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1 **Measurement of Acid Volatile Sulphide and Simultaneously Extracted**
2 **Metals in Sediment from Lake Albufera (Valencia, Spain)**

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5

6 **Abstract**

7 Lake Albufera (Valencia, Spain) is part of a legally protected wetland of international
8 importance. However, it has deteriorated as a result of urban, industrial and farming pollution.
9 It is highly eutrophic, and its sediment contains persistent pollutants, such as heavy metals. In
10 anoxic sediments, sulphides represent an important binding phase for heavy metals. In this
11 study, acid volatile sulphide (AVS) and simultaneously extracted metals (SEM) were
12 analysed in surface sediment extracted from Lake Albufera; organic matter and total metals
13 were also analysed. Twelve sites were sampled in each of three sampling campaigns
14 conducted in March and September 2007 and September 2008. The results revealed elevated
15 organic matter contents varying between 6.9 and 16.7 %. The concentrations of AVS in the
16 lake were high, ranging from 8.5 to 48.5 $\mu\text{mol/g}$; the lowest concentrations were found in the
17 central sites. The AVS results displayed significant differences between the samples from the
18 winter and summer of 2007 ($p < 0.05$) but not between the two summer samples. The results
19 obtained for SEM varied from 1.4 to 4.8 $\mu\text{mol/g}$. The difference SEM-AVS was less than zero
20 for all sampling locations and campaigns, indicating the existence of a sulphide pool able to
21 bind metals.

22

23 *Keywords:* heavy metals; AVS; SEM; Albufera; sediment.

24

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25 **1. Introduction**

26 Heavy metals are introduced into bodies of water via natural geochemical processes and
27 human activities. Once in the aquatic system, the heavy metals can be adsorbed by suspended
28 particles, settle and accumulate in the sediment. Accordingly, sediment plays a key role as a
29 sink for heavy metals (and other substances such as organic matter, nutrients and pesticides),
30 but, depending on environmental conditions, it may also act as source of the accumulated
31 substances. Among other factors, the bioavailability of heavy metals depends on chemical
32 speciation (Stumm and Morgan, 1996); therefore, the total metal concentration is not always
33 correlated with its toxic effects and its bioavailability (Di Toro et al., 1992). The level of
34 sediment contamination can be assessed via physico-chemical characterisation and the results
35 compared with values presented in numerical sediment quality guidelines (SQGs) that have
36 been empirically established based on biological exposure tests, field observations and other
37 studies. These guidelines are useful as a screening tool and help to prioritise particular
38 contaminants or areas of concern; however, it also seems advisable to include toxicity
39 bioassays when managing dredged sediment because, among other reasons, these guidelines
40 do not take into account the presence of ammonia, hydrogen sulphide or a lack of dissolved
41 oxygen (Casado-Martínez et al., 2006).

42 Another of the available methodologies is based on the theoretical equilibrium partitioning
43 model (EqP), which takes into account the factors influencing metal bioavailability in
44 anaerobic sediments. The EqP approach has been used to derive equilibrium partitioning
45 sediment benchmarks (ESBs) intended to protect benthic organisms (USEPA, 2005). This
46 model is based on the results of a water and sediment bioassay panel: water exposure
47 bioassays with different heavy metal concentrations and forms indicated that toxic effects are
48 correlated with divalent metal activity $\{Me^{2+}\}$. In addition, several sediment exposure
49 experiments demonstrated the equivalence of the response of aquatic organisms to metal

50 concentrations in interstitial water and in water-only exposure (USEPA, 2005). In anoxic
51 sediments, sulphide formation is considered the dominant mechanism limiting the solubility
52 of metals, although for copper the presence of organic ligands is also relevant (Förstner et al.,
53 1990). Finally, Di Toro et al. (1992) found no significant mortality for benthic organisms
54 when the molar concentration of sulphide present in the sediment, measured as acid volatile
55 sulphide (AVS), exceeded the molar concentration of the heavy metals simultaneously
56 extracted during the test (Σ SEM). The chemical basis is the rapid displacement of iron in iron
57 monosulphides ($\text{FeS}_{(s)}$) by divalent metals (Me^{2+}) to form more insoluble metal sulphides
58 ($\text{Me}^{2+} + \text{FeS}_{(s)} \rightarrow \text{MeS}_{(s)} + \text{Fe}^{2+}$). The Σ SEM value is the sum of metals in sulphides that are
59 less soluble than iron and manganese sulphides (commonly including NiS, ZnS, CdS, PbS and
60 CuS), which are at least partially soluble under the test conditions, and metals solubilised
61 from other phases. The AVS is an operationally defined term that includes the most easily
62 liberated sulphide fraction (Allen et al., 1993).

63 Since the development of this model, many studies have analysed AVS and SEM in
64 conjunction with biological assays. Lee et al. (2001, 2004) examined the toxicity of trace
65 metals to benthic organisms under more natural conditions than used in earlier bioassays by
66 employing more moderate metal concentrations and longer exposure times. They reported that
67 marine polychaetes accumulated metals predominately via the ingestion of contaminated
68 sediments and that dissolved metals in pore water had a minor influence on metal
69 bioaccumulation. Their results also supported the utility of the AVS criterion for predicting
70 non-acute toxicity when the difference between the values of SEM and AVS (SEM-AVS) is
71 less than zero but not for predicting the absence of metal bioaccumulation. De Jonge et al.
72 (2010) concluded that the relation between AVS and metal accumulation in aquatic
73 invertebrates is highly dependent on feeding behaviour and ecology. They found that AVS
74 appeared to influence the accumulation of Cr, Cu, Cd, Zn and Pb in the epibenthic taxa but

75 not in the benthic taxa; they also found no correlation between AVS and pore-water metal
76 concentrations. However, Speelmans et al. (2010) examined the influence of redox potential,
77 which is related to the hydrological regime, on Cu and Zn accumulation in *Tubifex tubifex* and
78 determined that flooded (and thus more reduced conditions) minimised the availability of
79 metals. Another consideration is that the variability of AVS due to several factors (e.g.,
80 changes in environmental conditions, biological activity or sediment resuspension) makes it
81 less appropriate the use of limited measurements of AVS-SEM to estimate the risk of metal
82 toxicity (Van Griethuysen et al., 2006). Of course, although sulphide has a positive effect in
83 that it limits the mobility of metals, it can itself be a direct cause of toxicity (De Lange et al.,
84 2008).

