

Linear alkylbenzene sulfonate (LAS) removal in constructed wetlands: The role of plants in the treatment of a typical pharmaceutical and personal care product

Thomas, Rhian; Gough, Rachel; Freeman, Christopher

Ecological Engineering

Published: 01/09/2017

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Thomas, R., Gough, R., & Freeman, C. (2017). Linear alkylbenzene sulfonate (LAS) removal in constructed wetlands: The role of plants in the treatment of a typical pharmaceutical and personal care product. *Ecological Engineering*, 106(part A), 415-422.

Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 Linear alkylbenzene sulfonate (LAS) removal in constructed wetlands: The role of plants

2 Rhian Thomas^{1*}, Rachel Gough¹, Christopher Freeman¹

3 ¹ School of Biological Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK

4 *Corresponding author: rh_tom@hotmail.com.

5 **Abstract**

6 Linear alkylbenzene sulphonate (LAS) is a major anionic surfactant used in detergents worldwide and
7 as such is a ubiquitous constituent of domestic and municipal wastewaters. Increasingly, constructed
8 wetlands are being employed as a low cost and sustainable alternative to traditional wastewater
9 treatment processes. Plants are known to play a vital role both directly and indirectly in the removal
10 of contaminants in wastewater treatment constructed wetlands. However, relatively little research
11 has been conducted into the manipulation of the plant component in order to optimise constructed
12 wetland performance. Furthermore, little is known about the role of plants in the removal of specific
13 contaminants including LAS. The present study investigated the effects of plant biomass and plant
14 species on LAS removal in a series of experimental subsurface flow wetlands. Our results confirm that
15 the presence of vegetation enhances LAS removal, with higher biomass systems associated with
16 higher LAS removal rates. Differences in LAS removal were also observed between different plant
17 species, although these were not found to be statistically significant.

18 **Key words:** biomass; constructed wetlands; enzymes; linear alkylbenzene sulphonate (LAS); plant
19 species; wastewater.

20 **Introduction**

21 Constructed wetlands are designed to mimic the biogeochemical characteristics and functions of a
22 natural wetland under more controlled conditions. On account of their natural nutrient cycling
23 capacity, the application of constructed wetlands for sewage treatment has been popular, with over
24 50,000 systems reported to be in operation in Europe (Wu *et al.* 2015), usually in a secondary or
25 tertiary capacity. The effectiveness of the wetland is based on various complex physical, chemical and
26 biological processes occurring in parallel between the substrate, plants and microorganisms. Surveys
27 on the removal of various pollutants in constructed wetlands have been conducted globally, e.g. 5-
28 day Biochemical Oxygen Demand (BOD₅) (Sankararajan *et al.* 2017), phosphate (Ramasahayam *et al.*
29 2014), nitrate (Wu *et al.* 2014) and metals (Šíma *et al.* 2016). To date however, relatively few studies
30 have considered the role of constructed wetlands in the removal of surfactants.

31 Surfactants are surface-active compounds that consist of both polar and non-polar parts (Swisher
32 1987). These compounds are widely used in detergents due to their unique surface-active properties
33 (Swisher 1987) and are therefore a major component of urban wastewaters. Linear alkylbenzene
34 sulphonate (LAS) is a major anionic surfactant used in detergents worldwide due to its effectiveness,
35 cost/performance ratio, versatility and environmental safety record (de Wolfe & Feijtel 1998). It is the
36 most widely used synthetic anionic surfactant and is therefore an omnipresent water contaminant
37 (Vymazal 2014). It can also be used as an indicator of the presence of other pharmaceuticals and
38 personal care products (PPCPs) in surface waters (Nakada *et al.* 2008). The surfactant was introduced
39 in the 1960s as a replacement for slowly degradable alkylbenzene sulphonate (ABS). Foaming
40 problems in sewage treatment plants, rivers and lakes mainly due to ABS are well documented (Jensen

41 1999). Since the foaming problems of the 1960s, regulations have been introduced stipulating that
42 surfactants released into the environment must exhibit high biodegradation capacities. Only limited
43 research has been conducted into the fate of LAS and other surfactants in wetlands; Inaba *et al.* (1988)
44 assessed LAS removal in a large-scale natural wetland system in Japan and reported seasonal variation
45 in LAS removal due to temperature-driven changes in biodegradation by bacteria and/or adsorption
46 on sediment particles. Longer alkyl chain homologues are reported to be removed to a greater extent
47 than shorter alkyl chains (Billore *et al.* 2002; Thomas *et al.* 2003). Research has also shown that
48 shallower beds, where more oxygenated conditions occur, are associated with the highest rates of LAS
49 degradation (Huang *et al.* 2004).

50 Plants are known to play a key role in various physical, chemical and biological processes in a wetland.
51 For example, they serve to stabilize the bed surface, insulate against freezing and frost through litter
52 production, prevent clogging, shield algae from incoming solar radiation, adsorb and store nutrients,
53 and prevent channeled flow (Brix 1997, Kadlec & Knight 1996). However, there remains a lack of
54 knowledge and quantitative data on the role of plants in wastewater treatment with information
55 mainly centered on nutrient rather than pollutant removal. Debate has arisen over the necessity of
56 plants and adverse impacts reported in some cases e.g. acid mine drainage treatment (King & Garey
57 1999). However, the impact plants have will depend on the individual constructed wetlands in terms
58 of their design, loading, type of treatment and environmental conditions (Vymazal 2009, Carballeira
59 *et al.* 2016). Published research suggests that greater LAS removal occurs when plants are present
60 (Federle & Schwab 1989). The present study aims to develop a more comprehensive understanding
61 of the role of plants in LAS removal by investigating the influence of plant biomass and plant species
62 on LAS removal in constructed wetlands.

63 This paper presents the results of a 6-month field based study comparing planted and unplanted
64 systems in mesocosm experimental subsurface flow wetlands. Plant biomass (zero, low and high)
65 effects were assessed at the same field site over a 15-day experiment. Finally, a microcosm laboratory
66 experiment was conducted to assess the effect of plant species on LAS removal. Substrate enzyme
67 activity was also compared between treatments.

