

1 ASSESSING METAL POLLUTION IN PONDS CONSTRUCTED FOR 2 CONTROLLING RUNOFF FROM RECLAIMED COAL MINES

3 Leticia Miguel-Chinchilla^{1*}

4 Eduardo González^{2,3,4}

5 Francisco A. Comín¹

6

7 ¹Pyrenean Institute of Ecology, Spanish National Research Council. Zaragoza, 50059 Spain

8 ²Université de Toulouse; INP, UPS; EcoLab; 31062 Toulouse, France

9 ³CNRS; EcoLab; 31062 Toulouse, France

10 ⁴Department of Biological Sciences, University of Denver. Denver, CO 80209 USA

11

12 * Corresponding author: leticia.m.ch@gmail.com

13

14

15 ABSTRACT

16 Constructing ponds to protect downstream ecosystems is a common practice in opencast coal mine
17 reclamation. As these ponds remain integrated in the landscape, it is important to evaluate the extent
18 of the effect of mine pollution on these ecosystems. However, this point has not been sufficiently
19 addressed in the literature. The main objective of this work was to explore the metal pollution in
20 manmade ponds constructed for runoff-control in reclaimed opencast coal mines over time. To do
21 so, we evaluated the concentration of 10 heavy metals in the water, sediment and *Typha* sp. in 16
22 runoff ponds ranging from 1 to 19 years old that were constructed in reclaimed opencast coal mines
23 of northeastern Spain. To evaluate degree of mining pollution, we compared these data to those from
24 a pit-lake created in a local un-reclaimed mine and to local streams as an unpolluted reference, as
25 well as comparing toxicity levels in aquatic organisms. The runoff ponds showed toxic
26 concentrations of Al, Cu and Ni in the water and As and Ni in the sediment, which were maintained
27 over time. Metal concentrations in runoff ponds were higher than in local streams, and macrophytes
28 showed high metal concentrations. Nevertheless, metal concentrations in water and sediment in
29 runoff ponds were lower than those in the pit-lake. This study highlights the importance of mining
30 reclamation to preserve the health of aquatic ecosystems and suggests the existence of chronic metal
31 toxicity in the ponds, potentially jeopardizing pond ecological functions and services.

32 **Key words:** runoff control; manmade ponds; heavy metals; reclamation; restoration; coal mining;
33 post-mining landscapes

34

35 **INTRODUCTION**

36 Surface coal mining operations remove the soil and rocks over the coal seam (overburden) to gain
37 access to the underlying coal. Despite reclamations, surface mining produces major environmental
38 disturbances due to excavation operations, including deforestation, topsoil loss, topological
39 alterations, soil compaction, hydrological changes and aquatic contamination (Shrestha and Lal
40 2006; Cravotta 2008; Pond et al. 2008; Palmer et al. 2010). Among all disturbances, the heavy metal
41 pollution derived from acidic mine drainage (hereafter called AMD) is one of the greatest threats to
42 the aquatic ecosystems because heavy metals degrade the aquatic habitat and are potentially toxic to
43 aquatic organisms (Dunbabin and Bowmer 1992; MacDonald et al. 2000; Cravotta 2008).

44

45 The main goal of mine reclamation is to isolate the pollutants from the ecosystems located
46 downstream of the mine sites. This objective should be achieved in combination with the final use of
47 the reclaimed mine (e.g., production of biomass for energy and sustain natural grassland and forest)
48 because the resulting ecosystem will remain integrated in the landscape (Bungart and Hüttl 2001;
49 Nicolau 2003; Gould 2012; Vickers et al. 2012). The control of the offsite effects are addressed by
50 the design of stable relief forms and safety structures, such as wetlands and ponds that guarantee the
51 non-emission of sediments and contaminants from restored sites to adjacent natural watercourses
52 (Sawtsky et al. 2000; Nicolau 2003; Wong 2003). These water bodies are normally constructed with
53 two objectives: the control of the runoff generated in the reclaimed mines and the specific treatment
54 of AMD.

55

56 The water bodies constructed to retain the runoff may be affected by metal pollution although water
57 has neutral pH and sediment does not show red, orange or yellow iron precipitates. In reclaimed
58 mines, coal mineral could be present in the overburden materials used to reconstruct the topography,
59 and metals could be released from the oxidation of pyrite present in the coal mineral (Johnson 2003;
60 Sheoran and Sheoran 2006; Cravotta 2008; Griffith et al. 2012). The manmade wetlands and ponds
61 remain integrated in the landscape after the end of mining reclamation and may provide new
62 ecological functions and services to society, such as aquatic fauna habitats and recreational areas.
63 But these functions ultimately depend on the quality of the ecosystem. Therefore, to know the extent
64 to which wetlands and ponds are affected by metal pollution is an important issue that nevertheless
65 was insufficiently addressed in the extant literature.

66

67 The main objective of this work was to explore the metal pollution in manmade ponds constructed
68 for runoff-control in reclaimed coal mines (hereafter called runoff ponds). Specifically, we were
69 interested in evaluating the temporal changes in metal concentrations to determine if toxic
70 concentrations of metals were present in the aquatic ecosystem. The temporal study of the metal
71 pollution for runoff ponds was done using a chronosequence approach. A comprehensive assessment
72 of the runoff ponds was conducted through a study of water, sediment and aquatic macrophytes,

73 which are three main factors involved in the process of heavy metal removal (Dunbabin and
74 Bowmer 1992; Sheoran and Sheoran 2006).
75

76 **MATERIAL AND METHODS**

77 **Site description**

78 The study was conducted in the Teruel coalfield, a mountainous area that is located in central-eastern
79 Spain (Fig. 1) and characterized by a continental Mediterranean climate. In this area, coal extraction
80 requires the construction of an external dump. As mining advances through the coalfield along the
81 coal seam, the overburden is used to refill the previously mined pits, and the post-mining topography
82 is created according to different reclamation objectives. We selected 16 manmade ponds created
83 during mine reclamation to control the runoff from reclaimed mines (Fig. 1B). Pond age ranged from
84 1 to 19 years old, so we used a space-for-time approach to evaluate metal pollution over time. Due to
85 logistic constraints in the samplings, chronosequences are frequently used instead of long data series
86 (Majer and Nichols 1998; Walker et al. 2010; Hart and Davis 2011). The runoff ponds were
87 designed mimicking natural ecosystems so that they would not require permanent human
88 management. The runoff ponds were oval shaped, and the area ranged from 0.2 to 3.9 ha with an
89 exception of one of them, which had an area of 22 ha. Littoral vegetation was dominated by *Typha*
90 sp. distributed in an average band of 1.5 m around the pond. All ponds were permanent and
91 endorheic; except for two ponds that were connected to a small stream, the water arrived from
92 superficial and sub-superficial runoff during rain events.

