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1 **Benthic fluxes of copper, complexing ligands and thiol compounds in shallow lagoon waters**

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7 **Keywords**

8 Heavy metals; speciation; ligands; thiols; benthic flux; salt marsh; Venice Lagoon

9 **Abstract**

10 Benthic fluxes of copper, copper complexing ligands and thiol compounds in the shallow
11 waters of Venice Lagoon (Italy) were determined using benthic chambers and compared to
12 porewater concentrations to confirm their origin. Benthic copper fluxes were small due to small
13 concentration differences between the porewaters and the overlying water, and the equilibrium
14 concentration was the same at both sites, suggesting that the sediments acted to buffer the copper
15 concentration. Thiol fluxes were ~10 x greater at 50 - 60 pmol cm⁻² h⁻¹, at the two sites.
16 Porewater measurements demonstrated that the sediments were an important source of the thiols
17 to the overlying waters. The overlying waters were found to contain at least two ligands, a strong
18 one, L1 (log K'_{CuL1} = 14.2) and a weaker one, L2 (log K'_{CuL2} = 12.5). The concentration of L1
19 remained relatively constant during the incubation and similar to that of copper, whereas that of
20 L2 was in great excess of copper, its concentration balanced by porewater releases and
21 breakdown, probably due to uptake by microorganisms, similar to that of the thiol compounds.
22 Similarity of the thiol and L2 concentrations and similar complex stability with copper suggest
23 that L2 was dominated by the thiols. The free copper concentration ([Cu[']]) in the Lagoon waters
24 was lowered by a factor of 10⁵ as a result of the organic complexation.

25 **Introduction**

26 Sediments can act as a source of copper (Klinkhammer, 1980), copper complexing
27 ligands(Skrabal et al., 2000), sulfur species and thiol compounds (Luther III et al., 1986). Metal
28 complexing ligands (Croot et al., 2000; Moffett and Brand, 1996) and thiol compounds are also
29 known to be released by marine phytoplankton (Dupont and Ahner, 2005; Leal et al., 1999) into
30 surface waters. Specific thiols like glutathione have been found in oceanic (Dupont et al., 2006;
31 Le Gall and van den Berg, 1998) and estuarine (Tang et al., 2004) waters. As thiol compounds
32 form stable complexes with copper(I) (Leal and Van den Berg, 1998) it is likely that the thiols are
33 part of the pool of copper complexing ligands in natural waters and could play a role in
34 controlling the bioactivity and biogeochemistry of copper.

35 In this study we investigated whether benthic interactions are an important source for thiols,
36 copper and copper-binding ligands in shallow surface waters as occurring in lagoons and salt
37 marshes. Venice Lagoon (Italy) was selected for this study as it is an extensive, shallow lagoon,
38 with salt marshes on the boundaries. Benthic interactions are likely to be relatively important to
39 its water composition due to a shallow water depth of typically 1 m. Benthic fluxes were
40 determined by placing large benthic chambers on the sediments and following changes in the
41 water composition as a function of time. Porewaters were collected from sediment cores to
42 identify the source of the thiols and copper, and the concentration difference with the overlying
43 water was used to estimate comparative thiol fluxes.

44 **Materials and Methods**

45 *Instrumentation and reagents*

46 Voltammetric equipment was a μ Autolab potentiostat attached to a Metrohm VA 663
47 electrode stand and controlled by a personal computer. The working electrode was a hanging
48 mercury drop electrode (HMDE), the reference electrode was double junction, Ag/AgCl, 3 M

49 KCl and the counter electrode was a glassy carbon rod.

50 Water used for dilutions was obtained from a Milli-Q (MQ) system. HCl and NH₃ were
51 purified by sub-boiling distillation on a quartz condenser. Copper standards were prepared by
52 dilution of atomic adsorption standards (Spectrosol, BDH) and acidified to pH 2.2 using 11 M
53 HCl. Stock solutions of 0.01 salicylaldehyde (SA) (BDH) were prepared in 0.1 M HCl.

54 *Speciation*

55 Copper, copper complexing ligands (Campos and van den Berg, 1994) and copper - thiol
56 interactions (Leal and Van den Berg, 1998) were determined by cathodic stripping voltammetry
57 (CSV). Copper complexing capacity titrations were at pH 7.8 (NBS scale), using 0.01 M HEPES
58 buffer, diluted from 1 M HEPES (N-2 hydroxyethylpiperazine-N'-2 ethanesulfonic acid, Merck)
59 and 0.55 M NaOH, using ligand competition against 2 μM SA (Campos and van den Berg, 1994).
60 Reactive copper is part of the total dissolved copper which is bound by the added SA, which is a
61 function of the competition between the added SA and the natural ligand. The reactive copper
62 concentration ($[Cu_{\text{reactive}}]$) was determined by CSV after deposition at -0.1 V, deposition time 90 s
63 and the scan used the square-wave mode. Uncalibrated reactive copper concentrations (peak
64 heights) were used to fit $[Cu_{\text{reactive}}]/[CuL]$ as a function of $[Cu_{\text{reactive}}]$; a linear least squares
65 regression was used unless curvature of a plot of $[Cu_{\text{reactive}}]/[CuL]$ as a function of $[Cu_{\text{reactive}}]$
66 showed the presence of more ligands, which were then fitted using iterative, linear, least-squares
67 regressions of the data. The sensitivity was corrected for the degree of unsaturation of the ligands
68 with copper at the end of the titrations (Leal and Van den Berg, 1998; Turoczy and Sherwood,
69 1997). The uncertainties in the values for the ligand concentrations and stability constants were
70 calculated from the standard deviation of the least-squares regressions.

71 Samples used for total dissolved copper were acidified to pH 2.5 and UV-digested for 1 h in
72 acid cleaned silica tubes, prior to CSV using 30 μM SA at pH 8.3 using 10 mM borate pH buffer.

73 The deposition potential was -1.1 V, the re-oxidation potential -0.1 V and the CSV scan was in a
74 negative direction using the square-wave modulation. The sensitivity was calibrated for each
75 sample by standard additions of copper.

76 Reactive thiol concentrations were determined by CSV as before (Leal et al., 1999).
77 Voltammetric parameters were as for reactive copper with calibration against glutathione which
78 was found to behave similarly to the unknown thiol species. Our method differed from that used
79 before in that the peak height of the total thiol concentration (I_{\max}) was determined after addition
80 of 100 μM EDTA to free any thiols bound by copper and other metals by lowering the, free, ionic
81 metal concentrations. The peak height of the reactive thiol species was measured during the
82 complexing ligand titrations along with reactive copper, and used to compute conditional stability
83 constants of the copper thiol complexes (Laglera and van den Berg, 2003). Stock solutions of
84 glutathione, thioacetamide, thiourea, cysteine (BDH) and 3-mercaptopropanoic acid (Fluka) were
85 prepared freshly before use by dissolution in MQ.

86 *Study sites*

87 Venice Lagoon, Italy, lies on the northern Adriatic coast and consists of salt marshes and
88 shallow waters. It is constituted of three main basins and several sub-basins. During the 1960s
89 and 1970s it was subjected to sizable anthropogenic inputs that have gradually impaired the
90 quality of the lagoon ecosystem (Pavoni et al., 1987). Significant amounts of these pollutants,
91 both organic and inorganic, have accumulated in the sediments and may constitute a potential
92 secondary source of contaminants. The water exchange rate is $140 \text{ km}^3 \text{ yr}^{-1}$, it has an area of 549
93 km^2 and an average depth of 1 m, causing a residence time of the water of about one day, but this
94 can increase to three days in areas of lower dynamics. In remote parts of the lagoon, atmospheric
95 deposition of contaminants is thought to dominate (Cochran et al., 1998) but run-off is the main
96 source near an industrial area (Donazzolo et al., 1984).

