

Biogeosciences Discuss., author comment AC2 https://doi.org/10.5194/bg-2021-165-AC2, 2021 © Author(s) 2021. This work is distributed under the Creative Commons Attribution 4.0 License.

## **Reply on RC2**

Sania Arif et al.

Author comment on "Composition and niche-specific characteristics of microbial consortia colonizing Marsberg copper mine in the Rhenish Massif" by Sania Arif et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-165-AC2, 2021

1. This study is just region research, how to attract global interests? Is there any implications for the other mining sites? Authors should highlight its "unique" in the Introduction.

The colonization of the similar Chloroflexi (KD96, Ktedonobacteria classes) is common at heavy metal contaminated sites and have been discussed in detail in the discussion section. The investigation of the Chloroflexi, Cyanobacteria and Actinobacteria stains may eventually be used to remediate the heavy metal contaminated sites.

2. Plz highlight the hypothesis. What is the scientific question in this study?

The last paragraph has been modified as per instructions from reviewer 1, the previous study has been added to highlight the hypothesis: to observe whether the mine waters enriched in transition metals may be toxic to microbial inhabitants or, conversely, support unique forms of metal respiration and enrich resistant microbial consortia under oligotrophic conditions.

3. Plz move the details of PCR to supporting information. **Corrected.** 

4. Just recommendation (Line 160): ASV is more widely acceptable than OTU.

Yes agreed, the study has already been commuted from OTUs.

5. Line 190: Provide the detail parameters for the metaG-analysis, such as what is the k-mer for assembling?

Information added in the text : Assembly was performed using metaSPAdes v.3.14.0 with kmers -k 21, 33, 55. Most of the metagenomic analysis parameters have been given in the announcements of the

https://journals.asm.org/doi/full/10.1128/MRA.01253-20.

6. Line 215 and Line 217: SO4? SO4-?

The charges for the ions have been skipped to avoid confusion because we are talking about analysed total concentrations not different specifically charged ions. With ICP-OES or ICP-MS we measured total amount of elements, e.g. Sr or Cu, but not their specific ions. One can't differentiate between species by the above techniques.

7. Too many colors, plz show the top taxonomies or the most important taxonomies. As instructed by reviewer 1, the taxonomy excel table has been made available online for easy viewing.

8. Sample names "MBS MB 1234" is confusing, plz use more readable ID.

Samples names MB were basically to distinct the biofilms samples collected around the copper plume from the other samples MBS. The denotations of the

## samples have been already used in the NCBI databases (as prerequisite for publication) and cannot be changed anymore.

9. Figure 7 is too complex, plz show the most important information.

**Figure 7 is modified as instructed by first reviewer, the GO terms are simplified.** 10. Fig8 and 9: The proposed pathway comes from all MAGs or just one MAG? What is the difference from already reported Copper-resistance pathway?

The proposed pathways are collected from all MAGs pathways map. Since the pathways are cumulative from the mixed microbiome, both copper-resistance pathways, the anaerobic Cus system and the aerobic CopA regulon are being observed. Moreover the sensors—CueR in Salmonella, and CsoR and RicR in Mycobacterium—which induce the expression of a number of copper-resistance mechanisms to counteract Cu toxicity and ensure survival are being observed. https://www.researchgate.net/publication/283819561\_Bacterial\_Copper\_Resis tance\_and\_Virulence.