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Wastewater reuse in agriculture: the effect of macrophyte-assisted vermifiltration treatment on seed germination and seedling development

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ABSTRACT

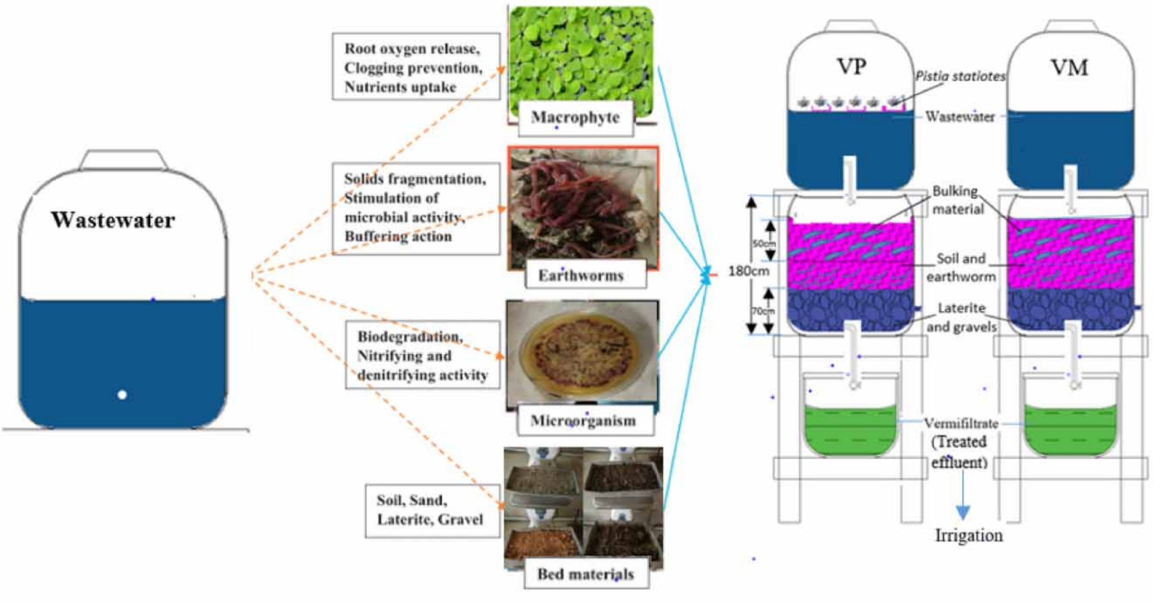
There is a growing need to reclaim wastewater for agricultural use due to freshwater limitation. Integrating macrophytes in vermifiltration improves the treatment efficiency. However, the effect of treated wastewater on seed germination and seedling development is not widely investigated. The study investigated the use of *Pistia stratiotes* in a macrophyte-assisted vermifiltration of domestic wastewater and assessed the effect on seed and seedling development of *Zea mays*, *Triticum aestivum* and *Sorghum bicolor*. Three irrigation treatments of water were applied: macrophyte-assisted vermifiltration (VP), no macrophyte vermifiltration (VM) and potable water (PW) as the control. Results showed that VP had a removal of 41–44% EC, 65–67% turbidity, 52–65% TDS, 67–70% TSS, 29–34% COD, 42–46% BOD, 67–70% N total, and 74–78% P available compared to VM. VP treatment reduced inhibition in the morphological, physiological and biochemical developments of seed and seedling growth. The use of macrophyte-vermifiltered wastewater significantly ($p < 0.05$) increased the percentage of germination and the radical length of all seed species increased as well. In terms of the seedling development, seedling mortalities were significantly ($p < 0.05$) reduced and more than 75% chlorophyll pigments estimations (C_{α}, C_6, $C_{\alpha+6}$ and C_{x+c}) were identified in all seedling species when macrophyte-vermifiltered wastewater was used to irrigate.

Key words: *Eisenia fetida*, *Pistia stratiotes*, seed germination, seedling development, vermifiltration, wastewater

HIGHLIGHTS

- Vermifiltration of domestic wastewater integrated with macrophytes proved effective.
- *Pistia stratiote* was suitable as a macrophyte in the water treatment process.
- Treated wastewater indicated a potential water source for irrigation.
- Water treatment reduced inhibition in seed growth and development.
- Macrophyte-assisted vermifiltration of domestic wastewater revealed a sustainable domestic wastewater treatment technology.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Freshwater scarcity is a problem reaching global alarming proportions and will become one of the most sensitive environmental issues in coming decades (Bhavini *et al.* 2020). This is because freshwater is a vital resource in sustaining both ecosystem health and human survival. Enormous generation of wastewater from rapid population growth, urbanization and industrialization contributes to this freshwater scarcity and depletion (Bayart *et al.* 2010). Sustainable wastewater management and technologies are therefore needed to reclaim wastewater for reuse (Anda & Shear 2021). Aside from water shortage caused by wastewater generation, pollutants in wastewater affect the quality of water bodies through complex mechanisms occurring concurrently when disposed of indiscriminately (Pfister *et al.* 2009). Disposal of wastewater without treatment releases biochemical oxygen demand (BOD), chemical oxygen demand (COD) and nutrients that cause dissolved oxygen (DO) depletion and eutrophication (Zheng *et al.* 2013). This is a contributing factor to the worldwide water quality degradation where most of the water bodies are now being contaminated with a heavy load of pollutants (Samal *et al.* 2019). Indiscriminate disposal of wastewater without treatment also causes deterioration of water bodies' ecology along with the loss of freshwater sources, making freshwater sources less available (Nsiah-Gyambibi *et al.* 2021). Agriculture is one of the sectors that is largely affected by freshwater scarcity (Tahiru *et al.* 2015). Due to freshwater scarcity, more than 50% of rural households in Sub-Saharan Africa (SSA) engage in traditional rain-fed agriculture (Scheiterle & Birner 2018). Production of cereals, such as maize, wheat and sorghum, which are major staple foods in Ghana, largely depends on rain-fed conditions due to water inaccessibility (Adu *et al.* 2021). Agricultural production depending on rain-fed conditions is unreliable and results in low outputs due to erratic rainfall exacerbated by climate change (Maaloul *et al.* 2019). This is a threat to food security (Tahiru *et al.* 2015). Therefore, one proposed approach is to reclaim and reuse wastewater generated from households and other sources after giving a certain level of treatment. However, this practice of reclaiming and reusing wastewater in agriculture has some drawbacks especially when the treatment method is expensive and inefficient (Zaied & Cheikh 2014). Recently, an international consortium reported that to be environmentally and economically sustainable, wastewater reclamation methods for agricultural usage should be eco-friendly and economical in ensuring high-quality treatment to remove pollutants that may have adverse impacts on plant productivity (Rekik *et al.* 2016). Plant productivity begins with seed germination, which depends largely on water quality (Chaabene *et al.* 2015; Pedrero *et al.* 2012). One approach to this wastewater reclamation is vermifiltration. Studies have identified vermifiltration as an economical and sustainable alternative wastewater reclamation technique (Sinha *et al.* 2008; Jiang *et al.* 2016; Singh *et al.* 2017). Vermifiltration is an aerobic treatment process that integrates earthworms in wastewater filtration process (Khwairakpam 2020). Vermifiltration comprises an earthworm active zone along with filter media bed that supports a microbial community. The vermifiltration technique combines the activities of earthworms and microbes. Wastewater passes through an initial layer where organic matter in the form of residues gets captured and is converted into humus-like material known as vermicompost by the earthworms (Singh *et al.* 2017). The earthworms do this through a biological transformation by passing the organic material through the digestive canal and breaking it down into smaller pieces allowing bacteria and fungi to feed on it to release nutrients (Luth 2011). In this case, the earthworms work as ecological engineers and the ecological engineering function of earthworms is used to reduce the pollution's effects. This is followed by a filtration through a filter media that supports biofilm growth leading to the degradation of contaminants such as BOD, COD and nitrogen derivatives (Samal *et al.* 2017b). *Eisenia fetida* is one of the common earthworm species employed in vermifiltration (Gunadi *et al.* 2002; Hughes *et al.* 2009). There is considerable evidence that contaminants are removed through vermifiltration as reclaimed wastewater gets stabilized for potential reuse (Nsiah-Gyambibi *et al.* 2022). During vermifiltration, the solids that are retained on filter beds are consumed by the earthworms and converted into the humus. The oxygen level is increased in the filter bed by the burrowing action of earthworms (Sinha *et al.* 2008; Jiang *et al.* 2016; Singh *et al.* 2018). Nowadays, researchers are focusing on integrating macrophytes in the vermifiltration system to improve the wastewater treatment efficiency and this is mostly known as a macrophyte-assisted vermifiltration system (MAV) (Chen *et al.* 2016). Morand *et al.* (2005) identified that integrating macrophytes to vermifiltration system is a further treatment that improves the treated wastewater quality. Bojcevska & Tonderski (2007) also reported that integrating macrophytes into the vermifiltration system as a further treatment increases not only nutrient reduction and biomass production, but also retention time to efficiently remove pollutants. Several previous studies are available on either utilization of vermifiltration or only macrophyte filtration system for removal of pollutants from wastewaters, but very few reports are available on utilizing the potentials of both systems to develop an effective integrated system (Samal *et al.* 2017b). Wider studies are needed to demonstrate the efficiency of MAV in treating wastewater and the effect of MAV effluents on seed germination and seedling development. *Pistia stratiotes* are free-floating aquatic macrophytes that are commonly used in a floating bed for pollutant removal in wastewater treatment systems (Samal *et al.* 2019). Xu *et al.* (2021) found *Pistia stratiotes* to be effective in removing large amounts of nutrients and pollutants in a wastewater treatment system. Therefore, the main aim of this study sought to investigate the use of *P. stratiotes* as macrophytes in a vermifiltration system. As an objective, the study further investigated the potential influence of the treated MAV wastewater as a source of irrigation on the morphological, physiological and biochemical developments of *Zea mays, Triticum aestivum* and *Sorghum bicolor* seed germination and seedling development.

