

High limits of quantification of antibiotic resistance genes may mask the impacts of water reuse in soils



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PORTO

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Summary

This study determined the limit of quantification (LOQ) values that can be achieved using current methodologies to quantify antibiotic resistance genes (ARGs) in soils. The LOQ values found can be considered extremely high to perform an accurate assessment of the impacts of ARGs discharges in soils.

Our key message is that ARGs accumulation will be noticeable only at very high doses and therefore the assessment of the impacts of ARGs discharges in soils, of associated risks of propagation and potential transmission to humans, must take into consideration this type of evidence.

The simplistic assumption that no detection corresponds to risk absence should be avoided.

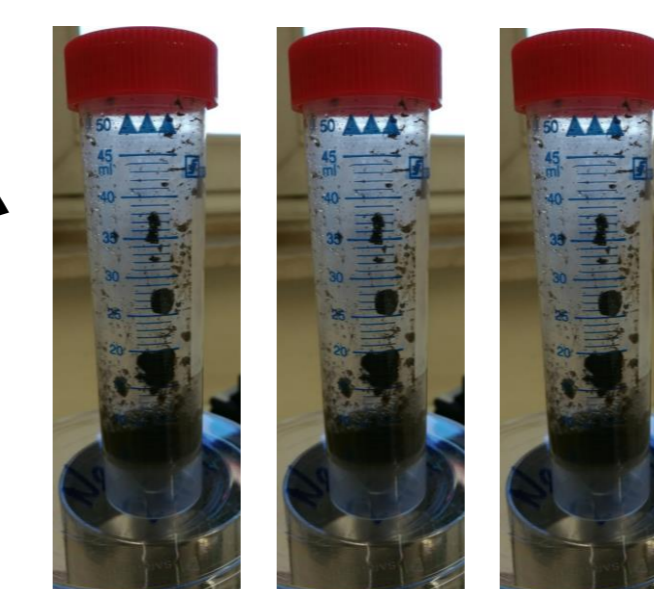
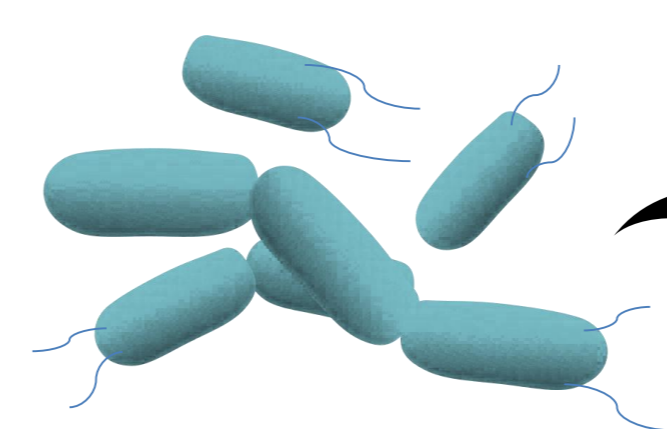
Fortunato et al., *Environmental Pollution*, in press.

Methods

Soil slurries: 10 g of soil

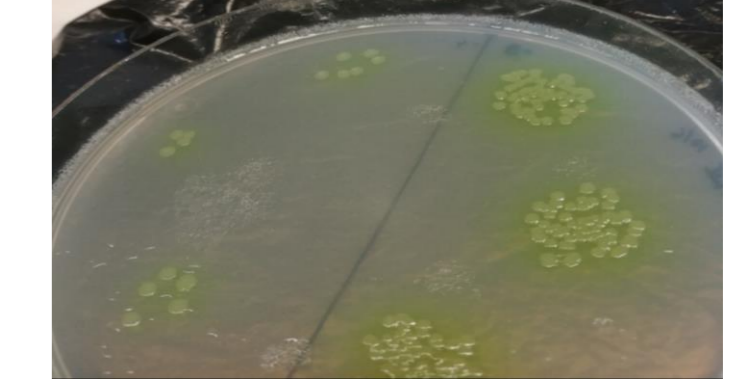
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10² to 10⁷ CFU/g of soil of wastewater antibiotic resistant bacteria (ARB) with known ARGs.



