Elsevier Editorial System(tm) for Science of the Total Environment
Manuscript Draft

Manuscript Number: STOTEN-D-20-07234R1

Title: Mercury and arsenic mobility in resuspended contaminated estuarine sediments (Asturias, Spain): a laboratory-based study

Article Type: Research Paper

Keywords: estuarine sediments, mercury, arsenic, contamination, resuspension, dredging

Corresponding Author: Dr. Stefano Covelli, Geological Sciences

Corresponding Author's Institution: University of Trieste

First Author: Efren García-Ordiales, Ph.D.

Order of Authors: Efren García-Ordiales, Ph.D.; Stefano Covelli, Geological Sciences; Greta Braidotti; Elisa Petranich; Elena Pavoni; Alessandro Acquavita; Lorena Sanz-Prada; Nieves Roqueñí; Jorge Loredo

Abstract: Estuarine sediments must be dredged in order to allow for navigation, and where these sediments are placed after dredging depends upon guidelines based only on the total concentration of contaminants. However, resuspension events could seriously affect the mobility and speciation of contaminants, including potentially toxic trace elements stored in sediments. The effects of resuspension on the cycling of mercury (Hg) and arsenic (As) between the sediment and water column was investigated in a mesocosm study. Four experiments were conducted in three estuaries in northern Spain based on samples collected from sites which have been impacted by now decommissioned Hg and As mines and periodically subjected to dredging activities. Designed to mimic the resuspension of particles, each of the experiments revealed that the release of Hg and As species does not depend on the total concentration in the sediments (16.3-50.9 mg kg-1, for As and 0.52-5.01 mg kg-1 for Hg). The contribution from porewaters and the subsequent reductive dissolution and/or desorption appears to be the main processes responsible for the abrupt increase in dissolved Hg and As species (maximum release of 427 % and 125 %, respectively). In some cases, As and Hg continued to remain at high concentrations in the water column even after the experiments were completed (after 24 h), thus testifying to their critical persistence in the dissolved form. Conversely, at the other sites, the restoration of pre-resuspension conditions were observed only a few hours after resuspension, mainly due to the role of Fe oxy-hydroxides which provides suitable surfaces for adsorption and/or co-precipitation processes involving dissolved Hg (maximum removal of -58 %) and As (maximum removal of -25 %) species. The results of this research could be helpful for policy making and to take appropriate decisions regarding dredging

Response to Reviewers: Responses to Reviewers comments:

Reviewer #1: The manuscript by García-Ordiales et al., attempts to evaluate the total Hg and As concentrations in both sediments and porewaters in a case study (Asturias, Spain), together with the effects of a mesocosm simulated resuspension in order to predict their behavior in real conditions. Such studies are of great importance for estuaries environmental quality and therefore research on monitoring approaches should be encouraged. However, while I enjoyed the flow of the paper, I could not overcome the sense that there are several issues that should be addressed prior to publication. In this context, the authors should provide more information concerning the methodological approach followed as well as to explain clearly which is the novelty of this work compared to others recently published (even from the same scientific team).

Graphical abstract: The graphical abstract is attractive to the readers and it does reflect the title of the paper and the key findings of the paper.

Thank you!

Highlights: The highlights are very general thus please add some specific/tangible results. To be honest, the last highlight seems so general in principle since e.g. "co-precipitation" is mentioned in the manuscript only 3 times, L. 316, 331 and in the conclusions.

Following your suggestion, the highlights have been rewritten and improved.

Introduction: The novelty of the study should be more highlighted. What is novel/new in this study, compared with other similar studies? What is the contribution of this investigation and it's major findings to understand this type of contamination in other parts in the world rather than in Spain (Asturias)? What kind of local and global knowledge the authors want to improve? Furthermore, which was the reason that the authors concentrated on Hg and As rather than other elements/metals that have been evaluated in their previous studies? These questions aren´t clear in the introduction and need further consideration/explanation.

Asturias is one of the most important Regions for mining and extraction activity of Hg and coal in Spain. Especially, the Nalòn River drainage basin has been severely affected by long term exploitation of sulphide ores (several mines north from Oviedo) and there are evidences that the sediments not only in the Nalòn estuary (García-Ordiales et al., 2019a, 2020), but also along the whole coastline (Forján et al., 2019; Sanz-Prada et al., 2020) are contaminated by Hg and As mainly by this source. On the basis of this comment and following your suggestions, we have improved the Introduction. In detail, we have added further information on the investigated area in order to clarify the reasons that we focused on the behaviour of Hg and As species. We have also better highlighted the primary aim of our study at the end of the Introduction (now Lines 107-128).
“The primary aim of this research was to investigate the effects of simulated resuspension events in replicate mesocosms to predict the behaviour of Hg and As associated with bottom sediments and porewaters and their fate in the water column after a perturbative event. A comparison among similar estuarine environments in Asturias affected by various sources of contamination and where dredging operations are periodically needed to allow navigation was provided. The occurrence and mobility of Hg and As in this area is of great concern due to their potential bioaccumulation in the aquatic trophic chain” with subsequent high risk of transfer to human beings through consumption of fish and,

especially, mussels. In addition, following the specific maritime policy established and directed for marine ecosystem protection and conservation by the EU Marine Strategy Framework Directive, we believe that this experimental approach could be applied to all coastal environments where information regarding the major geochemical processes that regulate PTE behaviour, mobility and fate is needed to support environmental management and risk assessment."

Materials and Methods: There are several places where the methods are poorly described and where it cannot be derived the reason why or/and what the authors have done. The clearest example is the description of the resuspension tests. There are quite several protocol leaching methods available in the literature, but it cannot be derived which one have the authors used or they have modified. However, since sealing of the top hole of the chamber was done, how do the authors conducted sampling without avoiding conduct with air or liberation of gases? In this context, how long was the benthic chamber left open and undisturbed between one sampling and the next one? Cover reposition before each sampling do not avoid the aforementioned problems. Moreover, how many bottom sediments and overlying water were collected? Please elaborate these points in detail.

Following your suggestions, we have improved the description of the methods section.
Regarding the protocol for the resuspension experiment, we have specified that we followed the experimental approach applied by Acquavita et al. (2012) (now Lines 184-186). We have also specified that the overlying water was carefully replaced with bottom water collected from the same sampling location in order to get the starting conditions as they effectively were and avoiding then any possible perturbative effect due to the transport of the chamber to the lab for the experiment (now Lines 189-193). This procedure was also successfully employed in other similar studies (e.g. Bertuzzi et al., 1996; Emili et al., 2011; Acquavita et al., 2012). We have added this information in the text.
The benthic chamber was left open and undisturbed between one sampling and the next one with the aim to simulate real environmental conditions where the water column is in direct contact with the atmosphere at the water-atmosphere interface. In addition, the benthic chamber was not sealed between one sampling and the next one in order to avoid possible oxygen consumption with subsequent alteration of the pyhsico-chemical boundary conditions of the system. Indeed, significant decrease in the oxygen concentration was pointed out by previous incubation experiments during which the benthic chamber was sealed (Emili et al., 2011, 2014). Although fluxes of trace elements at the water-atmosphere interface could occur, we retain that the liberation of gases (e.g. gaseous dissolved Hg0) may be negligible since the concentration of Hg0 is generally very low in sediments, water column and porewaters. Then, in order to allow the collection of the water just above the sediment-water interface (SWI), the cover was repositioned before each sampling. Indeed, the cover has two valves on its lid and one of them is connected to a tube that allows the sample collection at a defined depth, just above the SWI. Consequently, the cover has to be repositioned in order to be sure that the sampling is done at the same depth during the resuspension experiment. The sampling has to be done at the same depth during the experiment. This is extremely important for properly evaluate variations of the investigated parameters (Eh, dissolved and particulate PTEs) as well as the grain size composition of the suspended particles which tend to become finer with time due to settling of resuspended particles.

We have improved this description in the text (now Lines 198-213). The benthic chamber was left open and undisturbed between one sampling and the next one. This means that, for instance, the benthic chamber was left open for 30 min between the sample T0 (when shaking has stopped) and T1 (after 30 min). Then, the benthic chamber was left open for more 30 min between sample T1 (after 30 min) and T2 (after 1 h) and so on. Seven samples were collected within 24 h (T0 = 0, T1 = 30 min; T2 = 1h; T3 = 2h; T4 = 4h; T5 = 8h; T6 = 24h). This was already stated in the text (now Lines 208-209). For each sampling, the dissolved phase was separated from the suspended particles which were collected for both grain-size and chemical analyses. We have slightly modified the text in order to make this more clear (now Lines 211-213).
Bottom sediments were not collected during the resuspension experiment. Short sediment cores were collected in parallel with the benthic chamber at the same site to get a picture of the situation (sediment and porewater composition) before the resuspension event. The cores were extruded and sectioned into five slices (0-1, 1-2, 2-3.5, 3.5-5 and 5-7 cm) in a N2-filled chamber. This was already written at the beginning of the section 2.2 Experimental approach (now Lines 175-183).

Results and discussion: This section needs strengthening to improve clarity.
- The authors discuss about the possible sources of the effect of resuspension and the subsequent remobilisation and redistribution of As and Hg species. However, they only make general assumptions without providing arguments that support one or more of these assumptions in the specific area. Even though a plethora of data is provided for an in-depth geochemical study via geochemical modelling/kinetics only hypothesis and assumptions such as L. 358-359 for Hg " This could be attributed both to MeHg released from porewaters or to the mobilisation of bioavailable OM during resuspension " along with L. 384-385 for As " The prevalence of As(V) may be related to oxidation and/or adsorption processes mediated by Fe and Mn oxy-hydroxides, which represent very efficient oxidants of As(III) " are provided. How both outcomes derived and experimentally proved? The linkages presented here between are too loose, and would be strengthened considerably by a geochemical modelling analysis to conclusively demonstrate the crucial role of Fe in relation to As and Hg mobilisation and how this may affect pore water/sediment quality. A detailed geochemical modelling study will also help to identify the most critical parameters that affect either primary or secondary sources of iron and distinguish them on a real basis based on the obtained results.

In order to provide a statistical and modelling support to our hypothesis and assumptions through the different sectors of our article, we have added:
1. Principal component analysis (PCA) which was performed on both the sediment matrix (Fig. S1A) and porewaters (Fig. S1B).
We have added a brief explanation “2.4 Explorative multivariate data analysis” in the Materials and Methods section (now Lines 247-255).
We have improved the section 3.1. Sediment and porewater chemistry by adding further information provided by the PCA performed both on the sediment matrix and porewaters:
Regarding the sediment matrix, the PCA output (Fig. S1A, explaining 84.7% of the total variance) clearly denotes the differences among the different sites which are distinctly represented in the biplot although disparities in trace element concentrations along the sediment cores are not so evident.

As already reported in the text, no significant differences were observed for Hg at sites VV, SJ and SE (mostly lower than 1.0 mg kg-1), whereas the element was found to be one order of magnitude higher at site LL (3.73 ± 0.76 mg kg-1). This is also confirmed by the PCA output showing a quite evident relation between Hg and site LL (Fig. S1A). Slightly the same is also true in the case of Sulphur (which is higher at LL and VV), Fe and Mn (which showed the highest values at sites SJ and SE): these relationships are clearly supported by the PCA output. We have added this information in the text.

Regarding the porewater samples, oxidative conditions were found in the surnatant water (Eh ranged from 132 to 161 mV as well as quite low dissolved Hg, MeHg and As species, followed by Fe and Mn. This is clearly evident in the PCA output (explaining 71.1% of the total variance) highlighting a strong relation between the redox conditions and the surnatant water of all sites (Fig. S1B). In addition, the PCA output clearly highlighted strong correlations between As(III) and As(V) (r=0.919) and between As species and dissolved Fe (r=0.770 and r=0.757 for As(III) and As(V), respectively) (Fig. 2B). This suggested that the dissolution of Fe oxy-hydroxides could be responsible for the release of dissolved As species rather than Mn (r=0.171 and r=0.131 for As(III) and As(V), respectively). We have added this information in the text.

2. Equations describing the behaviour of dissolved As and Hg species as a function of time with the final aim to better describe if and after how long the system returns to the initial conditions (pre-resuspension) (Fig. S2).

The behaviour of dissolved As(III), As(V), Hg and MeHg during the resuspension experiment is described by a negative exponential equation:

C_Ti= C_T0 e^(-kx)

where CTi is the concentration at any time (Ti) during the resuspension experiment, CT0 is the concentration after shaking (T0) and k is the rate constant for the contaminant dispersion as a function of time (Fig. 6). The equation (1) was calculated until a decreasing trend was observed and was not applied when the concentration after shaking (T0) was higher than the concentration measured before the resuspension experiment (Tpre). Indeed, the final aim was to verify after how long the system gets the initial conditions (pre-resuspension).

We have added a brief explanation in the section 3.2.2 Behaviour of dissolved chemical species after resuspension (now Lines 417-426) and we have added further details in the sections Behaviour of dissolved mercury species (now Lines 441-445) and Behaviour of dissolved arsenic species (now Lines 500-509).

- Yes, we all know that L. 315-317 " In oxic conditions, Fe and Mn are present as oxyhydroxides and are often involved in adsorption processes, coprecipitation, but when reducing conditions occur there is a dissolution with the subsequent release of dissolved PTEs " but arguing that L. 329-330-" This could be related to the crucial role of Fe oxides as surface catalysts in the oxidation of Mn " is far too simple. It's much more complicated than that and therefore studies such as modelling, leaching tests and analytical characterization tests have been developed to take all possible factors and feedback mechanisms into account to answer these questions.

The first sentence you are referring to (now Lines 390-393) is at the beginning of the section 3.2.2 Behaviour of dissolved chemical species after resuspension and it is only introductory. We can agree with you saying that the role of Fe and Mn oxy-hydroxides can be more complex.

On the basis of your comment (second sentence you are referring to), we have slightly modified the text by adding a more detailed description of the role of Fe oxides in regulating dissolved Mn concentrations during a resuspension event (now Lines 403-416). Moreover, the mineralogical composition of the sediments from the Nalón River estuary is reported in García-Ordiales et al. (2017): it is mainly quartz, which is predominant in the coarse fractions, and kaolinite-smectite, illite, albite, and vermiculite in the finer fractions. The chemical concentrations in the sediments reported as oxides are clearly dominated by SiO2 (66.3%) and Al2O3 (8%), in addition to the significant presence of light amounts of Fe2O3 (3.7%), as expected based on the mineralogical analysis. Detected concentrations of MnO were consistently less than 1% and were not directly correlated with the fine fraction.

Although modelling, leaching tests and analytical characterisation tests (honestly, what do you mean with "analytical characterisation tests"? It is not properly clear for us) have been applied in other studies, the approach we have followed has been previously applied with success. This procedure allowed us to provide further information on As and Hg mobility in different environmental conditions.

-Another issue worthy of further discussion is the interrelated issue of contaminants and sediment cores surface/interparticle mass transfer. This is again related to the dynamic nature of "release from sediment to porewaters" and is closely linked to the issue of temporally "bonding" loss under different conditions that resulted in remobilisation and redistribution.

Unfortunately, the comment of the reviewer is not properly clear. If we have understood we are asked for a further discussion about the dynamics of the remobilisation of the elements from porewaters and sediment particles after the resuspension event. The simulation showed us that, not always, the release of trace elements from porewaters associated with desorption from particles is effective and there is also an antagonism between release/desorption and reprecipitation/readsorption processes. This is why we have introduced a simple modelling for calculating time necessary to go back to concentrations in dissolved form in the situation pre-resuspension.

Other comments
- Part of the conclusion is a direct repetition of the abstract e.g. "Part of the Hg and As released from porewaters and/or desorbed during resuspension was presumably removed by the subsequent adsorption onto the settling fine particles and/or co-precipitated in association with Fe and Mn oxy-hydroxides". Please modify the whole conclusion accordingly.

Please, we apologise for this inaccuracy and thank you for noticing it. We have decided to improve and modify the abstract also on the basis on the comments of the other Reviewers. We did not modify the conclusions since the Reviewer #3 stated that is well summarized but we have included an additional comment about the time necessary to restore the pre-resuspension As and Hg concentrations.

- Fig.1. Please provide GIS coordination grid in the provided map of the study area
We have added the following Table (Table 1) resuming the main characteristics of the investigated sampling sites including latitude and longitude.

- Some figures can be notably improved e.g. Fig.4 (split and use bigger graphs, not easily readable). Please make titles in both axes large enough such that the parameters are readable. Use bigger font size both in titles and legends.

Following your suggestion, we have improved and split the Fig. 4 in two figures: one for sites LL and VV (now Fig. 5) and one for SJ and SE (now Fig. 6).

- Limitation and scope of the study should be provided at the end. Limitations aren't weaknesses but can be possibilities for other studies - highlight them as necessary.

Following your suggestion, we have better highlighted the primary aim of our study at the end of the Introduction (now Lines 107-128) where we have also added further information on the investigated area in order to clarify the reasons that we focused on the behaviour of Hg and As (now Lines 95-106).
Regarding the limitations of our study, we had already stated in the text (see the last sentence of the Conclusions) that it would be useful to monitor the water column quality during dredging operations in situ and to compare the results with the mesocosm approach. In our opinion, this represent an extremely difficult task to perform since one has to study an open system which is much more complex compared to the mesocosm experiment.

- Authors should carefully revise the reference list as some citations are not reported and some others mentioned in the list are not present in the text e.g. L. 609 "García-Ordiales et al. 2020"

As suggested, we have carefully revised the reference list and deleted those citations that were not present in the text.

- L. 137. Full spot after "González-Fernández et al., 2018)", L. 175. Please change "hours" into "h), L.176. Please erase dot before parenthesis. Please choose "co-precipitation" or "coprecipitation" throughout the text.

We have modified the text following your suggestions. We have changed "hours" into "h" and coprecipitation" to "co-precipitation" throughout the text.

Reviewer #2: This paper investigates at lab conditions the remobilization of Hg and As after sediment resuspension, mimicking dredging. This type of papers are of scientific interest and fits well with the scope of the journal. My main concerns about the suitability of this work are related to its novelty and the absence of mineralogical/geochemical evidences to support their findings. Other works dealing with As and Hg mobility after sediment resuspension has been previously published. In addition, the authors discuss their analytical results without providing mineralogical composition of sediments or performing any sequential extraction to guess the affinity of As and Hg in the sediments. A statistical analysis of results could provide more robustness to result interpretation. On the other hand, the authors must take the most of data; for example, a geochemical modelling of porewater-sediment interaction could improve the quality of this paper.

First, there are a few papers dealing with Hg and, especially, As mobility after resuspension events associated mainly to dredging operations. A similar issue raised by the first reviewer and we have explained that the novelty of this paper is the idea to compare similar estuarine environments affected by different sources of contamination related to Hg and As. If the Nalòn River drainage basin has been severely impacted by long term exploitation of sulphide ores and coal the whole coastline further east is contaminated by Hg and As mainly by this source. The main ore mineral of these deposits is cinnabar in association with native Hg, stibnite [Sb2S3], and important amounts of As-bearing minerals such as realgar and pararealgar [As4S4], orpiment [As2S3], and arsenic-rich pyrite (Ordoñez et al. 2014).

In addition, we investigated also two other estuaries, Villaviciosa and Llanes, where the contamination is related to other anthropogenic sources (e.g. the Aviles port area) in addition to mining. A statistical analysis (PCA) has been added to the discussion to make our interpretation more robust.

We did not perform mineralogical analyses in our study but several information can be obtained from previous studies. From the geological point of view, at the basin level, the hydrographic system of the Nalòn River flows through Paleozoic bedrock from the Cambrian to the Upper Carboniferous periods (García-Ordiales et al., 2017). The western sector of the river basin is characterized by the presence of low-grade metamorphic silico-clastic rocks interbedded with a calcareous series of grey limestones and dolomites, whereas, in the eastern section, calcareous and siliciclastic series predominate. The Villaviciosa and Llanes estuaries belong to the second main set of rocks which is considered a mixed section with limestones, sandstones, quartzite and lutite of Permian-Mesozoic and Paleogene-Neogene periods.

The mineralogical composition of the sediments from the Nalón River estuary is reported in García-Ordiales et al. (2017): it is mainly quartz, which is predominant in the coarse fractions, and kaolinite-smectite, illite, albite, and vermiculite in the finer fractions. The total chemical concentrations in the sediments reported as oxides are clearly dominated by SiO2 (66.3%) and Al2O3 (8%), in addition to the significant presence of light amounts of Fe2O3 (3.7%), as expected based on the mineralogical analysis. Detected concentrations of MnO were consistently less than 1% and were not directly correlated with the fine fraction thus indicating that its variability is not directly related to the aluminosilicate minerals which, conversely, are correlated with total Fe concentrations.

Villaviciosa and Llanes located in the eastern area of Asturias also matched with another anomalous geological materials rich in As and Hg. This area is formed by materials from the ages between the Carboniferous and Jurassic in which hydrothermal processes generated ores (mainly fluorite) with metal sulphides rich in this element (Sanz-Prada et al., 2020). The same authors explained that regarding Hg, most of the sandy beach samples, whose concentrations were above the ERL (Effect Range Low) level, were from the eastern region where the lithology of the area is rich in carbonated rocks. Thus, it appeared that Hg concentrations could be related to this kind of geology because there are few industrial activities which could have affected this area. Forján et al. (2020) reported high concentrations of Hg and Ba on Vega beach, 30 km further east the Llanes estuary. Both elements belong to the fluorite ore paragenesis, mined in the surroundings. Hg was enriched in the finer fractions, and overall the main input was attributed to the mining waste discharged along the coast in the past. The district of Berbes, was in the 20th century, the most productive fluorite mining area not only of

Asturias, but also of Europe because of the outcropping of important mineral ores along much of its perimeter. The paragenesis of the mineralization in this area is composed by fluorite ($CaF2$) and abundant barite ($BaSO4$). Furthermore, cinnabar (HgS) is occasionally present in the form of fine inclusions in the fluorite and copper minerals (chalcopyrite, fahlore and Cu-carbonates are also abundant). Other potential contaminants are arsenic (As), related to the iron and copper sulfides in the area.
In literature, we can find results about speciation of As and Hg in fluvial sediments of the Nalon River drainage basin (Larios et al., 2012; Silva et al., 2014). The second authors report that "in spite of a higher As accumulation in the finest particle-size subsamples, As fractionation did not seem to depend on grain size since similar distribution profiles were obtained for the studied granulometric fractions. The presence of As was relatively low in the most mobile forms. Arsenic was predominantly linked to short-range ordered Fe oxyhydroxides, coprecipitated with Fe and partially with Al oxyhydroxides and associated with structural material in mine waste samples. Arsenic incorporated into short-range ordered Fe oxyhydroxides was the predominant fraction at stream sediment samples, representing more than 80 % of total As…Arsenic is retained in sediments mainly as highly stable amorphous iron oxyhydroxide–arsenic complexes, which can be present in a wide range of grain size classes."

Highlights:
Rewrite the highlight #2 as follows: "As and Hg released from porewaters due to dissolution of Fe-Mn oxy-hydroxides"

Following your suggestion, we have modified the highlight #2.

Rewrite the highlights #4 and #5

As you suggested, we have rewritten the highlights.

Line 31: not only contaminated sediments must be dredged to allow navigation.

Following your suggestion, we have modified the sentence and deleted the word "contaminated".

Lines 31-50: I miss here a quantification of changes in concentrations of elements following resuspension (e.g. 20% increase at t= X, etc.)

Following your suggestion and on the basis on the comments of Reviewer #3 we have modified the abstract providing a more generalised explanation of the main results of this research as well as maximum release and removal percentages observed for As and Hg species during the resuspension experiments.

Line 82: I do not see the point how investigating resuspension of estuarine sediments could contribute to improve air quality.
In estuarine environments, fluxes of trace elements at the water-atmosphere interface could occur (e.g. Floreani et al., 2019; O'Driscoll et al., 2019) and we retain that the processes involved in these fluxes are of concern. However, we agree with your observation. Since our research is primarily focused on the sediment-water interface we decided to delete the word "air" from that sentence.

Lines 82-85: the authors must explain the novelty of this study compared with previous similar experiments?

As already reported following a question of the reviewer #1, Asturias is one of the most important Regions for mining and extraction activity of Hg and coal in Spain. Especially, the Nalòn River drainage basin has been severely affected by long term exploitation of sulphide ores (several mines north from Oviedo) and there are evidences that the sediments not only in the Nalòn estuary (García-Ordiales et al. 2019, 2020), but also along the whole coastline (Forjan et al., 2019; Sanz-Prada et al. 2020) are contaminated by Hg and As mainly by this source. Besides, we investigated also two other estuaries where the contamination is related to other anthropogenic sources in addition to mining. The novelty is not the method itself but the comparison similar estuarine environments affected by different sources of contamination and where dredging operations are periodically needed to allow navigation.

Line 156: what the authors probably measured was ORP instead of Eh, right? And why did the authors not measure also pH? It is a critical parameter in metal solubility.

Technically, there is no difference between ORP and Eh. ORP is used for measurements in aquatic environments and Eh for water and other environmental systems. We measured Eh by means of a Pt/KCl−Ag/AgCl electrode calibrated with ZoBell solution (220 mV at 25°C). This description has been added to the text (now Lines 194-195).
We have added the Eh values in Table 2.
pH was measured in the surnatant water and in the water in the benthic chamber before and during the resuspension experiment (Table S2). Regarding the sediment samples, pH was measured only at sites SJ and SE (Table B).

Table B. pH values measured in the sediment cores collected at the Nalòn River estuary (sites SJ and SE).

Sediment layer (cm)	SJ	SE
Surnatant water	7.56	7.68
0-1	7.40	7.46
1-2	7.51	7.51
2-3.5	7.42	7.34
3.5-5	7.32	7.38
5-7	7.35	7.28

We agree with you saying that pH is also a critical parameter in metal solubility. However, slight variations with increasing depth were observed: 7.43 ± 0.09 at SJ and 7.44 ± 0.14 at SE.
In this context, Fiket et al. (2019), in a study strictly focused on the geochemistry of porewaters, collected two sediment cores: one for geochemical analysis and the other one for determination of physico-chemical parameters (Eh and pH). According to the authors, pH values in the sediment porewaters display more uniform distribution (from 7.18 to 7.83, RSD < 1.5%) along the depth profiles with respect to Eh. The authors also pointed out that the pH does not seem to have such an important influence in determining the levels of several trace elements in the sediment porewaters whereas Eh plays an important role.
For these reasons we decided not to include the pH values in our study.

Lines 165-167: why did the authors empty the water from the corers and take new water with a Niskin bottle? It seems to be more realistic to use the overlying water.

The overlying water was drained off from the benthic chamber (now Lines 189-193) and replaced with bottom water collected from the same sampling location using a Niskin bottle following the experimental approach applied by Acquavita et al. (2012). The overlying water was replaced in order to avoid any possible perturbation effect during the transport of the chamber from field to the laboratory and to get the undisturbed starting conditions. This procedure was also successfully employed in other similar studies (e.g. Bertuzzi et al., 1996; Emili et al., 2011; Acquavita et al., 2012). We have added these details in the text.

Lines 167-168: was dissolved oxygen monitored?

Unfortunately, the oxymeter was out of order.

Line 170: which is the reason for selecting the resuspension time as 10 min? based on previous studies or mimicking real dredgings?

We selected a resuspension time of 10 min following previous research (e.g. Acquavita et al., 2012; Bataillard et al., 2014). Indeed, we retain that 10 min of shaking may be enough to allow the resuspension of the surface sediments which usually is not compacted and quite fluid, also in terms of visual observation of the experiment. For instance, Saulnier and Mucci (2000) demonstrated that a significant amount of As is already released in solution after 6 min of resuspension.

Lines 199-200: the treatment of the solid sample with ascorbic and phosphoric + microwave acid was done for total composition determination? I see in lines 205-206, that total composition was performed by acid mixtures and microwave.

As already stated few lines above ("The As species were analysed using…"), the treatment you are referring to was performed for the analyses of the As species (As(III) and As(IV)) in the solid samples. Since we needed a solution for HPLC-ICP-MS analyses, 0.1 g of sample was placed together with an extracting agent (1 M H_3PO_4+0.1 M, ascorbic acid) in a microwave vessel (Ruiz-Chancho et al., 2005). The digested solution was then analysed.
We have slightly modified the sentence writing "The As species were directly measured in the porewater samples, whereas in the case of solids, 0.1 g of sample was placed together with an extracting agent (1 M H_3PO_4+0.1 M, ascorbic acid) in a microwave vessel. The digested solution was analysed following the aforementioned method." Now Lines 229-233.
The analytical determination of the total concentrations of As, Fe, Mn and S was also performed after acid digestion in a close microwave system but using aqua regia and HF, following the EPA method 3052. This was already written in the text.

Line 239: Eh or ORP?, please clear it up. If they are Eh values, I am not sure if those can be considered as oxidative conditions.

As explained in the comment related to line 156, we measured Eh. They are oxidative conditions compared to the reducing conditions found in the sediments.

Line 244: such Eh decrease causes a reductive dissolution of Fe/Mn oxides rather than desorption processes.

We have modified the sentence following your suggestion.

Lines 245-247: did the authors performed a statistical analysis to support this fact?

In order to provide statistical support to the traditional data interpretation, principal component analysis (PCA) was performed on both the sediment matrix (Fig. 2A) and porewaters (Fig. 2B). We have added a brief explanation "Explorative multivariate data analysis" in the Materials and Methods section.
Regarding your comment concerning porewaters, the PCA output (biplot explaining 71.1% of the total variance, Fig. 2B) highlighted a strong correlation between As species and dissolved Fe (r=0.919 between As(III) and As(V), r=0.770 between As(III) and Fe, r=0.757 between As(V) and Fe). In order to be consistent, r values reported in the text are referred to the entire set of data (LL, VV, SJ and SE). We have added this information in the text.
In order to provide you further information, we have also checked the correlations between dissolved As species and Fe at each sampling site. As species reached higher concentrations at the Nalòn estuary sites (SJ and SE) and showed strong correlations at all sites (r=0.941 at LL, r=0.689 at VV, r=0.971 at SJ, r=0.954 at SE). This is consistent with the results depicted in the PCA output. Weak correlation was found between dissolved As species and Fe at sites LL (r=0.346 and r=0.193 for As(III) and As(V), respectively) and VV (r=0.438 and r=0.470 for As(III) and As(V), respectively). Conversely, As species appear to be strong correlated with dissolved Fe at sites SJ (r=0.837 and r=0.890 for As(III) and As(V), respectively) and SE (r=0.526 and r=0.585 for As(III) and As(V), respectively).

Lines 258-260: does the authors have any evidence of the presence of these mineral phases in the sediments?

We did not perform any mineralogical analysis. As reported above, the mineralogical composition of the sediments from the Nalón River estuary is reported in García-Ordiales et al. (2017): it is mainly quartz, which is predominant in the coarse fractions, and kaolinite-smectite, illite, albite, and vermiculite in the finer fractions. The chemical concentrations in the sediments reported as oxides are clearly dominated by SiO2 (66.3%) and Al2O3 (8%), in addition to the significant presence of light amounts of Fe2O3 (3.7%), as expected based on the mineralogical analysis. Detected concentrations of MnO were consistently less than 1% and were not directly correlated with the fine fraction thus indicating that its variability is not directly related to the aluminosilicate minerals which, conversely, are correlated with Fe2O3. These data are consistent with the results we obtained from our analyses where Mn concentrations in the sediments are two orders of magnitude lower than Fe concentrations.

Lines 257-266: according to the author's belief, which redox pair may control the redox condition in the sediments, S(-II)-S(VI), Fe(III)-Fe(II), As(V)-As(III) or that of Mn?

The redox conditions in our sediments are mainly controlled by the redox pairs Fe(OH)3/Fe2+ considering the pH value of about 7.3-7.4 and the Eh negative values. In addition, reducing conditions favor the much more hazardous chemical form of arsenite (As+3) and this is visible in our porewater profiles where there is an increase of concentrations with depth (HAsO42-/H3AsO3). The redox pair SO42-/HS- can be present but our conditions are not strongly reducing to reach sulphate reduction. In these conditions, at the pH of the seawater and with available carbonate species, solid manganese could be present in form of rhodocrosite MnO2(s)+ HCO3 +3H+ +2e- = MnCO3(s)+2H2O whereas at the same conditions Fe is controlled more by the sulphides. Usually, the Eh-pH relations show relatively large field of stability for dissolved Mn2+ compared to the solid oxides MnO2.

Lines 278-281: again, does the authors have statistical evidence of this relatiship?

In order to provide statistical support to the traditional data interpretation, principal component analysis (PCA) was performed on both the sediment matrix (Fig. 2A) and porewaters (Fig. 2B). We have added a brief explanation "Explorative multivariate data analysis" in the Materials and Methods section.
Regarding your comment concerning the relationship between Hg and S in the sediment matrix, the PCA output (explaining 84.7% of the total variance, Fig. 2A) highlighted a strong correlation between Hg and S (r=0.628). Moreover, these elements also showed a clear relationship with the sediment core collected at site LL where the highest concentrations of Hg and S were observed. This is not surprising since most of the Hg of mining origin was present as cinnabar (HgS).
However, at site LL, no correlation was found between the two elements (r=0.297 at site LL) contrary to what was observed at sites VV (r=0.959), SJ (0.577) and SE (r=0.732). In this context, it is reasonable to expect that the occurrence of Hg at site LL may be related to a different local source which is currently under investigation.
Indeed, high concentrations of Hg were detected at the Vega beach (approximately 40 km west of site LL) due to the discharge of mine tailings from the district of Berbes which was one of the main fluorite mining areas in Europe where cinnabar was occasionally present (Symons et al., 2017; Forjan et al., 2019; Leuresse et al., 2019). According to Florjan et al. (2019), a marine outfall located 660 m away from the average low tide level has been build up for the discharge since 1990s. This suggests that fine suspended particles enriched in Hg could be transported by the ocean and shoreline currents and transported into the estuarine environment at site LL.
We have improved the text and added furhter information regarding the source of Hg at site LL (now Lines 341-352).

Line 295 onwards: please replace T1, T2…. the real time (15 min, 1h, 2h, etc.)

Following your suggestion, we have replaced T1-T6 with the real time and wrote "when shaking has stopped (T0)" and "before resuspension (Tpre-res) when necessary.

Lines 329-332: could the authors discard diffusion into the sediment of more metal-rich waters after the resuspension? As metal-rich porewaters seem to be diffused into the water column during the resuspension, the opposite effect could be also produced. Could the authors give their opinion on that?

We agree with you since also influxes of trace elements from the overlying water into the sediment could occur. Unfortunately, we did not investigate these processes and we actually do not have any experimental evidence to proof if a certain influx of trace elements could occur. However, we retain that influx of trace elements into the sediment during a resuspension event is a much more slow mechanism compared to other geochemical processes such as precipitation and adsorption on the surface of the resuspended particles.

Line 368: a lower?

We have modified the text following your suggestion.

Lines 370-371: which is the reason of this evolution?

At site SE, an increase of dissolved MeHg and a decrease of dissolved Hg were observed during the resuspension experiment. We retain that this evolution is the result of methylation processes which appear to prevail at site SE with respect to the other investigated sites. At site SE, the MeHg concentrations during the resuspension were found to be one order of magnitude higher than those measured in the sediment porewaters. This suggests that a certain amount of MeHg may be produced during the resuspension experiment and/or released from the resuspended fine particles due to desorption processes. Indeed, previous research at the Nalòn River estuary stated that the highest levels of MeHg in the surface sediments occurred at site SE (García-Ordiales et al., 2018). The same authors stated also that only low amounts of the THg are available for methylation. However, changes in environmental conditions (e.g. hydrological events) or anthropogenic activities (e.g. dredging) may alter the original unperturbed conditions with subsequent remobilization of PTEs from bottom sediments through resuspension. Moreover, at site SE the highest concentrations of inorganic Hg were observed (three orders of magnitude higher with respect to the other sites) as well as elevated amounts of SO42- and the increase of DOC due to OM degradation during the resuspension experiment. Since sediments can show different methylation potential (Bloom et al., 2003) which depends on several factors such as the bacterial activity, the physico-chemical boundary conditions, the occurrence of OM and the concentration of inorganic Hg available for methylation it is reasonable to suppose that sediment resuspension may promote methylation processes at site SE.
We have improved this discussion in the text (now Lines 464-480)

Lines 401-402: which is the cause of inhibition? The high concentration of sulphate or the availability of high concentrations of sulphate to be reduced instead of As(V).

The availability of high concentrations of sulphate to be reduced instead of As(V). On the basis of your comment we have modified the sentence as follows "Moreover, the availability of high concentrations of SO42- (>2,000 mg L-1) to be reduced can inhibit the reduction of As(V) to As(III)". Now Lines 518-520.

