

Peer Review File

Deep sea cold seeps are a sink for mercury and source for methylmercury



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Web links to the author's journal account have been redacted from the decision letters as indicated to maintain confidentiality

30th Nov 23

Dear Professor Peng,

Your manuscript titled "Deep sea cold seeps are a sink for mercury and source for methylmercury" has now been seen by 3 reviewers, whose comments are appended below. You will see that they find your work of some potential interest. However, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the manuscript for publication, but would be interested in considering a revised version that fully addresses these serious concerns.

We hope you will find the reviewers' comments useful as you decide how to proceed.

Should you decide to address these criticisms, please ensure that the following editorial thresholds are met:

- Present novel and firmly supported insights into Hg cycling processes in seafloor cold seeps based on your observations of Hg concentrations, isotopes in sediment, and bioaccumulative methylmercury and genetic markers measurements.
- Provide a comparison of the results for Hg cycling to the reference site and an in-depth discussion and calculation on the importance of the results for global mercury cycling and demonstrate that your claims are robustly supported.

If you choose to take up this option, please either highlight all changes in the manuscript text file or provide a list of the changes to the manuscript with your responses to the reviewers.

Please bear in mind that we will be reluctant to approach the reviewers again in the absence of substantial revisions.

If the revision process takes significantly longer than three months, we will be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

We are committed to providing a fair and constructive peer-review process. Please do not hesitate to contact us if you wish to discuss the revision in more detail.

Please use the following link to submit your revised manuscript, a point-by-point response to the reviewers' comments with a list of your changes to the manuscript text (which should be in a separate document to any cover letter), a tracked-changes version of the manuscript (as a PDF file) and any completed checklist:

[link redacted]

** This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage first **

Please do not hesitate to contact us if you have any questions or would like to discuss the required

revisions further. Thank you for the opportunity to review your work.

Best regards,

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EDITORIAL POLICIES AND FORMAT

If you decide to resubmit your paper, please ensure that your manuscript complies with our editorial policies and complete and upload the checklist below as a Related Manuscript file type with the revised article:

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For your information, you can find some guidance regarding format requirements summarized on the following checklist:(<https://www.nature.com/documents/commsj-phys-style-formatting-checklist-article.pdf>) and formatting guide (<https://www.nature.com/documents/commsj-phys-style-formatting-guide-accept.pdf>).

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

Summary:

Observations of Hg concentrations and isotopes in sediment are combined with measurements of bioaccumulative methylmercury (MMHg) and genetic markers of Hg transformations to interpret Hg cycling processes in seafloor cold seeps. The observations suggest previously unreported microbial processing of Hg in the subsurface of seabed cold seeps and shed light on the possibility that these processes may be important for understanding the global movement of Hg in the deep ocean environment. Genetic markers, Hg isotopes, Hg concentrations, and Hg bioaccumulation along with bulk sulfur and carbon geochemical patterns all point to important Hg cycling processes in the subsurface of the active seep that cause Hg and MeHg to become elevated in the surface sediment. While the details of the subsurface geomicrobial process remain unresolved, microbes carrying genetic markers of Hg methylation and demethylation processes belonging to known and novel clades of heterotrophic and autotrophic microbes were identified. The evidence for these bacterial Hg processes with isotopically enriched Hg in only the active seep area provide a plausible basis for the conceptual model put forward for Hg cycling in the deep sea cold seeps.

General comments:

The study was undertaken using established and recently developed methods to study mercury processes. Metagenomic analysis of genes relevant for Hg processing are the best tools available and Hg isotopes are becoming a more common method for inferring the effects of biogeochemical processes on Hg cycling. The objective of understanding whether seafloor cold seeps are hotspots for Hg processes is worthwhile and this objective was accomplished commendably. The study objective (and title) also includes the idea of characterizing the global importance of Hg processing at seafloor cold seeps; however, the current presentation does not put the total mass or turnover rate of Hg at seafloor cold seeps in an adequate global context.

The paper is very well organized and written and the figures are of excellent quality. There are a few small writing issues, but they do not distract considerably from the paper's meaning. I found the evidence and description of the isotope slopes very compelling. In addition to placing the masses of Hg and MeHg quantified at active seeps in a better global context, my other suggestion for improving the paper is to consider how a more spatially-resolved consideration of the depth-dependence of Hg and MeHg may allow for a fuller interpretation.

I believe the manuscript and its ideas will be of interest to Communications Earth & Environment's audience. I provide two general comments below with a few line-by-line comments in the following:

General comments:

The importance of depth-dependent patterns is discussed in detail in the sections of the paper about isotopes and microbes. However, the presentation of total Hg and methyl Hg concentrations in sediment refer to the supplemental figures and tables which average values over ~20 cm, I presume. It appears that many sediment samples from the active seep site (especially those below 12 cm) contained similar Hg and MMHg to the control and inactive seep site. The larger average concentrations at the active seep site (especially for MMHg) may be driven by a few large values. Aggregating Hg concentrations by depth currently limits the extent to which nuanced interpretations about mercury cycling can be made.

My second general comment is about the final paragraph which speculates about the importance of Hg found at seafloor cold seeps to the global Hg budget. It would be helpful to put these numbers in the context of some global or ocean mass balance.

Specific comments:

Line 72 to 74 – The values reported are, I assume, averages and standard deviations for all samples from 0 to ~22 cm in most cores. Does it matter that the number of samples in each core were different? Would it be helpful to report the average Hg concentration and related quantities as a 0 to 12 cm average and a separate 12 to 22 cm average? The plot in Supplementary Figure 2 shows a lot of variability in Hg and MeHg in one B core. Reporting the average Hg in shallow sediment separate from the average Hg in deep sediment (which seemed similar among sites) may help to accentuate the observed differences and introduce to the reader that processes below ~15 cm in the active seep sites are or could be more standard.

- Line 72, 97, 102 – Hg/TOC/MMHg concentrations are, ON AVERAGE, higher than... I think that clarifying that the authors understand that the conclusions we are drawing here are dependent on the average values of a variable that is quite

- Line 76 – Text should specify what the authors mean when they say “core-top layer.”

- Line 79 to 80 – over what depth were Hg concentration averaged in the comparison sites (Mariana

trench and Atacama trench)?

- Line 80 to 90 – since Hg in tissue is primarily MeHg, it might make sense to discuss tissue in a section after introducing MeHg in sediment.
- Line 98 to 99 – Another possibility is that a certain type of DOC was broken down in the active area, a type of DOC that Hg does not bind to as strongly as others.
- Line 106 – help with clarity: “...active seep area, the MMHg maxima also coincided with the depth of...”
- Line 110 – by “prefer,” I believe the authors mean “create conditions conducive to
- Line 114 – what is meant by “normal” values.
- Line 119 to 120 – I was unable to grasp the importance of this sentence.
- Line 123 – missing reference (REF)
- Line 136 to 137 – suggest replacing the phrase “generally keep stable” with “are variable but have no depth trend”
- Line 138 – I wonder if this figure reference is to Figure 3
- Line 159 to Line 167 – I find this line of reasoning very compelling
- Line 178 to 179 – are the authors referring to the sediment-water interface and/or deeper depths?
- Line 191 – “As time goes on,...” I wonder if there is some transport phenomenon also wrapped up in the depth patterns? A seep of this sort may spread the impact of the subsurface “dark processes” to other, shallower depths.
- Line 268 to 273 – These numbers are not compelling in support of the assertion that seeps are important sinks and sources of Hg and MeHg unless they are contextualized with some other mercury masses. For instance, how much Hg is in the ocean surface waters, or how many MG of Hg escape to the atmosphere?

