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Ecotoxicity of Pulp Mill Effluents from Different Prebleaching Processes

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The use of chlorine compounds by pulp mill industries to remove or decolorize residual lignin leads to a discharge of large amounts of chlorinated organic matter to the aquatic environment (Afonso et al. 1992; Owens 1991). There are two ways to minimize those impacts: (i) to remove toxic compounds from effluents (Afonso *et al.* 1992; Korczak *et al.* 1991; Kroiss *et al.* 1992), or (ii) to introduce new methodologies such as extended and oxygen delignifications and the replacement of chlorine by other chemicals (Axegard 1986, 1988; Bowen and Hsu 1990; Graves *et al.* 1993; Hurst 1993; Nutt *et al.* 1993; Pryke 1989).
Here, we compared the effluent toxicity of a pulp mill using chlorine dioxide as the oxidizing agent in the pulp bleaching process, before and after the introduction of oxygen delignification as a previous treatment.

The cladocerans *Daphnia magna* and *Ceriodaphnia dubia* (filter feeder organisms), and the mosquito larvae *Aedes aegypti* (a particulate feeder and air breathing organism) were used in acute toxicity testing. The role of the ingestion of suspended cellulose fibers present in the effluent as a pathway of toxicant (adsorbable organic halogens) intake was investigated. Futhermore, the effect of temperature on sublethal toxicity, using *C. dubia,* was also evaluated.

MATERIALS AND METHODS

Composite effluent samples, collected at the same sampling station, were provided by a pulp mill factory where *Eucalyptus globulus* wood was being processed. Samples were carried to the laboratory, at aproximately 4°C in glass vessels, and were frozen immediately upon arrival in the laboratory. Samples were collected before and after the introduction of oxygen delignification as a prebleaching process (codified as S1 and S2, respectively).

D. magna and *C. dubia* (Cladocera) were provided by adult females cultured in ASTM hard water (EPA 1991) with the organic supplement "Marinure 25" (Baird *et al.* 1989), and were fed daily with $5x10^6$ cells mL^{-1} of the green algae *Chlorella vulgaris.* Only juveniles less than 24 hr old, from 3rd, 4th and 5th broods were used in toxicity tests.

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First instar larvae of the mosquito *Aedes aegypti* (Diptera) were obtained from eggs laid by a stock population maintained in our laboratories, following the methodology described by Ribeiro *et al.* (1995).

The dilution water was prepared as described in Directive 92/32/EEC (EEC 1992) and used as control. A range-finding test (0.5, 5.0 and 50% of effluent) was carried out, in order to determine the definitive range of effluent dilutions (12, 24, 48, 69, 83 and 100%). Tests were performed in 175 mL and 42 mL glass vessels with 50 mL (for *D. magna*) and 20 mL effluent sample (*C. dubia* and *A. aegypti*). In each test dilution and control, 20 organisms were divided into four batches of five animals. The number of immobilised individuals in each vessel was recorded after 24 and 48 hr of exposure; *D. magna* and *A. aegypti* were also observed at 72 and 96 hr. Dissolved oxygen and pH were measured at 0, 48 and 96 hr. Tests were carried out at 20 ± 0.5°C, with a 16 hr light and 8 hr dark photoperiod.

Median effective dilution (ED_{50}) values and respective 95% confidence limits were calculated by probit analysis (Finney 1971). To further evaluate effluent partial toxicity, another equally designed test was performed with effluent samples after removing suspended solids (centrifugation during 30 min. at 4300 rpm).

Since acute toxicity was not found in S2 samples, sublethal endpoints were investigated, under two temperature regimes, using *C. dubia* juveniles. ASTM hard water (EPA 1991) was used as dilution water and control. The definitive range of effluent dilutions was 26, 36, 51, 71 and 100% of whole effluent (S2). Tests were performed using 15 mL of medium in 42 mL glass vessels. Ten replicates (one organism per replicate) were used for each test dilution and control. Medium renewal was done daily and organic supplement and food were added before the transference of the organisms. Chronic tests were performed simultaneously at two different temperatures, 20 and 25°C under the same photoperiod conditions (16 hr light : 8 hr dark). Tests ended when 60% of control females released the 3th brood (9 days at 25°C and 12 days at 20°C). Females were measured at the end of tests. Time to first brood, total number of eggs produced per female (juveniles and aborted eggs), and total number of viable eggs produced per female (juveniles), were also registered. One-way ANOVAs, Tukey multiple comparison tests, and t-tests were used for statistical analysis.

RESULTS AND DISCUSSION

During toxicity tests, dissolved oxygen decreased with exposure time and with increasing effluent percentage, ranging from 2.3 to 6.9 mg L^{-1}. The lowest value was recorded in *A. aegypti* experiments, after 96 hr of exposure, in non-diluted S1 samples. This low oxygen value was not considered as relevant because mosquito larvae are airbreathing organisms. Dissolved oxygen in cladoceran tests never fall below 4.1 mg L^{-1}, which was above the recommended limit for static acute testing: 3 mg L^{-1} (EEC 1992).