85 Despite these limitations on the use of EqP models to predict biological risk, measurements
86 of AVS and SEM are used to characterise sediments and AVS-SEM is also employed as
87 indicators of potential toxicity (Burton et al., 2007; Jingchun et al., 2010). In the present
88 study, we analysed the AVS and SEM contents at different sites in Lake Albufera (Valencia,
89 Spain) and the surrounding area. Lake Albufera is a hypertrophic, shallow body of water in
90 which the current conditions are very different from the lake's natural ecological status.
91 Therefore, it has been identified as a "heavily modified water body", and a programme of
92 measures has been enacted to achieve the objectives of the WFD there (Water Framework
93 Directive, 2000), i.e., "*protection and enhancement of all artificial and heavily modified*
94 *bodies of water, with the aim of achieving good ecological potential and good surface water*
95 *chemical status at the latest 15 years from the date of entry into force of Directive*". A
96 previous study (Peris, 1999) analysed the concentrations of total metals and persistent organic
97 pollutants in the lake and ran toxicity tests (using Microtox[®]) to analyse the sediment from
98 this lake. The test results, in which high concentrations of heavy metals do not always
99 coincide with toxicity, indicate that other factors can influence toxicity results.

100 The next step required is to extend our knowledge of the heavy metals in the sediment of
101 Lake Albufera. Thus, this study aimed to measure AVS and SEM and thereby provide
102 information regarding the current status of these metals. These parameters are also indicators
103 of the risk that metals will be released in more labile binding phases or into pore water as a
104 result of changes in environmental conditions; for example, due to improvements in water
105 quality or events that lead to sediment resuspension (storms, wind, dredging or biological
106 activity).

107

108 **2. Materials and methods**

109 2.1. Study area

110 Lake Albufera (Valencia, Spain) is a shallow coastal lake (with a mean water column
111 depth of one meter) that includes several reed islands and has a surface area of ~2,400
112 hectares. It is located approximately 10 km southeast of Valencia and is surrounded by a
113 metropolitan area of nearly 1.2 million people. This lake is part of the Natural Park of the
114 Albufera, established in 1986, and was added to the Ramsar List of Wetlands of International
115 Importance in 1990. It is also part of the Special Protection Area according to European
116 Directive 94/24/CE.

117 The ecological status of the lake has seriously deteriorated since the 1970s as a result of
118 diffuse and point pollution. The lake remains at a permanent hypertrophic level, with high
119 phytoplankton populations (at an annual mean of 100 $\mu\text{g Chl-}a/\text{L}$, with peaks at
120 approximately 300 $\mu\text{g Chl-}a/\text{L}$); cyanophytes predominate. As a result, dramatic daily
121 oscillations in pH and Dissolved Oxygen (DO) concentrations frequently occur. The mean
122 flow into the lake is approximately 5 m^3/s , and the mean hydraulic residence time is 0.15
123 year. The tributary and lake waters are rich in sulphates, with values of approximately 4,000
124 $\mu\text{mol/L}$ (Soria et al. 1987, Public Database of Confederación Hidrográfica del Júcar). The

125 lake is also vulnerable to silting; accumulations of sediment approximately one meter deep
126 have already been found, and sediment dredging has occasionally been proposed. However,
127 dredging has never been undertaken because it is uncertain what the collateral effects of such
128 a step would be.

129

130 2.2. Sediment sampling

131 Twelve samples of surface sediment (from a depth of 10 cm) were collected from the lake
132 area; there were nine sampling sites in the lake itself and three outside the lake (see Fig. 1).
133 Sites 1, 2, 3 and 4 are located at the north end of the lake, where the highest urban and
134 industrial pollution levels were detected. Sites 5 and 6 are reference sites located in the middle
135 of the lake, near the reed islands and far from areas affected by pollutant discharge, where a
136 lower concentration of heavy metals was previously reported (Peris, 1999). Sites 7, 10 and 11
137 are in the south, where agricultural pollution levels are high. The organic matter content in the
138 sediment in the lake is mainly produced by phytoplankton decay. Two of three outside sites
139 are irrigation channels, selected because the organic matter has an allochthonous origin: first,
140 the port of Catarroja to the north (PC), an area seriously polluted by urban and industrial
141 sewage; and second, the Overa channel (AO) to the south, which has been affected by
142 agricultural and urban pollution. The third outside location is an outflow channel (Gola de
143 Puchol, GP) which experiences changes in water quality due to its connection to the
144 Mediterranean Sea.

145 Samples from all locations were taken in late winter (22 February and 5 March 2007) and
146 in late summer (6 September 2007 and 4 September 2008) to study the effect of temperature.
147 The sampling took place between 9:00 am and 1:30 pm.

148 At each sampling site, three samples (0.5 L) were collected using a Van Veen grab
149 sampler. To avoid contact between the sediment and the air, the sediment was immediately

150 transferred to 100-mL polyethylene containers without headspace that were stored in an
151 icebox until their arrival at the laboratory, where they were frozen (-18°C) until analysis. The
152 physicochemical parameters of the water (temperature, pH, dissolved oxygen and
153 conductivity) were also measured from the surface down to the water-sediment interface at
154 20-cm intervals. Chlorophyll *a* data were obtained from the Conselleria de Medi Ambient,
155 Aigua, Urbanisme i Habitatge (CMAAUH) public database. The sulphate concentration was
156 measured at three sites (Sites 1, 6 and 11) to confirm that the water in the lake is rich in
157 sulphates.

