Aquatic Toxicology 112–113 (2012) 83–91

Contents lists available at SciVerse ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

Ecotoxicological impacts of effluents generated by oil sands bitumen extraction and oil sands lixiviation on *Pseudokirchneriella subcapitata*

T. Debenest*, P. Turcotte, F. Gagné**, C. Gagnon, C. Blaise

Environment Canada, Fluvial Ecosystem Research, 105 McGill Street, 7 floor, Montréal, Québec, H2Y 2E7, Canada

ARTICLE INFO

Article history:
Received 4 October 2011
Received in revised form 23 January 2012
Accepted 26 January 2012

Keywords:
Microalgae
Cell growth
Oil sands process affected water

ABSTRACT

The exploitation of Athabasca oil sands deposits in northern Alberta has known an intense development in recent years. This development has raised concern about the ecotoxicological risk of such industrial activities adjacent to the Athabasca River. Indeed, bitumen extraction generated large amounts of oil sands process-affected water (OSPW) which are discharged in tailing ponds in the Athabasca River watershed. This study sought to evaluate and compare the toxicity of OSPW and oil sands lixiviate water (OSLW) with a baseline (oil sands exposed to water; OSW) on a microalgae, *Pseudokirchneriella subcapitata*, at different concentrations (1.9, 5.5, 12.25, 25 and 37.5%, v/v). Chemical analyses of water-soluble contaminants showed that OSPW and OSLW were enriched in different elements such as vanadium (enrichment factor, EF = 66 and 12, respectively), aluminum (EF = 64 and 15, respectively), iron (EF = 52.5 and 17.1, respectively) and chromium (39 and 10, respectively). The toxicity of OSPW on cells with optimal intracellular esterase activity and chlorophyll autofluorescence (viable cells) (72 h-IC 50% < 1.9%) was 20 times higher than the one of OSW (72 h-IC 50% > 37.5%, v/v). OSLW was 4.4 times less toxic (IC 50% = 8.5%, v/v) than OSPW and 4.5 times more toxic than OSW. The inhibition of viable cell growth was significantly and highly correlated (<−0.7) with the increase of arsenic, beryllium, chromium, copper, lead, molybdenum and vanadium concentrations. The specific photosynthetic responses studied with JIP-test (rapid and polyphasic chlorophyll *a* fluorescence emission) showed a stimulation of the different functional parameters (efficiency of PSII to absorb energy from photons, size of effective PSII antenna and vitality of photosynthetic apparatus for energy conversion) in cultures exposed to OSPW and OSLW. To our knowledge, our study highlights the first evidence of physiological effects of OSPW and OSLW on microalgae.

1. Introduction

The oil sands deposits of Athabasca River area, located in northern Alberta (Canada), are the world's largest oil reserves with 27 billion m^3 of crude oil (Gosselin et al., 2010). These oil sands contain bitumen (8–14 wt%), fine sands, clay and water (3–5 wt%) (Gosselin et al., 2010). After surface mining excavation, bitumen is extracted from ore using Clark caustic hot water process in order to liberate bitumen from sand grains (Schramm and Smith, 1989; Schramm et al., 2000). The mixture obtained, which contains 60 wt% of bitumen, 30 wt% water and 10 wt% of fine solids, requires further cleaning with addition of solvents such as naphtha to produce concentrated bitumen (>99%) (Gosselin et al., 2010). After solvent recovery, large volume of process-affected water containing solids and inorganic ions (boron, chloride, lithium, sodium, strontium and sulfate), naphthenic acids (NAs) and alkylated polycyclic aromatic hydrocarbons (PAHs) were produced and further discharged into tailings ponds (Colavecchia et al., 2004; Van den Heuvel et al., 1999). The concentration of industrial infrastructures (ore-mining, bitumen extraction, and refinery) and liquid waste discharges (tailing ponds) adjacent to the Athabasca River raise concern about the aquatic ecotoxicological risk of oil sands related activities (Dowdeswell et al., 2010). Kelly et al. (2010) showed a release of priority pollutants (such as antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), selenium (Se), silver (Ag), titanium (Ti) and zinc (Zn)) in Athabasca River and watershed by the oil sands industrial activities. Van den Heuvel et al. (1999) suggested that drainage waters could be a source of oil sands related compounds (NAs) for a lake where no industry or human activities were reported. Holowenko et al. (2002) reported high concentrations of NAs (24 mg L^{-1}) in runoff waters from oil sands storage.

