Integrated Environmental Assessment of BKME Discharged to a Mediterranean River

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The Mediterranean climate of Central Portugal produces a bimodal precipitation pattern such that effluent dilution is high during the winter rainy period and low in summer. An integrated assessment strategy was used to investigate the effect of this seasonally distinct flow regime on the environmental effects of a largely eucalyptus-derived, bleached kraft mill effluent (BKME) that receives secondary treatment before discharge to the Vouga River. The assessment included the triad of various water chemistry measurements, laboratory bioassays, and field surveys of benthic invertebrate and periphytic diatoms. The BKME discharge increased water temperature, pH, colour, suspended solids, conductivity, chemical oxygen demand, plant nutrients (nitrogen and phosphorus) and AOX content in the downstream receiving waters during both seasons. However, differences were much more pronounced in summer during the natural low flow period. Ecotoxicity tests revealed a significant effluent effect only in summer. Attached diatoms exhibited reduced species diversity at effluent-affected sites only in summer. Invertebrate densities and diversity were reduced downstream of the effluent in both seasons, but more so in summer. The effluent discharge often increased downstream water temperature by 10 to 15°C, with summer temperatures regularly exceeding 30 to 35°C.

Key words: integrated assessment, benthic invertebrates, diatoms, microtox, BKME, river, Portugal

Introduction

Pulp and paper production is an important industry worldwide and the second largest manufacturing sector in Portugal. This activity can be highly polluting for rivers due to the high volume of effluent produced, the large number of potentially toxic chemical compounds in these effluents, and potentially detrimental physical properties such as high water temperature or colour (Kuisvasniemi et al. 1986; Davies et al. 1988; Bothwell 1992). Paper mill effluents have been shown to be toxic to invertebrates (Davies 1978; Petersen and Petersen 1984), zooplankton (Cooley 1977) and fish (Lehtinen 1989). They can cause a decrease in invertebrate
density and diversity (Scrimgeour 1989), and change the assemblage structure of periphytic algae (Amblard et al. 1990), macroinvertebrates and fish (Scrimgeour 1989). These biotic impacts have been attributed mainly to the combined effect of high suspended solids, biochemical oxygen demand (BOD), colour, pH and effluent toxicity (Walden 1976). In addition, phenolic compounds occurring in BKME (bleached kraft mill effluent) may bioaccumulate in food webs (Paasivirta et al. 1980).

Historically, BKME discharges were highly toxic, mainly due to resins and fatty acids. The use of secondary effluent treatments and, to a lesser extent, the advent of alternate bleaching technologies, has resulted in reduced effluent toxicity (Lehtinen 1989; Graves et al. 1993) but see Harris et al. (1992). However, even when toxicants are reduced to low levels, increased nutrient loading of phosphorus or nitrogen can lead to enrichment of the receiving system when these plant nutrients are limiting (Bothwell 1992). In addition, sublethal effects of these effluents have been noted, including the stimulation of growth in aquatic invertebrates (Lowell et al. 1995). The interaction of these nutrients and contaminant effects makes it difficult to provide general predictions on the effects of these effluents on food webs in receiving waters.

The impact of an effluent on the environment also depends upon the ratio of effluent volume to river discharge. In some geographic regions of Portugal, the Mediterranean climate produces a bimodal precipitation pattern which results in high winter and low summer flows. Because the impact of BKME discharge is related to dilution in the receiving waters, the biota of these rivers is likely to be most affected in summer when water temperatures are high and river discharge is low.

We investigated the impacts of this seasonally distinct flow regime on the environmental effects of a largely eucalyptus-derived BKME effluent that receives secondary treatment before being discharged into the Vouga River in Central Portugal. Our assessment integrated a triad of various water chemistry measurements, laboratory bioassays, and field surveys of benthic invertebrates and periphytic algae. The study was conducted at three sites upstream and three sites downstream of the BKME outfall during the winter and summer. Previous studies in the Vouga River basin have shown low water quality near the paper mill (Moreira et al. 1998).

