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Seawater influence monitored by NaCl on the growth of *Trametes versicolor*

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TITLE: NaCl monitoring seawater influence on the growth of *Trametes versicolor*

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FOOTNOTES TO THE TITLE: Salt effects on biodegradation of Tsunami wood debris

Key Words

Trametes versicolor, tsunami, biodegradation, Wood debris, Sodium chloride (NaCl),
salt effect

Abstract

There are only a few scientific data about the function of ecosystems after tsunami
disasters. The ecosystems help the environment to recover after a disaster and therefore
the research on its function is important. We estimated the seawater influences on wood
degradation after tsunami disaster by the growth of *Trametes versicolor*. NaCl in debris
was monitored to estimate the impact of tsunami on wood blocks. The debris from the

Great East Japan Earthquake on the pacific coast in March 2011 was used for the simulations. Its growth on debris was compared with those on seawater treated woods, and the amount of sodium chloride was examined to know the approximate amount of salts in the samples. As a result, this common white-rot fungus degraded wood debris in the same way as sound sapwood. Although the study was conducted at laboratory level, this is the first report from the real debris, which assessed the fungal decomposition ability of the ecosystem after tsunami disaster.

Introduction

Tsunami loads have been a huge problem in disaster areas. After the Great East Japan Earthquake on the pacific coast in March 2011, it is estimated that 20,188 thousand tons of debris accumulated. Since this type of debris was usually mixed with pollutants like anonymous chemicals and scrap metals (Karube et al. 2015), thus loads of classification and detoxification processes were required before recycling, incineration or landfill disposal for those debris (Ministry of the Environment, 2011). The processes took a time, and consequently most debris remained in disposal stations for ages. As for the Great East Japan Earthquake, in the end, 82 % was recycled, 12 % was incinerated and 6% was landfilled (Ministry of the Environment, 2015). According to the Reconstruction Agency in Japan, which was established after the Great East Japan Earthquake, half of the debris in Miyagi prefecture still remained in the cue for disposal

in 2013. About 14% of over two billion tons of the debris from Miyagi and Iwate prefectures was wood debris (Ministry of the Environment, 2015). The treatments of debris from the Great East Japan Earthquake were completed in 2014. The recovery of normal life in these area is the most urgent necessity after the natural disaster (Ministry of the Environment, 2011; Chandrasekhar et al., 2015), and therefore, there has not been many case studies of recoveries of ecosystem in tsunami disaster areas.

Here, we evaluated the fungal ability on decomposition of the wood debris from this huge disaster that occurred over a short time span. All analysis with debris was conducted in 2012 just after we received the debris from Miyagi prefecture. *Trametes versicolor* was employed as a model microbe since it is a common white-rot fungus, which attacks a wide range of woods. This fungus occurs commonly in forests and mountain sides but also attacks houses and buildings. It was used for the wood decaying test for industrial purpose (Japanese Industrial Standards JIS_2010). Since after a serious disaster there is no clear single factor which will affect biodegradation efficiency, we studied the influence of salt. We compared the data with that from the samples prepared with artificial seawater based on a NaCl parameter, sodium chloride contents. Salt inhibition on fungal growth is already reported but there is no detailed data after 1970s, and also there is no study using seawater.

The information of ecological cycles after tsunami disaster, especially near the urban area, will be helpful to reconstruct the area. In this study, *T. versicolor* attack level on wood debris was measured, and the data was compared with control blocks, which were treated with artificial seawater. The NaCl contents were used to get a comparative parameter. In order to estimate the tested debris condition, the influence of seawater immersion and washing were estimated also using NaCl contents as a parameter. This study provides the novel information about biodegradation of wood debris after tsunami disaster.

Materials and methods

Trametes versicolor

A white-rot fungus, *T. versicolor* (L. ex Fr.) Quel was used for the decay test, which is available in the Deterioration Organism Laboratory (DOL) of the Research Institute for Sustainable Humanosphere, Kyoto University, Japan. *T. versicolor* was pre-cultured in a 100 ml of nutrient solution (4.0 % glucose, 0.3 % peptone, and 1.5 % malt extract) for around a week until the fungus produced granules.