Lines 408-408: why is not possible here the oxidation of DOC and conversion into DIC?

We should take into consideration that only a small part of DOC is bioavailable or biodegradable by bacteria. The remaining part is recalcitrant to degradation as it is made by fulvic and humic acids and a myriad of macro- and micromolecular compounds also highly ramified and less active. This is why we think that dilution due to porewaters can explain the low concentrations after the resuspension but we cannot exclude a degradation to DIC although this process is not so fast and influent due to what reported above.

Lines 430-432: and associated to what?

At all the investigated sites, particulate PTEs showed decreasing trends as the result of the settling of the resuspended particles as a function of time. We have modified the sentence in order to better highlight this behaviour (now Lines 548-562).

Lines 438-439: how the authors could support this fact? Have the authors any information from sequential extraction or mineralogical evidences?

Unfortunately, we have no information from sequential extraction or mineralogical evidences.
The fact that Hg and MeHg were bound to the silty fraction is supported by the grain size spectra and composition (see Fig. 4) which tends to move to finer particles as a function of time. At site SE, these finer particles appear to be enriched in Hg and MeHg and, consequently, their concentrations (µg g-1) increase as the particles become finer. We already stated in the text that "by comparing the particulate PTE trends with TSM content, a preferential bound to the silty or clayey fraction was found (now Lines 553-554). We slightly modified the text specifying that the fact that particulate Hg and MeHg concentrations increased is consistent with the evolution of the grain size spectra and composition (Fig. 4) as a function of time and it indicates that Hg and MeHg were bound to both the silty and clayey fractions (now Lines 556-562).
Moreover, this fact is also confirmed by the significant correlations between the percentage of silt and particulate Hg and MeHg during the resuspension experiment at site SE:

Line 476: As(V) was totally removed? If so, I do not see how it could be higher than at Tpres-res

At site SJ, the concentrations of As(V) was 4.97 µg L-1 at the end of the experiment (after 24 h). This concentration is slightly lower to that measured immediately before resuspension (5.55 µg L-1) suggesting that the As(V) resealed during resuspension was removed from the solution. This was not observed at site SE where As(V) showed higher concentrations at the end of the experiment.
On the basis of your comment, we have modified the sentence in order to better explain the difference between the two sites and we have added further details regarding As(III): "At the end of the experiment, the concentration of As(V) at site SJ (4.97 µg L-1) was found to be slightly lower to that measured before the resuspension (5.55 µg L-1, Tpre-res) suggesting that the As(V) released during resuspension was removed after 24 h at SJ. On the contrary, this is not evident at site SE where As(V) concentration was still higher (56%) at the end of the experiment (15.5 µg L-1) than before the experiment (9.81 µg L-1, Tpre-res). Slightly the same was observed for As(III) which was removed after 8 h at SJ (<lod) and slightly higher (0.29 µg L-1 after 24 h) rather than before the resuspension (0.19 µg L-1, Tpre-res).

Lines 492-493: check this sentence

Thank you for notice this mistake. We have checked and corrected this sentence.

Reviewer #3: The authors investigated metal contents in marine sediments in Spain, and performed resuspension experiments in order to investigate the behavior of potentially toxic trace elements (PTEs) during sediment disturbance, ex dredging. The objectives and the experiments are interesting, and the manuscript is generally well written. However, I would like to suggest possible improvements and comments as follows.
Abstract: it is not generalized, with only fragmented explanation of results of each sediments. Abstract must clearly state the background, objectives and results with a flow of logic, and generalized explanation/interpretation should be provided, instead of independently stating results with each sediment. The conclusion is well summarized, so use some of those statements.

Following your suggestion we have modified the abstract providing a more generalised explanation of the main results of this research avoiding stating results regarding each sampling site.

Introduction: an explanation is lacking as to what is important research question. Sediment resuspension and PTE mobilization is well explained from L55 to 78 based on literature review, but no research question is provided. The explanation in L86-99 and L95-99 have some challenges regarding Hg and As resuspension in Nalon River estuary, but the explanation appear to be a bit weak. L100-L107 is a bit too general. There should be a strong, detailed statement as to what is unknown to date, and therefore what was investigated in this study. They must be explained well in accordance to the employed experimental methods.
Following your suggestion, we have improved the Introduction.
We have adding further information on the investigated area (now Lines 95-106) in order to expand the reasons that we focused on Hg and As. Indeed, Asturias is one of the most important Regions for mining and extraction activity of Hg and coal in Spain. Especially, the Nalòn River drainage basin has been severely affected by long term exploitation of sulphide ores (several mines north from Oviedo) and there are evidences that the sediments not only in the Nalòn estuary (García-Ordiales et al., 2019, 2020), but also along the whole coastline (Forjan et al., 2019; Sanz-Prada et al., 2020) are contaminated by Hg and As mainly by this source.
We have also better highlighted the aim of our study (now Lines 107-128).

L117-150: The description of the targeted three ports could be summarized in a Table, which would be easier to understand their different characteristics.

Following your suggestion, we have added a Table (Table 1) resuming the main characteristics of the investigated sampling sites.

L159: what inert atmosphere means? Which gas?

Inert atmosphere means filled with N2, as already specified few lines above. Following your suggestion, we have modified the sentence specifying that the extracted porewaters were recovered in the inert atmosphere chamber filled with N2.

L245-266: They should be explained in more details to facilitate understanding. Why it is inferred that Fe dissolution drove the release of As and Hg, rather than Mn? What is the reason? The surface sediment of site SE has the most As content among all. If As is mobilized to dissolved phase because of Fe dissolution, the why porewater of upper sediments had low As content but deeper sediments had high As in pore waters? Why porewater Fe (and Mn) had a peak in upper sediments? In L264-265, when Fe reduction occurs, As(V), which was adsorbed in the iron (hydr)oxides, would be immediately released to dissolved phase, but As(V) did not show immediate increase in the upper sediment pore water. Why?

First, Fe concentrations in the sediment is two orders of magnitude higher than Mn, also in the Nalòn R. estuary, where the concentrations of the two elements are the highest (SE and SJ). In these two sites, Fe concentrations are very high in porewaters from the first level (0-1 cm) at SE and from the second level (1-2 cm) at SJ. The dissolved Fe concentrations keep high throughout the porewater profile. It means that redox-conditions facilitate the reduction of the Fe oxy-hydroxides which must be an important component of the Nalòn estuarine sediments compared to the other two estuarine sampling sites. At VV and LL, indeed, Fe in the porewaters is far less abundant compared to SE and SJ but the profiles show peaks for Fe and Mn (not evident for LL) just below the sediment-water interface. We mean that the reduction of Fe and Mn oxy-hydroxides at VV and LL is relatively important only in upper sediments and not in the deeper layers as it is at SE and SJ. At these two sites, Arsenic should be present in association to Fe oxy-hydroxides. The role of Mn oxide phase in terms of reduction and release of adsorbed arsenate into porewaters (Masscheleyn et al., 1991a) appears definitely secondary. Silva et al. (2014) found As incorporated into short-range ordered Fe oxyhydroxides as the predominant fraction at stream sediment samples collected close to the mining sources in the Nalon River drainage basin. Arsenate may then be reduced to arsenite by bacteria in reducing sediments (Dowdle et al., 1996). This is what we observed throughout the sedimentary sequence at SE and SJ sites where Arsenate concentration in porewaters increases in parallel to Arsenite where the dissolved Fe is high. The solubility of As is less limited in estuarine sediments, which often have low concentrations of Sulphur, by formation of insoluble sulphide minerals. Therefore high-As waters are not expected where there is a high concentration of free sulphide (Moore et al., 1988). If sediment Eh drops below about -130 mV, the concentration of dissolved As may increase in sediment porewaters (Neff, 2002 and references therein). Sulphate reduction should not be particularly effective at these sites. This also evident at LL sites, where Hg concentrations in sediments are high but there is an increase in porewaters of dissolved Hg and MeHg with depth. If H2S had been present, Hg as As would have precipitated as sulphides and MeHg production would be inhibited.
On the basis of your comments and our explanations, we have modified/included some sentences in the text at Lines (275-327).

L252: "the metals" specify which metals.

Mercury and As. As suggested, we have specified it in the text.

Fig 2: the Eh values corrected for hydrogen-standard electrode?

No, it’s not necessary. We measured Eh by means of a Pt/KCl–Ag/AgCl electrode calibrated with ZoBell solution (220 mV at 25°C). This description has been added to the text in the Materials & Methods section.

Fig 3 and in the text: I would recommend not to use T1, T2,, but directly indicate 30min, 1h, etc.
Following your suggestion, we have replaced T1-T6 with the real time and wrote "when shaking has stopped (T0)" and "before resuspension (Tpre-res) when necessary. We have also modified the legend in the Fig. 4 by adding the time (T0==min, T1=30 min, T2=1 h, etc.)

Fig 4: the resolution is low.

Following your suggestion, we have improved and split the Fig. 4 in two figures: one for sites LL and VV (now Fig. 5) and one for SJ and SE (now Fig. 6).

3.2.2: Somehow Fig 4 and Fig 3 have similar temporal trends. How much possible that iron and manganese in the dissolved phase, along with other PTE, decreased simply by sedimentation, just like particulate phase in Fig 5? The dissolved samples are already filtered by 0.45 um filters, but some large fraction can be deposited during the first few hours? For example of As(V), given that As(V) has low solubility, it is easily bound to small particles (<0.45um). Therefore, in LL and VV, it is already bound to Fe or other adsorption sites in small particles at the beginning of the resuspension experiment, and deposited altogether?
In L382, how the the authors explain the initial high As(V) concentration immediately after resuspension? It is just a resuspension of As(V)-bound small particles or As(V) is mobilized from small particles? This may concern also the interpretation in 3.3.

In our opinion, the first part of your comment is not properly clear. Indeed, how can be possible that the concentration of a dissolved chemical species decreases simply by sedimentation? Do you mean that the decrease of dissolved iron is due to precipitation following sedimentation of this freshly formed solid phase?
The high concentrations of dissolved Fe could be due to the contribution of porewaters following resuspension. Dissolved Fe and Mn decreased over time at the Nalòn sites reaching concentrations lower than the beginning most likely due to oxidation processes and subsequent precipitation of Fe oxides. Although this was already stated, we have slightly modified the text (now Lines 398-411).
The large particles appear to be deposited during the initial stage of the experiment. Indeed, this is clearly evident from the grain size spectra (Fig. 4) which testify to sedimentation of large particles in the first 30 minutes (between T0 and T1). Then, the grain size spectra tend to move to finer particles as a function of time.
Regarding your comment concerning As(V) in LL and VV, are you talking about dissolved or particulate As(V)? It is not clear. If you are referring to dissolved As(V), a certain amount of As(V) could be bound to colloids (<0.45 µm). This could be verified by ultrafiltration. However, this technique needs large volume of water which is impossible to get from this experimental approach.
Moreover, As solubility decreases during oxidation events as a result of precipitation of As(V), which often occurs coupled with adsorption on the surface of Fe oxy-hydroxides. This was already stated in the text (now Lines 486-489).
The high concentrations of As(V) at the initial stage of the resuspension experiment (when shaking was stopped, T0) could be related to the contribution of porewaters. Indeed, sediment resuspension can affect the equilibrium of the chemical forms in the sediment matrix, inducing changes in the redox conditions, which in turn could favour the

desorption of As from Fe oxy-hydroxides, as well as the reductive dissolution of these minerals, with the subsequent release from sediment porewaters to the overlying water column. We have modified the text on the basis of these comments (now Lines 489-499).

Reviewer #4:
1. The paper is not well written.

Respectfully, we do not agree and it's not the opinion of the other three reviewers.

2. The paper will be far better suited for a different journal such as something more regional or perhaps even a aquatic toxicology journal.

We think that since the topic is related to the coastal marine environment and environmental issues it matches very well with this journal.

3. The paper is based on very small sample sizes with limited replication. I also question the papers reproducibility.

You can reproduce easily analysis but not experiments. Anyhow, we performed 4 experiments although in 4 different sites with the same approach and the reproducibility in terms of trends of parameters is evident.

4. STOTEN does not prioritze laboratory work except in cases where the work is of considerable interest to its broad readership.

Respectfully, this is a matter of the Editor

5. The paper has no hypotheseses presented.

The hypotheses have been rewritten

6. the Introdcution section is not complete with no set of specfic objectives identified.

The Introduction has been revised and improved

7. The grammar and syntax will need a major improvement.

Proofreading was provided by an expert professional mother tongue before submission. The same has been done with this revised version.

8. The paper has a considerable amount of speculation and is based on a vert small set of samples and the investigation has very low sptatitistical power.

The set of samples is not small. Statistical analysis has been added.

9. I found the conclusions in this investigation to not match up well with the data and again there is too much specualtion.

We do not agree. Abstract and Conclusion have been revised according to the useful suggestions of the other three reviewers.

10. I did not fins this paper to be in strong accordance withthe goals of the journal nor the quality expected of papers published by STOTEN.

As stated above at question n° 4.

11. The paper may become publishable, in a more specialized and/or regional journal, after some major revisions and a langauage wash by an expert in Enlgish language.

Proofreading was provided by an expert professional mother tongue before submission. The same has been done with this revised version.

Cover Letter

UNIVERSITÀ
DEGLI STUDI DI TRIESTE

dipartimento
di matematica
e geoscienze

Att. Damià Barceló
Co-Editor in Chief
Science of the Total Environment

Object: revised version article submission to Science of the Total Environment (Ms. Ref. No.: STOTEN-S-20-10086-2)

Dear Prof. Dr. Barceló,

The manuscript entitled ***"Mercury and arsenic mobility in resuspended contaminated estuarine sediments (Asturias, Spain): a laboratory-based study"***, coauthored by *García-Ordiales E., Covelli S., Braidotti G., Petranich E., Pavoni E., Acquavita A., Sanz-Prada L., Roqueñí N.* and *Loredo J.*, has been carefully revised and restructured following the constructive comments made by the reviewers. Respectfully, we did find some of the suggestions made by Reviewer #4 to be a bit vague. However, we have answered each of the questions posed and we believe that the manuscript has improved as a result of the comments and queries. We are grateful to all the reviewers for the time they dedicated to revising our work.

Briefly, as suggested by Reviewer #1, we have clarified some points in the Materials and Methods section where the description of the resuspension experiments is reported. As suggested by the first two reviewers, we have included some statistical analysis (Principal Component Analyses, PCA, Fig. S1 in the Supplementary Material) to support the discussion of our results on sediment and porewaters and simple modelling providing an explanation for the observed decrease of trace element concentrations in the dissolved phase after the simulated resuspension (Figure S2 in the Supplementary Material). In the Introduction section, we have better clarified the aim of our work as suggested by Reviewer#1. The Highlights, along with the Conclusions and Abstract sections, have been revised and adjusted following the suggestions of Reviewers #1, 2 and 3.

In addition, we have added comments and explanations in the "Revision Notes" section regarding minor changes for each point made by the reviewers.

My colleagues and I hope this revised version may be considered for publication in Science of the Total Environment.

Should you require any further information, please do not hesitate to contact us at any time convenient to you.

We look forward to hearing from you.

Best regards,

Stefano Covelli, Dr. Prof.
e-mail: covelli@units.it

Dipartimento di Matematica e Geoscienze - Via Edoardo Weiss 2, 34128 Trieste ITALY
Segreteria Tel. +39 040 558 2045/2264; Fax 2048 P. Iva 00211830328 - Cod. Fisc. 80013890324

Mercury and arsenic mobility in resuspended contaminated estuarine sediments (Asturias, Spain): a laboratory-based study

Efren García-Ordiales[1], Stefano Covelli[2,3]*, Greta Braidotti[2], Elisa Petranich[2], Elena Pavoni[2,4], Alessandro Acquavita[5], Lorena Sanz-Prada[1], Nieves Roqueñí[1], Jorge Loredo[1]

[1]ISYMA Research Group, University of Oviedo, Oviedo, Spain.

[2]Department of Mathematics and Geosciences, University of Trieste, Via E. Weiss 2, 34127 Trieste, Italy

[3]CoN.I.S.Ma. Consorzio Nazionale Interuniversitario per le Scienze del Mare, Piazzale Flaminio 9, 00196 Rome, Italy

[4]Department of Chemical and Pharmaceutical Sciences, University of Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy

[5]ARPA FVG Agenzia Regionale per la Protezione Ambientale del Friuli Venezia Giulia, Via Cairoli 14, Palmanova, 33057 Udine, Italy

*Corresponding author:

Stefano Covelli

Department of Mathematics and Geosciences

University of Trieste

Via Weiss, 2

34128 Trieste (Italy)

Phone: +39.040.5582031

Fax: +39.040.5582203

e-mail: covelli@units.it

Reviewers/Editor comments:

Reviewer #1: The manuscript by García-Ordiales et al., attempts to evaluate the total Hg and As concentrations in both sediments and porewaters in a case study (Asturias, Spain), together with the effects of a mesocosm simulated resuspension in order to predict their behavior in real conditions. Such studies are of great importance for estuaries environmental quality and therefore research on monitoring approaches should be encouraged. However, while I enjoyed the flow of the paper, I could not overcome the sense that there are several issues that should be addressed prior to publication. In this context, the authors should provide more information concerning the methodological approach followed as well as to explain clearly which is the novelty of this work compared to others recently published (even from the same scientific team).

Graphical abstract: The graphical abstract is attractive to the readers and it does reflect the title of the paper and the key findings of the paper.

Thank you!

Highlights: The highlights are very general thus please add some specific/tangible results. To be honest, the last highlight seems so general in principle since e.g. "co-precipitation" is mentioned in the manuscript only 3 times, L. 316, 331 and in the conclusions.

Following your suggestion, the highlights have been rewritten and improved.

Introduction: The novelty of the study should be more highlighted. What is novel/new in this study, compared with other similar studies? What is the contribution of this investigation and it's major findings to understand this type of contamination in other parts in the world rather than in Spain (Asturias)? What kind of local and global knowledge the authors want to improve? Furthermore, which was the reason that the authors concentrated on Hg and As rather than other elements/metals that have been evaluated in their previous studies? These questions aren´t clear in the introduction and need further consideration/explanation.

Asturias is one of the most important Regions for mining and extraction activity of Hg and coal in Spain. Especially, the Nalòn River drainage basin has been severely affected by long term exploitation of sulphide ores (several mines north from Oviedo) and there are evidences that the sediments not only in the Nalòn estuary (García-Ordiales et al., 2019a, 2020), but also along the whole coastline (Forján et al., 2019; Sanz-Prada et al., 2020) are contaminated by Hg and As mainly by this source. On the basis of this comment and following your suggestions, we have improved the Introduction. In detail, we have added further information on the investigated area in order to clarify the reasons that we focused on the behaviour of Hg and As species. We have also better highlighted the primary aim of our study at the end of the Introduction (now Lines 107-128).

"*The primary aim of this research was to investigate the effects of simulated resuspension events in replicate mesocosms to predict the behaviour of Hg and As associated with bottom sediments and porewaters and their fate in the water column after a perturbative event. A comparison among similar estuarine environments in Asturias affected by various sources of contamination and where dredging operations are periodically needed to allow navigation was provided. The occurrence and* *mobility of Hg and As in this area is of great concern due to their potential bioaccumulation in the aquatic trophic chain*" with subsequent high risk of transfer to human beings through consumption of fish and, especially, mussels. In addition, following the specific maritime policy established and directed for marine ecosystem protection and conservation by the EU Marine Strategy Framework Directive, we believe that *this experimental approach could be applied to all coastal environments where information regarding the major geochemical processes that regulate PTE behaviour, mobility and fate is needed to support environmental management and risk assessment*."

Materials and Methods: There are several places where the methods are poorly described and where it cannot be derived the reason why or/and what the authors have done. The clearest example is the description of the resuspension tests. There are quite several protocol leaching methods available in the literature, but it cannot be derived which one have the authors used or they have modified. However, since sealing of the top hole of the chamber was done, how do the authors conducted sampling without avoiding conduct with air or liberation of gases? In this context, how long was the benthic chamber left open and undisturbed between one sampling and the next one? Cover reposition before each sampling do not avoid the aforementioned problems. Moreover, how many bottom sediments and overlying water were collected? Please elaborate these points in detail.

Following your suggestions, we have improved the description of the methods section.

Regarding the protocol for the resuspension experiment, we have specified that we followed the experimental approach applied by Acquavita et al. (2012) (now Lines 184-186). We have also specified that the overlying water was carefully replaced with bottom water collected from the same sampling location in order to get the starting conditions as they effectively were and avoiding then any possible perturbative effect due to the transport of the chamber to the lab for the experiment (now Lines 189-193). This procedure was also successfully employed in other similar studies (e.g. Bertuzzi et al., 1996; Emili et al., 2011; Acquavita et al., 2012). We have added this information in the text.

The benthic chamber was left open and undisturbed between one sampling and the next one with the aim to simulate real environmental conditions where the water column is in direct contact with the atmosphere at the water-atmosphere interface. In addition, the benthic chamber was not sealed between one sampling and the next one in order to avoid possible oxygen consumption with subsequent alteration of the pyhsico-chemical boundary conditions of the system. Indeed, significant decrease in the oxygen concentration was pointed out by previous incubation experiments during which the benthic chamber was sealed (Emili et al., 2011, 2014).

Although fluxes of trace elements at the water-atmosphere interface could occur, we retain that the liberation of gases (e.g. gaseous dissolved Hg^0) may be negligible since the concentration of Hg^0 is generally very low in sediments, water column and porewaters. Then, in order to allow the collection of the water just above the sediment-water interface (SWI), the cover was repositioned before each sampling. Indeed, the cover has two valves on its lid and one of them is connected to a tube that allows the sample collection at a defined depth, just above the SWI. Consequently, the cover has to be repositioned in order to be sure that the sampling is done at the same depth during the resuspension experiment. The sampling has to be done at the same depth during the experiment. This is extremely important for properly evaluate variations of the investigated parameters (Eh, dissolved and particulate PTEs) as well as the grain size composition of the suspended particles which tend to become finer with time due to settling of resuspended particles.

We have improved this description in the text (now Lines 198-213).

The benthic chamber was left open and undisturbed between one sampling and the next one. This means that, for instance, the benthic chamber was left open for 30 min between the sample T_0 (when shaking has stopped) and T_1 (after 30 min). Then, the benthic chamber was left open for more 30 min between sample T_1 (after 30 min) and T_2 (after 1 h) and so on. Seven samples were collected within 24 h (T0 = 0, T1 = 30 min; T2 = 1h; T3 = 2h; T4 = 4h; T5 = 8h; T6 = 24h). This was already stated in the text (now Lines 208-209). For each sampling, the dissolved phase was separated from the suspended particles which were collected for both grain-size and chemical analyses. We have slightly modified the text in order to make this more clear (now Lines 211-213).

Bottom sediments were not collected during the resuspension experiment. Short sediment cores were collected in parallel with the benthic chamber at the same site to get a picture of the situation (sediment and porewater composition) before the resuspension event. The cores were extruded and sectioned into five slices (0-1, 1-2, 2-3.5, 3.5-5 and 5-7 cm) in a N_2-filled chamber. This was already written at the beginning of the section *2.2 Experimental approach* (now Lines 175-183).

Results and discussion: This section needs strengthening to improve clarity.

- The authors discuss about the possible sources of the effect of resuspension and the subsequent remobilisation and redistribution of As and Hg species. However, they only make general assumptions without providing arguments that support one or more of these assumptions in the specific area. Even though a plethora of data is provided for an in-depth geochemical study via geochemical modelling/kinetics only hypothesis and assumptions such as L. 358-359 for Hg " This could be attributed both to MeHg released from porewaters or to the mobilisation of bioavailable OM during resuspension " along with L. 384-385 for As " The prevalence of As(V) may be related to oxidation and/or adsorption processes mediated by Fe and Mn oxy-hydroxides, which represent very efficient oxidants of As(III) " are provided. How both outcomes derived and experimentally proved? The linkages presented here between are too loose, and would be strengthened considerably by a geochemical modelling analysis to conclusively demonstrate the crucial role of Fe in relation to As and Hg mobilisation and how this may affect pore water/sediment quality. A detailed geochemical modelling study will also help to identify the most critical parameters that affect either primary or secondary sources of iron and distinguish them on a real basis based on the obtained results.

In order to provide a statistical and modelling support to our hypothesis and assumptions through the different sectors of our article, we have added:

1. Principal component analysis (PCA) which was performed on both the sediment matrix (Fig. S1A) and porewaters (Fig. S1B).

We have added a brief explanation "*2.4 Explorative multivariate data analysis*" in the Materials and Methods section (now Lines 247-255).

We have improved the section *3.1. Sediment and porewater chemistry* by adding further information provided by the PCA performed both on the sediment matrix and porewaters:

Regarding the sediment matrix, the PCA output (Fig. S1A, explaining 84.7% of the total variance) clearly denotes the differences among the different sites which are distinctly represented in the biplot although disparities in trace element concentrations along the sediment cores are not so evident.

As already reported in the text, no significant differences were observed for Hg at sites VV, SJ and SE (mostly lower than 1.0 mg kg^{-1}), whereas the element was found to be one order of magnitude higher at site LL (3.73 ± 0.76 mg kg^{-1}). This is also confirmed by the PCA output showing a quite evident relation between Hg and site LL (Fig. S1A). Slightly the same is also true in the case of Sulphur (which is higher at LL and VV), Fe and Mn (which showed the highest values at sites SJ and SE): these relationships are clearly supported by the PCA output. We have added this information in the text.

Regarding the porewater samples, oxidative conditions were found in the surnatant water (Eh ranged from 132 to 161 mV as well as quite low dissolved Hg, MeHg and As species, followed by Fe and Mn. This is clearly evident in the PCA output (explaining 71.1% of the total variance) highlighting a strong relation between the redox conditions and the surnatant water of all sites (Fig. S1B). In addition, the PCA output clearly highlighted strong correlations between As(III) and As(V) (r=0.919) and between As species and dissolved Fe (r=0.770 and r=0.757 for As(III) and As(V), respectively) (Fig. 2B). This suggested that the dissolution of Fe oxy-hydroxides could be responsible for the release of dissolved As species rather than Mn (r=0.171 and r=0.131 for As(III) and As(V), respectively). We have added this information in the text.

2. Equations describing the behaviour of dissolved As and Hg species as a function of time with the final aim to better describe if and after how long the system returns to the initial conditions (pre-resuspension) (Fig. S2).

The behaviour of dissolved As(III), As(V), Hg and MeHg during the resuspension experiment is described by a negative exponential equation:

$$C_{Ti} = C_{T0}\, e^{-kx}$$

where C_{Ti} is the concentration at any time (T_i) during the resuspension experiment, C_{T0} is the concentration after shaking (T_0) and k is the rate constant for the contaminant dispersion as a function of time (Fig. 6). The equation (1) was calculated until a decreasing trend was observed and was not applied when the concentration after shaking (T_0) was higher than the concentration measured before the resuspension experiment (T_{pre}). Indeed, the final aim was to verify after how long the system gets the initial conditions (pre-resuspension).

We have added a brief explanation in the section *3.2.2 Behaviour of dissolved chemical species after resuspension* (now Lines 417-426) and we have added further details in the sections *Behaviour of dissolved mercury species* (now Lines 441-445) and *Behaviour of dissolved arsenic species* (now Lines 500-509).

- Yes, we all know that L. 315-317 " In oxic conditions, Fe and Mn are present as oxyhydroxides and are often involved in adsorption processes, coprecipitation, but when reducing conditions occur there is a dissolution with the subsequent release of dissolved PTEs " but arguing that L. 329-330-" This could be related to the crucial role of Fe oxides as surface catalysts in the oxidation of Mn " is far too simple. It's much more complicated than that and therefore studies such as modelling, leaching tests and analytical characterization tests have been developed to take all possible factors and feedback mechanisms into account to answer these questions.

The first sentence you are referring to (now Lines 390-393) is at the beginning *of the section 3.2.2 Behaviour of dissolved chemical species after resuspension* and it is only introductory. We can agree with you saying that the role of Fe and Mn oxy-hydroxides can be more complex.

On the basis of your comment (second sentence you are referring to), we have slightly modified the text by adding a more detailed description of the role of Fe oxides in regulating dissolved Mn concentrations during a resuspension event (now Lines 403-416). Moreover, the mineralogical composition of the sediments from the Nalón River estuary is reported in García-Ordiales et al. (2017): it is mainly quartz, which is predominant in the coarse fractions, and kaolinite-smectite, illite, albite, and vermiculite in the finer fractions. The chemical concentrations in the sediments reported as oxides are clearly dominated by SiO_2 (66.3%) and Al_2O_3 (8%), in addition to the significant presence of light amounts of Fe_2O_3 (3.7%), as expected based on the mineralogical analysis. Detected concentrations of MnO were consistently less than 1% and were not directly correlated with the fine fraction.

Although modelling, leaching tests and analytical characterisation tests (honestly, what do you mean with “analytical characterisation tests”? It is not properly clear for us) have been applied in other studies, the approach we have followed has been previously applied with success. This procedure allowed us to provide further information on As and Hg mobility in different environmental conditions.

-Another issue worthy of further discussion is the interrelated issue of contaminants and sediment cores surface/interparticle mass transfer. This is again related to the dynamic nature of "release from sediment to porewaters" and is closely linked to the issue of temporally "bonding" loss under different conditions that resulted in remobilisation and redistribution.

Unfortunately, the comment of the reviewer is not properly clear. If we have understood we are asked for a further discussion about the dynamics of the remobilisation of the elements from porewaters and sediment particles after the resuspension event. The simulation showed us that, not always, the release of trace elements from porewaters associated with desorption from particles is effective and there is also an antagonism between release/desorption and reprecipitation/readsorption processes. This is why we have introduced a simple modelling for calculating time necessary to go back to concentrations in dissolved form in the situation pre-resuspension.

Other comments

- Part of the conclusion is a direct repetition of the abstract e.g. "Part of the Hg and As released from porewaters and/or desorbed during resuspension was presumably removed by the subsequent adsorption onto the settling fine particles and/or co-precipitated in association with Fe and Mn oxy-hydroxides". Please modify the whole conclusion accordingly.

Please, we apologise for this inaccuracy and thank you for noticing it. We have decided to improve and modify the abstract also on the basis on the comments of the other Reviewers. We did not modify the conclusions since the Reviewer #3 stated that is well summarized but we have included an additional comment about the time necessary to restore the pre-resuspension As and Hg concentrations.

- Fig.1. Please provide GIS coordination grid in the provided map of the study area

We have added the following Table (Table 1) resuming the main characteristics of the investigated sampling sites including latitude and longitude.

Table 1. Resuming of the main characteristics of the investigated sampling sites along the Asturian coast: the Nalón River estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL).

Sampling site		San Juan (SJ)	San Esteban (SE)	Villaviciosa (VV)	Llanes (LL)
Location		port areas in the Nalón R. estuary		port area 60 km E of the Nalón R. estuary	port area 110 km E of the Nalón R. estuary
Latitude		43º 33´ 21.56" N	43º 33´ 4.90" N	43º 31´ 31.54" N	43º 25´13.04" N
Longitude		6º 4´ 37.51" W	6º 5´ 40" W	5º 23´ 21.20" W	4º 45´12.02" W
Depth (m)		3.20	4.50	2.40	3.80
Water circulation		open estuarine area	sheltered harbour	sheltered harbour	
Sediment grain size*	sand	26.4 %	4.92 %	3.91 %	27.4 %
	mud	73.6 %	95.1 %	96.1 %	72.6 %
As in sediments (μg g^{-1})		20.1 - 68.1**		average 11.3***	-
Hg in sediments (μg g^{-1})		0.10 - 1.33**		average 0.18***	> 3.81***

*: average grain size composition, this study; **: Garcìa-Oridales et al., 2018, 2019b; ***: unpublished data

- Some figures can be notably improved e.g. Fig.4 (split and use bigger graphs, not easily readable). Please make titles in both axes large enough such that the parameters are readable. Use bigger font size both in titles and legends.

Following your suggestion, we have improved and split the Fig. 4 in two figures: one for sites LL and VV (now Fig. 5) and one for SJ and SE (now Fig. 6).

- Limitation and scope of the study should be provided at the end. Limitations aren't weaknesses but can be possibilities for other studies - highlight them as necessary.

Following your suggestion, we have better highlighted the primary aim of our study at the end of the Introduction (now Lines 107-128) where we have also added further information on the investigated area in order to clarify the reasons that we focused on the behaviour of Hg and As (now Lines 95-106).

Regarding the limitations of our study, we had already stated in the text (see the last sentence of the Conclusions) that it would be useful to monitor the water column quality during dredging operations *in situ* and to compare the results with the mesocosm approach. In our opinion, this represent an extremely difficult task to perform since one has to study an open system which is much more complex compared to the mesocosm experiment.

- Authors should carefully revise the reference list as some citations are not reported and some others mentioned in the list are not present in the text e.g. L. 609 "García-Ordiales et al. 2020"

As suggested, we have carefully revised the reference list and deleted those citations that were not present in the text.

- L. 137. Full spot after "González-Fernández et al., 2018)", L. 175. Please change "hours" into "h), L.176. Please erase dot before parenthesis. Please choose "co-precipitation" or "coprecipitation" throughout the text.

We have modified the text following your suggestions. We have changed "hours" into "h" and coprecipitation" to "co-precipitation" throughout the text.

Reviewer #2: This paper investigates at lab conditions the remobilization of Hg and As after sediment resuspension, mimicking dredging. This type of papers are of scientific interest and fits well with the scope of the journal. My main concerns about the suitability of this work are related to its novelty and the absence of mineralogical/geochemical evidences to support their findings. Other works dealing with As and Hg mobility after sediment resuspension has been previously published. In addition, the authors discuss their analytical results without providing mineralogical composition of sediments or performing any sequential extraction to guess the affinity of As and Hg in the sediments. A statistical analysis of results could provide more robustness to result interpretation. On the other hand, the authors must take the most of data; for example, a geochemical modelling of porewater-sediment interaction could improve the quality of this paper.

First, there are a few papers dealing with Hg and, especially, As mobility after resuspension events associated mainly to dredging operations. A similar issue raised by the first reviewer and we have explained that the novelty of this paper is the idea to compare similar estuarine environments affected by different sources of contamination related to Hg and As. If the Nalòn River drainage basin has been severely impacted by long term exploitation of sulphide ores and coal the whole coastline further east is contaminated by Hg and As mainly by this source. The main ore mineral of these deposits is cinnabar in association with native Hg, stibnite [Sb_2S_3], and important amounts of As-bearing minerals such as realgar and pararealgar [As_4S_4], orpiment [As_2S_3], and arsenic-rich pyrite (Ordoñez et al. 2014).

In addition, we investigated also two other estuaries, Villaviciosa and Llanes, where the contamination is related to other anthropogenic sources (e.g. the Aviles port area) in addition to mining. A statistical analysis (PCA) has been added to the discussion to make our interpretation more robust.

We did not perform mineralogical analyses in our study but several information can be obtained from previous studies. From the geological point of view, at the basin level, the hydrographic system of the Nalòn River flows through Paleozoic bedrock from the Cambrian to the Upper

Carboniferous periods (García-Ordiales et al., 2017). The western sector of the river basin is characterized by the presence of low-grade metamorphic silico-clastic rocks interbedded with a calcareous series of grey limestones and dolomites, whereas, in the eastern section, calcareous and siliciclastic series predominate. The Villaviciosa and Llanes estuaries belong to the second main set of rocks which is considered a mixed section with limestones, sandstones, quartzite and lutite of Permian-Mesozoic and Paleogene-Neogene periods.

The mineralogical composition of the sediments from the Nalón River estuary is reported in García-Ordiales et al. (2017): it is mainly quartz, which is predominant in the coarse fractions, and kaolinite-smectite, illite, albite, and vermiculite in the finer fractions. The total chemical concentrations in the sediments reported as oxides are clearly dominated by SiO_2 (66.3%) and Al_2O_3 (8%), in addition to the significant presence of light amounts of Fe_2O_3 (3.7%), as expected based on the mineralogical analysis. Detected concentrations of MnO were consistently less than 1% and were not directly correlated with the fine fraction thus indicating that its variability is not directly related to the aluminosilicate minerals which, conversely, are correlated with total Fe concentrations.

