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Chronic arsenicosis and cadmium exposure in wild snowshoe hares (*Lepus americanus*) breeding near Yellowknife, Northwest Territories (Canada), part 1: Evaluation of oxidative stress, antioxidant activities and hepatic damage

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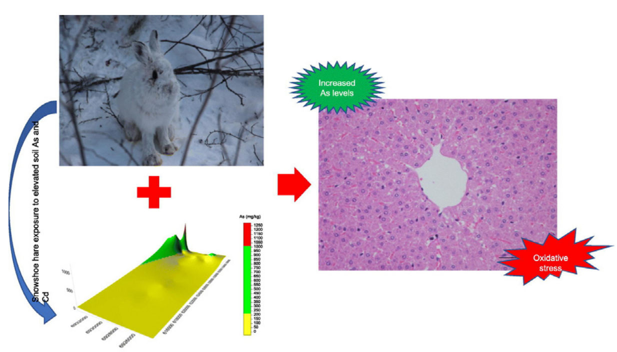
HIGHLIGHTS

- Chronic arsenicosis and Cd exposure were studied in wild snowshoe hares from the Giant mine area and reference location.
- Arsenic was elevated in nails of hares from the mine area compared to the reference site.
- No ocular lesion was observed but hepatic steatosis was common in hares from both sites.
- Hares from the mine area showed increased oxidative stress and reduced antioxidant capacity compared to the reference site.

GRAPHICAL ABSTRACT



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ABSTRACT

Previous gold mining activities and arsenopyrite ore roasting activities at the Giant mine site (1948 to 2004) resulted in the release of high amounts of arsenic and trace metals into the terrestrial and aquatic ecosystems of Yellowknife, Northwest Territories, Canada. While elevated levels of arsenic has been consistently reported in surface soils and vegetation near the vicinity of the Giant mine area and in surrounding locations, systematic studies evaluating the overall health status of terrestrial small mammals endemic to the area are lacking. The purpose of this present study was to evaluate and comparatively assess the biochemical responses and histopathological effects induced by chronic arsenic and cadmium exposure in wild snowshoe hares breeding near the city of Yellowknife, specifically around the vicinity of the abandoned Giant mine site and in reference locations. Analysis included measurement of total arsenic and cadmium concentration in nails, livers, kidneys, bones, stomach content of hares, in addition to histopathological evaluation of hepatic and ocular lesions. Biochemical responses were determined through measurement of lipid peroxidation levels and antioxidant enzymes activities (catalase, superoxide dismutase, glutathione peroxidase, and glutathione disulfide). The results revealed that arsenic concentration was 17.8 to 48.9 times higher in the stomach content, and in the range of 4 to 23 times elevated in the nails of hares from the mine area compared to the reference location. Arsenic and cadmium levels were also

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[1] Note: Dr. Amuno is an adjunct professor at the School of Environment and Sustainability, and his participation in this study was undertaken independently and apart from his current work with the Nunavut Impact Review Board (NIRB). The analysis and views expressed in the study remain solely those of the authors and do not constitute the views of NIRB.

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noted to be increased in the bones, renal and hepatic tissues of hares captured near the mine area compared to the reference site. Specifically, hares from the mine area showed nail cadmium levels that was 2.3 to 17.6 times higher than those from the reference site. Histopathological examination of the eyes revealed no specific ocular lesions, such as lens opacity (cataracts) or conjunctivitis; however, hares from both locations exhibited hepatic steatosis (fatty liver change). Lipid peroxidation levels were relatively increased and accompanied with reduced antioxidant enzyme activities in hares from the mine area compared to the hares from the reference site. The results of this preliminary study suggest that the snowshoe hares breeding near the vicinity of Yellowknife, including near the Giant mine area have been chronically exposed to elevated levels of arsenic and cadmium, which consequently led to the increased levels of oxidative stress and perturbation of antioxidant defense system in exposed animals. The results of this present study constitute the first observation of chronic arsenicosis in wild small mammal species in Canada.

