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Phytoremediation of olive mill wastewater with *Vetiveria zizanioides* (L.) Nash and *Cyperus alternifolius* L.

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ABSTRACT

Olive Mill Wastewater (OMW) contains high concentrations of contaminants, including organic, nitrogen, and phenolic compounds that are extremely harmful to the environment and human health. The key purpose of this study was to remove total organic carbon (TOC), total nitrogen (TN), and phenolic compounds (TP) from OMW using floating wetland planted with *Vetiveria zizanioides* (L.) Nash (vetiver) and *Cyperus alternifolius* L. (umbrella palm) species. A total of eighteen floating wetlands were constructed. Twelve tanks were planted with vetiver and umbrella palm while another six tanks were maintained as unplanted controls. Experiments were conducted with wastewater volume of 56 L for 67 days using 5% (OMW-5) and 15% (OMW-15) treatments of OMW in a greenhouse. The highest TOC, TN, and TP removal efficiencies were found to be 95.3 ± 0.01, 82.7 ± 2.55, and 98.8 ± 0.07% in umbrella palm planted OMW-5, while the removal efficiencies were 84.9 ± 0.38, 92.7 ± 0.37, and 38.9 ± 1.97% in vetiver planted OMW-5. Similarly, the TOC, TN, and TP removal efficiencies in OMW-15 were 89.3 ± 0.28, 40.86 ± 1.73, and 96.8 ± 0.18% with umbrella palm and 89.1 ± 0.70, 23.7 ± 1.27, and 92.1 ± 0.41% with vetiver. The plants accumulated trace elements, especially in the roots, with the order of Fe > Mn > Cu > Zn > B > Pb > Cr > Ni > Co > Cd for umbrella palm. The umbrella palm shoot phenol content was found to be 2358 ± 201 and 1421 ± 198 mg/kg in OMW-5 and OMW-15, respectively. Overall, this study revealed that floating wetlands planted with vetiver and umbrella palm species have the potential to be used as a green treatment method to treat diluted high strength OMW.

1. Introduction

Clean and adequate water become one of the crucial valuable resources. Growing industrialization and population results in the production of ever increasing amounts of wastewater. The natural water bodies are considerably contaminated by industrial wastewaters. Olive mill wastewater (OMW) is among those that causes concern because of a large amount of high-strength wastewater production seasonally in a relatively short period of time. In turn, their treatment, management, and storage are also challenging issues. Ninety-seven percent of the world's total olive oil is produced in the Mediterranean, e.g. Algeria, Egypt, Greece, Italy, Morocco, Portugal, Spain, Syria, Tunisia, and Turkey, resulting in an estimated annual OMW of 30 million m^3 (Halalsheh et al., 2021). The OMW is described as one of the most difficult

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industrial effluents to treat and dispose due to its complex physicochemical characteristics, with high biological oxygen demand (BOD), chemical oxygen demand (COD), total organic carbon (TOC), phenolic and polyphenolic compounds, nitrogen, sugar, lipid, strong unpleasant smell, dark brown color, total solids, and acidic pH (Hamimed et al., 2021). Phenolic compounds may pose serious health effects on humans, that can be both chronic and acute. Exposure to phenolic compounds can lead to coma, irregular breathing, respiratory arrest, tremor, and weakness of muscles at high doses, while irritation of the central nervous system, gastrointestinal, kidney, and liver may occur with chronic exposure to lower concentrations (Villegas et al., 2016). Phenolic compounds may affect groundwater and soil since it may decrease the soil porosity, affect germination of seeds, and may contaminate the groundwater by leaching through soil (Mandal et al., 2020). Removal of nitrogen species (ammonia, nitrite, nitrate, and organic bound N) from wastewater is also important to protect water resources as they cause eutrophication that result in depletion dissolved oxygen level in water bodies (Du et al., 2015). Contaminant levels notably depend on type of olive, harvesting time, type of fertilizer, type of soil, extraction method, and climatic conditions (Al-Bsoul et al., 2020). Because a large number of Small and Medium Enterprises (SMEs) are involved, direct discharge of OMW into surface waters and surrounding lands without pre-treatment had been an issue, and has been banned in many countries since the complex content of organic and phenolic compounds have ecotoxicological effects that impact the biodegradation processes and plant growth. The discharged untreated OMW may also reach groundwater sources through infiltration from soil and cause groundwater contamination (Bani-Hani et al., 2018) along with socio-economic impact due to its strong offensive smell. Therefore, there is a need for effective OMW treatment technologies that are sustainable for SMEs.

Several treatment technologies have been proposed to treat OMW, and minimize its harmful effects on the environment, such as adsorption, biological processes that include decomposition of chemicals by microorganisms, chemical precipitation, coagulation/flocculation, electrocoagulation, extraction, membrane technologies, oxidation, bioremediation, and hybrid processes (Lee et al., 2019). However, most of these techniques suffer from different shortcomings including, high operation-maintenance and energy costs, requirement of large space, high amount of sludge and toxic by-product formation, scaling and fouling, and insufficient removal efficiency (Hamimed et al., 2021; Lee et al., 2019).

Recently, constructed wetlands have gained a notable interest because it is a cost effective, environmental friendly, aesthetically attractive, and efficient treatment process (Panja et al., 2020a) for organic and inorganic matter, heavy metals, macro and micro nutrients, and color. In constructed wetlands, the removal of contaminants may occur with biological, chemical, and physical mechanisms. Their treatment performance mainly affected by system design, type of pollutants, plant species, weathering factors, retention time, and root zone interaction (Davamani et al., 2021). The floating wetlands are the most promising constructed wetland type due to its relatively lower cost, smaller area requirement, and ease of design compared with conventional wetlands (Chandanshive et al., 2020). In a floating wetland, the system is designed to place the plants over the surface of the wastewater considering the maximum contact of rhizosphere with the wastewater for effective treatment (Kale et al., 2015). The plant to be used is the main key parameter because it should be able to tolerate high concentrations of toxic contaminants.

To date, several studies have been conducted to treat soil and water using almost 400 different plant species (Richa et al., 2020). *Vetiveria zizanioides* (L.) Nash (vetiver) and *Cyperus alternifolius* L. (umbrella palm) have favorable features to tolerate high concentration of contaminants and extreme conditions, which makes them successful vegetative candidates in phytoremediation processes (Ali et al., 2020). The two plants have been tried for removal of heavy metals, organic compounds, nitrogen, and phosphorus in sewage wastewater (Parnian and Furze, 2021) showing their potential for municipal wastewaters.