Wood blocks

84 Standard wood blocks were prepared with sound sapwood of
85 *Cryptomeria japonica* D. Don (20 (R) x 20 (T) x 10 (L) mm).

86 In addition, two groups of seawater treated wood blocks were prepared, group
87 A as a model sample of waterborne debris such as floating driftwood or lumber and
88 group B as a model sample of seawater-washed log after tsunami. In group A, the wood
89 blocks were soaked in 200 ml artificial seawater (Daigo's artificial seawater SP for
90 marine microalgae medium, Nihon pharmaceutical Co. Ltd., Japan) with 100 g quartz
91 sands for 0, 1, 3 and 6 months. Those blocks were air-dried at room temperature for a
92 week before use. In group B, blocks were additionally treated with artificial seawater
93 according with the method of wood surface treatment in JIS K 1571-2010: They were
94 immersed in the seawater for 5 hours at 25 ± 2 °C and dried for 19 hours at 40 ± 2 °C
95 during 10 days before use.

96 Tsunami wood debris arrived from Natori city, Miyagi prefecture in 2012 July
97 (kindly supplied from Dr. K. Kashimura, Chubu University, Japan). They were from a
98 part of the debris in the cue of disposal treatment after the Great East Japan Earthquake
99 of March 11, 2011 for 16 months. Fifteen wood debris were randomly selected and cut
100 into 20 (R) x 20 (T) x 10 (L) mm as same as standard wood blocks (Fig. 1). The
101 condition of the debris did not allow us to identify its wood species, however it seemed

102 that there were at least five or six wood species in the debris. By identification on gross,
103 the 15 debris contained four hardwoods and 11 sapwoods containing *C. japonica*.

104

105 Seawater influence on the growth of *T. versicolor*

106 To learn the decomposition occurrence on waterborne debris, seawater
107 influence on growth of *T. versicolor* was examined with the wood blocks from group A.
108 Salt immersion into the blocks was estimated by the weight change before and after
109 soaking. Experiments were conducted with 12 repetitions for each soaking interval. A
110 monoculture decay test was conducted according to JIS K 1571-2010. All sample blocks
111 were weighed after oven drying at 60 ± 2 °C for 3 days and sterilized with gaseous
112 ethylene oxide prior to the decay test. A glass jar containing 250 g quartz sand (30-50
113 mesh) and 80 ml nutrient solution was autoclaved before use. Three ml of pre-cultured
114 liquid fungus suspension containing granules were inoculated to the glass jar medium
115 and incubated till the mycelia fully covered the surface of quartz sand in the jar. Thus,
116 wood samples were placed on the mycelial mat. They were incubated for 12 weeks at 26
117 ± 2 °C. Three samples were placed in one glass jar. Fungal attack to the wood samples
118 was estimated by comparing the oven-dried masses before and after the test. Hardness
119 was also measured by a durometer (hardness tester: Asker CL – 150H with DD2 tester,

120 Kobunshi keiki Co. Ltd., Japan).

121

122 Debris decay test

123 As for debris, blocks were obtained from both edges of 15 debris because each
124 edge could have different environmental effects such as one edge had been exposed to
125 the air and light but the other side had been buried. Therefore total 30 blocks were used
126 for a decay test. Sound sapwood of *C. japonica* was used as control test samples. For
127 positive control, 5 no-treated blocks were prepared. For negative control, 5 blocks from
128 group B were used. The monoculture decay tests were conducted as described above.

