Particle size characterization of oak wood leachate: chemical oxygen demand and toxicity distribution within different fractions

Henric Svensson, Yahya Jani, William Hogland and Marcia Marques

ABSTRACT

Oak wood leachate obtained from two storage facilities (storage pound and ditch) in a wood-based industry, and leachate generated by a laboratory leaching test, were characterized in seven categories regarding particle size distribution (PSD) (raw leachate, $\leq$20 μm, $\leq$10 μm, $\leq$1.2 μm, $\leq$13 nm, $\leq$5 nm and $\leq$2 nm). The PSD followed a normal distribution model with a correlation coefficient (r) varying from 82 to 88. Each fraction was analysed regarding chemical oxygen demand, polyphenols and acute toxicity in toxicity assays with *Artemia salina*, *Vibrio fischeri* and *Lactuca sativa*. Fractions with particles >1.2 μm were more toxic to *A. salina* and *V. fisheri* than fractions with particles $\leq$1.2 μm. No toxic effect was observed for *L. sativa*. The results suggest that polyphenols are the main toxic compounds in oak wood leachate. A conspicuous difference was found between field and laboratory samples.

Key words | oak, particle size distribution, toxicity, ultra filtration, wood leachate

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NOTATION

A	an empirical coefficient that represents the total concentration of a particulate matter in the water
b_H	endogenous decay rate (day^{-1})
d_p	particle size (m)
f_{Es}	soluble inert fraction of endogenous biomass
f_{EX}	particulate inert fraction of endogenous biomass
K_X	hydrolysis half saturation constant for S_H (g COD (g cell COD)$^{-1}$)
$N(d_p)$	particle size distribution
S_0	dissolved oxygen concentration (mg O_2 L^{-1})
S_s	readily biodegradable COD (mg COD L^{-1})
X_H	active heterotrophic biomass (mg cell COD L^{-1})
Y_H	heterotrophic yield coefficient (g cell COD (g COD)$^{-1}$)
β	an empirical coefficient that represents the power law
μ_H	maximum specific heterotrophic growth rate on S_s (day^{-1})

INTRODUCTION

Outdoor storage of logs by the wood industry and wood chips in bioenergy plants has increased during recent decades. The contact of logs and wood chips with irrigation water or rainfall generates contaminated leachate that becomes part of the stormwater runoff (Woodhouse & Duff 2004). Wood leachate contains organic and inorganic compounds found in soluble or insoluble form. The particle size distribution (PSD) (Figure 1) is a very important parameter to characterize any wastewater or stormwater and contributes to the decision about the most suitable treatment methods. As an example, the size range of the settleable pollutants in wastewater is commonly defined as being above 1×10^5 nm, which can be removed by choosing a chemical settling method (Dulekgurgen *et al.* 2006; Karahan *et al.* 2008). The correlation between the particle size and chemical parameters such as chemical oxygen demand (COD), colour, suspended solids and turbidity is also well known (Chavez *et al.* 2004).