**Materials and Methods**

**Study Area**

The Vouga River has a total length of 150 km, running from Serra da Lapa into the Atlantic Ocean at Aveiro, Portugal. Mean annual river discharge is 44 m$^3$/s, with a maximum of 150 m$^3$/s in February and a minimum of 3 m$^3$/s in August. However, occasional flows of 1.9 m$^3$/s have been reported. The volume of BKME discharged to the Vouga through a side channel ranges between 45,000 to 60,000 m$^3$/d (equal to 0.5–0.7 m$^3$/s). This corresponds to an effluent concentration in the river water up to 40%
in summer. The lower watershed drains through farmland while the upper watershed is forested mostly with *Eucalyptus globulus* and *Pinus pinaster* which are used primarily as furnish for pulp and paper mills. A paper mill located at the lower sector of the Vouga River produces approximately $500 \times 10^3$ kg (air dried) of bleached *Eucalyptus globulus* pulp, using two bleaching sequences $D_CE_O D_E P D_2$ and $D_CE_H D_E P D_2$ ($D_C$ is oxidation with 75% chloride dioxide and 25% chloride, $E_O$ is alkaline extraction with oxygen, $D$ is oxidation with chloride dioxide, $E_P$ is alkaline extraction with hydrogen peroxide, and $E_H$ is extraction with chloride acid). Before being discharged into the river, the effluent is subjected to primary and secondary treatments, and then retained for aeration for 2 days. The resulting effluent has a load of 3 to 5 ($10^3$ kg)/day of total suspended solids, 100 to 150 kg/day of AOX (adsorbable organic halogens) and 22 to 38 ($10^3$)kg/day of chemical oxygen demand.

To test for changes in the chemical, physical and biological parameters, we sampled at three upstream reference sites (sites 1, 3 and 4 located at 20, 10 and 5 km from the discharging point, respectively) and three downstream sites located at a backwater side channel where the effluent is discharged (site 5), 50 m downstream of its junction with the main river (site 6) and 2 km further downstream (site 7).

**Physical and Chemical Parameters**

Water samples (10 L/sampling site) were collected 5 times in the summer of 1995 (weekly from July 19 to September 12) and 3 times in the winter of 1996 (January 27 to February 22). At each site, water temperature, dissolved oxygen, pH and conductivity were measured with portable meters. Water samples were immediately transported to the laboratory where they were refrigerated at 4°C until the various analyses were performed (within 48 h). If this was not possible, samples were preserved with $H_2SO_4$ and $HNO_3$ when appropriate, and frozen for later analysis according to standard methods from APHA (American Public Health Association) and ISO (International Organization for Standardization).

Total suspended solids, chemical oxygen demand (COD—closed reflux method), nitrates and nitrites (ionic chromatography), phosphates (ascorbic acid method) and total nitrogen (Kjeldahl method) were determined according to standard methods from APHA (1995). Colour, total phosphorus, (oxidation with $HNO_3$ method) and organochlorides (AOX method) were determined using ISO procedures.

**Periphytic Diatoms**

During both summer and winter, plexiglass slides ($9 \times 3$ cm) were placed along the river margins at depth of 0.5 to 1 m for algal colonization. The slides were tied by fishing lines to trees in the margin ($n = 3$). At the end of 4 weeks the algae were scraped from the slides with a razor blade and preserved in a known volume of 70% alcohol. Diatom frustules were oxidized with a heated mixture of nitric acid and potassium per-
manganese, washed with distilled water and mounted on Naphrax resin. For quantitative studies, 1 mL of sample was allowed to dry on a cover slip, after which it was incinerated for 1 hour and mounted on Naphrax resin. At least 200 frustules (single valves were taken as half frustule) were counted; the numbers were converted to relative abundance for individual species (from qualitative slides) or number of cells/cm² (from quantitative slides). Diatoms were observed with a light microscope (30 fields, 40× magnification and between 200 to 300 frustules counted in each sample) and identified to species level.

