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Effect of the ebb-and-flow constructed wetland operation, media type and celery (*Apium graveolens* L.) growth on nutrient removal from a pre-treated brewery effluent

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ABSTRACT

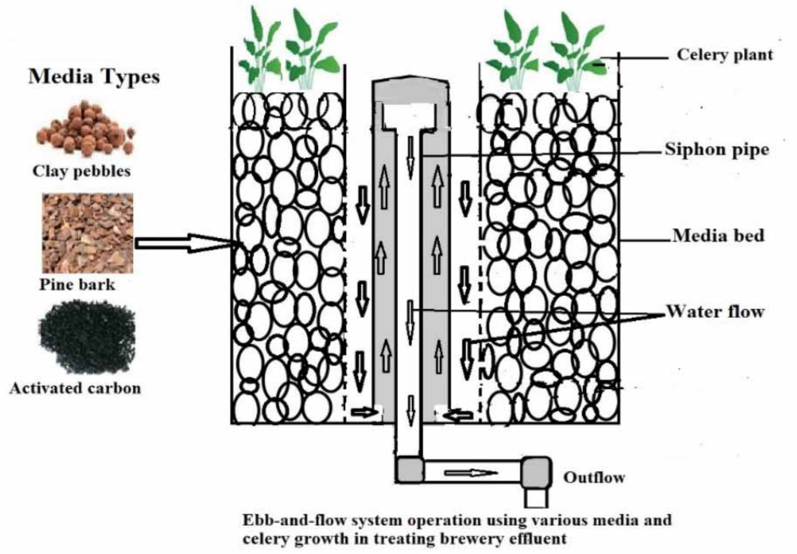
Breweries face a high cost of wastewater treatment to meet environmental discharge standards. Constructed wetland (CW) design and operation have been optimised for sustainable wastewater treatment. An ebb-and-flow CW was used to investigate the effect of media on nutrient removal from a brewery effluent. Eight media (clay pebbles, clay bricks, sand, bioballs, recycled plastic and gravel sizes), either alone or mixed, were tested. A 2:1:1 ratio of each media, pine bark and granular activated carbon, respectively, was used for mixed treatments. A uniform celery planting density of eight seedlings per m^2 and 12-min effluent retention time was used. There were significant differences between unmixed and mixed media on nutrient removal ($p < 0.05$). The unmixed media of clay pebbles had the highest mean COD reduction of 7.5% and ammonia 8.1%. Mixing resulted in better overall mean removal efficiency of nitrite-N (7.6%), nitrate-N (15.3%), total inorganic nitrogen (29.9%), orthophosphate (12.7%) and plant biomass (11158.5 gm^{-2}) than unmixed media. Media porosity, surface area and chemical composition and celery plant enhance nutrient removal through synergistic interactions. The study recommends unmixed clay pebbles, bioballs and 19-mm gravel for improved ammonia-N removal and mixed media for multiple pollutant removal and celery productivity.

Key words: celery, constructed wetland, ebb-and-flow, media, post-anaerobic digester effluent, salinity

HIGHLIGHTS

- Ebb-and-flow CW creates aerobic and anaerobic conditions for nutrient removal.
- Combining substrates has complementary properties for multiple nutrient removal.
- Celery is a halophyte plant adapted to saline conditions of the irrigating brewery effluent.
- Ebb-and-flow design, media and celery plant synergistically interact to remove nutrients.

GRAPHICAL ABSTRACT



INTRODUCTION

Globally, there has been growing concern about water shortage and pollution due to environmentally unfriendly effluent disposal (Wu *et al.* 2015). Constructed wetlands (CWs) have been used as a low-cost alternative to conventional wastewater treatment to enable re-use and minimise environmental pollution (Lee & Fletcher 2009; Götz *et al.* 2014). Ebb-and-flow is a constructed wetland (CW) technology for wastewater treatment that fills and drains effluent in a wetland bed (Behrends *et al.* 2000; Austin 2006). The fill and drain cycles promote alternate aerobic and anaerobic conditions that simultaneously remove multiple pollutants (Li *et al.* 2015; Zhang *et al.* 2021).

The CW components comprising a substrate, microorganism, plants and their interactive functions purify wastewater through chemical, physical and biological processes (Faulwetter *et al.* 2009; Vymazal 2011; Dordio & Carvalho 2013). The substrate/media provides a surface to which microorganisms attach and support plant growth, filtration, nutrient adsorption and chemical precipitation (García *et al.* 2010; Saeed & Sun 2011). Media are classified by their properties that influence contaminant removal. Physical properties comprise particle size, surface area, shape, porosity, hydraulic and electrical conductivity and mechanical strength. Chemical properties are ion exchange capacity and acid–base composition, while biological properties include electron donors and acceptors (Yang *et al.* 2018).

Natural, industrial and man-made products have shown potential as media for pollutant removal in CWs (Cheng *et al.* 2018; Wang *et al.* 2020). For example, activated carbon is a stable substrate with a large surface area and a high adsorption capacity; however, it has a high processing cost, thus is not viable for sustainable wastewater treatment (Fu *et al.* 2020; Marlena & Nowicki 2022). Recent studies focus on wetland substrate availability, cost-effectiveness and competitive pollutant removal efficiency (Yang *et al.*, 2018; Wang *et al.* 2020). The emerged substrates are from agricultural and industrial by-products such as construction wastes, tyre chips, clay bricks, clay aggregates, plastics and biochar (Liu *et al.* 2014; Lima *et al.* 2018; Sandoval *et al.* 2019; Wang *et al.* 2020). Organic wood mulch is a potential substrate providing organic carbon for biological pollutant removal processes, compared to sand which do not provide carbon (Saeed & Sun 2011, 2012; Yang *et al.* 2018).

Combining substrates is an economical strategy for achieving desirable properties for multiple pollutant removal. Fu *et al.* (2020) reported 96.2% total nitrogen removal in combined sand, activated carbon and ceramsite treating saline wastewater. Wu *et al.* (2019) reported above 90% removal of total nitrogen and phosphorus in layered zeolite, anthracite and bio-ceramic substrates in vertical CWs. These findings were attributed to the synergistic relationship between substrates, microorganisms and plants in pollutant removal. Researchers have examined the emerged substrates on a laboratory scale treating synthetic effluents (Kizito *et al.* 2017; Li *et al.*, 2019; Zhang *et al.* 2021); however, limited studies have used the actual wastewater. Moreover, the use of media alone or a combination has not been tested in treating brewery effluent.

Plant roots offer sites for the attachment of microbes and nutrient adsorption (Wu *et al.* 2015). A beneficial relationship exists between plant roots and the supporting substrate in transforming organic nutrients into simple forms for uptake

(Vymazal 2011). The length of time for contact between plant roots and the effluent influences organic matter breakdown and nutrient uptake (Stottmeister *et al.* 2003). Wetland plants are very productive and nutrient removal can be quantified as g m^{-2} biomass gain (Vymazal 2011).

Industrial effluents are rich sources of organic and inorganic nutrients essential for plant growth (Jones *et al.* 2016; Taylor *et al.* 2018). However, the high pH (9–12) and salinity (about 3,000 μS/cm in industrial effluent limits the bioavailability of nutrients for plants) (Power & Jones 2016; Zhao *et al.* 2021). Adjusting brewery effluent pH improved nutrient availability by successfully producing tomatoes, lettuce and cabbages (Power & Jones 2016; Taylor *et al.* 2018). Salt-tolerant plants, such as Swiss chard, maize, sunflower, sesame and lucerne, have been grown using a brewery effluent (Senthilraja *et al.* 2013; De Jong, 2019; Mabasa *et al.* 2021). Further research is needed for various plant crops to maximise nutrient uptake from industrial effluent.

