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1 Have decades of abiotic studies in
2 sediments been misinterpreted?

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11

12

13 **Abstract**

14

15 Sterilization techniques are largely employed to distinguish biotic and abiotic
16 processes in biogeochemical studies as they inhibit microbial activity. Since one century,
17 chemical sterilizers, supposed to preserve original environmental samples, have taken
18 precedence over physical sterilization techniques considered too destructive. Sodium azide
19 (NaN_3) is nowadays the most commonly used inorganic chemical sterilizer. It is sufficiently
20 purified to study trace metals, as well. Nevertheless, its (in)activity in physico-chemical
21 processes was never ascertained. Through the investigation of sediment resuspension in
22 seawater, the present work unequivocally demonstrated that NaN_3 can impact carbon and
23 trace metals' transfers by altering the redox balance and pH. Unlike decades of blind practice,
24 NaN_3 should be used with great care to track abiotic processes from organic matter rich and
25 reductive matrices.

26 **Keywords**

27 Sodium Azide; Sediment; Mixing; Impact; Organic Matter; Metals

28

29 1. Introduction

30 Since a century, more and more studies focused on the processes controlling the
31 contaminants' mobility at the solid/liquid interface, spanning from the fate of contaminants
32 linked to suspended matter along the salinity gradient (Cindrić et al., 2015; Gerringa et al.,
33 1998; Liu et al., 1998) to the resuspension of contaminated sediment (Dang et al., 2020;
34 Fangueiro et al., 2005). In addition, the diffusive flux of contaminants at the sediment-
35 seawater interface (Dang et al., 2014a) also interest researchers. A particular interest targeted
36 biotic or abiotic nature of such processes. In order to particularly address the questions,
37 researchers used different methods to inhibit biological activity. Physical techniques such as
38 autoclave and gamma irradiation were largely employed (Berns et al., 2008; Kelsey et al.,
39 2010; McNamara et al., 2003). Nevertheless, both techniques were singled out as matrix
40 destroyers, increasing surface area of clays as well as significantly altering the organic matter
41 structure (Berns et al., 2008; Kelsey et al., 2010; McNamara et al., 2003).

42 The use of chemical sterilization techniques (such as sodium azide (Cabrol et al.,
43 2017; Martin et al., 2018), mercury(II) chloride (Martin et al., 2018; Wang et al., 2011) and
44 antibiotics (Thiele-Bruhn and Beck, 2005)) gradually overthrown physical ones, which was
45 found advantageous in avoiding matrix modifications. Poisoning with sodium azide (NaN_3) is
46 now the most common sterilization method used in the studies of biotic/abiotic processes:
47 there are around 12000 published studies over the last 25 years, addressing NaN_3 use in the
48 determination of such processes in soil or sediment.

49 The impact of NaN_3 onto remobilization of organic matter and trace metals from
50 sediments was never studied. On the one hand, NaN_3 is known for long to interact with oxido-
51 reductive reactions (Hartzell and Beinert, 1976) and, more recently to change the optical
52 properties of the organic matter (Retelletti Brogi et al., 2019). On the other hand, trace metals
53 in soil and sediment are mostly linked to organic matter (OM) and/or exist in their reductive

54 forms. Therefore, it would be expected that sterilisation by NaN_3 would potentially impact the
55 transfer of trace metals due to their subsequent oxidation, thus biasing our perception of the
56 biological contribution in non-sterilized (real) conditions.

57 **2. Methods**

58 **2.1. Sampling and samples pre-treatment**

59 This work is questioning the relevance of NaN_3 use in remobilization experiments due to
60 its oxidizing power. The intrinsic effect of this poison onto remobilization of organic matter
61 and trace metals during sediment resuspension in seawater was evaluated. To avoid cross-
62 interactions with biotic interactions, sterilized seawater and sediment were mixed during 2
63 weeks with NaN_3 concentrations covering the range commonly encountered in the literature
64 (1 to 50 mM). At the end of incubation period, dissolved trace metals as well as dissolved
65 organic and inorganic carbon were quantified and compared to reference (initial seawater) and
66 control (no azide) values. In order to avoid potential contamination by the probes, pH and Eh
67 were monitored in the same mixtures prepared in separate set of bottles.