97 Two sites were studied, both in relatively contaminated waters: Tresse (Site 1), near the
98 industrial area of Marghera, and Campalto (Site 2), near to an area with a history of solid waste
99 disposal (Turetta et al., 2005) (Fig. 1). The experiment at Tresse was from 28 to 31 October 2002
100 and at Campalto from 25 to 27 May 2003; both started at 1:00 PM. The sediment from site 1 (the
101 first cm) had a mean organic carbon content of 0.4 % and was classified as silt clay loam. The
102 mean organic carbon concentration in the sediment of site 2 was 1.0 % which was classified as
103 silty clay.

104 *Benthic chambers*

105 Benthic chambers were constructed at Venice University with a volume of 90 L with
106 collapsible, polyethylene, sides to enable sample extraction without water exchange (Turetta et
107 al., 2005). The chambers (60 cm x 60 cm) had a water height of 25 cm and the water was mixed
108 slowly by a paddle (120 cm²) rotating at 10 rpm. The stirring ensured mixing of the internal water
109 in about 2 min with a diffusive boundary layer thickness of about 0.5 mm and a current speed
110 close to the bottom of about 2 cm s⁻¹, at which sediment resuspension should be minor (Tengberg
111 et al., 2005). Samples were collected by battery operated peristaltic pump. Oxygen, pH,
112 conductivity and temperature were monitored continuously using a multi-probe (YSI, model 556,
113 Ohio, USA).

114 Water samples for metal analyses were collected every 3 h and for metal speciation at 9 h
115 intervals. All samples were filtered using two-stage cartridge filters containing 0.45 µm and 0.2
116 µm membranes (Sartorius Sartobran 300) and collected in low density polyethylene (LDPE)
117 bottles. Speciation samples were subsequently stored frozen and other samples were acidified.
118 The sample bottles were cleaned by soaking sequentially in hot detergent and dilute (2 M) acid
119 and were stored partially filled with 0.01 M HCl. The bottles were rinsed with water from the
120 chamber before filling.

121 *Porewater sampling*

122 30 cm box cores were collected from the sediments at the sampling sites (June 2003) using
 123 a piston corer. The cores were sealed and stored in a nitrogen-flushed box to maintain an oxygen-
 124 free atmosphere. The overlying water column was sampled at the same time. The cores were
 125 sliced at intervals of 0.5-2 cm with more detail at the top of the core. The slices were centrifuged
 126 to extract the porewater, which was quickly filtered under an inert atmosphere and frozen until
 127 analysis. Copper in the porewaters was determined by ICP-SFMS (inductively coupled plasma -
 128 sector field mass spectrometry) (Turetta et al., 2005).

129 *Calculation of the benthic flux from the benthic chamber data*

130 Benthic fluxes were calculated from the change in the concentrations as a function of time
 131 where each change was corrected for the volume change after sampling:

$$132 \Delta \text{mol} \Delta t^{-1} = (\Sigma \text{mol}_{i(\text{rem})} + \text{mol}_i) \Delta t_i^{-1}$$

133 where $\Sigma \text{mol}_{i(\text{rem})}$ was the sum of the moles removed of the chamber as a result of sampling at time
 134 i , Δt_i the time lapsed during time interval i (h), and mol_i was the actual number of moles in the
 135 chamber at time i . The values for $\Delta \text{mol} \Delta t^{-1}$ were used to calculate the flux, F :

$$136 F = \Delta \text{mol} \Delta t^{-1} A^{-1}$$

137 where A was the sediment area (cm^2) covered by the chamber. The flux F ($\text{pmoles cm}^{-2} \text{h}^{-1}$) was
 138 obtained by linear regression of the data.

139 *Calculation of the benthic flux from the gradient at the sediment/water interface*

140 Estimates of the molecular diffusion of thiols across the sediment-water interface were
 141 calculated from the sediment/water gradient using the following equation (Berner, 1980):

$$142 F_d = -D_s \delta C \delta x^{-1}$$

143 where F_d = the diffusion flux of the species ($\text{pmol cm}^{-2} \text{h}^{-1}$), D_s = the diffusion coefficient of the
 144 species in sedimentary porewaters ($10^{-6} \text{cm}^2 \text{s}^{-1}$), $\delta C \delta x^{-1}$ = concentration gradient of the species

145 across the sediment-water interface (pmol cm^{-4}), and where $x = 0.05$ cm (diffusion boundary
146 layer thickness) was set by the stirring rate in the chamber. Uncertainties in the fluxes were
147 calculated from the standard deviation of the measured thiol concentrations. Differences in the
148 composition of porewater from the top 0.5 cm of the sediment and from surface water from a
149 depth of ~ 20 cm (total water depth ~ 1 m) were used to establish the gradients.

150 The porewater diffusion coefficient (D_s) was calculated using (Iversen and Jorgensen,
151 1993):

$$152 \quad D_s = D_0 (1 + n (1 - \phi))^{-1}$$

153 where parameter n depends on the type of sediment and the diffusing species: a value of 3 was
154 selected as before for similar sediments (Iversen and Jorgensen, 1993). Changing n in the
155 porosity range of the sample sites (0.5 – 0.75) created differences of less than 10 % in D_s per unit
156 of n . D_0 was the diffusion coefficient of the species at infinite dilution ($\text{cm}^2 \text{s}^{-1}$) (Li and Gregory,
157 1974) and ϕ was the porosity of the sediment (% volume/weight). The flux value included
158 tortuosity (constrained diffusion) and molecular diffusion within the porewaters, but did not
159 account for other processes such as adsorption, wave shear and bioturbation (Berner, 1980).

160 **Results and discussion**

161 *Temperature, oxygen and salinity*

162 The chambers were placed on the sediments, carefully pushed into the top of the sediments
163 and then the cover was closed; the stirrer was started and the measurements were initiated. The
164 chambers were fully submerged and the water depth was ~ 1 m. It is likely that the placing of the
165 benthic chamber and the starting of the stirrer would have disturbed the original gradients across
166 the sediment – water interface. However, this is an unavoidable problem with using benthic
167 chambers. In this case any effects were minimized by using a large chamber which was open at
168 the time of placing and by using a slow stirring rate. The absence of abrupt variability in the

169 major parameters (oxygen, pH) suggests that the water in the chamber had equilibrated within an
170 hour, possibly minutes. The oxygen concentration in both chambers dropped to $\sim 94 \mu\text{M O}_2$ (~ 30
171 % air saturation) during the first 12-15 h (Fig. 2), after which it continued to drop more gradually
172 at Tresse and became more or less constant at Campalto. The overall decrease was greatest at
173 Campalto which started from a higher concentration of oxygen ($219 \mu\text{M O}_2$ compared to $156 \mu\text{M}$
174 O_2 at Tresse). The temperature variation in the chambers can be used as a measure of possible
175 effects of solar radiation and from this it can be seen that the leveling off of the oxygen
176 concentration at Campalto, and the slower drop at Tresse, coincided with daylight, indicating that
177 further oxygen uptake was balanced by oxygen production by benthic or planktonic algae.

178 The pH dropped from 8 to 7.6 at Tresse and from 7.8 to 7.3 at Campalto presumably due to
179 diffusion of CO_2 out of the sediments, or bacterial activity in the chamber water itself. The drop
180 in pH paralleled that in O_2 which stabilized after 22 h at the Campalto site and gradually leveled
181 off at Tresse (Fig. 2). The parallel behaviour of O_2 and CO_2 is consistent with the release of CO_2
182 by heterotrophic organisms as they use oxygen, either in the benthic chamber itself or due to
183 diffusion of CO_2 from the sediments and O_2 into the sediments. Dissolved organic carbon (DOC)
184 showed comparatively small variability: at Tresse the decrease was from 160 at the beginning to
185 $150 \mu\text{M}$ carbon by the end of the experiment, and at Campalto from ~ 600 to $500 \mu\text{M}$ carbon
186 except for an outlier of $750 \mu\text{M}$ carbon after 25 h (Manodori et al., 2006). The decreases in DOC
187 in the benthic chambers were much less than those of dissolved oxygen indicating that the much
188 larger variations in O_2 and CO_2 were not related to bulk breakdown of the DOC in the chamber
189 but due to benthic processes.

