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Short communication

Toxicity of untreated wood leachates towards two saltwater organisms (*Crassostrea gigas* and *Artemia franciscana*)

G. Libralato*, C. Losso, A. Volpi Ghirardini

Environmental Sciences Department, University of Venice Cà Foscari, Campo della Celestia 2737/b, I-30122 Venice, Italy

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Abstract

Wood is widely used in the development of freshwater, estuarine and marine coastlines. Timbers last according to their content of naturally occurring preservatives (mostly phenols and aldehydes), produced to prevent decay from biotic agents. When untreated woods are exposed to aquatic media, leachates are generated with likely toxic effects on the target environment. The potential impact on saltwaters of leachates from some untreated timbers of both native and tropical species has been assessed. The leaching procedure was set up considering British Standard test methods for paints and OECD guidelines for wood preservatives emission scenarios. Toxicity was monitored via the acute toxicity test with the brine shrimp *Artemia franciscana* and the sub-chronic embryotoxicity test with the oyster *Crassostrea gigas*. Brine shrimps evidenced no toxic effects while oysters discriminated well among leachates: the tropical wood species showed similar or relatively lower toxic effects than the native ones according to both leaching cycles (24 and 72 h). The ecotoxicological data have been integrated with some physical and chemical parameters.

Keywords: Untreated wood leachate; Brine shrimp; Oyster; Bioassay

1. Introduction

Vast amounts of wood have been used over the centuries for piles, docks and bulkheads during human activities for developing freshwater, estuarine and coastal areas [1]. Wood is a particularly useful building material as it is a renewable resource, with relatively low harvesting costs and excellent strength-to-weight properties [2]. Timbers that are not naturally durable are treated with preservatives in order to prevent decay due to wood-boring organisms such as crustaceans, molluscs and fungi [3]. Recent studies [4,5] have shown that leachates containing both natural and synthetic wood preservatives are potentially harmful towards the aquatic biota. Leaching is the process by which soluble constituents dissolve from a solid material into a fluid by percolation or diffusion. When water-soluble materials come into contact with water, constituents in the solid phase dissolve into the liquid, forming a leachate. The extent to which the constituents dissolve in the contact liquid depends upon site- and material-specific conditions (chemical, physical and biological factors) and the length of time involved [6].

In some cases, leachates from untreated wood have been found to be more toxic towards fish and invertebrates than leachates from chromated copper arsenate (CCA) treated wood [3]. These adverse effects were thought to be due to naturally occurring extractives including aldehydes, phenols, terpinene, camphene and pinene [7,8].

Traditional timber species are now being substituted in Europe by tropical ones, which are more resistant to water and wood-boring agents. The potential impact of tropical wood (e.g. used as piles) on the biota of marine coastal and transitional waters is still unknown.

This research investigated the potential toxic effects on two saltwater organisms of untreated wood leachates leaking from timber storage areas and direct wood piling. The effects of native and tropical timber leachates were also compared. Eco-toxicological surveys were done using the static non-renewal acute toxicity test with *Artemia franciscana* (brine shrimp), indicated as test organism by the Italian regulatory authorities for wastewater monitoring, and the static non-renewal sub-chronic embryotoxicity test with the oyster *Crassostrea gigas*, internationally recognised as a good bioindicator species

* Corresponding author. Tel.: +39 0412347711/37; fax: +39 0415281494.
E-mail address: giovanni.libralato@unive.it (G. Libralato).

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[9–11]. Some physical and chemical characterisations were also performed on wood leachates to integrate the toxicity data.

2. Materials and methods

2.1. Background

The quality of Brazilian tropical wood leachates of *Pouteria guianensis* (commonly named Abiurana ferro) (AB), *Minquartia guianensis* (commonly named Acariquara) (AC) and *Eschweileria* spp. (commonly named Mata-Mata) (MM) were compared with that of native *Quercus* spp. (Oak) (RO) and *Picea abies* (Norway spruce) (PA). All wood was certified according to FSC international standards.

