Science of the Total Environment 562 (2016) 714–723

Contents lists available at ScienceDirect

Science of the Total Environment

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

Metals and metalloids in nestling tree swallows and their dietary items near oilsands mine operations in Northern Alberta

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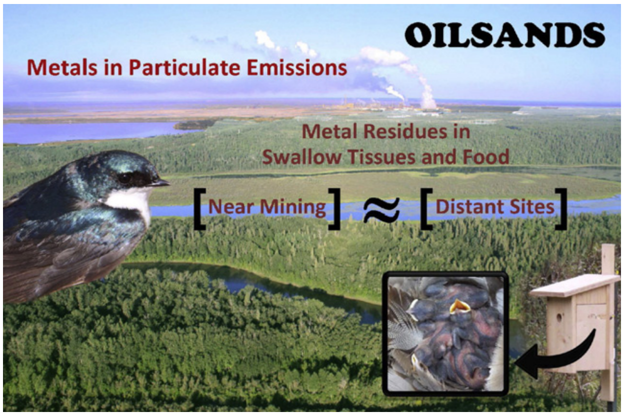
HIGHLIGHTS

- Oilsands mine operations may expose birds to metals and metalloid elements.
- Tree swallows ate up to 50% terrestrial insects, likely limiting exposure to metals.
- Annual variation in tissue element burden highlights need for multi-year data sets.
- Element variability in food is important when interpreting tissue concentrations.
- No evidence that nestling tree swallows accumulated metals approaching toxic levels

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 November 2015
Received in revised form 9 April 2016
Accepted 10 April 2016
Available online xxxx

Editor: D. Barcelo

Keywords:
Oilsands
Tree swallows
Metals
Tissue residues
Diet
Food web

ABSTRACT

Tree swallows (*Tachycineta bicolor*) nesting near oilsands development in northern Alberta are potentially exposed to elevated levels of metals. The objective of this study was to determine whether levels of metals and metalloid elements in dietary items and tissues of nestling tree swallows inhabiting areas near oilsands mine operations were higher compared to those of reference sites. We hypothesized that if there was increased, industry-related exposure to metals, it would be via the diet. We identified the invertebrate prey in the stomach contents of nestlings. We also collected invertebrates using Malaise traps near nest boxes, and analyzed those taxa found in the nestling diet to understand potential variability in metal exposure. For most elements, we found no significant differences in concentrations in the liver, kidney, or stomach contents between sites near to and far from oilsands operations. Concentrations of five elements were positively correlated among tissues and stomach contents. For invertebrates collected from Malaise traps, location differences occurred in some absolute elemental concentrations, which were most often highest at reference sites away from mining operations. We found no evidence that nestling tree swallows accumulated metals approaching toxic levels. Tree swallows consumed relatively high quantities of terrestrial insects, possibly limiting exposure to water borne, food-web-related contaminants. We suggest that annual variability associated with elemental exposure and dietary levels of elements be considered when interpreting concentrations in bird tissues.

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http://dx.doi.org/10.1016/j.scitotenv.2016.04.069

1. Introduction

The release of heavy metals into the environment is increasingly a concern in the Athabasca oilsands region north of Fort McMurray, Alberta, Canada (Timoney and Lee, 2009; Kelly et al., 2010; Schindler, 2010). Surface mining of the oilsands has been occurring since 1967, and bitumen production from mining is expected to increase from 0.9 million barrels per day (bpd) in 2010 to about 1.6 million bpd by 2020 (ERCB, 2011). A complex mixture of contaminants is derived from bitumen extraction and subsequent upgrading. Nickel and vanadium are found in the raw oilsands material (Research Council of Alberta, 1953; Har, 1981), and are present, along with other metals, in the by-products from bitumen production (Jervis et al., 1982; Holloway et al., 2005; Puttaswamy and Liber, 2012). Nickel, cadmium, lead, zinc, and other metals measured in particulates in snow were higher near oilsands mining operations (Kelly et al., 2010). Higher concentrations of metals have also been measured in lichen (*Hypogymnia physodes*) within 50 km of oilsands operations compared to further distances (Edgerton et al., 2012). Bitumen is converted to synthetic crude oil at upgrading facilities, and atmospheric deposition attributed to emissions from these upgraders is an important pathway of metal input to the environment (Bari et al., 2014), potentially contaminating surface waters (Barton and Wallace, 1979; Kelly et al., 2010; Puttaswamy and Liber, 2012).

Metal contamination in soil and surface water affects the species composition of plant, insect, bird, and small mammal communities (Heliovaara and Vaisanen, 1991; Eeva and Lehikoinen, 1996; Ruohomaki et al., 1996; Kiikkilä, 2003; Koptsik et al., 2003; Mukhacheva et al., 2010). However, there are few peer-reviewed studies that have directly examined the health effects of metals on wildlife in the oilsands region (see Rodríguez-Estival and Smits, 2016). Birds are often used to monitor the environment for metal contamination from industrial activities (Swiergosz et al., 1998; Eens et al., 1999; Bel'skii et al., 2005; Eeva et al., 2009; Berglund et al., 2010), and insectivorous birds in particular are useful for studying effects from metal exposure because the accumulation of metals can occur through the diet (Hunter and Johnson, 1982; Gochfeld and Burger, 1987; Eens et al., 1999).

Tree swallows (*Tachycineta bicolor*) have been used as an indicator of environmental exposure to contaminants in the oilsands region (Smits and Fernie, 2013; Cruz-Martinez et al., 2015), and contaminants have been shown to accumulate in nestlings (Custer, 2011). Tree swallows are an ideal model organism because they readily use nest boxes, allowing for appropriate sample sizes and standardization of methods (Jones, 2003). Tree swallows are semi-colonial, and birds will nest in close proximity to each other, allowing relatively high densities of birds within a small area (McCarty, 2001/2002). Broods of birds nesting in a common area are assumed to be exposed to the same contaminants, reducing the variability in dietary metal concentrations and making it possible to study the potential effects from metal exposure.

