THE USE OF *VETIVERIA ZIZANIOIDES* (L.) NASH AND *CYPERUS ALTERNIFOLIUS* L. BY FLOATING WETLAND TREATMENT TO IMPROVE WATER QUALITY

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Arzu YÜCEL

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We approve the thesis of **Arzu YÜCEL**

Examining Committee Members:

Prof. Dr. Aysun SOFUOĞLU
Department of Chemical Engineering
Izmir Institute of Technology

Prof. Dr. Delya SPONZA
Department of Environmental Engineering
Dokuz Eylül University

Asst. Prof. Dr. Altan ÖZKAN
Department of Environmental Engineering
Izmir Institute of Technology

4 July 2018

Prof. Dr. Aysun SOFUOĞLU
Supervisor, Department of
Chemical Engineering
Izmir Institute of Technology

Prof. Dr. Sait Cemil SOFUOĞLU
Co-Supervisor, Department of
Environmental Engineering
Izmir Institute of Technology

Prof. Dr. Sait Cemil SOFUOĞLU
Head of the Department of
Environmental Engineering

Prof. Dr. Aysun SOFUOĞLU
Dean of the Graduate School of
Engineering and Sciences

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ABSTRACT

THE USE OF *VETIVERIA ZIZANIOIDES* (L.) NASH AND *CYPERUS ALTERNIFOLIUS* L. BY FLOATING WETLAND TREATMENT TO IMPROVE WATER QUALITY

The olive mill wastewater (OMW), arises during the production process of olive oil, is an important problem in all Mediterranean countries. OMW shows seasonality, and contain high organic pollutant. Eventhough various methods have been studied for the treatment, olive oil producers neither can afford the cost of these methods, nor it can provide the discharge standards.

The capacity of phytoremediation of *Vetiveria zizanioide*s (L.) Nash (vetiver) and *Cyperus alternifolius* L. (umbrella palm) species was evaulated in the treatment of OMW by floating treatment wetland (FTW) method. The 5 and 15% concentrations of OMW (OMW-5 and OMW-15) were remediated by these plants, and unplanted sets as triplicates for 67 days to determine their organic and nitrogen reduction. Therefore, total organic carbon (TOC), total phenol and total nitrogen (TN) in the OMW were measured.

The amount of biomass at the beginning of the experiment were 2.5 and 4.0 kg OMW-5 and OMW-15 respectively. The highest biomass growth rate (149%) and total phenol accumulation in plant (439%) were observed in umbrella palm. The results showed that umbrella palm achieved 95%, 99%, 82% removal in TOC, total phenol, TN while these removals were 85%, 93%, 40% in vetiver planted OMW-5 tanks. Similarly, the removal in OMW-15 were comparable with 90, 97, 41 percentages with umbrella palm and 79, 92, and 21 percentages for vetiver respectively.

As a result, it seems that umbrella palm and vetiver have potential in diluted OMW treatment by FTW for future.

ÖZET

VETIVERIA ZIZANIOIDES (L.) NASH ve *CYPERUS ALTERNIFOLIUS* L. KÖK YÜZDÜRME YÖNTEMİ İLE SU KALİTESİNİ İYİLEŞTİRMEDE KULLANIM OLANAKLARI

Zeytinyağı üretim sürecinde oluşan atıksuyun (karasuyun) bertarafı, tüm Akdeniz ülkelerinde önemli bir sorundur. Mevsimsel olarak oluşan karasuyun organik kirliliği oldukça yüksek olup, arıtımı için çeşitli yöntemler üzerinde çalışılmaktadır. Ancak bu yöntemler deşarj standartlarını sağlamadığı gibi, ekonomik açıdan zeytinyağı üreticilerini zorlamaktadır.

Bu çalışmada, uygun maliyetli ve çevre dostu bitkisel arıtım yöntemi kullanılarak, *Vetiveria zizanioides* (L.) Nash (vetiver) ve *Cyperus alternifolius* L. (Japon şemsiyesi) bitkilerinin, yüzdürme yöntemi ile karasudaki organik karbon ve azot giderimi araştırılmıştır. Deney, bitkilerin uyum sağladığı %5 (OMW-5) ve %15 (OMW-15) oranlarındaki ham karasu seyreltilerek hazırlanan örneklerde, 67 gün süre ile üçer tekerrürlü olarak Japon şemsiyesi, vetiver ve bitkisiz kontrol grubu için yürütülmüştür. Belirli günlerde kalan suyun hacmi, sudaki toplam organik karbon (TOC), toplam fenol ve toplam azot (TN) miktarları ölçülmüştür.

OMW-5 ve OMW-15 gruplarında sırasıyla 2,5 ve 4,0 kg biyokütle ile başlatılan deneme sonunda, OMW-5'deki Japon şemiyesilerinde biyokütlenin (%149) ve biokütlede biriken toplam fenol miktarının (%439) en yüksek oranda arttığı görülmüştür.

OMW-5'de TOC, toplam fenol ve TN için en yüksek oranda azalma Japon şemsiyesinde, %95, %99 ve %82 sağlanmış,vetiverde ise bu oranlar %85, %93, %40 olarak görülmüştür. OMW-15'de ise Japon şemsiyesinde (TOC %90, toplam fenol %97, TN %41), vetiverde (TOC %79, toplam fenol %92, TN %21) ve kontrol bidonlarında (TOC %51, toplam fenol %81, TN % 0.08) OMW-5'e göre daha az olmakla birlikte yine aynı sıra ile azalmalar tespit edilmiştir.

Elde edilen çalışmalardan yola çıkarak seyreltilmiş OMW'nin arıtımında, Japon şemsiyesi ve vetiver bitkilerinin potansiyeli olduğu söylenebilir.

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CHAPTER 1

INTRODUCTION

Population growth, industrialization and urbanization in the 20th century brought new concepts of water management in many respects. While the world's population has tripled in the last century, water use has increased about six times (World Water Vision Report 2000). This growing demand for water resources will continue to increase over the coming years. For this reason, sustainability of the water has become one of the elements of stability in terms of the development of the countries. As regards of water management, many governments work closely with national and international institutions. For example, basin-based water management policy has been applied to solve the water and water pollution problems in Turkey. The watershed action plans have been prepared to prevent pollution and degradation of underground and surface water resources, followed by local implementation and control policy.

Treatment and disposal of wastewater (WW) for various purposes are one of the main issues of water management. WW emerges with activities such as industrial manufacturing, food production, maintenance and cleaning work, and domestic activities. The treatment of WW from food production is difficult due to the amount and/or strength of WW. Olive oil production is one of the food industry that faces this difficulty.

The olive tree, *Olea eurpoea* L., is one of the world's major economic plants. The cultivated plant, subsp. *Europaea*, is not known in the wild and must be considered an ancient cultigen. However, wild forms of this species with smaller, less oily fruits are known over a wide area, from Africa, Macaronesia, and Asia (Green, 2002). Total number of olive trees in the whole world is estimated approximately to be 800 million, cultivated over on 8.6 million hectares areas that in 95% are located in the Mediterranean region (Wingard, 2010). Olives grow best in warm temperatures, and cannot tolerate extreme climatic conditions. The Mediterranean region, owing to its mild climate, contains 98% of the olive harvest, and 95% of the olive oil production in the world. Harvesting and processing of olives are carried out between November and March (Republic of Turkey Ministry of Economy 2017).

The three largest producers of olive oil are Spain with 1.600.000 tons per year, Italy with 400.000 tons per year, and Greece with around 300.000 tons per year, which correspond to 67%, 17%, and 12% of the European production (IOOC 2013).

In the early 2000s, Turkey had 100 million olive trees. By the 2014/15 season, new plantings increased this number to 169 million. On average, Turkey has produced 170.000 tons of olive oil and 527.000 tons of table olives over the last five crop years. Around 76% of the olives produced in Turkey are pressed for oil, and the rest are reserved for table olives. Olive growing is concentrated around the regions of the Aegean, Marmara, and south-east Anatolia (Official Journal of the International Olive Council 2016).

Currently, commercial olive oil production in the Mediterranean area has been produced either continuous (centrifugation) or batch (traditional press) processes (Klen and Vodopivec, 2011). Both of the processes yield a liquid waste that is commonly referred as olive mill wastewater (OMW). OMW consists of the water contained in olive fruit, the added water required for washing the fruit, and for the wastewater from centrifugation process. Disposal and treatment of OMW are the main environmental problems of the olive oil industry. Many treatment methods have been studied: thermal methods (evaporation and burning), evaporation or irrigation from lagoons, flotation/ sedimentation, ultrafiltration, membrane filtration and reverse osmosis, anaerobic and aerobic biological treatment, and chemical treatment are among the proposed methods. Storage and evaporation in lagoons is the most common method for disposal of olive mill wastewaters for small and medium scale enterprises (SMEs). Although there are many proposed treatment methods, none has been widely accepted by the industry, and in turn, OMW is still an important environmental problem in almost all Mediterranean countries because of financial difficulties experienced by SMEs.

Instead of the expensive and high energy consuming methods, which are mentioned above, less expensive and more environment friendly remediation techniques are needed for OMW treatment. One of the interesting proposed solutions is called phytoremediation, as a restoration technology, is based on the use of vegetation for in situ treatment of contaminated soils, sediments, and water. Phytoremediation can be applied for any kind of contaminant or pollutant such as nutrients, organics, or metals depending on sequestration, degradation, and immobilization of the plant capacity through uptake of roots (Nikolopoulou and Kalogerakis, 2007).

Constructed wetlands containing emergent macrophytes growing hydroponically on a floating structure instead of being rooted in the sediments, termed as floating emergent macrophyte treatment wetlands (FTW) are an innovative variant of treatment wetland technology (Headley and Tanner, 2012). In this method, the roots of the plants are floating freely in the wastewater. Thus, the root system can be exposed homogenously to the contaminants avoiding local variations, which may occur in a soil system (Flocco et al., 2002). According to the literature, *Scirpus* spp., *Eleocharis* spp., *Cyperus* spp., *Carex* spp., *Juncus* spp., *Phragmites australis, Phalaris arundinacea, Glyceria maxima* and *Typha* spp., could be used as emergent macrophyte species; while *Eichhornia crassipes*, *Pistia stratiotes*, *Salvinia* spp., *Lemna* spp. and *Spirodella* spp. could be used as free-floating macrophytes (Ling, 2006; Brix and Schierup, 1989; Newete and Byrene, 2016).

In this study, *Vetiveria zizainoidies* and *Cyperus alternifolius* plants, which are prominent with the intake of high nitrogen, phosphorus, metal, and phenol in soil, wastewater, and surface waters, are studied by FTW approach as a viable alternative to the current practices to treat OMW. Total phenol and Total Organic Carbon (TOC) content measured before and during the experiments were used as indicators to estimate treatment performance.

This thesis has five chapters: a brief introduction about the study is given in Chapter 1; followed by Chapter 2 containing background information on the subject including the review of related literature; in Chapter 3 materials and methods used in the experiments are described; than obtained results are presented and discussed in Chapter 4; and finally conclusions based on the results of the study are stated in Chapter 5.

CHAPTER 2

BACKGROUND INFORMATION

2.1. Background of Olive Plant

Olive is a plant that produces high olive oil output under the preponderant climatic and ecological conditions of the Mediterranean Basin with the production being most particularly the zone between latitudes 30° and 45° North (Torres et al., 2017). Nonetheless over the last decades has bring on olive cultivation in the southern hemisphere, notably in Argentina, Chile, Perú and Australia (Sibbett and Ferguson, 2005). The distribution of olive oil production countries on the continents is given in the Figure 1 below.

Today, approximately 98% of olives are cultivated in Mediterranean Basin countries (Zamora et al., 2001). For this reason, olives and olive oil have an important place in the economy, diet and culture for the Mediterranean region countries such as Spain, Italy, and Greece, (produce about 77% of the total olive oil product), and Tunisia, Turkey, Morocco, Syria, and Egypt (El-Kholy, 2012).

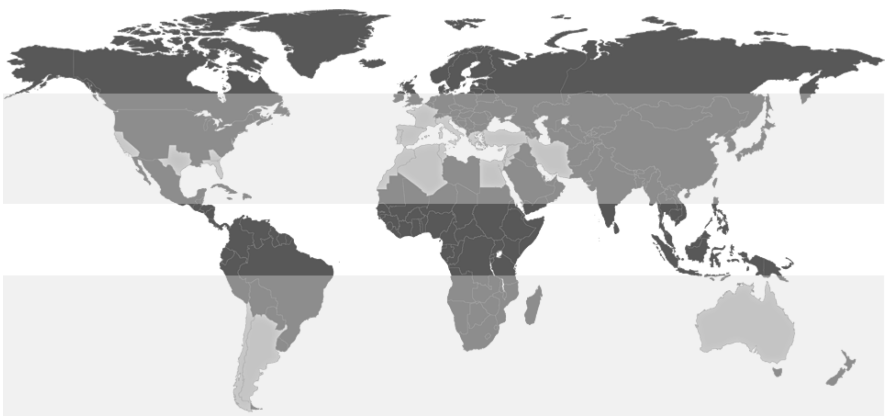

Figure 1. Geographical distribution of olive growing area.
(Source: web_01, californiaoliveranch.com)

2.2. Olive Oil Production Method

Olive oil production through physical methods has been conducted during November to February in Turkey. According to the General Directorate of Cooperatives of the Ministry of Customs and Trade's (2015), the production of olive oil between 2015 and 2016 was 175.000 tons. Currently olive oil is extracted from the processed olives either by means of a discontinuous press (classical process) or a solid/liquid centrifuge (continuous process) (Ergüder et al., 2000). In the continuous extraction system, the crushed and liquefied paste pass to centrifuge procedure. Depending on the separation method used in continuous operation, two technologies are employed: two- and three-phase processes. The principal characteristics of the three-phase extraction system are established, adding certain amount of water into the horizontal decanter for per unit of olives processed, as a result the system produces oil, olive mill wastewater (OMW) and solid wastes (known as olive pomace or olive cake). In contrast, the two-phase extraction, the horizontal decanter is designed to work without water, so that this system has two outputs. One of them is the vegetation water, that comes out together with the pomace (more wet than 3 phase*)* and the other one is the olive oil as a product. After these centrifugation procedures, the olive oil is canalized into a single vertical centrifuge via the filter and the remaining water is removed, the product is obtained. These two systems differ in terms of water demand and wastewater formation. Furthermore, the pollution loads of the wastewater are also higher in the 3-phase system. Olive oil production and water flow diagram seen in Figure 2. Even though conversion from there phase to two phase has been recommended by Ministry of Agriculture, currently 90% the oil producers in Turkey, uses 3 phase system (web_02).

2.3. Properties of Olive Mill Wastewaters

As mentioned above, OMW is a liquid waste that is disclosure during the production of olive oil. A two-phase plant, however, involves two phases (oil and water-solid mixture), and uses much less water compared to the three-phase process (Sengul et al., 2000). Both of the processes yield a liquid waste, which is commonly referred as OMW. The average amount of produced OMW is 1.2-1.8 m^3 per ton of olives with the continuous method, while it is 0.4-0.5 m^3 per ton of olives with the batch method (Tomati and Galli, 1992). OMW consists of some liquid water available in olive fruit, water required for washing the fruit, and the wastewater from centrifugation process. OMW composition show a very high variation depending on origin of the olive, harvesting time, annual climatic conditions, age of the olive tree, and the type of process involved in oil production etc. OMW is a complex mixture containing mainly water (83-92%), and contains organic matter (4-16%), and minerals (1-2%) ranges (Pe´rez et al., 1998).

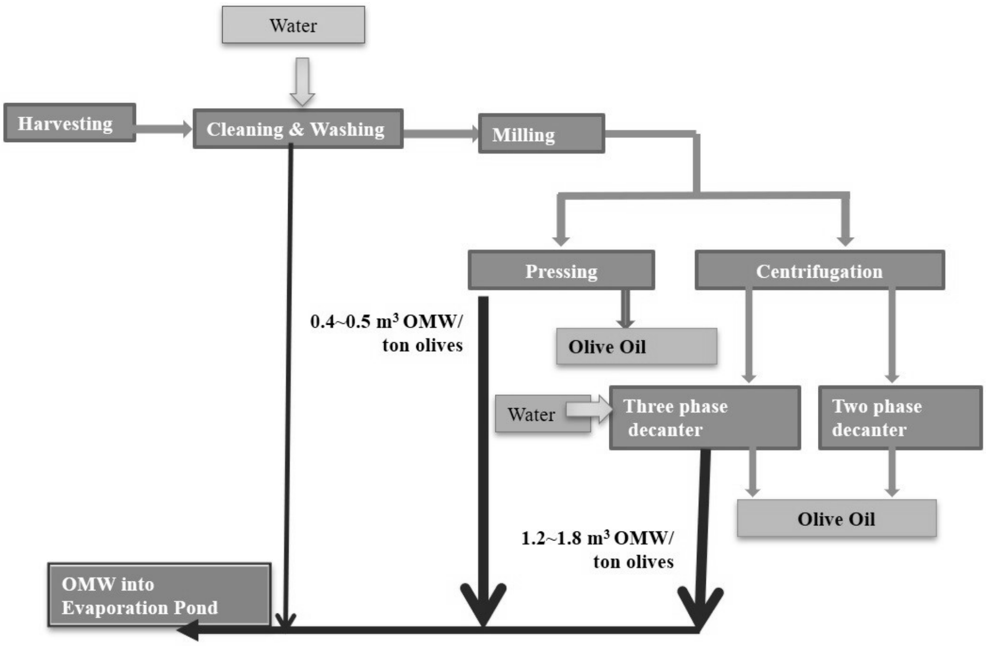

Figure 2. Olive oil production flow diagram.