Celery (*Apium graveolens* L.) is a perennial food crop of the family Umbelliferae (Kooti & Daraei 2017; Ashmawi 2019). The demand for celery stalks, leaves and seeds is rising as people become health-conscious (Ashmawi 2019; Stephen *et al.* 2020). Celery has bioactive compounds comprising flavonoids, alkaloids, phenolic acids, terpenoids and tannins, all with medicinal properties (Stephen *et al.* 2020). The high salt tolerance of celery is due to its ability to synthesise mannitol, a compatible solute, protecting the cell structure by increasing osmotic potential (Everard *et al.* 1994; Pardossi *et al.* 1999; Noiraud *et al.* 2000). Kotzen & Appelbaum (2010) reported good celery growth in an aquaponic system using brackish water of 4,500 μS cm^{-1} electrical conductivity. Despite the celery's intrinsic properties and salt tolerance, no study has examined celery's efficiency on nutrient removal from a brewery effluent in CWs.

This study forms a basis for choosing a media combination design for wastewater treatment in the ebb-and-flow CWs. This study compared the effect of a range of mixed and unmixed media planted with celery on nutrient removal from post-anaerobic digester (AD) effluent. Celery growth, survival and chemical composition were compared between media.

MATERIALS AND METHODS

Experimental site and wastewater treatment process

The study was conducted at Ibhayi Brewery (33.8378° S, 25.5419° E) in Port Elizabeth, Eastern Cape, South Africa. The region has a subtropical climate with a warm summer, temperature range of 25–35 °C and moderate winter seasons. The study period was 12 weeks during summer from October to December. South African brewery (SAB) effluent is treated in a commercial-scale AD and activated sludge units before being sent to a municipal sewer (Taylor *et al.* 2018). A portion of the post-AD effluent is sent to the Project Eden research facility for further treatment using alternative technologies such as primary facultative ponds, high-rate algal ponds and CWs (Jones *et al.* 2014). The research facility has six tidal wetland cells (320 m^2) serially connected and filled with 19–25 mm crushed gravel.

System components, design and operation

Forty-eight ebb-and-flow systems were positioned on a 320 m^2 tidal wetland cell. The systems were built using 100-L circular plastic tanks (0.45 m diameter and 0.55 m height). 50-mm polyvinyl chloride (PVC) pipe was installed on top of the systems as a water supply line, along with 25-mm water taps for each unit to trickle the effluent through the substrates. Post-AD effluent was pumped into the units using a submersible water pump (AquaDrive 670-SPECK Pump-6452NTL-A12X). A single-pass ebb-and-flow design with a uniform flow rate of 8.3 L/min was used. The outflow from the ebb-and-flow beds periodically drained into the tidal wetland.

A bell siphon is a mechanical device regulating water flow within a substrate by pressure and gravity. This device operates through filling and draining, creating aerobic and anaerobic cycles. During filling, water level rises to the maximum height of the standpipe, where low pressure created at the bell cap initiates draining by gravity. During draining, atmospheric air is sucked into the substrate to displace the draining water. The pressure difference created at the base and outlet pipe eventually breaks the siphon. The substrate bed is refilled and drained repeatedly (Figure 1).

Acquisition of substrate and plants for the experiment

One-week-old celery seedlings were purchased from Moorland Seedlings Pty Ltd, a commercial nursery in the Eastern Cape of South Africa. One hundred and ninety-two seedlings were grown in the ebb-and-flow beds. The media were purchased in Port Elizabeth, South Africa and were selected based on availability, cost and physicochemical properties (Table 1).

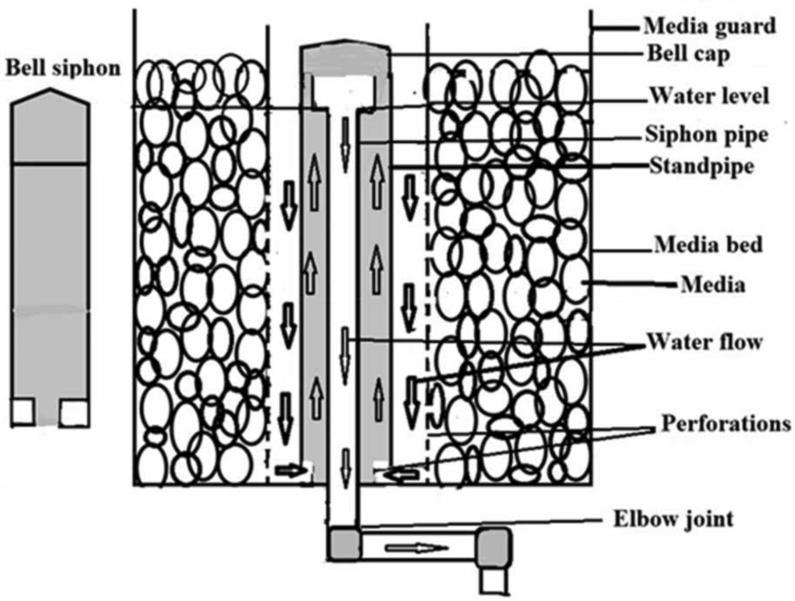


Figure 1 | The ebb-and-flow system and bell siphon sectional unit used in the experiment.

Table 1 | Media types, sizes and physicochemical characteristics

Nature	Type	Size (mm)	Physicochemical properties	Reference
Clays	Clay pebble Clay bricks	8 – 20 15 – 25	Highly porous, large surface area, rich in Si_4^+, Al^{3+} and Mg^{2+} cations Irregular surface with micropores	Lima *et al.* (2018); Gu *et al.* (2019)
Plastics	Bioballs Recycled plastics	10 10 – 20	Large surface area, lightweight, made of polyethene terephthalate, rough surfaces	Sandoval *et al.* (2019)
Stones	Gravel	7,13, 19	Large surface area, highly stable	Yang *et al.* (2018)
	Silica sand	1.05–2	Coarse, large surface area, rich in Ca^{2+} and SiO_2	Arias *et al.* (2001)
Carbon supplements	Activated carbon	3	Lightweight, large surface area, high adsorption capacity	Sanjrani *et al.* (2019); Fu *et al.* (2020)
	Pine bark	25	Lightweight, a fluffy texture, rich in organic carbon	(Saeed & Sun2013; Hua *et al.* 2016)

STUDY DESIGN

Eight media comprising clay pebbles, clay bricks, bioballs, recycled plastics, sand and gravel pebble sizes (7, 13, 19 mm) (Table 1) either alone or mixed were tested in 100-L experimental units. A 2:1:1 ratio of each media, pine bark and granular activated carbon, respectively, was used for the mixed treatments. A 12-min retention time (RT) was used as an extension for the recommended RT in the previous study, which treated AD brewery effluent at Ibhayi Brewery (De Jong 2019). Healthy celery seedlings with an average height of 4.64 ± 0.06 cm and a weight of 5.39 ± 0.60 g were planted at a uniform density of eight plants m^2. All treatments were in triplicates a randomised block design.

