



# Negligible microbial heterotrophic quantitative contribution onto trace metals remobilization during marine sediment resuspension - insights from a Mediterranean urbanized bay

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1 Negligible microbial heterotrophic  
2 quantitative contribution onto trace metals  
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## Abstract

Marine sediments are a sink for trace metals but also a potential source during sediment resuspension events. Understanding the factor that regulates this uptake or release of contaminant is of prime importance. While the impact of abiotic processes has been widely studied, the quantitative influence of microbial activities on metal cycling during sediment resuspension events is still largely unknown. This study was designed to quantify such microbial contributions on the cycling of a suite of metals (Al, As, Ba, Co, Cr, Cs, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sr, Ti, Tl, U, V and Zn) and evaluate the specific contributions of heterotrophic micro-organisms originating from either the seawater or the sediment. For that purpose, the sediment and seawater were selectively sterilized using either autoclave (sediment) or filtration under 0.2  $\mu\text{m}$  (seawater) prior to be mixed for 5 days in darkness. Dissolved concentration in trace elements were measured over time, along with physical-chemical parameters. The pH in the different conditions decreased all along the experiments while the redox potential decreased during up to 4 days before increasing back to its initial value. Three groups of trace metals were identified: metals whose dissolved concentrations (1) increased (Al, Ba, Co, Cs, Cu, Mn, Mo, Ni, Pb, Sb, Tl, U and Zn) as a consequence of the transfer from sediment, (2) decreased (Cr, Fe and Ti) as a consequence of transfer onto sediment, and (3) remained unchanged over time (As, Li, Rb, Sr and V). The sterilization of either sediment and/or seawater did not have a statistically significant impact onto the dynamics of the physical-chemical parameters, nor onto the metals' behavior (except Mn). Our results demonstrate (i) that marine sediment autoclaving prior to mixing with seawater did not disrupt the behavior of metals in the seawater / sediment mixing over the 5 days of experiments and (ii) that the microbial activity had a negligible influence on the variation of physical-chemical parameters or metals' transfers over the mixing time.

## Keywords

Marine sediment resuspension; metals mobility; microbial contribution; short-term

## 1. Introduction

Understanding the processes affecting the mobility of contaminants at the solid-liquid interface is key to assess the importance of sediment resuspension events in marine system as a potential source of pollution (Caille et al., 2003; Comber et al., 1996; Shipley et al., 2011; Zouch et al., 2018). When mixing occur, physical-chemical conditions such as redox potential, pH or solid/liquid ratio may change drastically compare to those in the sediment (Caille et al., 2003; Dang et al., 2020; Shipley et al., 2011). Microbial responses also vary significantly within hours to months, as demonstrated by studies focusing on the phytoplankton dynamics (Coclet et al., 2018; Lafabrie et al., 2013) or on the structure and functional properties of the prokaryotic community (Galand et al., 2016; Störmer et al., 2013; Won et al., 2017; Zouch et al., 2018). Planktonic active microbes can react very quickly to the large input of organic matter and nutrients in oxic water (Coclet et al., 2018; Lafabrie et al., 2013; Schäfer et al., 2001). On the other hand, the microbial flora of anoxic sediment could be largely inhibited by resuspension in oxic waters, with only dormant aerobic bacteria being able to develop after reactivation. It is clear that different successive microbial communities will occur after a sediment resuspension event. However, while the impact of abiotic drivers on the release and mobility of trace metals has been extensively studied, the impact of microbial communities is largely unknown.

Geochemical changes from sterilization techniques is a major scientific problem, as recently revealed for a widely used inorganic poison, sodium azide (Layglon et al., 2020). Physical techniques (autoclaving and gamma irradiation) are known to increase the surface area of clays and significantly alter the organic matter structure (Berns et al., 2008; Kelsey et al., 2010; McNamara et al., 2003), although the impact of such changes on metal transfers during seawater-sediment mixing has not been documented yet.

