

Interactive comment on “Linking dissolved organic matter composition to metal bioavailability in agricultural soils: effect of anionic surfactants” by M. C. Hernandez-Soriano and J. C. Jimenez-Lopez

M. C. Hernandez-Soriano and J. C. Jimenez-Lopez

m.hernandezsoriano@uq.edu.au

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Firstly, the authors would like to sincerely appreciate the time and effort of the anonymous referee. Responses to the comments and questions are provided below.

This paper is about the bioavailability of metals in soil. The bioavailability of Cd, Cu, Pb and Zn in three agricultural soils was essayed by the effect of greywater enriched in anionic surfactants (Aerosol 22 and Biopower) at field capacity and saturation regimes. The paper address relevant scientific questions within the scope of BG, but substantial

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conclusions are not yet reached. Response: The authors would like to clarify that the study examines the effect of two selected anionic surfactants representative of those compounds potentially present in greywater. Thus, aqueous solutions of the anionic surfactants were added to the soils. No greywater was used for this study.

The scientific experimental design (see below) and assumption are not valid or clearly outlined and therefore, the results are not sufficient to support the interpretations and conclusions. Response: This being a general remark the specific comments are addressed below.

Furthermore I am fully agree with Dr. Mostofa, in the sense that the authors give scarce credit to work and concepts related with the terminology. Response: The authors find it difficult to address this particular remark. The performance of the spectrofluorometric analysis, estimation of fluorescence indexes and subsequent interpretation of the results follow well established and recognized bibliography: e.g. Miller et al. 2009, Fellman et al. 2009&2010, Stutter et al. 2013, Zhou et al. 2013, Xue et al., 2012. A substantial number of relevant manuscripts have been considered. Accordingly, data discussion and linkage to the objectives of the work is supported by the mentioned bibliography. Nevertheless, the authors would be glad to extend the discussion concerning these results and/or clarify the proposed link between the characteristics of dissolved organic matter and the predicted bioavailability of metals.

This study presents serious methodological problems similar to those raised by the Anonymous Referee #1, but I'm not convinced that you can improve the statistical results by increasing the number of replicates ($N > 2$). Replicate a finding, it can reach the conclusion independently, because the variability associated can be estimated. By contrast, duplicate a finding, you cannot reach a conclusion independently, as they have reproduced the experimental conditions from the same portion of soil. This means that you do not have truly replicates or pseudo-replicates. You sampled three soil and made two portions (duplicates) from each and these sub-samples were used as replicates. I understand that many widespread type of experimental design in

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soil chemistry involves a single replicate per treatment which is neither surprising nor wrong. Indeed, replication is often unable to be realized when very large scale experimental unit are studied. A one replicates may be the only or best choice when gross effects of a treatment is expected, or when the cost of replication is very large. What is liable to objection is that your conclusions originate from unreplicated treatments justified by erroneous application of inferential statistics (ANOVA test). So, even when you have pseudo-replicates, you must justify your treatments, i.e., the contaminated water irrigation impact on metal speciation from the same soil sampled from two or more independent sites belonging to the same area (soil series). Response: The authors would kindly refer to responses provided to Referee #1 concerning the methodological approach. Overall, the range of combinations examined for the variables considered is sufficient to sustain the statistical analysis. It is described in section 2.5 that the statistical analysis performed is 'Principal Component Analysis', which was validated for the 'treatments replicates' considered. Also, it is clearly indicated that 'Dunnett's test was selected to determine significant differences between the treatments and the control while Tukey test was used to compare treatments. Kruskal–Wallis test was used when the variables did not meet the ANOVA normality assumption'. Thus, the 'erroneous application of inferential statistics (ANOVA test)' comment from the referee seems the product of a misunderstanding.

Specific comments M&M Soil description is very weak and it requires many inputs. Response: This being a general comment, the authors address specific questions below. There is no information of the soils origin and how they were sampled. Are the soils composed samples? Response: Indeed, composite samples were collected in the field, air dried (as indicated in section 2.2) and characterized prior to the study. The manuscript indicates that 'metal contamination in the soils assayed originated in atmospheric emissions from non-ferrous smelters' (section 2.1), while Table 1 summarizes the origin of the soil and relevant properties. The authors would be glad to provide specific coordinates for the sites upon request, although it seems not particularly relevant given the objectives of the study.

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Page 4, 20-25. You should describe the soil type. What kind of agriculture was practiced in these contaminated soils? Response: The authors would be glad to provide information on the cropping activities on the fields, however it seems a factor that would have little impact on the interpretation of the results – bioavailability of metals being more strongly related to physicochemical properties of the soil (Table 1) than to agricultural practices, which at most can be considered a random effect in the present study.

Page 5, 8-10. What do you mean by organic matter in soil solution in Table 1? Is it Dissolved Organic Matter? How did you extracted it? Response: Indeed, organic matter in solution is dissolved organic matter. The authors agree to rephrase footnote c to avoid further confusion. The dissolved organic matter was determined in the pore water, obtained and analysed as described in section 2.3.

What is the difference between DOC from pore water extraction described on Page 6 and dissolved organic matter? Response: To the best of the authors' knowledge, DOC just refers to the analytical quantification of organic carbon in the soil solution (pore water).

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