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

Thomas, Rhian; Gough, Rachel; Freeman, Christopher

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

2   Rhian Thomas[1]*, Rachel Gough[1], Christopher Freeman[1]

3   *[1] School of Biological Sciences, Bangor University, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK*
4   *Corresponding author: rh_tom@hotmail.com.

5   <u>**Abstract**</u>

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   <u>**Introduction**</u>

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 (BOD$_5$) (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 m^2 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 cm^3/ 5-10 cm depth) collected monthly from
84   each mesocosm.

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 cm^3) 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.

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.

Determination of LAS concentration

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 μm
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 CH$_3$OH: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.

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 μBondclone 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 C_{10}-C_{13} LAS homologues (with alkyl chain distributions of of C_{10}
126 15.8%; C_{11} 41.5%; C_{12} 30.1%; C_{13} 12.5%). LAS concentrations were derived by addition of the C_{10}-C_{13}
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 μM 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 Na_2HCO_3/1.8 mM Na_2CO_3. 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 rhizophere 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 BOD_5 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 effciciency. *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**

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

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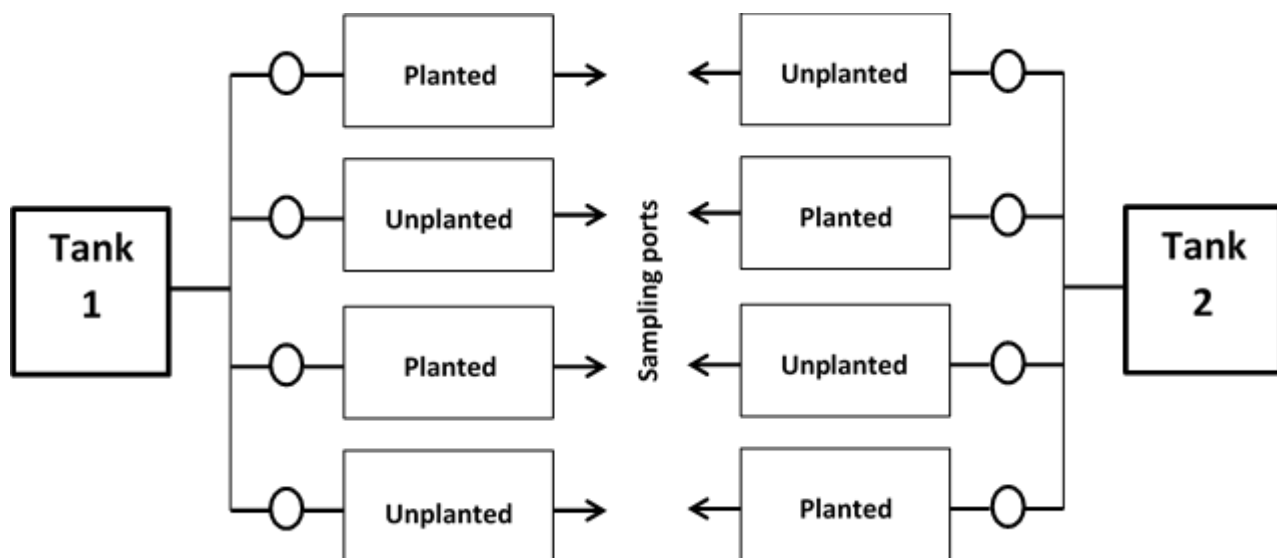
456   Figure 1. Constructed wetland mesocosm setup used for experiments 1 and 2

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})
$CuCl_2.2H_2O$	0.25
$Co(NO_3).6H_2O$	0.25
$Na_2B_4O_7.10H_2O$	0.25
$ZnCl_2.2H_2O$	0.25
$MnSO_4.H_2O$	1.00
K_2Mo_4	0.25
NH_4VO_3	0.10

