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Biodegradation of diuron in artificially contaminated water and seawater by wood colonized with the white-rot fungus *Trametes versicolor*

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Abstract

We investigated the potential of white-rot fungi for bioremediation of aqueous environments contaminated with diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea). First, diuron degradation activities of several white-rot fungi (*Ceriporia lacerata, Phanerochaete chrysosporium, Phanerochaete sordida, Trametes versicolor*) and a brown-rot fungus (*Gloeophyllum trabeum*) were evaluated under low- or high-nitrogen conditions. While *G. trabeum* showed hardly any degradation activity, white-rot fungi except for *C. lacerata* showed degradation activity, at least under some conditions. In particular, the activity of *T. versicolor* was high regardless of culture conditions (30-35% degradation at both levels of nitrogen). *T. versicolor* degraded diuron to two metabolites, 1-(3,4-dichlorophenyl)-3-methylurea and 1-(3,4-dichlorophenyl)urea, and did not accumulate the highly toxic metabolite 3,4-dichloroaniline. Moreover, the diuron content of artificially contaminated water dramatically decreased from 1.0 μM to 0.012 μM and artificially contaminated seawater (3.4% sea salt, w/v) decreased to 0.405 μM after incubation for 2 weeks with shaking on *T. versicolor*–colonized wood medium. These results indicated that wood medium colonized with white-rot fungi may be applicable for bioremediation and detoxification of diuron-contaminated aqueous environments.

1 **Introduction**

2 Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) is a nonhormonal-type
3 phenylurea class herbicide. Diuron binds to photosynthetic membranes, blocking electron
4 transfer, which is needed for plant growth. Diuron has a long dissipation half-life in soils
5 (90 days), high persistence during soil degradation and a long half-life during aqueous
6 photolysis [1]. Therefore, diuron is widely utilized for weed control in croplands and
7 urban areas, and is used as an antifouling paint biocide by the shipping industry. However,
8 diuron moves into groundwater and seawater via runoff due to its persistence and physical
9 characteristics [2]. Diuron contamination has been detected in rivers and seawater, where
10 the diuron concentration exceeds the levels of European Union guidelines at some sites
11 and in some seasons [3, 4].

12 Giacomazzi and Cochet [2] have summarized the toxic effects of diuron.
13 Diuron shows low toxicity for mammals, with slight or moderate toxicity to aquatic
14 invertebrates and fish. However, 3,4-dichloroaniline (3,4-DCA), one of diuron's main
15 degradation metabolites, is a much more toxic compound than diuron. 3,4-DCA shows
16 toxic and genotoxic effects on aquatic animals, as well as protozoans and bacteria.
17 Therefore, in aiming for diuron bioremediation, it is necessary to employ microorganisms
18 that never accumulate the ecotoxic 3,4-DCA.

19 Giacomazzi and Cochet [2] also reviewed diuron biodegradation. Several
20 bacteria partially degrade diuron to form 3,4-DCA via *N*-demethylation followed by
21 amide bond hydrolysis, and some bacteria are capable of degrading or mineralizing

22 diuron without accumulation of 3,4-DCA. Filamentous fungi also degrade diuron to form
23 some of the same metabolites as bacteria, and accumulate 3,4-DCA [5]. However, fungal-
24 bacterial consortia show more efficient mineralization of diuron and lower accumulation
25 of metabolites than the individual microorganisms [6]. Degradation of diuron by several
26 other white-rot as well as brown-rot fungi has been investigated; however, not all the
27 metabolites and metabolic pathways are known [7, 8]. In the degradation of diuron by
28 wood-rot fungi, *Phanerochaete chrysosporium* is the best investigated fungus. Coelho-
29 Moreira *et al.* [9] reported that the fungus degrades diuron to 1-(3,4-dichlorophenyl)-3-
30 methylurea (DCPMU) and DCPU, and that this *N*-demethylation reaction is significantly
31 inhibited by the addition of a cytochrome P450 (CYP) inhibitor. Although no 3,4-DCA
32 was detected as a metabolite, it was presumed that the urea bond of DCPU is cleaved to
33 form 3,4-DCA, because DCPMU and DCPU are also detected as metabolites of fungi
34 producing 3,4-DCA from diuron [2]. *P. chrysosporium* is also reportedly able to form
35 some metabolic products during mineralization of 3,4-DCA [10]. Recently, it has been
36 reported that the white-rot fungus *Ganoderma lucidum* can degrade diuron via the same
37 pathway without 3,4-DCA accumulation, and that both extracellular ligninolytic and CYP
38 enzymes degrade diuron [11]. These observations suggest that white-rot fungi degrade
39 diuron in collaboration with extracellular and intracellular enzymes, and do not
40 accumulate toxic metabolites. The proposed diuron metabolic pathway of white-rot fungi
41 was shown in Fig. 1.