In the wood industry, the composition of leachate depends on the structure and physical–chemical properties
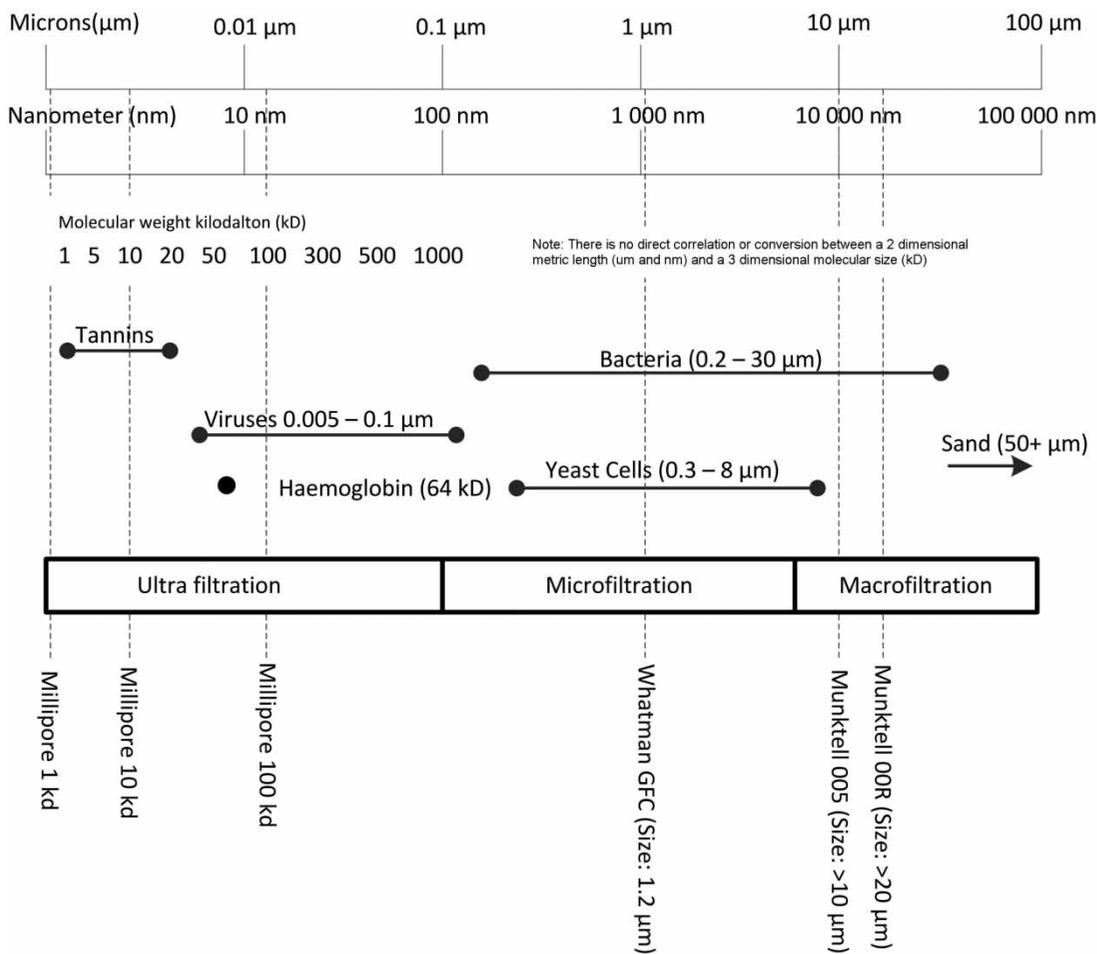
Figure 1 | The six filtration steps are marked with dotted lines on the scale, with some common materials, organisms and molecules classified by particle size in each category for reference purposes. Ultra filtration particle size is (0–100 nm), microfiltration (100-less than 10^4 nm) and macrofiltration (less than 10^4- higher than 10^5 nm).

of the wood species. A tree can produce different defence substances to protect itself against insects and diseases (Eyles *et al.* 2010). These defence substances can be easily transferred to the water phase by leaching, and its discharge can cause environmental impacts (Hedmark & Scholz 2008). Pedunculate oak (*Quercus robur*) trees produce a polyphenolic group called tannins. Trees are known to contain water soluble phenolic compounds (Scalbert *et al.* 1989) and mostly hydrolysable tannins or ellagitannins like esters of gallic acid (Puech *et al.* 1999). Tannins are large materials with a molar mass between 300 to 3,000 Da (3.0 kDa). However, tannins up to 20,000 Da (2.0 kDa) have been also found (Khanbabaee & van Ree 2001). These materials show phenolic reactions and have the ability to precipitates proteins. It is well known that tannins are toxic to microorganisms and ruminant animals (Mingshu *et al.* 2006; Bhat *et al.* 1998). No information has been found about the toxic effect of tannins on plants.