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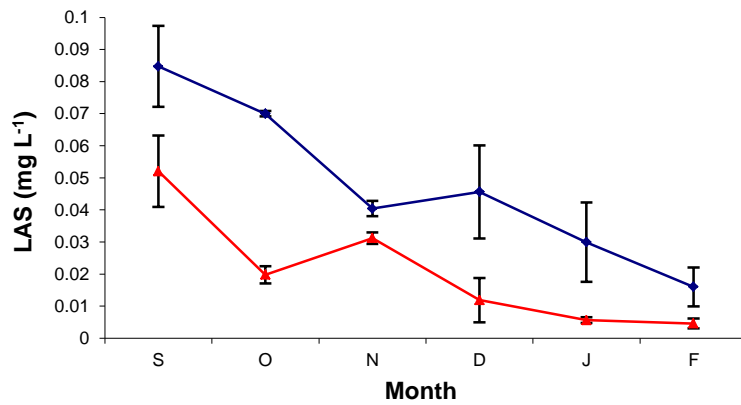
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461   Figure 2. Outflow LAS concentrations in planted (red) and unplanted (blue) mesocosms between
462   September and February. Error bars represent the standard error of the mean (n = 4).
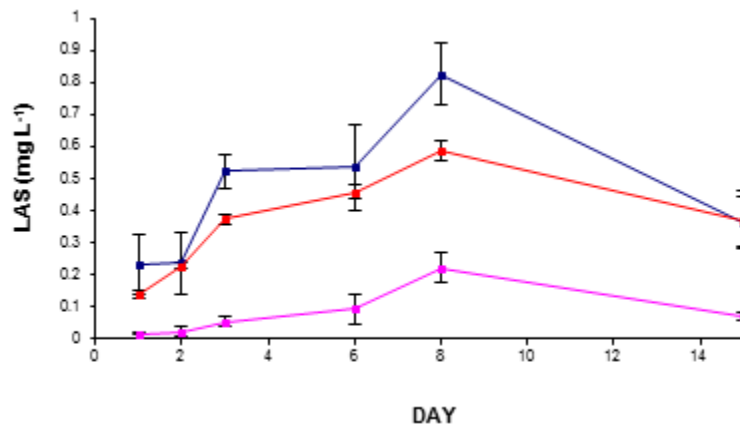
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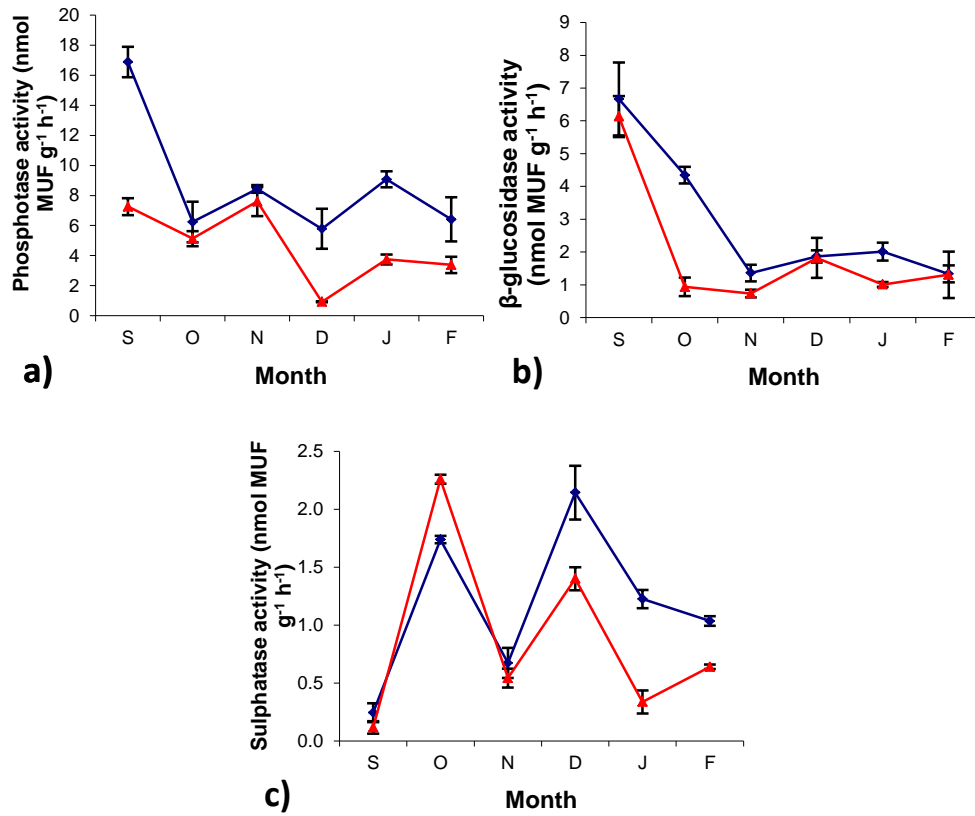
Figure 3. Enzyme activity in planted (red) and unplanted (blue) mesocosms between September and February showing phosphotase (a), β-glucosidase (b) and sulphatase (c). Error bars represent the standard error of the mean (n = 4).
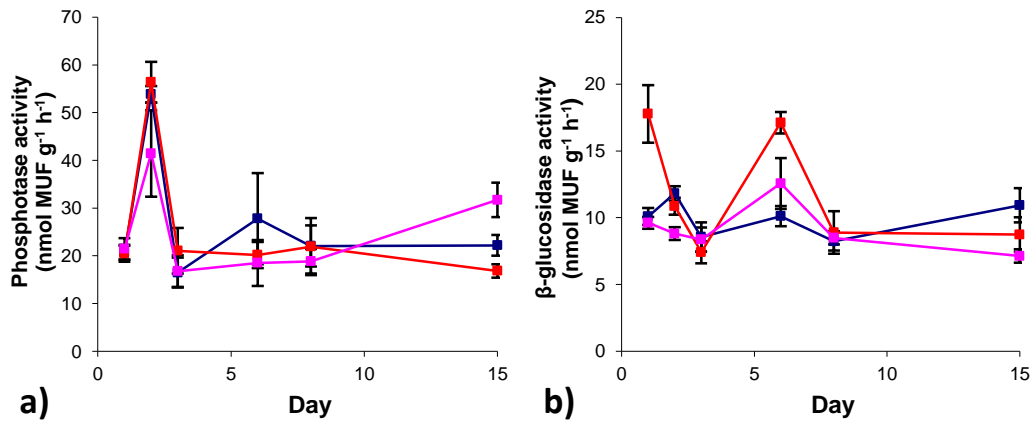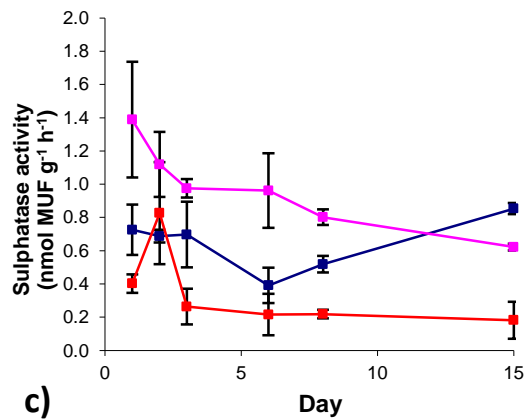
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473   Figure 4. Outflow LAS concentrations for high density (pink), low density (red) and control (blue)