42 Based on these observations, we considered that white-rot fungi might be useful

43 for bioremediation of diuron-contaminated environments. Therefore, we screened white-
44 rot fungi that are superior for diuron degradation, and determined the metabolic pathway
45 used. Fungal bioremediation of artificially diuron-contaminated water or seawater was
46 then demonstrated, suggesting the possible application of wood-rot fungi for remediation
47 of diuron-contaminated aqueous environments.
48
49 **Materials and methods**
50 **Fungal strains and chemicals**
51 The brown-rot fungus *Gloeophyllum trabeum* KU-41 (NBRC 111644), the
52 white-rot fungi *P. chrysosporium* ME-446 (ATCC 34541), *Phanerochaete sordida* YK-
53 624 (ATCC 90872), and new isolates *Ceriporia lacerata* K-70 and *Trametes versicolor*
54 K-41, which were isolated from natural decayed wood samples obtained from a mixed
55 forest in Shizuoka, Japan, were used. These isolates were identified based on their 18S
56 rRNA gene ITS and sequences (accession numbers: LC312412 and LC312413 for *C.*
57 *lacerate* K-70; LC312414 and LC312415 for *T. versicolor* K-41).
58 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (diuron) was purchased from Tokyo
59 Chemical Industry Co., Ltd., Japan. 1-(3,4-Dichlorophenyl) urea (DCPU) and 3,4-
60 dichloroaniline (3,4-DCA) were purchased from Wako Pure Chemical Industries, Ltd.,
61 Japan. All reagents had >98% purity, and were used without further purification. Atrazine
62 (Tokyo Chemical Industry) was used as an internal standard.
63

64 **Liquid cultivation and diuron degradation**

65 A disc (8.5 mm diameter) from the growing edge of mycelia on potato dextrose
66 agar (PDA) was put into a 50-ml glass vial containing 5 ml nitrogen-limited (LN) or high-
67 nitrogen (HN) basal III medium (containing 1% glucose, 20 mM 2,2-dimethylsuccinate
68 and 1.2 or 12 mM ammonium tartrate, respectively, pH 4.5) [12], and preincubated for 5
69 days at 30 °C under aerobic conditions. After preincubation, 50 µl of 10 mM diuron or
70 3,4-DCA (DMSO solution) was added to each culture, then the vial was sealed with a
71 butyl rubber stopper and aluminum seal. After a further 7 days of incubation, the culture
72 was homogenized following the addition of methanol (5 ml) and internal standard
73 (atrazine). The obtained homogenate was centrifuged (2,500 × *g* for 10 min) and the
74 resulting supernatant was filtered (Millex LG filter, pore size 0.20 µm, Merck Millipore
75 Ltd., Ireland). Then the concentrations of diuron and its metabolites were measured by
76 high-performance liquid chromatography (HPLC). The HPLC system (JASCO PU-2089
77 pump with a JASCO MD-2018 PDA detector) was equipped with an Inertsil ODS-3
78 column (4.6 × 250 mm) and eluted with 50% aqueous methanol. The maximum
79 absorbance of each compound (atrazine at 220 nm, diuron at 248 nm, DCPU at 210 nm,
80 and 3,4-DCA at 208 nm) were used for quantification. As necessary, the supernatant was
81 evaporated to dryness, followed by dissolving it in methanol (0.5 ml), and then analyzing
82 it by HPLC.

