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Oil sands process-affected water impairs feeding by *Daphnia magna*

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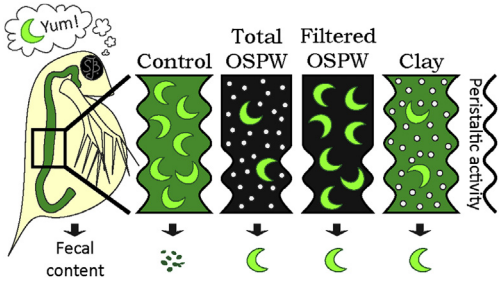
HIGHLIGHTS

- Suspended particles in OSPW reduce the gut capacity for food and digestion efficiency.
- Dissolved components of OSPW impair peristaltic activity and reduce clearance time.
- OSPW inhibits feeding of *D. magna* by impairing digestion efficiency, not ingestion.

GRAPHICAL ABSTRACT



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ABSTRACT

Growth in extraction of bitumen from oil sands has raised concerns about influences of this industry on surrounding environments. Water clearance rate (a surrogate of feeding rate by *Daphnia magna*) in water containing *D. magna* exposed to oil sands process-affected water (OSPW) and its principal components, dissolved component (DC) and suspended particulate matter (SPM), was reduced to 72, 29, and 59% of controls, respectively. This study also examined several possible mechanisms for the observed changes algal cell density (i.e., feeding rate). There was no change in the digestive enzymes trypsin or amylase when *D. magna* were exposed to DC or SPM; however, exposure to total OSPW reduced trypsin activity. Mandible rolling or post-abdominal rejections, which are indicators of feeding and palatability of food, were not affected by any exposures to OSPW. Beating of thoracic limbs, which provides water flow toward the feeding groove, was reduced by exposure to SPM or total OSPW. Peristaltic activity was reduced by exposure to DC, which then might result in reduced digestion time in *D. magna* exposed to DC, SPM or whole OSPW. All treatments caused an increase in numbers of intact algae cells in the hindgut and excreted material. These results suggest that both DC and SPM affect feeding of *D. magna* by impairing actions of the digestive system, but most probably not by reducing rates of ingestion.

1. Introduction

The oil sands mining industry in northern Alberta, Canada, is operating on the largest known deposit of bitumen in the world (ERCB, 2009). Both the current volume and rapid growth in

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extraction of bitumen, producing an estimated 3.95 million barrels per day by 2030 (CAPP, 2015), has increased concern about potential effects on the surrounding ecosystem (Dowdeswell et al., 2011; He et al., 2012). Oil sands process-affected water (OSPW), which is a byproduct of extraction of bitumen using the "Clark extraction process," is of main concern (Kavanagh et al., 2013).

Because industrial operators do not discharge OSPW back into the Athabasca River (Giesy et al., 2010), a large volume of OSPW produced throughout the years of mining, is currently stored on-site in tailings ponds (RSC, 2010). Recently, the Alberta Energy Regulator mandated minimizing the volume of liquid tailings stored in tailings ponds and requires that companies leave operation sites at a ready-to-reclaim state within ten years of the end of the mine's life (Government of Alberta (2015)). Fulfilling mandates and tailings reclamation requires knowledge of the chemical and physical characteristics of tailings, along with their effects on living organisms and the surrounding environment.

Oil sands process-affected water is a complex mixture of thousands of dissolved chemicals (organic and inorganic) and suspended particulate matter (SPM) (Del Rio et al., 2006; Debenest et al., 2012; Lengger et al., 2013). Depending on characteristics of source bitumen, extraction method, and age, chemical and physical compositions of specific OSPWs might be different. However, chemical characteristics and toxicity of three OSPW samples collected from three different tailing ponds of different companies were similar (Lari et al., 2016b). Although, specific compounds responsible for toxicity of OSPW are not well known, the dissolved organic fraction of OSPW, mainly naphthenic acids (NA) and polyaromatic hydrocarbons (PAH), are the main drivers of its toxicity (Anderson et al., 2012a, 2012b; Klamerth et al., 2015; Morandi et al., 2015; Lari et al., 2016b).

Several studies have investigated toxic effects of OSPW and its major components on a variety of aquatic invertebrates (Morandi et al., 2016). NAs of lesser molecular mass, isolated from OSPW were more potent at reducing survival of *Daphnia magna* Straus, 1820 than were NAs of greater molecular mass (Frank et al., 2009). In contrast, unfractionated, whole OSPW is not lethal to planktonic animals such as *D. magna* or *Chironomus dilutus* Shobanov, 1999 larvae at equivalent concentrations observed in holding ponds (Wiseman et al., 2013; Lari et al., 2016b). OSPW is also less potent at causing lethality than the water soluble fraction of crude oil (Lari et al., 2016a). During longer-term exposures to sub-lethal effects of OSPW or isolated NAs exhibited various potencies for reduction of rates of growth of various planktonic organisms (Goff et al., 2013; Wiseman et al., 2013; Lari et al., 2016b). Exposure of *D. magna* to small concentrations (IC50 = 5.34%) of OSPW for 24 h inhibited feeding (Lari et al., 2016b). That study also demonstrated that *D. magna* ingested SPM from suspensions of OSPW and suggested that SPM in OSPW reduced rate of feeding by *D. magna* by filling their gut and reducing their capacity to ingest particles of more nutritious foods (Lari et al., 2016b). However, the exact mechanism for effects of OSPW and contributions of its constituents, dissolved components (DC) vs. SPM, were not fully elucidated. For instance it was not determined whether filling the gut is the only mechanism by which SPM reduces the feeding rate or other mechanisms are also involved.

