *A. lumbricoides* (Fig. 7) than the reference section (16 ova L^{-1}) after 3 months of system establishment. These values were further reduced to 4 ova L^{-1}; removal rates were 82% on the planted section and 63% on the reference section of the rhizofilter. The removal of *A. lumbricoides* ova by the system was mostly observed by the heavy root network of the plants and the rhizofiltration unit substrate [21].

The coliform analysis in the different sections of the rhizofilter indicates the lower number of coliforms in the planted section compared with the reference section (Fig. 8). The highest inflow of coliforms was recorded during the month of October (26.0 MPN/100 × 10^{-5} mL) and lowest in August (5 MPN/100 × 10^{-5} mL). The average reductions were in the range of 2.0 MPN/100 × 10^{-5} mL in planted and 3.5 MPN/100 × 10^{-5} mL in the reference section during the warm season (October and November) and between 3.2 MPN/100 × 10^{-5} and 5.2 MPN/100 × 10^{-5} mL in the planted and reference sections, respectively. Faecal coliform bacteria may have been removed by the presence of macrophytes in the rhizofilter since removals were higher on the planted section as compared with the reference section. The mechanism of removal could be associated primarily with UV radiation and an element of higher temperatures on the sections of the rhizofilter that were exposed. The total coliforms could have been reduced by the presence of the macrophytes which excreted some inhibiting metabolites and the presence

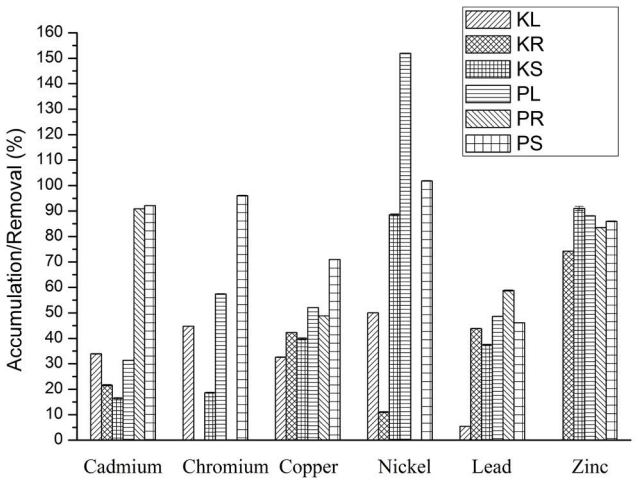

Fig. 5. Accumulation and removal (%) in metals concentration in the leaves (L), stem (S) and root (R) tissues of *K. nemoralis* (K) and *P. australis* (P) after 3 months of study on the rhizofiltration system establishment. Standard deviation whiskers represent the errors of means of three replicates. Accumulation and removal (%) were calculated according to the equation $(C_0 - C_1)/C_0 \times 100$), where C_0 and C_1 are the concentrations (mg L^{-1}) of metals at the commencement and after 3 months of the study, respectively.

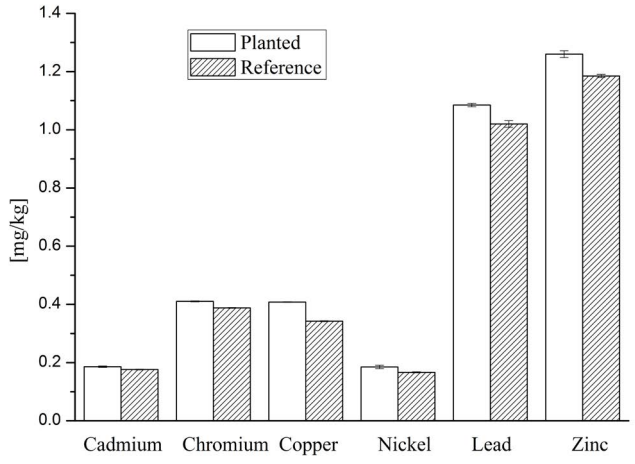

Fig. 6. Metals concentration on the sediment of planted and reference sections of the rhizofilter after 3 months of system establishment. Standard deviation whiskers represent the errors of means of three replicates.