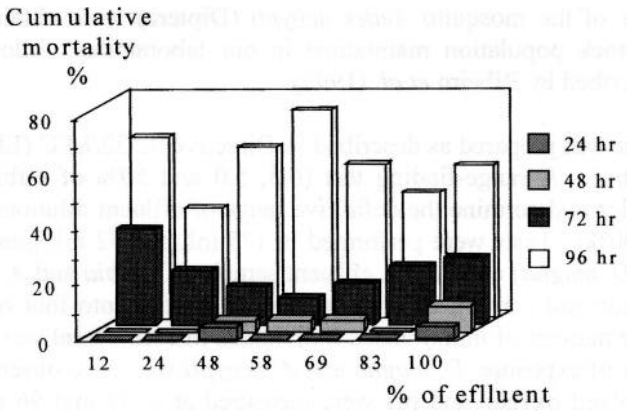

Figure 1. Cumulative mortality of *Aedes aegypti* larvae, exposed to S1 samples without cellulose fibers remotion.

In cladoceran acute tests with S1 samples, C. *dubia* presented a higher sensitivity (lower ED_{50} values) and and a higher discriminative power (closer confidence limits) than *D. magna* (Table 1). Significant mortality of *D. magna* (>10%) was only found after 48 hr of exposure, which is the time limit recommended for this test (EEC 1992). However, this test was carried out until 96 hr because starvation did not cause any mortality in the control, and, moreover, suspended cellulose fibers present in the effluent could be used as a food source by daphnids, which have cellulase in their digestive system (Schoenberg *et al.* 1984; Zanella and Berben 1980).

Table 1. Median lethal dilutions (in%) of S1 samples and respective 95 % confidence limits (inside brackets), in acute tests with *Ceriodaphnia dubia* (24 and 48 hr) and *Daphnia magna* (72 and 96 hr).

Organisms	24 h	48 h	72h	96 h
C. dubia	76.5 (75.9 - 77.2)	60.9 (60.8 - 61.0)		
D. magna	*	*	94.9 (82.9 - 108.6)	69.7 (62.3 – 78.1)

* Mortality less than 10%

Unexpectedly, the evaluation of ED_{50} values was not possible with *A. aegypti* larvae exposed to S1 samples, because cumulative mortality was not correlated with the percentage of effluent dilution (Fig. 1).

This result raised the hypothesis that ingestion of fibers present in the effluent could be an important pathway of toxicant intake. Thus, response varability between effluent dilutions could be due to the differences in ingestion rates, since the quantity of fibers was always high even in the highest dilution. This hypothesis was supported with the results of a test with non-diluted S1 effluent,

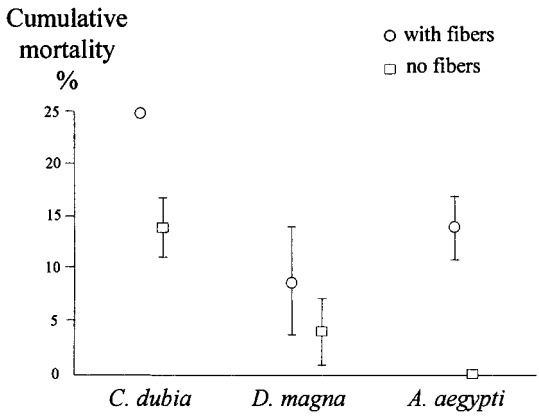

Figure 2. Cumulative mortality of *Ceriodaphnia dubia*, *Daphnia magna*, and *Aedes aegypti* larvae, exposed to non diluted S1 samples (mean and respective standard deviation), before (circles) and after (squares) removing the fibers by centrifugation.

before and after the remotion of fibers by centrifugation: a significantly lower mortality of *A. aegypti* and of *C. dubia* was found when fibers had been removed (*A. aegypti:* t=12.00, d.f.=6, $P<10^{-3}$; *C. dubia:* t=9.00, d.f.=6, $P<10^{-3}$) (Fig. 2). Suspended solids could contribute to a higher mortality of daphnids by reducing their mobility and, thus, their feeding ability (Owens, 1991). A similar trend was observed in *D. magna*, although no significant differences were detected by the t-test (t=1.852, d.f.=6, P=0.114), which was possibly due to the ability of daphnids to filter small and low density fibers not entirely removed by centrifugation.

Experiments with S2 samples did not reveal lethal effects in cladocerans or in mosquito larvae. This result was in accordance with other works, where chlorinated compounds are regarded as the main toxicant in pulp mill effluents (Wong *et al.* 1978; Axegård 1986; 1988; Heimburger *et al.* 1988; Earl and Reeve 1990; Graves *et al.* 1993; Haley *et al.* 1995). The application of oxygen, to depolimerise the lignin, decreases the quantity of chlorine dioxide used during the bleaching process, thus reducing the total amount of chlorinated compounds released in effluents, and, consequently, their toxicity (Pryke 1989).

