Growth and metal bioconcentration by conspecific freshwater macroalgae cultured in industrial waste water

The bioremediation of industrial waste water by macroalgae is a sustainable and renewable approach to the treatment of waste water produced by multiple industries. However, few studies have tested the bioremediation of complex multi-element waste streams from coal-fired power stations by live algae. This study compares the ability of three species of green freshwater macroalgae from the genus *Oedogonium*, isolated from different geographic regions, to grow in waste water for the bioremediation of metals. The experiments used Ash Dam water from Tarong power station in Queensland, which is contaminated by multiple metals (Al, Cd, Ni and Zn) and metalloids (As and Se) in excess of Australian water quality guidelines. All species had consistent growth rates in Ash Dam water, despite significant differences in their growth rates in "clean" water. A species isolated from the Ash Dam water itself was not better suited to the bioremediation of that waste water. While there were differences in the temporal pattern of the bioconcentration of metals by the three species, over the course of the experiment, all three species bioconcentrated the same elements preferentially and to a similar extent. All species bioconcentrated metals (Cu, Mn, Ni, Cd and Zn) more rapidly than metalloids (As, Mo and Se). Therefore, bioremediation *in situ* will be most rapid and complete for metals. Overall, all three species of freshwater macroalgae had the ability to grow in waste water and bioconcentrate elements, with a consistent affinity for the key metals that are regulated by Australian and international water quality guidelines. Together, these characteristics make *Oedogonium* a clear target for scaled bioremediation programs across a range of geographic regions.

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9 Introduction

10 The cultivation of algae in industrial effluent provides an effective form of bioremediation as

11 algae can bioconcentrate metals from waste water (Mehta and Gaur 2005; Troell et al. 2009;

12 Hubbe et al. 2011) while also capturing carbon and producing sustainable biomass (Roberts et

13 al. 2013). Integrated algal culture is particularly suited to coal-fired power stations as they are

14 significant sources of both metal-contaminated waste water and CO_2 (Roberts et al. 2013).

15 Water and CO_2 are two critical constraints to sustainable and cost effective aquatic biomass

16 production (McGinn et al. 2011; Slade and Bauen 2013). Therefore, integrating algal cultivation

17 with coal-fired power stations provides a path to biomass production with concomitant

18 bioremediation.

19 The success of the integrated aquaculture model relies on identifying species of algae that are

20 able to satisfy a number of criteria. Target species should preferably be robust to a range of

21 environmental conditions, competitively dominant, and widely distributed. Alternatively, robust

22 local strains are required to be isolated for region-specific applications and avoid the introduction

23 of non-endemic species. Green freshwater macroalgae are particularly strong candidates for

24 scaled bioremediation and biomass production as they satisfy these criteria (Lawton et al. 2013).

25 In addition, macroalgae require little specialised equipment to harvest and process, and are able

26 to bioconcentrate a wide range of metal ions from solution (Saunders et al. 2012; Roberts et al.

27 2013; Lawton et al. 2013). Previous experimental research has shown that species of the

28 freshwater genus of macroalgae, *Oedogonium*, can maintain high productivities in "clean" water

29 monocultures, readily outcompete co-occurring species under a variety of environmental

30 conditions, and are widely distributed throughout temperate, sub-tropical and tropical climates in

31 Australia (Lawton et al. 2014). *Oedogonium* is a genus of unbranched filamentous algae with a

32 worldwide distribution and grows attached to the substrate or as free-floating mats. The

33 widespread distribution of the genus, coupled with a filamentous growth form that is easy to

34 harvest, make species within the genus key candidates for scaled bioremediation and biomass

35 production.

36 The maintenance of high growth rates in biomass production is the cornerstone to developing

37 effective algal-based bioremediation (Roberts et al. 2013). There can be substantial variation in

38 growth rates between conspecific species of *Oedogonium* in small-scale laboratory cultures

39 (Lawton et al. 2014). However, it is unclear whether variation exists between conspecific species

40 in contaminated waste water and whether growth rates in clean water translates to similar

41 growth rates in waste water, and the bioconcentration of metals therein. The bioconcentration of

42 metal ions by macroalgae is a two phase process involving adsorption (rapid and passive

43 sorption of metal ions to cellular surfaces) followed by absorption (metabolically facilitated

44 internalisation of metal ions) (Genter 1996; Volesky 2007). The rate of bioremediation of metals

45 from industrial waste waters therefore varies depending upon the growth rate of the species,

46 with high growth rates having the dual benefit of delivering more rapid bioremediation and more

47 biomass for end-use applications (Roberts et al. 2013). Macroalgae have an ability to tolerate

48 high metal concentrations in water through the production of metal-binding phytochelatins

49 (Pawlik-Skowrońska 2001) and polyphosphate bodies (Nishikawa et al. 2003), or the

50 sequestration of metals in storage vacuoles (Hanikenne et al. 2005). While these are ubiquitous

51 properties of marine and freshwater species alike, co-occurring conspecifics can have

52 significantly different metal profiles *in situ* (Brown et al. 1999; Sawidis et al. 2001; Roberts et al.

53 2008) demonstrating the potential for significant variations in the suitability of conspecific species

54 for bioremediation applications.

55 We have previously demonstrated that *Oedogonium* can be cultivated in industrial waste water

56 from a coal-fired power station for the purposes of carbon capture and bioconcentration of a

57 range of metals (Al, Cd, Ni and Zn) and metalloids (As and Se for the purposes of this study)

58 using a locally isolated species (Roberts et al. 2013). However, a key uncertainty is whether

59 other clearly differentiated species within the genus *Oedogonium* from different geographic

60 locations have a similar capacity to grow and bioconcentrate metals and metalloids from waste

61 water to provide a bioremediation service. In this study we build upon previous experimental

62 research by measuring the growth rates and bioremediation of metals from a real-world

63 industrial effluent using multiple species of *Oedogonium*. We address two key questions. First,

64 do species of *Oedogonium* have a consistent ability to grow in metal-contaminated waste water

65 from a coal fired power station? Second, do species of *Oedogonium* bioconcentrate a similar

66 quantity and composition of metals from complex waste water? Together, these data provide a

67 basis to begin to assess the suitability of the genus *Oedogonium* more generally as a target for

68 scaled bioremediation programs across broad geographic regions.