Tannins are active proteins that interfere with digestion systems, making them hard to digest (Hättenschwiler & Vitousek 2000). Furthermore, they are known to interfere with nutrient availability, especially with nitrogen (Kanerva *et al.* 2006). It is also likely that these active protein features could affect other important functions inside organisms by affecting proteins. Tannins are often analysed by the Folin–Ciocalteu method, measured by spectrophotometry, correlated to gallic acid and reported as gallic acid equivalence or correlated to tannic acid and then reported as 'substances reducing Folin phenol reagent'. However, the term 'tannin and lignin' is often applied for this analysis. Considering that all reducing compounds are measured in the sample, the more appropriate term polyphenol (PP) found in (Georgé *et al.* 2005) was also used in this manuscript. Wastewater characterization has traditionally concentrated on the measurement of parameters such as total organic carbon (TOC) and COD. These parameters

give relevant information about the total organic materials; however, on their own, they do not inform organic compound distribution in terms of particle size, which might be of strategic importance for designing of treatment schemes.

Chromatographic methods are also useful, but they are time consuming, particularly when the content of the wastewater has not been described previously. Therefore, PSD is a useful evaluation method for the identification of the placement of COD in different fractions (Doğruel *et al.* 2011). PSD has been used for some time for the characterization of different wastewaters (Doğruel *et al.* 2011). PSD helps to understand the behaviour of suspended materials in a treatment system and to choose the treatment with the best expected removal performance. It is also a useful method for better understanding the biodegradation processes (Dulekgurgen *et al.* 2006). Doğruel *et al.* (2011) studied the effect of PSD on the biodegradation and treatability of leachate from organic waste. The experimental results showed that the COD of the leachate was 80,000 mg/L and the PSD analysis fitted the bimodal distribution (as shown by Equation (1)) with 60% of the COD below 2 nm and 25% above 1600 nm. The results obtained with the PSD could then explain the limitation of the lime and alum chemical treatment efficiency since only 30–35% of COD was removed from the 2 nm-sized particles. García-Mesa *et al.* (2010) characterized the wastewater in a biofilm reactor using PSD. The results showed that the PSD fitted a Bowers law equation (as shown by Equation (2)). High correlation coefficients between PSD and turbidity, and COD and suspended solids were also found. The experimental results proved that PSD is a very good tool to explain the performance of biodegradable treatment of the biofilm with a high correlation coefficient (82.14%). PSD has helped to find the best treatment method with a focus on the removal of toxicity encountered with oil mill effluents (Beccari *et al.* 2002). In the same way, it could assist understanding of the PSD of oak wood leachate to help find the best treatment method to solve the toxicity problems encountered with wood leachate

$$-\frac{dS_0}{dt} = \frac{1 - Y_H}{Y_H}.\mu H \frac{S_s}{K_s + S_s} X_H + (1 - f_{EX} - f_{Es}).b_H X_H \quad (1)$$

$$n(dp) = A.dp^{-\beta} \quad (2)$$

The aim of the present investigation was to assess chemical parameters and toxicity associated with particle size fractions in oak wood leachate obtained under laboratory conditions through controlled leaching tests, and in the field after rainfall and snowmelt have been in contact with logs and sawdust found outdoors in storage areas. To do that, water samples were sequentially fractioned with the help of filters in such a manner that each remaining fraction contained all particle sizes below the one removed. The fractions obtained in this way were analysed for TOC, polyphenols (PP), pH and colour. Different mathematical distribution models were applied to select the one that the data fitted best, based on the correlation coefficients. The species used in the toxicity assays were *Artemia salina*, *Vibrio fischeri* (Microtox®) and *Lactuca sativa*.

MATERIAL AND METHODS

The following types of waters containing contaminants leached from oak wood were investigated: (i) water collected outdoors from a pond that receives oak wood leachate generated due to log irrigation, named field sample point 1 (FSP-1); (ii) water collected outdoors from a ditch that receives leachate generated due to rainfall contact with oak wood chip piles (Svensson *et al.* 2012) (FSP-2); (iii) leachate generated via leaching test with oak sawdust under laboratory conditions, as previously described by Svensson (2013) laboratory leachate (LAB-L).

