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Selenium speciation and localization in chironomids from lakes receiving treated metal mine effluent

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HIGHLIGHTS

- Chironomid larvae are important vectors for selenium bioaccumulation.
- Chironomids exposed to waterborne selenium species were compared to field chironomids.
- Higher selenium levels led to a selenomethionine-like major form in tissues.
- Selenium primarily localized in the head capsule, brain, salivary glands and gut lining.
- Suggests selenomethionine is most readily accumulated, whether from food or water.

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ABSTRACT

A lake system in northern Saskatchewan receiving treated metal mine and mill effluent contains elevated levels of selenium (Se). An important step in the trophic transfer of Se is the bioaccumulation of Se by benthic invertebrates, especially primary consumers serving as a food source for higher trophic level organisms. Chironomids, ubiquitous components of many northern aquatic ecosystems, were sampled at lakes downstream of the milling operation and were found to contain Se concentrations ranging from 7 to 80 mg kg^{-1} dry weight. For comparison, laboratory-reared *Chironomus dilutus* were exposed to waterborne selenate, selenite, or seleno-DL-methionine under laboratory conditions at the average total Se concentrations found in lakes near the operation. Similarities in Se localization and speciation in laboratory and field chironomids were observed using synchrotron-based X-ray fluorescence (XRF) imaging and X-ray absorption spectroscopy (XAS). Selenium localized primarily in the head capsule, brain, salivary glands and gut lining, with organic Se species modeled as selenocystine and selenomethionine being the most abundant. Similarities between field chironomids and *C. dilutus* exposed in the laboratory to waterborne selenomethionine suggest that selenomethionine-like species are most readily accumulated, whether from diet or water.

1. Introduction

Selenium (Se), an important micronutrient required by most organisms, is toxic above a rather narrow beneficial range. Selenium has a tendency to biomagnify through the food chain, such that even relatively low levels in surface waters can lead to significantly higher levels in top predators (Hymer and Caruso, 2006). High Se exposure to oviparous animals has been shown to cause teratogenic effects (Lemly, 1993; Muscatello et al., 2006).

The present study involved a small lake system in northern Saskatchewan that has been receiving treated metal mine effluent for over 25 years (Wiramanaden et al., 2010a, 2010b). Mean surface water Se levels of 10.3 and 4.0 μg L^{-1} in lakes downstream of the effluent source (Wiramanaden et al., 2010a) exceeded the Canadian water quality guideline for protection of aquatic life of 1 μg L^{-1} (CCREM, 1987). Previous research has shown increased deformities in laboratory-raised juvenile northern pike from mothers collected downstream of the effluent source (Muscatello et al., 2006). Additional research by Muscatello and Janz (2009) studied Se concentrations within the food web of the same study area, finding that Se interactions are complex and not yet fully understood.

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An important step in the trophic transfer of Se is its bioaccumulation by benthic invertebrates, especially primary consumers serving as a food source for higher trophic level organisms. In the present study, chironomid larvae sampled from the sediment of the lake system were compared with *Chironomus dilutus* (Diptera: Chironomidae) reared in the laboratory under exposure to waterborne Se species at concentrations of 11 and 4 μg Se L^{-1}, based on the mean surface water concentrations at the field sites. Building on previous work (Wiramanaden et al., 2010a,b; Franz et al., 2011) the present study combines synchrotron X-ray fluorescence (XRF) imaging and X-ray absorption spectroscopy (XAS) to investigate the localization and speciation of Se in chironomids, providing insights into how Se is bioaccumulated through a northern aquatic food web.

2. Materials and methods

2.1. Field chironomid collection

Four sites were chosen for further investigation from the lake system previously described by Wiramanaden et al. (2010a,b). Sites F2 and F3 represent the inflow and outflow of Fox Lake, closer to the effluent source; sites U2 and U3 are in Unknown Lake, downstream of Fox Lake. Yeoung Lake, located in a separate lake system with a similar food web, was used as a reference lake (Phibbs et al., 2011). Chironomids of various species and ages were sampled on June 11–16, 2009, using a standard Ekman grab sampler. Sediment grabs were immediately sieved through a 500 μm sieve bucket and remaining material was placed in a cooler with site water. Within 24 h, chironomids, without a gut purge, were removed using PTFE coated tweezers (T5665 Sigma Aldrich), rinsed in 50 μm sieved site water, and sorted for XRF, XAS, and collision cell inductively coupled plasma mass spectrometry (ICP-MS) analysis. The best specimens for XRF imaging, chosen based on larger size (1–2 cm) and characteristic "bloodworm" redness, were preserved at 4 °C in 70% EtOH/filtered site water. Remaining chironomids were pooled into 2 mL Eppendorf tubes, frozen in liquid nitrogen to preserve speciation, and stored at −20 °C for later XAS and ICP-MS analysis. Since a late thaw in 2009 delayed chironomid life cycles, insufficient chironomids for ICP-MS were collected during June and additional chironomids were collected in July. Yeoung Lake yielded insufficient chironomids for ICP-MS analysis.

