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Chemosphere 63 (2006) 794–801

CHEMOSPHERE

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Responses of European eel (*Anguilla anguilla* L.) circulating phagocytes to an in situ closed pulp mill effluent exposure and its association with organ-specific peroxidative damage

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Received 28 October 2004; received in revised form 13 July 2005; accepted 31 July 2005
Available online 17 October 2005

Abstract

The effect of bleached kraft pulp mill effluent (BKPME) persistent compounds on phagocyte activities and its organ-specific influence in gill, kidney and liver was studied in European eel *(Anguilla anguilla* L.). Eels were caged and plunged at 3 different sites—50 m (site 1), 100 m (site 2) and 2000 m (site 3) away from the end of the closed BKPME discharging channel for 8 and 48 h. Gill, head kidney and peritoneum phagocytes oxidative burst activity (OBA) was measured by the nitroblue tetrazolium reduction assay whereas lipid peroxidation (LPO) in eel gill, kidney and liver was measured by thiobarbituric acid reaction. A significant gill OBA induction was found at 8 h on site 2, and on sites 1, 2 and 3 at 48 h exposure. However, in head kidney and peritoneal exudate phagocytes, OBA induction was significant only at sites 2 and 3 after 48 h exposure. In those particular sites, a significant increase in gill, kidney and liver LPO was measured that is assumed to result from OBA induction. Considering OBA and LPO, gill is the most affected tissue compared to kidney and liver. Gill vulnerability towards peroxidative damage was demonstrated at 8 h on site 2 and at 48 h on sites 1, 2 and 3, whereas in kidney was observed at sites 2 and 3 only at 48 h. Liver LPO increased at site 2 only after 48 h exposure. Our results demonstrate that the OBA activation pattern in gill and kidney is associated with the induced peroxidative damage extent in those organ, together with water pollution the exposure route, resulting from previous BKPME effluent sediment contamination which may affect the activation pattern of circulating fish phagocytes.

Keywords: *Anguilla anguilla* L.; Oxidative burst; Lipid peroxidation; Phagocytes activation; Peroxidative damage

1. Introduction

Bleached kraft pulp mill effluent (BKPME) is a complex mixture of environmentally active substances containing beyond 300 chemicals (Nestmann et al., 1980); however, not all the BKPME components have been identified (Mather-Mihaich and DiGiulio, 1991). The pulping and bleaching processes generate dissolved lignin,

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0045-6535/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2005.07.077

cellulose degradation products, and other wood extractives such as terpenoids, resin acids (RAs), phytosterols, and chlorophenolic compounds (Sjostrom, 1993). Various BKPME components are recognized as highly persistent in the environment and the risk for aquatic organisms may remain high even after the discharge suspension. For instance, the half-life of RAs was estimated to be 30 years in the sediment (Stuthridge and Tavendale, 1996). Other compounds such as retene, a RAs derivative, and chlorophenolics originated during pulp bleaching, are also fairly persistent in receiving aquatic ecosystems (Leppänen and Oikari, 1999; Leppänen et al., 1999).

BKPME exposed fish have shown a spectrum of effects such as physiological and histopathological alterations (Santos et al., 1993; Pacheco et al., 1993; Santos and Pacheco, 1995, 1996; Pacheco and Santos, 1999), genotoxicity (Ericson and Larsson, 2000; Maria et al., 2002) and carcinogenicity (Metcalfe et al., 1995). Among various fish immunological responses, phagocyte activities have proved their utility in fish defence against invading agents (Ahmad et al., 1998, 2004; Ellis, 1999; Fatima et al., 2000; Neumann et al., 2000). However, an imbalanced phagocyte function, induced by the presence of an excessive concentration of xenobiotics in the water, may predispose fish towards various types of toxicity. Thus, xenobiotic over activated phagocytes-induced oxidative burst phenomenon is an important source of reactive oxygen species (ROS) such as superoxide (O^{2-}), hydrogen peroxide (H_2O_2), singlet oxygen ($_1O^2$) and hydroxyl radicals ($^{\cdot}OH$) (Zelikoff et al., 1996; Lackner, 1998; Ellis, 1999; Fatima et al., 2000; Bols et al., 2001), besides NADPH oxidase, xanthine oxidase or respiratory chain reactions. The most typical reaction due to ROS overproduction and induced damage, involves the peroxidation of unsaturated fatty acids (Kappus, 1987; Fatima et al., 2000). Despite the relevance of these aspects, only few studies have been carried out showing BKPME effects on fish phagocytes. BKPME laboratory studies on snake head fish (*Channa punctatus* B.), demonstrated circulating phagocytes overactivation with increased ROS production, concomitantly associated with organ specific peroxidative damage in gill, kidney and liver, being suggested that these selective responses were due to differences in antioxidant status (Ahmad et al., 1998; Fatima et al., 2000; Ahmad et al., 2000).

In Portugal, the Cacia pulp mill factory produces mainly bleached pulp (approximate production 260 000 ton $year^{-1}$) using Tasmania blue gum (*Eucalyptus globulus*) and cluster pine (*Pinus pinaster*) (75% and 25%, respectively) as wood supply. Pulp bleaching is carried out primarily through chlorine dioxide. Subtil et al. (1984) have previously detected dehydroabietic acid in the sediment around the Cacia pulp mill effluent outlet. This effluent discharge into Vouga river lasted for 5 decades, contaminating the recipient waters and sediments, finally ending in May 2000 when it was diverted through a submarine outlet 2.5 km far from the sea coast (Fig. 1). Hence, the changed circumstances raise a question of the ecosystem health recovery.

As per the best of author's knowledge, there is a lack of studies on the ecosystem recovery and biological effects due to chemical persistence on BKPME affected areas. In this regard, recent works on Vouga river recovery assessment, showing eel tissue specific antioxidants responses, genotoxicity, provided evidence of the persistence of oxidants, genotoxic and pro-genotoxic

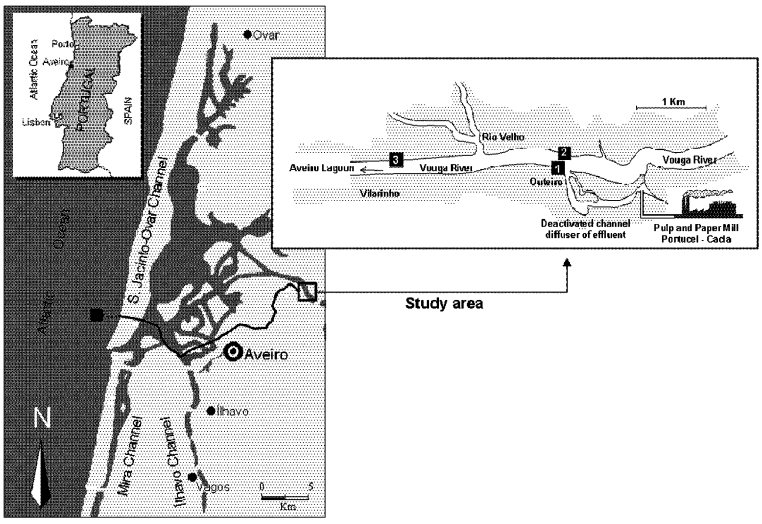

Fig. 1. Representation of eel caging sites on the Vouga river, Aveiro, Portugal: 50 m (site 1), 100 m (site 2) and 2000 m (site 3) away from the opening of deactivated BKPME discharging channel. The study area is contextualized in the Aveiro Lagoon and displays the present effluent discharging point (■).