69 **Materials and Methods**

70 *Biomass and effluent collection*

71 Three species of filamentous algae from the genus *Oedogonium* were used in the cultivation

72 experiments. One species was collected from the Ash Dam water (ADW) storage at Tarong

73 power station in October 2012 ('Tarong *Oedogonium*'), while the other two species were

74 collected from irrigation ditches in the Brandon sugarcane region. Attempts at species level

75   identification of the three species were made using taxonomic keys (Yee and Entwisle 2011),

76   however, the lack of clear morphological characteristics meant that the species could not be

77   identified beyond genus level. The species were therefore assessed using molecular techniques,

78   arguably the most effective approach to identifying cryptic species, and each was determined to

79   be a unique genotype, supporting its assignment as a unique species (Lawton et al. 2014). As

80   the three isolates could not be matched to extant species of *Oedogonium* on the basis of

81   classical taxonomic features they are hereafter referred to by their GenBank accession numbers.

82   The Tarong *Oedogonium* isolate (26.76°S, 151.92°E; temperate) has the accession number

83   KC606974, while the two strains isolated from the Brandon region (19.56°S, 147.36°E; tropical)

84   in far north Queensland, have the accession numbers KC701473 and KF606977. The strains

85   from the Brandon region were first collected in 2010 and have been maintained in culture at

86   James Cook University (JCU) for more than 2 years. All three species have been maintained in

87   stock cultures at the Marine and Aquaculture Research Faculties Unit (MARFU), JCU, Douglas

88   campus (19.33°S, 146.76°E), in dechlorinated town water (DTW) with f/2 media addition since

89   collection.

90   The ADW used in this study is an effluent created during ash disposal practices at a coal-fired

91   power station. ADW was sourced directly from the Tarong coal-fired power station in south-east

92   Queensland (26.76°S, 151.92°E) and transported to James Cook University (JCU), Townsville in

93   clean plastic 1000 L Intermediate Bulk Containers (IBCs) in November 2012. The ADW was then

94   stored at ambient temperature in 12,000 L storage tanks. A sub-sample of this water was taken

95   from the storage tank for this study in December 2012 and subject to elemental analysis to

96   quantify initial conditions in the effluent (Table 1). The ADW was provided by Stanwell Energy

97   Corporation.

98   *Experimental design*

99   A cultivation study was conducted to assess the growth of the three species of *Oedogonium*, as

100   well as the bioconcentration of metals and metalloids from the ADW. The experiments were

101   conducted within a climate controlled room (28°C, 100 µmol photons m^{-2} s^{-1} and a 12 light: 12

102   dark photoperiod). The experimental temperature was chosen to reflect the median daytime

103   temperature at the time of running the experiments. Stock cultures of each species were

104   maintained for five weeks in DTW with f/2 nutrient addition (0.1 g Aquasonic® f/2 growth media

105   L^{-1}) to acclimate them to the experimental conditions. The aim of the acclimation period was to

106   attain 'steady state' productivity, defined here as <5% change in Specific Growth Rate (SGR) for

107   two consecutive weeks prior to initiating the experiment. This was achieved on day 35 (Figure

108   S1), and from this time point onwards the three species were cultivated in either Ash Dam water

109   or DTW with f/2 addition as described below, for three consecutive growth periods of seven

110   days. The experimental period therefore spanned 21 days and consisted of three cultivation and

111   harvest cycles of equal duration as described below.

112   The three species of *Oedogonium* were cultured in 1 L Schott bottles at an initial stocking

113   density 0.5 g fresh weight (FW) L^{-1}. The FW was measured by gently blotting surface moisture

114   from the biomass and weighing it. The Schott bottles were stocked with biomass from one of the

115   three species and filled with either ADW or DTW amended with f/2 media, and then placed

116   randomly in the climate controlled room. The bottles were aerated with compressed air delivered

117   via a Pasteur pipette (at a flow rate of 0.2 L min^{-1}) to each replicate bottle, and they were

118   randomly redistributed on a daily basis to avoid possible light bias in the experimental area.

119   Each replicate bottle was harvested every seven days by pouring the contents through 20 µm

120   filter mesh. A sample of the water was filtered (0.45 µm) and retained for metal analysis. The

121   biomass was blot-dried with paper towel and weighed to the nearest 0.1 g. A sub-sample of this

122   biomass was returned to the bottle with new water and growth media to reset the stocking

123   density at 0.5 g FW L^{-1}. Any remaining biomass from each harvest was then dried in a

124   dehydrator for 48 hours at 60°C.

125   Growth rates for each of the treatments were calculated based upon the FW determined for

126   eight consecutive growth periods of seven days (t = Day 7). The Specific Growth Rate, SGR (%

127   FW d^{-1}), was calculated using the equation:

128       $SGR = Ln (W_f / W_i)/T*100$

129   Where W_f = the final weight (g FW) of biomass, W_i = the initial weight (g FW) of biomass, and T

130   = the number of days in culture (7).

131 *Elemental analysis*

132 The algal biomass and the two water sources (ADW and DTW) were each analysed for the

133 same 17 elements at the start of the experiment (Table 1). Water samples were collected using a

134 60 mL syringe and filtered (0.45 µm) to remove particulates. The concentrations of the 17

135 elements (Table 1) were also determined for all three species of *Oedogonium* grown in the Ash

136 Dam water treatments before exposure to Ash Dam water, and at each of the three time points

137 during the 21 day experimental period when *Oedogonium* was cultivated in Ash Dam water. All

138 biomass was prepared for the analysis by drying in a dehydrator for 48 h at 60°C, then digesting

139 100 milligrams (mg) of the dried algae in a Teflon digestion vessel with 3.0 milliliters (ml)

140 double distilled HNO_3 and 1.0 ml analytical grade H_2O_2. The solution was digested for 2

141 h then heated in a microwave oven to 180°C for 10 min, then diluted with Milli-Q water.

142 The concentrations of Al, As, Ba, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Se, Sr, V and Zn were

143 measured with a Bruker 820-MS Inductively Coupled Plasma Mass Spectrometer (ICP-MS). An

144 external calibration strategy was used for both instruments with a series of multi-element

145 standard solutions containing all of the elements of interest (three standard solutions were tested

146 containing 10, 25 and 50 µg L^{-1} of each element) and the results were reported after subtracting

147 the procedure blanks. The multi-element standards were obtained from Choice Analytical

148 (Sydney, Australia). Collisional Reaction Interface (CRI) was used for As (H_2) and V (He), while

149 ^{82}Se isotope was used for Se quantification, to eliminate polyatomic interferences for these

150 elements. A 1% HCl solution was spiked with 1 ppb As, Se and V and measured three times for

151 quality control; recovery between 98.5 and 110% indicated no significant interferences. All

152 analyses were conducted at the Advanced Analytical Centre at JCU, Townsville.