190 The oxygen flux ($\text{nmol cm}^2 \text{ h}^{-1}$), calculated from the change in the benthic chamber
191 composition as a function of time, was -86 (negative means into the sediments) at Tresse and -
192 163 at Campalto for the first 25 h of the experiment. These values are similar to oxygen fluxes

193 found in other estuarine sediments; for instance, a flux of $-150 \text{ nmol O}_2 \text{ cm}^{-2} \text{ h}^{-1}$ has been found
194 previously in estuarine sediments of sandy mud (Clavero et al., 1992). The oxygen flux decreased
195 after the first 25 h to $\sim 23 \text{ nmol cm}^{-2} \text{ h}^{-1}$ at Tresse, and became insignificant at Campalto, as the
196 chamber became equilibrated with the sediments and further oxygen decreases were offset by in-
197 situ oxygen production.

198 *Copper fluxes derived from the benthic chamber data*

199 The copper concentration in the chambers was initially greater at Tresse (40 nM) than at
200 Campalto (14 nM) (Fig. 3). With time the concentrations converged to similar levels at the two
201 sites: $\sim 24 \text{ nM}$ at Campalto and $\sim 19\text{-}24 \text{ nM}$ at Tresse, compared to outside chamber values of 40
202 nM (Tresse) and 24 nM (Campalto). Because of the greater than equilibrium concentration of
203 copper in the water at Tresse station, the copper flux was negative, $-4.4 \pm 0.2 \text{ pmol cm}^{-2} \text{ h}^{-1}$,
204 whereas it was positive $3.8 \pm 0.1 \text{ pmol cm}^{-2} \text{ h}^{-1}$ at Campalto (out of the sediments).

205 It is possible that 19 - 24 nM Cu is the steady state level for copper in these waters, a
206 balance of adsorption on particles, uptake by phytoplankton and other algae, releases or uptake
207 from the sediments and possible interactions with strong complexing ligands. This steady state
208 level (19 - 24 nM) is similar to levels of 8-21 nM found previously in the north of the lagoon
209 (Martin et al., 1994). It is likely that the proximity of an industrial zone (Porto Maghera) near
210 Tresse may have been the cause for the initially higher copper levels in the lagoon water at Tresse
211 than at Campalto. The steady state copper levels in the Venice Lagoon were greater than those (2-
212 15.5 nM) in San Diego Bay (Zirino et al., 1998), similar to those (10-25 nM), in the lagoons of
213 Jacarepaguá, Rio de Janeiro, Brazil (Fernandes et al., 1994), and less than those (31-95 nM) in
214 the Pom-Atasta Lagoon, Mexico (Vazquez et al., 1999), suggesting that, relative to other shallow
215 lagoon waters, Venice Lagoon does not suffer from major contamination with copper.

216 *Organic copper complexing ligands*

217 Curvature in the linearised form of the speciation data showed that copper complexation
218 was affected by more than one ligand (Fig. 3). A good fit of the data was obtained to a model
219 containing two ligands at both stations: L1 and L2, with complex stabilities ($\log K'_{\text{CuL1}}$ and \log
220 K'_{CuL2}) of 14.2 ± 0.5 and 12.5 ± 0.3 respectively (Table 1). The overall α -coefficient, α_{CuL} , was
221 10^6 (Table 1) predominantly as a result of complexation with L1, causing the free copper
222 concentration to be low with pCu values of 13.7 ± 0.2 ($\text{pCu} = -\log [\text{Cu}^{2+}]$). These levels are well
223 below where free copper affects marine microorganisms: for instance, cupric ion concentrations
224 >1 pM are thought to influence cyanobacteria (Brand et al., 1986).

225 The concentration of L1 was on average 17 ± 4 nM at the two stations, much less than that
226 (108 ± 51 nM) of L2 (Fig. 4). During the titrations L1 became saturated and subsequent copper
227 additions were bound to the weaker ligand (L2). The change in the slope (indicating saturation of
228 L1) occurred at pCu ~ 13.1 at both stations. Due to the higher copper concentrations at the start of
229 the Tresse experiment, L1 was largely saturated and L2 bound there more copper (up to 50 %)
230 than at Campalto. The difference between the complex stability of L1 and L2 was 1.7 log-units,
231 indicating that L1 and L2 are of a different nature or that different sites are responsible for copper
232 complexation.

233 The equilibrium concentration of copper at $\sim 19 - 22$ nM at both sites was similar to that of
234 L1, suggesting that this ligand may play an important role in keeping copper at a level
235 approximately equal to its concentration. Possibilities are that copper-complexed L1 is stabilized
236 from photochemical (Laglera and van den Berg, 2006), or bacterial breakdown, or because
237 copper-L1 species are released from porewaters, or a combination of these.

238 The concentration of L2 was much greater than that of copper. Because L2 forms weaker
239 complexes with copper than L1, L2 played a secondary role with respect to copper complexation

240 in these waters in spite of its higher concentration, whereas L1 was generally fully complexed
241 with copper. During the incubation in the chambers the concentration of L2 increased from 40 to
242 120 nM at Campalto, and from 120 to 160 nM at Tresse, as a result of diffusion from the
243 sediments as will be shown below, before dropping back to the original level near the end of the
244 experiment, probably due to photodegradation.

245 There is no previous published work on the chemical speciation of copper in this lagoon.
246 The ligand concentrations and complex stability are similar to those in estuarine waters or
247 productive shelf waters, but greater than those expected for deeper coastal waters. For instance at
248 the higher salinity end of the estuary of the river Scheldt the concentration of L1 was 12.9 nM
249 with a complex stability of $\log K'_{\text{CuL1}} = 14.8$ (Laglera and van den Berg, 2003); the concentration
250 of L2 in that water was 35 nM with a value for $\log K'_{\text{CuL2}}$ of 13.2. Guanabara Bay, a shallow
251 lagoon adjacent to Rio de Janeiro, had copper complexing ligands at greater concentrations of 40
252 - 300 nM and $\log K'_{\text{CuL}}$ values of 9.6 to 12.4 (Van Den Berg and De Luca Rebello, 1986). These
253 results are directly comparable as they had been determined at a similar detection window but
254 using a different ligand (catechol) as competing ligand. The much greater ligand concentrations
255 in Guanabara Bay were likely caused by a high primary productivity. Complex stabilities of \log
256 $K'_{\text{CuL1}} > 12.5$ have been reported for Gullmar Fjord, Sweden (Croot, 2003), and of $\log K'_{\text{CuL1}} >$
257 13.5 and $\log K'_{\text{CuL2}}$ of 9.0 – 9.6 in waters of San Francisco Bay (Donat et al., 1994). The complex
258 stabilities cover therefore several log-units, and the ligand concentrations several decades, in near
259 coastal or estuarine waters. Part of the reason for this variability may be that the analytical
260 methods have difficulties in discriminating between the ligands, but at the same time it is likely
261 that several ligands are present and that competition reactions with other metals can cause the
262 complex stabilities to vary.

263 *Thiols as a candidate for the natural ligands*

264 Thiol compounds are known to form strong complexes with copper (Leal and van den Berg,
265 1998) and have been shown to exist in estuarine waters (Laglera and van den Berg, 2003; Tang et
266 al., 2000) and salt marsh sediments (Luther III et al., 1986). For this reason a method (Laglera
267 and van den Berg, 2003) was used in this work that identifies thiol-type ligands directly from
268 their specific voltammetric response. The thiol peak was apparent in the voltammetric scans of all
269 the samples of this study, in the potential range -0.55 and -0.65V, typical for thiols (Al-Farawati
270 and Van Den Berg, 2001; Leal and Van den Berg, 1998), and sulphide species (Rozaan et al.,
271 2000). Stability of the peak indicated that the response was due to thiols, as the sulphide peak is
272 known to be unstable, except at very high concentration, decreasing rapidly in height due to
273 reaction with mercury traces in the voltammetric cell (Al-Farawati and van den Berg, 1997). The
274 sensitivity was calibrated with glutathione which matched the electrochemical characteristics of
275 the peak with respect to peak potential and shape and its response at different deposition
276 potentials. Copper additions caused the thiol peak to decrease and shift to a more negative
277 potential (Fig. 5 A) due to a change from a mercury-thiol species to a copper-thiol species on the
278 surface of the electrode with increasing copper concentration (Le Gall and van den Berg, 1993):



280 Copper-binding thiol concentrations and values for their complex stability (K_{CuThiol}) were
281 determined by fitting the voltammetric thiol response to the free, Cu^{2+} , concentration obtained
282 during the copper complexing capacity titrations (Laglera and van den Berg, 2003) and are shown
283 in Table 1.