The present study follows up on results of a previous (Lari et al., 2016b), and aims to: 1) determine mechanisms by which OSPW reduced water clearance rate by *D. magna*, as a model species and 2) investigate roles of DC and SPM. Potential causes of reductions in water clearance rate were investigated at three biological levels: behavioural (i.e. beating rate of the thoracic limbs, mandible rolling frequency, and rate of food rejection by the post-abdominal appendage), physiological (i.e. peristaltic activity and digestion efficiency) or biochemical (digestive enzyme activity).

2. Materials and methods

2.1. Test-chemicals

For this study, three major oil sands companies in the Athabasca region of northern Alberta, Canada, provided samples of OSPW. In studies of lethality and sub-lethal effects on feeding, growth, and reproduction, the three OSPW exhibited the same potency (Lari et al., 2016b). Profiles of relative abundances of dissolved organic species in the three OSPWs were also similar. Therefore, in the present study a mixture of all three OSPWs in equal proportions (v/v) was used. The OSPW mixture was stored in 20 L plastic buckets at 4 °C. The DC of OSPW was prepared by filtering the mixture through a 0.45 μm cellulose nitrate membrane filter (Whatman, Germany). The filtrate was dried at 60 °C for 48 h and weighed to determine the SPM content of OSPW mixture (0.686 g/L). Because filtration changes the mean size of filtered particles and small particles are trapped in filter pores, filtration residuum was not used for making SPM solution. Suspended particulate matter solution and reconstituted whole OSPW was made by adding sediment (<45 μm; Plainsman Clay Limited, Canada), at the same rate as the measured residue filtered from OSPW (0.686 g/L), to culture water and filtered OSPW, respectively.

2.2. Chemical characterization

Samples were extracted as described previously (Lari et al., 2016b). Briefly, 1 L of OSPW was filtered by use of a 0.7 μm glass fibre filter (Fischer Scientific, Ottawa, ON, Canada). Sample was acidified to pH 2 by use of concentrated H_2SO_4 (Fischer Scientific, Fair Lawn, NJ, USA). The acidified solution was extracted twice with 200 mL of dichloromethane (DCM) in a 2 L separatory funnel. The two extracts were combined and blown-down by use of nitrogen gas to approximately 5 mL, transferred to a pre-weighed vial, blown to near dryness and weighed. The extract was re-suspended in methanol for chemical profiling. Profiling of extracts by use of HPLC-ultrahigh resolution MS (Orbitrap), operated in positive and negative ion mode, was completed as described previously (Lari et al., 2016b). Approximately 100 mL of OSPW was sent to SGS Canada Inc. (Lakefield, Ontario, Canada) to measure total recoverable concentrations of vanadium (V), nickel (Ni), copper (Cu), cadmium (Cd), and zinc (Zn) by use of inductively coupled plasma mass spectrometry (ICP-MS). Above mentioned elements are the most relevant trace metals in OSPW.

2.3. Test animals

All *D. magna* used in the present study were from a culture held under controlled laboratory conditions as described previously (Lari et al., 2016b). Culture water was reconstituted from double deionized water by adding 0.096 mg/L $NaHCO_3$, 0.06 mg/L $CaSO_4.H_2O$, 0.06 mg/L $MgSO_4$, 0.012 mg/L KCl, 2.4 μg/L Na_2SeO_4 and 3.2 μg/L vitamin B12. The resultant culture water was moderately hard: hardness, 90 mg/L as $CaCO_3$; alkalinity: 165 mg/L as $CaCO_3$; pH, 8.3. To monitor sensitivity of the culture lethality caused by the reference toxicant sodium chloride (NaCl) was performed every month (Environment Canada, 1996). Neonates (i.e. ≤24 h old) from 3 to 5 week-old *D. magna* mothers were collected and raised at a density of approximately 80 individuals per liter of culture water, for 6 days (5th instar). Each group of 80 *D. magna* was fed *Raphidocelis subcapitata* daily at a density of 10^5 cell/L. All experiments described in the present study were conducted with these 6 day-old *D. magna*. Water in which *D. magna* were cultured, was also used for diluting OSPW and as the control water in all experiments.

2.4. Exposures of D. magna

Experiments were conducted on *D. magna* that were exposed to 10% equivalent (1/10X) concentrations of DC, SPM or unfractionated, whole OSPW. This equivalent concentration of OSPW was chosen based on the inhibition curve for effects of whole OSPW on the feeding by *D. magna* in an earlier study (Lari et al., 2016b; IC75 = 10% OSPW). A fourth group was exposed to *D. magna* culture water in the absence of OSPW as control. All groups were exposed to test solutions at the density of 5 individuals per 100 mL, with a fixed ration of *R. subcapitata* (5×10^5 cell/mL) as food. In order to prevent growth and reproduction of *R. subcapitata,* test beakers were kept in complete darkness during 24-h exposures.