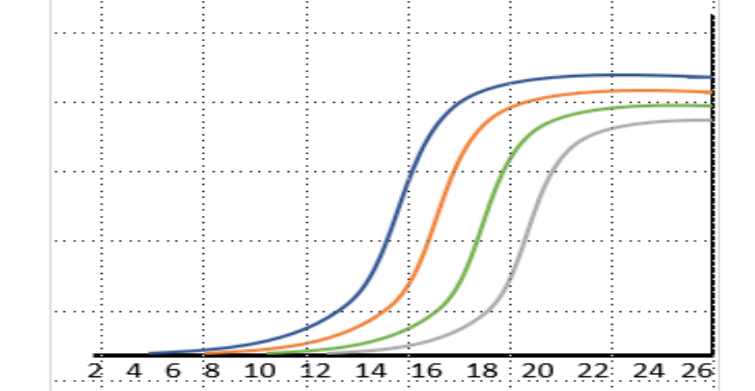
Enumeration of ARB on culture media

Culture dependent method



Quantification of ARGs by qPCR

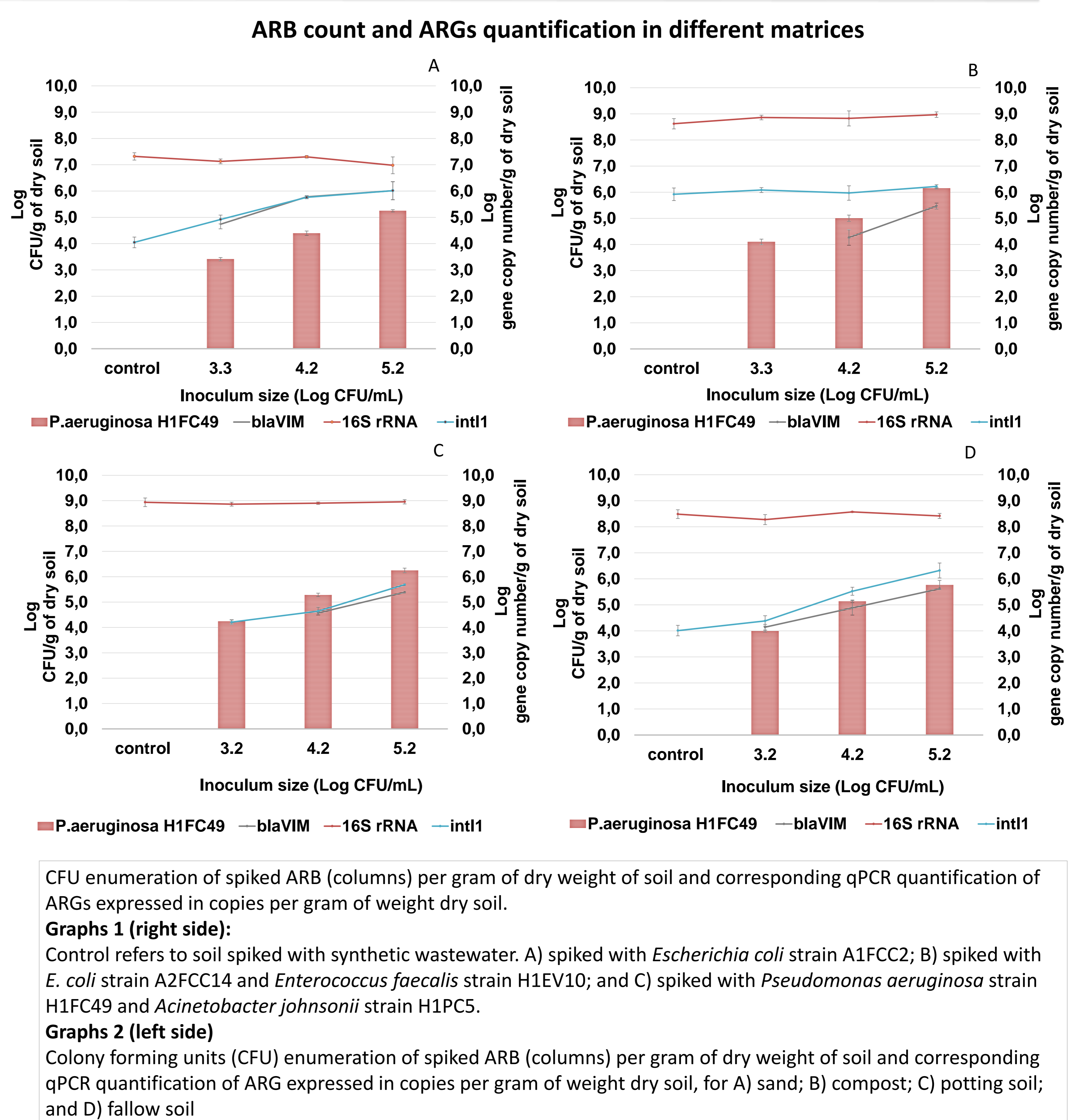
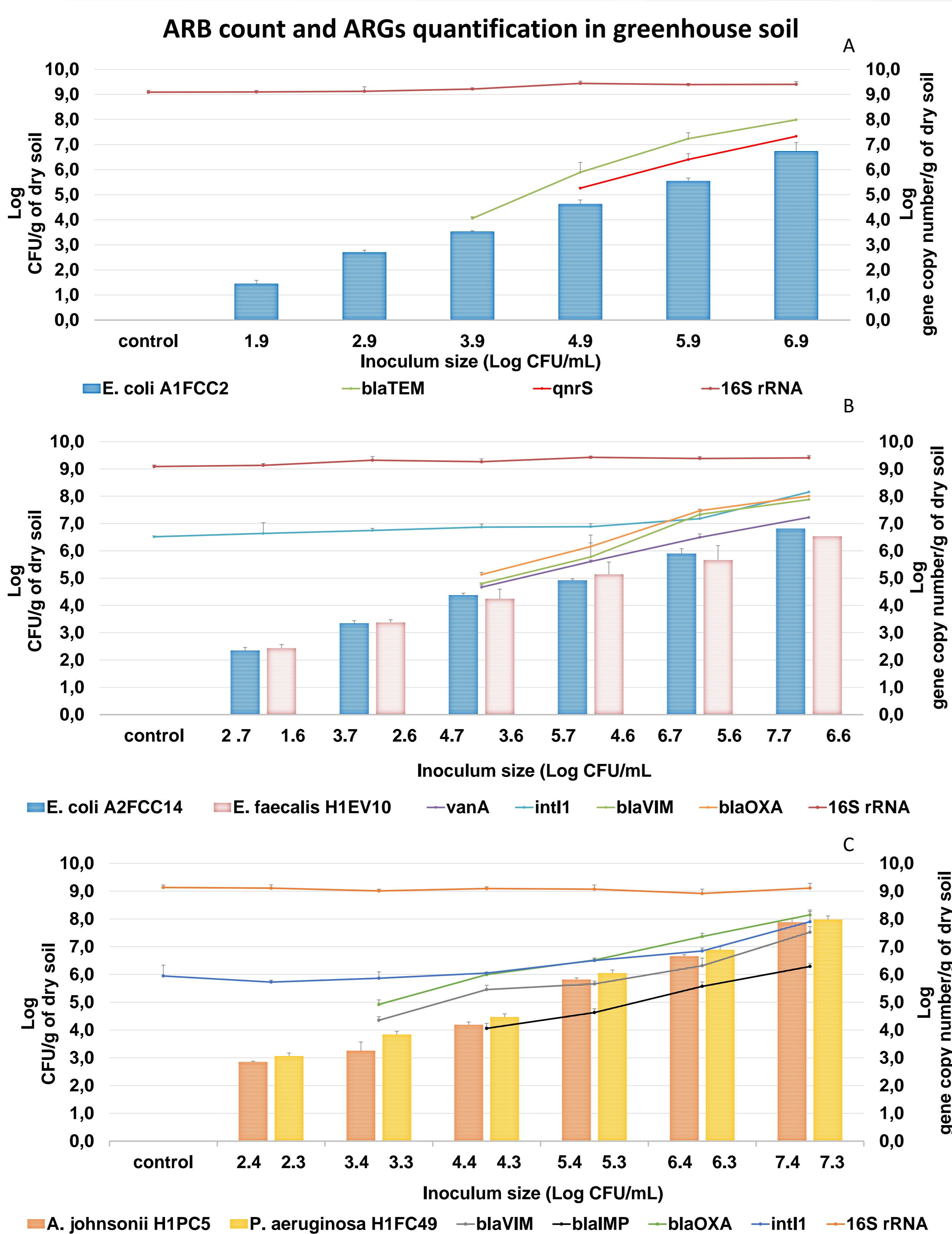
Culture independent method