Main organic constituents of OMW are sugars (1-8%), polyphenols and pectin's (1.0-1.5%), nitrogenous compounds (0.5-2.4%), fatty acids (0.5-1.5%), volatile acids and polyalcohol's, fats (0.02-1%) (Borja et al. 2002; Sassi et al. 2006; Tziotzios et al. 2007).

These wastewaters present high organic loads, usually indicated as chemical oxygen demand (COD), up to 100-200 g/L (Asses et al., 2000), and low biodegradability assessed by the ratio between COD and biochemical oxygen demand (BOD). As seen in the characteristics of some OMW samples collected from literature, the values shows variation based on cultivated area. Especially in the phenolic compounds content ranges from 0.4 to 29.5 g/L.

During the olive oil production, a large amount of phenolic compounds (~98%) remains in the olive oil by-products. The phenolic compounds are a large family of molecules sharing one common feature, at least one phenolic group, substituted in most of the cases with another functional group. Fifty phenol species that have already been identified in OMW classified in three important categories of phenolic compounds: (a) cinnamic acid derivatives, (b) benzoic acid derivatives, and (c) compounds related to tyrosol (Kapellakis et al., 2008). The toxicity of olive mill waste is mainly related to its high content of phenolic compounds which are distributed among oil (2%), OMW (53%) and pomace (45%) (Rodis et al., 2002).

Range of the polluting organic load per unit weight of processed olives, such as BOD, COD, suspended solids (SS), and phenolic compounds are reported to as 35-110 g/L, 45-170 g/L, 1-9 g/L, 0.5-24 g/L, respectively (Paraskeva and Diamadopoulos, 2006). Moreover, OMW possesses considerable amounts of mineral nutrients such as potassium (K_2O: 2.4-10.8 g/l) and phosphorus (P_2O_5: 0.3-1.5 g/l), and a wide-range of micronutrients (Ouzounidou et al., 2010).

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These wastewaters present high organic loads, usually indicated as COD, up to 100-200 g/L (Asses et al., 2000), and low biodegradability assessed by the ratio between COD and BOD. As seen in the characteristics of some OMW samples collected from literature, the values show some variations based on cultivated area. Especially in the phenolic compounds content ranges from 0.4 to 29.5 g/L. Characteristics of some OMW samples given in the literature are shown in Table 1.

Table 1. Characteristics of some OMW in literature.

	pH	COD (g/L)	BOD (g/L)	SS (g/L)	t-Phenol (g/L)	References
Turkey	4.9	140	-	137	29.5	(Yesilada et al., 1997)
Tunisia	5.3	74	14	54	9.4	(Mekki et al., 2012)
Tunisia	5.4	97	25	70	12.2	(Daâssi, et al., 2013)
Greece	5.1	90	38		5.1	(Niaounakis and Halvadakis, 2006)
Australia					1-8	(Nair and Markham, 2008)
Israel	5.0	153	71	51		(Nassar et al., 2014)
Iran	5	58.8	8	28.5	0.4	(Yazdanbakhsh et al., 2015)

In the studies, in order to clarify the distribution of organic matter in OMW samples, COD and Total Organic Carbon (TOC) parameters were measured by filtering samples through filters of 1200 ~ 1600 nm, 450 nm and 220 nm pore diameters in series.

It is assumed that the phase remaining below 450 nm in the filtration is the dissolved phase, it is found that the compounds forming the TOC in the OMW are considerably in dissolved form (Kiril et al., 2010; Dogruel et al., 2009). The general distribution of COD and BOD in TOC are shown in the Figure 3.

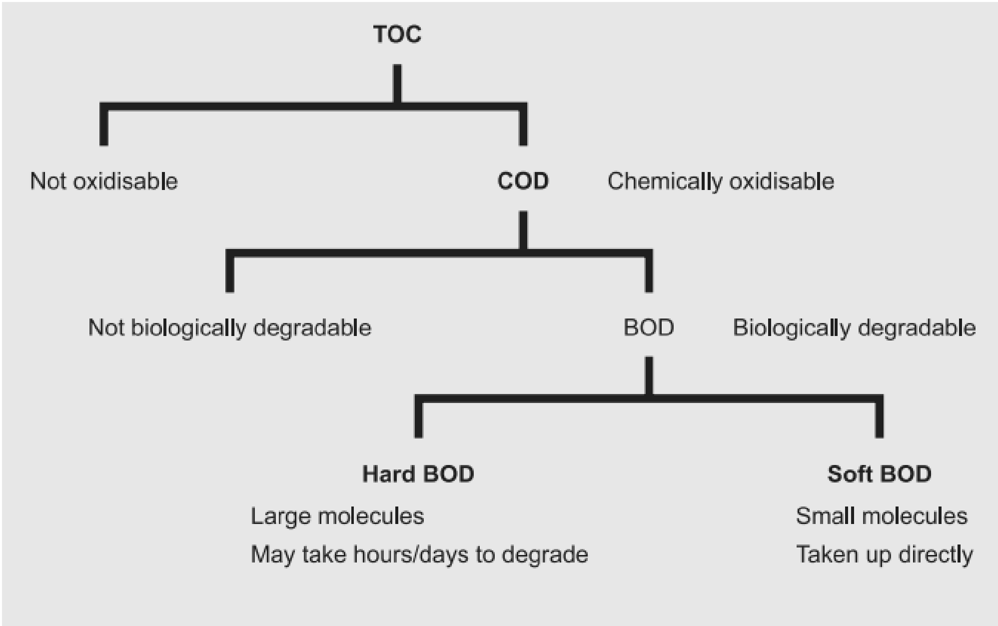


Figure 3. The relationship between the organic carbon fractions in wastewater. (Source: El Gohary et al., 2009)

Due to the effect of organic carbon content of OMW, there have been discharge limits for these contaminants. In the following Table 2 some relevant values for discharges to surface waters in Italy, Greece, Spain and Portugal are presented as set by national legislation (IPPC BREF, 2006b; IMPEL, 2003).

Similarly, OMW must be discharged, according to the "Water Pollution Control Regulation" in Turkey, by providing the discharge criteria in "Annex 1-Table 5-5", which are given below in Table 3.

2.4. Treatment Techniques of Olive Mill Wastewater

Due to scattered distribution of small enterprises around the country, presence of many pollutants, and seasonal release of OMW, treatment of this wastewater is difficult. Elimination and disposal of this waste are among the critical environmental problems associated with the olive oil industry. The development of cost-effective OMWs treatment technologies remains a priority, since OMWs are in most cases discharged directly to the environment without any other treatment performed.

Table 2. Discharge limits of OMWs to surface waters in some Mediterranean countries.

Parameters	Italy	Greece	Spain	Portugal
pH	5.5-9.5	6-9	5.5-9.5	6-9
BOD_5 (mg/L)	40	15-60	40-300	40
COD (mg/L)	80	45-180	160-500	150
TSS (mg/L)	160	25-100	30-300	60
Oil/Grease (mg/L)	20	5-40	20-40	15
Phenols (mg/L)	0.5	0.005-0.5	0.5-1	0.5
Total P (mg/L)	10	0.2-10	10-20	10
Total N (mg/L)	15	10-15	15-50	15
Free Cl (mg/L)	0.2	0.4-1	0.5	0.5
Nitrates (mg/L)	20	4-50	10-20	50
Nitrites (mg/L)	0.6	1-3	-	-

Table 3. Discharge standards according to the Water Pollution Control Regulation Food Industry (Olive Oil and Soap, Solid Oil Refining).

Parameters	Unit	Composite (2 Hour)	Composite (24 hour)
COD	(mg/L)	250	230
Oil and Gress	(mg/L)	60	40
pH	-	6-9	6-9

In the selection of the convenient method or combination of methods, the type and the composition of the wastewater is the primary factor. Various methods has been applied for OMW treatment, and also some has been still under investigation. These methods can be grouped as physical e.g. evaporation or irrigation for lagoons (Cabreraa et al., 1996), flotation, sedimentation, ultrafiltration, membrane filtration (Paraskeva et al., 2005; Kontosab et al., 2018), reverse osmosis (Ochando et al., 2012), chemical e.g.

precipitation, ion exchange, disinfection, and biological treatment e.g. activated sludge, and thermal methods (e.g. evaporation and burning).

Each of the method has advantages and disadvantages over other. Additionally considering the constraints in practice, the prominent disadvantages of these methods are high sludge formation and financial difficulties for small and medium scale enterprises (SMEs). Although there are many proposed treatment methods, the disposal of olive mill wastes on soil or in non-properly constructed evaporation ponds/lagoons are common and the cheapest practice. Disadvantages of disposal methods can be seen on Table 4.

Table 4. Summary of OMW treatment methods.

Methods	**Application**	**Disadvantages**
Physical methods	Sedimentation/settling or flotation	Unpleasant odor, high volume of settled sludge, it should be burn.
	Filtration, membrane technology (ultrafiltration, reverse osmosis)	High sludge volume, troublesome cleaning.
Thermal Process	Evaporation	Requirement of large area, long p eriod, low efficiency, risk for pollution of to affect the physical and chemical properties of soil, underground water and atmospheric pollution.
	Combustion	Emission of toxic substance, expensive.
Physicochemical methods	Adsorption, chemical oxidation	Cost and require advanced chemical .
	Coagulation, flocculation, adsorption	pH adjustment, high sludge formation, effluent water should be need secondary treatment.
Biological methods	Aerobic	Pretreatment is necessary for phenols, huge oxygen requirement will be high, high sludge formation.
	Anaerobic digestion	Dilution and pretreatment are necessary, otherwise it is toxic for the methane bacteria.
Combined Chemical and Biological Method		High operational cost.

Specific disposal areas evaporation ponds or lagoons is a practice spread in Greece. The present legislation in Greece does not allow application of untreated olive mill wastes to soil surface.

The Circular 2015/10 has been published in Turkey for the organization of wastewaters generated in olive oil facilities. Natural vaporization is the method applied in the removal of this wastewater, fortunately the lagoon structure predetermined.

According to the circular, the conditions of these lagoons must provide the defined requirements. In the lagoon where the ground and side walls must be impermeable, wastewater is allowed to evaporate in summer season and lime should be applied to prevent odor. A typical lagoon can be seen in the Figure 4. However, in general the lagoon type of treatment has negative environmental impacts on urban areas due to the foul odors, insect generation, leakages to soil and surface water, and silting with the remaining sludges.

Figure 4. OMW evaporation pond in İzmir.

In terms of both economical and convenience as a final solution of OMW treatment technology has not been developed yet. On the other hand the treatment of OMW is being studied with environmental friendly and cost-effective methods based remediation technologies. Bioremediation and phytoremediation are natural process in which microorganisms or plants metabolize contaminants through oxidative or reductive processes. As such, it uses relatively low-cost and simple techniques, which generally have high public acceptance and can often be carried out on site. However, remediation is not always suitable every contaminant types. Therefore the detailed pre-study of local conditions are required in order to identify if the contaminants could be biodegraded by microorganisms or plants, and the residual contaminant levels after remediation practices are admissible (Vidali, 2001). Origin of OMW is the production of olive oil from olives, due to being a part of nature, therefore possibility of biodegradability on this waste can be assumed. However, the OMW degradation rate, in particularly for the phenolic compounds, is much slower than the other substances (sugars or volatile acids with short chains). As a result, it is reported that OMW is not easily biodegradable (Preedy and Watson, 2010). Hence it is important to find a method that can increase biodegradability and applicable on site for SMEs. Many studies have been conducted in recent years in this direction.

For example, Goncalves et al. (2009) carried out a bioremediation study with yeast extract such as *Candida rugosa*, *Candida cylindracea* and *Yarrowia lipolytica* in OMW containing 100 g/L to 200 g/L COD from a three-phase centrifuge. The results of this study confirmed the *C. cylindracea* was the best strain both the lipase production and the COD reduction, tyrosol, hydroxytyrosol, oleuropein, syringic acid and vanillic acid did not show toxic effect the growth of yeasts cell. This result shows that OMW is also a resource to gain high-value products while is being degraded (Morillo et al., 2009).

2.5. Phytoremediation Techniques

Alternative methods of purification are increasing with the developing technology versus the conventional treatment methods. However, these new methods often increase the costs of energy, operation and maintenance, which are pushing the financial resources of local governments or businesses. The term phytoremediation “photo=plant and remediation=correct evil” is relatively new, coined in 1991 (EPA 2000). Phytoremediation is simply defined as the removal of soil or water pollutants by the plants, and keeping in the plant structure. All green plants in terrestrial and aquatic ecosystems are natural reservoirs for pollution control (Meagher, 2000). Therefore phytoremediation applications have being ongoing projects, because of its on-site applicability, cost-effectiveness and ecofriendly nature. (Lu et al., 2011).

Environmental Protection Agency (2000) classifies phytoremediation mechanisms under six major categories. These are rhizofiltration, rhizodegradation, phytostabilization, phytoextraction, phytovolatilization, phytodegradation. Each of this mechanism is summarized in terms of treatable contaminant, specific mechanisms of remediation and application media in Table 5. A schematic representation of these mechanisms is also given in the Figure 5.

Many phytoremediation studies have been published in the removal of organic contaminants such as polyaromatic hydrocarbons and (Aprill and Sims, 1990; Ukiwe et al., 2013) polychlorinated biphenyl from soil (Donnelly et al., 1994; Zeeb et al., 2006), trinitrotoluene from groundwater (Best et al., 1997; Wang et al., 2003), and soil (Makris et al., 2007), hydrocarbons in soil (Günther et al., 1996; McIntosh et al., 2017) and sediment (Ke et al., 2003) by using different plant species in Europe, North America and Asia. The metal and radionuclides contamination studies in groundwater (Tu et al., 2004; Vamerali et al., 2009), surface water (Fritioff and Greger, 2003) and wastewater (Lissy and Madhu, 2011) ensued that the removal capacity of both aquatic and terrestrial plants.

Table 5. Phytoremediation processes and mechanisms of contaminant removal.

Process	**Contaminant**	**Mechanism Involved**	**Media**
Rhizofiltration	Organics/ inorganics	adsorption, concentration and precipitation by roots	Aqueous
Rhizodegradation (Phytostimulation)	Organics	breakdown of contaminants through microbial activity on the rhizosphere	Soil, sediment, sludge, groundwater
Phytostabilization	Organics/ inorganics	sorption, precipitation, complexation, or metal valence reduction.	Soil, sediment, sludge
Phytoextraction (Phytoaccumulation)	Metals, radionuclides	uptake and translocation by roots into the aboveground portions of the plants.	Soil
Phytovolatilization	Organics/inorganics chlorinated solvents	uptake and transpiration by leaves.	Soil, sediment, sludges, groundwater
Phytotransformation (Phytodegradation)	Organics, chlorinated solvents, phenols, herbicides	the plants metabolic processes, or effect of enzymes produced by plants (free of microorganisms) to degrade pollutants.	Soil, groundwater, surface water

In addition to soil remediation, similar techniques have been applied to control pollution in rivers and streams, buffer strips are one of the phytoremediation model in natural ecosystems. An area of land maintained in permanent vegetation to control air, soil, and water quality, along with other environmental problems is called as a buffer strip. The vegetation in the buffer strip reduces the flow rate of the water, then sedimentation takes place, and the plants uptakes or adsorb some soluble pollutant in the water as nutrients. Research studies on the pollutant removal capability of the buffers have being performed on pilot-scale. The buffer trapping efficiency ranged for total phosphorus and nitrate-nitrogen as 27% to 96% and 7% to 100% respectively (Dillaha et al., 1989; Lee et al., 2000).

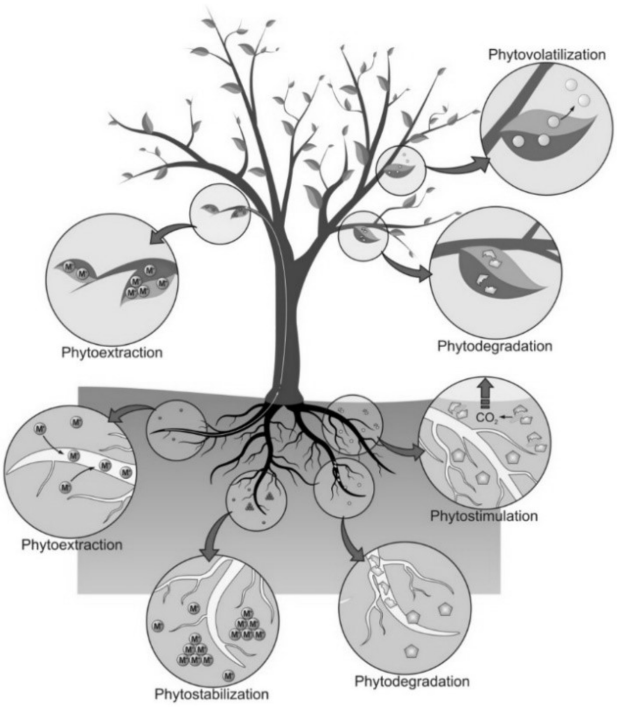


Figure 5. Schematic representation of phytoremediation.
(Source: Favaset al., 2014).