2.5. Inhibition of feeding

Studies of feeding were accomplished by use of five replicates for each treatment. Treatment solutions were sampled prior to the addition of *D. magna*, and held in the same condition as the exposures without adding *D. magan*. After 24 h of exposure in complete darkness, *D. magna* were removed, the test solution was vigorously shaken, and density of remaining algae cells was enumerated by use of a Neubauer chamber (Marienfeld, Germany) and light microscope (Eclipse 80i, Nikon, Japan). In order to calculate consumption of food, density of the remaining algae was compared to the density of algae in the sample without animals.

2.6. Activities of thoracic limb, mandible, and peristalsis and frequency of rejection of food

Rates of beating of thoracic limbs, mandible rolling frequency, peristaltic activity of the gut, and frequency of rejection were measured by use of previously described methods (Lari et al., 2017). Briefly, after a 24 h exposure (as described in section 2.4) individual *D. magna* were mounted inside a test chamber by gently adhering the back of its carapace with petroleum jelly (Vaseline, USA) to a holder inside the chamber. A 1 mL/min flow of test solution, identical to the exposure solution, was maintained throughout the test. Individual *D. magna* were given 10 min to acclimate to test conditions. Afterward, responses of individuals were recorded for 30 s at 60 frames per second (FPS) for measuring the beating rate of the thoracic limbs (reciprocating beats of the first pair), mandible rolling (semicircular reciprocating movement), and frequency of reverse peristaltic contractions (upward moving contractions) of the gut and 2 min at 30 FPS for measuring the rejection frequency. The recording system consisted of a digital camera (FDK 23UP1300, Imaging Source, Germany) with a macro zoom lens (0.3~1 × 1:45; MLM3X-MP, Computar, Japan) and was placed at a distance of 15 cm from the test chamber. The videos were reviewed in slow motion at 30% of normal speed to measure the beating rate of the thoracic limbs and mandible rolling and reviewed at normal rate to measure the peristalsis activity and rejection frequency using VLC media player. Fifty individuals (20 for thoracic limb and mandible activity, 20 for peristaltic activity, and 10 for post-abdominal rejection tests) were exposed to each dilution of OSPW. In some video recordings mandibles were not properly visible such that mandible rolling of those individuals could not be measured.

2.7. Activities of digestive enzymes

Digestive activity was assessed by use of kits for measuring activities of amylase (ab102532, Abcam, USA) and trypsin (ab102531, Abcam, USA). Following a 24 h exposure (as described in section 2.4), approximately 10 mg (10–15 individuals) of *D. magna* (24 h exposed as described in section 2.4) per replicate were rinsed in phosphate buffer solution (PBS), dried, crushed and diluted with reaction buffer. Samples were then centrifuged at 15,000 g for 15 min and the supernatant was collected to be used in each treatment and split between three wells of a 96 well plate analyzed by a microplate reader (Varioskan Flash, Thermo Scientific, Finland).

2.8. Gut contents

Contents of guts of *D. magna* (after 24 h of exposure as described in section 2.4) were studied to investigate presence of SPM in the gut as well as efficiency of digestion of ingested algal cells. To determine presence of SPM in gut, five individuals from each group were rinsed with distilled, de-ionized water (ddH_2O), their carapace and antennae were removed by dissection and the remaining soft parts were pressed onto a scanning electron microscope (SEM) 18 mm carbon mount (TED PELLA, INC., USA). A qualitative feature, energy-dispersive X-ray spectroscopy (EDX), on a tabletop SEM (TM-1000, Hitachi, Japan) was used to detect the presence of aluminum (Al) and silica (Si) as indicators of SPM in guts of *D. magna* (Lari et al., 2016b).

In order to investigate efficiency of the digestive system for processing ingested algae, content of hindguts of each of 10 individuals were combined then diluted in 20 μL culture water, and the number of intact algae cells was counted using a Neubauer chamber. Feces of individuals from all groups were also examined for intact algal cells. Feces were collected from individual *D. magna* by placing them on concave microscope slides (VWR, USA), then examining them under a light microscope (Eclipse 80i, Nikon, Japan). Using a standard glass capillary tube (World Precision Instruments, USA), the first fecal output produced was collected. Because the amount of feces was not measurable and only portions of feces were collected immediately, numbers of algal cells were not quantified. Only presence or absence of intact algal cells was determined.

2.9. Data analyses

Analyses of feeding behaviour and digestive enzymes were done in R 3.2.1 (R-Core-Team, 2015). Assumptions of parametric statistical tests were tested. The Shapiro-Wilk test was used to test for normality of frequency distributions while Bartlett's test was used to assess homogeneity of variances. For each endpoint, differences among main effects (treatments) were determined by use of ANOVA with a Dunnett's *post-hoc* multiple range test, except for inhibition of feeding which was examined by use of Tukey's *post-hoc* analysis.

3. Results

3.1. Chemical characterization of OSPW sample

Concentration of Cd and Cu were more and less than Canadian Council of Ministers of the Environment (CCME) criteria for protection of aquatic life, respectively. No CCME criterion was available for V, Ni, or Zn (Table 1). Characteristics of organic compounds in

Table 1
Trace metal content of the OSPW sample (N = 3).