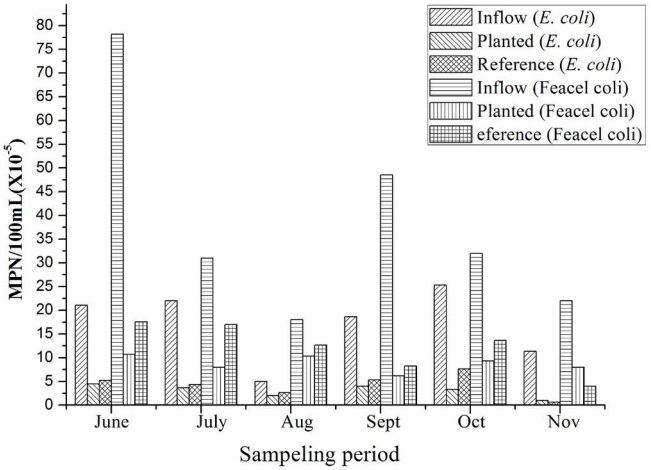

Fig. 8. Concentration of *E. coli* and faecal coliforms within the inflow, planted and reference sections over a 6-month period covering cold and warm seasons. Standard deviation whiskers represent the errors of means of three replicates.

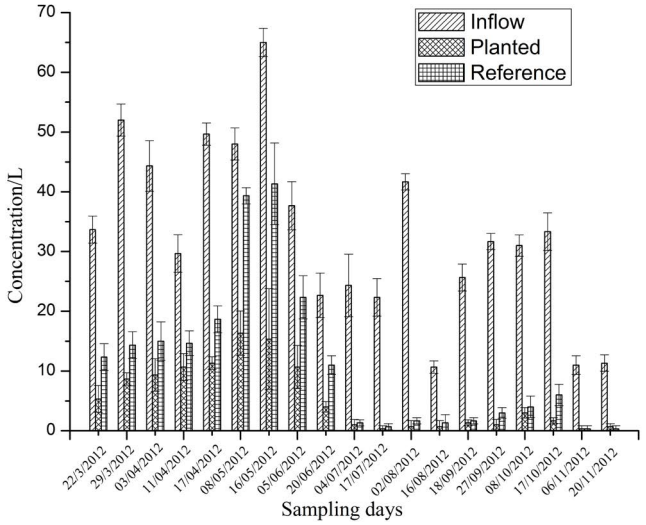

Fig. 7. Concentration and seasonal variations on ova of *Ascaris lumbricoides* at the inflow, planted and reference sections of the rhizofilter during the first year of study from March to November 2012. The variances were considered using ANOVA. Standard deviation whiskers represent the errors of means of three replicates.

of the macrophytes could have also stimulated the growth of some preying microorganisms within the rhizosphere [32].