1. Introduction

The occurrence of gold mineralization in the geology of Yellowknife was instrumental to the growth and development of the mining industry in the Northwest Territories (Canada) with the Giant mine (1948 to 2004) being one the most prominent gold producer in the region (Andrade et al., 2010; van Hees et al., 1999; Wagemann et al., 1978). During the early phase of mining operations at the Giant mine site, roasting of arsenopyrite-bearing gold ores resulted in the stack release of an estimated 20,000 tonnes (t) of roaster generated arsenic trioxide (As_2O_3) into natural environment and caused extensive and widespread contamination of the local terrestrial and aquatic ecosystems (Jamieson, 2014; Bromstad, 2011; Bright et al., 1994). In addition, approximately 237,000 t of As_2O_3 was stored in the underground chambers of the Giant mine, which till date continue to be an ongoing source of environmental contamination (Jamieson, 2014). In addition, the contamination of the local environment with mining-derived contaminants also increased human health risk concerns among Yellowknife residents and aboriginal communities due to concerns regarding the potential entry of metals or metalloids into the human food chain, and the associated health effects (Jamieson, 2014). While many previous studies have already reported elevated levels of As in the soils, vegetation, surface water and aquatic biota of the Yellowknife area for several decades (Wagemann et al., 1978; Hocking et al., 1978; Hutchinson et al., 1982; Jamieson, 2014), information is still limited regarding the current status of contaminant bioaccumulation, especially with reference to As and Cadmium (Cd), and exposure-related effects in terrestrial wildlife species inhabiting the area (Koch et al., 2005; Saunders et al., 2009; Saunders et al., 2011).

Small mammals are important biological component of the terrestrial ecosystem and they have been used in many studies as sentinel species for monitoring metal bioavailability in the natural environment, as well as the toxicological effects of polluted areas (Saunder et al., 2009; Amuno et al., 2016). Although elevated concentration of As and other metals have been detected in the terrestrial ecosystem of the Yellowknife area for many decades; however, till date no attempt has been made to specifically investigate the biochemical effects, including histopathological changes associated with wildlife exposure to elevated levels of As and other trace metals in the natural environment (Shore and Douben, 1994; Sánchez-Chardi and Nadal, 2007). This line of scientific inquiry is important because many furbearer species, including snowshoe hares are still being trapped for their pelt and meat around the Yellowknife area, and there are limited studies regarding contaminant accumulation, including exposure-related effects in small mammals inhabiting the area. Given that small mammals, such as snowshoe hares have limited home range and exhibit geophagy (soil eating behavior) they are likely to accumulate higher concentration of As and other trace metals directly from the natural environment, which may subsequently result in elevated tissue concentration of contaminants. Chronic environmental exposure of wildlife to As and other trace metals has been associated with several severe biological effects, such as oxidative stress and perturbation of antioxidant defense system, including histopathological effects (Amuno et al., 2016; Sánchez-Chardi and Nadal, 2007). Furthermore, human consumption of animals with elevated tissue concentration of As and trace metals may increase the contaminant burden in exposed population, causing the development of metabolic diseases and cancers (Bordeleau et al., 2016; Ogwok et al., 2014). As a result of the long history of As contamination in the Yellowknife area and the risk for wildlife exposure, there is an urgent need to develop a monitoring program, in order to assess tissues concentration of As and other trace metals in exposed wildlife species, in order to support conservation initiatives and determine which species are at risk of chronic toxicity.