The present study aimed to both evaluate the influence of sediment autoclaving and quantify the contribution of benthic and planktonic heterotrophic microbes on metal transfers (between sediment and seawater, one way or another) during the resuspension of marine sediment in seawater. For that purpose, four different resuspension experiments were conducted: (1) a control experiment with unprocessed seawater and sediment, (2) both compartments were sterilized prior to mixing (in order to evaluate the whole microbial contribution) and (3 and 4) selective sterilizations of either water or sediment were performed. Dissolved oxygen concentrations, pH, Eh and dissolved metals concentrations were measured after several contact time up to five days, since in most of the cases, sediment resuspension does not extend beyond this duration in the natural environment.

## 2. Methods

### 2.1. Sampling and samples pre-treatment

For the purpose of this work, the sediment was sampled in an enclosed area of Toulon Bay (in the naval harbor, coordinates 43.11454 N, 5.885418 E), ensuring the sampling of heavily-contaminated fine sediment (Cossa et al., 2017; Misson et al., 2016; Wafo et al., 2016). The sediment core was sampled with an Uwitec interface corer equipped with PVC® tubes of 90 mm in diameter. The first 30 cm of the sediment core was homogenized under inert atmosphere, in order to prepare a composite sediment.

In addition, seawater was sampled in a less contaminated part of the bay (43.08528 N, 6.02002 E). Twenty-five litres of surface (-0.5 m) seawater were sampled using a 4.2 L horizontal van Dorn type water sampler (Wildco, model Beta) and stored at ambient temperature into a clean 25 L LDPE bottle (Nalgene) previously rinsed three times with water from the site. The experiments were conducted within 4h.

All materials (sampling devices, bottles, tanks, filters and syringes) were previously acid-cleaned (10% v / v HCl, pro analysis, Fisher Scientific) and fully rinsed with Milli-Q water (18.2 MΩ, Millipore) in the laboratory to avoid contamination.

## **2.2. Laboratory resuspension experiments**

This work was carried out in order to study to what extent microbial activity can impact geochemical transfers during sediment resuspension events. Four different experiments were performed: 1- sediment and seawater remained unprocessed to assess the geochemical remobilization in natural samples (later referred as “control condition”); 2- both sediment and seawater were sterilized to assess the abiotic contribution in the geochemical transfers (later referred as all sterilized); 3- the sediment was sterilized while the seawater remained unprocessed (later referred as sterilized sediment) to assess the microbial contribution from benthic microorganisms by comparison to control condition and 4- the seawater was sterilized while the sediment remained unprocessed (later referred as “sterilized seawater”) to assess the planktonic microbial contribution by comparison to control condition.

Seawater sterilization was ensured by two successive filtrations using syringe filters (0.2 µm cellulose acetate filters) (Black et al., 2012; Laturus et al., 2000; Pouliquen et al., 2007) while sediment sterilization was performed by autoclaving sediment twice for 20 minutes, observing a cooling period until the sediment were at room temperature after each operation (Otte et al., 2018; Tuominen, 1994). Precautions were taken to minimize both microbial and chemical contaminations during the experiments by using sterile material and by sampling and manipulating under a clean laminar flow hood.

Assuming that the development of residual microbes after sterilization would take place after a certain lag time, the experiments were carried out only on a short time scale to limit the risks induced by the development of residual microorganisms. Cabrol et al. (2017) demonstrated in similar experiments but without sterilization that the density of free-living prokaryotes was still increasing after four days. Thus, the experiments lasted 5 days to focus on the normal development period of microorganisms in the mixing.

For each experiment, the sediment was mixed with the seawater (with a ratio corresponding to 2.5 g of wet sediment for 1 L of seawater, *i.e.*  $\sim 1.2$  g of dry sediment for 1 L of seawater) into 1 L FEP bottles. All experiments were performed in triplicates. All the bottles were submitted to continuous overhead shaking (15 rpm; Heidolph Reax 20) in an opaque box to prevent sunlight influence. Water-sediment mixture was sub-sampled after 30 min, 1 hour, 1, 2, 3, 4 and 5 days of contact time.

Each sub-sample was filtered through a 0.2  $\mu\text{m}$  cellulose acetate syringe filter (Sartorius) and stored into HDPE bottles, acidified to 0.2%  $\text{HNO}_3$  s.p. (Merck) and kept at ambient temperature until the analysis of dissolved trace metal concentrations.