After observation of the effectiveness of plants in soil with buffer strips, plants have been applied for the treatment of natural contaminated areas or to treat some of the polluted water with engineered system. Constructed wetlands (CWs) have been seen in Europe and the United States to treat wastewaters since the 1970's (Ohlendorf et al., 1999). CWs are engineered systems that have been designed and constructed to utilize the natural processes involving wetland vegetation, soils, and the associated microbial assemblages to assist in treating wastewaters. CW's are designed to benefit from the advantages being offered by same processes that take place in natural wetlands, but it do so in a more controlled media. CWs may be classified according to the life form of the dominant species, as free-floating, floating leaved, rooted emergent and submerged macrophytes, this classification is schematized in the Figure 6 (Brix and Schierup, 1989).

The uptake and removal process is explained by the oxygen uptake of the root. In aquatic media, the plants transmit to atmospheric oxygen to their root up to 120 cm and form oxygen-rich zones within the water. As a results oxygenated and oxygen-free zones formation have been occurred in water. Those different zones allow different chemical phenomena to take place and thus provide a rapid breakdown of pollutants (Soran, 1992). In the presence of oxygen, microorganisms metabolizes the pollutant for plant uptake of organic substances to their stem and leaf structure as a nutrients. The general schematic of how nutrients and oxygen transferred and used in the plant is given in Figure 7.

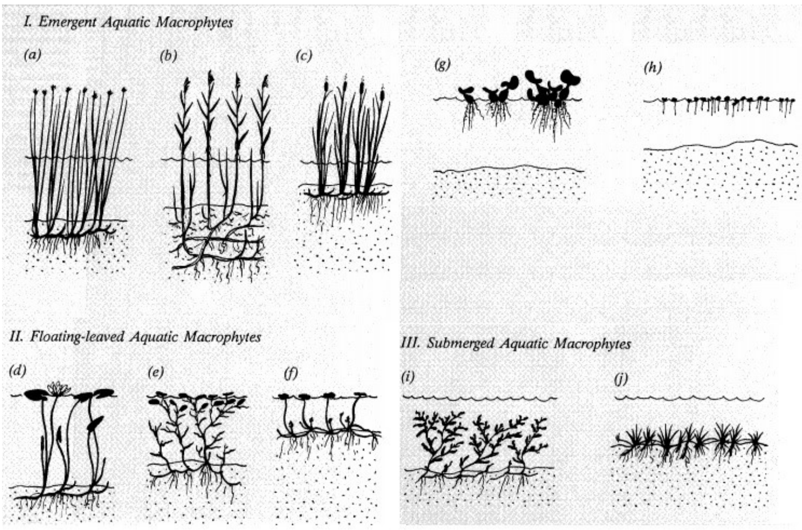


Figure 6. Classification of aquatic macrophytes based on growth form.
(Source: Brix and Schierup, 1989)

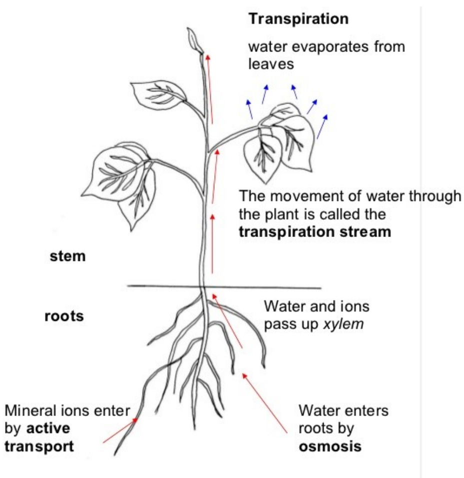


Figure 7. Nutrient, water transfer in plant
(Source: www.slideshare.net/scuffruff transport-in-flowering-plants-1)

Any terrestrial and aquatic plant species can be used in the application of phytoremediation technology due to their extensive potential for detoxification, degradation, and removal capacity of contaminants from the environment (Prasad and Hagemeyer, 1999). Plant properties, such as endogenous, genetic, biochemical and physiological, are important factors to improve soil and water quality in the environment. While selecting plant species in phytoremediation applications, featured parameters are growth of plant in natural environment, adaptation to climatic condition and existing vegetation, resistance to insects and other diseases, increase of mass throughout the year, use of high amount of nutrients and accumulation and make effective root structure. Plants provide pollutant removal in three ways: (a) enhancement of degradation by excretion of enzymes into the soil, water which are caused by microbial activities; (b) intra- or extracellular catalysis within the root and shoot tissues by plants enzymes; (c) transfer of the pollutant through the roots and shoots by sorption mechanism to plant tissues or volatilization into the atmosphere through plant pores (Vroblesky et al., 1999).

Several plant species used for phytoremediation especially to decrease soil pollution, therefore, there is an extensive knowledge about the removal capacity of those plant species used in the studies (Vogelmann et al., 2016). These plant species have also been used for wastewater treatments, and after efficiently applications their usage has become widespread (Table 6).

Many plants adaptation in the treatment of polluted water lead the development of phytoremediation application in the rehabilitation of other surface water such as urban runoff, urban drainage waters, eutrophic lakes etc. (Revitt et al., 1999; Ideet al., 2000).

Floating islets designed for ornamental purposes were thought to be capable of biological treatment in these surface waters. Based on this, the floating plantation structures was used the removal of pollutants which are causing eutrophication i.e. nitrogen and phosphorus in the water. Constructed wetlands containing emergent macrophytes growing hydroponically on a floating texture instead of rooting the soil, its termed floating emergent macrophyte treatment wetlands (FTW). FTW schematic diagram, which is adapted from (Headley and Tanner, 2008) is given in Figure 8.

In the FTW the major pollutant reduction mechanisms has been explained biologically by uptake of plants directly, and the microbial decomposition due to the presence of microorganism available on the floating panel and plant roots, and lastly root systems filtering out sediment and associated pollutants.

Table 6. Some aquatic plants used in constructed wetlands.

Family	**Scientific name**	**Common name**
Alismataceae	*Sagitaria lancifolia*	Bulltongue arrowhead
Araceae	*Pistia stratiotes*	Water lettuce
Araceae	*Lemna valdiviana*	Duckweed
Araceae	*Spirodela sp.*	Duckweed
Ceratopphyllaceae	*Ceratophyllum demersum*	Hornwort
Cyperaceae	*Scirpus californicus*	Bulrushes
Cyperaceae	*Eleocharis sphacelata*	Spike sedge
Cyperaceae	*Cyperus giganteus*	Umbrella plant
Cyperaceae	*Cyperus involucratus*	Umbrella plant
Cyperaceae	*Scirpus validus*	Soft stem bulrush
Hydrocharitaceae	*Elodea canadensis*	Waterweed
Hydrocharitaceae	*Egeria densa*	Waterweed
Juncaceae	*Juncus effusus*	Soft rush
Marantaceae	*Thalia dealbata*	Alligator
Marantaceae	*Thalia geniculata*	Alligator
Poaceae	*Phragmites communis*	Common reed
Pontederiaceae	*Eichhornia crassipes*	Water hyacinth
Typhaceae	*Typha angustifolia*	Carrow leaved cattail
Typhaceae	*Typha domingensis*	Southern cattail
Typhaceae	*Typha latifolia*	Broad leaved cattail

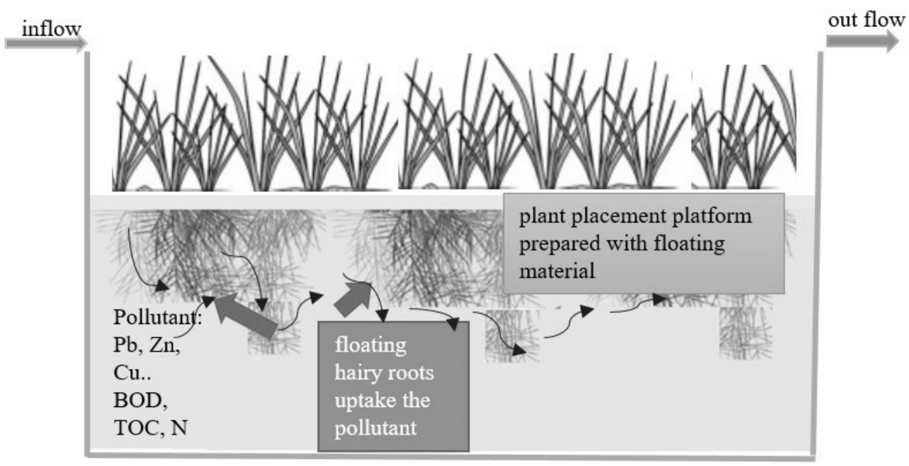


Figure 8. Schematic of a typical FTW.

This technique has been applied for many pollutants. These are water quality improvement and storm water control (Revitt et al., 1997; Kerr-Upal et al., 2000; Li et al., 2010), piggery effluent treatment (Liao et al., 2003; Hubbard et al., 2004), metals in road runoff treatment (Borne et al., 2014), nutrient removal (Keize-Vlek et al., 2014; Xu et al., 2017), TOC removal some textile dyes (Anamaria et al., 2015; Almaamary et al., 2017; Kah et al., 2016), oil well wastewater (Rehman et al., 2018), with various aquatic and emergent plants.

2.5.1. Kinetics

In order to investigate the phytoremediation potential rate, kinetic models can be used in experimental data. Many models are used to fit the kinetic experiments. The majority of the models of constructed wetlands are based on influent and effluent data. The most common reaction is used as first order equations (Equation 2.1).

$$C_t = C_o e^{-kt} \quad (2.1)$$

where C_t and C_0 are the pollutant concentration at time t and 0, respectively, k is degradation rate constant *(1/h),* and *t* is the unit in hours.

2.5.2. *Vetiveria zizanioides* (L.) Nash

Vetiveria zizanioides (L.) Nash commonly known as Vetiver is a perennial grass belonging to Poaceae family, it's able to live in wetlands or a semi-submerged state during long periods, even without being an aquatic plant. Vetiver has eleven species and naturally found in tropical Asian countries, the Pacific Islands and Australia (Grimshaw and Helfer, 1995). It is grown mainly for essential oils and pharmaceuticals, and also are use in particularly superior in erosion control. The use of in wastewater treatment was first proposed in Australia in 1995 (Truong, 2000). Researchers investigate the use of vetiver, which is still using in erosion control areas, into domestic wastewater, industrial wastewater and surface water treatment studies (Dalton et al., 1996; Boonsong and Chansiri, 2008; Yeboah et al., 2015).

Vetiver is a plant that survive even when exposed to high heavy metal ratios, and show strong resistant in the worst environmental conditions (Truong, 1999; Xia and Shu, 2001). It has been successfully used in salt-alkaline (pH: 9.5) coal mines and in high acid (pH: 2.07) gold mine sites in Australia. Its long, hard and dense root structure can reach 3 meters in length (Truong and Baker, 1998).

Vetiver was first fetched to Turkey from the Cataña region of Sicily in 1998, and started to be produced in İzmir Karşıyaka in 2001 (Kilci et al.; 2004). They found that *Vetiveria zizanioides* (L.) Nash's adapted 100% in eight sites of eleven trial sites under Aegean Region conditions during vegetation periods. They showed the potential of this plant for future use.

Hart et al. (2003) studied the removal of nitrogen at an effluent flow rate of 20 L/min, one square meter of long rooted hydroponic vetiver. They have shown the ability of total nitrogen removal of 30.000 mg in eight days, and with light excluded in the recirculation tank can treat 3.575 mg total phosphorus in eight days from domestic sewage. They concluded that vetiver reduced nitrogen and phosphorus considerably more than other plants. In another study, *in vitro* grown vetiver plantlets under aseptic conditions were shown to remove almost all of the phenol from the medium in a period of 4 days when concentration of phenol was less than 200 mg/L (Singh et al., 2008). It has also been observed that vetiver was effective in the removal of organics measured as oxidizable (COD) and biodegradable (BOD) at low concentrations from palm oil mill effluents (Darajeh et al., 2014).

Darajeh et al. (2016) examined the removal of BOD and COD capacity of vetiver at low, medium and high ratio of palm oil mill wastewater (POME) under aerobic and anaerobic conditions by floating technique. 96% and 94% BOD and COD removal were obtained in POMEs using 30 vaccine vetiver plants for 4 weeks. The initial BOD value of 50 mg/L in the 5% POME which was reduced to 2 mg /L on 13rd day with 15 vaccine vetiver plants.

Vetiver plant has also a few other uses, e.g. as forage for livestock, ornamentals, handicraft and art works, medicinal application, construction-related activities (roof thatch, hut). However, vetiver come to the forefront of with its oil, which is popular especially in perfumery (Lavania, 2003). The oil is amber-brown in color, and it's described as a sweet, woody and smoky fragrance. Vetiver oil is obtained from the plant's roots by distillation. It has been found that it contains more than 100 compounds. The compounds of vetiver are khusimene, delta-selinene, beta-vetivenene, cyclocopacamphan-12-ol (epimers A and B), vetiselinenol, khusimol, isovalencenol, khusimone, alpha-vetivone and beta-vetivone have commercial importance (Choua et al., 2012). Therefore, vetiver draws attention as an aromatherapy product.

2.5.3. *Cyperus Alternifolius* L.

The other plant can be used for phytoremediation for OMW is *Cyperus alternifolius*, known as the umbrella palm. Umbrella palm is a perennial plants, and belonging to family of *Cyperaceae*. This emergent aquatic plant has several stems growing directly upward from roots and an umbrella form bunch of leaves at the top of each stem, and growing up to 2 m tall. Native distribution seems in Madagascar, Mauritius, and Reunion Islands. Freshwater, pond, lake and river are i.e. aquatic media is native habitat for umbrella palm. It is one of the most commonly used Cyperus species for landscaping purposes. Well-developed root system securely anchors the plant in wet soil. The improved root system enhances the phytoremediation potential to umbrella palm.

Kantawanichkul et al. (2013) comprised the efficiency of domestic wastewater treatment by vertical flow constructed wetland systems under different hydraulic loading rates (HLR) with umbrella palm and vetiver. They found that the removal efficiency of the umbrella palm was higher than vetiver for both COD and TN parameter at the lowest HLR. The removal efficiency of nitrogen in terms of total Kjeldahl nitrogen (TKN) was 65% for umbrella palm, and 56% for vetiver at 27.4 mg/L initial load of NH_4^+-N and 20 cm/d of HLR. Nitrogen accumulation in plant biomass was also higher in umbrella palm than vetiver for each HLR.

After the screening of some plants, Dolphen and Thiravetyan (2015) showed that *Cyperus alternifolius* had the highest removal efficiency in wastewater containing monoethanolamine, compared to the species of *Ehinodorus cordifolius, Thalia geniculata, Acorus calamus, and Dracaena sanderiana*. Therefore, they studied the degradation of monoethanolamine (MEA), diethanolamine (DEA), and triethanolamine (TEA) by *C. alternifolius*. Plants degraded TEA into DEA, then into MEA, and then further into acetic acid. The practice of umbrella palm in wastewater containing monoethanolamine revealed that plant could exactly uptake MEA at day 5 from an initial MEA concentration of 18 mM. The study concluded that umbrella palm has the potential to remove ethanolamines and can be applied to ethanolamine-contaminated wastewater.

Huang et al. (2017) aimed to determined removal of nutrients of *Canna indica* L, *Iris tectorums, Lythrum salicaria L.,* and *Cyperus alternifolius* in an aquatic environment. Those the four plants were planted in floating devices in a pond, and the unplanted one set as a control pond. The results suggested that combination of four emergent species removal efficiencies for suspended solids (SS), turbidity, total nitrogen (TN), total phosphorus (TP) were 82.5%, 87.5%, 75%, 82.9% respectively, and higher than the control pond. According to water quality classification, planted ponds water body level successfully had reach from V grade to III grade. In the study, it has been shown that with the combination of plants which have different flowering period's highly effective nutrient removal can be achieved, concurrently visually permanent and pleasant urban aesthetic can be achieved.

In the study of Mustapha et al. (2018), the performance of pilot-scale vertical subsurface flow constructed wetlands planted with three local plants*, Typha latifolia, Cyperus alternifolius,* and *Cynodon dactylon*, in terms of removing heavy metals was conducted. Experiment was carried out secondary treatment of petroleum refinery wastewater. The best heavy metal remedies ordered *Typha latifolia, Cyperus alternifolius,* and *Cynodon dactylon* plantation respectively. The researchers reported that these three species of plants could be used for removal of heavy metals in secondary refinery wastewater.

2.5.4. Phytoremediation Studies on Olive Mill Wastewater

Despite existing laws and regulations in the countries which are producing olive oil, OMW origin contaminated agricultural land, soil and water pollution still exist. Nonetheless, olive mill remains infer from problem can be evaluated as a by-product. In the LIFE Project of European Union (LIFE07 INF/IT/438), European awareness raising campaign for an environmentally sustainable olive mill waste management was aimed. The project groups have presented currently possible eco-innovative technologies. Bio fuel pallet, composting and phytoremediation are also some of the alternatives of eight solutions technologies for OMW management determined in the OLEICO + project.

Hence phytoremediation technology requires large application areas depending on the production amount and hence volume of OMW to treat.

Olive oil production usually takes place in the fall and winter season, as a result OMW has been produced luckily vegetation in the environment occurs at the spring season. This can be considered to make the phytoremediation applicable. Nitrogen together with water is the nutrient element that is found scarce. Therefore, it often comes out as a nutrient element that controls plant growth (Cepel, 1996; Gardiner and Miller, 2008; Fageria, 2009).

