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Effects of ozone, ultraviolet and peracetic acid disinfection of a primary-treated municipal effluent on the Immune system of rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Municipal sewage effluents are complex mixtures that are known to compromise the health condition of aquatic organisms. The aim of this study was to evaluate the impacts of various wastewater disinfection processes on the immune system of juvenile rainbow trout (*Oncorhynchus mykiss*). The trout were exposed to a primary-treated effluent for 28 days before and after one of each of the following treatments: ultraviolet (UV) radiation, ozonation and peracetic acid. Immune function was characterized in leucocytes from the anterior head kidney by the following three parameters: phagocytosis activity, natural cytotoxic cells (NCC) function and lymphocyte (B and T) proliferation assays. The results show that the fish mass to length ratio was significantly decreased for the primary-treated and all three disinfection processes. Exposure to the primary-treated effluent led to a significant increase in macrophage-related phagocytosis; the addition of a disinfection step was effective in removing this effect. Both unstimulated and mitogen-stimulated T lymphocyte proliferation in fish decreased dramatically in fish exposed to the ozonated effluent compared to fish exposed to either the primary-treated effluent or to aquarium water. Stimulation of T lymphocytes proliferation was observed with the peracetic acid treatment group. In conclusion, the disinfection strategy used can modify the immune system in fish at the level of T lymphocyte proliferation but was effective to remove the effects on phagocytosis activity.

1. Introduction

Urban wastewaters comprise complex mixtures of chemicals and biological agents that could harm aquatic organisms living in the vicinity of the effluent outfall area. They contain a number of chemicals such as heavy metals, polyaromatic hydrocarbons, polar polycyclic organic chemicals (bisphenol A, phthalates and pharmaceutical and personal care products), microorganisms and perhaps even compounds derived from nanotechnology. Urban wastewaters are usually treated by screening to remove solids and suspended materials by the aid of precipitating agents (alun or ferric chloride and surfactants). The process allows also aeration to eliminate volatile compounds as well as a preliminary biological degradation. This so-called primary-treated effluent is then further processed to remove pathogenic microorganisms and aerobically degrade potentially toxic organic matter. Such treatments include ozonolysis, photo-catalysis (ultraviolet radiation), chemical oxidation (e.g. peracetic acid) and biological aeration. In a previous study, ozone treatment (>20 mg/L) of a primary-treated effluent was effective in achieving a targeted reduction in fecal coliform levels (Gehr et al., 2003). Other studies show that ozonation has the potential to remove several contaminants in addition to microorganisms. However, ozone treatment introduces oxidative chemical modifications (Swietlik and Sikorska, 2004) that could modulate or even possibly increase the toxic potential of municipal wastewaters toward aquatic biota. Since these effluents are rich in dissolved organic matter such as humic acids and proteinaceous compounds, the mobility and fate of heavy metals and other chemicals could be affected during the ozone treatment process. The mechanism of action of UV radiation consists of damaging the genetic material of various microorganisms to the point of inhibiting survival, growth and proliferation (Gehr and Nicell, 1996). Because of their capacity to repair UV-induced damage, incomplete UV-disinfection processes (by absorbance of the effluent matrix, for example) may lead to bacterial regrowth (Gehr and Nicell, 1996). Ultraviolet treatment can become prohibitive at higher effluent volume and flow rates, as a greater number of mercury lamps are required, with obvious restrictions on how they can be disposed. Chemical disinfectants like peracetic acid (PAA) are commonly used in Europe for various wastewater applications, including disinfection (Baldry and French, 1989), secondary treatment of effluents in coastal waters (Lefebvre et al., 1992; Sanchez-Ruiz et al., 1995) and

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reclamation of municipal wastewaters for agriculture (Liberti and Notarnicola, 1999). While it offers interesting advantages—low cost, simple operation and ease of start-up (Wagner et al., 2002), to name a few—the efficacy of chemical disinfection varies depending on the organic load of the wastewater, not to mention the extensive safety requirements that accompany the use and storage of peracetic acid (Wagner et al., 2002).

The environmental discharge of effluents from a wastewater treatment plant (WTP) is a major concern. It was estimated that 50% of all fish species in Switzerland are affected by compromised water quality from wastewater discharges (Escher et al., 1999). The estrogenic properties of wastewater of both industrial and domestic origin are well recognized (Folmar et al., 1996; Purdom et al., 1994; Vos et al., 2000). These endocrine (estrogenic) alterations are manifested as vitellogenin production, reduced gonad growth, testicular abnormalities, inhibition of spawning, and intersex (Allen et al., 1999; Harries et al., 1996; Lye et al., 1997; Routledge et al., 1998; Waring et al., 1996). Little is known about the effects of wastewater discharges on wildlife. Nevertheless, sewage effluent can increase mortality or reduce growth in exposed organisms (Escher et al., 1999; Grizzle et al., 1988). The physiological alterations provoked by sewage effluent exposure, such as the occurrence of infectious diseases, skin erosion, hyperplasia, hypertrophy, necrosis, inflammation, leukocyte infiltration and induction of cytochrome P4501A1 activity, have been demonstrated in skin, fins, jaw, gills, liver and kidney (Burkhard-Holm et al., 1997; Bucher and Hofer, 1993; Escher et al., 1999; Gagné and Blaise, 1999; Grizzle et al., 1988; Kosmala et al., 1998).