DATA COLLECTION

Water quality sampling and analysis

Water temperature (°C), dissolved oxygen (DO) ($mg\ L^{-1}\ O_2$), pH, and electrical conductivity ($mS\ cm^{-1}$) were measured daily at the inlets and outlets using a portable Lutron PDO-519 DO meter, pH Lutron-electrode meter Model: PH-220 and Lutron conductivity meter, code PCD-432, respectively. Water samples were collected weekly at the inlet and outlet and analysed for chemical oxygen demand (COD), ammonia-N, nitrite-N, nitrate-N and orthophosphate by using a spectrophotometer (DR2800-01B1, Hach (Pty) Ltd, USA). The nutrient removal efficiency was calculated as percentage values (Equation (1)).

$$\text{Nutrient removal}(\%) = \frac{C_e - C_i}{C_i} \times 100 \tag{1}$$

where C_i is the inflow concentration, C_e is the outflow concentration.

Total inorganic nitrogen is calculated using (Equation (2)).

$$\text{Total inorganic nitrogen}(\%) = [NH_4^+\text{–}N] + [NO_2^-\text{–}N] + [NO_3^-\text{–}N] \tag{2}$$

Celery production

The initial wet-weight of the seedlings was measured to the nearest 0.01 g using a Radwag weighing balance (WLC 6/A/A2/C/2, code WL-217-0014, LLC, Poland). Plant height was measured weekly using a measuring tape (cm). The chlorophyll concentration index (CCI) was measured at the start and end of the experiment on the topmost leaves using a chlorophyll content meter (CCM-200, Opti-Sciences Inc., USA). Plants were monitored for stress symptoms and observations were captured in photographs. At the end of the trial, three plants from each replicate were harvested and weighed as wet-weight (g), oven-dried at 70 °C for 3 days and recorded as dry-weight according to Reuben *et al.* (2016) (Equation (3)).

$$\text{The gross weight of dry biomass} = \frac{\text{Dry sample weight}}{\text{Fresh sample weight}} \times \text{Gross fresh weight} \tag{3}$$

Celery leaf chemical analysis

Celery leaves were sampled from each replicate at the start and end of the experiment for elemental analysis. The leaves were analysed for concentrations of nitrogen, potassium, sodium, phosphorus, aluminium, calcium, copper, iron, manganese, magnesium and zinc, at the Department of Agriculture, Elsenburg, South Africa commercial laboratory. Celery leaves were analysed using the standardised procedures by the Agricultural Laboratory Association of Southern Africa (ALASA 1998). Nitrogen was determined using the standard Kjeldahl method. Leaf samples were ashed in a Neytech Muffle Furnace (Vulcan 3-1750A) overnight at 460 °C to determine the concentration of leaf potassium, sodium, phosphorus, aluminium, calcium, copper, iron, manganese, magnesium and zinc. The samples were heated for 30 min in a sample-to-acid ratio of 1:1 consisting of 32% hydrochloric acid. 50 mL of distilled water was added to the mixture and filtered to determine the element concentrations using inductively coupled plasma atomic emission spectroscopy (ICP-OES, Vista MPX, Varian Inc., USA; Sithole, pers. comm.).

STATISTICAL ANALYSIS

All data were checked for equality of variance and normality of residuals using Levene's and Shapiro–Wilk's tests. If the assumptions were not met, data were log- or arcsine-transformed. The means of the weekly data for water quality variables, celery height, chlorophyll concentration and dry and wet biomass were compared between treatments using multifactor ANOVA. The inflow and outflow physicochemical variables and nutrient removal efficiencies were compared over time using repeated measures ANOVA at an alpha error of $p < 0.05$. The significantly different means were compared using Tukey's *post hoc* test. The Kruskal–Wallis test compared the percentage of plant mortality between treatments.

RESULTS

The mean inflow concentration of water parameters from post-AD effluent into the ebb-and-flow system is presented in Table 2.

The overall DO levels increased in the outflow of the ebb-and-flow systems of different media with ranges of 1.6–3.2 mg L^{-1}. The interaction between weeks, media and mixed did not affect the outflow DO concentration (repeated measures ANOVA, F $(77, 352) = 0.9$, $p = 0.61$). The highest mean outflow DO was in unmixed clay pebbles (2.33 ± 0.15 mg L^{-1})

Table 2 | The ranges of inflow concentrations of the post-anaerobically digested effluent into the ebb-and-flow system ($n = 624$)

Water parameter	Inflow values
Temperature °C	23.3 ± 0.12
Dissolved oxygen (mg L^{-1})	1.05 ± 0.01
Chemical oxygen demand (mg L^{-1})	109.7 ± 1.09
Ammonia-N (mg L^{-1})	24.91 ± 0.25
Nitrite-N (mg L^{-1})	0.06 ± 0.11
Nitrate-N (mg L^{-1})	0.04 ± 0.11
Orthophosphate (mg L^{-1})	16.05 ± 0.14
Conductivity (mS cm^{-1})	2.92 ± 0.16
pH range	8.06–8.4

and bioballs (2.32 ± 0.13 mg L^{-1}), followed by 19 mm gravel (2.31 ± 0.03 mg L^{-1}), and lowest in sand (2.08 ± 0.07 mg L^{-1}). Clay bricks, gravel (7 and 13 mm) and recycled plastic media had a mean DO concentration range of 2.20 ± 0.11 –2.30 ± 0.14 mg L^{-1}. The temperature range was 18.1–28.5 °C. There were slight differences between the inflow and outflow conductivity and pH levels.

Media significantly differed in reducing COD and ammonia-N removal (multifactor ANOVA, $p < 0.01$). Unmixed media had higher means of $6.55 \pm 0.13\%$ and $7.22 \pm 0.14\%$ than mixed treatments, with $5.86 \pm 0.10\%$ and $6.47 \pm 0.11\%$ for COD and ammonia-N removal, respectively (Figure 2i and ii). The unmixed clay pebbles had the highest mean COD reduction of $7.51 \pm 0.22\%$ and ammonia $8.13 \pm 0.23\%$, followed by bioballs and 19 mm gravel media and lowest in sand media with $5.03 \pm 0.12\%$ and $5.35 \pm 0.16\%$, respectively.

An interaction between media and mixing influenced nitrite-N, nitrate-N, total inorganic nitrogen and orthophosphate removal, multifactorial ANOVA ($p < 0.001$, Figure 2iii–vi). The mixed media had significantly higher overall mean nitrite-N removal of $7.53 \pm 0.05\%$ than unmixed with $6.48 \pm 07.03\%$. Mixed bioballs ($7.93 \pm 0.33\%$) had the highest mean nitrite-N removal followed by 7 mm gravel ($7.81 \pm 0.21\%$); lowest was in all unmixed media which did not differ between treatments, $p > 0.05$, Figure 2iii.

The mean nitrate-N and total inorganic nitrogen (TIN) removal significantly differed between media treatments $p < 0.001$. Mixed media had mean removal of $15.89 \pm 0.1\%$ and $29.93 \pm 0.83\%$ higher than unmixed with $13.35 \pm 0.09\%$ and $26.59 \pm 0.22\%$ for nitrate-N and TIN, respectively. Nitrate-N and TIN removal did not differ between mixed media $p > 0.05$, Figure 2iv–v. The unmixed clay pebbles mean nitrate and TIN removal were $15.26 \pm 0.47\%$ and $28.68 \pm 1.06\%$, respectively. The lowest means were in unmixed sand with $12.62 \pm 0.58\%$ and $25.11 \pm 0.94\%$, respectively. The mean orthophosphate removal differed between media and mixing, F (1, 32) = 12608.2, $p < 0.001$, Figure 2vi, with higher means in mixed ($12.90 \pm 0.11\%$) than unmixed media ($8.61 \pm 0.21\%$). Mixed media of clay pebbles, clay bricks and sand had the highest mean orthophosphate removal of 13%, while mixed gravel and plastic media had a mean range of 12.53–12.74%. Orthophosphate removal was lowest in the unmixed plastic and gravel media with 7.6 ± 0.13–$9.7 \pm 1.18\%$ (Figure 2vi).