To produce the particle size fractions, vacuum filtration was carried out in the following sequence: Munktell filter paper grade 005 (20 μm), Munktell 00R (10 μm), and Watchman GF/C (1.2 μm). Next, the remaining water was filtrated with Millipore stirred Cell Model 8400, which is a nitrogen gas powered filtration cell. Filtration in the ultra-range is measured in Dalton, which is not an SI unit. To be able to make mathematical calculations and comparisons, Dalton was approximately converted to metric. The ultra-filtration discs were then used in the following order: Millipore 100 kDa (approximately 13 nm), 10 kDa (approximately 5 nm) and 1 kDa (approximately 2 nm). After each filtration step, 50 mL of water was removed and used for chemical analyses.

Three distribution models (normal distribution model, binomial distribution model and Poisson distribution model) were tested with the experimental data to find the one that fitted best. To do that, a programme was built up in Matlab and all the experimental data were tested based on the calculation of the correlation coefficient by the analysis of variance (ANOVA) statistical method.

Chemical analyses

COD was analysed with Hach Lange test kits LCK 114 (150–1000 mg/L) in a Dr Lange 5,000 spectrophotometer. Phenolic substances were analysed with Folin–Ciocalteu reagent according to Georgé *et al.* (2005) and reported as total content of polyphenols (in mg/L gallic acid equivalents). Colour was measured by Hach Lange spectrometer Dr 5,000 according to Method 8,025 and expressed as mg/L P-co.

Toxicity assays

When conducting toxicity assays, it is important to use more than one species. The test battery approach (TBA) including at least three different trophic levels, according to Ren & Frymier (2003) was applied. The bioindicators selected were: *A. salina, V. fischeri* (Microtox®) and *L. sativa*. All samples had the pH adjusted with sodium hydroxide to 6–8 before the toxicity assays. EC_{50} concentrations (that cause 50% mortality) were calculated using the software GraphPad prism 5.

A. salina

The toxicity assay was performed as previously described (Svensson *et al.* 2005). Potassium dichromate ($K_2Cr_2O_7$) was used as the reference chemical. The EC_{50}-value was calculated, and variation coefficients were calculated. To ensure that any toxic effect was not due to acidic pH, the water's pH was adjusted with NaOH to a range of 6–8.

V. fischeri (Microtox® test)

The test was performed according to the Microtox® standard method. The EC_{50} values were established after 15 minutes of exposure.

L. sativa

Lettuce seeds (Sonette) were used in the germination test (OPPTS 850.4,200 standard). In total, 10 seed (in triplicate) were placed in Petri dishes with a filter paper and 8 mL of water. Tap water was used in the control. The Petri dishes were wrapped with parafilm and placed in a dark control room (20–25 °C) for 4 days. After this period, the germination was considered successful in those cases where the plantlet had reached more than 20 mm.

Table 1 | Chemical and toxicological characterization of the water samples. Mean values (standard deviation)

	FSP-1	FSP-2	LAB-L
pH	6.9	4.3	3.2
COD mg/L	216 (13)	5,098 (323)	2,919 (320)
PP mg/L	35 (8)	842 (87)	1,249 (134)
PP/COD ratio	0.16	0.17	0.43
V. fischeri (EC50)	n.t.	58 (9)	22 (3)
A. salina (EC50)	n.t.	70 (0)	80 (0)
L. sativa (EC50)	n.t.	n.t.	n.t.

PP = polyphenols; n.t. = no toxic effect detected.

RESULTS

Chemical characterization

The sample characterizations are presented in Table 1. The PP/COD ratios of FSP 1 and 2 are similar, but much lower than that of the laboratory leachate (LAB-L). The polyphenols group is a minor component in the old wood leachates found in the storage facilities (FSP-1 and FSP-2) but a major component in the leachate released by leaching tests in the laboratory.

The PSD analysis

The effects of PSD on the concentration of COD and polyphenol are shown in Table 2. Normal, binomial and Poisson distribution models were applied to describe the PSD of FSP-1, FSP-2 and LAB-L.

The statistical results show that the normal distribution model gave the best fit to the experimental results with high correlation coefficients such as: FSP-1 ($r = 83\%$), FSP-2 ($r = 82\%$) and LAB-L ($r = 88\%$).