2.2. Sample preparation and handling

Due to the absence of a specific protocol, the test method chosen for this study was in accordance with the determination of release rates of biocides from antifouling paints—general method for extraction of biocides [12], fitted and integrated according to the Emission Scenario Document for Wood Preservatives of the Organisation for Economic Cooperation and Development [13].

Untreated wood samples, free of damage, knots, visible resin, moulds, stains or wood destroying fungi and xylophages in general, were prepared for the leaching procedure.

A predetermined ratio was maintained between wood block surface area (A_s) and water leaching solution volume (V_{ls}), corresponding to the immersion of a wood block of 0.1 m × 0.1 m × 0.1 m in 1 L of water ($A_s/V_{ls} = 0.6\,cm^{-1}$) [13]. The blocks were cut to the required dimensions (0.03 m × 0.03 m × 0.065 m) with a circular saw. The leaching procedure consisted of agitating the blocks fixed to a Jar Test (mod. ISCO, Vittadini, Italy) at 60 rpm at 20 °C in a leaching medium consisting of aerated artificial sea water [9].

Two leaching cycles were taken into account, in order to observe a potential minimum variation in toxic effects with leaching time. The first cycle lasted 24 h, the leaching fluid was then completely renewed and the leaching procedure continued for a further 48 h (72 h cycle). Wood blocks were not substituted. A 48 h leaching cycle (24 h + 24 h) was not considered because, in a previous study [14], its leachate showed no significant dissimilarity from the 24 h leachate. The moisture content of fresh wood was determined by weighing the blocks before and after drying at 60 °C for 24 h [8].

pH values were measured just after the blocks were first immersed in the leaching fluid, then 3, 24 and 72 h after the leaching procedure started (perpHecT LogR meter, model 330, Orion, Beverly, MA, USA). pH and salinity of samples were checked before the start of each bioassay. pH values below 7.5 were corrected by adding NaOH 0.1 M aliquots. In order to simulate low tide, just one natural drying period was considered, lasting 6 h between the end of the 24 h cycle and the beginning of the 72 h cycle. Dissolved oxygen (DO) was determined by a WTW multiparametric device. Leachate chemical oxygen demand (COD) was performed according to [15]. Leachate samples were stored in 100 mL PE containers at −18 °C for later ecotoxicological analyses.

2.3. Ecotoxicological analyses

Conditioned oysters (*C. gigas*) were purchased from an English hatchery (Guernsey Sea Farm Ltd., UK). The embryotoxicity test was performed according to the method proposed by [16]. Adults were induced to spawn by thermal stimulation (temperature cycles at 18 and 28 °C). Good quality gametes were selected from the best males and females and filtered at 32 μm (sperm cells) and 100 μm (eggs) to remove impurities. After fertilisation, egg density was determined by counting four subsamples of known volume. Fertilised eggs, added to test solutions in order to obtain a density of 60–70 eggs/mL, were incubated for 24 h at 24 °C and fixed with buffered formalin. Hundred larvae were counted, distinguishing between normal larvae (D-shape) and abnormalities (malformed larvae and prelarval stages). Test results acceptability was based on negative control for a percentage of normal D-shape larvae ≥80% [17]. A first screening phase assessed samples' toxicity levels, exposing embryos to 6, 12, 25, 50, 75 and 100% of leachates. On the basis of previous information, the second analysis screened lower dilutions: 0.37, 0.75, 1.5, 3, 6, 12, 25 and 50% of leachates. In addition, a reference toxicant (6, 12, 18, 24 and 36 μg/L of $Cu(NO_3)^2{\cdot}3H_2O$, nominal concentration) and dilution water control were assessed. Three replicates were tested for each sample.

