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Toxicological assessment and management options for boat pressure-washing wastewater

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ABSTRACT

Boats are washed periodically for maintenance in order to remove biofoulants from hulls, which results in the generation of wastewater. This study aimed at evaluating the cyto/genotoxic and mutagenic properties of wastewater produced by pressure washing of boats. The chemical characterisation of this wastewater showed that Cu, Zn, V, Cr, Fe, Pb, and select organic contaminants exceeded the maximum allowable values from 1.7 up to 96 times. The wastewater produced negative effects on human lymphocytes resulting in decreased cell viability after 4 and 24 h of exposure. Chromosome aberration, micronucleus, and comet assay parameters were significantly higher after 24 h of exposure. At the same time, the *Salmonella typhimurium* test showed negative for both TA98 and TA100 strains at all of the concentrations tested. After the treatment of wastewater using electrochemical methods/ozonation during real scale treatment plant, removal rates of colour, turbidity and heavy metals ranged from 99.4% to 99.9%, while the removal of total organic carbon (TOC) and chemical oxygen demand (COD) was above 85%. This was reflected in the removal of the wastewater's cyto/genotoxicity, which was comparable to negative controls in all of the conducted tests, suggesting that such plants could be implemented in marinas to minimise human impact on marine systems.

1. Introduction

The fouling of biological material in vessel hulls is a major issue in modern ship transport. If not specially protected, a boat bottom can gather up to 150 kg of fouling per 1 m^2 within six months time, which subsequently increases fuel consumption by 40–50% and shipping costs by up to 70% (Yebra et al., 2004).

In the last several years, the development of ship hull protection has improved the management of biofouling. This includes: slippery surface coating, enzymes that prevent bacteria from attaching to the hull, electricity, more frequent hull cleaning, and antifouling paints based on heavy metals such as copper (Oreščanin et al., 2012; Yebra et. al, 2004).

At the same time, copper-based antifouling paints are considered an important anthropogenic source of copper in the aquatic environment, where approximately 2 kg of copper per boat is released annually (Boxall et al., 2000; Jones and Bolam, 2007). Once a year, each boat is dry docked and washed as part of regular maintenance, which generates roughly 100 L of boat pressure-washing wastewater (BPWW) containing antifouling paint (Oreščanin et al., 2012). Therefore, this study set out to evaluate the toxic potential of such wastewater from the aspects of mutagenicity and cyto/genotoxicity.

First, a chemical characterisation of wastewater produced from high pressure washing of boat paints was conducted in order to detect the amount of heavy metals released into the environment. Using two different bacterial strains, we tried to determine whether such BPWW poses a threat from the aspect of mutagenicity.

The chromosome aberrations, comet, and micronucleus tests on human and animal cells have been used in a large number of environmental studies (Au et al., 2001; Almeida et al., 2013; Frenzilli et al., 2009; Gajski et al., 2012; Gerić et al., 2012) and the present study highlights their possible application in the detection of cyto/genotoxicity induced by BPWW. Finally, we will demonstrate toxicological evidence of a possible cost-effective solution for purifying BPWW using electrochemical treatment with simultaneous ozonation. According to the UN Water Development

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Report (2012), as much as 80% of wastewater is released into the environment without any treatment therefore similar purification methods could be implemented in marinas around the world.

2. Materials and methods

2.1. Wastewater treatment and analysis

Wastewater was treated in a full-scale plant (Fig. 1) developed according to the patented technology invented by Oreščanin et al. (2013) (patent no. WO 2013/144664). BPWW was collected in the Kaštela Marina, Kaštel Gomilica, Croatia.

One thousand litre of wastewater were pumped into one of the three settlement tanks of the treatment plant. After 1 h, the coarse particles settled and 200 L of wastewater was pumped into the reaction vessel equipped with two sets of electrode plates (iron and aluminium). Each set consisted of nine plates separated by an electro insulator (Oreščanin et al., 2011). The surface of each plate was 450 cm^2, the distance among the plates was 10 mm, the applied current and voltage were 45 A and 12 V, respectively. Treatment with the iron electrode set lasted 15 min followed by 20 min of treatment with aluminium electrode plates. Simultaneously with electrochemical treatment, wastewater was ozonated with an ozone generator at a rate of 3500 mg/h. Finally, the suspension of electrochemically generated flocks and water was mixed by ozone bubbles for an additional 20 min, while the settlement time lasted 30 min.

The analysis of heavy metals in original and treated wastewater was done by energy dispersive X-ray spectrometry as previously described (Oreščanin et al., 2011). pH value was determined by a PHT-027 water quality multiparameter monitor (Kelilong Electron, China). The colour, turbidity, chemical oxygen demand (COD) and total organic carbon (TOC) were determined by HACH DR890 colorimeter (Hach Company, USA). For sample digestion, a DRB 200 reactor (Hach Company) was used. All measurements were done in pentaplicates.