Since OMW has a high concentration of potassium and significant degree of nitrogen, phosphorus, calcium, magnesium, and iron, (Nikolopoulou and Kalogerakis 2007) were studied the potential of phytoremediation capacity for poplar trees. They placed in impermeable membrane at a depth of 5 meters, over the 50 * 50 m^2 area, and planted two years old poplar trees. During the 90 days, the area irrigated by the ditch which was in depth of 40 cm with OMWs, then monitored the amount of water removed by these trees. It was stated that the remaining water in the drainage canal was not polluting for groundwater. The high amount of water (0.185 cm/day) consumed by the poplar tree is also convenient for phytoremediation.

Chartzoulakis et al. (2010) applied of fresh OMW to the soil surface of an olive orchard during winter time, and reported that the potential use of OMW as a soil amendment, had positive effects on soil properties (total organic C, total N, available P, exchangeable K, and available trace elements). Besides they conducted another experiment for the possibility of groundwater pollution in large lysimeters, the composition of drainage water at a soil depth of 2 m. not altered by the application of OMW.

Removal of polyphenols and Fe, Cu, Zn and Ni by phytoremediation from OMW with Barley (*Hodeum vulgare* L.) was examined by Yosef, (2016). Water containing 50% and 100% OMW, fresh water, and synthetic water that prepared with polyphenol and heavy metals were used to inseminating of barley. The barleys were irrigated with 50%, 100% OMW and fresh water, they were germinated and grown. However, irrigated with 100% OMW the size of the barleys elongated less. It was found that polyphenol, Fe and Zn were reduced, but there was no significant decrease in Cu and Ni concentration. The uptake of polyphenols ratios were 0.31-0.19-0.26 while the Fe uptakes were 0.42, 0.49 and 0.27 in 50%, 100% OMW and frees water areas, respectively.

It is reported that phenol disturbs the metabolic and growth processes in plants, and different plant species have different tolerances to phenol (Flocco et al., 2002).

Herouvim et al. (2011) constructed pilot-scale CW to treat pre-treated OMW in a Greece. The pilot-scale CWs were filled with various porous media, and two series of them were planted with common reeds while the third one was unplanted as a control. Mean influent concentrations were 14 120 mg/L, 2841 mg/L and 506 mg/L while removal performance achieved about 70%, 70% and 75% for COD, phenols, and TKN respectively. In this study COD, phenol and TKN removal seemed to be significantly higher in the planted series.

Yalcuk et al. (2010) examined the performance of a vertical subsurface CWs in treating OMW on pilot scale wetlands by *Typha latifolia* and *Cyperus alternatifolius*. Concentration based average removal efficiencies were 73.46, 73.91, 71.40% for COD 49.06, 37.38, 39.02% for NH_4-N; and 95.43, 95.93, 94.47% for PO_4-P by *T. latifolia*, *C. alternatifolius* planted and control reactors respectively. Again Yalcuk (2011) also studied the phenol removal of OMW in the planted/unplanted CWs in the packed bed reactor with zeolites and sand at two different percentage combinations on a laboratory scale. *Cyperus alternatifolius* was used as the plant for both systems. The avarage phenol concentration was 22.89 mg/L while hydraulic retention time was about 3 days. The phenol removal values varied in respect with the filling material percentages. The removal percentages of CWs were reported as 89.6±2.61 and 74.55±14.17 in planted, 83.21±5.10 and 73.44±19.07 in unplanted reactors. This study informed that uptake of the phenol performance may faster in the umbrella palm planted wetlands.

Kalyvas et al. (2018) investigated the ability of *P.vittata* to extract As, Pb, and Zn from polluted soil by mining activities, with under the effect of EDTA, OMW, and their combination. OMW treatment substantially reinforced plant uptake when combined with EDTA. OMWs assisted the healthier growth of the plants after the second application, and balanced the initially negative impact of OMW.

Darajeh et al. (2014) conducted an experiment for the phytoremediation of the palm oil effluents (POME) which was in the range of high strength of BOD and COD. The wastewater contacted with vetiver plants under hydroponic conditions for 2 weeks. Vetiver plants with well-developed root and shoots were able to reduce the BOD up to 96% in low concentration POME (%10 diluted) and 62% in high concentration POME (undiluted), while control sets (without plant) only was able to reduce 15% of BOD. The

COD removal ratio was 92% in low concentration POME, and 39% in undiluted POME, while control samples just shows 12% removal.

In another phytoremediation study for POME, the organic pollution as COD, nutrients (NH_3-N and TP) and suspended solids removal capacities were tested with *Cyperus Alternifolius*. COD and TSS removal rate were reported as 96% and 91%, respectively, in the presence of umbrella plant for 21 days (Sa'at et al., 2017).

The study which was conducted with phytoremediation of pig farm wastewaters by umbrella palm and vetiver, the COD, BOD, NH_4^+-N and phosphorus removal were investigated by placing 3.65 kg of plants in wastewater. After 8 days of contact time, with an initial COD concentration was 825 mg/L and the COD removal rate were reported as 66 and 64% respectively (Liao et al., 2003).

CHAPTER 3

MATERIALS AND METHODS

In these chapter materials, analytical methods, experimental set-up and procedures used in this study are described.

3.1. Materials

3.1.1. Olive Mill Wastewater

The OMW samples used in the study were obtained from a local olive cooperative in 09/12/2016. The mill uses a three-phase continuous process for oil production. According to the report of facility that the OMW given to these experiments belongs to the species of olive known as Ayvalık.

3.1.2. Preparation of Plant Cultivation

Vetiveria zizanioidies and *Cyperus Alternifolius* species were propagated by cutting in Aegean Forestry Research Institute, and rooted in soil environment. It was observed that the vegetation started in the beginning of February 2017, and the plants were removed from soil, and taken into a hydroponic solution with (N,P,K: 15:15:15) fertilizer until adequate root and shoot development ready for the experiment. Dark-colored containers were used so that roots of plants did not receive sunlight. After observing that the plants were new rooted in the water media, they were taken to diluted OMW containers on 2nd of March, and adapted until the 10th of May. Diluted OMW were prepared in 20 liters of water from 5 to 40% at various ratios (called as OMW-5, OMW-10, OMW-20, OMW-30, OMW-40) to investigate the adaptation of plant with varying concentration. pH was adjusted around neutral value (6-7). Tap water was used for dilution.

3.2. Analysis of Parameters

In this section analytical methods used for the total organic carbon (TOC), total nitrogen (TN), total phenol and the content of the phenolic compound and plant analysis are described.

3.2.1. Analysis of Total Organic Carbon and Total Nitrogen in OMW

Many methods have been developed to measure the amount of organic matter in wastewater. Currently, the most common methods of the forms for organic substance identification are BOD, COD, and TOC methods. Organic carbons measurements were preferred in this study in order to determine the TOC content of the samples.

TOC was determined using TOC–L analyzer (Shimadzu, TOC-VCPH Total Organic Carbon Analyzer). This analyzer measures the 680°C combustion catalytic oxidation method, convert total carbon into CO_2, range of 4 µg/L to 30.000 mg/L, through coordination with non-dispersive infrared (NDIR) according to Standard Methods 5310B. Total Carbon (TC) is measured by injecting a portion, the water is vaporized and TC, the organic carbon and the inorganic carbon (IC), is converted to carbon dioxide. The carbon dioxide is carried with the carrier gas stream from the combustion tube to a NDIR detector, and concentration of carbon dioxide is measured (Figure 9.). The TC concentration of the sample is obtained by using the calibration curve prepared with standard solutions. IC is measured by injecting a portion of the sample into an IC reaction chamber filled with phosphoric acid solution. All IC is converted to carbon dioxide and concentration. In order to stay in the range of the method, 0-100 mg/L, and ultra-pure waters were used when dilution was required.

3.2.2 Phenol Extraction of OMW

Liquid–liquid extraction with ethyl acetate was carried out on OMW samples. Total phenolic compound content in OMW was carried out by phenolic extract which were prepared according to (Garcia et al., 2000) procedures. OMW samples were acidified to pH 2 with all. Then, in order to remove the lipid fraction, 10 mL of the samples were washed with 15 mL of hexane. The mixture was centrifuged for 5 min at 3000 rpm. The phases were separated, and the washing was repeated successively two times. Extraction of phenolic compounds was then carried out with 10 mL ethyl acetate addition followed by centrifuging for 5 min at 3000 rpm. The phases were separated, and the extraction was repeated successively three times. Then, ethyl acetate was evaporated under vacuum at 40°C in a rotary evaporator (Buchi B-490) for 30 min. The dry phenol residue was dissolved in 3 mL of methanol, and this solution was used for quantification and fractionation of phenolic compounds.

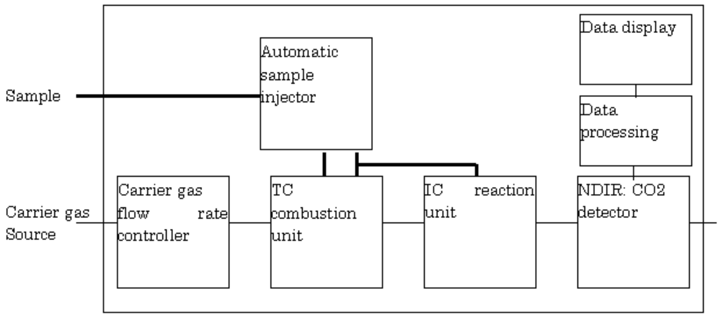


Figure 9: Schematic diagram of TOC measurement by Shimadzu TOC-L analyzer.

To determine the reproducibility of the extraction method, the standard deviation of the method was calculated as 146 mg/L (n=10) for a total phenol mean value of 7484 mg/L resulting in a coefficient of variation value of 2%.

3.2.3. Phenol Extraction of Plant Tissues

The extraction was carried out on vetiver and umbrella palm plants root and shoot. Samples of fresh green shoots were used. Samples washed with deionized water and then dried at 37 °C for 3 consecutive days. The dried shoots and root were ground for extraction. A mixture of methanol and water (10 ml, 4:1 v/v) was added the plants shoot and root powder (10 g) and the mixture was kept under agitation for 24 hours. The solution was filtered by GF/F filter paper. The solvent of the extracted medium was removed by using the rotary evaporator at 40 °C, under vacuum.

3.2.4. Analysis of Total Phenolic Contents in OMW and Plant Tissues

Total phenolics were determined by the Folin–Ciocalteu method (Singleton and Joseph 1965). Two hundred microliters of 1:10 diluted sample were added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 ml of sodium carbonate (75 g/L) were added. After 2 hours of incubation at room temperature, the absorbance was measured at 765 nm (Thermo Scientific Evolution 300 UV- visible spectrophotometer). Gallic acid (0–500 mg/L) was used for calibration of a standard curve show in Figure 10. The results were expressed as gallic acid equivalents (GAE)/ g extract and dry weight of plant material. The both OMW and water samples triplicate measurements were taken and mean values are reported.

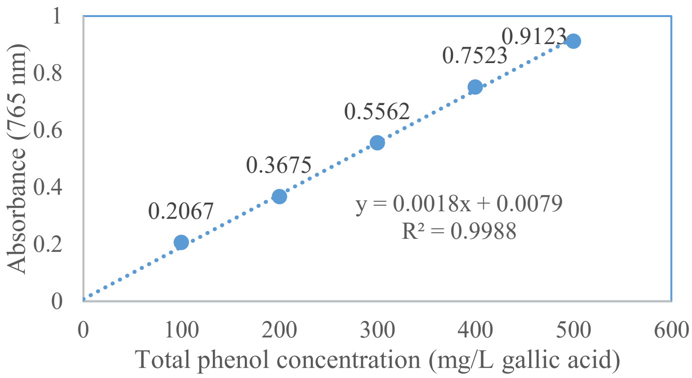


Figure 10. Calibration curve for total phenolic compound content.

3.2.5. Analysis of Phenolic Compounds in OMW

Tyrosol, hydroxy tyrosol, vanillic acid, syringic acid, and p-coumaric acid which are likely to be present in the OMW according to the literature were measured by HLPC. Operating conditions of the HPLC are given in Table 7.

3.2.6. pH

pH values of the samples measured at sites using portative WTW pH meter at the determinate days. As soon as the OMW samples brought to the lab, and the pH value was measured again using a Thermo Orion 3 Star pH-meter.

Table 7. Operating Conditions for HPLC Analysis.

Variable	**Property**	
Device	Agilent 1100 series HPLC	
Column	Inertsil ODS3V (GL Science)	
Column size	4.6 mm x 150 mm x 5 µm	
Moving phase flow rate	1 mL/min	
Column temperature	30ºC	
Detector	DAD	
Wavelength	280 nm	
Carrier phase A	%70 : %30 (ACN) : (MeOH)	
Carrier phase B	% 0.1 (o-H_3PO_4)	
Injection volume	20 µL	
Used Filter	0.45µm PTFE	
HPLC Gradient program applied in the analysis		
	Carrier phase A	Carrier phase B
t (minute)	(70:30, ACN:MeOH)	(% 0.1 o-H_3PO_4)
0	5	95
25	30	70
35	50	50
55	5	95
60	5	95

3.2.7. Measurement of Growth Parameters and Macro- Micronutrients Analysis of Plant

Three growth parameters root length, stem heights and total biomass amount, were measured at the beginning, and end of the experiment. Root lengths were determined by measuring from the root collar to the tip of the tap root. The stem heights were determined by measuring from the root collar to the stem top. The biomass values at the beginning and end of the experiment were weighed as a total wet weight, additionally, dry weight was also taken separately for roots and shoots at the end of experiment.

To determine the wet weights, plant samples were weighed on a precision scale to gram (±0.1), then incubated at 65-70 ^{0}C for 24-42 hours for the removal of water which is called as air-dried weight, and then ground for rest of analyses. The milled plant samples were subjected to moisture determination (TS ISO 11465) at 105 °C for 24 hours.

Thus, the results of the whole plant analysis were calculated according to the moisture correction coefficients obtained and corrected according to the weight at 105 °C. The dried plant samples were digested by wet-burning method using microwave combustion system, freeze analysis using H_2O_2 and HNO_3, in order to carry out macro and micro elements and heavy metal analysis. The analyzes of K, Ca, Mg, Na, Fe, Cu, Zn, Mn and heavy metals were analyzed according to ICP-OES (Perkin Elmer Optima 2100 DV) and ICP-MS (Perkin Elmer Nexion) principle. The P analysis was made according to the vanadomolybdo phosphoric acid colorimetric method (yellow method) at wavelength of 470 nm (Thermo Scientific-Evolution 300 UV-VIS). In the C, N and S analyzes, the pulverized samples were directly analyzed. C, N, S analyzes were made according to Dumas (dry burning) method.

3.2.8. Experimental 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 orto phosphoric acid 85%were purchased from Merck.

3.3. Experimental Set-Up and Sampling

In this section set-up of experiments and the sampling method used in this study are described.

3.3.1. Experiments

Two types of experiments were conducted. Primarily, the tolerance level of dilution was determined by the results of different olive mills wastewater dilution values. As a result, the phytoremediation experiment were carried out in two different OMW dilution ratios 5% and 15%, respectively and coded as OMW-5 and OMW-15.Due to availability of the plants, the experiments were conducted by using 2.5 and 4 kg of umbrella palm and vetiver plant groups formed from individuals which had been adapted to OMW, for placement in OMW-5 and OMW-15 respectively. With and without plants in triplicates according to schematic explanation of the procedure can be seen in Figure 11. The experiment was carried out in the greenhouse environment. Dark colored, 60 L plastic tanks were used. In the beginning of the experiments 56 L diluted OMW was added in each of the tanks. Tap water (TW) was used to prepare OMW-5 and OMW-15 dilutions. The surface of tanks was covered with greenhouse nylon to reduce evaporation.

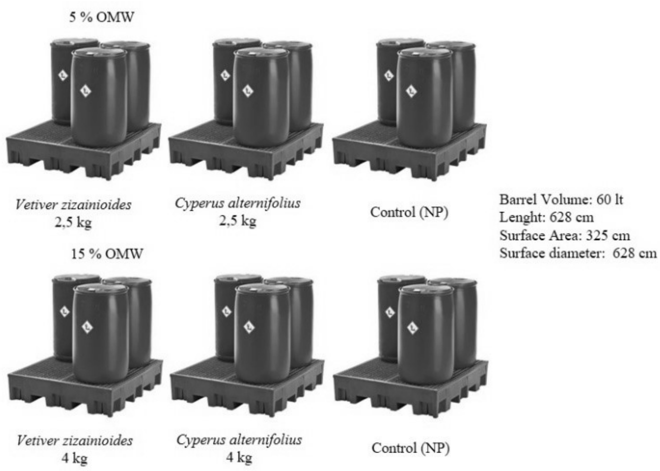


Figure 11. Experimental design

The pH values and the amount of evaporation in the tanks were recorded on a daily basis. During the experiment, no nutrients and water were added. Water samples were taken on 1^{st}, 9^{th}, 17^{th}, 25^{th}, 45^{th}, 53^{rd}, 59^{th} and 67^{th} days for the monitoring of total phenol, total carbon as TOC TN analysis. At the end of the study, the plant measurements were used to calculate the treatment performance of the plants.