Villaviciosa and Llanes located in the eastern area of Asturias also matched with another anomalous geological materials rich in As and Hg. This area is formed by materials from the ages between the Carboniferous and Jurassic in which hydrothermal processes generated ores (mainly fluorite) with metal sulphides rich in this element (Sanz-Prada et al., 2020). The same authors explained that regarding Hg, most of the sandy beach samples, whose concentrations were above the ERL (Effect Range Low) level, were from the eastern region where the lithology of the area is rich in carbonated rocks. Thus, it appeared that Hg concentrations could be related to this kind of geology because there are few industrial activities which could have affected this area. Forján et al. (2020) reported high concentrations of Hg and Ba on Vega beach, 30 km further east the Llanes estuary. Both elements belong to the fluorite ore paragenesis, mined in the surroundings. Hg was enriched in the finer fractions, and overall the main input was attributed to the mining waste discharged along the coast in the past. The district of Berbes, was in the 20th century, the most productive fluorite mining area not only of Asturias, but also of Europe because of the outcropping of important mineral ores along much of its perimeter. The paragenesis of the mineralization in this area is composed by fluorite (CaF_2) and abundant barite ($BaSO_4$). Furthermore, cinnabar (HgS) is occasionally present in the form of fine inclusions in the fluorite and copper minerals (chalcopyrite, fahlore and Cu-carbonates are also abundant). Other potential contaminants are arsenic (As), related to the iron and copper sulfides in the area.

In literature, we can find results about speciation of As and Hg in fluvial sediments of the Nalon River drainage basin (Larios et al., 2012; Silva et al., 2014). The second authors report that “in spite of a higher As accumulation in the finest particle-size subsamples, As fractionation did not seem to depend on grain size since similar distribution profiles were obtained for the studied granulometric fractions. The presence of As was relatively low in the most mobile forms. Arsenic was predominantly linked to short-range ordered Fe oxyhydroxides, coprecipitated with Fe and partially with Al oxyhydroxides and associated with structural material in mine waste samples. Arsenic incorporated into short-range ordered Fe oxyhydroxides was the predominant fraction at stream sediment samples, representing more than 80 % of total As…Arsenic is retained in sediments mainly as highly stable amorphous iron oxyhydroxide–arsenic complexes, which can be present in a wide range of grain size classes.”

Highlights:

Rewrite the highlight #2 as follows: "As and Hg released from porewaters due to dissolution of Fe-Mn oxy-hydroxides"

Following your suggestion, we have modified the highlight #2.

Rewrite the highlights #4 and #5

As you suggested, we have rewritten the highlights.

Line 31: not only contaminated sediments must be dredged to allow navigation.

Following your suggestion, we have modified the sentence and deleted the word “contaminated”.

Lines 31-50: I miss here a quantification of changes in concentrations of elements following resuspension (e.g. 20% increase at t= X, etc.)

Following your suggestion and on the basis on the comments of Reviewer #3 we have modified the abstract providing a more generalised explanation of the main results of this research as well as maximum release and removal percentages observed for As and Hg species during the resuspension experiments.

Line 82: I do not see the point how investigating resuspension of estuarine sediments could contribute to improve air quality.

In estuarine environments, fluxes of trace elements at the water-atmosphere interface could occur (e.g. Floreani et al., 2019; O’Driscoll et al., 2019) and we retain that the processes involved in these fluxes are of concern. However, we agree with your observation. Since our research is primarily focused on the sediment-water interface we decided to delete the word “air” from that sentence.

Lines 82-85: the authors must explain the novelty of this study compared with previous similar experiments?

As already reported following a question of the reviewer #1, Asturias is one of the most important Regions for mining and extraction activity of Hg and coal in Spain. Especially, the Nalòn River drainage basin has been severely affected by long term exploitation of sulphide ores (several mines north from Oviedo) and there are evidences that the sediments not only in the Nalòn estuary (García-Ordiales et al. 2019, 2020), but also along the whole coastline (Forjan et al., 2019; Sanz-Prada et al. 2020) are contaminated by Hg and As mainly by this source. Besides, we investigated also two other estuaries where the contamination is related to other anthropogenic sources in addition to mining. The novelty is not the method itself but the comparison similar estuarine environments affected by different sources of contamination and where dredging operations are periodically needed to allow navigation.

Line 156: what the authors probably measured was ORP instead of Eh, right? And why did the authors not measure also pH? It is a critical parameter in metal solubility.

Technically, there is no difference between ORP and Eh. ORP is used for measurements in aquatic environments and Eh for water and other environmental systems. We measured Eh by means of a Pt/KCl–Ag/AgCl electrode calibrated with ZoBell solution (220 mV at 25°C). This description has been added to the text (now Lines 194-195).

We have added the Eh values in Table 2.

pH was measured in the surnatant water and in the water in the benthic chamber before and during the resuspension experiment (Table S2). Regarding the sediment samples, pH was measured only at sites SJ and SE (Table B).

Table B. pH values measured in the sediment cores collected at the Nalòn River estuary (sites SJ and SE).

Sediment layer (cm)	SJ	SE
Surnatant water	7.56	7.68
0-1	7.40	7.46
1-2	7.51	7.51
2-3.5	7.42	7.34
3.5-5	7.32	7.38
5-7	7.35	7.28

We agree with you saying that pH is also a critical parameter in metal solubility. However, slight variations with increasing depth were observed: 7.43 ± 0.09 at SJ and 7.44 ± 0.14 at SE.

In this context, Fiket et al. (2019), in a study strictly focused on the geochemistry of porewaters, collected two sediment cores: one for geochemical analysis and the other one for determination of physico-chemical parameters (Eh and pH). According to the authors, pH values in the sediment porewaters display more uniform distribution (from 7.18 to 7.83, RSD < 1.5%) along the depth profiles with respect to Eh. The authors also pointed out that the pH does not seem to have such an important influence in determining the levels of several trace elements in the sediment porewaters whereas Eh plays an important role.

For these reasons we decided not to include the pH values in our study.

Lines 165-167: why did the authors empty the water from the corers and take new water with a Niskin bottle? It seems to be more realistic to use the overlying water.

The overlying water was drained off from the benthic chamber (now Lines 189-193) and replaced with bottom water collected from the same sampling location using a Niskin bottle following the experimental approach applied by Acquavita et al. (2012). The overlying water was replaced in order to avoid any possible perturbation effect during the transport of the chamber from field to the laboratory and to get the undisturbed starting conditions. This procedure was also successfully employed in other similar studies (e.g. Bertuzzi et al., 1996; Emili et al., 2011; Acquavita et al., 2012). We have added these details in the text.

Lines 167-168: was dissolved oxygen monitored?

Unfortunately, the oxymeter was out of order.

Line 170: which is the reason for selecting the resuspension time as 10 min? based on previous studies or mimicking real dredgings?

We selected a resuspension time of 10 min following previous research (e.g. Acquavita et al., 2012; Bataillard et al., 2014). Indeed, we retain that 10 min of shaking may be enough to allow the resuspension of the surface sediments which usually is not compacted and quite fluid, also in terms of visual observation of the experiment. For instance, Saulnier and Mucci (2000) demonstrated that a significant amount of As is already released in solution after 6 min of resuspension.

Lines 199-200: the treatment of the solid sample with ascorbic and phosphoric + microwave acid was done for total composition determination? I see in lines 205-206, that total composition was performed by acid mixtures and microwave.

As already stated few lines above ("The As species were analysed using…"), the treatment you are referring to was performed for the analyses of the As species (As(III) and As(IV)) in the solid samples. Since we needed a solution for HPLC-ICP-MS analyses, 0.1 g of sample was placed together with an extracting agent (1 M H_3PO_4+0.1 M, ascorbic acid) in a microwave vessel (Ruiz-Chancho et al., 2005). The digested solution was then analysed.

We have slightly modified the sentence writing "The As species were directly measured in the porewater samples, whereas in the case of solids, 0.1 g of sample was placed together with an extracting agent (1 M H_3PO_4+0.1 M, ascorbic acid) in a microwave vessel. The digested solution was analysed following the aforementioned method." Now Lines 229-233.

The analytical determination of the total concentrations of As, Fe, Mn and S was also performed after acid digestion in a close microwave system but using aqua regia and HF, following the EPA method 3052. This was already written in the text.

Line 239: Eh or ORP?, please clear it up. If they are Eh values, I am not sure if those can be considered as oxidative conditions.

As explained in the comment related to line 156, we measured Eh. They are oxidative conditions compared to the reducing conditions found in the sediments.

Line 244: such Eh decrease causes a reductive dissolution of Fe/Mn oxides rather than desorption processes.

We have modified the sentence following your suggestion.

Lines 245-247: did the authors performed a statistical analysis to support this fact?

In order to provide statistical support to the traditional data interpretation, principal component analysis (PCA) was performed on both the sediment matrix (Fig. 2A) and porewaters (Fig. 2B). We have added a brief explanation "Explorative multivariate data analysis" in the Materials and Methods section.

Regarding your comment concerning porewaters, the PCA output (biplot explaining 71.1% of the total variance, Fig. 2B) highlighted a strong correlation between As species and dissolved Fe (r=0.919 between As(III) and As(V), r=0.770 between As(III) and Fe, r=0.757 between As(V) and Fe). In order to be consistent, r values reported in the text are referred to the entire set of data (LL, VV, SJ and SE). We have added this information in the text.

In order to provide you further information, we have also checked the correlations between dissolved As species and Fe at each sampling site. As species reached higher concentrations at the Nalòn estuary sites (SJ and SE) and showed strong correlations at all sites (r=0.941 at LL, r=0.689 at VV, r=0.971 at SJ, r=0.954 at SE). This is consistent with the results depicted in the PCA output. Weak correlation was found between dissolved As species and Fe at sites LL (r=0.346 and r=0.193 for As(III) and As(V), respectively) and VV (r=0.438 and r=0.470 for As(III) and As(V), respectively). Conversely, As species appear to be strong correlated with dissolved Fe at sites SJ (r=0.837 and r=0.890 for As(III) and As(V), respectively) and SE (r=0.526 and r=0.585 for As(III) and As(V), respectively).

Lines 258-260: does the authors have any evidence of the presence of these mineral phases in the sediments?

We did not perform any mineralogical analysis. As reported above, the mineralogical composition of the sediments from the Nalón River estuary is reported in García-Ordiales et al. (2017): it is mainly quartz, which is predominant in the coarse fractions, and kaolinite-smectite, illite, albite, and vermiculite in the finer fractions. The chemical concentrations in the sediments reported as oxides are clearly dominated by SiO_2 (66.3%) and Al_2O_3 (8%), in addition to the significant presence of light amounts of Fe_2O_3 (3.7%), as expected based on the mineralogical analysis. Detected concentrations of MnO were consistently less than 1% and were not directly correlated with the fine fraction thus indicating that its variability is not directly related to the aluminosilicate minerals which, conversely, are correlated with Fe_2O_3. These data are consistent with the results we obtained from our analyses where Mn concentrations in the sediments are two orders of magnitude lower than Fe concentrations.

Lines 257-266: according to the author's belief, which redox pair may control the redox condition in the sediments, S(-II)-S(VI), Fe(III)-Fe(II), As(V)-As(III) or that of Mn?

The redox conditions in our sediments are mainly controlled by the redox pairs $Fe(OH)_3/Fe^{2+}$ considering the pH value of about 7.3-7.4 and the Eh negative values. In addition, reducing conditions favor the much more hazardous chemical form of arsenite (As^{+3}) and this is visible in our porewater profiles where there is an increase of concentrations with depth ($HAsO_4^{2-}/H_3AsO_3$). The redox pair SO_4^{2-}/HS^- can be present but our conditions are not strongly reducing to reach sulphate reduction. In these conditions, at the pH of the seawater and with available carbonate species, solid manganese could be present in form of rhodocrosite $MnO_2(s)+ HCO_3 +3H^+ +2e^- = MnCO_3(s)+2H_2O$ whereas at the same conditions Fe is controlled more by the sulphides. Usually, the Eh-pH relations show relatively large field of stability for dissolved Mn2+ compared to the solid oxides MnO_2.

Lines 278-281: again, does the authors have statistical evidence of this relatiship?

In order to provide statistical support to the traditional data interpretation, principal component analysis (PCA) was performed on both the sediment matrix (Fig. 2A) and porewaters (Fig. 2B). We have added a brief explanation “Explorative multivariate data analysis” in the Materials and Methods section.

Regarding your comment concerning the relationship between Hg and S in the sediment matrix, the PCA output (explaining 84.7% of the total variance, Fig. 2A) highlighted a strong correlation between Hg and S (r=0.628). Moreover, these elements also showed a clear relationship with the sediment core collected at site LL where the highest concentrations of Hg and S were observed. This is not surprising since most of the Hg of mining origin was present as cinnabar (HgS).

However, at site LL, no correlation was found between the two elements (r=0.297 at site LL) contrary to what was observed at sites VV (r=0.959), SJ (0.577) and SE (r=0.732). In this context, it is reasonable to expect that the occurrence of Hg at site LL may be related to a different local source which is currently under investigation.

Indeed, high concentrations of Hg were detected at the Vega beach (approximately 40 km west of site LL) due to the discharge of mine tailings from the district of Berbes which was one of the main fluorite mining areas in Europe where cinnabar was occasionally present (Symons et al., 2017; Forjan et al., 2019; Leuresse et al., 2019). According to Florjan et al. (2019), a marine outfall located 660 m away from the average low tide level has been build up for the discharge since 1990s. This suggests that fine suspended particles enriched in Hg could be transported by the ocean and shoreline currents and transported into the estuarine environment at site LL.

We have improved the text and added furhter information regarding the source of Hg at site LL (now Lines 341-352).

Line 295 onwards: please replace T1, T2…. the real time (15 min, 1h, 2h, etc.)

Following your suggestion, we have replaced T1-T6 with the real time and wrote “when shaking has stopped (T0)” and “before resuspension (Tpre-res) when necessary.

Lines 329-332: could the authors discard diffusion into the sediment of more metal-rich waters after the resuspension? As metal-rich porewaters seem to be diffused into the water column during the resuspension, the opposite effect could be also produced. Could the authors give their opinion on that?

We agree with you since also influxes of trace elements from the overlying water into the sediment could occur. Unfortunately, we did not investigate these processes and we actually do not have any experimental evidence to proof if a certain influx of trace elements could occur. However, we retain that influx of trace elements into the sediment during a resuspension event is a much more slow mechanism compared to other geochemical processes such as precipitation and adsorption on the surface of the resuspended particles.

Line 368: a lower?

We have modified the text following your suggestion.

Lines 370-371: which is the reason of this evolution?

At site SE, an increase of dissolved MeHg and a decrease of dissolved Hg were observed during the resuspension experiment. We retain that this evolution is the result of methylation processes which appear to prevail at site SE with respect to the other investigated sites. At site SE, the MeHg concentrations during the resuspension were found to be one order of magnitude higher than those measured in the sediment porewaters. This suggests that a certain amount of MeHg may be produced during the resuspension experiment and/or released from the resuspended fine particles due to desorption processes. Indeed, previous research at the Nalòn River estuary stated that the highest levels of MeHg in the surface sediments occurred at site SE (García-Ordiales et al., 2018). The same authors stated also that only low amounts of the THg are available for methylation. However, changes in environmental conditions (e.g. hydrological events) or anthropogenic activities (e.g. dredging) may alter the original unperturbed conditions with subsequent remobilization of PTEs from bottom sediments through resuspension. Moreover, at site SE the highest concentrations of inorganic Hg were observed (three orders of magnitude higher with respect to the other sites) as well as elevated amounts of SO_4^{2-} and the increase of DOC due to OM degradation during the resuspension experiment. Since sediments can show different methylation potential (Bloom et al., 2003) which depends on several factors such as the bacterial activity, the physico-chemical boundary conditions, the occurrence of OM and the concentration of inorganic Hg available for methylation it is reasonable to suppose that sediment resuspension may promote methylation processes at site SE.

We have improved this discussion in the text (now Lines 464-480)

Lines 401-402: which is the cause of inhibition? The high concentration of sulphate or the availability of high concentrations of sulphate to be reduced instead of As(V).

The availability of high concentrations of sulphate to be reduced instead of As(V). On the basis of your comment we have modified the sentence as follows “Moreover, the availability of high concentrations of SO_4^{2-} (>2,000 mg L^{-1}) to be reduced can inhibit the reduction of As(V) to As(III)”. Now Lines 518-520.

Lines 408-408: why is not possible here the oxidation of DOC and conversion into DIC?

We should take into consideration that only a small part of DOC is bioavailable or biodegradable by bacteria. The remaining part is recalcitrant to degradation as it is made by fulvic and humic acids and a myriad of macro- and micromolecular compounds also highly ramified and less active. This is why we think that dilution due to porewaters can explain the low concentrations after the resuspension but we cannot exclude a degradation to DIC although this process is not so fast and influent due to what reported above.

Lines 430-432: and associated to what?

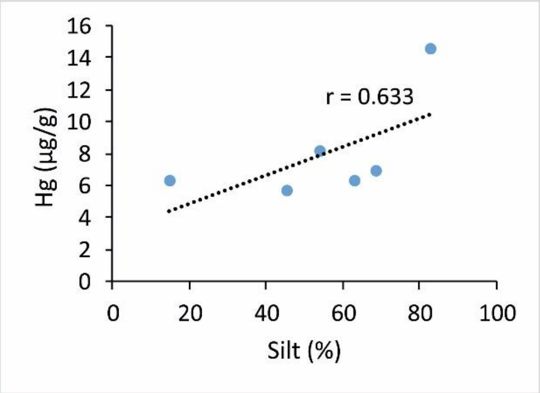
At all the investigated sites, particulate PTEs showed decreasing trends as the result of the settling of the resuspended particles as a function of time. We have modified the sentence in order to better highlight this behaviour (now Lines 548-562).

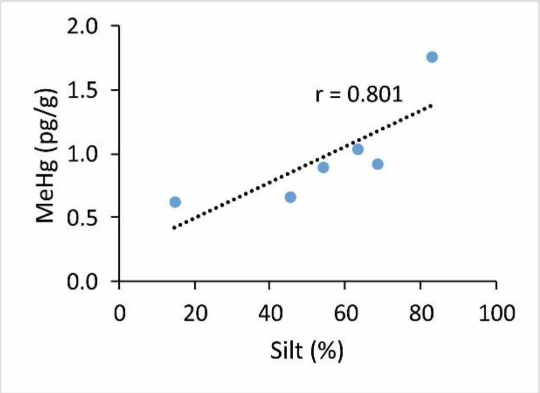
Lines 438-439: how the authors could support this fact? Have the authors any information from sequential extraction or mineralogical evidences?

Unfortunately, we have no information from sequential extraction or mineralogical evidences.

The fact that Hg and MeHg were bound to the silty fraction is supported by the grain size spectra and composition (see Fig. 4) which tends to move to finer particles as a function of time. At site SE, these finer particles appear to be enriched in Hg and MeHg and, consequently, their concentrations (µg g^{-1}) increase as the particles become finer. We already stated in the text that "by comparing the particulate PTE trends with TSM content, a preferential bound to the silty or clayey fraction was found (now Lines 553-554). We slightly modified the text specifying that the fact that particulate Hg and MeHg concentrations increased is consistent with the evolution of the grain size spectra and composition (Fig. 4) as a function of time and it indicates that Hg and MeHg were bound to both the silty and clayey fractions (now Lines 556-562).

Moreover, this fact is also confirmed by the significant correlations between the percentage of silt and particulate Hg and MeHg during the resuspension experiment at site SE:





Line 476: As(V) was totally removed? If so, I do not see how it could be higher than at Tpres-res

At site SJ, the concentrations of As(V) was 4.97 µg L^{-1} at the end of the experiment (after 24 h). This concentration is slightly lower to that measured immediately before resuspension (5.55 µg L^{-1}) suggesting that the As(V) resealed during resuspension was removed from the solution. This was not observed at site SE where As(V) showed higher concentrations at the end of the experiment.
On the basis of your comment, we have modified the sentence in order to better explain the difference between the two sites and we have added further details regarding As(III): "At the end of the experiment, the concentration of As(V) at site SJ (4.97 µg L^{-1}) was found to be slightly lower to that measured before the resuspension (5.55 µg L^{-1}, $T_{pre\text{-}res}$) suggesting that the As(V) released during resuspension was removed after 24 h at SJ. On the contrary, this is not evident at site SE where As(V) concentration was still higher (56%) at the end of the experiment (15.5 µg L^{-1}) than before the experiment (9.81 µg L^{-1}, $T_{pre\text{-}res}$). Slightly the same was observed for As(III) which was removed after 8 h at SJ (<lod) and slightly higher (0.29 µg L^{-1} after 24 h) rather than before the resuspension (0.19 µg L^{-1}, $T_{pre\text{-}res}$).

Lines 492-493: check this sentence

Thank you for notice this mistake. We have checked and corrected this sentence.

Reviewer #3: The authors investigated metal contents in marine sediments in Spain, and performed resuspension experiments in order to investigate the behavior of potentially toxic trace elements (PTEs) during sediment disturbance, ex dredging. The objectives and the experiments are interesting, and the manuscript is generally well written. However, I would like to suggest possible improvements and comments as follows.

Abstract: it is not generalized, with only fragmented explanation of results of each sediments. Abstract must clearly state the background, objectives and results with a flow of logic, and generalized explanation/interpretation should be provided, instead of independently stating results with each sediment. The conclusion is well summarized, so use some of those statements.

Following your suggestion we have modified the abstract providing a more generalised explanation of the main results of this research avoiding stating results regarding each sampling site.

Introduction: an explanation is lacking as to what is important research question. Sediment resuspension and PTE mobilization is well explained from L55 to 78 based on literature review, but no research question is provided. The explanation in L86-99 and L95-99 have some challenges regarding Hg and As resuspension in Nalon River estuary, but the explanation appear to be a bit weak. L100-L107 is a bit too general. There should be a strong, detailed statement as to what is unknown to date, and therefore what was investigated in this study. They must be explained well in accordance to the employed experimental methods.

Following your suggestion, we have improved the Introduction.

We have adding further information on the investigated area (now Lines 95-106) in order to expand the reasons that we focused on Hg and As. Indeed, Asturias is one of the most important Regions for mining and extraction activity of Hg and coal in Spain. Especially, the Nalòn River drainage basin has been severely affected by long term exploitation of sulphide ores (several mines north from Oviedo) and there are evidences that the sediments not only in the Nalòn estuary (García-Ordiales et al., 2019, 2020), but also along the whole coastline (Forjan et al., 2019; Sanz-Prada et al., 2020) are contaminated by Hg and As mainly by this source.

We have also better highlighted the aim of our study (now Lines 107-128).

L117-150: The description of the targeted three ports could be summarized in a Table, which would be easier to understand their different characteristics.

Following your suggestion, we have added a Table (Table 1) resuming the main characteristics of the investigated sampling sites.

Table 1. Resuming of the main characteristics of the investigated sampling sites along the Asturian coast: the Nalón River estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL).

Sampling site		San Juan (SJ)	San Esteban (SE)	Villaviciosa (VV)	Llanes (LL)
Location		port areas in the Nalón R. estuary		port area 60 km E of the Nalón R. estuary	port area 110 km E of the Nalón R. estuary
Latitude		43º 33´ 21.56" N	43º 33´ 4.90" N	43º 31´ 31.54" N	43º 25´13.04" N
Longitude		6º 4´ 37.51" W	6º 5´ 40" W	5º 23´ 21.20" W	4º 45´12.02" W
Depth (m)		3.20	4.50	2.40	3.80
Water circulation		open estuarine area	sheltered harbour	sheltered harbour	
Sediment grain size*	sand	26.4 %	4.92 %	3.91 %	27.4 %
	mud	73.6 %	95.1 %	96.1 %	72.6 %
As in sediments (µg g^{-1})		20.1 - 68.1**		average 11.3***	-
Hg in sediments (µg g^{-1})		0.10 - 1.33**		average 0.18***	> 3.81***

*: average grain size composition, this study; **: Garcìa-Oridales et al., 2018, 2019b; ***: unpublished data

L159: what inert atmosphere means? Which gas?

Inert atmosphere means filled with N_2, as already specified few lines above. Following your suggestion, we have modified the sentence specifying that the extracted porewaters were recovered in the inert atmosphere chamber filled with N_2.

L245-266: They should be explained in more details to facilitate understanding. Why it is inferred that Fe dissolution drove the release of As and Hg, rather than Mn? What is the reason? The surface sediment of site SE has the most As content among all. If As is mobilized to dissolved phase because of Fe dissolution, the why porewater of upper sediments had low As content but deeper sediments had high As in pore waters? Why porewater Fe (and Mn) had a peak in upper sediments? In L264-265, when Fe reduction occurs, As(V), which was adsorbed in the iron (hydr)oxides, would be immediately released to dissolved phase, but As(V) did not show immediate increase in the upper sediment pore water. Why?

First, Fe concentrations in the sediment is two orders of magnitude higher than Mn, also in the Nalòn R. estuary, where the concentrations of the two elements are the highest (SE and SJ). In these two sites, Fe concentrations are very high in porewaters from the first level (0-1 cm) at SE and from the second level (1-2 cm) at SJ. The dissolved Fe concentrations keep high throughout the porewater profile. It means that redox-conditions facilitate the reduction of the Fe oxy-hydroxides which must be an important component of the Nalòn estuarine sediments compared to the other two estuarine sampling sites. At VV and LL, indeed, Fe in the porewaters is far less abundant compared to SE and SJ but the profiles show peaks for Fe and Mn (not evident for LL) just below the sediment-water interface. We mean that the reduction of Fe and Mn oxy-hydroxides at VV and LL is relatively important only in upper sediments and not in the deeper layers as it is at SE and SJ. At these two sites, Arsenic should be present in association to Fe oxy-hydroxides. The role of Mn oxide phase in terms of reduction and release of adsorbed arsenate into porewaters (Masscheleyn et al., 1991a) appears definitely secondary. Silva et al. (2014) found As incorporated into short-range ordered Fe oxyhydroxides as the predominant fraction at stream sediment samples collected close to the mining sources in the Nalon River drainage basin. Arsenate may then be reduced to arsenite by bacteria in reducing sediments (Dowdle et al., 1996). This is what we observed throughout the sedimentary sequence at SE and SJ sites where Arsenate concentration in porewaters increases in parallel to Arsenite where the dissolved Fe is high. The solubility of As is less limited in estuarine sediments, which often have low concentrations of Sulphur, by formation of insoluble sulphide minerals. Therefore high-As waters are not expected where there is a high concentration of free sulphide (Moore et al., 1988). If sediment Eh drops below about -130 mV, the concentration of dissolved As may increase in sediment porewaters (Neff, 2002 and references therein). Sulphate reduction should not be particularly effective at these sites. This also evident at LL sites, where Hg concentrations in sediments are high but there is an increase in porewaters of dissolved Hg and MeHg with depth. If H_2S had been present, Hg as As would have precipitated as sulphides and MeHg production would be inhibited.

On the basis of your comments and our explanations, we have modified/included some sentences in the text at Lines (275-327).

L252: "the metals" specify which metals.
Mercury and As. As suggested, we have specified it in the text.

Fig 2: the Eh values corrected for hydrogen-standard electrode?

No, it’s not necessary. We measured Eh by means of a Pt/KCl–Ag/AgCl electrode calibrated with ZoBell solution (220 mV at 25ºC). This description has been added to the text in the Materials & Methods section.

Fig 3 and in the text: I would recommend not to use T1, T2,, but directly indicate 30min, 1h, etc.

Following your suggestion, we have replaced T1-T6 with the real time and wrote “when shaking has stopped (T0)” and “before resuspension (Tpre-res) when necessary. We have also modified the legend in the Fig. 4 by adding the time (T0==min, T1=30 min, T2=1 h, etc.)

Fig 4: the resolution is low.

Following your suggestion, we have improved and split the Fig. 4 in two figures: one for sites LL and VV (now Fig. 5) and one for SJ and SE (now Fig. 6).

3.2.2: Somehow Fig 4 and Fig 3 have similar temporal trends. How much possible that iron and manganese in the dissolved phase, along with other PTE, decreased simply by sedimentation, just like particulate phase in Fig 5? The dissolved samples are already filtered by 0.45 um filters, but some large fraction can be deposited during the first few hours? For example of As(V), given that As(V) has low solubility, it is easily bound to small particles (<0.45um). Therefore, in LL and VV, it is already bound to Fe or other adsorption sites in small particles at the beginning of the resuspension experiment, and deposited altogether?

In L382, how the the authors explain the initial high As(V) concentration immediately after resuspension? It is just a resuspension of As(V)-bound small particles or As(V) is mobilized from small particles? This may concern also the interpretation in 3.3.

In our opinion, the first part of your comment is not properly clear. Indeed, how can be possible that the concentration of a dissolved chemical species decreases simply by sedimentation? Do you mean that the decrease of dissolved iron is due to precipitation following sedimentation of this freshly formed solid phase?

The high concentrations of dissolved Fe could be due to the contribution of porewaters following resuspension. Dissolved Fe and Mn decreased over time at the Nalòn sites reaching concentrations lower than the beginning most likely due to oxidation processes and subsequent precipitation of Fe oxides. Although this was already stated, we have slightly modified the text (now Lines 398-411).

The large particles appear to be deposited during the initial stage of the experiment. Indeed, this is clearly evident from the grain size spectra (Fig. 4) which testify to sedimentation of large particles in the first 30 minutes (between T0 and T1). Then, the grain size spectra tend to move to finer particles as a function of time.

Regarding your comment concerning As(V) in LL and VV, are you talking about dissolved or particulate As(V)? It is not clear. If you are referring to dissolved As(V), a certain amount of As(V) could be bound to colloids (<0.45 μm). This could be verified by ultrafiltration. However, this technique needs large volume of water which is impossible to get from this experimental approach.

Moreover, As solubility decreases during oxidation events as a result of precipitation of As(V), which often occurs coupled with adsorption on the surface of Fe oxy-hydroxides. This was already stated in the text (now Lines 486-489).

The high concentrations of As(V) at the initial stage of the resuspension experiment (when shaking was stopped, T0) could be related to the contribution of porewaters. Indeed, sediment resuspension can affect the equilibrium of the chemical forms in the sediment matrix, inducing changes in the redox conditions, which in turn could favour the desorption of As from Fe oxy-hydroxides, as well as the reductive dissolution of these minerals, with the subsequent release from sediment porewaters to the overlying water column. We have modified the text on the basis of these comments (now Lines 489-499).

Reviewer #4:

1. The paper is not well written.

Respectfully, we do not agree and it’s not the opinion of the other three reviewers.

2. The paper will be far better suited for a different journal such as something more regional or perhaps even a aquatic toxicology journal.

We think that since the topic is related to the coastal marine environment and environmental issues it matches very well with this journal.

3. The paper is based on very small sample sizes with limited replication. I also question the papers reproducibility.

You can reproduce easily analysis but not experiments. Anyhow, we performed 4 experiments although in 4 different sites with the same approach and the reproducibility in terms of trends of parameters is evident.

4. STOTEN does not prioritze laboratory work except in cases where the work is of considerable interest to its broad readership.

Respectfully, this is a matter of the Editor

5. The paper has no hypotheseses presented.

The hypotheses have been rewritten

6. the Introdcution section is not complete with no set of specfic objectives identified.

The Introduction has been revised and improved

7. The grammar and syntax will need a major improvement.

Proofreading was provided by an expert professional mother tongue before submission. The same has been done with this revised version.

8. The paper has a considerable amount of speculation and is based on a vert small set of samples and the investigation has very low sptatitistical power.

The set of samples is not small. Statistical analysis has been added.

9. I found the conclusions in this investigation to not match up well with the data and again there is too much specualtion.

We do not agree. Abstract and Conclusion have been revised according to the useful suggestions of the other three reviewers.

10. I did not fins this paper to be in strong accordance withthe goals of the journal nor the quality expected of papers published by STOTEN.

As stated above at question n° 4.

11. The paper may become publishable, in a more specialized and/or regional journal, after some major revisions and a langauage wash by an expert in Enlgish language.

Proofreading was provided by an expert professional mother tongue before submission. The same has been done with this revised version.

Mercury and arsenic mobility in resuspended contaminated estuarine sediments (Asturias, Spain): a laboratory-based study

Efren García-Ordiales[1], Stefano Covelli[2,3]*, Greta Braidotti[2], Elisa Petranich[2], Elena Pavoni[2,4], Alessandro Acquavita[5], Lorena Sanz-Prada[1], Nieves Roqueñí[1], Jorge Loredo[1]

[1]ISYMA Research Group, University of Oviedo, Oviedo, Spain.
[2]Department of Mathematics and Geosciences, University of Trieste, Via E. Weiss 2, 34127 Trieste, Italy
[3]CoN.I.S.Ma. Consorzio Nazionale Interuniversitario per le Scienze del Mare, Piazzale Flaminio 9, 00196 Rome, Italy
[4]Department of Chemical and Pharmaceutical Sciences, University of Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy
[5]ARPA FVG Agenzia Regionale per la Protezione Ambientale del Friuli Venezia Giulia, Via Cairoli 14, Palmanova, 33057 Udine, Italy

*Corresponding author:

Stefano Covelli

Department of Mathematics and Geosciences

University of Trieste

Via Weiss, 2

34128 Trieste (Italy)

Phone: +39.040.5582031

Fax: +39.040.5582203

e-mail: covelli@units.it

Field Code Changed

~~April~~ June 2020

Formatted: English (United States)

ABSTRACT

~~Contaminated e~~Estuarine sediments must be dredged in order to allow for navigation, and where these sediments are placed after dredging depends upon guidelines based only on the total concentration of contaminants. However, resuspension events could seriously affect the mobility and speciation of contaminants, including potentially toxic trace elements stored in sediments.

The effects of resuspension on the cycling of mercury (Hg) and arsenic (As) between the sediment and water column was investigated in a mesocosm study. Four experiments were conducted in three estuaries in~~of~~ northern Spain~~,~~ based on samples collected from sites which have been impacted by now decommissioned Hg and As mines and periodically subjected to dredging activities. Designed to mimic the resuspension of particles, each of the experiments revealed that the release of Hg and As species does not depend on the total concentration in the sediments (16.3-50.9 mg kg^{-1}, for As and 0.52-5.01 mg kg^{-1} for Hg). The contribution from porewaters and the subsequent reductive dissolution and/or desorption appears to be the main processes responsible for the ~~An~~ abrupt increase in ~~particulate and~~ dissolved Hg and As species (maximum release of 427 % and 125 %, respectively)~~ was observed following a resuspension event which involved both contaminated resuspended particles and the release of these species from porewaters~~. ~~At San Esteban, in the Nalón River estuary~~In some cases, ~~the concentrations of~~ As and Hg ~~in the water column~~ continued to remain~~ed~~ at high concentrations in the water column even after the experiments were completed (after 24 h~~ours~~), thus testifying to their critical persistence in the dissolved form. ~~A similar trend was observed for Hg in the port of Llanes.~~ Conversely, at the other sites, ~~the effects of resuspension lasted only a few hours and were followed by~~ the restoration of pre-resuspension conditions were observed only a few hours after resuspension, mainly due to the role of Fe oxy-hydroxides which provides suitable surfaces for adsorption and/or co-precipitation processes involving dissolved Hg (maximum removal of -58 %) and As (maximum removal of -25 %) species. ~~Part of the Hg and As released from porewaters and/or desorbed during resuspension was presumably removed by the subsequent adsorption onto the settling fine particles and/or co-precipitated in association with Fe and Mn oxy-hydroxides.~~ The results of this research could be helpful for policy making and to take appropriate decisions regarding dredging.

Keywords: estuarine sediments, mercury, arsenic, contamination, resuspension, dredging

1. Introduction

Estuaries are crucial areas for sediment transfer between fluvial and marine systems, often forming sinks for sediment moving downstream, alongshore or landwards (Ridgway and Shimmield, 2002). A wide range of human activities take place in these sites (i.e., ports, industrial, urban and recreational settlements) and, as a result, estuarine waters receive dissolved and particulate contaminants, including potentially toxic trace elements (PTEs). Fine-grained sediments often become a repository for contaminants by reducing their toxicity (Eggleton and Thomas, 2004 and references therein). However, ~~N~~natural events such as tidal currents, wave action, storm surge and bioturbation (Sanford et al., 1991; Arfi et al., 1993; Kalnejais et al., 2007), along with anthropogenic activities ~~such as~~including dredging, shipping and trawling (Schoellhamer, 1996; Lewis et al., 2001), may be responsible for the resuspension of sediment particles. In particular, dredging and disposal are common practices to maintain navigation channels and access to port areas, but they may impact upon aquatic communities (Roberts, 2012) since contaminants can be released in dissolved form in the water column (Stephens et al., 2001; Caplat et al., 2005). When resuspended in the oxic water column, anoxic sediments may result in variable desorption rates of PTEs previously co-precipitated with/or adsorbed to Fe and Mn sulphides (Simpson et al., 1998;

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Caetano et al., 2003). The released Fe and Mn are quickly reprecipitated, acting as scavengers for PTEs and other contaminants (Caetano et al., 2003; Jones-Lee and Lee, 2005). This precipitation process will involve any particle available with a layer of Fe oxy-hydroxide, previously mobilised by the oxidation of sulphides, and PTEs will bind to the newly created adsorption surfaces of the settling particles (Goossens and Zwolsman, 1996). The extent of sediment resuspension and dispersal depends on the water movements in nearby dredging/disposal areas (Eggleton and Thomas, 2004). The modification of the physico-chemical properties (i.e., pH and redox conditions) can affect not only the mobility, but also the bioavailability of toxic compounds with adverse effects on aquatic organisms (Kim et al., 2004, 2006; Cotou et al., 2005; Bocchetti et al., 2008). Investigation into the potential mobility and bioavailability of contaminants in estuarine sediments is a challenging but essential task, since the understanding of the processes involved in the recycling of these contaminants between solid and aqueous phases may contribute to the preservation of water~~/air~~ quality by informing environmental policies. To better evaluate these issues, most research has taken the form of laboratory experiments which simulate the dredging effects using different time intervals (e.g. Van Den Berg et al., 2001; Caetano et al., 2003; Cantwell et al., 2008; Monte et al., 2015; de Freitas et al., 2019).