Reviewer #2 (Remarks to the Author):

The deep ocean is the most understudied reservoir in regard to the global Hg cycle, and this manuscript provides novel data on Hg storage in cold seep sediments. The inclusion of Hg isotope ratios and Hg-cycling gene abundance enhances the values of this work. My only concern is the small sample size. The authors present Hg data from only one sediment core from each of their sites (active, inactive, reference), two animals (one from the active site, one from the inactive site), and discuss the abundance and presence of Hg-cycling genes in inactive and active cold seep areas, but do not compare these results to a reference site.

Line 24: “Here we show that significant...”

Line 45: I wouldn’t say that inorganic Hg is “readily” transformed. Percent MMHg in marine environments is generally less than 20% and there are many factors that inhibit inorganic Hg methylation.

Line 63: The supplementary data shows that only 5 of these cores were used. Total Hg was measured in 5 different cores, MMHg and Hg isotopes were measured in three cores, and Hg-cycling genes were measured in 3 cores. Genes were extracted from active and inactive sediments, not background sediments.

Line 72: Specify that ‘average’ Hg concentrations are 1.5 x greater...important here as Hg was measured at various intervals within the core.

Line 74: Add statistics here to show that Hg concentrations at active seeps are significantly greater than inactive and reference sites (i.e., provide p-values)

Line 76: Add (0-2 cm) after “The core-top layer”

Line 78: “significantly” is a term generally reserved to communicate the relevance of a statistical test (i.e., a t-test showing average concentrations are significantly higher with a reported p-value)

Line 80: More data is needed here to support conclusions. Only one animal at each site was analyzed and there is no information on the size or age comparison of the two samples. Older and/or larger animals may have higher Hg concentrations because they have eaten more prey and hence bioaccumulated more Hg. More information is needed to hear to conclude that the concentration difference is the result of the two different environments.

Line 88-90: This statement is confusing (concentration differences between G. Haimaensis and 0-2 cm and between 0-2 cm and bottom of core are similar), and I’m not sure how the comparison is relevant.

Line 110: “prefer Hg-methylation” is not the best word choice. Explain that MMHg maxima were found in the region where the activity of known methylators was evident

Line 120: It’s interesting that Hg in bivalves was primarily inorganic – any insight or comment on this finding?

Line 272: How does this value (3,060 Mg Hg) compare to current estimates of Hg stored in deep sea sediments?

Reviewer #3 (Remarks to the Author):

Presented Hg concentrations and isotope ratios in sediments from an active methane seep area are interesting and provide support for the idea of upward transport of Hg⁰. The authors imply that this Hg⁰ is strictly from the reduction of deposited Hg²⁺, but could it be from a deeper source in the seep? The case for cold seeps as a sink for Hg could be stronger. The decreasing concentrations of Hg with depth in the active sediments in Fig. 2 make the case for a net water column source in the upper sediments, but wouldn't upward transport of Hg⁰ be a net source to the ocean?

Depth profiles of the abundances of the two Hg methylation genes show that there was more *hgcA* near the surface, but higher amounts of *hgcB* deeper in the active site sediment cores. What could explain this? Do the mag results provide any clues?

The schematic model in Fig. 5 is not very clear. First, why do the inputs of Hg in the upper left lead first to Hg⁰ and then Hg²⁺? Is this supposed to be occurring in the atmosphere? Second, isn't Hg⁰ also supplied from below along with methane in cold seeps? Third, the proposed geochemistry of Hg in the sediments is not shown. The box on the left should show representative profiles of Hg species in the sediment, and not such a large area of overlying water, which is not relevant. Fourth, while the Hg transformations part on the right is generally fine, “light” and “heavy” are vague (use $\delta^{202}\text{Hg}$ with up and down arrows), and a decrease in $\delta^{202}\text{Hg}$ should be shown next to the arrow from the Hg-demethylators to Hg²⁺, not from MeHg to the microbe. It is not clear from the picture why *C. marrisinica* doesn't accumulate MeHg. The sediment part of the figure should be larger and include active and inactive areas with the respective bivalves located in each.

Check global estimates of Hg and MMHg in cold seeps (266-272). Based on the numbers given, I get 3.7 Mg Hg and 0.014 Mg MMHg in Hama and 3328 Mg Hg and 12.6 Mg MMHg globally.

Other comments

24 Reword for clarity: "Here we show that significant Hg and methylmercury (MMHg) enrichments and occurrence of Hg metabolizing microbes"

25 "MeHg" is used in Fig. 5.

35 Suggest: "We estimate that 3,060 Mg Hg and 14.7 Mg MMHg are stored in cold seeps globally."

36 Suggest: "In summary, we propose that cold seeps function as a previously unrecognized sink for Hg and source for MMHg in the deep ocean."

53 "it is uncertain whether the deep ocean has the capacity"

56 Reword for clarity. Chemosynthetic organisms are the base of the food web, and they support sulfate reduction and methanogenesis, not the other way around.

59 "anaerobic methane oxidizing archaea"

76 Is this the global average deep sediment Hg concentration?

76 Check wording of "The core-top layer of the active seep area is three times higher (164 ng g⁻¹) than the reference area... and significantly higher than those"

85 Suggest "in the South China Sea."

98 "the ability of TOC to sorb Hg in the active seep area is higher than that of background marine sediments"

110 "favor Hg-methylation"

111 Suggest: "In the inactive seep area, however,"

115 "This result is consistent with the observation of many dead clams (*C. marissinica*) in this area"

119 "The concentration of MMHg in bivalves from the Haima cold seep was below the limit of detection"

121 Evidence of what?

123 Missing citation

135-136 Check wording of "while their values in the reference" and "generally keep stable"

142 Clarify "both"

148 "are significantly correlated"

152 What was the average d₂₀₀Hg in the active site?

163 "nuclear volume effects" of "nuclear field effects"

172 Only two? Likely many possible scenarios may explain this observation.

246 "The thriving seep ecosystem, which supports animal (e.g., *G. haimaensis*) supplies a large amount.."

248 Cut "absorptions"

249 Reword "would be continuously being accumulated here."

250 Clarify "it"; "part of the inorganic Hg(II) is converted into MMHg"

252 "might also occur"

255 "peak under certain conditions"

258 "abiotic dark redox transformation of Hg"

259 Reword for accuracy. The oxidation of Hg⁰ as it moves up will enrich remaining Hg⁰ in 202 and result in higher d₂₀₂ values of produced Hg(II) higher in the sediment column.

271 "If we assume.."

269 Clarify that "extra" Hg and MMHg refers to the difference between active and reference sediments.

275 Reword "along with the exploration scale increases."

623 "microbially-mediated"

In the caption to Fig 4a, clarify which sites the three cores are from or refer readers to the explanations of B1, etc. in Fig. 1.

Response to referees

Reviewer #1

Summary:

Observations of Hg concentrations and isotopes in sediment are combined with measurements of bioaccumulative methylmercury (MMHg) and genetic markers of Hg transformations to interpret Hg cycling processes in seafloor cold seeps. The observations suggest previously unreported microbial processing of Hg in the subsurface of seabed cold seeps and shed light on the possibility that these processes may be important for understanding the global movement of Hg in the deep ocean environment. Genetic markers, Hg isotopes, Hg concentrations, and Hg bioaccumulation along with bulk sulfur and carbon geochemical patterns all point to important Hg cycling processes in the subsurface of the active seep that cause Hg and MeHg to become elevated in the surface sediment. While the details of the subsurface geomicrobial process remain unresolved, microbes carrying genetic markers of Hg methylation and demethylation processes belonging to known and novel clades of heterotrophic and autotrophic microbes were identified. The evidence for these bacterial Hg processes with isotopically enriched Hg in only the active seep area provide a plausible basis for the conceptual model put forward for Hg cycling in the deep sea cold seeps.