The algal data set was analyzed by correspondence analysis followed by correlation (Spearman) between ordination axes and environmental parameters (Digby and Kempton 1989), and cluster analysis (Digby and Kempton 1989) using the Bray-Curtis dissimilarity index (relative abundance values).

**Macroinvertebrates**

Macroinvertebrate samples were collected from river substrates using a 0.3 × 0.3 m kick net with 0.5-mm mesh size. Sampling was distributed across all major microhabitats in the river. A sample consisted of 5 cumulative sampling units of 1 m each. All samples were preserved in 4% formalin, sorted out at the laboratory and the specimens identified to genus or species. Dipterans were identified to subfamily level.

**Ecotoxicity Tests**

Algal growth inhibition tests using the green alga *Selenastrum capricornutum* and the bioluminescent bacterium, *Vibrio fischeri* (formerly named *Photobacterium phosphoreum*—Microtox system) were used to test the effects of the river water on living organisms. Growth inhibition of *S. capricornutum* is widely used in toxicity tests (Nyholm and Kallqvist 1989; Lewis 1990), including bioassays with paper mill effluents (Amblard et al. 1990; Hornstrom 1990).

Tests were carried out twice in summer and once in winter. Water samples were refrigerated and analyzed within 48 h of collection (Microtox) or filtered and stored at -20°C until analysis (algal bioassays). The algal bioassays were carried out according to EPA protocols (Lewis et al. 1992). After 96-h exposures, algal growth was quantified as absorbance at 750 nm (5-cm light path). Results were expressed in terms of 96 h IC₂₅ (%), the concentration of sample inhibiting population growth by 25%, whenever adverse effects were registered. We also compared algal growth in 100% sample and control (site 4), and computed a growth index—GI (96 h):

\[
\text{GI (96)} = \frac{\text{Abs}_{750} (\text{sample})}{\text{Abs}_{750} (\text{control})}
\]  

where \(\text{Abs}_{750} (\text{sample})\) is sample absorbance at 750 nm (5-cm light path), and \(\text{Abs}_{750} (\text{control})\) is control absorbance at 750 nm (5-cm light path).

Microtox tests were carried out in the microtox analyzer (model 500) according to the protocol provided by the manufacturer. The 100% test procedure was used and an initial concentration of 90% of sample. Results
were expressed as %S (15 min), % of stimulation relative to the control, after 15 minutes of exposure:

\[
%S \text{ (15 min)} = \frac{(I_t - I_0)}{I_0} \cdot 100
\]  

(2)

where \( I_t \) is light level in the sample, and \( I_0 \) is light level in the control.

To test for the thermal effect of the effluent on the macroinvertebrate assemblages, 96 adults of the freshwater shrimp *Atyaephyra desmarestii*, an abundant species at reference sites, were collected and randomly allocated into 12 (8 specimens in each) flasks containing 500 mL of unpolluted filtered stream water. Six flasks were kept at 20°C (environmental temperature) whereas the water temperature of the other 6 flasks was increased 2°C per day to 36°C, a temperature recorded at the site closest to the effluent output. The survivorship of invertebrates was recorded during the experimental period of 9 days.

**Results**

Effluent discharge caused a decrease in dissolved oxygen and an increase in the river temperature, colour, total suspended solids, conductivity, BOD₅, nitrites, Kjeldahl nitrogen, phosphates, total phosphorus, chloride and organochlorides (Fig. 1; Table 1). These effects were more intense in summer when pH and nitrates also increased. The high concentration of the major parameters analyzed rapidly declined downstream of the effluent output, so that in winter the water quality at 2 km downstream of the side channel was similar to reference sites.

Macroinvertebrate diversity in the river was low with a maximum of 52 taxa identified. Reference sites had higher numbers of taxa than sites located downstream of the effluent output during both summer and winter (Fig. 2). While upstream macroinvertebrate assemblages were dominated mainly by Baetidae, Simuliidae, Naididae (winter), *Micronecta* sp. and *Physa* sp. (summer), the downstream sites were dominated by Tubificidae and Naididae (mainly *Slavina appendiculata*).