158

159 2.3. Sediment analysis

160 AVS and SEM were analysed using the cold-acid purge-and-trap methodology described
161 in Allen et al. (1993), with slight modifications made to adapt it to this system. The procedure
162 involves reacting a sediment sample (with a dry weight of approximately 1 g, handled and
163 weighed in a nitrogen chamber) with 1 M HCl at room temperature for 40 minutes. The AVS,
164 released during reaction, is sparged from the acid solution with nitrogen gas at a flow rate of
165 250 mL/min and trapped in a 0.5 M NaOH solution. The amount of sulphide trapped in the
166 NaOH solution is measured spectrophotometrically (using the methylene blue method) at a
167 wavelength of 670 nm. The SEM remaining in the reaction mixture is filtered through a
168 prerinsed 0.45- μ m filter and measured using a flame atomic absorption spectrometer (Philips
169 PU9100X). The recovery of sulphide was tested using a standardised sodium sulphide
170 solution; the averaged percentage recovery for this system was 92 ± 2 ($n = 5$). The degree of
171 sediment moisture was determined by drying the sediment at 105°C for 24 hours, and the
172 results were expressed in dry weight. The organic matter content was measured according to
173 the degree of loss on ignition (LOI) for 1 h at 600°C. For the samples taken in September
174 2008, Total Organic Carbon (TOC) and the total concentration of heavy metals were also

175 determined. TOC was determined using the Walkley-Black procedure (as described in
176 Schumacher, 2002). Total metal content was evaluated based on the digestion of the sediment
177 (fraction < 150 µm) in aqua regia (according to UNE 77322); one replicate was analysed. The
178 metal analysis procedure was checked using Certified Reference Material CRM 320 from the
179 Community Bureau of Reference (BCR), and the data were all within 10% of the certified
180 values: Cd (98%), Cu (100%), Ni (92%), Pb (95%) and Zn (109%). All reagents were of
181 analytical or Suprapur quality and all materials were acid-cleaned prior to use.

182

183 2.4. Statistical analysis

184 The average values and standard deviations were assessed for each location based on the
185 three replicate analyses. These average values were analysed using SPSS 15.0 software, with
186 a nonparametric test conducted for several related samples (Friedman's test) and pairwise
187 comparisons (Wilcoxon's test) performed to examine the variations in AVS, SEM and
188 organic matter among the samplings; statistical significance was indicated by a probability of
189 type I error of 5% or less ($p \leq 0.05$). The averages and relative standard deviations (RSD) of
190 the nine in-lake sample values were also calculated to evaluate the degree of spatial
191 heterogeneity. The Pearson correlation coefficient was computed to study the relationship
192 between the sulphide data and the organic matter data.

193

194 3. Results and discussion

195 3.1. Physico-chemical parameters of the water column

196 The main results indicating the physicochemical parameters of the samples are summarised
197 below (see Table 1). The level of dissolved oxygen measured in March indicates that there
198 was a high level of photosynthetic activity at the surface at that time (with a mean Chl-*a* value
199 of approximately 161.7 µg Chl-*a*/L and a %DO saturation > 100). However, in September

200 (when the mean Chl-*a* value was approximately 60.1 µg Chl-*a*/L), no evidence of oxygen
201 oversaturation was found; most samples were under 96 %. A dissolved oxygen value of 34 %
202 was found in the Catarroja Port (PC) as a result of high pollution. At each sampling point, a
203 dissolved oxygen profile was created for each 20 cm of depth, and anoxic conditions were
204 always observed at the sediment-water interface. As a result of the level of phytoplankton
205 productivity, pH values up to 9.2 were observed. Conductivity in the lake was high (2,070 to
206 3,960 µS/cm) but was lower at the irrigation channels (1,414 to 1,804 µS/cm). These higher
207 conductivity levels are the result of elevated evaporation and high residence time due to low
208 inflows. The average concentration of sulphate was 3,750 µmol/L, consistent with previously
209 reported values (Soria et al., 1987).

210

211 3.2. Organic matter in sediment

212 The organic matter content (as shown in Fig. 2) was high in the entire sediment of the lake
213 (at approximately 9 %) and was quite homogeneous in all but the central locations (Sites 5
214 and 6). The high level of organic content seems to be a function of the high concentration of
215 phytoplankton and of the environmental conditions that normally promote net sedimentation.
216 The maximum values in the lake were obtained at Site 5 (11.65 to 16.68 %) due to the
217 abundant vegetal content in the samples. Outside the lake, the maximum was found at Site PC
218 (16.54 and 16.46 %); this is attributed to the historically high wastewater loads at the site. The
219 variations in the organic matter content of the sampling collections were on average not
220 significant ($p > 0.05$). To gather new information regarding the characteristics of the
221 sediment, the TOC was analysed based on the 2008 samples (see Table 2). The TOC of the
222 sediment samples varied between 0.88 and 4.34 %. A dimensionless factor of 1.33 is
223 commonly applied to TOC results to correct for incomplete oxidation (Schumacher, 2002).
224 The conversion factor for TOC and LOI ranged from 2.1 to 3 g OM/g C for most samples

225 (excluding Sites 2, AO and GP, which had much higher rates), with an average of 2.61 g
226 OM/g C. This is slightly higher than the values cited in the literature, which range from 1.724
227 to 2.5 (Schumacher, 2002) or 2.13 (Besser et al., 2008), probably due to more complete
228 ignition.

229

230 3.3. Acid Volatile Sulphide (AVS)

231 The AVS concentrations at different locations in the lake were heterogeneous (Fig. 3), with
232 a relative standard deviation (RSD) of approximately 50 % in 2007 and 35% in 2008. The
233 minimum values were found at the central sites, Sites 5 and 6 (8.45 to 14.19 $\mu\text{mol AVS/g}$),
234 whereas the maximum AVS was found at Site 2 (32.66 to 45.50 $\mu\text{mol/g}$). The AVS values
235 decreased significantly ($p < 0.05$) from March 2007 to September 2007 at the locations in the
236 lake, and no significant differences were observed between September 2007 and September
237 2008. This decrease was unexpected because AVS concentrations tend to increase with
238 temperature (Leonard et al., 1993; Grabowski et al., 2001; Van Griethuysen et al., 2006).
239 However, Zheng et al. (2004) also found lower summer concentrations.

