

The Appropriateness of Using Aquatic Snails as Bioindicators of Toxicity for Oil Sands Process-Affected Water

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Abstract: Canada's oil sands mining activity produces large volumes of oil sands process-affected water (OSPW), and there have been increasing concerns regarding the potential environmental impacts associated with this material. Developing an understanding of the toxicity of OSPW is critical to anticipating and mitigating the potential risks and effects of the oil sands industry on surrounding ecosystems. The composition of OSPW is highly variable and is influenced by a range of factors. While numerous research projects have been conducted on the toxicity of OSPW, much remains unknown about its impact on various biota. Freshwater gastropods (snails and slugs) are an ecologically crucial aquatic group, and members of this taxa have been used as bioindicators in a range of ecological settings. The literature suggests freshwater snails could be used as an indicator of toxicity in monitoring programs associated with oil sands development. This mini-review explores the use of snails as bioindicators in aquatic systems affected by oil sands development, focusing on how snails may respond to potential constituents of concern in systems exposed to OSPW.

Keywords: snail (aquatic/freshwater); bioindicator; toxicity effects; oil sands; process water; OSPW



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1. Introduction

Oil sands process-affected water (OSPW) is a term used to denote those waters whose composition has been altered by bitumen extraction or material transport processes [1]. OSPW is produced in considerable quantities by the oil sands industry [2] and represents a complex mixture of chemical compounds and elements. Several substances in OSPW, including naphthenic acids (NAs), polycyclic aromatic hydrocarbons (PAHs), metals, and ions [3], may contribute to acute and chronic toxicity in aquatic environments. Although no releases of any OSPW into the aquatic environment have been approved to date, seepage from tailing ponds is still an ongoing concern [4]. Considering the increasing inventories of OSPW from the oil sands industry, sustainable water management practices may require returning treated water to the environment, even in the short-term [5]. Therefore, it is important to determine the potential environmental impact of OSPW on aquatic systems in the oil sands region to inform better risk assessment and management practices related to oil sands extraction and processing [6]. While the specific toxicological effects of OSPW on aquatic organisms are not well understood [7], it is possible that a bioindicator approach may be used to address environmental concerns associated with oil sands development. Bioindicator species can effectively reflect the nature and/or magnitude of environmental changes [8]. Bioindicators can be used to monitor the overall adverse effects of OSPW without needing to know the combined impacts of individual components and how they interact (e.g., synergetic or antagonistic), though it can be challenging to select and identify appropriate bioindicators based on their sensitivity to individual constituents of concern [9].

Freshwater gastropods (Mollusca: Gastropoda; snails and slugs) are broadly distributed in various aquatic environments and represent significant biomass in freshwater ecosystems, such as small streams and springs [10]. Snails are crucial to many food webs, both as decomposers and as consumers [11]. Freshwater snails are regularly utilized as ecotoxicological models for laboratory and in situ studies [12] since they can be easily accommodated within relatively little space and are easy to manipulate/breed [13]. In addition, freshwater snails have a relatively short life cycle, which minimizes the time required to complete testing procedures. For example, the great or common pond snail (*Lymnaea stagnalis*), has a lifespan of only 2 to 2.5 years. The objective of this mini-review is to explore the potential utility of snails as bioindicators of OSPW toxicity.

2. Bioindicator

Ecotoxicological bioindicators can warn of potential impacts to the survival of individual species and act as part of an early warning system [14]. The risk associated with OSPW, because it is a complex mixture, cannot be adequately evaluated using those toxicity tests traditionally employed for risk assessment [15]. The compositional complexity of OSPW, combined with the high cost associated with traditional toxicity assays, suggests the utilization of bioindicators as a means of assessing ecological status. Bioindicators can also be combined with other assessments of toxicity (e.g., rainbow trout toxicity assays) to estimate the risks of OSPW to freshwater ecosystems. For example, potential bioindicators could be incorporated into ecotoxicological analysis for water quality assessment.

A bioindicator is any living organism or community that reflects an important characteristic of the surrounding environment [16]. Bioindicators are an essential tool for detecting the environmental impact of industrial processes and materials [17]. A highquality bioindicator should satisfy specific criteria, including a well-characterized biology, the ability to generate an early warning of impact to ecosystem health, wide distribution in the relevant environment, embodiment of an essential function in the ecosystem, and the presence of a well-established link between the toxicological endpoint and the level of a pollutant [18]. By these criteria, some aquatic snails have the potential to be considered ideal bioindicators. For example, the biology of a few snail species, such as Biomphalaria glabrata [19], are well-studied. Some species are known to respond to environmental contaminants; for example, esterase activity of *Lymnaea natalensis* is considered an early warning signal of pollutants exposure [20]. The relationship between the degree of metal exposure and a measurable effect has been largely established for some freshwater snails, such as Biomphalaria alexandrina [21] and Pomacea canaliculate [22]. Mollusks have been recognized as ideal bioindicators for decades [23], and snails, which are widespread in freshwater ecosystems, can represent up to 60% of the total biomass of macroinvertebrates in some freshwater ecosystems [24].