2. METHODS AND MATERIALS

2.1. Study location

The study was carried out at the Environmental Engineering Laboratory, Department of Civil Engineering at Kwame Nkrumah University of Science and Technology in Kumasi. Kumasi is a city in the Ashanti region of Ghana with a tropical forest belt between latitude 6.400 and 6.350 N and longitude 1.30 and 1.35 W. Kumasi is at 250–399 m above sea level with an average ambient temperature of 25–28 °C, which is the optimum temperature range for earthworm species (Tripathi & Bhardwaj 2004). The experiments were performed in the months of April–June.

2.2. The source of the wastewater and experimental setup

Domestic wastewater was sampled from a household septic tank within the Oforikrom sub-metro in Kumasi Metropolitan Assembly (KMA) located in Ghana. Two vermifiltration experimental setups were constructed labelled VP and VM. VP had a macrophyte, *P. stratiotes*, commonly known as water lettuce, planted in the influent collection chamber while VM had no macrophyte as shown in Figure 1. *P. stratiotes* was used as the macrophyte primarily on availability and economic uses. *P. stratiotes* was obtained from a stream and cultured in tap water for 24 hours. About 0.25 m^2 patches of cultured *P. stratiotes* were stocked in the influent collection container of VP. The influent collection chamber and the filtration setup were constructed with 60.0 cm^3 polyethylene terephthalate barrels. The filtration setup consisted of filter bed material, earthworms, wastewater distributor and drain system. The filter bed had three layers. The top layer (50 cm thick) was made up of coconut coir (6–8 mm) as a bulking material with an empty space of 5 cm at the top for aeration purpose. The middle layer (55 cm thick) consisted of sand (1–2 mm, 10 cm thick), gravels (6–8 mm) and mature vermicompost. This active

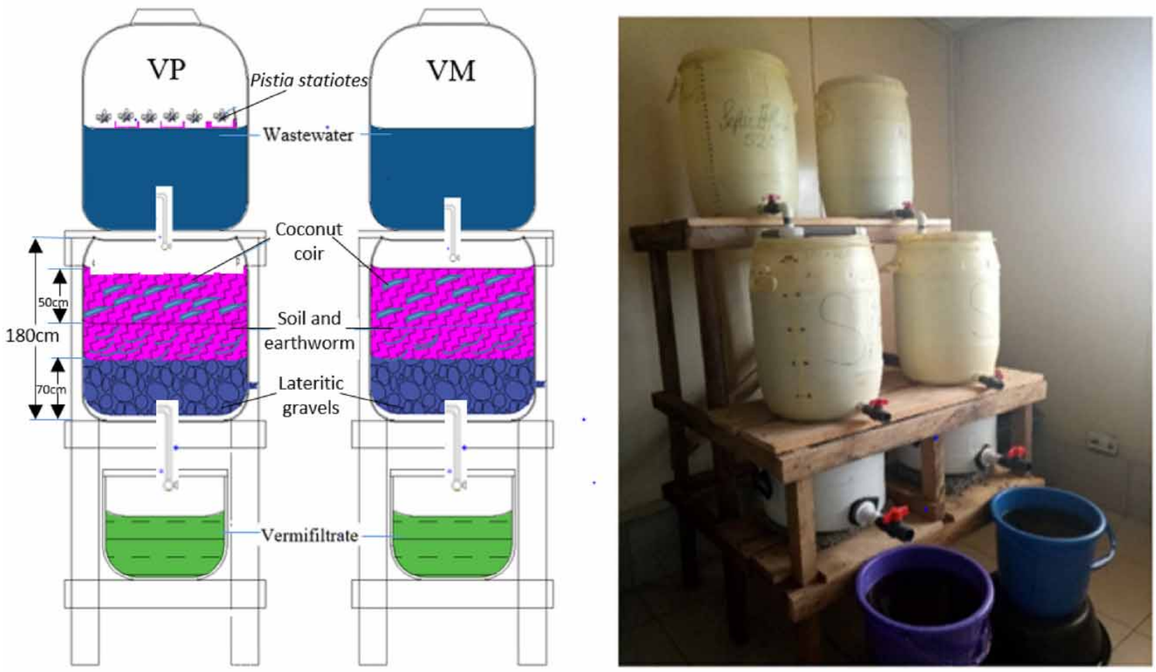


Figure 1 | Vermifiltration experimental setups.

layer was the earthworm packing bed where four hundred and fifty (live weight ~255–275 mg) clitellated earthworms species were added following the stocking density calculations used by Khwairakpam 2020. The earthworm species used was *E. fetida* and they were obtained from a breeding stock cultured in the laboratory at a temperature of 25 °C. The bottom-most supporting layer consisted of coarse layer of lateritic hardpan gravels (12–14 mm, 15 cm thick).

The experimental setups were allowed to acclimatize for seven days before the experiments. After the stabilization phase, they were allowed to run for 5 weeks continuously to get enough treated water for the irrigation experiment. Wastewater was pumped using a 0.5HP single stage laboratory vacuum pump and distributed over the top of filter bed at a constant hydraulic loading rate (HLR) of $0.339\,\mathrm{m^3\,m^{-2}\,d^{-1}}$. Infiltration of wastewater in the filtration setups were by gravitational flow in a vertical flow system (VFS) through a shower head of 1–2 mm perforations for its uniform distribution. Treated water was collected at the bottom and stored in clean containers for seed irrigation. Samples of raw wastewater (influent) and treated water (effluent) were analysed for physico-chemical parameters.

The physico-chemical parameters analysed were pH, electrical conductivity (EC), turbidity, total dissolved solids (TDS), DO, TSS, COD, BOD, total nitrogen (N_{tot}) and phosphorus (P_{avail}) on samples. pH, EC, turbidity, TDS, and DO were measured using Palintest multi portable meter. TSS of samples were determined following the standard protocol of filtration and gravimetric oven drying method (APHA 2005). COD measurements were made using high-range ampoules (HACH Chemical) with a spectrophotometer (HACH, DR5000) (Hur *et al.* 2010). BOD was determined following the Lovibond Water Testing protocol of BOD-System BD 600 method. N_{tot} was measured by Persulfate Digestion Method 10072 of the TNT protocol. P_{avail} was determined using the Molybdovanadate with Acid Persulfate Digestion Hach Method 10127 (Hach Company, Loveland, Colorado, USA).