While there is a growing body of knowledge regarding the metal accumulation trends in ungulates and carnivores from the Canadian arctic, there is still scarcity of data regarding the biochemical responses and histopathological effects of contaminant exposure in small mammal species inhabiting post-mining areas of the arctic (de Jong et al., 2017; Amuno et al., 2016). For example, Amuno et al. (2016) assessed heavy metal bioaccumulation and histopathological changes in arctic hares from the vicinity of the former Nanisivik lead-zinc mine and a reference location in near the community of Arctic Bay, Nunavut (Canada). The study specifically noted that organ tissues of hares from the mine area showed a relatively higher metal content and severe histopathological changes compared to the hares from the reference location. In the Siberian arctic, Allen-Gil et al. (2003) utilized lemmings for monitoring metal bioavailability and the toxicological effect of the world's largest smelting complex located in Norilsk, Russia. In Northern Alaska, Allen-Gil et al. (1997) monitored the bioavailability of select heavy metals, organochlorine pesticides and polychlorinated biphenyl in the terrestrial environment using arctic ground squirrels as sentinel species for assessing environmental quality. These previous studies have demonstrated the relevance of the use of small mammals as sentinel species for monitoring the quality of the natural environment, and for assessing the potential effects of contaminant exposure in the terrestrial ecosystem due to industrial activities. Recognizing that the background levels of As in the soils and vegetation around the Yellowknife area are significantly elevated, there are growing concerns and speculations that many terrestrial herbivores inhabiting the area may be chronically exposed to high concentration of As and trace metals, which may result in adverse health outcomes and diseases in exposed animals (Koch et al., 2000; Reimer et al., 2003). Despite this concern, no study has investigated the biochemical responses and histopathological effects associated with chronic As and Cd exposure in wild terrestrial animals from the Yellowknife area.

The objective of this research project is twofold. The first is to determine the current levels of As and Cd accumulation in select organ tissues of snowshoe hares captured within and around the vicinity of the abandoned Giant mine and in a reference site approximately 20 km from the City of Yellowknife. This study was specifically interested in comparing As and Cd accumulation in various tissues and organs of snowshoe hares breeding near the former mining area with those from the reference location to determine whether any significant differences exist between the two groups. The second was to assess the effect of chronic As and Cd exposure on biomarkers of oxidative stress and antioxidant capacity, as well as to determine whether current exposure levels induces histopathological changes in ocular and hepatic tissues of snowshoe hares from both locations. Ocular tissues were specifically selected for this investigation because both chronic As and Cd exposure has been shown to induce conjunctivitis and lens opacity (cataracts), which has further been associated with visual degeneration, altered animal behaviour, reduced fitness and increased predation risk (Baidya et al., 2006; See et al., 2007; Wang et al., 2016). The liver was also selected for this study because previous investigations have reported the hepatotoxic effect of both As and Cd exposure in animal and human populations (Rikans and Yamano, 2000; Mazumder, 2005).

2. Status of the Giant mine site

After the closure of the Giant mine in 2004, the care and maintenance of the mine became the full responsibility of the Government of Canada through the Department of Indigenous and Northern Affairs Canada (INAC), to specifically address various environmental issues left behind by the previous mine operators, including the 237,000 t of arsenic trioxide dust currently stored in fourteen underground chambers at the Giant Mine site ("Giant Mine Remediation Project", 2017). INAC on behalf of the Government of Canada, and the Government of the Northwest Territories are the Co-Proponents of the Giant Mine Remediation Project, which is funded through the Federal Contaminated Sites Action Plan ("Giant Mine Remediation Project", 2017). The remediation project is currently focused on the long-term containment and management of the stored underground arsenic trioxide waste, demolition and removal of on-site buildings, water management and treatment, and the remediation of all surface areas including the tailings ponds at the Giant Mine site ("Giant Mine Remediation Project", 2017). After considering a wide range of technical solutions to manage the potential environmental impacts associated with the underground arsenic trioxide dust, the Giant Mine Project Team determined that the preferred method was to permanently freeze the dust and the rock around each underground chamber ("Giant Mine Remediation Project", 2017).

