

Fate of *Pseudomonas aeruginosa* and *bla_{VIM}* in soil under selective pressure by copper and zinc



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Summary

Besides the natural resistance to antimicrobials present in soil, antibiotic-resistant bacteria (ARB) and antibiotic resistance genes (ARGs) can be discharged in soil through irrigation or other agricultural practices, which can also be a source of other contaminants such as metals. It is hypothesized that metals may generate selective pressure enhancing the survival of bacteria and/or of their ARGs

Objective

This study aims to assess the survival of a multidrug-resistant *Pseudomonas aeruginosa* strain in soil contaminated with metals as well as the fate of the *bla_{VIM}* gene.

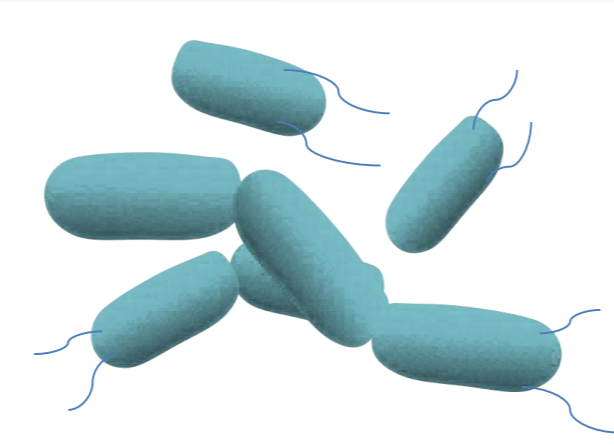
Methods

Soil slurries: 10 g of agricultural soil

without metals aging (control)

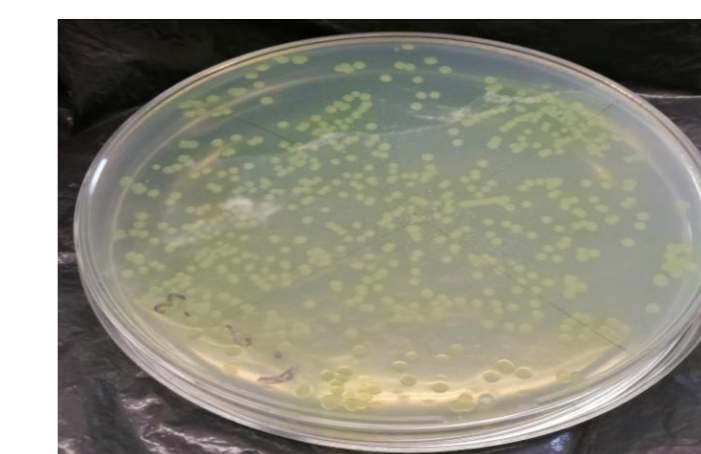
aged 1 month with copper and zinc sulfate (20 mM)

aged 1 month with copper and zinc nitrate (20 mM)

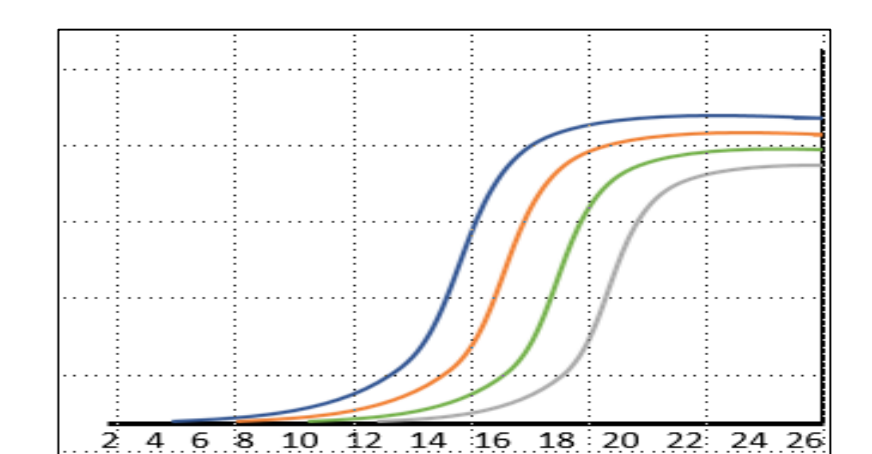


Spiked with a suspension (10^7 CFU/g of soil) of *P. aeruginosa*, harboring *bla_{VIM}* and *int1* genes.