83 To determine the effect of salt at seawater concentration on diuron degradation,
84 *T. versicolor* K-41 was preincubated in 5 ml HN medium containing 0% or 3.4% sea salt

85 (Sigma-Aldrich). Diuron was added to the culture in the same manner as described above,
86 and the culture was incubated further.
87
88 **Enzyme assay**

89 The culture supernatants on day 5 were obtained from LN and HN cultures.
90 The supernatants were used for assays of ligninolytic enzyme activities. Manganese
91 peroxidase (MnP), lignin peroxidase (LiP) and laccase activities were measured as
92 described previously [13]. Activities were calculated from absorbance change at 469 nm
93 (for MnP and laccase, 2,6-dimethoxyphenol oxidation, ε_{469} = 49,600 M^{-1} cm^{-1}) and 310
94 nm (for LiP, oxidation of veratryl alcohol, ε_{310} = 9,300 M^{-1} cm^{-1}) [14].
95
96 **Metabolite identification**

97 Metabolites were obtained from 14-day cultures (140 vials) of *T. versicolor* K-
98 41 incubated on HN medium. Filtrate was separated from the culture homogenate
99 prepared as described above, and was extracted with ethyl acetate. In this case, the
100 internal standard was not added to the culture, and quantification was not performed. A
101 concentrated ethyl acetate fraction was fractionated by silica gel column chromatography
102 (silica 60 N, 800 g, φ60 × 500 mm) eluted with *n*-hexane, ethyl acetate, and methanol
103 (hexane/EtOAc/MeOH=6/4/0, 5/5/0, 4/6/0, 3/7/0, 2/8/0, 1/9/0, 0/10/0, 0/9/1, 0/8/2 (1 l
104 each) and then 0/0/10 (3 l)). Fractions were collected every 100 ml. Fractions containing
105 metabolites (fractions 62-69 for product I, fractions 85-100 for product II) were further

106 purified by HPLC on a Develosil C30-UG-5 column (4.5 × 250 mm; Nomura Chemical
107 Co., Ltd., Japan) using 40% aqueous methanol. Electrospray ionization (ESI) mass
108 spectrometry and ^{1}H-nuclear magnetic resonance (NMR) spectrometry were used the
109 same equipment as previously described [15]. Diuron metabolites were ESI-mass
110 analyzed in negative ionization mode, and were dissolved in CD_3OD for NMR analysis.
111

112 **Fungal cultivation on wood substrate, and diuron removal from artificially**
113 **contaminated water**

114 A mycelial plate (PDA, 9 cm i.d.) was homogenized with 25 ml distilled water.
115 Homogenate and additional distilled water were mixed well with wood substrate (180 g
116 beech wood meal and 20 g wheat bran meal) to meet a solid content of 28%. The wood
117 substrate was incubated for 2 weeks at 30 °C with *T. versicolor* K-41 to allow colonization.

118 Artificial contaminated water (containing 10 mM $CaCl_2$ to prevent pesticide
119 sorption as described by Li et al. [16]) and artificial contaminated seawater (containing
120 3.4% (w/v) sea salt), both artificially contaminated with 1 µM diuron, were prepared. *T.*
121 *versicolor* K-41–colonized wood medium was added to 20 ml of artificially contaminated
122 water and seawater at 5% (dry w/v). The suspension was rotated at 120 rpm at 30 °C for
123 2 weeks under ambient atmosphere. At the end of the reaction, the suspension was
124 centrifuged at 8,000 × *g* for 10 min to separate supernatant and a precipitate. After the
125 addition of internal standard, the supernatant was evaporated, then extracted with ethyl
126 acetate to remove hydrophilic impurities. The extract was dried and redissolved in