240 In the present study, the differences in AVS between March and September can be
241 explained by changes in the amount and the biodegradability of organic matter, given that the
242 other main factors are not limiting. The sulphate concentration was sufficiently high that AVS
243 production was not limited (in the review by Du Laing et al., 2009, a concentration below 30
244 $\mu\text{mol}\cdot\text{L}^{-1}$ is cited as that limiting sulphate reduction), and the concentration of dissolved
245 oxygen at the interface was so low that it did not limit sulphide production in September. In
246 the March sampling, the levels of easily biodegradable OM could be high due to its slow
247 consumption by bacteria during the winter because of the low temperature; in addition, the
248 sedimentation of phytoplankton is greater than in September because its concentration in the
249 water is higher in March. When the temperature begins to increase in February, sulphate-

250 reducing bacteria (SRB) can grow rapidly by feeding on this easily degradable OM reserve. In
251 the September sampling, the easily degradable fraction may be low due to high levels of
252 bacterial activity during the previous hot months. Therefore, the substrate may limit the
253 growth of bacteria. Combined with the oxidation of part of the AVS measured in March under
254 maximum oxygenation conditions, this may have given rise to the lower AVS concentrations
255 in September. It is also worth mentioning that the results are influenced by the mobility of the
256 sediment in the lake: because it is a shallow lagoon, the sediment can easily be resuspended
257 and then aerated on windy days.

258 **Figure 4** shows the OM data versus the AVS data. We observed a positive correlation ($R^2 =$
259 0.72) between the two variables at nine of the twelve sites, in accordance with Di Toro (2001)
260 and Hernández-Crespo et al. (2010), which indicates that sulphide production is related to the
261 presence of organic matter. It must be highlighted that the relationship is strongly determined
262 by outside sites. Sites 5, 6 and 2 are exceptions and were not included in the correlation. A
263 combination of factors may explain the unique nature of Sites 5 and 6. First, the reeds
264 growing near these sites promote the oxidation of AVS. The cause is twofold: the roots
265 release oxygen, and evapotranspiration induces an increase in the flow of dissolved species
266 (e.g., dissolved oxygen) from the overlying water into the sediment (Choi et al., 2006).
267 Second, the samples from these locations include significant amounts of vegetal organic
268 matter with decay rates of approximately 0.0027 d^{-1} (Longhi et al. 2008); this rate is much
269 lower than the rate of the fast-decay fraction (0.35 d^{-1} , Di Toro, 2001).

270 At Site 2, which is located at the mouth of the Massanassa dry creek (an ephemeral
271 stream), the highest AVS level may be due to factors other than simple organic matter
272 content, such as grain size or organic matter reactivity. This stream reaches peak flows of 538
273 m^3/s (10/24/2000), with high velocities that can modify the sediment in its surroundings
274 (TYPSA, 2004). When the lake is flooded, a large volume of new suspended and settleable

275 solids (mainly clays) replaces the previous OM deposits (first the superficial and rapidly
276 decaying deposits and then, depending on the intensity of the flood, the deeper and more
277 refractory deposits). When the flooding ends and the water returns to the hypertrophic level, a
278 thin layer of phytoplanktonic OM begins to spread over the sediment surface. The overall
279 concentration of OM is low, but it is fresh and readily biodegradable. Consequently, sulphide
280 production increases.

281 The fact that the outer sites (AO, PC and GP) showed higher AVS levels is also related to
282 the organic matter decay rate: readily biodegradable organic matter is present at these
283 locations (wastewater origins in AO and PC) and in the outflow channel (GP) because
284 phytoplankton from the lake can decay quickly when they comes into contact with highly
285 saline seawater. At these sites, the level of AVS increased from March to September of 2007,
286 reflecting the effect of temperature, and decreased from September 2007 to September 2008,
287 showing the influence of OM type.

288

289 3.4. Simultaneously Extracted Metals (SEM) and Total Metals

290 The Σ SEM and Σ Total Metals concentrations are shown in **Fig. 5**. Based on all the
291 sampling campaigns, the Σ SEM values in the lake ranged from 1.39 to 4.83 $\mu\text{mol/g}$. These
292 results uniformly indicate high spatial variability (RSD: 35%). However, Site 11 presented
293 much higher Σ SEM results than the remaining sites due to its high concentration of Zn. In
294 fact, when this site was excluded, the RSD decreased to 22%. Among the data for the external
295 sites, the extremely high values for Σ SEM found at Site PC are especially important to note;
296 they are seven times higher than the overall average levels at the lake. Finally, temporal
297 variations in Σ SEM were not significant inside the lake; however, the outside sites show
298 evidence of a decreasing temporal trend.

299 Based on the total metal results, the sites in the north zone (Sites 1 to 4) and Site 11 were
300 the most polluted, undoubtedly because these zones have historically received point and
301 diffuse loads with heavy metals. The reference sites (5 and 6) showed Σ Total Metals results
302 significantly lower than those of sites from the north zone (1 to 4). However, this distinction
303 was not as clear based on the Σ SEM results. The Σ SEM values for Sites 5 and 6 were not
304 much lower than those of the north zone because the extraction percentages (Σ SEM/ Σ Total
305 Metal \times 100) in the north zone were considerably lower than in the remaining sites. Because of
306 these differences, we concluded that Σ SEM should not be used as an indicator of pollution
307 levels. Finally, we can establish two zones that are clearly different with regard to their
308 extraction percentages: the north zone (1–4) at 62% and the central-south zone (5–7 and 10–
309 11) at 85%.

310 The mean extraction percentages ($SEM_{Me}/Total\ Metal\times 100$) were Cd, 57%; Cu, 3%; Ni,
311 70%; Pb, 84%; and Zn, 95%. These are similar to the average results obtained in other
312 studies: 71–81% for Cd, 1–39% for Cu, 35–88% for Ni, 76–82% for Pb and 40–67% for Zn
313 (Peng et al. 2004; Fang et al. 2005; Besser et al. 2008). These high extraction percentages (for
314 all metals except for copper) indicate that heavy metals in the lake are mainly associated with
315 AVS (Peng et al. 2004; Fang et al. 2005) or other more reactive phases.