2.3. Seed germination and growth

Garden soil, obtained from a vegetable farm, was used in pots for seed germination. Seeds of *Zea mays, Triticum aestivum* and *Sorghum bicolor* were obtained from the CSIR-Crops Research Institute. Seeds were prepared by having their surface disinfected using 2% sodium hypochlorite for 10.0 min and then thoroughly washed using chlorhydric acid 10 − 3 normal solution for two 5-minute times to avoid fungal contaminations. The seeds were cleaned by rinsing with sterile distilled water. Uniform sized disinfected seeds were transferred into pots containing agricultural soils and grown in a greenhouse at 25.0 °C with a 16-h daily photoperiod (under continuous light conditions (230 μmol m^{-2} s^{-1})) and 8 h of dark. Three sources of irrigation water were used to irrigate pots. The three treatments include macrophtye-assisted vermifiltered wastewater (VP), no macrophyte vermifiltered wastewater (VM) and potable water (PW) as the control. Seeds were watered every 24 hours and parameters were monitored for a 15-day germinating period to get a wide assessment of treatments on the development of seedlings. Treatments were in triplicates and a seed was considered to be successfully germinated if the root system was visible and measurable (at least 1.0 mm). Sampling on seed germination, morphological, physiological and biochemical growth attributes were assessed. Seed germination was assessed using the following germination indices: germination potential, germination speed, mean germination time, mean daily germination, peak value of germination and seedling mortality. Germination indices were estimated using Equations (1)–(6).

Germination potential (GP) was estimated using Equation (1)

$$GP = \left(\frac{\text{Germinated seeds}}{\text{Total seeds}}\right) \times 100 \tag{1}$$

Germination speed (GS) was estimated using Equation (2)

$$GS = \frac{n1}{d1} + \frac{n2}{d2} + \frac{n3}{d3} + \cdots \tag{2}$$

where, n = number of germinated seeds, d = number of days.

Mean germination time (MGT) was estimated using Equation (3)

$$MGT = \frac{\text{n1} \times \text{d1} + \text{n2} \times \text{d2} + \text{n3} \times \text{d3} + \ldots}{\text{total number of days}} \tag{3}$$

where, n = number of germinated seeds, d = number of days.

Mean daily germination (MDG) was estimated using Equation (4)

$$MDG = \frac{\text{Total number of germinated seeds}}{\text{total number of days}} \tag{4}$$

Peak value of germination (PV) was estimated using Equation (5)

$$PV = \frac{\text{Highest seed germinated}}{\text{number of days}} \tag{5}$$

Seedling mortality (SM) was estimated using Equation (6)

$$SM = \frac{\text{Number of non} - \text{germinated seeds}}{\text{number of days}} \times 100 \tag{6}$$

Parameters used in measuring morphological development included root length (cm), shoot length (cm), number of leaves per plant, the number of secondary roots and fresh and dry weight of roots and shoots of seedlings. The shoot and root weight were determined by using an electronic balance. Root length and root area were determined using the Root Law Software Program (Washington State Research Foundation USA). Physiological development was estimated using photosynthetic pigments (chlorophyll ɑ ($C_ɑ$), chlorophyll ϐ ($C_ϐ$), total chlorophyll ($C_{ɑ+ϐ}$) and total carotenoids (C_{x+c})), total soluble phenolics content. Estimation of photosynthetic pigments was carried out by the method as given by Heath & Packer (1968). The contents of chlorophyll ɑ ($C_ɑ$), chlorophyll ϐ ($C_ϐ$), total chlorophyll ($C_{ɑ+ϐ}$) and total carotenoids (C_{x+c}) were determined in a whole–pigment extract of green plant tissue by UV–vis spectroscopy. After extraction of samples by 96% ethanol, absorbance was measured at 470 (A470), 663 (A663) and 645 (A645) nm. Chlorophyll and carotenoid contents were estimated using the Equations (7)–(10). The method described by Wolfe *et al.* (2006) was used for the extraction and quantification of total soluble phenolics content in leaves of seedlings. The total soluble phenolics content was expressed as μg gallic acid eq./gram leaf tissue.

$$C_{\mathrm{a}} = 12.70\ \mathrm{A663} - 2.69\ \mathrm{A645} \tag{7}$$

$$C_{\mathrm{b}} = 22.90\ \mathrm{A645} - 4.68\ \mathrm{A663} \tag{8}$$

$$C_{\mathrm{a+b}} = 20.21\ \mathrm{A645} + 8.02\ \mathrm{A663} \tag{9}$$

$$C_{x+c} = \frac{(1000\ \mathrm{A470} - 1.9\ \mathrm{Ca} - 63.14\ \mathrm{Cb})}{214} \tag{10}$$

The parameters used in assessing the biochemical development were hydrogen peroxide (H_2O_2) concentration, total soluble proteins, amylase activity, lipid peroxidation levels, catalase enzyme concentration and superoxide dismutase enzyme concentration. H_2O_2 concentration was estimated using the protocol described by Cheeseman (2006). H_2O_2 concentration was expressed as μmol g^{-1} fresh weight. Soluble protein content was estimated according to Chaabene *et al.* (2015). Briefly, 0.5 g fresh tissue samples were harvested and homogenized using a mortar and a pestle in 1.5 ml 0.1 M potassium phosphate buffer, pH 7.0. After centrifugation of the homogenate at 13,000.0 g and 4.0 °C for 15.0 min, concentration of supernatant soluble proteins was determined spectrophotometrically. Amylase activity was assayed according to Miller (1959). Reaction mixture contained 1% w/v starch, 100 mM phosphate buffer pH 7.0 and protein sample extract in a final volume of 1.0 ml. The mixture was then incubated at 37.0 °C and pH 7.0 for 10.0 min. Reducing sugars released by the action of putative amylase activity on starch are measured using the dinitrosalycylic acid (DNS) method. The amount of enzyme capable to produce 1.0 μmol glucose min^{-1} reducing sugar equivalent was set to represent one unit of amylase activity in our experimental conditions. Lipid peroxidation was analysed by the procedure of Heath & Packer (1968). Fresh vegetal material (roots and leaves) was homogenized using 5.0 mL TCA (0.1% v/v) per 1.0 g fresh plant material. After centrifugation at 10,000 g for 15 min at 4 °C, the supernatant was added to 4 mL premixed TCA (20%) and TBA (2–thiobarbutiric acid, 0.5%). The homogenate was then incubated at 95.0 °C for 30.0 min and the reaction stopped by incubation on ice. After 15 min centrifugation at 10,000 g, the absorbance of the sample was measured at 532 nm.

2.4. Statistical analysis

One- way ANOVA was used to analyse the data and when significant effects were detected, the groups were compared using a post-hoc Tukey's HSD test. The level of significance used for all statistical tests is 5% ($p < 0.05$).

3. RESULTS

3.1. Physico-chemical changes of wastewater after vermifiltration

pH of the wastewater was observed to be acidic with significant ($p < 0.05$) high concentrations of EC, turbidity, TD, TSS, COD, BOD, N_{tot} and P_{avail} compared to the portable water which was used as a control (Table 1). Vermifiltration of wastewater increased the pH to 7.2–7.4 demonstrating the natural buffering capacity of earthworms during vermifiltration as observed in previous studies (Wang *et al.* 2010; Furlong *et al.* 2014; Singh *et al.* 2018). Vermifiltration of the wastewater also accounted for the following % reductions; 41–44% EC, 65–67% turbidity, 52–65% TDS, 67–70% TSS, 29–34% COD, 42–46% BOD, 67–70% N_{tot}, and 74–78% P_{avail} in VP effluents and 39–42% EC, 62–64% turbidity, 52–65% TDS, 65–68% TSS, 25–27% COD, 41–44% BOD, 60–64% N_{tot}, and 54–58% P_{avail} in VM effluents. DO however increased in VP (600%) and VM (500%).