Metal	Mean concentration ± SD (μg/L)
Ni	17.1 ± 0.3
V	20.2 ± 0.2
Zn	19.0 ± 0.2
Cu	7.3 ± 0.1
Cd	0.11 ± 0.02

OSPW are represented graphically (Fig. 1). When detected by use of positive ion mode, oxygen- and nitrogen-containing compounds predominated, while sulfur-containing chemical species contributed little to total mass spectral response. Multi-oxygen containing chemical species ($O_{5\text{-}6}^{+}$) were the most abundant chemical classes, each representing >20% of total intensity, respectively. Conversely, mono-, di- and tri-oxygenated species contributed minor signals, representing less than 5 percent of total mass spectral response. Nitrogen-containing compounds accounted for approximately 50% of total mass spectral response and were enriched in N^+, ON^+ and O_2N^+ containing chemical classes. When characterized by use of negative ion mode, a similar distribution of classes of heteroatoms was observed as oxygen and nitrogen containing compounds predominated, representing greater than 30 and 67% of total mass spectral response, respectively. However, in contrast to positive ion mode, $O_{2\text{-}4}^{-}$ containing species accounted for greater than 28% of total intensity. Interestingly, the O_2^- chemical class, traditionally classified as naphthenic acids, contributed less than 8% of relative intensity. Comparison of nitrogen containing compounds revealed a similar profile to positive ion mode as the sample was enriched in N^-, ON^- and O_2N^- containing chemical species. Because the greater filter size used in chemical characterization might alter the profile of chemical classes compared to the biological assays the characterization is necessarily operationally defined.

3.2. Inhibition of feeding

Compared to the control group, all three treatments reduced water clearing rate of *D. magna* [$F(3,16) = 106.26$, $p < 0.001$; Fig. 2]. The order of inhibition was: whole OSPW (72%) > SPM (59%) > DC (29%). Inhibition of feeding by SPM was significantly greater than that of DC ($p < 0.001$), which suggests that SPM is more effective at reducing feeding rate than DC.

3.3. Movement of thoracic limb, mandible, peristalsis and frequency of rejection

None of the treatments had significant effects on frequency of post-abdominal rejection [$F(3,28) = 1.10$, $p = 0.36$; Fig. 3] or rate of mandible rolling [$F(3, 47) = 0.48$, $p = 0.70$; Fig. 4] of *D. magna*, relative to that of the control. Treatments changed rate of beating of the thoracic limbs [$F(3,36) = 21.76$, $p < 0.001$; Fig. 4] and peristaltic activity [$F(3, 74) = 7.81$, $p < 0.001$; Fig. 5]. Compared to the control group, exposure to DC did not alter rate of beating of thoracic limbs, while both SPM and whole OSPW reduced it. Peristaltic activity of the gut was reduced in individuals exposed to DC, but not in those exposed to SPM or whole OSPW.

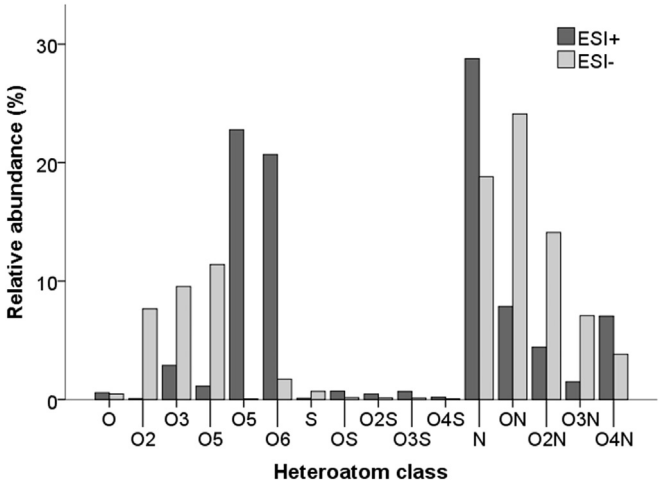

Fig. 1. Distribution of classes of heteroatoms detected mode in the sample of oil sands process-affected water by use of HPLC ultrahigh resolution mass spectrometry (Orbitrap; HPLC-UHRMS) run in positive (ESI+) and negative (ESI-).

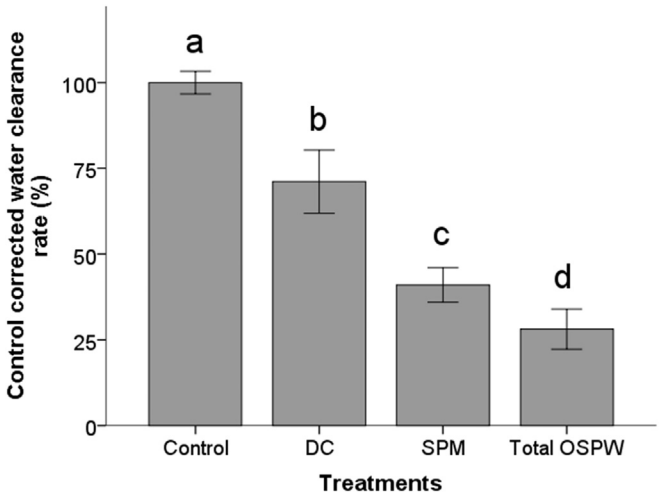

Fig. 2. Effects of treatments on grazing by *Daphna magna*: OSPW: oil sands process-affected water; DC: dissolved component of OSPW; SPM: suspended particulate matter in OSPW. n = 5, Confidence limits: ± 2 SE.