153 *Statistical analysis*

154 As the aim of the experiment was to compare long-term growth rates for the three species of

155 *Oedogonium* in Ash Dam water with DTW, the mean growth rate was determined for each

156 replicate over the course of the three week experimental period containing the ADW treatments.

157 The mean growth rate was then analysed by two-factor analysis of variance (ANOVA) for the

158 main experimental factors of water source (Ash Dam water and DTW) and species. Where

159 necessary the growth data were log transformed (Quinn and Keough 2002). Residual

160 histograms and scatter plots of residuals vs. estimates were assessed to determine normality

161 and homogeneity of variance respectively (Quinn and Keough 2002). Post-hoc comparisons for

162 main effects and their interactions were made using Tukey's HSD multiple comparisons.

163   The concentrations of metals in the algal biomass were first subject to multivariate ordination

164   with non-metric Multi-dimensional Scaling (nMDS) to assess multivariate patterns in the

165   temporal profile of metal bioconcentration from Ash Dam water by the three species. The mean

166   data for the metal concentration within the biomass (mg kg^{-1}) were reassembled in a Bray-Curtis

167   similarity matrix, focusing specifically on elements for which there are existing Australian and

168   New Zealand Environmental Control Council (ANZECC) water quality criteria (see Table 1 for

169   ANZECC water quality criteria). Mean metal contents of the three species were then contrasted

170   for individual metals with a one-way ANOVA with the factor 'species'. The elemental

171   concentration of the algal biomass was also converted to a bioconcentration factor (BCF) for

172   elements that have stipulated ANZECC trigger values. The BCF is the ratio of the element

173   concentration in the water, to the final concentration in the biomass (DeForest et al. 2007). The

174   bioconcentration factor (BCF) was calculated using the equation:

175    $BCF = C_b / C_w$

176   Where, C_b is the concentration of the element in the dry macroalgal biomass (mg kg^{-1}) and C_w is

177   the initial concentration of the element in the water phase (mg L^{-1}). The BCF is therefore a

178   dimensionless ratio that expresses uptake of elements relative to availability in the water

179   sample.

180   **Results**

181   *Elemental profile of Ash Dam water and DTW*

182   The elemental composition of the Ash Dam water was complex with several metals and

183   metalloids at concentrations significantly higher than the relevant ANZECC water quality criteria

184   (Table 1). The concentrations of Al, As, Cd, Mg, Mn, Mo, Ni, Se, Sr, V and Zn were all

185   significantly higher in Ash Dam water than DTW (two-tailed *t* test, P < 0.05), with the

186   concentration of Al, As, Cd, Ni, Se and Zn exceeding the ANZECC trigger values designated for

187   the protection of aquatic life at the 95% level (Table 1). The Ash Dam water is therefore a

188   complex industrial effluent with multiple metal (Al, Cd, Ni and Zn) and metalloid (As and Se)

189   targets for bioremediation. The remainder of the results section will focus on those elements that

190   exceed ANZECC water quality criteria as the aim of the study is to ascertain interspecific

191   variation in the potential for bioremediation.

192 *Between-species variation in growth rates*

193 'Steady state' productivity (defined here as < 5% change in SGR for two consecutive weeks)

194 was attained after 5 weeks of acclimation for all three species (Figure S1). Acclimation to 'steady

195 state' productivity was conducted to minimise any anomalous growth rates during the

196 experiments as a result of changing photo-regime (µmol photons m^{-2} s^{-1}) and photoperiod

197 associated with the biomass being sourced from stock cultures and subsequently exposed to

198 controlled experimental conditions. During this period, one of the *Oedogonium* species

199 (KC701473) had a significantly higher mean productivity (23% SGR) than the other two species

200 (~20% SGR for both KF606977 and KC606974; Figure S1).

201 After acclimation, all three species were exposed to either Ash Dam water or DTW with nutrient

202 addition. There was a significant 'species x water source' interaction for the mean growth rates

203 of the three species of *Oedogonium* ("Species x Water Source": $F_{2,12}$ = 4.95, P = 0.027). The

204 growth rate of one species of *Oedogonium* (KC701473) was significantly higher than the growth

205 rates of the two other species in DTW (Figure 1). KC701473 had a mean SGR of 26% in DTW,

206 in comparison to the other species that had a mean SGR of 20% (KF606977) and 16%

207 (KC606974) over the three week experimental period (Figure 1).

208 The overall growth rates for each species were reduced in Ash Dam water relative to those in

209 DTW, with the greatest reduction for KC701473 (decreasing from 26% in DTW to 7.5% in Ash

210 Dam water) (Figure 1). However, in contrast to the significant difference observed in growth

211 between the three species when grown in DTW, there was no difference in the growth rates

212 between the three species of *Oedogonium* in Ash Dam water (Figure 1). There was an apparent

213 trend towards higher SGR for the *Oedogonium* from the Brandon region (KF606977) and Tarong

214 (KC606974) than KC701473 in Ash Dam water (Figure 1), but these differences were not

215 statistically significant (Figure 1).

216 *Patterns in metal and metalloid bioconcentration*

217 All three species had low concentrations of most elements at the end of the acclimation period in

218 DTW, although KC701473 had a significantly higher K content than the other two *Oedogonium*

219 species (Supplementary Materials Table S1). There were no significant differences between the

220 three species of *Oedogonium* with respect to mean bioconcentration of metals or metalloids over

221 the course of the experiment (Figure 2). The only elements for which there was a significant

222 difference in bioconcentration between species of *Oedogonium* were Cd and As. Cd was

223 bioconcentrated to a significantly higher concentration in KF606977 and KC606974 than

224 KC701743 (Figure 3b, Table 2), while As was bioconcentrated to a significantly higher

225 concentration in KC701743 than the other two species (Figure 3e, Table 2). The remaining

226 elements showed no significant differences in their bioconcentration between the three species

227 of *Oedogonium*. (Figure 3, Table 2). The majority of elements that did not initially exceed

228 ANZECC criteria also had similar concentrations in the three species of *Oedogonium* at the

229 conclusion of the experiment (Supplementary Materials Figure S2 and Table S2).