93

94 In addition to the 16 runoff ponds, we sampled one pit-lake from an un-reclaimed coal mine within
95 the same study area. Pit-lakes, which are formed when water fills excavated mining pits, are
96 typically deep lakes with vertical walls. The pit-lake surface was 7.6 ha, and the pit-lake was narrow,
97 deep, without a littoral zone and vegetation and enclosed by steep rock walls averaging 10 m high.
98 The low water level and steep banks, typical characteristics of the un-reclaimed pit-lakes in the study
99 area, make sampling extremely difficult and dangerous, and these conditions prevented us from
100 including other pit-lake replicates in the study. The sampled pit-lake was older than 25 years old.

101

102 Finally, unpublished data of metal content in local streams were also considered to compare with
103 data collected in the runoff ponds and used as a reference. Stream water and sediment samples were
104 collected from 26 points along the mainstream (Martín River) and their principal tributaries in spring
105 and summer of 2008 (Fig. 1A). Only dissolved metals in water and total metals in sediment are
106 available.

107

108 **Sample collection and processing**

109 *Water*

110 In the spring and summer of 2009, the water conductivity ($\mu\text{S cm}^{-1}$) and pH were measured *in situ* at
111 each pond using calibrated portable probes (WTW Multiline P4, Weilheim, Germany). All the
112 material used for metal analysis was soaked in 10% HNO_3 (Sastre et al. 2002). The water was
113 collected using polyethylene bottles at a depth of 15-20 cm. Samples were placed in portable
114 refrigerators and immediately transported to the lab for further analyses. In the lab samples were
115 stored in a refrigerator until the analysis were done. The alkalinity (mg l^{-1}) was determined by pH
116 potentiometric automatic titration with 0.004 N H_2SO_4 (Metrohm, Herisau, Switzerland) within the
117 24h after collection. The water samples were filtered through pre-ashed and deionizer water cleaned
118 $0.45\mu\text{m}$ cellulose acetate filters. The dissolved SO_4^{2-} (mg l^{-1}) was determined by ion chromatography
119 (Metrohm 861 Advanced Compact IC, Herisau, Switzerland). In addition, we determined ten
120 dissolved trace metals (mg l^{-1}), Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn, using Inductively
121 Coupled Plasma Optical Emission Spectrometry (ICP-OES iCAP6300Duo; Thermo Fisher,
122 Waltham, MA, USA). After filtering, water aliquots for metal analysis were immediately acidified to
123 $\text{pH} < 2$ with ultrapure HNO_3 and kept at 4°C until analysis. The analyses performed in the lab
124 followed standard American Public Health Association methods (APHA et al. 1992). Solution
125 standards were appropriately diluted and used to calibrate the ICP-OES before metal determinations.
126 Quality control for metal analysis was attained by including blanks and sample duplicates. The limits
127 of detection in to water samples were Al= 0.04 mg l^{-1} , As= 0.08 mg l^{-1} , Cd= 0.01 mg l^{-1} , Cr= 0.01
128 mg l^{-1} , Cu= 0.01 mg l^{-1} , Fe= 0.02 mg l^{-1} , Mn= 0.002 mg l^{-1} , Ni= 0.03 mg l^{-1} , Pb= 0.07 mg l^{-1} and Zn=
129 0.005 mg l^{-1} .

130

131 *Sediment*

132 One composite sample of sediment was collected from three points in the littoral zone of each pond,
133 where *Typha* sp. grows, in the spring and summer of 2009. Sediment samples were taken using a
134 hand corer sediment sampler 50 mm dia. The sampling was limited to the superficial sediment (i.e.,
135 top ~ 10 cm) because most physical and chemical changes in sediments occur within the upper 10 cm
136 layer (Meyer et al. 2008). The sediment pH and conductivity ($\mu\text{S cm}^{-1}$) were measured in the lab in a
137 solution of 10 g of fresh sediment dispersed in deionized water (pH : 2.5:1 g ml^{-1} , conductivity: 5:1 g
138 ml^{-1}) after shaking for 30 min. The collected sediments were air dried and sieved into fractions. The
139 < 2 mm fraction was used to determine the total sulfur (%) using an elemental analyzer (LECO SC-
140 144DR; Leco Instruments, St. Joseph, MI, USA). The $< 63 \mu\text{m}$ fraction was used to determine the
141 total and extractable metals (mg kg^{-1} DW): Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn (Förstner and
142 Wittmann 1983; Casas et al. 2003). Total metal concentration was analyzed per the US
143 Environmental Protection Agency 3051A Method (US EPA 2007), and the extractable metals were
144 obtained using acetic acid (Davidson et al. 1994; Rauret 1998; Pueyo et al. 2001). In both cases,
145 sediment digestion was performed by microwave extraction (Speedwave MWS-3, Berghof,
146 Germany). Total and the extractable metals were determined using the ICP-OES following

147 American Public Health Association methods (APHA et al. 1992), in the same way as in water
148 samples. The limits of detection to sediment were 0.01 mg l⁻¹ for the 10 studied metals.

