EFFICIENCY OF A CONSTRUCTED WETLANDS SYSTEM FOR WASTEWATER TREATMENT: REMOVAL OF BACTERIAL INDICATORS AND ENTERIC VIRUSES

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ABSTRACT

Sewage contains various pathogenic microorganisms, which entail a potential risk to human health and could be minimised using the advanced processes of wastewater treatment. Constructed Wetlands (CWs) have been identified as alternative systems for wastewater treatment, which could also meet the overall socio – economical and environmental requirements of small communities due to their low operational and maintenance cost.

Within that scope, this study aimed to evaluate the effectiveness of CWs for the removal of pathogenic microorganisms and viruses, which are commonly found in domestic and municipal sewage. Initially a pilot-scale system using a freewater surface (FWS) wetland, a horizontal subsurface flow (HSF) wetland (with three different kinds of plants) and a sand filter at the end was developed to process primary domestic wastewater from the city of Heraklion (Crete). In addition bacterial indicators of fecal pollution like Total Coliforms (TC), *Escherichia coli* and Enterococci, F-RNA coliphages and enteric viruses (Adenoviruses, Enteroviruses) were monitored to measure the treatment efficiency of the CWs system. Adenoviruses (HAdV) and Enteroviruses (EV) were concentrated from the outlet of HSF using electropositive non-woven filter media. Elution and reconcentration of the viruses was performed according to EPA Method 1615, while polymerase chain reaction (PCR) methods were used to identify and quantify the viruses in the samples.

Results indicated a significant removal efficiency of the fecal indicators. A 4 log reduction regarding *E. coli* and Enterococci from inlet to outlet of the system was achieved while this reduction was lower for TC which were almost 10^4 CFUs/100mL. Enteric viruses were also detected at all sampling points across the system, where comparing to EV the presence of HAdV was more frequent as HAdV are more resistant under environmental stress and adverse environmental conditions. Positive detection was 60% for the HAdV and 20% for EV of all samples examined. Bacteriophages were also detected at the sampling points of the system. Finally it should be highlighted that HAdV and bacteriophages could be employed as possible indicators of the microbiological quality of recreational waters.

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REMOVAL EFFICIENCY OF CONSTRUCTED WETLANDS WITH TWO DIFFERENT WETLAND HALOPHYTES FOR Cd, Ni AND Zn POLLUTED WATER TREATMENT

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ABSTRACT

The deliberate use of wetlands as biological treatment systems for effluent purification has developed rapidly over the last decades with the scientific documentation of the role of plants in surface water, groundwater or wastewater treatment. Constructed treatment wetlands are engineered systems, designed and constructed to utilize the natural functions of wetland vegetation, soils and their microbial populations to treat contaminants in waters or waste streams. In this study, heavy metal contaminated water remediation by constructed wetlands with halophytic plantation is investigated based on the special interest for halophytic plants regarding their ability to tolerate a wide variety of stresses except salt, such as heat, drought and heavy metals.

The specific aim of this study is to investigate the performance of two halophyte-based CWs for the removal of Cd, Ni and Zn from polluted water. For that purpose, two 65L-continuous, horizontal flow with complete water recirculation wetlands were constructed. Each was planted with a halophytic plant species; the first with *Juncus acutus* L. plants and the second with *Halimione portulacoides* L. plants. Plants roots were fully covered with gravels creating a gravel bed. The contaminated water was pumped onto the gravel bed at a rate of 1 L/h -the water level in the CW remained constant at all times- and the initial concentrations of Cd, Ni and Zn were 20 μg/L, 400 μg/L and 4 mg/L, respectively. Water samples were collected at the inlet and outlet of the CW every 24 h and taken to the laboratory within 2 h for same-day processing. Influent and effluent water samples were analyzed for pH and ORP, filtered and Cd, Ni and Zn concentration measurement was performed by an ICP-MS.

The results indicated that both CWs are able to treat heavy metal polluted water. *J. acutus*-CW and *H. portulacoides*-CW were found able to achieve more than 85% reduction of the influent Ni and Zn concentrations in every cycle reaching concentrations well below the limits for wastewater reuse in EU, while for Cd both wetlands achieved total reduction within a few days.

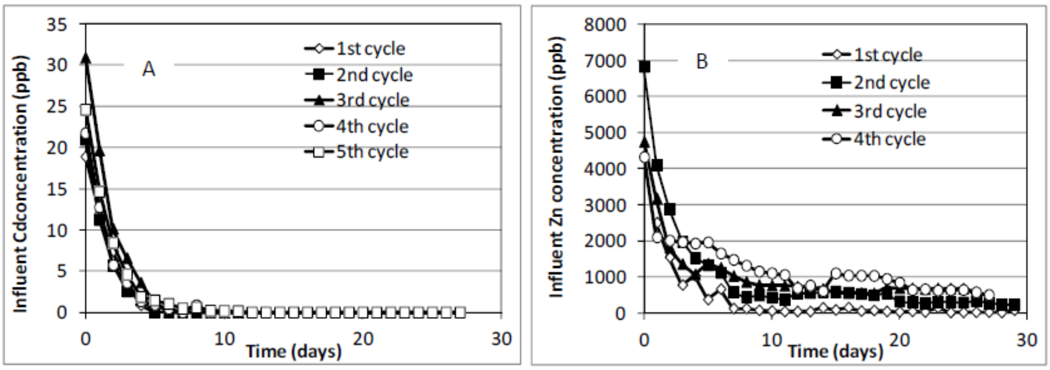

Figure: A. Concentration of Cd in the influent of the CW in which J. acutus plants are grown, B. Concentration of Zn in the influent of the CW in which H. portulacoides plants are grown