Sampled weekly



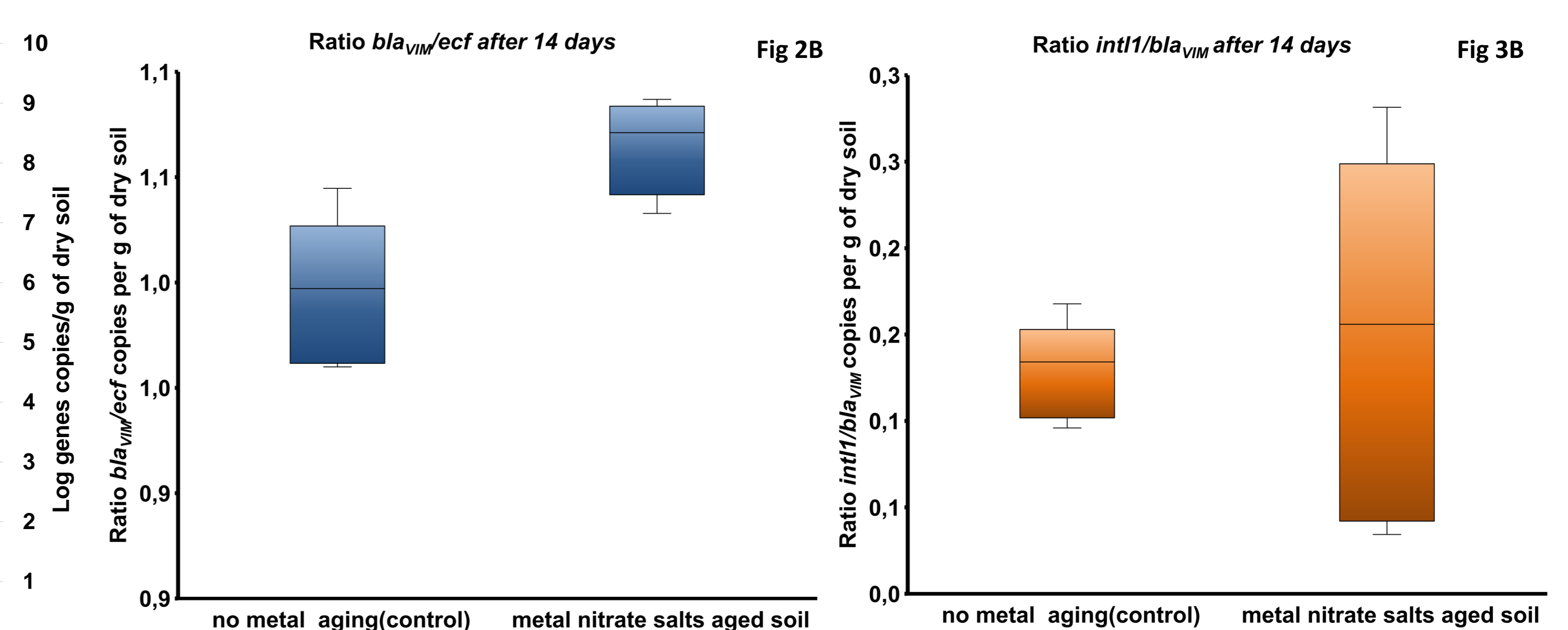
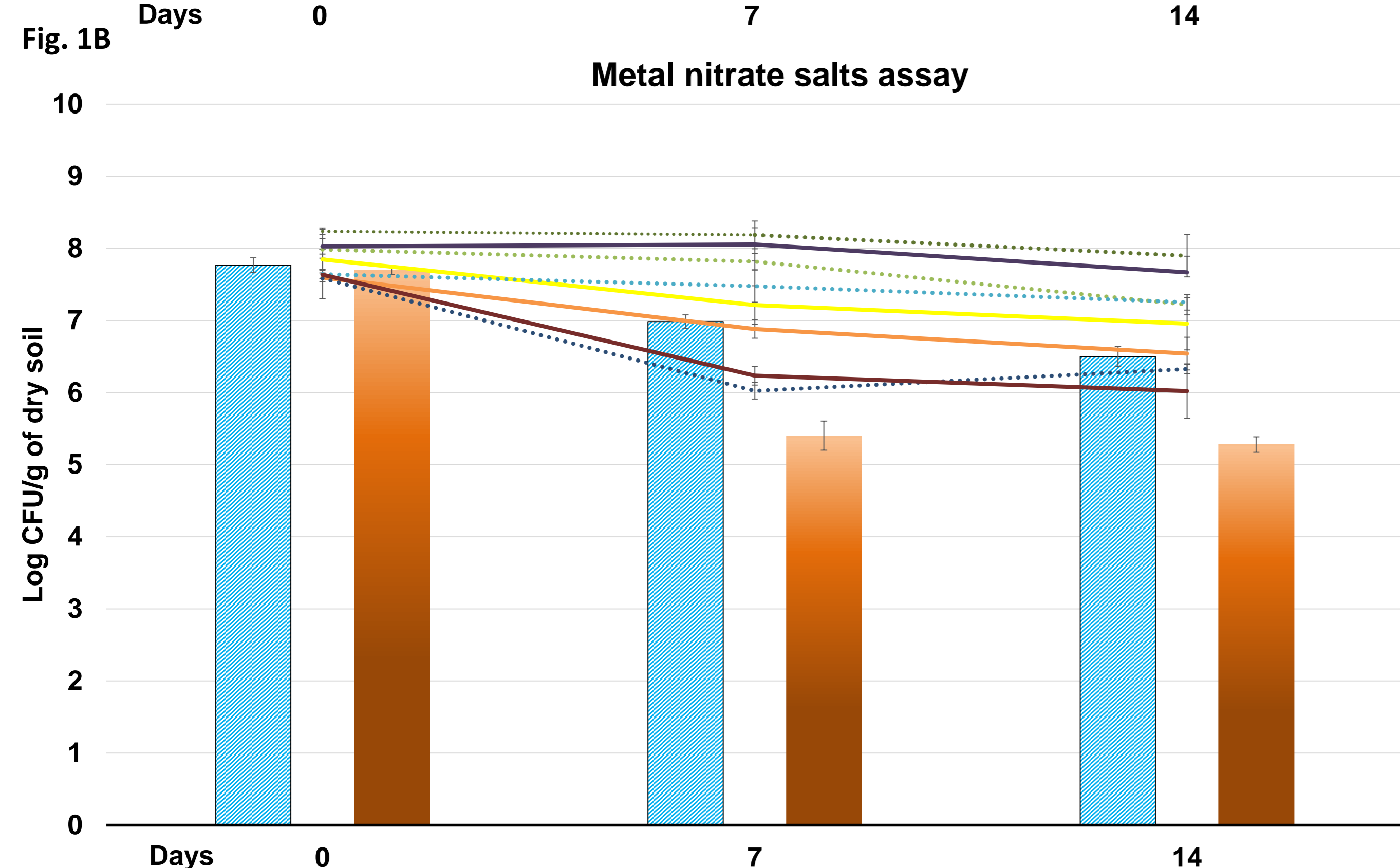