[Answer: Thank you for your positive feedback on our manuscript.](#)

General comments:

The study was undertaken using established and recently developed methods to study mercury processes. Metagenomic analysis of genes relevant for Hg processing are the best tools available and Hg isotopes are becoming a more common method for inferring the effects of biogeochemical processes on Hg cycling. The objective of understanding whether seafloor cold seeps are hotspots for Hg processes is worthwhile and this objective was accomplished commendably. The study objective (and title) also includes the idea of characterizing the global importance of Hg processing at seafloor cold seeps; however, the current presentation does not put the total mass or turnover rate of Hg at seafloor cold seeps in an adequate global context.

[Answer: Thank you for pointing out the need to contextualize the total mass and turnover rate of mercury \(Hg\) at seafloor cold seeps within a global framework. In response to your feedback, we](#)

have revised the manuscript to include the following content, which aims to address this aspect more comprehensively:

"To appropriately contextualize our findings on a global scale, we have extrapolated the data from the Haima seep area to estimate the potential Hg and methylmercury (MMHg) storage across global seep systems. If we assume that other cold seeps have a comparable scale of active area with Haima, then we estimate that an additional 2,835 Mg of Hg and 9 Mg of MMHg are stored in cold seeps globally. According to Hayes et al. (2021), the global deep-sea burial fluxes of Hg are approximately 220 Mg Hg per year, and Zhang et al. (2020) report a global burial flux of 9 Mg MMHg per year. Based on these figures, the total amount of additional Hg and MMHg stored in global cold seep surface sediments appears to correspond to about 13 years' and 1 year's worth of the global deep-sea annual burial flux for Hg and MMHg, respectively."

Zhang, Y.; Soerensen, A. L.; Scharup, A. T.; Sunderland, E. M.. A global model for methylmercury formation and uptake at the base of marine food webs. *Global Biogeochemical Cycles*, **2020**, 34, e2019GB006348.

Hayes, C. T.; Costa, K. M.; Anderson, R. F.; Calvo, E.; Chase, Z.; Demina, L. L.; et al.. Global ocean sediment composition and burial flux in the deep sea. *Global Biogeochemical Cycles*, **2021**, 35, e2020GB006769.

The paper is very well organized and written and the figures are of excellent quality. There are a few small writing issues, but they do not distract considerably from the paper's meaning. I found the evidence and description of the isotope slopes very compelling. In addition to placing the masses of Hg and MeHg quantified at active seeps in a better global context, my other suggestion for improving the paper is to consider how a more spatially-resolved consideration of the depth-dependence of Hg and MeHg may allow for a fuller interpretation.

Answer: Based on your comments, in the revised manuscript, we have described the masses of Hg and MeHg in these sediment cores as a 0 to 12 cm average and a separate 12 to 22 cm average.

"In this study, Hg is evidently enriched in the upper sediment column from the active area. The average concentrations of Hg in sediments above 12 cm from the active seep area ($97.22 \pm 37.17 \text{ ng g}^{-1}$, $n=10$) are 2.5 and 2.3 times higher than those of inactive seep area ($42.83 \pm 4.18 \text{ ng g}^{-1}$, $n=6$) and the reference site ($39.53 \pm 6.68 \text{ ng g}^{-1}$, $n=14$) (Supplementary Figure 2 and Supplementary Table 1), respectively. In contrast, sediment samples below 12 cm from the active seep area have similar Hg level to the reference and inactive seep site."

With respect to the importance of Hg found at seafloor cold seeps to the global Hg budget, we investigated the buried fluxes of Hg and MMHg in global deep-sea sediments, and calculated the global share of cold seep sediments in the oceanic Hg cycle as follow:

“To appropriately contextualize our findings on a global scale, we have extrapolated the data from the Haima seep area to estimate the potential Hg and methylmercury (MMHg) storage across global seep systems. If we assume that other cold seeps have a comparable scale of active area with Haima, then we estimate that an additional 2,835 Mg of Hg and 9 Mg of MMHg are stored in cold seeps globally. According to Hayes et al. (2021), the global deep-sea burial fluxes of Hg are approximately 220 Mg Hg per year, and Zhang et al. (2020) report a global burial flux of 9 Mg MMHg per year. Based on these figures, the total amount of additional Hg and MMHg stored in global cold seep surface sediments appears to correspond to about 13 years’ and 1 year’s worth of the global deep-sea annual burial flux for Hg and MMHg, respectively.”

General comments:

The importance of depth-dependent patterns is discussed in detail in the sections of the paper about isotopes and microbes. However, the presentation of total Hg and methyl Hg concentrations in sediment refer to the supplemental figures and tables which average values over ~20 cm, I presume. It appears that many sediment samples from the active seep site (especially those below 12 cm) contained similar Hg and MMHg to the control and inactive seep site. The larger average concentrations at the active seep site (especially for MMHg) may be driven by a few large values. Aggregating Hg concentrations by depth currently limits the extent to which nuanced interpretations about mercury cycling can be made.

Answer: Thanks very much. As you said, many sediment samples from the active seep site (especially those below 12 cm) contained similar Hg and MMHg to the control and inactive seep site.

In the revised manuscript, we have rewritten this part as follow:

“In this study, Hg is evidently enriched in the upper sediment column from the active area. The average concentrations of Hg in sediments above 12 cm from the active seep area ($97.22 \pm 37.17 \text{ ng g}^{-1}$, $n=10$) are 2.5 and 2.3 times higher than those of inactive seep area ($42.83 \pm 4.18 \text{ ng g}^{-1}$, $n=6$) and the reference site ($39.53 \pm 6.68 \text{ ng g}^{-1}$, $n=14$) (Supplementary Figure 2 and Supplementary Table 1), respectively. In contrast, sediment samples below 12 cm from the active seep area have similar Hg level to the reference and inactive seep site.”

My second general comment is about the final paragraph which speculates about the importance of Hg found at seafloor cold seeps to the global Hg budget. It would be helpful to put these numbers in the context of some global or ocean mass balance.

Answer: Thanks very much for this suggestion.

We queried the buried fluxes of Hg and MMHg in global deep-sea sediments, and calculated the global share of cold seep sediments in the oceanic Hg cycle as follow:

“To appropriately contextualize our findings on a global scale, we have extrapolated the data from the Haima seep area to estimate the potential Hg and methylmercury (MMHg) storage across global seep systems. If we assume that other cold seeps have a comparable scale of active area with Haima, then we estimate that an additional 2,835 Mg of Hg and 9 Mg of MMHg are stored in cold seeps globally. According to Hayes et al. (2021), the global deep-sea burial fluxes of Hg are approximately 220 Mg Hg per year, and Zhang et al. (2020) report a global burial flux of 9 Mg MMHg per year. Based on these figures, the total amount of additional Hg and MMHg stored in global cold seep surface sediments appears to correspond to about 13 years’ and 1 year’s worth of the global deep-sea annual burial flux for Hg and MMHg, respectively.”

Specific comments:

Line 72 to 74 – The values reported are, I assume, averages and standard deviations for all samples from 0 to ~22 cm in most cores. Does it matter that the number of samples in each core were different? Would it be helpful to report the average Hg concentration and related quantities as a 0 to 12 cm average and a separate 12 to 22 cm average? The plot in Supplementary Figure 2 shows a lot of variability in Hg and MeHg in one B core. Reporting the average Hg in shallow sediment separate from the average Hg in deep sediment (which seemed similar among sites) may help to accentuate the observed differences and introduce to the reader that processes below ~15 cm in the active seep sites are or could be more standard.