68 **Methods**

69 ***Experiment 1: LAS removal in planted and unplanted mesocosm systems***

70 Eight identical sub-surface flow wetland mesocosms (Figure 1) were constructed at an outdoor site in
71 Abergwyngregyn, north Wales (grid ref. SH 655736). The mesocosms measured 1.95 m (l) x 0.65 m (w)
72 x 0.4 m (d) and were filled with gravel (approximately 5-10 mm diameter) to a depth of 0.38 m. Four
73 of the mesocosms were planted with *Phragmites australis* at a density of 4 plants per m² and the
74 remaining 4 mesocosms were left unplanted. The design incorporated 2 large storage tanks, each
75 connected *via* smaller storage tanks to 4 mesocosms arranged in parallel. Flow was controlled using a
76 tap and ball cock valve system. Inflow rates of 35 L day⁻¹ of 5 mg L⁻¹ LAS in distilled water were applied
77 on a continuous basis to mimic full-scale operational constructed wetlands flow and LAS loading at a
78 typical wastewater treatment plant. The theoretical hydraulic residence time (nHRT) was 13.8 days.
79 Outflow water from each mesocosm was sampled on a monthly basis over a 6-month period
80 (September 2000 to February 2001). The addition of LAS-spiked water began at the start of September
81 2000 with the first samples taken at the end of the month. LAS concentration measurements were

82 conducted on outflow water. Enzyme (phosphatase, β -glucosidase and sulphatase) measurements
83 were conducted on 5 replicate gravel grab samples (150 cm³/ 5-10 cm depth) collected monthly from
84 each mesocosm.

85 ***Experiment 2: Effect of plant biomass on LAS removal***

86 The mesocosms described in experiment 1 were also used to investigate the effect of plant biomass
87 (zero, low and high) on LAS removal. Artificial sewage (see Table 1) containing 10 mg L⁻¹ LAS (simulating
88 normal – high LAS loading) was loaded onto the mesocosms continuously over a 15 day period (6-20th
89 June 2001) at a rate of 35 L day⁻¹ (nHRT = 13.8 days). Sampling of outflow water began 1 day after the
90 start of the treatment, with subsequent samples collected every 2 days. LAS concentration
91 measurements were conducted on outflow water. As with experiment 1, gravel substrate samples
92 (150 cm³) were also collected for enzyme (phosphatase, β -glucosidase and sulphatase) analyses. KBr
93 (1.5 mg L⁻¹ Br⁻) was added to the water supply tank at the start of the experiment, as a chemical tracer
94 to assess the hydraulic retention time.

95 ***Experiment 3: Effect of plant species on LAS removal***

96 Small-scale replicate wetland microcosms were built to compare the effect of 5 different plant species
97 on LAS removal. The microcosms were constructed from transparent plastic beakers (11.5 cm
98 diameter x 13 cm depth) filled with gravel substrate and planted with a single specimen (*Phragmites*
99 *australis*, *Typha latifolia*, *Salix viminalis*, *Iris* and *Juncus effusus*). Unplanted microcosms acted as a
100 control. The microcosms (4 replicates per treatment) were stored in a temperature-controlled room
101 maintained at 12°C. 350 mL of artificial sewage containing 10 mg L⁻¹ LAS was added to each microcosm
102 at the beginning of the experiment. Sampling of water began 1 day after the addition of the sewage
103 solution and continued on a daily basis until day 4, after which samples were collected every 4 days.
104 Filters (cut off 2.5 mL Plastipak™ syringes packed with glass wool) were inserted as a sample port in
105 the top of each microcosm. LAS concentration measurements were conducted on collected water.

106 ***Determination of LAS concentration***

107 *LAS Analytical Procedure*

108 Quantification of LAS was based on the procedure developed by Matthijs & De Henau (1987), but
109 modified slightly to improve selectivity. Prior to analysis, samples were filtered through a 0.2 μ m
110 Whatman membrane filter. Solid Phase Extraction (SPE) was used to isolate and concentrate the LAS
111 in the aqueous samples before HPLC analyses. Each sample was initially passed through a Hypersep
112 C18 SPE column and then eluted with methanol onto a Hypersep SAX Anion Exchange SPE column
113 (both Thermo Fisher Scientific, Waltham MA, US). LAS was then eluted into a glass vial with 3 mL of
114 CH₃OH:HCl solution (80:20) and evaporated to dryness at 75°C under a gentle stream of nitrogen. The
115 samples were stored in a dry state at <4°C before analyses. In order to minimise contamination all
116 glassware was washed in methanol then conditioned with LAS solution for 24 h prior to use to reduce
117 loss of surfactant to the glass surface.

118 *HPLC Analyses*

119 Separation of LAS homologues was achieved by reversed phase separation using a Dionex DX-300
120 HPLC system (Thermo Fisher Scientific, Waltham MA, US) equipped with a μ Bondclone C18 analytical
121 column (Phenomenex, Torrance CA, US). Measurement was using a LS50 fluorescence spectrometer
122 (PerkinElmer, Waltham MA, US) (excitation λ = 232 nm; emission λ = 290 nm; slit width = 10 nm). The
123 mobile phase was a 22:78 distilled water:methanol solution containing sodium perchlorate buffer
124 (0.0875 M) with the flow rate set to 2 mL min⁻¹. Calibration standards were Nansa HS 80/S
125 alkylbenzene sulfonic acids containing C₁₀-C₁₃ LAS homologues (with alkyl chain distributions of of C₁₀
126 15.8%; C₁₁ 41.5%; C₁₂ 30.1%; C₁₃ 12.5%). LAS concentrations were derived by addition of the C₁₀-C₁₃
127 LAS homologue concentrations.

128 ***Enzyme assays***

129 Activities of three hydrolytic enzymes (β -glucosidase, sulphatase and phosphatase) were determined
130 in 5 replicate gravel samples from each mesocosm using fluorogenic methylumbelliferyl (MUF)
131 substrates (Freeman *et al.* 1995). 2 mL of cellosolve (ethylene glycol monoethyl ether) was used to
132 pre-dissolve all MUF substrates for each assay as substrates have minimal solubility in pure water.
133 Cellosolve does not affect enzyme activity (Hoppe, 1983).

134 In a plastic stomacher bag, 7 mL of MUF substrate was added to 1 g of gravel sample, homogenised
135 using a Seward Stomacher 80 Laboratory Blender and incubated at field temperature for 1 h. The
136 reaction was terminated by centrifuging the mixture at 10,000 rpm for 5 min. 0.5 mL of supernatant
137 was then added to 2.5 mL of deionised water and fluorescence determined with a LS50 fluorescence
138 spectrometer (PerkinElmer, Waltham MA, US) (excitation λ = 330 nm; emission λ = 450 nm; slit width
139 = 2.5 cm). Calibration curves were constructed using 0-100 μ M MUF-free acid solution and assayed as
140 above.

141 ***Determination of KBr tracer***

142 A Dionex DX-120 Ion Chromatograph equipped with an IonPac AS4A anion analytical column was used
143 to measure the concentration of bromide. The eluent was 1.7 mM Na₂HCO₃/1.8 mM Na₂CO₃. The
144 column was calibrated using standard Dionex solutions and a flow rate of 1 mL min⁻¹ was used.