Exposure to sewage effluents can disrupt the immune function of young life stages and compromise the survival of adults while increasing the incidence of disease in wildlife (Vos et al., 2000; Fournier et al., 2000). Higher rates of parasitic and bacterial infections were found in trout exposed to effluent in the Alte Aare River, suggesting that their immune systems might have been compromised (Escher et al., 1999). Moreover, an increased prevalence of tumors was shown in fish living in the vicinity of a municipal/industrial wastewater outfall (Black and Baumann, 1991). To the best of our knowledge, studies evaluating the (non)specific immunity status of fish exposed to sewage effluent are lacking. Phagocytosis is a key function in ensuring immune defence and it is performed principally by macrophagic and granulocytic cells (Anderson and Zeeman, 1995). Rainbow trout can exhibit both innate and specific immune responses (Watts et al., 2001; Zapata et al., 1996). The innate immune function consists of internalizing, killing and digesting foreign microorganisms (Secombes, 1996). Other innate functions performed by "natural killer or cytotoxic" cells (NCC) are known to combat tumor establishment and virus-infected cells (Evans and Jaso-Friedmann, 1994; Faisal et al., 1991). In fish, analogous cells known as non-specific or NCC demonstrated spontaneous cytotoxicity against fish and mammalian cancerous cell lines, virus-infected cells and protozoan parasites (Evans and Jaso-Friedmann, 1994; Zapata, 1981). NCC cells recognize bind and kill target cells by involving both apoptotic and necrotic mechanisms (Greenlee et al., 1991). Specific immune responses can be conveniently assessed by a number of assays using flow cytometry (Brousseau et al., 1998). The mitogenic activity assay is commonly used to evaluate the capability of B and T lymphocytes to divide and replicate in order to protect the host against (a)biotic constituents. It represents a measure of adaptive immunity implicating antigen-antibody interactions such as antigen presentation and antibody production (Manning and Nakanishi, 1996).

The purpose of this study was to examine the effects of a primary-treated effluent after disinfection by three different methods (ozonation, ultraviolet and peracetic acid treatments) on the immune system of rainbow trout (*Oncorhynchus mykiss*). Special attention was given to evaluating the efficacy of each treatment method in removing the effects associated with primary-treated effluent on phagocytic activity, lymphocyte T proliferation and NCC capacity.

2. Materials and methods

2.1. Trout maintenance

Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from Aquipro fish farm in Quebec. The specimens were left to acclimate at a temperature of 15 °C and 12 h/12 h (day/night) for two weeks in 90-L glass aquaria containing chlorine-free tap water. The fish (body mass of 7 ± 1 g) were fed daily at a rate between 1 and 2% body weight with commercial G1 food (Aquipro). Half the aquarium water was changed with fresh or effluent-containing aquarium water every second day (semi-static conditions).

2.2. Experimental design

The fish were divided into five groups of 15 fish ($N = 15$) and placed in 60-L tanks. One group was exposed to control conditions (unchlorinated tap water). The other four were exposed to a 10% concentration of the primary-treated effluent and after each disinfection strategy in the primary-treated effluent from the City of Montréal (Québec, Canada). The fish were exposed to the effluent before and after disinfection by UV radiation, ozonation and peracetic acid, respectively, for a period of 28 days at 15 °C.

2.3. Effluent disinfection

A volume of 200 L of Montreal effluent was collected and subsequently separated into three 50-L fractions for each treatment. The UV disinfection process was performed at the municipal wastewater pilot plant and pilot-plant scale. The effluents were disinfected using a medium-pressure system with a dose of 30 mJ/cm^2 and a quick contact time. Ozone disinfection was performed using a bubbling system to deliver ozone to the effluent. Wastewaters were disinfected using a dose of 18 mg/L O_3 and a contact time of 18 min. The peracetic acid was supplied by Solvay Interox Inc. (Houston, TX, USA) and the disinfection was carried out using the protocol of Wagner et al. (2002). Briefly, a dose of 2 mg/L of peracetic acid was applied to the primary-treated effluent and allowed to stand for two hours to remove any residual or unstable peroxy-compounds in the effluent matrix. For each process, samples destined for bacteriological analysis (total and fecal coliforms (CEAEQ, 2005) and *Enterococci* bacterial counts (CEAEQ, 2006), coliphage (CEAEQ, 2003) and presence of intestinal parasite *Clostridium perfringens* (EPA, 1996) were collected before and after the disinfection using described methods (Payment et al., 2000). Each treatment was separately divided into 1- and 4-L polypropylene bottles (Nalgene Brand Products, Rochester, NY, USA). All aliquots were maintained at −4 °C for no longer than three days until analysis.

2.4. Preparation of head kidney cell suspensions and leukocytes

At the end of exposure time, fish were anesthetized by the addition of 0.1% of MS-222 (Boreal Laboratories, ON, Canada) and measured for weight and length. Anterior kidneys were removed aseptically and mashed with a 2-mL glass grinder (Wheaton Scientific, NJ, USA) containing 1 mL of RPMI 1640 sterile culture medium (Bio Media, QC, Canada) supplemented with heparin (10 U/mL), buffered at pH 7.4 with 10 mM HEPES, penicillin (100 U/mL)/streptomycin (100 mg/mL) (Bio Media) and 10% (v/v) fetal bovine serum (FBS). The cellular suspension was then transferred into a sterile polypropylene tube (17–120 mm) (Sarstedt, NC, USA) and the final volume was adjusted to 5 mL with RPMI. The cellular suspension (5 mL) was laid over 5 mL of Lympholyte-Poly gradient (Cedarlane Laboratories, ON, Canada) into a sterile 15-mL polypropylene tube. The suspension was centrifuged at