Algal taxa richness was higher in winter (\( n = 165 \)) than in summer (\( n = 142 \)). In winter, the total number of diatom taxa was relatively unaffected below the effluent output, whereas in summer there was a reduction in the number of taxa in the 2 sites located immediately downstream of the effluent output. A reduction in algal cell densities was also observed at site 5 (Fig. 2) only in summer. A correspondence analysis of the algal data set segregated the summer from the winter samples and reference from impacted sites (Fig. 3A). Axis 1 was correlated with temperature (\( R_s = 0.91; P < 0.01 \)), dissolved oxygen (\( R_s = -0.86; P < 0.01 \)), colour (\( R_s = 0.73; P < 0.05 \)), pH (\( R_s = 0.66; P < 0.05 \)) and total phosphorus (\( R_s = 0.66; P < 0.05 \)), whereas axis 2 was correlated with phosphates (\( R_s = 0.64; P < 0.05 \)). Cluster and correspondence analyses were seasonally consistent with segregation of winter and summer samples. Moreover, cluster analysis denoted that seasonal effects were stronger than pollution-relating effects on the structure of algal assemblages. The segregation of clean/impacted sites in cluster analysis was clear in summer but not in winter (Fig. 3B).
Effluent discharge appeared to affect the abundance of several common algal taxa. For example, *Surirella linearis*, *Gomphonema parvulum* and *Hannaea arcus*, were common (>5% relative abundance) species at reference sites, but absent at sites with effluent exposure. In contrast, *Achnanthes exigua*, *Eunotia bilunaris* and *Nitzschia inconspicua* were more abundant in impacted river reaches.

Ecotoxicological methods provided evidence that the effluent acted to enrich the riverine ecosystem in the winter, but led to potential toxic effects in summer. Mill effluent significantly increased algal growth in winter.

![Graphs showing chemical and physical parameters in the Vouga River](image)

**Fig. 1.** Mean values (± maximum and minimum) of several chemical and physical parameters in the Vouga River at reference (1, 3, 4) and impact sites (5, 6, 7) during winter (▲) and summer (●); n = 3.
Table 1. Chemical and physical data of Vouga River above (sites 1, 3 and 4) and below (sites 5, 6 and 7) the paper mill effluent output—mean ± S.E.; n = 15 and 9 for summer and winter, respectively, except when indicated in superscript

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Above</th>
<th>Below</th>
<th>Above</th>
<th>Below</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dissolved oxygen (mg/L)</td>
<td>7.2±0.2</td>
<td>2.2±0.6</td>
<td>10.1±0.3</td>
<td>8.4±0.3</td>
</tr>
<tr>
<td>Oxygen (% sat)</td>
<td>83±2</td>
<td>26±7</td>
<td>91±3</td>
<td>78±2</td>
</tr>
<tr>
<td>pH</td>
<td>6.5±0.1</td>
<td>7.2±0.0</td>
<td>6.4±0.2</td>
<td>6.7±0.1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>22.4±0.4</td>
<td>29.7±1.1</td>
<td>11.8±0.2</td>
<td>14.8±1.0</td>
</tr>
<tr>
<td>Conductivity (μS/cm)</td>
<td>107±2</td>
<td>1219±143</td>
<td>78.2±6.4</td>
<td>289±73</td>
</tr>
<tr>
<td>Colour (Pt-Co *10^3)</td>
<td>62±3</td>
<td>2280±325</td>
<td>11±3</td>
<td>374±138</td>
</tr>
<tr>
<td>Total suspended solids (mg/L)</td>
<td>11.9±1.0</td>
<td>38.8±7.9</td>
<td>8.9±1.1</td>
<td>16.5±2.0</td>
</tr>
<tr>
<td>Chemical oxygen demand (mg/L)</td>
<td>20.7±5.0</td>
<td>397±79</td>
<td>12.9±2.9</td>
<td>54±15.3</td>
</tr>
<tr>
<td>Organochloride (mg/L)</td>
<td>0.016±0.001</td>
<td>1.167±0.181</td>
<td>0.012±0.002</td>
<td>0.239±0.087</td>
</tr>
<tr>
<td>Phosphates (mg/L PO_4)</td>
<td>0.034±0.003</td>
<td>0.174±0.044</td>
<td>0.048±0.009</td>
<td>0.077±0.011</td>
</tr>
<tr>
<td>Chlorides (mg/L Cl)</td>
<td>11.89±0.18</td>
<td>130.04±30.08</td>
<td>8.75±0.63</td>
<td>22.51±3.95</td>
</tr>
<tr>
<td>Nitrites (mg/L NO_2)</td>
<td>0.010±0.001</td>
<td>0.026±0.004</td>
<td>0.013±0.002</td>
<td>0.048±0.010</td>
</tr>
<tr>
<td>Nitrites (mg/L NO_3)</td>
<td>2.14±0.05</td>
<td>4.96±0.92</td>
<td>4.56±0.30</td>
<td>4.78±0.32</td>
</tr>
<tr>
<td>Kjeldahl (mg/L N)</td>
<td>0.31±0.26</td>
<td>2.59±0.61</td>
<td>0.31±0.03</td>
<td>0.88±0.24</td>
</tr>
<tr>
<td>Total phosphorus (mg/L P)</td>
<td>0.079±0.011</td>
<td>0.418±0.09</td>
<td>0.057±0.004</td>
<td>0.100±0.017</td>
</tr>
</tbody>
</table>

