Response to the review of the manuscript: Hydrological tracers for assessing transport and dissipation processes of pesticides in a model constructed wetland system

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Answers to referee #2

Comment 1: *The authors present an interesting and well written paper on transport and dissipation processes of different substances in a constructed wetland at the lab scale. This is a highly relevant topic that is within the scope of HESS and of interest for a broader audience. The experiments and results are highly interesting and are largely presented clearly. A few points in the analysis and interpretation, however, should be revised to be less speculative and more supported by the results. This requires mainly further elaboration of the discussion, as detailed in the specific comments below.*

Response 1: We appreciate the positive feedback, thoughtful comments and constructive suggestions from the reviewer that will help us improve the manuscript. We next detail the reviewer's comments (in italics) and our answers on how we will address the comments in the revised manuscript. Some long comments have been subdivided into several comments

General comments

1. Recovery

Comment 2: *A main concern is the low recovery of most of the substances. Except for bromide and SRB, less than a third of the applied masses were detected in the investigated compartments (the data on SRB suggests a recovery between 48 and 105% - Fig. 8). Because it is ambiguous to judge the parts that have not been observed, care needs to be taken in drawing conclusions on transport and dissipation from the data. The authors often did well in this regard and addressed possible pathways of the substances' fates by deduction and use of available literature. Sometimes they overachieved a bit, and some aspects deserve further clarification.*

Response 2: We thank the reviewer for pointing this out. We are aware of the difficulty of drawing conclusions on transport and dissipation from the data when some observations in certain parts could not been made. That is why we have been very careful when interpreting the results. In any case, we agree with the comments of the reviewer and further clarification of the results in terms of transport and dissipation will be done in the revised version.

Comment 3: *I would appreciate if the authors discussed possible reasons for low recovery in more detail.*

Response 3: Thank you for this suggestion. The low recoveries of the solutes is precisely a key point in our study and despite the limitations of the experiment, we have very detailed data that can help us better address this question. We agree with your assessment. As such, we will discuss it in greater depth in the revised version: in particular we will discuss recent scientific studies that have shown transformation of UR in contrast to SRB. Br as a salt can anyhow be treated to be chemically inert. Pesticides are known to be affected by biochemical degradation, however most knowledge stems from unsaturated soil and not from wetland sediments.

Comment 4: *What about the formation of other transformation products – is this likely, are other TP known that might be formed under the given circumstances?*

Response 4: We are grateful for this comment. Indeed, although met-ESA and met-OA are reported to be the major metabolites of metazachlor, it is possible that other transformation products formed in our system. However, such minor compounds were most likely below the limit of detection and therefore could not be identified. This information will be mentioned in the revised version.

Comment 5: *If sorption is a major pathway, why have the substances not been detected in sediment/plants?*

Response 5: We are sorry for not making this point clear enough. The hydrological tracers were detected in the sediment/plants. Only the pesticides and their TPs could not be measured in this compartment because a quantitative method was lacking. We are aware of the importance of such information in unveiling the fate of the solutes. However, this again points to the advantage of using tracers instead of pesticides, as they are easier to be measured. The data provided in our study allowed us to build an overall view of the solutes behavior with great spatial and temporal detail. Moreover, we believe that our study represents a first approximation in this regard, and further experiments need to be done. We will enlarge the discussion in this point.

Comment 6: *Which other pathways are possible, especially for the substances that are not likely to be adsorbed or degraded?*

Response 6: We thank the referee for this important comment. In our study, we have speculated that plant uptake could be an important dissipation pathway. Mineralisation of the compounds to CO_2 may be another possible pathway, although according to literature (EFSA, 2008) the mineralisation of the compounds is generally minimal and slow. As for possible volatilisation from aqueous systems/soil water, only limited losses can be expected, based on the same literature. This information will be discussed more in depth in the revised version.

Comment 7: *Can the expected degradation/mineralisation be quantified using literature values, and contrasted with the measurements?*

Response 7: We appreciate the observation, but an exact quantification would imply the application of modeling approaches. As already mentioned in the response to comment 8 of reviewer 1, modeling the data to do predictions is a distinct topic and not the purpose of the present manuscript. Therefore, it will be treated separately in a forthcoming study.

Comment 8: *With regard to the transformation/degradation of UR:*

(1) How much of the degradation was possibly due to photolysis in the inlet container or at the system's surface?

(2) Could you estimate photolysis rates quantitatively?

(3) Microbial degradation of UR seems not to be enhanced after the first application, as illustrated by the similar recovery rate in the second part. If the system has not been exposed to UR before, and microbial decay was a major pathway, the first application of tracer probably would have fostered the microbial community capable of degrading the dye (Käss 1998), which would have enhanced microbial decay in the second run.

