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An Integrated Approach for the Assessment of Constructed Wetlands Operational Efficiency, using both Chemical and Biological Data

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Abstract

The present study evaluated the operational efficiency of a constructed wetland (CW) located at Andritsaina/Krestena municipality (Western Greece, Peloponnese, Greece), using a battery of tests and bioassays. Specifically, raw WWs entering the CW basins with broadleaf cattail *Typha latifolia* (raw-WWs) and biologically treated WWs effluents ending up in the adjustment tank were randomly collected in October, December 2018, and January 2019. Thereafter, freshwater algae (i.e., *Chlorococcum* sp., *Scenedesmus* sp.), invertebrates (i.e., *Thamnocephalus platyurus*, *Brachionus calyciflorus*), higher plant species (i.e., *Sorghum saccharatum*, *Lepidium sativum* and *Sinapis alba*) and human lymphocytes were treated with WWs for determining critical toxic endpoints in any case. All data were further interpreted with physicochemical parameters, like conductivity (Cond), COD (total and dissolved), total suspended solids (TSS) and volatile suspended solids (VSS), commonly measured in both raw- and treated-WWs samples. The results showed that almost all chemical parameters measured in treated-WWs were lower than those occurred in raw-WWs, and significantly related with the obtained critical toxic endpoints in all cases. Those preliminary findings give rise to the importance of using a battery of bioassays as useful tools for assessing CWs treatment process efficiency thus contributing to the environmental sustainability and human health.

Keywords: bioassays; chemical analysis; constructed wetlands, operational efficiency.

1. Introduction

Constructed wetlands (CWs) are considered important in rural areas for the treatment of domestic/household wastewaters (WWs) (**Gikas and Tsihrintzis, 2014**). In this context, CWs systematic monitoring for ensuring environmental and human sustainability is of great concern. Given that the efficacy of both physico-chemical and biological processes that take place into CWs operational units, is routinely performed via chemical analysis, but less attempt has been performed for estimating WWs biological effects, the present study evaluated the operational efficiency of a CW located at Andritsaina/Krestena municipality (Western Greece, Peloponnese, Greece), using both chemical and biological data from (a) raw WWs entering the CW basins with broadleaf cattail *Typha latifolia* (raw-WWs) and (b) biologically treated WWs effluents ending up in the adjustment tank, collected in October, December 2018, and January 2019.

2. Materials and Methods

2.1. Sampling and physicochemical characterization of WWs

Raw- and biologically treated WWs were collected monthly (October, December 2018 and January 2019). pH, conductivity and salinity values were measured *in situ* using a HQ40 multimeter (Hach Lange GmbH, Germany), while chemical oxygen demand COD (total and dissolved COD), total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to well-known procedures (**Eaton et al. 1995**).

2.2. Bioassays

72h algal biotests, using the freshwater microalgae species *Chlorococcum sp.* SAG 22.83 & *Scenedesmus rubescens* SAG 5.95 (Göttingen, Germany), cultured in BG-11 medium, were performed according to the **OECD 201 (2011)**. Cell numbers, growth (μ) and inhibition (%I) rates were assessed, while 72h IC_{50} values (Probit analysis, p<0,05) were determined in both cases. Higher plant species (*Sorghum saccharatum*, *Lepidium sativum* and *Sinapis alba*) were used for assessing germination index (GI), while acute toxicity tests (24h LC_{50}) were performed using instars larvae of (a) the fairy shrimp *T. platyurus*, and (b) neonates of the rotifer *B. calyciflorus* (Screening Toxicity test supplied by MicroBio Tests Inc.; Thamnotoxkit F™ and Rotoxkit F respectively). Additionally, the cytokinesis block micronucleus (CBMN) assay, using cytochalasin B (Cyt-B) in human lymphocytes was performed, following standard procedures (**OECD, 2014**) for assessing (a) the frequency of micronucleus (MN) being present in binucleated cells, (b) the cytokinesis block proliferation index (CBPI) and (c) the replication index (RI).

2.3. Statistical analysis

All data are means± standard deviation from 4 different measurements. After checking for homogeneity, differences among abiotic parameters were tested by ANOVA (Bonferroni test, $p < 0.05$, IBM SPSS 24 Inc. software package), while differences among biotic parameters were tested non-parametrically, using Mann Whitney *U* test ($p < 0.05$). Pearson rank correlation analysis ($N = 24$, $p < 0.05$) was performed to investigate significant relationships among biological and physicochemical parameters tested, while PCA was performed for underlying abiotic variables that explain the pattern of correlations within the full set of the abiotic variables observed and explain most of the variance observed. The Factor scores of the first two extracted components of the most significantly related parameters were used to evaluate potential site- and month-related differences (**Tsarpali and Dailianis, 2012**).

3. Results and Discussion

According to the results, almost all chemical parameters measured in treated-WWs were lower than those occurred in raw-WWs, thus indicating an efficient CW treatment process, followed by a concomitant attenuation of its toxic potency as revealed by the determination of critical toxic endpoints in all cases. The fact that physicochemical parameters were significantly correlated with critical toxic endpoints (see for example Figure 1), give rise to the importance of using a battery of bioassays as useful tools for assessing CWs treatment process efficiency through the evaluation of wastewaters' toxic potential, thus contributing to the environmental sustainability and human health.

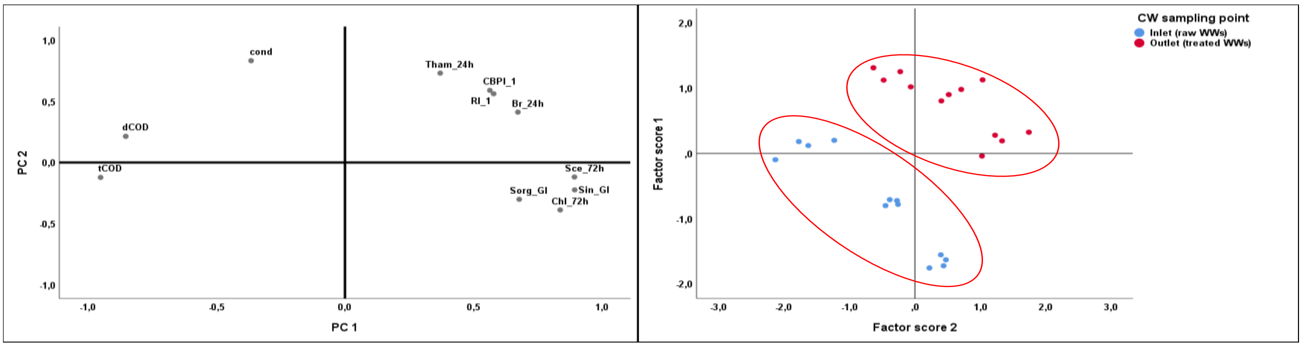

Figure 1. 2D-plot of the principal component weights and scatter plot of the first two PC factor scores extracted of abiotic and biotic parameters measured in WWSs samples collected the sampling periods. The first two components account for 74.4% of the variability of the original data.

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