Among PTEs, mercury (Hg) and arsenic (As) are well known for the recognised toxicity of their different chemical species and diffusion in the environment (i.e., industrial settlements, mining activity). There are several studies on Hg (and methylmercury, MeHg) remobilisation after resuspension from bottom sediments (e.g. Bloom and Lasorsa, 1999; Conaway et al., 2003; Kim et al., 2004; Benoit et al., 2009; Acquavita et al., 2012; Seelen et al., 2018; Zhu et al., 2018). In particular, Bloom and Lasorsa (1999) found that approximately 5% of MeHg bound to the sediment and less than 1% of total Hg were released during a laboratory mixing experiment, but the release can be limited in quantity and time due to the dilution of the Hg species in the water body and their reburial in the solid phase (Acquavita et al., 2012).

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

The Asturias coast (northern Spain) represents one of the most impacted regions of Spain due to several anthropogenic activities, including long-term mining of sulphide ore, which have severely affected the Nalòn River drainage basin, the main hydrographical system (3692 km^2) of the region (Loredo et al., 1999; Loredo, 2000; Fernández-Martínez et al., 2005). The sources and distribution of trace elements, especially As and Hg, have been investigated in several environmental matrices including soils, mine tailings, fresh and ground waters and estuarine sediments (e.g. Loredo et al., 2010; Ordóñez et al., 2014; Silva et al., 2014; García-Ordiales et al., 2018). In the Nalón River estuary, in spite of the high levels of As and Hg in the fine sediments, ~~the high presence of sulphides and~~ the redox conditions appear to govern the speciation cycles of these elements, thus reducing the formation of the most toxic species, such as As(III) and MeHg (García-Ordiales et al., 2018). In addition, there is evidence along the Asturian coasts that other minor estuarine systems may be potentially affected by Hg and As contamination (Forján et al., 2019; García-Ordiales et al., 2019a, 2020; Sanz-Prada et al., 2020). ~~However, no experimental evidence is available on the effects of possible resuspension events.~~

~~In this study, the total Hg and As concentrations in the sediments and porewaters, together with~~ The primary aim of this research was to investigate the effects of ~~a mesocosm~~ simulated resuspension events in replicate mesocosms ~~were investigated~~ to predict the behaviour of Hg and As associated with bottom sediments and porewaters and their fate ~~these PTEs~~ in the water column after a perturbative event. A comparison among~~ at four selected sites.~~ similar estuarine environments in Asturias affected by various sources of contamination and where dredging operations are periodically needed to allow navigation was provided. The occurrence and mobility of Hg and As in this area is of great concern due to their potential bioaccumulation in the aquatic trophic. Within the European Union, the Water Framework Directive (WFD, European Parliament, Council of the European Union, 2000) sets “good status” objectives for water bodies throughout the member states. The status is simply based on chemical and ecological criteria. A classification system has

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: English (United States)

Formatted: Not Highlight

Formatted: Not Highlight

been developed to decide upon chemical status, with threshold values known as "Environmental Quality Standards" (EQS). A specific maritime policy was later established and directed for marine ecosystem protection and conservation by the EU Marine Strategy Framework Directive (MSFD) (European Parliament, Council of the European Union 2008). The MSFD specifically requests that member states implement monitoring programs for the assessment of the environmental status of marine waters. The results of this experimental study ~~obtained following this experimental approach~~ may be useful in providing the scientific background to help policy-makers take appropriate decisions regarding dredging and to control ~~As and Hg~~PTE contamination levels. ~~Controlling As and Hg levels~~This is especially relevant due to the biological richness of estuaries, their significance as sites of a wide variety of food intended for human consumption (fisheries, aquaculture, mussel collection), and the possible implications for the health and economy of affected areas. This experimental approach could be applied to all coastal environments where information regarding the major geochemical processes that regulate PTE behaviour, mobility and fate is needed to support environmental management and risk assessment.

Formatted: Not Highlight

2. Materials and Methods

2.1. Study area

The experimental sites are located in three estuaries found in eastern central Asturias (Fig. 1), which has one of the best-preserved coastline environments in Spain. As part of this conservation policy, these estuaries are supported by several environmental protection programmes and strategic EU initiatives such as the Habitats Directive and the Natura Network 2000 (92/43/EC). Each of these estuaries has diverse characteristics (Table 1).

The Nalón River estuary

The Nalón River empties into the largest drainage basin in the northern Spain. Its estuary has been subject to very high levels of contamination due to mining which took place over a period of 150 years (García-Ordiales et al., 2019a). The riverine flow to the estuary is high, showing a notable water column stratification that may extend several kilometres (Ceñal and Flor, 1993). The estuary has two port areas which are exposed to the main channel flows, which in turn affects the grain size of the sediments. The San Juan (SJ) port area is quite open and the accumulated bottom sediments are mostly sandy, whereas the San Esteban (SE) port area has a protection dock which acts as a sedimentary trap for silt and clay (García-Ordiales et al., 2018). García-Ordiales et~~.~~ al. (2018, 2019b) reported high concentrations of As (20.1~~0~~-68.1~~0~~ μg g^{-1}) and Hg (0.10-1.33 μg g^{-1}) in bottom sediments and the potential transfer to biota.

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

The Villaviciosa estuary

The Ria de Villaviciosa (VV) is located approximately 60 km east of the Nalón estuary. The drainage basin extends for 160 km^2 and the estuarine area is 12.6 km^2. The freshwater flow rate is low and the tidal influence is strong, producing a total mixing, the absence of water column stratification and a high sedimentary accumulation rate (Flor et al., 1996). Recently, as a consequence of wastewater discharge, mussel collection has been banned. Other potential anthropogenic contamination sources are not known. Conversely, in nearby areas there are different outcrops of bituminous rocks and hydrothermal ores of fluorite that could potentially be the sources of As and Hg (Iglesias and Loredo, 1994; González-Fernández et al., 2018). Preliminary sediment analyses performed by the regional port authority have reported average concentrations of 11.3 μg g^{-1} for As and 0.18 μg g^{-1} for Hg (unpublished data).

Formatted: Not Highlight

Formatted: Not Highlight

The Llanes port

The Llanes (LL) port is approximately 110 km east of the Nalón estuary and 50 km east of VV estuary. Similar to VV, the tidal influence in LL is high, producing a lack of stratification in the water column due to the almost null contributions of freshwater from the inflowing Carroced creek. The sedimentary dynamics are controlled by an access gate between the old (inner) and new (outer) port areas. The tidal flow restriction produced by the gate has converted the old port area, where sediments were collected for this study, into a sedimentary trap of fine sediments similar to SE. Over the last 15 years, sediment monitoring conducted by the regional port authority has shown the presence of high Hg concentrations (up to 3.81 µg g^{-1}) (unpublished data). Potential local sources of Hg are currently under investigation.

2.2. Experimental approach

A scuba diver collected undisturbed short sediment cores by pushing a Plexiglass tube (30 cm length; 16 cm i.d.) into the bottom sediment. Upon collection and transport to the laboratory, these cores were extruded and sectioned into five slices (0-1, 1-2, 2-3.5, 3.5-5 and 5-7 cm) in a N_2-filled chamber to preserve the original redox conditions. At the same time, the redox potential (Eh) was measured in the surnatant water and the corresponding slices. These slices were homogenised and split to determine water content. The remaining sediment was centrifuged at *in situ* temperature and the extracted porewaters, recovered in the inert atmosphere (N_2-filled chamber), were filtered (0.45 µm pore size, i.d. = 33 mm, Millex-HV), collected in acid pre-cleaned vials and stored in a deep-freeze until analyses.

In parallel, bottom sediments and the overlying water were collected by a scuba diver using a cylindrical Plexiglas chamber as a sampler (h = 25 cm, i.d. = 24 cm, wall thickness = 0.6 cm) following the experimental approach applied by Acquavita et al. (2012). The average depth of sediments inside the chamber was 10 cm with approximately 15 cm of overlying water (ratio

Formatted: Not Highlight

water/sediment = 1.5). Careful transportation of the chamber to the laboratory minimised perturbation at the sediment-water interface (SWI). Here the overlying water was drained off and carefully replaced with bottom water collected from the same sampling location using a Niskin bottle. This step was taken in order to exclude any possible perturbative effect on the water inside the chamber possibly occurring during transportation (e.g. Bertuzzi et al., 1996; Emili et al., 2011; Acquavita et al., 2012). The basic physico-chemical parameters (T, pH, Eh) were measured using a portable multiprobe (Crison MP41). Eh measurements were performed by means of a Pt/KCl–Ag/AgCl electrode calibrated with ZoBell solution (220 mV at 25ºC).

The simulated resuspension was performed using a mechanical horizontal shaker (130-150 cycles min^{-1}, t = 10 min) after sealing the top hole of the chamber. Periodic sampling of the resulting mixture between dissolved and particulate phases was then performed. In detail, the benthic chamber was left open and undisturbed between one sampling and the next to simulate real environmental conditions where the water column is in direct contact with the atmosphere. In order to allow the collection of water just above the SWI, ~~and~~ the cover was repositioned before each sampling. In detail, the cover has two valves on its lid, one being connected to a tube (0.5 cm diameter) that allows for water sample collection at approximately 5 cm just above the SWI. This is important to properly evaluate variations of the dissolved and particulate concentration of chemical species as a function of time, as well as the grain size composition of the suspended particles which tend to become finer after the resuspension event. Using a plastic syringe, water lying on top of the sediment was syphoned off via one of the two valves on the lid of the chamber and placed in a pre-treated borosilicate container (V_f = 600 mL). Seven samples were recovered within 24 h~~ours~~ (T_0 = 0, T_1 = 30 min; T_2 = 1h; T_3 = 2h; T_4 = 4h; T_5 = 8h; T_6 = 24h~~.~~) and the main physico-chemical parameters were promptly measured. Samples were then centrifuged (10,000 rpm min^{-1}, t = 10 min; Eppendorf 5804) to separate the dissolved phase from the suspended particles. The solid fraction ~~latest~~ were collected for both grain size and chemical analyses. The dissolved phase was

Formatted: English (United States)

Formatted: English (United States), Not Highlight

Formatted: English (United States)

subsequently filtered (0.45 µm pore size, i.d. = 33 mm, Millex-HV) and divided for the necessary analytical aliquots.

2.3. Analyses of samples

Grain-size analyses on bottom sediments and resuspended particles were performed following the method outlined by García-Ordiales et al. (2017). The sediment aliquots were oxidised (H_2O_2=3%, t=24 h) to remove most of the organic matter (OM). Successively, the solution was wet-sieved through a 2-mm sieve to remove coarse, shelly fragments. The fraction < 2000 µm was recovered and analysed using a laser difractometer (Fritsch Anaysette 22 Laser-Particle Sizer Microtec).

Formatted: Not Highlight

The measurement of the isotopic composition of Hg was carried out via gas chromatography combined with inductively coupled plasma mass spectrometry (7890A Agilent GC and HP 7500c Agilent ICP-MS). The determination of the elemental species concentrations was carried out by species-specific isotope dilution mass spectrometry. All sample preparation procedures were previously developed and validated for water (Amouroux et al., 1998, Rodríguez-González et al., 2002, Bouchet et al., 2011) and sediments (~~Rodríguez-González et al., 2007; ~~Rodríguez Martin-Doimeadios et al., 2003; Rodríguez-González et al., 2007). The mathematical approach of the methods was described in detail in Rodríguez-González et al. (2013).

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

The As species were analysed using a mobile phase of 2 M phosphate buffered saline (PBS)/0.2 M EDTA (pH 6.0) in a separation column with a 1260 Infinity high-performance liquid chromatograph (HPLC) coupled with ICP-MS. The As species were directly measured in the porewater~~Liquid~~ samples~~ were directly measured~~, whereas in the case of solids, 0.1 g of sample was placed together with an extracting agent (1 M H_3PO_4+0.1 M, ascorbic acid) in a microwave vessel (Ruiz-Chancho et al., 2005). The digested solution was analysed following the aforementioned method. The

Formatted: Not Highlight

accuracy of the results was verified by comparing the total As concentration to the sum of all the determined species concentrations. The recovery of As speciation ranged from 95% to 106%.

Total As, Fe, Mn and S concentrations in dissolved and solid phases (bottom sediments and resuspended sediment particles) were determined by ICP-MS and ICP-OES. For solids, samples were previously digested by *aqua regia* + HF in a microwave vessel (EPA method 3052). Samples were analysed in batches, and the accuracy of the elemental determination was verified using the CRM042-056 and other internal laboratory standards. Recovery percentages of the different elements were in the range of 93–106%, and the RSD was less than 7%. The dissolved organic carbon (DOC) was determined using the TOC-V CSH (Shimadzu) instrument.

2.4. Explorative multivariate data analysis

Principal component analyses (PCA) was employed as an unsupervised exploratory chemometric tool for the visual identification of relationships among the samples (PC scores and score plots), within variables (PC loadings and loading plots), and between samples and variables (biplots) (Oliveri et al., 2020). In order to minimise systematic differences between variables, data matrices were pre-processed (column autoscaling) before the multivariate analysis (Oliveri et al., 2019). Multivariate analysis was performed using the CAT (Chemometric Agile Tool) package, based on the R platform (The R Foundation for Statistical Computing, Vienna, Austria) and freely distributed by Gruppo Italiano di Chemiometria (Italy) (Leardi et al., 2019).

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

3. Results and Discussion

3.1. Sediment and porewater chemistry

Grain-size distribution was found to be different among the sampling sites and rather constant with increasing depth (Table ~~1~~2, Fig. S1A). According to Shepard’s (1954) classification, the sediment

Formatted: Not Highlight

consisted mostly of silt, which was found to be higher than 90% at VV and SE, followed by SJ and LL which appeared to be less homogeneous with depth. The clayey fraction was rather constant with depth and generally poorly represented, whereas the sandy fraction clearly prevailed at LL and SJ (Table 2~~1~~, Fig. S1A).

The Asturian estuarine systems are known to be affected by high levels of anthropogenic pressure (García-Ordiales et al., 2018, 2019a, b), thus the sediments collected displayed elevated concentrations of both As and Hg (Table 2~~1~~). Although disparities in trace element concentrations along the sediment cores collected at the investigated sites are not particularly evident, the PCA output (explaining 84.7% of the total variance) clearly denotes the differences among the various sites and are distinctly represented in the biplot (Fig. S1A). Arsenic was found to be rather homogeneous downcore, with the lone exception being site SE, where As rapidly decreased from 50.9 mg kg^{-1} (0-1 cm) to 31.8 mg kg^{-1} (1-2 cm). No significant differences were observed for Hg at sites VV, SJ and SE (mostly lower than 1.0 mg kg^{-1}), whereas the element was found to be one order of magnitude higher at site LL (3.73 ± 0.76 mg kg^{-1}) as also confirmed in the PCA output showing a relatively clear relationship between Hg and site LL (Fig. S1A). Silva et al. (2014) reported that As incorporated into short-range ordered Fe oxy-hydroxides was the predominant fraction in the stream sediment samples of the Nalòn River drainage basin. This fraction represented more than 80 % of the total As concentration, although realgar and pararealgar [As_4S_4], orpiment [As_2S_3], and arsenic-rich pyrite were the original sulphide ore deposits (Ordoñez et al., 2014).

Similarly, Fe, Mn and S were rather constant moving downcore, except for the weak increase observed for Mn at SE. Comparable S concentrations were found among sites, although slightly higher values were detected at LL and VV. Conversely, Fe and Mn showed the highest concentrations at the Nalón River estuary sites, especially at SE (Fig. S1A).

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Estuarine sediments suffer intense chemical, physical and biological reactions due to the interaction between the solid phase and porewaters, promoting the formation of new and altered minerals and/or changes in the porewater composition (Beck et al., 2008; Oliveri et al., 2016). In this context, Fe and Mn oxy-hydroxides represent suitable adsorptive phases for PTEs, especially under oxidising conditions (Turner et al., 2004). Conversely, under conditions of oxygen depletion, Fe and Mn oxy-hydroxides may act as secondary oxidant sources during OM degradation (Froelich et al., 1979) and their subsequent reduction/dissolution could be responsible for the release of dissolved PTEs in porewaters.

Oxidative conditions were found in the surnatant water (Eh ranged from 132 to 161 mV) as well as quite low total dissolved Hg, MeHg and As (III and V) species, followed by Fe and Mn (Fig. 2). This is clearly evident in the PCA output (explaining 71.1% of the total variance) highlighting a strong relationship between the redox conditions and the surnatant water collected from all sites (Fig. S1B). Although some differences occurred among the sites, OM degradation and slow oxygen diffusion through the sediment drove reductive conditions just below the SWI. Redox potential (Eh) decreased downcore at all sites (up to almost - 400 mV) ~~as well as~~whereas dissolved As and Hg species (at LL) and MeHg (0-2 cm) at site SE ~~(Fig. 2)~~increased with depth (Fig. 2 and S1B), due to ~~desorption and/or~~reductive dissolution of Fe ~~and Mn~~ oxy-hydroxides (Smedley and Kinniburgh, 2002; Fiket et al., 2019). In detail~~deed~~, the strong correlations ~~similar trend~~ between As(III) and As(V) (r = 0.919) and between As species and dissolved Fe (r =0 .770 and r = 0.757 for As(III) and As(V), respectively) highlighted in the PCA output (Fig. S1B) ~~observed at site SE~~ could be explained as ~~the~~ Fe dissolution, rather than Mn (r = 0.171 and r = 0.131 for As(III) and As(V), respectively), drove the release of As ~~and Hg~~ species especially at the Nalòn estuary sites (SE and SJ). This is also consistent with the fact that the role of Mn oxide in the reduction and subsequent release of adsorbed As(V) into porewaters definitely appears to be secondary (Masscheleyn et al., 1991) confirming that As should be mainly released from Fe oxy-hydroxides at sites SJ and SE.

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Conversely, at site VV, a marked release of dissolved Fe and Mn in the upper sediment (520-1,360 μg L^{-1} and 299-828 μg L^{-1}, respectively) appears not to have affected the dissolved Hg and As species that, in fact, showed constant trends with depth (Fig. 2). Moreover, no variability was noticed in the sediments downcore for As, Hg, Fe and Mn. It is possible that their behaviour in the sediment did not result in Fe and Mn oxy-hydroxides precipitation. Due to highly reducing conditions (- 400 mV), ~~the metals~~Hg and As could be present as insoluble or very low soluble sulphur compounds, as also suggested by the strong correlation between Hg and S in the sediment matrix (r = 0.628, Fig. S1A) (Fang and Chen, 2015~~6~~; Wang et al., 2016; Petranich et al., 2018).

Formatted: Not Highlight

Dissolved As species were found to be high in the Nalón ~~River~~ estuary (SJ and SE, Fig. S1B), where both As(V) and As(III) notably increased with depth reaching concentrations of 231 and 67.3 μg L^{-1} at SE whereas~~:~~ lower values were found at SJ (179 and 32.9 μg L^{-1}, for As(V) and As(III), respectively). In this context, simultaneous dissolution and desorption processes involving Fe ~~and Mn~~ oxy-hydroxides, carbonate fraction and/or the degradation of OM (Wang et al., 2016 and references therein) ~~and soluble As sulphur complexes~~, may have led to elevated amounts of As(V), which in turn might be rapidly reduced by bacteria to the more labile and toxic As(III) (Dowdle et al., 1996; Smedley and Kinniburgh, 2002; Pfeifle et al., 2018). Indeed, dissolved Fe ~~and Mn~~ increased in the first cm (up to 4,131 ~~and 604~~ μg L^{-1} at SJ and 6,469 ~~and 451~~ μg L^{-1} at SE, respectively) attesting to the transition to anoxic conditions (Fiket et al., 2019). This is in agreement with the trend described for As species, since the reduction of As(V) usually occurred after Fe(III) and before sulphate reduction according to the diagenetic sequence (Froelich et al., 1979; Smedley and Kinniburgh, 2002).

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

As also confirmed in the PCA output (Fig. S1B), ~~T~~total dissolved Hg gradually increased with depth only at LL (from 2.18 to 13.3 pg L^{-1} in the surnatant water and deepest layer, respectively). In this site, MeHg was found to be extremely low (0.40 ± 0.26 pg L^{-1}) and represented only 8.26 and

6.64 % of the dissolved Hg pool in the surnatant water and downcore, respectively. Moreover, a constant increase downcore was observed (from 0.18 to 0.88 pg L^{-1}) whereas slightly elevated amounts of MeHg were found in the 1-2 cm level at sites SJ and SE (Fig. 2 and S1B). Although Hg and MeHg generally displayed extremely low concentrations, the MeHg profile found at site SE suggests that methylation processes mediated by sulphate reducing bacteria (Hines et al., 2017) cannot be completely excluded and could be favoured under low rates of sulphate reduction (Gilmour et al., 1992). The Log K_D of Hg (expressed in L kg^{-1}), which describes the partitioning between the solid and dissolved phases (Hammerschmidt et al., 2004), was quite constant with depth, varying between 8.18 ± 0.02 (SE) and 9.13 ± 0.13 (VV), thus suggesting the prevalence of the element in the solid phase (Table S1). The extremely negative values of Eh can favour the occurrence of sulphate reduction allowing for sulphide precipitation (Hines et al., 1997). ~~;~~However, although ~~this was evident at site LL, where~~ the highest ~~elevated~~ concentrations of both Hg (5.01 mg kg^{-1}) and S (1.20 %) were detected at site LL, no correlation was found between the two elements (r = 0.297 at site LL), contrary to what was observed at sites VV (r = 0.959), SJ (r = 0.577) and SE (r = 0.732). This suggests that the occurrence of Hg at site LL may be related to a different local source currently under investigation. Indeed, Forján et al. (2019) pointed out high concentrations of Hg at Vega beach (approximately 40 km west of site LL) due to the discharge of mine tailings from the district of Berbes which was one of the main fluorite mining areas in Europe where cinnabar was occasionally present (Iglesias and Loredo, 1994; Symons et al., 2017; Levresse et al., 2019). On account of the marine outfall, which has been built up for the discharge since 1990s (Forján et al., 2019), it is reasonable to expect that ocean and shoreline currents play a crucial role in transporting fine suspended particles enriched in Hg into the Llanes estuary (LL).

The sulphate reduction may also have influenced the low levels of dissolved As(III) in the deepest layer at VV and LL (0.07 and 9.42 µg L^{-1}), since the reduction of As(V) is inhibited when sulphate reduction occurs (Moore et al., 1998; Frohne et al., 2011; Burton et al., 2013). At these sites, the

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: English (United States)

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: English (United States)

Formatted: Not Highlight

Formatted: Not Highlight

reduction of Fe and Mn oxy-hydroxides appear to be relatively notable only in the upper sediments. ~~Similarly,~~Conversely, dissolved Fe and Mn showed the highest concentrations in porewaters only below 2 cm at SE and SJ as well as in the corresponding sediment cores (Fig. 2 and 1SB), thus indicating a higher release from sediment to porewaters at the Nalón~~ River~~ estuary sites.

Sulphate profiles showed an almost stable trend, except for LL, where they decreased downcore. Together with the increase of Hg and the elevated DOC concentrations, this may have favoured methylation processes at LL, thus explaining the increase in dissolved MeHg in the deeper layers (Fig. 2 and S1B).

3.2. Resuspension experiments

3.2.1. Physico-chemical parameter variability

Total suspended matter (TSM) content was markedly high just after artificial perturbation was stopped ~~immediately after the resuspension events~~(T_0) (Fig. 3), especially at SE and LL (4.88 and 3.42 g L^{-1}, respectively), and decreased abruptly after 30 min~~t T_1~~ (Table S2) at all sites until the end of the experiments. This trend suggests that the effect of resuspension is limited at the initial stage of the experiments, when the highest turbidity was followed by the rapid deposition of the coarser fractions (Van Den Berg et al., 2001).

Total suspended matter (TSM) grain size distribution was determined only at the Nalón ~~River~~ estuary (SJ and SE) (Fig. 3). At site SE, coarse and fine silt predominated at T_0, followed by the finer fraction (very fine silt) after 30 min~~at T_1~~ and by the clayey fraction after 8 h~~at T_5~~. At site SJ, the distribution was trimodal and fine sand, coarse and fine silt prevailed at T_0. Due to the low amount of TSM (close to the lod of the instrument), the distribution was irregular in the rest of the experiment but similar to that described for SE (Fig. 3).

Formatted: Subscript

Formatted: Not Highlight

The pH varied in a narrow range at all the sites (7.08-7.86) with the exception of SE (7.04-8.02) (Table S2). The Eh values testified to the occurrence of oxidising conditions throughout all the experiments with a decreasing trend from pre-resuspension to the initial stage of the experiment (T_0) at LL and SJ, and an increasing trend at VV and SE (Table S2). Generally, there were no marked variations (almost constant) and the values at the end of the experiment were lower than the beginning (Fig. 3). Despite the reductant conditions found in the upper levels of the sediment cores (Fig. 2), the oxidising environment in the mesocosm experiment suggested that the direct contribution from porewaters was negligible (Van Den Berg et al., 2001) or had hidden any dilution processes between porewaters and the water into the chamber.

Formatted: Not Highlight

3.2.2 Behaviour of dissolved chemical species after resuspension

The behaviour of PTEs during a resuspension event is influenced by physico-chemical parameters and by dissolved Fe and Mn occurrence. In oxic conditions, Fe and Mn are present as oxy-hydroxides and are often involved in adsorption processes, co-precipitation, but when reducing conditions occur there is a dissolution with the subsequent release of dissolved PTEs (Gagnon et al., 1997).

Formatted: Not Highlight

Noteworthy differences were found among the sites, both in terms of concentration and trend during the experiments (Fig. 4 and 5). Iron and Mn varied in a wide range being the highest concentrations at SE (up to 7,180 and 2,760 µg L^{-1}, respectively) followed by SJ. Conversely, at LL and VV there was a low variability and the concentrations were significantly lower.

A common behaviour at all the sites was the increasing trend in the earliest phase of resuspension, since the disturbance events involving anoxic sediments usually lead to the release of dissolved Fe and Mn (Saulnier and Mucci, 2000; Caetano et al., 2003). Considering dissolved Fe, for instance, the maximum concentrations found at SE (7,180 µg L^{-1} after 30 min~~at T_1~~) and SJ (5,100 µg L^{-1} at

Formatted: Not Highlight

T_0) could be due to the contribution of porewaters following resuspension. In fact, at SE, Fe was found to be higher than 6,000 µg L^{-1} in the porewater extracted from the first centimetre (Fig. 2). Iron~~Fe~~ and Mn decreased over time at the Nalón estuary sites, reaching concentrations lower than the beginning, most likely due to oxidation processes and subsequent precipitation of Fe oxides. According to Caetano et al. (2003), adsorption and co-precipitation in association with Fe oxides represents the main processes responsible for the removal of dissolved Mn from the solution. Indeed, ~~This could be related to the crucial role of~~ Fe oxides appear to play a crucial role as surface catalysts in the oxidation of Mn (Caetano et al., 2003 and references therein). Indeed, due to the oxidising conditions measured in the chamber (Eh > 120 mV), adsorption and co-precipitation with freshly formed Fe oxides are likely the main processes which remove Mn from the solution.

Formatted: Not Highlight

Formatted: Not Highlight

Conversely, at the other sites, Fe varied more (LL) or less (VV) markedly during the experiments, especially until 4 h~~T_4~~ (Fig. 4), whereas Mn was almost constant at both sites during the resuspension experiment (24 h)~~from T_0 to T_6~~. Similar behaviour was observed by Caetano et al. (2003), who stated that following resuspension, dissolved Mn remains in solution over a longer period of time than Fe and then decreases slowly to reach stable levels after one week (Saulnier and Mucci, 2000).

Formatted: Not Highlight

Formatted: Not Highlight

At the four investigated sites, the behaviour of dissolved Hg, MeHg, As(III) and As(V) during the resuspension experiment is discussed in the following paragraphs and described by a negative exponential equation (1):

$$(1)\ C_{Ti} = C_{T0}\, e^{-kx}$$

where C_{Ti} is the concentration at any time (T_i) during the resuspension experiment, C_{T0} is the concentration at T_0 and k is the rate constant for the contaminant dispersion as a function of time (Fig. S2). The equation (1) was calculated until a decreasing trend was observed and was not applied when the concentration at T_0 was higher than the concentration measured before the resuspension experiment (T_{pre}). Indeed, the final aim was to verify how long it took for the samples to return to their initial pre-resuspension.

Behaviour of dissolved mercury species

Although the highest Hg concentrations in sediments and porewaters were found at LL (avg. 3.90 mg kg^{-1} and 6.85 pg L^{-1}, respectively), the release of total dissolved Hg at this site (1.18-6.22 pg L^{-1}) was found to be comparable with those observed at sites SE (1.90-4.50 ng L^{-1}) and SJ (2.95-5.90 ng L^{-1}). The lowest values were found at site VV (0.41-1.27 pg L^{-1}). This could indicate greater Hg mobility at the Nalón estuary sites, in agreement with previous research (Garcí~~i~~a-Ordiales et al., 2018). In addition, MeHg at these sites was found to be twenty times higher (3.54-9.97 pg L^{-1} at SJ and 4.13-6.93 pg L^{-1}at SE, respectively) than at LL (0.12-0.33 pg L^{-1}) and VV (< lod-0.14 pg L^{-1}) (Fig. 4 and 5). Mercury concentrations at SE and SJ were similar to those reported by Acquavita et al. (2012) in a lagoon environment contaminated by Hg from mining and industrial origin. The same authors reported notably higher (avg. 226 and 51 pg L^{-1}, respectively) MeHg concentrations than our study, likely due to a sudden release from porewaters at T_0 ~~immediately after resuspension~~. Total dissolved Hg and MeHg increased after resuspension and then decreased, in all the investigated sites, until the end of the experiment. According to the equation given above (1), dissolved Hg and MeHg reached concentrations comparable to those measured before the resuspension experiment (T_{pre}) after 6 h at sites LL, VV and SJ (Fig. S2). The only exception was the dissolved Hg at site LL, where Hg was found to be slightly higher at the end of the experiment (1.87 pg L^{-1} after 24 h) with respect to the initial stage (1.18 pg L^{-1} before the experiment). These trends are similar to those for dissolved Fe and Mn, thus suggesting a potential removal (scavenging) of Hg species due to Fe ~~and Mn~~ oxy-hydroxide formation (Gagnon et al., 1997;

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Acquavita et al., 2012; Petranich et al., 2018). The only exception was SE, where Hg and MeHg slightly decreased from 4.50 ng L^{-1} and 5.08 pg L^{-1} (pre-resuspension, $T_{pre-res}$) to 3.10 ng L^{-1} and 4.13 pg L^{-1} (T_0), respectively (Fig. 5~~4~~). Successively, Hg decreased to the same levels observed at other sites, whereas MeHg progressively increased to 7.25 pg L^{-1} (after 4 h~~T_4~~) and remained almost constant until the end of the experiment (after 24 h)~~T_6~~. This could be attributed both to MeHg released from porewaters (Fig. 2) or to the mobilisation of bioavailable OM during resuspension, which may have favoured the metabolic activity of sulphate-reducing bacteria involved in Hg methylation (Gilmour et al., 1992) as suggested by the high levels of DOC (11.2-17.6 mg L^{-1}, Fig. 5~~4~~).

Formatted: Not Highlight

Since significant changes in the association with Hg binding phases could influence methylation and the subsequent transfer of MeHg from the sediment to biota (Kim et al., 2006), the MeHg/Hg ratio (%) in the dissolved phase after resuspension was calculated. Despite the low MeHg levels (< lod-0.33 pg L^{-1}), VV and LL displayed the highest average ratio (~~9.2~~11.5 and 4.79~~8~~ %, respectively), if compared to SJ and SE (0.15 and 0.22 %, respectively). This is due to Hg that was at most one order of magnitude higher than MeHg at LL and VV, whereas at SJ and SE Hg was three orders of magnitude higher. A lower MeHg/Hg ratio was reported by Acquavita et al. (2012) where Hg levels (2.95-15.2 ng L^{-1}) were far higher than MeHg (0.15-0.30 ng L^{-1}): here the ratio increased at the end of the experiment. In our study, this occurred only at SE, where increasing MeHg concentrations and decreasing Hg concentrations over time were observed and could be the result of methylation processes (Fig. 5~~4~~). Indeed, the MeHg concentrations during resuspension were found to be one order of magnitude higher than those measured in the sediment porewaters, suggesting that a certain amount of MeHg may be produced during the experiment and/or released from the resuspended fine particles due to desorption processes. This is in agreement with previous research at the Nalòn estuary which has found that which has found that the highest levels of MeHg in the surface sediments occurred at site SE (Gracía-Ordiales et al., 2018). The same authors also

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

found that low amounts of THg are available for methylation under unperturbed conditions. However, resuspension events may alter the original conditions with the subsequent remobilisation of MeHg from the surface of the resuspended particles. Moreover, at site SE the highest concentrations of inorganic dissolved Hg were observed, as well as elevated concentrations of SO_4^{2-}, as well as an increase in DOC due to OM degradation during the resuspension experiment. Since sediments can show different methylation potential (Bloom et al., 2003) depending on several factors such as bacterial activity, the occurrence of OM and the concentration of inorganic Hg available for methylation (Gilmour et al., 1992; Bloom and Lasorsa, 1999), it is reasonable to hypothesise that sediment resuspension may promote methylation processes at site SE.

Behaviour of dissolved arsenic species

The behaviour of As is of particular interest as it was found to be readily bioavailable in surface sediments of the Nalón River estuary (García-Ordiales et al., 2019a, b). The release of As(III) from sediments to porewaters is enhanced under reducing conditions (Bataillard et al., 2014) due to desorption processes (Wilkin and Ford, 2006; Beak et al., 2008). Conversely, As solubility decreases during oxidation events as a result of precipitation of As(V), which often occurs coupled with adsorption on the surface of Fe(III) oxy-hydroxides (Saulnier and Mucci, 2000; Amirbahman et al., 2006; Root et al., 2007; Jeong et al., 2010). Thus, resuspension events involving anoxic sediments strongly affect the release of dissolved As (Saulnier and Mucci, 2000). Indeed, sediment resuspension processes can interfere with the equilibrium of these chemical forms, inducing changes in the redox conditions, which in turn could favour the desorption of As from Fe oxy-hydroxides, as well as the reductive dissolution of these minerals, with a subsequent release from sediment porewaters to the overlying water column (Smedley and Kinniburgh, 2002).

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

This could explain the high As(V) concentrations ~~immediately after resuspension (~~ atT_0~~)~~, whereas the release of As(III), with concentrations up to ten times lower than As(V), was evident only at SE and SJ (Fig. 5~~4~~). The prevalence of As(V) may be related to oxidation and/or adsorption processes mediated by Fe ~~and Mn~~ oxy-hydroxides, which represent very efficient oxidants of As(III) (Saulnier and Mucci, 2000; Amirbahman et al., 2006; Root et al., 2007; Jeong et al., 2010).