284 The total thiol concentrations were determined by adding EDTA (100 μM , $\log \alpha_{\text{CuEDTA}} = 6$)
285 to each sample to mask ambient copper from the thiol to obtain the overall thiol peak current
286 (I_{max}), where the α -coefficient indicates the ratio of complexed over free copper
287 ($[\text{CuEDTA}]/[\text{Cu}^{2+}]$). The EDTA addition was found to increase the reactive thiol concentration

288 by 20-25% compared to that in the presence of 10 μM SA ($\log \alpha_{\text{CuSA}} \sim 5$) in line with expectation
289 for a high complex stability of the thiol species. The CSV scans for the total thiol determinations
290 were similar to the scan shown at the lowest copper concentration in Fig. 5A, but then with a
291 smaller peak height for copper and a greater peak height for the thiol.

292 Plots of I_p / I_{max} showed that the thiol compounds started to bind copper at 10^{-14} Cu^{2+} and
293 were saturated at 10^{-12} M Cu^{2+} consistent with a value for $\log K'_{\text{Cuthiol}}$ near 13 (Fig. 5 B). The
294 fitted $\log K'_{\text{Cuthiol}}$ values were similar at the two study sites with an average of 13.1 ± 0.2 . The
295 complex stability of the copper thiols is near that of the CuL_2 species ($\log K'_{\text{CuL}_2} = 12.5 \pm 0.3$).
296 The alpha-coefficient for copper binding by the thiols, $\alpha_{\text{Cu-thiol}} = [\text{CuThiol}] / [\text{Cu}^{2+}]$, was $\sim 10^{6.1}$
297 (for 100 nM thiols with average complex stability), compared to a value of $\alpha_{\text{CuL}_2} = 10^{5.5}$ for 100
298 nM of L2 and $\alpha_{\text{CuL}_1} = 10^{6.5}$ for 20 nM of L1. The higher value for Cu^{2+} complexation with L1 is
299 due to the high stability of the L1 species, but it does not reflect that L1 is mostly saturated with
300 copper causing any excess copper to be bound with L2.

301 *Identification and quantification of the thiol compounds*

302 The CSV response of the thiols in Venice Lagoon was compared to that of several thiols
303 (thioacetamide, thiourea, cysteine and glutathione) and glutathione was found to give a good
304 match. 100 nM additions caused a small 10 mV negative shift in peak position and a slight
305 broadening of the peak. The thiol peak height is known to be dependent on pH, deposition
306 potential as well as on concentration (Laglera and van den Berg, 2003; Le Gall and van den Berg,
307 1993) so this shift was not unusual. The CSV response for the thiols in Venice Lagoon varied
308 upon variation of the deposition potential similarly to that of glutathione, with a much smaller
309 change in response than for thiourea or thioacetamide consistent with a response due to mercury
310 species typical for glutathione (Hg(II) -glutathione) (Leal and Van den Berg, 1998). Because of its
311 similar response characteristics, glutathione was used to calibrate the sensitivity for the thiol

312 determinations.

313 *Fluxes of thiols from the benthic chamber data*

314 Thiol concentrations in the chambers at both sites were found to initially increase with time
315 and subsequently decrease after about 40 h. At Tresse the thiols increased from 60 nM to 120 nM
316 (glutathione equivalents), and at Campalto from 100 to 160, so the overall increase was the same
317 magnitude. The thiol concentration in the external water was 40 nM at Tresse and 110 nM at
318 Campalto. The average thiol fluxes were $63 \pm 1 \text{ pmol cm}^{-2} \text{ h}^{-1}$ at Campalto and $51 \pm 1 \text{ pmol cm}^{-2}$
319 h^{-1} at Tresse for the first 25 h of the experiment. The decreasing thiol concentrations at the end of
320 the experiment may be due to activities of suspended or benthic organisms, or to photochemical
321 breakdown as the chamber was exposed to sunlight in these very shallow waters and as thiols and
322 other ligands are known to be liable to photochemical breakdown (Laglera and van den Berg,
323 2006; Shank et al., 2006). Biological breakdown may also have played a role as thiols are a
324 known substrate for marine bacteria (Tripp et al., 2008; Visscher and Taylor, 1993),
325 phytoplankton (Ietswaart et al., 1994) and the microphytobenthic community generally (Linares
326 and Sundback, 2006).

327 *Thiols in the sedimentary porewaters*

328 The thiol concentrations (60 - 170 nM) during these benthic chamber experiments were
329 much greater than those (0.7 - 3.6 nM) typical for the deeper coastal waters of the North Sea (Al-
330 Farawati and Van Den Berg, 2001). In view of the similar increase in both chambers it is likely
331 that benthic processes are the source of the much greater thiol concentrations in these marsh
332 waters. For this reason porewaters were subsampled from sediment box-cores at the experimental
333 sites. The cores were collected about 2 weeks after the benthic chamber experiment, but this
334 should not affect the basic findings as the benthic processes are established over long time scales.

335 The porewaters were found to contain extremely high (micromolar) thiol concentrations

336 (1.2 μM at a depth of 2 cm, 6 μM at 8 cm) much greater than in the overlying waters (~ 100 nM)
337 (Fig. 4). The large difference explains that the high apparent thiol flux in the benthic chamber
338 could easily have originated from the sediments, not from in-situ production in the chamber. The
339 thiol identity (similar to glutathione as based on the electrochemical characteristics) was the same
340 in the porewaters as in the overlying water, confirming that the porewater thiols are the source of
341 the thiols in the overlying water.

342 The porewater concentrations of the thiols increase with depth, which is consistent with an
343 in-situ production by reaction of sulfide with sedimentary organic matter (Vairavamurthy and
344 Mopper, 1987). Similar trends but at higher levels (0.1-2.5 mM organic thiol) have been observed
345 previously in salt-marsh porewaters (Luther III et al., 1986). Our benthic chamber experiments
346 demonstrate that the porewaters are the main source of thiols in the overlying waters, and that the
347 steady state level of thiols is a balance of porewater releases and biological uptake and
348 photochemical reactions in the surface waters.

349 Porewater fluxes were estimated from the concentration difference between the top of the
350 porewaters and the overlying waters (Table 2). These thiol fluxes are much greater than those
351 from the benthic chamber data (7x at Tresse and 1.3x at Campalto), suggesting that there is a
352 rapid loss of the thiols in the overlying water, which may be due to rapid uptake by
353 microorganisms, or photolysis, of the thiols. It is also possible that a benthic boundary diffusive
354 sublayer (Morse, 1974; Sundby et al., 1986) inhibited transport from the benthic interface to the
355 overlying water.

356 Although the gradient in the sediment core itself is evidence for an outward flux, the
357 calculation of the magnitude of the flux is subject to large uncertainty due to the difficulty of
358 finding the thiol concentration at the surface of the sediments which is responsible for the flux
359 into the overlying water in case of a steep gradient inside the sediment. The difference in the thiol

360 fluxes at Tresse and Campalto is probably due to this uncertainty, so the best we can establish
361 from the porewater data is a) that the porewaters are a source of thiols to the overlying waters
362 because the porewater thiol concentration is much greater than in the overlying water, and b) that
363 the flux must be large in view of the large gradient in the sediments and because of the
364 concentration difference with the overlying water.