129

130 NaCl contents

131 Salt contents in debris and group B wood blocks were unknown, therefore they
132 were estimated by contents of sodium ion as below. Approximately 0.1 g fractions were
133 collected from each sample block. They were kept in a melting pot and heated at 150 °C
134 for 3 hours in 60 % nitric acid to remove all organic materials. Ash remaining in the
135 melting pot was dissolved in distilled water, whose amount was 100 times volume of the
136 initial fractions. The ahs-containing solutions were filtered (DISMIC-13 cp, 0.20 μm,
137 Advantec, Japan). NaCl contents in the wood samples were estimated by Na content in

the digested solution using Inductively Coupled Plasma Optical Emission Spectrometer (ICP spectrometer) (SPS-7800 Plasma Spectrometer, SII - Seiko Instruments Inc., Japan). A small amount, about 10 cm³, was injected into the instrument to measure its contents. The standard solutions were made by a commercial sodium standard solution (Na 1000 ppm, Nacalai tesque). To estimate the unpredictable Na contents in the seawater treated wood blocks and debris, standard curves were obtained with 0, 1, 10 and 100 ppm standard solutions to cover the wide range of concentration, and the data under 10 ppm Na contents were double-checked with 0, 1 and 10 ppm standard curves.

The Na estimation with ICP were conducted on the sample blocks of group B used for decay test and debris. The middle parts of each debris were used to measure the salt contents. 15 debris blocks were used to estimate NaCl contents in the debris.

Statistics

Kruskal-Wallis test was used to analyze the decaying rate of debris and controls. On the other hand, the weight loss and hardness change before and after decay test was analyzed with Wilcoxon test. Na contents in the debris were compared with the controls by the Mann-Whitney test. Mann-Whitney test was also applied to examine the

hardness change by seawater immersion with each soaking interval. JMP 9.0 software was used for all analyses.

Results

Seawater influence on fungal attack of *T. versicolor*

This decomposition test with group A blocks was conducted to learn the impact of seawater immersion on biodegradation. Longer immersion in the artificial seawater drove salt accumulation into the wood blocks (Fig. 2A, $p < 0.001$, $X^2 = 40.976$, Kruskal-Wallis test). The weight increase of sample blocks after 1, 3 and 6 months immersion were observed and it indicated that minerals in the artificial seawater penetrated gradually into the wood blocks (Fig. 2A). On the other hand, hardness did not changed by this soaking treatment ($p = 0.485$, $X^2 = 2.446$, Kruskal-Wallis test).

T. versicolor attack caused a significant weight loss in all samples but not hardness change (Fig. 2BC) (0 month: $p < 0.001$, $X^2 = 17.280$ in weight loss parameter and $p < 0.001$, $X^2 = 11.608$ in hardness parameter, 1 month: $p < 0.001$, $X^2 = 14.533$ in weight loss parameter and $p = 0.002$, $X^2 = 9.545$ in hardness parameter, 3 month: $p < 0.001$, $X^2 = 17.295$ in weight loss parameter and $p = 0.644$, $X^2 = 0.213$ in hardness parameter, 6 month: $p = 0.008$, $X^2 = 7.053$ in weight loss parameter and $p = 0.312$, $X^2 =$

1.022 in hardness parameter, Mann-Whitney test). The intense fungal attack was observed on the surface of 1 month seawater soaking samples (Supple. 1), but their degradation level was as same as control blocks (Fig. 2B). Though the considerable weight loss was occurred in all samples, the decay rate decreased with the immersion period ($p < 0.001$, $F = 27.580$, linear regression analysis), which reflected the hardness change with 3month and 6 month immersion samples (Fig. 2C). It meant that longer immersion in the seawater considerably made *T. versicolor* difficult to attack wood.

Debris decay test

The decay level of tsunami wood debris was compared with naïve (positive control) and group B (negative control) blocks to estimate the potential biodegradation of debris in the nature. *T. versicolor* attacked the debris without any difficulties. The mass loss from the decay test in wood debris was 17.6 ± 2.18 (average $\pm$ standard error, SE) wt%, while it was 21.2 ± 1.57 wt% in no-treated sample blocks. Hardness value decreased by fungus attack ($p < 0.001$, $X^2 = 15.196$, Wilcoxon test). There was no significant difference in decaying levels among wood debris, no-treated and seawater surface-treated wood blocks ($p = 0.194$, $X^2 = 3.282$, Kruskal-Wallis test). At the initial stage of incubation, group B wood blocks were less attacked (Supple. 2) however the

192 these blocks even got slightly more attack after 12 weeks incubation (Mass loss, $26.5 \pm$
193 2.17 wt%).