Although numerous studies were conducted on the wastewater treatment performance of vetiver plants, to our best knowledge, it was not investigated for OMW treatment. Therefore, this work aimed to contribute by demonstrating a new area of potential use for vetiver and umbrella palm plants, to propose a new sustainable alternative for treatment of OMW that can be effective on macro and micro nutrients, toxic elements (potentially toxic elements, PTEs, here on), organic compounds, and phenolic compounds. In this perspective, there is also a potential for producing a valuable biomass. Specific objectives of this study were (i) to investigate the use of floating-constructed-wetland planted with vetiver and umbrella palm to remove TOC, nitrogen, and phenolic compounds from OMW, and (ii) to evaluate effect of dilution ratio on the treatment performance on OMW, nutrient and PTEs uptake capacities, and plant growth.

2. Material and methods

2.1. Chemicals

Folin–Ciocalteu's phenol reagent 2N (F9252), trans p-coumaric acid (55823), vanillic acid (68854), 2-(3,4-dihydroxyphenyl) (91404), 4-hydroxytphenly ethanol (79058), hydrochloric acide at 37%-extra (07102) were purchased from Sigma Aldrich. Ethyl acetate at 99.5% grade; methanol, acetonitrile, n-hexane at HPLC grade, sodium sulfate anhydrous and ortho-phosphoric acid at 85% grade were purchased from Merck.

2.2. Characterization of raw olive mill wastewater (OMW)

OMW was obtained from a local olive mill facility located in Izmir, Turkey. OMW samples were stored in a polyethylene container in a refrigerator (at 4 °C). The OMW was analyzed before phytoremediation process to evaluate the total phenol (TP), total nitrogen (TN), and total organic carbon (TOC) concentrations since these parameters may be considered as the main pertinent components. The OMW was at a pH of 5.12 (±0.001), COD of 103 (±3.53) g/L, TN of 0.51 (±0.001) g/L, TOC of 23.0 (±0.28) g/L, and TP of 13.2 (±0.56) g/L. 4-hydroxytphenly ethanol, 2-(3,4-Dihydroxyphenly) ethanol, vanillic acid, and trans p-coumaric acid concentrations were 270 (±16.97), 85.5 (±1.34), 28.9 (±6.22), and 17.4 (±3.11) mg/L, respectively.

2.3. Acclimatization of Vetiveria zizanioides (L.) Nash and Cyperus alternifolius L.

The plants of vetiver and umbrella palm were picked from Aegean Forestry Research Institute in Izmir, where they are grown for research. The plants were placed in soil environment to let them adapt and rooted for two weeks. For further acclimatization, the plants were removed from soil and placed in a 60 L dark-colored plastic tanks containing 56 L of hydroponic solution with fertilizer (N:P:K = 15:15:15 and pH = 6–7) without sunlight, for six weeks until sufficient fresh shoot and root development were observed in solution (Hoagland and Arnon, 1950; Panja et al., 2020b; Mirzaee et al., 2021). Then, the specified amount of cultivated plants (2.5 and 4.5 kg) were harvested for use in the experiments.

2.4. Experimental set up and procedure

A total of 18 dark-colored tanks with dimensions of 40 cm in diameter, 60 cm in depth, and 60 L in volume were constructed, and operated in a greenhouse at batch mode. The surface of tanks was sheltered using greenhouse nylon to prevent evaporation of water sample and intrusion of sunlight. Control tanks were prepared in two different configurations: Unplanted OMW and planted with tap water to measure volatilization and growth respectively (in the Supplementary Material (SM) **Fig. S1**).

Primarily, the tolerance level of plants to different dilutions were investigated at five levels; 5% (OMW-5), 10% (OMW-10), 20% (OMW-20), 30% (OMW-30), and 40% (OMW-40). Tap water was used for dilution. pH was adjusted to 6–7. Experiments were conducted at dilution ratios of 5 and 15% because the lowest feasible dilution ratio was determined to 15% (see Section 3.1) after a preliminary experiment. After the acclimatization period, a specified amount of OMW samples (OMW-5 and OMW-15) was measured for 56 L for each tank where a specified amount of vetiver were used for treatment of 56 L OMW, then another tank was filled with the OMW without vetiver as the control, and these procedures were repeated with the umbrella palm. According to preliminary experiments, the amount of plants required for an efficient treatment was determined as 2.5 kg in OMW-5 and 4 kg in OMW-15. Experiments were conducted in simultaneous triplicates of both cases and controls to determine the TP, TN, TOC, micro and macro nutrient, and PTE removal performance of the plants. No fresh OMW sample and nutrients were added through the experimental runs to obtain a constant pattern of contaminant removal. The OMW in each tank was sampled over a 67-day period, on days 1, 9, 17, 25, 45, 53, 59, and 67. The amount of evaporation by measuring the water level, and pH were measured daily.

2.5. Analytical methods

pH, COD, TOC, TN, and phenolic compounds (tyrosol, hydroxy tyrosol, vanillic acid, syringic acid, and p-coumaric acid) were measured in raw and treated OMW samples. The details of these analytical methods used for the above parameters are presented in **SM Text S1**. Information on moisture, micronutrients, macronutrients, and PTE analyses are presented in **SM Text S2** (ISO, 1993).

Plant growth (stem heights, root length, and biomass amount) were determined at the beginning and end of the experimental runs. The plant biomass amounts were as total wet weight at the beginning and end of the experiments, while the dry weight was also determined for shoots and roots at the end of each experiment. For dry weight, the plant samples were weighed and incubated at 65–70 °C for 24–42 h to remove water before final weighing. The specific growth rate (SGR) period is defined as the rate of increase of biomass of a cell population per unit of biomass concentration (Bhatia, 2015). The SGR of plants were calculated using dry weight differences between the beginning and end of the experiments. SGR was determined using Eq. (1) (Seroja et al., 2018):

$$\mathrm{SGR}\ (\%) = (\ln X_f - \ln X_i)\,/(t - t_0) \times 100 \tag{1}$$

where, X_i and X_f are the initial and final dry weights of the plants in grams, respectively; t_0 is the initial time of observation (day); and t is the treatment duration.

Table 1
The tolerance of plants in different concentrations of OMW.

Parameter	OMW-5	OMW-10	OMW-20	OMW-30	OMW-40
pH	6.55	6	7.78	7.61	7.22
t-phenol (mg/L)	900	1400	3620	5470	10160
TOC (mg/L)	717	1062	4484	10110	12093
***Cyperus alternifolius* L.**					
Plant shoot color	Green	Green	Green–yellow	Green–yellow	Yellow
Plant root color	White	White	White–brown	Brown	Brown
New root and shoot	Yes	Yes	Yes	No	No
***Vetiveria zizanioides* (L.) Nash**					
Plant shoot color	Green	Green–yellow	Yellow	Yellow	Yellow
Plant root color	White–brown	White–brown	Brown–black	Black	Black
New root and shoot	Yes	Yes	No	No	No

3. Results and discussion

3.1. Plant tolerance to OMW

The OMW dilution ratio and plant species are the two most important parameters on treatment of OMW using phytoremediation. Therefore, the effect of OMW dilution on vetiver and umbrella palm was investigated in this study. The two species were exposed to OMW at different dilution ratios for 67 days, and the impacts of OMW on plant development, growth, and tolerance were monitored (Table 1). As anticipated, the TP and TOC concentrations were increased with increasing OMW concentration. The highest TP and TOC concentrations were found to be 10,160 and 12,093 mg/L at OMW-40, while the lowest TP and TOC concentrations were 900 and 717 mg/L at OMW-5.