68 For the purpose of this work, the sediment was sampled in an enclosed area of Toulon
69 Bay (harbour of La Seyne-sur-Mer, 43.1026; 5.8827), ensuring the use of fine and heavily
70 contaminated sediment (Coclet et al., 2018; Dang et al., 2018, 2015b, 2015a, 2014b; Pougnet
71 et al., 2014; Tessier et al., 2011). The seawater was sampled in a more open and less
72 contaminated part of the bay (43.0948; 5.9276) in order to maximize our ability to quantify
73 transfers.

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

77 The sediment was collected with a grab sampler (Tall Ekman Grab, Wildco 5.3 L) and
78 twenty-five liters of seawater were sampled on top of the water column (-0.5 m) using a 4.2 L
79 horizontal van Dorn type water sampler (Wildco, model Beta). Collected water was stored
80 into clean 25 L LDPE bottle (Nalgene) previously rinsed three times with water from the site.
81 Subsamples of 24 mL of filtered seawater (0.2 μm cellulose acetate syringe filter, Sartorius)
82 were stored in pre-calcinated (4 h, 450 $^{\circ}\text{C}$) glass tubes (equipped with Teflon / silicon septum,
83 Wheaton), poisoned with NaN_3 (final concentration 1 mM, Sigma Aldrich) and stored at 4 $^{\circ}\text{C}$,
84 in order to analyse Dissolved Organic Carbon (DOC) and Dissolved Inorganic Carbon (DIC)
85 concentrations. Similarly, subsamples of 8 mL of filtered seawater were stored into HDPE
86 bottles and acidified with 0.2% HNO_3 s.p. (Merck) to determine dissolved metal
87 concentrations.

88 **2.2. Laboratory resuspension experiments**

89 In order to evaluate the intrinsic effect of NaN_3 onto the behavior of trace metals as
90 well as DOC and DIC when sediment is resuspended into seawater, the seawater was filtered
91 under 0.2 μm (bacterial cut-off pore size; Durapore filter 47 mm) and the sediment was
92 autoclaved in order to avoid azide-dependent interactions with biotic or biota-related
93 transfers.

94 The sediment was mixed with the seawater (with a ratio corresponding to 130 g of wet
95 sediment for 1 L of seawater, *i.e.* to 50 g of dry sediment for 1 L of seawater) into 500 mL
96 FEP bottles. Different conditions were tested: without addition of NaN_3 and with a range of
97 concentrations of sodium azide added into the mixture (1, 5, 10 or 50 mM, final
98 concentrations). All experiments were performed in triplicates. All the bottles were submitted
99 to continuous overhead shaking (15 rpm; Heidolph Reax 20) in an opaque box to prevent
100 sunlight alteration. Water-sediment mixture was sub-sampled after 2 weeks of contact time.

101 Samples were taken with syringes equipped with Teflon tubes. Each sub-sample was filtered
102 and stored as described above for seawater prior to DOC, DIC and trace metals analysis.

103 In parallel, the same mixtures were reproduced in 1 L HDPE bottles in order to
104 monitor pH and the redox potential (Eh) through the experiment using PHC101 and MTC101
105 multiparameter probes (Hach).

106 **2.3. Chemical analyses**

107 Trace metals concentrations were obtained by High Resolution Inductively Coupled
108 Plasma Mass Spectrometer (Element 2, HR ICP-MS, Thermo). All samples were spiked with
109 an Internal Standard (Indium). A certified reference material (CASS-5, Nearshore seawater
110 reference material for trace metals, National Research Council Canada) was used as a quality
111 control of HR ICP-MS measurements. For HR ICP-MS measurement, filtered seawater
112 samples were diluted 10 times. Matrix matching calibration (CASS-5) was used for
113 concentration quantification.

114 DOC and DIC concentrations were determined using a TOC-VCSH analyzer
115 (Shimadzu). The measurement is based on a high temperature catalytic oxidation followed by
116 an infrared detection. The analysis was validated using certified reference material (SUPER
117 05, Canada).

118 **2.4. Statistical analyses**

119 All statistical analyses and heatmap visualizations were performed using RStudio (R
120 Core Team, 2015) and packages *agricolae*, *ggpubr*, *gridExtra*, *Hmisc*, *reshape2* and *stats*. The
121 influence of sodium azide on trace metals and dissolved organic carbon remobilization were
122 assessed through one-way ANOVA and Tukey post-hoc comparisons. Correlations between
123 DOC and dissolved trace metal concentrations were assessed by Spearman's correlation tests.