145 ***Statistical Analysis of Results***

146 Statistical analyses were conducted using Minitab™ version 13.1 (Minitab Inc. 2000). Differences
147 between planted and unplanted treatments were assessed *via* paired t-tests. For differences between
148 more than two treatments (species and biomass), repeated measures ANOVA tests were applied. For
149 significant ANOVA results, the Tukey post-hoc test was used to identify were significant differences
150 between groups lay.

151 **Results**

152 ***Planted vs. unplanted mesocosms***

153 *LAS removal*

154 High LAS degradation was observed from the start of the experiment and increased with time (Figure
155 2). Outflow water LAS concentration in the unplanted mesocosms (mean 0.05 mg L⁻¹) was consistently
156 higher than in the planted mesocosms (mean 0.02 mg L⁻¹). This difference was found to be statistically
157 significant ($p < 0.01$). However, high LAS removal rates (>95%) were observed in both systems
158 throughout the course of the experiment.

159 *Enzyme activity*

160 Enzyme activity was significantly higher for phosphatase, than β -glucosidase and sulphatase, by a
161 minimum of a 2-fold factor in the planted ($F = 6.04$, $p < 0.01$) and unplanted ($F = 12.79$, $p < 0.001$)
162 mesocosms (Figure 3). Unplanted systems exhibited higher mean enzyme activity in comparison to
163 planted microcosms but this was only significant for phosphatase ($p < 0.05$). Both phosphatase and β -
164 glucosidase activity decreased from the initial activity measured in September, especially for the
165 unplanted systems (Figure 3a and 3b). In contrast, an increase in sulphatase activity from initial levels
166 after LAS addition was observed (Figure 3c).

167 ***Effect of plant biomass on LAS removal***

168 *LAS*

169 High LAS removal (>95%) was observed in all treatments with LAS concentrations increasing initially,
170 and then decreasing in the last 7 days (Figure 4). Mean LAS removal rates varied as follows: high-
171 biomass (0.08 mg L⁻¹)>low-biomass (0.36 mg L⁻¹)>unplanted (0.45 mg L⁻¹). Statistical analysis identified
172 significant differences in LAS removal rates between treatments ($F = 8.26$, $p < 0.01$). However, the
173 post-hoc test revealed no significant differences between the unplanted and low biomass planted
174 treatments.

175 *Enzyme activity*

176 Highest activity in all treatments was observed for phosphatase, followed by β -glucosidase and
177 sulphatase, respectively (Figure 5). No statistically significant correlations were identified between
178 enzymes in different treatments, except for phosphatase ($p < 0.05$), reflecting the large fluctuations
179 in activity measured. The only statistically significant differences between planted and unplanted
180 treatments was observed for sulphatase ($F = 15.192$, $p < 0.001$). Compared with the levels reported
181 for experiment 1, approximately 4-fold higher phosphatase and β -glucosidase activity was observed.
182 However, in contrast, lower sulphatase activity was observed. This was more prominent in the planted
183 (low-biomass -30%, high-biomass -60%) than unplanted (-20%) mesocosms.

184 *Tracer study*

185 Figure 6 shows the tracer study results which provide an indication of the diffusion rate. Faster
186 recovery was observed in the unplanted, compared with the low and high-biomass mesocosms,
187 respectively. The control/high-biomass comparison was statistically significant ($F = 7.756$, $p < 0.01$). A
188 slower initial diffusion rate was exhibited for the planted treatments in comparison to unplanted.

189 ***Effect of plant species on LAS removal***

190 LAS

191 The mean concentrations measured after 12 days in the six treatments were, in descending order, the
192 gravel control (1.83 mg L⁻¹), *Typha* (0.23 mg L⁻¹), *Iris* (0.12 mg L⁻¹), *Juncus* (0.10 mg L⁻¹), *Salix* (0.09 mg
193 L⁻¹) and *Phragmites* (0.08 mg L⁻¹) (Figure 7). A marked and statistically significant difference in LAS
194 concentration was observed between the unplanted and each of the planted treatments ($p < 0.001$).
195 No significant difference was identified between planted treatments.

196 **Discussion**

197 ***LAS removal in planted and unplanted systems***

198 LAS

199 The high LAS removal rates observed both in the planted and unplanted systems suggest that
200 constructed wetlands have high potential for LAS degradation under optimal conditions. However,
201 significantly higher LAS outflow concentration in the unplanted system suggests that the presence of
202 vegetation enhances LAS treatment efficiency. This is consistent with the findings of previous studies
203 which report higher removal of organic matter (Allen *et al.* 2002), nutrients (Heritage *et al.* 1995),
204 heavy metals (Doyle & Otte 1997) and ammonia (Sikora *et al.* 1995) in planted systems. Federle &
205 Schwab (1989) reported a higher rate of LAS mineralization with microbiota associated with aqueous
206 plants in the rhizosphere than in nearby root-free sediment. This may be explained by several possible
207 plant mechanisms facilitating microbial activity, including rhizosphere oxygen release, rhizosphere and
208 root attachment sites for bacterial growth, DOC root release enhancing bacterial activity and plant
209 uptake (Brix 1994, Brix 1997). The high removal efficiency observed in the unplanted systems suggests
210 that related physical processes such as adsorption or formation of biofilms on the gravel surface
211 contribute significantly to LAS removal. Indeed Fountoulakis *et al.* (2009) report that adsorption onto
212 media is the main mechanism for LAS removal in their pilot constructed wetland systems.

213 The observed general decline in LAS concentration with time could be explained by the acclimatisation
214 of the microcosm bacterial community to the surfactant. Microbial communities acclimated by pre-
215 exposure to the surfactant are enriched in organisms capable of degrading the compound, resulting
216 shifts in community structure with increasing dominance in populations of these organisms (Federle
217 & Pastwa 1988). Previous adaptation accelerated by initial LAS degradation is reported (Larson &
218 Payne 1981, Palmisno *et al.* 1991, Federle & Pastwa 1988, Branner *et al.* 1999, Jensen 1999). Brown
219 (1995) suggests that the bacterial population can increase its capacity to degrade surfactants by, for
220 example, population growth potentially increasing the number of degraders, an increase in the
221 amount of enzyme per cell biosynthesised, or random genetic mutations increasing biodegradation
222 activity or creating new activity. Terzic *et al.* (1992) reported that the composition of a mixed bacterial
223 culture rather than total number of bacteria determined biodegradation efficiency. Larson & Payne
224 (1981) reported a shorter half-life with a 10-fold faster rate for degradation tests with river sediment
225 collected closest to the vicinity of the effluent from a sewage treatment plant, similarly suggesting
226 adaptation of communities receiving higher LAS concentrations. Shimp *et al.* (1989) reported greater

227 numbers of LAS degrading microorganisms, reduced lag period and higher degradation in water
228 samples collected from an effluent exposed site compared with a pristine control site.