316 The SEM_{Me} results are shown in **Table 3**. Among the metals studied, Zn and Ni had the
317 most representative values for Σ SEM, with 76% and 17%, respectively, whereas the results
318 for Pb, Cd and Cu were less important in this regard. The percentages were similar for total
319 metal concentrations except for copper, which was more representative in total metal (18%)
320 than in SEM (3%). These results may be explained by copper's high affinity to other binding
321 phases, such as organic matter (Allen et al., 1993; Fang et al., 2005), or by the fact that CuS is
322 not completely dissolved in the process (Allen et al., 1993).

323

324 3.4. Differences between SEM and AVS

325 The differences between SEM and AVS are shown in Fig. 6. The values for Σ SEM were
326 smaller than those for AVS for all sampling sites and dates. The differences between SEM
327 and AVS in the lake ranged from -6.08 to -46.55 $\mu\text{mol/g}$, with a large degree of spatial
328 variation (RSD: 57 % in 2007 and 39 % in 2008). The spatial and temporal variation in SEM–
329 AVS has been linked to AVS concentrations because these values are considerably greater
330 than those for SEM. The sites with lower AVS contents (Sites 5 and 6) were closest to
331 exceeding the capacity of metal binding by sulphides, with molar sulphide excess varying
332 between -6.08 and -11.97 $\mu\text{mol/g}$. In contrast, the site with the highest SEM concentration
333 (PC) had a molar excess of AVS (-113.18 and -165.44 $\mu\text{mol/g}$). However, due to the dynamic
334 nature of sulphide and the continued disturbance of the sediment by the movement of boats,
335 the excessive heavy metal content of this location requires special attention.

336 Although the differences between SEM and AVS were less than zero, we cannot fully
337 assess the risk of toxic metals because to do so we would need to use additional parameters
338 such as biological accumulation tests or bioassays. Nevertheless, we can confirm the presence
339 of a sulphide pool acting to bind metals, although this does not mean that metals are
340 necessarily bound to sulphides; the extraction method is not selective, and metals bound to
341 other phases can be extracted, including carbonates and Fe-Mn-oxyhydroxides (Fang et al.,
342 2005).

343

344 4. Conclusions

345 It is known that the total metal concentration in sediments from Lake Albufera is high. We
346 now report that this sediment also contains significant concentrations of sulphides (8.5–48.5
347 $\mu\text{mol/g}$) due to the high levels of organic matter in the sediment (6.9–16.7%) and sulphates in
348 the tributary waters. Inside the lake, AVS levels decreased between first two sample periods

349 but not from the second to the third, when they even increased at some sites. These results
350 indicate that the situation is stable with a certain degree of variation that is due mainly to
351 changes in temperature and organic matter reactivity. In each of the three sampling
352 campaigns, there was sulphide available to bind metals; the differences between SEM and
353 AVS were less than zero. This becomes important when consider what precautions should be
354 taken in future sediment sampling or biological tests. It also allows us to predict the
355 consequences of dredging activities: a strong oxygen demand and the possible release of
356 metals or their transition into other, more labile phases. Total metal content was high at two
357 sites in the lake (Sites 1 and 11) and very high in the PC channel. Despite the efforts to reduce
358 pollution levels in the lake, there are still significant pollution loads, as can be seen at sites
359 like the PC channel, which is a potential source of heavy metals and nutrients.

360

361 **References**

- 362 Allen H.E., Fu G., Deng B. 1993. Analysis of Acid Volatile Sulfide (AVS) and Simultaneously Extracted Metals
363 (SEM) for the estimation of potential toxicity in aquatic sediments. *Environ. Toxicol. Chem.* 12, 1441-
364 1453.
- 365 Besser J.M., Brumbaugh W.G., Ivey C.D., Ingersoll C.G., Moran P.W. 2008. Biological and chemical
366 caharacterization of metal bioavailability in sediments from lake Roosevelt, Columbia river, Washington,
367 USA. *Arch. Environ. Contam. Toxicol.* 54, 557-570.
- 368 Burton G.A., Green A., Baudo R., Forbes V., Nguyen L.T.H., Janssen C.R., Kukkonen J., Leppanen M., Maltby
369 L., Soares A., Kapo K., Smith P., Dunning J. 2007. Characterizing sediment acid volatile sulphide
370 concentrations in European streams. *Environ. Toxicol. Chem.* 26, 1-12.
- 371 Casado-Martínez M.C., Buceta J.L., Belzunce M.J., Del Valls T.A. 2006. Using sediment quality guidelines for
372 dredged material management in commercial ports from Spain. *Environ. Int.* 32, 388-396.
- 373 Choi J.H., Park S.S., Jaffé P.R. 2006. Simulating the dynamics of sulphur species and zinc in wetland sediments.
374 *Ecol Model* 199, 315-333.

375 De Jonge M., Blust R., Bervoets L. 2010. The relation between Acid Volatile Sulfides (AVS) and metal
376 accumulation in aquatic invertebrates: Implications of feeding behaviour and ecology. *Environ. Pollut.* 158,
377 1381-1391.

378 De Lange H.J., Van Griethuysen C., Koelmans A.A. 2008. Sampling method, storage and pretreatment of
379 sediment affect AVS concentrations with consequences for bioassay responses. *Environ. Pollut.* 151, 243-
380 251.

381 Directive 2000/60/EC of the European Parliament and of the Council establishing a framework for the
382 Community action in the field of water policy. EU Water Framework Directive. OJ L 327 on 22 December
383 2000.

384 Di Toro, D.M.; Mahony, J.D.; Hansen D.J.; Scott, K.J.; Carlson, A.R.; Ankley, G.T. 1992. Acid volatile sulfide
385 predicts the acute toxicity of Cadmium and Nickel in sediments. *Environ. Sci. Technol.* 26, 96-101.