124 **3. Results and Discussions**

125 **3.1. Resuspension experiment without Sodium Azide**

126 In resuspension experiment performed without NaN_3 , Eh and pH slightly decreased
127 after 2 weeks (**Figure 1.A**) if compared to the initial seawater: from 259 to 221 mV and from
128 7.99 to 7.03, respectively. Similar variations were previously observed in biotic
129 sediment/water mixing experiments (Cabrol et al., 2017; Dang et al., 2014a; Pía Di Nanno et
130 al., 2007; Zouch et al., 2018), but our experimental design demonstrates the abiotic control of
131 such processes.

132 Sterilized sediment and water mixing increased dissolved organic carbon (DOC) and
133 dissolved inorganic carbon (DIC) concentrations from 2.4 to 30 mg.L^{-1} and from 27 to 73
134 mg.L^{-1} , respectively (**Figure 1.B**). Previous observations of DOC increase were reported for
135 sediment resuspension in seawater with a sediment/seawater ratio ranging from 1 to 100 g.L^{-1}
136 (Kieber et al., 2006; Komada and Reimers, 2001; Shipley et al., 2011). Particulate organic
137 carbon (POC) pool was assumed to be converted in DOC (Koelmans and Prevo, 2003;
138 Komada and Reimers, 2001), thus potentially explaining the observed DOC increase in our
139 experiment. Observed DIC increase could be related to dissolution of particulate carbonates as
140 a consequence of the pH drop. Indeed, the dissolution of particulate carbonates at pH higher
141 than that recorded in our experiment have already been demonstrated (Comeau et al., 2017).

142 Through sediment resuspension in the water column, remobilization of trace metals
143 comes out either as a removal (adsorption or co-precipitation onto particles) or as a desorption
144 from particles (Calmano et al., 1993; Dang et al., 2020, 2015b; Xie et al., 2019). In our study,
145 after 2 weeks of contact time without addition of NaN_3 , most of the analyzed elements (Li,
146 Mo, Sb, Cs, Pb, U, V, Mn, Co, Ni, Cu, Zn, Sr, Ba and As) had undergone a desorption from
147 the particles (representative examples are shown in **Figure 1.C and D**). Such desorption was
148 probably due to the oxidation of the sediment particles. Indeed, the oxidation will impact the
149 organic matter which will release metals previously bound to it. In addition, reduced inorganic
150 metals (linked to sulfides) will also be oxidized and therefore released in the dissolved

151 phase. Other metals (Al, Fe and Ti) had been adsorbed onto the particles (representative
152 examples are shown in **Figure 1.E and F**). This adsorption was probably related to Fe and
153 Mn oxidation, which will generate oxy-hydroxide and become new carrier phases for metals
154 (Dang et al., 2020).

155

156 **3.2. Geochemical transfers are poisoned by the use of NaN₃**

157 Along with increasing NaN₃ concentration, pH and Eh increased compared to the
158 control condition (**Figure 1.A**), Eh even reaching above-reference values with the highest
159 NaN₃ concentrations. This clearly shows the strong oxidant power of NaN₃. Moreover, pH
160 increase is probably due to the reaction between azoture ions (N₃⁻) and H₂O molecules,
161 forming HN₃ and OH⁻. This can be easily demonstrated by adding 50 mM sodium azide into
162 milli-Q whose pH increased from 5.8 to 7.4.