230 While the mean bioconcentration of metals and metalloids did not differ between the three

231 species by the end of the experiment, there were minor differences in the temporal pattern of

232 metal bioconcentration. The clustering of the three species with respect to the vector orientation

233 in the MDS supports a temporal sequence in metal uptake and that this differs between the three

234 species. In the first week following the transfer of the cultures to Ash Dam water, all three

235 species bioconcentrated a subset of elements from Ash Dam water (Mn, Pb and Fe, Figure 4),

236 however, KC606974 and KF606977 bioconcentrated the metals Cd, Ni and Zn much more

237 rapidly in the first week than KC701473 (Figure 4). By week two, KC701473 had a similar

238 element profile to the other species having bioconcentrated the trace elements Al, Cd, Ni, Se

239 and Zn, while KC606974 clustered with the group of element vectors of Se, Ni, Zn, Cd, Al and B

240 and had the highest internal concentrations of these elements in week 2 (Figure 4). In the third

241 week of cultivation, the elemental profile of the *Oedogonium* KC606974 was similar to its initial

242 (post-acclimation) composition, while the other two *Oedogonium* species (KC701473 and

243 KC606974) continued to have higher concentrations of elements (Figure 4).

244 These patterns are also clearly reflected in the temporal sequence of bioconcentration of metals

245 (Al, Cd, Ni and Zn) and metalloids (As, Se) that exceeded ANZECC criteria by the three species

246 of *Oedogonium*. The four metals (Al, Cd, Ni and Zn) were bioconcentrated more rapidly by

247 *Oedogonium* isolate KC606974 and KF606977 than KC701473. There were significantly higher

248 internal concentrations of Al, Cd, Ni and Zn in these two species after one week of exposure to

249 Ash Dam water (Figure 5a-d). However, the internal concentrations of Al, Cd and Ni in the three

250 species converged and were not different by the end of the experiment, while Zn remained

251 higher in KF606977 than the other two species (Figure 5d). In contrast, bioconcentration of As

252 differed between the three species, with significantly higher As contents in KC701473 than

253 KF606977 and KC606974 by the end of the experiment (Figure 5e). There were no significant

254 differences in the rate or extent of bioconcentration of Se by the three *Oedogonium* species

255 (Figure 5f).

256 *Metal and metalloid bioconcentration factors*

257 The element bioconcentration factors were very consistent between the three species of

258 *Oedogonium*. For all species, the highest BCF was attained for Cu, Mn, Zn and Ni with values

259 ranging from 709-1418 for Zn, 889-1256 for Ni, 6938-8943 for Cu, and 5892-16719 for Mn

260 (Tables 4 and 5). Conversely, Al, Se and Mo all had the lowest BCF with values of 3-4 for Mo,

261 79-93 for Se and 259-351 for Al (Tables 4 and 5). The ranking of the BCF amongst elements

262 was, however, relatively consistent with the same four elements (Cu, Mn, Zn and Ni) having the

263 highest BCF for all three species, while the same three elements (Al, Se and Mo) had the lowest

264 BCF for all species (Table 4).

265 **Discussion**

266 To be a suitable candidate for bioremediation applications, a target group of macroalgae should

267 be widely distributed, robust to variable environmental conditions, be able to out-compete other

268 species in intensive culture, and grow at high rates. The genus *Oedogonium* is already known to

269 meet these criteria from individual studies investigating species within the genus (Lawton et al.

270 2013). However, it remains unresolved as to whether individual species within the genus also

271 have a similar capacity to grow and bioconcentrate metals and metalloids from waste water to

272 provide a bioremediation service – given that there is substantial conspecific variation in growth

273 rates and in metal concentrations for macroalgae more generally (Lawton et al. 2014). Our

274 results clearly demonstrate that the individual species within the genus *Oedogonium* that were

275 assessed in this study have an equal capacity to grow in a complex industrial waste stream, and

276 to sequester metals and metalloids from this waste stream to provide a bioremediation service.

277 The specific growth rates (SGR) of *Oedogonium* averaged approximately 12% d^{-1} in complex

278 waste water containing multiple elements that require remediation. Interestingly, all three species

279 of *Oedogonium* had a similar SGR in Ash Dam water, despite quite significant differences in

280 SGR when the same species were cultivated in DTW. These differences were evident during

281 both the acclimation and experimental cultivation periods in DTW. The consistently lower growth

282 rates of *Oedogonium* in Ash Dam water demonstrate that the growth rates of individual species

283 in clean-water cultures cannot be used to predict their productivities in metal-contaminated

284 waste waters, for which there was a relatively consistent reduction in SGR of approximately

285 50%. Furthermore, the species-level traits that confer more rapid growth rates in DTW do not

286 confer more rapid growth rates in industrial effluent. The SGR of all species was higher in DTW,

287 and for this reason site-specific evaluations of potential growth rates in industrial effluents will be

288 required. In general, our data support the prediction that the species of the genus *Oedogonium*

289 are strong candidates for metal-bioremediation programs given their ability to deliver consistent

290   growth in complex effluents while accumulating metals and metalloids over time. However, this
291   conclusion needs to be tempered in that individual species will always require trials to ensure
292   their efficacy in complex waste streams, in ambient environmental conditions, and that the
293   bioremediation of waste streams will, by nature of the composition of the waste stream and the
294   endemic flora, be influenced by site-specific water quality profiles.

295   In addition to having similar growth rates, the three species of *Oedogonium* also had very similar
296   patterns of metal bioconcentration. While there were minor differences in the temporal pattern of
297   the bioconcentration of some elements between the three species, the mean bioconcentration of
298   ANZECC-listed elements over the course of the experiments did not differ for the majority of
299   elements. Each of the species was able to bioconcentrate internal metal and metalloid
300   concentrations orders of magnitude above their concentrations in the Ash Dam water. The three
301   species also had very similar elemental composition over the course of the experiment, and
302   delivered consistent element-specific bioconcentration factors (BCFs) for each of the ANZECC-
303   listed elements. The metals Cu, Mn, Zn and Ni were bioconcentrated to a significantly higher
304   degree by the species of *Oedogonium* than the metalloids Mo and Se, supporting what has
305   previously been published for single species (Saunders et al. 2012; Roberts et al. 2013). This
306   evidence adds to a clear consistency amongst studies that live algal cultures are effective at
307   sequestering metal cations, but are less effective at sequestering metalloids that occur as
308   oxyanions, particularly Se (as SeO_4^{2-}), As (as AsO_4^{3-}) and Mo (as MO_4^{2-}) (Roberts et al. 2013).
309   The algal cell wall has a natural affinity for dissolved cations and this is exploited in the use of
310   dried biomass as a biosorbent for dissolved metals (Kidgell et al. in press). In contrast, dissolved
311   metalloids do not show a natural affinity for algal cell walls which may explain the limited uptake
312   in these experiments. Additionally, there may be significant competition for uptake sites in the
313   complex effluent tested in these experiments and the bioconcentration of the metalloids may be
314   expected to increase in the absence of competing ions.