386 Di Toro D.M. 2001. Sediment flux modeling. John Wiley & Sons Intersciences Series, New York.

387 Du Laing G., Rinklebe J., Vandecasteele B., Meers E., Tack F.M.G. 2009. Trace metal behaviour in estuarine
388 and riverine floodplain soils and sediments: A review. *Sci. Total Environ.* 407, 3972-3985.

389 Fang T., Xiangdong L., Zhang G. 2005. Acid volatile sulfide and simultaneously extracted metals in the
390 sediment cores of the Pearl River Estuary South China. *Ecotoxicol. Environ. Saf.* 61, 420-431.

391 Förstner U., Ahlf W., Calmano W., Kersten M., Schoer J. 1990. Assessment of metal mobility in sludges and
392 solid wastes. In, *Metal speciation in the environment*. Springer-Verlag Berlín Heidelberg New York.

393 Grabowski L.A., Houpis J.L., Woods W.I., Johnson K.A. 2001. Seasonal bioavailability of sediment-associated
394 heavy metals along the Mississippi river floodplain. *Chemosphere* 45, 643-651.

395 Hernández-Crespo C., Martín M., Ferrís M., Oñate M., Torán M. 2010. Spatial variation of Acid Volatile Sulfide
396 (AVS) and Simultaneously Extracted Metals (SEM) in sediments from Beniarrés, Amadorio and Guadalest
397 reservoirs (Alicante, Spain). 20th SETAC Europe Annual Meeting. Science and Technology for
398 Environmental Protection, Seville (Spain).

399 Jingchun L., Chongling Y., Spencer K.L., Ruifeng Z., Haoliang L. 2010. The distribution of acid-volatile sulfide
400 and simultaneously extracted metals in sediments from a mangrove forest and adjacent mudflat in
401 Zhangjiang, China. *Mar. Pollut. Bull.* doi:10.1016/j.marpolbul.2010.03.029

402 Lee J.S., Lee B.G., Yoo H., Koh C.H., Luoma S.N. 2001. Influence of reactive sulfide (AVS) and supplementary
403 food on Ag, Cd and Zn bioaccumulation in the marine polychaete *Neanthes arenaceodentata*. *Mar. Ecol.*
404 *Prog. Ser.* 216, 129-140.

405 Lee J.S., Lee B.G., Luoma S.N., Yoo H. 2004. Importance of equilibration time in the partitioning and toxicity
406 of zinc in spiked sediment bioassays. *Environ. Toxicol. Chem.* 23, 65-71.

407 Leonard E.N., Mattson V.R., Benoit D.A., Hoke R.A., Ankley G.T. 1993. Seasonal variation of acid volatile
408 sulphide concentration in sediment cores from three northeastern Minnesota lakes. *Hydrobiologia* 271, 87-
409 95.

410 Longhi D., Bartoli M., Viaroli P. 2008. Decomposition of four macrophytes in wetland sediments: Organic
411 matter and nutrient decay and associated benthic processes. *Aquat Bot* 89, 303-310.

412 Peng S.H., Wang W.X, Li X., Yen Y.F. 2004 Metal partitioning in river sediments measured by sequential
413 extraction and biomimetic approaches. *Chemosphere* 57, 839-851.

414 Peris E. 1999. Caracterización de los materiales de fondo del lago de la Albufera evaluación del nivel de
415 aterramiento y caracterización mineralógica de los materiales así como de la carga contaminante persistente
416 residente en el lago. Departamento de Ingeniería de la Construcción. Universidad Politécnica de Valencia.

417 Public database of Confederación Hidrográfica del Júcar (http://www.chj.gob.es/Redesdecalidad/red_ica.aspx).

418 Schumacher B.A. 2002. Methods for the determination of total organic carbon (TOC) in soils and sediments.
419 PhD. Ecological Risk Assessment Support Center. U.S. Environmental Protection Agency, Las Vegas, US.

420 Soria J.M., Miracle M.R., Vicente E. 1987. Aporte de nutrientes y eutrofización de la Albufera de Valencia.
421 *Limnetica* 3, 227-242.

422 Speelmans M., Lock K., Vanthuyne D.R.J., Hendrickx F., Du Laing G., Tack F.M.G., Janssen C.R. 2010.
423 Hydrological regime and salinity alter the bioavailability of Cu and Zn in wetlands. *Environ. Pollut.* 158,
424 1870-1875.

425 Stumm W., Morgan J.J. 1996. *Aquatic Chemistry, Chemical Equilibria and Rates in Natural Waters*. 3rd Ed John
426 Wiley & Sons Intersciences Series, New York.

427 Técnica y Proyectos S.A. (TYPSA) 2004. Estudio para el desarrollo sostenible de l' Albufera de Valencia.
428 Confederación Hidrográfica del Júcar. Ministerio de Medio Ambiente.

429 UNE 77322:2003 – Calidad del suelo. Extracción de elementos traza solubles en *agua regia* (ISO 11466:1995).

430 U.S.EPA. 2005. Procedures for the derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the
431 protection of benthic organisms: metal mixtures (Cadmium, Copper, Lead, Nickel, Silver and Zinc). EPA-
432 600-R-02-011. Office of Research and Development. Washington DC 20460.

433 Van Griethuysen C., De Lange H.J., Van den Heuvel M., De Bies S.C., Gilissen F., Koelmans A.A. 2006.
434 Temporal dynamics of AVS and SEM in sediment of shallow freshwater floodplain lakes. *Appl. Geochem.*
435 21, 632-642.

436 Zheng L., Xu X.Q., Xie P. 2004. Seasonal and vertical distributions of Acid Volatile Sulfide and metal
437 bioavailability in a shallow subtropical lake in China. *Bull. Environ. Contam. Toxicol.* 72, 326-334.

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441 **Figure legends:**

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443 **Fig. 1.** Location of study area and Lake Albufera sampling sites.

444 **Fig. 2.** Mean organic matter content, expressed in %, at different sampling sites on different
445 dates. Error bars represent the standard deviations in triplicate analyses.