163 With NaN₃ addition, DOC concentration increased by a factor 1.8 when compared to
164 the control condition (from 30.3 to 54 mg.L⁻¹) whereas DIC concentration decreased by a
165 factor 2.6, returning to the reference value (27 mg.L⁻¹) (**Figure 1**). NaN₃-related DOC
166 increase had already been documented (Koelmans and Prevo, 2003; Pett, 1989; Shipley et al.,
167 2011). Pett (1989) explained this observation either by an alteration of algae cell walls'
168 permeability or a release of enzymes by azide-killed bacteria. However, in the present study,
169 the sediment was autoclaved and the seawater was filtered so the hypothesis of Pett could
170 only fit with our control condition. Moreover, considering an average of 5 x 10⁸ bacterial cells
171 in each g of humid sediment (Whitman et al., 1998) and an average of 20 fg C per cell (Pedler
172 et al., 2014), the bacterial-decay-related DOC increase should not have risen the concentration
173 above 2.6 mg.L⁻¹, which remains far from the 29 and 54 mg.L⁻¹ recorded in the control
174 condition and with the largest NaN₃ addition, respectively. Thus, we hypothesize that the
175 increase in DOC concentration was related to POC alteration by NaN₃. Considering the

176 oxidant power highlighted above, such alteration could consist in an oxidation of the
177 originally reduced pool of sediment POC.

178 Phytoplankton usually uses DIC for the photosynthesis. Yet, in the present study, all
179 experiments were performed with sterilized sediment and water samples and protected from
180 light, therefore any contribution from photosynthesis to DIC decrease can be ruled out. The
181 NaN_3 -induced pH increase could have strongly contributed to modify the carbonate system
182 and thus DIC concentrations. An acidification caused the transformation of CO_3^{2-} into HCO_3^- ,
183 leading to the dissolution of CaCO_3 (Honisch et al., 2012; Pedler et al., 2014). Then, the
184 addition of NaN_3 concentration above 10 mM promoted pH values similar to the seawater
185 reference and appeared to inhibit this phenomenon. Thus, the use of azide clearly modified
186 the carbonates system in our experiment by inducing pH increase.

187 When NaN_3 was used in remobilization experiment, dissolved concentrations of most
188 of the trace metals studied were statistically related to NaN_3 concentration (some examples
189 are shown on **Figure 1C, D, E and F**) indicating strong effect of NaN_3 on their behavior.
190 Only As, Sb, and Ti were not affected by NaN_3 addition. The positive (Al, Co and V) or
191 negative correlation (Ba, Co, Cs, Fe, Li, Mn, Ni, Pb, Sr, U and Zn) with NaN_3 is probably
192 linked the carrier phases and their respective specific alterations. **Figure 2** gathers trace
193 metals according to their behavior and their link with DOC concentration considering all
194 experimental conditions. Three groups can be deciphered: (1) Al, Mo and V were strongly
195 and positively linked to DOC concentration, (2) Ba, Co, Cs, Fe, Li, Mn, Ni, Pb, Sr, U and Zn
196 were strongly negatively linked to DOC concentrations and (3) As, Cu, Sb and Ti were not
197 related to DOC and tend to show singular behaviors. Nevertheless, this behavior might not be
198 directly linked to organic carbon fate. Trace metals strongly negatively linked to DOC were
199 removed onto particles with NaN_3 additions. This removal might be explained by the increase
200 of Fe and Mn oxides, generated by the oxidant power of NaN_3 , becoming new carrier phases

201 for trace metals. Thus, NaN_3 -related organic carbon transformations as well as higher Fe/Mn
202 oxide precipitation were the main driving forces influencing transfers of trace metals during
203 sediment resuspension and reveal the incompatibility of millimolars of NaN_3 with
204 geochemical studies.

205

206 **Conclusion**

207 Sodium azide was largely employed during the last century to inhibit microbial
208 activity in biogeochemical studies in order to identify the contribution of biotic processes
209 affecting transfers of trace metals and/or carbon without considering the intrinsic effect of
210 NaN_3 . The present study clearly demonstrated that the addition of NaN_3 at concentrations
211 commonly used in sediment resuspension studies (1-50 mM) strongly impacted both pH and
212 Eh, DOC/DIC and trace element behaviors. Oxidative power of NaN_3 caused increase of
213 DOC, whereas increase of pH diminished solubility of carbonates, leading to a decrease in
214 DIC concentrations. Furthermore, oxidative power of NaN_3 promoted formation of the
215 various carrier phases (i.e. oxides and organic matter) which were having strong influence on
216 the behavior of trace metals during such experiments. An increase of NaN_3 caused enhanced
217 transfer of some trace metals from solid phase to the dissolved form, while for others a
218 decrease in dissolved concentration was observed. As a conclusion, the authors want to
219 highlight the potentially unexpected strong effects of azide on physico-chemical properties of
220 environmental matrices and recommend banishing its use for biogeochemical studies in
221 organic and reductive matrices.