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MICROBIAL COMMUNITY STRUCTURE AND DIVERSITY IN SUBSURFACE FLOW CONSTRUCTED WETLANDS

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ABSTRACT

Microorganisms have an important role in the treatment performance of constructed wetlands (CWs). Recent studies have shown that microbial communities show seasonal variations in response to changing environmental conditions, while vegetation has been recognised as an important driver of microbial community composition. To test how these factors affect the treatment performance of CWs, we planned an experiment with pilot subsurface flow CWS fed with synthetic municipal wastewater. Six pilot CWs were installed, duplicates of the following treatments: i) no vegetation, ii) planted with *Typha latifolia*, and iii) planted with *Phragmites australis*. Analyses of COD, TKN, NO_3^--N, and NH_4^+-N were carried out on a weekly basis at the inlet and the outlet to evaluate the treatment efficiency of CWs in terms of N and C removal. Microbial community composition was examined by amplicon sequencing of the V4-V5 regions of the 16S rRNA gene. Bacteria outnumbered Archaea (98.6% to 0.4%), while at the level of Phylum, *Proteobacteria* dominated the other phyla (66%) followed by *Bacteroidetes* (11.5%), *Cyanobacteria* (4.9%), *Verrucomicrobia* (2.7%), and *Acidobacteria* (2%). Within *Proteobacteria* the class of β-*Proteobacteria* was the most abundant (28.7%), followed by γ-*Proteobacteria* (25.6%) which mainly include microorganisms with a copiotrophic lifestyle thriving on readily available organic matter.

Regarding the alpha-diversity, analysis of variance revealed a significant influence of the sampling time on the evenness (F=25.4, p<0.001), but not on the richness (F=1.9, p>0.05) of microbial communities. Vegetation did not affect either of these variables (F=1.5, p>0.05; F=0.4, p>0.05). Stepwise multiple regression analysis identified TKN as a significant variable for the community richness, explaining 16.5% of the total variance, while COD and NO_3^--N were found to be significant variables for community evenness explaining 50.2% of the total variance.

Regarding the beta-diversity, principal coordinates analysis using the phylogenetic UniFrac distances indicated a significant grouping according to the sampling time, with the first two axes explaining 67.8% of the total variance. Permutation analysis of variance estimated that sampling time explained 45.7% of the community variability (F=5.5, p<0.001), while vegetation explained only 4.9% (F=1.5, p>0.05). In addition, distance-based redundancy analysis showed 17.6% of the total variation could be explained by the chemical parameters monitored.

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MATHEMATICAL MODELING OF OLIVE MILL WASTEWATER RHIZODEGRADATION WITH THE HALOPHYTE *JUNCUS ACUTUS* FOR WATER REUSE

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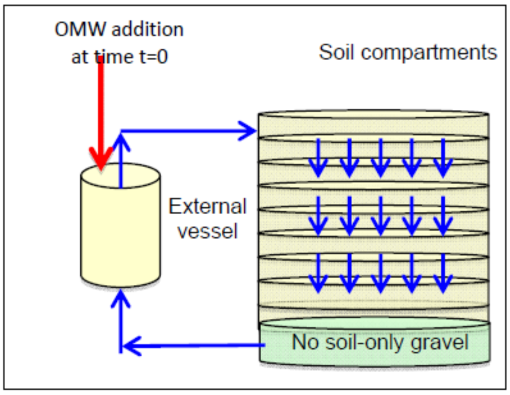
ABSTRACT

Olive mill wastewater (OMW) constitutes a major ecological issue, especially in Mediterranean olive-growing countries, where large volumes of the order of 30×10^6 m^3 are produced every year over a short periods of time and a large portion is discharged untreated or partially treated to the environment. In this work we consider the treatment of OMW through a phytoremediation process using halophytes. Phytoremediation is an emerging technology that is generally considered effective, inexpensive and environmentally friendly. In this phytoremediation system two processes are dominant for the reduction of the COD. The main degradation process is microbial degradation in the rhizosphere (known as rhizodegradation) as the OMW is recycled through the soil. The COD removal process is also linked to evapotranspiration whereby the COD entering the roots and moving to leaves is degraded by plant's oxidative enzymes or by endophytic bacteria. In general, the soil absorbs organic compounds from the recycled OMW, which are subsequently desorbed as the COD in the OMW is reduced. In this work we assume that thermodynamic equilibrium is reached at all times as COD is slowly reduced. Furthermore, halophytic species were employed as they are of special interest for phytoremediation research since these plants are naturally better adapted to cope with environmental stress.

In this work the potential of the halophyte *Juncus Acutus* to degrade the organic matter of OMW has been investigated experimentally as well as through modeling of a rhizodegradation pilot, emulating a larger facility. More specifically, a pilot unit containing two grown-up *Juncus Acutus* plants has been operated over a period of two months treating OMW through recirculation of the OMW from an external storage tank. Two experimental runs have been conducted with OMW of different strength (about 5,500 and 8,500 mg/L COD respectively) and the dynamics of the remediation process have been monitored by frequent sampling and chemical analysis. According to the experimental results, the phytoremediation process has been proven to be very effective as a COD reduction of the order of 98% has been accomplished within a period of 23 days when the starting concentration of COD is 8,500 mg/L. The treated OMW can be reused for the irrigation of crops (e.g., corn) or trees (e.g., olive trees, vineyards, etc).



A mathematical model has also been developed in the MATLAB® environment to describe the dynamics of the overall OMW reduction process, considering microbial degradation in the rhizosphere zone (rhizodegradation) as the main COD removal mechanism, taking also into account COD removal through plant up-take during evapotranspiration (phytodegradation) and, COD equilibrium adsorption to the soil. The model calibration has been performed with raw data from the two experimental runs of the pilot unit and the simulations match the experimental data for both runs satisfactorily.

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