3.2. Effects of vermifiltrates on seeds germination in *Z. mays, T. aestivum* and *S. bicolor*

3.2.1. Morphological effects

Alterations in the morphological development on seed germination in *Z. mays*, *T. aestivum* and *S. bicolor* seeds irrigated with irrigation water treatments were recorded in terms of percentage of germination and radicle length (Figure 2). Seeds irrigated with PW demonstrated the highest germination percentage in all germination days compared to VP and VM (Figure 2(a)–2(c)). Germination percentage in PW seeds were significantly higher than VP and VM except with few exceptions (*Z. mays* day 7, day 9 and day 13; *T. aestivum* day 1, day 5 and day 13; *S. bicolor* day 1, day 3 and day 15). In terms of the two vermifiltered wastewater treatments, VP effect on *Z. mays* was not significantly different ($p > 0.05$) from VM except on day 5, 13 and 15 (Figure 2(a)). *T. aestivum* and *S. bicolor* showed different patterns (Figure 2(b) and 2(c) respectively), whereas in *T. aestivum*, VP

Table 1 | Physicochemical characteristics of wastewater, VP, VM and PW of physicochemical parameters for irrigation water and abundance in plant tissues, $n = 3$

Parameters	Wastewater	VP	VM	PW
pH	6.6–6.9	7.2–7.4	7.2–7.4	6.8–7.2
EC (ms cm^{-1})	7.50 ± 1.60a	4.21 ± 1.10b	4.50 ± 0.80b	0.15 ± 1.30c
Turbidity (NTU)	102.20 ± 4.50a	33.30 ± 4.10b	37.25 ± 3.70c	10.30 ± 3.20d
TDS (mg l^{-1})	1,340.40 ± 56.42a	424.30 ± 20.50b	466.20 ± 30.10c	680.20 ± 8.10d
DO (mg l^{-1})	0.20 ± 0.04a	1.40 ± 0.20b	1.20 ± 0.22b	3.60 ± 0.50c
TSS (mg l^{-1})	106.60 ± 4.20a	37.50 ± 2.50b	43.30 ± 1.10c	<1
COD (mg l^{-1})	236.30 ± 10.40a	166.13 ± 2.20b	176.40 ± 3.25c	<10
BOD (mg l^{-1})	120.40 ± 8.50a	62.17 ± 7.50b	67.30 ± 10.20b	Undetected
N_{tot} (mg l^{-1})	41.40 ± 5.20a	12.30 ± 1.60b	16.10 ± 2.40c	1.44 ± 0.80d
P_{avail} (mg l^{-1})	36.70 ± 1.50a	8.40 ± 1.50b	15.21 ± 2.10c	0.30 ± 1.20d

Data presents mean ± standard error. Values with different letters are significantly different among the treatments at $P < 0.05$ using the Tukey's HSD test at $p < 0.05$.

germination percentage were significantly higher ($p < 0.05$) than VM in all germination days except day 1 and 11, in *S. bicolor*, VP germination percentage were significantly higher ($p < 0.05$) than VM in all germination days except day 1, 3 and 15. Changes in the radical length was used to assess the impact of irrigation treatments on seeds vigor during germination as shown in Figure 2(d)–2(f). In *Zea mays* (Figure 2(d)), treatments demonstrated significant differences ($p < 0.05$) in radical length where PW had the longest length followed by VP and VM, except in day 3. In *T. aestivum* (Figure 2(e)), there was no significant difference ($p > 0.05$) in radical length between treatments on day 1, 3, 5 and 15. *T. aestivum* radical length of PW seedlings were not significantly different ($p > 0.05$) from VP (except day 7, 9 and 13) and VM (except day 7, 9, 11 and 13). *S. bicolor* showed similar seedling vigor in radical length (Figure F) compared with *T. aestivum* with some few exceptions (VP showed significant differences ($p < 0.05$) compared with VM in day 7, 11 and 13).

3.2.2. Physiological effects

Physiological transformation data revealed that PW recorded the highest significant seed germination in all seeds compared to VP and VM (Figure 3(a)–3(c)). Although VP recorded a higher speed germination compared to VM in all seeds, the difference was not significant ($p > 0.05$). The order of mean germination time in all seeds were PW > V*P* > VM, where PW recorded no significant difference ($p > 0.05$) with VP but was significantly higher ($p < 0.05$) than VM except in *Z. mays* (Figure 3(a)). The mean daily germination followed similar pattern in the order PW > V*P* > VM with significances occurring only in *S. bicolor* (Figure 3(c)). The treatment effect on peak value was also in the order PW > VP > VM with no significant differences ($p > 0.05$) in all seeds. In terms of seedling mortality, PW demonstrated the least and was significantly lower ($p < 0.05$) that VP and VM in all seeds. VP however did not show any significant difference ($p > 0.05$) in seedling mortality compared with VM in all seeds.

3.2.3. Biochemical effects

3.2.3.1. Soluble protein content. The use of vermifiltrates in seed irrigation reduced soluble protein content compared to the control (Figure 4(a)–4(c)). The control PW recorded the most soluble protein content significantly different ($p < 0.05$) from VP (except *Z. mays* day 5; *T. aestivum* day 5; *S. bicolor* day 0 and 3) and VM (except *Z. mays* day 0 and 5; *T. aestivum* day 3; *S. bicolor* day 0). Soluble protein content in VP treatments was significantly higher ($p < 0.05$) than VM in all seeds except *Z. mays* day 1, 4 and 5; *T. aestivum* day 1 and 2; *S. bicolor* day 0, 1, 4 and 5.

3.2.3.2. H_2O_2 levels. H_2O_2 levels were higher in germinating seeds when irrigated with vermifiltered wastewater compared to the portable water (Figure 4(d)–4(f)). In *Z. mays*, except day 0, 2 and 3, H_2O_2 were significantly higher ($p < 0.05$) in VP compared to VM (Figure 4(d)). *T. aestivum* revealed a different trend where there was no significant differences ($p > 0.05$) in H_2O_2 among the three irrigation treatments occurring only on day 2