The efficiency of media in removing nutrients increased with similar trends to maximum values, after which it decreased (Figure 3i–vi). The unmixed media had higher mean COD reduction and ammonia-N removal than the mixed media (repeated measures ANOVA, $p < 0.001$, Figure 3i, ii). Mixed media had higher removal for nitrite-N, nitrate-N and orthophosphate ($p < 0.001$, Figure 3iii–vi).

CELERY HEIGHT AND CHLOROPHYLL INDEX

The celery height and CCI differed between media (Multifactorial ANOVA, $p < 0.05$) (Figure 4i, ii). Celery grown in bioballs media died 4 weeks after planting, and no growth parameters were recorded. The longest mean celery was in mixed (62.11 ± 0.70 cm) than unmixed media (52.72 ± 1.34 cm) and lowest in unmixed recycled plastic (38.76 ± 3.69 cm) (Figure 4i). The sand and 7 mm gravel media did not differ in mean celery height. All other unmixed media had a mean height of 55.3–57.6 cm (Figure 4i).

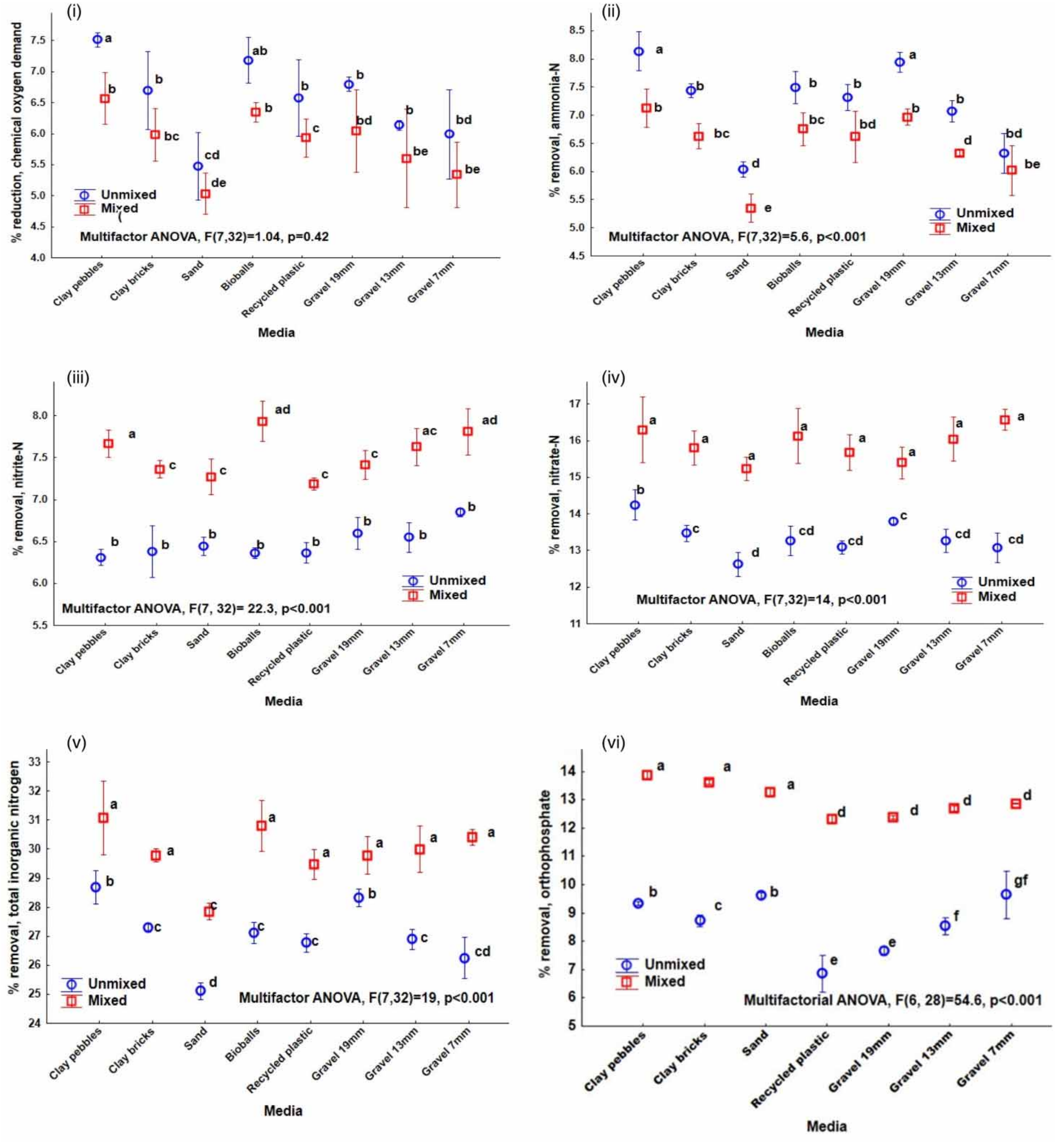


Figure 2 | Means (±95% confidence interval) of nutrients (i–vi) ammonia-N, nitrite-N, nitrate-N and orthophosphate removal efficiency in the mixed and unmixed media of ebb-and-flow system. Treatments with letters a–f next to the mean were significantly different (Multifactorial ANOVA, $p < 0.001$).

The mixed media had a higher CCI (70.23 ± 0.58) than the unmixed (63.45 ± 1.44) (Figure 4ii). The mixed 7 mm gravel (75.73 ± 1.53) had the highest mean chlorophyll index followed by clay pebbles, clay bricks, sand and gravel 13 and 19 mm with a mean range of 70.76 ± 0.54–71.73 ± 0.79. The lowest was in unmixed recycled plastic media (48.33 ± 4.45). Unmixed clay pebbles, clay bricks and gravel (7, 13 and 19 mm) had a CCI range of 65.96 ± 0.70–68.31 ± 0.90 (Figure 4ii).