127 methanol. The precipitate was extracted twice with methanol (20 ml). The methanol-
128 soluble fraction was purified and redissolved in methanol in the same manner as the
129 supernatant. Recovery of diuron was analyzed by HPLC. Wood substrate without any
130 inoculum (abiotic) and autoclaved colonized wood medium (sterilized) were used as
131 controls.
132
133 **Results and Discussion**
134 **Selection of diuron-degrading wood-rot fungi and their metabolites**
135 To develop a bioremediation method for aqueous contamination with diuron,
136 we screened for wood-rot fungi that show higher diuron degradation activity and do not
137 accumulate toxic compounds such as 3,4-DCA. Figure 2 shows diuron degradation by
138 wood-rot fungi after a 7- and 14-day incubation period in LN and HN media. Generally,
139 increasing nitrogen concentration in liquid culture promotes growth of white-rot fungi. In
140 contrast, the ligninolytic activity of some white-rot fungi is inhibited under HN conditions,
141 and secondary metabolism, including ligninolytic activity, is stimulated by nitrogen
142 limitation [17]. *G. trabeum*, used as a representative brown-rot fungus, slightly degraded
143 diuron in HN medium, but not in LN medium. On the other hand, the white-rot fungus *P.*
144 *chrysosporium* had degraded almost 20% of the diuron in both media at 7 days, and *P.*
145 *sordida* showed diuron-degrading activity only in LN medium. In diuron metabolism by
146 *P. chrysosporium* and *G. lucidum*, the following facts have been shown; i) diuron
147 degradation was correlated with ligninolytic activity on nitrogen-limited and wood

148 culture, ii) laccase was able to degrade diuron, and iii) diuron metabolism was
149 considerably inhibited by addition of CYP inhibitors [9, 11, 18]. These facts indicate that
150 ligninolytic enzymes are not mandatory to diuron degradation of white rot fungi, and also
151 suggest that white rot fungi can degrade diuron under the condition in which the CYP
152 isozyme involved in diuron metabolism is expressed. Therefore, as shown in Fig. 2, there
153 is considered that diuron degradation of white rot fungi was not always influenced by
154 nitrogen concentration in the culture medium. There are some reports describing the
155 degradation of neonicotinoid herbicides by *P. sordida,* and the metabolites are
156 presumably formed via *N*-demethylation or *N*-nitro reduction catalyzed by CYPs [15, 19].
157 However, no metabolites were detected in single cultures of these two *Phanerochaete*
158 species based on HPLC in the present study. *T. versicolor* K-41 was one of the best fungi
159 for diuron degradation, and showed higher diuron degradation in HN than LN medium
160 after a 7-day incubation period (Fig. 2). Bending and co-authors [7] reported diuron
161 degradation by a wide range of wood-rot fungi including *T. versicolor*, but did not report
162 any correlation between ligninolytic activity and diuron degradation. Thus, there is still a
163 lack of information on metabolites and the mechanisms of diuron degradation by *T.*
164 *versicolor*. Leatham and Kirk [17] described strong stimulation of ligninolytic activity of
165 *T. versicolor* under nitrogen limited conditions and inhibition of activity by high nitrogen
166 concentration, though moderate ligninolytic activity remained. In this study, *T. versicolor*
167 K-41 was showed MnP and laccase activity under HN condition (1.5 ± 0.6 U/l and 3.2 ±
168 0.2 U/l, respectively), while the higher activities were presented under LN condition (2.4

169 U/l MnP and 5.2 ± 0.1 U/l laccase). Trace levels of LiP activity were detected from both
170 cultures. Nevertheless, diuron degradation activities under both nitrogen condition were
171 showed almost same level. Therefore, it was presumed that *T. versicolor* K-41 is able to
172 degrade diuron by ligninolytic extracellular and non-ligninolytic intracellular enzymes,
173 as described above for other white-rot fungi. *T. versicolor* K-41 showed higher diuron
174 degradation activity regardless to nitrogen concentration, the degradation rates of *T.*
175 *versicolor* K-41 were the equally as *P. sordida* under LN condition and *P. chrysosporium*
176 under HN condition. Although mycelial growth is expected to vary depending on culture
177 condition, it is important to show the high degradation activity regardless of condition in
178 order to apply for bioremediation use. Therefore, *T. versicolor* K-41 was used for
179 subsequent experiments.