194

195 NaCl contents in the debris

196 To obtain the comparative parameter, NaCl contents in the debris and group B
197 samples were measured by the detection of sodium ion. Na contents were significantly
198 low from the negative controls ($p = 0.001$, $X^2 = 10.714$, Mann-Whitney test). The debris
199 samples contained $0.12 - 3.3$ mg / g (average: 1.14 mg / g) Na and the group B blocks
200 contained $15.17 - 25.71$ mg / g (average: 19.32 mg / g). Na contents in non-treated
201 wood blocks were below the measurable limit. The results were similar with 0, 1 and 10
202 ppm standard curve.

203

204 **Discussions**

205 This study demonstrated the salt effects on the growth of *T. versicolor*. The real
206 debris from the Great East Japan Earthquake was also analyzed. The inhibition on the
207 fungal growth by seawater was observed, however the results indicated that debris
208 certainly contained salt, but not at the critical level. According to the data from ICP
209 spectrometer, the debris contained average 1.14 mg/g Na and they were attacked by the

210 white-rod wood decaying fungi as same as the control sound sapwood.

211 It is well known that salt has a function to inhibit the fungal growth (Tresner
212 and Hayes 1971; Sato et al. 2014), on the other hand, there are few reports of tsunami
213 influence on fungal growth the nature. Seawater normally has 3.5 % salt content. Our
214 experiments demonstrated that wood blocks could accumulate around 5 % salt by 3
215 month if they were soaked into seawater. The growth rate of *T. versicolor* was
216 significantly delayed if the wood blocks contained this amount of salt (Fig. 2, Supple. 3).
217 Seawater contains many ions, but NaCl is one of the representative component of
218 seawater. The results matched with the former study using 29 *T. versicolor* strains,
219 which added sodium chloride into media (Tresner and Hayes 1971). Moreover the
220 hardness increase of wood blocks with the salt contents was observed in this study. It
221 would be amenable with the fact that NaCl treatment improves tensile strength of
222 fiberboard (Uraki et al. 2005). NaCl is also known to change the water movement inside
223 of woods (Yang et al, 2011). It seemed, however, that biodegradation process would not
224 be really disturbed by salt after a real disaster. This is probably because that fungal
225 tolerance to NaCl is varied in wide range (Tresner and Hayes 1971). It is highly possible
226 that salt-sensitive microbes simply do not colonize on the debris after tsunami disaster
227 (Higashijima et al. 2012). Actually Sato et al. (2014) isolated the fungi, which were

228 tolerant to 15-20 % salt from the paper objects in Tohoku regions at 5 months after the
229 Great East Japan Earthquake. Debris were often left uncontrolled in the nature after
230 Tsunami disaster. Our samples, which were placed in debris dump for 16 months,
231 contained about 0.001 % Na, which indicated that it contained 0.003 % salt (calculated
232 from NaCl / Na proportion). This would be partly because of wash-off effect of rain
233 (Pérez-Rodríguez et al. 2015; Konoplev et al. 1996; Nasrabadi et al. 2011). The wash-
234 off function of fresh water was successfully confirmed with seawater treated wood
235 blocks (Supple. 4), and we think that same washing-off procedure occurred on the
236 debris due to rain.

237 In the test, the debris was simply classified and numbered by a chunk. Most of
238 them were materials from residential constructions. The 15 debris seemed to contain
239 four hardwood and 11 sapwood containing one *C. japonica*. This variation made
240 difficult to assess the quality of these debris for their different densities and unknown
241 wood preservation treatment before the disaster. Therefore we simply compared the data
242 with well-studied sapwood *C. japonica* as an implementing option to evaluate the debris
243 condition. Sapwood generally has lower natural durability and it is used as a control
244 wood in the decaying test of JIS K 1571-2010. Even with careful consideration about
245 this alternative, our results supported the sufficient ability of microbe to decompose the

246 waterborne organic debris after Tsunami disaster. Decaying scale would be much larger
247 in the nature than this experiments, however it should not be a matter because our
248 additional test with larger wood blocks still supported this conclusion that debris would
249 be decomposed by microbes as same as normal logs in the nature (Supple. 5).

