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The effects of municipal effluents on oxidative stress, immunocompetence and DNA integrity in fathead minnow juveniles

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ABSTRACT

The purpose of this study was to compare the toxicity of two major municipal effluents subjected to different treatment processes: a physico-chemically treated effluent (high-risk effluent) and a biofiltered/ UV-disinfected effluent (low-risk effluent). Juvenile fathead minnows were exposed to increasing concentrations of the high-risk and low-risk effluents in semi-static conditions for 16 weeks at 25 °C. At the end of the exposure period, juveniles were collected for immunocompetence (leukocyte density and phagocytosis), oxidative stress (catalase and superoxide dismutase activity) and DNA damage (COMET assay) assessments. The data revealed that DNA damage was the most sensitive biomarker, with increases at concentrations between 3.5% and 5%, and that the treatment processes had no influence on the intensity of genotoxic effects. A greater increase in oxidative stress enzymes was observed with the high-risk effluent as compared to the low-risk effluent, suggesting that oxidative stress was dependent on the treatment process applied. At the immunocompetence level, a biphasic pattern of response was found in both the high-risk and low-risk effluents. Indeed, in fish exposed to the low-risk effluent, an initial increase in leukocyte density and phagocytosis activity was followed by a subsequent decrease in these effects with increasing concentrations of the effluent. In the high-risk effluent, the initial increase and subsequent drop in leukocyte density occurred at lower concentrations while phagocytosis activity increased only at the highest concentration. In conclusion, the occurrence of DNA damage was not affected by the 2 types of effluents; however, oxidative stress and immunocompetence were more strongly influenced by the high-risk effluent than the low-risk one.

KEYWORDS: wastewater treatments, fathead minnow juveniles, DNA damage, phagocytosis, oxidative stress

1. INTRODUCTION

Wastewaters released from municipal treatment plants are recognized as sources of pollution for aquatic ecosystems. These effluents contain a large number of “traditional” contaminants, such as heavy metals, pesticides and polycyclic aromatic hydrocarbons, as well as “emerging” contaminants like pharmaceuticals, personal care products, hormones and flame retardants [1-3]. Although municipal wastewater treatment plants (WWTPs) are effective at reducing the levels of total suspended solids and nutrients (phosphates and ammonia) in

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effluents, emerging contaminants are removed with varying levels of efficiency [4, 5]. These contaminants are considered pseudo-persistent since they are released on a continuous basis and therefore could pose a risk to organisms that are not as competent as mammals at metabolizing/eliminating them. Hence, knowledge of the associated long-term toxicity in aquatic organisms is needed to better protect the environment, given that targeted chemical analysis approaches are not able to predict the toxic outcome of municipal effluents [2, 6].

Municipal effluents are known to induce many toxic effects in aquatic organisms. Some of the contaminants detected in WWTP effluents can affect the endocrine system and reproduction in fish [7-9]. Although some literature reports [10, 11] have focused on the effects of pollutants on the reproductive system, many pollutants found in treated effluents can alter other important physiological systems, such as the nervous and immune systems, and genetic material. Moreover, estrogenic compounds can also alter immunocompetence and produce inflammation in fish, reducing their ability to fight infection and threatening their health status [12]. For example, feral fish were collected downstream of a major municipal discharge point and immune impairment was observed in *Notropis hudsonius* minnow populations [13]. In another study, an increase in the activity of enzymes involved in oxidative stress defense was found in caged fathead minnows at the effluent-plume site [14]. A literature survey encompassing 48 endocrine-disrupting compounds showed that half of them impacted the immune system and more than two-thirds of them appeared to be carcinogenic or mutagenic [10]. Municipal effluents have been reported to be a significant source of genotoxic compounds that can lead to heritable mutations linked to reduced fertility in fish [1, 15]. Therefore, genotoxicity endpoints should also be examined in long-term toxicity investigations on municipal effluents. Genotoxicity was also observed in freshwater mussels caged downstream of a major municipal effluent plume [16]. As stated previously, very little is known about the effect of the long-term toxicity of municipal effluents on immunocompetence and damage at the genome level in comparison with the effects on the endocrine system, reproduction and oxidative stress.

The purpose of this study was to examine the long-term impacts of two types of wastewater treatments on *Promelas pimephales* juveniles, at the immunotoxic, genotoxic and oxidative stress levels. The juvenile stage was chosen in this study for two reasons: First, because exposure to toxic substances during early life-stages can produce harmful effects in fish, later on at the immunocompetence and genotoxic levels, even after exposure has ceased [17]. And second, because the 21-day survival test in juveniles is a recommended test for municipal effluents in Canada. The hypothesis guiding our study was that effluent toxicity depends on the treatment process applied (the null hypothesis is that the treatment process has no influence on the long-term toxic effects). To test this, two different treatment processes were examined: the “high-risk” effluent, to which a primary, physico-chemical treatment is applied, and the “low-risk” effluent, to which more extensive primary/secondary treatments are applied. This study compares the long-term toxicity of two differently treated municipal effluents in fathead minnow juveniles, at the oxidative, immunotoxic and genotoxic levels.