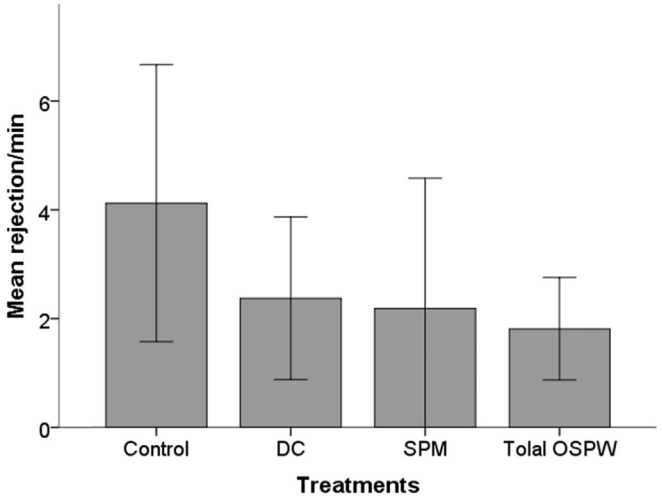

Fig. 3. Effect of treatments on frequencies of post-abdominal rejection by *Daphnia magna*: OSPW: oil sands process-affected water; DC: dissolved component of OSPW; SPM: suspended particulate matter in OSPW. n = 10, Confidence limits: ± 2 SE.

3.4. Digestive enzymes activity

There were no differences in activities of amylase among exposures [$F(3,26) = 2.198$, $p = 0.11$; Fig. 6]. However, trypsin activity was significantly [$F(3, 60) = 7.18$, $p < 0.001$; Fig. 6] less in individuals exposed to whole OSPW exposure as compared to those exposed to either the control or DC.

3.5. Contents of guts

The EDX measurements of *D. magna* exposed to SPM or whole OSPW showed clear peaks for both Al and Si, while neither Al nor Si was detected in *D. magna* exposed to DC or culture water

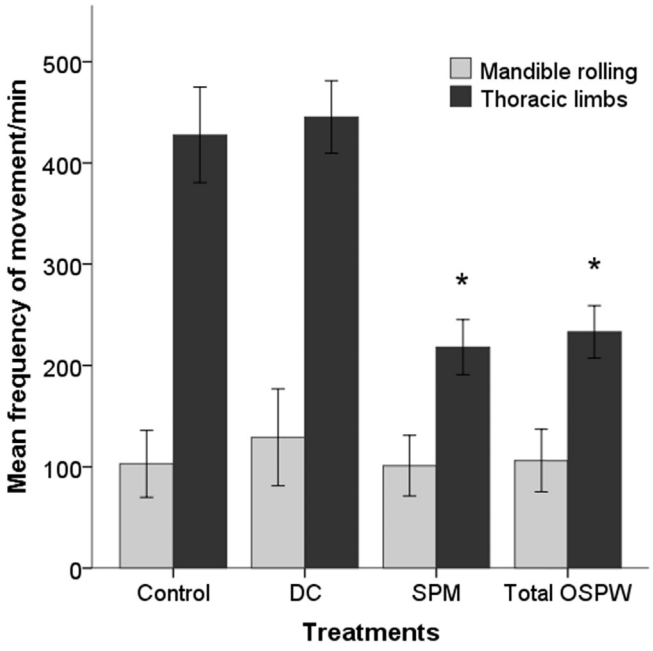

Fig. 4. Effects of treatments on rates of beating of thoracic limbs (n = 20) and mandible rolling (n = 13) of *Daphnia magna*. OSPW: oil sands process-affected water; DC: dissolved component of OSPW; SPM: Suspended particulate matter in OSPW. Asterisks (*) show a significant difference from the control group. Confidence limits: ± 2 SE.

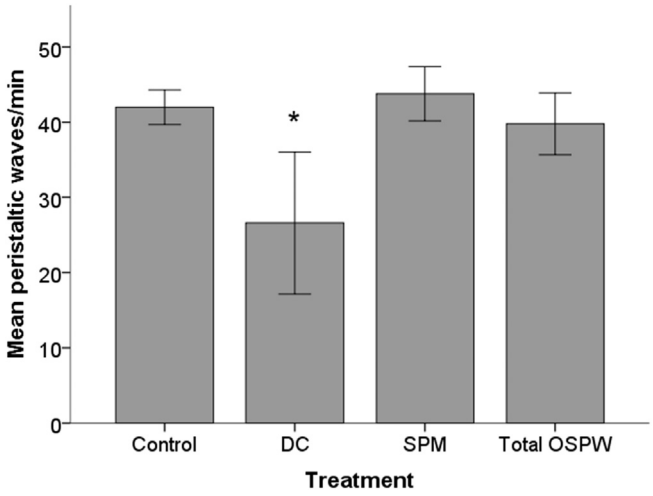

Fig. 5. Effects of treatments on peristaltic activity of *Daphnia magna*: OSPW: oil sands process-affected water; DC: dissolved component; SPM: Suspended particulate matter. Asterisks (*) show a significant difference with the control group. n = 20, Confidence limits: ± 2 SE.