149

150 *Vegetation (Typha sp.)*

151 In the summer of 2009, we collected three plants of *Typha* sp. at the perimeter of each runoff pond.
152 The plants were carefully washed using tap and distilled water, cleaned by immersion in 0.01 M
153 ethylenediaminetetraacetic acid (EDTA) to remove any absorbed metals and finally rinsed with
154 deionized water (Carranza-Alvarez, Alonso-Castro et al. 2008). Later, the plants were dried to
155 constant weight at 60°C. The metal concentration in the tissues of *Typha* sp. varied, with root >
156 rhizome > leaf (Dunbabin and Bowmer 1992; Sasmaz et al. 2008), so the dried plants were separated
157 into leaf, root and rhizome and grounded in a laboratory mixer mill (MM400 Retsch, Haan,
158 Germany) using teflon recovered grinding jars and balls. The plant material was digested with
159 nitric acid to solubilize metals (Meeravali and Kumar 2000; Sastre et al. 2002) using a microwave
160 system to reduce the total analysis time and the risk of sample contamination (Nadkarni 1984; Smith
161 and Arsenault 1996; Sastre et al. 2002). Finally, Al, As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn were
162 determined in ICP-OES following APHA et al. (1992) methods as describe above for the water
163 samples. The limits of detection to plant tissues were 0.01 mg l⁻¹ for the ten studied metals. The
164 metal concentration in the plant tissues was expressed in mg kg⁻¹ DW.

165

166 **Data analyses**

167 *Water and sediment*

168 Changes in the water and sediment characteristics in the runoff ponds were assessed over time by
169 linear mixed-effect models (LMM). The LMM allowed for the detection of trends in the change of
170 the environmental variables and control for the effect of the sampling season. For each variable, we
171 built a model in which the fixed factor was the age of the ponds, and sampling season was used as
172 random variable. LMM analyses were conducted with R software v.2.15.1 (R Core Team 2012). We
173 used the function “lme” of the “nlme” package (Pinheiro et al. 2012). The “lme” function assumes
174 that both the random effects and the errors follow normal distributions. All the water and sediment
175 variables, except pH, were transformed with the log(x+1) to improve model fitting.

176

177 The metal toxicity in the runoff ponds was evaluated using several criteria obtained from the
178 literature. To evaluate water pollution, we used the criteria established by the Environmental
179 Protection Agency of United States for the indefinite (CCC: Criterion Continuous Concentration)
180 and brief (CMC: Criteria Maximum Concentration) exposure of aquatic organisms without
181 producing unacceptable effects (US EPA 2002). In addition, we used the toxic concentration of
182 dissolved metals to plants defined by Markert (1992). The sediment metal pollution was evaluated
183 using the quality guidelines for freshwater ecosystems proposed by MacDonald et al. (2000) that
184 provide an accurate basis to predict the absence of sediment toxicity (TEC: Threshold Effect

185 Concentration) and the presence of sediment toxicity (PEC: Probable Effect Concentration).
186 Moreover, Kruskal-Wallis analyses between metal concentration of runoff ponds and streams were
187 conducted with R software v.2.15.1 (R Core Team 2012), to evaluate the mining effect over aquatic
188 ecosystems located in reclaimed areas. We used the function “kruskal.test” of the “stats” package
189 (Hollander and Wolfe 1973). In addition, the metal concentrations in the runoff ponds were
190 compared to the metal concentration in the pit-lake created within the un-reclaimed coal mine.

191

192 *Aquatic macrophytes*

193 We assessed the influence of mining on the macrophytes by comparing the metal concentration of
194 the *Typha* sp. to the average concentration of metals in plants growing in natural ecosystems
195 following Markert (1992). We did not use a chronosequence approach to analyze metal content in
196 *Typha* sp. because the time that each plant was exposed to the metals in the runoff pond could not be
197 determined.

198

199 **RESULTS**

200 **Metal pollution evaluation**

201 In runoff ponds, heavy metals were rarely detected in water. As, Cd, Fe and Pb were below the limits
202 of detection and Cr was only detected in the pit-lake, therefore were not shown in the figures. Water
203 Al, Cu, Mn, Ni and Zn concentrations were above detection limits and shown in Figure 2. The water
204 in the runoff ponds had a neutral pH (7.67 ± 0.16). The water pH in the pit-lake was acidic ($3.48 \pm$
205 0.06) and alkalinity was not detected. The concentrations of sulfate, Al, Cu, Mn, Ni and Zn in the pit
206 lake were several orders of magnitude higher than the runoff ponds (Fig. 2 from (a) to (i)).
207 According to the criteria proposed by the US EPA (2002), the dissolved Al, Cu and Ni
208 concentrations in the runoff ponds were above CCC criteria and only the Cu concentration were
209 above CMC criteria. Ni was in the same order of magnitude than CCC, Al was four times greater
210 than the CCC criteria, and mean Cu was six times greater than CCC and four times greater than
211 CMC. The pit-lake showed Al, Cr, Cu, Ni, and Zn metal concentrations above CCC criteria and Al,
212 Cr, Cu and Zn concentrations above CMC criteria. In fact, the differences between the pit-lake and
213 reference levels were much higher than between the runoff ponds and the reference levels (Fig. 2
214 from (e) to (i)); notably, the Al and Cu metal concentrations in the pit-lake were more than 100 times
215 greater. Among all metals in runoff ponds and the pit-lake, only Al in the pit-lake (Fig. 2(e))
216 exceeded the toxicity values established for plants in Markert (1992).

217

218 Total metal concentrations in sediment were detected in a high percentage of samples (from 60 to
219 100 %) for most of the studied metals, with the exception of Cd, which was below its limit of
220 detection. Extractable concentrations of metals in sediments were detected in less than 50% of
221 samples and As, Cd, Cr and Pb were below limit of detection (data not shown in figures).