It was concluded that the OMW-5, OMW-10, and OMW-20 applications showed no inhibition on umbrella palm, allowing growth of fresh white roots and green shoots forth thanks to relatively low TP and TOC content and sufficient nutrient content. On the other hand, there was a negative impact of the OMW-30 and OMW-40 applications on the plant causing green–yellow and yellow shoot color, brown root color and no growth of fresh roots and shoots, which was most probably due to the higher TP and TOC concentrations in OMW-30 and OMW-40 samples, which unbalances the protein concentration and decreases the productivity of plants with photosynthesis inhibition (Rusan et al., 2015). Briefly, the growth and tolerance of umbrella palm decreased with increasing TP and TOC concentrations. For instance, the plant shoot color changed from green to yellow with increasing TP and TOC concentrations from 900 (OMW-5) to 10160 mg/L (OMW-40) and 717 (OMW-5) to 12093 mg/L (OMW-40), respectively. Furthermore, the visual observations about the impact of OMW-5 and OMW-40 on growth of umbrella palm are presented in **SM Fig. S2a** and **S2c**. The root and leaf chlorosis and no production of fresh shoots and roots were visually observed with decreasing OMW dilution ratio from OMW-5 to OMW-40.

The application of OMW-20, OMW-30, and OMW-40 samples indicated considerable physiological or morphological inhibition effect on the vetiver. Exposure to low OMW dilution ratios resulted with black plant root color, yellow plant shoot color, and no production of fresh roots and shoots. On the other hand, the plant showed a fresh root–shoot production and green shoots in OMW-5 and OMW-10. At the end of the operating time of 67 days, the morphological changes in vetiver, which were exposed to OMW-5 and OMW-40, are presented in **SM Fig. S2b** and **S2d**. Comparison of the two plants showed that umbrella palm can tolerate higher amounts of OMW. For instance, the root color of umbrella palm and vetiver plants were observed as white–brown and brown–black in OMW-20, respectively. Moreover, there was no fresh shoot and root production for vetiver with OMW-20, while there was new shoot and root formation for umbrella palm plant. These results revealed that umbrella palm is a promising plant that can be utilized for relatively higher concentrations of OMW application compared with vetiver. Umbrella palm produced new roots and shoots while existing roots well tolerated the OMW-20, whereas vetiver did not tolerate well and produced no roots and shoots. For OMW-10, however, vetiver well tolerated and produced shoots and roots while umbrella palm showed no inhibition on growth. Due to the difference in responses of the two plants at 10 and 20%, a dilution ratio of 15% was selected as the upper level, and the rest of the experiments were performed using OMW-5 and OMW-15 samples.

3.2. Variation in pH

The initial pH of OMW is another important operating parameter affecting plant growth and OMW removal efficiency. Therefore, a preliminary study was conducted to investigate the effect of pH on vetiver and umbrella palm plants. For experiments, the pH of tap water was adjusted from 4 to 9, and growth of plants was monitored. Results revealed that both of the plants continued their growth in pH range of 6–8. At lower and higher pH values, the growth of plants decreased most probably due to the alkalosis and acidosis. It has also been reported that the exposure of plants to extreme conditions (acidic and/or alkali) causes oxidative stress by the formation of hydroxyl radicals and hydrogen peroxide, which leads to considerable detrimental impacts on plants including, alteration of gene expression and inhibition of enzyme and

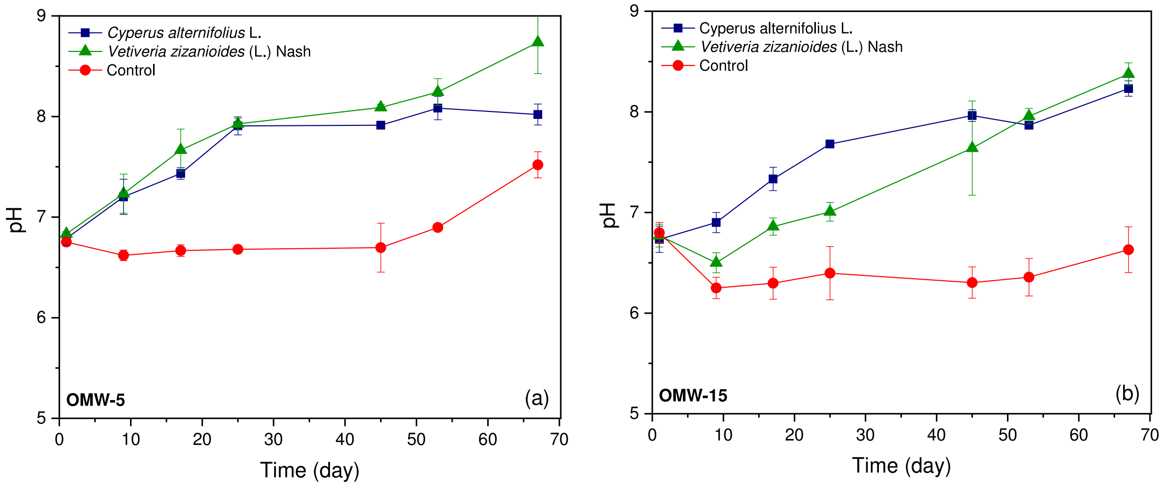

Fig. 1. Variation in pH in (a) OMW-5 and (b) OMW-15 for 67 days.

protein secretion activity (Thakur et al., 2021). Darajeh et al. (2016) studied treatment of palm oil mill wastewater using *Chrysopogon zizanioides* (L.), and reported that the maximum removal performance of the plant was achieved at pH 5 while it was decreased with increase to pH of 7, and dipping down to no removal at pH of 12. Consequently, they reported that the pH value in the range of 5–9 is suitable for plants to treat wastewater. In accordance, pH of OMW-5 and OMW-15 samples were not adjusted at the beginning of the experiments since the initial pH of the solutions was found to be around 7. The pH, in general, tended to increase both for OMW-5 and OMW-15, and controls (Fig. 1).