The highest concentrations of As species, released from porewaters, were found at SE. Generally, As(V) ~~species~~ markedly decreased at LL and VV ~~reaching~~reaching pre-resuspension conditions after 4-5 h (Fig. S2).~~,~~ ~~and t~~To a lesser extent, something similar was observed at SJ ~~and SE~~, where ~~until T_6, reaching~~ As(V) and As(III) concentrations comparable with the beginning (pre-resuspension, $T_{pre\text{-}res}$) were reached after 18 and 9 h for As(V) and As(III), respectively (Fig. S2). On the contrary, ~~with~~ the ~~exception~~ concentration of As(V) at SE~~, where at T_6 the concentrations~~ after 24 h was higher than that seen ~~at~~ before the experiment ~~$T_{pre\text{-}res}$~~ (15.3 and 9.81 µg L^{-1} at T_6 and $T_{pre\text{-}res}$, respectively). A~~Conversely, a~~t the same site, As(III) showed a sharp initial increase during the first 30 min of resuspension ~~from $T_{pre\text{-}res}$ to T_0~~ (0.19 to 2.77 µg L^{-1} at $T_{pre\text{-}res}$ and T_0, respectively) reaching concentrations comparable to those measured before the experiment after 7-8 h, and then decreased over time until the end of the experiment ~~T_6~~ (0.29 µg L^{-1} after 24 h) (Fig. 5 and S2). This behaviour can be attributed to adsorption processes onto Fe ~~and Mn~~ oxy-hydroxides (Smedley and Kinniburgh, 2002) ~~, which showed a similar trend~~ (Fig. 5~~4~~), and to the formation of As(V), which consistently showed high concentrations throughout the experiment as a consequence of As(III) oxidation (Bataillard et al., 2014) sustained by the oxidising conditions measured in the chamber (Table S2).

On the contrary, the rapid initial decrease in As(V) at LL and VV until 4 h after resuspension~~from T_0 to T_4~~, could be due to adsorption processes on Fe oxy-hydroxides (Fig. 4~~4~~), since dissolved Fe showed comparable behaviour during the experiments. Regarding the As(III), values < lod were

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

likely related to the scarce contribution from porewaters, where very low concentrations were found (0.12-9.42 µg L^{-1} at LL and < lod and 0.07 µg L^{-1} at VV). Moreover, the availability of high concentrations of SO_4^{2-} (> 2,000 mg L^{-1}) to be reduced can inhibit the reduction of As(V) to As(III) (~~Chen et al., 2002;~~ Burton et al., 2013). Bacterial activity also controls the As(V)/As(III) ratio (Bataillard et al., 2014): in oxic and abiotic conditions the As(V) form prevails, whereas in biotic conditions, As is preserved from total oxidation, thus both chemical forms can be present. This feature deserves to be more carefully investigated and should be taken into consideration in future studies.

Formatted: Not Highlight

Formatted: Not Highlight

Even DOC can be involved in adsorption processes on the suspended particulate matter. In our study, DOC concentrations immediately decreased at T_0~~after resuspension~~, likely caused by the dilution with porewaters, and remained almost constant until 4 h after resuspension~~T_4~~. Successively, DOC increased (at VV, SJ and SE) until the end of the experiment (after 24 h)~~T_6~~ and reached values lower than the beginning, with the exception of LL where a slight decrease was seen ~~from~~ between ~~T_4~~ 4 ~~to~~and 24 h after resuspension~~T_6~~ (13.2 and 10.3 mg L^{-1}, respectively).

3.2.3. Behaviour of the particulate phase after resuspension

Sediment resuspension events can determine the recycling of particles as well as the reintroduction of contaminants into the water column through the SWI (Bloesch, 1995). However, if the event occurred over a short period of time (24 h~~ours~~), the release of dissolved species is limited and most of the PTEs remain bound to the particulate phase (Van Den Berg et al., 2001; Cantwell and Burgess, 2004; Cantwell et al., 2008; Bataillard et al., 2014). According to Guerra et al. (2009), even in heavily contaminated sites (e.g. Pialassa Baiona Lagoon, Italy), dredging does not always have dramatic effects on the environmental quality of the aquatic environment. This could be the case in our study where resuspension was simulated for a limited time.

Formatted: Not Highlight

Formatted: Not Highlight

Formatted: Not Highlight

The Log K_D values for Hg and MeHg (K_D = [PHg]/[DHg], L kg^{-1}) were significantly higher at LL and VV (see Table S3), suggesting that these species remain preferentially partitioned in the particulate phase also during resuspension events, as previously stated by Kim et al. (2004). However, it is interesting to note that Log K_D at SJ and SE showed a decrease of two orders of magnitude if compared to that calculated for core sediments, thus confirming the evidence reported in Garcí~~i~~a-Ordiales et al. (2018). The Log K_D of As species was generally low and did not show noticeable differences among the sites. Conversely, Fe and Mn appeared to be mainly associated with the dissolved phase (Log K_D, 0.81-4.08).

Formatted: Not Highlight

Formatted: Not Highlight

~~Particulate PTEs showed decreasing trends at all the sites (Fig. 5), with the exception of~~ Although Hg and As(V) at SJ (which saw an increase during the first 30 min after resuspension~~from T_0 to T_1~~), and As(V) at SE (which saw an increase between 30 min and 1 h after resuspension~~from T_1 to T_2~~), particulate PTEs showed decreasing trends at all the sites (Fig. 6).~~.~~ This behaviour ~~has~~was also ~~been~~ reported for Zn and Cu~~d~~ by Vidal-Durà et al. (2018) as the result of the settling of the resuspended particles as a function of time.~~.~~

Formatted: Not Highlight

By comparing the particulate PTE trends with TSM content, a preferential bond to the silty or clayey fraction was found. At SE, the TSM rapidly decreased in the first 4 h~~ours~~ after resuspension, from 4.88 (coarse silt) to 0.31 mg L^{-1} (very fine silt), as well as particulate Hg and MeHg, from 14.6 µg g^{-1} and 1.76 pg g^{-1} (T_0) to 5.65 µg g^{-1} and 0.67 pg g^{-1} (after 4 h,~~T_4~~), respectively. However, as the particles in suspension became finer, particulate Hg and MeHg concentrations slightly increased up to 6.29 µg g^{-1} and 0.83 pg g^{-1} (after 24 h~~T_6~~), respectively. This is consistent with the evolution of the grain size spectra and composition as a function of time (Fig. 3) and indicates that Hg and MeHg were bound to both the silty and clayey fractions, in addition to Fe. On the contrary, As(V), As(III) and Mn showed the highest concentrations in the early phase of the experiment, thus were preferentially bound to the silty fraction (Fig. 3).

At SJ, the clay prevailed in suspension as early as 1 hour after resuspension~~ (T_2)~~, when particulate Hg reached the highest concentration (9.18 µg g^{-1}), and particulate MeHg (0.98 pg g^{-1}) was slightly lower than the beginning (1.29 pg g^{-1}) and decreased as particles settled down (Fig. 6~~5~~). As observed at site SE, Hg was found to be bound to both grain size fractions, whereas MeHg, Fe and Mn were preferentially bound to the silty fraction. Conversely, particulate As(V) and As(III) increased as the particles in suspension became finer.

3.3. Assessment of arsenic and mercury species released-removal after simulated resuspension events

The amounts of Hg, MeHg, As(V) and As(III) released during the experiments were estimated assuming that the resuspension events involved only the first 3.5 cm of the sedimentary sequence (based on visual observations), considering the size of the chamber and the water content in each layer of the sediment cores. Furthermore, the concentrations at $T_{pre-res}$ were converted into the amount (mass) of each chemical species. By adding the amounts of elements released by porewaters to those of the water column before resuspension ($T_{pre-res}$), and dividing by the water volume in the chamber (6.79 L), the chemical species concentrations that should have been found following the resuspension in the water column (T_0) were estimated (Table S4).

The concentrations measured at LL, VV and SJ (only for Hg species promptly re-precipitated/re-adsorbed) at T_0 were higher than those estimated for the sole porewater contribution, thus there was also an active desorption from the resuspended sediment. For the Nalón River estuary, the ~~T_0~~ concentrations of Hg and As species at T_0 were found to be lower than the estimated concentrations at SE. In addition, the percentages of As and Hg species in the water column until the end of the experiment (after 24 h)~~from T_0 to T_6~~, with respect to the dissolved concentrations before resuspension were calculated (Table S4 and Fig. 7~~6~~). Positive values for Hg and MeHg (excluding the sampling performed after 8 h~~T$_5$~~) at LL and, in the first hour, at VV, suggested a further release from resuspended sediments. In detail, at LL the percentage of Hg released at T$_0$ was especially high (335%) and remained so (58%) after 24 h~~ours~~. Surprisingly, a scarce release ~~after resuspension~~ at T$_0$ was found at SE where the concentration ~~at T$_0$~~ was approximately 30% lower than ~~at~~ before the experiment (T$_{pre-res}$) and beca~~o~~me even lower (-58%) after 24 h (Fig. 7~~6~~). The time of removal for MeHg from the water column was quite variable among the sites, however, the MeHg levels were almost constant thus suggesting the enhancement of methylation processes after resuspension as observed by Acquavita et al. (2012). As suggested by Bloom and Lasorsa (1999), the mixing of surface sediments associated with fresh sulphate and organic carbon with contaminated deep sediments may be a favourable environment for MeHg production: if these conditions occur, unacceptably high levels of MeHg could result, however, this does not appear to be the case in this study.

Formatted: Not Highlight

Formatted: Not Highlight

At the end of the experiment, the concentration of As(V) at site SJ (4.97 µg L^{-1}) was found to be slightly lower than that measured before the resuspension (5.55 µg L^{-1}, T$_{pre-res}$) suggesting that the As(V) released during resuspension was removed at the end of the experiment (after 24 h)~~ours~~ at SJ. On the contrary, this was not evident at site SE, where ~~but its~~ As(V) concentration was still higher (56%) at the end of the experiment (15.5 µg L^{-1}) than before the experiment ~~at~~ (9.81 µg L^{-1}, T$_{pre-res}$) ~~at the end of the experiment at site SE,~~. Something similar was observed for As(III) which was removed after 9 h at SJ (<lod) and slightly higher (0.29 µg L^{-1} after 24 h) than before resuspension (0.19 µg L^{-1}, T$_{pre-res}$) ~~whereas As(III) released at SJ was removed 8 hours following resuspension~~.

Conclusions

Mainly due to the legacy of mining activities, Hg and As accumulated in sediments found on the Asturian coasts could be problematic in the ~~event~~ case of resuspension due to natural (e.g. storm surges) or anthropogenic events (e.g. dredging). A~~In this work a~~ preliminary characterisation of the area (sediment cores) was ~~performed~~ provided for the~~at~~ selected sites. Furthermore, to investigate the effect of resuspension and the subsequent remobilisation and redistribution of As and Hg species, a mesocosm experiment (simulated resuspension under controlled conditions) was performed. The major findings can be summarised as follows:

- The bottom sediments were mostly constituted of silt at SE and VV, whereas sand prevailed at LL and SJ. As concentration demonstra~~r~~ted a wider range than Hg moving downcore with rather homogeneous profiles. However, for both elements the levels were quite high and exceeded the standard set by the EU. Increasing negative Eh values moving downcore were measured at all the sites.

- Several dissolved species were determined in porewaters (As(V), As(III), Hg and MeHg). Generally, Hg concentrations were rather constant with depth~~ for~~. In the case of As species, an increase due to the dissolution and desorption from Fe, ~~and~~ rather than Mn, oxy-hydroxides coupled with the transition from oxic to anoxic conditions occurred. These processes could have led to high amounts of As(V), which in turn might be rapidly reduced to the more labile and toxic As(III). The highest As(V) and SO_4^{2-} concentrations detected in porewaters at the Nalón River estuarine sites could testify to a scarce sulphate reduction which usually inhibits the reduction of As(V). Moreover, the low rates of sulphate reduction could also explain the pronounced increase in MeHg at the same sites (SJ and SE).

- Particulate and dissolved PTE concentration increased when the artificial perturbation stopped ~~immediately after resuspension~~ and together TSM rapidly decreased over time. The abrupt increase in dissolved PTEs can be mainly ascribed to the porewater contribution and the effects of resuspension lasted only a few hours until pre-resuspension conditions were restored. Part of the Hg and ~~As~~ MeHg released from porewaters and/or desorbed during resuspension was ~~presumably~~ removed after 6 h at sites LL, VV and SJ (with the exception of Hg at site LL) by the re-adsorption onto the settling fine particles and/or co-precipitation with Fe ~~and Mn~~ oxy-hydroxides. In the case of As species, the restoration of the pre-resuspension concentrations of As(III) was reached after 7-9 h at the Nalòn estuary sites. Something similar was observed for As(V) which showed concentrations comparable to the beginning after 4-5 h at LL and VV and after 18 h at SJ, whereas the re-adsorption and/or re-precipitation was incomplete at site SE.

~~- However, in some cases the re-adsorption and/or re-precipitation was incomplete.~~

These results suggest that resuspension events of contaminated estuarine sediments can be critical for the surrounding environment, resulting in a worsening of the quality for water and biota. The consequences, however, are not unequivocal, strongly depending on site characteristics and, as seen in the mesocosm experiments, appear to be limited in time. In this context, the restoration of pre-resuspension conditions is observed a few hours after resuspension, but in some sites precautions should be taken when planning to dredge.

Further investigation could be helpful in order to elucidate the behaviour of other pollutants such as POPs and TBT, which commonly affect estuarine and coastal areas. Moreover, it would be useful to monitor the water column quality during dredging operations in *situ* and to compare the results with the mesocosm approach.

Acknowledgments

This study was co-supported by the Spanish Ministry of Economy, Industry and Competitiveness through the Research Projects METRAMER [grant number: MINECO-13-CGL2013-44980-R] and ECOMER [grant number: MINECO-18-CGL2017-84268-R] and the Asturias Ministry of Education and Science [grant number: FC- 15-GRUPIN14-067]. The authors are very grateful to José Manuel Rico for his valuable support in sampling operations. Karry Close is warmly acknowledged for proofreading the manuscript. The anonymous reviewers are sincerely acknowledged for their critical reviews and useful suggestions which improved the quality of the manuscript.

References

Formatted: Italian (Italy)

Acquavita, A., Covelli, S., Emili, A., Berto, D., Faganeli, J., Giani, M., Horvat, M., Koron, N., Rampazzo, F., 2012. Mercury in the sediments of the Marano and Grado Lagoon (northern Adriatic Sea): sources, distribution and speciation. Estuar. Coast. Shelf S. 113, 20–31.

Amirbahman, A., Kent, D.B., Curtis, G.P., Davis, J.A., 2006. Kinetics of sorption and abiotic oxidation of arsenic(III) by aquifer materials. Geochim. Cosmochim. Ac. 70, 533–547.

Amouroux, D., Tessier, E., Pécheyran, C., Donard, O.F.X., 1998. Sampling and probing volatile metal(loid) species in natural waters by in-situ purge and cryogenic trapping followed by gas chromatography and inductively coupled plasma mass spectrometry (P-CT-GC-ICP/MS). Anal. Chim. Acta 377, 241–254.

Arfi, R., Guiral, D., Bouvy, M., 1993. Wind induced resuspension in a shallow tropical lagoon. Estuar. Coast. Shelf S. 36, 587–604.

Bataillard, P., Grangeon, S., Quinn, P., Mosselmans, F., Lahfid, A., Wille, G., Joulian, C., Battaglia-Brunet, F., 2014. Iron and arsenic speciation in marine sediments undergoing a resuspension event: the impact of biotic activity. J. Soils Sediments 14, 615–629.

Beak, D.G., Wilkin, R.T., Ford, R.G., Kelly, S.D., 2008. Examination of arsenic speciation in sulfidic solutions using X-ray absorption spectroscopy. Environ. Sci. Technol. 42, 1643–1650.

Beck, M., Dellwig, O., Schnetger, B., Brumsack, H.J., 2008. Cycling of trace metals (Mn, Fe, Mo, U, V, Cr) in deep pore waters of intertidal flat sediments. Geochim. Cosmochim. Ac. 72, 2822–2840.

Benoit, J.M., Shull D.H., Harvey, R.M., Beal, S.A., 2009. Effect of bioirrigation on sediment-water exchange of methylmercury in Boston Harbor, Massachusetts. Environ. Sci. Technol. 43, 3669–3674.

Bertuzzi, A., Faganeli, J., Brambati, A., 1996. Annual variation of benthic nutrient fluxes in shallow coastal waters (Gulf of Trieste, northern Adriatic Sea). Mar. Ecol. 17, 261–278.

Bloesch, J., 1995. Mechanisms, measurement and importance of sediment resuspension in lakes. Mar. Freshwater Res. 46, 295–304.

Bloom, N.S., Lasorsa, B.K., 1999. Changes in mercury speciation and the release of methyl mercury as a result of marine sediment dredging activities. Sci. Total Environ. 237–238, 379–385.

Bloom, N.S., Preus, E., Katon, J., Hiltner, M., 2003. Selective extractions to assess the biogeochemically relevant fractionation of inorganic mercury in sediments and soils. Anal. Chim. Acta 479, 233–248.

Bocchetti, R., Fattorini, D., Pisanelli, B., Macchia, S., Oliviero, L., Pilato, F., Pellegrini, D., Regoli F., 2008. Contaminant accumulation and biomarker responses in caged mussels, *Mytilus galloprovincialis*, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbours areas. Aquat. Toxicol. 89, 257–266.

Bouchet, S., Tessier, E., Monperrus, M., Bridou, R, Clavier, J, Thouzea, G, Amouroux, D., 2011. Measurements of gaseous mercury exchanges at the sediment–water, water–atmosphere and

Formatted: Italian (Italy)

Formatted: English (United States)

Formatted: English (United States)

Formatted: Italian (Italy)

Formatted: Italian (Italy)

Formatted: English (United States)

sediment–atmosphere interfaces of a tidal environment (Arcachon Bay, France). J. Environ. Monitor. 13, 1351–1359.

Burton, E.D., Johnston, S.G., Kraal, P., Bush, R.T., Claff, S., 2013. Sulfate availability drives divergent evolution of arsenic speciation during microbially mediated reductive transformation of schwertmannite. Environ. Sci. Technol., 47, 2221–2229.

Caetano, M., Madureira, M.J., Vale, C., 2003. Metal remobilisation during resuspension of anoxic contaminated sediment: short-term laboratory study. Water Air Soil Pollut. 143, 23–40.

Cantwell, M.G., Burgess, R.M., 2004. Variability of parameters measured during the resuspension of sediments with a particle entrainment simulator. Chemosphere 56, 51–58.

Cantwell, M.G., Burgess, R.M., King, J.W., 2008. Resuspension of contaminated field and formulated reference sediments Part I: evaluation of metal release under controlled laboratory conditions. Chemosphere 73, 1824–1831.

Caplat, C., Texier, H., Barillier, D., Lelievre, C., 2005. Heavy metals mobility in harbour contaminated sediments: the case of Port-en-Bessin. Mar. Pollut. Bull. 50, 504–511.

Ceñal, R.C., Flor, G., 1993. Evolución reciente del estuario del Nalón (Asturias). Cuaternario y Geomorfología 7, 23-34.

~~Chen, M., Ma, L.Q., Harris, W.G., 2002. Arsenic concentrations in Florida surface soils: influence of soil type and properties. Soil Sci. Soc. Am. J. 66, 632–640.~~

Conaway, C.H., Squire, S., Mason, R.P., Flegal, A.R., 2003. Mercury speciation in the San Francisco Bay estuary. Mar. Chem. 80, 199–225.

Cotou, E., Gremare, A., Charles, F., Hatzianestis, I., Sklivagou, E., 2005. Potential toxicity of resuspended particulate matter and sediments: environmental samples from the Bay of Banyuls-sur-Mer and Thermaikos Gulf. Cont. Shelf Res. 25, 2521–2532.

de Freitas, A.R., de Castro Rodrigues, A.P., Monte, C.N., Freire, A.S., Santelli, R.E., Machado, W., Sabadini-Santos E., 2019. Increase in the bioavailability of trace metals after sediment resuspension. SN Applied Sciences 1:1288 https://doi.org/10.1007/s42452-019-1276-8.

Dowdle, P.R., Laverman, A.M., Oremland, R.S., 1996. Bacterial dissimilatory reduction of arsenic (V) to arsenic (III) in anoxic sediments. Appl. Environ. Microbiol. 62, 1664–1669.

Eggleton, J., Thomas, K.V., 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ. Int. 30, 973–980.

Emili, A., Koron, N., Covelli, S., Faganeli, J., Acquavita, A., Predonzani, S., De Vittor, C., 2011. Does anoxia affect mercury cycling at the sediment-water interface in the Gulf of Trieste (northern Adriatic Sea)? Incubation experiments using benthic flux chambers. Appl. Geochem. 26, 194–204.

European Parliament, Council of the European Union (2000) Water Framework Directive. 2000/60/EC.

European Parliament, Council of the European Union (2008) Establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). 2008/56/EC.

Fang, T.H., Chen, Y.S., 2015. Arsenic speciation and diffusion flux in Danshuei Estuary sediments, Northern Taiwan. Mar. Pollut. Bull. 101, 98–109.

Fernández-Martínez, R., Loredo, J., Ordóñez, A., Rucandio, M.I., 2005. Distribution and mobility of mercury in soils from an old mining area in Mieres, Asturias (Spain). Sci. Total Environ. 346, 200–212.

Fiket, Ž., Fiket, T., Ivanić, M., Mikac, N., Kniewald, G., 2019. Pore water geochemistry and diagenesis of estuary sediments—an example of the Zrmanja River estuary (Adriatic coast, Croatia). J. Soils Sediments 19, 2048–2060.

Formatted: Italian (Italy)

Formatted: English (United States)

Formatted: Italian (Italy)

Formatted: Hyphenate, Tab stops: Not at 0.5 cm

Formatted: Font: (Default) Times New Roman, 12 pt, English (United Kingdom)

Formatted: English (United Kingdom)

Formatted: English (United Kingdom)

Flor, G., Fernández Pérez, L.A., Menéndez Fidalgo, R., Martínez Cueto-Felgueroso, E.M., Rodríguez Casero, G., 1996. Dynamics and sedimentation of the mesotidal estuary of Villaviciosa (Asturias, Northern Spain). Revista de la Sociedad Geológica de España 9, 3–4.

Forján, R., Baragaño, D., Boente, C., Fernández-Iglesias, E., Rodríguez-Valdes, E., Gallego, J.R., 2019. Contribution of fluorite mining waste to mercury contamination in coastal systems. Mar. Pollut. Bull. 149, 110576.

Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N.A., Heath, G.R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., Maynard, V., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochim. Cosmochim. Ac. 43, 1075–1090.

Frohne, T., Rinklebe, J., Diaz-Bone, R.A., Du Laing, G., 2011. Controlled variation of redox conditions in a floodplain soil: impact on metal mobilization and biomethylation of arsenic and antimony. Geoderma 160, 414–424.

Gagnon, C., Pelletier, E., Mucci, A., 1997. Behaviour of anthropogenic mercury in coastal marine sediments. Mar. Chem. 59, 159–176.

García-Ordiales, E., Flor-Blanco, G., Roqueñí, N., Covelli, S., Cienfuegos, P., Fontolan, G., Loredo, J., 2020. Anthropocene human footprint in the Nalón estuarine sediments (northern Spain). Mar. Geol. ~~https://doi.org/10.1016/j.margeo.2020.106167~~424, 106167.

García-Ordiales, E., Cienfuegos, P., Roqueñí, N., Covelli, S., Flor-Blanco, G., Fontolan, G., Loredo, J., 2019a. Historical accumulation of potentially toxic trace elements resulting from mining activities in estuarine salt marshes sediments of the Asturias coastline (northern Spain). Environ. Sci. Pollut. Res. 26, 3115–3128.

García-Ordiales, E., Roqueñí, N., Rico, J.M., Cienfuegos, P., Alvarez, R., Ordoñez, A., 2019b. Assessment of the toxicity toward Vibrio fischeri in sediments of a mining impacted estuary in the north of Spain. Sci. Total Environ. 660, 826–833.

Formatted: Italian (Italy)

García-Ordiales, E., Covelli, S., Rico, J.M., Roqueñí., N., Fontolan, G., Flor-Blanco, G., Cienfuegos, P., Loredo, J., 2018. Occurrence and speciation of arsenic and mercury in estuarine sediments affected by mining activities (Asturias, northern Spain). Chemosphere 198, 281–289.

García-Ordiales, E., Loredo, J., Covelli, S., Esbrí, J.M., Millán, R., Higueras, P., 2017. Trace metal pollution in freshwater sediments of the world's largest mercury mining district: sources, spatial distribution, and environmental implications. J. Soils Sediments. 17, 1893–1904.

Gilmour, C.C., Henry, E.A., Ralph, M., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. 26, 2281–2287.

González-Fernández, B., Rodríguez-Valdés, E., Gallego, J.R., 2018. Contaminación de un acuífero por metales pesados de origen geogénico: importancia del control geológico e hidrogeológico de los sondeos de captación de aguas. Geogaceta 64, 59–62.

Goossens, H., Zwolsman, J.J.G., 1996. An evaluation of the behaviour of pollutants during dredging activities. Terra et Aqua 62, 20–28.

Guerra, R., Pasteris, A., Ponti, M., 2009. Impacts of maintenance channel dredging in a northern Adriatic coastal lagoon. I: Effects on sediment properties, contamination and toxicity. Estuar. Coast. Shelf S. 85, 134–142.

Hammerschmidt, C.R., Fitzgerald, W.F., Lamborg, C.H., Balcom, P.H., Visscher, P.T., 2004. Biogeochemistry of methylmercury in sediments of Long Island Sound. Mar. Chem. 90, 31–52.

Hines, M.E., Covelli, S., Faganeli, J., Horvat, M., 2017. Controls on microbial mercury transformations in contaminated sediments downstream of the Idrija mercury mine (West Slovenia) to the Gulf of Trieste (northern Adriatic). J. Soils Sediments 17, 1961–1971.

Hines, M.E., Faganeli, J., Planinc, R., 1997. Sedimentary anaerobic microbial biogeochemistry in the Gulf of Trieste, Northern Adriatic Sea: influences of bottom water oxygen depletion. Biogeochemistry 39, 65–86.

Iglesias, J. G., Loredo, J., 1994. Geological, geochemical and mineralogical characteristics of the Asturias Fluorspar District, Northern Spain. Explor.~~ation &~~ Min.~~ing~~ Geol.~~ogy Journal~~ 3, 31-37.

Jeong, H.Y., Han, Y.S., Park, S.W., Hayes, K.F., 2010. Aerobic oxidation of mackinawite (FeS) and its environmental implication for arsenic mobilization. Geochim. Cosmochim. Ac. 74, 3182–3198.

Jones-Lee, A, Lee, G.F., 2005. Role of iron chemistry in controlling the release of pollutants from resuspended sediments. Remediation 16, 33–41.

Kalnejais, L.H., Martin, W.R., Signell, R.P., Bothner, M.H., 2007. Role of sediment resuspension in the remobilization of particulate-phase metals from coastal sediments. Environ. Sci. Technol. 41, 2282−2288.

Kim, E.H., Mason, R.P., Porter, E.T., Soulen, H.L., 2004. The effect of resuspension on the fate of total mercury and methylmercury in a shallow estuarine ecosystem: a mesocosm study. Mar. Chem. 86, 121−137.

Kim, E.H., Mason, R.P., Porter, E.T., Soulen, H.L., 2006. The impact of resuspension on sediment mercury dynamics, and methylmercury production and fate: A mesocosm study. Mar. Chem. 102, 300−315.

Leardi, R., Melzi, C., Polotti, G., 2019. CAT (Chemometric Agile Tool), freely downloadable from http://gruppochemiometria.it/index.php/software (accessed September 3rd, 2019).

Levresse, G., Tritlla, J., Rosique, A.R., Cardellach, E., Rollion-Bard, C., Pironon, J., Sandoval, S.J., 2019. Hydrocarbons in silica: PVTX properties of fluids and the genesis of diamond quartz from Caravia-Berbes Fluorite district (Asturias, Spain). Mar. Petrol. Geol. 102, 1–15.

Lewis, M.A., Weber, D.E., Stanley, R.S., Moore, J.C., 2001. Dredging impact on an urbanized Florida bayou: effects on benthos and algal-periphyton. Environ. Pollut. 115, 161–171.

Formatted: English (United Kingdom)

Loredo, J., 2000. Historic Unreclaimed Mercury Mines in Asturias (Northwestern Spain): Environmental Approaches. Assessing and Managing Mercury from Historic and Current Mining Activities, pp. 175–180.

Loredo, J., Ordóñez, A., Gallego, J., Baldo, C., Garcia-Iglesias, J., 1999. Geochemical characterisation of mercury mining spoil heaps in the area of Mieres (Asturias, northern Spain). J. Geochem. Explor. 67, 377–390.

Loredo, J., Petit-Domínguez, M.D., Ordóñez, A., Galán, M.P., Fernández-Martínez, R., Alvarez, R., Rucandio, M.I., 2010. Surface water monitoring in the mercury mining district of Asturias (Spain). J. Hazard. Mater. 176, 323–332.

Masscheleyn, P.H., Delaune, R.D., Patrick Jr., W.H., 1991. Arsenic and selenium chemistry as affected by sediment redox potential and pH. J. Environ. Qual. 20, 522–527.

Moore, J.N., Ficklin, W.H., Johns, C., 1988. Partitioning of arsenic and metals in reducing sulfidic sediments. Environ. Sci. Technol. 22; 432–437.

Monte, C.N., Rodrigues, A.P.C, Cordeiro, R.C., Freire, A.S., Santelli, R.E., Machado, W., 2015. Changes in Cd and Zn bioavailability upon an experimental resuspension of highly contaminated coastal sediments from a tropical estuary. Sustain. Water Resour. Manag. 1, 335–342.

Oliveri, E., Salvagio Manta, D., Bonsignore, M., Cappello, S., Tranchida, G., Bagnato, E., Sabatino, N., Santisi, S., Sprovieri, M., 2016. Mobility of mercury in contaminated marine sediments: Biogeochemical pathways. Mar. Chem. 186, 1–10.

Oliveri, P., Malegori, C., Casale, M., 2020. Chemometrics: Multivariate Analysis of Chemical Data, in: Pico, Y. (Ed.), Chemical Analysis of Food, 2nd Edition. Elsevier.

Oliveri, P., Malegori, C., Simonetti, R., Casale, M., 2019. The impact of signal pre-processing on the final interpretation of analytical outcomes – A tutorial. Anal. Chim. Acta 1058, 9–17.

Formatted: Italian (Italy)

Ordóñez, A., Silva, V., Galán, P., Loredo, J., Rucandio, I., 2014. Arsenic input into the catchment of the River Caudal (Northwestern Spain) from abandoned Hg mining works: effect on water quality. Environ. Geochem. Health 36, 271–284.

Petranich, E., Croce, S., Crosera, M., Pavoni, E., Faganeli, J., Adami, G., Covelli, S., 2018. Mobility of metal(loid)s at the sediment-water interface in two tourist port areas of the Gulf of Trieste (northern Adriatic Sea). Environ. Sci. Pollut. Res. 25, 26887–26902.

Pfeifle, B.D., Stamm, J.F., Stone, J.J., 2018. Arsenic geochemistry of alluvial sediments and pore waters affected by mine tailings along the Belle Fourche and Cheyenne River floodplains. Water Air Soil Poll. 229, 183.

Ridgway, J., Shimmield, G., 2002. Estuaries as repositories of historical contamination and their impact on shelf seas. Estuar. Coast. Shelf Sci. 55, 903–928.

Roberts, D.A., 2012. Causes and ecological effects of resuspended contaminated sediments (RCS) in marine environments. Environ. Int. 40, 230–243.

Rodríguez-González, P., Bouchet, S., Monperrus, M., Tessier, E., Amouroux, D., 2013. In situ experiments for element species-specific environmental reactivity of tin and mercury compounds using isotopic tracers and multiple linear regression. Environ. Sci. Pollut. R. 20, 1269–1280.

Rodríguez-González, P., Monperrus, M., García Alonso, J.I., Amouroux, D., Donard, O.F.X., 2007. Comparison of different numerical approaches for multiple spiking species-specific isotope dilution analysis exemplified by the determination of butyltin species in sediments. J. Anal. Atom. Spectrom. 22, 1371–1382.

Rodríguez-González. P., Ruiz Encinar, J., García Alonso, J.I., Sanz-Medel, A., 2002. Determination of butyltin compounds in coastal sea-water samples using isotope dilution GC-ICP-MS. J. Anal. Atom. Spectrom. 17, 824–830.

Rodríguez Martin-Doimeadios, R.C., Monperrus, M., Krupp, E., Amouroux, D., Donard, O.F.X., 2003. Using speciated isotope dilution with GC− inductively coupled plasma MS to determine

Formatted: English (United States)

Formatted: English (United Kingdom)

and unravel the artificial formation of monomethylmercury in certified reference sediments. Anal. Chem. 75, 3202–3211.

Root, R.A., Dixit, S., Campbell, K.M., Jew, A.D., Hering, J.G., O’Day, P.A., 2007. Arsenic sequestration by sorption processes in high-iron sediments. Geochim. Cosmochim. Ac. 71, 5782–5803.

Ruiz-Chancho, M.J., Sabé, R., López-Sánchez, J.F., Rubio, R., Thomas, P., 2005. New approaches to the extraction of arsenic species from soils. Microchim. Acta 151, 3–4, 241–248.

Sanford, L.P., Panageotou, W., Halka, J.P., 1991. Tidal resuspension of sediments in northern Chesapeake Bay. Mar. Geol. 97, 87–103.

Sanz-Prada, L., García-Ordiales, E., Roqueñi, N., Grande Gil, J.A., Loredo, J., 2020. Geochemical distribution of selected heavy metals in the Asturian coastline sediments. Mar. Pollut. Bull. 156, 111263.

Saulnier, I., Mucci, A., 2000. Trace metal remobilization following the resuspension of estuarine sediments: Saguenay Fjord, Canada. Appl. Geochem. 15, 191–210.

Schoellhamer, D.H., 1996. Anthropogenic sediment resuspension mechanisms in a shallow microtidal estuary. Estuar. Coast. Shelf Sci. 43, 533– 548.

Seelen, E.A., Massey, G.M., Mason, R.P., 2018. Role of sediment resuspension on estuarine suspended particulate mercury dynamics. Environ. Sci. Technol. 52, 7736–7744.

Shepard, F.P., 1954. Nomenclature based on sand–silt–clay ratios. J. Sediment Petrol. 24, 151–158.

Silva, V., Loredo, J., Fernández-Martínez, R., Larios, R., Ordóñez, A., Gómez, B., Rucandio, I., 2014. Arsenic partitioning among particle-size fractions of mine wastes and stream sediments from cinnabar mining districts. Environ. Geochem. Health 36, 831–843.

Simpson, S.L., Apte, S.C., Batley, G.E., 1998. Effect of short-term resuspension events on trace metal speciation in polluted anoxic sediments. Environ. Sci. Technol. 32, 620–625.

Formatted: Italian (Italy)

Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Appl. Geochem. 17, 517–568.

Stephens, S.R., Alloway, B.J., Parker, A., Carter, J.E., Hodson, M.E., 2001. Changes in the leachability of metals from dredged canal sediments during drying and oxidation. Environ. Pollut. 114, 407–413.

Symons, D.T.A., Kawasaki, K., Tornos, F., Velasco, F., Rosales, I., 2017. Temporal constraints on genesis of the Caravia-Berbes fluorite deposits of Asturias, Spain, from paleomagnetism. Ore Geol. Rev. 80, 754–766.

~~Sutherland, R.A., 2000. Bed sediment-associated trace metals in an urban stream, Oahu, Hawaii. Environ. Geol. 39, 611–627.~~

Turner, A., Millward, G.E., Le Roux, S.M., 2004. Significance of oxides and particulate organic matter in controlling trace metal partitioning in a contaminated estuary. Mar Chem 8:179-192

Van Den Berg, G.A., Meijers, G.G.A., Van Der Heijdt, L.M., Zwolsman, J.J.G., 2001. Dredging-related mobilisation of trace metals: a case study in the Netherlands. Water Res. 35, 1979–1986.

Vidal-Durà, A., Burke, I.T., Stewart, D.I., Mortimer, R.J.G., 2018. Reoxidation of estuarine sediments during simulated resuspension events: effects on nutrient and trace metal mobilisation. Estuar. Coast. Shelf Sci. 207, 40–55.

Wang, H., Liu, R., Wang, Q., Xu, F., Men, C., Shen, Z., 2016. Bioavailability and risk assessment of arsenic in surface sediments of the Yangtze River estuary. Mar. Pollut. Bull. 113, 125–131.

Wilkin, R.T., Ford, R.G., 2006. Arsenic solid-phase partitioning in reducing sediments of a contaminated wetland. Chem. Geol. 228, 156–174.

Zhu, W., Song, Y., Adediran, G.A., Jiang, T., Reis, A.T., Pereira, E., Skyllberg, U., Björn, E., 2018. Mercury transformations in resuspended contaminated sediment controlled by redox conditions, chemical speciation and sources of organic matter. Geochim. Cosmochim. Ac. 220, 158–179.

Formatted: English (United Kingdom)

Figure captions

Fig. 1. The study area along the Asturian coast where sediment samples for resuspension experiments were collected from the following sites: the Nalón River Estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL).