222 Recapitulation of the occurring processes is schematized on **Figure 3**.

223

224

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235

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360 **Figures and tables captions**

361 **Figure 1.** Variation of pH and Eh (A), DOC and DIC (B), Pb and Ni (C), Cu and Mo (D), Al
362 (E) and Fe (F) as a function of azide concentration. Column plots represent the mean value of
363 triplicated experiments after 2 weeks of sediment and seawater continuous mixing. Error bars
364 represent standard deviation. Horizontal lines represent initial values in seawater. Letters on
365 the plots represent the significant variability between conditions coming from Tukey post-hoc
366 comparisons.

367 **Figure 2.** DIC concentration as a function of the recorded pH during the experiment. This
368 representation includes the 5 experimental conditions after 2 weeks of sediment resuspension
369 as well as the measurement in initial seawater.

370 **Figure 3.** Heatmap of correlation intensity between studied trace metals and DOC.
371 Correlations were calculated from 15 values corresponding to 5 triplicated azide
372 concentrations. The color scale corresponds to Spearman's correlation coefficients. 1, 2 and 3
373 stars represent a pvalue <0.05 , <0.01 and <0.001 respectively.

374 **Figure 4.** Schematic conclusion representing the different processes occurring during the
375 experiments.

376

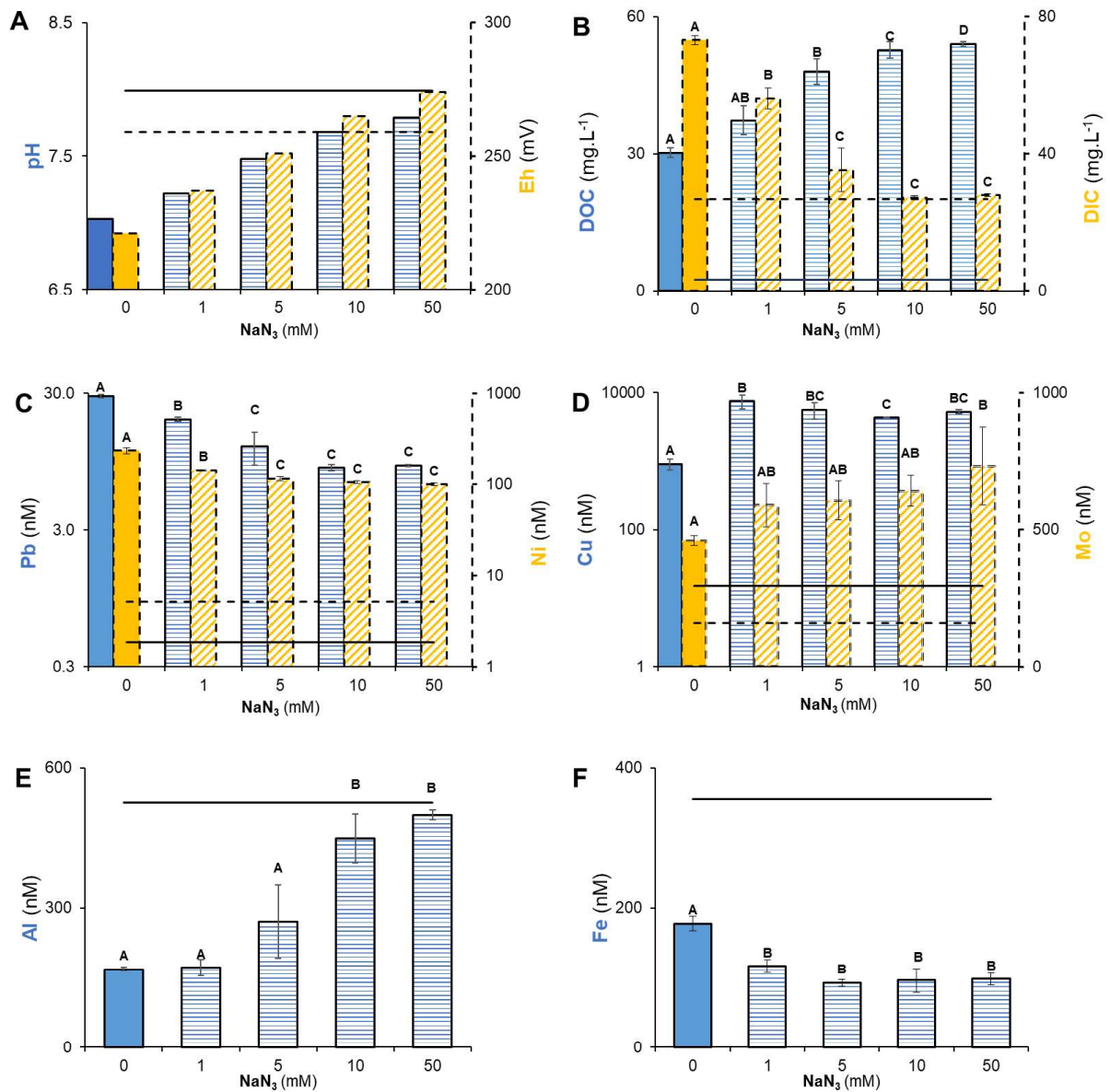


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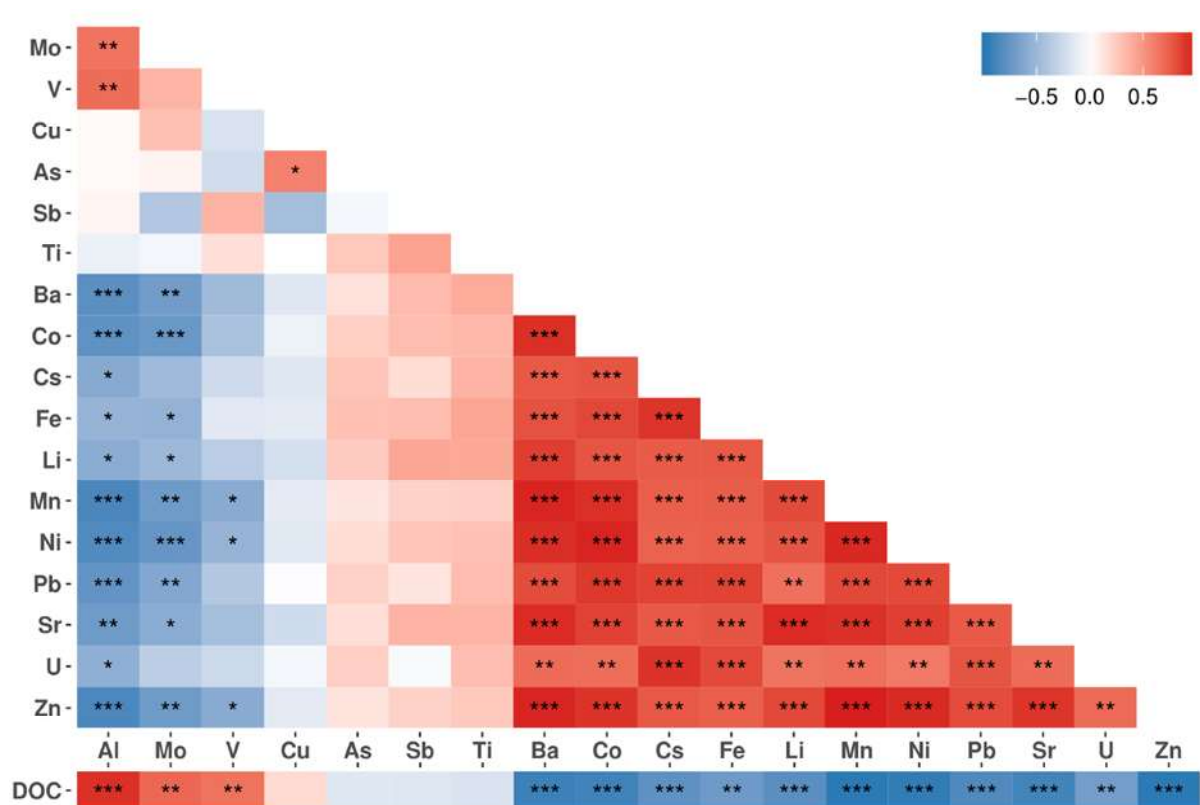


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Figure3
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