2.2. Laboratory chironomids

Chironomus dilutus was chosen as the test organism because it was readily available from an in house culture maintained at the Toxicology Centre (University of Saskatchewan). It is representative of local midge species that serve as a food source for predators and thus represents a vector of Se bioaccumulation through the food chain. Its extensive characterization and relatively short life cycle also make it ideal for contaminant exposure studies (e.g. Benoit et al., 1997). *Chironomus dilutus* cultures were raised in one of two water exposures spiked with sodium selenate (Na_2SeO_4), sodium selenite (Na_2SeO_3), or seleno-DL-methionine ($C_5H_{11}NO_2Se$) as described below. Selenate and selenite are the most abundant bioavailable Se forms in surface water (Besser et al., 1993); selenomethionine is readily found in both living and decaying organisms. One water exposure was a 10-d uptake in dechlorinated water (DCW), followed by a 10-d depuration in DCW with no added Se. The other exposure was a 10-d uptake in BW (Barnstead NANOpure Diamond water, 18.2 MΩ cm) used to eliminate DCW as a trace Se source (0.5 μg Se L^{-1}; City of Saskatoon Water Treatment Plant, 2009).

Chironomus dilutus larvae were reared similarly to the methods of Franz et al. (2011) using glass beakers (300 mL Pyrex) containing 96 ± 10 g of washed silica sand (Granusil silica fillers) and 200 mL of Se spiked water with constant aeration. Consistent with field-collected chironomids, *C. dilutus* did not undergo a gut purge prior to their analysis. Exposure concentrations of 11 and 4 μg Se L^{-1} were based on June 2009 average surface water concentrations in Fox and Unknown Lake of 10.3 ± 0.4 and 3.9 ± 0.3 μg Se L^{-1}, respectively, collected and measured using ICP-MS as described by Wiramanaden et al. (2010a). Stock 110 mg Se L^{-1} solutions of Na_2SeO_4 (Sigma–Aldrich, Missouri, USA), Na_2SeO_3 (Alfa Aesar, Massachusetts, USA), and $C_5H_{11}NO_2Se$ (Sigma–Aldrich, Missouri, USA) in BW were prepared on day 0. Every 3 d for a water change, the stock solution was diluted 1:10 followed by 1:1000 or 1:2750 for representative Fox or Unknown Lake Se water concentrations, respectively. Chironomid food was prepared by blending 10 g of fish flakes (Bio Flakes, Sera, Heinsburg, Germany) in 100 mL BW on day 0. Aliquots of the mixture were frozen, then diluted 1:10 every day to feed 1 mL to each beaker containing 10 chironomids. Each treatment was performed in triplicate to yield sufficient chironomids for ICP-MS, XRF and XAS analysis. At 10 or 20 d as applicable, 1–2 chironomids were removed and preserved for XRF imaging as described below. Remaining chironomids for XAS and ICP-MS analysis were frozen and stored at −80 °C.

Dissolved oxygen was measured daily (Orion 3 Star DO Portable, DO Probe 081010MD, Thermo Scientific). Other water quality measurements were made on days 0, 3, 6 and 10 for the uptake phase and on days 13, 16 and 20 for the depuration phase. The 10-d uptake in BW and the 10-d uptake followed by a 10-d depuration in DCW showed the following respective means ± SD: dissolved oxygen (7.5 ± 0.4, 8.3 ± 0.2 mg mL^{-1}), conductivity (30 ± 12, 432 ± 4 μS cm^{-1}), pH (7.5 ± 0.1, 8.2 ± 0.0), alkalinity (13 ± 4, 111 ± 3 mg L^{-1} as $CaCO_3$), and hardness (78 ± 4, 137 ± 1 mg L^{-1} as $CaCO_3$). Ammonia was below the detection limit (<0.02 mg L^{-1}) for both water treatments (Ammonia Nitrogen Test Kit, Low Range Method, La Motte, USA).

2.3. Inductively coupled plasma mass spectrometry (ICP-MS)

Chironomids were dried for several days in a drying oven at 60 °C until masses were constant, then were cold digested using nitric acid (5 mL Omnitrace Ultra, EM Science) and hydrogen peroxide (1.5 mL Super Pure; EMD Chemicals). Samples were measured using ICP-MS (X Series II Thermo Electron Corporation, Waltham, MA, USA) and analyzed for ^{80}Se using collision cell technology (Wiramanaden et al., 2010a). Triplicate analysis of the standard reference material Tort-2 (Lobster hepatopancrease, NRC) was within the certified value of 5.63 ± 0.67 mg kg^{-1} (Wiramanaden et al., 2010a).

2.4. X-ray absorption spectroscopy (XAS)

Frozen chironomids for XAS analysis were crushed to a homogenized powder using an agate mortar and pestle cooled in liquid nitrogen to minimize tissue Se speciation changes. Homogenization ensured that XAS was representative of the average speciation and allowed more tissue to be packed, resulting in improved signal-to-noise. Frozen, homogenized chironomids were packed tightly into 2 mm path length cuvettes, sealed with a drop of glycerol and stored in liquid nitrogen until data acquisition.

XAS was collected on beamlines 7-3 and 9-3 at the Stanford Synchrotron Radiation Lightsource (SSRL) using a Si(220) double crystal monochromator. A 15 keV cutoff was achieved by adjusting the angle of the upstream Rh-coated vertically collimating mirror; 9-3 additionally has a downstream Rh-coated focusing mirror. Upstream slits defined a 1.0 × 8.0 mm^2 (height × width) beam.