^a n = 6.
^b n = 8.
but not in summer (t = 0.01; n = 48; P > 0.05) as illustrated by Selenastrum tests (Fig. 4). In contrast, effluent caused an inhibition of bioluminescence in *Vibrio fischeri* in summer (t = 3.29; n = 16; P < 0.05), but not in winter (t = 0.89; n = 10; P > 0.05; Fig. 4).

High summer temperatures (36°C) appear to be lethal for some reference site invertebrates such as the shrimp *Atyaephyra desmarestii*. For

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**Fig. 2.** Mean number of macroinvertebrates (A), algal taxa (B), and density of periphytic algal cells (C) in the Vouga River at reference (1, 3, 4) and impact (5, 6, 7) sites during winter (△) and summer (●).
this species, mortality was 100% when water temperature reached 34°C whereas in control invertebrates kept at 20°C mortality was 4%.

**Discussion**

Limiting the environmental effects of BKME discharges to rivers with highly seasonal flow regimes is extremely difficult. Our work demonstrates that for a typical river in a Mediterranean climate, the Vouga River, Portugal, high winter flows may provide adequate dilution to minimize toxic and thermal effects (although not nutrient enrichment), while low summer flows result in inadequate effluent dilution, thereby producing potentially severe environmental stresses. The environmental impacts on the Vouga, and likely other similar rivers, are further complicated because the low flow term is a period of naturally high water temperature which produces low dissolved oxygen concentration and thermal stress.
Except for the site immediately below the outfall, and in comparison with summer, the effluent had a relatively lower effect on stream chemistry during winter. Furthermore, the BKME appears to have low toxicity in winter. However, results of the *Selenastrum* tests suggest that algal nutrients (P and N) in the BKME have the potential to enrich the river in winter. However, in situ sampling did not support this enrichment hypothesis, since the densities of diatoms were similar both up- and down-stream of the effluent discharge. This suggests that factors other than nutrients may be limiting the primary producers. As the winter period is marked by a highly coloured effluent and high suspended solids, we hypothesize that light availability may have limited algal growth at downstream sites.