The objective of our study was to determine if the levels of metals and other metalloid elements in nestling tree swallows inhabiting areas near oilsands mine operations were higher compared to reference sites. We used nestling tree swallows because adults spend only a short period in the study area and may be exposed to metals during migration or on wintering grounds, and accumulation in adults may be affected by age and health. We hypothesized that if there was increased exposure to metals, it would be via the diet because contaminant exposure through foraging has been most frequently documented (McCarty, 2001/2002), compared to exposure from inhalation or drinking water. We measured the element burden in bird tissues and in the food items provided to the nestlings by adults, as determined from stomach contents. We predicted that higher levels of metals would occur in the tissues of nestlings near oilsands mining operations compared to birds in reference sites (distant from mining operations) that would reflect the natural environmental conditions. If the nestlings were being exposed to metal contaminants through food, then we expected that metal levels in tissues would be correlated to the metal levels in the diet. Adult females excrete metals into eggshells (Burger, 1994; Dauwe et al., 1999), with some transfer of metals to the eggs. However, a minimal portion of the body load is excreted into the eggs (Dauwe et al., 2005), and is diluted through the rapid gain of mass during post-hatching development of the nestlings from <2 g as eggs which includes shell mass (Whittingham et al., 2007), to 22 g (unpublished data) as fledglings.

Tree swallows forage primarily on invertebrates with aquatic larval stages (Mengelkoch et al., 2004). They consume brachyceran, nematoceran and cyclorrhaphan Diptera, as well as Trichoptera, Ephemeroptera, Odonata, and Hemiptera (McCarty and Winkler, 1999; Smits et al., 2000). Tree swallows generally forage within a few hundred metres of their nest through the breeding season (Quinney and Ankney, 1985; McCarty and Winkler, 1999; McCarty, 2002; Mengelkoch et al., 2004), and therefore, any measured metal accumulations in nestlings can be attributed to local exposure from foods brought to them by adults (Echols et al., 2004). We collected invertebrate samples near nest locations to measure metal concentrations in dietary items to understand the potential metal exposure from the tree swallow diet.

2. Methods

2.1. Study sites

Our study was conducted in 2012 and 2013 with populations of tree swallows using nest boxes near Fort McMurray (Zone 12 V, 0476688 Easting, 6287042 Northing, Datum NAD83), Alberta, Canada. We sampled nestlings at six sites; Sites 1, 2, and 3 were within 5 km of active oilsands mining operations and were exposed to aerial emissions and potentially to contaminants in water (Fig. 1). The reference sites (Sites 4, 5, and 6) were located 60 to 65 km south of the active mining area. The prevailing winds in the region are generally west to east (Vickers et al., 2001). Therefore, we considered reference sites to be unexposed to contaminants from upgrader emissions and mining activities. Each site had 20 to 30 nest boxes, and was near an open water body, wetland or pond. Near operations, Site 1 was beside a pond built in 1993 to support tailings research, and contained fine tailings that have settled to the bottom with a cap of natural surface water. The area around the pond has naturally revegetated. Site 2 was a reclaimed area and on the edge of a wetland that formed following reclamation in 2003. Site 3 was also reclaimed from an overburden dump in 1983, and now supports a mature upland forest and wetland habitat. Reference Site 4 was on the edge of an old borrow pit that provided gravel fill for road construction, and has naturally revegetated and filled with water. Site 5 was adjacent to a beaver pond and a natural drainage system. Site 6 was on a grass-sedge wetland on the edge of Maqua Lake.

2.2. Tissue sampling

We monitored tree swallow nest-boxes regularly from the initiation of nest building beginning about 20 May in both 2012 and 2013. We inspected nest boxes daily or every second day during egg laying to determine the date of clutch completion. Nest boxes were left undisturbed during incubation until hatching, and then checked daily until all nestlings had hatched. We considered day zero to be when half or more of the eggs had hatched. At the age of 14 days, one to three nestlings were randomly collected from each nest box for tissue sampling. The laying order was not known, and bias from laying order was avoided by selecting nestlings at random. Three nestlings were collected from larger brood sizes (six or more nestlings) to improve sample sizes. Nestlings were anesthetized with isoflurane, and then euthanized by cervical dislocation. Once nestlings were euthanized, the liver and kidney were removed and immediately frozen in liquid nitrogen. The stomach was removed and kept on ice until being transferred to a freezer at the end of each day. The protocols used in this study were approved by the Animal Care Committee at the University of Calgary (LESACC protocol number BI11R-27), in compliance with standards set by the Canadian

