

Treated wastewater irrigation promotes the spread of antibiotic resistance genes in soil and subsurface environments.

Treated wastewater (TWW) irrigation is a useful counter-measure against the depletion of freshwater (FW) resources. However, the TWW contains several contaminants of emerging concern, such as antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs). Consequently, TWW irrigation might promote the spread of antimicrobial resistance in soil and subsurface environments. In the first part of the present work, it was tested whether the ARG load and irrigation intensity define the influence of TWW irrigation on ARG spread dynamics in soil, using a multiphase approach. The approach consisted of monitoring surveys in a real-scale TWW irrigated field and laboratory-scale soil microcosms irrigated with TWW or FW. The genes *sull*, *qnrS*, *bla_{OXA-58}*, *tet(M)* and *intI1* were significantly more abundant in the TWW irrigated field soil, whereas *bla_{CTX-M-32}* and *bla_{TEM}*, the least abundant genes in the TWW irrigation, showed higher abundance in the non-irrigated soil. The relative abundance of *sull*, *qnrS*, *bla_{OXA-58}*, *tet(M)* and *intI1* correlated with TWW irrigation intensity and decreased during irrigation breaks. Microcosm experiments verified observations from the field study. In the second part, the hypothesis that TWW irrigation increases ARG prevalence in subsoil pore-water was tested using a similar multiphase approach consisting of sampling in a real-scale TWW-irrigated field and microcosm experiments. Despite the TWW irrigation bacterial load, the bacterial load of subsoil pore water was independent of irrigation intensity in the field study and irrigation water type in the microcosms. Among the tested genes in the field study, *sull* and *intI1* exhibited constantly higher relative abundances and their abundance positively correlated with increasing irrigation intensity. The controlled microcosm experiments verified the observed field study results as well. The relative abundance of several genes, including *sull* and *intI1*, increased significantly when irrigating with TWW compared to FW irrigation. Overall, TWW irrigation promoted the ARG and *intI1* spread in the soil and subsoil pore-water bacteria, while the bacterial load of soil and subsoil pore-water was independent of irrigation water type. Thus the combined results from the real-scale agricultural field and the controlled lab microcosms indicate that the dissemination of ARGs in soil and subsurface environments needs to be taken into account during TWW irrigation scenarios.