180 A peak (product I, 17.70 min) having a UV spectrum resembling diuron was
181 detected in an ethyl acetate extract of culture homogenate of *T. versicolor* K-41 after
182 HPLC, as shown in Fig. 3. Product I showed a molecular ion peak at *m/z* 203 [M -H]- and
183 resulting chlorine substitution peaks at *m/z* 205 and 207. A fragment ion peak at *m/z* 160
184 [M -$CONH_2$]- and chlorine substitution peaks at *m/z* 162 and 164 were observed. From
185 these results and the ^{1}H-NMR spectrum, product I was estimated as DCPU, and was
186 finally identified by comparison with authentic standard. In addition to product I, product
187 II (24.0 min) was detected as a minor peak on HPLC in a 50-100 times concentrated
188 extract without internal standard. Because product II presented almost the same retention
189 time as the internal standard (atrazine, 23.6 min), it could not be detected in quantification

190 experiments with internal standard by HPLC (Fig. 3). Product II showed a relatively weak
191 molecular ion peak at *m/z* 217 [M -H]- (and two ion peaks indicating chlorine
192 substitution), and a fragment ion peak at *m/z* 160 [M -$CONHCH_3$] was observed. Its ^{1}H-
193 NMR spectrum was identical to one previously reported [20], and therefore product II
194 was identified as DCPMU. On the other hand, although 3,4-DCA was not detectable by
195 HPLC of unconcentrated supernatant, a slight amount (2.3 ± 0.2 μM) was detected in
196 concentrated extract prepared from a 14-day culture (Fig. 3). Therefore, to reveal whether
197 3,4-DCA is accumulated or is a transient metabolite, a degradation experiment for 3,4-
198 DCA was performed. In that experiment, the concentration of 3,4-DCA decreased to
199 below the lower detection limit after a 2-day incubation, which indicated that 3,4-DCA
200 is just one of the intermediates produced during diuron metabolism of *T. versicolor* K-41,
201 and that the fungus is able to metabolize 3,4-DCA quickly under these experimental
202 conditions, the same as *P. chrysosporium* [10].
203
204 **Diuron removal from artificially contaminated water**
205 Because diuron is typically present in river and seawater [3, 4], diuron
206 degradation by *T. versicolor* K-41 under hypersaline conditions was investigated. The
207 recovery of diuron and DCPU from normal and hypersaline (3.4% sea salt) cultures of *T.*
208 *versicolor* K-41 is shown in Fig. 4. Diuron degradation and DCPU production by *T.*
209 *versicolor* were inhibited by the addition of sea salt. DCPU yields from nonsaline and
210 hypersaline cultures incubated with diuron for an additional 14 days were 10.8 ± 4.1 and

211 2.0 ± 0.4 μM, respectively. And respective 2.3 ± 0.2 and 1.8 ± 0.1 μM of 3,4-DCA were
212 recovered from the cultures. No DCPMU was detectable from extracts of single cultures.
213 However, diuron degradation in hypersaline conditions was 60% of that under nonsaline
214 conditions. Total recoveries of diuron and its metabolites were 55% from nonsaline
215 culture and 70% from hypersaline culture. The phenomenon that the total recovery rate
216 of diuron and its metabolites decreases by fungus treatment has been also observed in *P.*
217 *chrysosporium* and *G. lucidum* [9, 11].

218 Next, to estimate the remediation potential of *T. versicolor* K-41 in a diuron-
219 contaminated aqueous environment, the efficiency of diuron removal from artificially
220 diuron-contaminated water and seawater was evaluated. In 2007, European-wide
221 environmental quality standards for 33 high-priority hazardous substances including
222 diuron were published [21]. Annual average and maximum allowable concentrations of
223 diuron for surface water have been set at 0.2 and 1.8 μg/L, respectively. However,
224 because of experimental limitations to quantitation, we chose a diuron concentration in
225 artificially contaminated water of 1 μM, a much higher concentration than allowable
226 under these standards.

