

Efficiency of Constructed Wetlands in Mitigating Antibiotic Resistance Concern: Safe Water Reuse for Agriculture and Sustainable Food Production

Francesco Guarino, Annamaria Gentile, Patrizia Iannece, Angela Cicatelli and Stefano Castiglione

University of Salerno dept Chemistry and Biology "A. Zambelli", Salerno, Italy

Abstract

Nowadays, drugs are commonly released in wastewater and derived by human treatments and/or for livestock, or by personal products care, and included the Contaminants of Emerging Concern (CECs). Among CECs, antibiotics can promote the selection and diffusion of antibiotic resistance bacteria (ARB) and genes (ARGs). Because of their incomplete removal by conventional wastewater plants, CECs, and in particular ARBs and ARGs, are found in the effluents, posing risks to the environment and public health, mainly related to water reuse. Here, we tested Constructed Wetlands (CWs), characterized by a coupled water flow (vertical and horizontal flow in series - HCW), for civil wastewater treatment to evaluate the efficacy of ARBs/ARGs removing in the perspective of water reuse and safe food production. To this aim, a pilot-scale plant configuration HCW was designed with an innovative combination of multilayer and exploiting the plant biodiversity, that allow to reduce the CW surface of roughly five fold respect to the common literature indications. The experimentation has foreseen: i) physico-chemical (pH, COD, N, Cl, antibiotics, etc.) and biological (BOD, coliforms, enterococci) characterization of the HCW influent and effluent; ii) an UV-ray irradiation treatment of an aliquot of the effluents ; iii) evaluation of the presence of ARB/ARGs in the influents and effluents (after HCW or HCW + UV treatments) through cultural and molecular methods; iv) quantification of ARGs by real-time PCR analysis on influent and effluent ; v) use of the effluents (collected after HCW or HCW + UV treatments) for irrigation of lettuces to verify the ARB/ARGs transfer to the rhizosphere or to the leaves. The results revealed that HCW treatment was effective to obtain water of high quality for irrigation purposes, as confirmed by the physico-chemical and biological parameters assayed on the effluents deriving from HCW pilot plant. Moreover, cultural methods showed a significant decrease of ARBs (in terms of CFUs) in the effluents, after 48 hours of treatment, and their total removal after the UV irradiation. Moreover, genes quantification carried out on the effluents, collected after HCW or HCW + UV treatments, demonstrated the ARG removal up to 97% and 99%, respectively. Finally, after irrigation of lettuce with HCW effluents, the microbiological analyses on the rhizosphere highlighted the presence of few ARBs and ARGs, while they were absent in

the leaves. Instead, in the case of lettuce irrigated with HCW effluent, also treated with UV, no ARBs were found both in plant organs or in their rhizosphere. In conclusion, we demonstrated that: the new HCW configuration ensure the water quality achievement for its reuse for agriculture, also reducing or completely removing the ARBs and ARGs input to soils; the limited number of ARBs and ARGs found in the rhizosphere of lettuce irrigated with HCW effluents were not translocated to the leaves, resulting safe for consumers.

Keywords: Antibiotic resistance; water reuse; constructed wetlands; nature based solutions

Acknowledgments: This research was funded by the “National Biodiversity Future Center—NBFC”—National Recovery and Resilience Plan (NRRP), Mission 4 Component 2 Investment 1.4—Call for tender No. 3138 of 16 December 2021, rectified by Decree n.3175 of 18 December 2021 of Italian Ministry of University and Research funded by the European Union—Next Generation EU, Project code CN_00000033

ACCEPTED