222 Concentration of Al, As, Cr, Cu, Fe, Mn, Ni, Pb and Zn, to both, total and extractable fractions, were
223 shown in Figure 3. In the sediment, the pH was neutral in the runoff ponds (7.32 ± 0.19) and acidic
224 (4.73 ± 1.23) in the pit-lake, and the total sulfur was three times higher in the pit-lake than in the
225 runoff ponds (Fig. 3 (a) and (c) respectively). Despite these results, we did not detect differences in
226 the total and extractable heavy metals among runoff ponds and the pit-lake (Fig. 3 from (d) to (l)). In
227 both water bodies, only As and Ni exceeded the quality guidelines established by MacDonald et al.
228 (2000) for sediments in freshwater ecosystems. Notably, mean Ni was 20 times higher than the
229 threshold value of TEC criteria, and approximately 10 times higher than PEC in both the runoff
230 ponds and the pit-lake.

231

232 Metal concentration in streams are shown in Table 1. Al, Cd, Cu, Ni, Pb and Zn were below the limit
233 of detection in water samples and Cd in sediment samples. The comparison of runoff ponds and
234 stream metal concentration showed that dissolved Al, Cu, Ni were present in runoff pond but absent
235 in streams and also that Mn showed a higher concentration in runoff ponds (Kruskal-Wallis test, $P <$
236 0.001). Dissolved Fe was present in streams and absent in runoff ponds, but was only detected in
237 20% of the stream samples. Total sediment concentration of As, Cu, Fe and Zn were higher in runoff
238 ponds than in streams (Kruskal-Wallis test, $P < 0.001$, $P = 0.014$, $P = 0.002$ and $P = 0.018$
239 respectively). Al total concentration in sediment was significantly higher in the streams (Kruskal-
240 Wallis test, $P = 0.028$) although mean concentrations in runoff ponds and in streams were in the
241 same order of magnitude.

242

243 Finally, concentration of metals in plants were found in most of samples in a high percentage, with
244 the exception of Cd and Cr that were below limits of detection. Metal concentration in plants (except
245 Cd and Cr) were shown in Figure 4. The *Typha* sp. exceeded the average metal concentration of Al,
246 As, Fe and Ni in a great percentage of samples, especially in the roots and rhizomes where was near
247 100% (Fig. 4 a, b, d and f). It is noteworthy that Fe was more than 100 times higher than the mean
248 concentration in plants defined by Markert (1992).

249

250 **Changes over time**

251 Runoff ponds had similar values in the heavy metals carried by water, as well as in pH, conductivity
252 alkalinity and sulfate concentration, regardless of age (LMM analysis, $P > 0.05$). The conductivity,
253 total sulfur, As, Fe and Zn in the sediment increased with pond age (LMM analysis: $t = +2.687$, $P =$
254 0.012 ; $t = +3.572$, $P = 0.001$; $t = +2.662$, $P = 0.013$; $t = +4.284$, $P < 0.001$; and $t = +2.169$, $P = 0.038$
255 respectively). Despite the increase over time, toxic levels of As in the sediment to the aquatic
256 organisms were maintained above TEC and below PEC thresholds.

257

258 **DISCUSSION**

259 **Ponds exhibit toxic metal concentrations for aquatic life**

260 The references used to detect metal toxicity in the aquatic organisms showed potentially toxic levels
261 of Al, Cu and Ni in water and As and Ni in sediment (MacDonald et al. 2000; US EPA 2002). In
262 addition, the higher metal concentration in runoff ponds than in local streams evidenced the effect of
263 metal pollution from coal mining. Ponds are generally considered to be good metal sinks (Dunbabin
264 and Bowmer 1992; Mitsch and Wise 1998; Sheoran and Sheoran 2006; Merricks et al. 2007).
265 However, the case of the Teruel coalfield showed that the pond's ability to retain metals in the
266 sediment did not completely reduce the water's metal concentration below levels toxic to the aquatic
267 organisms. CCC, CMC, PEC and TEC criteria consider the negative effects that heavy metals could
268 cause for a wide range of aquatic organisms (plankton, macroinvertebrates and fishes). Therefore,
269 the persistent metal concentration above toxic levels over time may compromise the composition
270 and development of the entire aquatic community. For example, metal pollution in aquatic sites
271 could reduce the taxonomic richness of macroinvertebrate communities and induce a shift toward
272 more tolerant taxa (Clements 1994; David 2003; Merricks et al. 2007; Loayza-Muro et al. 2010).
273 Indeed, a low macroinvertebrate biodiversity was found in the studied runoff (Miguel-Chinchilla
274 2013; Miguel-Chinchilla et al. 2014). In addition, in fishes, heavy metals may restrict the presence of
275 some species, reduce embryonic survival and increase the frequency of body malformations and
276 deaths (Dubé et al. 2005; Lindberg et al. 2011; Witeska et al. 2013).

277

278 Although metal concentrations in the runoff ponds were not above levels considered to be toxic to
279 plants (Markert 1992), the effects of the high metal concentrations were reflected in the study of the
280 *Typha* sp. We detected Al, As, Fe, and Ni concentrations in the *Typha* sp. that were well above
281 concentrations considered to be normal for plants (Markert 1992) and higher than the metal
282 concentration of *Typha* sp. growing in non-polluted sites (Babcock et al. 1983; Samecka-Cymerman
283 and Kempers 2001). *Typha* sp. is known to accumulate high amounts of metals in their tissues
284 (McNaughton et al. 1974; Ye et al. 2003; Demirezen and Aksoy 2004), and our results are consistent
285 with these previous findings. This accumulation of metals in the plant tissues could be a long-term
286 problem because when plants die, metals in their tissues return to the ecosystems into detritus and
287 during decomposition become more available, particularly to deposit feeders (Dorgelo et al. 1995;
288 Weis and Weis 2004). Remarkably, *Typha* sp. was almost the only macrophyte in the runoff ponds.
289 This observation suggests that other plants were less competitive than *Typha* and unable to establish
290 due to prevalent heavy metal levels in the runoff ponds, indicating that ponds ultimately suffered
291 from undesirable heavy metal pollution.