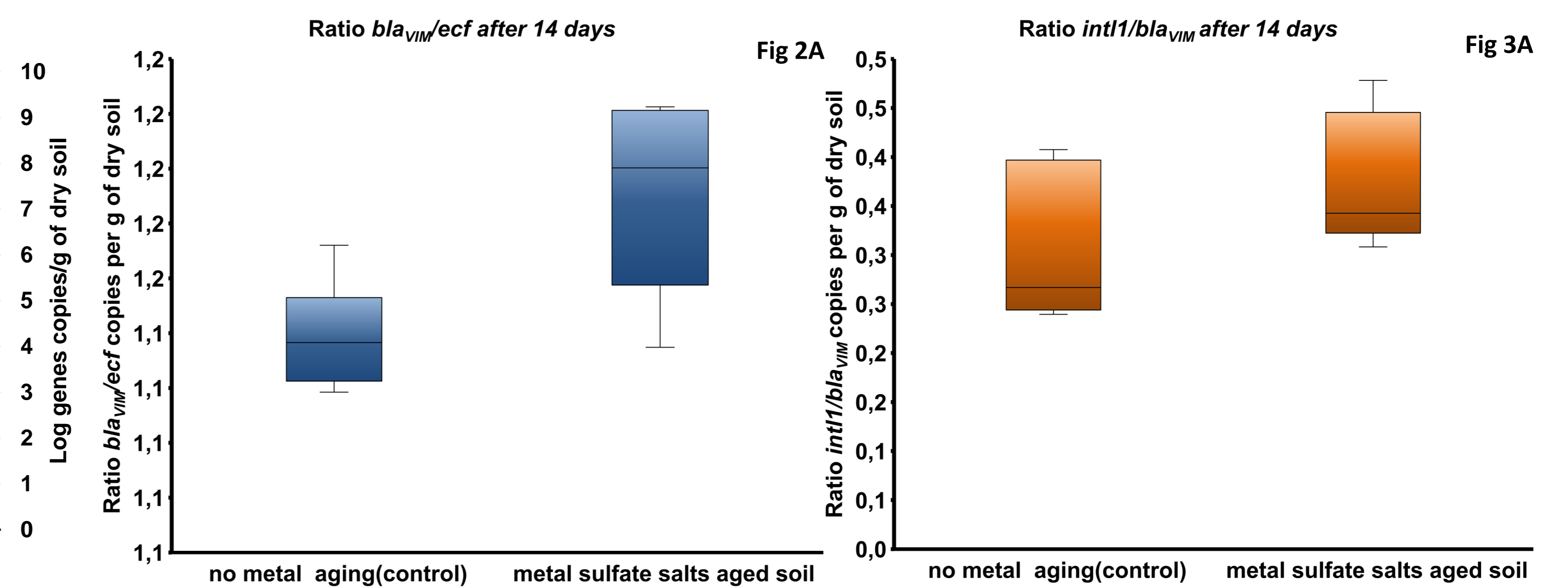
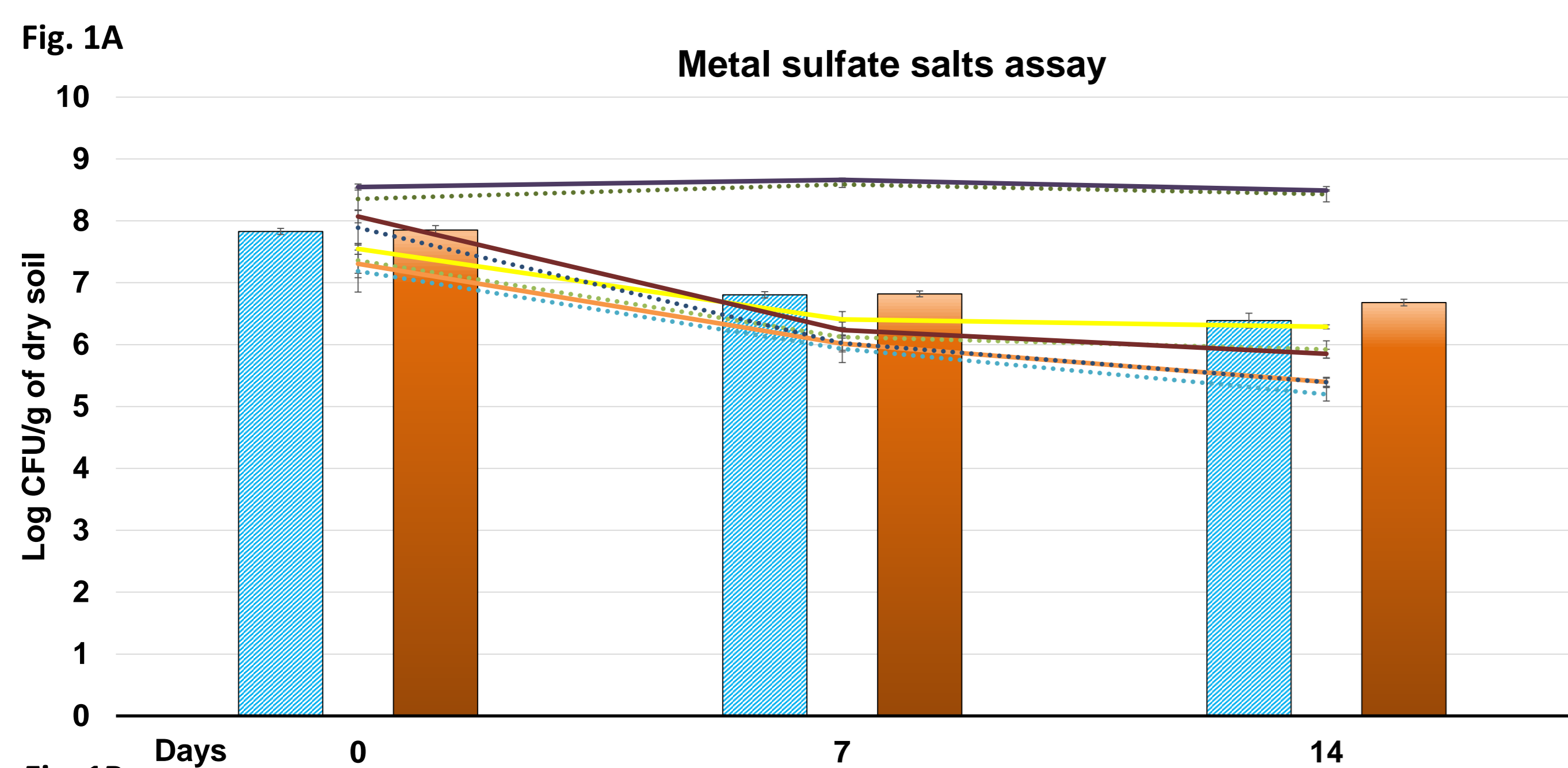
Colonies enumeration on:
P.C.A. (Plate Count Agar)
Cetrimide agar + nalidixic acid (15 mg/L)