315   The only significant differences between species of *Oedogonium* with respect to the
316   bioconcentration of elements were for the ANZECC metalloid As and the ANZECC metal Cd.
317   KC701473 had a significantly higher content of As than the other two species, but a lower
318   content of Cd. There is some evidence that the presence of As in contaminated soils can reduce
319   the bioconcentration of Cd by terrestrial plants (Sun et al. 2009). Reduced Cd bioconcentration
320   in the presence of As could be a result of competition for common binding sites on the algal cell
321   wall by the two elements. Indeed, similar antagonistic relationships have been documented in
322   marine seaweeds where the presence of Cu can impede Cd bioconcentration (Roberts et al.
323   2008). The variance between the three species of *Oedogonium* with respect to As

324   bioconcentration suggests it may be possible to identify species that are more effective at

325   accumulating this metalloid, but there may be a trade-off of reduced Cd bioconcentration. There

326   was also a slight difference in the temporal profile of metal bioconcentration, with the Tarong

327   isolate KC606974 having rapid initial bioconcentration of the metals Al, Cd, Ni and Zn, followed

328   by a slower rate of bioconcentration in the later stages of the experiment. This could be

329   indicative of an efficient metal detoxification pathway in this isolate that was originally collected

330   from Tarong Ash Dam. Green freshwater macroalgae can develop efficient metal detoxification

331   mechanisms such as the production of metal-binding phytochelatins to sequester and detoxify

332   contaminants in cellular vacuoles, and this property is most pronounced in isolates that have

333   been exposed to contaminants for a long period of time (Pawlik-Skowrońska 2001; Pawlik-

334   Skowrońska 2003). Regardless, all species had low BCFs for metalloids, and bioconcentrated

335   them at a slow rate relative to their availability in the effluent, and this finding is consistent with

336   previous research that has highlighted the relatively slow rates of metalloid bioconcentration by

337   green macroalgae relative to the rates at which metals are bioconcentrated (Roberts et al.

338   2013). Therefore, the three species of *Oedogonium* delivered equal growth rates and

339   bioconcentrated the same elements preferentially such that the over-arching bioremediation

340   potential of the three species was effectively the same. The genus *Oedogonium* therefore has a

341   consistent ability to sequester a range of metals and metalloids from complex effluents for

342   broad-spectrum remediation applications and, furthermore, it is possible to predict the key

343   metals that will be most effectively remediated by conspecific species.

344   Importantly, we have demonstrated that species within the cosmopolitan algal genus

345   *Oedogonium* have a consistent ability to bioconcentrate metals from waste water. One could

346   therefore isolate native *Oedogonium*, potentially from the waste water source requiring

347   remediation as we have done, and use these species to remediate waste water in intensive on-

348   site cultivation. Notably, the *Oedogonium* KC606974 from Tarong used during this study is

349   endemic at the Tarong power station (Stanwell Energy) and this provides an example of the

350   capacity to use a native species for culture within the industrial waste stream (Ash Dam water

351   from Tarong) to deliver the first step in a potentially larger integrated waste management

352   process.

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Table 1_(on next page)

Table 1

Elemental composition of dechlorinated town water and Ash Dam water.

Element	Dechlorinated town water (µg L^{-1} ± SE)	Ash dam water (µg L^{-1} ± SE)	95A% ANZECC trigger value (µg L^{-1})
Aluminium	10 ± 5.8	**123.3 ± 13.3** [a]	**55**
Arsenic	1 ± 0.6	**33.7 ± 1.2** [a]	**24**
Barium	< LOD	< LOD	ID
Cadmium	0.1 ± 0.1	**2.5 ± 0.0** [a]	**0.2**
Chromium	< LOD	< LOD	1.0
Cobalt	< LOD	< LOD	ID
Copper	2 ± 1.2	1 ± 0.0	1.4
Iron	50 ± 58.9	50 ± 0.0	ID
Lead	1 ± 0.6	1 ± 0.0	3.4
Magnesium	2 ± 1.2	92.7 ± 0.7 [a]	ID
Manganese	1 ± 0.6	4 ± 0.0 [a]	1900
Molybdenum	1 ± 0.6	1280 ± 35.1 [a]	ID
Nickel	1 ± 0.6	**34.7 ± 0.3** [a]	**11**
Selenium	10 ± 5.8	**70 ± 0.0** [a]	**11**
Strontium	56.7 ± 32.7	2243 ± 63.3 [a]	ID
Vanadium	10 ± 5.8	843.3 ± 17.6 [a]	ID
Zinc	8 ± 4.6	**55 ± 0.6** [a]	**8.0**

[a] Significantly greater than dechlorinated town water (two-tailed t-test, $P < 0.05$)

< LOD - less than Level of Detection (1 µg L^{-1} for most elements)

ID = insufficient data to calculate ANZECC 95% trigger values

All data are mean concentrations ± S.E.

Bold values exceed the ANZECC trigger value.

Table 2

Analysis of variance for ANZECC metals (Al, Cd, Ni and Zn) and metalloids (As and Se) concentrations in the three Oedogonium species after cultivation in ADW.

Factor	df	Aluminium			Cadmium			Nickel			Zinc			Arsenic			Selenium		
		MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P	MS	F	P
Species	2	271.8	0.73	0.52	1.14	5.82	**0.04**	156.9	0.77	0.50	1141.6	3.66	0.09	178.1	7.41	**0.02**	10.1	0.67	0.55
Residual	6	372.8			0.20			203.4			312.7			24.0			14.9		

Bold values are statistically significant (P < 0.05).

Table 3

Table 3

Bioconcentration factors for ANZECC-listed elements in Ash Dam water.