Certified brine shrimp cysts (*A. franciscana*) were purchased from specialist dealers. The acute test was performed according to the [15] protocol. Brine shrimp cysts were hatched in artificial salt water (Instant Ocean®, 35‰) at 25 °C, pH between 7.00 and 8.30 and constant lighting (3000 lux) for 1 h. The bioassay was performed in a disposable multiwell test plate with 24 (6 × 4) test wells where three replicates of 10 instars II–III larvae were incubated in 2 mL samples at 25 °C in darkness. Larval mortality was estimated after 24 h by counting the dead larvae (i.e. those which exhibited no internal or external movement during 10 s observation). The test was considered suitable if the mortality in the control did not exceed 10%. $CuSO_4{\cdot}5H_2O$ was used as reference toxicant. Serial test dilutions of samples were 12, 25, 50 and 100%. Three replicates per dilution were considered.

2.4. Data analyses

For the embryotoxicity test with *C. gigas* and *A. franciscana*, data were expressed as EC50 and LC50 values, respectively, based on the percentages of abnormalities or dead organisms, calculated by Trimmed Spearman-Karber statistical methods [18]. The data expressed as EC50/LC50 were transformed into Toxicity Units (TU50 = 100/EC50(LC50)) to reveal the direct relationship between toxic effects and measurement system used. Toxicity data expressed as percentage (%) of effect were adjusted according to Abbott's formula [19].

Table 1
Physico-chemical parameters measured in the wood leachates after 24 and 72 h leaching cycles (W = water content; DO = dissolved oxygen; COD = chemical oxygen demand)

Samples	W (%)	24 h			72 h		
		DO (mg O_2/L)	pH	COD (mg O_2/L)	DO (mg O_2/L)	pH	COD (mg O_2/L)
AB	9.6	6.63	7.77	1203	6.67	7.86	996
MM	11.6	6.52	7.54	1286	6.66	7.61	1120
RO	10.6	6.64	4.29[a] (8.00)	1411	6.63	4.01[a] (7.80)	1452
AC	6.9	6.65	5.84[a] (7.42)	1161	6.28	6.44[a] (7.45)	1120
PA	10.5	6.62	7.56	622	6.45	7.85	954

Tropical timbers are AB = Abiurana ferro, MM = Mata-Mata, AC = Acariquara and native timbers are RO = Oak and PA = Norway spruce.
[a] pH adjusted with NaOH 0.1 M.

3. Results and discussion

3.1. Physico-chemical parameters

Physical and chemical parameters are given in Table 1. Wood water content ranged between 6.9% and 11.6%. DO levels were always above 6 mg O_2/L. Leachates pH values were lower than 7.5 for both 24 and 72 h cycles for RO and AC. They were therefore adjusted to acceptable pHs for an ecotoxicological comparison with the other leachates. COD values did not change significantly after 24 and 72 h of leaching procedure, ranging from 622 to 1411 mg O_2/L at 24 h, and from 954 to 1452 mg O_2/L at 72 h. For both tropical and native species, COD was up to six times lower than wood leachate COD produced from *Populos tremuloides* Michx. [20], but similar to that found on leachates obtained from woody wastes [10]. Salinity levels were in the best range of 33–35‰.

3.2. Ecotoxicological analyses

For oysters, dilution water quality control showed an average of 80 ± 6% of well-developed larvae, according to the acceptability limit of [17]. Reference toxicant reported an EC50 value of 9.01 μg/L (8.29–9.78 μg/L), comparable with those previously found and that reported by [17] (5–13 μg/L).

For brine shrimps, the negative control showed a mortality of 5 ± 1%, according to test acceptability and the reference toxicant reported an EC50 value of 5.1 (4.6–5.6) mg/L, comparable with [15].