Several ecotoxicological studies have been reported which used freshwater snail species as bioindicators [25]. For instance, *Lymnaea stagnalis* has been widely adopted in ecotoxicological studies and pollution biomonitoring programs [26], especially for investigating the toxicological effects of aquatic contaminants [27]. Some relevant studies using freshwater snails in ecotoxicological assessments of analytes relevant to OSPW are provided in Table 1. Interestingly, more investigations focus on potentially toxic metals than organic compounds, perhaps due to the technical challenges associated with the quantification and characterization of organic materials.

The sensitivity of aquatic invertebrates to OSPW has been reported to be between that of fish and macrophytes [28]. A recent species sensitivity distribution comparison—expressed as critical target lipid body burdens (CTLBBs)—was developed for organisms exposed to OSPW-related organics [29]. The results showed that the relative species sensitivity of *Lymnaea stagnalis* (log CTLBB = 1.83 μ mol/g lipid) is close to the mean of 14 tested aquatic species (log CTLBBs = 1.85 μ mol/g lipid), including invertebrates, algae, and bacteria [30]. A biomimetic extraction method by solid-phase microextraction (BE–SPME) has been developed to predict toxicity during exposure to complex organic mixtures [31]. Pairing BE-SPME analysis results with toxicity response/endpoints of snails could validate this screening tool for estimating the toxicity of OSPW in the future.

3. Effect of Naphthenic Acids

NAs refer to an extensive and diverse group of polar organic compounds present in OSPW [32]. Reviews of the composition and toxicity of OSPW have confirmed NAs as the primary source of toxicity (both acute and chronic) to a variety of fish and invertebrates [33]; it is possible that this toxicity can be alleviated through the process of biodegradation [34].

Research on the toxicity of commercial NAs to the pond snail, Physa heterostropha, can be traced back to the 1960s [35], and investigations of the adverse impact of NAs on the physiological and behavioral functions of snails (e.g., Lymnaea stagnalis [36]) are still ongoing. Recently, the embryonic development of Lymnaea stagnalis has emerged as a tool in the study of toxicity associated with NAs derived from OSPW [37]. Although reported EC50 (concentration associated with 50% effect, 31 mg/L) for NAs (solution pH 7.2–7.6) in Lymnaea stagnalis is higher than in fathead minnow (Pimephales promelas 1.7-12.4 mg/L for hatching success, solution pH 8.2-8.4 [38]), this snail species was suggested as a bioindicator to monitor the acute toxicity of surfactants in aquatic ecosystems [39]. This point is especially pertinent to the effects of OSPW, given that the mechanism of toxicity for NAs may be associated with their surfactant properties [40]. The age of OSPW, as well as the character of the oil sands materials with which it has been in contact are important determinants of its NA molecular structure [41]. The analytical difficulties associated with NAs [42], as well as the qualitative differences between commercially available and OSPW-extracted NA mixtures [43], are still challenges for the quantification and comparison of toxicity data between species.

4. Accumulation of Polycyclic Aromatic Hydrocarbons (PAHs)

PAHs are a concern in the oil sands area of Alberta [44]. Mortality was not detected in the freshwater snail, *Physella virgata*, subsequent to acute exposure to a fluoranthene (a PAH) within the limit of its water solubility [45]. However, subsequent investigations of PAH toxicity in the *Lymnaeidae* family suggested that these freshwater snails may be useful bioindicators for this class of compounds [46]. Recently, the freshwater snail *Lanistes carinatus* was reported as a bioindicator for crude oil pollution based on its capacity to bioaccumulate PAHs [47]. The biota sediment accumulation factor (BSAF) values observed in field-collected freshwater brown snails in Nigeria ranged between 0.22 and 11.72 among 12 individual PAH compounds [48].

5. Effect of Other Organic Compounds

Other organic compounds found in the oil sands environment include BTEX (benzene, toluene, ethylbenzene, xylenes) and their alcohol derivatives such as phenol [49]. To the authors' best knowledge, fewer studies have been conducted on organic compounds than on metals and other trace elements. The effects of benzene, xylene, and styrene on the mortality of *Amphimelania holandri* and *Lymnaea stagnalis* have been explored, but it was found that freshwater snails were less sensitive to these compounds than certain crustacean species are [50]. Ethylbenzene was believed to be the source of potential genotoxicity in the freshwater snail *Bellamya aeruginosa* [51], but further evaluation is required to confirm this supposition.

6. Bioaccumulation of Metals

Metals exist as free cations or as inorganic/organic complexes in water and do not degrade like organic compounds. Many metals have the potential for bioaccumulation and biomagnification in aquatic organisms [52] and studies of the ability of snails to bioaccumulate metals have been reported for years [53]. For example, it was reported in the 1970s that *Physa gyrina* can rapidly accumulate cadmium (Cd) [54]. Over the past two decades, toxicological investigations involving freshwater snails have focused on sensitivity to metals [27]. Pulmonated (i.e., air breathing) freshwater snails are sensitive to both acute and chronic metals exposures [55], which makes these taxa particularly relevant as bioindicators informing future water quality criteria [56] and metals risk assessments [57].