365 The porewater concentration of copper of 16-20 nM (Fig. 4B) was similar to or slightly less
366 than in the overlying water in the benthic chambers, except for high values (83 nM at Tresse and
367 47 nM at Campalto) at the sediment surface (this had been sampled separately using an in-situ
368 pump, close to the sediment surface). The porewater concentrations are similar to those found in
369 other coastal sediments (Ciceri et al., 1992; Westerlund et al., 1986). The much greater levels at
370 the sediment surface are possibly associated with a fluffy layer of organic-rich material
371 (Hlawatsch et al., 2002) enriched in copper, which could have been sucked up into the sampling
372 tube. These high values were not used for the porewater flux calculations as it is likely that this
373 copper was not free but associated with flocs of organic matter, but they are of interest as it
374 illustrates the variability at the sediment surface. The lack of a clear gradient in the sediments
375 suggests that any copper flux into, or out of the overlying waters is small. Any copper diffusing
376 out of, or into these sediments would have to pass through, and could be trapped by, this fluffy
377 layer. It will be interesting to investigate the copper speciation exactly at the interface in further
378 work, as stabilization of copper at this location, rather than the deeper porewater concentration,
379 could control the copper concentration in the overlying waters.

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507 measurement of Cu(II)(aq) in seawater at pH 8 with the jalpaite ion-selective
508 electrode. *Marine Chemistry* 61, 173-184.
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514 **Table 1** Copper speciation and ligand concentrations in Venice Lagoon. C_{L1} and C_{L2} (nM), K'_{CuL1}
 515 and K'_{CuL2} were determined by copper complexing capacity titrations, while $K'_{CuThiol}$ was
 516 determined from the cathodic stripping voltammetry (CSV) response for thiols during the copper
 517 complexing capacity titrations; thiol concentrations were separately measured and are in
 518 glutathione equivalents. The standard deviation of the log $K'_{CuThiol}$ values is ± 0.1 ($n = 12$).

Site / time from start (h)	C_{Cu} (nM)	C_{L1} (nM)	Log K'_{CuL1}	C_{L2} (nM)	Log K'_{CuL2}	pCu	Log α_{CuL}	Thiols (nM)	Log $K'_{CuThiol}$
Tresse / 6	24.4	22.9 \pm 1.9	14.8 \pm 0.6	41 \pm 1	12.8 \pm 0.1	13.8	6.2	62	13.1
Tresse / 17	23.0	17.3 \pm 2.2	13.9 \pm 0.3	41 \pm 4	12.8 \pm 0.2	13.3	5.6	77	13.0
Tresse / 29	21.7	20.2 \pm 0.4	14.7 \pm 0.1	97 \pm 10	12.4 \pm 0.1	13.8	6.1	113	13.2
Tresse / 41	19.1	13.7 \pm 0.5	15.0 \pm 0.3	116 \pm 1	12.4 \pm 0.1	13.7	6.0	120	13.3
Tresse / 53	18.8	12.4 \pm 1.1	14.3 \pm 0.4	33 \pm 2	13.0 \pm 0.2	13.5	5.7	57	13.1
Campalto /2	16.5	15.1 \pm 4.0	13.8 \pm 0.2	122 \pm 2	12.7 \pm 0.1	13.9	5.9	97	13.3
Campalto /12	10.3	14.3 \pm 1.8	13.7 \pm 0.1	127 \pm 6	12.6 \pm 0.1	14.0	6.0	125	13.2
Campalto /23	13.9	17.7 \pm 2.1	13.5 \pm 0.1	168 \pm 14	12.5 \pm 0.1	13.8	5.9	152	13.4
Campalto /36	18.0	25.2 \pm 0.4	13.7 \pm 0.2	169 \pm 4	12.1 \pm 0.1	13.7	5.9	154	13.5
Campalto /48	20.7	12.7 \pm 0.3	14.2 \pm 0.1	167 \pm 1	12.2 \pm 0.1	13.4	5.8	139	13.5
Campalto/60	24.2	19.7 \pm 0.2	14.2 \pm 0.1	106 \pm 2	12.4 \pm 0.1	13.5	5.9	94	13.2

519 **Table 2.** The measured flux (F_m in $\text{pmol cm}^{-2}\text{h}^{-1}$) from the benthic chamber data and the modelled
 520 diffusion flux (F_d in $\text{pmol cm}^{-2}\text{h}^{-1}$) from porewater data. Diffusion coefficients used for the
 521 calculation: thiol: $14.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ and copper: $7.3 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. The values shown are the mean \pm
 522 SD for 3 repeated analyses.

Site name	Thiol		Copper	
	Tresse	Campalto	Tresse	Campalto
F_m	51 ± 1	63 ± 1	-4.4 ± 0.2	3.8 ± 0.1
F_d	344 ± 2	84 ± 8	-2.1 ± 0.1	-3.8 ± 0.2

523

524

525 **Figure Captions**

526 Figure 1. Map showing the locations of the sample sites in Venice Lagoon, Italy.

527

528 Figure 2. Temperature, pH and dissolved oxygen (μM), inside the benthic chambers during the
529 experiments. A) Tresse: 28 – 31 October 2002 (salinity 29.4); B Campalto: 25 – 27 May 2003
530 (salinity 28.6). Solid black bars show night time hours.

531

532 Figure 3. Complexing capacity titration of copper in the presence of $2 \mu\text{M}$ SA. A) Curvature in
533 the plot of reactive copper as a function of total copper showed the presence of complexing
534 ligands; B) linearization of the data: curvature shows the presence of at least two ligands. A good
535 data fit was obtained to a two-ligand model.

536

537 Figure 4. Changes in the concentrations of copper, ligands and thiols (glutathione equivalents) as
538 a function of time in the benthic chambers at Tresse (A) and Campalto (B). Mean and standard
539 deviation values are shown for 3 repeated analyses are shown.

540

541 Figure 5. Effect of varying the copper concentration on the response for the thiol species. A) CSV
542 scans for copper and thiols in Lagoon water showing the decrease in the thiol peak (which is an
543 Hg-thiol species) as the copper-SA peak increases; the copper-SA peak is off scale at -0.35 V ; B)
544 change in the thiol peak height ($R = \text{relative peak height} = i_p / i_{p_{\text{max}}}$) as a function of $p\text{Cu}$ showing
545 that the thiols started to bind copper from $p\text{Cu} \sim 14$, becoming saturated at $p\text{Cu} \sim 12.5$. Individual
546 $p\text{Cu}$ values were calculated from $[\text{Cu}_{\text{reactive}}] / \alpha_{\text{CuSA}}$.

547

548 Figure 6. Concentrations of dissolved thiols (glutathione equivalents) (A) and copper (B) in
549 porewaters from sediment cores taken at the study sites. Mean values and standard deviations for
550 3 repeated analyses are shown ($n = 3$).