Formatted: Font: (Default) Times New Roman, 12 pt

Fig. 2. Vertical profiles for both As and Hg species, Fe, Mn, DOC, SO_4^{2-} in dissolved form in porewaters and Eh values in the four sediment cores collected from the Nalón River estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL).

Fig. 3. Trend of total suspended matter (TSM) concentrations during resuspension experiments at all sites (above) and grain-size distribution of TSM at San Esteban (SE) and San Juan (SJ) in the Nalón River Estuary (below).

Fig. 4. Behaviour of As(V), As(III), Hg, MeHg, Fe, Mn, DOC and SO_4^{2-} in the dissolved phase of the water column during resuspension experiments at the port of Llanes (LL) and at the Villaviciosa estuary (VV). ~~Note that Hg, MeHg, Fe and Mn at San Juan (SJ) and San Esteban (SE) show a different scale and As(III) is only present at these two sites. ~~Note that the term “pre” that follows the chemical element in each graph (e.g. As(V) pre) indicates the PTE concentration before the resuspension event (at time $T_{pre\text{-}res}$).

Fig. 5. Behaviour of As(V), As(III), Hg, MeHg, Fe, Mn, DOC and SO_4^{2-} in the dissolved phase of the water column during resuspension experiments at the Nalón River estuary (SJ and SE). Note that Hg, MeHg, Fe and Mn at San Juan (SJ) and San Esteban (SE) show a different scale and As(III) is only present at these two sites. Note that the term “pre” that follows the chemical element

in each graph (e.g. As(V) pre) indicates the PTE concentration before the resuspension event (at time $T_{pre-res}$).

Fig. 6~~5~~. Behaviour of As(V), As(III), Hg, MeHg, Fe and Mn in the particulate phase of the water column during resuspension experiments at all investigated sites, expressed using different scales.

Fig. ~~6~~7. Percentages of removal (negative values) and/or release (positive values) of As and Hg species in the water column from T_0 to T_6 with respect to the dissolved concentrations before resuspension ($T_{pre-res}$).

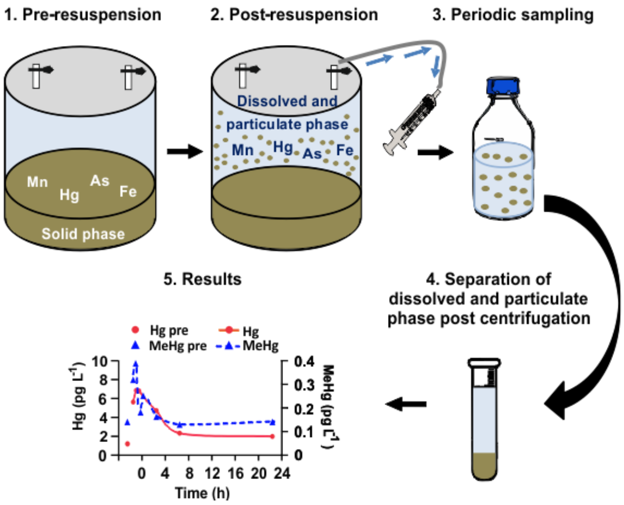
Supplementary Material - Figure captions

Fig. S1. Biplots summarising the results of PCA performed on A) grain size composition and total concentrations of As, Hg, Fe, Mn and S in the sediment cores and B) physico-chemical parameters and dissolved concentrations of Hg, MeHg, As(III), As(V), Fe, Mn, SO42- and DOC in the porewaters collected from the port of Llanes (LL), the Villaviciosa estuary (VV) and the Nalòn River estuary (SJ and SE).

Fig. S2. Behaviour of dissolved Hg, MeHg, As(V) and As(III) as a function of time during the resuspension experiments at the Nalón River estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL). Note As(III) is only present at these two sites. Note that the term “pre” indicates the PTE concentration before the resuspension event (at time $T_{pre-res}$).

***Graphical Abstract**

Graphical abstract



***Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)**

Highlights

- The behaviour of As and Hg species during sediment resuspension was investigated
- As and Hg occurrence in porewaters are related to dissolution of Fe oxy-hydroxides
- Particulate and dissolved As and Hg increased following the resuspension
- Fe oxy-hydroxides strongly regulate the mobility of As and Hg in the water column
- The restoration of pre-resuspension conditions was mainly reached after few hours

Mercury and arsenic mobility in resuspended contaminated estuarine sediments (Asturias, Spain): a laboratory-based study

Efren García-Ordiales[1], Stefano Covelli[2,3]*, Greta Braidotti[2], Elisa Petranich[2], Elena Pavoni[2,4], Alessandro Acquavita[5], Lorena Sanz-Prada[1], Nieves Roqueñí[1], Jorge Loredo[1]

[1]ISYMA Research Group, University of Oviedo, Oviedo, Spain.
[2]Department of Mathematics and Geosciences, University of Trieste, Via E. Weiss 2, 34127 Trieste, Italy
[3]CoN.I.S.Ma. Consorzio Nazionale Interuniversitario per le Scienze del Mare, Piazzale Flaminio 9, 00196 Rome, Italy
[4]Department of Chemical and Pharmaceutical Sciences, University of Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy
[5]ARPA FVG Agenzia Regionale per la Protezione Ambientale del Friuli Venezia Giulia, Via Cairoli 14, Palmanova, 33057 Udine, Italy

*Corresponding author:
Stefano Covelli
Department of Mathematics and Geosciences
University of Trieste
Via Weiss, 2
34128 Trieste (Italy)
Phone: +39.040.5582031
Fax: +39.040.5582203
e-mail: covelli@units.it

June 2020

ABSTRACT

Estuarine sediments must be dredged in order to allow for navigation, and where these sediments are placed after dredging depends upon guidelines based only on the total concentration of contaminants. However, resuspension events could seriously affect the mobility and speciation of contaminants, including potentially toxic trace elements stored in sediments. The effects of resuspension on the cycling of mercury (Hg) and arsenic (As) between the sediment and water column was investigated in a mesocosm study. Four experiments were conducted in three estuaries in northern Spain based on samples collected from sites which have been impacted by now decommissioned Hg and As mines and periodically subjected to dredging activities. Designed to mimic the resuspension of particles, each of the experiments revealed that the release of Hg and As species does not depend on the total concentration in the sediments (16.3-50.9 mg kg^{-1}, for As and 0.52-5.01 mg kg^{-1} for Hg). The contribution from porewaters and the subsequent reductive dissolution and/or desorption appears to be the main processes responsible for the abrupt increase in dissolved Hg and As species (maximum release of 427 % and 125 %, respectively). In some cases, As and Hg continued to remain at high concentrations in the water column even after the experiments were completed (after 24 h), thus testifying to their critical persistence in the dissolved form. Conversely, at the other sites, the restoration of pre-resuspension conditions were observed only a few hours after resuspension, mainly due to the role of Fe oxy-hydroxides which provides suitable surfaces for adsorption and/or co-precipitation processes involving dissolved Hg (maximum removal of -58 %) and As (maximum removal of -25 %) species. The results of this research could be helpful for policy making and to take appropriate decisions regarding dredging.

Keywords: estuarine sediments, mercury, arsenic, contamination, resuspension, dredging

1. Introduction

Estuaries are crucial areas for sediment transfer between fluvial and marine systems, often forming sinks for sediment moving downstream, alongshore or landwards (Ridgway and Shimmield, 2002). A wide range of human activities take place in these sites (i.e., ports, industrial, urban and recreational settlements) and, as a result, estuarine waters receive dissolved and particulate contaminants, including potentially toxic trace elements (PTEs). Fine-grained sediments often become a repository for contaminants by reducing their toxicity (Eggleton and Thomas, 2004 and references therein). However, natural events such as tidal currents, wave action, storm surge and bioturbation (Sanford et al., 1991; Arfi et al., 1993; Kalnejais et al., 2007), along with anthropogenic activities including dredging, shipping and trawling (Schoellhamer, 1996; Lewis et al., 2001), may be responsible for the resuspension of sediment particles. In particular, dredging and disposal are common practices to maintain navigation channels and access to port areas, but they may impact upon aquatic communities (Roberts, 2012) since contaminants can be released in dissolved form in the water column (Stephens et al., 2001; Caplat et al., 2005). When resuspended in the oxic water column, anoxic sediments may result in variable desorption rates of PTEs previously co-precipitated with/or adsorbed to Fe and Mn sulphides (Simpson et al., 1998; Caetano et al., 2003). The released Fe and Mn are quickly reprecipitated, acting as scavengers for PTEs and other contaminants (Caetano et al., 2003; Jones-Lee and Lee, 2005). This precipitation process will involve any particle available with a layer of Fe oxy-hydroxide, previously mobilised by the oxidation of sulphides, and PTEs will bind to the newly created adsorption surfaces of the settling particles (Goossens and Zwolsman, 1996). The extent of sediment resuspension and dispersal depends on the water movements in nearby dredging/disposal areas (Eggleton and Thomas, 2004). The modification of the physico-chemical properties (i.e., pH and redox conditions) can affect not only the mobility, but also the bioavailability of toxic compounds with adverse effects on aquatic organisms (Kim et al., 2004, 2006; Cotou et al., 2005; Bocchetti et al., 2008).

Investigation into the potential mobility and bioavailability of contaminants in estuarine sediments is a challenging but essential task, since the understanding of the processes involved in the recycling of these contaminants between solid and aqueous phases may contribute to the preservation of water quality by informing environmental policies. To better evaluate these issues, most research has taken the form of laboratory experiments which simulate the dredging effects using different time intervals (e.g. Van Den Berg et al., 2001; Caetano et al., 2003; Cantwell et al., 2008; Monte et al., 2015; de Freitas et al., 2019).

Among PTEs, mercury (Hg) and arsenic (As) are well known for the recognised toxicity of their different chemical species and diffusion in the environment (i.e., industrial settlements, mining activity). There are several studies on Hg (and methylmercury, MeHg) remobilisation after resuspension from bottom sediments (e.g. Bloom and Lasorsa, 1999; Conaway et al., 2003; Kim et al., 2004; Benoit et al., 2009; Acquavita et al., 2012; Seelen et al., 2018; Zhu et al., 2018). In particular, Bloom and Lasorsa (1999) found that approximately 5% of MeHg bound to the sediment and less than 1% of total Hg were released during a laboratory mixing experiment, but the release can be limited in quantity and time due to the dilution of the Hg species in the water body and their reburial in the solid phase (Acquavita et al., 2012).

The Asturias coast (northern Spain) represents one of the most impacted regions of Spain due to several anthropogenic activities, including long-term mining of sulphide ore, which have severely affected the Nalòn River drainage basin, the main hydrographical system (3692 km^2) of the region (Loredo et al., 1999; Loredo, 2000; Fernández-Martínez et al., 2005). The sources and distribution of trace elements, especially As and Hg, have been investigated in several environmental matrices including soils, mine tailings, fresh and ground waters and estuarine sediments (e.g. Loredo et al., 2010; Ordóñez et al., 2014; Silva et al., 2014; García-Ordiales et al., 2018). In the Nalón River estuary, in spite of the high levels of As and Hg in the fine sediments, redox conditions appear to govern the speciation cycles of these elements, thus reducing the formation of the most toxic species, such as As(III) and MeHg (García-Ordiales et al., 2018). In addition, there is evidence along the Asturian coasts that other minor estuarine systems may be potentially affected by Hg and As contamination (Forján et al., 2019; García-Ordiales et al., 2019a, 2020; Sanz-Prada et al., 2020).

The primary aim of this research was to investigate the effects of simulated resuspension events in replicate mesocosms to predict the behaviour of Hg and As associated with bottom sediments and porewaters and their fate in the water column after a perturbative event. A comparison among similar estuarine environments in Asturias affected by various sources of contamination and where dredging operations are periodically needed to allow navigation was provided. The occurrence and mobility of Hg and As in this area is of great concern due to their potential bioaccumulation in the aquatic trophic chain. Within the European Union, the Water Framework Directive (WFD, European Parliament, Council of the European Union, 2000) sets “good status” objectives for water bodies throughout the member states. The status is simply based on chemical and ecological criteria. A classification system has been developed to decide upon chemical status, with threshold values known as “Environmental Quality Standards” (EQS). A specific maritime policy was later established and directed for marine ecosystem protection and conservation by the EU Marine Strategy Framework Directive (MSFD) (European Parliament, Council of the European Union 2008). The MSFD specifically requests that member states implement monitoring programs for the assessment of the environmental status of marine waters. The results of this experimental study may be useful in providing the scientific background to help policy-makers take appropriate decisions regarding dredging and to control PTE contamination levels. This is especially relevant due to the biological richness of estuaries, their significance as sites of a wide variety of food intended for human consumption (fisheries, aquaculture, mussel collection), and the possible implications for the health and economy of affected areas. This experimental approach could be applied to all coastal environments where information regarding the major geochemical processes that regulate PTE behaviour, mobility and fate is needed to support environmental management and risk assessment.

2. Materials and Methods

2.1. Study area

The experimental sites are located in three estuaries found in eastern central Asturias (Fig. 1), which has one of the best-preserved coastline environments in Spain. As part of this conservation policy, these estuaries are supported by several environmental protection programmes and strategic EU initiatives such as the Habitats Directive and the Natura Network 2000 (92/43/EC). Each of these estuaries has diverse characteristics (Table 1).

The Nalón River estuary

The Nalón River empties into the largest drainage basin in the northern Spain. Its estuary has been subject to very high levels of contamination due to mining which took place over a period of 150 years (García-Ordiales et al., 2019a). The riverine flow to the estuary is high, showing a notable water column stratification that may extend several kilometres (Ceñal and Flor, 1993). The estuary has two port areas which are exposed to the main channel flows, which in turn affects the grain size of the sediments. The San Juan (SJ) port area is quite open and the accumulated bottom sediments are mostly sandy, whereas the San Esteban (SE) port area has a protection dock which acts as a sedimentary trap for silt and clay (García-Ordiales et al., 2018). García-Ordiales et al. (2018, 2019b) reported high concentrations of As (20.1-68.1 µg g^{-1}) and Hg (0.10-1.33 µg g^{-1}) in bottom sediments and the potential transfer to biota.

The Villaviciosa estuary

The Ria de Villaviciosa (VV) is located approximately 60 km east of the Nalón estuary. The drainage basin extends for 160 km^2 and the estuarine area is 12.6 km^2. The freshwater flow rate is low and the tidal influence is strong, producing a total mixing, the absence of water column stratification and a high sedimentary accumulation rate (Flor et al., 1996). Recently, as a consequence of wastewater discharge, mussel collection has been banned. Other potential anthropogenic contamination sources are not known. Conversely, in nearby areas there are different outcrops of bituminous rocks and hydrothermal ores of fluorite that could potentially be the sources of As and Hg (Iglesias and Loredo, 1994; González-Fernández et al., 2018). Preliminary sediment analyses performed by the regional port authority have reported average concentrations of 11.3 µg g^{-1} for As and 0.18 µg g^{-1} for Hg (unpublished data).

The Llanes port

The Llanes (LL) port is approximately 110 km east of the Nalón estuary and 50 km east of VV estuary. Similar to VV, the tidal influence in LL is high, producing a lack of stratification in the water column due to the almost null contributions of freshwater from the inflowing Carroced creek. The sedimentary dynamics are controlled by an access gate between the old (inner) and new (outer) port areas. The tidal flow restriction produced by the gate has converted the old port area, where sediments were collected for this study, into a sedimentary trap of fine sediments similar to SE. Over the last 15 years, sediment monitoring conducted by the regional port authority has shown the presence of high Hg concentrations (up to 3.81 µg g^{-1}) (unpublished data). Potential local sources of Hg are currently under investigation.

2.2. Experimental approach

A scuba diver collected undisturbed short sediment cores by pushing a Plexiglass tube (30 cm length; 16 cm i.d.) into the bottom sediment. Upon collection and transport to the laboratory, these cores were extruded and sectioned into five slices (0-1, 1-2, 2-3.5, 3.5-5 and 5-7 cm) in a N_2-filled chamber to preserve the original redox conditions. At the same time, the redox potential (Eh) was measured in the surnatant water and the corresponding slices. These slices were homogenised and split to determine water content. The remaining sediment was centrifuged at *in situ* temperature and the extracted porewaters, recovered in the inert atmosphere (N_2-filled chamber), were filtered (0.45 µm pore size, i.d. = 33 mm, Millex-HV), collected in acid pre-cleaned vials and stored in a deep-freeze until analyses.

In parallel, bottom sediments and the overlying water were collected by a scuba diver using a cylindrical Plexiglas chamber as a sampler (h = 25 cm, i.d. = 24 cm, wall thickness = 0.6 cm) following the experimental approach applied by Acquavita et al. (2012). The average depth of sediments inside the chamber was 10 cm with approximately 15 cm of overlying water (ratio water/sediment = 1.5). Careful transportation of the chamber to the laboratory minimised perturbation at the sediment-water interface (SWI). Here the overlying water was drained off and carefully replaced with bottom water collected from the same sampling location using a Niskin bottle. This step was taken in order to exclude any possible perturbative effect on the water inside the chamber possibly occurring during transportation. (e.g. Bertuzzi et al., 1996; Emili et al., 2011; Acquavita et al., 2012). The basic physico-chemical parameters (T, pH, Eh) were measured using a portable multiprobe (Crison MP41). Eh measurements were performed by means of a Pt/KCl–Ag/AgCl electrode calibrated with ZoBell solution (220 mV at 25ºC).

The simulated resuspension was performed using a mechanical horizontal shaker (130-150 cycles min^{-1}, t = 10 min) after sealing the top hole of the chamber. Periodic sampling of the resulting mixture between dissolved and particulate phases was then performed. In detail, the benthic chamber was left open and undisturbed between one sampling and the next to simulate real environmental conditions where the water column is in direct contact with the atmosphere. In order to allow the collection of water just above the SWI, the cover was repositioned before each sampling. In detail, the cover has two valves on its lid, one being connected to a tube (0.5 cm diameter) that allows for water sample collection at approximately 5 cm just above the SWI. This is important to properly evaluate variations of the dissolved and particulate concentration of chemical species as a function of time, as well as the grain size composition of the suspended particles which tend to become finer after the resuspension event. Using a plastic syringe, water lying on top of the sediment was syphoned off via one of the two valves on the lid of the chamber and placed in a pre-treated borosilicate container (V_f = 600 mL). Seven samples were recovered within 24 h (T_0 = 0, T_1 = 30 min; T_2 = 1h; T_3 = 2h; T_4 = 4h; T_5 = 8h; T_6 = 24h) and the main physico-chemical parameters were promptly measured. Samples were then centrifuged (10,000 rpm min^{-1}, t = 10 min; Eppendorf 5804) to separate the dissolved phase from the suspended particles. The solid fractions were collected for both grain size and chemical analyses. The dissolved phase was subsequently filtered (0.45 µm pore size, i.d. = 33 mm, Millex-HV) and divided for the necessary analytical aliquots.

2.3. A*nalyses of samples*

Grain-size analyses on bottom sediments and resuspended particles were performed following the method outlined by García-Ordiales et al. (2017). The sediment aliquots were oxidised (H_2O_2=3%, t=24 h) to remove most of the organic matter (OM). Successively, the solution was wet-sieved through a 2-mm sieve to remove coarse, shelly fragments. The fraction < 2000 µm was recovered and analysed using a laser difractometer (Fritsch Anaysette 22 Laser-Particle Sizer Microtec).
The measurement of the isotopic composition of Hg was carried out via gas chromatography combined with inductively coupled plasma mass spectrometry (7890A Agilent GC and HP 7500c

Agilent ICP-MS). The determination of the elemental species concentrations was carried out by species-specific isotope dilution mass spectrometry. All sample preparation procedures were previously developed and validated for water (Amouroux et al., 1998, Rodríguez-González et al., 2002, Bouchet et al., 2011) and sediments (Rodríguez Martin-Doimeadios et al., 2003; Rodríguez-González et al., 2007). The mathematical approach of the methods was described in detail in Rodríguez-González et al. (2013).

The As species were analysed using a mobile phase of 2 M phosphate buffered saline (PBS)/0.2 M EDTA (pH 6.0) in a separation column with a 1260 Infinity high-performance liquid chromatograph (HPLC) coupled with ICP-MS. The As species were directly measured in the porewater samples, whereas in the case of solids, 0.1 g of sample was placed together with an extracting agent (1 M H_3PO_4+0.1 M, ascorbic acid) in a microwave vessel (Ruiz-Chancho et al., 2005). The digested solution was analysed following the aforementioned method. The accuracy of the results was verified by comparing the total As concentration to the sum of all the determined species concentrations. The recovery of As speciation ranged from 95% to 106%.

Total As, Fe, Mn and S concentrations in dissolved and solid phases (bottom sediments and resuspended sediment particles) were determined by ICP-MS and ICP-OES. For solids, samples were previously digested by *aqua regia* + HF in a microwave vessel (EPA method 3052). Samples were analysed in batches, and the accuracy of the elemental determination was verified using the CRM042-056 and other internal laboratory standards. Recovery percentages of the different elements were in the range of 93–106%, and the RSD was less than 7%. The dissolved organic carbon (DOC) was determined using the TOC-V CSH (Shimadzu) instrument.

2.4. Explorative multivariate data analysis

Principal component analyses (PCA) was employed as an unsupervised exploratory chemometric tool for the visual identification of relationships among the samples (PC scores and score plots), within variables (PC loadings and loading plots), and between samples and variables (biplots) (Oliveri et al., 2020). In order to minimise systematic differences between variables, data matrices were pre-processed (column autoscaling) before the multivariate analysis (Oliveri et al., 2019). Multivariate analysis was performed using the CAT (Chemometric Agile Tool) package, based on the R platform (The R Foundation for Statistical Computing, Vienna, Austria) and freely distributed by Gruppo Italiano di Chemiometria (Italy) (Leardi et al., 2019).

3. Results and Discussion

3.1. Sediment and porewater chemistry

Grain-size distribution was found to be different among the sampling sites and rather constant with increasing depth (Table 2, Fig. S1A). According to Shepard's (1954) classification, the sediment consisted mostly of silt, which was found to be higher than 90% at VV and SE, followed by SJ and LL which appeared to be less homogeneous with depth. The clayey fraction was rather constant with depth and generally poorly represented, whereas the sandy fraction clearly prevailed at LL and SJ (Table 2, Fig. S1A).

The Asturian estuarine systems are known to be affected by high levels of anthropogenic pressure (García-Ordiales et al., 2018, 2019a, b), thus the sediments collected displayed elevated concentrations of both As and Hg (Table 2). Although disparities in trace element concentrations along the sediment cores collected at the investigated sites are not particularly evident, the PCA output (explaining 84.7% of the total variance) clearly denotes the differences among the various sites and are distinctly represented in the biplot (Fig. S1A). Arsenic was found to be rather homogeneous downcore, with the lone exception being site SE, where As rapidly decreased from

50.9 mg kg^{-1} (0-1 cm) to 31.8 mg kg^{-1} (1-2 cm). No significant differences were observed for Hg at sites VV, SJ and SE (mostly lower than 1.0 mg kg^{-1}), whereas the element was found to be one order of magnitude higher at site LL (3.73 ± 0.76 mg kg^{-1}) as also confirmed in the PCA output showing a relatively clear relationship between Hg and site LL (Fig. S1A). Silva et al. (2014) reported that As incorporated into short-range ordered Fe oxy-hydroxides was the predominant fraction in the stream sediment samples of the Nalòn River drainage basin. This fraction represented more than 80 % of the total As concentration, although realgar and pararealgar [As_4S_4], orpiment [As_2S_3], and arsenic-rich pyrite were the original sulphide ore deposits (Ordoñez et al., 2014).

Similarly, Fe, Mn and S were rather constant moving downcore, except for the weak increase observed for Mn at SE. Comparable S concentrations were found among sites, although slightly higher values were detected at LL and VV. Conversely, Fe and Mn showed the highest concentrations at the Nalón River estuary sites, especially at SE (Fig. S1A).

Estuarine sediments suffer intense chemical, physical and biological reactions due to the interaction between the solid phase and porewaters, promoting the formation of new and altered minerals and/or changes in the porewater composition (Beck et al., 2008; Oliveri et al., 2016). In this context, Fe and Mn oxy-hydroxides represent suitable adsorptive phases for PTEs, especially under oxidising conditions (Turner et al., 2004). Conversely, under conditions of oxygen depletion, Fe and Mn oxy-hydroxides may act as secondary oxidant sources during OM degradation (Froelich et al., 1979) and their subsequent reduction/dissolution could be responsible for the release of dissolved PTEs in porewaters.

Oxidative conditions were found in the surnatant water (Eh ranged from 132 to 161 mV) as well as quite low total dissolved Hg, MeHg and As (III and V) species, followed by Fe and Mn (Fig. 2). This is clearly evident in the PCA output (explaining 71.1% of the total variance) highlighting a strong relationship between the redox conditions and the surnatant water collected from all sites

(Fig. S1B). Although some differences occurred among the sites, OM degradation and slow oxygen diffusion through the sediment drove reductive conditions just below the SWI. Redox potential (Eh) decreased downcore at all sites (up to almost - 400 mV) whereas dissolved As and Hg species (at LL) and MeHg (0-2 cm) at site SE increased with depth (Fig. 2 and S1B), due to reductive dissolution of Fe oxy-hydroxides (Smedley and Kinniburgh, 2002; Fiket et al., 2019). In detail, the strong correlations between As(III) and As(V) (r = 0.919) and between As species and dissolved Fe (r =0 .770 and r = 0.757 for As(III) and As(V), respectively) highlighted in the PCA output (Fig. S1B) could be explained as Fe dissolution, rather than Mn (r = 0.171 and r = 0.131 for As(III) and As(V), respectively), drove the release of As species especially at the Nalòn estuary sites (SE and SJ). This is also consistent with the fact that the role of Mn oxide in the reduction and subsequent release of adsorbed As(V) into porewaters definitely appears to be secondary (Masscheleyn et al., 1991) confirming that As should be mainly released from Fe oxy-hydroxides at sites SJ and SE.

Conversely, at site VV, a marked release of dissolved Fe and Mn in the upper sediment (520-1,360 µg L^{-1} and 299-828 µg L^{-1}, respectively) appears not to have affected the dissolved Hg and As species that, in fact, showed constant trends with depth (Fig. 2). Moreover, no variability was noticed in the sediments downcore for As, Hg, Fe and Mn. It is possible that their behaviour in the sediment did not result in Fe and Mn oxy-hydroxides precipitation. Due to highly reducing conditions (- 400 mV), Hg and As could be present as insoluble or very low soluble sulphur compounds, as also suggested by the strong correlation between Hg and S in the sediment matrix (r = 0.628, Fig. S1A) (Fang and Chen, 2015; Wang et al., 2016; Petranich et al., 2018).

Dissolved As species were found to be high in the Nalón estuary (SJ and SE, Fig. S1B), where both As(V) and As(III) notably increased with depth reaching concentrations of 231 and 67.3 µg L^{-1} at SE whereas lower values were found at SJ (179 and 32.9 µg L^{-1}, for As(V) and As(III), respectively). In this context, simultaneous dissolution and desorption processes involving Fe oxy-hydroxides, carbonate fraction and/or the degradation of OM (Wang et al., 2016 and references therein), may have led to elevated amounts of As(V), which in turn might be rapidly reduced by bacteria to the more labile and toxic As(III) (Dowdle et al., 1996; Smedley and Kinniburgh, 2002; Pfeifle et al., 2018). Indeed, dissolved Fe increased in the first cm (up to 4,131 µg L^{-1} at SJ and 6,469 µg L^{-1} at SE, respectively) attesting to the transition to anoxic conditions (Fiket et al., 2019). This is in agreement with the trend described for As species, since the reduction of As(V) usually occurred after Fe(III) and before sulphate reduction according to the diagenetic sequence (Froelich et al., 1979; Smedley and Kinniburgh, 2002).

As also confirmed in the PCA output (Fig. S1B), total dissolved Hg gradually increased with depth only at LL (from 2.18 to 13.3 pg L^{-1} in the surnatant water and deepest layer, respectively). In this site, MeHg was found to be extremely low (0.40 ± 0.26 pg L^{-1}) and represented only 8.26 and 6.64 % of the dissolved Hg pool in the surnatant water and downcore, respectively. Moreover, a constant increase downcore was observed (from 0.18 to 0.88 pg L^{-1}) whereas slightly elevated amounts of MeHg were found in the 1-2 cm level at sites SJ and SE (Fig. 2 and S1B). Although Hg and MeHg generally displayed extremely low concentrations, the MeHg profile found at site SE suggests that methylation processes mediated by sulphate reducing bacteria (Hines et al., 2017) cannot be completely excluded and could be favoured under low rates of sulphate reduction (Gilmour et al., 1992). The Log K_D of Hg (expressed in L kg^{-1}), which describes the partitioning between the solid and dissolved phases (Hammerschmidt et al., 2004), was quite constant with depth, varying between 8.18 ± 0.02 (SE) and 9.13 ± 0.13 (VV), thus suggesting the prevalence of the element in the solid phase (Table S1). The extremely negative values of Eh can favour the occurrence of sulphate reduction allowing for sulphide precipitation (Hines et al., 1997). However, although the highest concentrations of both Hg (5.01 mg kg^{-1}) and S (1.20 %) were detected at site LL, no correlation was found between the two elements (r = 0.297 at site LL), contrary to what was observed at sites VV (r = 0.959), SJ (r = 0.577) and SE (r = 0.732). This suggests that the occurrence of Hg at site LL may be related to a different local source currently under investigation. Indeed, Forján et al. (2019) pointed out high concentrations of Hg at Vega beach (approximately 40 km west of site LL) due to the discharge of mine tailings from the district of Berbes which was one of the main fluorite mining areas in Europe where cinnabar was occasionally present (Iglesias and Loredo, 1994; Symons et al., 2017; Levresse et al., 2019). On account of the marine outfall, which has been built up for the discharge since 1990s (Forján et al., 2019), it is reasonable to expect that ocean and shoreline currents play a crucial role in transporting fine suspended particles enriched in Hg into the Llanes estuary (LL).

The sulphate reduction may also have influenced the low levels of dissolved As(III) in the deepest layer at VV and LL (0.07 and 9.42 µg L^{-1}), since the reduction of As(V) is inhibited when sulphate reduction occurs (Moore et al., 1998; Frohne et al., 2011; Burton et al., 2013). At these sites, the reduction of Fe and Mn oxy-hydroxides appear to be relatively notable only in the upper sediments. Conversely, dissolved Fe and Mn showed the highest concentrations in porewaters only below 2 cm at SE and SJ as well as in the corresponding sediment cores (Fig. 2 and 1SB), thus indicating a higher release from sediment to porewaters at the Nalón estuary sites.

Sulphate profiles showed an almost stable trend, except for LL, where they decreased downcore. Together with the increase of Hg and the elevated DOC concentrations, this may have favoured methylation processes at LL, thus explaining the increase in dissolved MeHg in the deeper layers (Fig. 2 and S1B).

3.2. Resuspension experiments

3.2.1. Physico-chemical parameter variability

Total suspended matter (TSM) content was markedly high just after artificial perturbation was stopped (T_0) (Fig. 3), especially at SE and LL (4.88 and 3.42 g L^{-1}, respectively), and decreased abruptly after 30 min (Table S2) at all sites until the end of the experiments. This trend suggests that the effect of resuspension is limited at the initial stage of the experiments, when the highest turbidity was followed by the rapid deposition of the coarser fractions (Van Den Berg et al., 2001). Total suspended matter (TSM) grain size distribution was determined only at the Nalón estuary (SJ and SE) (Fig. 3). At site SE, coarse and fine silt predominated at T_0, followed by the finer fraction (very fine silt) after 30 min and by the clayey fraction after 8 h. At site SJ, the distribution was trimodal and fine sand, coarse and fine silt prevailed at T_0. Due to the low amount of TSM (close to the lod of the instrument), the distribution was irregular in the rest of the experiment but similar to that described for SE (Fig. 3).

The pH varied in a narrow range at all the sites (7.08-7.86) with the exception of SE (7.04-8.02) (Table S2). The Eh values testified to the occurrence of oxidising conditions throughout all the experiments with a decreasing trend from pre-resuspension to the initial stage of the experiment (T_0) at LL and SJ, and an increasing trend at VV and SE (Table S2). Generally, there were no marked variations (almost constant) and the values at the end of the experiment were lower than the beginning (Fig. 3). Despite the reductant conditions found in the upper levels of the sediment cores (Fig. 2), the oxidising environment in the mesocosm experiment suggested that the direct contribution from porewaters was negligible (Van Den Berg et al., 2001) or had hidden any dilution processes between porewaters and the water into the chamber.

3.2.2 Behaviour of dissolved chemical species after resuspension

The behaviour of PTEs during a resuspension event is influenced by physico-chemical parameters and by dissolved Fe and Mn occurrence. In oxic conditions, Fe and Mn are present as oxy-hydroxides and are often involved in adsorption processes, co-precipitation, but when reducing conditions occur there is a dissolution with the subsequent release of dissolved PTEs (Gagnon et al., 1997).

Noteworthy differences were found among the sites, both in terms of concentration and trend during the experiments (Fig. 4 and 5). Iron and Mn varied in a wide range being the highest concentrations at SE (up to 7,180 and 2,760 µg L^{-1}, respectively) followed by SJ. Conversely, at LL and VV there was a low variability and the concentrations were significantly lower.

A common behaviour at all the sites was the increasing trend in the earliest phase of resuspension, since the disturbance events involving anoxic sediments usually lead to the release of dissolved Fe and Mn (Saulnier and Mucci, 2000; Caetano et al., 2003). Considering dissolved Fe, for instance, the maximum concentrations found at SE (7,180 µg L^{-1} after 30 min) and SJ (5,100 µg L^{-1} at T_0) could be due to the contribution of porewaters following resuspension. In fact, at SE, Fe was found to be higher than 6,000 µg L^{-1} in the porewater extracted from the first centimetre (Fig. 2). Iron and Mn decreased over time at the Nalón estuary sites, reaching concentrations lower than the beginning, most likely due to oxidation processes and subsequent precipitation of Fe oxides. According to Caetano et al. (2003), adsorption and co-precipitation in association with Fe oxides represents the main processes responsible for the removal of dissolved Mn from the solution. Indeed, Fe oxides appear to play a crucial role as surface catalysts in the oxidation of Mn (Caetano et al., 2003 and references therein). Indeed, due to the oxidising conditions measured in the chamber (Eh > 120 mV), adsorption and co-precipitation with freshly formed Fe oxides are likely the main processes which remove Mn from the solution.

Conversely, at the other sites, Fe varied more (LL) or less (VV) markedly during the experiments, especially until 4 h (Fig. 4), whereas Mn was almost constant at both sites during the resuspension experiment (24 h). Similar behaviour was observed by Caetano et al. (2003), who stated that following resuspension, dissolved Mn remains in solution over a longer period of time than Fe and then decreases slowly to reach stable levels after one week (Saulnier and Mucci, 2000).

At the four investigated sites, the behaviour of dissolved Hg, MeHg, As(III) and As(V) during the resuspension experiment is discussed in the following paragraphs and described by a negative exponential equation (1):

$$(1)\ C_{Ti} = C_{T0}\, e^{-kx}$$

where C_{Ti} is the concentration at any time (T_i) during the resuspension experiment, C_{T0} is the concentration at T_0 and k is the rate constant for the contaminant dispersion as a function of time (Fig. S2). The equation (1) was calculated until a decreasing trend was observed and was not applied when the concentration at $T_{0,}$ was higher than the concentration measured before the resuspension experiment (T_{pre}). Indeed, the final aim was to verify how long it took for the samples to return to their initial pre-resuspension conditions.

Behaviour of dissolved mercury species

Although the highest Hg concentrations in sediments and porewaters were found at LL (avg. 3.90 mg kg^{-1} and 6.85 pg L^{-1}, respectively), the release of total dissolved Hg at this site (1.18-6.22 pg L^{-1}) was found to be comparable with those observed at sites SE (1.90-4.50 ng L^{-1}) and SJ (2.95-5.90 ng L^{-1}). The lowest values were found at site VV (0.41-1.27 pg L^{-1}). This could indicate greater Hg mobility at the Nalón estuary sites, in agreement with previous research (García-Ordiales et al., 2018). In addition, MeHg at these sites was found to be twenty times higher (3.54-9.97 pg L^{-1} at SJ and 4.13-6.93 pg L^{-1}at SE, respectively) than at LL (0.12-0.33 pg L^{-1}) and VV (< lod-0.14 pg L^{-1}) (Fig. 4 and 5). Mercury concentrations at SE and SJ were similar to those reported by Acquavita et al. (2012) in a lagoon environment contaminated by Hg from mining and industrial origin. The same authors reported notably higher (avg. 226 and 51 pg L^{-1}, respectively) MeHg concentrations than our study, likely due to a sudden release from porewaters at T_0.