(Supplementary Fig. 1). These results indicate that during these exposures *D. magna* took up SPM from their environment. The color of *D. magna*'s gut exposed to SPM and total OSPW changed from green to brown (Supplementary Fig. 2) over the course of the exposure. Numbers of intact algal cells in hindguts of *D. magna* exposed to all three types of fractions of OSPW were significantly greater than those of individuals in the control group [$F(3, 36) = 14.17$, $p < 0.001$; Fig. 7]. Mean numbers of intact algae cells in *D. magna* exposed to DC, SPM and total OSPW treatments were 11.5-, 13.5- and 23-fold greater than that in those of the control group, respectively, suggesting that all three treatments prevented

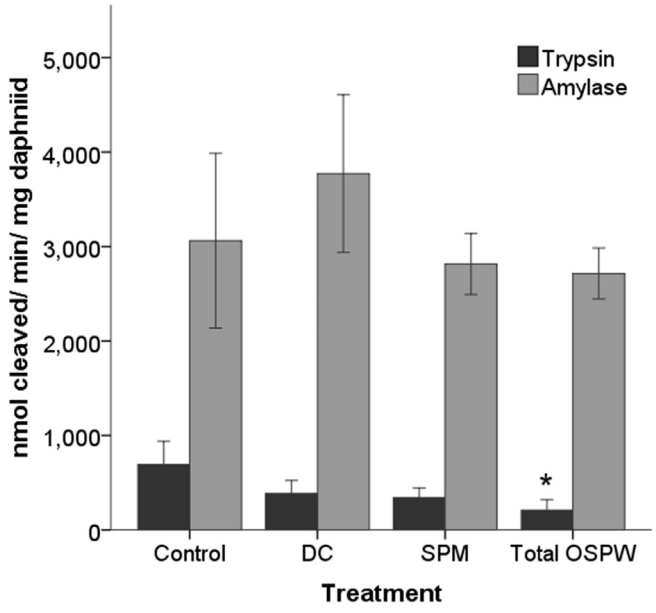

Fig. 6. Effects of treatments on activities of digestive enzymes in guts of *Daphnia magna*: OSPW: oil sands process-affected water; DC: dissolved component of OSPW; SPM: suspended particulate matter in OSPW. Asterisk (*) shows a significant difference with the control group. Trypsin (n = 16), Amylase (n = 8), Confidence limits: ± 2 SE.

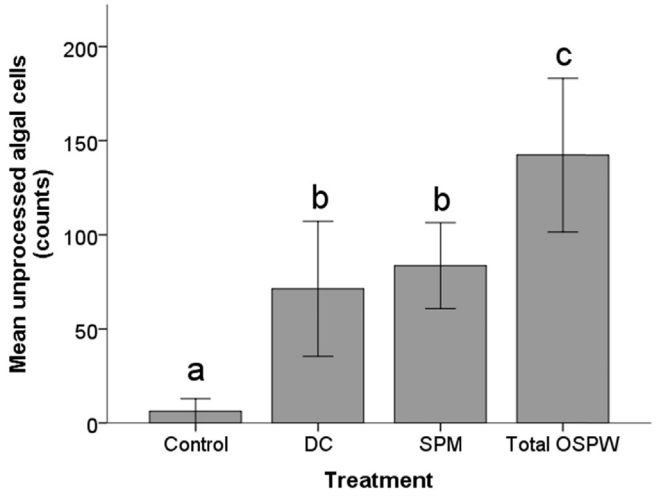

Fig. 7. Effects of treatments on numbers of unprocessed algal cells in hindguts of *Daphnia magna*: OSPW: oil sands process-affected water; DC: dissolved component of OSPW; SPM: Suspended particulate matter in OSPW. n = 10, Confidence limits: ± 2 SE.

D. magna to from digesting a significant number of ingested algal cells. Examining feces of *D. magna*, demonstrated that intact algal cells in feces of all ten specimens exposed to each of the three treatments, while on only one occasion were intact algal cells observed in feces of *D. magna* in the control group. The feces of *D. magna* in treatments was not in the form of fecal pellets and the excreted material, including the algal cells, was suspended in the water.

4. Discussion

OSPW has been shown to reduce rates of feeding of *D.* magna (Lari et al., 2016b). In the same study, authors reported ingestion of SPM from OSPW by *D. magna*. The present study was designed to