Objective

Demonstrate that the limit of quantification (LOQ) and limit of detection (LOD) of ARGs in soil slurries is influenced by intrinsic technical factors, being generally too high to assess human health risks

Results



CFU enumeration of spiked ARB (columns) per gram of dry weight of soil and corresponding qPCR quantification of ARGs expressed in copies per gram of weight dry soil.

Graphs 1 (right side):

Control refers to soil spiked with synthetic wastewater. A) spiked with *Escherichia coli* strain A1FCC2; B) spiked with *E. coli* strain A2FCC14 and *Enterococcus faecalis* strain H1EV10; and C) spiked with *Pseudomonas aeruginosa* strain H1FC49 and *Acinetobacter johnsonii* strain H1PC5.

Graphs 2 (left side)

Colony forming units (CFU) enumeration of spiked ARB (columns) per gram of dry weight of soil and corresponding qPCR quantification of ARG expressed in copies per gram of weight dry soil, for A) sand; B) compost; C) potting soil; and D) fallow soil

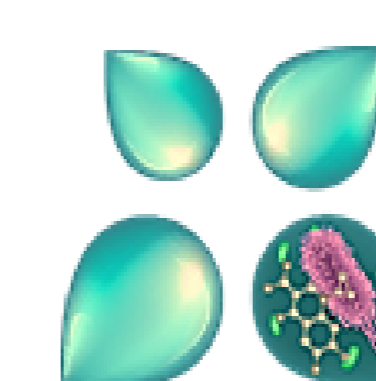
Conclusions

- The observed **LOQ** values ranged, for all analyzed ARGs and types of soil or related matrices, **4-5 log-units** per gram of dry soil.
- In average, **10⁴ CFUs/g dry soil** were necessary to achieve a reliable qPCR quantification of an ARG in the soil.
- With this study, we demonstrate that even when ARGs are not being detected or quantified in soils, the risks cannot be neglected since that soil sample may contain at least **one thousand** of a given **ARG** per gram of soil dry weight.

Acknowledgements

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ANSWER