227 Because these fungi need utilizable saccharides for survival, we used wood as
228 a source of nutrients and as an immobilized carrier for the fungi for bioremediation of
229 aqueous contaminants. Wood colonized with *T. versicolor* K-41 was suspended in water
230 or seawater artificially contaminated with diuron to evaluate the adsorption and co-
231 metabolism of diuron relative to uninoculated wood substrate and sterilized controls. As

232 shown in Fig. 5, calculated recovery rates of diuron from abiotic and sterilized controls
233 were 93.7-105.5% of the initial amount (1 μM). In these experiments, 20% or less of the
234 diuron was recovered from the aqueous phase of controls; the other 80% was absorbed to
235 the wood substrate. Additionally, it seemed that sterilized mycelium was not involved in
236 diuron adsorption, but that sea salt slightly affected diuron sorption to woody materials.
237 Wood adsorption of aromatic pesticides having a structure similar to diuron has been
238 reported [22]. We speculated that some components of sea salts affected diuron
239 adsorption to the woody materials, because it has been reported that adsorption of diuron
240 to soil is greatly affected by the salt type and concentration [16].

241 During treatment of artificially contaminated water with *T. versicolor* K-41-
242 colonized wood medium, diuron was almost completely degraded (98.7%) during a 2-
243 week incubation period (Fig. 5). The fungus might have been able to degrade diuron
244 effectively due to adsorption of diuron to the medium. In artificially contaminated
245 seawater, on the other hand, the degradation rate of diuron was lower than in
246 contaminated water without saline, as observed in liquid cultures. However, more than
247 half of the diuron was degraded by the *T. versicolor* K-41 colonizing the wood medium.
248 No metabolites were confirmed by HPLC after fungal treatment of the contaminated
249 water and seawater. The absence of metabolite detection was presumably due to the low
250 initial amount of diuron, so that the amount of metabolites did not reach the level
251 detectable by HPLC. These results suggested that *T. versicolor* K-41–colonized wood
252 medium can be utilized for bioremediation of diuron-contaminated aqueous environments,

253 especially under salt-free conditions.

254 Several diuron degrading filamentous fungi have been isolated from
255 environments such as diuron-treated fields, and they are also able to degrade the related
256 phenyl urea herbicides [23]. Some reports have also described filamentous fungi that are
257 capable of growth in soil contaminated with diuron and may be useful for bioremediation
258 of diuron-contaminated soil [24, 25]. On the other hand, a wide range of wood-rot fungi,
259 including the edible mushroom *Pleurotus ostreatus*, have the capability to degrade diuron
260 and related herbicides [7, 8]. Nevertheless, the growth of wood-rot fungi is usually
261 inhibited when introduced into a soil environment, which is different from wood in many
262 respects due to the low amount of available nutrients and competitive indigenous
263 microorganisms [26]. However, utilization of lignocellulosic material such as wood
264 allows growth of white-rot fungi in soil environments. Actually, there are several reports
265 on successful soil remediation by applying lignocellulosic materials and white-rot fungi
266 [26]. These observations suggest that white-rot fungi may be applicable for
267 bioremediation in several environments by establishing conditions appropriate for their
268 growth. In the present study, diuron degradation in artificially contaminated water was
269 demonstrated by the white-rot fungus *T. versicolor* K-41 after it had colonized wood meal,
270 which was utilized as both a source of nutrients and an immobilizing carrier. In natural
271 environments, various factors are involved in biodegradation of organic pollutants;
272 therefore, additional study is needed for evaluation of the effectiveness of fungal-
273 colonized wood in bioremediation of actual polluted aqueous environments. Still, there

274 is a possibility that inexpensive bioremediation methods for aqueous pollution utilizing
275 spent mushroom wastes of edible or medicinal mushrooms (such as *P. ostreatus* and *T.*
276 *versicolor*), which are currently discarded as industrial wastes, can be developed if
277 suitable procedures are established by future studies.