474   plant biomass treatments. Error bars represent the standard error of the mean (n = 4).
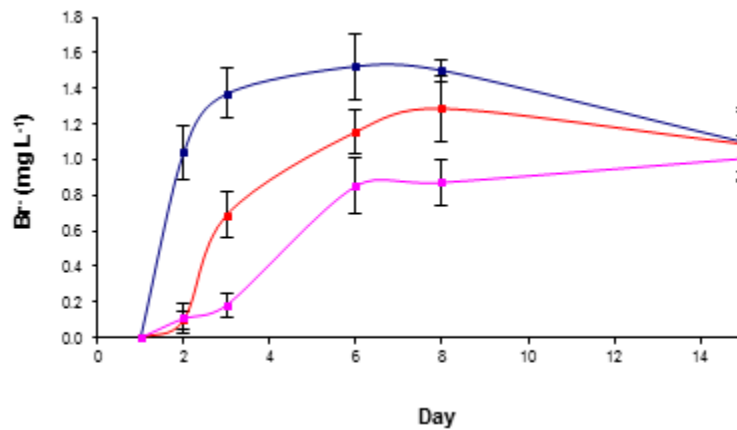
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477   Figure 5. Enzyme activity in high density (pink), low density (red) and control (blue) plant biomass
478   treatments showing phosphotase (a), β-glucosidase (b) and sulphatase (c). Error bars represent the
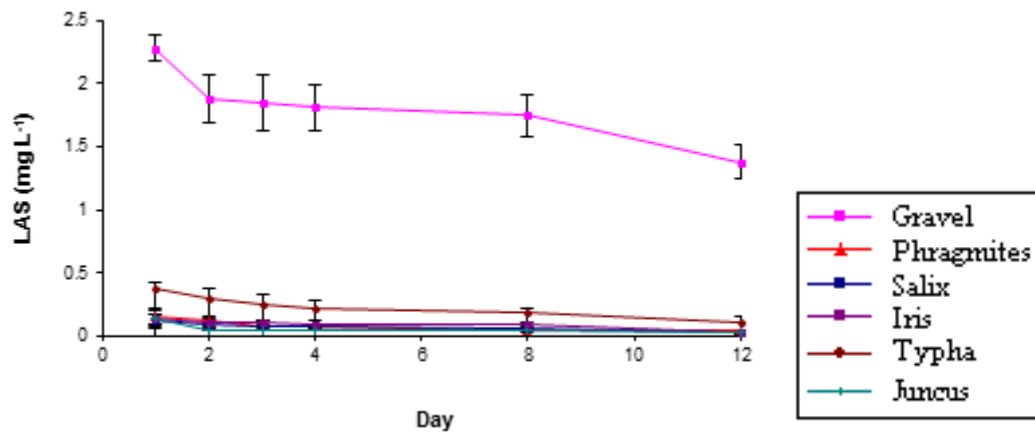479   standard error of the mean (n = 4).
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481
482   Figure 6. Br⁻ tracer study results showing Br⁻ concentration for the high density (pink), low density
483   (red) and control (blue) plant biomass treatments. Error bars represent the standard error of the
484   mean (n = 4).

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485
486   Figure 7. Outflow LAS concentrations planted mesocosms and gravel control. Error bars represent
487   the standard error of the mean ($n = 4$).