OMW-5 and OMW-15 samples demonstrated an initial neutral pH of almost 7.0. A negligible pH change (6.8–7.5) was observed in control groups. On the other hand, pH was increased with both of the plants both in OMW-5 and OMW-15. The pH of the OMW-5 sample with vetiver plant considerably increased from 6.83 to 8.74 at the end of the experiment, while for umbrella palm the increase was from 6.8 to 7.95. Similar trend observed for pH values of OMW-15 samples. At the end of the experiments, the pH values of the OMW-5 and OMW-15 samples were stable between 7.5–8.0, while pH in the control groups was stable between 6.8–7.5, indicating that the plants increased the pH of the medium slightly. The slight increase in pH for both plants occurred most probably due to the photosynthesis. Overall, the results indicated that there were no detrimental impact of pH on the all groups during the experiments. These results were in good agreement with literature. Effendi et al. (2020) studied ammonia and orthophosphate removal from tilapia cultivation wastewater using *Vetiveria zizanioides*. They reported that pH of the wastewater was increased from 6.9 to 7.0 at the end of the treatment process. *Vetiveria zizanioides* was used for compost leachate treatment; the effluent pH was found to be 7.4 after treatment, while initial pH of the wastewater was 6.2 (Bakhshoodeh et al., 2017). Mahmoudpour et al. (2021) investigated phytoremediation performance of vetiver grass (*Chrysopogon zizanioides* (L.) Roberty) for synthetic sewage wastewater treatment. They found that neutral initial pH (7.02) was changed into alkaline (8.15) at a phytoremediation time of 31 days, while, the change in pH was not as large for phytoremediation of a paper mill wastewater with vetiver (*Chrysopogon zizanioides*), which increased from 8.18 to 8.53 (Davamani et al., 2021).

3.3. Water uptake capacity

Water usage in the phytoremediation experiments is generally described using water uptake by plants and evaporation from water surface. The water uptake by plants is described using transpiration rates. Transport of water across cell membranes is a basis requirement for regulating plant–water interaction. The insufficient water uptake is one of the most important environmental stress on plants causing decrease in photosynthesis and growth, hormonal variation, and oxidative damage, which are directly related to tissue dehydration (Aroca et al., 2012). When plants being dehydrated, leaves start closing their stomata, which cause stress-induced growth reduction. Therefore, plant water uptake is an important aspect in phytoremediation experiments. However, the phytoremediation experiments in this study was conducted in the hydroponic environment, thus plants did not face any water stress.

The amount of remaining water in tanks was measured during the experiments. Volumetric change in water in the control and the planted tanks are presented in Fig. 2a and 2b. Moreover, the temperature was measured during the experimental period and it was found that the temperature ranged from 25 °C (May 9th) to 40 °C (June 30th) leading to the inference that the decrease in water amount occurred most probably due to evaporation in addition to consumption by plants. Therefore, the water consumption by plants was determined by subtracting the water loss in the control tanks, which was the evaporation from free surfaces (Fig. 2c and 2d).

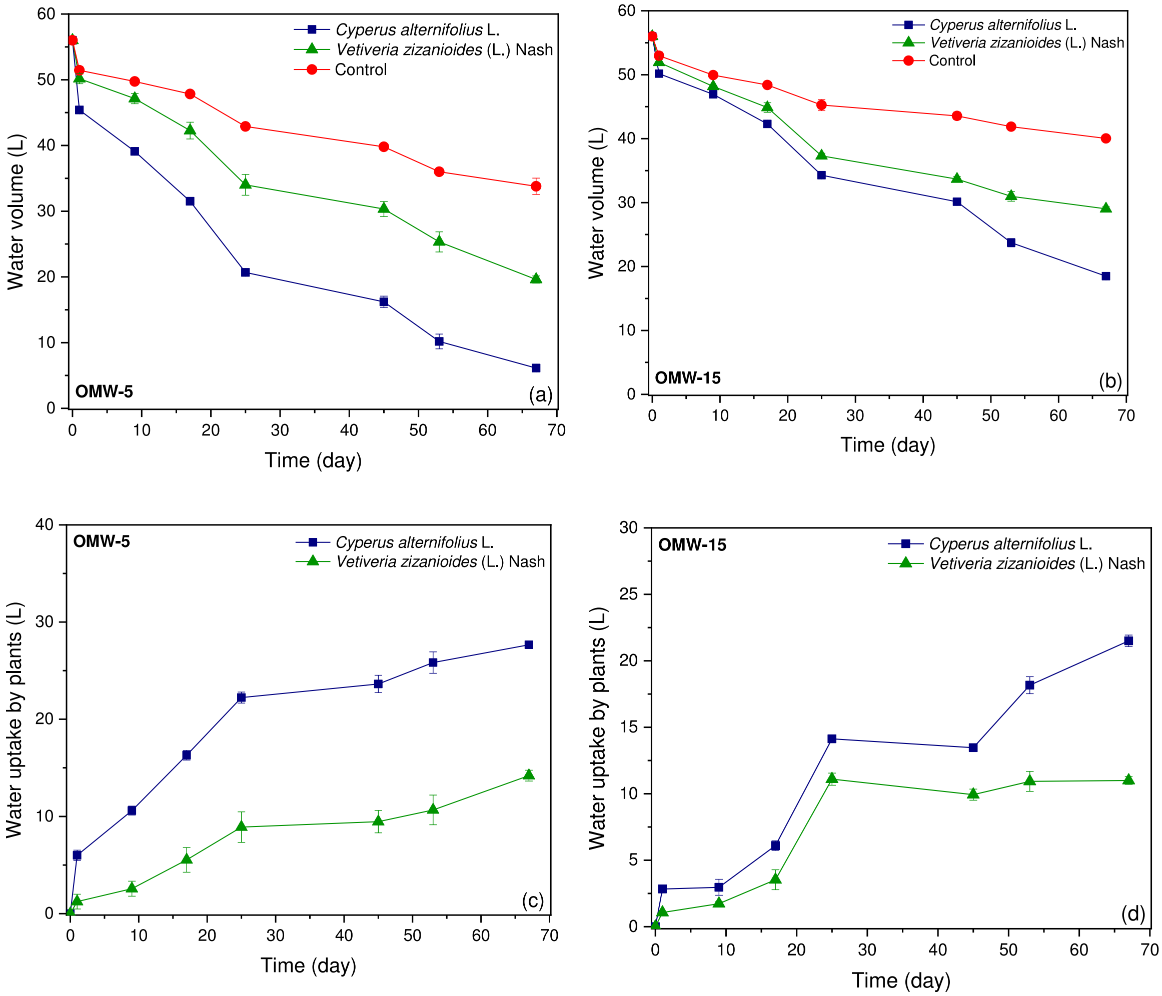

Fig. 2. Water volume change during the experiment in (a) OMW-5 and (b) OMW-15, and water consumption by plants in (c) OMW-5 and (d) OMW-15.