(4) Can alteration of fluorescent properties be a reason for apparent loss of UR (pH values are given in Table 2, but apparently only one measurement)?

Response 8-(1): We thank the reviewer for raising this important point. Possible photolysis of the compounds in the inlet container was discarded, because this part of the system was covered to avoid exposure to light. As for the system's surface, we have assumed that photolysis of UR most likely took place. We will try to estimate a possible loss in the revised version.

Response 8-(2): One could estimate how much UR was lost during the first saturation phase according to the concentrations measured at the vegetated part of the uppermost layer. Light decay can be estimated assuming first order loss and half-lives from literature. This information will be included in the revised version.

Response 8-(3): Regarding the possible microbial degradation of UR, we speculated that the missing percentage of the final mass balance was mostly due to abiotic degradation (i.e., photodegradation). Nevertheless, we have also hypothesized that possible microbiological degradation of UR took place, but to a lesser extent. The fact that it was not enhanced after the first application could be due to the probable existence of other preferred substrates for microbial degradation. These preferentially utilized compounds would have limited the degradation of alternative substrates such as UR. This is an important point that will be enlarged in the discussion of the revised version, since we also expected a more intense biodegradation of UR during the second execution of our experiment.

Response 8-(4): The possible alteration of the fluorescent properties of UR is ruled out because the pH of the samples was always raised with buffer solution during the measurements. Unfortunately, the pH of the sand could only be measured at the end of the experiment when the sediment samples could be extracted. That is why we only provide one measurement.

Comment 9: *The different substances were mixed in one solution for application, but possible interactions of the applied substances are not discussed.*

(1) Can interactions of the different organic compounds be ruled out?

(2) Testing a reference sample of the injection solution repeatedly over time could give further hints on interactions or degradation rates of the mixture.

Response 9-(1): It is true that the possible interaction between the applied substances is not discussed in the manuscript and cannot be completely ruled out. The solution was prepared immediately prior to the injection. Tracer concentrations were measured inside the solution during the same day and a couple of days after and no significant changes were observed. On the other hand, the pesticides were mixed in one solution in the laboratory and no interaction of the substances was detected. We will discuss this point in the revised version.

Response 9-(2): Testing the injection solution over a long time was not considered. On the one hand because it was not possible to prevent the solution from being degraded by unknown microorganisms, and on the other hand, because the results of a possible degradation/interaction in a bottle could not be transferred to a system with different conditions and greater complexity.

2. Flow paths and preferential flow

Comment 10: *The flow paths during the tracer application are not completely clear to me.*

(1) From Fig. 3 it appears as if the tracer solution was applied to the surface near the inlet container by letting the inlet container overflow.

(2) The arrows indicate vertical movement downwards near the inlet, and vertical movement upwards elsewhere. Is the ponding on the surface from surface flow from the inlet, or from upward flow through the soil? In case of the former: Has air been entrapped in the system?

Response 10-(1): We apologize for the confusion. In fact, the arrows on the surface of Fig. 3-1) only indicate the direction of the flow but do not represent the actual movement of the water during the injection and therefore can be misleading. These arrows will be eliminated in the revised version.

Response 10-(2): That is correct, the injection solution was applied to the surface near the inlet by letting the inlet container overflow. Due to the low flow rate the solution moved first downward near the inlet and then upward as the system was filling up. The ponding on the surface is coming from the upward flow. Therefore, possible entrapment of air in the system can be ruled out. We will clarify the flow paths by a more detailed description in the revised version.

Comment 11: *A great part of the transport in the experiment has been attributed to preferential flow in the upper and lowermost layer. Given the coarse texture of the soil, hydraulic conductivity will be high and lead to fast regular flow rates already.*

(1) Is the observed breakthrough still considered preferential when compared with expected flow rates using conductivity and hydraulic gradient?

(2) If preferential flow is an issue, how would that influence the spatial distribution of substances in the sediment, and in turn the recovery of substances from sampling sediment?

Response 11-(1): We thank the referee for this important comment. Yes, we consider that the obtained breakthrough curves are due to non-uniform movement of water through the soil as a consequence of the system design and the presence of plants. This statement is based on the obvious differences in concentrations observed and the faster arrival of solutes to the lower and uppermost layers compared to the middle layers.

Mean flow velocity can be estimated if we assume uniform flow, but a comparison with expected velocities for each curve cannot be made without modeling the data. And, as already indicated in previous comments, this was not the purpose of the present manuscript.

Response 11-(2): Indeed, the observed distribution of substances in the sediment and their recovery is in agreement with the formation of preferential flow in the upper and lowermost layers.

3. Correlation analysis

Comment 12: *Much of the interpretation is based on a correlation analysis.*

(1) Please describe in bit more detail what was correlated – I expect you used measured concentration time series?