229 *Enzyme activity*

230 Measurement of enzyme activity showed that greatest activity occurred for phosphatase, confirming
231 the findings of previous studies (Freeman *et al.* 1995, Chappell & Goulder 1992). The low enzyme
232 activity observed overall may reflect the low nutrient and organic carbon availability in the system.
233 The decrease in phosphatase and β -glucosidase activity over time, especially for the unplanted
234 systems, may suggest toxicity or lack of suitable substrate and nutrients.

235 Surprisingly, planted systems exhibited lower enzyme activity than unplanted systems. Previous
236 studies have shown higher phosphatase activity in planted compared with unplanted systems (Khan
237 1970, Neal 1973, Kiss *et al.* 1974 as quoted in Speir & Ross 1978). This effect may be indirect and
238 caused by changes in organic matter and microbial populations brought about by the plants with
239 highest activity when growth was most intensive (Speir & Ross 1978).

240 ***Effect of plant biomass on LAS removal***

241 *LAS*

242 The occurrence of highest LAS removal in the high biomass systems suggests that wetlands with a high
243 plant biomass ratio will promote LAS removal to a greater degree than comparative low plant biomass
244 ratio wetlands. Knaebel & Vestal (1992) reported that the amount of above ground plant biomass
245 correlated positively with the initial rates of mineralization. Wiessner *et al.* (2002) reported that the
246 total size of the root system did not significantly affect the amount of oxygen root release but was
247 governed by the size of the above ground biomass.

248 KBr was chosen as a tracer in this study due to its stability and ease of analysis (Tanner *et al.* 1998).
249 Tanner *et al.* (1998) reported similar curves in a rain-free tracer study using bromide to those shown
250 here. The results suggest slower diffusion rates in the planted mesocosms, especially in the high
251 biomass treatment. The slower the diffusion rate, the greater the contact time between
252 microorganisms and LAS within the wetland, promoting greater biodegradation. Greater removal has
253 been reported in wetland systems with longer retention times. For example, greater removal of TN,
254 TP and COD is reported with a 5 day retention time compared with a 2.5 day retention time (Breen
255 1997). Tanner (1994) reported a positive correlation between removal and retention time. Fisher
256 (1990) and Marstener *et al.* (1996) found that plant roots markedly affected the hydraulic flow profiles
257 in the upper layers of gravel wetlands in comparison to unplanted controls (as quoted in Tanner *et al.*
258 1998). On the other hand, the faster diffusion rate for the unplanted control may suggest potential
259 short-circuiting. Gravel substrate can cause problems with non-uniform and short-circuiting flow of
260 wastewater through the wetland (King *et al.* 1997). Factors, such as clogging by solid particles, can
261 lead to preferential flow paths occurring. However, channelling within planted wetlands has also been
262 reported (Bavour *et al.* 1988 as quoted in King *et al.* 1997).

263 *Enzyme activity*

264 No significant difference between treatments in enzyme activity was observed, except for sulphatase
265 which was highest in the unplanted mesocosms. This contradicts the hypothesis that planted

266 mesocosms would exhibit higher activities. A comparison of the activity of different enzymes
267 (phosphatase> β -glucosidase>sulphatase) supports findings reported previously (Freeman *et al.* 1995).
268 Higher phosphatase and β -glucosidase activities compared with the planted/unplanted experiment
269 reflect the higher nutrient input with levels more reflective of operational wetlands. The stimulated
270 activity may also reflect the warmer temperature (Kang & Freeman 1998). Higher activity associated
271 with plant growth mechanisms reported elsewhere (Speir & Ross 1978) is unlikely as the increase was
272 observed in both planted and unplanted mesocosms.

273 Lower sulphatase activity compared with the planted/unplanted experiment may suggest inhibition
274 of this enzyme. Inhibition may be due to specific effects on microbial growth and subsequent enzyme
275 synthesis or possible modification of the active site of the enzyme protein (Dinesh *et al.* 1995).
276 Possible inhibition of enzyme activity and subsequent nutrient cycling by LAS is suggested elsewhere
277 (Jensen 1999). The greater reduction observed in the planted compared with the unplanted
278 mesocosms may suggest plant mechanisms enhancing the inhibitory effect. However, no further
279 conclusions may be drawn from the data.

280 ***Effect of plant species on LAS removal***

281 *LAS*

282 The high LAS removal rates observed in this laboratory-scale experiment (>98% in planted systems)
283 again highlights the potential for high LAS removal in constructed wetland systems. This study also
284 confirms greater LAS removal in planted treatments in comparison to the unplanted gravel control.
285 Though small in an operational context, the difference between planted systems in terms of net
286 percentage removal were significant.

287 Variations in treatment by different plant species have been reported previously (Allen *et al.* 2002,
288 Zhu & Sikora 1995) and several studies support the order of treatment efficiency reported here. For
289 example, greater removal by *Phragmites* than *Typha* for ammonia and BOD (Gersberg *et al.* 1986),
290 ammonium and nitrate (Zhu & Sikora 1995) and TN and TP (House *et al.* 1994) has been reported.
291 Gersberg *et al.* (1986) attributed this result to the enhanced ability of *Phragmites* to pass oxygen into
292 the root-zone. However, Burgoon *et al.* (1990) found *Typha* removed a significantly larger percentage
293 of BOD₅ and total phosphate than *Phragmites* and Coleman *et al.* (2001) reported that *Typha*
294 outperformed *Juncus* in wastewater treatment.

295 Although this experiment was conducted under controlled conditions, it is recognised that the effect
296 of environmental conditions on treatment performance may vary between plant species. For example,
297 *Phragmites* has an optimal pH of 2-8, *Typha* of pH 4-10 and *Juncus* of pH 5-7.5 (Reed *et al.* 1995). Plant
298 biomass will also influence treatment efficiency. *Phragmites* is reported to have much deeper root
299 penetration in gravel than *Typha* (Reed *et al.* 1995).

300 The effect of using a mixture of species on treatment efficiency has also been investigated with some
301 evidence of improved performance using mixtures compared with monocultures (Coleman *et al.*
302 2001). However, the issue of competition between species is also important since this can cause a
303 shift in species assemblage. Coleman *et al.* (2001) found that *Typha* was the superior competitor in
304 plant mixture mesocosms, whereas *Juncus* is unlikely to be competitive (Tanner 1996).

305 **Conclusion**

306 This study investigated the importance of plants in LAS removal in small-scale experimental wetlands.
307 The effect of plant biomass and species was assessed and a comparison of substrate enzyme activity
308 conducted between treatments. All of the experiments confirmed that the presence of plants
309 significantly enhanced LAS removal. No clear relationship between the presence/absence of plants
310 and analysed substrate enzyme activity was evident from the data. However, for the longer-term
311 experiment (experiment 1, lasting 6 months) and the plant species experiment (experiment 3) LAS
312 removal increased with time, possibly due to acclimatisation of the microcosm bacterial community
313 to the surfactant. Further research is necessary to develop a more comprehensive understanding of
314 the mechanisms involved in the degradation of LAS and the role of plants therein. In particular,
315 research should focus on the relative importance of biological and physical processes as well as their
316 interaction, and the impact of environmental parameters such as temperature, pH and oxygen
317 availability.