2. MATERIALS AND METHODS

2.1. Experimental setup and fish exposure

Adult fathead minnows from Aquatic Research Organisms (Hampton, NH, USA) were acclimated for 2 months prior to exposure to the effluents in aquariums at a 2:1 female-to-male ratio at 25 °C under constant aeration. Each aquarium contained polyethylene tiles (a 10 cm cylinder cut in half) to permit egg deposition by the females and rearing by the males. Any eggs laid were removed from the tiles on a daily basis. Juveniles (N = 8) were exposed to two municipal effluents for 16 weeks in triplicate in semi-static conditions. 7-day-old larvae were used to expose juveniles to the effluents for 16 weeks. The exposure concentrations were 1.25, 2.5, 5, 10 and 20% (v/v) and these concentrations were refreshed every 3 days during the 16-week exposure period. The effluent samples were collected weekly in the mid-morning from the 2 wastewater treatment plants. The high-risk effluent originated from a primary physico-chemical treatment and the low-risk effluent from

combined biofiltration and UV-disinfection steps. Treatment of the high-risk effluent involved the use of ferrous chloride and anionic polymers to assist with the flocculation of suspended solids. The basic chemical characteristics of the effluents are presented in table 1.

2.2. Fish tissue sampling

At the end of the exposure period (16 weeks), all fish were ethically euthanized in 4 L of 50 mg/L tricaine methanesulfonate (buffered to pH 7.4 with 1 M $NaHCO_3$). Fish were immediately placed on ice for dissection. Fish weight and fork length were determined prior to tissue sampling. The liver was removed, weighed and stored at -85 °C for oxidative stress assessment as described in the next section. Leukocytes were isolated from the anterior kidney for immunocompetence and genotoxicity assessments. The pronephros was collected in sterile Petri dishes containing 1 mL of RPMI (Roswell Park Memorial Institute medium) 1640 cell culture media with 10 U/mL heparin, 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazine ethane sulfonic acid)-NaOH (pH 7.4), 100 U/mL penicillin, 100 mg/mL streptomycin, and 10% fetal bovine serum (BioMedia, Canada). For each fish, the kidney was gently crushed on ice with the rubber end of a 1-mL sterile syringe. The leukocytes were layered onto 51% Percoll solution (Sigma-Aldrich, ON, Canada) and centrifuged at 400 × *g* for 30 min at 20 °C (Sigma-Aldrich, ON, Canada). Leukocytes were found at the cell culture media/Percoll solution interface and were washed twice in phosphate-buffered saline (PBS: 140 mM NaCl, 5 mM KH_2PO_4, 1 mM glucose, pH 7.4) by successive resuspension and centrifugation steps. The cells were then resuspended in RPMI without heparin for cell counting and initial viability determinations using Trypan blue dye (0.004%) exclusion under a microscope with a hemocytometer. Only cell suspensions with initial viability of > 95% were used. The cell suspensions were adjusted to 5 mL with the RPMI media without serum or heparin. Samples were appropriately stored in 15-mL polypropylene sterile tubes at 4 °C in the dark for subsequent analysis the same day.

Table 1. Physico-chemical properties of the municipal effluents.

	High-risk effluent	**Low-risk effluent**
Type of treatment	Physico-chemical process	Biofiltration and UV disinfection process
Flow rate	10	1
Equivalent inhabitants	7	1
	High-risk effluent	**Low-risk effluent**
BOD_5	46 $mg.L^{-1}$	19 $mg.L^{-1}$
P_{tot}	0.4 $mg.L^{-1}$	1 $mg.L^{-1}$
Total fecal coliforms	4.8 * 10^5/100 mL	1.9 * 10^4/100 mL
Total nitrogen ($NH4^+$; mg/L)	6.7	12.4
Nitrates	< 1 mg/L	< 2 mg/L
Suspended matter (mg/L)	18.4	15.8
pH Conductivity ($uS * cm^{-1}$)	6.9 700-800	7.4 N.D.

N.D. Not determined.
1: To ensure anonymity, only the relative population number and flow rates were provided. For example, the high-risk effluent has a population 7 times greater and flow rates 10 times greater than those of the low-risk effluent.