446 **Fig. 3.** Mean AVS concentration, expressed in $\mu\text{mol/g}$, at different sampling sites on different
447 dates. Error bars represent the standard deviations in triplicate analyses. The secondary axis is
448 for the PC site.

449 **Fig. 4.** Correlation between AVS concentrations ($\mu\text{mol/g}$) and organic matter contents (%) in
450 surface sediments from Lake Albufera. The equation applies to all sites except for Sites 2, 5
451 and 6.

452 **Fig. 5.** Mean $\sum\text{SEM}$ and $\sum\text{Total Metals}$ concentration, expressed in $\mu\text{mol/g}$, at different
453 sampling sites on different dates. Error bars represent the standard deviations in triplicate
454 analyses. The secondary axis is for the PC site.

455 **Fig. 6.** Differences between SEM and AVS at different sampling sites on different dates. The
456 secondary axis is for the PC site.

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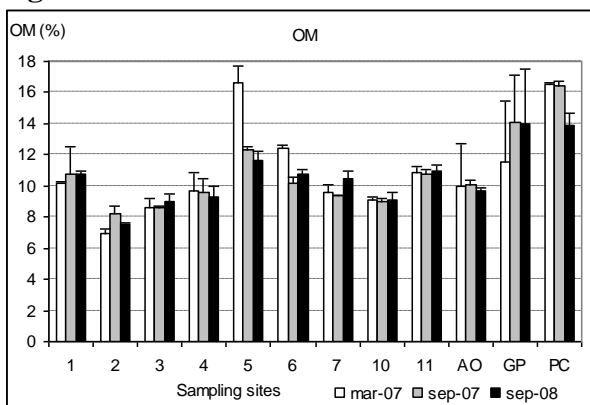
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464 **Fig. 1**



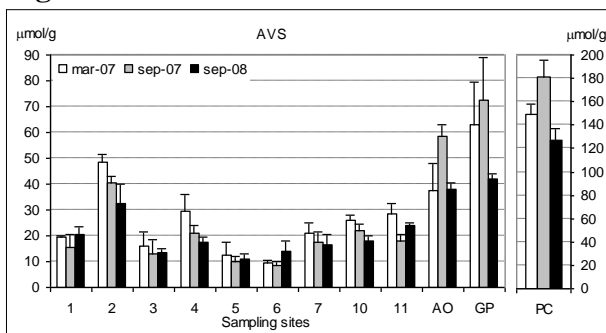
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Fig. 2



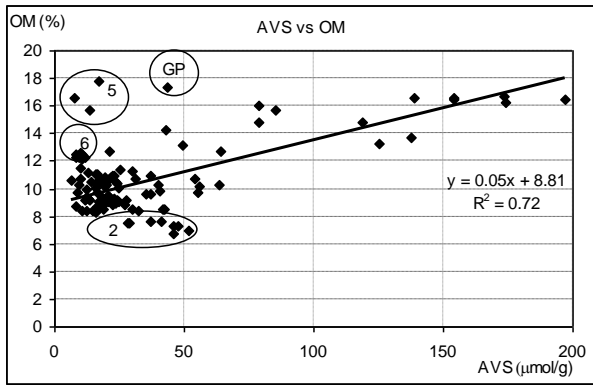
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Fig. 3



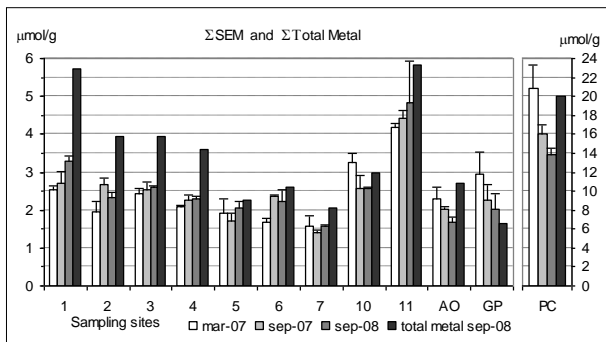
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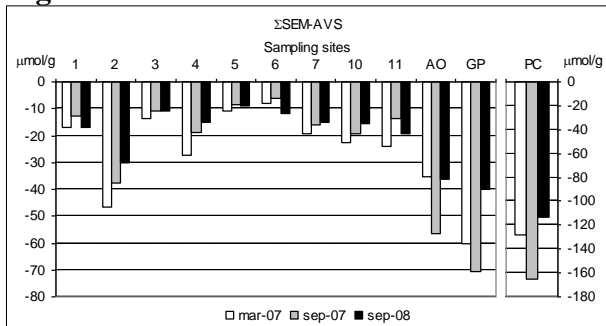
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Fig. 5



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Fig. 6



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Table 1. Values at the top of the water column (0–20 cm deep) for T, pH, Chl-*a*, conductivity and percentage of saturation of dissolved oxygen measured during both sample collection periods: March (Mar.) and September (Sep.). Chl-*a* data were extracted from a public database of CMAAUH. N.a.: not available.