278

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353

354 **Figure Legends**

355

356 Fig. 1 Proposed Diuron metabolic pathway of white rot fungi. Diuron: 3-(3,4-dichlorophenyl)-
357 1,1-dimethylurea, DCPMU: 1-(3,4-dichlorophenyl)-3-methylurea, DCPU: 1-(3,4-
358 dichlorophenyl) urea, DCA: 3,4-dichloroaniline.

359

360 Fig. 2. Diuron recovery from fungal cultures grown on LN and HN media after 0, 7 and
361 14 days of incubation (black, gray and white bar, respectively). Values are means
362 ± standard deviation of triplicate samples.

363

364 Fig. 3. High-performance liquid chromatograms (210 nm, maximum absorption
365 wavelength of DCPU) of concentrated extracts obtained from whole HN cultures
366 of *T. versicolor* K-41 that were incubated with diuron. A and B show
367 chromatograms of extracts from nonsaline cultures incubated with diuron for an
368 additional 0 and 14 days, respectively. C shows a chromatogram of extracts from
369 hypersaline cultures incubated with diuron for an additional 14 days.

370

371 Fig. 4. Time courses of diuron degradation and DCPU accumulation by *T. versicolor* K-
372 41 under hypersaline and nonsaline conditions. The filled and open symbols are
373 indicated diuron degradation and DCPU accumulation under hypersaline
374 (diamonds) and nonsaline (circles) conditions, respectively. Values are means ±

375 standard deviation of triplicate samples.

376

377 Fig. 5. Recovery of diuron from artificially contaminated water (containing 10 mM
378 $CaCl_2$) and seawater (containing 3.4% sea salts) incubated for 2 weeks with a wood
379 substrate (abiotic), and sterilized and unsterilized (live) wood medium colonized
380 with *T. versicolor* K-41. Each column indicates total diuron recovery, and the gray
381 portion represents the aqueous-soluble portion.

382

383 **Figure**

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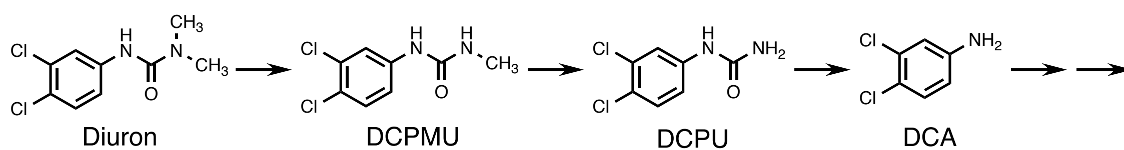

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389 Fig. 1

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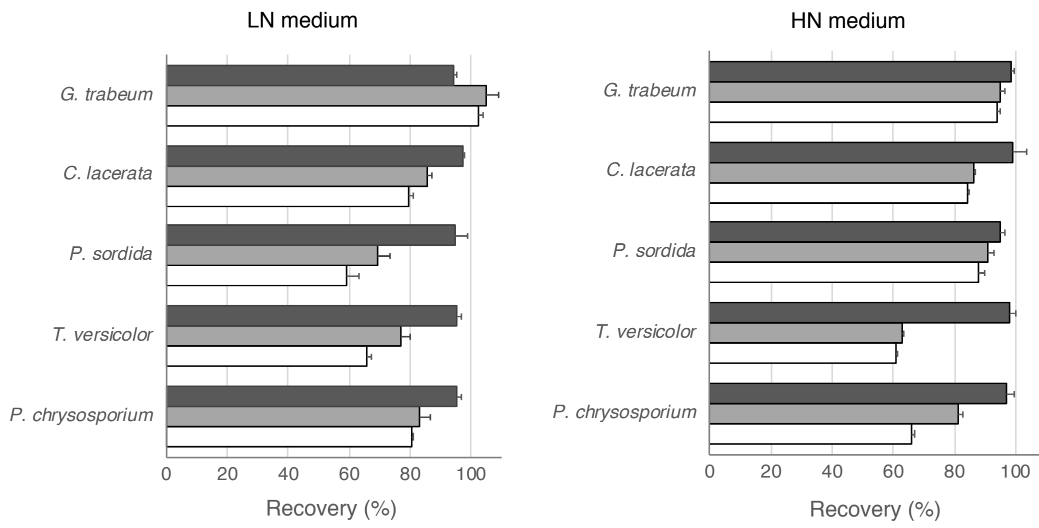

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396 Fig. 2.

