Plastic contamination and water quality assessment of urban wastewaters

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Introduction

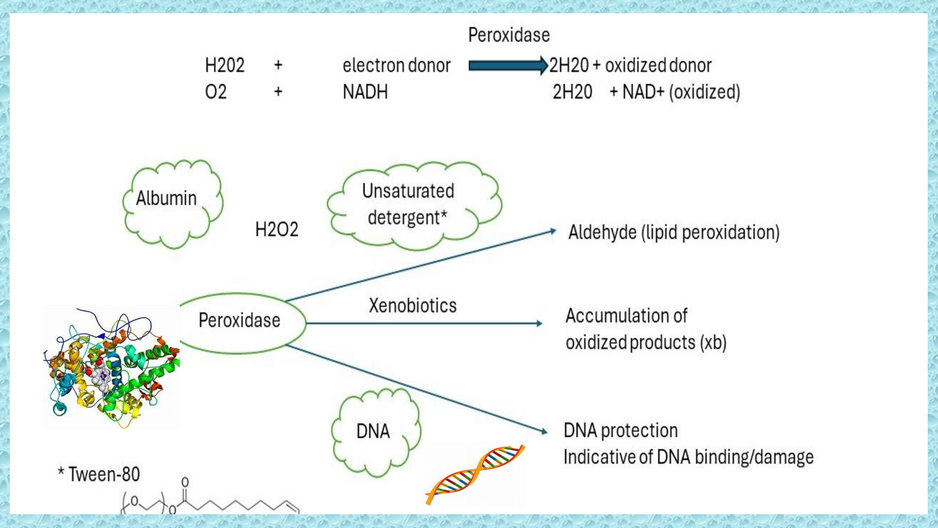
Municipal wastewaters are produced from domestic and industrial waste products from cities iincluding hospital wastewaters, solid waste disposal sites and road runoffs leachates; Moreover, the intensity of rainfall events from global warming are exceeding the capacity of some treatment plants to handle and treat excess volumes (Min, 2011; Eum et al., 2014);

In addition to existing contaminants (PAHs, metals/elements, pesticides, pharmaceutical and personal care products), micro and nano-sized plastics are found in treatment plant effluents (Uoginte et al., 2022; Sivarajah et al., 2023).

Recent studies revealed that wastewaters do contain **microplastics** with 43% remaining in dissolved organic fraction in effluents. The most commonly found plastics was polystyrene (PS), polypropylene (PP) and polyethylene terephthalate (PET). N**anoplastics** (NPs; 100-1 nm range) in effluents are less understood at the present time. They are found between 0.01-10 µg/L (Okoffo and Thomas, 2024) corresponding to 3-10% of NPs in the untreated influent. NPs were reported to cause oxidative stress and genotoxicity in various organisms.

There is a need for quick, cheap and alternative (fish) methods to screen for toxic compounds in municipal effluents. Enzyme biosensors using **peroxidase** represent a case for wastewater quality assessments and predicts trout (fish) toxicity .

Principle of the peroxidase toxicity assay (Perotox)



Gagné F, Smyth SA, André C 2024. Plastic Contamination and Water Quality Assessment of Urban Wastewaters. J Environ Toxicol Res 1, 1-14.

- Per inhibitions predicted toxic effluents in fish
- DNA protection was associated to 70% of genotoxic effluents (SOS chromotest) (Gagné and Blaise, 1997).

Objectives

1) The purpose of this study was therefore to test the Perotox assay in various municipal wastewaters following 5 different treatment processes from 7 Canadian cities.
2) The influence of DNA on Per activity was also examined to determine potential DNA binding or genotoxic agents in municipal effluents.
3) In parallel, the organic carbon fraction of the municipal wastewater extracts was examined for the presence of Pahs, nanoplastics (PsNPs) using fluorometric methods.

4) An attempt was made to examine the influence of population size, treatment type and of plastics on the Perotox assay.

Methods

Municipal effluent sampling. Wastewaters from 7 townships differing in population size were collected as 24h-composite for 3 days. A 1 L was stored at 4°C in the dark. The untreated wastewaters (influent) were also collected.