The same mixtures were reproduced in parallel, in 1 L HDPE bottles in order to monitor pH and the redox potential (Eh) through the experiment, avoiding any contamination in FEP bottles. For that, we used PHC101 and MTC101 multi-parameter probes (Hach).

Daily aeration was ensured to avoid oxygen-depletion-related variations in the experiment by opening HDPE and FEP bottles few minutes.

### **Chemical analyses**

The concentrations of dissolved Al, As, Ba, Co, Cr, Cs, Cu, Fe, Li, Mn, Mo, Ni, Pb, Rb, Sb, Sr, Ti, Tl, U, V and Zn were measured by High Resolution Inductively Coupled Plasma Mass Spectrometer (Element 2, HR ICP-MS, Thermo), with a 10-fold dilution for seawater samples to reduce salt-matrix effect. All samples were spiked with an Internal Standard (Indium). A certified reference material (CASS-5, Nearshore seawater reference material for metals, National Research Council Canada) was used as a quality control of HR ICP-MS measurements. Two separate CASS-5 control samples were measured after every 10-15 samples, giving at the end 6-10 of control measurements (depending on the sequence length). Matrix matching calibration (in 10-fold diluted CASS-5 sample) was used for concentration quantification. Determined concentrations of metals in CASS-5 sample were within 10% of the certified values.

### **2.3. Statistical analyses**

The influences of sterilization conditions and contact time on metals transfers were assessed through two-way repeated measure ANOVA and pairwise comparisons. The p-value was adjusted with Bonferroni method. Correlations between dissolved metals concentrations were assessed by Spearman's correlation tests. All statistical analyses and heatmap visualizations were performed using RStudio (R Core Team, 2015) and packages *agricolae*, *ggpubr*, *gridExtra*, *Hmisc*, *reshape2*, *rstatix* and *stats*.

### 3. Results and Discussions

#### 3.1. Physical-chemical parameters short-term variations are not linked to microbial activities

In the control experiment, pH significantly decreased over time ( $p\text{-value} = 2.17 \times 10^{-10}$ ) from a starting value of 7.99 to a final value of 7.76 (**Figure 1. A**). Eh decreased over time up to 4 days ( $p\text{-value} = 1.18 \times 10^{-6}$ ) from a starting value of 180 mV to a final value of 154 mV (**Figure 1. B**). This Eh decrease could be due to reduced species input with sediment addition. At the end of the experiment (after 5 days of mixing) the Eh value returned to the initial value ( $p\text{-value} > 0.05$ ) (**Figure 1. B**), probably due to regular aeration of the mixture. Such results were already reported in biotic sediment / seawater mixing experiments (Cabrol et al., 2017; Dang et al., 2014; Pía Di Nanno et al., 2007; Zouch et al., 2018). The pH decrease over the time was presumed to be due to the development / respiration of microorganisms having suddenly access to a large pool of bioavailable organic matter (Dang et al., 2020). Although a microbial activity could have led to dissolved oxygen depletion (Moriarty, 1997), in our experiments  $O_2$  didn't significantly vary ( $p\text{-value} > 0.05$ ) and remained within the range 8.58 to 8.84  $mg.L^{-1}$  (**Figure 1. C**), showing that the daily opening of the bottles during our experiment was sufficient to prevent microbial inhibition by oxygen depletion.

The results obtained in experiments 2 and 3 where sediment was autoclaved showed no statistical difference ( $p\text{-value} > 0.05$ ) compared to the control condition. Such observation tends to demonstrate that sediment autoclaving did not disrupt the processes controlling pH, Eh and  $O_2$  variations in this study.

Furthermore, the results obtained with the selective sterilizations showed that sterilization of sediment and / or seawater did not affect the variation of pH, Eh and  $O_2$  too ( $p\text{-value} > 0.05$ ). This conclusion implies that the variation of pH and Eh is mainly due to abiotic processes, which is in agreement with a previous work conducted with a different sterilization method (autoclaving and inorganic poison) and sediment originating from the same bay (Layglon et al., 2020). Thereafter, the pH decrease, commonly recorded during sediment resuspension experiments, could be linked to the increasing concentration of dissolved acid substances through abiotic processes

rather than to microbial mineralization of organic substrates. Further confrontation of this result to the resuspension of a wide panel of sediment quality is however required to determine the abiotic processes leading to pH decrease.