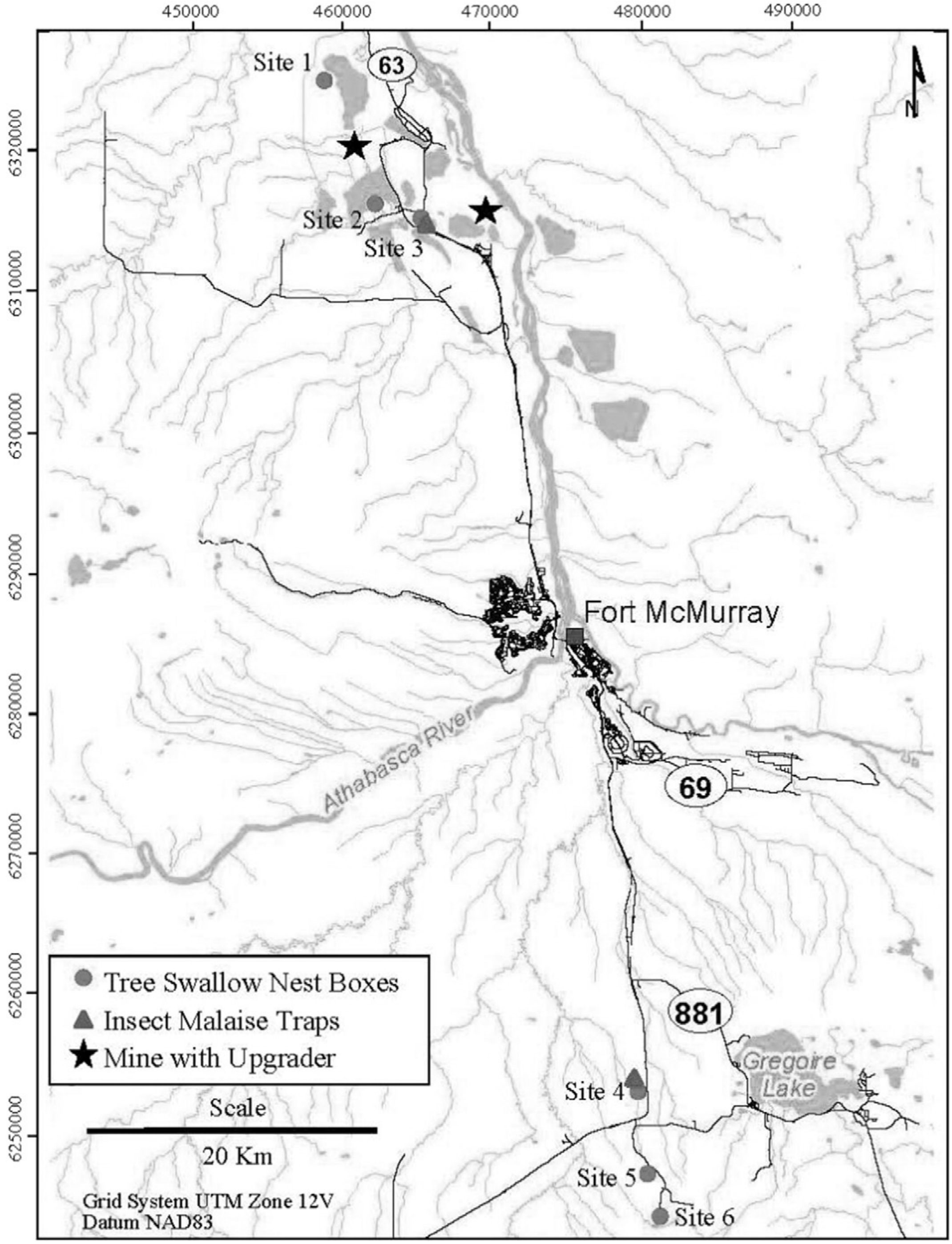

Fig. 1. Study sites where tree swallow and invertebrate samples were obtained near oilsands mining operations north of Fort McMurray, Alberta, Canada and reference sites to the south, during 2012 and 2013.

Council on Animal Care, and tissues from the nestlings were shared among three research teams to maximize data collected from each bird. We were issued a Canadian Wildlife Service Permit (11-AB-SC015) and Alberta Wildlife Research Permit (#51072) and Collection Licence (#49386) for this study.

Liver and kidney samples were shipped on dry-ice to the Prairie Diagnostic Services (PDS) accredited laboratory at the Western College of Veterinary Medicine in Saskatoon, Saskatchewan, for metal analysis. We removed stomach contents to identify food items to the lowest taxonomic level possible. We placed the stomach contents in a sterile petri dish and items were viewed and sorted individually under a compound microscope. We visually estimated the percent volume of each taxon by determining their proportional contribution to the total volume of the stomach contents. Trace amounts were given a value of 1% as an indication of presence. Similar methods are used to estimate the proportion of food items in the diet of birds (Todd et al., 1998; Wheelwright, 1986) as well as bats (Kunz and Whitaker, 1982). Following identification, the contents of each stomach sample were oven dried at 40 °C for 48 to 72 h. The dried samples were then shipped to the PDS laboratory for metal analysis.

2.3. Invertebrate sampling

We used a Malaise trap to collect flying insects at Site 3 near mine operations and at reference Site 4. The traps were installed near the nest boxes and in close proximity to open water and tall shrubs that provided cover for insects. Insects were collected over three weeks, with one sample bottle per week, providing three samples per location, beginning 15 June when nestlings started hatching, until fledging. We sorted the insects from each sample bottle and those we had identified in the nestling diet were analyzed for metals. Insect samples were oven-dried at 40 °C for 48 to 72 h, and shipped to the PDS laboratory for analysis.

2.4. Laboratory analyses

Elemental analysis of liver, kidney, stomach contents, and invertebrate samples involved digestion with HNO_3 in a pressurized Microwave-Accelerated Reaction System (MARS) following the manufacturer's instructions (CEM Corporation). The 14 metal and metalloid elements measured were vanadium (V), chromium (Cr), nickel (Ni), arsenic (As), strontium (Sr), molybdenum (Mo), cadmium (Cd), thallium (Tl), lead (Pb), iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), and selenium (Se). Elements were quantified using an Inductively Coupled Plasma-Mass-Spectrometer (ICP-MS, Thermo Jarrell-Ash Corporation, Franklin, MA, USA). Liver and kidney samples were wet digested, and results are therefore presented on a wet-weight basis. Invertebrates in the stomach contents and Malaise trap samples were oven dried and results are expressed per dry-weight. Stock standards used by PDS were purchased from SCP Science or VWR International, and the standard reference material (powder-dried liver) was purchased from the National Institute of Standards and Technology. A new calibration curve for each element was generated from the stock standards prior to analyzing each sample batch. The limit of detection (LOD) for each element was: V (10 ppt), Cr (10 ppt), Ni (17 ppt), As (80 ppt), Sr (3 ppt), Mo (10 ppt), Cd (6 ppt), Tl (0.70 ppt), Pb (2.6 ppt), Fe (0.9 ppt), Co (1.35 ppt), Cu (36 ppt), Zn (0.36 ppb), and Se (172 ppt) (PDS Laboratory personal communication). The reported LOD concentrations for V, Ni, As, Tl, and Se were two orders of magnitude lower than the concentrations measured in tissues and insect samples in our study, and were three or more orders of magnitude lower for the other elements. Percent recovery of six of the elements (Mo, Fe, Co, Cu, Zn, Se) in the standard reference material ranged from 84 to 113%, and were similar to those from in-house controls. Standard reference materials were not available for the other elements, and in-house controls and calibration curves were applied.