4. Conclusion

The study demonstrated the potential of the rhizofilter in pollutant removal to some acceptable levels. This was initially achieved through constant monitoring of the functionality of the system in general pollutants removal. The effective functioning of the system is dependent on well-regulated flows and optimum macrophyte growth which was achieved at 80% cover within 3 months of system establishment. The physicochemical parameters were effectively reduced by the system proving the system had achieved optimum pollutant removal efficiency. There was an increase in pH from 6.90 ± 0.08 pH units to 7.53 ± 0.01 pH units and 7.23 ± 0.07 pH units in the planted and reference sections, respectively, was observed. There was a reduction in BOD and COD by 79% and 75%, respectively. Suspended solids were reduced by 86% in the planted section and 59.8% in the reference section. EC was reduced by 7.7% in the planted section and 0.83% in the reference section. Total dissolved solids were reduced by 11.5% in the planted section and 3.5% in the reference section while the temperature was reduced by 11.9% in the planted section and 1.2% in the reference section, while dissolved oxygen was raised by 10% in the planted section and 5% in the reference section. Turbidity was reduced by 9.7 NTU in the planted section and 9.1 NTU in the reference section, while alkalinity was reduced by 46.3% in planted and 45.5% in reference sections of the rhizofilter. Considering the entire rhizofilter, heavy metals were accumulated at varying concentrations in the planted and reference section of the rhizofilter. The entire rhizofiltration system was found to have an average pathogen removal ratio between 45% and 98% for the various pathogens detected in the influent wastewater. *Ascaris lumbricoides* was reduced by 77% in the planted section and 53% in the reference section. Cadmium levels also observed to be increased by 33% and by 21% on the root system of *K. nemoralis* which indicate the efficiency of system in removing heavy metals from the wastewater. A vertical flow system such as the one used in this study has the potential of functioning efficiently without minimal clogging challenges because the raw sewage is settled first before being allowed to flow into the system.

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References

[1] C.A. Odinga, F.M. Swalaha, F.A.O. Otieno, K.R. Ranjith, F. Bux, Investigating the efficiency of constructed wetlands in the removal of heavy metals and enteric pathogens from wastewater, Environ. Technol. Rev., 2 (2013) 1–16.

[2] M. Sundaravadivel, S. Vigneswaran, Constructed wetlands for wastewater treatment, Crit. Rev. Env. Sci. Technol., 31 (2001) 351–409.

[3] H. Ali, E. Khan, M.A. Sajad, Phytoremediation of heavy metals—concepts and applications, Chemosphere, 91 (2013) 869–881.

[4] L. Boutilier, R. Jamieson, R. Gordon, C. Lake, Modeling *E. coli* fate and transport in treatment wetlands using the water quality analysis and simulation program, J. Environ. Sci. Health., Part A, 46 (2011) 680–691.

[5] Q. Mahmood, A. Pervez, B.S. Zeb, H. Zaffar, H. Yaqoob, M. Waseem, Zahidullah, S. Afsheen, Natural treatment systems as sustainable ecotechnologies for the developing countries, Biomed Res. Int., 2013 (2013) 1–19.

[6] R. Krishna, M.H. Fulekar, B. Patakg, Rhizofiltration: a green technology for remediation of heavy metals, Int. J. Innov. Bio-Sciences, 2 (2012) 193–199.

[7] A. Mustafa, Constructed wetland for wastewater treatment and reuse: a case study of developing country, Int. J. Environ. Sci. Dev., 4 (2013) 20.

[8] I.A. Qureshi, A.R. Ghumman, H.N. Hashmi, Performance evaluation of constructed wetland for wastewater treatment, Life Sci. J., 10 (2013) 1139–1145.

[9] J. Garcia, D.P.L. Rousseau, J. Morato, E.L.S. Lesage, V. Matamoros, J.M. Bayona, Contaminant removal processes in subsurface-flow constructed wetlands: a review, Crit. Rev. Env. Sci. Technol., 40 (2010) 561–661.

[10] T.T. Vu, H. Kim, V.K. Tran, Q. Le Dang, H.T. Nguyen, H. Kim, I.S. Kim, G.J. Choi, J.-C. Kim, In vitro antibacterial activity of selected medicinal plants traditionally used in Vietnam against human pathogenic bacteria, BMC complement, Altern. Med., 16 (2015) 32.

[11] M. Parmar, L.S. Thakur, Heavy metal Cu, Ni and Zn: toxicity, health hazards and their removal techniques by low cost adsorbents: a short overview, Int. J. Plant Anim. Environ. Sci., 3 (2013) 2231–4490.

[12] M. Thayaparan, S.S. Iqbal, P.K.D. Chathuranga, M.C.M. Iqbal, Rhizofiltration of Pb by *Azolla pinnata*, Int. J. Environ. Sci., 3 (2013) 1811.