Total dissolved Hg and MeHg increased after resuspension and then decreased, in all the investigated sites, until the end of the experiment. According to the equation given above (1), dissolved Hg and MeHg reached concentrations comparable to those measured before the resuspension experiment (T_{pre}) after 6 h at sites LL, VV and SJ (Fig. S2). The only exception was the dissolved Hg at site LL, where Hg was found to be slightly higher at the end of the experiment (1.87 pg L^{-1} after 24 h) with respect to the initial stage (1.18 pg L^{-1} before the experiment). These trends are similar to those for dissolved Fe and Mn, thus suggesting a potential removal (scavenging) of Hg species due to Fe oxy-hydroxide formation (Gagnon et al., 1997; Acquavita et al., 2012; Petranich et al., 2018). The only exception was SE, where Hg and MeHg slightly decreased from 4.50 ng L^{-1} and 5.08 pg L^{-1} (pre-resuspension, $T_{pre\text{-}res}$) to 3.10 ng L^{-1} and 4.13 pg L^{-1} (T_0), respectively (Fig. 5). Successively, Hg decreased to the same levels observed at other sites, whereas MeHg progressively increased to 7.25 pg L^{-1} (after 4 h) and remained almost constant until the end of the experiment (after 24 h). This could be attributed both to MeHg released from porewaters (Fig. 2) or to the mobilisation of bioavailable OM during resuspension, which may have favoured the metabolic activity of sulphate-reducing bacteria involved in Hg methylation (Gilmour et al., 1992) as suggested by the high levels of DOC (11.2-17.6 mg L^{-1}, Fig. 5).

Since significant changes in the association with Hg binding phases could influence methylation and the subsequent transfer of MeHg from the sediment to biota (Kim et al., 2006), the MeHg/Hg ratio (%) in the dissolved phase after resuspension was calculated. Despite the low MeHg levels (< lod-0.33 pg L^{-1}), VV and LL displayed the highest average ratio (11.5 and 4.79 %, respectively), if compared to SJ and SE (0.15 and 0.22 %, respectively). This is due to Hg that was at most one order of magnitude higher than MeHg at LL and VV, whereas at SJ and SE Hg was three orders of magnitude higher. A lower MeHg/Hg ratio was reported by Acquavita et al. (2012) where Hg levels

(2.95-15.2 ng L^{-1}) were far higher than MeHg (0.15-0.30 ng L^{-1}): here the ratio increased at the end of the experiment. In our study, this occurred only at SE, where increasing MeHg concentrations and decreasing Hg concentrations over time were observed and could be the result of methylation processes (Fig. 5). Indeed, the MeHg concentrations during resuspension were found to be one order of magnitude higher than those measured in the sediment porewaters, suggesting that a certain amount of MeHg may be produced during the experiment and/or released from the resuspended fine particles due to desorption processes. This is in agreement with previous research at the Nalòn estuary which has found that the highest levels of MeHg in the surface sediments occurred at site SE (Gracía-Ordiales et al., 2018). The same authors also found that low amounts of THg are available for methylation under unperturbed conditions. However, resuspension events may alter the original conditions with the subsequent remobilisation of MeHg from the surface of the resuspended particles. Moreover, at site SE the highest concentrations of inorganic dissolved Hg were observed, as well as elevated concentrations of SO_4^{2-}, as well as an increase in DOC due to OM degradation during the resuspension experiment. Since sediments can show different methylation potential (Bloom et al., 2003) depending on several factors such as bacterial activity, the occurrence of OM and the concentration of inorganic Hg available for methylation (Gilmour et al., 1992; Bloom and Lasorsa, 1999), it is reasonable to hypothesise that sediment resuspension may promote methylation processes at site SE.

Behaviour of dissolved arsenic species

The behaviour of As is of particular interest as it was found to be readily bioavailable in surface sediments of the Nalón River estuary (García-Ordiales et al., 2019a, b). The release of As(III) from sediments to porewaters is enhanced under reducing conditions (Bataillard et al., 2014) due to desorption processes (Wilkin and Ford, 2006; Beak et al., 2008). Conversely, As solubility decreases during oxidation events as a result of precipitation of As(V), which often occurs coupled with adsorption on the surface of Fe(III) oxy-hydroxides (Saulnier and Mucci, 2000; Amirbahman et al., 2006; Root et al., 2007; Jeong et al., 2010). Thus, resuspension events involving anoxic sediments strongly affect the release of dissolved As (Saulnier and Mucci, 2000). Indeed, sediment resuspension processes can interfere with the equilibrium of these chemical forms, inducing changes in the redox conditions, which in turn could favour the desorption of As from Fe oxy-hydroxides, as well as the reductive dissolution of these minerals, with a subsequent release from sediment porewaters to the overlying water column (Smedley and Kinniburgh, 2002). This could explain the high As(V) concentrations at T_0, whereas the release of As(III), with concentrations up to ten times lower than As(V), was evident only at SE and SJ (Fig. 5). The prevalence of As(V) may be related to oxidation and/or adsorption processes mediated by Fe oxy-hydroxides, which represent very efficient oxidants of As(III) (Saulnier and Mucci, 2000; Amirbahman et al., 2006; Root et al., 2007; Jeong et al., 2010).

The highest concentrations of As species, released from porewaters, were found at SE. Generally, As(V) markedly decreased at LL and VV reaching pre-resuspension conditions after 4-5 h (Fig. S2). To a lesser extent, something similar was observed at SJ, where As(V) and As(III) concentrations comparable with the beginning (pre-resuspension, $T_{pre\text{-}res}$) were reached after 18 and 9 h for As(V) and As(III), respectively (Fig. S2). On the contrary, the concentration of As(V) at SE after 24 h was higher than that seen before the experiment (15.3 and 9.81 $\mu g\ L^{-1}$ at T_6 and $T_{pre\text{-}res}$, respectively). At the same site, As(III) showed a sharp initial increase during the first 30 min of resuspension (0.19 to 2.77 $\mu g\ L^{-1}$ at $T_{pre\text{-}res}$ and T_0, respectively) reaching concentrations comparable to those measured before the experiment after 7-8 h, and then decreased over time until the end of the experiment (0.29 $\mu g\ L^{-1}$ after 24 h) (Fig. 5 and S2). This behaviour can be attributed to adsorption processes onto Fe oxy-hydroxides (Smedley and Kinniburgh, 2002) (Fig. 5), and to the formation of As(V), which consistently showed high concentrations throughout the experiment as a consequence of

As(III) oxidation (Bataillard et al., 2014) sustained by the oxidising conditions measured in the chamber (Table S2).

On the contrary, the rapid initial decrease in As(V) at LL and VV until 4 h after resuspension, could be due to adsorption processes on Fe oxy-hydroxides (Fig. 4), since dissolved Fe showed comparable behaviour during the experiments. Regarding the As(III), values < lod were likely related to the scarce contribution from porewaters, where very low concentrations were found (0.12-9.42 μg L^{-1} at LL and < lod and 0.07 μg L^{-1} at VV). Moreover, the availability of high concentrations of SO_4^{2-} (> 2,000 mg L^{-1}) to be reduced can inhibit the reduction of As(V) to As(III) (Burton et al., 2013). Bacterial activity also controls the As(V)/As(III) ratio (Bataillard et al., 2014): in oxic and abiotic conditions the As(V) form prevails, whereas in biotic conditions, As is preserved from total oxidation, thus both chemical forms can be present. This feature deserves to be more carefully investigated and should be taken into consideration in future studies.

Even DOC can be involved in adsorption processes on the suspended particulate matter. In our study, DOC concentrations immediately decreased at T_0, likely caused by the dilution with porewaters, and remained almost constant until 4 h after resuspension. Successively, DOC increased (at VV, SJ and SE) until the end of the experiment (after 24 h) and reached values lower than the beginning, with the exception of LL where a slight decrease was seen between 4 and 24 h after resuspension (13.2 and 10.3 mg L^{-1}, respectively).

3.2.3. Behaviour of the particulate phase after resuspension

Sediment resuspension events can determine the recycling of particles as well as the reintroduction of contaminants into the water column through the SWI (Bloesch, 1995). However, if the event occurred over a short period of time (24 h), the release of dissolved species is limited and most of the PTEs remain bound to the particulate phase (Van Den Berg et al., 2001; Cantwell and Burgess,

2004; Cantwell et al., 2008; Bataillard et al., 2014). According to Guerra et al. (2009), even in heavily contaminated sites (e.g. Pialassa Baiona Lagoon, Italy), dredging does not always have dramatic effects on the environmental quality of the aquatic environment. This could be the case in our study where resuspension was simulated for a limited time.
The Log K_D values for Hg and MeHg (K_D = [PHg]/[DHg], L kg^{-1}) were significantly higher at LL and VV (see Table S3), suggesting that these species remain preferentially partitioned in the particulate phase also during resuspension events, as previously stated by Kim et al. (2004). However, it is interesting to note that Log K_D at SJ and SE showed a decrease of two orders of magnitude if compared to that calculated for core sediments, thus confirming the evidence reported in García-Ordiales et al. (2018). The Log K_D of As species was generally low and did not show noticeable differences among the sites. Conversely, Fe and Mn appeared to be mainly associated with the dissolved phase (Log K_D, 0.81-4.08).

Although Hg and As(V) at SJ (which saw an increase during the first 30 min after resuspension), and As(V) at SE (which saw an increase between 30 min and 1 h after resuspension), particulate PTEs showed decreasing trends at all the sites (Fig. 6). This behaviour was also reported for Zn and Cu by Vidal-Durà et al. (2018) as the result of the settling of the resuspended particles as a function of time.

By comparing the particulate PTE trends with TSM content, a preferential bond to the silty or clayey fraction was found. At SE, the TSM rapidly decreased in the first 4 h after resuspension, from 4.88 (coarse silt) to 0.31 mg L^{-1} (very fine silt), as well as particulate Hg and MeHg, from 14.6 μg g^{-1} and 1.76 pg g^{-1} (T_0) to 5.65 μg g^{-1} and 0.67 pg g^{-1} (after 4 h,), respectively. However, as the particles in suspension became finer, particulate Hg and MeHg concentrations slightly increased up to 6.29 μg g^{-1} and 0.83 pg g^{-1} (after 24 h), respectively. This is consistent with the evolution of the grain size spectra and composition as a function of time (Fig. 3) and indicates that Hg and MeHg were bound to both the silty and clayey fractions, in addition to Fe. On the contrary, As(V), As(III) and Mn showed the highest concentrations in the early phase of the experiment, thus were preferentially bound to the silty fraction (Fig. 3).

At SJ, the clay prevailed in suspension as early as 1 hour after resuspension, when particulate Hg reached the highest concentration (9.18 µg g^{-1}), and particulate MeHg (0.98 pg g^{-1}) was slightly lower than the beginning (1.29 pg g^{-1}) and decreased as particles settled down (Fig. 6). As observed at site SE, Hg was found to be bound to both grain size fractions, whereas MeHg, Fe and Mn were preferentially bound to the silty fraction. Conversely, particulate As(V) and As(III) increased as the particles in suspension became finer.

3.3. Assessment of arsenic and mercury species released-removal after simulated resuspension events

The amounts of Hg, MeHg, As(V) and As(III) released during the experiments were estimated assuming that the resuspension events involved only the first 3.5 cm of the sedimentary sequence (based on visual observations), considering the size of the chamber and the water content in each layer of the sediment cores. Furthermore, the concentrations at $T_{pre-res}$ were converted into the amount (mass) of each chemical species. By adding the amounts of elements released by porewaters to those of the water column before resuspension ($T_{pre-res}$), and dividing by the water volume in the chamber (6.79 L), the chemical species concentrations that should have been found following the resuspension in the water column (T_0) were estimated (Table S4).

The concentrations measured at LL, VV and SJ (only for Hg species promptly re-precipitated/re-adsorbed) at T_0 were higher than those estimated for the sole porewater contribution, thus there was also an active desorption from the resuspended sediment. For the Nalón River estuary, the concentrations of Hg and As species at T_0 were found to be lower than the estimated concentrations at SE. In addition, the percentages of As and Hg species in the water column until the end of the experiment (after 24 h), with respect to the dissolved concentrations before resuspension were calculated (Table S4 and Fig. 7). Positive values for Hg and MeHg (excluding the sampling performed after 8 h) at LL and, in the first hour, at VV, suggested a further release from resuspended sediments. In detail, at LL the percentage of Hg released at T_0 was especially high (335%) and remained so (58%) after 24 h. Surprisingly, a scarce release at T_0 was found at SE where the concentration was approximately 30% lower than before the experiment ($T_{pre\text{-}res}$) and became even lower (-58%) after 24 h (Fig. 7). The time of removal for MeHg from the water column was quite variable among the sites, however, the MeHg levels were almost constant thus suggesting the enhancement of methylation processes after resuspension as observed by Acquavita et al. (2012). As suggested by Bloom and Lasorsa (1999), the mixing of surface sediments associated with fresh sulphate and organic carbon with contaminated deep sediments may be a favourable environment for MeHg production: if these conditions occur, unacceptably high levels of MeHg could result, however, this does not appear to be the case in this study.

At the end of the experiment, the concentration of As(V) at site SJ (4.97 µg L^{-1}) was found to be slightly lower than that measured before the resuspension (5.55 µg L^{-1}, $T_{pre\text{-}res}$) suggesting that the As(V) released during resuspension was removed at the end of the experiment (after 24 h) at SJ. On the contrary, this was not evident at site SE, where As(V) concentration was still higher (56%) at the end of the experiment (15.5 µg L^{-1}) than before the experiment (9.81 µg L^{-1}, $T_{pre\text{-}res}$). Something similar was observed for As(III) which was removed after 9 h at SJ (<lod) and slightly higher (0.29 µg L^{-1} after 24 h) than before resuspension (0.19 µg L^{-1}, $T_{pre\text{-}res}$).

Conclusions

Mainly due to the legacy of mining activities, Hg and As accumulated in sediments found on the Asturian coasts could be problematic in the case of resuspension due to natural (e.g. storm surges) or anthropogenic events (e.g. dredging). A preliminary characterisation of the area (sediment cores) was provided for the selected sites. Furthermore, to investigate the effect of resuspension and the subsequent remobilisation and redistribution of As and Hg species, a mesocosm experiment (simulated resuspension under controlled conditions) was performed. The major findings can be summarised as follows:

- The bottom sediments were mostly constituted of silt at SE and VV, whereas sand prevailed at LL and SJ. As concentration demonstrated a wider range than Hg moving downcore with rather homogeneous profiles. However, for both elements the levels were quite high and exceeded the standard set by the EU. Increasing negative Eh values moving downcore were measured at all the sites.
- Several dissolved species were determined in porewaters (As(V), As(III), Hg and MeHg). Generally, Hg concentrations were rather constant with depth. In the case of As species, an increase due to the dissolution and desorption from Fe, rather than Mn, oxy-hydroxides coupled with the transition from oxic to anoxic conditions occurred. These processes could have led to high amounts of As(V), which in turn might be rapidly reduced to the more labile and toxic As(III). The highest As(V) and SO_4^{2-} concentrations detected in porewaters at the Nalón River estuarine sites could testify to a scarce sulphate reduction which usually inhibits the reduction of As(V). Moreover, the low rates of sulphate reduction could also explain the pronounced increase in MeHg at the same sites (SJ and SE).
- Particulate and dissolved PTE concentration increased when the artificial perturbation stopped and together TSM rapidly decreased over time. The abrupt increase in dissolved PTEs can be mainly ascribed to the porewater contribution and the effects of resuspension

lasted only a few hours until pre-resuspension conditions were restored. Part of the Hg and MeHg released from porewaters and/or desorbed during resuspension was removed after 6 h at sites LL, VV and SJ (with the exception of Hg at site LL) by the re-adsorption onto the settling fine particles and/or co-precipitation with Fe oxy-hydroxides. In the case of As species, the restoration of the pre-resuspension concentrations of As(III) was reached after 7-9 h at the Nalòn estuary sites. Something similar was observed for As(V) which showed concentrations comparable to the beginning after 4-5 h at LL and VV and after 18 h at SJ, whereas the re-adsorption and/or re-precipitation was incomplete at site SE.

These results suggest that resuspension events of contaminated estuarine sediments can be critical for the surrounding environment, resulting in a worsening of the quality for water and biota. The consequences, however, are not unequivocal, strongly depending on site characteristics and, as seen in the mesocosm experiments, appear to be limited in time. In this context, the restoration of pre-resuspension conditions is observed a few hours after resuspension, but in some sites precautions should be taken when planning to dredge.

Further investigation could be helpful in order to elucidate the behaviour of other pollutants such as POPs and TBT, which commonly affect estuarine and coastal areas. Moreover, it would be useful to monitor the water column quality during dredging operations in *situ* and to compare the results with the mesocosm approach.

Acknowledgments

This study was co-supported by the Spanish Ministry of Economy, Industry and Competitiveness through the Research Projects METRAMER [grant number: MINECO-13-CGL2013-44980-R] and ECOMER [grant number: MINECO-18-CGL2017-84268-R] and the Asturias Ministry of

Education and Science [grant number: FC- 15-GRUPIN14-067]. The authors are very grateful to José Manuel Rico for his valuable support in sampling operations. Karry Close is warmly acknowledged for proofreading the manuscript. The anonymous reviewers are sincerely acknowledged for their critical reviews and useful suggestions which improved the quality of the manuscript.

References

Acquavita, A., Covelli, S., Emili, A., Berto, D., Faganeli, J., Giani, M., Horvat, M., Koron, N., Rampazzo, F., 2012. Mercury in the sediments of the Marano and Grado Lagoon (northern Adriatic Sea): sources, distribution and speciation. Estuar. Coast. Shelf S. 113, 20–31.

Amirbahman, A., Kent, D.B., Curtis, G.P., Davis, J.A., 2006. Kinetics of sorption and abiotic oxidation of arsenic(III) by aquifer materials. Geochim. Cosmochim. Ac. 70, 533–547.

Amouroux, D., Tessier, E., Pécheyran, C., Donard, O.F.X., 1998. Sampling and probing volatile metal(loid) species in natural waters by in-situ purge and cryogenic trapping followed by gas chromatography and inductively coupled plasma mass spectrometry (P-CT-GC-ICP/MS). Anal. Chim. Acta 377, 241–254.

Arfi, R., Guiral, D., Bouvy, M., 1993. Wind induced resuspension in a shallow tropical lagoon. Estuar. Coast. Shelf S. 36, 587–604.

Bataillard, P., Grangeon, S., Quinn, P., Mosselmans, F., Lahfid, A., Wille, G., Joulian, C., Battaglia-Brunet, F., 2014. Iron and arsenic speciation in marine sediments undergoing a resuspension event: the impact of biotic activity. J. Soils Sediments 14, 615–629.

Beak, D.G., Wilkin, R.T., Ford, R.G., Kelly, S.D., 2008. Examination of arsenic speciation in sulfidic solutions using X-ray absorption spectroscopy. Environ. Sci. Technol. 42, 1643–1650.

Beck, M., Dellwig, O., Schnetger, B., Brumsack, H.J., 2008. Cycling of trace metals (Mn, Fe, Mo, U, V, Cr) in deep pore waters of intertidal flat sediments. Geochim. Cosmochim. Ac. 72, 2822–2840.

Benoit, J.M., Shull D.H., Harvey, R.M., Beal, S.A., 2009. Effect of bioirrigation on sediment-water exchange of methylmercury in Boston Harbor, Massachusetts. Environ. Sci. Technol. 43, 3669–3674.

Bertuzzi, A., Faganeli, J., Brambati, A., 1996. Annual variation of benthic nutrient fluxes in shallow coastal waters (Gulf of Trieste, northern Adriatic Sea). Mar. Ecol. 17, 261–278.

Bloesch, J., 1995. Mechanisms, measurement and importance of sediment resuspension in lakes. Mar. Freshwater Res. 46, 295–304.

Bloom, N.S., Lasorsa, B.K., 1999. Changes in mercury speciation and the release of methyl mercury as a result of marine sediment dredging activities. Sci. Total Environ. 237–238, 379–385.

Bloom, N.S., Preus, E., Katon, J., Hiltner, M., 2003. Selective extractions to assess the biogeochemically relevant fractionation of inorganic mercury in sediments and soils. Anal. Chim. Acta 479, 233–248.

Bocchetti, R., Fattorini, D., Pisanelli, B., Macchia, S., Oliviero, L., Pilato, F., Pellegrini, D., Regoli F., 2008. Contaminant accumulation and biomarker responses in caged mussels, *Mytilus galloprovincialis*, to evaluate bioavailability and toxicological effects of remobilized chemicals during dredging and disposal operations in harbours areas. Aquat. Toxicol. 89, 257–266.

Bouchet, S., Tessier, E., Monperrus, M., Bridou, R, Clavier, J, Thouzea, G, Amouroux, D., 2011. Measurements of gaseous mercury exchanges at the sediment–water, water–atmosphere and sediment–atmosphere interfaces of a tidal environment (Arcachon Bay, France). J. Environ. Monitor. 13, 1351–1359.

Burton, E.D., Johnston, S.G., Kraal, P., Bush, R.T., Claff, S., 2013. Sulfate availability drives divergent evolution of arsenic speciation during microbially mediated reductive transformation of schwertmannite. Environ. Sci. Technol., 47, 2221–2229.

Caetano, M., Madureira, M.J., Vale, C., 2003. Metal remobilisation during resuspension of anoxic contaminated sediment: short-term laboratory study. Water Air Soil Pollut. 143, 23–40.

Cantwell, M.G., Burgess, R.M., 2004. Variability of parameters measured during the resuspension of sediments with a particle entrainment simulator. Chemosphere 56, 51–58.

Cantwell, M.G., Burgess, R.M., King, J.W., 2008. Resuspension of contaminated field and formulated reference sediments Part I: evaluation of metal release under controlled laboratory conditions. Chemosphere 73, 1824–1831.

Caplat, C., Texier, H., Barillier, D., Lelievre, C., 2005. Heavy metals mobility in harbour contaminated sediments: the case of Port-en-Bessin. Mar. Pollut. Bull. 50, 504–511.

Ceñal, R.C., Flor, G., 1993. Evolución reciente del estuario del Nalón (Asturias). Cuaternario y Geomorfología 7, 23-34.

Conaway, C.H., Squire, S., Mason, R.P., Flegal, A.R., 2003. Mercury speciation in the San Francisco Bay estuary. Mar. Chem. 80, 199–225.

Cotou, E., Gremare, A., Charles, F., Hatzianestis, I., Sklivagou, E., 2005. Potential toxicity of resuspended particulate matter and sediments: environmental samples from the Bay of Banyuls-sur-Mer and Thermaikos Gulf. Cont. Shelf Res. 25, 2521–2532.

de Freitas, A.R., de Castro Rodrigues, A.P., Monte, C.N., Freire, A.S., Santelli, R.E., Machado, W., Sabadini-Santos E., 2019. Increase in the bioavailability of trace metals after sediment resuspension. SN Applied Sciences 1:1288 https://doi.org/10.1007/s42452-019-1276-8.

Dowdle, P.R., Laverman, A.M., Oremland, R.S., 1996. Bacterial dissimilatory reduction of arsenic (V) to arsenic (III) in anoxic sediments. Appl. Environ. Microbiol. 62, 1664–1669.

Eggleton, J., Thomas, K.V., 2004. A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events. Environ. Int. 30, 973–980.

Emili, A., Koron, N., Covelli, S., Faganeli, J., Acquavita, A., Predonzani, S., De Vittor, C., 2011. Does anoxia affect mercury cycling at the sediment-water interface in the Gulf of Trieste (northern Adriatic Sea)? Incubation experiments using benthic flux chambers. Appl. Geochem. 26, 194–204.

European Parliament, Council of the European Union (2000) Water Framework Directive. 2000/60/EC.

European Parliament, Council of the European Union (2008) Establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). 2008/56/EC.

Fang, T.H., Chen, Y.S., 2015. Arsenic speciation and diffusion flux in Danshuei Estuary sediments, Northern Taiwan. Mar. Pollut. Bull. 101, 98–109.

Fernández-Martínez, R., Loredo, J., Ordóñez, A., Rucandio, M.I., 2005. Distribution and mobility of mercury in soils from an old mining area in Mieres, Asturias (Spain). Sci. Total Environ. 346, 200–212.

Fiket, Ž., Fiket, T., Ivanić, M., Mikac, N., Kniewald, G., 2019. Pore water geochemistry and diagenesis of estuary sediments – an example of the Zrmanja River estuary (Adriatic coast, Croatia). J. Soils Sediments 19, 2048–2060.

Flor, G., Fernández Pérez, L.A., Menéndez Fidalgo, R., Martínez Cueto-Felgueroso, E.M., Rodríguez Casero, G., 1996. Dynamics and sedimentation of the mesotidal estuary of Villaviciosa (Asturias, Northern Spain). Revista de la Sociedad Geológica de España 9, 3–4.

Forján, R., Baragaño, D., Boente, C., Fernández-Iglesias, E., Rodríguez-Valdes, E., Gallego, J.R., 2019. Contribution of fluorite mining waste to mercury contamination in coastal systems. Mar. Pollut. Bull. 149, 110576.

Froelich, P.N., Klinkhammer, G.P., Bender, M.L., Luedtke, N.A., Heath, G.R., Cullen, D., Dauphin, P., Hammond, D., Hartman, B., Maynard, V., 1979. Early oxidation of organic matter in pelagic sediments of the eastern equatorial Atlantic: suboxic diagenesis. Geochim. Cosmochim. Ac. 43, 1075–1090.

Frohne, T., Rinklebe, J., Diaz-Bone, R.A., Du Laing, G., 2011. Controlled variation of redox conditions in a floodplain soil: impact on metal mobilization and biomethylation of arsenic and antimony. Geoderma 160, 414–424.

Gagnon, C., Pelletier, E., Mucci, A., 1997. Behaviour of anthropogenic mercury in coastal marine sediments. Mar. Chem. 59, 159–176.

García-Ordiales, E., Flor-Blanco, G., Roqueñí, N., Covelli, S., Cienfuegos, P., Fontolan, G., Loredo, J., 2020. Anthropocene human footprint in the Nalón estuarine sediments (northern Spain). Mar. Geol. 424, 106167.

García-Ordiales, E., Cienfuegos, P., Roqueñí, N., Covelli, S., Flor-Blanco, G., Fontolan, G., Loredo, J., 2019a. Historical accumulation of potentially toxic trace elements resulting from mining activities in estuarine salt marshes sediments of the Asturias coastline (northern Spain). Environ. Sci. Pollut. Res. 26, 3115–3128.

García-Ordiales, E., Roqueñí, N., Rico, J.M., Cienfuegos, P., Alvarez, R., Ordoñez, A., 2019b. Assessment of the toxicity toward Vibrio fischeri in sediments of a mining impacted estuary in the north of Spain. Sci. Total Environ. 660, 826–833.

García-Ordiales, E., Covelli, S., Rico, J.M., Roqueñí., N., Fontolan, G., Flor-Blanco, G., Cienfuegos, P., Loredo, J., 2018. Occurrence and speciation of arsenic and mercury in estuarine sediments affected by mining activities (Asturias, northern Spain). Chemosphere 198, 281–289.

García-Ordiales, E., Loredo, J., Covelli, S., Esbrí, J.M., Millán, R., Higueras, P., 2017. Trace metal pollution in freshwater sediments of the world's largest mercury mining district: sources, spatial distribution, and environmental implications. J. Soils Sediments. 17, 1893–1904.

Gilmour, C.C., Henry, E.A., Ralph, M., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. 26, 2281–2287.

González-Fernández, B., Rodríguez-Valdés, E., Gallego, J.R., 2018. Contaminación de un acuífero por metales pesados de origen geogénico: importancia del control geológico e hidrogeológico de los sondeos de captación de aguas. Geogaceta 64, 59–62.

Goossens, H., Zwolsman, J.J.G., 1996. An evaluation of the behaviour of pollutants during dredging activities. Terra et Aqua 62, 20–28.

Guerra, R., Pasteris, A., Ponti, M., 2009. Impacts of maintenance channel dredging in a northern Adriatic coastal lagoon. I: Effects on sediment properties, contamination and toxicity. Estuar. Coast. Shelf S. 85, 134–142.

Hammerschmidt, C.R., Fitzgerald, W.F., Lamborg, C.H., Balcom, P.H., Visscher, P.T., 2004. Biogeochemistry of methylmercury in sediments of Long Island Sound. Mar. Chem. 90, 31–52.

Hines, M.E., Covelli, S., Faganeli, J., Horvat, M., 2017. Controls on microbial mercury transformations in contaminated sediments downstream of the Idrija mercury mine (West Slovenia) to the Gulf of Trieste (northern Adriatic). J. Soils Sediments 17, 1961–1971.

Hines, M.E., Faganeli, J., Planinc, R., 1997. Sedimentary anaerobic microbial biogeochemistry in the Gulf of Trieste, Northern Adriatic Sea: influences of bottom water oxygen depletion. Biogeochemistry 39, 65–86.

Iglesias, J. G., Loredo, J., 1994. Geological, geochemical and mineralogical characteristics of the Asturias Fluorspar District, Northern Spain. Explor. Min. Geol. 3, 31-37.

Jeong, H.Y., Han, Y.S., Park, S.W., Hayes, K.F., 2010. Aerobic oxidation of mackinawite (FeS) and its environmental implication for arsenic mobilization. Geochim. Cosmochim. Ac. 74, 3182–3198.

Jones-Lee, A, Lee, G.F., 2005. Role of iron chemistry in controlling the release of pollutants from resuspended sediments. Remediation 16, 33–41.

Kalnejais, L.H., Martin, W.R., Signell, R.P., Bothner, M.H., 2007. Role of sediment resuspension in the remobilization of particulate-phase metals from coastal sediments. Environ. Sci. Technol. 41, 2282−2288.

Kim, E.H., Mason, R.P., Porter, E.T., Soulen, H.L., 2004. The effect of resuspension on the fate of total mercury and methylmercury in a shallow estuarine ecosystem: a mesocosm study. Mar. Chem. 86, 121−137.

Kim, E.H., Mason, R.P., Porter, E.T., Soulen, H.L., 2006. The impact of resuspension on sediment mercury dynamics, and methylmercury production and fate: A mesocosm study. Mar. Chem. 102, 300−315.

Leardi, R., Melzi, C., Polotti, G., 2019. CAT (Chemometric Agile Tool), freely downloadable from http://gruppochemiometria.it/index.php/software (accessed September 3rd, 2019).

Levresse, G., Tritlla, J., Rosique, A.R., Cardellach, E., Rollion-Bard, C., Pironon, J., Sandoval, S.J., 2019. Hydrocarbons in silica: PVTX properties of fluids and the genesis of diamond quartz from Caravia-Berbes Fluorite district (Asturias, Spain). Mar. Petrol. Geol. 102, 1–15.

Lewis, M.A., Weber, D.E., Stanley, R.S., Moore, J.C., 2001. Dredging impact on an urbanized Florida bayou: effects on benthos and algal-periphyton. Environ. Pollut. 115, 161–171.

Loredo, J., 2000. Historic Unreclaimed Mercury Mines in Asturias (Northwestern Spain): Environmental Approaches. Assessing and Managing Mercury from Historic and Current Mining Activities, pp. 175–180.

Loredo, J., Ordóñez, A., Gallego, J., Baldo, C., Garcia-Iglesias, J., 1999. Geochemical characterisation of mercury mining spoil heaps in the area of Mieres (Asturias, northern Spain). J. Geochem. Explor. 67, 377–390.

Loredo, J., Petit-Domínguez, M.D., Ordóñez, A., Galán, M.P., Fernández-Martínez, R., Alvarez, R., Rucandio, M.I., 2010. Surface water monitoring in the mercury mining district of Asturias (Spain). J. Hazard. Mater. 176, 323–332.

Masscheleyn, P.H., Delaune, R.D., Patrick Jr., W.H., 1991. Arsenic and selenium chemistry as affected by sediment redox potential and pH. J. Environ. Qual. 20, 522–527.

Moore, J.N., Ficklin, W.H., Johns, C., 1988. Partitioning of arsenic and metals in reducing sulfidic sediments. Environ. Sci. Technol. 22; 432–437.

Monte, C.N., Rodrigues, A.P.C, Cordeiro, R.C., Freire, A.S., Santelli, R.E., Machado, W., 2015. Changes in Cd and Zn bioavailability upon an experimental resuspension of highly contaminated coastal sediments from a tropical estuary. Sustain. Water Resour. Manag. 1, 335–342.

Oliveri, E., Salvagio Manta, D., Bonsignore, M., Cappello, S., Tranchida, G., Bagnato, E., Sabatino, N., Santisi, S., Sprovieri, M., 2016. Mobility of mercury in contaminated marine sediments: Biogeochemical pathways. Mar. Chem. 186, 1–10.

Oliveri, P., Malegori, C., Casale, M., 2020. Chemometrics: Multivariate Analysis of Chemical Data, in: Pico, Y. (Ed.), Chemical Analysis of Food, 2nd Edition. Elsevier.

Oliveri, P., Malegori, C., Simonetti, R., Casale, M., 2019. The impact of signal pre-processing on the final interpretation of analytical outcomes – A tutorial. Anal. Chim. Acta 1058, 9–17.

Ordóñez, A., Silva, V., Galán, P., Loredo, J., Rucandio, I., 2014. Arsenic input into the catchment of the River Caudal (Northwestern Spain) from abandoned Hg mining works: effect on water quality. Environ. Geochem. Health 36, 271–284.

Petranich, E., Croce, S., Crosera, M., Pavoni, E., Faganeli, J., Adami, G., Covelli, S., 2018. Mobility of metal(loid)s at the sediment-water interface in two tourist port areas of the Gulf of Trieste (northern Adriatic Sea). Environ. Sci. Pollut. Res. 25, 26887–26902.

Pfeifle, B.D., Stamm, J.F., Stone, J.J., 2018. Arsenic geochemistry of alluvial sediments and pore waters affected by mine tailings along the Belle Fourche and Cheyenne River floodplains. Water Air Soil Poll. 229, 183.

Ridgway, J., Shimmield, G., 2002. Estuaries as repositories of historical contamination and their impact on shelf seas. Estuar. Coast. Shelf Sci. 55, 903–928.

Roberts, D.A., 2012. Causes and ecological effects of resuspended contaminated sediments (RCS) in marine environments. Environ. Int. 40, 230–243.

Rodríguez-González, P., Bouchet, S., Monperrus, M., Tessier, E., Amouroux, D., 2013. In situ experiments for element species-specific environmental reactivity of tin and mercury compounds using isotopic tracers and multiple linear regression. Environ. Sci. Pollut. R. 20, 1269–1280.

Rodríguez-González, P., Monperrus, M., García Alonso, J.I., Amouroux, D., Donard, O.F.X., 2007. Comparison of different numerical approaches for multiple spiking species-specific isotope dilution analysis exemplified by the determination of butyltin species in sediments. J. Anal. Atom. Spectrom. 22, 1371–1382.

Rodríguez-González. P., Ruiz Encinar, J., García Alonso, J.I., Sanz-Medel, A., 2002. Determination of butyltin compounds in coastal sea-water samples using isotope dilution GC-ICP-MS. J. Anal. Atom. Spectrom. 17, 824–830.

Rodríguez Martin-Doimeadios, R.C., Monperrus, M., Krupp, E., Amouroux, D., Donard, O.F.X., 2003. Using speciated isotope dilution with GC− inductively coupled plasma MS to determine and unravel the artificial formation of monomethylmercury in certified reference sediments. Anal. Chem. 75, 3202–3211.

Root, R.A., Dixit, S., Campbell, K.M., Jew, A.D., Hering, J.G., O’Day, P.A., 2007. Arsenic sequestration by sorption processes in high-iron sediments. Geochim. Cosmochim. Ac. 71, 5782–5803.

Ruiz-Chancho, M.J., Sabé, R., López-Sánchez, J.F., Rubio, R., Thomas, P., 2005. New approaches to the extraction of arsenic species from soils. Microchim. Acta 151, 3–4, 241–248.

Sanford, L.P., Panageotou, W., Halka, J.P., 1991. Tidal resuspension of sediments in northern Chesapeake Bay. Mar. Geol. 97, 87–103.

Sanz-Prada, L., García-Ordiales, E., Roqueñi, N., Grande Gil, J.A., Loredo, J., 2020. Geochemical distribution of selected heavy metals in the Asturian coastline sediments. Mar. Pollut. Bull. 156, 111263.