292

293 The accumulation of metals in the pond may not necessary indicate that ponds will be their final sink
294 (Dunbabin and Bowmer 1992). Even if ponds have an endorheic character (similar to our study
295 case), possible negative effects over downstream ecosystems should be considered. For example,
296 metal exportation outside the reclaimed mines could happen during high storm events (Mitsch and

297 Gosselink 2000; Griffith et al. 2012) and through the food web (Braune et al. 1999; Parker 2004;
298 Croteau et al. 2005).

299

300 **Metal pollution persists in runoff ponds over time**

301 The temporal approach used in this study indicates that metal pollution in the runoff ponds persisted
302 even 19 years after their construction. Similarly, other studies revealed that heavy metal pollution in
303 streams close to reclaimed coal mines was detected even more than 20 years after mining
304 reclamation finished (Hartman et al. 2005; Clements et al. 2010; Hopkins et al. 2013). The increase
305 of total sulfur, As, Fe and Zn in sediments over time may evidence a continuous introduction of
306 metals into the runoff ponds. When coal mine spoils are exposed to natural weathering conditions,
307 the pyretic minerals contained in the coal are oxidized in presence of oxygen and water, producing
308 sulfuric acid and releasing heavy metals (Johnson 2003; Sheoran and Sheoran 2006; Cravotta 2008).
309 Thus, sulfates and metals could move from the reclaimed mines into the runoff ponds during rain
310 events dissolved in the water runoff. Moreover, soil and overburden erosion during storm events
311 could mobilize sulfur and metals in suspension.

312

313 Despite continuous metal introduction into the runoff ponds, the pH, alkalinity, SO_4^{2-} and dissolved
314 heavy metals in water and the extractable metals in sediment did not change over time. These results
315 suggest that during the studied period, represented by the chronosequence, the runoff ponds had a
316 chemical equilibrium that may be favored by carbonate parent material that provides extensive
317 internal buffering capacity (Pond et al. 2008; Bernhardt and Palmer 2011; Griffith et al. 2012).
318 Nevertheless, the observed chemical equilibrium in the runoff ponds may be altered by sediment pH
319 changes in the future. This can sometimes occur when reduced sediment become oxidized, then
320 stored metals may be released to more mobile forms (Gambrell 1994).

321

322 **Relevance of mine reclamation**

323 The concentration of heavy metals in the water of the runoff ponds was significantly lower than the
324 concentration of heavy metals found in the pit-lake located in the un-reclaimed mine. Although the
325 pit-lake was formed in a limestone area more than 25 years ago, it had a low pH and high
326 concentrations of sulfate and heavy metals typical in this type of lakes (Blodau 2006; Yucel and
327 Baba 2012). The concentrations of most of the studied metals in the water of the pit-lake were above
328 the levels considered to be toxic for the aquatic organisms. Moreover, the combination of the acidic
329 pH and the toxic level of Al could contribute to the absence of macrophytes and aquatic plants in the
330 pit-lake (Markert 1992; Samecka-Cymerman and Kempers 2001; Brix et al. 2002). Therefore, if
331 opencast coal mines had not been reclaimed in our study area, it is expectable that acidic and metal
332 polluted lakes with poor conditions to sustain a biological community would have formed. In
333 accordance to previous works (Younger 2001; Rodrigue et al. 2002; Wei et al. 2011), our study
334 highlighted the relevance of opencast coal mine reclamation.

335

336 **CONCLUSIONS AND MANAGEMENT IMPLICATIONS**

337 Currently, the study of mining pollution is primarily focused on the study of downstream
338 ecosystems, and on constructed wetlands and ponds to specific AMD treatment. Nevertheless,
339 reclaimed mines also support aquatic ecosystems that could be highly affected by mining as shown
340 by our results. Even though reclamation of opencast coal mines plays a key role in reducing metal
341 pollution and obtaining functional ecosystems in post-mining landscapes, mine reclamation does not
342 completely avoid metal pollution, supporting higher concentration in the constructed runoff ponds
343 than in local streams. Metal concentrations were maintained over time, and in some cases even
344 increased, indicating that metal pollution may be a chronic problem. This is a relevant issue that may
345 determine the future land use of the reclaimed mines. Monitoring and control of post-mining areas
346 are therefore necessary on a long-term basis.

347

348 Runoff ponds are efficient tools to reduce mining pollution of natural ecosystems under the
349 condition that runoff is managed through endorheic basins. Nevertheless, we advocate a more
350 ambitious goal in restoration of opencast coal mines: metal pollution in manmade ponds should be
351 targeted to be reduced to the lowest level possible. The reclamation of opencast coal-mining
352 landscapes should focus on maintaining self-organized ecosystems in combination with the final use
353 of the post-mining area (Hobbs and Norton 1996). Topography, soil, water and vegetation should be
354 collectively considered in mining management projects to design landscapes that reduce the mining
355 pollution not only downstream but also *within* the reclaimed mines (Nicolau 2003). The selection of
356 the overburden with the best physical and chemical characteristics in the most superficial layers and
357 the design of a topography that minimizes erosion are important points to consider in post-mining
358 area management to reduce the metal pollution in constructed ponds and their associated aquatic and
359 terrestrial ecosystems.

360

361 **ACKNOWLEDGMENTS**

362 This work was supported by a research and assistance agreement between Endesa Foundation and
363 the Pyrenean Institute of Ecology-CSIC. The regional government of Aragón, Spain supported the
364 PhD studies of the first author. We are grateful to our fieldwork assistants as well as the Endesa S.A.
365 and their employees in the Teruel mines. Finally we would like to thank to the anonymous reviewer
366 for their helpful comments on a previous version of this manuscript.