Element	Ash dam water (mg L^{-1})	Bioconcentration factor		
		KF606977	KC701473	KC606974
Al	0.1233	276	259	351
As	0.0337	481	504	721
Cd	0.0025	730	288	642
Cu	0.001	6938	8943	8884
Pb	0.001	346	438	1009
Mn	0.004	5892	16719	12739
Mo	1.28	3	3	3
Ni	0.0347	889	929	1256
Se	0.07	83	79	93
Zn	0.055	1418	709	1033

Table 4_(on next page)

Table 4

Bioconcentration factors in order of decreasing magnitude.

Species	Bioconcentration factor
KF606977	Cu > Mn > Zn > Ni > Cd > As > Pb > Al > Se > Mo
KC701473	Mn > Cu > Ni > Zn > As > Pb > Cd > Al > Se > Mo
KC606974	Mn > Cu > Ni > Zn > Pb > As > Cd > Al > Se > Mo

Figure 1

Figure 1

Specific Growth Rate of the three *Oedogonium* species in Ash Dam Water and Dechlorinated Town Water. Data are means ± SE.
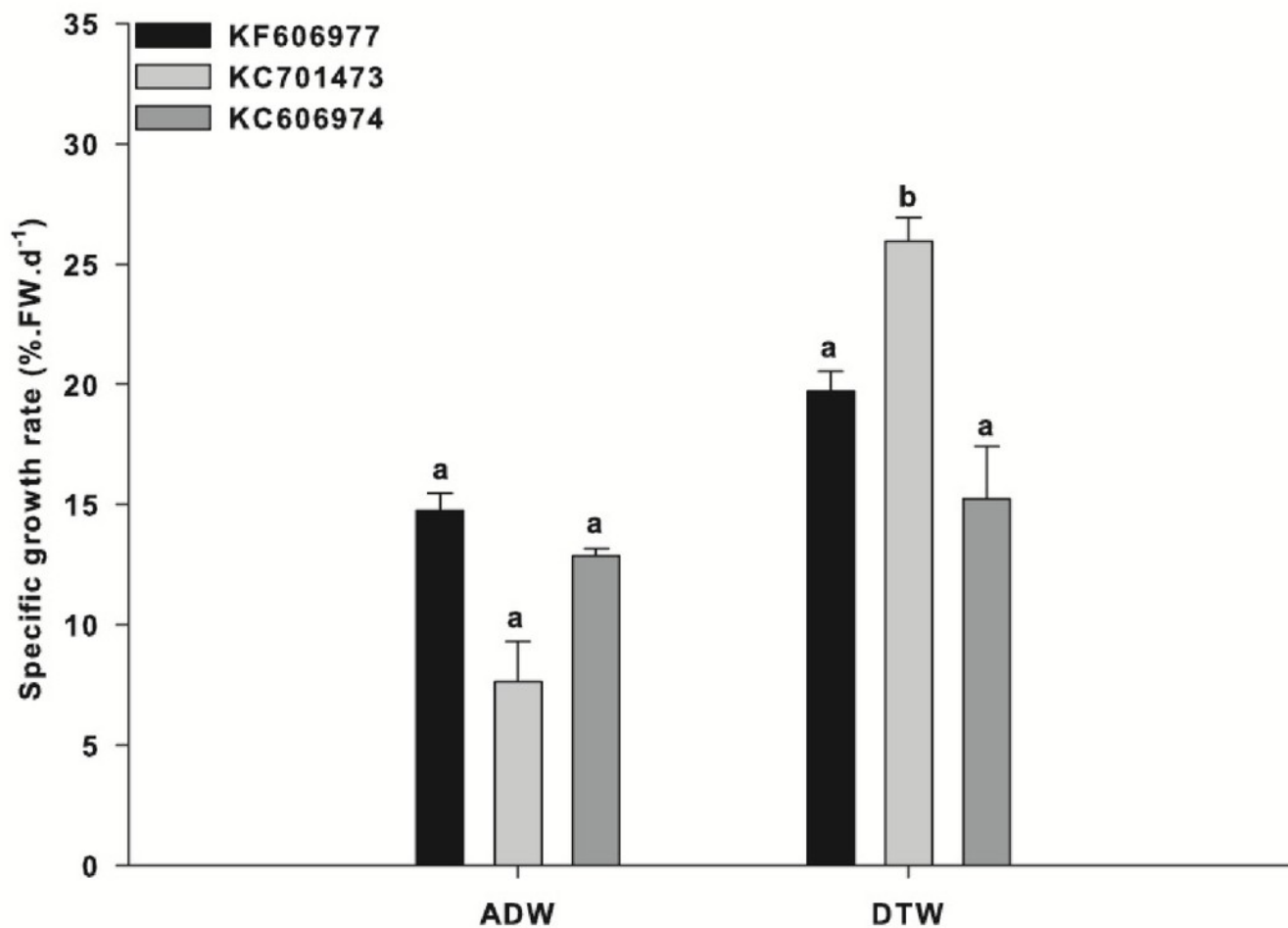
Figure 2