The results show that there are differences between the types of contaminated waters. Both field-sampling points (FSP1, FSP2) had roughly the same distribution even though they did not have the same concentrations. FSP 1 and 2 had over 50% of the COD in the fraction ≤1.2 μm, while the spread of particle sizes is much higher in the LAB-Leachate. It should be noted that around 50% of the polyphenols in FSP 1 and 2 were bigger than expected; over 50% of the polyphenols were found to have a molecular size over 100 kda (0.013 μm).

The PP to COD ratio was considerably lower in the raw FSP 1 and 2 compared to the LAB-L (Figure 2). Whereas

Table 2 │ Particle size distribution of FSP-1, FSP-2 and LAB-L. Weight measured as COD

Particle size (μm)	FSP-2		FSP-1		LAB-L	
	Weight (mg)	Accumulated	Weight (mg)	Accumulated	Weight (mg)	Accumulated
20–10	410	0.07	2.3	0.01	66.7	0.02
10–1.2	0	0.07	7.0	0.04	213.3	0.09
1.2–0.013	3335	0.68	118.2	0.57	283.3	0.19
0.013–0.005	70	0.69	0.0	0.57	536.7	0.37
0.005–0.002	445	0.77	65.1	0.85	944.0	0.69
0.002	1260	1.00	33.0	1.00	926.0	1.00
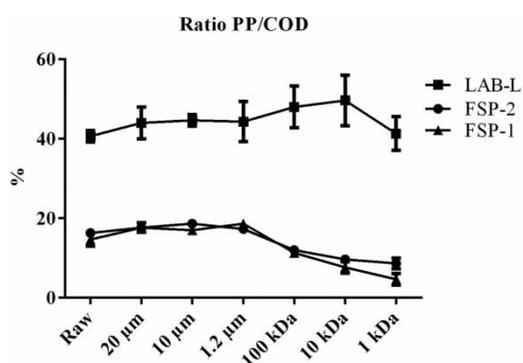
Figure 2 │ PP/COD ratios in the water fraction after each filtration step.

40% of the COD in the laboratory leachate was formed by polyphenols, in both field samples PP corresponded to approximately 15% of the COD (Figure 2). During fractionation, as soon as the large particle sizes were removed, a tendency for reduction in the PP/COD ratio was observed (Figure 2), which makes sense due to the relatively high molecular sizes of PP.

The toxicity analysis

Only FSP 2 and LAB-L were toxic to *A. salina* and *V. fischeri*. No sample had a toxic effect on *L. sativa* germination. Therefore, EC$_{50}$ values for FSP-2 and the laboratory leachate were calculated only for *A. salina* and *V. fischeri*, Table 1. FSP-2 was considerably more toxic to *V. fischeri* (Microtox) than the laboratory leachate (*T*-test *P*-value 0.0002). For *A. salina*, the toxicity levels of both samples (FSP-2 and LAB-L) were considered similar (*T*-test *P*-value 0.4339).

The effects of filtration on wood chips and leachate water are presented in Figure 3. The toxic effect drops significantly for wood chips after the 100 kDa filter in both Microtox and Artemia tests, whereas the drop is not that significant for leachate water.

DISCUSSION

Particle size distribution: The PSD gives information not visible in chemical characterization, based on parameters such as COD. The oak wood leachate generated in the laboratory by leaching differs in terms of size distribution from samples collected in the field. About 50% of COD found in samples collected in the field is in the fraction ≤100 kDa (approximately 0.013 μm). Meanwhile, only 10% of COD is removed from the same water fraction after filtration of the laboratory leachate. It is also the 100 kDa filter that removes the highest amount of PP found in field samples, while the amount of PP in the laboratory leachate is more equally distributed along fractions. The PP/COD ratio showed differences, and one possible explanation is that the degradation in the field is higher for PP compounds smaller than 100 kDa, resulting in a higher proportion of this PP left in samples from the field.