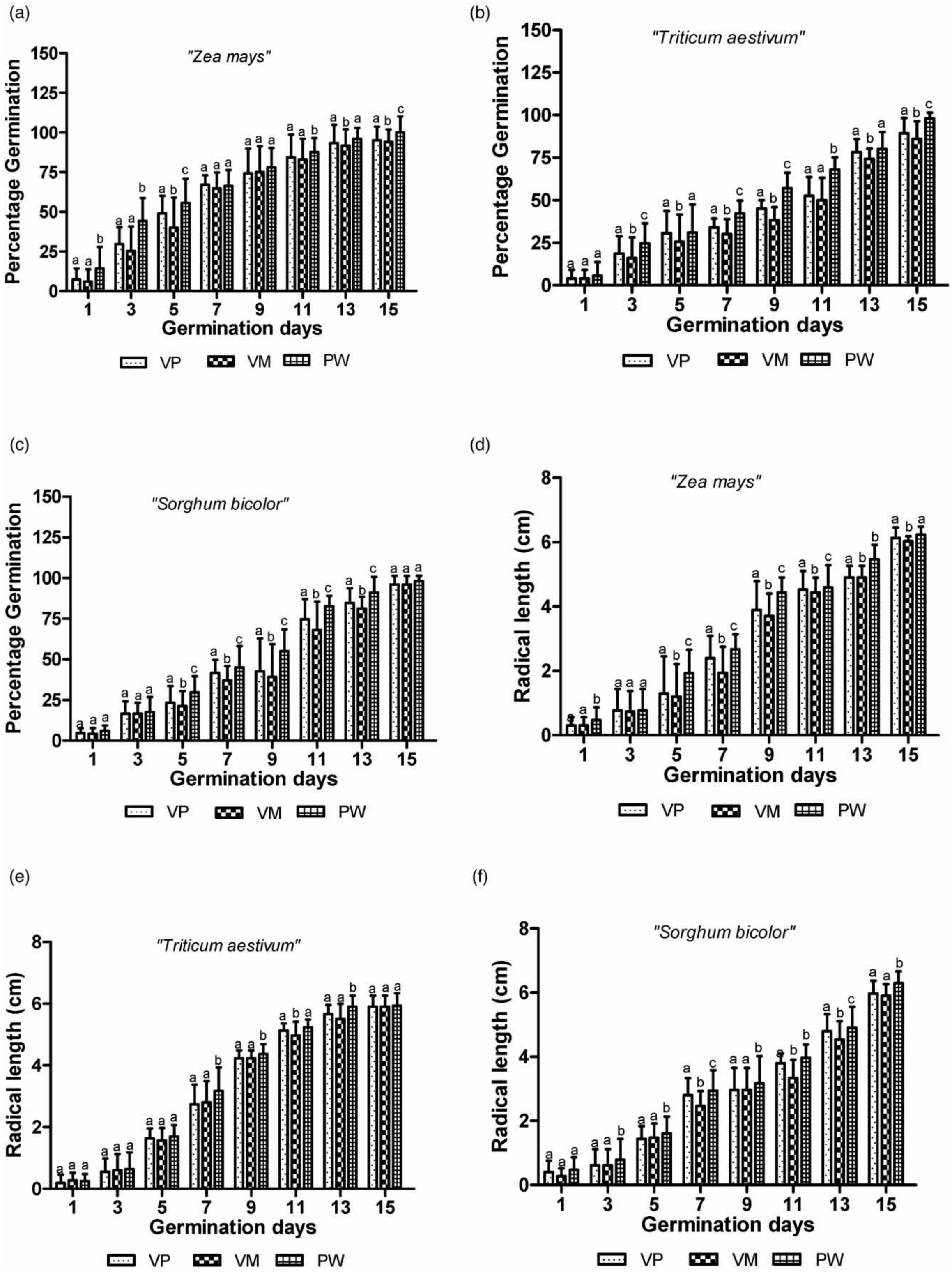


Figure 2 | Percentage of germination and radicle length of *Z. mays* (a and d, respectively), *T. aestivum* (b and e, respectively) and *S. bicolor* (c and f, respectively) irrigated with VP, VM and control (PW). Data presents mean ± standard error. Bars labelled with different letters are significantly different among the treatments at $P < 0.05$ using the Tukey's HSD test. In each bar groups, bars labelled with the same letter are not significantly different from each other according to Tukey's HSD at $p < 0.05$.

(Figure 4(e)). In *S. bicolor*, H_2O_2 in VP was not significantly different ($p > 0.05$) from VM except on day 4 and 5 (Figure 4(f)). VP however showed no significant difference with PW in all sampling days after day 0 ($p < 0.05$).

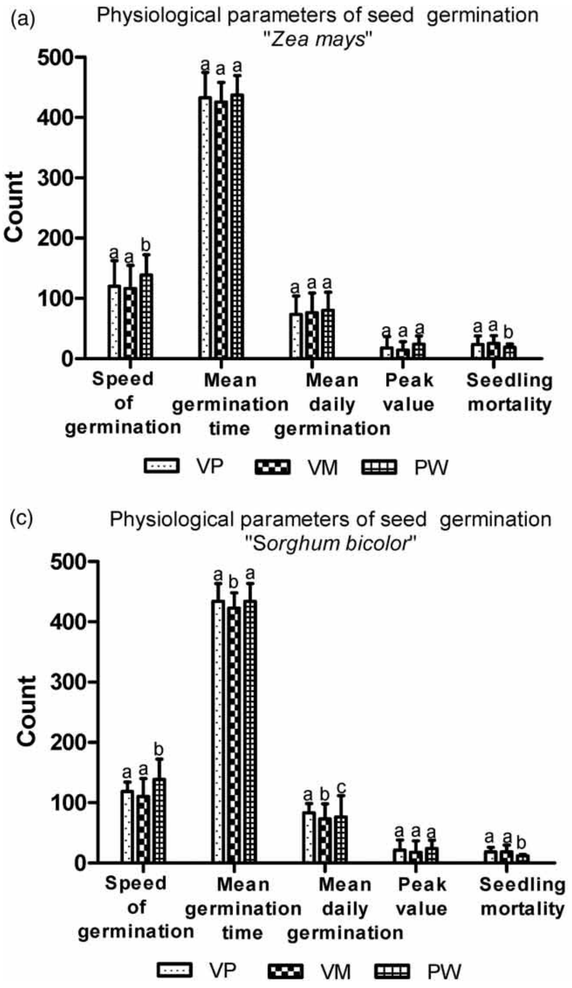


Figure 3 | Physiological parameters of seed germination of *Z. mays* (a), *T. aestivum* (b) *S. bicolor* (c) irrigated with VP, VM and control (PW). Data presents mean ± standard error. Bars labelled with different letters are significantly different among the treatments at $P < 0.05$ using the Tukey's HSD test. In each bar groups, bars labelled with the same letter are not significantly different from each other according to Tukey's HSD at $p < 0.05$.

3.2.3.3. Amylase activity. Amylase activity in seeds decreased with germinating days as shown in Figure 4(g)–4(j). Amylase activity in *Z. mays* showed no significant difference ($p > 0.05$) in VP treatments compared to VM except on day 4 (Figure 4(g)). Aside day 2 of monitoring, amylase activity levels in VP were also not significantly different from control values ($p > 0.05$). In *T. aestivum*, amylase activity was significantly disturbed ($p < 0.05$) in all sampling days when vermifiltered wastewater was used to irrigate seeds compared to the control (Figure 3(h)). However, VP demonstrated no significant disturbances compared to VM only on day 2 ($p > 0.05$). Different trend was observed in *S. bicolor* (Figure 4(j)) where VP was significantly different ($p < 0.05$) from VW in all germinating days except day 0 ($p > 0.05$).

3.3. Effects of vermifiltrates on seedling development in *Z. mays, T. aestivum* and *S. bicolor* seedlings development

3.3.1. Morphological effects

The morphological effects of vermifiltered wastewater on seedling development in terms of root and shoot length, number of leaves, number of secondary roots, fresh and dry weight of roots and shoots and fresh and dry weight of leaf were monitored after two weeks of irrigation (Table 2). In *Z. mays*, all morphological parameters of VP were not significantly different ($p > 0.05$) compared to VM. VP and VM were however significantly different ($p < 0.05$) compared to PW in the root length, number of secondary roots and leaf fresh weight. Similar trend was recorded in *T. aestivum* where all morphological parameters in VP were not significantly different ($p > 0.05$) compared to VM except in root length ($p > 0.05$) and leaf fresh weight ($p > 0.05$). VP and VM in