Answer: In the revised manuscript, we have rewritten this part as follow:

“In this study, Hg is evidently enriched in the upper sediment column (0-12 cm) from the active area. The average concentrations of Hg in sediments above 12 cm from the active seep area ($97.22 \pm 37.17 \text{ ng g}^{-1}$, $n=10$) are 2.3 and 2.4 times higher than those of inactive seep area ($42.83 \pm 4.18 \text{ ng g}^{-1}$, $n=6$) and the reference site ($39.53 \pm 6.68 \text{ ng g}^{-1}$, $n=14$) (Supplementary Figure 2 and Supplementary Table 1), respectively. In contrast, sediment samples below 12 cm from the active seep area have similar Hg level

[to the reference and inactive seep site.”](#)

- Line 72, 97, 102 – Hg/TOC/MMHg concentrations are, ON AVERAGE, higher than... I think that clarifying that the authors understand that the conclusions we are drawing here are dependent on the average values of a variable that is quite

[Answer: We have revised it according to your comment.](#)

- Line 76 – Text should specify what the authors mean when they say “core-top layer.”

[Answer: The “core-top layer” means 0-2cm. We have added this information to our revised manuscript.](#)

- Line 79 to 80 – over what depth were Hg concentration averaged in the comparison sites (Mariana trench and Atacama trench)?

[Answer: These data are from Sanei et al \(2021\). There are two cores from Mariana trench, and their depths are 14 and 9.5 cm, respectively. While the concentration of Hg 400 ng g⁻¹ presenting in the 0-2 cm of sediment core from Atacama trench.](#)

[Sanei, H.; Outridge, P.M.; Oguri, K. et al. High mercury accumulation in deep-ocean hadal sediments. *Sci Rep* **2021**,11, 10970.](#)

- Line 80 to 90 – since Hg in tissue is primarily MeHg, it might make sense to discuss tissue in a section after introducing MeHg in sediment.

[Answer: In this study, we found that the concentration of MMHg in the tissue of bivalves from the Haima cold seep were below the limit of detection \(<0.002 ng g⁻¹\) after several detections. So we didn’t discuss MMHG in tissue after introducing MMHg in sediment. And the phenomenon that low or below detection limits of MMHG in organisms from hydrothermal or cold seep environments also appeared in Yuan et al. \(2023\).](#)

[Yuan, J.; Liu, Y.; Chen, S.; Peng, X.; Li, Y.-F.; Li, S.; Zhang, R.; Zheng, W.; Chen, J.; Sun, R.; Heimbürger-Boavida, L.-E., Mercury Isotopes in Deep-Sea Epibenthic Biota Suggest Limited Hg Transfer from Photosynthetic to Chemosynthetic Food Webs. *Environmental Science & Technology* **2023**, 57, \(16\), 6550-6562.](#)

- Line 98 to 99 – Another possibility is that a certain type of DOC was broken down in the active area, a type of DOC that Hg does not bind to as strongly as others.

[Answer: Thanks for this comment. This is possible.](#)

[We have added this explanation in the revised manuscript. This sentence has been changed into “This suggests that the ability of TOC to sorb Hg in the active seep area is higher than that of background marine sediments, or TOCs that do not bind strongly to mercury are preferentially degraded in the active”](#)

[area](#)”

- Line 106 – help with clarity: “...active seep area, the MMHg maxima also coincided with the depth of...”

[Answer: Thanks very much. We have revised it according to your comments.](#)

- Line 110 – by “prefer,” I believe the authors mean “create conditions conducive to”

[Answer: We have revised it according to your comments.](#)

- Line 114 – what is meant by “normal” values.

[Answer: We have replaced “normal” into “relative high”.](#)

[We originally wanted to express that the values here are normal values comparable to those of the background area, relative to the extremely depleted values in the active area.](#)

- Line 119 to 120 – I was unable to grasp the importance of this sentence.

[Answer: We did not detect MMHG in bivalve tissues. This sentence has been moved to earlier in the content of this paragraph.](#)

- Line 123 – missing reference (REF)

[Answer: We have added the related references.](#)

- Line 136 to 137 – suggest replacing the phrase “generally keep stable” with “are variable but have no depth trend”

[Answer: Thanks very much. We have revised it according to your comments.](#)

- Line 138 – I wonder if this figure reference is to Figure 3

[Answer: Both Figure 2 and 3 can see this result. We have added Figure 3 in the revised manuscript.](#)

- Line 159 to Line 167 – I find this line of reasoning very compelling

[Answer: Thanks for your positive comments.](#)

- Line 178 to 179 – are the authors referring to the sediment-water interface and/or deeper depths?

[Answer: It depends on the depth of the redox layer in the sedimentary column. Usually slightly below the sediment-water interface. This sentence has been changed into:](#)

[“Secondly, a significant amount of Hg⁰ rising from the deep would be oxidized to Hg\(II\) through dark reactions in absence of light slightly below the sediment-water interface.”](#)

- Line 191 – “As time goes on,” I wonder if there is some transport phenomenon also wrapped up in the depth patterns? A seep of this sort may spread the impact of the subsurface “dark processes” to other,

shallower depths.

Answer: Thanks for this insightful comment. We would like to clarify here. Some other secondary processes, such as microbial reduction, adsorption and desorption, also might wrap up in the depth pattern. However, above processes cannot induce the negative transition for $\Delta^{199}\text{Hg}$ in deep sediments. The organic matter-driven dark reduction is speculated to be the most reasonable mechanism. The dark reduction was occurred at the shallower depth and deeper depth consistently, but the more negative shift was observed at the deeper depth with elevated dark reduction happened.

- Line 268 to 273 – These numbers are not compelling in support of the assertion that seeps are important sinks and sources of Hg and MeHg unless they are contextualized with some other mercury masses. For instance, how much Hg is in the ocean surface waters, or how many MG of Hg escape to the atmosphere?

Answer: Thanks for this suggestion. In the revised manuscript, we have investigated the buried fluxes of Hg and MMHg in global deep-sea sediments, and calculated the global share of cold seep sediments in the oceanic Hg cycle as follow:

“To appropriately contextualize our findings on a global scale, we have extrapolated the data from the Haima seep area to estimate the potential Hg and methylmercury (MMHg) storage across global seep systems. If we assume that other cold seeps have a comparable scale of active area with Haima, then we estimate that an additional 2,835 Mg of Hg and 9 Mg of MMHg are stored in cold seeps globally. According to Hayes et al. (2021), the global deep-sea burial fluxes of Hg are approximately 220 Mg Hg per year, and Zhang et al. (2020) report a global burial flux of 9 Mg MMHg per year. Based on these figures, the total amount of additional Hg and MMHg stored in global cold seep surface sediments appears to correspond to about 13 years’ and 1 year’s worth of the global deep-sea annual burial flux for Hg and MMHg, respectively.”

❑ Reviewer #2

The deep ocean is the most understudied reservoir in regard to the global Hg cycle, and this manuscript provides novel data on Hg storage in cold seep sediments. The inclusion of Hg isotope ratios and Hg-cycling gene abundance enhances the values of this work. My only concern is the small sample size. The authors present Hg data from only one sediment core from each of their sites (active, inactive, reference), two animals (one from the active site, one from the inactive site), and discuss the abundance and presence

of Hg-cycling genes in inactive and active cold seep areas, but do not compare these results to a reference site.

Answer: Thanks for these useful comments. We agree with you that the overall sample size is small.

We would like to point out that a total of six cores were used in this study, including three cores from the reference area (A2, A3 and A4), two cores from the active area (B1 and B2) and one core from the inactive zone (C1). It is just that, considering the conditions of limited funds, Hg isotopes were examined for one core in the reference area, two cores in the active area and one core in the inactive area. For the metagenomic data, two cores in the active area and one core in the inactive area were examined. However, the similarity of data characteristics exhibited by the same environment, and the differences exhibited by sample data in different environments are pronounced, so we still consider conclusion is reasonable.