367

368

369 **REFERENCES**

- 370 APHA, AWWA, WPCF (1992) Métodos normalizados para el análisis de aguas potables y
371 residuales. Ediciones Díaz de Santos
- 372 Babcock MF, Evans DW, Alberts JJ (1983) Comparative uptake and translocation of trace elements
373 from coal ash by typha latifolia. *Sci Total Environ* 28:203–214. doi: 16/S0048-
374 9697(83)80019-9
- 375 Bernhardt ES, Palmer MA (2011) The environmental costs of mountaintop mining valley fill
376 operations for aquatic ecosystems of the Central Appalachians. *Ann N Y Acad Sci* 1223:39–
377 57. doi: 10.1111/j.1749-6632.2011.05986.x
- 378 Blodau C (2006) A review of acidity generation and consumption in acidic coal mine lakes and their
379 watersheds. *Sci Total Environ* 369:307–332. doi: 10.1016/j.scitotenv.2006.05.004
- 380 Braune B, Muir D, DeMarch B, et al. (1999) Spatial and temporal trends of contaminants in
381 Canadian Arctic freshwater and terrestrial ecosystems: a review. *Sci Total Environ* 230:145–
382 207. doi: 10.1016/S0048-9697(99)00038-8
- 383 Brix H, Dyhr-Jensen K, Lorenzen B (2002) Root-zone acidity and nitrogen source affects Typha
384 latifolia L. growth and uptake kinetics of ammonium and nitrate. *J Exp Bot* 53:2441 –2450.
385 doi: 10.1093/jxb/erf106
- 386 Bungart R, Hüttl R. (2001) Production of biomass for energy in post-mining landscapes and nutrient
387 dynamics. *Biomass Bioenergy* 20:181–187. doi: 10.1016/S0961-9534(00)00078-7
- 388 Casas JM, Rosas H, Sole M, Lao C (2003) Heavy metals and metalloids in sediments from the
389 Llobregat basin, Spain. *Environ Geol* 44:325–332.
- 390 Clements WH (1994) Benthic Invertebrate Community Responses to Heavy Metals in the Upper
391 Arkansas River Basin, Colorado. *J North Am Benthol Soc* 13:30–44. doi: 10.2307/1467263
- 392 Clements WH, Vieira NKM, Church SE (2010) Quantifying restoration success and recovery in a
393 metal-polluted stream: a 17-year assessment of physicochemical and biological responses. *J*
394 *Appl Ecol* 47:899–910. doi: 10.1111/j.1365-2664.2010.01838.x
- 395 Cravotta C (2008) Dissolved metals and associated constituents in abandoned coal-mine discharges,
396 Pennsylvania, USA. Part 1: Constituent quantities and correlations. *Appl Geochem* 23:166–
397 202. doi: 10.1016/j.apgeochem.2007.10.011
- 398 Croteau MN, Luoma SN, Stewart AR (2005) Trophic Transfer of Metals along Freshwater Food
399 Webs: Evidence of Cadmium Biomagnification in Nature. *Limnol Oceanogr* 50:1511–1519.
400 doi: 10.2307/3597695
- 401 David CPC (2003) Establishing the impact of acid mine drainage through metal bioaccumulation
402 and taxa richness of benthic insects in a tropical Asian stream (the Philippines). *Environ*
403 *Toxicol Chem* 22:2952–2959. doi: 10.1897/02-529
- 404 Davidson CM, Thomas RP, McVey SE, et al. (1994) Evaluation of a sequential extraction procedure
405 for the speciation of heavy-metals in sediments. *Anal Chim Acta* 291:277–286.

406 Demirezen D, Aksoy A (2004) Accumulation of heavy metals in *Typha angustifolia* (L.) and
407 *Potamogeton pectinatus* (L.) living in Sultan Marsh (Kayseri, Turkey). *Chemosphere* 56:685–
408 696. doi: 10.1016/j.chemosphere.2004.04.011

409 Dorgelo J, Meester H, Velzen C van (1995) Effects of diet and heavy metals on growth rate and
410 fertility in the deposit-feeding snail *Potamopyrgus jenkinsi* (Smith) (Gastropoda:
411 Hydrobiidae). *Hydrobiologia* 316:199–210. doi: 10.1007/BF00017437

412 Dubé MG, MacLatchy DL, Kieffer JD, et al. (2005) Effects of metal mining effluent on Atlantic
413 salmon (*Salmo salar*) and slimy sculpin (*Cottus cognatus*): using artificial streams to assess
414 existing effects and predict future consequences. *Sci Total Environ* 343:135–154. doi:
415 10.1016/j.scitotenv.2004.09.037

416 Dunbabin JS, Bowmer KH (1992) Potential use of constructed wetlands for treatment of industrial
417 wastewaters containing metals. *Sci Total Environ* 111:151–168. doi: 10.1016/0048-
418 9697(92)90353-T

419 Förstner U, Wittmann GTW (1983) *Metal pollution in the aquatic environment*. Springer

420 Gambrell R (1994) Trace and toxic metals in wetlands - A review. *J Environ Qual* 23:883–891.

421 Gould SF (2012) Comparison of Post-mining Rehabilitation with Reference Ecosystems in
422 Monsoonal Eucalypt Woodlands, Northern Australia. *Restor Ecol* 20:250–259. doi:
423 10.1111/j.1526-100X.2010.00757.x

424 Griffith MB, Norton SB, Alexander LC, et al. (2012) The effects of mountaintop mines and valley
425 fills on the physicochemical quality of stream ecosystems in the central Appalachians: A
426 review. *Sci Total Environ* 417–418:1–12. doi: 10.1016/j.scitotenv.2011.12.042

427 Hart TM, Davis SE (2011) Wetland development in a previously mined landscape of East Texas,
428 USA. *Wetl Ecol Manag* 19:317–329. doi: 10.1007/s11273-011-9218-2

429 Hartman KJ, Kaller MD, Howell JW, Sweka JA (2005) How much do valley fills influence
430 headwater streams? *Hydrobiologia* 532:91–102. doi: 10.1007/s10750-004-9019-1

431 Hobbs RJ, Norton DA (1996) Towards a Conceptual Framework for Restoration Ecology. *Restor*
432 *Ecol* 4:93–110. doi: 10.1111/j.1526-100X.1996.tb00112.x

433 Hollander M, Wolfe DA (1973) *Nonparametric statistical methods*. John Wiley & Sons, New York

434 Hopkins RL, Altier BM, Haselman D, et al. (2013) Exploring the legacy effects of surface coal
435 mining on stream chemistry. *Hydrobiologia* 713:87–95. doi: 10.1007/s10750-013-1494-9

436 Johnson DB (2003) Chemical and microbiological characteristics of mineral spoils and drainage
437 waters at abandoned coal and metal mines. *Water Air Soil Pollut Focus* 3:47–66.