318 **Acknowledgements**

319 We would like to acknowledge the advice and assistance provided by Dr Kay Fox and Dr Naheed
320 Rehman at Unilever.

321 **References**

- 322 Allen WC, Hook PB, Biederman JA & Stein OR, 2002. Temperature and wetland species effects in
323 wastewater treatment and root zone oxidation. *Journal of Environmental Quality* 31(3), 1010-1016.
- 324 Billore SK, Ram H, Singh N, Thomas R, Nelson RM & Pare B, 2002. Treatment performance evaluation
325 of surfactant removal from domestic wastewater in a tropical horizontal subsurface constructed
326 wetland. *Proceedings of the 8th International Conference Wetland Systems for Water Pollution*
327 *Control*. University of Dar es Salaam, Tanzania, pp. 393–399.
- 328 Branner U, Merete M & Jorgensen C, 1999. Degradation of linear alkylbenzene sulfonate in soil
329 columns. *Environmental Toxicology and Chemistry* 18(8), 1772-1778.
- 330 Breen PF, 1997. The performance of vertical flow experimental wetland under a range of operational
331 formats and environmental conditions. *Water Science and Technology* 35(5), 167-174.
- 332 Brix H, 1994. Functions of macrophytes in constructed wetlands. *Water Science and Technology*
333 29(4), 71-78.
- 334 Brix H, 1997. Do macrophytes play a role in constructed treatment wetlands?. *Water Science and*
335 *Technology* 35(5), 11-17.
- 336 Brix H, Arias CA & del Bubba M, 2001. Media selection for sustainable phosphorus removal in
337 subsurface flow constructed wetlands. *Water Science and Technology* 44(11-12), 47-54.
- 338 Brown D, 1995. Introduction to surfactant biodegradation. In: *Biodegradability of surfactants* (Eds.
339 DR Karsa & MR Porter) pp. 1-27 Blackie Academic and Professional, London.
- 340 Burgoon PS, Reddy KR & DeBusk TA, 1990. Domestic wastewater treatment using emergent plants
341 cultured in gravel and plastic substrates. In: *Proceedings of the International Conference on the use*

342 of Constructed Wetlands in Water Pollution Control (Eds. PF Cooper & BC Findlater) Pergamon Press,
343 Oxford.

344 Carballeira T, Ruiz I & Soto M, 2016. Effect of plants and surface loading rate on the treatment
345 efficiency of shallow subsurface constructed wetlands. *Ecological Engineering* 90, 203-214.

346 Chappell KR & Goulder R, 1992. Epilithic extracellular enzyme activity in acid and calcareous
347 headstreams. *Archive fur Hydrobiologie* 125, 129-148.

348 Coleman J, Hench K, Garbutt K, Sexstone A, Bissonnette G & Skousen J, 2001. Treatment of domestic
349 wastewater by three plant species in constructed wetlands. *Water Air and Soil Pollution* 128(3-4),
350 283-295.

351 De Wolfe W & Feijtel T, 1998. Terrestrial risk assessment for linear alkyl benzene sulfonate (LAS) in
352 sludge-amended soils. *Chemosphere* 36(6), 1319-1343.

353 Dinesh R, Ramanathan G & Singh H, 1995. Influence of chloride and sulfate ions on soil enzymes.
354 *Journal of Agronomy and Crop Science* 175(2), 129-133.

355 Doyle MO & Otte ML, 1997. Organism-induced accumulation of iron, zinc and arsenic in wetland
356 soils. *Environmental Pollution* 98(1), 1-11.

357 Dunbabin JS & Bowmer KH, 1992. Potential use of constructed wetlands for treatment of industrial
358 wastewaters containing metals. *Science of the Total Environment* 111, 151-168.

359 Federle TW & Pastwa GM, 1988. Biodegradation of surfactants in saturated subsurface sediments: A
360 field study. *Ground Water* 26(6), 761-770.

361 Federle TW & Schwab BS, 1989. Mineralization of surfactants by microbiota of aquatic plants.
362 *Applied and Environmental Microbiology* 55(8), 761-770.

363 Fountoulakis MS, Terzakis S, Kalogerakis N & Manios T, 2009. Removal of polycyclic aromatic
364 hydrocarbons and linear alkylbenzene sulfonates. *Ecological Engineering* 35, 1702-1709.

365 from domestic wastewater in pilot constructed wetlands and a gravel filter

366 Freeman C, Liska G, Ostle NJ, Jones SE & Lock MA, 1995. The use of fluorogenic substrates for
367 measuring enzyme activity in peatlands. *Plant and Soil* 175, 147-152.

368 Gersberg RM, Elkins BV, Lyon SR & Goldman CR, 1986. Role of aquatic plants in wastewater
369 treatment by artificial wetlands. *Water Research* 20(3), 363-368.

370 Hammer DA & Knight RL, 1994. Designing constructed wetlands for nitrogen removal. *Water Science*
371 *and Technology* 29(4), 15-27.

372 Heritage A, Pistillo P, Sharma KP & Lantzke IR, 1995. Treatment of primary-settled urban sewage in
373 pilot-scale vertical flow wetland filters: comparison of four emergent macrophyte species over a 12-
374 month period. *Water Science and Technology* 32(3), 295-304.

375 House CH, Broome SW & Hoover MT, 1994. Treatment of nitrogen and phosphorus by a constructed
376 upland-wetland wastewater treatment system. *Water Science & Technology* 29(4), 177-184.

377 Huang Y, Lattore A, Barceló D, García J, Aguirre P, Mujeriego R & Bayona JM, 2004. Factors affecting
378 linear alkylbenzene sulfonates removal in subsurface flow constructed wetlands. *Environmental*
379 *Science & Technology* 38, 2657–2663.

380 Inaba K, Iwasaki K & Osami Y, 1988. A method for behaviour analyses of synthetic chemicals in the
381 aquatic environment using their adsorption constants – a study of linear alkylbenzenesulfonate in
382 wetland. *Environmental Technology Letters* 9, 1387-1392.

383 Jensen J, 1999. Fate and effects of linear alkyl benzene sulfonates (LAS) in the terrestrial
384 environment. *Science of the Total Environment* 226, 93-111.

