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Ecotoxicological Assessment of Two Pulp Mill Effluent, Biobio River Basin, Chile

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The control of ecotoxicological quality of industrial and urban effluent based on individual chemical compounds does not guarantee an adequate protection for the aquatic life. The presence of chemical compounds itself, do not allow to predict their biological effects when aditivity or sinergic interactions occur between the components of a mixture (Enserink et al. 1991), when the components toxicity is not known and the chemical characterization has not been carried out (Fisher et.al. 1990). The toxicity bioassays is a biological tool used to evaluate the risk of xenobiotics, it gives a global answer for chemical agents dissolved in industrial and domestic effluents (Fisher et al. 1990).

An industrial activity with severe impacts on the aquatic systems are the pulp mills (Hansson, 1987). Among these, pulp mills with Kraft process is of concern since they use chlorine and dioxide chlorine for pulp bleaching (Kovacs et al. 1995). In this process, highly toxic organochlorine compounds are formed. In order to eliminate the toxicity of effluents the substitution of chlorine for chlorine dioxide has been proposed (McUbbin and Folke 1995). However, there is not clear evidence of its effectiveness for the effluent (O´Connor 1994). In Chile, 83% of the pulp production is located in the Biobío river basin (Parra et al. 1993). The majority of these kraft processing industries discharge their effluents direct or indirectly to the Biobío river, constituting a risk for the biological community that inhabit this ecosystem. The objectives of this research are: 1) to evaluate the physico-chemical characteristic of the effluents and 2) to evaluate the ecotoxicological quality of the effluents of two pulp mills by toxicity bioassays using *Photobactereum phosphoreum* (microtox), the cladocera *Daphnia pulex*, larvae of *Chironomus piger* and the freshwater microalgae *Scenedesmus spinosus*.

MATERIALS AND METHODS

Four samples were obtained of effluents belonging to two pulp mills denominated: mills A and B. The samples of effluents were refrigerated

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and transported to laboratory for the chemical analysis and toxicity bioassays.

The followings physico-chemical parameters were measured in each sample: pH, dissolved oxygen, turbidity, conductivity and temperature with an apparatus HORIBA U-10. Adsorbibles Organic Halogens (AOX), Extractables Organic Halogens (EOX), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD). The bioassays with *Scenedesmus spinosus* (Chlorophyceae). The growth was measured reading the fluorescence each 24 h during four days with a Turner Designs 10 fluorometer, adapted for readings "in vivo". The growth was expressed as k (div./day). The bioassays with *Daphnia pulex* were carried out with organisms of < 24 h. The bioassays with *Chironomus piger* were carried out with the second instar. The bioassays with *Photobacterium phosphoreum* (microtox), were carried out according to (Arbuckle & Alleman, 1992). The ecotoxicological indexes determined were the LC(E,I) 50 with the Probit program. These indexes were converted to Units of Toxicity (U.T).

RESULTS AND DISCUSSION

The results obtained show important differences in the physico-chemical characteristics of the two pulp mill effluents (Table 1). The temperature in the effluent from mill A ranges from 18 to 23,9ºC while in mill B goes from 32 to 39,1ºC. The pH was the other parameter that showed large differences: in the mill A was lightly alkaline (7.47 - 7.67), while in the mill B presented acid characteristics (3.22 - 4.6). Similar situation was observed in the other parameters (oxygen, conductivity and turbidity). The effluent from mill A seems to have a better environmental quality since complies with the Chilean norms 92 for discharges on superficial waters. This must be the result of a primary treatment plant implemented in mill A which produces a stabilized effluent. Mill B do not neutralize their industrial liquid residues, resulting in an effluent very hazardous for aquatic life as is evidenced by the analyses of the bioassays.

The concentrations of organochlorine compounds measured as AOX, showed similar pattern of differences between both industries (Fig. 1). The value in mill A fluctuated between 2,4 mg/l and 22 mg/ l, while in mill B was between 13,1 and 113 mg/l. The value of the organochlorine compounds measured as EOX (Fig. 2) was high in mill B.

In mill A this type of compounds, apart from being present at much lower concentrations, had a slight variation between samples, in contrast to mill B where marked variations were observed. The EOX concentrations detected in the mills represented < 1% of the AOX. The correlation between these parameters was significant (Table 2).

Table 1. Values of environmental parameters measured in effluents.