Toxicity data are reported in Table 2 as percentage of effect (%) and TU50. For oysters, all leachate samples displayed potential toxicity (Table 2). Twenty-four hour leachates showed higher toxicity levels than 72 h leachates, suggesting that the 24 h leaching cycle released more ecotoxicological compounds, as indicated by [8,21]. Leachate toxicities showed that AB < MM ≈ RO < AC < PA at 24 h and AB ≈ AC ≈ MM ≈ RO < PA at 72 h. From 24 to 72 h, toxic effects were reduced by more than 50% for all samples except for PA, which maintained almost the same toxicity values (3% reduction). *Vibrio fischeri* revealed similar toxicity values in wood leachates from paper mills, due mostly to the presence of unsaturated fatty acids and resinous acids [22,23]. For brine shrimp (Table 2), only two leachates (RO and AC) were toxic at 24 h, while at 72 h samples displayed only a percentage of effect (≤14%). RO and AC 24 h leachate toxicities were detected in brine shrimps and oysters, which both evidenced greater toxicity of AC leachate than RO. At 72 h, RO and AC leachate toxicity substantially disappeared in *A. franciscana*. Tropical timber leachate toxicities AB and MM were lower than those of traditional wood species RO and PA. AC displayed lower toxicity than PA at 24 h, but higher than RO, which was greatly reduced in the 72 h leaching cycle. Oysters showed that PA leachate could be a toxicity hot spot at both 24 and 72 h. PA toxicity as detected by *C. gigas* was not confirmed by *A. franciscana*, which appeared to be much less sensitive than oysters at discriminating leachate toxicities between the two leaching cycles. It is also possible to classify samples according to Tonkes' classification [24], based on the percentage sample volume that induces EC50 (<1 vol.% = very acutely toxic; 1–10 vol.% = moderately acutely toxic; 10–100 vol.% = minor acutely toxic; and >100 vol.% = not acutely toxic). For oysters, leachates were from minor acutely toxic (AB at 24 h; AB and MM at 72 h) to moderately acutely toxic (MM, RO, AC and PA at 24 h; RO, AC and PA at 72 h). For brine shrimps, RO and AC were both considered as moderately acutely toxic at 72 h, whereas all other samples showed to be not acutely toxic.

The comparison between physical and chemical parameters and ecotoxicological data, and between COD and toxicity data, did not display any significant correlation ($p < 0.05$).

Table 2
Wood leachates sub-chronic embryotoxicity displayed by *C. gigas* and acute toxicity showed by *A. franciscana*

Samples	24 h		72 h	
	% effect	TU50	% effect	TU50
C. gigas				
AB		8.01 (7.20–8.92)		2.40 (2.20–2.60)
MM		14.73 (13.79–15.75)		6.45 (6.02–6.91)
RO		16.00 (14.41–17.76)		7.06 (6.59–7.53)
AC		26.11 (24.81–27.40)		3.97 (3.52–4.49)
PA		55.56 (52.63–59.17)		53.76 (50.76–57.14)
A. franciscana				
AB	–		–	
MM	31 ± 7		14 ± 9	
RO		47.57 (41.65–54.75)	–	
AC			9 ± 3	
PA	–	78.21 (75.58–84.09)	4 ± 2	

Data are expressed as % of effect (after Abbott's formula adjustment) and TU50; –: no effect. % of effect was not reported when samples showed TU50 values.

4. Conclusions

Wood leachates from tropical (AB, MM and AC) and native timber species (RO and PA) showed some acute toxic effects (*A. franciscana*) after a 24 h leaching period and widespread sub-chronic toxic effects (*C. gigas*) after both 24 and 72 h leaching periods. A high toxicity reduction (>50%) was observed in oysters from 24 to 72 h leachates. Oyster embryotoxicity bioassay was demonstrated to be more suitable for marine organisms protection than brine shrimp acute test towards wood leachates. The use of *P. guianensis*, *M. guianensis* and *Eschweileria* spp. of tropical timber for transitional and coastal marine water piles should be no more harmful than that of the traditional native species *Picea abies* and *Quercus* spp. Leakages from coastal timber storage areas should be collected and treated to avoid any potential impact on seawater organisms.

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