2.5. Data analyses

We conducted statistical analyses using SAS software 9.3 (SAS, 2008. SAS/STAT® 9.3 User's Guide. SAS Institute Inc.). We log-transformed the element concentrations in tissues, stomach contents, and insect samples to achieve normal distributions. Element concentrations were analyzed with generalized linear models with the Identity link function (GENMOD procedure in SAS). We used the GENMOD procedure to accommodate differences in variance in element concentrations among locations. For tissues and stomach contents, each model contained year, location, and the interaction between year and location as fixed effects. Given that three sites were used to define each location (near operations and reference), variability among sites was accounted for by including 'site within location' as a nested effect (site (location)) in each model. Brood was included as a repeated measure to account for correlation among nestlings within a nest box. A Spearman Rank correlation was used to test correlations in metal levels among the liver, kidney, and stomach contents. The level of statistical significance was $p < 0.05$.

3. Results

We sampled a total of 98 nestling tree swallows from 38 broods. In 2012 and 2013, respectively, we sampled 16 and 31 nestlings at reference sites, and 23 and 28 nestlings near oilsands operations. Of the 28 nestlings that were collected near operations in 2013, six were collected following several days of heavy rain and cool weather. The stomach contents in these six nestlings contained minimal food, indicating that they had not been fed recently. Two stomachs were completely empty and were not included in the analysis, while the other four contained various amounts of grass (likely nest material), and undigested food items of adequate amounts to include in the analyses.

We identified insects from nine orders in the stomachs of nestling tree swallows (Table 1). Coleoptera and diptera consistently contributed large proportions to the diet in both years and both areas (near mining and reference). Ephemeroptera were common, especially in birds from reference sites, while odonates were relatively common in birds from sites near mining. The most abundant taxa in the diet were not the same in the two years.

For 12 of the 14 elements, there were no significant location differences in concentrations in liver tissue between reference sites and near operations (Table 2). Co was higher at reference sites ($\chi^2 = 3.90$, $p = 0.048$). In 2012, Pb was higher at reference sites ($\chi^2 = 8.31$, $p = 0.004$), but no difference was found in 2013. Year differences occurred in liver for eight elements; V, Ni, As, Cd, and Co were higher in 2013 than 2012 (V, $\chi^2 = 19.95$, $p < 0.001$; Ni, $\chi^2 = 10.77$, $p = 0.001$; As, $\chi^2 = 10.92$, $p = 0.001$; Cd, $\chi^2 = 11.06$, $p = 0.001$; Co, $\chi^2 = 6.86$, $p = 0.009$) at both reference sites and near operations, while Sr, Cu, and Zn were higher in 2012 (Sr, $\chi^2 = 6.50$, $p = 0.011$; Cu, $\chi^2 = 9.04$, $p = 0.003$; Zn, $\chi^2 = 15.54$, $p < 0.001$).

In kidney, there were no significant location differences for 12 of the 14 elements (Table 3). Cd was higher in kidney at reference sites ($\chi^2 = 5.37$, $p = 0.021$). In 2012, Se was higher at reference sites than near operations ($\chi^2 = 6.00$, $p = 0.014$). Year differences were found for V, Cd, and Zn, and levels were significantly higher in 2013 compared to 2012 (V, $\chi^2 = 21.74$, $p < 0.001$; Cd, $\chi^2 = 16.22$, $p < 0.001$; Zn, $\chi^2 = 10.64$, $p = 0.001$) at reference sites and near operations, while As was higher in 2012 ($\chi^2 = 8.92$, $p = 0.003$). Across locations, liver and kidney were positively correlated for V, As, Tl, and Fe in 2013, and for Cd, Co, and Se in both years (Table 4).

Significant location differences were found for metal concentrations in the stomach contents for 6 of the 14 elements (Table 5). Stomach contents from reference sites were higher in As, Cd, Co, Cu, and Se compared to near operations (As, $\chi^2 = 6.98$, $p = 0.007$; Cd, $\chi^2 = 6.59$, $p = 0.010$; Co, $\chi^2 = 5.51$, $p = 0.019$; Cu, $\chi^2 = 5.27$, $p = 0.022$; Se, $\chi^2 = 6.16$, $p = 0.013$). Ni was higher in stomach contents near operations ($\chi^2 = 5.12$, $p = 0.024$). Year differences were found for V, Cr, Ni, As, and Tl in the stomach contents, and were higher in 2013 compared to 2012 at reference sites and near operations (V, $\chi^2 = 16.54$, $p < 0.001$; Cr, $\chi^2 = 7.80$, $p = 0.005$; Ni, $\chi^2 = 12.16$, $p = 0.001$; As, $\chi^2 = 7.25$, $p = 0.007$; Tl, $\chi^2 = 7.54$, $p = 0.006$). Metal concentrations in the stomach contents were positively correlated with levels in the liver for As and Cd in 2012, for Mo, Fe, and Co in 2013, and for Tl and Se in both years (Table 4). Element concentrations in the stomach contents and kidney were positively correlated for Cd in 2012, for Cr, Tl, Pb, and Fe in 2013, and for Co and Se in both years. Mo and Cu were negatively correlated between stomach contents and kidney in 2013. The levels of Ni, Sr, and Zn in the stomach contents were not correlated with levels in kidney or liver. V was correlated only between liver and kidney.

We sorted invertebrates collected from Malaise traps, and analyzed nine taxonomic groups that we found in the nestling diet: Coleoptera (beetles), Odonata (Zygoptera (damselflies)), five from the Order Diptera (Muscomorpha (flies), Syrphidae (hoverflies), Tipulidae (crane flies), Tabanidae (horse flies), and Culicidae (mosquitoes)), Lepidoptera (moths), and Hymenoptera (Ichneumonidae (parasitic wasps)). Ephemeroptera were not captured in the Malaise traps and comparisons could not be made with samples from near operations. Although we did not have sufficient replicates to conduct statistical analyses, absolute element concentrations varied and were most often (83 of 126 cases) highest at reference sites (Table 6). This pattern was seen

Table 1
Mean percent by volume (minimum and maximum percent) of invertebrates consumed by 14-day old tree swallow nestlings near Fort McMurray, Alberta, Canada in 2012 and 2013 as determined by analysis of stomach contents.