2.3. Oxidative stress assessment

Catalase (CAT: EC 1.11.1.6) and superoxide dismutase (SOD: EC 1.15.1.1) were analyzed using commercial kits (Cayman Chemical, Ann Arbor, MI, USA). Freshly thawed liver samples were homogenized with a 2-ml glass tissue grinder in 9 volumes (w/v) of homogenization buffer (20 mM HEPES, pH 7.4, 1 mM EDTA, 0.1% Triton X-100). The homogenate was centrifuged at 10000 × *g* for 20 min in accordance with the kit manufacturer's directions, and an aliquot of supernatant was taken and diluted in the appropriate sample buffer. Samples were stored again at -85 °C and thawed for immediate analysis of enzyme activity with a plate reader. Absorbance was read at 540 nm for CAT and at 450 nm for SOD. CAT activity was expressed as U/mg proteins, where U was defined as nmol H_2O_2 consumed/min. SOD activity was expressed as U/mg proteins, where U = µmol $O_2^{\bullet-}$ formed/min.

2.4. Immunocompetence assessment

Immunocompetence was determined from the following endpoints: leukocyte concentration, viability and phagocytosis activity. Leukocyte concentration was microscopically counted at 200 × enlargement with a hemocytometer (Bright-Line, PA, USA). Cell mortality was evaluated by flow cytometry using the propidium iodide (PI) exclusion test as described elsewhere [18]. Briefly, 4 µL of PI (100 µg/mL) was added to 500 µL of each cell suspension and analyzed by flow cytometry (FACSCalibur, Becton Dickinson, ON, Canada) for DNA-PI fluorescence at > 600 nm using an argon laser as the excitation source. Phagocytosis activity was measured using fluorescent latex microspheres (1.8-µm diameter; Fluoresbrite®, Polysciences, USA). Immunoactivity was operationally defined as the percentage of cells that engulfed at least one bead, and immunoefficiency as the percentage of cells that engulfed at least three fluorescent beads. Leukocytes were incubated in complete-RPMI in the dark for 18 h at 18 °C, with a 1:100 cell-beads ratio. After the incubation period, cell suspensions were layered over 4 mL of RPMI supplemented with 3% bovine serum albumin (BSA; Sigma) and 10% foetal bovine serum (FBS) and centrifuged at 150 × *g* for 8 min to separate unbound beads from cells. Cells were then fixed in 0.5 mL of 0.5% formaldehyde in phosphate-buffered saline (Hematall, Becton Dickinson, CA, USA) prior to flow cytometric analysis measuring the frequency distribution histogram of cells that exhibit yellow-green fluorescence in the FL-1 channel (λ = 530 nm). A total of 10,000 cells were analyzed for leukocyte viability and phagocytosis activity and efficiency assessments.

2.5. COMET assay

DNA damage was assessed on leukocytes by the COMET assay, which was performed under alkaline conditions according to Devaux *et al*. [19]. After the exposure period, isolated leukocytes were mixed with an equal volume of 1% low melting point agarose prepared in PBS at 37 °C. A 40-µL aliquot was spread on a frosted microscope slide pre-coated with normal agarose (1%) and covered with a 22 × 22 mm coverslip (two gels per slide). Coverslips were removed after agarose polymerization (4 °C, 10 min), and the slides were left in a lysing solution (2.5 M NaCl, 0.1 M EDTA, 10 mM Tris, 10% DMSO, 1% Triton X100; pH 10) for 1 h at 4 °C in the dark. The slides were placed in an electrophoresis buffer (0.3 M NaOH and 1 mM EDTA) for 30 min to allow the DNA to unwind. Electrophoresis was carried out at 25 V (0.61 V/cm) and 300 mA for 24 min. Slides were then washed three times for 5 min each time with a neutralization buffer (0.4 M Tris–HCl, pH 7.5) and dried in absolute ethanol. After staining with diluted SYBR Green solution (1/10,000), each slide was viewed using a fluorescence microscope (X 400) equipped with a CCD camera. Fifty cells per gel were analyzed with the KOMET 6.0 software (Andor).

2.6. Statistical analysis

The exposure aquariums contained N = 8 juvenile fish and 3 aquaria per exposure concentration were used. The data were expressed as mean with standard error. The data were checked for normality and homogeneity of variance with the Shapiro-Wilk and Levene's tests, respectively. When the data were not normally distributed or heterogeneous, they were log-transformed. The effects of the effluent type (high-risk or low-risk) and concentration were examined using factorial 2-way analysis of variance, and significant differences between

treatments were evaluated using Fisher's least square difference test. Correlations between biomarkers were determined using the Pearson product-moment correlation test. Significance was set at $\alpha = 0.05$ using the Statistica software package (version 8, Statsoft Inc., France).