The amount of water consumed by vetiver and umbrella palm plants was calculated to be 14.2 ± 0.45 and 27.7 ± 0.04 L in OMW-5. Results revealed that the umbrella palm plant consumed almost two times more water than vetiver. These results were in good agreement with the literature. For instance, Chandra et al. (2017) reported that the vetiver grass may uptake more water than other common wetland plants, such as *Typha* spp., *Phragmites australis*, and *Schoenoplectus validus*. Similar trends observed in the planted OMW-15 in terms of changes in water volume. The water uptake by vetiver and umbrella palm plants was 11.0 ± 0.3 and 21.5 ± 0.4 L for OMW-15, respectively. The water uptake capacities of both plants were slightly decreased in OMW-15 compared with OMW-5, most probably due to adverse effects of the relatively higher contaminant concentrations on the plants. Overall, the results confirmed that the type of plant could be an important parameter considering the use of treated water for alternative usages such as domestic and/or agricultural purposes.

3.4. Olive mill wastewater treatment

Vetiver and umbrella palm plants can remove inorganic and organic nutrients, and PTEs from wastewaters through bioaccumulation, sorption, and biodegradation of organic substrates for their growth. The removal performance of the plants was determined by measuring TOC, TP, and TN concentrations in OMW, and nutrient and PTEs in plant tissues.

3.4.1. Organic carbon

The treatment performance of vetiver and umbrella palm species for organic matter removal efficiency was investigated in the OMW-5 and OMW-15 using TOC as the indicator. The initial TOC concentration was found to be 1,132 ± 58 and

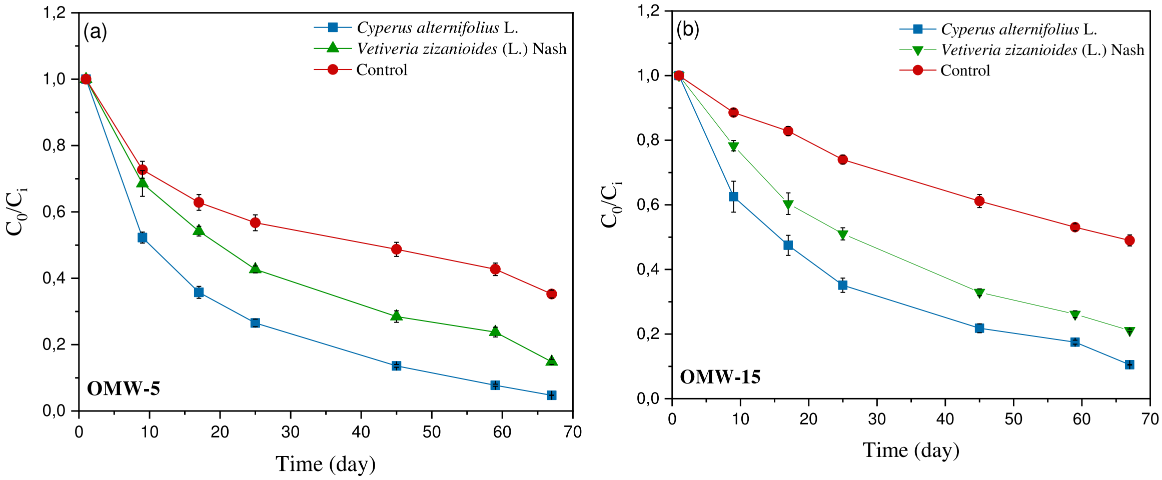

Fig. 3. Relative effluent TOC concentration of *Vetiveria zizanioides* (L.) Nash and *Cyperus alternifolius* L. species in (a) OMW-5 and (b) OMW-15.

3,168 ± 108 mg/L in OMW-5 and OMW-15, respectively. Comparison of the plants on TOC removal efficiency at OMW-5, OMW-15, and control samples are presented in Fig. 3.

Umbrella palm plant substantially removed TOC within the 67-day period at 95.3 ± 0.01% and 89.3 ± 0.28% for OMW-5 and OMW-15, respectively. Although not as much, removal was considerably high with vetiver at 84.9 ± 0.38% and 79.1 ± 0.7%, respectively for OMW-5 and OMW-15. It is thought that due to tolerance and growth (increase of biomass) of the plant in OMW-5, the reduction of TOC increased over the time from 32 to 95% for umbrella palm and 9 to 85% for vetiver. Mass of the umbrella palm increased by 149% indicating that its tolerance to OMW was better than vetiver at lower amount of OMW presence. The results showed that TOC removal efficiency decreased slightly for OMW-15, which may be explained with the higher contamination causing burn in the plants tissues and acting as stressor agents (Niu et al., 2012).

A decrease in the TOC concentration was observed in both of the control tanks. In control of OMW-5, the decrease was found to be 62.9 ± 1.52% at the end of the experimental period, while the TOC removal was 55.9 ± 2.88% in control tank of OMW-15. The TOC decrease in control tanks could be occurring by biodegradation with the microbial consortium of the wastewater, which plays an important role in degradation of organic carbon (Wang and Sample, 2014). Panja et al. (2020a) studied treatment of domestic wastewater using vetiver grass (*Chrysopogon zizanioides*), who reported that the TOC content in unplanted control tanks was significantly decreased (9%–20%). Chang et al. (2012) reported that the microbial colonization governed their control systems, resulting with higher nutrient removal than planted floating wetlands.

3.4.2. Phenolic compounds

Phenolic compounds constitute an important part of the organic portion of OMW, which are the most toxic compounds of this type of wastewater. The levels of phenolic compounds in OMW are sufficient to cause inhibition of biodegradability as well as toxicity on microorganisms and plants, which makes them one of the key factors for OMW treatment using phytoremediation considering phytotoxicity (Barbera et al., 2013). More than 50 types of phenolic compounds have been identified in OMW, among them hydroxytyrosol and tyrosol are reported as the most commonly present ones. TP content in raw OMW was found to be 13,200 mg/L. The TP concentrations at the beginning of the experiments were 219 and 611 mg/L in OMW-5 and OMW-15, respectively. The TP measurements were performed three times during the experiments, at the 1st, 25th, and 67th days (**SM Fig. S3**).

The results showed that higher TP removal efficiency was substantially achieved in the first 25 days in both planted OMW-5 and OMW-15 tanks. At the 25th day, the average TP removal efficiencies in OMW-5 were 93.9 ± 0.57, 82.1 ± 1.6, and 52.3 ± 2.34% for umbrella palm, vetiver, and control, respectively. All five targeted phenolic compounds (tyrosol, hydroxy tyrosol, vanillic acid, syringic acid, and p-coumaric acid) were below detection limit in the 25th day samples. TP removal by both of the plants continued in the 25th to 67th day period of the experiment (98.8 ± 0.07% for umbrella palm and 92.7 ± 0.37% for vetiver) while it was not the case in the controls (55.4 ± 0.65%). Similar TP removal efficiency trend observed for both plants in OMW-15 (92.8 ± 0.71%, 83.2 ± 1.69%, and 51.6 ± 6.13% in umbrella palm, vetiver, and control, respectively, on the 25th day, and 96.8 ± 0.18%, 92.1 ± 0.41%, and 53.2 ± 3.21%, respectively, on the 67th day). These results showed that there is an important benefit provided by the presence of plants as these planted systems achieved a considerable increase of almost 40% in the TP removal efficiency, and planted tanks presented a higher removal within 25 days compared to the controls. Removal of phenolic compounds may occur with four key mechanisms: biodegradation, plant uptake, sorption, and volatilization (Yalcuk, 2011).