385 Kadlec RH & Knight RL, 1996. *Treatment wetlands*, Lewis Publishers, Boca Raton.

386 Kang H & Freeman C, 1998. Measurements of cellulose and xylosidase activities in peat using a
387 sensitive fluorogenic compound assay. *Communications in Soil Science and Plant Analysis*
388 29(17&18), 2769-2774.

389 Khan SU, 1970. Enzymatic activity in a grey wooded soil as influenced by cropping systems and
390 fertilisers. *Soil Biology and Biochemistry* 2, 137-139.

391 King GM & Garey MA, 1999. Reffic iron reduction by bacteria associated with the roots of freshwater
392 and marine macrophytes. *Applied and Environmental Microbiology* 65(10), 4393-4398.

393 King AC, Mitchell CA & Howes T, 1997. Hydraulic tracer studies in a pilot scale subsurface flow
394 constructed wetland. *Water Science and Technology* 35(5), 189-196.

395 Knaebel DB & Vestal JR, 1992. Effects of intact rhizosphere microbial communities on the
396 mineralization of surfactants in surface soils. *Canadian Journal of Microbiology* 38, 643-653.

397 Larson RJ & Payne AG, 1981. Fate of the benzene ring of linear alkylbenzene sulfonate in natural
398 waters. *Applied and Environmental Microbiology* 41(3), 621-627.

399 Matthijs e & De Henau, 1987. Determination of LAS. *Tenside Surfactants Detergents* 22, 193-198.

400 Nakada N, Kiri K, Shinohara H, Harada A, Kuroda K, Takizawa S & Takada H, 2008. Evaluation of
401 pharmaceuticals and personal care products as water-soluble molecular markers of sewage.
402 *Environmental Science & Technology* 42(17), 6347-53.

403 Neal JR, 1973. Influnc of selected grasses and herbs on soil phosphate activity. *Canadian Journal of*
404 *Soil Science* 53, 119-121.

405 Palmisno AC, Schwab BS, Maruscik DA & Ventullo RM, 1991. Seasonal-change in mineralization of
406 xenobiotics by stream microbial communities. *Canadian Journal of Microbiology* 37(12), 939-948.

407 Ramasahayam SK, Guzman L, Gunawan G & Viswanathan T, 2014. A comprehensive review of
408 phosphorus removal technologies and processes. *Journal of Macromolecular Science, Part A.* 51(6),
409 538-45.

410 Reed SC, Crites RW & Middlebrooks EJ, 1995. Natural systems for wastewater management and
411 treatment, McGraw-Hill Inc., London.

412 Sankararajan V, Neelakandhan N & Chandrasekaran S, 2017. Modeling of constructed wetland
413 performance in BOD5 removal for domestic wastewater under changes in relative humidity using
414 genetic programming. *Environmental Monitoring and Assessment* 189(4), 164.

415 Shimp RJ, Schwab BS & Larson RJ, 1989. Adaption to a quaternary ammonium surfactant by
416 suspended microbial communities in a model stream. *Environmental Toxicology and Chemistry* 8(8),
417 723-730.

418 Sikora FJ, Tong Z, Behrends LL, Steinberg SL & Coonrod HS, 1995. Ammonium removal in constructed
419 wetlands with recirculating subsurface flow: Removal rates and mechanisms. *Water Science and*
420 *Technology* 32(2), 193-202.

421 Šíma J, Svoboda L & Pomijová Z, 2016. Removal of Selected Metals from Wastewater Using a
422 Constructed Wetland. *Chemistry & biodiversity* 13(5), 582-90.

423 Speir TW & Ross DJ, 1978. Soil phosphate and sulphatase. In: *Soil enzymes* (ed. RG Burns) pp. 198-
424 250, Academic Press, London.

425 Swisher RD, 1987. *Surfactant biodegradation*, Marcel Dekker Inc., New York, 2nd edn.

426 Tanner CC, 1994. Treatment of dairy farm wastewaters in horizontal and up-flow gravel-bed
427 constructed wetlands. *Water Science and Technology* 29(4), 85-93.

428 Tanner CC, 1996. Plants for constructed wetland treatment systems – a comparison of growth and
429 nutrient uptake of eight emergent species. *Ecological Engineering* 7, 59-83.

430 Tanner CC, Sukias JPS & Upsdall MP, 1998. Relationship between loading rates and pollutant removal
431 during maturation of gravel-bed constructed wetlands. *Journal of Environmental Quality* 27, 448-458.

432 Terzic S, Hrsak D & Ahel M, 1992. Primary biodegradation kinetics of linear alkylbenzene sulfonate in
433 estuarine waters. *Water Research* 26(5), 585-591.

434 Thomas R, Freeman C, Rehman N & Fox K, 2003. Removal of linear alkylbenzene sulphonate (LAS) in
435 constructed wetlands. In: Vymazal, J. (Ed.), *Wetlands-Nutrients, Metals and Mass Cycling*. Backhuys
436 Publishers, Leiden, The Netherlands, pp. 35–47.

437 Vymazal J, 2009. The use constructed wetlands with horizontal sub-surface flow for various types of
438 wastewater. *Ecological Engineering* 35, 1-17.

439 Vymazal J, 2014. Constructed wetlands for treatment of industrial wastewaters: A review. *Ecological*
440 *Engineering* 73, 724-751.

441 Wiessner A, Kusch P & Stottmeister U, 2002. Oxygen release by roots of *Typha latifolia* and *Juncus*
442 *effuses* in laboratory hydroponic systems. *Acta Biotechnologica* 22(1-2), 209-216.

443 Wu S, Kuschk P, Brix H, Vymazal J & Dong R, 2014. Development of constructed wetlands in
444 performance intensifications for wastewater treatment: a nitrogen and organic matter targeted
445 review. *Water Research* 57, 40-55.

