Quantification of plasmid- and integron-associated antibiotic resistance genes along a treated wastewater-soil continuum

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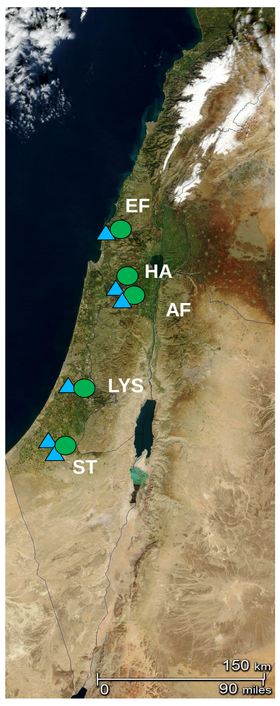
Background: Due to severe water scarcity, over 80% of urban wastewater in Israel is recycled and used for irrigation. Wastewater treatment plants (WWTP) are considered to be reservoirs of genetic determinants that encode for antibiotic resistance, and these may be transferred from treated wastewater (TWW) through soil to irrigated crops, thus contributing to the global spread of antibiotic resistance. Using a quantitative PCR approach, we tracked the abundance and distribution of six "mobile" antibiotic resistant genes (ARGs), and *intI*1 (a proxy for anthropogenic pollution), to evaluate ARG and *intI*1 abundance in WWTP effluents and their persistence in soil. Effluents from different stages of six WWTP and corresponding irrigated soils from **commercial agricultural fields and orchards** and **experimental lysimeters** were targeted.



Aerial image of sampling sites (left): sampled fields (●) and WWTPs (▲) and lysimiters plots (below)

Abundance of *bla*$_{OXA10}$ in various WWTP effluents, storage reservoirs and freshwater samples

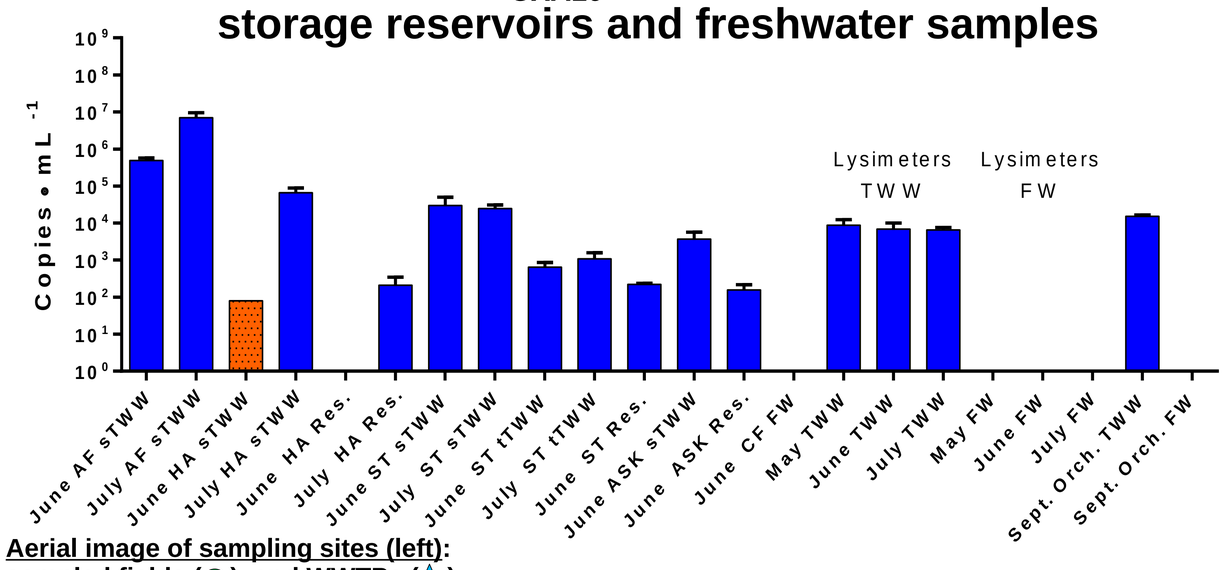


Fig. 1 sampled points: AF, HA, EF (orch.), ST, Lys.