### **3.2. Transfer of metals are mostly due to abiotic processes during sediment resuspension**

Beyond Toulon Bay, sediment resuspension can occur in any shallow marine environment following strong wind episodes. In harbors, sediment resuspension might also be induced by boat mooring and maneuver or dredging. During sediment resuspension in the water column, metals may either remain undisturbed by the mixing, or undergo transfers consisting in a removal by or a desorption from particles (Calmano et al., 1993; Dang et al., 2020, 2015; Layglon et al., 2020). Such processes are element dependent and follow different kinetics (Dang et al., 2020; Monnin et al., 2018).

In the control condition, three groups of metals showing three behaviors over time were identified through correlation analysis (**Supplementary Information**). The first group gathered metals whose dissolved concentration increased over time: Al, Ba, Co, Cs, Cu, Mn, Mo, Ni, Pb, Sb, Tl, U and Zn (a representative example is presented in **Figure 2. A**). Such progressive desorption was previously attributed to sulfide oxidation and organic matter mineralization (Dang et al., 2020; Layglon et al., 2020; Monnin et al., 2018; Saulnier and Mucci, 1999). The oxidation of sulfide phase was attributed to the extended aeration of the sediment, therefore such oxidation was mostly attributed to abiotic processes (Caille et al., 2003). The organic matter oxidation / mineralization was mostly attributed to microbial activity which is enhanced when organic matter is released from anoxic to oxic media (Aller, 1998; Bastviken et al., 2004; Hulthe et al., 1998).

The second group gathered metals whose dissolved concentration decreased over time: Cr, Fe and Ti (a representative example is presented in **Figure 2. B**). During resuspension, such decrease (adsorption) could mostly be due to the addition of carrier phases with sediment addition as well as Fe oxy-hydroxides precipitation (Dang et al., 2020; Layglon et al., 2020). Over time, Fe and Mn generally spontaneously and abiotically precipitate under oxy-hydroxides forms in presence of oxygen (Atkinson et al., 2007; Dang et al., 2020; Hirst and Aston, 1983; Monnin et al., 2018; Vdović et al., 2006; Vidal-Durà et al., 2018). However, in our experiment, dissolved Mn did not decrease over time, its dynamic differs from Fe.

The third group gathered metals whose dissolved concentration did not appear to change significantly over time: As, Li, Rb, Sr and V (a representative example is presented in **Figure 2. C**, metals with the same behavior over time are presented in **SI**).



The results obtained with the selective sterilizations showed that seawater sterilization did not affect the behavior of metals in our experiments ( $p\text{-value} > 0.05$ ) (**Figure 2 et 3**). In the experiments where the sediment was sterilized, the behavior of the metals tested (except Mn) showed no statistical difference when compared to control condition whatever the sampling time ( $p\text{-value} > 0.05$ ). This is in contradiction with the observations of Chifflet et al., (2019) who showed that autoclaving sediment / seawater mix strongly impacted the dissolved concentration of several metals. However, since autoclaving is based on an increase in temperature and pressure, and as the solubility constant depends on temperature and pressure as well as sediment / seawater ratio, an increase in solubilization of the metals at high temperatures may have introduced some equilibrium changes. Thus, to study the microbial contribution on the geochemical transfer of metals, sediment should be autoclaved separately prior to mixing in seawater to reduce temperature and pressure impact. Although sediment alterations are inevitable by autoclaving, this study tends to demonstrate that separate sterilization avoids altering the transfers. Although autoclaving sediment is a disapproved method, it seems to be much more appropriate, when studying metals transfers during sediment resuspension experiments, than the use of azide as previously demonstrated (Layglon et al., 2020). Hence, results suggest that the bacterial activity on the geochemical transfer of the metals is negligible during short term mixing (5 days) when compared to the influence of abiotic processes, a behavior that should now be better deciphered for modelling-based sediment management in contaminated areas.