It was not expected that PP should be removed by filtration with 100 kDa filters. Tannin and lignin are two major groups of wood extractives detected with the Folin–Ciocalteu method. Tannins are usually not reported as having large molecule size and lignin has low solubility in water (Sjöström 1993); therefore, lignin should not be able to be a major component of the wastewater. However, thousands of phenolic compounds have been identified in wood extractives (Sjöström 1993). The explanation for the fractionation pattern is that tannins and/or lignins with size >100 kDa are present; or, it is possible that during storage, further polymerization of polyphenols might have occurred. According to Schaumann (2006), different models have been formulated since the late 90s to show how humic substances aggregate or form supra-molecules. Aggregated molecules are held together by planar-π-door-planar-π*-accepter or by cation bridges (Schaumann 2006). Therefore, the differences
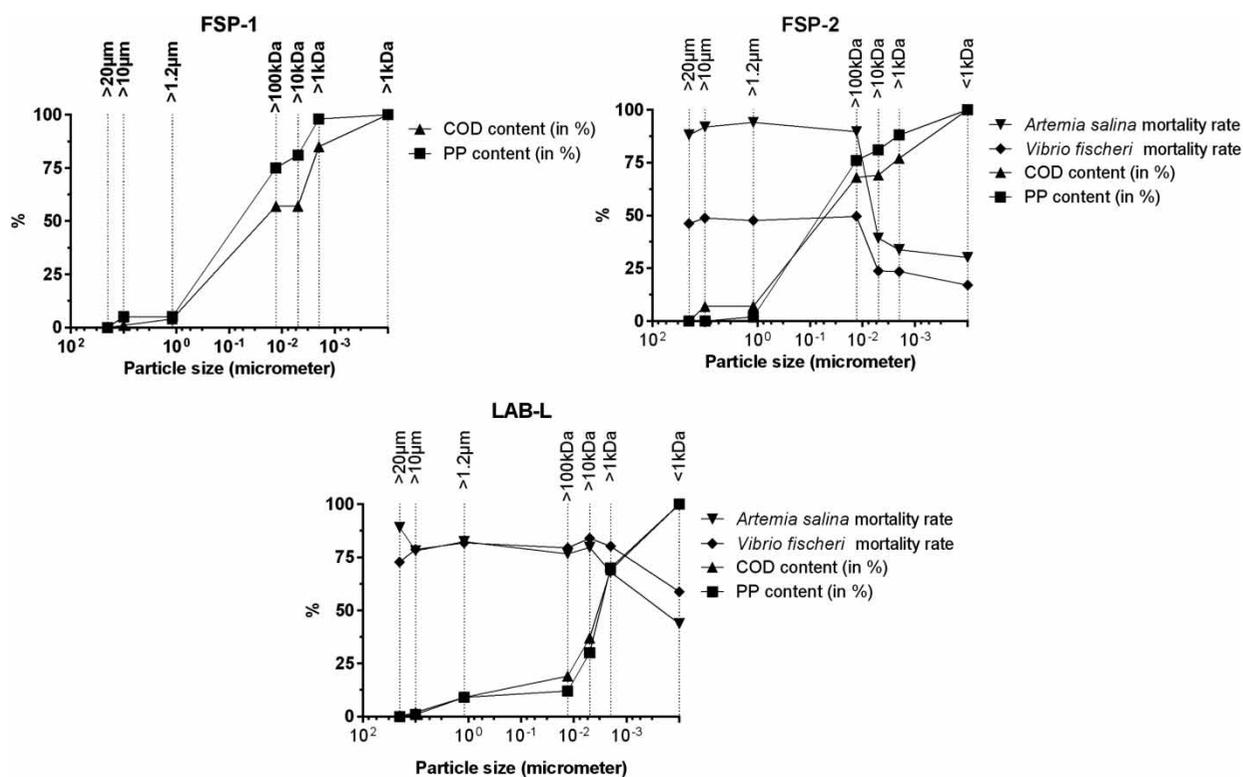
Figure 3 | Distribution (in %) of COD, PP and toxicity to *A. salina and V. fischeri* (mortality in %) and germination (reduction in %) of *L. sativa* for different particle size fractions of oak leachate after sequential filtration. Leachate obtained via leaching test with oak sawdust under laboratory conditions (LAB-L); water collected outdoors from a ditch in a log storage area (FSP-2); water collected outdoors from a pond that receives runoff from sawdust storage (FSP-1).

in particle sizes between field samples and laboratory leachate could also be due to the higher amount of cations found in the field samples compared to the laboratory samples. A third possibility is that the charge in polarity might affect the retention in the filter (Van der Bruggen *et al.* 1999), which could affect the result.