Nevertheless, studies with more samples from more locations and covering larger spatial gradients in the future will be necessary to determine if the trends presented here are universal for Hg cycles in global deep sea cold seeps.

Line 24: “Here we show that significant...”

Answer: Thanks. We have revised it according to your comment.

Line 45: I wouldn't say that inorganic Hg is “readily” transformed. Percent MMHg in marine environments is generally less than 20% and there are many factors that inhibit inorganic Hg methylation.

Answer: We have rewritten this sentence into “In aquatic ecosystems, inorganic Hg can be transformed into methylmercury (MMHg)”.

Line 63: The supplementary data shows that only 5 of these cores were used. Total Hg was measured in 5 different cores, MMHg and Hg isotopes were measured in three cores, and Hg-cycling genes were measured in 3 cores. Genes were extracted from active and inactive sediments, not background sediments.

Answer: Thanks for pointing out this. We have corrected it.

Line 72: Specify that ‘average’ Hg concentrations are 1.5 x greater...important here as Hg was measured at various intervals within the core.

Answer: We have revised it according to your comments.

Line 74: Add statistics here to show that Hg concentrations at active seeps are significantly greater than inactive and reference sites (i.e., provide p-values)

Answer: Following the advice of another reviewer, we have placed the comparison primarily in the upper part (above 12cm) of sediment cores. This sentence has been rewritten as follows:

“In this study, Hg is evidently enriched in the upper sediment column from the active area. The average concentrations of Hg in sediments above 12 cm from the active seep area ($97.22 \pm 37.17 \text{ ng g}^{-1}$, $n=10$) are 2.3 and 2.4 times higher than those of inactive seep area ($42.83 \pm 4.18 \text{ ng g}^{-1}$, $n=6$) and the reference site ($40.35 \pm 6.49 \text{ ng g}^{-1}$, $n=14$) (Supplementary Figure 2 and Supplementary Table 1), respectively.”

The result of T test showed the Hg concentrations at active area are significantly greater than those in the inactive area ($p < 0.01$) and reference site ($p < 0.001$).

Line 76: Add (0-2 cm) after “The core-top layer”

Answer: We have revised it according to your comments.

Line 78: “significantly” is a term generally reserved to communicate the relevance of a statistical test (i.e., a t-test showing average concentrations are significantly higher with a reported p-value)

Answer: We have replaced “significantly” with “much” in the revised manuscript.

Line 80: More data is needed here to support conclusions. Only one animal at each site was analyzed and there is no information on the size or age comparison of the two samples. Older and/or larger animals may have higher Hg concentrations because they have eaten more prey and hence bioaccumulated more Hg. More information is needed to hear to conclude that the concentration difference is the result of the two different environments.

Answer: Thanks for this reminder. We agree with you that more data is needed.

To fill this gap, we cite the example of Site F for additional proof of this phenomenon. “Yuan et al. reported that the bathymodiolin mussel *Bathymodiolus aduloides* (592 to 920 ng g^{-1}) living in the center of the cold seep had a much higher Hg concentration than those of *Gigantidas platifrons* (46 to 173 ng g^{-1}) living more distant”.

Furthermore, we have added the size comparison of the two samples in the revise manuscript. As show in the supplementary Figure 1, a marker pen ($\sim 13.5 \text{ cm}$ long) is used as a scale, and we can see that the size of *G. haimaensis* ($\sim 8 \text{ cm}$) living in the active seep area are much smaller than those of *C. marissinica* ($\sim 18 \text{ cm}$) living in the inactive seep area. This sentence “*G. haimaensis* collected from the active area was approximately 8 cm long, while *C. marissinica* collected from the inactive area was approximately 13 cm long” has been added in the Sampling section of this revised manuscript.

Line 88-90: This statement is confusing (concentration differences between *G. Haimaensis* and 0-2 cm and between 0-2 cm and bottom of core are similar), and I'm not sure how the comparison is relevant.

Answer: This sentence has been removed from the revised manuscript.

Line 110: “prefer Hg-methylation” is not the best word choice. Explain that MMHg maxima were found in the region where the activity of known methylators was evident.

Answer: We have replaced the word “prefer” with “create conditions conducive to” according to the other reviewer’s suggestion.

The whole sentence has been rewritten into “This may be because the dynamic microbial sulfate reduction and anaerobic methanotrophic activities in the SMTZ create conditions conducive to the activity of known Hg methylators”.

Line 120: It’s interesting that Hg in bivalves was primarily inorganic – any insight or comment on this finding?-

Answer:

For these two animal samples, we conducted several examinations and finally confirmed that the MMHG in their tissue samples was indeed below the detection limit. And the phenomenon that MMHG is low or below detection limits in organisms from hydrothermal or cold seep environments also reported in Yuan et al. (2023). We speculate that this may be related to shorter food chains resulting in a less pronounced biomagnification effect in hydrothermal fields and cold seeps environments.

Yuan, J.; Liu, Y.; Chen, S.; Peng, X.; Li, Y.-F.; Li, S.; Zhang, R.; Zheng, W.; Chen, J.; Sun, R.; Heimbürger-Boavida, L.-E., Mercury Isotopes in Deep-Sea Epibenthic Biota Suggest Limited Hg Transfer from Photosynthetic to Chemosynthetic Food Webs. *Environmental Science & Technology* **2023**, 57, (16), 6550-6562.

Line 272: How does this value (3,060 Mg Hg) compare to current estimates of Hg stored in deep sea sediments?

Answer: In response to another reviewer's comments, we have recalculated the total Hg content of the upper part of sediment column (above 12 cm), then this value has been updated to 2,835 Mg Hg. And we have added some content to show the importance of the storage of Hg in cold seeps as follow:

“To appropriately contextualize our findings on a global scale, we have extrapolated the data from the Haima seep area to estimate the potential Hg and methylmercury (MMHg) storage across global seep systems. If we assume that other cold seeps have a comparable scale of active area with Haima, then we estimate that an additional 2,835 Mg of Hg and 9 Mg of MMHg are stored in cold seeps globally

According to Hayes et al. (2021), the global deep-sea burial fluxes of Hg are approximately 220 Mg Hg per year, and Zhang et al. (2020) report a global burial flux of 9 Mg MMHg per year. Based on these figures, the total amount of additional Hg and MMHg stored in global cold seep surface sediments appears to correspond to about 13 years' and 1 year's worth of the global deep-sea annual burial flux for Hg and MMHg, respectively."

❑ Reviewer #3

Presented Hg concentrations and isotope ratios in sediments from an active methane seep area are interesting and provide support for the idea of upward transport of Hg⁰. The authors imply that this Hg⁰ is strictly from the reduction of deposited Hg²⁺, but could it be from a deeper source in the seep? The case for cold seeps as a sink for Hg could be stronger. The decreasing concentrations of Hg with depth in the active sediments in Fig. 2 make the case for a net water column source in the upper sediments, but wouldn't upward transport of Hg⁰ be a net source to the ocean?

Answer: Thank you for your insightful suggestion.

We acknowledge the possibility that the cold seep fluids could transport mercury from deeper sources within the seep. To better represent this concept in our study, we have included a new illustration in Figure 5 depicting the potential deep Hg sources.

