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Raw and biologically treated paper mill wastewater effluents and the recipient surface waters: Cytotoxic and genotoxic activity and the presence of endocrine disrupting compounds

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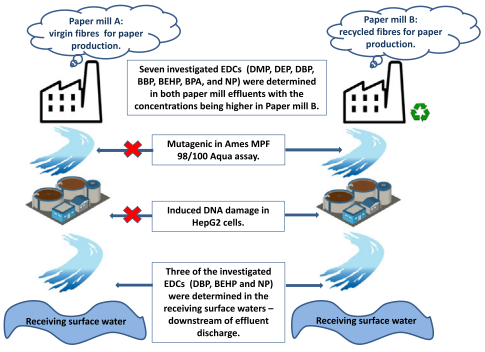
HIGHLIGHTS

- EDCs were detected in raw and biologically treated paper mill effluents.
- DBP, BEHP, and NP were determined in recipient surface waters.
- Paper mill (uses recycled fibres for paper production) effluents were mutagenic.
- Paper mill (uses virgin fibres for paper production) effluents were not mutagenic.
- Paper mill discharge contributed to genotoxity of recipient surface waters.

GRAPHICAL ABSTRACT



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ABSTRACT

Paper mill effluents are complex mixtures containing different toxic compounds including endocrine-disrupting (EDCs) and genotoxic compounds. In the present study non-concentrated raw and biologically treated wastewaters from two paper mill plants with different paper production technologies i) Paper mill A uses virgin fibres, and ii) Paper mill B uses recycled fibres for paper production and the corresponding receiving surface waters, were assessed for their cytotoxic/genotoxic activity with SOS/*umuC*, Ames MPF 98/100 Aqua, and comet assay with human hepatoma HepG2 cells. In addition the levels of seven selected EDCs were quantified in wastewater samples and receiving surface waters. All investigated EDCs were confirmed in raw and biologically treated effluents from both paper mills with concentrations being markedly higher in Paper mill B effluents. In the receiving surface waters three of the studied EDCs were determined downstream of both paper mills effluent discharge. The wastewater samples and the recipient surface water samples from Paper mill A were not mutagenic for bacteria and did not induce DNA damage in HepG2 cells. On the contrary, half of the raw wastewater samples from Paper mill B were mutagenic whereas biologically treated wastewater and the recipient surface water samples were negative. In HepG2 cells most of the raw and biologically treated wastewater samples from Paper mill B

Abbreviations: AFB1, aflatoxin B1; AOX, adsorbable organic halogen compounds; BaP, benzo [a] pyrene; BBP, benzyl butyl phthalate; BEHP, bis(2-ethylhexyl) phthalate; BOD, biochemical oxygen demand; BPA, bisphenol A; BSTFA, *N,O*-bis(trimethyl-silyl)trifluoroacetamide; COD, chemical oxygen demand; DBP, dibutyl phthalate; DEP, diethyl phthalate; DMP, dimethyl phthalate; DMSO, dimethyl sulfoxide; EDC, endocrine disrupting compound; EDTA, ethylenediaminetetraacetic acid; G, grow rate; GC–MS, gas chromatography–mass spectrometry; IR, induction ratio; MNNG, 1-methyl-3-nitro-1-nitrosoguanidine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NP, nonylphenol; OD, optical density; ONPG, *ortho*-nitrophenyl-β-galactoside; PCP, pentachlorophenol; SPE, solid phase extraction; TGA, tryptone, glucose and ampicillin; U, relative unit.

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as well as surface water samples collected downstream of Paper mill B effluent discharge induced DNA damage. The results confirmed that genotoxic contaminants were present only in wastewaters from Paper mill B that uses recycled fibres for paper production, and that the combined aerobic and anaerobic wastewater treatment procedure efficiently reduced contaminants that are bacterial mutagens, but not those that induce DNA damage in HepG2 cells. This study highlights that in addition to chemical analyses bioassays are needed for a comprehensive toxicological evaluation of complex wastewater samples.

1. Introduction

For humans, surface water is required as a source of drinking water, for agricultural activities, for industrial use and for sports and leisure as well. It is, therefore, important to acquire and/or maintain high quality of surface waters. The quality of surface waters can be influenced by many toxic contaminants originating from industry, agriculture and private households (Claxton et al., 1998; White and Rasmussen, 1998; Gavrilescu et al., 2015).