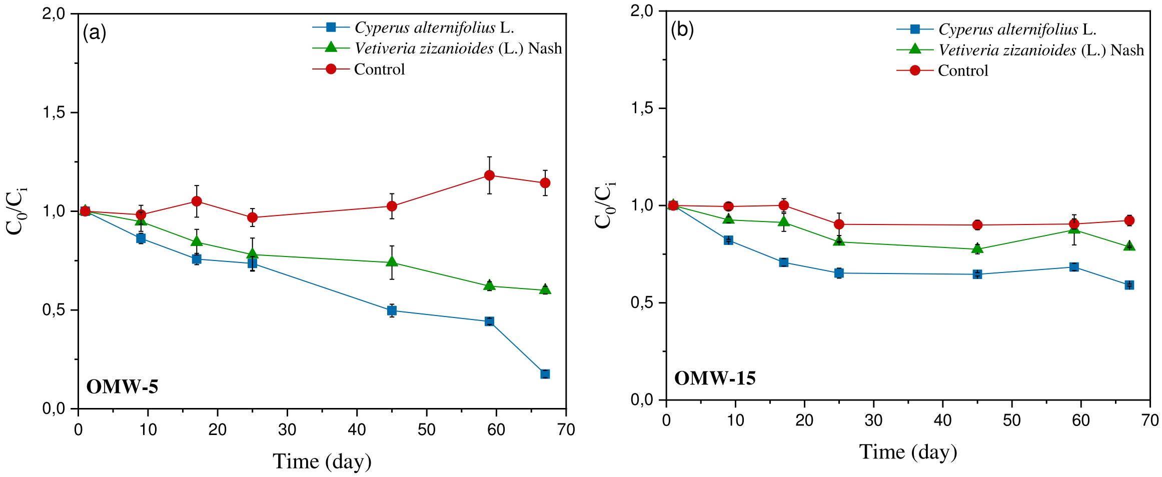

Fig. 4. Relative effluent TN concentrations in (a) OMW-5 and (b) OMW-15.

TP content of plants in OMW-5, OMW-15, and controls (umbrella palm planted tap water) was also analyzed at the end of the experiment. TP values were 326 ± 37.4, 250 ± 124.7, and 74.7 ± 2.52 mg/kg in OMW-5, OMW-15, and control, respectively, in roots of umbrella palm, while the levels in shoots were 2,358 ± 201, 1,421 ± 198, and 1,131 ± 130 mg/kg, respectively. Phenolic compounds accumulated more (5.6 and 7.2 folds) in the shoots compared to the roots. The difference between the two dilution ratios indicate that phenolic-compound accumulation capacity of the plant was decreased with increasing OMW concentration most probably due to their toxicity at higher concentrations. Accumulation of phenolic compounds in roots and shoots of the vetiver plant were lower than those in umbrella palm, with 269 ± 38.6 and 811 ± 74.5 mg/kg, 186 ± 26.4 and 815 ± 40.8 mg/kg, and 116 ± 19.4 and 663 ± 41.4 mg/kg for OMW-5, OMW-15, and control, respectively. In contrast to the umbrella palm, a higher accumulation was observed in vetiver roots than in the shoots. Mojiri et al. (2017) reported that phenolic compounds accumulated more in roots that in shoots of water hyacinth and *Typha* plants for synthetic wastewater treatment. Overall, at the end of the 67-day period, both of the plants accumulated TP in comparison to the controls, both in roots (340%) and shoots (109%) in umbrella palm, whereas only in roots (141%) but not in shoots (22%) in vetiver, showing that both plants could be quite effective in removing phenolic compounds from OMW.

3.4.3. Total nitrogen removal

Besides organic and phenolic compounds, OMW contains considerable amount of nitrogen compounds such as ammonium, nitrate, and nitrite. Uncontrolled disposal of nitrogen compounds containing OMW in water resources, could cause eutrophication with oxygen level reduction and loss of aquatic species and/or health problems to humans (Hodaifa et al., 2020). The initial TN concentrations were measured to be 26.6 ± 1.24 mg/L and 72.6 ± 5.10 mg/L in OMW-5 and OMW-15, respectively.

The results showed that the TN concentration could be decreased with both plants at both dilution ratios (Fig. 4). On the other hand, slight increase in TN concentration was observed, most probably suggesting nitrification of organic nitrogen in the OMW-5 during the long operating time of 67 days (Gupta et al., 2012). Moreover, degradation of organic matter could be inhibiting the rate of nitrification thus disrupting bacterial growth, inhibiting oxidation of ammonia, and causing accumulation of TN (Chen et al., 2005). The highest TN removal of 82.7 ± 2.54% was achieved using umbrella palm in OMW-5, while the removal was 38.9 ± 1.97% for vetiver. On the other hand, the TN removal efficiency decreased with increasing OMW concentration for both plants. In the high OMW dose, the nitrogen compounds probably cause plant stress (Karpouzas et al., 2009). The removal efficiency decreased from 82.7 ± 2.54 to 40.9 ± 1.73% with decreasing dilution rate from OMW-5 to OMW-15 using umbrella palm, which may be due to toxicity caused by pollutants such as PTEs and phenolic compounds at higher concentrations. Similarly, the removal efficiency decreased from 38.9 ± 1.97 to 23.7 ± 1.27% with vetiver. But still, the observed TN removal efficiencies with the plants were considerably higher compared to the controls (1.94 ± 0.65 – 7.33 ± 1.52%). In floating wetlands, TN removal mechanism include ammonium-nitrogen volatilization, nitrification–denitrification, plant uptake, adsorption by plants roots from rhizosphere (Li et al., 2021). The difference in TN removal efficiencies observed in the planted and control systems imply that accumulation and adsorption are the plausible mechanisms.

The levels of removal efficiency are comparable to those reported in the literature. Worku et al. (2018) reported that the highest ammonium-nitrogen removal efficiencies were 35 and 58% for initial nitrogen concentrations of 4 and 11 mg/L, respectively, in treatment of brewery wastewater with a vetiver-grass hydroponic system. Nitrogen removal efficiency of 85% was also reported using floating wetland planted with vetiver for secondary treated domestic wastewater (Akbarzadeh et al., 2015). Davamani et al. (2021) performed hydroponic phytoremediation of paperboard mill wastewater using *Chrysopogon zizanioides*, reaching a maximum TN removal efficiency of 59%.