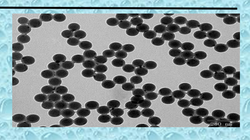
Effluent SPE extraction The influents/effluents were filtered on 0.8 µm pore cellulose filter and 250 mL was passed through a reverse phase C18 cartridge (500 mg; Supelco, USA). After washing with MilliQ water (10 ml), the material was eluted with 5 mL ethanol and concentrated to 0.5 mL under nitrogen stream (500 X concentrate). This fraction is considered the dissolved organic matter.

Characterisation of the dissolved organic matter

The total levels of the **dissolved organic matter** (DOC) was determined by the spectrometric methodology at 254 nm (Brandstetter et al., 1996);

The levels of **light, medium and heavy polyaromatic hydrocarbons** (Pahs) were determined using fixed wavelength fluorometry (Aas et al., 1995).

.....*Methods*



The levels of **polystyrene nanoplastics (PsNPs)** were determined using a **molecular rotor probe** 9(dicyanovinyl)-julolidine as described (Gagné, 2019). Ffluorescence were taken at 450 nm excitation and 620 nm emission. Standard solutions of polystyrene nanoparticles were used for calibration. Levels of plastic-like substances (PS/PVC, PP) in the organic matter matrix (i.e. the C18 extract) were also determined using the copper fluorescence quenching methodology (Lee et al., 2021). The data were expressed as ug PP or PS/PVC-equivalents/ mg DOC.

Peroxidase toxicity assay

Direct assay. The reaction media was composed of peroxidase and albumin at 0.1 µg/mL in 0.1 X PBS-tween 80 (0.001%) in a total volume of 160 µL. After adding 20 µL of the ethanolic extract for 5 min, 20 µL of 1 µM DCFDA and 0.01 % H_2O_2 were added. The reaction was allowed to proceed at 25°C for 30 min with readings taken at each 2-3 min interval for fluorescein (excitation 485 nm/emission 530 nm).

DNA protection assay. The same procedure was repeated with the ethanol extract pre-incubated for 5 min with 1 µg/mL DNA to determine the influence of DNA on Per reaction rates. The **DNA protection index is defined as: Per activity with DNA/Per activity.** Blanks consisted of ethanol only and $CdNO_3$ (0.1 µg/L) was used as a positive control.