3. RESULTS AND DISCUSSION

Fish were exposed for 16 weeks starting at the 7-day larval stage (juveniles). Juvenile survival rates were similar to control values (> 90%) (results not shown) after 16 weeks. With regard to fish weight, 2-way analysis of variance (ANOVA) revealed a significant interaction between effluent type and concentration ($p < 0.01$). The high-risk effluent did not influence juvenile weight (Figure 1). The low-risk effluent induced a significant increase in weight in response to the 2.5% and 5% effluent concentration, in comparison with the controls and the high-risk effluent at the same concentrations. The hepatic somatic index (liver weight/fish weight) did not significantly change. The levels of SOD and CAT activity were also determined in juveniles. SOD activity was not influenced by either effluent type or concentration factors (Figure 2A). However, CAT activity was significantly influenced by both exposure concentrations and effluent type (Figure 2B). A significant induction of CAT activity was observed in fish exposed to the low-risk effluent at the highest concentration (20%) relative to the controls and to the high-risk effluent at the corresponding concentration. These results differ from the induction of CAT and SOD activity observed in wild perch populations collected near (4 and 10 km away) the discharge point of a high-risk effluent [20]. Other studies also reported oxidative stress in fish exposed to a primary-treated effluent [21, 22]. This indicates that adaptation of young fish to long-term exposure (16 weeks) to high-risk effluents (primary treated) may involve mechanisms other than CAT and SOD activities, such as xenobiotic efflux mechanisms, which are also involved in xenobiotic resistance. Indeed, increased gene expression of multidrug resistance pump genes was observed in hepatocytes exposed to various municipal effluents and to environmental toxins [23, 24]. Further research is needed to identify these adaptation mechanisms and should include bioavailability data on potential "oxidative stress contaminants" in municipal wastewaters.

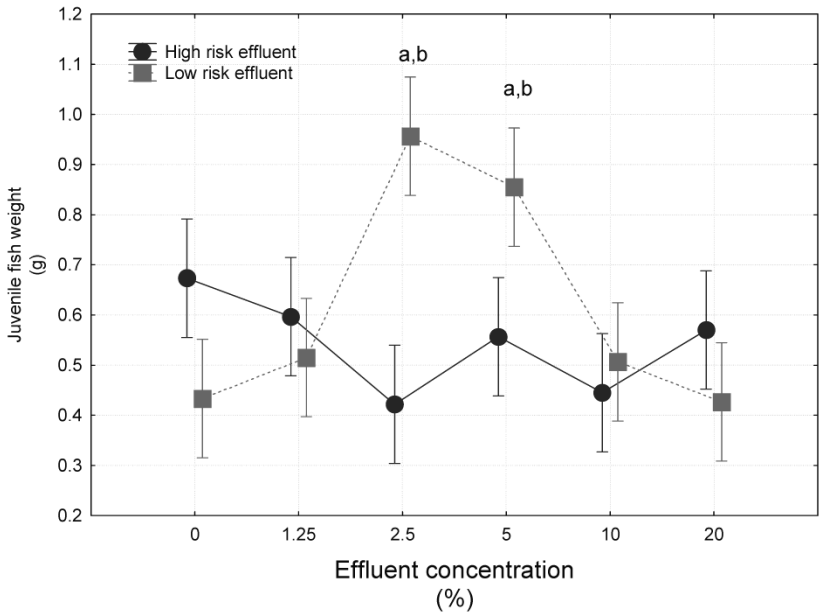

Figure 1. Juvenile weight changes after exposure to the high-risk and low-risk effluents for 16 weeks.
Adult fathead minnows (FHM) were exposed to increasing concentrations of high-risk and low-risk municipal effluents for 16 weeks. The data are expressed as the mean with the standard error. The letter 'a' indicates significance from controls and 'b' indicates a significant difference between the high-risk and low-risk effluents.