expand on those results in order to determine the mechanism by which OSPW modulates consumption of food by *D. magna* and to determine relative potencies of each component of OSPW (i.e. DC and SPM) on feeding of *D. magna*. The DC sample impaired rate of feeding of *D. magna*, while the effect of SPM was significantly greater, which suggests that SPM more effectively reduces feeding of *D. magna* than did DC. Identifying chemical classes contributing to the observed toxicity was difficult due to complexity of the mixture. The DC sample had greater abundances of nitrogen containing chemical species and lesser abundances of NAs. However, it is important to note that the larger filter size used for chemical characterization might have affected the response of various chemical classes as it is known that ion suppression and chemical interactions can affect characterization (Bataineh, 2006). In *Daphnia*, movement of thoracic limbs directs water toward the feeding fans and oral groove, where food particles are trapped and collected (Smirnov, 2013). Particles collected in the food groove are then directed toward the mouth by the mandibles (Smirnov, 2013). Rates of beating of thoracic limbs were reduced in individuals exposed to SPM or whole OSPW, but not in those exposed to DC. In contrast to dissolved constituents of OSPW, contaminants such as dissolved cadmium (45 μg Cd/L) can reduce rates of beating of thoracic limbs and consequently rates of feeding of *D. magna* (Lari et al., 2017). Exposure of *D. magna* to either of the insecticides cypermethrin (0.1 μg/L) or azoxystrobin (0.5 mg/L) caused lesser rates of beating of thoracic (Friberg-Jensen et al., 2010). Reduction of rates of beating of thoracic limbs of *Daphnia* in response to exposure to SPM or whole OSPW, which has been observed in several studies, suggests that increase in the concentration of particulate matter including food (Lari et al., 2017; Peñalva-Arana et al., 2007) and suspended minerals (Kirk, 1991; Lari et al., 2017) reduce the beating rates of thoracic limbs. Thus, reduction of rates of beating of thoracic limbs might not be due to toxicity, but, rather a response to the amount of particulate matter. Thus, this confounds the results and interpretation of effects of whole OSPW on rates of feeding of *D. magna*.

Rates of feeding by *Daphnia* are directly correlated with rates of rolling of the mandible (Murtaugh, 1985). Contaminants such as metals (Lari et al., 2017), pesticides (Gliwicz and Sieniawska, 1986; Friberg-Jensen et al., 2010), crude oil (Wong et al., 1983), and biotoxins (Ghadouani et al., 2004; Rohrlack et al., 2005) can reduce rates of mandible rolling. Exposure to none of the fractions of OSPW used in the present study (i.e. DC, SPM, and total OSPW) altered rates of rolling of mandibles of *D. magna*. Results of the study reported here are in contrast with those reported previously (Kirk, 1991), where a reduction in rate of rolling of the mandible in response to exposure to 50 mg/L particles was observed. However, the results are consistent with results of previous studies (Lari et al., 2017) that suggested that particulate matter in the surrounding environment is less likely to affect rates of rolling of mandibles. Intakes of particles by *Daphnia* spp. and consequently mandible rolling are directly proportional to concentrations of particles, unless a maximum rate of intake is reached (Smirnov, 2013). For instance, adding algal cells to water increased mandible rolling in *D. magna*; on the other hand, increasing the concentration of algae from 0.5 to 5 million cells/L did not change mandible rolling (Lari et al., 2017).

Daphnia spp. select particles based on size (Burns, 1968; Geller and Müller, 1981) and probably electric charge (Gerritsen and Porter, 1982). *D. magna* even ingest silver nanoparticles, which exert their toxicity immediately (Asghari et al., 2012). In response to larger size (Webster and Peters, 1978) or abundance (Porter et al., 1982) of particles, *Daphnia* increase frequency of post-abdominal rejection. Frequency of post-abdominal rejection did not change by experimental treatment, suggesting that particles in treatments were within the size and abundance limits of *D. magna* for ingesting particles. However, presence of particles in the medium increases the frequency of post-abdominal rejection in *Daphnia pulex* (see Kirk, 1991). EDX measurements of contents of guts found Al and Si (main elements of soil) in guts of *D. magna* exposed to SPM or total OSPW, which suggests that *D. magna* ingested SPM. Results of EDX and post-abdominal rejection tests along with the above-mentioned studies that suggest low selectivity of *D. magna*, corroborate the result of the mandible rolling test that showed no reduction in response to particulate matter.

In *Daphnia* spp., peristalsis of the gut controls transportation of food (Smirnov, 2013). A strong reverse peristalsis was first observed in 1925 in *D. magna* that were fed (Rankin, 1925). These reverse peristaltic waves originate as weak waves at the rectal end, and their intensity increases as the waves travel through, and vanish in the middle of the gut (personal observation). Peristaltic activity mixes contents of the gut with digestive enzymes and keeps ingested substances in the gut for a sufficient period to allow for digestion and nutrient uptake to take place. Results of studies of peristalsis showed that the presence of DC inhibits peristaltic activity while SPM and total OSPW did not. The reason that DC and total OSPW act differently toward suppressing peristalsis is not clear. A possible explanation might be binding of toxicants in DC with SPM; however, further investigation is required. Reduction of peristaltic wave frequency reduces the efficiency of the gut in mixing its contents and decreases the clearance time, which is already short (31–35 min) (Esipova, 1971 reviewed in Smirnow, 2013) in *D. magna*.

Several contaminants can suppress activities of digestive enzymes of *Daphnia* (De Coen and Janssen, 1997; Zellmer et al., 2006; Houde et al., 2013). The two enzymes investigated in the present study (i.e. amylase and trypsin) are major digestive enzymes in guts of *Daphnia* (Agrawal et al., 2005; Zellmer et al., 2006; Houde et al., 2013). Activity of trypsin was reduced in *D. magna* exposed to OSPW, however, amylase nor trypsin was changed in response to exposure of *D. magna* to DC or SPM, which suggests that these two components of OSPW were not toxic to digestive enzyme activity in *D. magna* at concentrations in OSPW studied.