[13] A. Baysal, N. Ozbek, S. Akman, Determination of Trace Metals in Waste Water and Their Removal Processes, Waste Water - Treatment Technologies and Recent Analytical Developments, InTech Open, London, UK, 2013.

[14] A. Franco, L. Rufo, V. de la Fuente, Metal concentration and distribution in plant tissues of Nerium oleander (Apocynaceae, Plantae) from extremely acidic and less extremely acidic water courses in the Rio Tinto area (Huelva, Spain), Ecol. Eng., 47 (2012) 87–91.

[15] C. Chaidez, M. Soto-Beltran, C.P. Gerba, A.H. Tamimi, Reduction of risk of Salmonella infection from kitchen cleaning clothes by use of sodium hypochlorite disinfectant cleaner, Lett. Appl. Microbiol., 59 (2014) 487–492.

[16] M. Al-Jaboobi, M. Bouksaim, M. Tijane, S. El-Ariqi, Agricultural quality evaluation of wastewater, used in Yemen vegetables production, Middle East J. Sci. Res., 16 (2013) 667–677.

[17] N. Rani, P. Vajpayee, S. Bhatti, S. Singh, R. Shanker, K.C. Gupta, Quantification of Salmonella Typhi in water and sediments by molecular-beacon based qPCR, Ecotoxicol. Environ. Saf., 108 (2014) 58–64.

[18] J. Vymazal, Plants used in constructed wetlands with horizontal subsurface flow: a review, Hydrobiologia, 674 (2011) 133–156.

[19] G. Geller, Horizontal subsurface flow systems in the German speaking countries: summary of long-term scientific and practical experiences; recommendations, Water Sci. Technol., 35 (1997) 157–166.

[20] G.A. Di Luca, M.A. Maine, M.M. Mufarrege, H.R. Hadad, G.C. Sánchez, C.A. Bonetto, Metal retention and distribution in the sediment of a constructed wetland for industrial wastewater treatment, Ecol. Eng., 37 (2011) 1267–1275.

[21] G.D. Gikas, V.A. Tsihrintzis, A small-size vertical flow constructed wetland for on-site treatment of household wastewater, Ecol. Eng., 44 (2012) 337–343.

[22] A.D. Eaton, L.S. Clesceri, A.E. Greenberg, M.A.H. Franson, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Vol. 21, 2005, p. 1600.

[23] A. Hou, E.A. Laws, R.P. Gambrell, H.-S. Bae, M. Tan, R.D. Delaune, Y. Li, H. Roberts, Pathogen indicator microbes and heavy metals in Lake Pontchartrain following Hurricane Katrina, Environ. Sci. Technol., 40 (2006) 5904–5910.

[24] P.K. Rai, Heavy metal pollution in aquatic ecosystems and its phytoremediation using wetland plants: an ecosustainable approach, Int. J. Phytorem., 10 (2008) 133–160.

[25] S.V. Ravva, C.Z. Sarreal, R.E. Mandrell, Identification of protozoa in dairy lagoon wastewater that consume *Escherichia coli* O157: H7 preferentially, PLoS One, 5 (2010) e15671.

[26] C.S.C. Calheiros, A. Teixeira, C. Pires, A.R. Franco, A.F. Duque, L.F.C. Crispim, S.C. Moura, P.M.L. Castro, Bacterial community dynamics in horizontal flow constructed wetlands with different plants for high salinity industrial wastewater polishing, Water Res., 44 (2010) 5032–5038.

[27] S.G. Abdelhakeem, S.A. Aboulroos, M.M. Kamel, Performance of a vertical subsurface flow constructed wetland under different operational conditions, J. Adv. Res., 7 (2016) 803–814.

[28] U. Stottmeister, A. Wießner, P. Kuschk, U. Kappelmeyer, M. Kästner, O. Bederski, R.A. Müller, H. Moormann, Effects of plants and microorganisms in constructed wetlands for wastewater treatment, Biotechnol. Adv., 22 (2003) 93–117.