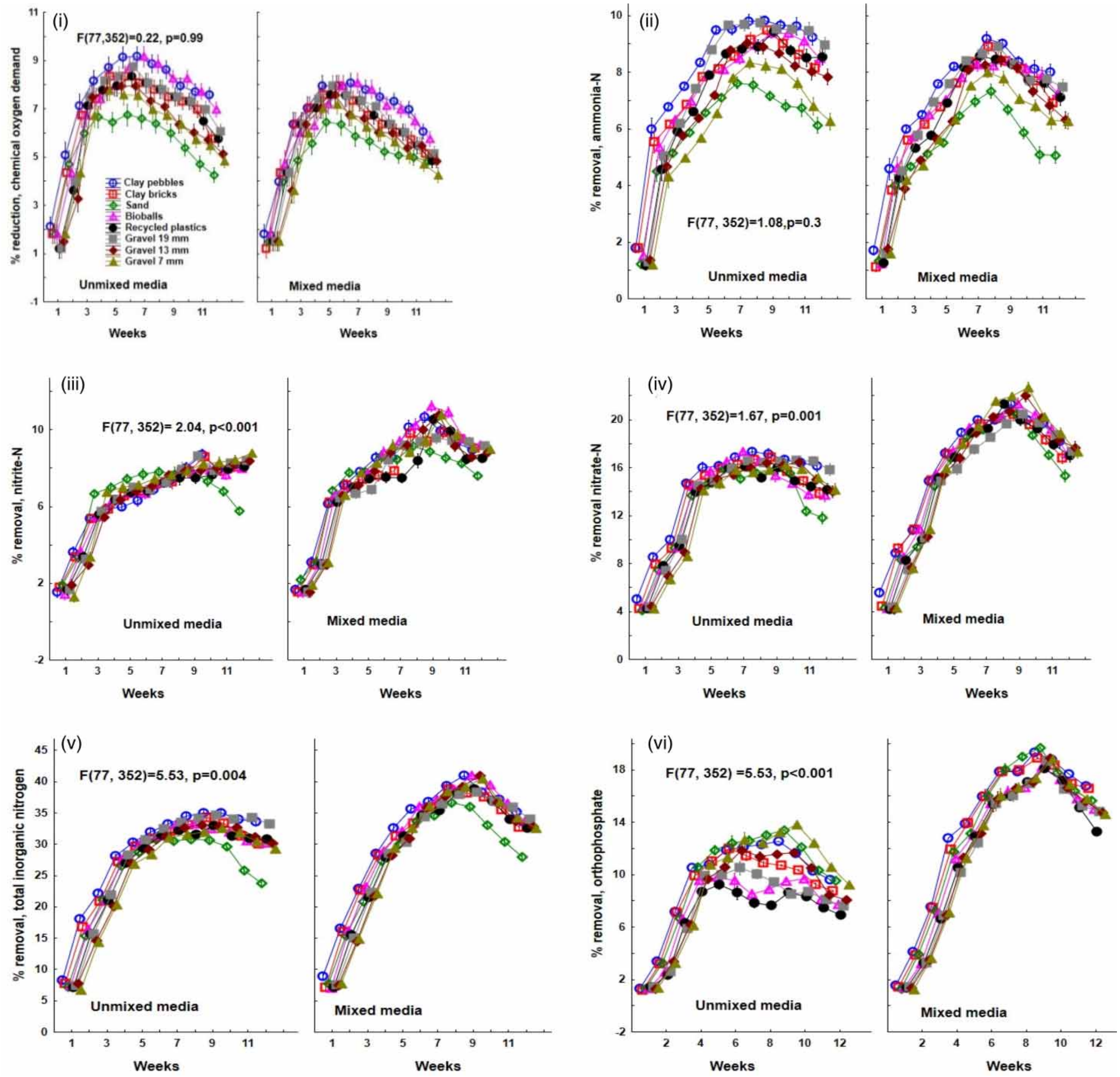


Figure 3 | The removal efficiency of nutrients (i–v) chemical oxygen demand, ammonia-N, nitrite-N, nitrate-N and orthophosphate from post-AD effluent using mixed and unmixed media of ebb-and-flow system for 12 weeks (Repeated measures ANOVA, *F*-statistics for the interactions of media, mixing and time).

CELERY PLANT BIOMASS

The celery biomass increased from seedlings ($5.85 \pm 0.03\ \mathrm{g}^{-2}$) to final weight ($9229.43 \pm 383.38\ \mathrm{g}^{-2}$) in all media. Media significantly differed (multifactor ANOVA, $p < 0.001$) for celery biomass (Figure 5i, ii). The mixed media had 11158.48 ± 411 f g^{-2} and $1157.14 \pm 45.05\ \mathrm{g}^{-2}$ higher than unmixed (7300.38 ± 248.05) and (751 ± 24.46) wet and dry biomass, respectively. The mean wet biomass did not differ between mixed clay pebbles, clay bricks and sand with a mean range of 13241.3 ± 423.70 to $13458.3 \pm 336\ \mathrm{g}^{2}$. The mixed sand and gravel range was 8350.6 ± 160.20 to $10685.3 \pm 452\ \mathrm{g}^{-2}$. The unmixed recycled plastic had the lowest mean wet biomass of $64{,}841 \pm 423.70\ \mathrm{g}^{-2}$, corresponding to the dry biomass, respectively (Figure 5i, ii).

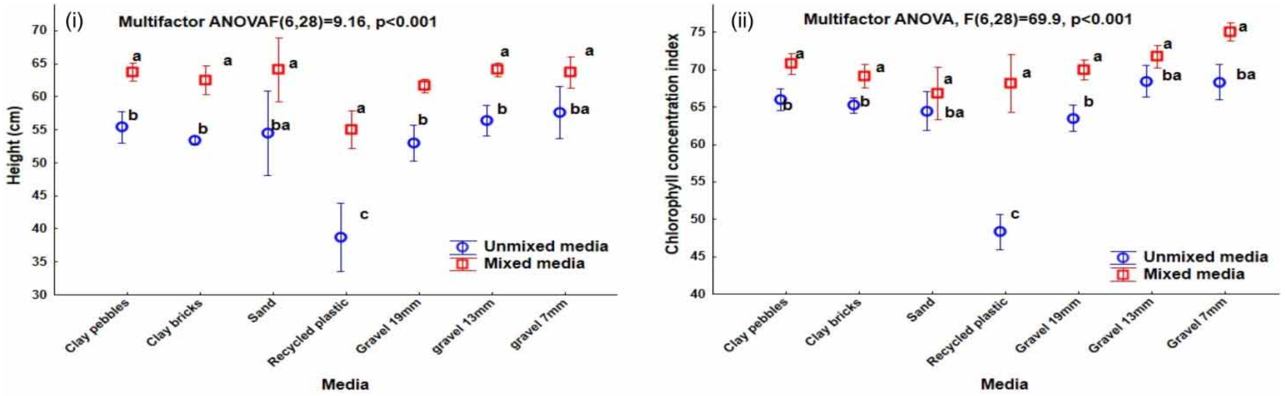


Figure 4 | Means ($\pm$95% confidence interval) of celery height and CCI (i– ii) in the unmixed and mixed media irrigated with post-AD brewery effluent for 12 weeks in the ebb-and-flow systems. Treatments with similar letters next to the mean are not significantly different.

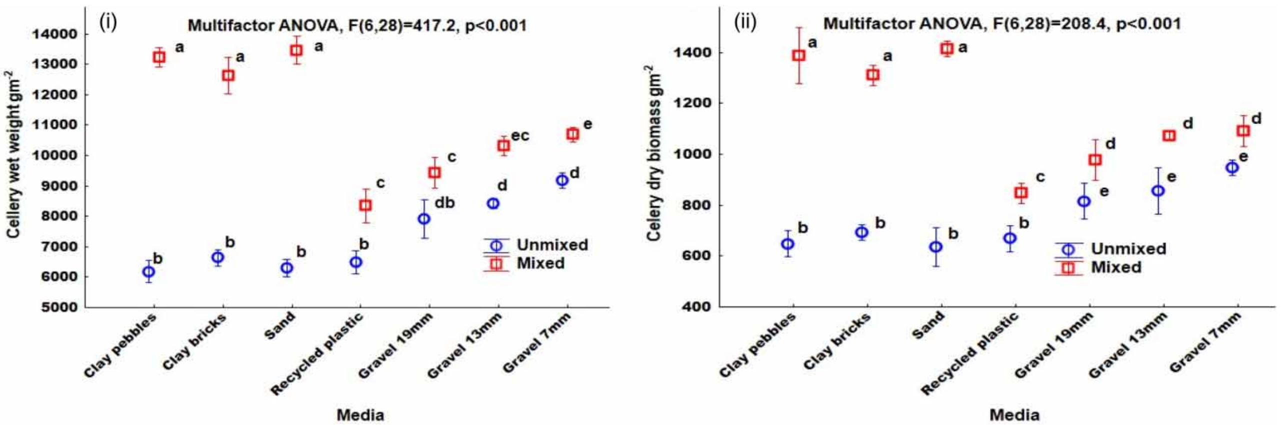


Figure 5 | The mean ($\pm$95% confidence interval) wet and dry biomass of celery grown in unmixed and mixed media and irrigated by post-AD brewery effluent for 12 weeks in ebb-and-flow systems.