438 Lindberg TT, Bernhardt ES, Bier R, et al. (2011) Cumulative impacts of mountaintop mining on an
439 Appalachian watershed. *Proc Natl Acad Sci* 108:20929–20934. doi:
440 10.1073/pnas.1112381108

441 Loayza-Muro RA, Elías-Letts R, Marticorena-Ruiz JK, et al. (2010) Metal-induced shifts in benthic
442 macroinvertebrate community composition in Andean high altitude streams. *Environ Toxicol*
443 *Chem* 29:2761–2768. doi: 10.1002/etc.327

444 MacDonald DD, Ingersoll CG, Berger TA (2000) Development and evaluation of consensus-based
445 sediment quality guidelines for freshwater ecosystems. *Arch Environ Contam Toxicol* 39:20–
446 31.

447 Majer, Nichols (1998) Long-term recolonization patterns of ants in Western Australian rehabilitated
448 bauxite mines with reference to their use as indicators of restoration success. *J Appl Ecol*
449 35:161–182. doi: 10.1046/j.1365-2664.1998.00286.x

450 Markert B (1992) Presence and significance of naturally-occurring chemical-elements of the
451 periodic system in the plant organism and consequences for future investigations on inorganic
452 environmental chemistry in ecosystems. *Vegetatio* 103:1–30.

453 McNaughton SJ, Folsom TC, Lee T, et al. (1974) Heavy Metal Tolerance in *Typha Latifolia* without
454 the Evolution of Tolerant Races. *Ecology* 55:1163–1165.

455 Meeravali NN, Kumar SJ (2000) Comparison of open microwave digestion and digestion by
456 conventional heating for the determination of Cd, Cr, Cu and Pb in algae using transverse
457 heated electrothermal atomic absorption spectrometry. *Fresenius J Anal Chem* 366:313–315.
458 doi: 10.1007/s002160050061

459 Merricks TC, Cherry DS, Zipper CE, et al. (2007) Coal-mine hollow fill and settling pond influences
460 on headwater streams in southern West Virginia, USA. *Environ Monit Assess* 129:359–378.

461 Meyer CK, Baer SG, Whiles MR (2008) Ecosystem Recovery Across a Chronosequence of Restored
462 Wetlands in the Platte River Valley. *Ecosystems* 11:193–208. doi: 10.1007/s10021-007-9115-
463 y

464 Miguel-Chinchilla L (2013) Physicochemical and Macroinvertebrate Community Trends in
465 Manmade Ponds Constructed in Reclaimed Opencast Coal Mines. Universidad de Alcalá

466 Miguel-Chinchilla L, Boix D, Gascón S, Comín FA (2014) Macroinvertebrate biodiversity patterns
467 during primary succession in manmade ponds in north-eastern Spain. *J. Limnol.* 73:

468 Mitsch WJ, Gosselink JG (2000) *Wetlands*, 3rd ed. Wiley

469 Mitsch WJ, Wise KM (1998) Water quality, fate of metals, and predictive model validation of a
470 constructed wetland treating acid mine drainage. *Water Res* 32:1888–1900. doi:
471 10.1016/S0043-1354(97)00401-6

472 Nadkarni RA (1984) Applications of microwave oven sample dissolution in analysis. *Anal Chem*
473 56:2233–2237. doi: 10.1021/ac00276a056

474 Nicolau JM (2003) Trends in relief design and construction in opencast mining reclamation. *Land*
475 *Degrad Dev* 14:215–226.

476 Palmer M, Bernhardt E, Schlesinger W, et al. (2010) Mountaintop Mining Consequences. *Science*
477 327:148–149. doi: 10.1126/science.1180543

478 Parker GH (2004) Tissue metal levels in Muskrat (*Ondatra zibethica*) collected near the Sudbury
479 (Ontario) ore-smelters; prospects for biomonitoring marsh pollution. *Environ Pollut* 129:23–
480 30. doi: 10.1016/j.envpol.2003.10.003

481 Pinheiro J, Bates D, DebRoy S, et al. (2012) nlme: Linear and Nonlinear Mixed Effects Models. R
482 Package Version 3.1-111

483 Pond GJ, Passmore ME, Borsuk FA, et al. (2008) Downstream effects of mountaintop coal mining:
484 comparing biological conditions using family- and genus-level macroinvertebrate
485 bioassessment tools. *J North Am Benthol Soc* 27:717–737. doi: 10.1899/08-015.1

486 Pueyo M, Rauret G, Luck D, et al. (2001) Certification of the extractable contents of Cd, Cr, Cu, Ni,
487 Pb and Zn in a freshwater sediment following a collaboratively tested and optimised three-
488 step sequential extraction procedure. *J Environ Monit* 3:243–250. doi: Article

489 R Core Team (2012) R: A language and environment for statistical computing. R foundation for
490 Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria

491 Rauret G (1998) Extraction procedures for the determination of heavy metals in contaminated soil
492 and sediment. *Talanta* 46:449–455.

493 Rodrigue JA, Burger JA, Oderwald RG (2002) Forest Productivity and Commercial Value of Pre-
494 Law Reclaimed Mined Land in the Eastern United States. *North J Appl For* 19:106–114.

495 Samecka-Cymerman A, Kempers AJ (2001) Concentrations of heavy metals and plant nutrients in
496 water, sediments and aquatic macrophytes of anthropogenic lakes (former open cut brown
497 coal mines) differing in stage of acidification. *Sci Total Environ* 281:87–98.

498 Sasmaz A, Obek E, Hasar H (2008) The accumulation of heavy metals in *Typha latifolia* L. grown in
499 a stream carrying secondary effluent. *Ecol Eng* 33:278–284.