250 Ecological services for human life have been highlighted nowadays and we
251 have just realized that research on its beneficial and detrimental effects is important (Li
252 et al., 2014). Nevertheless ecosystem service has both useful and difficult aspects for us
253 and though it is not easy to control, it can greatly help the recovery process after a
254 tsunami disaster (Costanza et al. 1997; Gómez-Baggethun and Barton 2013; Jansson
255 2013). Moreover, the knowledge of the ecological condition after the disaster will be
256 important for encouraging victims to resume their normal life. Toyofuku et al. (2014)
257 reported that the biotic resilience in Tohoku area was quicker than they had expected
258 and it means that there would be active interactions between living organisms and
259 debris. These debris will stay in nature for a certain time. Generally metal and plastic
260 debris would have a slower impact than organic materials even though they can cause a
261 serious environmental problem. In this study, we examined the wood debris but we must
262 pay high attention also on the metal debris, especially on heavy metals. We must find
263 the way to use well the ecosystem and nature to deal with a natural disaster in this

264 modern world.

265 Natural disaster causes more and more serious problems nowadays because of
266 the urbanization. Many difficulties exceed human efforts. Eventually we can not help
267 relying on ecological purification systems to get back the healthy environment after
268 disasters. When the scale of natural disaster is larger than our assumption, even nature
269 itself needs a long time for the recovery. Therefore assessments after natural disaster in
270 the city area are essential not only for the human habitability but also for the
271 sustainability of natural eco-systems. For the prompt recovery of nature after tsunami
272 disaster, further study on ecosystems is essential.

273

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281

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336

337 **Figure legends**

338 Fig. 1 Tsunami wood debris from Natori city, Miyagi prefecture, Japan in 2012 July.

339 A: Arrived debris. Radioactivity was at natural level. B: 15 debris samples. C: 20 (R) x
340 20 (T) x 10 (L) mm sample block used for a decay test.

341

342 Fig. 2 Seawater influence on fungal attack of *T. versicolor*.

343 A: Salt (wt%) absorbed into the wood blocks by the immersion treatment before using
344 for decay test (n = 12) is illustrated with □ and solid line. NaCl contents in the debris,
345 which was calculated with ICP-indicated Na contents by NaCl molecular weight, before
346 using for decay test is shown with ◆ (n = 30). B: The mass loss by decaying test (wt%)
347 on *C. japonica* wood blocks immersed in the seawater for 0, 1, 3, and 6 months (n = 12).
348 The data from debris is shown with gray column (n = 30). C: Hardness change of *C.*
349 *japonica* wood block conditions after decay test. The hardness data from debris is
350 illustrated with gray column for the data before decay test and with dotted column after
351 decay test (n = 28). Vertical bar indicates standard error. Letters at the top of the column
352 indicate the results of the Tukey-Kramer HSD test.

353

354 Supplemental data

355 Supple. 1 Photos of the decay test with 1 month seawater immersed samples to see *T.*
356 *versicolor* attack. A: Fungal attack on wood block at 1 week after inoculation. Right jar
357 was for control and left jar was for test. B: Surface of control wood block after 12 week
358 fungus attack. C: Surface of 1 month seawater soaked wood block after 12 week fungus
359 attack.

360

361 Supple. 2 Decay test with *T. versicolor*.

362 *T. versicolor* attacked A: sound sapwood with no treatment (positive control), B: sound
363 sapwood treated with artificial seawater (negative control), C: wood debris from
364 tsunami disaster (test). Scale bar at the bottom of the photos indicated 2 cm.