Site	T (°C)			pH			Phytoplankton (µg Chl- <i>a</i> /L)		Conductivity (µS/cm)			% Sat. OD		
	Mar. 07	Sep. 07	Sep. 08	Mar. 07	Sep. 07	Sep. 08	Mar. 07	Sep. 07	Mar. 07	Sep. 07	Sep. 08	Mar. 07	Sep. 07	Sep. 08
1	18.2	24.6	24.0	8.0	7.6	7.5	101.0	33.6	2240	2800	2210	88.4	70.7	79.1
2	17.3	24.4	23.7	8.7	7.6	7.6	101.0	33.6	2480	2910	2240	121.9	80.7	94.9
3	16.8	25.2	24.3	8.7	7.9	7.6	116.7	96.4	2820	3370	2340	108.9	102.1	57.1
4	16.3	25.2	24.0	9.2	8.2	7.2	157.0	62.2	2460	3660	2740	124.6	91.3	60.9
5	12.3	24.6	25.4	8.6	7.9	8.6	N.a.	N.a.	2540	3550	2280	132.4	87.2	78.4
6	15.7	24.3	25.7	8.9	8.1	8.3	253.2	93.7	2460	3870	2360	104.7	83.2	71.9
7	12.5	24.0	25.9	8.7	7.7	8.1	166.5	59.4	2430	2790	2180	129.6	74.2	96.4
10	16.0	24.8	25.0	9.1	8.2	7.7	251.9	49.4	2430	3960	2270	129.2	105.3	54.1
11	15.9	24.7	24.7	8.9	8.2	7.7	146.0	52.5	2420	3520	2070	116.2	102.8	91.8
AO	13.6	24.0	24.8	7.8	7.4	7.4	N.a.	N.a.	1638	1520	1414	92.5	59.9	55.6
PC	14.8	21.2	25.6	7.9	7.1	7.5	N.a.	N.a.	1804	1556	2490	88.1	33.9	75.7
GP	12.4	22.3	27.4	8.3	8.4	8.7	N.a.	N.a.	2710	2430	4010	110.8	124.4	110.3

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Table 2. Total Organic Carbon (TOC) (% C), TOC corrected and conversion factor for OM and TOC.

Site	TOC (%)	TOC·1.33 (%C)	Factor (g OM/g C)
1	3.20	4.26	2.6
2	0.88	1.17	6.4
3	2.42	3.22	2.9
4	2.65	3.52	2.9
5	4.34	5.77	2.1
6	3.28	4.36	2.5
7	2.67	3.55	2.8
10	3.13	4.16	2.3
11	2.70	3.59	3.0
AO	1.90	2.53	3.8
PC	4.33	5.76	2.4
GP	1.70	2.26	4.5

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Table 3. Simultaneously Extracted Metals (SEM_{Me}, mg/kg) at different sampling sites on different dates. M:

547 mean, SD: standard deviation (n = 3). N.a.: not available.

Site sampling:			1	2	3	4	5	6	7	10	11	AO	PC	GP
Cu	Mar. 07	M	3.58	0.59	0.85	1.01	1.74	1.36	1.17	1.39	1.86	1.28	12.16	1.88
		SD	0.39	0.11	0.05	0.31	0.52	0.09	0.1	0.22	0.18	0.23	0.15	0.43
	Sep. 07	M	3.08	11.3	11.46	10.33	12.17	11.13	7.78	8.36	6.75	6.82	37.78	24.39
		SD	2.15	1.49	1.03	0.61	1.1	0.34	0.19	1.68	0.65	1.02	6.56	6.77
	Sep. 08	M	4.17	0.55	2.55	0.68	0.83	0.78	0.67	0.70	0.64	0.55	0.79	0.83
		SD	2.76	0.00	0.21	0.03	0.03	0.11	0.02	0.02	0.02	0.01	0.04	0.24
Zn	Mar. 07	M	134.25	105.98	131.04	111.16	91.37	95.12	79.26	191.79	255.36	138.33	1023.52	174.19
		SD	7.75	14.33	11.37	3.41	17.59	6.05	14.66	11.86	8.35	18.34	137.45	36.3
	Sep. 07	M	130.43	136.87	112.52	100.31	70.92	89.04	52.14	108.55	233.66	85.64	756.74	87.07
		SD	17.15	14.3	9.1	9.81	8.31	3.32	4.13	10.1	10.83	4.85	30.84	18.99
	Sep. 08	M	168.99	124.22	135.85	113.82	93.68	101.4	70.60	134.03	272.23	83.76	690.13	110.52
		SD	4.18	8.75	6.23	0.81	10.07	22.49	0.34	1.49	68.39	6.79	9.66	29.04
Ni	Mar. 07	M	15.32	12.13	15.84	16.55	23.85	6.79	16.56	13.85	9.29	6.39	178.55	9.38
		SD	1.66	1.67	1.54	0.11	5.8	0.43	3.55	2.16	0.89	1.13	62.05	2.14
	Sep. 07	M	30.77	21.92	34.38	30.01	21.5	45.86	24.87	41.86	40.59	32	119.67	28.46
		SD	1.84	3.96	5.38	1.46	6.18	1.72	2.75	10.28	2.48	2.61	18.97	4.68
	Sep. 08	M	26.55	18.27	21.12	26.02	32.45	33.85	23.45	27.01	32.08	18.93	93.54	13.81
		SD	12.17	0.70	2.51	2.71	9.94	5.36	1.42	0.61	2.99	0.93	29.70	3.97
Pb	Mar. 07	M	31.83	22.98	28.49	18.14	17.41	13.59	11.75	13.85	18.57	12.77	397.63	18.76
		SD	1.47	5.37	5.43	4.41	5.22	0.86	1.03	2.16	1.78	2.25	27.85	4.28
	Sep. 07	M	29.94	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	N.a.	385.5	N.a.
		SD	1.91										24.45	
	Sep. 08	M	35.71	23.47	21.50	19.60	13.96	15.96	13.00	10.82	20.64	15.08	349.39	13.14
		SD	1.44	0.64	2.87	0.40	0.51	1.26	4.12	0.44	3.75	0.66	8.68	3.16
Cd	Mar. 07	M	0.52	0.3	0.85	0.71	0.87	0.68	0.59	0.69	0.93	0.64	1.16	0.94
		SD	0.18	0.05	0.05	0.22	0.26	0.04	0.05	0.11	0.09	0.11	0.16	0.21
	Sep. 07	M	0.99	0.73	0.99	0.92	0.65	1.04	0.95	1.04	1.01	0.44	1.09	0.62
		SD	0.15	0.01	0.04	0.03	0.03	0.06	0.03	0.03	0.06	0.01	0.54	0.2
	Sep. 08	M	0.90	0.66	1.14	1.13	0.93	1.23	1.12	1.31	1.44	0.98	1.57	0.92
		SD	0.07	0.00	0.1	0.17	0.06	0.21	0.06	0.17	0.16	0.12	0.08	0.17

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