Genes quantified by qPCR:
bla_{VIM}, *ecf*, *int1*

Results

Abundance of ARB and ARGs in soil slurries aged in the absence (control) or presence metal salts (copper and zinc sulfate or nitrate at concentration 20 mM)



■ *P.aeruginosa* CFU/g of soil no metals
■ *P.aeruginosa* CFU/g of soil metals
● *bla_{VIM}* no metal
● *bla_{VIM}* metal
● *ecf* no metal
● *ecf* metal
● *int1* no metal
● *int1* metal
● 16s rRNA inoculum no metal
● 16s rRNA inoculum metal

TOP Fig 1A *P. aeruginosa* colony forming units (CFUs) and gene copy number per g of dry soil in controls and in the presence of 20 mM of copper and zinc sulfate.

Fig 2A ratio between the ARG *bla_{VIM}* and the housekeeping gene *ecf*; **Fig 3A** ratio between *int1* and the *bla_{VIM}* gene.

BOTTOM Fig 1B as above using 20 mM of copper and zinc nitrate.

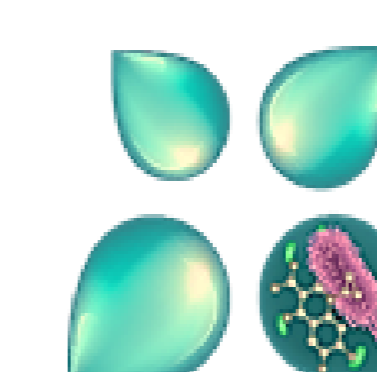
Conclusions

- P. aeruginosa* and hosted *bla_{VIM}* and *int1* genes persist in soil slurries irrespective of the metal salts presence.
- After 14 days of incubation, in presence of the metals, the *P. aeruginosa* housekeeping gene *ecf* decreased more than the ARG *bla_{VIM}*
- Further assays are ongoing to define the impact of the soil contamination with metals salts on the soil bacterial communities.
- These results suggest that antibiotic resistance selection may occur at both molecular and cellular levels. The molecular mechanisms behind these variations will be further discussed.

Acknowledgements

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ANSWER