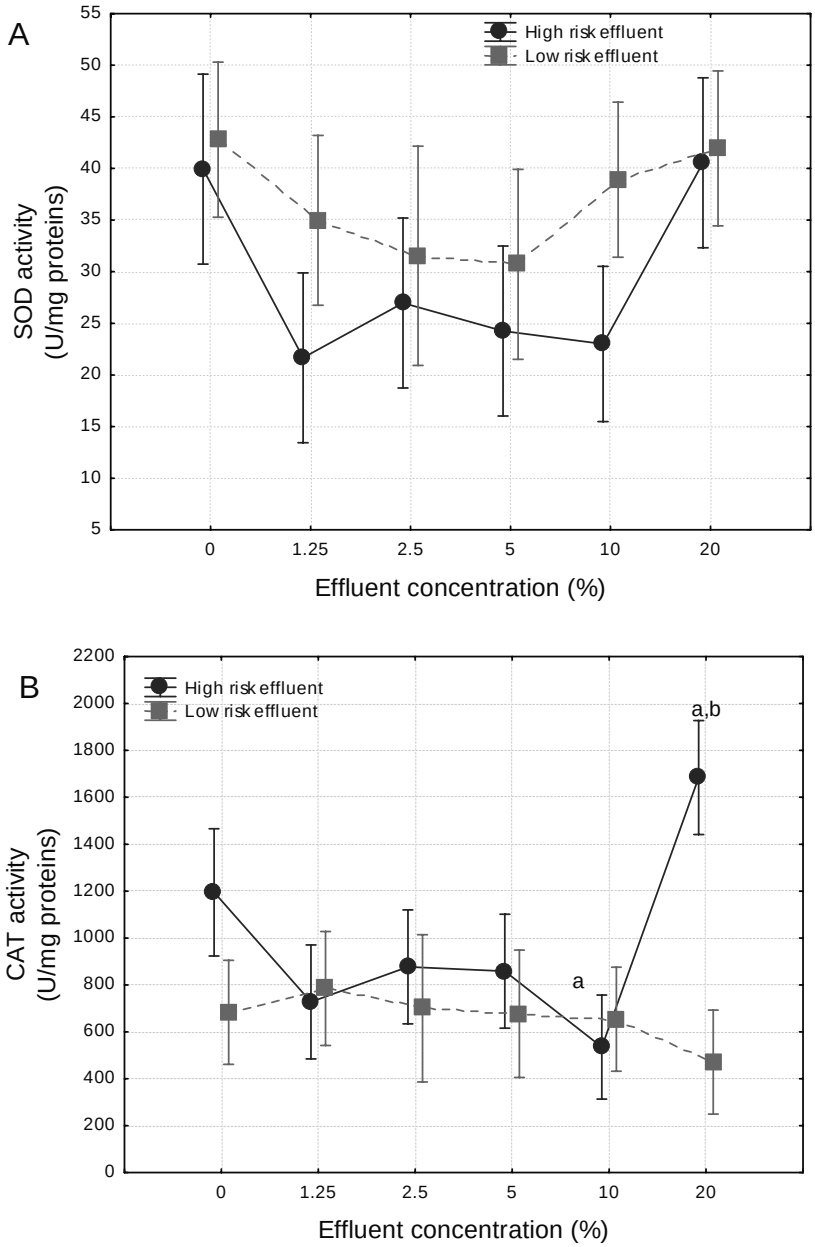

Figure 2. Change in oxidative stress enzyme activity after 16 weeks.
Juveniles were exposed for 16 weeks to the municipal effluents. The SOD (A) and CAT (B) activities were determined. The data are expressed as the mean with the standard error. The letter 'a' indicates significance from controls and 'b' indicates a significant difference between the high-risk and low-risk effluents.

Genotoxicity was determined by the COMET assay as shown in figure 3. Two-way ANOVA revealed that the exposure concentration was the only significant factor (no influence of the treatment process). A clear concentration-response relationship was found for both effluents, which suggests that both effluents had genotoxic effects in juvenile fish. In addition, no correlation was observed between DNA damage and oxidative stress enzymes, which suggests that genotoxicity was not mediated by oxidation mechanisms (Table 2). This corroborates earlier studies highlighting the genotoxic properties of industrial and municipal effluents [25, 26]. The observed genotoxicity was not associated with