Several species of freshwater snails, including *Viviparus acerosus* [58], *Lymnaea stagnalis* [59], and *Physa acuta* [60], have great potential to serve as bioindicators of metal pollution. For example, *Physa acuta* was shown to be a suitable bioindicator of metal toxicity under laboratory conditions [61]. Subsequent research has confirmed this species to be useful for metal bio-uptake research [62]. In addition, *Lymnaea stagnalis* was reported to be one of the most sensitive species to a 96 h copper exposure [53], and a good bioindicator of mercury contamination in aquatic ecosystems [63]. A recent study revealed that the BSAF in *Melanoides tuberculata* was more than two for six metals, resulting in this species being classified as a macro-concentrator for Mn, Fe, Cu, Zn, Cd, and Pb [64].

7. Bioindication of Salt and Nutrients

OSPW is generally dominated by sodium, chloride, bicarbonate, ammonium, and sometimes sulfate ions [49]. *Leptoxis ampla* was found to be sensitive to chloride with a LC50 (lethal concentration associated with 50% mortality) value of 3414 μ g/L, which was the lowest LC50 value among four species reported, including two gastropod and two bivalve species [65]. *Lymnaea stagnalis*, at early developmental stages, was reported to be a very sensitive bioindicator of ammonia pollution [66]. An in situ evaluation in Canada has reported that tissues of lymnaeid snails collected from a natural pond exhibited lower trace element concentrations than those collected from microcosms where petroleum coke was used as sediment. However, the authors speculated that this might be due to the differences between the natural wetland and microcosms [67].

8. Discussion

From the literature review, *Lymnaea stagnalis* appears to be widely used to assess several toxicants associated with OSPW. The *Lymnaea stagnalis* Reproduction Test was adopted by the OECD (Organization for Economic Cooperation and Development) under its Guidelines for the Testing of Chemicals in 2016 [68]. However, mollusk toxicity evaluation is not emphasized in the biological test methods and guidance documents published by Environment Canada. Certain species of freshwater snails, such as *Lymnaea stagnalis* and *Physa acuta*, could be considered as standard toxicology model organisms in Canada. In addition, incorporating case studies using native snail species in field monitoring programs could address some concerns of Indigenous communities and other stakeholders about the potential long-term effects of oil sands activities. Overall, the literature supports the potential for aquatic snails to act as bioindicators of toxicity associated with OSPW exposure. Field-scale investigations with OSPW from different operators, in addition to laboratory testing focusing on OSPW-derived compounds (especially NAs), may be useful in further evaluating the utility of these organisms as bioindicators.

Table 1. Selected studies using freshwater snails in ecotoxicological assessments of analytes relevant to oil sands processaffected water (OSPW).

Species (Family)	Age	Chemical Compounds	Effect	Measured Response Type	Exposure Duration (Days)	Toxicological Values (µg/L)	Measured Level in OSPW (µg/L) ¹
Potamopyrgus antipodarum (Hydrobiidae)	NR ³	Cadmium	Mortality	LC50	2	1000–4000 [69]	1.6
Somatogyrus sp. (Hydrobiidae)	Adult	Potassium	Ventilation/ movement	EC50	4	7285 [70]	12,000

Species (Family)	Age	Chemical Compounds	Effect	Measured Response Type	Exposure Duration (Days)	Toxicological Values (μg/L)	Measured Level in OSPW (µg/L) ¹		
	Embryo	Naphthenic acids	Embryonic development	EC50	28	31,000 [37]	53,000		
	Embryo	Ammonia	Mortality	LC50	1	24,270 [66]	6400		
	Juvenile	Silver	Growth	EC10	14	1.48 [56]	0.17		
	Juvenile	Cadmium	Growth	EC10	14	12.0 [56]	1.6		
	Juvenile	Copper	Growth	EC10	14	3.71 [56]	2.5		
	Juvenile	Copper	Mortality	LC20	4	18 [53]	2.5		
	Juvenile	Nickel	Growth	EC10	14	115 [56]	120		
	Juvenile	Lead	Growth	EC10	14	4.00 [56]	1.2		
	Juvenile	Zinc	Growth	EC10	14	223 [56]	20		
	NR ³	Mercury	Mortality	LC50	4	203.92 [63]	0.047		
<i>Radix auricularia</i> (Lymnaeidae)	Embryo	Cadmium	Embryonic development	LC50	4	58.26 [71]	1.6		
<i>Aplexa hypnorum</i> (Physidae)	Adult	Acenaphthene	Mortality	LC50	4	>2040 [72]	<0.11		
Physa gyrina	Adult	Naphthalene	Mortality	LC50	2	5020 [73]	< 0.075		
(Physidae)	Immature	Cadmium	Mortality	TL50 ²	4	430 [54]	1.6		
Biomphalaria glabrata (Planorbidae)	Embryo	Chromium	Embryonic development	EC50	1	5760 [25]	4.4		
<i>Leptoxis ampla</i> (Pleuroceridae)	Juvenile	Chloride	Mortality	LC50	4	3414 [65]	139,000		

Table 1. Cont.

¹ Median values were derived from Table 6-1 of reference [49]; ² TL50 = 50% tolerance limits; ³ NR = not reported.

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