3.5. Tissue nutrient and PTE concentrations

The shoots and roots of the plants were analyzed at the beginning and end of the 67-day experimental period to determine the amount of elements accumulated by the plants. The concentrations of micro–macro nutrients and PTEs in roots and shoots of vetiver and umbrella palm are presented in Table 2, showing that all three cases, i.e. increasing, stable, and decreasing concentrations, occurred. A slight increase (greater than one standard deviation, >1 SD) was observed in C, N, and S contents in both plants shoots and roots. In umbrella palm, the C content increase was found as 6.5 and 8.1% in shoots, respectively for OMW-5 and OMW-15, while the increase was found to be 0.6 and 1.1% in roots. Similar trends observed in vetiver exposed to OMW-5 and OMW-15. On the other hand, a notable increase (greater than two standard deviations, >2 SDs) was observed for the PTE concentrations in plant tissues. A considerable increase (>2 SDs) was observed in Fe content in shoots and roots of both plants. The Fe content increase in umbrella palm was determined to be 41 and 336% in shoots and roots, respectively in OMW-15. Similarly, the Fe increase was 153 and 296% in shoots and roots for vetiver in OMW-15.

These findings indicated that the uptake of PTEs was higher in roots than shoots most probably due to the low transport from root to shoots (Borralho et al., 2020). This phenomenon explains that the plants have greater ability to be rhizofiltrator than phytoextractor. Moreover, there was no considerable difference between the two plants and OMW dilution rates on PTEs and nutrient uptake in the plant tissues. Among the PTEs, the concentration of Fe is the highest in both plants due to the fact that plants need considerable amount of Fe at the beginning of the growth (Darajeh et al., 2014). Results showed good agreement with the literature, which reported that Fe present a higher accumulation capacity in vetiver, compared to the other metals (Borralho et al., 2020; Suelee et al., 2017). Suelee et al. (2017) reported that the highest accumulation in vetiver from synthetic wastewater observed for Fe with 19,197 and 1,549 mg/kg in the roots and leafs, respectively. Overall, amount of accumulation was in the order of Fe > Mn > Cu > Zn > B > Pb > Cr > Ni > Co > Cd and Fe > Zn > Mn > Cu > B > Pb > Cr > Ni > Co > Cd in roots of umbrella palm and vetiver, respectively. Consequently, both plants have nutrient and PTE removal potential from OMW.

3.6. Growth potential

The growth of umbrella palm and vetiver plants in diluted OMW along with controls (in tap water) at the beginning and end of the experiments were investigated visually and analytically. Shoots of umbrella palm were green and the roots were fresh. New white roots were produced in OMW-5 during the experiments (**SM Fig. S4a**). On the other hand, shoot and roots of the plant were severely damaged by yellowing and drying in OMW-15 (**SM Fig. S4b**). For vetiver, greenness of shoots was lost over time both in OMW-5 and OMW-15 (**SM Fig. S4c** and **S4d**). The number of green shoots of vetiver started to decrease after 45 days in OMW-5 while it occurred sooner, after 17 days, in OMW-15. Formation of new roots in OMW-5 continued during the experiment, while in OMW-15 did not show any new root growth. These results revealed that the plants exposed to higher doses of OMW, developed stress symptoms similar to those observed by Panja et al. (2019), e.g. drying leaf, decreasing of biomass, and mild chlorosis.

The change in the SGR and root–shoot lengths of the two plants are presented in Table 3. Umbrella palm showed considerable increase in shoots, roots, and SGR. At the lower OMW concentration, there was a higher SGR (1.4%) while it was 0.2% in OMW-15. The shoot and root lengths were also increased by 4.3% and 163% in OMW-5, respectively. A similar case was observed in OMW-15 and control. However, in the control, the root length was found to be 1.6 times lower than those in both experimental dilutions, which was most probably due to high organic and nutrient content of the OMW that enhance the growth of roots and shoots (Delis et al., 2015). A notable increase in roots of plants, was reported in the literature, in the range of 12.3–19.3 cm that may be provided by a greater nutrient uptake (Effendi et al., 2017). Consequently, the nutrients in the OMW resulting from degradation of organic compounds could be sorbed by the plants with longer roots. For vetiver plant, the SGR values were found to be 0.4 and −0.3% in OMW-5 and OMW-15, respectively. The initial shoot length was decreased by 15.5% and 27.7% in OMW-5 and OMW-15, while root lengths presented negligible increase. Overall, the growth of vetiver was negatively affected under OMW treatment compared with umbrella palm in agreement with the literature: In a hydroponic system that was designed to study the root growth of different species (i.e. *Canna indica* L., *Cyperus alternifolius* L., *Pennisetum purpureum* Schum., *Vetiveria zizanioides* (L.) Nash, *Acorus calamus* L., *Hymenocallis littoralis* (Jacq.), Salisb. *Phragmites communis* Trin. and *Typha angustifolia* L.) in wastewater, average number of roots for species with fibril roots, i.e. *C. indica*, *C. Alternifolius*, *P. purpureum*, and *V. zizanioides*, reached 1871, 1309, 1231, and 985 per plant, respectively, while the species of rhizomatic roots (*H. littoralis*, *P. communis*, and *T. angustifolia*) had only 28, 291, and 168 roots, respectively, except for *A. calamus* with 1709, after ten weeks of cultivation (Chen et al., 2007).

4. Conclusion

Phytoremediation using floating wetlands planted with umbrella palm and vetiver presented a great potential in removing TOC, TN, and TP in an olive mill wastewater (OMW). The plants tolerated up to 20% and 10% OMW well, respectively, and showed growth in 5% and 10% OMW. The treatment achieved removal efficiency of 95.3 ± 0.01, 82.7 ± 2.55, and 98.8 ± 0.07% for TOC, TN, and TP with umbrella palm in OMW-5, while the levels were 84.9 ± 0.38,

Table 2
Nutrient and PTE contents in *Cyperus alternifolius* L. and *Vetiveria zizanioides* (L.) Nash tissues (C, N, and S in %, others in mg/kg).