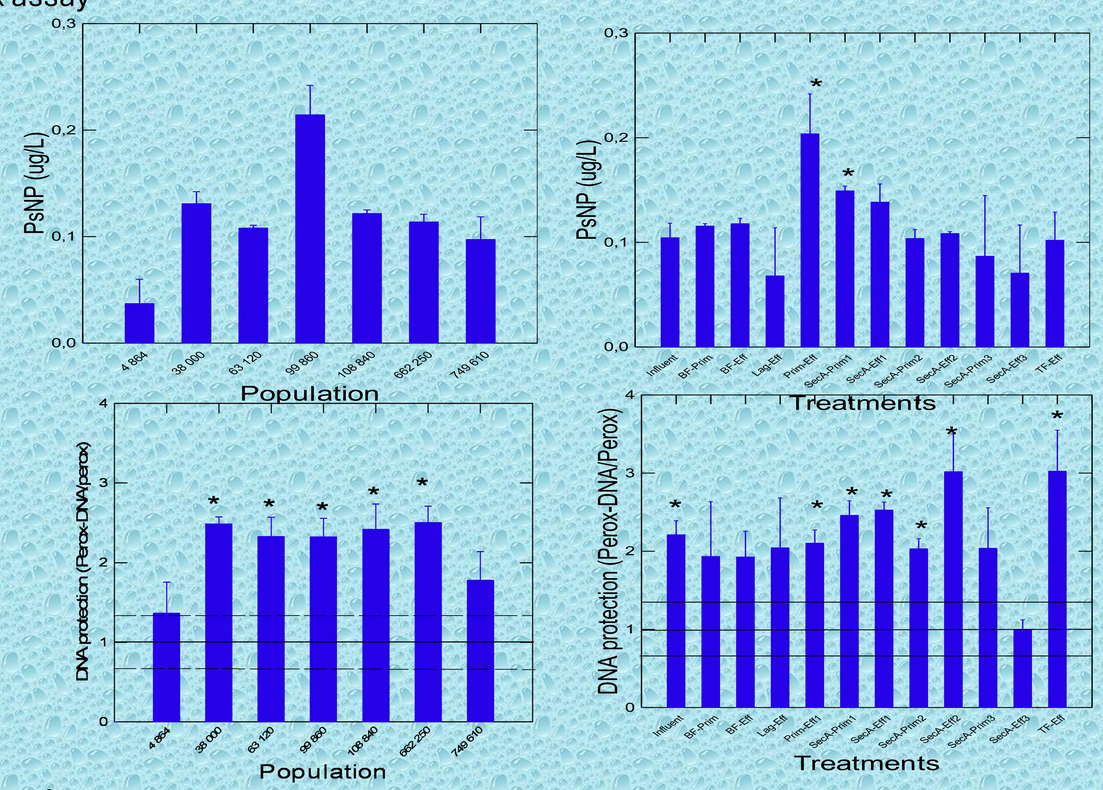
Results

1) DOC characterization

Townships	Population	Treatment	Comments
1	4 864	Aerated lagons (Lag)	Only the influent (Inf) and final effluent (Eff) were sampled
2	38000	Secondary activated sludge (SecA)	Influent (Inf), primary (Prim) and the final Eff) effluents
3	63120	Secondary activated sludge (SecA)	Influent (Inf), primary (Prim) and the final Eff) effluents
4	99 860	Primary (Prim)	Influent (Inf), primary (Prim) and the final Eff) effluents
5	108 840	Biological filtration (BF)	Influent (Inf), primary (Prim) and the final Eff) effluents
6	662 250	Trickling filter (TF)	Influent (Inf), primary (Prim) and the final Eff) effluents
7	749 610	Secondary activated sludge (SecA)	Influent (Inf), primary (Prim) and the final Eff) effluents

Treatments	OC mg/L	Light Pahs µg/L	Med Pahs µg/L	High Pahs µg/L	Humic/ fluvic acid (RFU/mg OC)
Influent (untreated)	1.13± 0.01	2.2± 0.4	0.26± 0.08	0.03± 0.006	0.18± 0.05
SecA-Prim1	1.17± 0.01	2.7± 0.8	**0.10± 0.03***	0.02± 0.009	**0.094± 0.02***
SecA-Eff1	1.18±0.013	**4.7± 1.4***	**0.07± 0.01***	0.005± 0.004	**0.04± 0.01***
SecA-Prim2	1.17± 0.03	**5.6± 1***	**0.12± 0.03***	**0.014± 0.002***	**0.05± 0.03***
SecA-Eff2	1.18± 0.003	**5.0± 0.8***	0.17± 0.01	0.03± 0.009	**0.048± 0.001***
SecA-Prim3	1.11± 0.02	**4.9± 1.8***	0.18± 0.06	**0.06± 0.03***	0.2± 0.02
SecA-Eff3	1.07± 0.006	1.6± 0.4	**0.13± 0.03***	0.03± 0.008	0.09± 0.04
TF-Eff	1.17 ± 0.01	3.3± 1.7	0.07±0.02*	0.03± 0.01	**0.07± 0.02***
Prim-Eff	1.1± 0.005	**6.4± 2***	**0.7± 0.1***	**0.08± 0.005***	**0.58± 0.06***
BF-Prim	1.1± 0.008	1± 0.6	0.13± 0.003	0.02±0.006	0.12± 0.01
BF-Eff	1.075 ±0.007	2.2± 0.9	0.17± 0.02	0.032±0.004	0.16± 0.01
Lag-Eff	1.1± 0.3	0.4± 0.04	0.15± 0.06	0.02± 0.01	**0.06± 0.02***

2) Perotox assay



Conclusions

Inhibition of the Per reaction was observed in most samples (90%) leading to reduced elimination rates of peroxide, which can initiate toxicity.

In a previous study, the Perotox inhibition assay was significantly correlated with trout toxicity data-96h LC50 (Gagné and Blaise, 1997) and selected metals/elements (r=0.6).

In the present study, toxicity occurred at 1-50 X concentration suggesting no toxicity in fish; this was corroborated with the absence of toxicity with the trout 96h LC50 assay.

A low but significant correlation with plastic levels (r=-0.4) was found with the inhibition potential.

The DNA protection index was found in nearly 60 % of the effluents suggesting genotoxic compounds in these effluents.

The study also revealed that effluents using biofiltration and aeration processes were generally less harmful towards the Perotox and DNA protection assays.

The Perotox assay represents a cost-effective alternative for the rapid and cheap screening for municipal wastewater quality.

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