500 Sastre J, Sahuquillo A, Vidal M, Rauret G (2002) Determination of Cd, Cu, Pb and Zn in
501 environmental samples: microwave-assisted total digestion versus aqua regia and nitric acid
502 extraction. *Anal Chim Acta* 462:59–72. doi: 10.1016/S0003-2670(02)00307-0

503 Sawtsky L, McKenna G, Keys MJ, Long D (2000) Towards Minimising the Long-term Liability of
504 Reclaimed Mine Sites. In: Haigh MJ (ed) *Reclaimed Land Eros. Control Soils Ecol.* Taylor &
505 Francis, pp 21–36

506 Sheoran AS, Sheoran V (2006) Heavy metal removal mechanism of acid mine drainage in wetlands:
507 A critical review. *Miner Eng* 19:105–116. doi: 10.1016/j.mineng.2005.08.006

508 Shrestha RK, Lal R (2006) Ecosystem carbon budgeting and soil carbon sequestration in reclaimed
509 mine soil. *Environ Int* 32:781–796. doi: 10.1016/j.envint.2006.05.001

510 Smith FE, Arsenault EA (1996) Microwave-assisted sample preparation in analytical chemistry.
511 *Talanta* 43:1207–1268. doi: 10.1016/0039-9140(96)01882-6

512 US EPA (2002) National Recommended Water Quality Criteria: 2002. Office of Water, EPA-822-R-
513 02-047. United States Environmental Protection Agency, Washintong, DC, USA. Accessed
514 23 Jun 2013

515 Vickers H, Gillespie M, Gravina A (2012) Assessing the development of rehabilitated grasslands on
516 post-mined landforms in north west Queensland, Australia. *Agric Ecosyst Environ* 163:72–
517 84. doi: 10.1016/j.agee.2012.05.024

518 Walker LR, Wardle DA, Bardgett RD, Clarkson BD (2010) The use of chronosequences in studies
519 of ecological succession and soil development. *J Ecol* 98:725–736. doi: 10.1111/j.1365-
520 2745.2010.01664.x

521 Wei X, Wei H, Viadero Jr. RC (2011) Post-reclamation water quality trend in a Mid-Appalachian
522 watershed of abandoned mine lands. *Sci Total Environ* 409:941–948. doi:
523 10.1016/j.scitotenv.2010.11.030

524 Weis JS, Weis P (2004) Metal uptake, transport and release by wetland plants: implications for
525 phytoremediation and restoration. *Environ Int* 30:685–700.

526 Witeska M, Sarnowski P, Ługowska K, Kowal E (2013) The effects of cadmium and copper on
527 embryonic and larval development of ide *Leuciscus idus* L. *Fish Physiol Biochem Adv Online*
528 *Publ.* doi: 10.1007/s10695-013-9832-4

529 Wong M. (2003) Ecological restoration of mine degraded soils, with emphasis on metal
530 contaminated soils. *Chemosphere* 50:775–780. doi: 10.1016/S0045-6535(02)00232-1

531 Ye ZH, Lin ZQ, Whiting SN, et al. (2003) Possible use of constructed wetland to remove
532 selenocyanate, arsenic, and boron from electric utility wastewater. *Chemosphere* 52:1571–
533 1579. doi: 10.1016/S0045-6535(03)00497-1

534 Younger PL (2001) Mine water pollution in Scotland: nature, extent and preventative strategies. *Sci*
535 *Total Environ* 265:309–326. doi: 10.1016/S0048-9697(00)00673-2

536 Yucel DS, Baba A (2012) Geochemical Characterization of Acid Mine Lakes in Northwest Turkey
537 and Their Effect on the Environment. *Arch Environ Contam Toxicol* 64:357–376. doi:
538 10.1007/s00244-012-9843-7

539

540

541 Table 1: Mean and standard deviation of metal concentrations of streams located in the river basin where
 542 mines are located. Dissolved metals in water (mg l⁻¹) and total metals in sediment (mg kg⁻¹) are shown.
 543 ND= no data.

544
 545

	Water (mg l ⁻¹)		Total Sediment (mg kg ⁻¹)	
	Average	SD	Average	SD
Al	<0.04	-	21237.85	9447.13
As	ND	-	10.06	4.02
Cd	<0.01	-	<2.50	-
Cr	<0.01	-	26.69	9.79
Cu	<0.01	-	3.52	3.32
Fe	0.011	0.028	22764.96	5310.63
Mn	0.024	0.077	364.01	163.58
Ni	<0.03	-	199.90	301.44
Pb	<0.07	-	22.31	9.08
Zn	<0.05	-	46.75	43.08

546 Figure 1: Study site. Section A: location of the study site (inset) and Martín river basin showing sampling
547 points in the local streams. Section B: Mining area showing the 16 runoff ponds constructed in reclaimed
548 opencast coal mines and the pit-lake located in an un-reclaimed opencast coal mine.

549

550 Figure 2: Scatter plots of the water characteristics in relation to the runoff pond age. Circles represent
551 spring data, and squares represent summer data. The pit-lake (age = 25 yr) values were shown in the grey
552 area of the graphic. Dashed lines indicate reference criteria to metal pollution CMC: Criteria Maximum
553 Concentration (US EPA 2002) CCC: Criterion Continuous Concentration (US EPA 2002); PTC: average
554 toxic concentration to plants (Markert 1992). When a range of references exists, H represents the high
555 value and L represents the low value.

556

557 Figure 3: Scatter plots of the sediment characteristics in relation to runoff pond age. Circles represent
558 spring data, and squares represent summer data. The pit-lake (age = 25 yr) values were shown in the grey
559 area of the graphic. Dashed lines indicate reference criteria to metal pollution TEC: threshold effect
560 concentration and PEC: probable effect concentration (MacDonald et al. 2000).

561

562 Figure 4: Metal concentration in *Typha* sp. root, rhizome and leaf tissues. Dashed lines indicate the
563 averaged range of metal concentrations for plants (Markert 1992), with H indicating the high and L the low
564 value of the range. M indicates a unique mean value when a range of references does not exist.