3.3.2. Sampling

20 ml polystyrene test tubes were used the OMW sampling. Temperature and pH measurements were performed at site, and kept under + 4 C until analysis at the laboratory. Required dilutions for each parameter and required extraction were prepared at the beginning of the analyses.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Characteristics of Raw OMWs

In the literature, olive mill wastewaters (OMW) show differences even within Mediterranean area. While pH values were in a narrow range around five, the other characteristic parameters vary considerably. Therefore, determination of characteristics of OMW is important, which also reflect the strength of the wastewater. The wastewater composition and variation in the 12-day period is reported in Table 8. Notable change was observed in some of the phenolic compounds, i.e., 4-hydroxytphenly ethanol, vanillic acid, and coumaric acid, there was no considerable change in the other parameters. It is known that some of the phenolic compounds could be converted to other metabolites or oxidized.

Table 8. The results of preliminary adaptation of the plants to diluted OMW.

Parameter	**OMW (15.12.2017)**	**OMW (27.12.2017)**
pH	5.12	5.13
t-Phenol (g/L)	13.2±0.56	10.4±0.30
4-Hydroxytphenly ethanol (mg/L)	270	294
2-(3,4-Dihydroxyphenyl)ethanol (mg/L)	85.5	87.4
Vanillic acid (mg/L)	28.9	20.1
Syringic acid (mg/L)	-	-
trans p-coumaric acid (mg/L)	17.4	21.8
Total Nitrogen (TN) (g/L)	0.51	0.50
Chemical Oxygen Demand COD (g/L)	103	98
Total Organic Carbon TOC (g/L)	23.0	23.4

4.2. Adaptation of Vetiver and Umbrella Palm in Olive Mill Wastewaters

In general, not every plant grows in every environment because tolerance mechanisms for every plant are different for the adaptation to accumulate or exclude any of the problematic chemical to maintain their growth (Sabir et al., 2015). It is reported that if a plant will be used for contaminant removal, understanding the mechanism of accumulation and tolerance of contaminants by the plant are important due to complexity of this phenomena. For example, the metal adaptation is explained as, the movement of metals across the root membrane, then loading and translocation of metals through the xylem, and sequestration and detoxification of metals at cellular, and whole plant levels in the accumulator plants. Therefore, the plant adaptation of different kind of waste is mainly required for the effective removal of contaminants.

Adaptation of the plants to OMW was conducted at the beginnings of March. It was observed that umbrella palm was well adapted with growth of new/fresh white root shoots and green shoots forth in OMW-5, OMW-10 and OMW-20. However, vetiver plants have shown anew white root and green shoot forth only in OMW-5 diluted media. In the other diluted OMW that within the individuals 60% of the shoots turned to yellow colors and existing roots began to blacken, but they have stayed alive. Growth observations of the plants in OMW dilutions are presented in Table 9.

Table 9. Observations of plant growth exposed to OMW

	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	1 400	3620	5470	1 016
TOC (mg/L)	717	1062	4484	10110	12093
Umbrella palm					
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
Vetiver					
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

The images in Figure 12 represent the general state of the plants at the lowest and highest diluted OMW's. Taken into account, the livability of the plants, further experiments were conducted with OMW-5 and OMW-15 samples.

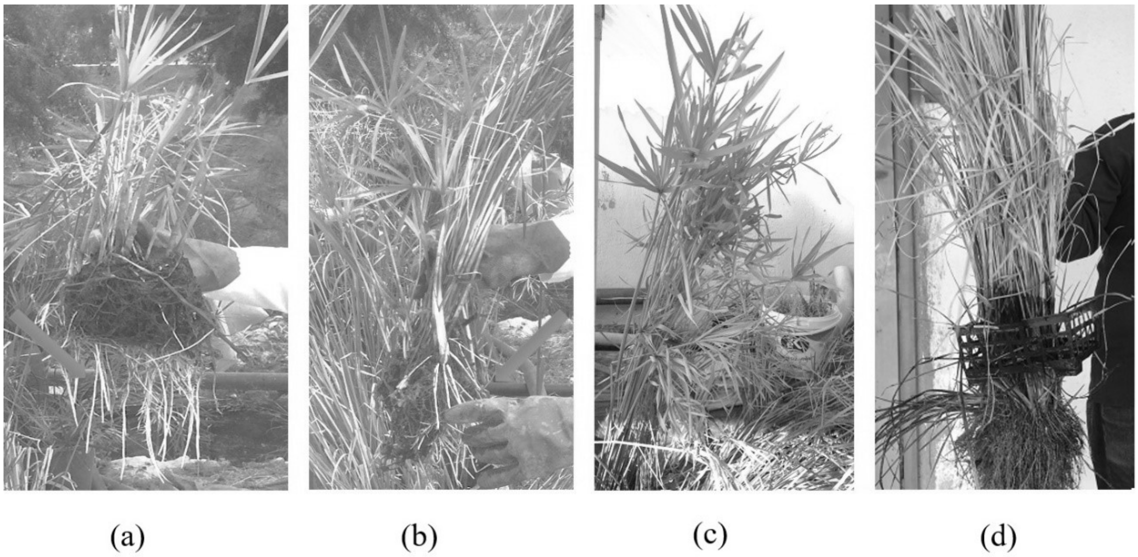


Figure 12. Plant adaptation in OMW-5 (a) umbrella palm, (b) vetiver and in OMW-40 (c) umbrella palm, (d) vetiver.

4.3. pH Measurement

As is known the pH factor of soil or water reflects its acidity level, which is important to consider, since species of plants require at different levels of pH for growth properly. The pH value has an effect on the growth of plants and the intake of water and therefore nutrients. The level of pH is determined the dispersion of other macro and micro nutrients in the medium, and an unproper circumstances can inhibit a plant's ability to absorb them. Plants generally develop better in soil and water media at 5 to 7 pH values (Arnon and Johnson, 1942; Truong, 2006). Researchers examined the pH effect on capacity of vetiver's remediation, and reported that the vetiver was tolerant to pH 3.5-11.5.

At the beginning of the study, a preliminary study was conducted to monitor the effect of pH on these plants. Therefore, pH was adjusted from 4 to 9 in tap water, and the growth of umbrella palm and vetiver growth were observed. Both species continued their development around pH 6-8. Since the pH of solutions at the beginning of the experiment was around 7 in OMW-5 and OMW-15, pH values was not adjusted in the beginning and duration of the experiment.

pH of control group did not change at initial and final reading pH (6.8-7.5), but the pH values increased in planted groups, during the experiment. The significant pH change occurred in the umbrella palm planted OMW-15 solution (from 6.85 to 8.23), and in OMW-5 for vetiver (6.33 to 9). The change in pH values over time is given in the Figure 13.

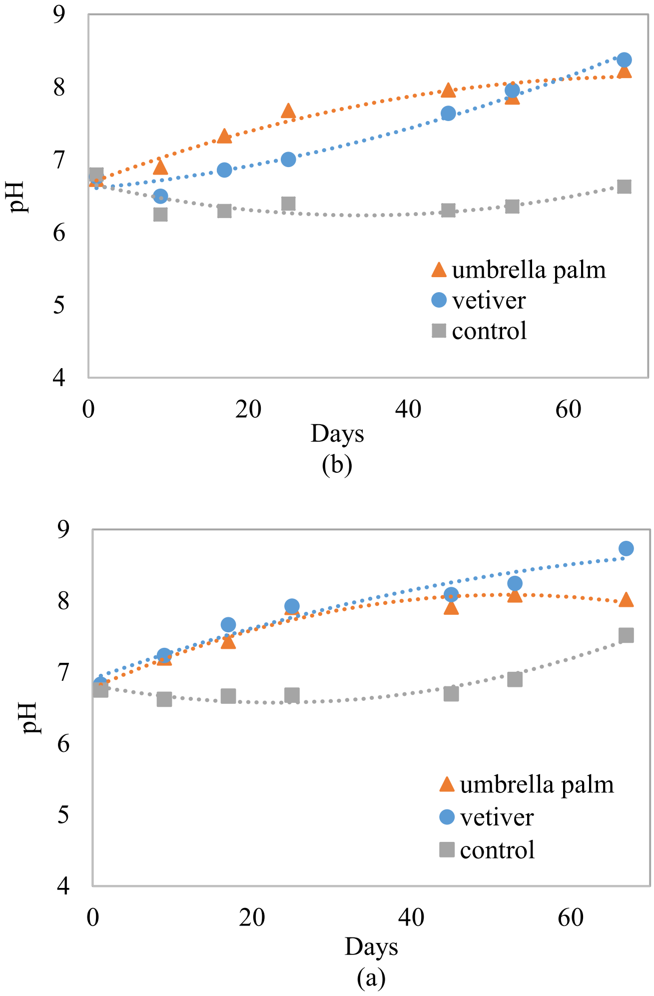


Figure 13. Variation of average pH values in (a) OMW-5, (b) OMW-15.

4.4. Water Uptake

Due to constant loss of water through plant leaves, there must be a certain amount of continuous moisture available in the soil for survival of plants (Tardieu et al.,2017; Kubota, 2017). Water use in the experiment can be described by evaporation from water surface and water uptake by plants. The water uptake by the plant released to the atmosphere is called as transpiration. There are a number of factors that determine transpiration rates which also is part of water uptake. Transport of water across through cell membranes is a basis requirement for regulating plant-water situation. Many specific stress factors affect the mechanism of hydraulic conductivity of roots, including salinity, drought, root temperature and oxygen demand (Aroca et al., 2012). However, the experiment was run in the hydroponic environment, therefore plants did not have any stress due to water.

Two types of water absorption are available in plants: passive and active. In the passive transport, the aerial parts of the plant due to loss of water in transpiration creates a tension or low water potential of several atmospheres in the xylem channels while active water absorption occurs due to forces present in the root which is through living cells activation in metabolic condition.

At the beginning of the experiment, all tanks were filled with 56 L. of diluted OMW. During the phytoremediation experiments, the amount of remaining water in each tank was measured. Cumulative reduction in the amount of water in the control and the experiment tanks are shown in Figure 14. During the experiment period the temperature ranged from 25 ºC (May 9th, 2018) to 40 ºC (June 30th, 2018).

The decrease in the water amount occurred due to plant consumption and evaporation therefore the consumption of water by plants was calculated by subtracting the water loss in the control tanks which was the evaporation from free surfaces (Figure 15). The amount of evaporation was relatively higher in the control tank of OMW-5 (22.2±1.01L), than OMW-15 (16±0.3 L), it was assumed that the remaining water- used by plants. It is observed that umbrella palm consumed 27.7±0.04L and vetiver 14.2±0.45 L of water in OMW-5, and it were 14.8±0.4, 11.0±0.3L for OMW-15. While at low OMW content, umbrella palm consumed faster and almost all of the volume.

Chandra et al. (2017) were discussed that high rates of water uptake and transpiration could result in efficient transport of compounds from roots to stem causes rapid growth and large biomass production. It was reported that under wetland conditions or high water supply vetiver can use more water than other common wetland plants such as *Typha* spp., (approximately 7.5 times more) and *Phragmites australis* and *Schoenoplectus validus.* This was explained due to structural differences in root system.

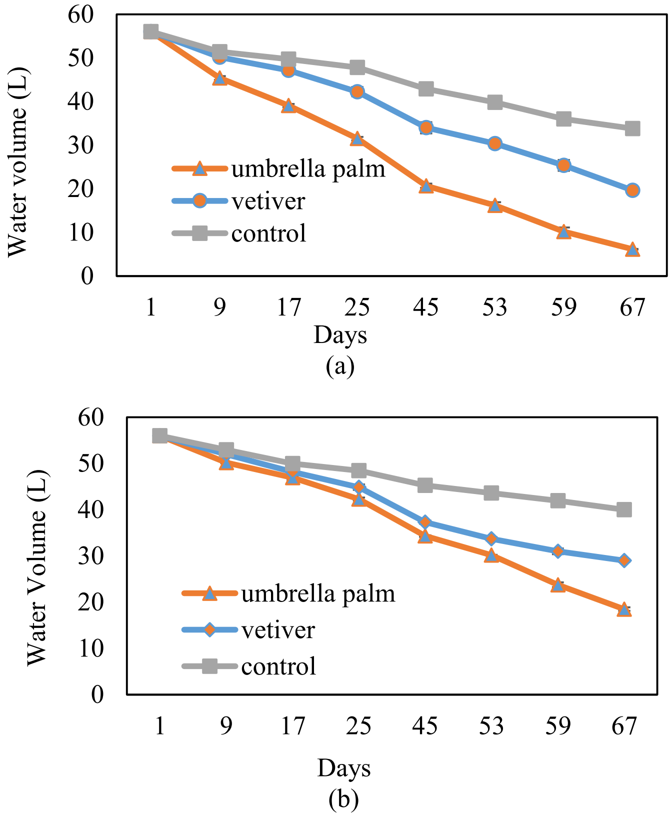


Figure 14. Water volume change during the experiment in (a), OMW-5 (b) OMW-15. Error bars show one standard deviation.

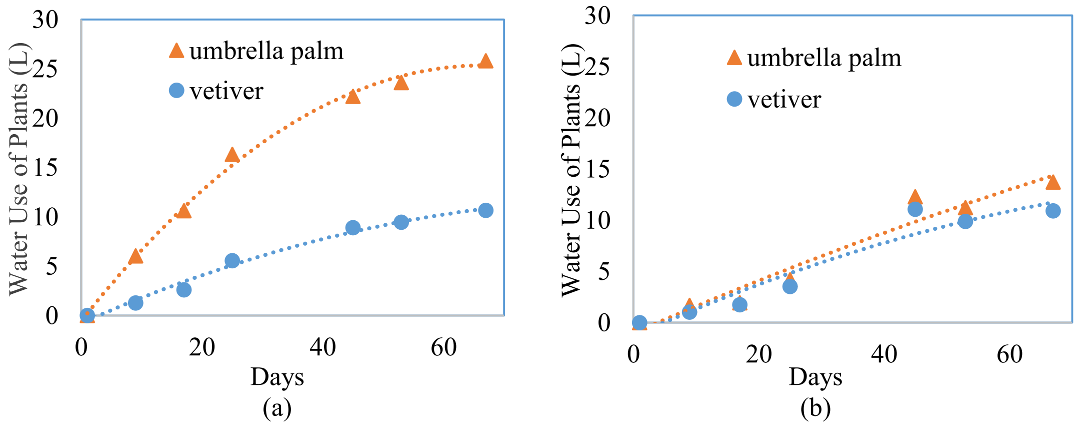


Figure 15. Water consumption by plants in (a) OMW-5, and (b) OMW-15.

The researchers with reference to importance of the evapotranspiration in CWs for wastewater treatment used a pilot plant at a horizontal subsurface system in Italy (Tuttolomondo et al., 2015). Two separate planted areas were made for two types of plants with *Arundo donax* L. giant, and *Cyperus alternifolius* L. individually. The aim of the study was to evaluate, how different macrophytes use up the water at the outflow of CWs under same environmental, growth and hydraulic conditions. In the two years of tests, giant reed was found to have higher cumulative evapotranspiration values than umbrella palm. For both macrophytes, evapotranspiration values were found to be higher during the vegetative season, when the plants reached maximum growth. Water use efficiency was rather low on average, at 0.94 g/L for giant reed, and 0.66 g/L for umbrella palm. Their results showed that greater or lesser amount of water at the outflow of the CWs is always dependent upon the evapotranspiration rate of the species and it is essential to be identified.

When the contaminant removal is similar for different species, the water requirement for future use of treated effluent is a key factor, selection species could be an important parameter based on water uptake capacity of the species. For example, if phytoremediated water will be used for agricultural irrigation, the one has less water uptake capacity could be the better alternative. In terms of water consumption in this study umbrella palm consumed 1.9 times more water than the vetiver in OMW-5.

4.5. Contaminant Removal

4.5.1 Organic Carbon Removal

Organics in water may measure as COD, BOD, and TOC. The removal of organics in literature commonly reported as based on the method application in preference. TOC analysis gradually began to take the place the analysis of BOD and COD, on the grounds that the BOD measurement requirement for 5-days, and generation of also hazardous waste because of the chemicals used in the COD experiment (Bourgeois et al., 2001). TOC test methods were initially developed in the late 1960's to rapidly determine the organic content of waters. To link up a relationship between TOC and COD or BOD, the type of wastewater, and the amount at the beginning are significant. For instance, Dubber and Gray (2010) investigated the correlation of BOD_5, COD and TOC in the wastewater from municipal wastewater treatment plants. They found out a strong linear relationship in influent water for BOD and COD correlated, but not strong relationship found after settlement in water. In the outflow water, rather weak correlation was determined. Between COD and TOC, there was a strong relationship between inflow and outflow.

Badawya et al. (2009) investigated the OMW treatment with photocatalytic oxidation process in Egypt, while Belaid et al., (2006) focused on the electrochemical treatment of OMW in Tunisia. They measured the efficiency of these methods by TOC, phenol and COD parameters. The ratio of COD/TOC were 2.6 in both studies while in the OMW coagulation and electrocoagulation study of Kartal et al. (2008) the COD / TOC ratio was calculated as 2.9. The COD / TOC ratio of OMW used in this study was found 4.32. It was already mentioned, olive shows variation based on geographical region which reflects in the content of wastewater as well.