Wood leachate from cedar trees has been found to be hard to biodegrade in wetland systems (Tao *et al.* 2006). This has also been observed for oak wood leachate (Svensson *et al.* 2011). One explanation for the recalcitrant behaviour could be the size of molecules found in the wood leachate. Big molecules need to be broken down in an extracellular environment (hydrolyzed), which is a slow process (Henze *et al.* 2001); or, they need to be settled from the water phase. Doig *et al.* (2006) found that both processes are important for wood leachate when they examined water from a log-sorting yard in British Columbia. Settling without biological activity over 13 days removed 25% of COD, and settling with biological activity removed 33%. Their results also indicated that during storage of wood leachate where flocculation occurred, some big particles ($>1\,\mu m$) created larger polymers that were not settable. This could explain the large polyphenol particles discussed above.

Size distribution comparison between oak wood leachate and municipal wastewater shows significant differences. Municipal wastewater was found to have only around 12% of organic matter in particles sized between 100 and 1 kDa (Doğruel *et al.* 2011), while oak leachate had up to 50% of COD content within a 100 to 1 kDa size range. This could explain some of the oak wood leachate's resilience to biodegradation, since in most cases only monomers and oligomers with a molecular size smaller than 1 kDa are able to cross the bacteria membrane (Cadoret *et al.* 2002); therefore, intercellular breakdown is ruled out.

The normal distribution model was found to give the best fit to the experimental results.

Toxicity: P were expected to be the main toxic component in the studied wastewater, since oak wood leachate is known to be rich in PP (Svensson *et al.* 2013). However, tannins were expected to be found in the particle size range of 1–20 kDa, while more than 50% of the polyphenols were found to be bigger than 100 kDa. It was demonstrated that large molecules ($>100\,kDa$) are responsible for more than 50% of the mortality of *A. salina* in the field sample, while more than 50% of the mortality of *A. salina* in contact with LAB-L is caused by substances smaller than 1 kDa. For

V. fischeri, the mortality is higher in leachate water and the toxic effect is caused by smaller substances (<1 kDa). However, the toxicity clearly follows the concentration of PP suggesting that these molecules are the main toxicant in this water.

As mentioned previously, based on size distribution, it is hard to determine what kind of substance PP bigger than is 100 kDa. Both tannins and lignin are candidates, tannins are known to be toxic; however, lignin is also reported as being toxic (Libralato *et al.* 2011).

No toxic effect to *L. sativa* was observed in the seed germination test. Tannins are not expected to cause toxic effects to seeds. However, PP-rich wastewaters, such as olive-mill wastewater, have been proven to be toxic to seed germination (Beccari *et al.* 2002, Casa *et al.* 2003)). The results illustrate the importance of TBA when working with and interpreting toxicity data.

CONCLUSIONS

PSD highlights important aspects of wastewater characterization not shown by chemical characterization. For example, significant differences were found between laboratory leachate and field samples, while similar PSDs were found when field samples were compared, irrespective of quite different COD contents. The results suggest that leachate produced by leaching procedures in laboratory conditions is not always comparable to what happens outdoors. Oak wood leachate was not toxic towards *L. sativa* but clearly toxic to *V. fisheri* and *A. salina*. Furthermore, this study show that big molecules, above 100 kDa, have an effect on toxicity in the oak leachate, which is in contrast to the most common results (smaller molecules are more toxic than larger ones). The normal distribution model fitted the experimental results with a high correlation coefficient with an average of higher than 80%.

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First received 12 February 2014; accepted in revised form 19 May 2014. Available online 30 May 2014