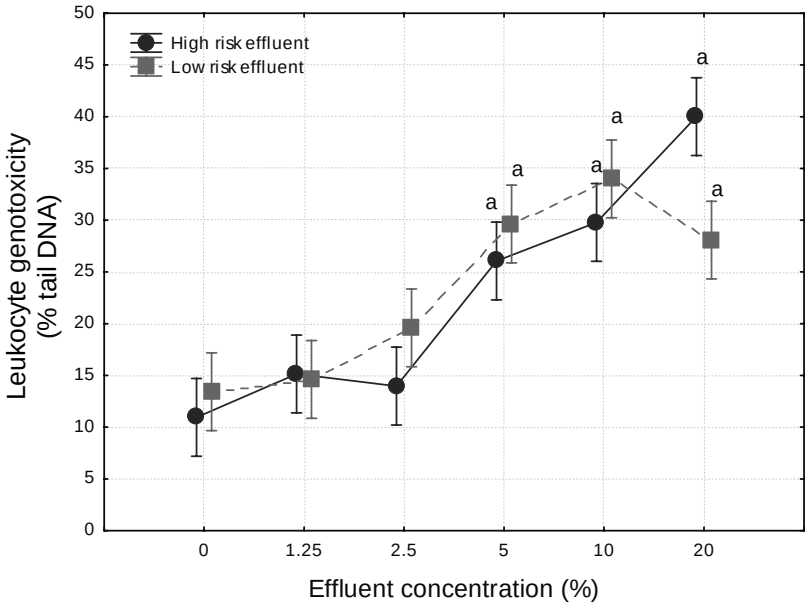

Figure 3. DNA damage in juvenile tissues exposed to the effluents for 16 weeks.
DNA damage was determined using the COMET assay in juvenile minnows exposed to the effluents. The data represent the mean with the standard error. The letter 'a' indicates significance from controls.

Table 2. Correlation analysis between biomarkers.

	COMET	**Fish weight**	**SOD**	**CAT**	**Leukocyte viability**	**Leukocyte conc.**	**Phagocytosis activity**	**Phagocytosis efficiency**
COMET	1	0.03 p > 0.1	0.06 p > 0.1	0.10 p > 0.1	-0.1 p > 0.1	0.04 p > 0.1	0.08 p > 0.1	0.06 p > 0.1
Fish weight		1	*-0.20* *p < 0.1*	**-0.28** **p < 0.05**	-0.01 p > 0.1	0.07 p > 0.1	-0.09 p > 0.1	-0.08 p > 0.1
SOD			1	**0.54** **p < 0.05**	-0.07 p > 0.1	-0.07 p > 0.1	0.15 p > 0.1	0.11 p > 0.1
CAT				1	0.14 p > 0.1	-0.09 p > 0.1	0.01 p > 0.1	-0.06 p > 0.1
Leukocyte viability					1	0.19 p > 0.1	-0.06 p > 0.1	-0.05 p > 0.1
Leukocyte concentration						1	-0.11 p > 0.1	-0.14 p > 0.1
Phagocytosis activity							1	**0.93** **p < 0.001**

Significant correlations are in **bold**. Marginally significant (0.05 < p < 0.1) correlations are in *italic*.

loss of leukocyte viability since this parameter was not significantly affected by the effluents (mean viability was 91% ± 10% for the high-risk effluent and 86% ± 13% for the low-risk effluent). For both the effluents, a significant increase in DNA damage was observed at concentrations between 2.5% and 5% (i.e., threshold concentration of 3.5%). The maximum fold induction was 3.6-fold and 2.1-fold at the highest concentration for the high-risk and low-risk effluents, respectively. In a previous study, an effluent similar to the high-risk effluent of the present study was shown to display

significant genotoxic activity in the alkaline precipitation assay and DNA metabolism endpoints (dehydrofolate reductase, aspartate transcarbamoylase, xanthine oxidoreductase changes) in freshwater mussels exposed to the effluent for 14 days [27]. In another study [16], caged mussels placed 20 km downstream of the plume of an effluent similar to the high-risk one for 8 weeks also had increased DNA damage based on the COMET assay, which agrees with our findings. It is noteworthy that intensity in DNA damage observed at a 2.5% concentration corresponded to increased DNA damage in mussels placed 20 km from the discharge point of the high-risk effluent. This is the case for the low-risk effluent as well given its concentration-response slopes similar to those of the high-risk effluent (Table 1). Based on the relative flow rates and assuming a similar dispersion rate in the environment, the flow rate of the high-risk effluent is 10 times greater than that of the low-risk effluent, which indicates a shorter distance of impact for the low-risk effluent, perhaps in the order of 10 times less (representing circa 2 km). However, this relationship should be validated directly at each site. Nevertheless, the genotoxic properties of the effluents were observed regardless of the wastewater treatment types, at least those examined here.

Based on the chemical characterization of these effluents, several genotoxic compounds were found, although their concentrations were not systematically higher in the high-risk effluent samples [28]. This is consistent with the similar dose-response slopes for the high-risk and low-risk effluent discharges but does not support the difference observed in the maximum induction responses. It appears that the genotoxicity of municipal effluents is impervious to the treatment process, and reduction of target organic contaminants by the treatment process has no effect on the resulting genotoxicity of the treated effluent [6].