Sample	Parameters	Effluent A	Effluent B
Sample 1	T °C	23.9	33.5
	pH	7.47	3.22
	Cond. μcm^{-1}	1.01	1.95
	O_2 (mg/l)	8.56 (100)	6.93 (97)
	Turbidity	25	29
Sample 2	T °C	19	39.1
	pH	7.67	4.47
	Cond. μcm^{-1}	1.17	1.71
	O_2 (mg/l)	8.77 (97)	4.95 (80)
	Turbidity	21	39
Sample 3	T °C	18	32
	pH	7.6	4.6
	Cond. μcm^{-1}	1.2	1.7
	O_2 (mg/l)	7.13 (77)	2.24 (30)
	Turbidity	20	50
Sample 4	T °C	20	33
	pH	7.6	3.7
	Cond. μcm^{-1}	1.01	1.4
	O_2 (mg/l)	8.5 (96)	6.62 (94)
	Turbidity	-	-

( ): % oxigen

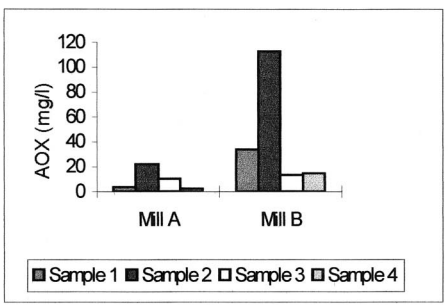

Figure 1. Concentrations of organo-chlorine compounds measured as AOX, in effluents

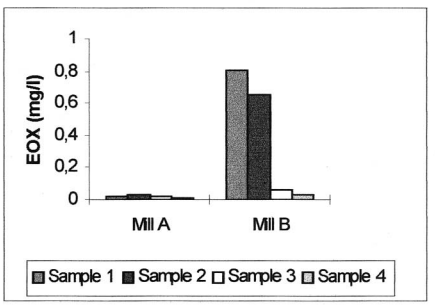

Figure 2. Concentrations of organo-chlorine compounds measured as EOX, in effluents

The biodegradable organic material in the effluent, measured as biological oxygen demand (Fig. 3) was high in the mill B, detecting a value of 744 mg/l in the third sample, while in mill A the highest was 210 mg/l in the first sample, the half of the registered for the same period in the mill B (400 mg/l). A decrease of the levels of BOD toward the term of the study was observed. The similar situation occurred with the COD (Fig. 4).

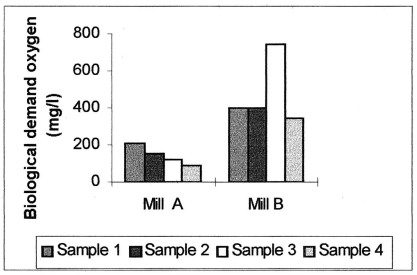

Figure 3. Biological oxygen demand (mg/l), measured in effluents.

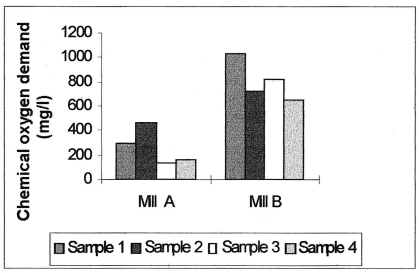

Figure 4. Chemical oxygen demand (mg/l), measured in effluents

This result can be consequence of the primary treatment applied to the effluent of nill A which would reduce the organic charge by retaining mainly the suspended solids. Such a system is absent in mill B. Furthermore, mill A uses hard wood only (Eucalyptus) that have lower content of lignin than the soft wood (Pinus) used in mill B. On the other hand, mill A carried out, during the study, a process of substitution of chlorine for chlorine dioxide C:D (20/80). This set of differences between both pulp mills is probably setting in mill A lower levels of organochlorine compounds measured as AOX and EOX. The relationship between these parameters was similar to those presented by Folke *et al.* (1991), where the compounds measured as EOX represented < 1% of the AOX.

Table 2 shows that the AOX were significantly correlated with the toxicity *in C. piger*, *D. pulex* and *S. spinosus*, but not with *P. phosphoreum*. This indicates that only some bioassays show toxicity for certain level of AOX and that exist other non clorated toxic compounds.

Some bioassays show similar answers to similar chemical agents like the test with *C. piger*, *D. pulex* and *P. phosphoreum* (15 minutes), while the response of *S. spinosus* were not correlated with them. This fact confirms the necessity to make bioassays with batteries of organisms as indicated by Perssone (1991,1992).

The EOX were not correlated significantly with the toxicity, then this chemical parameter would not be a good indicator of toxic effect, which could be due to an incomplete separation of all compounds with toxic properties from the sample through the method of extraction for the chemical analyses.

Table 2. Correlation (Pearson) between chemical and biological variables in effluents.