On the other hand, bitumen deposits are naturally a source of chemicals and trace metals and metalloids in Athabasca River

* Corresponding author. Tel.: +1 514 496 5024.
** Corresponding author. Tel.: +1 514 496 7105.
E-mail addresses: tdebenest@yahoo.fr, timothee.debenest@ec.gc.ca (T. Debenest), francois.gagne@ec.gc.ca (F. Gagné).

0166-445X/$ – see front matter.
doi:10.1016/j.aquatox.2012.01.021

(Colavecchia et al., 2004; Kelly et al., 2010). The evaluation of oil sands process-affected water (OSPW) and oil sands drainage water toxicities according to natural toxicity baseline is thus a major scientific issue to circumscribe the aquatic toxic risk of oil sands extraction related activities (Dowdeswell et al., 2010). Colavecchia et al. (2004) showed, for Fathead minnows (*Pimephales promelas*) exposed to oil sands and sediments from tailing ponds, a decrease of hatching success, an increase of malformations and larval mortality in comparison with river sediments. The toxicity of OSPW was thus mainly evaluated on fish, this organism being known to be particularly sensitive to NAs (e.g. Colavecchia et al., 2007; Kavanagh et al., 2011; Lister et al., 2008; Nero et al., 2006; Peters et al., 2007). However, metals are also a key group of abundant substances that are released by oil sands related activities (Kelly et al., 2010). These contaminants are indeed more subject to water solubilisation than organic contaminants and thus are more easily released from oil sands. High concentrations of trace metals such as Al, As, Cr, Cu, Fe, Pb, Mo, Ti and Zn, were also detected in different OSPWs (Allen, 2008; Van den Heuvel et al., 2000). OSPWs were also specifically enriched in V (38–104 $\mu g\,L^{-1}$ in tailing pond, Environment Canada unpublished data).

However, little is known about the physiological effects of these effluents on microalgae. Owing to their position at the base of food chain, microalgae are very attractive scientific models to evaluate the toxicity of such waste waters. Toxic effects on these organisms may indeed disturb the productivity of the entire aquatic ecosystem, microalgae being a source of food for many higher organisms. Leung et al. (2003) studied the changes of species composition in phytoplanktonic communities from different tailing ponds but did not explore the physiological responses of microalgae. Moreover, some soluble elements detected in OSPW are known to be either essential elements (V; Meisch and Bielig, 1975; Meisch and Benzschawel, 1978) or strong inhibitors (Al, Cu; Franklin et al., 2001; Saçan et al., 2007) of microalgae growth and photosynthesis. However, microalgae were never used to assess the enrichment of these trace metals in OSPW.

In this context, an experiment was carried out to study the physiological effects of OSPW, oil sands lixiviate water (OSLW) and oil sands exposed to water (OSW) on microalgae The purpose of this research was to evaluate the impact of effluents generated by oil sands related activities (waste discharge: OSPW and lixiviation: OSLW) according to a baseline (OSW) in order to appraise the environmental toxic risk of such activities for the aquatic environment and the relative impacts of releases of their water-soluble contaminants. Different parameters were assessed in microalgae. The esterase activity and the chlorophyll autofluorescence were measured with a flow cytometer and the photosynthesis efficiency with a Plant Efficiency Analyzer based on the time-resolved kinetics of chlorophyll fluorescence.