Sample	Initial	OMW-5	OMW-15	Initial	OMW-5	OMW-15	Initial	OMW-5	OMW-15	Initial	OMW-5	OMW-15
Parameter	Root of *Cyperus alternifolius* L.			Shoot of *Cyperus alternifolius* L.			Root of *Vetiveria zizanioides* (L.) Nash			Shoot of *Vetiveria zizanioides* (L.) Nash		
C	36.8 ± 3.4	43.3 ± 2.6	44.9 ± 3.72	43.9 ± 0.57	45.3 ± 0.56	45.0 ± 1.1	47.1 ± 3.77	50.4 ± 1.69	50 ± 0.1	45 ± 1.02	46 ± 0.56	46.7 ± 1.14
N	1.15 ± 0.23	1.85 ± 0.18	1.59 ± 0.09	1.44 ± 0.37	1.96 ± 0.13	2.10 ± 0.29	0.80 ± 0.17	1.75 ± 0.61	1.30 ± 0.07	0.92 ± 0.22	1.95 ± 0.13	0.83 ± 0.13
S	0.26 ± 0.01	0.48 ± 0.04	0.51 ± 0.11	0.23 ± 0.09	0.44 ± 0.02	0.27 ± 0.02	0.26 ± 0.09	0.59 ± 0.03	0.5 ± 0.08	0.141 ± 0.05	0.44 ± 0.02	0.12 ± 0.05
B	3.18 ± 0.77	61 ± 10	51 ± 8	2.1 ± 0.60	33 ± 4	20 ± 3	1.1 ± 0.34	29 ± 5	23 ± 5	1.24 ± 0.36	13 ± 0	15 ± 4
Ca	9151 ± 915	22267 ± 394	35103 ± 412	7335 ± 513	5056 ± 141	6500 ± 749	5955 ± 618	11762 ± 2402	12511 ± 2892	3563 ± 384	5056 ± 141	5207 ± 1056
Cd	0.1 ± 0.03	0.4 ± 0.05	0.8 ± 0.08	0.04 ± 0.01	0.07 ± 0.02	0.2 ± 0.03	0.02 ± 0.007	0.3 ± 0.08	0.3 ± 0.03	0.07 ± 0.001	0.07 ± 0.02	0.09 ± 0.01
Co	0.2 ± 0.01	1.4 ± 0.2	2.7 ± 0.4	0.01 ± 0.001	0.08 ± 0.02	0.1 ± 0.04	0.2 ± 0.07	1.2 ± 0.1	1.5 ± 0.03	0.03 ± 0.01	0.1 ± 0.05	0.2 ± 0.04
Cr	1.5 ± 0.3	8.6 ± 0.4	15.4 ± 0.04	0.3 ± 0.03	1.8 ± 0.2	3.2 ± 0.4	3.2 ± 0.4	10.1 ± 1.8	7.9 ± 0.4	2.2 ± 0.6	3.2 ± 0.3	4.3 ± 0.4
Cu	83 ± 7	119 ± 3	75 ± 3	2.7 ± 0.6	4.4 ± 0.4	11 ± 1	16 ± 3	123 ± 24	193 ± 69	2 ± 0.1	7 ± 1	15 ± 5
Fe	1275 ± 139	1368 ± 159	5557 ± 3	142 ± 36	134 ± 11	200 ± 28	1816 ± 297	3366 ± 221	7189 ± 1426	178 ± 46	211 ± 56	451 ± 10
K	17341 ± 948	20321 ± 1978	25845 ± 3012	33487 ± 3223	30278 ± 821	35691 ± 4700	3266 ± 477	8723 ± 18	13091 ± 582	16455 ± 3028	30278 ± 821	16705 ± 1045
Mg	3629 ± 168	10422 ± 936	8231 ± 1310	2555 ± 172	1784 ± 222	2475 ± 229	1581 ± 280	3718 ± 953	2565 ± 248	2132 ± 222	1784 ± 222	2475 ± 800
Mn	120 ± 15	217 ± 9	374 ± 48	59 ± 12	236 ± 36	204 ± 11	115 ± 30.2	150 ± 33	193 ± 55	61 ± 8	96 ± 10	101 ± 10
Na	3717 ± 584	11658 ± 290	5110 ± 563	1404 ± 287	2149 ± 307	2800 ± 419	1309 ± 290	3369 ± 376	1888 ± 438	982 ± 255	2149 ± 307	2473 ± 397
Ni	1.1 ± 0.2	8.4 ± 0.4	12.2 ± 1.6	0.2 ± 0.05	1.8 ± 0.08	2.6 ± 0.4	1.6 ± 0.2	7.4 ± 0.3	11.4 ± 2.5	0.6 ± 0.2	2.6 ± 0.3	2.6 ± 0.5
P	638 ± 241	1693 ± 60	2300 ± 411	830 ± 156	2300 ± 655	2433 ± 361	535 ± 77	2193 ± 395	1871 ± 525	1137 ± 245	2301 ± 656	1344 ± 90
Pb	1.1 ± 0.2	18.9 ± 0.2	32.6 ± 3.4	0.6 ± 0.05	3.1 ± 0.4	3.6 ± 0.4	0.6 ± 0.05	10.6 ± 0.3	11.7 ± 1.8	1.0 ± 0.2	2.7 ± 0.3	3.6 ± 0.2
Zn	86 ± 12	108 ± 28	127 ± 28	31 ± 3	20 ± 1	52 ± 12	15.7 ± 3.3	241 ± 76	175 ± 52	26 ± 8	30 ± 4	32 ± 5

Table 3
Change in plants shoot and root lengths (cm), and specific growth rate (%) in OMW-5, OMW-15, and controls.

Parameter	OMW-5	OMW-15	Control	OMW-5	OMW-15	Control
	Cyperus alternifolius L.			*Vetiveria zizanioides* (L.) Nash		
Initial shoot length	92.7 ± 7.50	92.7 ± 7.50	92.7 ± 7.50	128.3 ± 16.1	128.3 ± 16.1	128.3 ± 16.1
Final shoot length	96.7 ± 7.64	91.7 ± 1.53	111.7 ± 11.5	108.3 ± 2.89	92.7 ± 2.52	126.7 ± 16.1
Initial root length	55	55	55	45	45	45
Final root length	145 ± 13.2	152.3 ± 15.0	91 ± 3.61	61.6 ± 2.89	55 ± 3.0	69 ± 18.2
SGR	1.4	0.2	0.62	0.4	−0.3	0.37

92.7 ± 0.37, and 38.9 ± 1.97%, respectively, with vetiver. The removal efficiency was slightly lower in OMW-15 for TOC and TP whereas it was considerably lower for TN. The plants accumulated PTEs, to a higher degree in roots compared to shoots, showing mitigation potential. The amount of accumulation in the roots was in the order of Fe> Mn> Cu> Zn> B> Pb> Cr> Ni> Co> Cd in umbrella palm and Fe> Zn> Mn> Cu> B> Pb> Cr> Ni> Co> Cd in vetiver. Consequently, pilot scale experimental work conducted in this study demonstrate a realistic potential for floating wetlands planted with vetiver and umbrella palm for OMW treatment.

CRediT authorship contribution statement

Aysegul Yagmur Goren: Writing – original draft, Visualization. **Arzu Yucel:** Writing – original draft, Methodology, Investigation, Formal analysis. **Sait C. Sofuoglu:** Supervision, Writing – review & editing. **Aysun Sofuoglu:** Supervision, Writing – review & editing, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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