The sex steroid 17β-Estradiol (E2) has been found to be genotoxic in fish erythrocytes based on the COMET and the micronucleus tests [29]. Bisphenol A, known to exert detrimental reproductive effects in fish, also increased chromosomal aberrations and DNA damage in the MCF-7 human tumor cell line [30]. Furthermore, pharmaceutical products, including venlafaxine, fluoxetine, carbamazepine and diclofenac, known to be present in the two tested effluents, induced DNA damage in zebrafish and in blue mussels [31, 32]. In a 2012 study, more than half of a set of 11 organic fractions from treated effluents demonstrated a significant increase in DNA damage, indicating that they can be considered genotoxic, regardless of the treatment process applied [33]. That study also revealed that the origin of the effluents could also influence genotoxicity and effluents from pharmaceutical industries were genotoxic as well [33]. More extensive biological treatments (tertiary-treated effluents) were able to eliminate toxicity (mortality) but had no effect on genotoxicity [34]. Acute toxicity was completely removed by an anoxic/oxic process, whereas the genotoxicity of the treated effluent was increased relative to the original effluent, which suggests that the treatment process produced genotoxic compounds adding to the initial genotoxicity of untreated wastewaters entering the wastewater treatment plant. In another study, in addition to genotoxicity, a mixed secondary effluent was found to be estrogenic. Although 20-70% of the measured endocrine-disrupting compounds were removed by ozonation, the estrogenic potential of the effluent was reduced by only 20% and still revealed the presence (formation?) of genotoxic compounds [35].

Immunocompetence in juveniles was determined by the following measurements: leukocyte density, phagocytosis activity and efficiency (Figure 4). A two-way factorial ANOVA of leukocyte density revealed a significant interaction between effluent type and concentration (Figure 4A). The concentration of leukocytes in the head kidney followed a biphasic response with an initial increase at low concentrations followed by a decrease at higher concentrations. At the lowest effluent concentration used (1.25%), the responses were stronger with the high-risk effluent compared to the low-risk one. An increase in leukocyte number was considered a first-response against invading microorganisms in feral fish collected from a bacteria-rich municipal discharge site [13]. No significant changes in leukocyte viability were observed by the exposure concentrations for both the high and low-risk effluents. Phagocytosis activity and efficiency were also determined in isolated leukocytes (Figures 4B and 4C). Two-way ANOVA revealed a significant interaction