Mn was the only impacted element by sediment sterilization. Indeed, dissolved Mn concentration was higher in the mixing after three days in experiments where the sediment was sterilized ( $p\text{-value} = 4.19\text{e}^{-14}$ ) (**Figure 3**). Such differentiation between the control experiment and the experiment where the sediment was sterilized could have different origins. Higher dissolved Mn concentration after autoclaving was already observed by Wolf et al., (1989) and it was ascribed to the enhanced Mn oxides dissolution due to the autoclave treatment. Reduction of Mn oxides related to organic matter degradation by bacteria as described by Magen et al., (2011) is excluded in our case because such mechanism was associated to anaerobic conditions, whereas our samples were fully oxygenated. Furthermore, selective extraction revealed that Mn in our samples was mainly in the form of sulfide and not oxide (Layglon et al., *in prep*). In previous studies, Mn was found to be mainly oxidized through biologically-mediated pathways (Learman et al., 2011). Therefore, in this work the inhibition of the microbial community responsible of such oxidation would lead to an increase of dissolved Mn as observed in the experiments where the sediment was sterilized. Thus, it could be hypothesized that the increase in dissolved Mn in the experiments where the sediment was sterilized was not due to Mn oxides dissolution after autoclave treatment, but to the removal of benthic microorganisms preventing Mn oxidation. If the oxidation of Mn (II) was mainly biologically mediated by

plankton, the dissolved Mn concentration in our experiments should be higher in the sterilized seawater condition in comparison to the control condition. However, only the sterilization of seawater did not affect the dissolved concentration of Mn when compared to the control condition (p-value > 0.05). Thus, plankton did not seem to regulate Mn behavior during sediment resuspension.

## **Conclusion**

The present study clearly reports an absence of disturbance of both the physical-chemical parameters and geochemical transfers in sediment / seawater mixing after sediment autoclaving. Results suggest that the decrease in pH, hitherto proposed as resulting from the degradation of organic matter by microbial activity generating acid substances, could be largely due to abiotic processes. Abiotic processes also appeared to be the main drivers of the remobilization of potentially toxic trace metals. Furthermore, this work tends to demonstrate that the microbial influence on metals' behavior remains negligible, metals transfers between sediment and water mainly relying on abiotic processes at least during the first 5 days. Similar studies carried out with sediments of various quality and various microbial communities should be performed to assess the generality of these results.

## **Ethics approval and consent to participate**

Not applicable

## **Consent for publication**

Not applicable

## **Authors' contributions**

N.L., B.M, V.L and S.M designed the study. N.L, S.D'O. and N.G prepared the experiments and collected the samples. N.L., S.D'O. and D.O. performed the analysis. N.L., B.M, V.L and S.M interpreted the raw and collected data. N.L., B.M, V.L and S.M wrote the original draft. All the authors read, revised and approved the manuscript.

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## **Availability of data and materials**

Not applicable

**Conflict of Interest Statement**

☒ 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:

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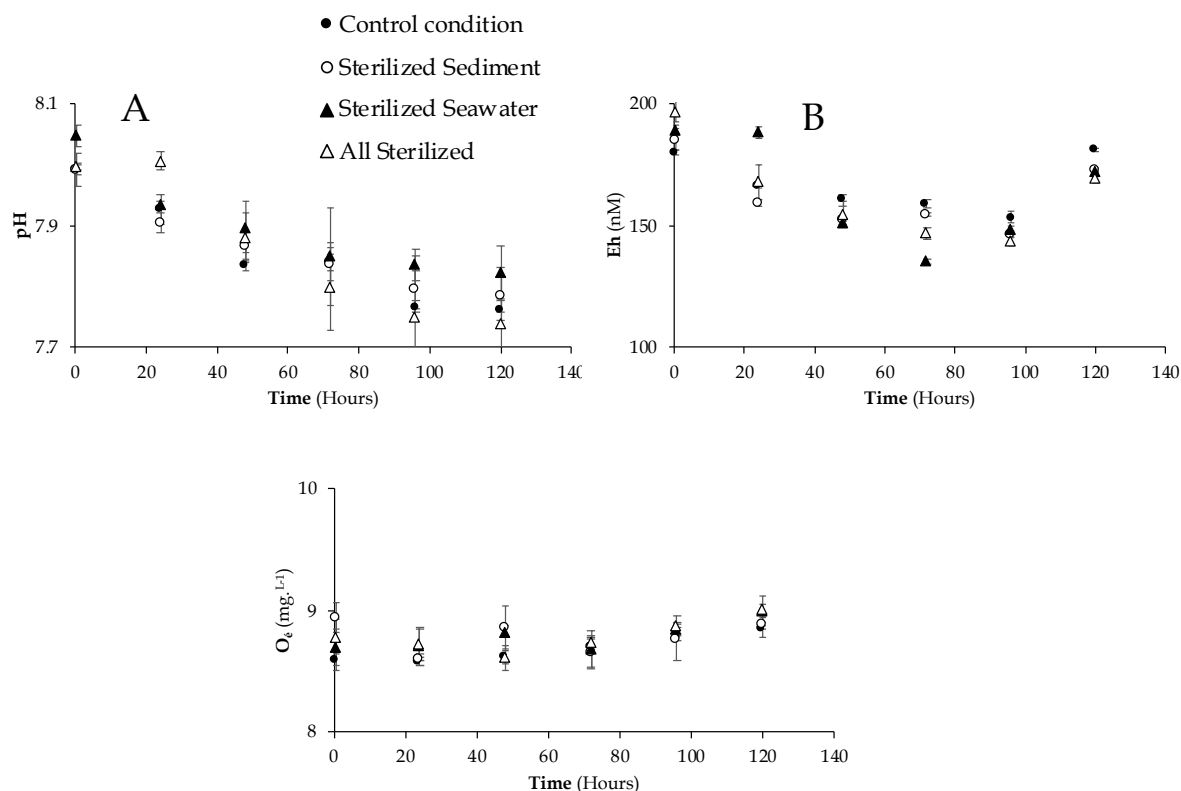
This work is dedicated to Cédric Garnier. We will always remember him as an outstanding scientist, a committed colleague and friend who left us too soon.