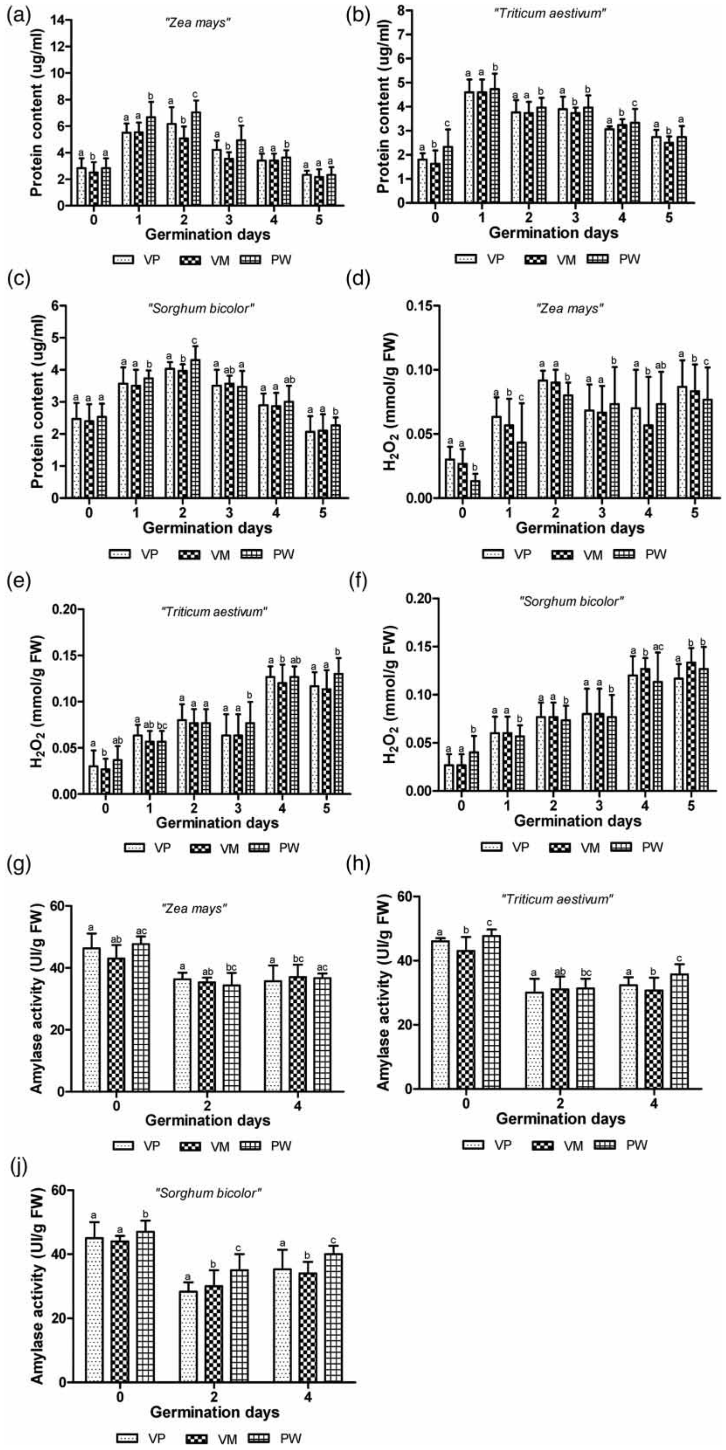


Figure 4 | Total protein content, H_2O_2 accumulation and amylase activity of *Z. mays* (a, d, g), *T. aestivum* (b, e, h) and *S. bicolor* (c, f, j) irrigated with VP, VM and control (PW). Data presents mean ± standard error. Bars labelled with different letters are significantly different among the treatments at $P < 0.05$ using the Tukey's HSD test. In each bar groups, bars labelled with the same letter are not significantly different from each other according to Tukey's HSD at $p < 0.05$.

T. aestivum seedlings showed significant differences ($p < 0.05$) compared to PW in all morphological parameters except in shoot length, root dry weight, shoot fresh weight and shoot dry weight. VP and VM in *S. bicolor* seedlings were also significantly different ($p < 0.05$) from PW in all morphological parameters except in shoot length, number of secondary roots, leaf fresh weight, shoot fresh weight and shoot dry weight. In terms of germination percentage, *Z. mays* recorded 100% efficiency with PW in contrast to VP and VM that recorded 80–90%

Table 2 | Morphological parameters of *Z. mays*, *T. aestivum* and *S. bicolor*, irrigated (VP, VM) and control (PW)

	Z. mays			***T. aestivum***			***S. bicolor***		
Parameters	**VP**	**VM**	**PW**	**VP**	**VM**	**PW**	**VP**	**VM**	**PW**
Root length (cm)	3.74 ± 0.20a	3.62 ± 0.30a	4.40 ± 0.35b	4.10 ± 1.10a	3.62 ± 1.20b	4.50 ± 1.05a	3.60 ± 1.25a	3.55 ± 1.85a	4.49 ± 0.88b
Shoot length (cm)	3.33 ± 0.14a	3.30 ± 0.10a	3.57 ± 0.25a	3.21 ± 0.43a	3.19 ± 0.38a	3.47 ± 0.31a	3.17 ± 0.35a	3.07 ± 0.49a	3.62 ± 0.16a
Number of leaves	3.15 ± 0.10a	3.13 ± 0.12a	3.30 ± 0.12a	2.95 ± 0.61a	2.86 ± 0.60a	3.15 ± 0.15b	2.87 ± 0.58a	2.88 ± 0.78a	3.09 ± 0.10b
Number of secondary roots	5.02 ± 0.15a	4.93 ± 0.15a	5.27 ± 0.12b	4.63 ± 1.60a	4.86 ± 1.82b	5.03 ± 0.47c	5.17 ± 1.50a	4.88 ± 1.40a	4.96 ± 0.48a
Root fresh weight (100*mg)	0.62 ± 0.10a	0.67 ± 0.16a	0.70 ± 0.12a	0.60 ± 0.10a	0.65 ± 0.20a	0.63 ± 0.12b	0.64 ± 0.12a	0.58 ± 0.07b	0.68 ± 0.16a
Root dry weight (100*mg)	0.46 ± 0.01a	0.43 ± 0.06a	0.40 ± 0.12a	0.28 ± 0.14a	0.25 ± 0.18a	0.30 ± 0.15a	0.21 ± 0.07a	0.24 ± 0.24b	0.28 ± 0.17c
Leaf fresh weight (100*mg)	4.44 ± 0.25a	4.55 ± 0.20a	5.03 ± 0.23b	4.46 ± 1.31a	4.28 ± 1.20b	5.07 ± 0.31c	4.30 ± 1.23a	4.36 ± 1.45a	4.26 ± 0.10a
Leaf dry weight (100*mg)	3.23 ± 0.06a	3.17 ± 0.15a	3.13 ± 0.35a	2.63 ± 1.30a	2.78 ± 1.50a	2.90 ± 0.47b	2.82 ± 1.40a	2.15 ± 1.60b	2.88 ± 0.23b
Shoot fresh weight (g)	0.46 ± 0.03a	0.35 ± 0.01b	0.44 ± 0.04a	0.40 ± 0.10a	0.38 ± 0.10a	0.38 ± 0.15a	0.36 ± 0.06a	0.33 ± 0.21a	0.37 ± 0.16a
Shoot dry weight (g)	0.04 ± 0.00a	0.04 ± 0.00a	0.04 ± 0.01a	0.04 ± 0.01a	0.03 ± 0.02a	0.04 ± 0.01a	0.04 ± 0.01a	0.04 ± 0.02a	0.04 ± 0.01a

Data presents mean ± standard error. Values with different letters are significantly different among the treatments at $P < 0.05$ using the Tukey's HSD test at $p < 0.05$.

efficiency (Figure 5(a)). A similar trend was recorded in *T. aestivum* (Figure 5(b)) and *S. bicolor* (Figure 5(c)) with 80–90% germination for VP and VM versus 100% for the control PW.

3.3.2. Physiological effect

Chlorophyll pigment (C_{α}, C_6 and $C_{\alpha+6}$) and carotenoid (C_{x+c}) contents estimation in seedlings revealed the physiological responses of seedling species to the irrigation treatments (Figure 6(a)–6(c)). In *Z. mays*, all treatments demonstrated significant differences ($p < 0.05$) in all chlorophyll pigment and carotenoid contents except C_{α}, where VP showed no significant difference ($p > 0.05$) compared to VM (Figure 6(a)). *T. aestivum* (Figure 6(b)) and *S. bicolor* (Figure 6(b)) recorded similar physiological effect with significant differences ($p < 0.05$) in all chlorophyll pigment and carotenoid contents among all treatments.

3.3.3. Biochemical effects

3.3.3.1. H_2O_2 levels. H_2O_2 levels in the roots and shoots of seedling species (Figure 6(d)–6(f)) were not significantly different ($p > 0.05$) between irrigation treatments except with few exceptions, where H_2O_2 levels in the shoots of VP of *Z. mays* and *T. aestivum* were significantly lowered ($p < 0.05$) compared to VM. H_2O_2 levels in the shoots of both VP and VM of *S. bicolor* were also significantly higher ($p < 0.05$) than PW.

4. DISCUSSION

Physico-chemical characterization revealed that the wastewater used in this study was acidic, had low DO and high EC, turbidity, TD, TSS, COD, BOD and N_{tot} levels compared to the portable water (Table 1). The higher acidity of the wastewater could be attributed to the presence of urea sourced from urine and excreta that is involved in amino acid metabolism and the production of ammonium ions (Mesdaghinia *et al.* 2004). The pH of the wastewater generally increased after vermifiltration and this is in line with earlier studies that have recorded similar results (Xing *et al.* 2010; Furlong *et al.* 2014; Nsiah-Gyambibi *et al.* 2022). Rise in pH after