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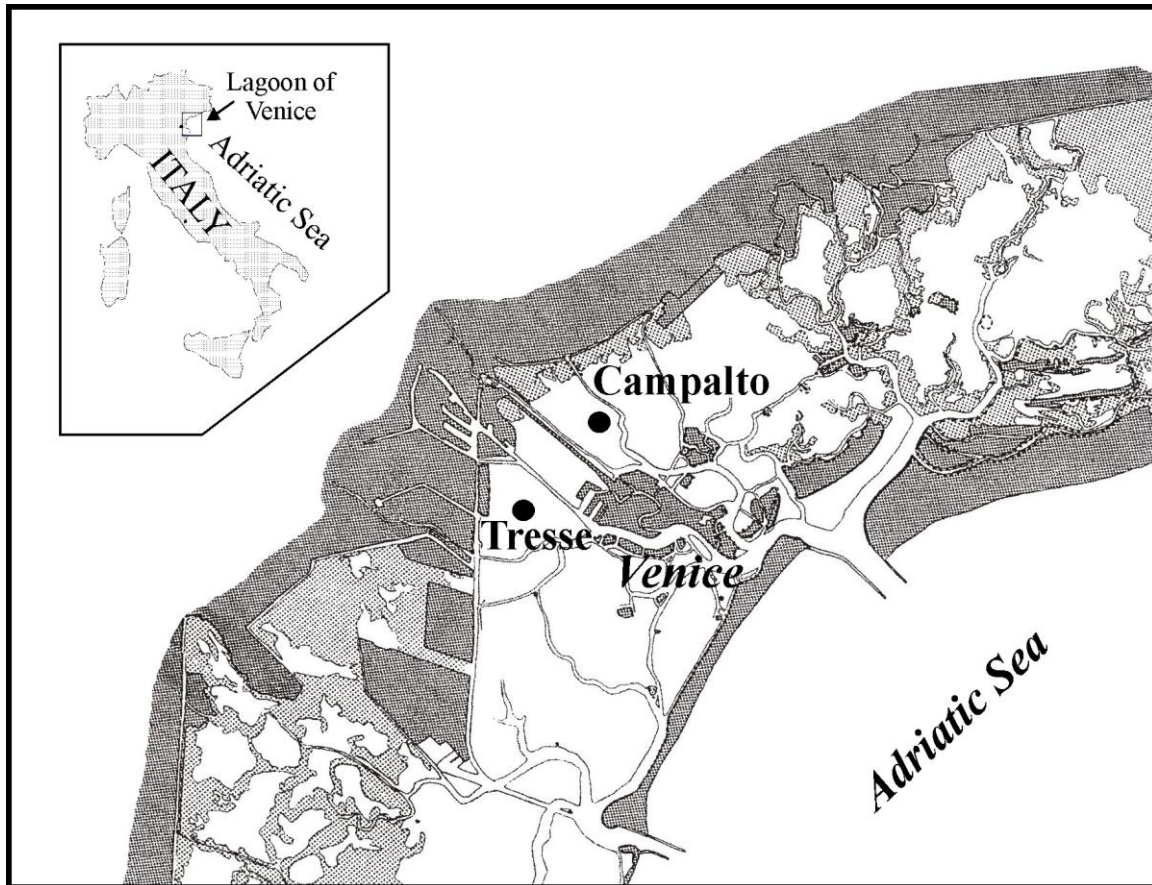


Figure 1.

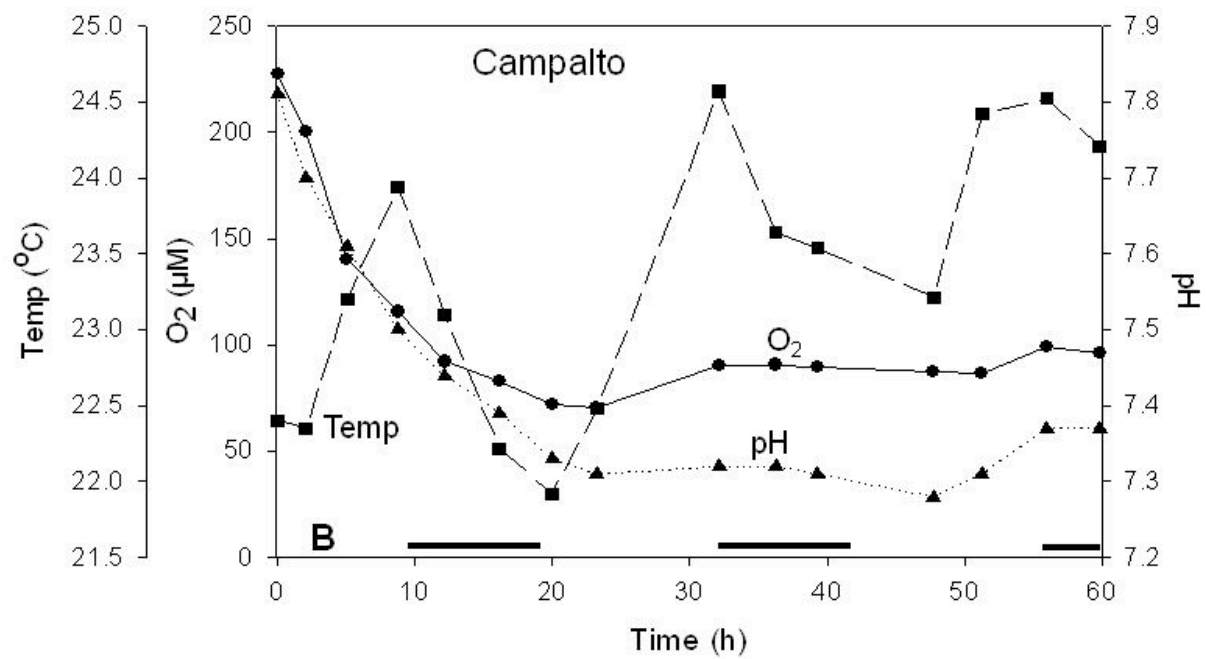
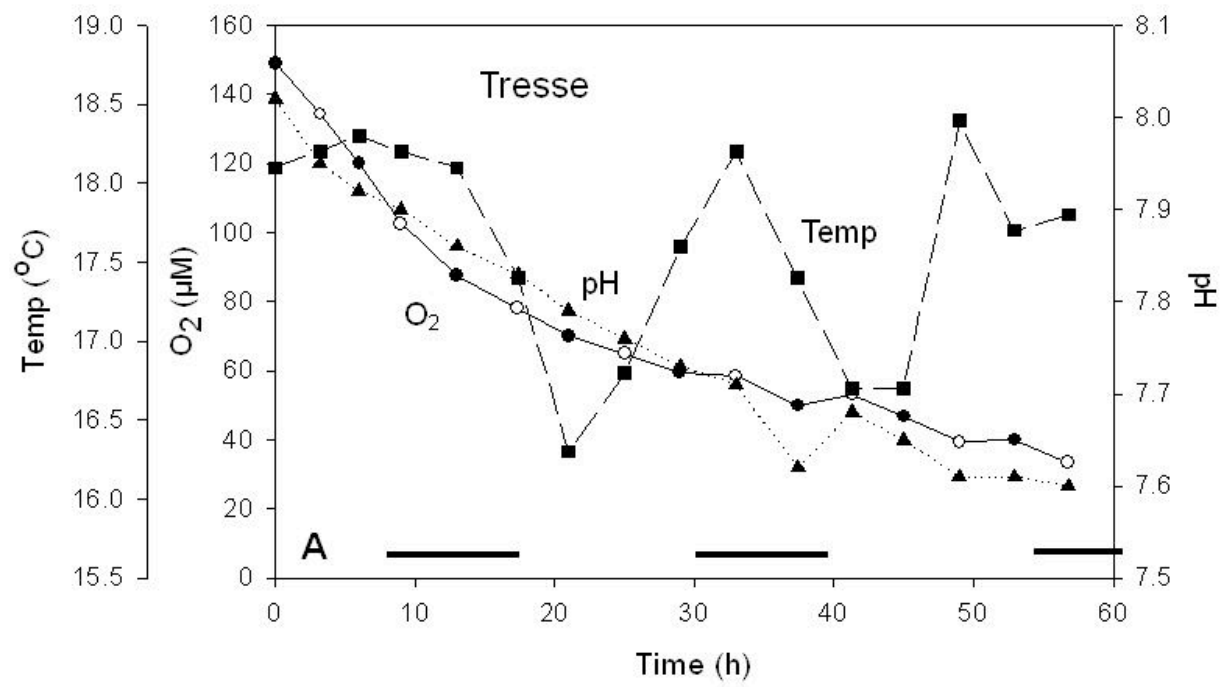