365

366 Supple. 3 NaCl influence on growth of *T. versicolor*.

367 The standard data for the comparison between debris decaying rate and control
368 wood decaying rate based on sodium chloride contents.

369 A: Fungus growth (diameter) in 90 mm petri dishes on PDA medium contained 0 %
370 NaCl: ○, 1 % NaCl: □ 3 % NaCl: ◇ 5 % NaCl: ● 10 % NaCl: ■ Vertical bar indicates
371 standard error. B: Photo of fungus growth at 3 month after inoculation.

372 To get the simplest standard of salt effects on the growth of *T. versicolor*, NaCl
373 influence on growth of *T. versicolor* was observed on a medium. Potato dextrose agar
374 (PDA: potato extract, 0.4 %; glucose, 2.0 %; agar, 1.5 %) contained 0, 1, 3, 5 and 10 %
375 NaCl was prepared in 90 mm petri dishes and a pre-cultured 8 mm disk of *T. versicolor*
376 was placed in the middle of each petri dish. Fungal growth was measured for 12 weeks
377 at 26 ± 2 °C. Experiments were conducted with 10 repetitions for each NaCl
378 concentration.

379 As it is well known that the NaCl added to PDA affected negatively on *T.*
380 *versicolor* growth (Supple. 4A) ($p < 0.001$, $F = 47.808$ in the parameter of growth speed
381 difference along with NaCl contents, linear regression analysis). It took 7 days, 3 weeks
382 and 2 months for the mycelia of *T. versicolor* to cover the media with 0, 1 and 3 % NaCl
383 contained media respectively. Approximately 67.5 % surface of medium was covered by
384 mycelia at three month interval with 5 % NaCl contained PDA, and no growth was
385 observed with 10 % NaCl contained PDA (Supple. 4B).

386

387 Supple. 4 NaCl loss test by washing to simulate the function of rain.

388 The wash-off effect by rain had been examined to monitor the conditional
389 influence of the debris. A: washing condition with the indication of fractions obtained

390 parts. B: Salt reduction rate in wood blocks after washing at 7th day (n = 5). Salt
391 quantities were estimated with Na contents detected by ICP spectrometer (SPS-7800
392 Plasma Spectrometer, SII - Seiko Instruments Inc., Japan). Vertical bar indicates
393 standard error. Letters at the top of the column indicate the results of the Tukey-Kramer
394 HSD test.

395 Samples treated with artificial seawater were used to simulate the washing function on
396 salt reduction by rain. Wood sample blocks were placed on the plastic mesh so that all
397 water was running away from a test arena. Those blocks were showered with 10 ml, 100
398 ml and 500 ml distilled water every day for 7 days. Fractions were obtained at 7th day
399 and they were treated with nitric acid, and then adjusted with distilled water. Salt
400 residue in the wood samples were estimated by Na content in the digested solution
401 using ICP spectrometer. A small amount, about 10 cm³, was injected into the instrument
402 to measure its contents of Na. Standard curve was obtained with 0, 1, 10 and 100 ppm
403 standard solution adjusted by a commercial sodium standard solution (Na 1000 ppm,
404 Nacalai tesque). Some of detected values exceeded 100 ppm, however the data
405 demonstrated that a water shower had a certain effect on washing out NaCl from the
406 wood blocks ($p < 0.001$, $F = 12.100$, linear regression analysis). This wash-off effect by
407 rain had been examined to monitor the conditional influence of the debris.

408

409 Supple. 5 Mass loss from the larger scale decay test with 20(R) x 20(T) x 110(L) mm

410 wood block (n=5).

411 Debris no. 1-5 were used for tests and control samples were made with sound sapwood

412 *C. japonica*. Decay level was same in control and test samples ($p = 0.502819$, T test).

413

414

415

Fig 1

Fig 2

Supple 1

Supple 2

Mass loss(%) of middle sized wood blocks (n = 5)		
	Control	Debris
% (wt)	8.00	5.60
SE	0.015	0.031