Taxa	Reference (%)		Near operations (%)		Larval habitat
	2012 ($n = 16$)	2013 ($n = 31$)	2012 ($n = 23$)	2013 ($n = 28$)	
Coleoptera	28 (5–75)	10 (0–30)	37 (0–75)	14 (5–65)	Terrestrial
Ephemeroptera	25 (0–90)	46 (0–100)	0	10 (0–85)	Aquatic
Diptera	27 (0–85)	25 (0–95)	9 (0–25)	31 (0–85)	Terrestrial/aquatic
Tipulidae	0	<1 (0–15)	0	4 (0–40)	Aquatic
Odonata	<1 (0–10)	2 (0–50)	30 (0–80)	9 (0–80)	Aquatic
Hymenoptera	3 (0–25)	4 (0–35)	6 (0–30)	6 (0–40)	Terrestrial
Lepidoptera	<1 (0–3)	1 (0–10)	5 (0–90)	1 (0–10)	Terrestrial
Trichoptera	1 (0–10)	3 (0–65)	0	2 (0–30)	Aquatic
Hemiptera	<1 (0–5)	1 (0–10)	<1 (0–5)	1 (0–10)	Terrestrial
Neuroptera	0	0	0	<1 (0–5)	Terrestrial
Other	2 (0–20)	<1 (0–5)	1 (0–15)	<1 (0–1)	
Unknown	13 (0–35)	8 (0–55)	13 (0–60)	16 (0–50)	

consistently in Cd. Elements expected to be higher near operations, such as V and Ni, showed no consistent pattern. Although Ephemeroptera were not captured, four stomach samples from reference sites contained >90% Ephemeroptera. These four samples had higher than average levels of V, Fe, Cu, and Se, and lower than average levels of Sr and Zn. All remaining elements were similar to the average element concentrations measured in other taxa.

4. Discussion

We predicted that metal and metalloid levels would be greater in nestling tree swallows near oilsands mining operations. However, we did not find significant location differences in concentrations for most elements in liver, kidney, or stomach contents, and when location differences occurred for As, Cd, Pd, Co, Cu, and Se, concentrations were higher at reference sites, away from mining operations. We discuss the variation in metal and metalloid concentrations, and assess whether levels were high enough to be of concern in terms of toxicity to tree swallows.

The concentrations of elements in tissues differed between years, being higher in 2013 than in 2012. We do not know the reasons for the year differences, although 2013 was much wetter than 2012, and spring rains resulted in regional flooding and nest-box abandonment across the study area. Increased surface runoff may have affected metal uptake in nestling food and tissues. The linkages and potential transfer of toxicants between aquatic and terrestrial ecosystems have been well documented (e.g. Schulz et al., 2015), and high rainfall and overland runoff can increase concentrations of some metals in sediments (Fonseca et al., 2013). Another possibility is that aerial emissions from oilsands operations differed between years, although this is not readily determined (NPRI, 2014).

We hypothesized that if nestling tree-swallows were exposed to metals, it would be via their diet. Concentrations of some elements were positively correlated among liver, kidney, and stomach contents, supporting our hypothesis. Three of these (Se, Tl, Co) were the same elements related to lower body condition and smaller testes size in deer mice (*Peromyscus maniculatus*) from a reclaimed oilsands site (Rodríguez-Estival and Smits, 2016). Contrary to our hypothesis, we found no correlations in the levels of Ni, Sr, and Zn among tissues and stomach contents, and negative correlations for Mo and Cu. At low concentrations, many elements are readily metabolized and excreted (Lui et al., 2008), and thus do not accumulate in tissues, potentially resulting

Table 2
Metal concentrations (wet-weight) (mean ± SD, range) in liver of 14-day old tree swallow nestlings ($n = 90$) at reference and near-operations sites in 2012 and 2013.

Element	Reference 2012 ($n = 15$)	Near operations 2012 ($n = 23$)	Reference 2013 ($n = 25$)	Near operations 2013 ($n = 27$)
V (ppb)	20 ± 10 (8–43)	24 ± 22 (7–117)	213 ± 73 (115–384)	236 ± 98 (100–469)
Cr (ppb)	220 ± 42 (155–288)	322 ± 79 (188–478)	280 ± 77 (186–564)	332 ± 116 (219–827)
Ni (ppb)	13 ± 5 (6–24)	19 ± 31 (5–158)	40 ± 30 (8–115)	36 ± 30 (9–126)
As (ppb)	23 ± 13 (2–51)	16 ± 11 (2–38)	49 ± 11 (31–69)	30 ± 12 (9–54)
Sr (ppb)	124 ± 67 (48–260)	132 ± 60 (58–313)	80 ± 40 (47–217)	97 ± 38 (49–185)
Mo (ppm)	0.7 ± 0.2 (0.5–1.1)	0.8 ± 0.1 (0.5–1.1)	0.7 ± 0.2 (0.2–1.0)	0.8 ± 0.1 (0.6–1.0)
Cd (ppb)	21 ± 8 (2–36)	8 ± 5 (4–24)	43 ± 23 (9–106)	20 ± 17 (3–63)
Tl (ppb)	1 ± 0.4 (0.7–2.2)	2.1 ± 1.0 (0.8–5.4)	3.2 ± 2.6 (0.3–11.5)	2.9 ± 1.9 (0.1–6.8)
Pb (ppb)	45 ± 68 (8–270)	7 ± 8 (2–34)	9 ± 15 (2–76)	7 ± 8 (2–45)
Fe (ppm)	397 ± 120 (183–531)	526 ± 219 (179–1033)	391 ± 174 (106–682)	479 ± 196 (140–776)
Co (ppb)	11 ± 3 (4–17)	8 ± 2 (5–13)	16 ± 5 (6–25)	14 ± 8 (8–46)
Cu (ppm)	10 ± 5 (5–18)	9 ± 3 (5–19)	8 ± 4 (2–24)	6 ± 2 (4–12)
Zn (ppm)	36 ± 6 (25–45)	39 ± 9 (25–62)	25 ± 4 (20–40)	26 ± 4 (21–34)
Se (ppm)	1.9 ± 0.7 (0.3–3.3)	0.6 ± 0.2 (0.5–1.6)	1.3 ± 0.3 (0.8–1.9)	1.0 ± 0.4 (0.4–1.7)

Table 3
Metal concentrations (wet-weight) (mean ± SD, range) in kidney of 14-day old tree swallow nestlings ($n = 89$) at reference and near-operations sites 2012 and 2013.