Figure 3

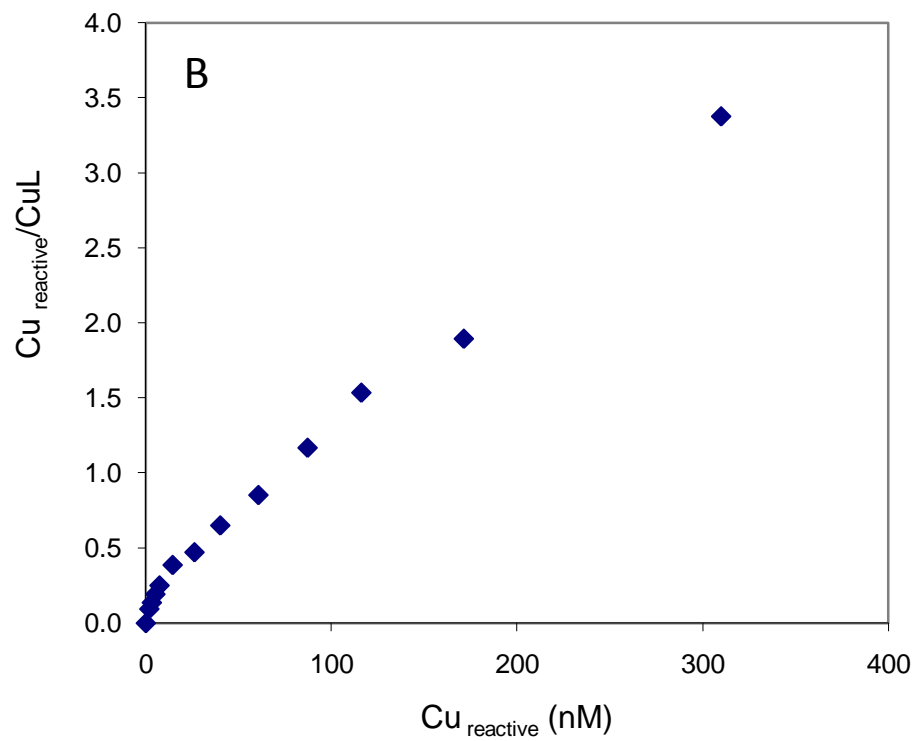
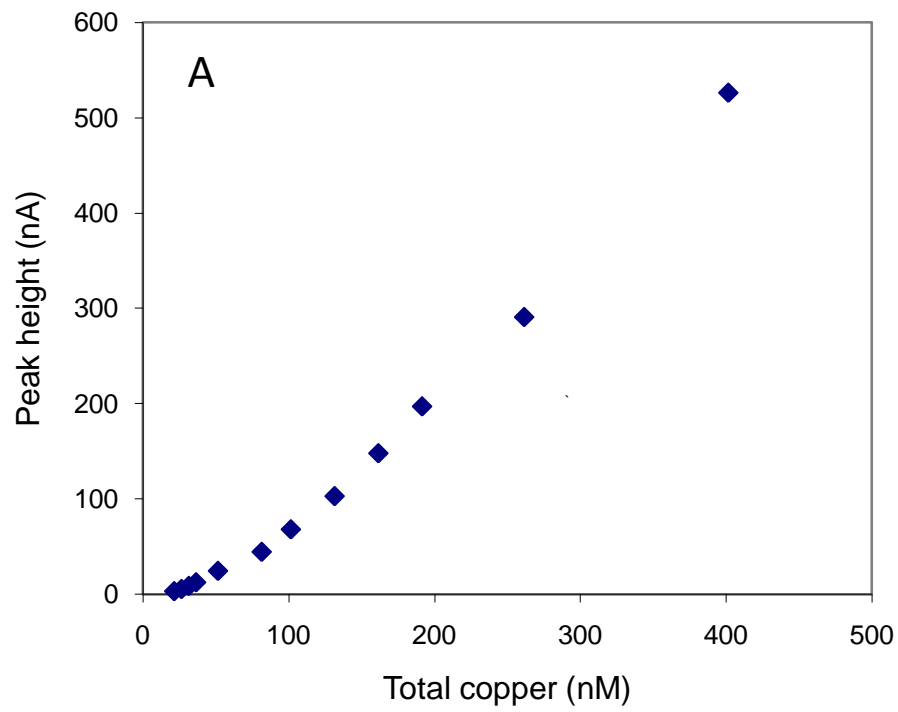


Figure 4

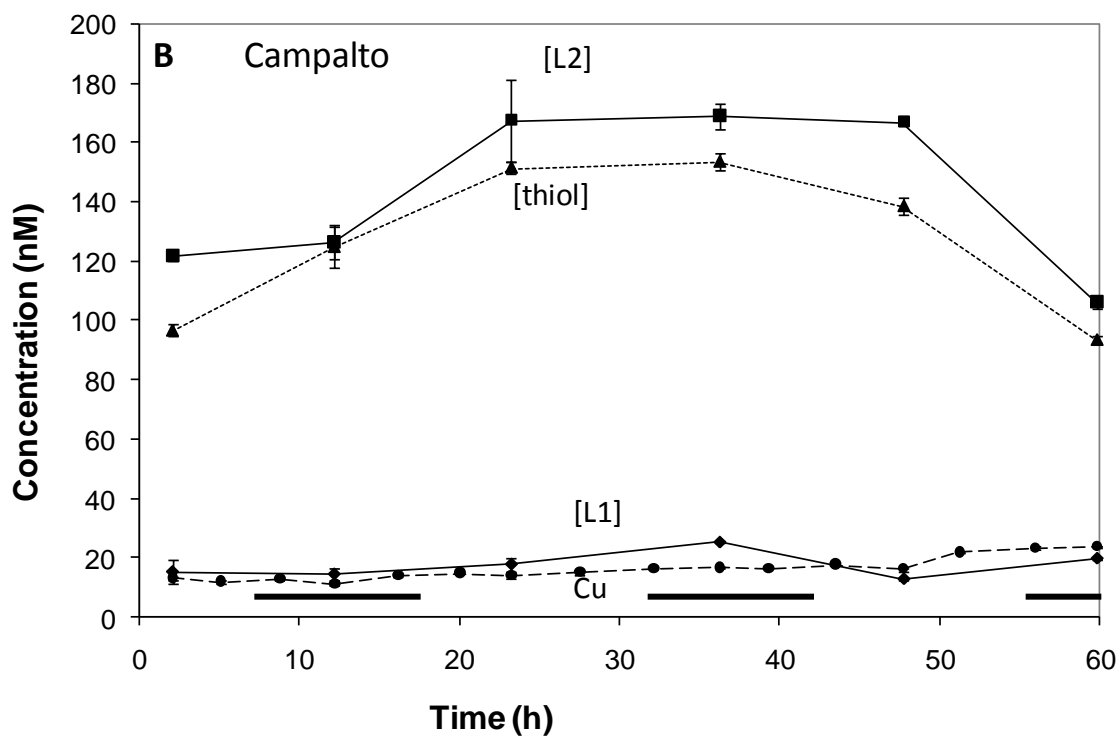
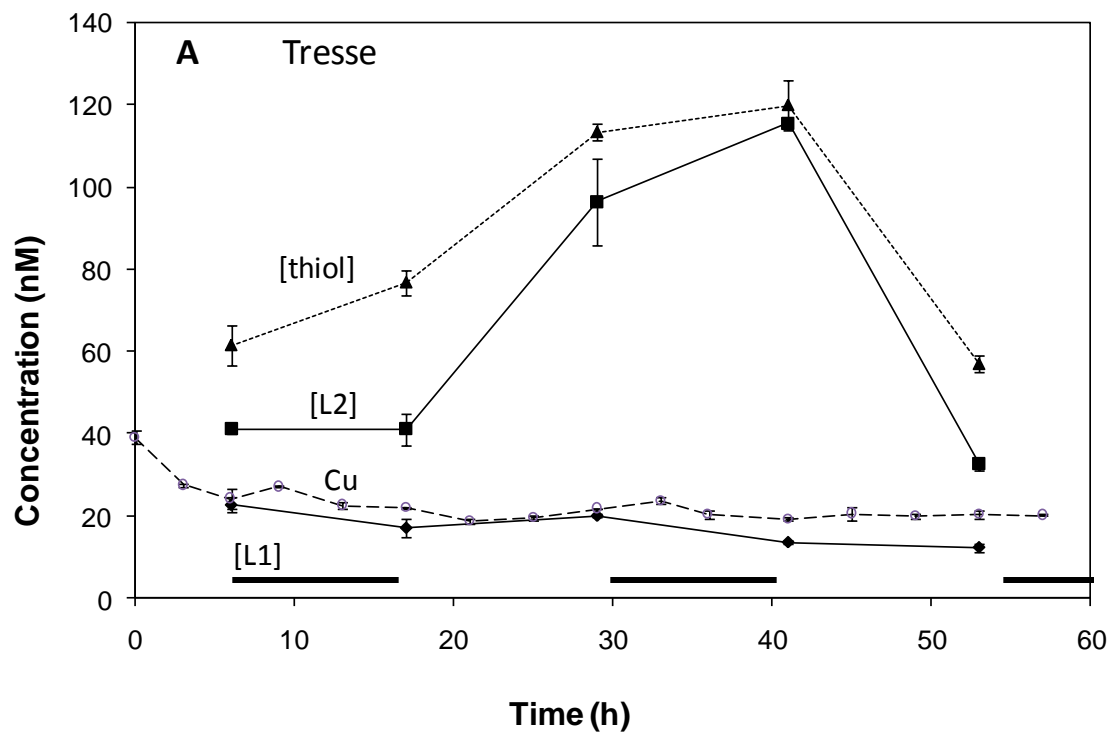