Initial average TOC concentrations were 1132±58 mg/L and 3168 ±108 mg/L in trials of OMW-5 and OMW-15, respectively. Accordingly, average TOC load per gram of fresh plant weight were calculated as 25.46±0.22 and 25.87±1.04 mg TOC/g wet weight in OMW-5, 45.30±1.44 and 44.01±0.88 mg TOC/g wet weight in OMW-15 respectively for umbrella palm and vetiver planted. Those initial values changed over time therefore, the increase in plant mass and decrease in TOC during the experiment taken into account. The changes in the loads from the first to 67th days of the experiment are given in Table 10.

Table 10. Average load of TOC on the plants.

	Umbrella palm	**Vetiver**	**Umbrella palm**	**Vetiver**
mg TOC / g plant	**OMW-5**		**OMW-15**	
1st day	25	26	45	44
67th day	0.5	3	4	11

The TOC value was measured by ppm in the appropriate diluted samples on the control days without any delay. This measured value was multiplied by volume of water available in tanks and the total TOC mass was calculated as grams. The calculated total amount of TOC is given in the Table 11.

Removal of TOC was calculated using the following Equation 4.1:

$$Removal\ (\%) = \left[\frac{C_0 - C_t}{C_0}\right] \times 100 \qquad (4.1)$$

where C_0 and Ct represent the pollutant concentration at time t and 0 and final concentration, respectively.

The removal of total TOC mass were 95% and 85% for umbrella palm and vetiver, while it was 65% in control tank for OMW-5. It can be seen that total organic carbon content of OMW reduction was higher both umbrella palm and vetiver than that of the control sets. This reduction were slightly less both in planted and control tanks in OMW-15 groups compared to OMW-5, with 90%, 79%, and 51% for umbrella palm, vetiver, and control tanks respectively (Figure 16).

Table 11. Change in TOC mass (g) during the experiment.

Dates	09.05.17	17.05.17	25.05.17	02.06.17	22.06.17	30.06.17	14.07.17
Samples	**OMW-5 (g TOC)**						
Umbrella palm-1	63.56	32.37	21.14	16.78	9.01	5.21	3.03
Umbrella palm-2	64.37	35.15	23.49	18.01	8.63	4.85	3.06
Umbrella palm -3	63.01	32.28	23.63	15.87	8.32	4.73	2.94
Vetiver-1	62.27	45.14	34.34	26.88	18.80	16.07	9.82
Vetiver-2	68.28	43.21	35.60	28.15	17.83	15.28	9.46
Vetiver-3	63.48	44.40	35.09	27.69	18.44	14.68	9.36
Control-1	61.55	46.92	40.72	36.90	31.83	27.71	22.68
Control-2	72.07	50.62	43.76	39.06	33.89	29.09	24.58
Control-3	67.26	48.17	41.45	37.69	31.87	28.74	23.38
	OMW-15 (g TOC)						
Umbrella palm-1	175.76	106.86	85.31	60.17	38.79	31.80	18.58
Umbrella palm-2	178.69	123.40	90.46	68.19	41.67	31.68	18.88
Umbrella palm -3	189.19	109.20	81.80	62.36	37.77	31.46	19.54
Vetiver-1	178.71	137.64	103.09	86.59	59.70	48.95	38.57
Vetiver-2	178.34	137.92	104.02	92.09	56.16	44.24	36.71
Vetiver-3	171.03	137.74	111.28	90.60	58.00	44.83	36.20
Control-1	172.17	150.57	141.73	130.09	110.26	93.56	87.40
Control-2	168.14	151.43	142.50	124.80	100.50	89.35	78.50
Control-3	169.74	149.71	138.20	122.61	101.32	87.74	84.00

It is thought that the reduction of TOC increased over the time from 32-95% for umbrella palm and 9-85% for vetiver due to adaptation and growth (increase of biomass) of the plant in OMW-5. The umbrella palm plant biomass increased as 149% implying that the tolerance of OMW was better for umbrella plant in lower amount of OMW presence.

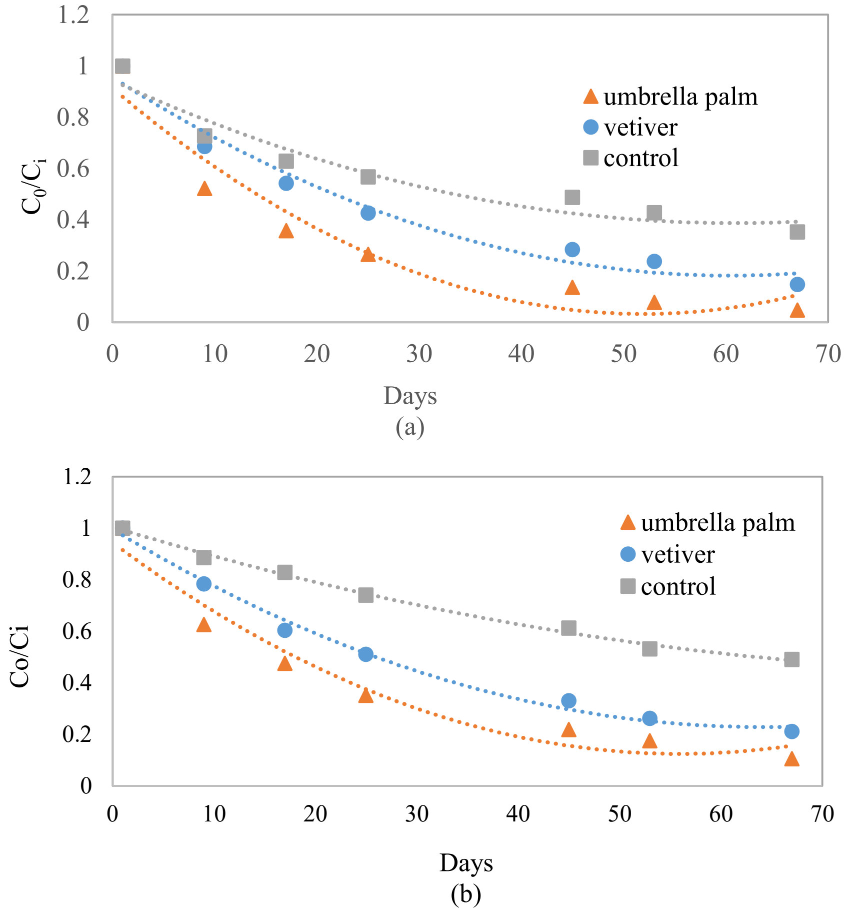


Figure 16. TOC removal in (a) OMW-5 and (b) OMW-15.

Similar studies were conducted for the removal of organic carbon (Table.12). One of them is the study of palm oil mills effluents which were treated in constructed wetland with floating root technique. *Cyperus alternifolius* and *Chrysopogon zizanioides* L. (species of Vetiver) were used as plant species, COD was monitored to estimate removal rate. Removal of COD ranged from 30-96% (Darajeh, 2014 and 2016; Sa'at, 2014) in undiluted and diluted samples. While it was 65% for both plant species in pig farm wastewater with floating technique (Liao et al., 2003). Kapellakis et al. (2012) achieved approached 90% COD treatment efficiency for OMW by recirculation in CWs, which are planted with *phragmites australis*. Yalcuk et al. (2010) achieved 73.46, 73.91% COD removal efficiencies by *Typha latifolia* and *Cyperus alternatifolius* in a vertical subsurface CWs.

Almaamary et al. (2017) investigated that the possibility of *Scirpus grossus*, genus of aquatic, grass-like species in the family *Cyperaceae*, for degradation of a basic dye, methylene blue at the different dye concentrations up to 1000 mg/L. The highest TOC removal occurred at in 200 mg/L (63%), while the lowest was at 1000 mg/L as 26% after 72 days.

Table 12. Literature comparison of removal of organic carbon with similar plants used in this study.

Effluent	Type of System	Dilution Rate (%)	Initial Conc. (mg/L)	Retention Time	Plant Species	Removal (%)	References
POME	CW*	none	COD 790	21	*Cyperus alternifolius*	96	Sa'at,2017
POME	CW	none	COD 810	14	*Chrysopogon zizanioides L.*	30-39	Darajeh, 2014
POME	CW	10		14	*Chrysopogon zizanioides L.*	74-92	Darajeh, 2014
POME	FR* w/ aeration	none	COD 750	27	*Chrysopogon zizanioides L.*	66	Darajeh, 2016
POME	FR w/aeration	10	COD 115	14	*Chrysopogon zizanioides L.*	64	Darajeh, 2016
Pig Farm	FR	none	COD 825	8	*Umbrella palm*	66	Liao and Wu, 2003
Pig Farm	FR	none	COD 825	8	*Vetiver*	64	Liao and Wu, 2003
OMW	CW		COD 2881	3	*Typha latifolia, C.alternatifolius*	73-74	Yalcuk et al. (2010)
OMW	CW		COD 14120		*Phragmites australis*	70	Herouvim et al. (2011)
OMW	CW		COD 6680		*Phragmites australis*	80-90%	Kapellakis et al. (2012)
OMW	FR	5	TOC 1132	64	*Umbrella palm- Vetiver*	95-85	This study
OMW	FR	15	TOC 3168	64	*Umbrella palm- Vetiver*	90-79	This Study

*CW: Constructed wetland; FR: Floating root

4.5.2. Removal Kinetics

The kinetics of the total organic carbon removal was evaluated. The data was plotted time versus concentration for zero, first and second order reactions. As seen in the Figure 17, the best fit was obtained from first order reaction kinetics. Therefore it could be said that TOC reduction in diluted OMW followed the first-order rate law. The rate constant of TOC removal in OMW-5 was 0.0018 (1/h) for umbrella palm, and 0.0011 (1/h) for vetiver while without plants it was found as 0.0006 1/h. This value for OMW-

15 was 0.0013 (1/h) in the umbrella palm planted, and 0.0010 (1/h) in vetiver planted tanks while it was found as 0.0004 1/h for the control tanks.

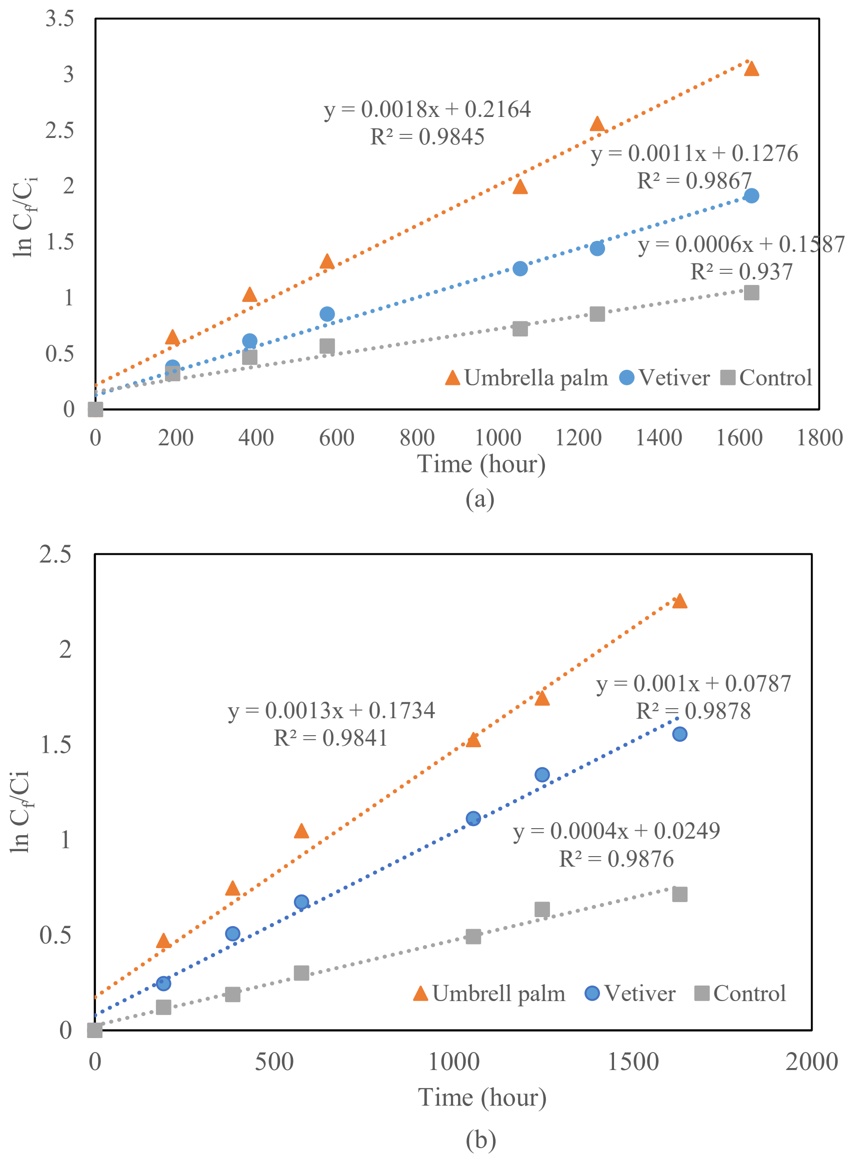


Figure 17. TOC removal kinetics modeled by first order reaction (a) in OMW5, (b) in OMW15.

4.5.3. Removal of Total Phenol

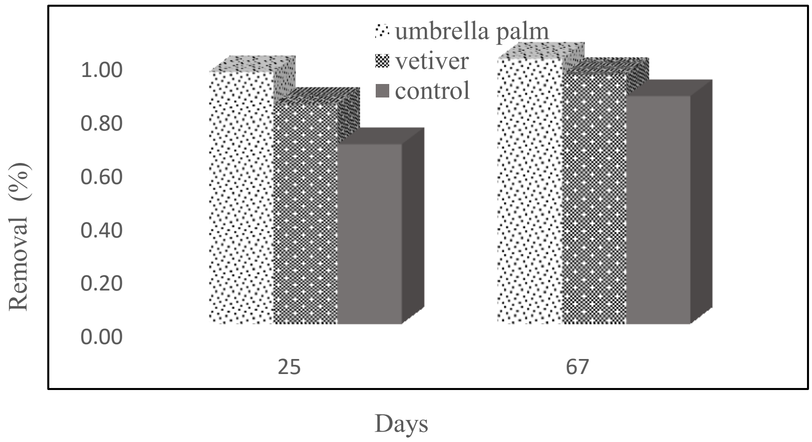
Olive mill wastewater (OMW) generally has high organic load due to the presence of phenolic compounds. More than 50 types of phenolic compounds have been identified in OMW, among them hydroxytyrosol and tyrosol are reported as the most commonly present phenolic compounds. Phenrat et al. (2017) conducted experiments to investigate the feasibility of using vetiver plantlets on a floating treatment technique with aeration to degrade 500 mg/L phenol in illegally dumped industrial wastewater. The plant with aeration degraded the phenol less than 32 days while it was 235 days with only aerated microbial degradation.

In this study, OMW sample was obtained on 09/12/2016, and the total phenol measured as 13.2 g/L and 10.4 g/L in raw OMW samples on 15/12/2016 and 27/12/2016 respectively. This OMW sample was stored at the container in the greenhouse until the vegetation started on May. Immediately before the experiment, total phenol measured to determine the initial total phenol, 4000 mg /L. There was no difference in the concentrations of TOC and TN during the storage from December to May. The first TOC and TN value were 23 g/L and 0.5 g/L, they were measured as 18.2 g/L and 0.52 g/L in May. This decrease in total phenol value was also observed in other studies in the literature. Akardere (2012) monitored, the activities of enzymes that catalyzes the degradation of phenolic compounds during the bioremediation of OMW by *Rhodotorula glutinis* and *Debaryomyces hansenii* yeasts. The variation in total phenol and some phenolic compounds were investigated, individually. At different time measurement, total phenol content decreased, even it was stored at + 4 °C. The decrease was attributed to air oxidation of the phenolic compounds during the storage.

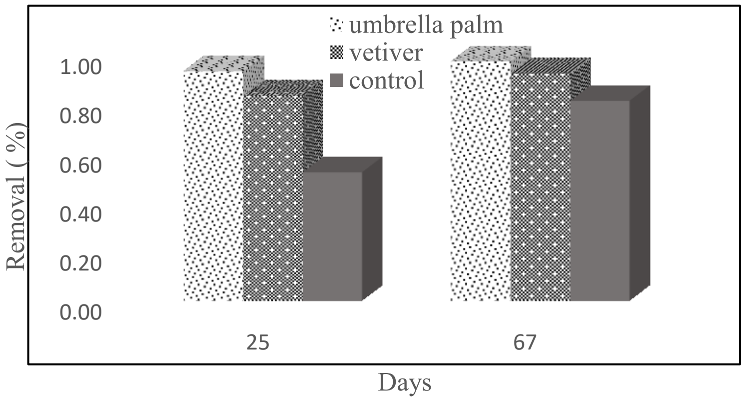
In our study, the initial total phenol concentrations were 219 mg/L, and 611 mg/L based on the dilution of OMW-5 and OMW-15. The average loading of phenol per gram of fresh plant weight was calculated as 5.5 mg/g wet weight and 9.8 mg/ g wet weight in umbrella palm and vetiver planted groups respectively, at the beginning of the experiment.

Total phenol measurements were conducted 3 times during the experiments, as 1^{st}, 25^{th} and 67^{th} days. The results of 25^{th} day and 67^{th} total phenol amount showed that higher amount of phenolics substantially removed in first 25 days both in OMW-5 and OMW-15. The higher removal was achieved by the plants in OMW-5 both on 25^{th} day (93%, 82% and 52% in umbrella palm, vetiver and control) and on 67^{th} day (97%, 92%, 81%) Figure 18.