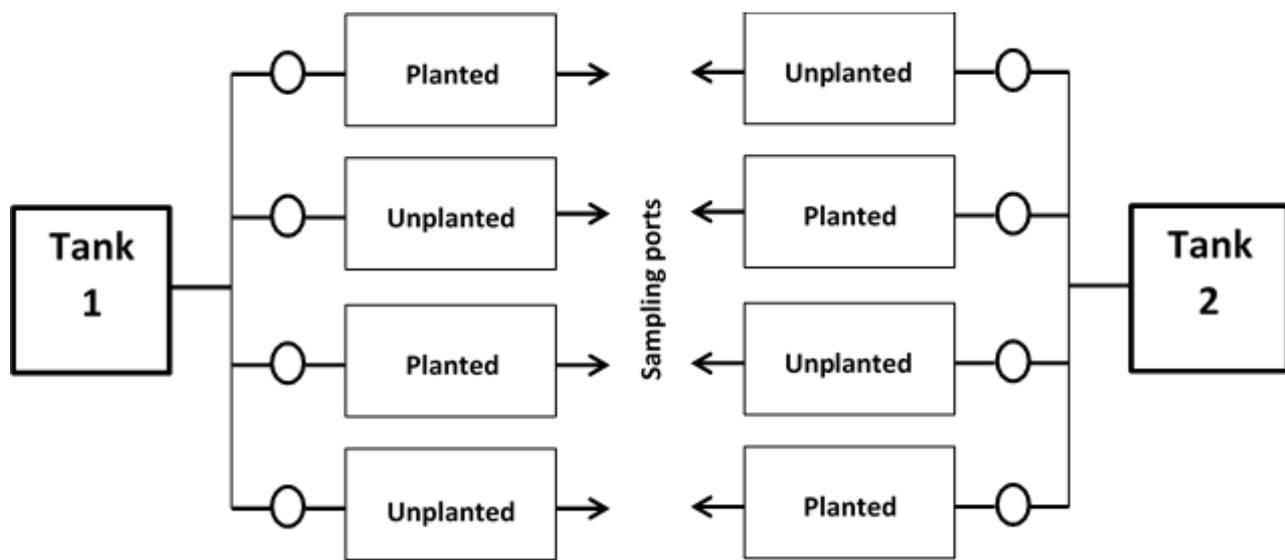
446 Wu H, Zhang J, Ngo HH, Guo W, Hu Z, Liang S, Fan J & Liu H, 2015. Review on the sustainability of
447 constructed wetlands for wastewater treatment: Design and operation. *Bioresource Technology* 175,
448 594-601.

449 Zhu T & Sikora FJ, 1995. Ammonium and nitrate removal in vegetated and unvegetated gravel bed
450 microcosm wetlands. *Water Science and Technology* 32(3), 219-228.

451

452

453



455

456

Figure 1. Constructed wetland mesocosm setup used for experiments 1 and 2

457

458

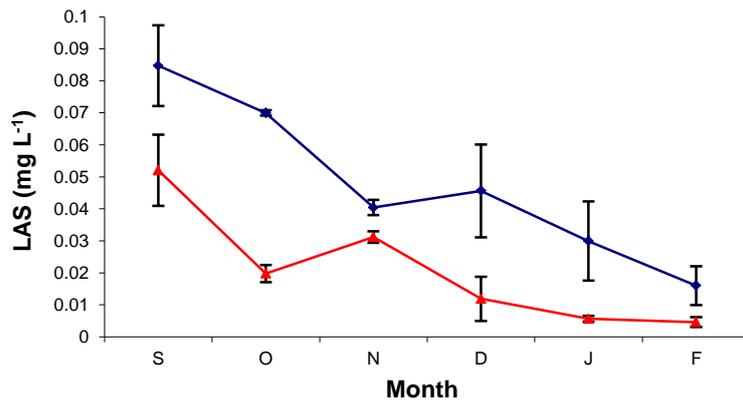
Table 1. Recipe for artificial sewage solution

Ingredient	Concentration (mg L ⁻¹)
Peptone	70
Urea	25
Sucrose	35
Soluble starch	35
Ammonium sulphate	140
Mixed acids	105
Potassium hydrogen phosphate	28
Ferrous ammonium sulphate	21
Trace metals solution	1 mL

Acid	Concentration (mg L ⁻¹)
Sodium acetate	136
Sodium propionate	28
Sodium butyrate	12
Sodium benzoate	100
Sodium citrate	44

Metal	Concentration (mg L ⁻¹)
CuCl ₂ .2H ₂ O	0.25
Co(NO ₃).6H ₂ O	0.25
Na ₂ B ₄ O ₇ .10H ₂ O	0.25
ZnCl ₂ .2H ₂ O	0.25
MnSO ₄ .H ₂ O	1.00
K ₂ Mo ₄	0.25
NH ₄ VO ₃	0.10

459



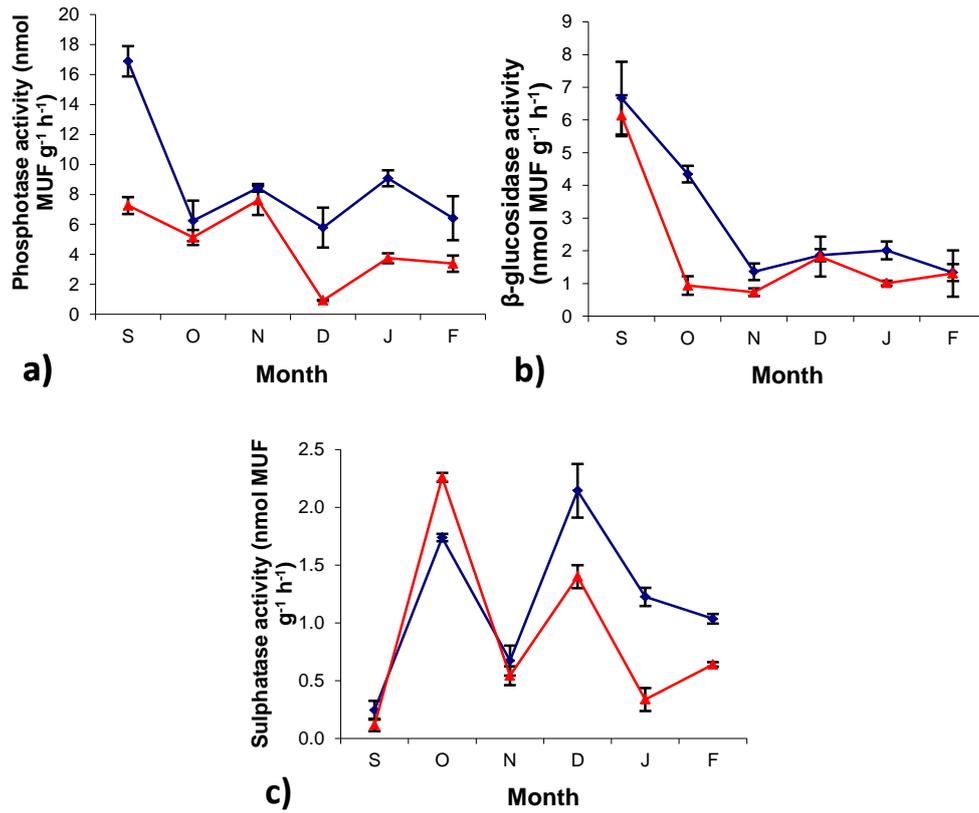
460

461 Figure 2. Outflow LAS concentrations in planted (red) and unplanted (blue) mesocosms between
 462 September and February. Error bars represent the standard error of the mean ($n = 4$).