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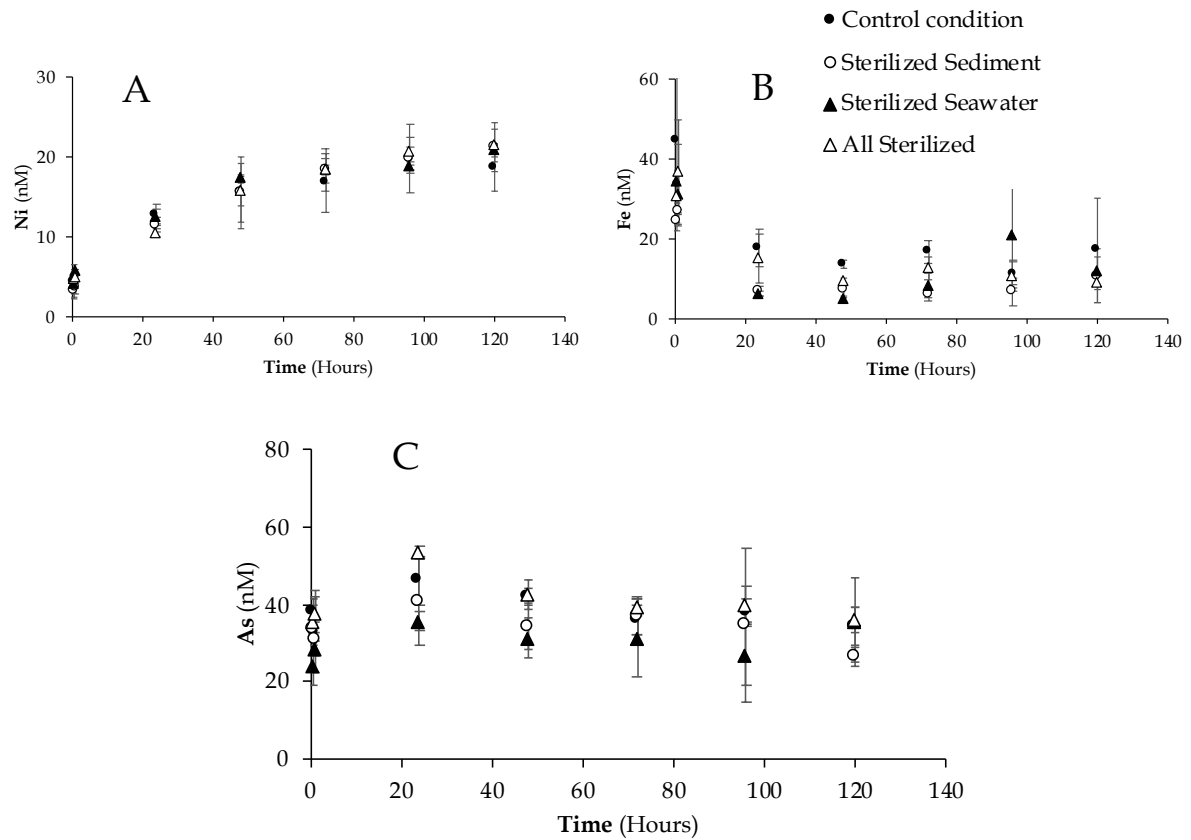
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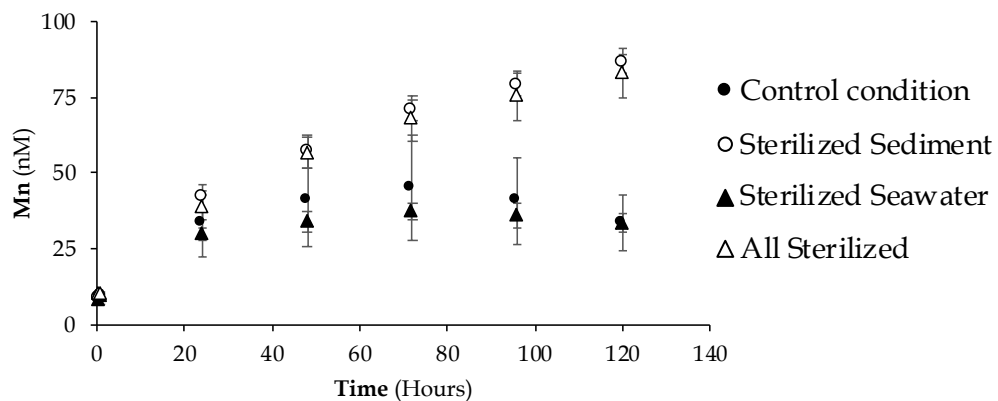
## Figures and tables captions



**Figure 1.** Variation of pH (A), Eh (B) and O<sub>2</sub> (C) during the resuspension experiment in the control condition. Only control condition is represented since there was no significant difference between conditions over time and since points were largely overlapping.



**Figure 2.** Metals whose dissolved concentrations increased (A), decreased (B), and remained unchanged (C) in the control condition after 30 min, 1 h, 1, 2, 3, 4 and 5 days of resuspension experiment. The metals displayed here belong to larger groups discriminated statistically in SI. Only control condition is represented since there was no significant difference between conditions over time and since points were largely overlapping.

