

Reviewer 1

The authors Vanesa Santas-Miguel et al investigate the microbial antibiotic tolerance under heavy metal pollution from soils with and without prior heavy metal pollution. In a very complex microcosms experiment of repeated incubations the authors demonstrate that antibiotic and heavy metal tolerance is linked to heavy metal pollution heredity. This combined effect of antibiotics and heavy metals on soil microbial growth is attributed by the authors as co-tolerance. This work depicts interesting relationships among heavy metals and antibiotics on microbial growth, and those relationships are quantified as toxicity index, PICT and IC50.

1. The authors should revise their manuscript carefully for English grammar and specifically the use of clause sentences. Furthermore **the authors should introduce the current state of the art with relevant data and numbers and later on elaborate and interpret their findings on their discussion.**

Thank you for your comment. The English was carefully checked and corrected, with changes marked in red fonts. In addition, the current state of art and the overall discussion were improved, also marking changes in color fonts.

Also, the authors should briefly explain why the selected heavy metals are of importance.

Thank you for your comment. The explanation was added in Lines 40-45, using color fonts.

2. Equally the authors should explain their experimental design in a concise and clear way. For example, the addition of an experimental design schematic

would greatly illustrate their complex experiment design of numerous incubations.

The experimental design was clarified and a scheme of the metal spiking procedure added as Figure S1 in Supporting Information.

3. Adding on that aspect, the authors should express their opinion and justification why so little soil (1.7 g) was used for the subsequent incubations.

The reason is that this amount of soil has been shown to be enough to extract soil bacteria and perform bacterial community tolerance to any metal. In addition, please take into account that three replications were used in each case.

4. Please comment why sterile water was used instead of PBS to extract soil microbes, which is rather common in soil biology. The authors should consider that any solution will partly extract intact microbial cells from soil and discuss how this affects their interpretation.

The reason of using that procedure is that, after being designed by Bååth (1994) and modified subsequently by Bååth et al. (2001), it has been shown to be highly effective to estimate bacterial growth. In fact, this is a method widely used in the last 20 years, and indeed it includes an extraction of soil bacteria with distilled water, not with PBS.

Bååth. E., 1994. Thymidine and leucine incorporation in soil bacteria with different cell size. *Microb. Ecol.* 27, 267-278.

Bååth. E., Petterson. M., Söderberg. K.H., 2001. Adaptation of a rapid and economical microcentrifugation method to measure thymidine and leucine incorporation by soil bacteria. *Soil Biol. Biochem.* 33, 1571-1574.

5. It is also essential to differentiate between total and bioavailable heavy metal concentrations. This should be made clear to the reader and also it should be discussed later on in the manuscript.

Thank you for your comment. Information on two types of bioavailable metals was added to the “material and methods” section, and it was also considered in the “discussion” section (Lines 119-121, 145-151 and 253-257).

6. L145 is very interesting that those number differ from others, however the authors should discuss why there are differences.

The differences may be attributed to variations in physicochemical characteristics. Some details of soils from other works were added.

7. Please also explain the logical arguments when comparing soil microbial data with those of higher organisms in lines 158 and 161. Is there a common mode of action or biological link between them? Can those numbers actually be compared?

Heavy metals may be toxic for both, soil microorganisms and soil fauna, and also for aquatic organisms. For that reason, it is interesting to determine whether toxicity sequences (i.e. which are the most toxic metals for soil microbes) are the same or not, for soil fauna and aquatic organisms. Our results, as well as data from other manuscripts, show that Cu and Cd are the

most toxic heavy metals for a number of organisms, including soil microorganisms, soil fauna and aquatic organisms.

8. The authors should discuss their reasoning behind selecting 1000 mg/kg for their polluted soil treatment. In their introduction it is illustrated that heavy metals and antibiotics accumulate in soil slowly- slowly by repeated depositions. One could argue that a polluted soil might have lower levels of pollutants. The authors should justify their approach.

Thank you for this and all your other constructive comments. The rationale of 1000 mg kg⁻¹ selection was added in the “introduction” section. Lines 90-100.

Reviewer 2

Dear Authors,

The manuscript is about a very interesting and important scientific topic, and I have only a comment about the soil characteristics, as we are studying heavy metals, so we must to have more detailed information about the soil texture, EC, carbonate and other macro elements.

Thank you very much for your comment. Line 125-127 the method used for the analysis of electrical conductivity (EC) has been added. In line 133 was added the electrical conductivity value.

On the other hand, this type of acidic soil does not present carbonates. However, it seems to us a very interesting comment.

The main characteristics of the soil studied are found in Table S1 of Supplementary Material (Line 16). We have added the EC of the soil.

And a recommendation for future work, your research could be more interesting and important using more types of soil, with different pH, texture and calcium carbonate, to link also the effect of soil properties on the heavy metals availability and so their effect on microorganisms.

Thank you very much for your comment. In the future we would like to expand the number of soils and see if there are correlations between the different soil characteristics and the toxicity of heavy metals used show on the growth of soil bacterial communities.

Thanks and best regards