2. Materials and methods

2.1. Bitumen extraction and effluent preparation

Oil sands samples were collected in 2011 in Fort McMurray (Alberta, Canada) by Environment Canada along Athabasca River shore. Based on different published processes (Schramm et al., 2000; Schramm and Smith, 1989; Sanford and Seyer, 1979), an industrial bitumen extraction from oil sands was reproduced in laboratory. In glass beaker, 550 mL of deionized water was heated up to 75 °C after addition of 0.12 g of sodium hydroxide giving a pH value of 11. Oil sands (250 g) were added and the mixture was bubbled during 10 min. Aerated bitumen floating on the surface of water was continuously collected. At the end of the extraction, OSPW was collected and centrifuged at 2700 × *g* for 20 min. The supernatant was frozen at −20 °C until analysis. In parallel, 550 mL of deionized water was added to 250 g of oil sand in glass bottle. Two reaction vessels were thus set up. One was allowed to stand for 24 h at room temperature to evaluate the baseline leaching of compounds from oil sands (oil sands with water: OSW). The other one was mixing (30 rpm) during 24 h at room temperature to simulate oil sands lixiviation (oil sands lixiviate water: OSLW). Similarly to OSPW, effluents were centrifuged at 2700 × *g* for 20 min and the supernatants were frozen at −20 °C.

2.2. Effluent characterization

The different effluents were then filtered (GF/C, 0.7 μm, Waterman, London, UK) and dried in vacuum chamber, due to the presence of bitumen droplets, to assess the total suspended solids (TSS) content. The organic matter (OM) content was measured after ignition of dried filters at 550 °C during 20 min.

Subsample of oil sand lixiviation and extract were acid-digested according to protocol, method 3030 E of standard methods (Clesceri et al., 2005). Hydrogen peroxide 1:20 (v/v) was added to the digestion solutions to prevent interferences of organic carbon at high concentrations. Solutions were analyzed for metals by ICP-MS (Xserie II, ThermoFisher, Waltham, MA, USA). Reproducibility was better than 5% for all the measured metals.

2.3. Cell culture and effluent exposures

Pseudokirchneriella subcapitata (UTEX 1648 strain) was obtained from the University of Texas and cultured in autoclaved AAP (algal assay procedure) medium. The flasks were placed in an incubator (Innova 44 R Orbital Shaker, New Brunswick Scientifics, USA) under continuous illumination (40–50 $\mu mol\,m^{-2}\,s^{-1}$ of photosynthetically active radiation) provided by white fluorescent lamps (Sylvania® Gro Lux F15W, Germany) at 25 ± 1 °C and with constant rotary agitation (100 rpm). Exponentially growing cells were diluted with fresh medium to achieve samples with a cell density around 20–25 cells μL^{-1}. Microalgae were then exposed to effluents to reach final cell density of 10–12.5 cells μL^{-1} and effluent tested concentrations (1.9, 5, 12.25, 25 and 37.5%, v/v). Culture volume was 25 mL. The pH was adjusted to 7.5 according to the Athabasca River pH (Gueguen et al., 2011) and the biological test method for a freshwater alga published by Environment Canada (2007). Microalgae were exposed to effluents for 72 h.

2.4. Flow cytometric analyses

Cells were incubated with fluorescein diacetate (FDA; CAS N. 596.09.8; 13 $mg\,L^{-1}$ final concentration) during 15 min in darkness to assess the intracellular esterase activity and the chlorophyll autofluorescence with a three-color Guava EasyCyte Plus System cytometer (Guava Technologies Inc. Hayward, USA) using a laser emitting at 488 nm and a micro-plate reader as described by Debenest et al. (2010). The effects of effluents on microalgae growth were thus investigated with two physiological parameters (esterase activity and chlorophyll autofluorescence) to compare the physiological responses cell by cell and to provide more accurate toxicity appraisal Debenest et al. (2010). Data obtained were displayed on bi-dimensional red *vs* green cytograms to discriminate algal cells from the other particles present in effluent (fine elements) (Fig. 1). Markers were set up on control populations and applied afterwards to the treated populations in order to discriminate cells with optimal esterase activity and chlorophyll autofluorescence (M1), cells with optimal chlorophyll autofluorescence but reduced esterase activity (M2) from cells with optimal chlorophyll autofluorescence but no esterase activity (M3) (Fig. 1)