2.2. Ames mutagenicity test

Salmonella typhimurium TA98 and TA100 strains were kindly provided by Maron and Ames (University of California, CA, USA). The modified standard plate incorporation procedure–preincubation assay was used (Maron and Ames, 1983) where 100 µL of *Salmonella typimurium* strains (TA98 and TA100) was incubated with different concentrations of BPWW (1%, 5%, 25%, 50%, and original) for 20 min at 37 °C. Subsequently, 2 mL of top agar was added to minimal Vogel-Bonner plates and incubated at 37 °C for 72 h. Methyl methane sulphonate (MMS, Kodak, NY, USA) was used as positive control without metabolic activator and 2-aminoantracene (2-AA, Sigma, Chemical Company, MO, USA) with S9 activation. The induction of mutation was obtained after dividing the number of induced revertants with the number of spontaneous revertants (Durgo et al., 2009). Each experiment was repeated twice and three plates were taken for each concentration. A positive result was considered to be achieved when the number of revertants exceeded the corresponding control value 2.5 times.

2.3. Cell treatment

Peripheral blood was taken from a healthy, female, non-smoking donor. The study was a part of a project approved by the Institutional Ethics Committee and observed the ethical principles of the Declaration of Helsinki. A volume of 100 µL of untreated and purified boat washing wastewater was then added to a blood volume of 900 µL, whereas distilled water was used for negative control. A total of 1 mL of each sample was placed into the incubator at 37 °C in an atmosphere with 5% CO_2 (Heraeus Heracell 240 incubator, Germany) and incubated for 4 and 24 h.

2.4. Cell viability test

Cell viability test was performed according to Duke and Cohen (1992). The HPBLs were isolated using modified Ficoll-Histopaque centrifugation technique (Singh, 2000), stained with 2 µL of acridine orange/ethidium bromide (Sigma) and analysed using an Olympus BX51 microscope (Japan). Depending on the staining, 100 cells per repetition were classified either viable (uniform green) or non-viable (uniform orange) and four repetitions were done per sample.

2.5. Chromosome aberrations test

The chromosome aberration test was performed in agreement with IPCH guidelines (Albertini et al., 2000) with minor modifications (Gajski et al., 2014). Whole blood cultures were established, where 0.5 mL of each sample was added to 6 mL of RPMI 1640 medium (Gibco, Life Technologies, USA) containing antibiotics, 1 mL foetal bovine serum (Gibco), and 0.1 mL phytohaemagglutinin (Remel Europe, UK). The cultures were placed at 37 °C and 5% CO_2 and incubated for 48 h. Colchicine (Sigma, 0.004%) was added 4 h prior to the harvest which arrested lymphocytes division in metaphase. When the incubation period expired, the cultures were fixed in freshly prepared ice cold methanol/acetic acid (3:1 v/v) dropped onto slides and air-dried. After staining with 5% Giemsa solution (Merck, Germany), a total of 200 metaphases per

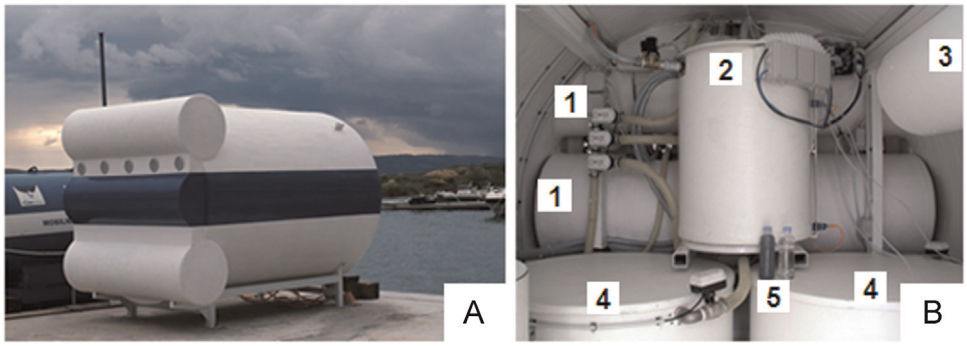

Fig. 1. A full-scale plant for boat pressure washing wastewater treatment installed in Kaštela Marina, Kaštel Gomilica, Croatia (capacity 5 m^3/day). (A) exterior view describing the location of the plant in the Kaštela Marina. (B) interior view of the plant showing: two receiving horizontal tanks for coarse particle separation (1000 L each) (1), reaction vessel with iron and aluminium electrode plates (200 L) (2), corona discharge based ozone generator (3), two sedimentation tanks for the separation of purified water from the sludge (300 L each) (4), and untreated and purified water samples (5).