3. Historical records of arsenic poisoning in wildlife around Yellowknife

Aboriginal community members started reporting on the severity of As contamination in the Yellowknife area in the 1950s although with very limited data regarding wildlife health and exposure-related effects (Sandlos and Keeling, 2016). While some elders from the Yellowknives Dene First Nations, including local residents have shared memories of sled dogs, cattle, chickens and moose dying after foraging and drinking As-contaminated water from the vicinity of the mine area, it is important to note here that none of these reported cases of suspected incidences of arsenic poisoning was supported by any toxicology data, or documentation of the levels of total As concentration in the organ tissue or any pathological evidence, in order to conclusively determine whether As exposure was the cause of animal mortalities. To the best of our knowledge, this current study is among the first scientific investigation to present evidence of the toxic effects of chronic arsenicosis and Cd exposure in terrestrial wildlife from the Yellowknife area.

4. Clinical assessment and pathological diagnoses of chronic arsenicosis

Arsenicosis is a term used for denoting chronic health condition due to prolonged ingestion of As above the safe dose for at least 6 months (Dhar et al., 2005; Chappell et al., 2003). The diagnosis of arsenicosis in the study area followed the definition and criteria described in previous studies (Saha, 2003; Bertin et al., 2013; Mandal, 2017; Datta et al., 2012; Nain and Smits, 2012) and adapted to include the following:

(1) Arsenic exposure data: Given that snowshoe hares are herbivores with geophagic (soil eating) behaviour, environmental As exposure data mainly focused on the assessment of As concentration in surface soil and vegetation of the study area. Previous investigation of 98 lakes within a 30 km radius of the City of Yellowknife already confirmed that As concentration exceeded the federal drinking water guideline of 10 μg/L for many lakes within 12 km of the Giant mine area (Palmer et al., 2015).
(2) Biomarker of prolonged Arsenic exposure: Evidence of prolonged As exposure was obtained by measuring total As levels in nails of snowshoe hares from both the mine area and reference location.
(3) Arsenic bioaccumulation status: The status of As bioaccumulation was obtained by measuring total As levels in select biological compartments, such as stomach content, liver, kidney and bones of snowshoe hares from the study area.

Supportive features included evaluation of biochemical responses (oxidative stress and antioxidant capacity) and histopathological evaluation of ocular and hepatic lesions.

5. Material and methods

5.1. Sampling

A wildlife research permit and ethical clearance (NWTWCC #2016-016) for wildlife handling was obtained from the Department of Environment and Natural Resources, Government of the Northwest Territories (GNWT). A research licence was also obtained from the Aurora Research Institute prior to the commencement of the study. Snowshoe hares were trapped within and around the vicinity of Yellowknife with the assistance of an experienced local furbearer hunter from October through December 2016 using humane trapping techniques, in order to minimize physical stress and harm on the animals. 10 sub-adult hares (6 females and 4 males) were randomly trapped in locations within 1–3 kilometres (km) radius of the abandoned Giant mine site in Yellowknife (Fig. 1). Another 10 sub-adult hares (4 females and 6 males) were trapped at various locations of about 20 km radius from the Giant mine area, which was considered in this study as the reference site. All the hares utilized for the study were within the same age range of 1 to 2 years. In addition, soil and vegetation (willows) samples were also collected from the mine area and analyzed for As and Cd. Since access to the Giant mine site was not possible at the time of the fieldwork, a certain location (62°30′0.39″N; 114°21′34.36″W) proximal to the mine area was used as a central point for estimating the distances of the sampling points to the Giant mine area. In order to establish the naturally occurring background levels of trace elements for the site, additional soil and willow samples were collected from the reference location and analyzed. The GPS locations of where each soil, willows and animal was collected were recorded in relation to distance from the Giant mine area. The hares were euthanized on the field by the hunter using cervical dislocation and dissected for the removal of organ tissues, such as livers, kidneys, eye globes, stomach content, bones and nails. Tissue samples were stored in prepared vials for determination of total As and Cd. The samples for biochemical analysis were excised in the field and immediately frozen. All biological samples were stored in a cooler filled with ice to facilitate transportation of tissues for laboratory analyses. Current data indicate that the home range size of snowshoe hares varies depending on food availability but is generally between 0.05 to 0.2 squared km (5–20 ha), with males having larger home ranges than females (Macdonald, 2017).