(2) Sorption is significant for some of the substances. How would retardation affect the results of the correlation analysis?

Response 12-(1): We apologize for the lack of detail in this section and the revised version will include a better description. That is correct, we used measured concentration time series for the correlations.

Response 12-(2): We have hypothesized that the shape of the breakthrough curves will be affected by the retardation of the solutes resulting in non-significant or non-existent correlations particularly with Br. We will clarify this dependence in the revised version.

Specific comments

Comment 13: *P6, LL 33-35: This part is unclear.*

(1) How does the design of the inlet cause preferential flow towards the bottom?

(2) And what is meant with "plants channel flow to the surface" – flow from lower layer to the soil surface? Or do you mean enhanced infiltration from the surface?

Response 13-(1): We appreciate this comment. Obviously, we did not make this point clear enough and will clarify this in the revised version. The inflow system (by overflowing the inlet reservoir) and the low flow rate are believed to be the origin of preferential flow. Such design caused the injection solution to slide down the inlet glass wall channeling the water towards the bottom.

Response 13-(2): As for the plants, we hypothesized that they likely facilitated the transport of solutes along the root channels from the bottom to the surface layer. Besides this, the plants may have also introduced heterogeneities in the medium that have contributed to the formation of preferential flows. However, in agreement with comment 16 (see below), there could be other explanations for this phenomenon that are not necessarily related to the presence of plants. This will be addressed in the revised version.

Comment 14: *P6, LL39-40: Consider rewriting sentence. Br- had almost complete recovery and was found in plants and roots, so you may delete "possibly", and refer to Fig. 8 and not only the lack of measured Br- in pore water.*

Response 14: Thank you for this suggestion. We agree with your assessment. As such, we will rewrite the sentence as proposed.

Comment 15: *P7, L4: "Early Breakthrough" – compare with expected flow velocity (see comment above)*

Response 15: We appreciate this remark. Yet, as stated in comment 11-(1), a comparison between the estimated mean flow velocity and the expected flow velocities for each curve is not possible without applying modeling approaches.

Comment 16: *P7 L12: "absence of BTC in middle layer" and "early BTC in uppermost layer" "confirmed the influence of plants" is too strong as a statement. Other explanations are possible for these observations – preferential flow without the influence of plants (fingering), bias in the observations, etc.*

Response 16: We agree, and the statement will be corrected so that other possible explanations to the observations will be discussed.

Comment 17: *P7 L 14 "evidenced" – too strong as well. It might be a hint but could also be that the degradation is just a function of time, and transport over that time ended in the vegetated part, opposite from the inlet.*

Response 17: We thank the reviewer for pointing this out. This sentence will be corrected too.

Comment 18: *P7 L23-27/Table 4: How significant are the differences in recovery of Br- given in Table 4, which is the basis for your argumentation here? The differences do not appear large enough to justify the conclusion.*

Response 18: We are grateful for this comment. The percentage of total Br recovered from the different depths was used to support the statement about the possible affectation of the system's performance due to changes in the density of the roots and/or spatial distribution. However, we agree that the differences in recovery between the first and second run are not big enough to justify such conclusion. Therefore, we will refrain from this conclusion in the revised version.

Comment 19: *P8, L 36: Please explain "low leaching potential" as a property of a substance – does that mean high sorption?*

Response 19: We appreciate this comment. "low leaching potential" means that the substance is less likely to move through the soil, but not only because of sorption, as this index is based on the chemical's adsorption (Koc) and persistence (DT50) in the soil. We will clarify this in the revised version.

Comment 20: *P9, L 38: "could be identified" – an unambiguous identification was unfortunately not possible in the experiment, but valuable hints / indications were collected*

Response 20: We thank the reviewer for this suggestion. The sentence in the revised version will be changed as proposed.

Comment 21: *P10, L 5: "biochemical transformation had a major contribution" – only <10 % of the parent substance were found as TP, so it is not possible to say which was a major contribution*

Response 21: While it is true that only <10 % of metazachlor was found as TPs; the recoveries of this pesticide were the lowest among the solutes. This result, together with the physicochemical properties of metazachlor could be a hint that transformation/mineralisation might have played an important role in its dissipation. Nevertheless, it is true that we do not have enough information to justify such statement. Therefore, we will be more careful with this statement in the revised version.

Comment 22: *Fig 4: How do you explain the obvious differences in Br- breakthrough between the first and second run?*

Response 22: We thank the referee for pointing this out. This difference has been attributed to possible changes in the density of the roots and/or spatial distribution over the experiment. This statement is based on the greatest development of the roots observed in the system at the end of the study (see Fig. 5 below). We will include this discussion in our revised version.