Element	Reference 2012 ($n = 16$)	Near operations 2012 ($n = 20$)	Reference 2013 ($n = 25$)	Near operations 2013 ($n = 28$)
V (ppb)	20 ± 6 (9–31)	27 ± 12 (13–57)	292 ± 120 (173–749)	330 ± 137 (99–582)
Cr (ppb)	289 ± 48 (232–381)	274 ± 62 (199–395)	294 ± 56 (195–441)	307 ± 60 (246–510)
Ni (ppb)	55 ± 42 (14–178)	61 ± 34 (29–153)	58 ± 30 (16–168)	56 ± 48 (9–162)
As (ppb)	77 ± 13 (46–95)	31 ± 11 (13–62)	53 ± 18 (9–84)	35 ± 16 (10–75)
Sr (ppb)	228 ± 227 (109–1024)	478 ± 636 (106–2449)	534 ± 653 (73–2500)	403 ± 373 (92–1468)
Mo (ppm)	0.6 ± 0.1 (0.5–0.7)	0.6 ± 0.1 (0.4–0.8)	0.5 ± 0.1 (0.3–0.9)	0.6 ± 0.1 (0.4–0.8)
Cd (ppb)	23 ± 6 (15–31)	7 ± 5 (3–23)	54 ± 18 (24–86)	30 ± 26 (2–94)
Tl (ppb)	9 ± 2 (6–11)	10 ± 4 (7–22)	16 ± 10 (6–41)	16 ± 7 (6–34)
Pb (ppb)	17 ± 20 (3–85)	15 ± 15 (3–63)	14 ± 14 (3–55)	16 ± 25 (2–121)
Fe (ppm)	76 ± 15 (52–102)	88 ± 26 (45–144)	84 ± 66 (42–388)	86 ± 31 (52–196)
Co (ppb)	17 ± 3 (11–24)	12 ± 3 (8–19)	17 ± 8 (7–47)	14 ± 5 (9–32)
Cu (ppm)	4 ± 0.3 (3–5)	4 ± 0.6 (3–5)	4 ± 1 (2–9)	4 ± 0.5 (3–5)
Zn (ppm)	16 ± 2 (13–21)	17 ± 3 (13–26)	21 ± 5 (13–33)	18 ± 2 (15–27)
Se (ppm)	2.5 ± 0.5 (1.6–3.4)	0.7 ± 0.2 (0.5–1.4)	1.6 ± 0.5 (0.8–2.3)	1.2 ± 0.4 (0.5–1.9)

in the lack of correlation observed for some elements. We did not investigate metal exposure via other pathways such as absorption or inhalation, as contaminant exposure through the diet is thought to be most important (McCarty, 2001/2002). If alternative pathways of exposure were influential, we would expect even less correlation between element levels in the diet and tissues.

The diet of nestling tree swallows varied with location, similar to results found for tree swallows elsewhere (Beck et al., 2013). Although the invertebrates consumed by tree swallows in our study were similar to those in other studies, the proportions differed. In particular, Coleoptera was consumed in higher quantities than previously reported (Kraus, 1989; Johnson and Lombardo, 2000; Custer et al., 2005; Beck et al., 2013). It is possible that the differences in diet between years may have contributed to the different levels of metals in the two years. However, in both years, elemental concentrations in the stomach contents were generally consistent with, or lower than, reported normal levels in the diet of tree swallows in Minnesota (Custer et al., 2006).

We found no evidence that invertebrates sampled from near operations had consistently greater concentrations of metals compared to those from reference sites. V and Ni are considered the best indicators of exposure to the products of oilsands operations, as they tend to be found in association with bitumen and bitumen production (Zhao et al., 2000, 2002). Aeshnidae (dragonfly) nymphs exposed to oilsands petroleum coke treatments and waste-water, accumulated over two times the V and Ni we found in invertebrates (Baker et al., 2012), with the exception of Culicidae and Ichneumonoidea, which had higher concentrations in reference samples. Chironomid larvae exposed to oilsands petroleum coke had high concentrations of V (913 ppm) (Baker et al., 2012), far exceeding the levels we measured. In Ontario, reference concentrations of Ni in gypsy moth larvae (*Lymantria dispar*) were up to twice as high (0.4–7.2 ppm) as levels we measured (Bagatto and Shorthouse, 1996). Taken together, these results indicate that invertebrates in our study were exposed to levels of elements that were naturally present and variable, rather than to increased levels due to oilsands operations.

Table 4
Spearman Rank correlation coefficients (rs) for relationships of element concentrations between liver, kidney and stomach contents of 14 day-old tree swallow nestlings in 2012 and 2013 near Fort McMurray, Alberta, Canada. Bold italicized font indicates a statistically-significant correlation ($p < 0.05$).

	2012			2013		
	Liver-kidney ($n = 35$)	Liver-stomach ($n = 38$)	Kidney-stomach ($n = 35$)	Liver-kidney ($n = 52$)	Liver-stomach ($n = 52$)	Kidney-stomach ($n = 53$)
V	0.25	−0.10	0.11	***0.44***	0.15	0.08
Cr	−0.13	−0.01	−0.09	0.16	0.08	***0.31***
Ni	0.06	−0.14	0.13	0.20	0.08	0.03
As	0.35	***0.41***	0.14	***0.57***	0.26	0.21
Sr	0.25	−0.18	−0.06	0.27	−0.10	−0.24
Mo	0.06	−0.06	−0.12	−0.28	***0.29***	***−0.30***
Cd	***0.76***	***0.62***	***0.71***	***0.33***	0.15	0.23
Tl	0.33	***0.42***	−0.01	***0.74***	***0.66***	***0.45***
Pb	0.08	0.28	0.19	−0.05	−0.12	***0.31***
Fe	0.07	−0.08	−0.05	***0.45***	***0.40***	***0.32***
Co	***0.50***	0.30	***0.47***	***0.64***	***0.42***	***0.35***
Cu	0.28	−0.13	−0.08	0.11	0.01	***−0.31***
Zn	−0.28	−0.17	−0.04	−0.07	−0.18	−0.15
Se	***0.71***	***0.56***	***0.56***	***0.80***	***0.38***	***0.30***

Table 5
Metal concentrations (dry-weight) in stomach contents (mean ± SD, range) of 14-day old tree swallow nestlings ($n = 96$) at reference and near-operations sites in 2012 and 2013.