In the sublethal test with S2 samples, the body length of *C. dubia* decreased with increasing effluent percentage, at both 20 and 25°C. Same values of the No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) (26 and 36% of effluent, respectively) were determined (Table 2) at both temperatures. Nevertheless, at 25°C females presented a higher value of body length (non-diluted effluent: t=-3.23, d.f.=15, P=0.006) in a shorter period of time than at 20°C (9 days and 12 days, respectively).

Table 2. *Ceriodaphnia dubia* growth, exposed to S2 samples. NOEC and LOEC values determined with a Tukey multiple range test (homogeneous groups indicated by a,b,c, and d) performed with the body size values, obtained at the end of the tests (20 and 25°C).

			25°C	
% of effluent	mean (± sd) (mm)		mean (± sd) (mm)	
Control	1.01 (±0.05)	a	1.05 (±0.06)	a
26	0.96 (±0.04)	a	1.00 (±0.03)	b
36	0.91 (±0.02)	b	0.91 (±0.05)	b
51	0.87 (±0.03)	b	0.85 (±0.06)	b, c
71	0.82 (±0.03)	c	0.93 (±0.03)	c
100	0.67 (±0.04)	d	0.75 (±0.05)	d

The pre-reproductive stage (time to the first brood release) was delayed with increasing effluent percentage, mainly for the females exposed at 25°C (Table 3). This delay could be related with higher growth rates: energetic gains were mainly used in somatic growth instead of reproductive processes. The fecundity per female increased with effluent dillution with equal NOEC and LOEC values (36 and 51% of effluent, respectively) for both 20 and 25°C conditions (Fig. 3).

Table 3. The No Observed Effect Concentration (NOEC) and the Lowest Observed Effect Concentration (LOEC) for *Ceriodaphnia dubia* reproduction (time to first reproduction, fecundity and viability of produced eggs), exposed to S2 samples, at 20 and 25°C.

	Time to 1st brood		**Eggs production**		**Eggs viability**	
	20°C	25°C	20°C	25°C	20°C	25°C
NOEC	71%	51%	36%	36%	26%	36%
LOEC	100%	71%	51%	51%	36%	51%

The number of viable eggs also decreased with effluent increasing percentage, not only due to the reduction in egg production, but also due to embryonic mortality. Unviable eggs were registered in all effluent dillutions, at 20°C showing that, for this temperature, eggs development was more sensitive than their production rate (Fig. 4). At 25°C, same NOEC and LOEC values were determined for fecundity and eggs viability (Fig. 4).

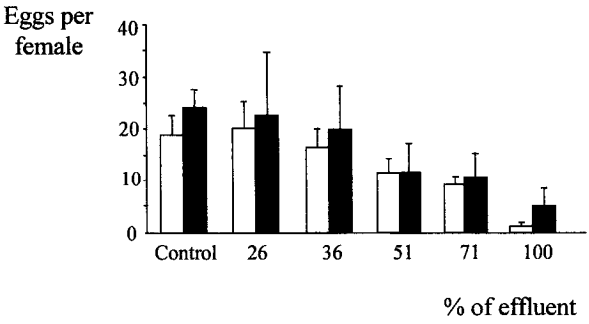

Figure 3. Fecundity of *Ceriodaphnia dubia* (mean and standard deviation) exposed to S2 samples, at 20 (white bars) and 25°C (stripped bars).

The number of viable eggs also decreased with increasing effluent percentage, not only due to the reduction in egg production, but also due to embryonic mortality. Non viable eggs were registered in all effluent dillutions, at 20°C, showing that, for this temperature, eggs development was more sensitive than their production rate (Fig. 4). At 25°C same NOEC and LOEC values were determined for fecundity and eggs viability.

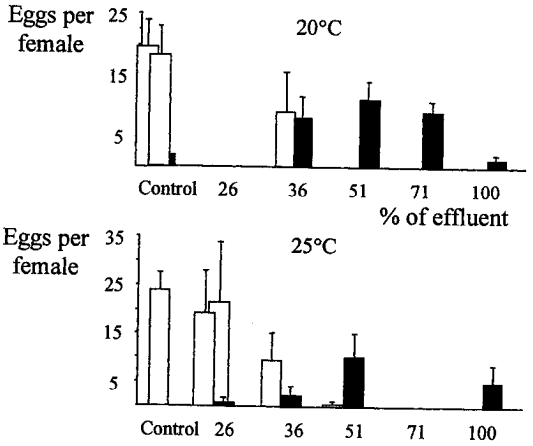

Figure 4. Number of viable (white bars) and non viable eggs (stripped bars) produced by *Ceriodaphnia dubia* exposed to S2 samples, at 20 and 25°C.

In conclusion, the introduction of oxygen delignification as a prebleaching process resulted in a strong decrease of the effluent ecotoxicty, eliminating acute effects on tested organisms. Nevertheless, a significant effluent dilution is still needed to avoid sublethal effects on reproduction of *C. dubia.*

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