Mean concentration of a) summed ANZECC metals and b) summed ANZECC metalloids in the three *Oedogonium* species cultured in Ash Dam Water at time 0 (black bars) and after three weeks of cultivation (grey bars). Data are means ± SE.
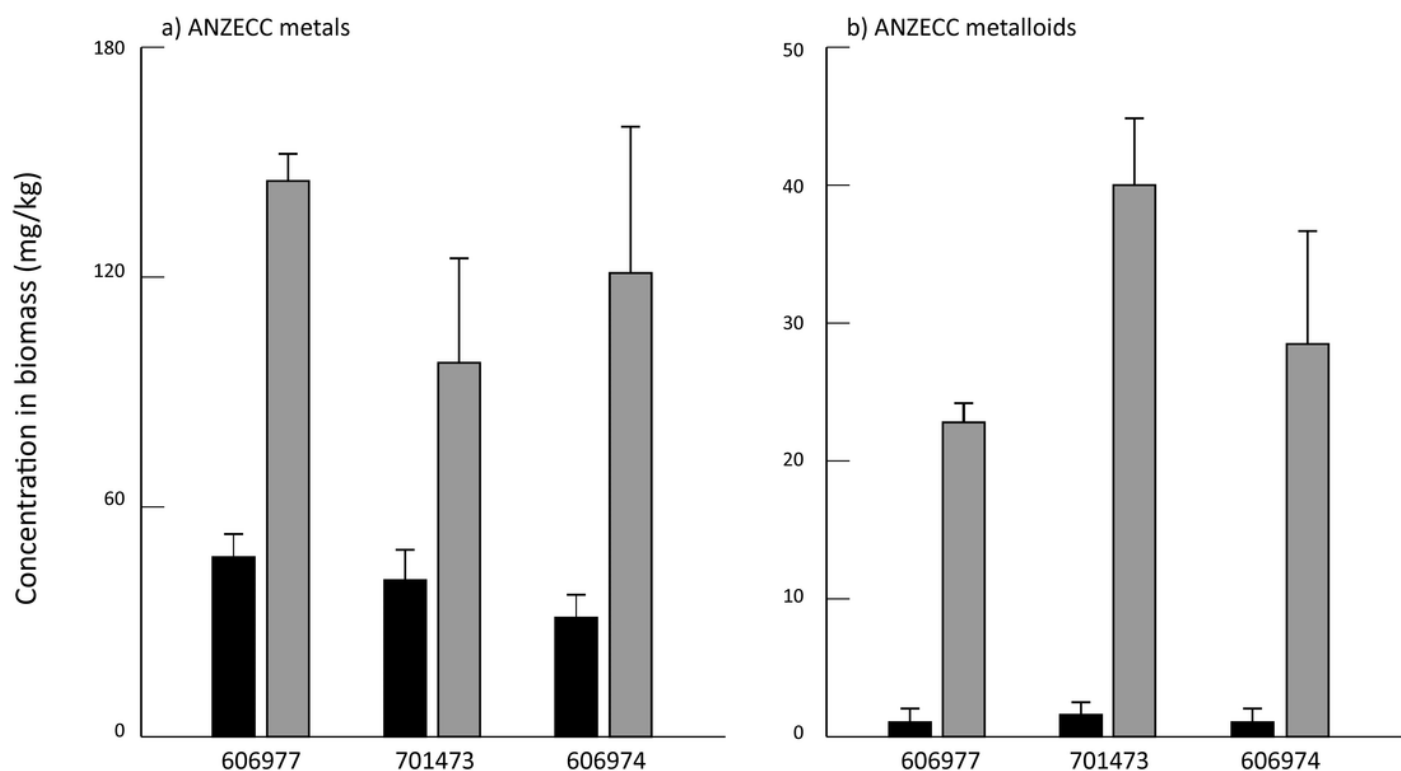
Figure 3

Figure 3

Mean concentration of metals (Al, Cd, Ni and Zn) and metalloids (As and Se) in the three *Oedogonium* species in Ash Dam Water at time 0 (black bars) and after three weeks of cultivation (grey bars). Data are means ± SE.
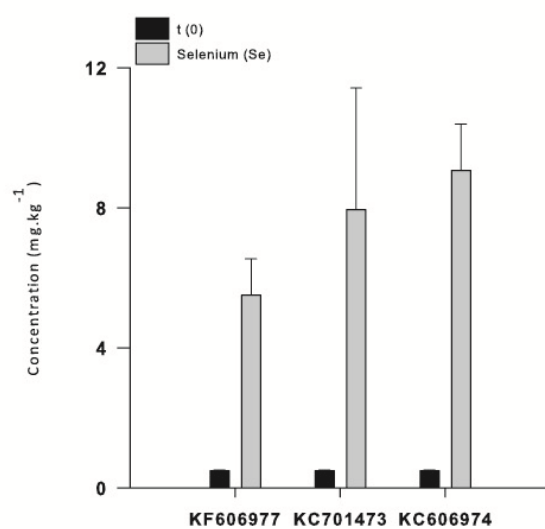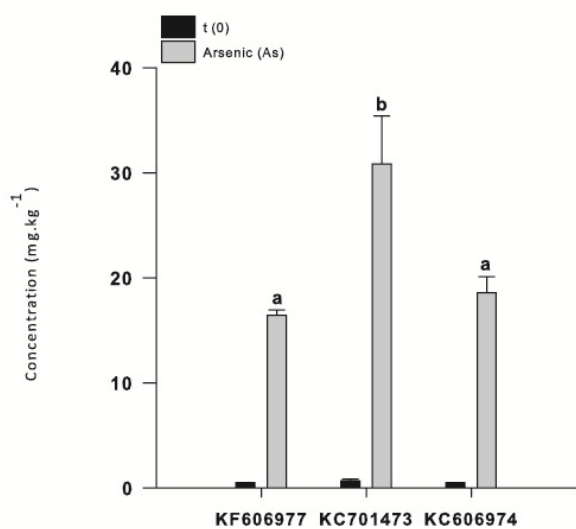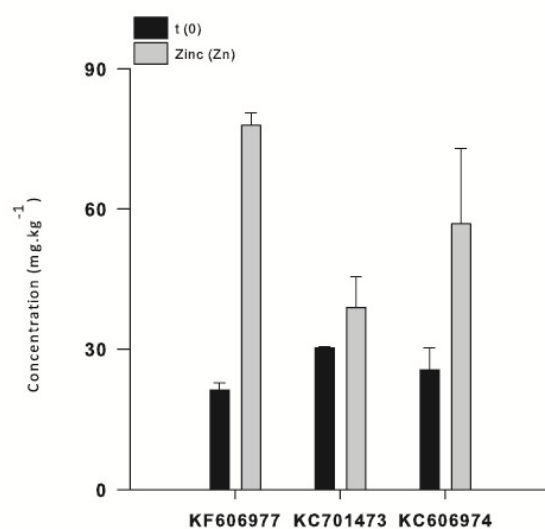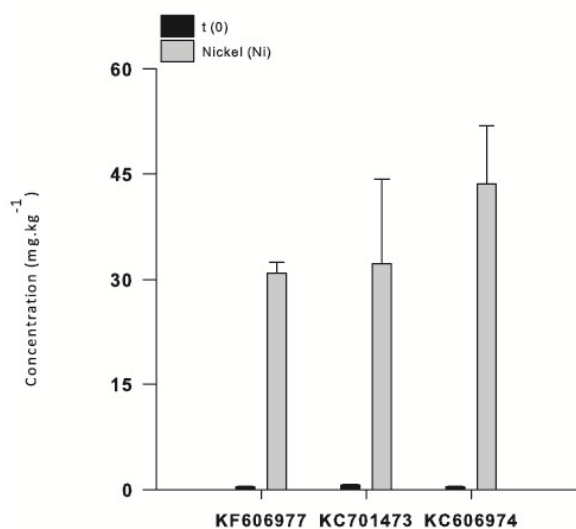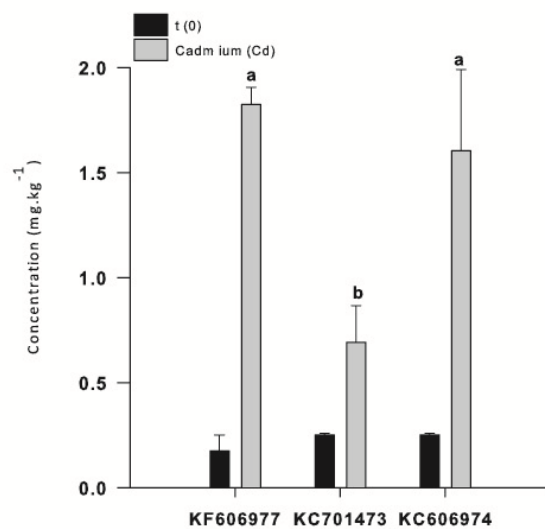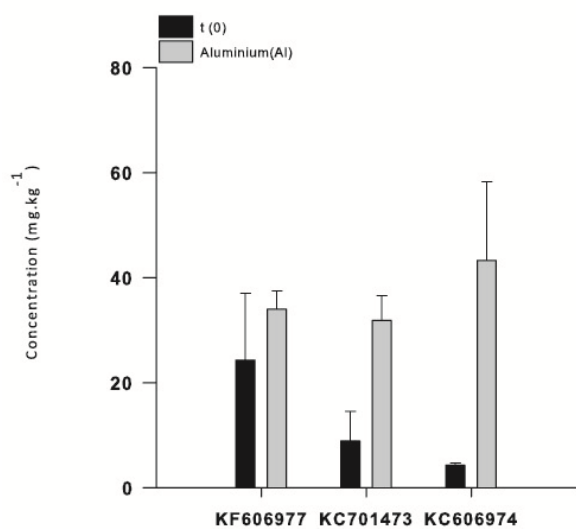
Figure 4