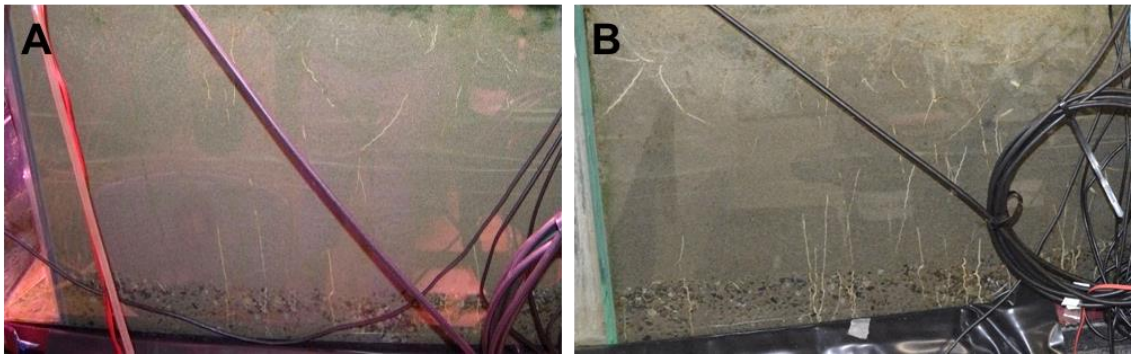

Fig. 5. Front view of the root system in the vegetated part of the model constructed wetland before the first run A) and at the end of the second run B).

Comment 23: *Fig 7: Recovery of TP in % - how can the total amount be known?*

Response 23: We apologize for the lack of clarity in this case. The recovery of TPs has been calculated according to the total amount of parent compound injected. This will be defined in the revised paper.

Comment 24: *Fig. 8: Please comment on the large error bar for SRB:*
(1) Which indicates recovery to be between 48 and 105 %.
(2) Would more sediment samples have reduced this uncertainty?
(3) How does this uncertainty influence your interpretation?

Response 24-(1): We appreciate this comment. The recovery measured in the sediment of the vegetated part has a large error bar due to the heterogeneous distribution of the tracer. That is, almost 99% of the tracer measured in the vegetated part is located in the uppermost layer. This heterogeneous distribution indicates that the tracer was transported preferentially to this layer, as discussed in the manuscript.
Response 24-(2): We thank the referee for raising this important question. In fact, we collected a great number of sediment samples to reduce the uncertainty**:** A total of 16

sediment cores (four per longitudinal and four per lateral transect) that were divided into four fractions, each representing a different sampling depth (0-8 cm, 9-20 cm, 21-32 cm, 33-42 cm). This gives a total number of 64 sediment samples. We think that this number is adequate for the system.

Response 24-(3): In our case, we believe that it does not constitute a major factor of uncertainty. As indicated in the previous response, we have a measurement for each longitudinal/lateral transect and sampling depth, covering practically the whole sediment. This gives us a detailed picture of the distribution of the tracers in the system.

Technical comments

Comment 25: *P2, L32: 100 mg L -> 100 mg L^{-1}*

Response 25: The indicated change will be made in the revised version.

Comment 26: *P2, L35: Please give the dimensions of the constructed wetland system also without inlet/outlet.*

Response 26: The dimensions will be provided in the revised version.

Comment 27: *P5, L11: resulted curves -> resulting curves*

Response 27: The indicated change will be made in the revised version.

Comment 28: *P5, L13, and elsewhere throughout the text: Br -> Br^-*

Response 28: The indicated change will be made in the revised version.

Comment 29: *P7, L26: "was most likely" -> "were most likely"*

Response 29: The indicated change will be made in the revised version.

Comment 30: *P8, L28: "were classified": classified for what (recovery rate, I presume?)*

Response 30: Yes, that is right, the classification is for the recovery rate. We apologize for the confusion. The sentence will be improved.

Comment 31: *Fig 4: Consider duplicating the figure and display vegetated and non-vegetated parts separately, which would make distinguishing these parts a lot easier*

Response 31: Thanks for the suggestion. We agree with your assessment, and as already indicated in the response to the comment 35 from reviewer 1, we will duplicate the figure to show separately the vegetated and non-vegetated parts.

References

Comment 32: Käss, W. (1998): Tracing technique in geohydrology, 581 pp., Balkema, Rotterdam, The Netherlands.

Response 32: The indicated change will be made in the revised version.

Literature

European Food Safety Authority (EFSA).: Conclusion regarding the peer review of the pesticide risk assessment of the active substance metazachlor, EFSA Journal, 6(7), 145r, 2008.

European Food Safety Authority (EFSA).: Conclusion regarding the peer review of the pesticide risk assessment of the active substance penconazole, EFSA Journal, 6(10), 175r, 2008.