[29] O. Shelef, A. Gross, S. Rachmilevitch, The use of Bassia indica for salt phytoremediation in constructed wetlands, Water Res., 46 (2012) 3967–3976.

[30] M.H. Sayadi, R. Kargar, M.R. Doosti, H. Salehi, Hybrid constructed wetlands for wastewater treatment: a worldwide review, Proc. Int. Acad. Ecol. Environ. Sci., 2 (2012) 204.

[31] D. Singh, A. Tiwari, R. Gupta, Phytoremediation of lead from wastewater using aquatic plants, J. Agric. Technol., 8 (2012) 1–11.

[32] S.Y. Lee, M.C. Maniquiz, J.Y. Choi, S.M. Jeong, L.H. Kim, Seasonal nutrient uptake of plant biomass in a constructed wetland treating piggery wastewater effluent, Water Sci. Technol., 67 (2013) 1317–1323.

[33] O.S. Adefemi, E.E. Awokunmi, Uptake of heavy metals by tomato (*Lycopersicum esculentus*) grown on soil collected from dumpsites in Ekiti State, South West, Nigeria, Int. J. Chem., 5 (2013) 70.

Supplementary information

Table S1
Variations in flow rates at the various sampling points within the rhizofilter

Sampling taps	Site	Flow rate (L min^{-1})	Flow rate (L min^{-1})
A	Planted section	0.551331	0.017011
B		0.782616	0.019686
D		0.490782	0.009909
E		0.394296	0.009026
F	Reference section	0.712971	0.0068
G		0.528174	0.015379
I		0.673334	0.018073
J		0.871006	0.003293

S1. Method

S1.1. Methods for metals detection

S1.1.1. Preparation of water samples for analysis

Triplicate samples were filtered through 0.45 μm Whatman No. 1 filter paper for total dissolved metals and acidified using 1:1 ratio of concentrated trace-metal grade nitric acid to pH of 2 [23]. A portion of the sample (45 mL) was carefully transferred into the microwave Teflon tube (Milestone START D). Each Teflon tube was covered with a safety shield. A 3 mL sample of reagent grade HNO_3 (65%) and 2 mL of HCl was carefully added to the reaction mixture in a fume cupboard. The solutions were carefully mixed to ensure homogeneity and digested for 30 min. using a microwave digester system (Milestone START D) equipped with a revolving carousel with a capacity of 12, 100 mL type quartz tubes to a maximum temperature of 165°C ± 5°C. The reaction mixture was evaporated on a hot plate and the residue diluted to 50 mL using double distilled water. The combination of the two strong acids is recommended since the wastewater sample is composed of readily oxidizable organic matter. Blank samples were prepared with each batch and treated in the same manner as the experimental samples. Metal analysis was done on the clear supernatant using the inductively coupled plasma-optical emission spectroscopy (ICP-OES) according to a method by Luca et al. [20].

S1.2. Preparation of plant material for analysis

The specimens were washed with distilled water to remove attached soil particles and placed in a sieve to drain out excess water. The plant specimens were further immersed in a solution of HCl (0.01 M) in order to remove any exterior metal deposits. The material was then rinsed with double

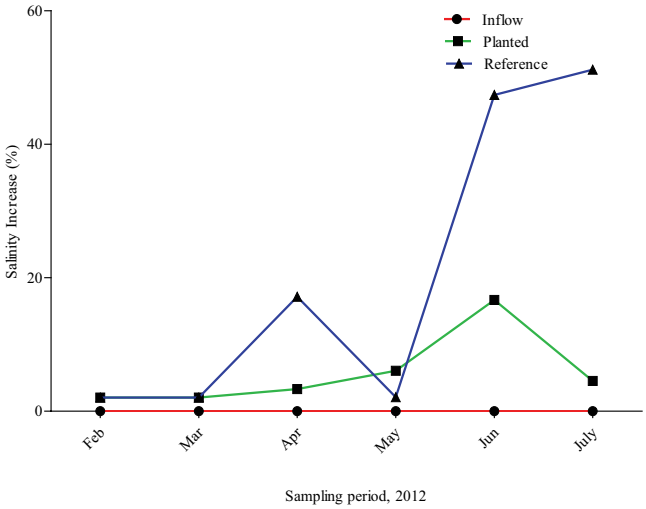

Fig. S2. Variations (%) in salinity at the inflow, planted and reference sections covering both warm (February – April) and cold (May – August) seasons during the study period.