Our observations indicate that sediment samples from deeper layers (below 12 cm) at the active seep site show Hg concentrations is similar to those at the reference and inactive seep sites. However, the upper sediments (above 12 cm) in the active area exhibit significantly higher Hg levels compared to the background zone. This pattern suggests an enhanced accumulation of Hg in the upper layers of the active seep, potentially indicative of upward transport. While the data support the presence of elevated Hg in the upper sediments of the active seep, they do not conclusively point to the upward transport of Hg⁰ as a net source to the ocean. Our study, therefore, stops short of firmly establishing cold seeps as a significant source of Hg to the ocean, based on the current dataset. This aspect of the cold seep Hg model remains an intriguing area for future investigation, and we hope our study will inspire further research in this direction.

Depth profiles of the abundances of the two Hg methylation genes show that there was more hgcA near the surface, but higher amounts of hgcB deeper in the active site sediment cores. What could explain this?

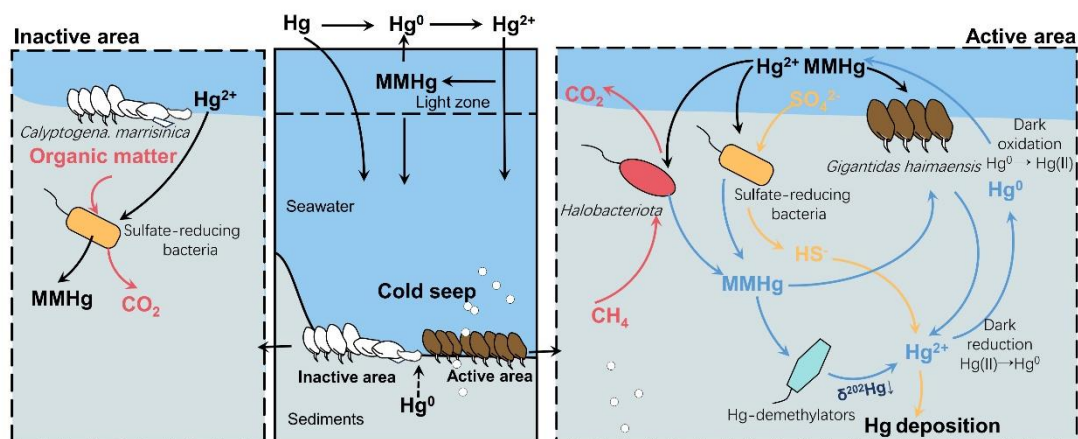
Do the mag results provide any clues?

Answer: The observed depth profile differences in the abundances of *hgcA* and *hgcB* genes in sediment cores from the active site can be explained by the annotation process of *hgcB*. The annotation of *hgcB* requires the principle of co-localization, as relying solely on Hidden Markov Model (HMM) annotation might mistakenly label it as a 4Fe-4S motif, leading to an apparent higher abundance compared to *hgcA*. Therefore, the results for *hgcB* may not be accurate. Following practices in other studies, we have decided to exclude the *hgcB* data from our analysis and focus solely on the data for *hgcA*.

The schematic model in Fig. 5 is not very clear. First, why do the inputs of Hg in the upper left lead first to Hg⁰ and then Hg²⁺? Is this supposed to be occurring in the atmosphere? Second, isn't Hg⁰ also supplied from below along with methane in cold seeps? Third, the proposed geochemistry of Hg in the sediments is not shown. The box on the left should show representative profiles of Hg species in the sediment, and not such a large area of overlying water, which is not relevant. Fourth, while the Hg transformations part on the right is generally fine, "light" and "heavy" are vague (use $\delta^{202}\text{Hg}$ with up and down arrows), and a decrease in $\delta^{202}\text{Hg}$ should be shown next to the arrow from the Hg-demethylators to Hg²⁺, not from MeHg to the microbe. It is not clear from the picture why *C. marrisinica* doesn't accumulate MeHg. The sediment part of the figure should be larger and include active and inactive areas with the respective bivalves located in each.

Answer:

1) The process that the inputs of Hg in the upper left occur in the atmosphere. 2) As mentioned above, we think it is quite possible that cold seep fluids bring Hg from a deeper source. To make the cold seep Hg model more plausibly, we have added an illustration of the deep Hg sources in Fig. 5. 3) The box in the middle is an overall conceptual diagram. To show the details of proposed Hg geochemistry in the sediment columns, we plotted them into the right box (active site) and left box (inactive site), respectively. The blue area means seawater, and gray area means sediments. 4) We have used $\delta^{202}\text{Hg}$ with up and down arrows in the revised Figure 5 according to your comments. 5) We have revised it and contained active and inactive areas with the respective bivalve according to your comments.



Check global estimates of Hg and MMHg in cold seeps (266-272). Based on the numbers given, I get 3.7 Mg Hg and 0.014 Mg MMHg in Hama and 3328 Mg Hg and 12.6 Mg MMHg globally.

Answer: Thanks.

We have recalculated the values of global estimates of Hg and MMHg in cold seeps. According to that the upper 12 cm of sediment cores in the active area is more enriched in Hg and MMHg, then an additional 3.15 Mg Hg and 0.01 Mg MMHg will be preserved in Hama, and an additional of 2,835 Mg Hg and 9 Mg MMHg being stored in the global seep systems.

Other comments

24 Reword for clarity: "Here we show that significant Hg and methylmercury (MMHg) enrichments and occurrence of Hg metabolizing microbes"

Answer: Reworded as: “Here we demonstrate substantial enrichments of mercury (Hg) and methylmercury (MMHg), along with the presence of microbes capable of metabolizing Hg”.

25 "MeHg" is used in Fig. 5.

Answer: It has been corrected in the revised Figure 5.

35 Suggest: "We estimate that 3,060 Mg Hg and 14.7 Mg MMHg are stored in cold seeps globally."

Answer: We have revised it according to your comments.

36 Suggest: "In summary, we propose that cold seeps function as a previously unrecognized sink for Hg
and source for MMHg in the deep ocean."

Answer: We have revised it according to your comments.

53 "it is uncertain whether the deep ocean has the capacity"

Answer: We have revised it according to your comments.

56 Reword for clarity. Chemosynthetic organisms are the base of the food web, and they support sulfate reduction and methanogenesis, not the other way around.

Answer: We have revised it according to your comments.

59 "anaerobic methane oxidizing archaea"

Answer: We have revised it according to your comments.

76 Is this the global average deep sediment Hg concentration?

Answer: Thanks for this reminder. This sentence has been rewritten into as follows:

"Our reference site corresponds well with the deep basin sediment Hg concentrations ($38 \pm 21 \text{ ng g}^{-1}$) in the Atlantic Ocean".

76 Check wording of "The core-top layer of the active seep area is three times higher (164 ng g^{-1}) than the reference area... and significantly higher than those"

Answer: This sentence has been changed into:

"The concentration of Hg in core-top layer (0-2 cm) of the active seep area is three times higher (164 ng g^{-1}) than those of the reference area (51.1 ng g^{-1}) (Figure 1 and Supplementary Table 1). This value is also much higher than those of recently reported Hg-rich Mariana hadal trench zone⁵ ($89.0 \pm 33.0 \text{ ng g}^{-1}$) but lower than maximum concentrations in Atacama trench (up to 400 ng g^{-1})²²."

85 Suggest "in the South China Sea."

Answer: We have revised it according to your comments.

98 "the ability of TOC to sorb Hg in the active seep area is higher than that of background marine sediments"

Answer: We have revised it according to your comments.

110 "favor Hg-methylation"

Answer: Thanks for this helpful comment.

We have revised it into "create conditions conducive to the activity of known Hg methylators" according to another reviewer's comment.

111 Suggest: "In the inactive seep area, however,"

Answer: We have revised it according to your comments.

115 "This result is consistent with the observation of many dead clams (*C. marissinica*) in this area"

Answer: We have revised it according to your comments.

119 "The concentration of MMHg in bivalves from the Haima cold seep was below the limit of detection"

Answer: We have revised it according to your comments.