Figure 4

Nonmetric multidimensional scaling bi-plot of biomass elemental profiles through time.
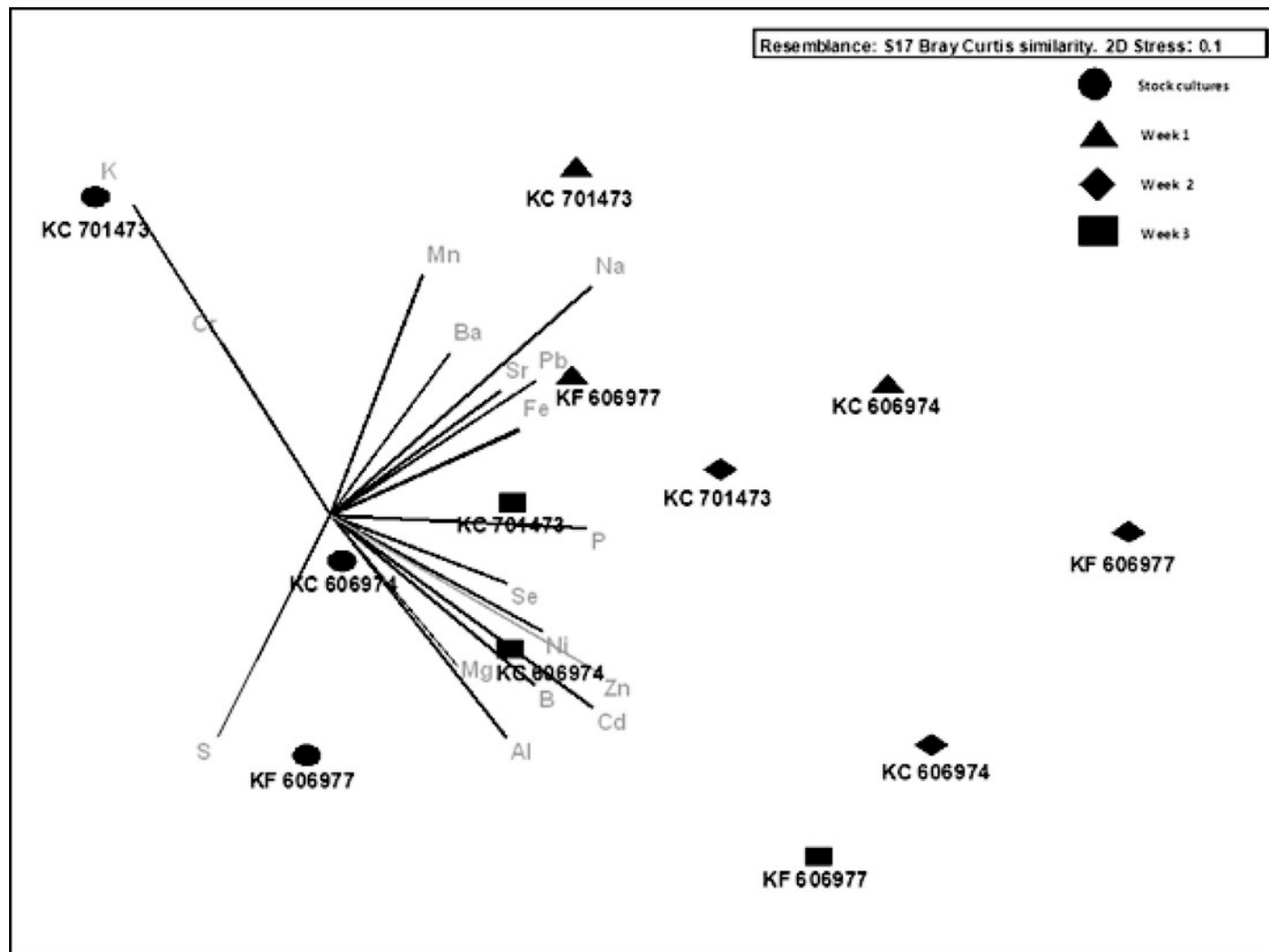
Figure 5

Figure 5

Bioaccumulation of metals (Al, Cd, Ni and Zn) and metalloids (As and Se) by the three *Oedogonium* species from Ash Dam Water through time. Data are means ± SE.