LEAF CHEMICAL ANALYSIS

The seedlings' composition and elemental uptake of celery grown in the media treatments in an ebb-and-flow system for 12 weeks are illustrated in Figure 6i–xi. The percentage uptake of nitrogen, calcium, zinc, manganese and sodium increased from seedlings in celery leaves grown in all media treatments. Leaf potassium, copper, iron and aluminium decreased in the media, except for unmixed sand with increased leaf iron (Figure 6vi). The mean leaf sodium concentration increased from seedlings 13,040 $\pm$ 62.5 to 37824.3 $\pm$ 2121.7 mg kg^{-1} and 41047.6 $\pm$ 1822.9 mg kg^{-1} in unmixed and mixed media, respectively (Figure 6ix).

An interaction between media and mixing did not affect the uptake of leaf nitrogen, calcium, potassium, manganese and sodium (multifactor ANOVA, $p > 0.05$, Figure 6i, iii, iv, vii, ix). The mean leaf nitrogen was lowest in unmixed recycled plastic media (2.33 $\pm$ 0.01%). Leaf nitrogen did not differ in clay pebbles, clay bricks and sand media treatments (Figure 6i). Leaf calcium, potassium, manganese and sodium concentrations did not differ between treatments (Figure 6iii, iv, vii, ix).

Leaf phosphorus, potassium and magnesium were below 5% in all media. Unmixed sand had the highest mean leaf iron of 247.63 $\pm$ 11.8 mg kg^{-1} (Figure 6vi). The leaf zinc, copper and aluminium concentrations were similar in all media (Figure 6vii, x, xi).

CELERY HEALTH AND STRESS SYMPTOMS

The mortality of seedlings in the first month of planting was significantly higher in unmixed than mixed media (Kruskal–Wallis H (1, $N = 48$, $=12.1$, $p < 0.05$)). There was unequal variation in percentage mortality between media

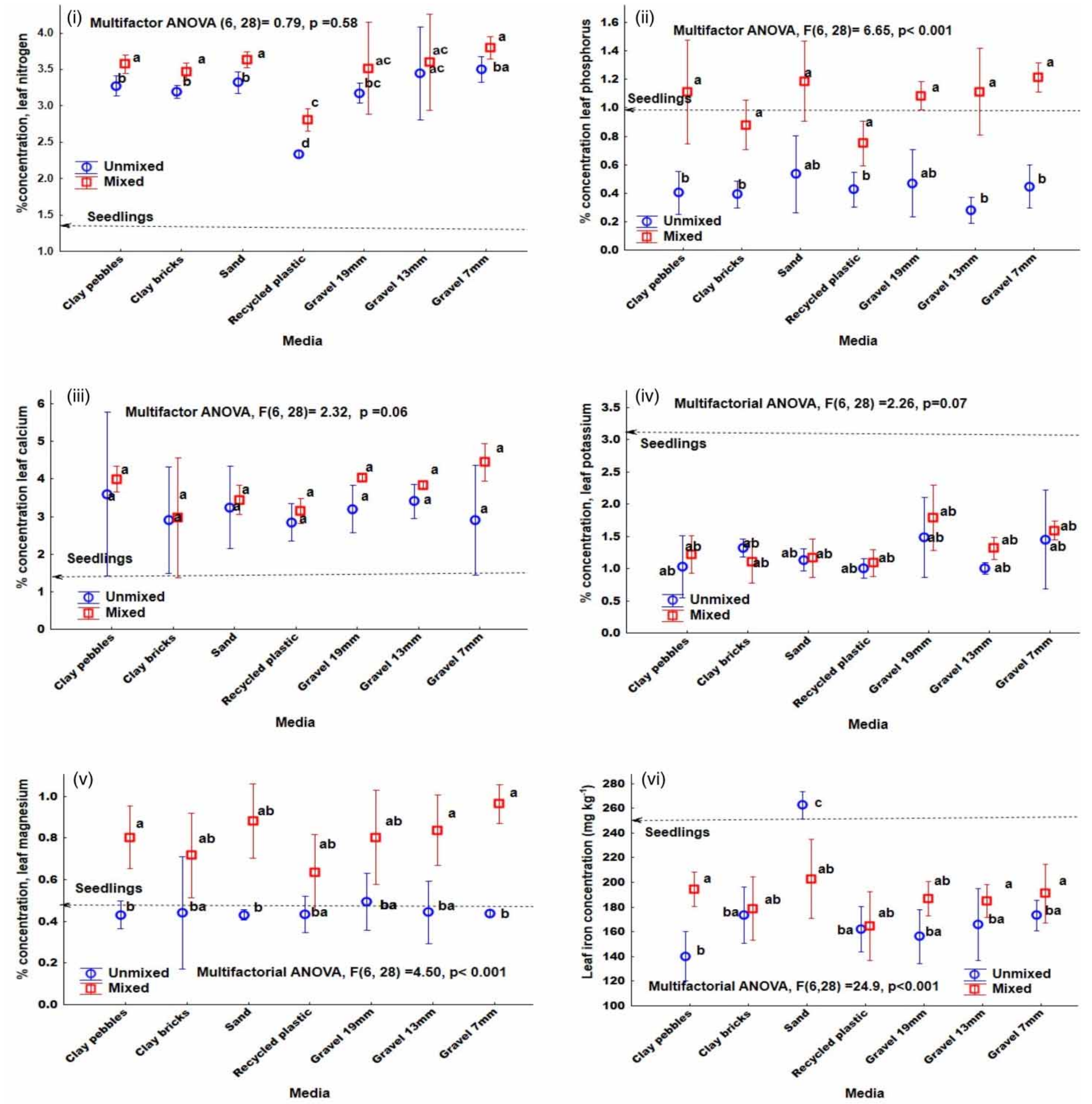


Figure 6 | Mean (±95% confidence interval) of (i–xi) element uptake in celery leaves grown in the mixed and unmixed media of ebb-and-flow system and irrigated by post-AD brewery effluent for 12 weeks. The dotted line designates the seedlings' element concentration. *(continued).*

(Kruskal–Wallis H (7, $N = 48$, $= 8.7$, $p = 0.28$)). The pale yellow top leaves were observed among all media (Plate 1 (a–d)). Plants grown in recycled plastics had short stature, chlorosis, shrink and hard leaves with burnt tips. Plate 1 (b, e), and pale yellow in the unmixed clay bricks and gravel media (Plate 1 (a, c, d)).