Element	Reference 2012 ($n = 16$)	Near operations 2012 ($n = 23$)	Reference 2013 ($n = 31$)	Near operations 2013 ($n = 26$)
V (ppb)	647 ± 396 (83–1608)	731 ± 540 (155–2449)	2534 ± 1618 (587–6459)	3262 ± 2658 (555–11,600)
Cr (ppb)	997 ± 201 (642–1377)	996 ± 210 (533–1243)	1423 ± 711 (274–3293)	1962 ± 1408 (735–7154)
Ni (ppb)	668 ± 608 (88–2264)	2034 ± 4504 (163–16,900)	2849 ± 2916 (52–13,900)	6997 ± 10,516 (358–40,700)
As (ppb)	149 ± 197 (44–815)	105 ± 104 (7–473)	282 ± 153 (95–756)	133 ± 71 (15–321)
Sr (ppm)	8.3 ± 16.8 (0.6–67.3)	19.6 ± 24.8 (0.6–69.0)	17.5 ± 31.3 (0.2–150.5)	9.9 ± 18.0 (0.6–69.1)
Mo (ppm)	1.2 ± 0.4 (0.7–2.4)	0.9 ± 0.5 (0.2–1.9)	1.0 ± 0.4 (0.3–2.1)	1.2 ± 0.8 (0.2–3.7)
Cd (ppb)	680 ± 200 (420–1211)	190 ± 150 (53–625)	666 ± 291 (122–1339)	395 ± 307 (8–1000)
Tl (ppb)	2 ± 1 (0.1–3)	3 ± 4 (0.02–13)	15 ± 13 (1–52)	19 ± 17 (3–83)
Pb (ppb)	131 ± 76 (38–370)	150 ± 100 (34–460)	160 ± 176 (10–655)	125 ± 90 (27–449)
Fe (ppm)	451 ± 624 (214–2786)	286 ± 233 (58–1101)	461 ± 444 (91–2003)	702 ± 577 (25–2304)
Co (ppb)	337 ± 227 (123–868)	177 ± 129 (21–522)	305 ± 253 (51–1165)	206 ± 140 (29–532)
Cu (ppm)	82 ± 72 (24–304)	19 ± 9 (2–43)	47 ± 22 (14–90)	30 ± 15 (3–72)
Zn (ppm)	139 ± 61 (53–262)	115 ± 56 (15–227)	91 ± 58 (13–235)	129 ± 71 (11–282)
Se (ppm)	1.5 ± 0.7 (0.2–2.8)	0.5 ± 0.3 (0.1–1.2)	1.6 ± 0.7 (0.6–3.0)	1.0 ± 0.4 (0.3–1.8)

The concentrations of elements we measured in invertebrates were highly variable. Variation within species may be related to an individual's size, age, sex, and developmental stage (Hare et al., 1989; Hare, 1992; Hare and Campbell, 1992). Metals may also accumulate or be lost through ecdysis of the cuticle during invertebrate development (Timmermans and Walker, 1989), and the gut contents of invertebrates can represent up to 65% of the total body metal content (Chapman, 1985; Hall et al., 1988; Gower and Darlington, 1990). When exposure to metals from environmental contamination is high, the levels measured in insects reflect relative exposure levels (Dauwe et al., 2004; Bel'skii and Belskaya, 2013).

Malaise traps are ineffective in catching Ephemeroptera (Didham et al., 2012) and we did not catch any, despite the fact they were consumed by nestling tree swallows, especially at reference sites. Stomach samples that contained mostly Ephemeroptera had higher than average levels of V, Fe, Cu, and Se, likely influencing the levels of these elements measured in reference nestlings.

In addition to there being no increase in element levels in tree swallows near oilsands operations compared to reference sites, the elemental concentrations we measured in nestlings were not indicative of exposure to higher than normal levels. For example, although limited data are available on V toxicity in birds, levels in Canada geese (*Branta canadensis*) found dead from exposure to toxic levels of V (Rattner et al., 2006), were over 100 times higher in liver and kidney than the range in our study. The dose that resulted in mild intestinal hemorrhage in an experimental study was over 20 times higher (Rattner et al., 2006) than the maximum V concentration we measured in dietary items. The Ni levels we measured in liver and kidney were near the lowest levels reported for birds in unpolluted environments (20 to 1300 ppb), and well below levels in polluted environments (100 to 20,000 ppb; Outridge and Scheuhammer, 1993).

Table 6
Mean element concentrations (ppb dry-weight) ($n = 3$) for Coleoptera, Culicidae, Ichneumonoidea, Lepidoptera, Muscomorpha, Syrphidae, Tabanidae, Tipulidae, and Zygoptera in 2013 for samples from reference sites and near oilsands operations. BD signifies level below detection.