Concentrations of intact, undigested, algal cells in hindguts of *D. magna* from treatments were 11.5- to 23-fold greater than that of the control group. Intact algal cells were observed in feces of *D. magna* from all three experimental treatments, but were rare to non-existent in the controls. Since the feces was not pelletized, the excreted intact algal cells were suspended in the water again. Results of the two studies of algae in the hindgut and feces of *D. magna* clearly illustrate that both particulate and dissolved components of OSPW diminish efficiency of the digestive system of *D. magna*. Particulate matter in OSPW does not paralyze the digestive system of *D. magna*. However, SPM becomes the dominant type of particle in the water and consequently dominant type of particle that *D. magna* ingested. It also increased total concentration of particulate matter, including food and mineral particulate matter in water. In this situation, SPM is the main type of particle that *D. magna* ingested which filled the lumen of the gut, reducing the available space for food. Consequently, food algae made up a small portion of the material that *D. magna* ingested, which, in turn, resulted in faster clearance time because of reduced peristaltic activity. When *Daphnia* spp. are exposed to high concentrations of particles, they display luxury feeding behaviour. In this situation they ingest high quantity of particles but less of the food gets digested. Moreover, reduced peristaltic activity and faster clearance time reduces time of contact of undigested food with digestive enzymes, thereby reducing digestive efficiency.

Although both SPM and DC of OSPW reduced consumption of food by *D. magna,* the inhibitory effect of the SPM was significantly

greater than that of DC, suggesting that filtering SPM from OSPW may considerably reduce inhibitory effects of OSPW on feeding of *D. magna* and perhaps other filter-feeding zooplanktons. The results of the mechanistic survey illustrate that neither SPM nor DC impair the food intake but impair the digestion of the ingested food. The mechanisms by which they impair the effective digestion of food in *D. magna* are different but both result in excreting undigested food.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.02.088.

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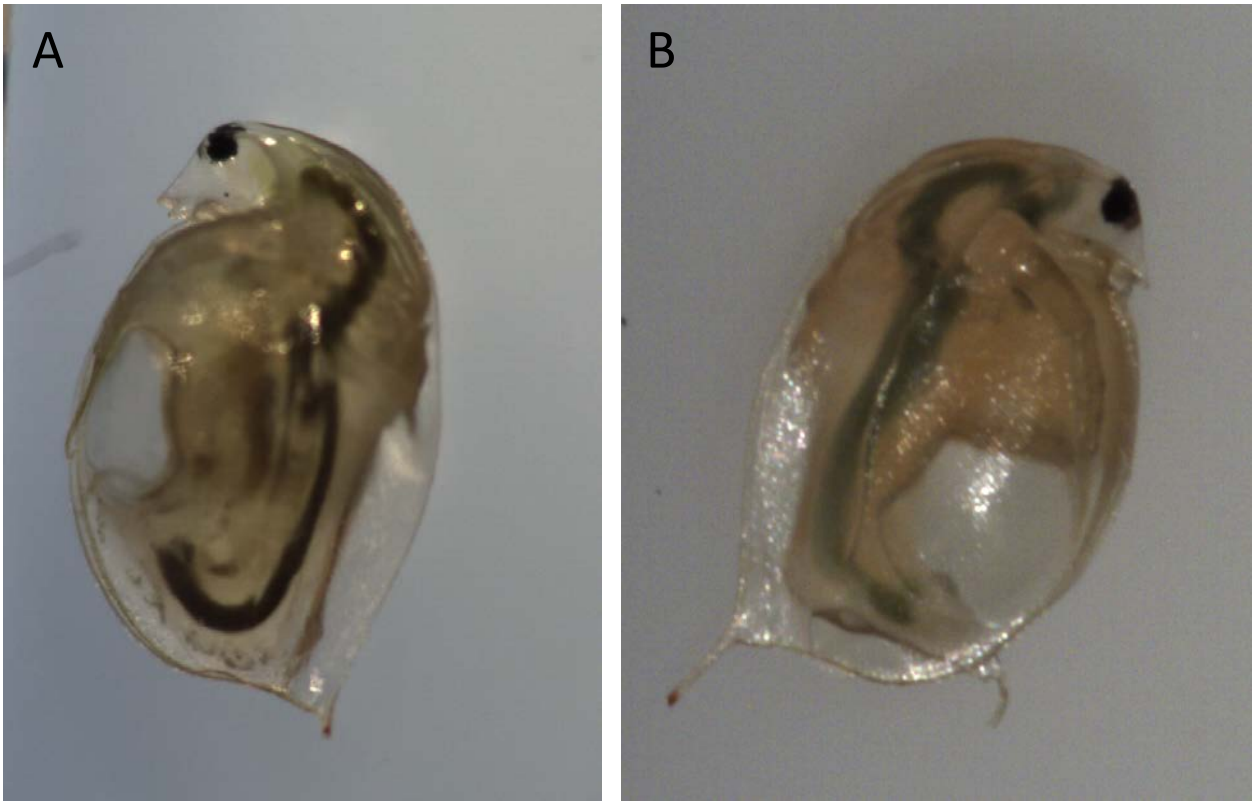

Appendix B: Samples of the gut color of *Daphnia magna* exposed to: 1) total oil sands process-affected water (OSPW), 2) *D. magna* culture water (control).