DISCUSSION

The ebb-and-flow wetland operation strategy and choice of substrate and celery growth influenced nutrient removal from a post-AD effluent. The higher outflow DO concentration than the inflow was attributed to subsequent system drain cycles,

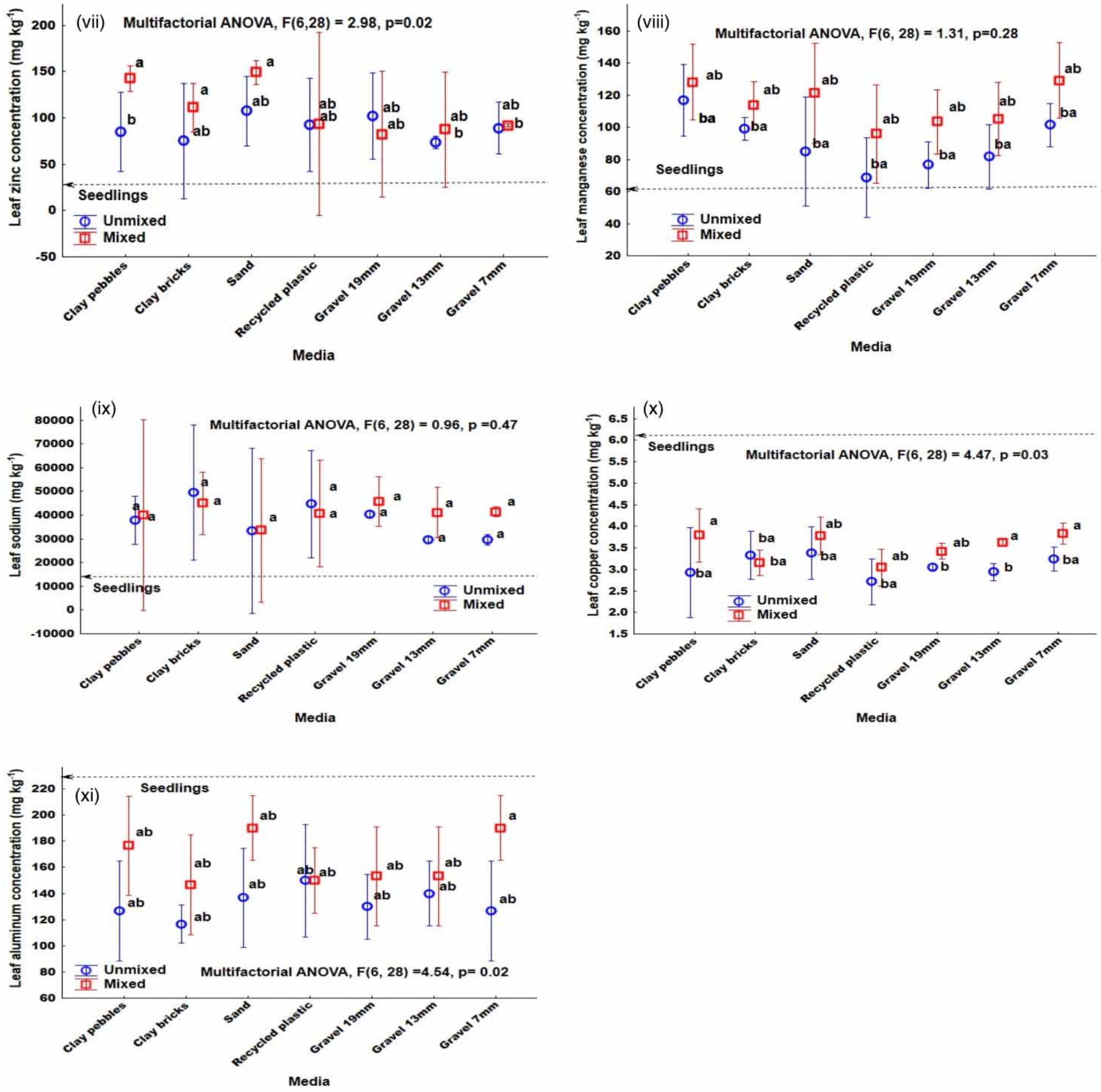


Figure 6 | continued.

contributing to oxidation, transformation and degradation of nutrients in the ebb-and-flow CWs. Austin (2006) and Behrends *et al.* (2000) reported that the draining phase of a tidal CW rapidly diffuses atmospheric oxygen in the biofilm of the substrate. The rate of diffusion varies depending on media porosity and surface area (Akratos & Tsihrintzis 2007; Almuktar *et al.* 2018; Fu *et al.* 2020).

The highest COD reduction and ammonia removal in unmixed clay pebbles, followed by bioballs and 19 mm gravel could be attributed to the physical properties of these media. Clay pebbles and bioballs are lightweight and less compacted, clay pebbles have air spaces that trap more oxygen, for the oxidation of COD and ammonia-N. The 19-mm gravel is dense with large interstitial spaces and better effluent drainage during the ebb-and-flow operation. Gravel media porosity and effluent drainage decreased with increased pebble surface area. Sand is compacted with limited air spaces and poor effluent drainage. Highly porous media are linked to better aeration, while compacted media is related to low O_2 levels. Aerobic and anaerobic conditions created by the ebb-and-flow operations in the substrate transform ammonia-N to nitrite-N and nitrate-N through adsorption and desorption processes (Austin 2006; Gregory *et al.* 2012). Moreover, oxygen distribution within the substrate is stratified; the surface and middle layers are highly aerated, while the bottom layer can be anoxic (Saeed & Sun 2012; Huang *et al.* 2013), creating a range of environmental conditions for enhanced nutrient removal.

The superior performance of mixed media in removing nitrite, nitrate, total nitrogen and orthophosphate is due to varied porosities and surface area, creating varied DO conditions facilitating oxidation, transformation and nutrient removal. A combination of substrates' physical and chemical properties has complementary benefits. For example, granular activated carbon has a large surface area creating more adsorption sites thus contributing to nutrient removal (Wu *et al.* 2019; Fu *et al.* 2020). The supplemented pine bark media is a carbon source for denitrification (Saeed & Sun 2013; Hua *et al.* 2016). Saeed & Sun (2011) reported 97.8% total nitrogen removal in combined gravel and organic wood mulch in vertical wetlands with *Phragmites australis* plants. Fan *et al.* (2013) reported a simultaneous removal of 99% ammonia-N and 90% total nitrogen with intermittent aeration at an influent COD: N ratio of 1: 10 in vertical CWs. The difference between these studies could be synthetic wastewater treated on a laboratory scale while the current study used a unique brewery effluent with varied effluent concentrations over time.

The low orthophosphate removal in unmixed media could be associated with high competition of nutrients on the limited adsorption sites. The mixed media of clay and sand have aluminium (Al^{3+}), calcium (Ca^{2+}), iron (Fe^{3+}) and magnesium (Mg^{2+}) exchangeable cations while granular activated carbon has more adsorption sites (Rittmann *et al.* 2011; Fu *et al.* 2020; Marlena & Nowicki 2022). Recently, studies have indicated that denitrifying polyphosphate accumulating organisms (dPAO) can store phosphorus in the cells under alternate aerobic and anaerobic conditions (Winkler & Straka 2019; Shukla *et al.* 2020). Therefore, the identification and isolation of dPAO strains should be considered in future studies of ebb-and-flow operations.

Nutrient removal increased with similar trends in all media, followed by a phase of reducing efficiency. The increased nutrient removal phase could be due to progressive biofilm formation on the substrate improving microorganism activity. The alternate aerobic and anaerobic conditions favour the growth of diverse microorganisms depending on the media type used (Liu *et al.* 2020; Zhang *et al.* 2021).