A major effect of BKME in summer was to raise river water temperature below the outfall and, as noted by Nalewajko and Dunstall (1994), high temperature may inhibit or reduce photosynthesis. High temperature can also increase the rate of decomposition processes in rivers (Maloney and Lamberti 1995), possibly leading to higher oxygen con-

![Fig. 4. Bioluminescent activity of *Vibrio fischeri* (A) and growth index (B) of *Selenastrum capricornutum* in the Vouga River water at reference (ref) and plume (5, 6, 7) effluent discharge sites; light bars = winter, dark bars = summer. GI = 1 (dotted line), there is no effect; GI > 1, stimulation; GI < 1, inhibition.](image-url)
sumption within the ecosystem. This mechanism combined with the lower capacity of water to hold oxygen at high temperatures, likely produces the marked reductions in dissolved oxygen observed downstream of the effluent discharge. Summer oxygen values in impacted areas were generally below 6 mg/L, values that may have sublethal effects on invertebrates and fish (Chapman 1986; Lowell and Culp 1996). In addition, these oxygen concentrations are lower than minimal requirements in some countries (CCREM 1987).

Multivariate analyses of algal assemblages indicate that the major impact of the effluent was related to increased water temperature, nutrients, colour, and lower dissolved oxygen. Diatom taxonomic richness at the BKME outfall was 35% lower than the reference sites in summer. Richness quickly recovered to reference site values at the downstream sites, although these sites were dominated by pollution tolerant taxa (e.g., *Nitzchia palea* and *Achantes exigua* accounted for 71% of all diatoms). We noted that the increase in taxa 2 km below the BKME discharge was, in part, due to the influence of brackish estuarine water which allowed immigration of marine taxa (e.g., *Actinoptychus undulatus*, *Cocconeis scutellum*). High flow in winter appeared to attenuate the direct effects of the effluent on algal growth. Thus, algal assemblages were good bioindicators for BKME effects, as previously observed by Amblard et al. (1990).

Macroinvertebrate assemblages were also affected by the BKME discharge in summer as a result of the combined effect of low dissolved oxygen, higher temperature and organic enrichment. No major changes in river natural physical conditions (depth, current, substrate) occurred in the study section. The water temperature at the site immediately below the effluent outfall was lethal to the shrimp *Atyaephyra desmarestii*, and, presumably, is lethal or sublethal for many other invertebrates found at the reference sites, but eliminated from impacted stream reaches. Moreover, macroinvertebrate assemblages at impacted sites were dominated by Tubificidae and Naididae. These organisms have been referred to as indicators of pollution when present in large numbers (Spellerberg 1991). Macroinvertebrate richness was also low in winter despite the improved environmental conditions relative to summer.

In summary, the triad approach allowed a more complete assessment of the environmental effects of BKME discharge. For example, the use of independent toxicity bioassays revealed the potential for enrichment of algal growth in winter and, in summer, weak effluent toxicity and severe thermal stresses. These bioassays, combined with field measurements of physicochemical conditions, essentially provided the basis for explaining the impacts of BKME on riverine algal and macroinvertebrate assemblages. Because BKME discharge may result in a variety of ecological effects, we support the suggestions of Chapman (1996) that integrated monitoring approaches provide a comprehensive understanding of the effects of effluents on receiving waters. Finally, due to the strongly seasonal discharge regimes in Mediterranean climates, effluent loading scenarios must compensate for minimum discharge levels in summer. Strategies which may help reduce environmental effects in these types of
rivers include the extension of discharge outfall zones to water bodies with greater assimilation capacity (e.g., piping effluent to marine environments) or the incorporation of effluent holdback strategies during periods of low river discharge.

Acknowledgments

This research was financed by JNICT (Portugal) project number PEAM/C/TAI/256/93. We thank TECNOCEL/RAIZ for all the laboratory facilities at Eixo, Aveiro, especially to Carlos A. Valente and Leonor Guedes for their precious help. Portucel Industrial (Cacia) kindly provided data on river and effluent discharges. We also thank Nuno Coimbra for his collaboration in the taxonomy of macroinvertebrates and in the multivariate analyses. Data on the river discharge was provided by the “Direcção Regional do Ambiente e Recursos Naturais do Centro/Direcção de Serviços da Água–Divisão dos Recursos Hídricos. Finally, we thank Fátima Matos from Portucel Industrial, Cacia, for a critical manuscript review. Fátima Santos (Department of Botany, University of Coimbra) kindly provided the *Selenastrum capricornutum* culture.

References


