MIXED HEAVY METAL TOLERANCE AND ACCUMULATION IN THE WETLAND HALOPHYTE *JUNCUS ACUTUS* L.

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ABSTRACT

Halophytic species are widespread in Mediterranean basin and are known to tolerate various environmental stresses including heavy metals. *Juncus acutus* L. which is a perennial wetland halophytic plant with a wide ecological range has proven before to be tolerance to various environmental stresses including heavy metal stress by zinc and hexavalent chromium. In this study, the mixed metal tolerance and accumulation ability of the plant is investigated in order to explore its potential for mixed heavy metal phytoremediation.

For that purpose, a 5 months (October to April) pot experiment was conducted (six pots per treatment) with *J. acutus* plants collected from the Souda Bay in Chania and planted to the same amount of a typical surface soil collected from an agricultural area in Chania. After a sufficient adaptation period, the experiment started with the addition of 10 ppm Cd, 150ppm Ni, 500ppm Zn and 500ppm Pb, in one dose as aqueous solutions. The experiment took place in an open air area under natural light conditions with temperatures ranging from 5.3 to 31.1 °C. The soil was always over-saturated, as the pots were irrigated with water to achieve a water layer above the soil surface in order to simulate wetland conditions. At the end of the experimental period, measures of metals concentrations in plant tissues and in water of the soil pores and measurements of plant biomass, chlorophyll content, shoot water content and shoot proteins were performed. Moreover, plants were, also, assayed for activities of antioxidant enzymes such as the guaiacol peroxidase (GPX) and catalase (CAT).

The results suggest that *J. acutus* is not a hyperaccumulator of the examined metals (Table); however, it showed high tolerance to mix heavy metal pollution since all plants showed no visible toxicity symptoms such as chlorosis, necrosis, or root inhibition. Moreover, shoot proteins, chlorophyll content and shoot water content were not found to be statistically affected by the presence of metals and in addition, plant' s biomass was increased in the presence of metals. Furthermore, GPX activity and CAT activity of exposed plants to metals was not statistically elevated in comparison with the controls.

Table. Metal concentration in *J. acutus* tissues (ppm). Values represent means ± standard error (n =6).

		Cd	Pb	Ni	Zn
Control	Shoots	n.d.	1,25 ± 0,08	n.d.	15,66 ± 2,3
	Roots	n.d.	5,98 ± 0,9	12,78 ± 6,6	28,64 ± 5,6
Metal treatment	Shoots	1,59 ± 0,6	84,94 ± 30,7	100,22 ± 18,7	200,28 ± 39,5
	Roots	12,77 ± 1,9	626,35 ± 106,5	259,65 ± 36,4	476,14 ± 55,9

All the above suggest that *Juncus acutus* L. is a heavy metal tolerant plant that could be used in phytoremediation strategies for revegetation of mixed heavy metal polluted areas.

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EVALUATION OF A PILOT-SCALE CONSTRUCTED WETLAND WITH *JUNCUS ACUTUS* L. FOR THE REMOVAL OF BISPHENOL A FROM SECONDARY-TREATED WASTEWATER

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ABSTRACT

Previous studies have shown the ability of halophytes to grow in soil polluted by heavy metals and toxic ions and also the tolerance of *J. acutus* endophytic bacteria to heavy metals and bisphenol A [BPA, 4-[1-(4-hydroxyphenyl)-1-methylethyl]phenol). Moreover, several endophytic strains of *J. acutus* have the potential to enhance not only bioremediation of bisphenol A but also plant growth. Thus *J. acutus* was demonstrated as an appropriate species for implementing phytoremediation strategies in areas with contaminated groundwater. Application of the phytoremediation strategy in secondary treated municipal wastewater is now investigated.

The aim of this study was to evaluate the removal of the phenolic estrogen BPA, in a small pilot-scale constructed wetland system fed with secondary-treated municipal wastewater, spiked with known amounts of BPA. For that reason a constructed wetland system, planted with *J. acutus*, was designed and operated in the WWTP of city of Chania, Crete, Greece. The unit was running in a horizontal flow with a surface area of $1m^2$. In the evaluation of the CW, removal efficiencies of BPA was monitored and several parameters were measured; Chemical Oxygen Demand (COD), Biochemical oxygen demand (BOD), pH, Total Suspended Solids (TSS), Total Dissolved Solids (TDS), Total Organic Carbon (TOC), Total Phosphorus (TP) and Dissolved Nitrogen. Experiments were conducted at different hydraulic residence times (HRTs) and robustness of the system was also tested by changing the BPA concentration of the influent stream from 20ppb to 200ppb. The results indicated significant removals of BPA, while plants did not show any toxicity symptoms.

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INSIGHTS ON THE ROLE OF VEGETATION ON NITROGEN REMOVAL IN SUBSURFACE FLOW CONSTRUCTED WETLANDS

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ABSTRACT

In this study we investigate the role of vegetation on nitrogen cycling in Constructed Wetlands (CWs) subsurface flow fed with synthetic municipal wastewater. Six pilot CWs were set, duplicates of the following treatments, without vegetation and planted with *Typha latifolia* and *Arundo donax.* Analyses of COD, TKN, NO_3^--N, and NH_4^+-N were carried out at the inlet and the outlet of CWs in a weekly basis to investigate their treatment efficiency in terms of N removal. We also monitored the abundance of ammonia oxidizing organisms and denitrifiers through qPCR to provide insights on the pathways operating. Our findings revealed a seasonal impact of the vegetation on N removal rates between 67th and the 178th day of the experiment, when CWs planted with *T. latifolia* showed higher removal rates for TNK and NH_4^+-N compared to the unplanted or planted with *A. donax* CWs. Before and after this interval there was no effect of vegetation on the treatment performance of CWs. Analysis of variance revealed that there was not any significant effect of vegetation on the abundance of gene copies of ammonia oxidizers and dentrifiers. Ammonia oxidizing bacteria (AOB) dominated over ammonia oxidizing archaea throughout the study period implying that they had a higher contribution on nitrification. This hypothesis is strengthened by the significant correlation between AOB and NH_4^+-N removal rate and denitrification genes. Finally, denitrification genes found to be highly abundant indicating denitrification as the major pathway of N removal.

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