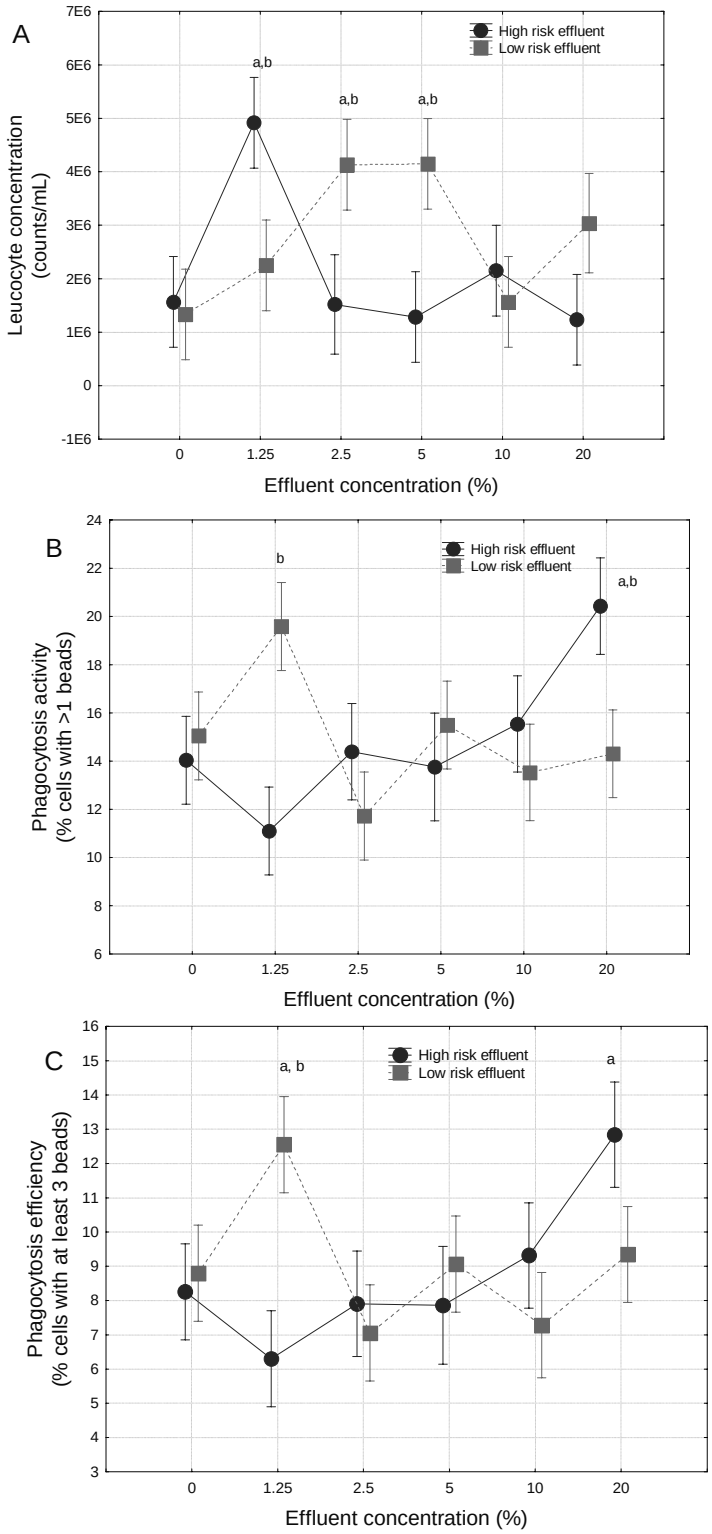

Figure 4. Immunocompetence evaluation.
Immunocompetence was determined by observing the changes in leukocyte density (A), phagocytosis activity (B) and efficiency (C). The data represent the mean with the standard error. The letter 'a' indicates significance from controls and 'b' indicates a significant difference between the high-risk and low-risk effluents.

between effluent type for both phagocytosis activity and efficiency parameters. Phagocytosis activity was significantly increased at the highest concentration (20%) and at the lowest concentration (1.25%) in the high-risk effluent compared to the controls and the low-risk effluent at the same concentrations. The increase in phagocytosis is thought to be associated with the increased presence of microorganisms and suspended matter, while dampening of phagocytosis could result from the presence of contaminants, including estrogenic compounds [36, 37]. In a study involving spottail shiner minnows, when phagocytosis was corrected against bacterial loadings, significant inhibition of phagocytosis was found in fish located downstream of a dispersion plume from a primary-treated effluent [13]. Biphasic changes in the immunocompetence in fish exposed to a primary-treated municipal effluent similar to the high-risk effluent were also reported. Rainbow trout exposed for 45 days to a physico-chemically treated effluent showed inhibited phagocytosis at low concentrations (0.03%) but increased phagocytosis at higher concentrations (3%) [38]. Increased phagocytosis in rainbow trout was observed after 28 days of exposure to the effluent, followed by a decrease at day 90 [12]. Although the reason for these biphasic changes is unclear, one possible explanation relates to the interplay between particles and the microbial and chemical composition of the effluent as mentioned above. Another possibility relates to the interplay between innate and acquired immunity in fish, whereby the fish takes time to produce antibodies which can more specifically target particles/microbes that are recognized by the immune system (non-self). The modulation of the innate immune response (i.e., phagocytosis and cytokine production) may be attributable to the bacterial load of the effluents [13, 21]; however, municipal effluents are complex mixtures of contaminants that can either exacerbate or suppress phagocytosis in fish. For example, a 30-day exposure to 1 and 5 µg/L of cadmium or mercury resulted in inhibition of phagocytosis [39]. Pharmaceuticals or compounds acting on the opiate and estrogen receptors were shown to silence the immune system in fish [36]. However, a study examining the immunotoxicity of organic extracts (the particles/microorganisms removed) from wastewaters from 12 cities across Canada before and after 6 different treatment processes revealed both decreases and increases in phagocytosis activity; the initial untreated wastewaters were found to have a greater influence than the type of treatment process applied [23]. This suggests that the chemical components of the effluent could produce either inhibition or stimulation of phagocytosis and that generalization to all the different treatment processes is not possible at the present time. In another study, exposure of rainbow trout to a high-risk effluent before and after removal of fine particles/ microorganisms by filtration on a 0.2-µm pore membrane for 28 days was undertaken to assess the effect of particles and the dissolved components of the effluents on phagocytosis [12]. The study revealed that the fine particles fraction of the effluent strongly increased phagocytosis activity while the dissolved fractions only marginally increased it. This supports the contention that the particle phase has more of an impact on phagocytosis, although the contribution of the dissolved fractions in these complex mixtures cannot be ignored.

4. CONCLUSION

In conclusion, long-term exposure of juvenile fish to high-risk and low-risk municipal effluents led to genotoxic effects and immunotoxicity to some extent. DNA damage was strongly induced at low effluent concentrations and the two treatment processes had little influence on the response. Immunocompetence followed a biphasic response which was more evident in the low-risk effluent, suggesting that phagocytosis activity and efficiency initially increased and then decreased as the concentration of the effluents increased. Although treatment of municipal wastewaters seems to mitigate these responses, especially with more extensive treatments such as those applied to the low-risk effluent, the effluents still have the potential to disrupt aquatic life in nearby urban environments.

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CONFLICT OF INTEREST STATEMENT

The authors do not have any financial or other conflicts of interest.

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