Pulp and paper industry is the sixth largest industrial polluter discharging a variety of solid, liquid and gaseous wastes into the environment (Ali and Sreekrishnan, 2001). Most of the pollutants from pulp and paper industry are discharged into the environment via wastewater, thus the major concern is the pollution of surface water bodies since large volumes of wastewaters (>12 m^3) are generated for each ton of paper produced (Ali and Sreekrishnan, 2001; Balabanič and Krivograd Klemenčič, 2011; Lindholm-Lehto et al., 2015). Paper mill effluents exhibit characteristics of hazardous wastes because they contain considerable amounts of fibres, fillers and toxic chemicals (Raj et al., 2014; Hermosilla et al., 2015; Lindholm-Lehto et al., 2015). These compounds come from wood extractives or additives, which are used in the paper production processes (Ali and Sreekrishnan, 2001). Among different pollutants present in paper mill wastewaters, endocrine-disrupting compounds (EDCs) are one of the most critical class of contaminants since they can cause adverse effects in the living organisms due to their interference with the endocrine system (Rigol et al., 2002; Balabanič et al., 2011; Gore et al., 2015). A number of studies showed endocrine disruption related consequences of EDCs in wildlife, such as birds, amphibians, reptiles and fish (Kidd et al., 2007; Flint et al., 2012; Kabir et al., 2015). In humans, EDCs are suspected to cause cryptorchidism, hypospadias, abnormalities in sperm and increased hormone-related cancers (e.g. testicular and prostate cancer, female breast cancer) (Balabanič et al., 2011; Gore et al., 2015). Among chemicals known as EDCs used in paper production are phthalates, which are employed in paper production process mostly as softeners in additives, glues and printing inks. Polycyclic aromatic hydrocarbons (PAHs) may also be found in printing inks. Further on, alkylphenols (nonylphenol) are constituents of widely used defoamers, cleaners, and emulsifiers, while pentachlorophenol (PCP) is the major component of some biocides used in pulp and paper industry (Yu et al., 2007; Balabanič and Krivograd Klemenčič, 2011; Balabanič et al., 2012).

Another group of hazardous contaminants in paper mill wastewaters are genotoxic compounds, which can induce DNA damage and mutations in wildlife and humans. Chronic exposure to low doses of genotoxic compounds may affect biodiversity, while in humans it may increase the risk for cancer development (Filipič et al., 1995; Ohe et al., 2004; Žegura et al., 2009). A number of studies showed that paper mill effluents are often genotoxic (Rao et al., 1995; Claxton et al., 1998; Pokhrel and Viraraghavan, 2004), while more and more published data show that certain EDCs (e.g. nonylphenol) in addition to the disruption of endocrine system induce also genotoxic effects (Frassinetti et al., 2011; Tiwari et al., 2012; Xin et al., 2015).

Genotoxic compounds and EDCs when released into the environment may affect aquatic organisms or even enter the human food chain through contaminated drinking water or food (Guenther et al., 2002). Therefore, to protect the environment and human health it is very important to regulate the discharges of the pollutants into the environment properly. According to the Urban Wastewater Treatment Directive (UWTD, 91/271/EEC) and the more recent Water Framework Directive (WFD) (Directive 2000/60/EC), all industrial water pollution sources must be regularly controlled for the presence of numerous compounds that are toxic, bio-accumulative or function as endocrine disruptors. However, only for some of these pollutants limit values in surface waters are established.

The pollution level of the effluents from the papermaking industry has been so far evaluated mainly by the cumulative parameters, such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), suspended solids (SS), conductivity, total phosphorus, total nitrogen and adsorbable organic halogen compounds (AOX) (Pokhrel and Viraraghavan, 2004; Leiviska et al., 2008; Lindholm-Lehto et al., 2015); however, little is known about the types and the concentrations of individual toxic compounds such as EDCs in the paper mill effluents (Balabanič and Krivograd Klemenčič, 2011; Dykstra et al., 2015). The main objective of this study was to quantify the levels of seven selected EDCs in a) raw (untreated), and b) biologically treated effluents from two Slovenian paper mills employing different paper production processes (Paper mill A uses virgin fibres, and Paper mill B uses recycled fibres as the raw material for paper production) as well as c) their recipient surface waters. The EDCs included in the study were dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), benzyl butyl phthalate (BBP), bis(2-ethylhexyl) phthalate (BEHP), bisphenol A (BPA), and nonylphenol (NP). The second objective of this study was to determine cytotoxic and mutagenic/genotoxic activities of the same water samples as used for chemical analyses. For this purpose we applied three bioassays, namely bacterial SOS/*umuC* assay (ISO/CD: 13829:2000), and Ames MPF™ 98/100 Aqua assay (Xenometrix, 2010) that are standardized for genotoxicity and mutagenicity testing, respectively, of non-concentrated aquatic samples, and comet assay with human hepatoma HepG2 cells as the eukaryotic test system. The HepG2 cells were selected because owing to their endogenous expression of a variety of xenobiotic metabolizing enzymes they are particularly useful for detecting the combined effects of chemicals in complex mixtures (Mersch-Sundermann et al., 2004).

2. Materials and methods

2.1. Wastewater and recipient surface water sampling

Grab wastewater samples were collected from raw (untreated) and biologically treated wastewaters from two Slovenian paper mills (Paper mill A and Paper mill B), and their recipient surface waters 50 m upstream and 50 m downstream the paper mill effluent discharges. Sampling locations are presented in Table 1. Paper mill A (water consumption 286 m^3/h, wastewater amount 277 m^3/h) is manufacturing label papers, base papers for vacuum metallizing, high-gloss label papers, base papers for siliconising and flexible packaging papers from fresh cellulose fibres and has on site aerobic biological wastewater treatment plant with retention time 48 h. Fresh cellulose fibres used for paper production in Paper mill A are not produced in Slovenia. Paper mill B (water consumption 287 m^3/h, wastewater amount 273 m^3/h) is manufacturing carton board from 100% recycled fibres of grammages between 200 and 500 g/m^2 used in food, cosmetics,