ARGs and class I integron dynamics along a secondary, tertiary, reservoir water continuum

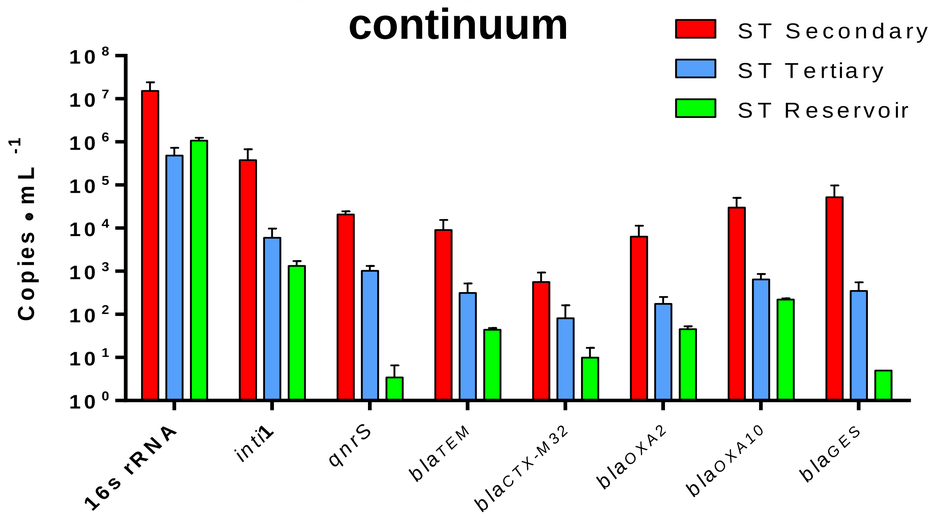


Fig. 2

The seven targeted genes displayed different patterns of distribution in the different treated wastewater and freshwater samples. *bla*$_{OXA10}$ (Fig. 1) and *bla*$_{GES}$ (β-lactamase-encoding genes) were strongly associated with wastewater derived samples, but were completely absent in freshwater samples. Interestingly, absolute and relative abundances of the tested ARGs dropped significantly between tertiary effluents and storage reservoirs (Fig. 2), despite the slight increase in total bacterial abundance.