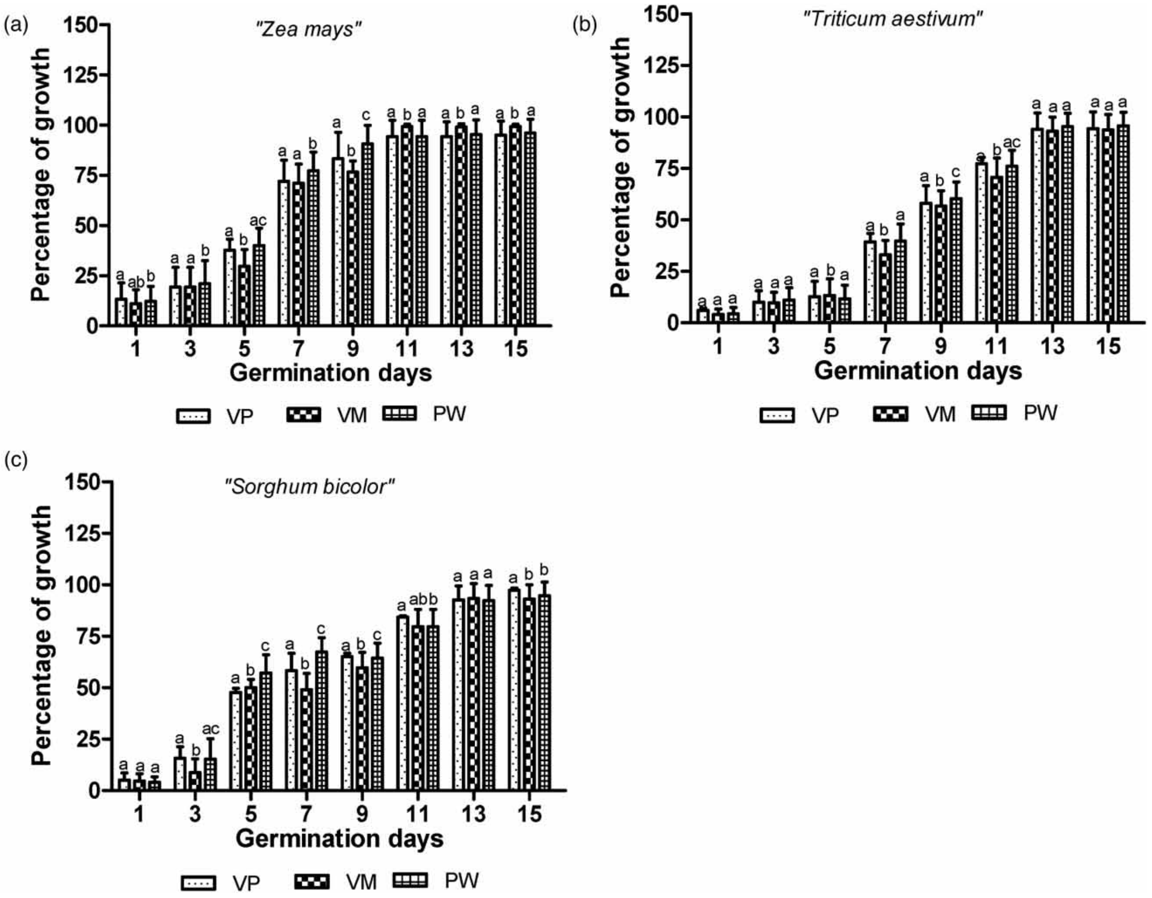


Figure 5 | Percentage of seedling growth, representative seedlings grown in pots and representative whole seedlings of *Z. mays* (a), *T. aestivum* (b) and *S. bicolor* (c), irrigated with VP, VM and control (PW). Data presents mean ± standard error. Bars labelled with different letters are significantly different among the treatments at $P < 0.05$ using the Tukey's HSD test. In each bar groups, bars labelled with the same letter are not significantly different from each other according to Tukey's HSD at $p < 0.05$.

vermifiltration is thought to be due to neutralization of wastewater acidity by the intestinal calcium secretions of earthworms and through the production of ammonia from ammonification (Edwards & Lofty 1997). Release of exchangeable cations when the wastewater is filtered through the filter media could have also shifted the pH toward neutrality (Woomer *et al.* 1994). Results also showed that there were significant reductions ($p < 0.05$) in TSS, TDS, COD, BOD and nutrients (N_{tot} and P_{avail}) after vermifiltration. A vermifilter bed with filter and bulking materials supported the earthworm growth in providing food source by sorption mechanism from the wastewater, and a microbial layer is formed because of low porosity (Wang *et al.* 2010; Liu *et al.* 2013; Singh *et al.* 2017). Comparing the two vermifiltration systems' (VP and VM) results showed that effluents from the two treatments were significantly different ($p < 0.05$) in TSS, TDS, COD, BOD and nutrients (N_{tot} and P_{avail}). VP recorded lower concentrations compared to VM and this could be due to the presence of the macrophytes. These results are similar with previous studies (Lin *et al.* 2002; Chen *et al.* 2016). Sinha *et al.* (2008) also recorded similar removal efficiencies (BOD by over 90%, COD by 80–90%, TDS by 90–92% and TSS by 90–95%) and concluded that earthworms' body works as a biofilter to remove the contaminants from wastewater by the general mechanism of ingestion, absorption through body walls and biodegradation of pollutants from wastewater. In vermifiltration, suspended solids are trapped on top of the vermifilter and processed by earthworms or used by microbes for immobilization (Sinha *et al.* 2008). Earthworms consume retained suspended particles in the filter during ingestion and significantly reduce COD and BOD causing a significant reduction in nutrients concentration (Li *et al.* 2009; Wang *et al.* 2011). Nitrogen are removed during vermifiltration through the nitrification process when earthworms aerate the system through its burrowing action and create favourable microenvironment conditions for the growth of aerobic nitrobacteria (Samal *et al.* 2017b). Lin *et al.* (2002)