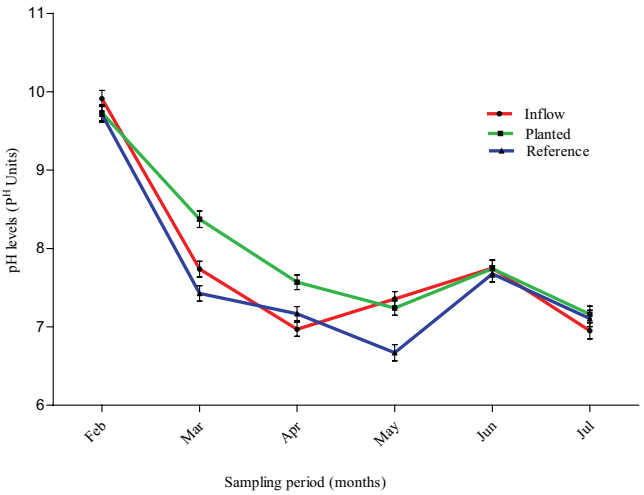

Fig. S1. Temporal mean pH variations covering hot and cold seasons during the study. Whiskers represent standard deviations (n = 3).

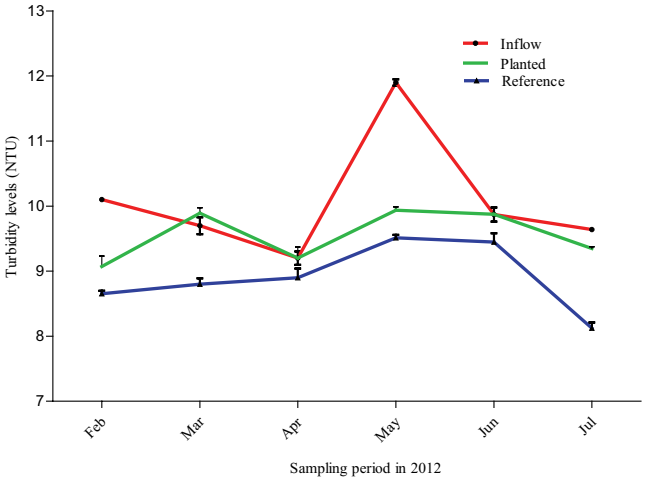

Fig. S3. Variations in turbidity covering both warm (February – April) and cold (May – August) seasons in Durban during the study period. The standard deviation whiskers represent the errors of means.

distilled water for about 1 min. The material was then partitioned into leaves, stems and roots and then shredded using a pair of scissors and secateurs. The sample was air dried to remove excess water and then dried at 80°C for 24 h. The material was blended to a fine powder using a Milestone start D Mellerware 50 g capacity blender.

The homogenized dried plant material (0.3 g) was weighed in triplicate and placed in a microwave Teflon tube. The tube was introduced into the Teflon safety shield and 8 mL of HNO_3 (65%) followed by 2 mL of H_2O_2 (30%) were carefully added and mixed in a fume cupboard. The solution was gently swirled to ensure homogeneity. The Teflon tube was tightly closed and placed in the microwave (Milestone START D) cavity and digested for 35 min at 180°C. The residue was evaporated on a hot plate at 50°C and diluted using double distilled water to a volume of 50 mL. The metal content was determined using inductively coupled plasma optical emission spectrometer (ICP-OES) as described by Rai.