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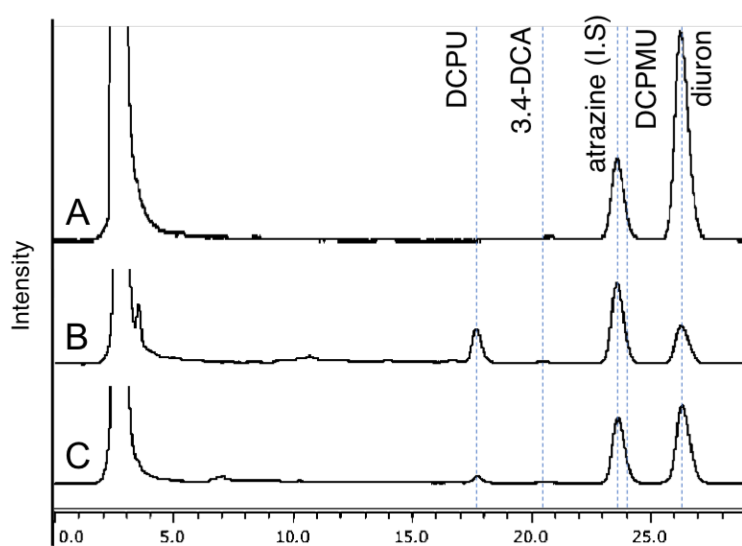

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403 Fig. 3.

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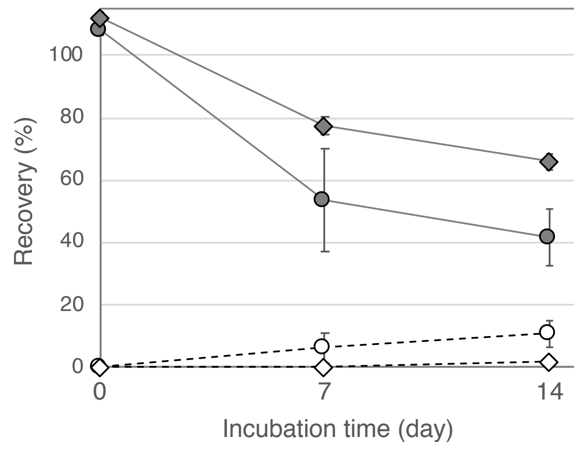

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411 Fig. 4.

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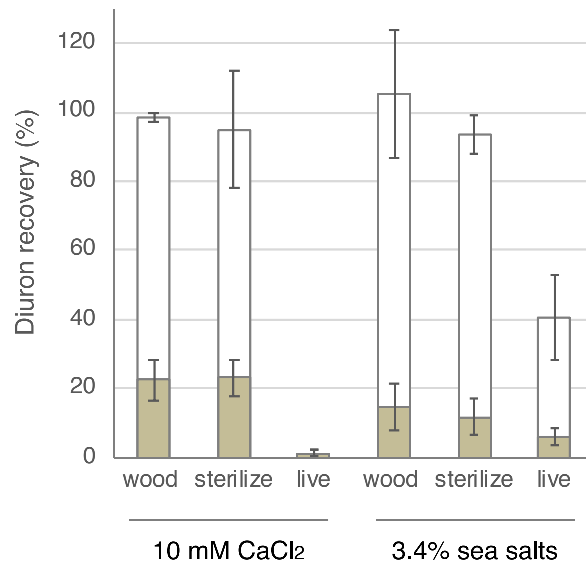

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419 Fig. 5.

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