Saulnier, I., Mucci, A., 2000. Trace metal remobilization following the resuspension of estuarine sediments: Saguenay Fjord, Canada. Appl. Geochem. 15, 191–210.

Schoellhamer, D.H., 1996. Anthropogenic sediment resuspension mechanisms in a shallow microtidal estuary. Estuar. Coast. Shelf Sci. 43, 533– 548.

Seelen, E.A., Massey, G.M., Mason, R.P., 2018. Role of sediment resuspension on estuarine suspended particulate mercury dynamics. Environ. Sci. Technol. 52, 7736–7744.

Shepard, F.P., 1954. Nomenclature based on sand–silt–clay ratios. J. Sediment Petrol. 24, 151–158.

Silva, V., Loredo, J., Fernández-Martínez, R., Larios, R., Ordóñez, A., Gómez, B., Rucandio, I., 2014. Arsenic partitioning among particle-size fractions of mine wastes and stream sediments from cinnabar mining districts. Environ. Geochem. Health 36, 831–843.

Simpson, S.L., Apte, S.C., Batley, G.E., 1998. Effect of short-term resuspension events on trace metal speciation in polluted anoxic sediments. Environ. Sci. Technol. 32, 620–625.

Smedley, P.L., Kinniburgh, D.G., 2002. A review of the source, behaviour and distribution of arsenic in natural waters. Appl. Geochem. 17, 517–568.

Stephens, S.R., Alloway, B.J., Parker, A., Carter, J.E., Hodson, M.E., 2001. Changes in the leachability of metals from dredged canal sediments during drying and oxidation. Environ. Pollut. 114, 407–413.

Symons, D.T.A., Kawasaki, K., Tornos, F., Velasco, F., Rosales, I., 2017. Temporal constraints on genesis of the Caravia-Berbes fluorite deposits of Asturias, Spain, from paleomagnetism. Ore Geol. Rev. 80, 754–766.

Turner, A., Millward, G.E., Le Roux, S.M., 2004. Significance of oxides and particulate organic matter in controlling trace metal partitioning in a contaminated estuary. Mar Chem 8:179-192

Van Den Berg, G.A., Meijers, G.G.A., Van Der Heijdt, L.M., Zwolsman, J.J.G., 2001. Dredging-related mobilisation of trace metals: a case study in the Netherlands. Water Res. 35, 1979–1986.

Vidal-Durà, A., Burke, I.T., Stewart, D.I., Mortimer, R.J.G., 2018. Reoxidation of estuarine sediments during simulated resuspension events: effects on nutrient and trace metal mobilisation. Estuar. Coast. Shelf Sci. 207, 40–55.

Wang, H., Liu, R., Wang, Q., Xu, F., Men, C., Shen, Z., 2016. Bioavailability and risk assessment of arsenic in surface sediments of the Yangtze River estuary. Mar. Pollut. Bull. 113, 125–131.

Wilkin, R.T., Ford, R.G., 2006. Arsenic solid-phase partitioning in reducing sediments of a contaminated wetland. Chem. Geol. 228, 156–174.

Zhu, W., Song, Y., Adediran, G.A., Jiang, T., Reis, A.T., Pereira, E., Skyllberg, U., Björn, E., 2018. Mercury transformations in resuspended contaminated sediment controlled by redox conditions, chemical speciation and sources of organic matter. Geochim. Cosmochim. Ac. 220, 158–179.

Figure captions

Fig. 1. The study area along the Asturian coast where sediment samples for resuspension experiments were collected from the following sites: the Nalón River Estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL).

Fig. 2. Vertical profiles for both As and Hg species, Fe, Mn, DOC, SO_4^{2-} in dissolved form in porewaters and Eh values in the four sediment cores collected from the Nalón River estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL).

Fig. 3. Trend of total suspended matter (TSM) concentrations during resuspension experiments at all sites (above) and grain-size distribution of TSM at San Esteban (SE) and San Juan (SJ) in the Nalón River Estuary (below).

Fig. 4. Behaviour of As(V), As(III), Hg, MeHg, Fe, Mn, DOC and SO_4^{2-} in the dissolved phase of the water column during resuspension experiments at the port of Llanes (LL) and at the Villaviciosa estuary (VV). Note that the term “pre” that follows the chemical element in each graph (e.g. As(V) pre) indicates the PTE concentration before the resuspension event (at time $T_{pre\text{-}res}$).

Fig. 5. Behaviour of As(V), As(III), Hg, MeHg, Fe, Mn, DOC and SO_4^{2-} in the dissolved phase of the water column during resuspension experiments at the Nalón River estuary (SJ and SE). Note that Hg, MeHg, Fe and Mn at San Juan (SJ) and San Esteban (SE) show a different scale and As(III) is only present at these two sites. Note that the term “pre” that follows the chemical element in each graph (e.g. As(V) pre) indicates the PTE concentration before the resuspension event (at time $T_{pre\text{-}res}$).

Fig. 6. Behaviour of As(V), As(III), Hg, MeHg, Fe and Mn in the particulate phase of the water column during resuspension experiments at all investigated sites, expressed using different scales.

Fig. 7. Percentages of removal (negative values) and/or release (positive values) of As and Hg species in the water column from T_0 to T_6 with respect to the dissolved concentrations before resuspension ($T_{pre\text{-}res}$).

Supplementary Material - Figure captions

Fig. S1. Biplots summarising the results of PCA performed on A) grain size composition and total concentrations of As, Hg, Fe, Mn and S in the sediment cores and B) physico-chemical parameters and dissolved concentrations of Hg, MeHg, As(III), As(V), Fe, Mn, SO42- and DOC in the porewaters collected from the port of Llanes (LL), the Villaviciosa estuary (VV) and the Nalòn River estuary (SJ and SE).

Fig. S2. Behaviour of dissolved Hg, MeHg, As(V) and As(III) as a function of time during the resuspension experiments at the Nalón River estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL). Note As(III) is only present at these two sites. Note that the term “pre” indicates the PTE concentration before the resuspension event (at time $T_{pre\text{-}res}$).

Tables

Table 1. Resuming of the main characteristics of the investigated sampling sites along the Asturian coast: the Nalón River estuary (SJ and SE), the Villaviciosa estuary (VV) and the port of Llanes (LL).

Sampling site		San Juan (SJ)	San Esteban (SE)	Villaviciosa (VV)	Llanes (LL)
Location		port areas in the Nalón R. estuary		port area 60 km E of the Nalón R. estuary	port area 110 km E of the Nalón R. estuary
Latitude		43º 33´ 21.56" N	43º 33´ 4.90" N	43º 31´ 31.54" N	43º 25´13.04" N
Longitude		6º 4´ 37.51" W	6º 5´ 40" W	5º 23´ 21.20" W	4º 45´12.02" W
Depth (m)		3.20	4.50	2.40	3.80
Water circulation		open estuarine area	sheltered harbour	sheltered harbour	
Sediment grain size*	sand	26.4 %	4.92 %	3.91 %	27.4 %
	mud	73.6 %	95.1 %	96.1 %	72.6 %
As in sediments (µg g^{-1})		20.1 - 68.1**		average 11.3***	-
Hg in sediments (µg g^{-1})		0.10 - 1.33**		average 0.18***	> 3.81***

*: average grain size composition, this study; **: Garcìa-Oridales et al., 2018, 2019b; ***: unpublished data

Table 2. Grain size composition, Eh values and concentrations of As, Hg, Mn, Fe and S in the sediment cores collected from the port of Llanes (LL), the Villaviciosa estuary (VV) and the Nalón River Estuary (SJ and SE).

Sampling site	sediment layer cm	sand %	silt %	clay %	Eh mV	As mg kg^{-1}	Hg mg kg^{-1}	Mn mg kg^{-1}	Fe %	S %
LL	0-1	11.8	85.5	2.64	-191	23.9	3.12	101	1.41	1.12
	1-2	33.1	65.5	1.40	-235	23.2	3.29	108	1.50	1.19
	2-3.5	36.8	62.0	1.22	-318	23.0	3.44	109	1.49	1.10
	3.5-5	27.0	71.4	1.58	-341	22.7	3.79	114	1.71	1.40
	5-7	28.1	70.4	1.54	-355	26.3	5.01	124	1.67	1.21
VV	0-1	4.23	93.2	2.54	-153	20.2	0.77	194	2.51	0.81
	1-2	4.06	92.8	3.11	-164	17.6	0.69	190	2.50	0.89
	2-3.5	4.38	92.5	3.07	-175	17.3	0.68	193	2.57	1.01
	3.5-5	2.46	94.4	3.16	-185	16.3	0.57	191	2.57	1.14
	5-7	4.42	92.8	2.81	-191	16.5	0.58	192	2.58	1.20
SJ	0-1	35.8	62.8	1.35	-87	26.8	0.79	411	2.32	1.01
	1-2	32.0	66.6	1.41	-134	25.5	0.58	386	2.37	1.00
	2-3.5	26.3	72.1	1.63	-241	26.1	0.52	447	2.62	0.94
	3.5-5	23.7	74.0	2.32	-187	27.8	0.59	474	2.79	0.97
	5-7	14.1	83.7	2.17	-307	27.3	0.57	469	2.62	0.88
SE	0-1	5.00	92.2	2.78	-118	50.9	0.80	463	3.38	0.62
	1-2	5.01	92.5	2.51	-232	31.8	0.75	499	3.48	0.66
	2-3.5	4.58	92.8	2.61	-263	35.1	0.83	484	3.32	0.84
	3.5-5	4.26	93.0	2.70	-375	32.1	0.67	554	3.03	0.62
	5-7	5.77	91.7	2.51	-348	34.6	0.83	637	3.28	0.82

Figure 1

Click here to download high resolution image

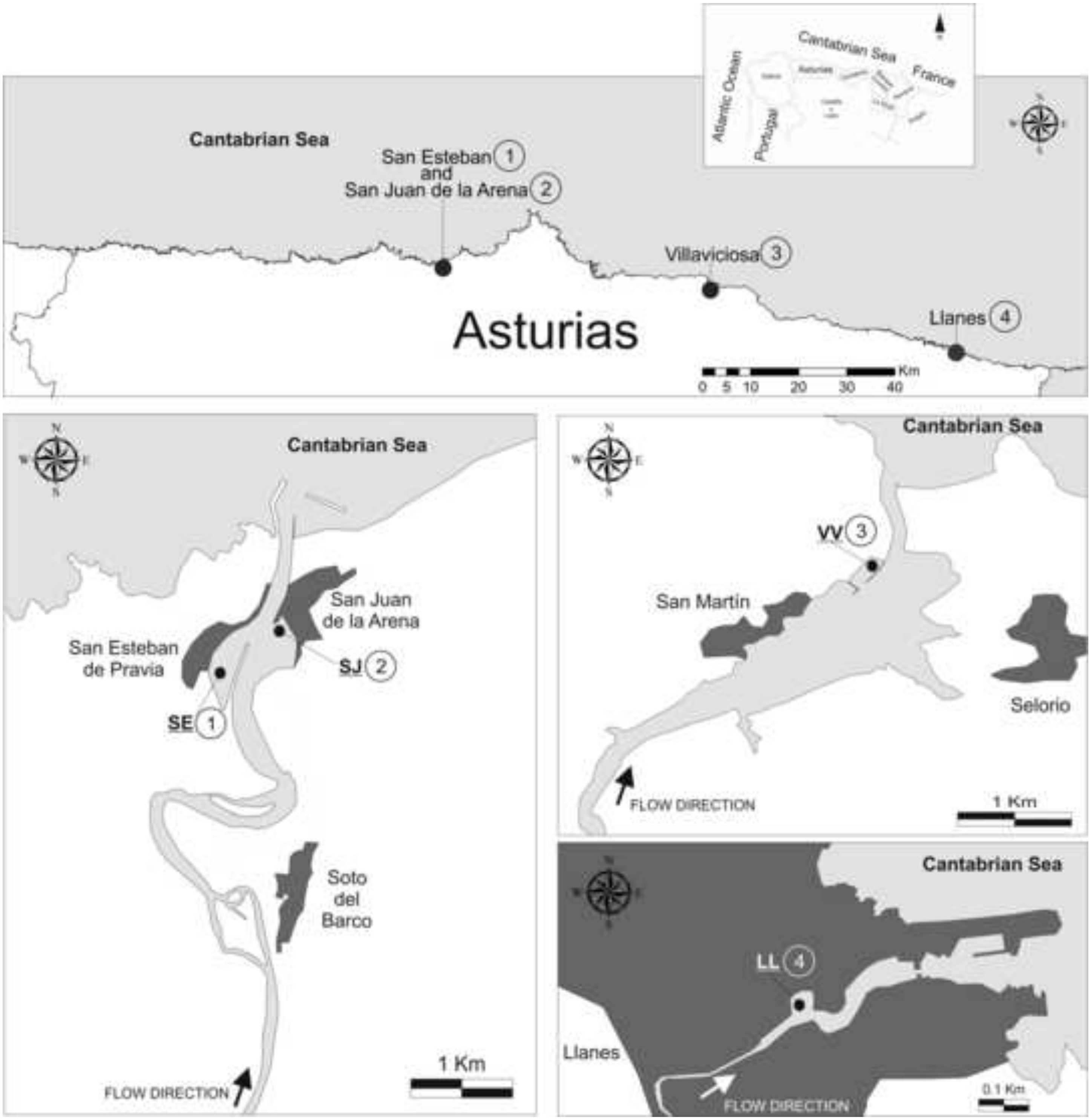


Figure 2 revised

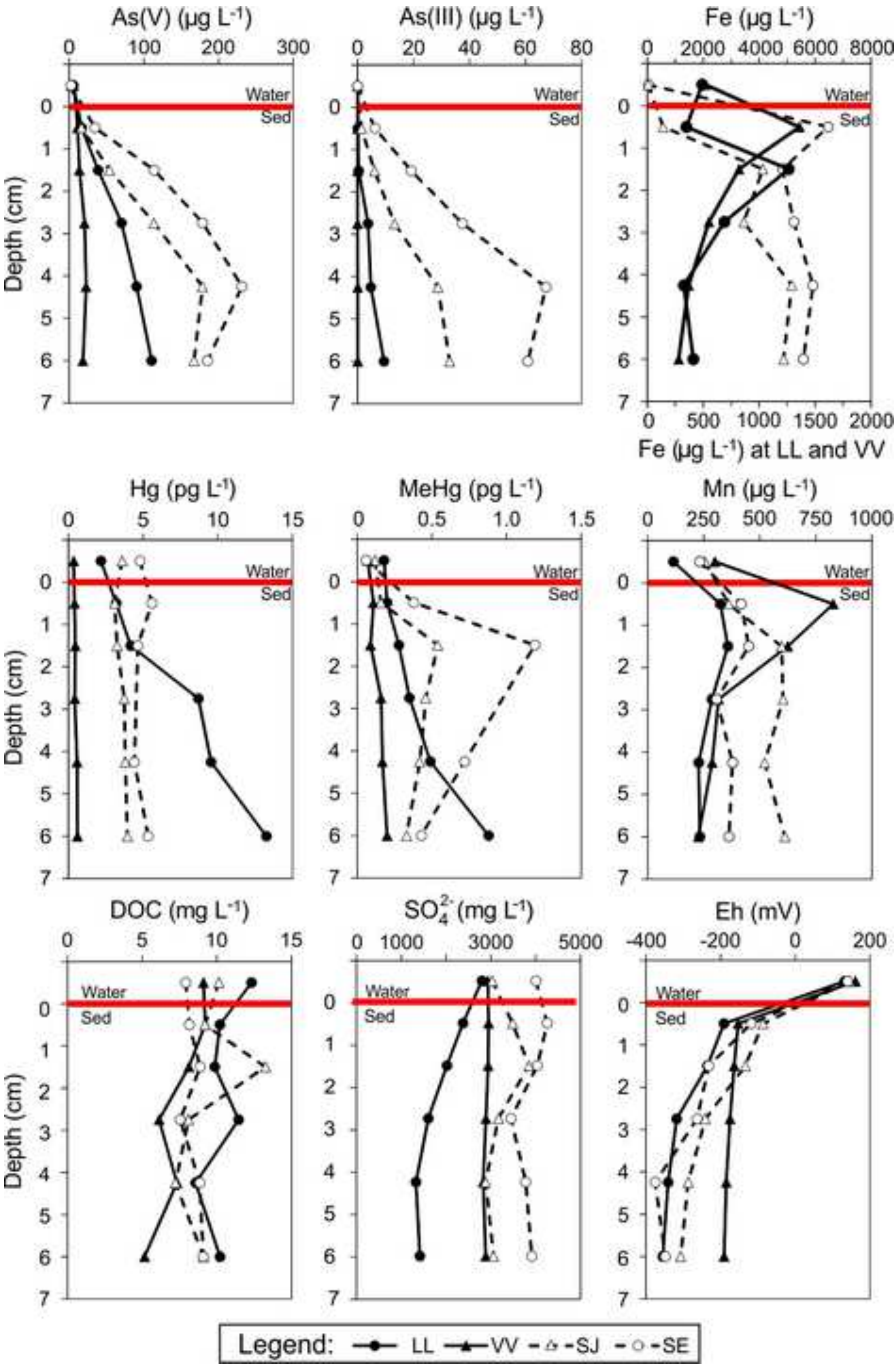


Figure 3 revised

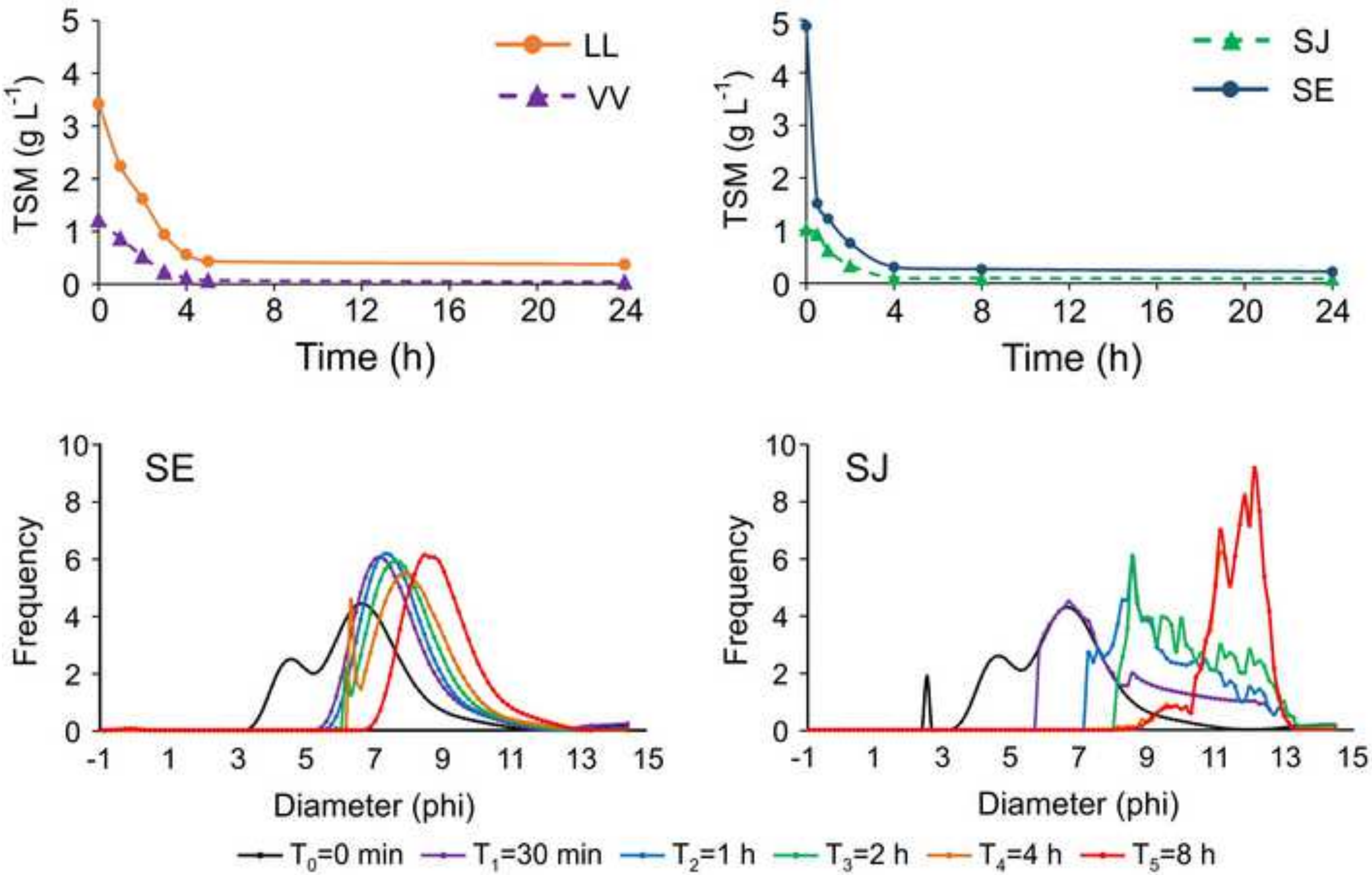


Figure 4 revised

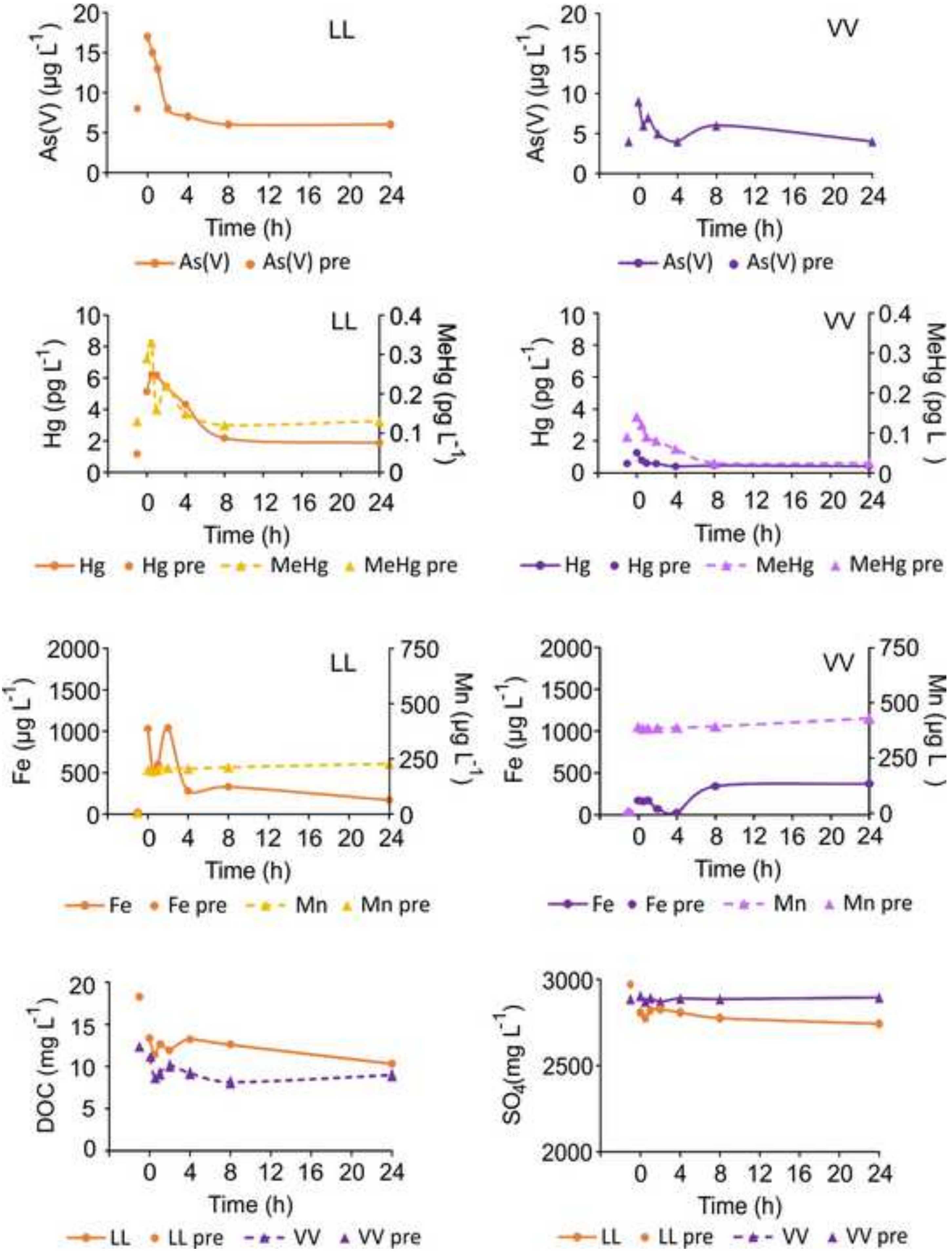


Figure 5 revised

Click here to download high resolution image

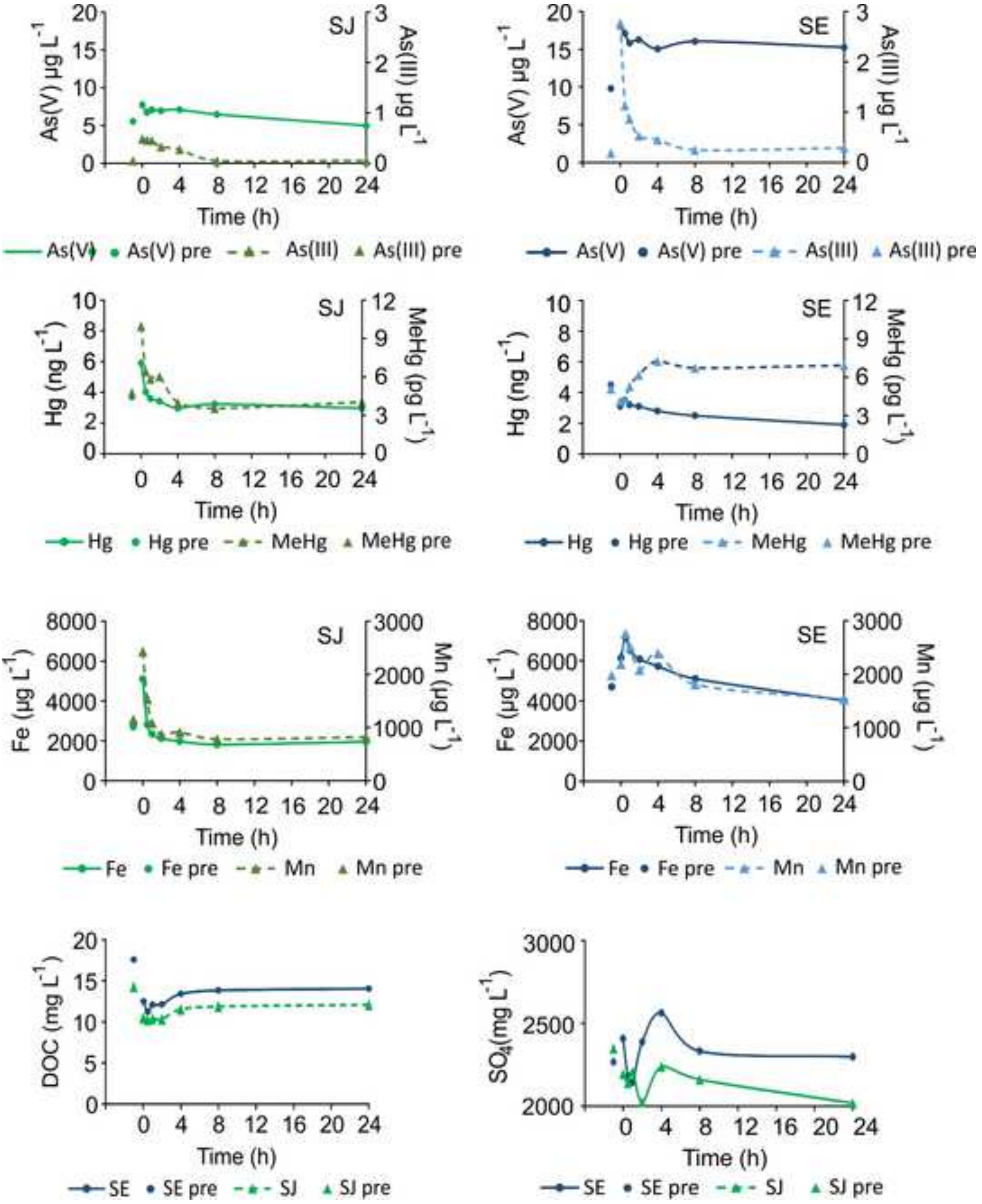


Figure 6 revised

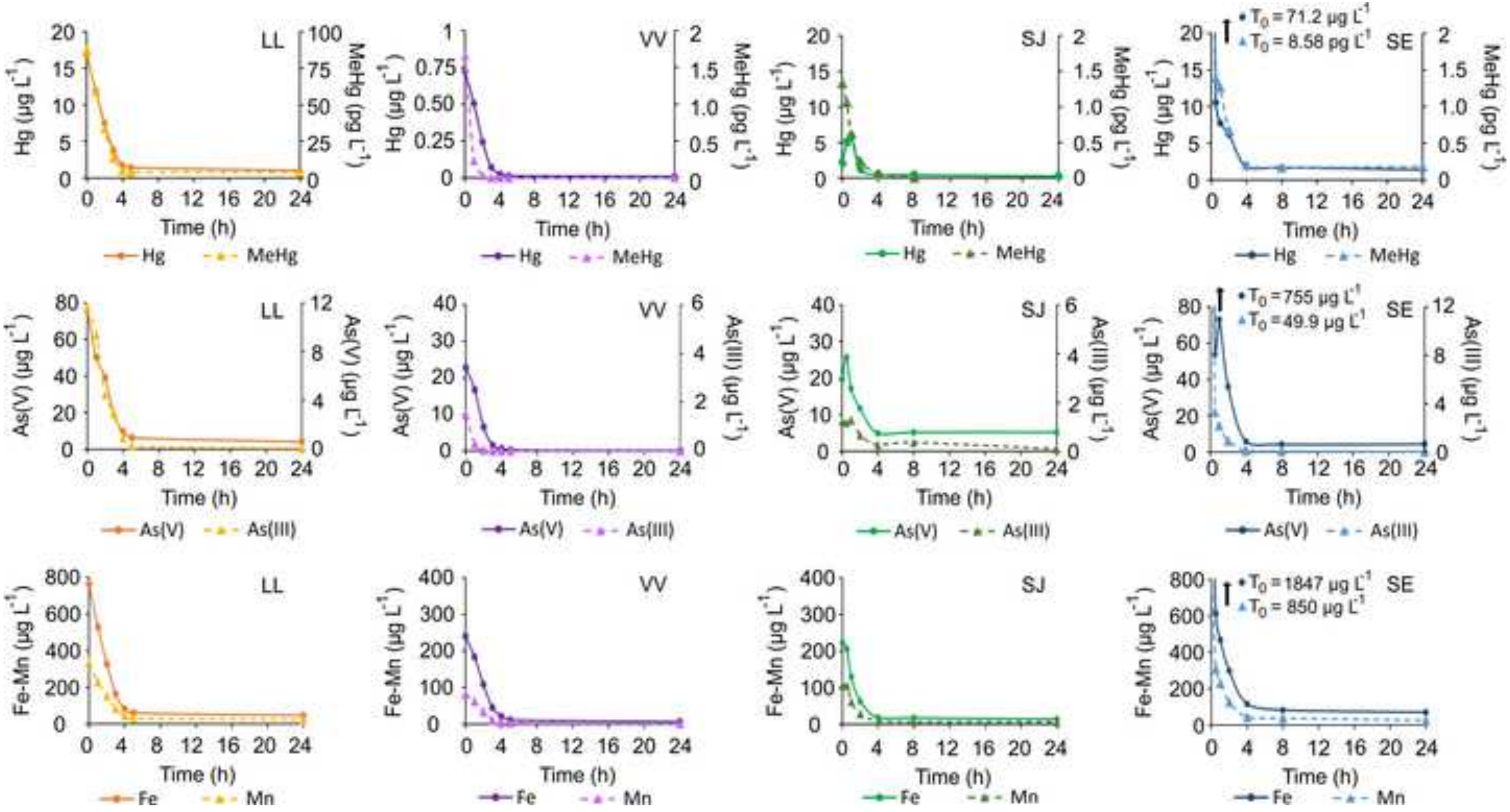
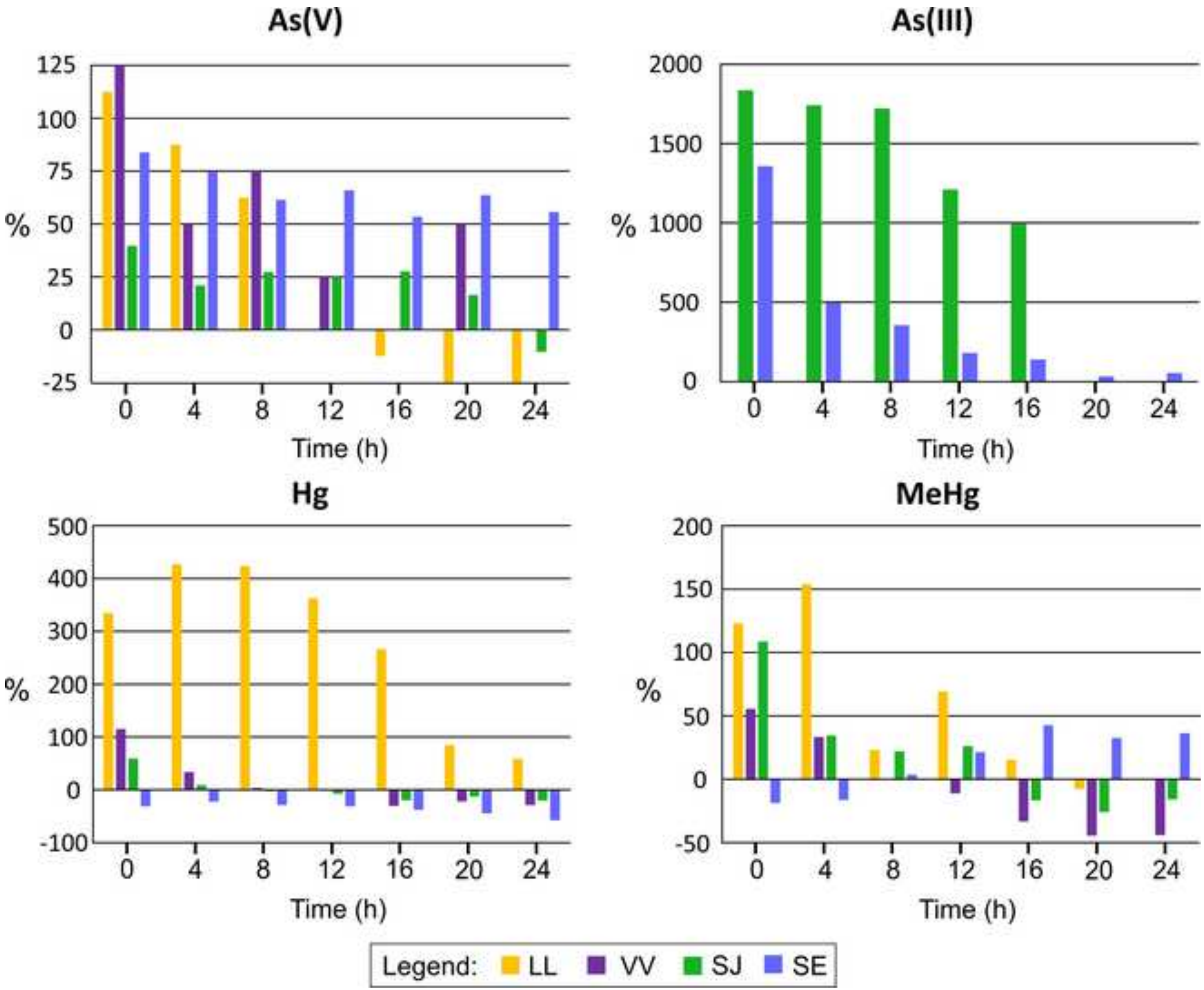


Figure 7 revised



***conflict of Interest Statement**

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Credit Author Statement

Garcia Ordiales E.: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Supervision

Covelli S.: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization, Writing - Review & Editing

Braidotti G.: Methodology, Investigation, Resources

Petranich, E.: Investigation, Writing - Original Draft, Visualization, Writing - Review & Editing

Pavoni E.: Formal analysis, Visualization, Investigation, Resources, Writing - Original Draft, Visualization, Writing - Review & Editing

Acquavita A.: Investigation, Writing - Original Draft

Sanz-Prada L.: Methodology, Investigation, Resources

Roqueñí N.: Supervision, Project administration

Loredo J.: Supervision, Project administration