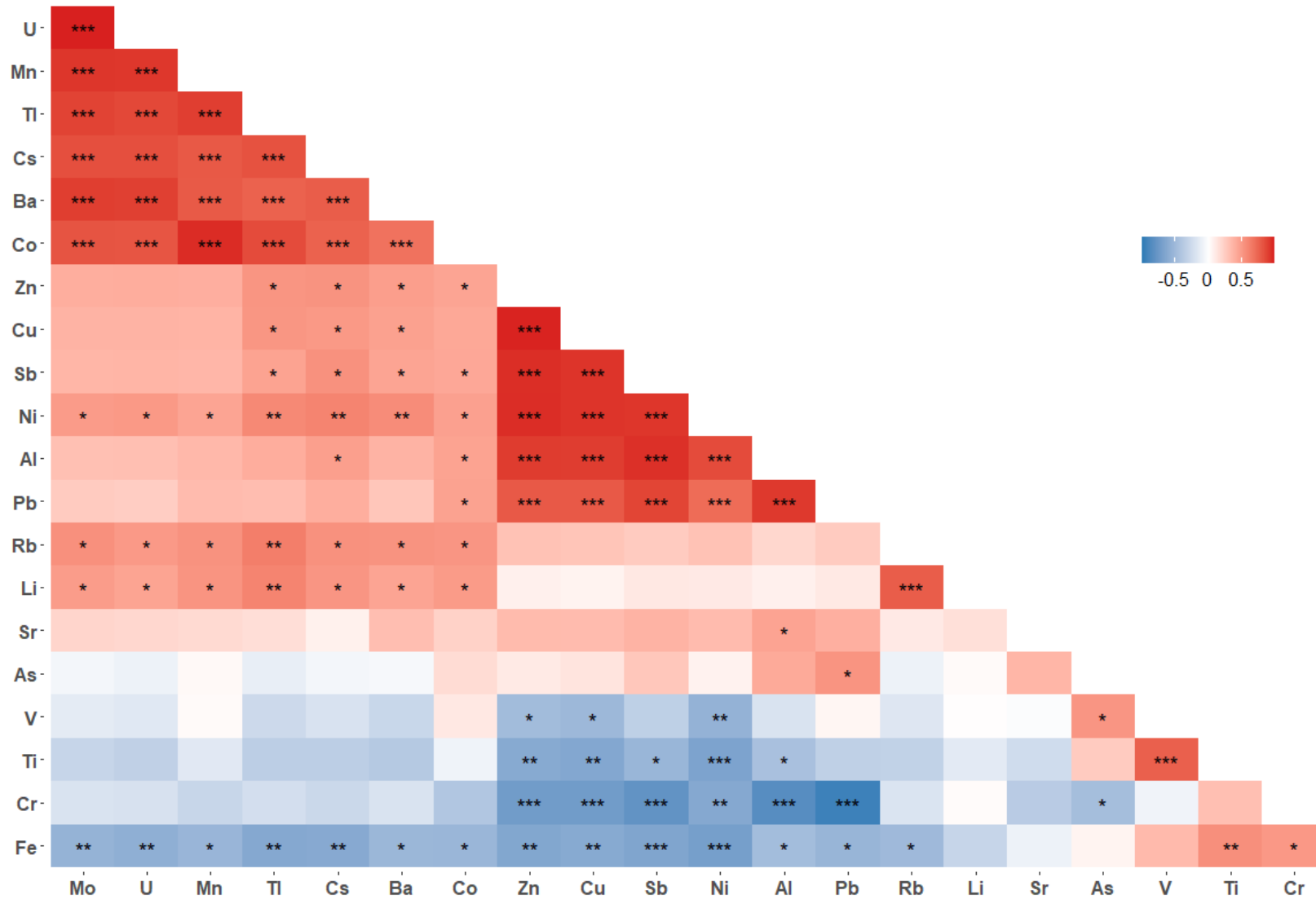


**Figure 3.** Variation of dissolved Mn during the resuspension experiments throughout different sterilization conditions after 30 min, 1 h, 1, 2, 3, 4 and 5 days of mixing.

## **Supplementary Information**

In order to have a quick overview of metals having the same behavior over the 5 days of experiment in the control condition (i.e. sediment and water continuous mixing at a ratio of 1 g.L<sup>-1</sup> eq. DW), Spearman's correlation tests were performed from 21 measurements corresponding to 7 triplicated sampling times. The heatmap correlation clearly showed three main groups, one with the metals ranging from Mo to Pb, a second with the metals ranging from Rb to V and a third one with those ranging from Ti to Fe. The first cluster corresponded to metals whose concentration was increasing over time during the seawater-sediment mixing experiment. The second main cluster represented the metals whose dissolved concentration didn't change while the third one represented the metals whose dissolved concentration decreased over time.





**SI 1.** Heatmap of correlation intensity between studied metals in the control condition. The color scale corresponds to Spearman's correlation coefficients. 1, 2 and 3 stars represent a pvalue <0.05, <0.01 and <0.001 respectively.