It is observed that faster phenol removal occurred in the first 25 days, which was more evident for umbrella palm. TOC and the phenol removal was similar in both dilution groups. This may be the result of the phenol's oxidation by air or other factors rather than the tolerance of the plants. On the other hand, it is also known that the behavior of phenolic substances becomes more complicated as the number of phenolic substances in a mixture increases (Gienfreda, et al. 2003).



(a)



(b)

Figure 18. Total phenol removal in (a) OMW-5 and (b) OMW-15.

In the wetlands, phenol removal can be attributed to four main processes; biodegradation, sorption, plant uptake, and volatilization. The primary mechanism is biodegradation. Microorganisms responsible for phenol removal are favored by the presence of oxygen which is present at higher concentrations in planted CWs (Herouvim et al., 2011).

Phenol removal I the literature were varied from 70 to 90 % with different initial concentrations and plant species. Kapellakis et al. (2012) run experiments at 1:10 diluted OMW with *phragmites* *australis* planted in free water surface CW. While initial total phenol was 1065 mg/L, the removal was reached up to 87%. Herouvim et al. (2011)

achieved, 70% phenol removal in CWs that planted with *phragmites australis*, at 2841 mg/L initial load. Another study was conducted by Del Bubba et al. (2004) to determine the performances of a pilot-scale reed bed for OMW treatment. They were monitored influent and effluent pollutant such as COD, total Kjeldahl nitrogen (TKN), and polyphenols. Pre-treated, and diluted OMW COD, and polyphenols were fed to the reed bed. The removal for COD was 74.1±17.6% and polyphenols 83.4±17.8% respectively. In the pilot scale system, they return the effluent for the improvement of the wastewater quality. Thus the parameters outlet concentrations were compatible for discharge to surface water according to Italian regulations.

Wang et al. (2014) conducted a study with *Polygonum orientale* to remove phenol in the concentration between 5-100 mg/L synthetic phenol solutions. The *P.orientale* plant was exposed to these solutions under the laboratory conditions for 13 days. They determined the inhibition concentration for phenol on *P. orientale* as 100 mg/L, and they found the maximum adsorption capacity was 0.030 mg /L. Similarly, Lee and Ahmad (2017) aimed to determine the phenol remediation capacity of *Ipomoea aquatica* Forssk. First, adaptation have been applied to the plantlet and observed the plantlets morphology. They found out plantlet's roots in 100 mg/L phenol were significantly longer than other the control plantlet, and *I. aquatica* Forssk. was able to survive in 300 mg/L phenol dosage. After 12 days of growth the plantlets were able to completely remove 50 and 100 mg/L phenol from the water spiked with phenol at 0.05 g/L.

Vetiveria zizanioides L. Nash.planlets when the medium was supplemented with 50 and 100 mg/L phenol removed all phenols in 4 days (Singh et al., 2008); *Vicia sativa* removed in 7 days, while alfalfa removed 100 mg/L phenol in 30 days (Flocco et al., 2002). It could be said that the removal varies with plant type and dosage of contaminants.

4.6. Removal of Total Nitrogen

Both free-floating hydrophytes and emergent macrophytes absorb nutrients in dissolved forms from the substrate or water column, via their roots and through the underside of their leaves (Fang et al., 2008). Nitrogen is one of the primary macronutrients among others such as phosphorus (P), and potassium (K) to grow plants. Plants absorb nitrogen in the forms of nitrate (NO_3^-) or ammonium (NH_4^+) ion, and the form of nitrogen could have different effects on growth and nitrogen uptake mechanism of plants. (Vince and Zoltán, 2011). The ammonium ions can be directly absorbed by plant roots or as a result of nitrate ion reduction. It is further assimilated into the amide amino group of glutamine by the glutamine synthetase, and into glutamic acid by the glumate synthase. These two enzymes can take part in the assimilation of the ammonium ions (Daubresse et al., 2010). Taking advantage of the use of nitrogen by wetland plants, the excess nitrogen in water can be bound in plant biomass, and then it will be used, as a result the removal of the nitrogen have provided. The TN treatment efficiency ranges between 77% to 99 % both CWs and FTW by free floating (i.e. water hyacinth, water lettuce, *M. korsakowii, Ipomoea aquatica*), and submerged aquatic plants (i.e. *Myriophyllum spicatum),* and emergent plants (i.e. *Carex virgate*, *Cyperus ustulatus*) related by residence time of the treatment (Li et al., 2007; Tanner et al., 2012; Fu and He, 2013; Zhang, et al. 2016).

Similar to TOC measurements, TN measurements were carried out under same conditions, and samples were taken on the same days. Initially, average TN concentrations were 26.55±1.24 mg/L and 72.64±5.1 mg/L in OMW-5 and OMW-15 respectively. Average TN load per gram of fresh plant weight were 0.61 and 0.99 mg/ g wet weight, and 0.58 and 1.02 mg/ g wet weight in vetiver and umbrella palm tanks at the beginning in OMW-5 and OMW-15 dilutions, respectively. The change in the TN load on plants during the experiment are shown in Table 13.

This measured value was multiplied by the amount of water in the tanks and the total TN mass was calculated as grams. The calculated total amount of TN is given in Table 14. The decreases in total TN mass were 82% and 40% for umbrella palm and vetiver while -0.14% in control at OMW-5. There was not decrease in the control group, whereas there was a higher decrease in the umbrella palm than in the vetiver. This reduction were occurred less both in planted tanks at the OMW-15 groups compared to OMW-5, with 41%, 21%, and 0.08 % for umbrella palm, vetiver, and control tanks respectively, (Figure 19).

Table 13. Average load of TN on the plants.

	Vetiver	Umbrella palm	Vetiver	Umbrella palm
mg TN/g plant	%5 OMW		%15 OMW	
1st.day	0.61	0.58	0.99	1.02
67th day	0.28	0.44	0.92	0.52

Table 14. Reduction in the total TN mass during the experiment.

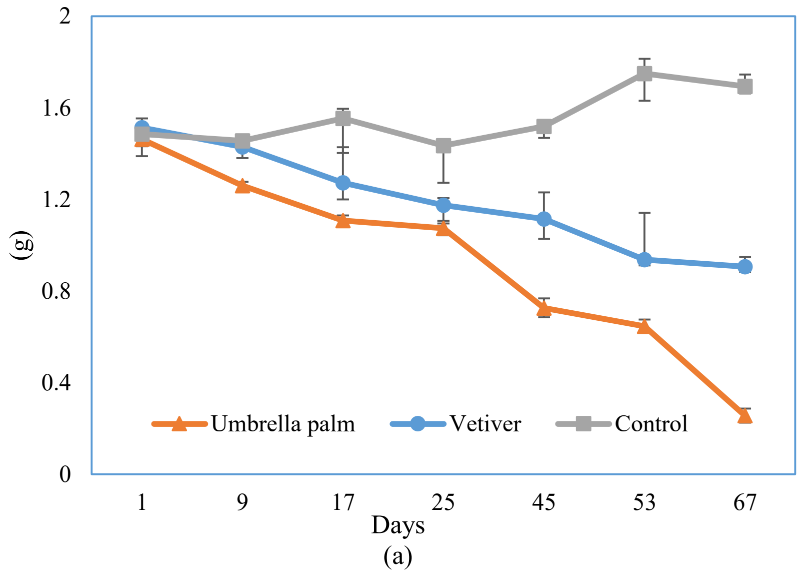
Dates	09.05.17	17.05.17	25.05.17	02.06.17	22.06.17	30.06.17	14.07.17
Samples	**OMW-5 (g TN)**						
Umbrella Palm -1	1.48	1.28	1.13	1.08	0.68	0.68	0.25
Umbrella Palm -2	1.42	1.27	1.12	1.11	0.70	0.6	0.22
Umbrella Palm -3	1.48	1.23	1.07	1.03	0.80	0.66	0.30
Vetiver-1	1.44	1.42	1.34	1.29	1.23	0.93	0.90
Vetiver-2	1.63	1.43	1.30	1.14	1.08	0.97	0.94
Vetiver-3	1.48	1.44	1.17	1.10	1.03	0.91	0.88
Control-1	1.38	1.45	1.59	1.42	1.54	1.79	1.68
Control-2	1.54	1.45	1.58	1.46	1.50	1.81	1.76
Control-3	1.54	1.47	1.49	1.43	1.52	1.65	1.64
	OMW-15 (g TN)						
Umbrella Palm -1	4.01	3.27	2.90	2.76	2.62	2.82	2.35
Umbrella Palm -2	3.99	3.30	2.87	2.53	2.59	2.75	2.39
Umbrella Palm -3	4.22	3.46	2.87	2.67	2.68	2.78	2.48
Vetiver-1	3.92	3.63	3.50	3.19	3.09	3.44	3.06
Vetiver-2	4.00	3.63	3.47	3.24	3.18	3.87	3.16
Vetiver-3	3.91	3.69	3.82	3.17	2.90	3.04	3.08
Control-1	4.12	4.06	3.98	3.97	3.83	3.87	3.91
Control-2	4.13	4.22	4.33	3.79	3.71	3.63	3.85
Control-3	4.33	4.22	4.27	3.58	3.77	3.88	3.84

Many studies reported that the major nitrogen treatment mechanisms in constructed wetlands, include microbial interactions around the plant root with nitrogen, sedimentation, chemical adsorption, and plant uptake (Khatiwada and Polprasert,1999; Spieles and Mitsc, 2000). None of the reported phytoremediation studies have used vetiver or umbrella palm on OMW treatment. Due to the lack of studies with these techniques, the results were compared with other studies, that have been used different macrophytes and domestic and industrial wastewaters such as pig farm, palm oil mills effluents etc.

In the study of Kapellakis et al. (2012) the removal for TN was 87% with an initial Total Kjeldahl Nitrogen of 136.80 mg/L.

After 8 days of remediation of POME by free floating species of water hyacinth and water lily TN reduced up to 88% 83.47% respectively (Hadiyanto et al., 2013).

Kantawanichkul et al. (2013) found that the removal efficiency of the umbrella palm (65%) was higher than vetiver (56%) in domestic wastewater treatment by vertical flow constructed wetland.



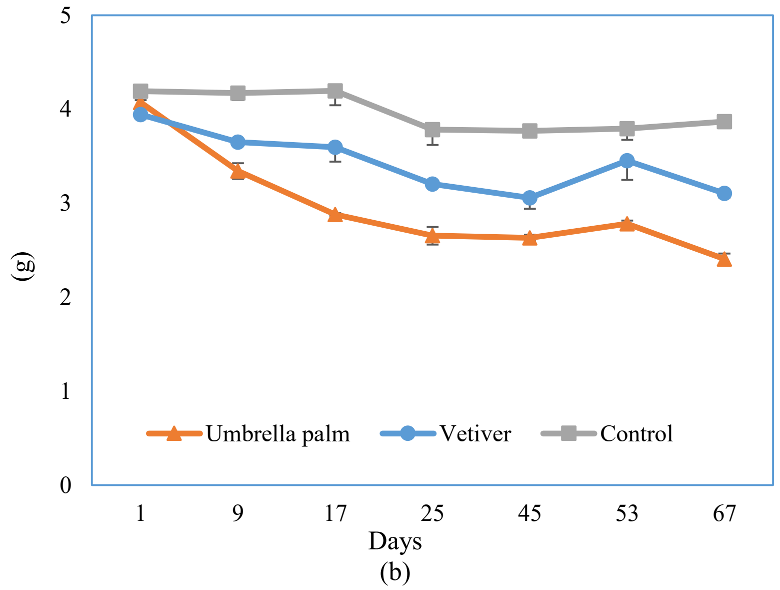


Figure 19. TN removal in (a) OMW-5, and (b) OMW-15. Error bars show one standard deviation.

4.7. Plant Growth and Analysis

The experiment was completed at the end of the 67 th day due to consumption of all wastewater by the plant. After the experiment was completed, water in plant roots were drained, then total wet weights of roots were weighed. Root and shoot lengths were measured. Then the plants were cut and separated root and shoots, and their heights and dry weights were measured individually.

4.7.1. Plant Growth

It was observed that the shoot of the umbrella palm groups were green, and the roots of them were alive and new white roots were present during the experiment. Unfortunately, in the vetiver group, the lost greenness of shoots over time compared to the initial look were observed in both the OMW-5 and the OMW-15. The number of green shoots decreased after 45 days in OMW-5, and after 17 days in OMW-15 in shoots of vetiver. In the vetiver roots, the formation of new roots in OMW-5 continued during the experiment, while in OMW-15 did not show any new root growth (Figure 20 and Figure 21).

In classical growth analysis, relative growth ratio (RGR) is calculated as the ratio of dry or fresh weight difference between the beginning and end of the experiment. The formula is given as follows;

$$RGR = \frac{W_t - W_0}{W_t} \tag{4.3}$$

The fresh weights of plants measured at the beginning, and the end the experiment are listed in Table 15. Besides, the dry weights of the plants were measured as a result of the experiment, and the change in the dry matter ratios were determined.

RGR increase rate were calculated as 0.60 and 0.14 for umbrella palm, and 0.22 and -0.19 for vetiver in OMW-5 and OMW-15 respectively, slower growth ratio was obtained for OMW-15 for both species.Besides, the dry weights of the plants were measured at the end of the experiment, and the change in the dry matter ratios were varied between 0.22 and 0.14 for umbrella palm, that was higher than the vetiver (0.14 and 0.08) in OMW-5 and OMW-15 respectively.

During the experiment, growth of a group of plants both umbrella palm and vetivers were also stayed in tap water (TW), and the changes in OMW-5 and OMW-15 were compared with the plant in tap water. The change in the root and shoot length of vetiver and umbrella palm given in Table 16. Change in length of roots were as 263%, 276%, 165% for umbrella palm, and 137%, 122%, 127% for vetiver in OMW-5 OMW-15 and control respectively over the time. The longest roots were found in umbrella palm both in OMW-5 and OMW-15.

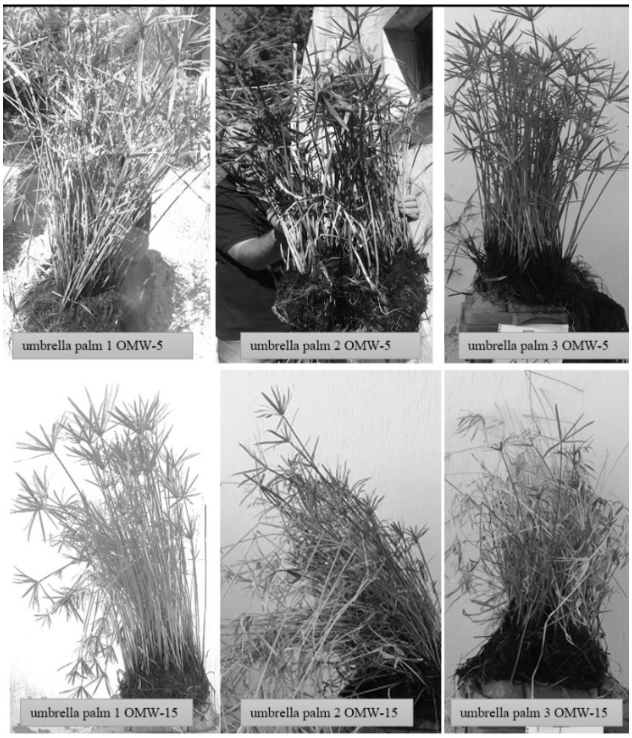


Figure 20. Appearance of umbrella palm groups at the end of experiment.

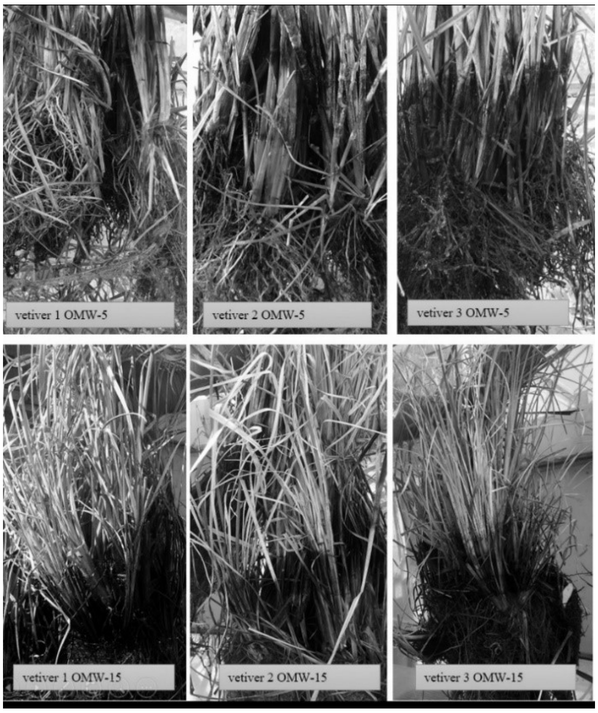


Figure 21. Appearance of vetiver groups at the end of experiment.

Table 15. Fresh weight of the plants at the beginning and the end of the experiment in OMW-5 and OMW-15.