121 Evidence of what?

Answer: This has been changed into "Mercury isotope fractionation in cold seep environments".

123 Missing citation

Answer: The related citation has been added.

135-136 Check wording of "while their values in the reference" and "generally keep stable"

Answer: It has been changed into as follows:

"while their values in the reference site (0.02‰ to -0.83‰) and inactive seep area (-0.21‰ to -0.64‰) are variable but have no depth trend in the vertical profile".

142 Clarify "both"

Answer: We have added the information in the revised sentence:

"the two cores (B1 and B2) both".

148 "are significantly correlated"

Answer: Corrected.

152 What was the average $\Delta^{200}\text{Hg}$ in the active site?

Answer:

The average $\Delta^{200}\text{Hg}$ values in the active site is $0.01\text{‰} \pm 0.03\text{‰}$. We have added this information in the revised manuscript.

163 "nuclear volume effects" of "nuclear field effects"

Answer: It is "nuclear volume effects".

172 Only two? Likely many possible scenarios may explain this observation.

Answer: This sentence has been revised into as follow: "Among many possible scenarios for this, two of them are provided here".

246 "The thriving seep ecosystem, which supports animal (e.g., *G. haimaensis*) supplies a large amount.."

Answer: Corrected.

248 Cut "absorptions"

Answer: Removed.

249 Reword "would be continuously being accumulated here."

Answer: It has been rewritten as follows:

"would continue to be accumulated here".

250 Clarify "it";

Answer: "It" has been replaced by "the surface sedimentary environments".

250 "part of the inorganic Hg(II) is converted into MMHg"

Answer: Corrected.

252 "might also occur"

Answer: Corrected.

255 "peak under certain conditions"

Answer: Corrected.

258 "abiotic dark redox transformation of Hg"

Answer: Corrected.

259 Reword for accuracy. The oxidation of Hg⁰ as it moves up will enrich remaining Hg⁰ in ²⁰² and result in higher ²⁰² values of produced Hg(II) higher in the sediment column.

Answer: "as Hg⁰ undergoes oxidation while ascending through the sediment column, the remaining Hg⁰ becomes enriched in ²⁰² isotope, leading to increased $\delta^{202}\text{Hg}$ values in the produced Hg(II) at higher sediment layers."

271 "If we assume.."

Answer: Corrected.

269 Clarify that "extra" Hg and MMHg refers to the difference between active and reference sediments.

Answer: This sentence has been changed to "an additional 3.15 Mg Hg and 0.01 Mg MMHg will be preserved in the active seep sediments, compared to the same area scale of the reference area".

275 Reword "along with the exploration scale increases."

Answer: This sentence has been changed to "as more and more marine cold seeps will be discovered accompanying growth in seafloor survey coverage".

623 "microbially-mediated"

Answer: Corrected.

In the caption to Fig 4a, clarify which sites the three cores are from or refer readers to the explanations

of B1, etc. in Fig. 1.

Answer: The related information has been added in the revised caption to Fig 4a as follow: “B1 and B2 were collected from the active area, and C1 were collected from the inactive area”.

27th Mar 24

Dear Professor Peng,

Your manuscript titled "Deep sea cold seeps are a sink for mercury and source for methylmercury" has now been seen by our reviewers, whose comments appear below. In light of their advice we are delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment under the open access CC BY license (Creative Commons Attribution v4.0 International License).

We therefore invite you to revise your paper one last time to address the remaining concerns of our reviewers. At the same time we ask that you edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

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Please review our specific editorial comments and requests regarding your manuscript in the attached "Editorial Requests Table".

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Mojtaba Fakhraee, PhD
Editorial Board Member
Communications Earth & Environment

Clare Davis, PhD
Senior Editor
Communications Earth & Environment

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REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

Review of COMMSENV-23-1542A - REVISED

Title: Deep sea cold seeps are a sink for mercury and source for methylmercury

Summary:

The authors have, in my view, done an excellent job revising the paper according to the feedback from reviewers. All of my substantive concerns have been adequately addressed in the revised manuscript.

General comments:

I appreciate the thought that was put into the global Hg budget calculations and the depth-dependent trends. My only substantive suggestion is to soften the language related to the interpretation of global storage. The ideas are good and I believe the calculations make sense, AND they are still speculation so should be presented as such. I provide some suggestions for this below and include clarifying points for a couple small editorial pieces related to the revisions made in response to my prior comments.

Specific comments:

Line 85 to 86 - Suggest including the depth over which concentrations from other sites are reported:

e.g. "...Mariana hadal trench zone (89.0 ± 33.0 ng g⁻¹, top~10 cm) but ... Atacama trench (up to 400 ng g⁻¹, top 2 cm)."

Line 109 to 110 – I have two suggestions for the sentence about the predominantly inorganic Hg in bivalve tissue:

(1) the low MMHg is not adequately contextualized – it is a surprising finding (at least for me) and the "below detection limit" does not seem to do a good job of drawing out the context of the surprising finding. I would suggest something like "Consistent with Yuan et al. 2023, the methylmercury content of bivalves was extremely low: below detection limits (0.002 ng/g) and less than 1 % of total mercury. The low methylmercury content has been attributed to..."

(2) the sentence's new location feels slightly out of place – I know it would then be in a paragraph about total mercury rather than methyl mercury, but I think this information would feel more natural to include when the tissue Hg concentration is first introduced at the end of the first results paragraph.

Line 114 – insert the word "maximum" to describe what about MMHg corresponded with the depth of Hg/TOC maximum: "In the active seep area, the MMHg maximum also coincided with..."

Line 123 – the adjective "relative" should be "relatively," though a clearer approach to phrasing this idea may be: "...and values of $\delta^{13}\text{C-DIC}$ (-5.95‰) are much higher than those found in the surface sediment of the active area and similar to those of the reference area".

Line 286 to 292 – I'm glad this has been included, I suggest softening language (see ALL CAPS in the following) to reflect that these are, at best, order-of-magnitude estimates:
"then we estimate that an additional 2,835 Mg Hg and 9 Mg MMHg are (COULD BE) stored in cold seeps globally. According to Hayes et al.²¹, the global deep-sea burial fluxes of Hg are approximately (ON THE ORDER OF) 220 Mg Hg per year, and Zhang et al. report a global burial flux of (ABOUT) 9 Mg MMHg per year. Based on these figures, the total amount of additional Hg and MMHg stored in global cold seep surface sediments appears to (COULD) correspond to about 13 years' and 1 years' worth of the global deep-sea annual burial flux for Hg and MMHg, respectively

Reviewer #3 (Remarks to the Author):

The authors have adequately addressed reviewer concerns in revising their paper. A few additional suggested edits are listed below.

39 "cold seeps globally."

56 "However, it is uncertain whether the deep ocean"

77 "In this study, Hg concentrations are significantly higher in the upper sediment"

112 "organic carbon that does not bind strongly to mercury are preferentially degraded."

115 "were, on average, higher"

116 "However, concentrations of MMHg in the tissue of bivalves from the Haima cold seep were below the limit of detection"

292 "an additional 3.15 Mg Hg"

Reviewer #4 (Remarks to the Author):

I have reviewed the paper, including the author's responses to the comments by the reviewers. I was generally impressed with this work and the writing/paper. I believe the authors, in this revised version, have placed their inferences from the work in better context and have included some needed phrases and potential caveats. I also believe the authors have sufficiently addressed many, but not all, of the reviewer's concerns. However, on balance, I would give the authors good marks for their serious consideration of the reviewer's comments.

Unless I missed it there is no quality assurance information provided for the analyses. I suggest results for reference materials/recoveries, blanks, and replicates be added or at least summarized for the THg and MMHg analyses.