		V	Cr	Ni	As	Sr	Mo	Cd	Tl	Pb	Fe	Co	Cu	Zn	Se
Order Coleoptera	Reference	716	1262	758	100	18,700	410	4347	BD	60	137,930	395	58,870	218,830	1070
	Near operations	166	480	239	211	2387	310	229	2	37	130,700	225	31,770	156,000	780
Family Culicidae	Reference	758	1630	3291	65	101,033	2080	238	4	102	188,030	1662	11,570	175,670	580
	Near operations	166	1122	511	247	28,033	1300	167	2	94	217,870	6725	12,820	151,600	670
Superfamily Ichneumonoidea	Reference	85	869	1850	185	1290	950	523	2	31	125,400	187	35,170	121,000	4250
	Near operations	134	1032	175	67	1321	410	263	3	33	123,530	113	30,300	113,130	580
Order Lepidoptera	Reference	72	1056	146	118	1753	510	1058	1	51	74,530	88	13,370	266,230	840
	Near operations	154	828	192	116	2035	510	227	1	134	91,170	74	16,670	181,670	290
Infraorder Muscomorpha	Reference	189	733	226	228	3506	440	4190	3	88	159,530	247	18,730	188,730	1330
	Near operations	154	770	264	57	3258	470	1280	3	83	194,430	121	19,570	171,570	840
Family Syrphidae	Reference	195	884	340	137	2259	340	721	3	84	201,600	188	17,170	121,300	350
	Near operations	203	702	222	81	2026	300	595	2	82	209,400	70	15,730	125,300	190
Family Tabanidae	Reference	98	1070	220	413	25,233	410	848	4	95	215,170	204	21,670	348,870	960
	Near operations	241	972	283	146	37,633	390	225	3	87	248,330	575	19,300	367,000	620
Family Tipulidae	Reference	725	1326	527	42	4779	770	327	BD	78	198,400	513	24,030	201,600	740
	Near operations	327	936	333	39	4936	300	36	BD	65	171,130	831	14,570	125,800	910
Suborder Zygoptera	Reference	171	1456	355	135	3613	500	1248	5	175	156,300	68	29,200	176,770	2040
	Near operations	394	1596	256	152	3324	380	373	5	93	154,330	31	19,830	136,430	1210

Tissue levels > 1 ppm total Cr are evidence of exposure to Cr contamination (Eisler, 2000a). The levels we found in tree swallow tissues were well within the range reported in other insectivorous passerines in an unpolluted environment (0.1–1.0 ppm; Llacuna et al., 1995). Adverse effects have been documented from exposure to 10 ppm of hexavalent Cr in the diet of sensitive wildlife species (Eisler, 2000a). In our study, insect taxa reached only 2 ppm total Cr, while nestling stomach contents contained a maximum of 7 ppm total Cr.

The levels of Mo, Cd, Tl, and Pb we recorded were generally at or below normal levels found in previous studies (Franson et al., 1995; Custer et al., 2005; Mochizuki et al., 2005; Custer et al., 2006; Custer et al., 2009; Franson and Pain, 2011; Wayland and Scheuhammer, 2011), and in the case of Mo, below reported effects levels (Eisler, 2000b).

Few interpretive data for Sr in avian tissues are available. Tissue levels in our study were higher than those in cavity-nesting birds, including tree swallows (Custer et al., 2006; Custer et al., 2009), but as we found no difference between sites, the levels likely reflect natural geological levels in the oilsands region, rather than increased exposure due to mining operations. Similarly, we found higher levels of Co in liver and stomach contents at reference sites compared to near mining operations. Hepatic Co was higher than reported normal levels (Custer et al., 2009), but lower than in great tits (*Parus major*) nesting near a heavy-metal smelter (Dauwe et al., 2005).

In our study, hepatic and renal Fe, Cu, and Zn were not different between reference and near operations sites, and although they were higher than those described in tree swallows from across the United States (Custer et al., 2001, 2005, 2006, 2009, 2013), they were still far below experimental levels causing acute toxicity in birds fed high doses of Zn (Gasaway and Buss, 1972), Fe (Olsen et al., 2006), or Cu and Zn (Mondal et al., 2010).

We found normal levels of hepatic As (Albert, 2006) and Se (Ohlendorf and Heinz, 2011), while renal levels of these two elements at reference sites were slightly higher than normal. Se levels in stomach contents and insects were similar to or lower than normal levels (Ohlendorf and Heinz, 2011).

Tree swallows foraged extensively on terrestrial insects, likely limiting their exposure to trace elements from aquatic sources. In other studies, metal pollution in streams originating from the mining of mineral deposits (Kraus et al., 2014) and coal mining (Paetzold et al., 2011) reduced the emergence of aquatic insects. If there was a reduction in aquatic prey in our study area, it might account for the increase in consumption of terrestrial prey. However, terrestrial prey items appeared to be consumed in similar amounts near mining and at reference sites. Greater integration of aquatic and terrestrial food webs in ecosystem studies has been emphasized (Soininen et al., 2015), an important consideration in studies involving predators such as tree swallows which feed in riparian areas.

Although the composition of the diet of nestling tree swallows varied by location and year, our findings indicate that the low levels of elements absorbed from the diet are unlikely to compromise nestling health in our study area. These findings are in contrast to those in deer mice, in which the higher levels of Co, Se, and Tl were correlated with reduced body condition (Rodríguez-Estival and Smits, 2016). While the use of reclaimed sites, and contact of the mice with soil and vegetation that originated from various sources complicates comparisons to our study, the interspecific differences highlight the value of detailed investigations to better understand routes of contaminant exposure for a variety of potentially susceptible wildlife.

In conclusion, the principal finding of our study was that the concentrations of individual metal elements in nestling tree swallows and their food were not elevated near oilsands mining operations, and were below levels of toxicological concern. We found no evidence that oilsands mine operations are exposing tree swallows to levels of metals and metalloids above those attributed to the local geology. A study on the chemical composition of lichen in the oilsands region suggested that the accumulation pattern of some elements is not consistent from year to year, at times being very apparent and at other times being difficult to detect (Puckett, 2015). We too found variation in metal concentrations from year to year, emphasizing that annual variation associated with elemental exposure, and a cross-ecosystem, food-web approach needs to be considered in future toxicological studies in the oilsands region.

Acknowledgments

Special thanks go to K. Foster (Owl Moon Environmental Inc.), J. Swann and L. Cruz-Martinez (University of Calgary), and G. Treen and M. Hamilton (Environment Canada) for providing assistance with field data collection. L. Harder provided helpful advice regarding statistical analyses. Thanks also to R. Lein, H. Habibi and several anonymous reviewers for comments that improved drafts of this manuscript. Funding was provided by an Industrial Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC) and Syncrude Canada Ltd., (AID 418927) a grant from the Alberta Conservation Association, and a grant from NSERC (RGPIN/39695-2013) to RMRB.

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