	Initial Weight	Final Weight	Final Root Weight	Final Shoot Weight
Samples	**OMW-5 (g)**			
Umbrella Palm-1	2500	5560	4870	690
Umbrella Palm-2	2500	6420	5720	700
Umbrella Palm-3	2500	6660	5920	740
Vetiver-1	2500	3200	2270	930
Vetiver-2	2500	3100	2130	970
Vetiver-3	2500	3270	2150	1120
	OMW-15 (g)			
Umbrella Palm-1	4000	4850	3720	1130
Umbrella Palm-2	4000	4970	3910	1060
Umbrella Palm-3	4000	4150	3180	970
Vetiver-1	4000	3500	2400	1100
Vetiver-2	4000	3520	2420	1100
Vetiver-3	4000	3100	2070	1030

Table 16. Change in plant roots lengths.

Initial Root Length (cm)	Final Root Length (cm)		
OMW-5, OMW-15, Tap Water (TW)	**OMW-5**	**OMW-15**	**Control**
Umbrella palm			
55	130	135	88
55	155	160	95
55	150	162	90
Vetiver			
45	60	58	60
45	60	55	57
45	65	52	55

Previous phytoremediation studies stated that contaminant removal capabilities correlated with plant root numbers, and root surface area in CWs (Kyambadde et al., 2004; Cheng et al., 2009). This function of the roots may have a close relationship to root morphology and structure. Roots of plant are elementarily classified into taproots and fibrous roots. The taproot system has deep rooted by comparison with fibrous root. The taproot system provides the plant go down deep into the soil and obtain water in deep sources. Fibrous root grows more shallowly, and have many fine branching roots, called hairy root. Root development is very effective on ion absorption.

The selected species in this study which are umbrella palm and vetiver also have fibrosis root structure. Their root system was arborized vertically into the media, whether it is the main root, secondary root or their fibrous brachiation. Lai et al. (2011) studied in small-scale wetlands with 35 emergent wetland plants, which have different type of roots indicating the differences in morphological, structural, and eco-physiological properties. Fibrous-root plants were showed higher photosynthesis capacity, greater radial oxygen loss, and higher nutrient removal rates, there as they may have better decontamination ability when compared to thick-root plants. The roots of vetiver is economically the most useful part of the plant.

The photographs showing the developing of plants at the roots are given in Figure 22. When the vetiver is harvested, its roots are very important for the reason of aromatic essential oils at perfumery and medicine sectors.

(a) (b) (c) (d)

Figure 22. The root appearance of umbrella palm (a) before, (b) after and vetiver (c) before, (d) after in OMW-5.

Chen et al. (2007) was designed a hydroponic culture system to study the root growth of eight 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 with the plants which have two different root system. The results referred that fibril roots were grown faster than that the rhizomatic roots. After ten weeks of cultivation, while average root number of species with fibril roots, *C. indica*, *C. alternifolius P. purpureum* and *V. zizanioides* were reached 1871, 1309, 1231, and 985 per plant, respectively, the species of rhizomatic roots (*H. littoralis*, *P. communis* and *T. angustifolia*) had only 28, 291 and 168 in roots respectively, except *A. calamus*, it had 1709. The average root length of the fibril roots was greater than the rhizomatic roots till the eighth week, whereas the two type roots of length were shared similarity after the eighth week.

After the experiment plants were measured with respect to the highest stem from the root to the tip of each plant and calculated the averages. The heights of roots were longer in plants in treatment tanks, while the shorter roots were observed in the controls (Table 17). It can be concluded that plants can metabolize the pollutants and used them for their growth.Umbrella palm and vetiver's tolerance towards the OMW-5 were demonstrated in terms of water consumption, cumulative shoot and root growing and dry weight.

Table 17. The plant shoot lengths.

Initial Shoot Length (cm)	**Final Shoot Length (cm)**		
OMW-5, OMW-15, TW	**OMW-5**	**OMW-15**	**Control**
Umbrella palm			
85	90	92	105
93	95	93	105
100	105	90	125
Vetiver			
135	110	95	120
110	105	93	145
140	110	90	115

4.7.2. Total Phenolic Content in Plants

Total phenol content of plants was determined by Folin-Ciocalteu assay and results were expressed as mg gallic acid equivalent per kg of plant (mg GAEq /kg). The concentrations of total phenolic compounds are shown on Figure.23. At the end of 67th day compared to the plants in control (tap water), total phenol amount in the umbrella palm was higher in OMW-5 sample (root 340%, shoots 109%). The total phenol content of vetiver was also increased up to 141% in root while it was 22% in shoot.

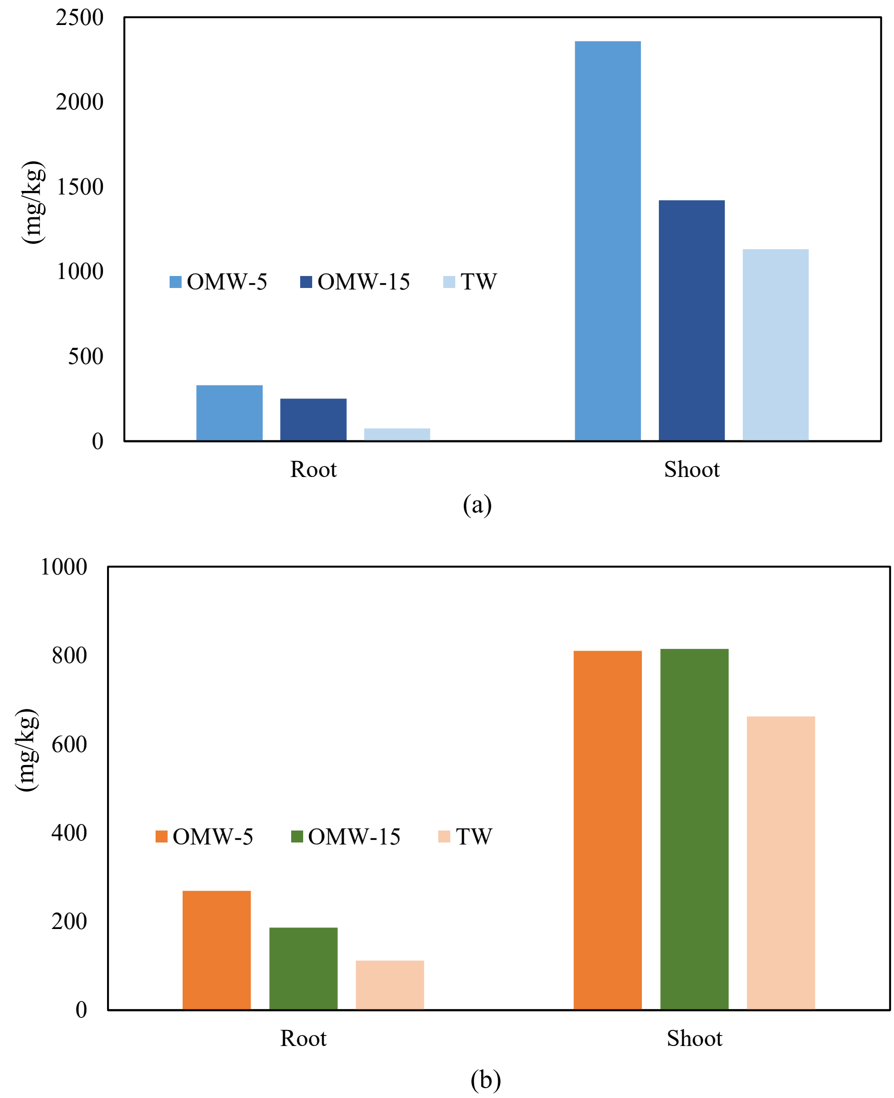


Figure 23. Total phenol in the root and shoot of (a) umbrella palm, and (b) vetiver.

It is reported the contaminants from soil or the environment taken by the plants is called phytoextraction which requires an ability to translocate contaminant from roots to shoots or leaves. The ratio of translocation is called as translocation factor (TF). When TF>1, this is the indicator that of plant translocates metals effectively from roots to shoots/leaves. Translocation of the contaminants in the plant usually is determined by either C_{leaves}/C_{root} or C_{shoots}/C_{roots} ratios. The solubility and concentration of organic pollutants could be effective in translocation of pollutants in the plants, but it is also reported that this effect could be minor importance when compared with the physical and chemical properties of organic pollutants such as molecular mass and hydrophobicity related to partitioning coefficients K_{ow} and K_{oa}. K_{oa}, is the partition coefficient between octanol and air, is not the factor that effects our experimental work. Therefore, only K_{ow} will be discussed. K_{ow} is the partition coefficient between octanol and water. It was mentioned that the molecular mass of an organic pollutant is below 1000, and high Kow value for organic pollutant could show the absorption ability of the pollutants by plant roots. Even though, the strongest effect is reported as root extractable lipid content, environmental media factors are also included as indirect factors: background electrolyte type, dissolved organic carbon (DOC) concentration, pH, and organic matter content (Zhang, et al. 2017).

In this study, TF values for umbrella palm and vetiver were found bigger than 1, as 7.2 and 3, implying that phenolic compounds were mostly found in shoots. Interestingly, TF for vetiver in the OMW-15 were found higher than OMW-5 as 4.4 and 3, which shows that vetiver could be more successful for translocating the phenolic compounds in OMW-15.

Zhang et al. (2015) found out that there was positive correlation between translocation factors (TFs) and log Kow during translocation from root to stem. However, it was mentioned that the accumulation mechanism in the leaf was concentration-dependent. It is found that translocation is also plant and compound dependent mechanism. It is found that even similar structured chemical group translocation could results differently in plants. For example Wang et al., (2011) studied polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) translocation in maize. While PBDEs showed higher accumulation in maize roots, PCBs of the same halogen-substitutions due to the higher partition coefficients (log K_{ow}) showed less.

4.7.3 Macro Micronutrients Analysis of Plant

The corresponding plant shoots and roots were analyzed to determine the amount of elements accumulated by the plant species. The concentration of macro and micro nutirents and metals are presented in Table 18 and 19.

Table 18. Multi-element analysis in umbrella palm plants.

Samples	Initial	OMW-5	OMW-15	Initial	OMW-5	OMW-15
Parameters	**Root of Umbrella Palm**			**Shoot of Umbrella Palm**		
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
C (%)	36.8±3.40	43.3±2.6	44.86±3.72	43.92±0.57	45.3±0.56	45±1.1
S (%)	0.26±0.01	0.48±0.04	0.51±0.11	0.23±0.09	0.439±0.02	0.27±0.02
P (mg/kg)	638±241	1693±60	2300±411	830±156	2300±655	2433±361
K (mg/kg)	17341±948	20321±1978	25845±3012	33487±3223	30278±821	35691±4700
Ca (mg/kg)	9151±915	22267±394	35103±412	7335±513	5056±141	6500±749
Mg(mg/kg)	3629±168	10422±936	8231±1310	2555±172	1784±222	2475±229
Na (mg/kg)	3717±584	11658±290	5110±563	1404±287	2149±307	2800±419
Fe (mg/kg)	1275±139	1368±159	5557±3	142±36	134±11	200±28
Zn (mg/kg)	86±12	108±28	127±28	31±3	20±1	52±12
Cu (mg/kg)	83±7	119±3	75±3	2.7±0.6	4.4 ±0.4	11±1
Mn(mg/kg)	120±15	217±9	374±48	59±12	236±36	204±11
B (mg/kg)	3.18±0.77	61±10	51±8	2.1±0.60	33±4	20±3
Pb (mg/kg)	1.1±0.2	18.9±0.2	32.6±3.4	0.6±0.05	3.1±0.4	3.6±0.4
Cr (mg/kg)	1.5±0.3	8.6±0.4	15.4±0.04	0.3±0.03	1.8±0.2	3.2±0.4
Ni (mg/kg)	1.1±0.2	8.4±0.4	12.2±1.6	0.2±0.05	1.8±0.08	2.6±0.4
Co (mg/kg)	0.2±0.01	1.4±0.2	2.7±0.4	0.01±0.001	0.08±0.02	0.1±0.04
Cd (mg/kg)	0.1±0.03	0.4±0.05	0.8±0.08	0.04±0.01	0.07±0.02	0.2±0.03

Among the plants, maximum contents of metals in roots were observed as in the order of Mn (374 mg/kg), Pb (32.6 mg/kg), Cr (15.4 mg/kg), Ni (12.2 mg/kg), Co (2.7 mg/kg), and Cd (0.8 mg/kg) in OMW-15 and while B (61mg/kg) was observed in OMW-5 for umbrella palm. The other metals have reached the highest concentrations in vetiver, as Zn (241 mg/kg) in OMW-5 and Fe (7189 mg/kg) and Cu (193 mg/kg) in OMW-15 respectively.It was observed that uptake of metals was maximum in roots than the shoots. That indicates low mobility from roots to shoots.

The decreasing trend of various metals in roots of umbrella palm was Fe> Mn> Cu> Zn> B> Pb> Cr> Ni> Co> Cd while it was Mn> Fe> B> Zn> Cu> Pb > Cr> Ni> Co> Cd for shoots.

Table 19- Multi-element analysis in vetiver plants.

Samples	Initial (n=7)	OMW-5 (n=3)	OMW-15 (n=3)	Initial (n=7)	OMW-5 (n=3)	OMW-15 (n=3)
Parameters	**Root of VETIVER**			**Shoot of Vetiver**		
N (%)	0.80±0.17	1.75±0.61	1.30±0.07	0.92±0.22	1.95±0.13	0.83±0.13
C (%)	47.1±3.77	50.4±1.69	50±0.1	45±1.02	46±0.56	46.7±1.14
S (%)	0.26±0.09	0.59±0.03	0.5±0.08	0.141±0.05	0.44±0.02	0.12±0.05
P (mg/kg)	535±77	2193±395	1871±525	1137±245	2301±656	1344±90
K (mg/kg)	3266±477	8723±18	13091±582	16455±3028	30278±821	16705±1045
Ca (mg/kg)	5955±618	11762±2402	12511±2892	3563±384	5056±141	5207±1056
Mg (mg/kg)	1581±280	3718±953	2565±248	2132±222	1784±222	2475±800
Na (mg/kg)	1309±290	3369±376	1888±438	982±255	2149±307	2473±397
Fe (mg/kg)	1816±297	3366±221	7189±1426	178±46	211±56	451±10
Zn (mg/kg)	15.7±3.3	241±76	175±52	26±8	30±4	32±5
Cu (mg/kg)	16±3	123±24	193±69	2±0.1	7±1	15±5
Mn (mg/kg)	115±30.2	150±33	193±55	61±8	96±10	101±10
B (mg/kg)	1.1±0.34	29±5	23±5	1.24±0.36	13±0	15±4
Pb (mg/kg)	0.6±0.05	10.6±0.3	11.7±1.8	1.0±0.2	2.7±0.3	3.6±0.2
Cr(mg/kg)m	3.2±0.4	10.1±1.8	7.9±0.4	2.2±0.6	3.2±0.3	4.3±0.4
Ni (mg/kg)	1.6±0.2	7.4±0.3	11.4±2.5	0.6±0.2	2.6±0.3	2.6±0.5
Co (mg/kg)	0.2±0.07	1.2±0.1	1.5±0.03	0.03±0.01	0.1±0.05	0.2±0.04
Cd (mg/kg)	0.02±0.007	0.3±0.08	0.3±0.03	0.07±0.001	0.07±0.02	0.09±0.01

In the literature, it is found that eventhough metal concentrations in soil, and water have not been high, the amount of heavy metals in aquatic plants could be high, which is the indication of the plant being an accumulator plant for the specific metal. Therefore, bioaccumulation factor (BAF) is commonly used to calculate the efficiency of a plant species to accumulate a metal into the structure of the plant from the surrounding medium (Ladislas et al., 2012). The BAF of different metals from water to the plants was calculated using the equation given by Wilson and Pyatt (2007).

$$BAF\ (\%) = \left[\frac{Cplant_t - Cplant_0}{C_{water}}\right] \times 100 \qquad (4.2)$$

In this thesis, this calculation was not conducted due to lack of specific analysis of OMW-5 and OMW-15, only olive mills wastewater analysis was conducted. However the Table 19 was examined, both plants have ability to uptake the some of heavy metals.

CHAPTER 5

CONCLUSION

This study demonstrates the importance of macrophytes in the reduction of organic carbon, phenolic compounds and nitrogen concentrations in the olive mills wastewater. Structurally two different macrophytes *Cyperus alternifolius* L. (Umbrella palm) and *Vetiveria zizanioides* (L.) Nash (Vetiver) used and they were shown promising results in the treatment of olive mills wastewater.

Average TOC removal was achieved up to 92% by both plants, while umbrella palm showed higher reduction due to its structure.

The plants indicated different behavior for the contaminants when translocation factors were analyzed for these contaminants. Increase in the concentration of phenolic compounds (t-phenol) in OMW, plants response changed. For instance, increase in translocation factor for umbrella plant was observed while it was decreased for vetiver when contaminant concentration increased in wastewater. It is believed that Vetiver is an aromatic medicinal plants and can be used for further purposes.

In addition, the plants also have the ability to tolerate some of the heavy metals, such as Cr, Pb etc., present in OMW.

The results of this study indicated that floating umbrella palm could be employed in treatment of OMW, and that further research should be conducted to reveal the involved removal mechanisms and their kinetics. Future studies can be combined with bioremediation to increase the efficiency of the phytoremediation. The appropriate pH range for plant species can be elaborated. Different studies should be carried out for the adaptation of plants to OMW which could lead better performance.

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