A decreased nutrient removal efficiency could be associated with excessive biofilm formation and pollutants concentrating on the adsorption sites over time, blocking and saturating the media (Hua *et al.* 2013; Vymazal 2018; Zhou *et al.* 2020). Liu *et al.* (2014) reported media saturation in a long-term operation of tidal flow CWs treating high ammonium effluent, consistent with this study. The brewery's effluent composition varies over time depending on the season of beer production. Moreover, the high conductivity and pH of the post-AD effluent can influence the performance of the ebb-and-flow in this study. Therefore, screening and layering of substrates have been identified as anti-blockage mechanisms for enhanced spatiotemporal wastewater treatment (Wu *et al.* 2019; Liu *et al.* 2020).

CELERY MEAN HEIGHT, CHLOROPHYLL CONCENTRATION AND BIOMASS

Celery growth in height, chlorophyll content and biomass at the end of the study indicates that celery adapted to post-AD irrigating effluent, media types and ebb-and-flow operation. However, celery grown bioballs media died 4 weeks after planting while those in recycled plastic media had the lowest mean height. The plastic media are lightweight with irregular spaces that cannot support celery roots in the ebb-and-flow systems. The similar celery height in sand and 7-mm gravel media can be related to their density and stability, supporting celery roots. Moreover, its large surface area increased the surface contact, enhancing nutrient retention capacity for celery uptake (Calheiros *et al.* 2008; Ge *et al.*, 2015).

The CCI indicates plant health and productivity. A higher chlorophyll concentration and above-ground biomass of celery grown in mixed media could be attributed to the irrigating effluent nutrient composition and supplemented media properties. The unmixed media solely relied on the nutrients from the irrigating effluent, which could be deficient in organic carbon, an essential plant growth promoter. Wu *et al.* (2015) reported that industrial effluents have varied organic matter content and limited labile carbon depending on the raw materials and pretreatment processes. Therefore, post-AD effluent supplemented with organic carbon can improve celery productivity.

LEAF CHEMICAL COMPOSITION

At the end of the study, leaf nitrogen, calcium, zinc, manganese and sodium concentrations were higher than in the seedlings in all media. The increased leaf nitrogen content could be attributed to the uptake of ammonia from the inflowing wastewater and nitrate as transformed ammonia products by the ebb-and-flow operation. The forms of nitrogen for plant uptake are ammonia and nitrate-N, with ammonia-N being preferred (Vymazal 2007). An increase in nutrient uptake corresponded to celery height, CCI and above-ground biomass in mixed media treatments.

The increased leaf sodium and calcium uptake is expected due to high concentrations of dissolved salts from the effluent source. Sodium hydroxide, sodium chloride, caustic soda and calcium sulphate are used as pH buffers and cleaning detergents in brewing upstream activities, increasing the effluent salinity (Simate *et al.* 2011; Cilliers 2012). Celery is a halophyte adapted to saline conditions by synthesising mannitol, a photosynthetic product that increases osmotic potential (Everard *et al.* 1994; Noiraud *et al.* 2000). High salinity levels increase the uptake of calcium and sodium ions in the plant cell (Zhao *et al.* 2021). Similarly, Pardossi *et al.* (1999) reported a high salt tolerance in celery, by comparing salinity levels of 50, 100 and 300 mM sodium chloride on celery growth and mineral content. Therefore, the high sodium and calcium contents of celery grown in this study increase celery's potential use as a bio-indicator and phytoremediator of a saline effluent quality.

The high leaf iron content in unmixed sand could be due to the composition of ferric iron, an exchangeable cation increasing the uptake (Arias *et al.* 2001). The decreased leaf phosphorus from the seedlings stage could be due to competition between plant uptake, microorganisms' activities and adsorption on the media. Phosphorus binding properties could increase adsorption on surfaces with high cation exchange in clay pebbles, sand and granular activated carbon (GAC) (Vohla *et al.* 2011; Lima *et al.* 2018). A decrease in leaf potassium, copper and aluminium could be related to the high salinity of irrigating effluent hindering the uptake of these microelements. Sodium and potassium are monovalent cations, at high salinity sodium ions limit the uptake of potassium ions at plant roots (Pardossi *et al.* 1999; Jouyban 2012). Potassium ions are essential for enzyme activities and maintaining cell turgidity, a deficiency limits plant growth and metabolism (Jouyban 2012). This is the first study of celery grown in a brewery effluent and adjusting the post-AD effluent pH and salinity could improve nutrient bioavailability and uptake.

Celery plant health and stress symptoms

Plant health and stress symptoms exhibited by visual indicators relate to plant productivity. Celery grown in bioballs media died while plants in recycled plastic stress symptoms. The bioballs and recycled plastic are lightweight media limiting the support of plant roots during ebb-and-flow operation (Yang *et al.* 2018). The top leaves chlorosis could be due to low phosphorus, potassium and iron uptake.

The pale yellow celery leaves grown in unmixed clay bricks could be caused by excessive iron uptake. Clay bricks are rich in ferric iron and are susceptible to cation exchange (Gu *et al.* 2019). Aslam & Chouhan (2023) reported iron toxicity in soils high in pH to limit the uptake of manganese, zinc and copper thus, causing chlorosis, necrosis and slow growth. This can be mitigated by adjusting soil pH, using iron-tolerant plants and chelation. A decrease in leaf copper, iron and aluminium concentrations could be a deficiency from the irrigating effluent. Rakocy *et al.* (2006) recommended supplementing the limiting nutrients to meet plant requirements in a hydroponic system. Overall, this study demonstrated the removal efficiency of comparable media and celery growth on nutrient removal as a potential for treating post-AD brewery effluent.

CONCLUSION

The ebb-and-flow operation, media type and celery growth influenced post-AD effluent treatment in a single-pass system design. The unmixed media of clay pebbles had the highest mean COD reduction of 7.51% and ammonia at 8.13%, followed by bioballs and 19 mm gravel. Mixing improved the removal efficiency of nitrite-N (7.6%), nitrate-N (15.3%), TIN (29.9%), orthophosphate (12.7%) and plant biomass (11158.48 g^{-2}). Bioballs and recycled plastics cannot support celery growth in the ebb-and-flow CW. Media porosity, surface area, chemical composition and celery plant enhanced nutrient removal through synergistic interactions.

RECOMMENDATION

The study recommends unmixed clay pebbles, bioballs and 19-mm gravel for improved ammonia-N removal and mixed media for multiple pollutant removal and celery productivity. Further studies should explore the ebb-and-flow system's performance under a wider range of environmental conditions. Future studies should examine the spatiotemporal effects of substrate layering on nutrient removal. The optimal salinity level, celery planting density and nutrients in a brewery effluent should be determined.

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AUTHORS CONTRIBUTIONS

Dr R. T. and Prof. H. K. conceptualised the whole article, described the study design and prepared data analysis. E. O. supported in experiment set-up, data collection and analysis and writing. Prof. D. L., Prof. J. M., Dr. S. A. and Dr. N. M. reviewed manuscript drafts.

DATA AVAILABILITY STATEMENT

All relevant data are available from https://docs.google.com/spreadsheets/d/1ulhQxwLet_ins7_biZtzbbrIPiWhuLzS/edit?usp=sharing&ouid=101275453203082863687&rtpof=true&sd=true.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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