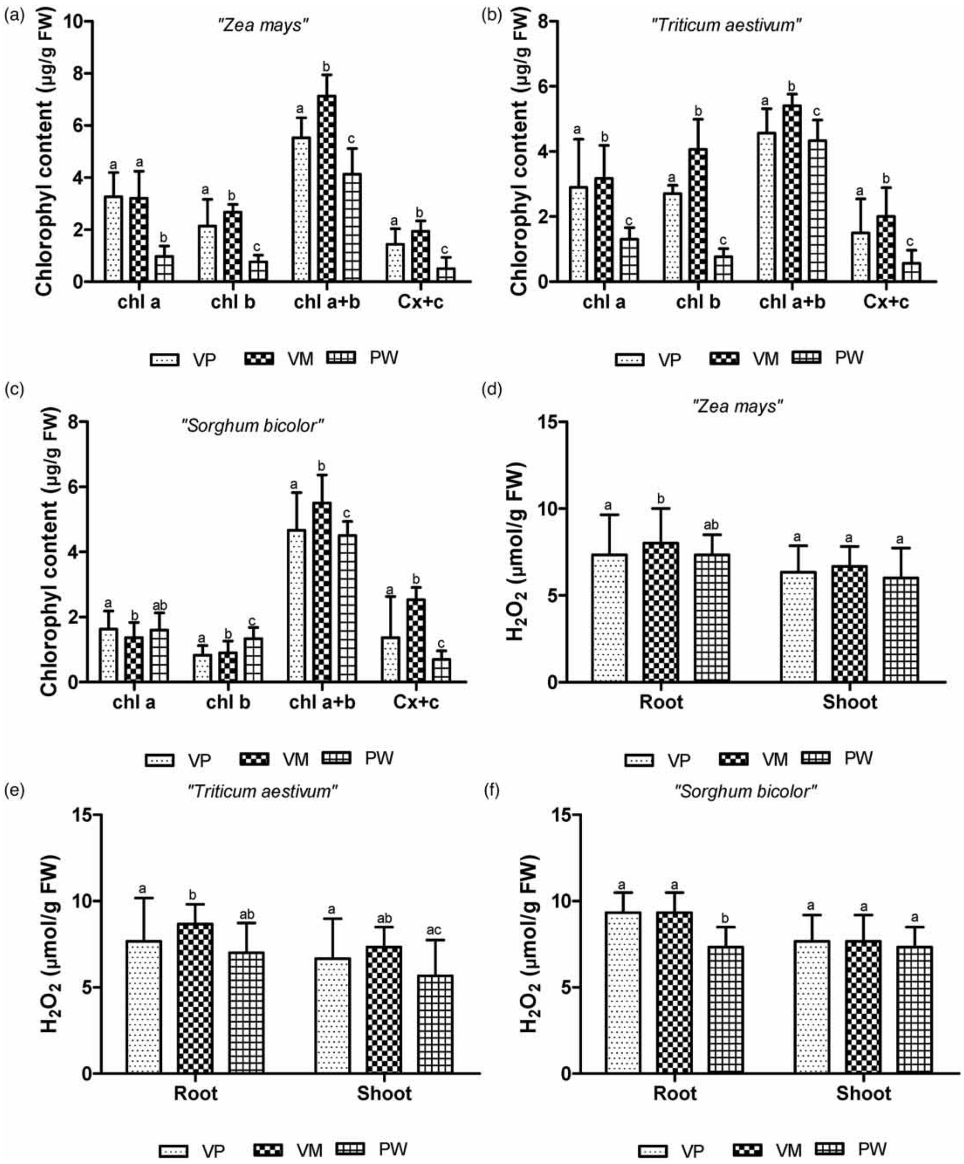


Figure 6 | Chlorophyll a, b, total chlorophyll and carotenoids content of *Z. mays* (a), *T. aestivum* (b) and *S. bicolor* (c), irrigated with VP, VM and control (PW). H_2O_2 content of roots and shoots of *Z. mays* (d), *T. aestivum* (e) and *S. bicolor* (f), irrigated with VP, no macrophyte-assisted vermifiltrate (VM) and control (PW). Data presents mean ± standard error. Bars labelled with different letters are significantly different among the treatments at $P < 0.05$ using the Tukey's HSD test. In each bar groups, bars labelled with the same letter are not significantly different from each other according to Tukey's HSD at $p < 0.05$.

reported that macrophyte roots release both oxygen and supporting microbes that are capable of biodegrading adsorbed contaminants. Macrophyte basically make a uniform distribution of microbes and oxygen in the filter bed through its rooting system and also maintains porosity in both top and bottom zone of the filter for aerobic degradation of contaminants (Samal *et al.* 2017b). Macrophytes integrated in vermifiltration systems moreover provide active layers for earthworm inoculation zone to facilitate ammonification and nitrification (Samal *et al.* 2018). The ammonification and nitrification typically occurred in these active layers due to high activity of earthworms and its associated gut microbes. *P. stratiotes* have been found to possess recognized roots that aid in achieving increased values of dissolved oxygen for biofilm formation that contribute to the uptake of nutrients, which also results in BOD and COD reduction (Anawar & Chowdhury 2020).

Tolerance of seeds and seedlings to abiotic stresses such as water quality stress is very complex, due to the intricate nature of interactions between stress factors and various morphological, physiological and biochemical changes affecting seed germination and seedling development (Razmjoo *et al.* 2008). Results on the morphological monitoring of seed germination showed that when vermifiltered wastewater (VP and VM) were used as irrigation treatments, there were visible inhibitory effects on germination percentage and radical growth of *Z. mays*, *T. aestivum* and *S. bicolor* compared to the control. The use of vermifiltered wastewater as irrigation water lowered the germination percentage and radical growth of all seed species. Results from the study clearly showed consistent varying dynamics of morphological development of seed species. Compounds in the effluents could have contributed to these varying dynamics by producing residual inhibitory effects on the germination percentages and radical growths. Similar results were recorded on the physiological development of seed germination. These inhibitory effects in VP treatments were significantly lower than VM in all germination days except a few. Results are consistent with previous studies (Rui *et al.* 2007; Samal *et al.* 2017b) and this could be due to the removal of wastewater contaminants caused by the presence of the macrophyte. Jaleel *et al.* (2009) reported that water quality is one of the major abiotic stresses that adversely affect seed germination, crop growth and yield. Chaabene *et al.* (2015) also reported that contaminates in wastewater are capable of altering seed water absorption which is a major factor for germination success. The water parameters related to the loss of water absorption could be the presence of suspended and dissolved solids (Gupta 2009). These contaminants or compounds present in the effluents could have contributed to the physiological development of seeds by causing inefficient mobilization of seed reserves that alters seed water absorption to cause reductions or delays in seed germination (Jaleel *et al.* 2008). Soluble protein content, H_2O_2 levels and amylase activity in the seeds submitted to different irrigation treatments were used to monitor the biochemical alterations in the seed germination. Vermifiltrates (VP and VM) reduced soluble protein content, H_2O_2 levels and amylase activity in seeds compared to the control. PW treatments recorded the most soluble protein content, H_2O_2 levels and amylase activity significantly different ($p < 0.05$) from VP and VM except in few cases. Results in present study is consistent with previous evidences (Gupta *et al.* 2007; Fendri *et al.* 2013; Chaabene *et al.* 2015; Rekik *et al.* 2016) and this could be due to compounds in the effluents resulting in water quality stress and toxicity increase that could have affected the physiological development of seeds.

To widely assess the effect of vermifiltered wastewater on the growth parameters of seedlings, germination days were extended to two weeks. Compared with the control, results clearly showed that the vermifiltered wastewater reduced root and shoot length, number of leaves, number of secondary roots, fresh and dry weight of roots and shoots and fresh and dry weight of leaf in all plant species (Table 2). However, with few exceptions, these reductions were not significantly different ($p < 0.05$) between the two vermifiltered treatments (VP and VM). Results on seedling physiological development in terms of Chlorophyll pigment (C_a, C_b and C_{a+b}) and carotenoid (C_{x+c}) production showed that PW exerted the least production inhibition. Previous studies have recorded similar results (Brix 1994; Samal *et al.* 2017a, 2017b) and this could be due to the compounds presented in the effluents which might have resulted to water quality stress. Compounds in effluents causing water quality stress inhibits cell enlargement and cell division that contributes to seedling physiological and biochemical development (Shao *et al.* 2008). Development of optimal leaf area is an important photosynthetic indicator for the production of photosynthetic pigments because these pigments are mainly responsible for harvesting light (Jaleel *et al.* 2009). Changes in photosynthetic pigments are of paramount importance to seedling growth and therefore an essential indicator in assessing water quality stress in seedling development. Water quality stress, among other changes, also has the ability to reduce the tissue concentrations of chlorophyll pigments and carotenoids (Kiani *et al.* 2008). Both chlorophyll a and b are prone to water quality stress and contaminants or compounds in the effluent are capable of blocking both stomatal and non-stomatal openings (Farooq *et al.* 2009). Compounds in effluents are capable of slowing down cell growth by affecting various biochemical processes such as respiration, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters (Jaleel *et al.* 2008). The effect of the compounds in the effluent on the biochemical development of seedlings in terms of hydrogen peroxide productions followed similar pattern as observed in the physiological development. With the exception of a few occasions, PW recorded the highest H_2O_2 concentrations followed by VP and VM respectively. Compounds in the effluent could have resulted to these intricacies of biochemical development because the compounds are capable of causing low turgor pressure that greatly suppresses cell growth for the production of H_2O_2 (Jaleel *et al.* 2008). However, removal of contaminants by macrophyte roots through osmotic regulation could have attributed to the differences in biochemical effect between VP and VM as this can enable the maintenance of cell turgor pressure to facilitate cell growth and production of H_2O_2 (Shao *et al.* 2008).

5. CONCLUSION

Vermifiltration of wastewater integrated with macrophyte (*P. stratiotes*) proved effective in the removal of contaminants. Integrating macrophytes to the vermifiltration system in this study proved efficient in the removal of contaminants or compounds from the influents. From the present study, by comparison, there was more reduction of contaminants in the VP than VM and this reduced the water quality stress on the morphological, physiological and biochemical developments of seed germination and seedling growth. This study has demonstrated that integrating macrophyte to the vermifiltration system used produced effluents that had consistent varying effects on the morphological, physiological and biochemical developments of seed germination and seedling growth. *P. stratiotes* was found to be a suitable macrophyte integrated into the vermifiltration system. This is because compounds found in the effluents produced from *P. stratiotes*–assisted vermifiltration system was greatly reduced when compared with the influent. The effluents from the VP can therefore be utilized as irrigation water indicating a potential use for agricultural purpose. Based on these preliminary results, a large-scale application of VP is realistic and can be considered as an important source of irrigation water with a continuous monitoring of the water quality. However, a careful selection of the plant species from seed germination assays extended to seedling development should be of central importance to revalorize this treatment approach. Meanwhile, this study recommends further investigations into the effect of continuous use of VP wastewater as irrigation on soil fertility to determine the residual effects and accumulation in plants.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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