I also think it may be worth noting in the paper that others (reference below) have found somewhat different results for a cold seep in the northern Gulf of Mexico, though there may be sampling, analysis, or other factors contributing these differences.

Brown G., Sleeper K., Johnson M., Blum J., Cizdziel J.* (2013) Mercury concentrations, speciation, and isotopic composition in sediment from a cold seep in the northern Gulf of Mexico, Marine Pollution Bulletin 77:308-314. <https://doi.org/10.1016/j.marpolbul.2013.09.030>

In summary, I believe the paper should proceed to the publication stage, but I recommend some of my suggestions be considered.

Review of COMMSENV-23-1542A - REVISED

Title: Deep sea cold seeps are a sink for mercury and source for methylmercury

Summary:

The authors have, in my view, done an excellent job revising the paper according to the feedback from reviewers. All of my substantive concerns have been adequately addressed in the revised manuscript.

General comments:

I appreciate the thought that was put into the global Hg budget calculations and the depth-dependent trends. My only substantive suggestion is to soften the language related to the interpretation of global storage. The ideas are good and I believe the calculations make sense, AND they are still speculation so should be presented as such. I provide some suggestions for this below and include clarifying points for a couple small editorial pieces related to the revisions made in response to my prior comments.

Specific comments:

Line 85 to 86 - Suggest including the depth over which concentrations from other sites are reported: e.g. "...Mariana hadal trench zone (89.0 ± 33.0 ng g⁻¹, top~10 cm) but ... Atacama trench (up to 400 ng g⁻¹, top 2 cm)."

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Response to referees

Reviewer #1

Summary:

The authors have, in my view, done an excellent job revising the paper according to the feedback from reviewers. All of my substantive concerns have been adequately addressed in the revised manuscript.

[Answer: Thank you for your positive feedback.](#)

General comments:

I appreciate the thought that was put into the global Hg budget calculations and the depth-dependent trends. My only substantive suggestion is to soften the language related to the interpretation of global storage. The ideas are good and I believe the calculations make sense, AND they are still speculation so should be presented as such. I provide some suggestions for this below and include clarifying points for a couple small editorial pieces related to the revisions made in response to my prior comments.

[Answer: Thank you for pointing out this. We have softened the language related to the interpretation of global storage in the revised manuscript according to your comment.](#)

Specific comments:

Line 85 to 86 - Suggest including the depth over which concentrations from other sites are reported: e.g. "...Mariana hadal trench zone (89.0 ± 33.0 ng g⁻¹, top~10 cm) but ... Atacama trench (up to 400 ng g⁻¹, top 2 cm)."

[Answer: We have revised it according to your comment.](#)

Line 109 to 110 – I have two suggestions for the sentence about the predominantly inorganic Hg in bivalve tissue:

(1) the low MMHg is not adequately contextualized – it is a surprising finding (at least for me) and the "below detection limit" does not seem to do a good job of drawing out the context of the surprising finding. I would suggest something like "Consistent with Yuan et al. 2023, the methylmercury content of bivalves was extremely low: below detection limits (0.002 ng/g) and less than 1 % of total mercury. The low methylmercury content has been attributed to..."

(2) the sentence's new location feels slightly out of place – I know it would then be in a paragraph about total mercury rather than methyl mercury, but I think this information would feel more natural to include when the tissue Hg concentration is first introduced at the end of the first results paragraph.

Answer: We have revised it according to your comment. And we have put two sentence in the end of this paragraph as follows:

“In addition, consistent with Yuan et al. (2023)²³, the methylmercury content of bivalves was extremely low: below detection limits (0.002 ng/g) and less than 1% of total mercury. This may be related to shorter food chains resulting in a less pronounced biomagnification effect in hydrothermal fields and cold seeps environments”.

Line 114 – insert the word “maximum” to describe what about MMHg corresponded with the depth of Hg/TOC maximum: “In the active seep area, the MMHg maximum also coincided with...”

Answer: We have revised it according to your comment.

Line 123 – the adjective “relative” should be “relatively,” though a clearer approach to phrasing this idea may be: “...and values of $\delta^{13}\text{C-DIC}$ (-5.95‰) are much higher than those found in the surface sediment of the active area and similar to those of the reference area”.

Answer: We have revised it according to your comment.

Line 286 to 292 – I'm glad this has been included, I suggest softening language (see ALL CAPS in the following) to reflect that these are, at best, order-of-magnitude estimates:

“then we estimate that an additional 2,835 Mg Hg and 9 Mg MMHg are (COULD BE) stored in cold seeps globally. According to Hayes et al.²¹, the global deep-sea burial fluxes of Hg are approximately (ON THE ORDER OF) 220 Mg Hg per year, and Zhang et al. report a global burial flux of (ABOUT) 9 Mg MMHg per year. Based on these figures, the total amount of additional Hg and MMHg stored in global cold seep surface sediments appears to (COULD) correspond to about 13 years' and 1 years' worth of the global deep-sea annual burial flux for Hg and MMHg, respectively

Answer: Thanks very much for these helpful comments. We have revised it according to your comment.

Reviewer #3

The authors have adequately addressed reviewer concerns in revising their paper. A few additional suggested edits are listed below.

[Answer: Thanks for this positive comment.](#)

39 "cold seeps globally."

[Answer: We have revised it according to your comment.](#)

56 "However, it is uncertain whether the deep ocean"

[Answer: We have revised it according to your comment.](#)

77 "In this study, Hg concentrations are significantly higher in the upper sediment"

[Answer: We have revised it according to your comment.](#)

112 "organic carbon that does not bind strongly to mercury are preferentially degraded."

[Answer: We have revised it according to your comment.](#)

115 "were, on average, higher"

[Answer: We have revised it according to your comment.](#)

116 "However, concentrations of MMHg in the tissue of bivalves from the Haima cold seep were below the limit of detection"

[Answer: We have revised it according to your comment.](#)

292 "an additional 3.15 Mg Hg"

[Answer: We have revised it according to your comment.](#)

Reviewer #4

I have reviewed the paper, including the author's responses to the comments by the reviewers. I was generally impressed with this work and the writing/paper. I believe the authors, in this revised version, have placed their inferences from the work in better context and have included some needed phrases and potential caveats. I also believe the authors have sufficiently addressed many, but not all, of the reviewer's concerns. However, on balance, I would give the authors good marks for their serious consideration of the reviewer's comments.

Unless I missed it there is no quality assurance information provided for the analyses. I

suggest results for reference materials/recoveries, blanks, and replicates be added or at least summarized for the THg and MMHg analyses.

Answer: The related information for the quality assurance and quality control of total Hg and MMHg analyses has been added in the method section. Thanks for these helpful comments.

I also think it may be worth noting in the paper that others (reference below) have found somewhat different results for a cold seep in the northern Gulf of Mexico, though there may be sampling, analysis, or other factors contributing these differences.

Brown G., Sleeper K., Johnson M., Blum J., Cizdziel J.* (2013) Mercury concentrations, speciation, and isotopic composition in sediment from a cold seep in the northern Gulf of Mexico, Marine Pollution Bulletin 77:308-314.
<https://doi.org/10.1016/j.marpolbul.2013.09.030>

Answer:

We have read this article.

Their research did not uncover variances in Hg geochemistry and stable isotopes between the cold seeps and background regions, possibly due to the utilization of a box-sampler for sediment core collection. This method may not precisely target and retrieve sediment samples from cold seeps at significant water depths due to current influences. Consequently, the samples they attribute to the seep area are likely sourced from the background area just adjacent to the seep, rather than from the actual seep site.

In summary, I believe the paper should proceed to the publication stage, but I recommend some of my suggestions be considered.

Answer:

Thanks very much. We have revised this manuscript according to your comments.