Figure 5

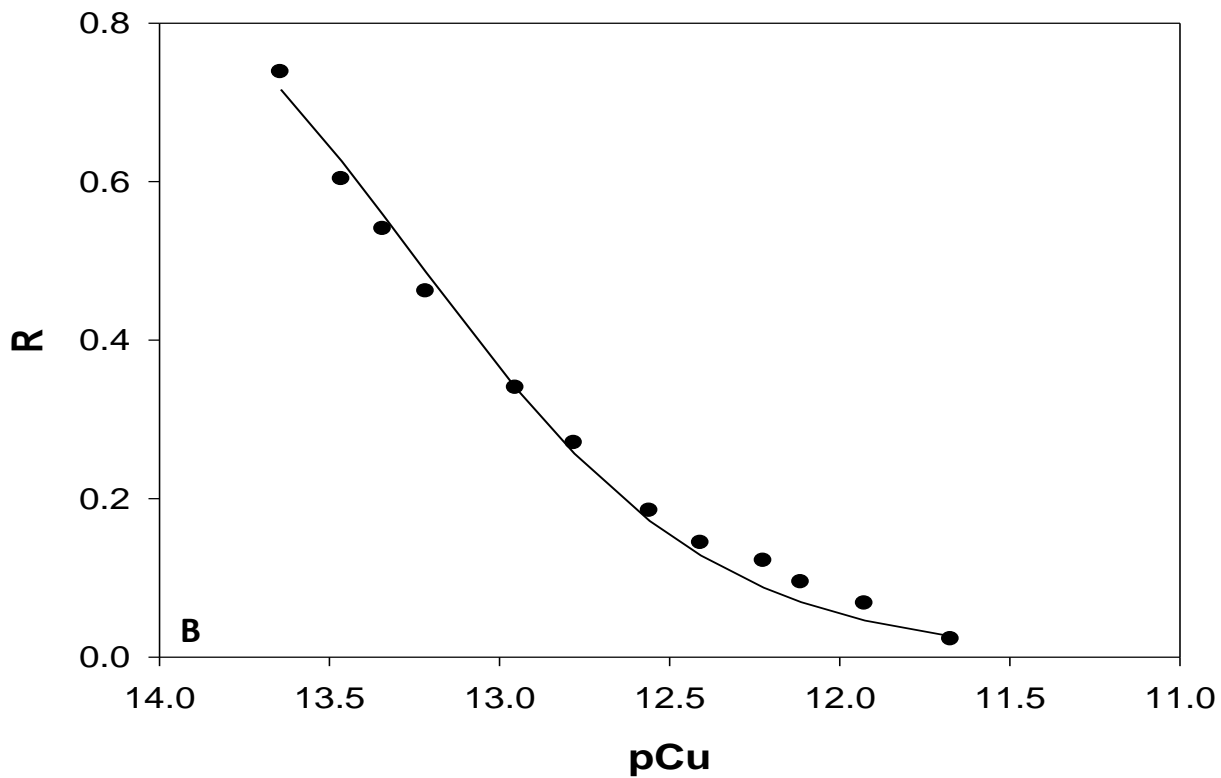
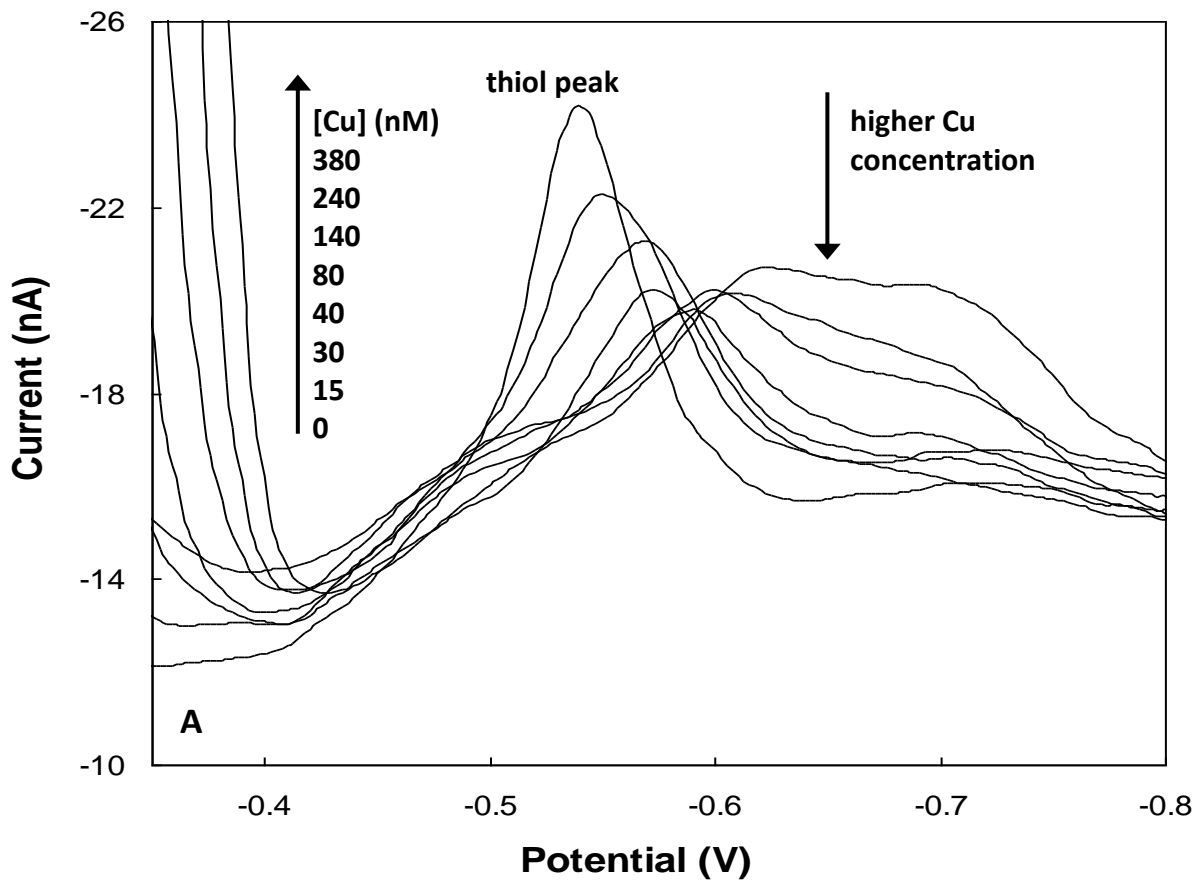


Figure 6