	AOX	*C.piger*	*D.pulex*	DBO	DQO	EOX	Microtox 5 min.	Microtox 15 min	*S.spinosus*
AOX	1.000								
C. piger	0.812*	1.000							
D.pulex	0.878*	0.916*	1.000						
DBO	0.249	0.262	0.535	1.000					
DQO	0.443	0.531	0.608	0.777*	1.000				
EOX	0.719*	0.636	0.612	0.293	0.695	1.000			
Microtox 5 min	0.227	0.688	0.594	0.320	0.340	-0.011	1.000		
Microtox 15 min	0.412	0.808*	0.730*	0.335	0.388	0.130	0.981*	1.000	
S.spinosus	0.780*	0.482	0.547	-0.130	-0.095	0.310	0.024	0.183	1.000

* significativo ($p<0,05$)

The effluent of mill B was more toxic than the effluent from mill A. The most sensitive species to mill B were *P. phosphoreum* and *S. Spinosus*. *D. pulex* showed a variable response. In two bioassays the toxicity units were < 1, which mean that the LC50 was superior to 100% of the effluent. *C. piger* was the species less sensitive (Fig. 5a,b).

The effluent of mill A provoked toxics effects only on *S. spinosus*. The low toxicity of mill A could be consequence of the substitution of chlorine with dioxide chlorine in the proportion C:D (20:80). Munro *et al.* (1990) and Nelson (1995) found that with 52% and 70% of substitution with chlorine dioxide, highly toxic compounds such as 2,3,7,8 TCDD and 2,3,7,8 TCDF decrease their concentration to undetectable levels (< 2 ppt) which are considered of very low toxicity.

Another aspect of considerable importance for the toxicity of the effluent is the type of wood utilized in the process. While mill B utilizes soft (Pinus) and hard (Eucalyptus) wood, mill A utilizes only hard wood. When soft wood is employed a great amount of organochlorine compounds are formed (chlorophenols, guaiacols, vanillins and catechols) (Hing Bui *et al.*, 1989). The 90% of toxicity in fish could be attributed to 3,4,6 trichloroguaiacol and tetrachloroguaiacol (Folke *et al.*, 1991).

To assess the ecotoxicological effect of an environmental sample the utilization of a battery of species is recomended, since important variations in the sensibility to the same chemical agents exist between species (Persoone, 1992).

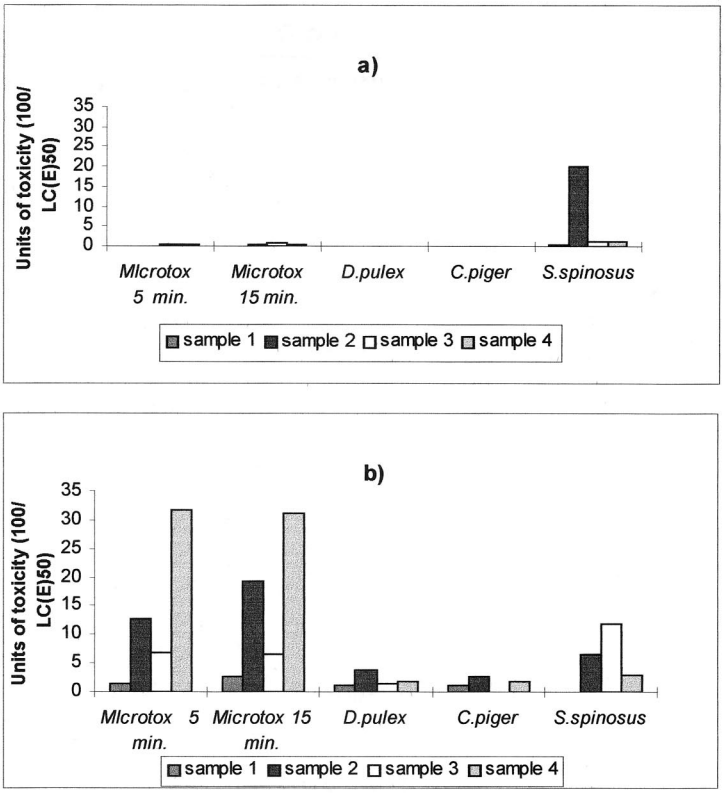

Figure 5a,b Levels of toxicity in the effluent, expressed in units of toxicity (u.t.), a) mill A and b) mill B.

We found that the most sensitive species to the chemical agents present in the effluent samples was *P. phosphoreum* followed by *S. spinosus*. Similarly, Firth & Backman (1990) found that in toxicity evaluations of treated and not treated pulp mill effluents, *P. phosphoreum* was more sensitive than *Oncorhynchus mykiss* and *Ceriodaphnia dubia*. The high sensitivity of *P. phosphoreum* could be due to its procarion constitution, lacking membranes that may act as barriers that difficult the interaction between pollutants and the intracellular organelles, affecting their function. The low toxicity on *C. piger*, could be explained by the presence of nutrients in the effluent that mask the toxic effects of the chemical agents (Lowell *et al.*,1995).

Our research show that pulp mills provoke toxic effects on aquatic organism of different trofic levels. The bioassays in order of sensitivity were *P. Phosphoreum* and *S. spinosus* followed of *D. pulex* and *C. piger.* This result indicates the importance to consider the ecotoxicological assessment in the quality evaluation of pulp mills effluent.

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