Normalized abundance of *intI*1 and targeted ARGs in irrigated soils

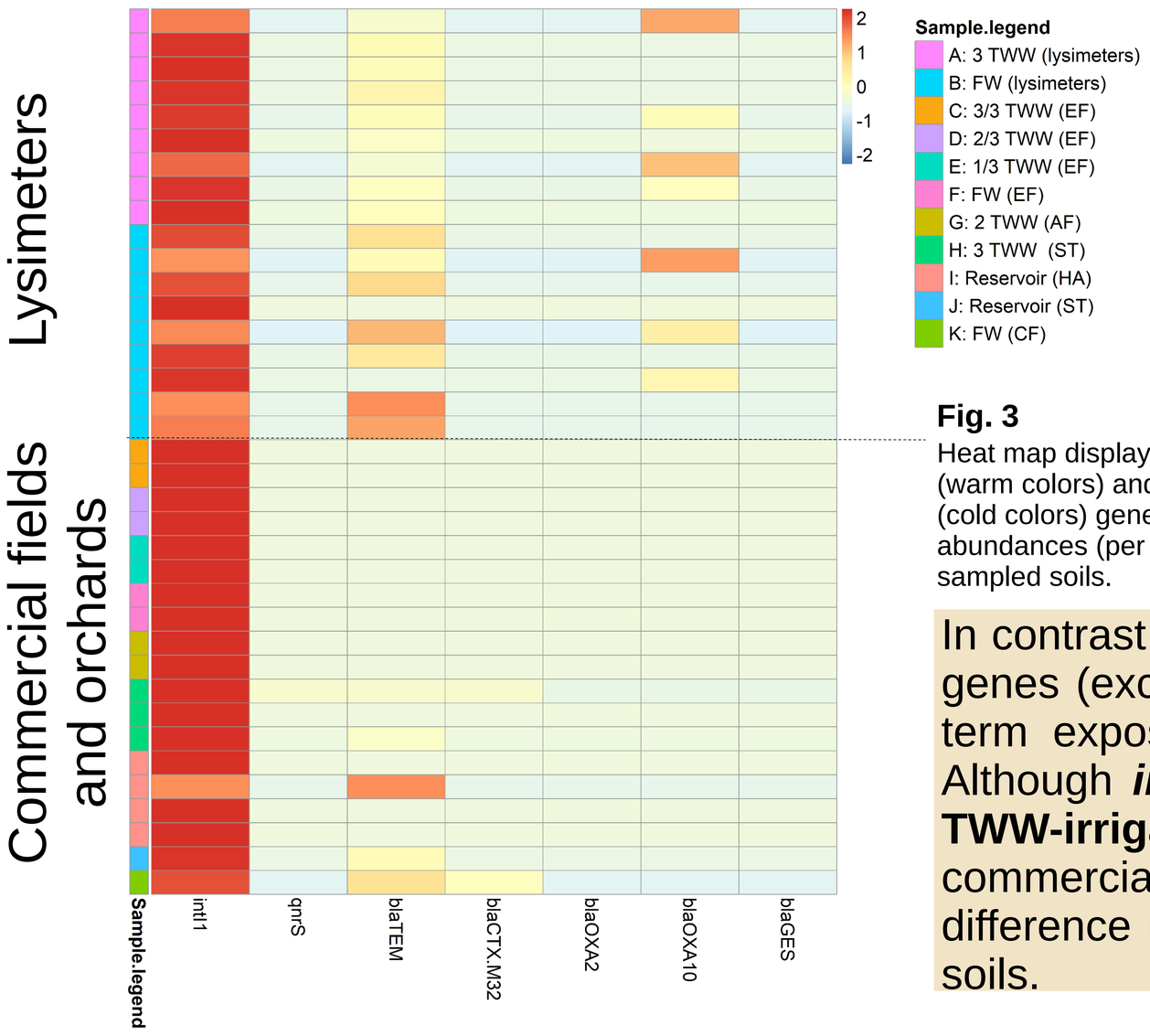


Fig. 3
Heat map displaying high (warm colors) and low (cold colors) gene copy abundances (per g soil) in sampled soils.

Normalized abundance of *intI*1 in lysimeter soils

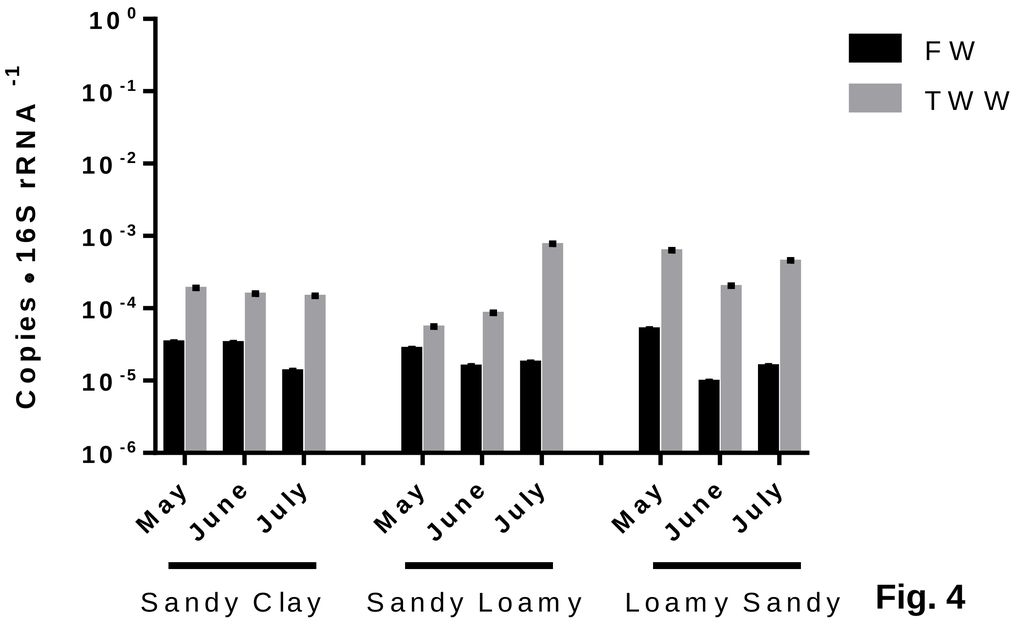


Fig. 4

In contrast to wastewater, in soil samples nearly all the targeted genes (excluding *intI*1) were below detection limit despite long-term exposure to high levels of TWW-borne ARGs (Fig. 3). Although ***intI*1 was significantly more abundant ($p<0.05$) in TWW-irrigated soils in the lysimeters (Fig. 4)**, in most of the commercial fields and orchard soils there was no significant difference in *intI*1 abundance between TWW- and FW-irrigated soils.

CONCLUSIONS: TWW is a profuse source of clinically-relevant ARGs; however, evidence from an array of field sites and experimental lysimeters indicates that these genes do not readily transfer to irrigated soils despite long-term irrigation. We hypothesize that strong ecological barriers limit the persistence of effluent bacteria in soil and the transmission of effluent-derived ARGs to soil microbial communities.

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ANSWER