463

464

465



466

467

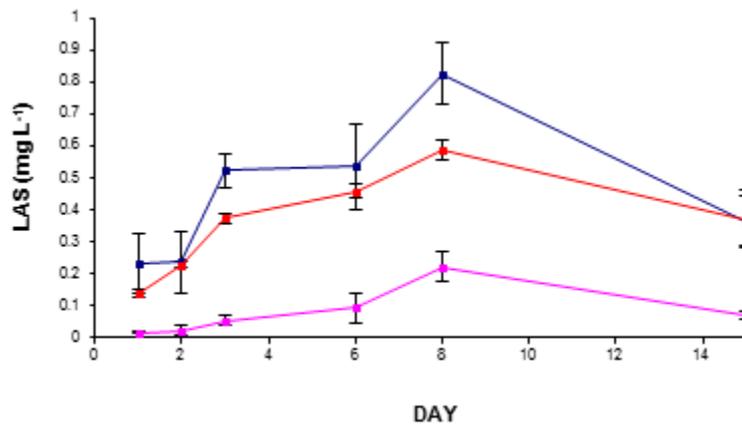
468

469

470

471

Figure 3. Enzyme activity in planted (red) and unplanted (blue) mesocosms between September and February showing phosphatase (a), β -glucosidase (b) and sulphatase (c). Error bars represent the standard error of the mean ($n = 4$).

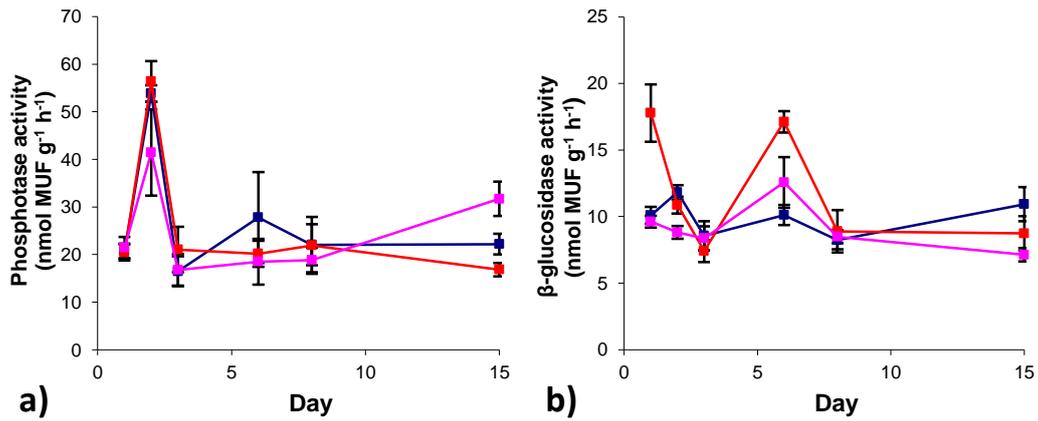


472

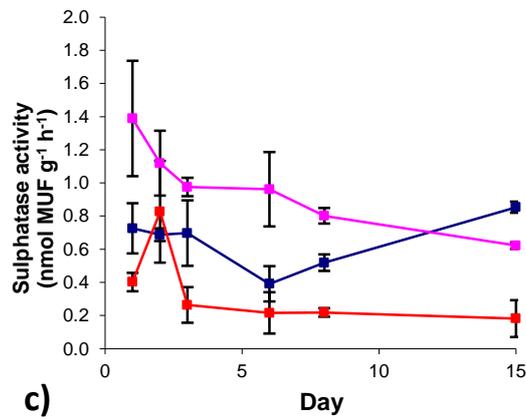
473

474

Figure 4. Outflow LAS concentrations for high density (pink), low density (red) and control (blue) plant biomass treatments. Error bars represent the standard error of the mean ($n = 4$).



475



476

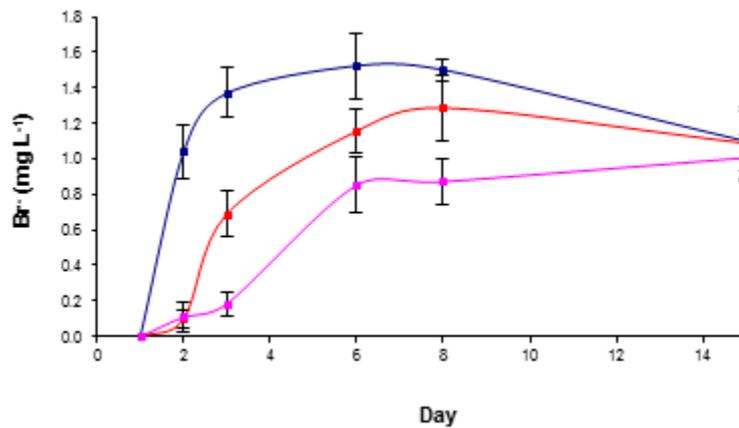
477

Figure 5. Enzyme activity in high density (pink), low density (red) and control (blue) plant biomass treatments showing phosphatase (a), β -glucosidase (b) and sulphatase (c). Error bars represent the standard error of the mean ($n = 4$).

478

479

480



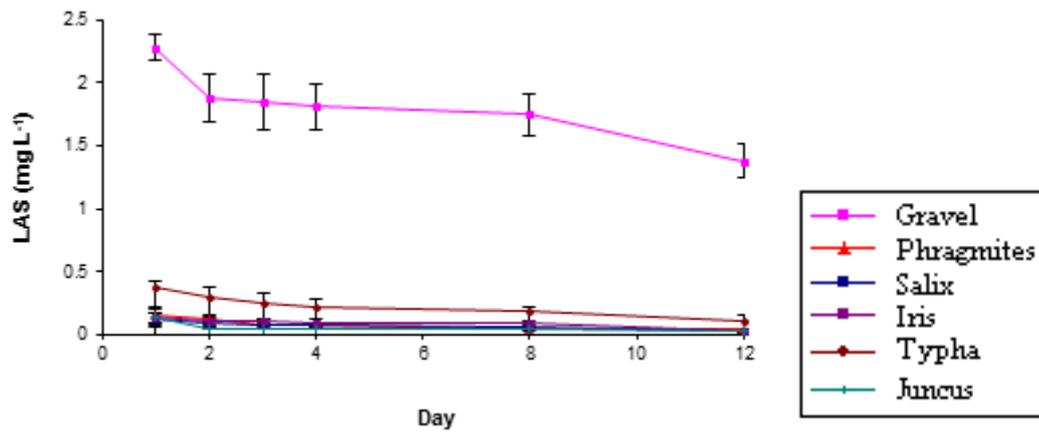
481

482

Figure 6. Br^- tracer study results showing Br^- concentration for the high density (pink), low density (red) and control (blue) plant biomass treatments. Error bars represent the standard error of the mean ($n = 4$).

483

484



485

486

487

Figure 7. Outflow LAS concentrations planted mesocosms and gravel control. Error bars represent the standard error of the mean ($n = 4$).