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Fig. 1. Map of the study area showing the mine area and reference location.

5.2. Trace element analysis in tissue, soil and vegetation

All of the tissue samples (~1.0 g) except the nails and bones were digested in 15 mL polyethylene vials containing 1 N nitric acid (Ultrapure, Merck, Canada), with a tissue weight to acid volume ratio of 1:5, at 60 °C for 48 h (Amuno et al., 2016). The nail and bone samples were digested similarly in concentrated nitric acid (16 N; Ultrapure, Merck, Canada) (Chen et al., 1999). Prior to digestion, all tissue samples were rinsed in deionized water to eliminate external contamination, and then blotted dry. The concentrations of metals in digested tissues were measured in a graphite furnace atomic absorption spectrometer (Analyst 800, Perkin Elmer Ltd., USA) after appropriate dilutions with 0.2% nitric acid. The quality control and quality assurance of the analytical method were maintained by using certified standards for each element, the standard addition and recovery procedure, and a certified reference material (DOLT-4; National Research Council of Canada). The reference material was digested and analyzed concurrently with the tissues samples, and the recovery of metals varied between 96 and 104%. The detection limits for both As and Cd in animal tissue samples was 0.005 μg/g. The trace element levels in animal tissues are presented on a wet weight basis. The top soils collected were shipped to a commercial laboratory for trace element analysis similar to the procedures outlined in Amuno et al. (2016). Trace elements were determined (VG101 package, ACME laboratories, Vancouver) in willow samples using a 1 g split digested in nitric acid followed by Aqua Regia and analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Specifically, raw vegetation samples were dried and macerated, and subsequently digested in aqua regia at 95 °C for 2 h, and with the resultant sample solutions diluted and analyzed by ICP-MS. A control reference material (CRM) was inserted after every 10th sample. Acme also inserted their own vegetation CRM samples (V16) after every 20th sample. Reagent blanks were used to correct instrument readings. The detection limits for As and Cd for soils and vegetation were 0.01 mg/kg and 0.001 mg/kg, respectively. Surfer 13 mapping software was used to plot a three-dimensional surface that approximated the natural variability and anthropogenic input of As across the study area (Amuno, 2013). Spatial interpolation of As in surface soil was done in accordance with the procedures outlined in Surfer 13 User's Guide (Golden Software).

5.3. Histology

Eye globes and liver sections of snowshoe hare were collected promptly in the field and immersed in 10% formalin at 10:1 fluid to tissue ratio. Liver sections were dehydrated through a series of alcohols and xylene and then sectioned and embedded into paraffin wax. Blocks were sectioned and routinely stained with haematoxylin and eosin, and further examined with light microscopy for any ultrastructural changes and abnormalities. Following formalin fixation of the eye tissues, the globes were examined grossly and measured with a caliper. The globes were sectioned with a brain blade in a vertical plane. Each half of the globe was examined grossly for occurrences of abnormalities. Six micron sections were harvested and stained routinely with haematoxylin and eosin and periodic Schiff and Luxol fast blue stains.

5.4. Measurement of cellular thiol redox balance (GSH:GSSG ratio)

Hepatic concentration of reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) was determined using the procedures outlined in Jamwal et al. (2016). The GSH and GSSG content was expressed as μg mg^{-1} of protein. In addition, the GSH content of each replicate was divided by its corresponding GSSG content and expressed as a ratio. The Bradford method was used for estimating the protein content of the samples (Bradford, 1976).

5.5. Measurement of antioxidant enzyme activities and lipid peroxidation levels

For the measurement of enzyme activities, the frozen livers were thawed and homogenized on ice using a hand-held homogenizer. The homogenate was then centrifuged at 25,000 $\times g$ for 20 min at 4 °C and supernatant was collected for enzymatic analysis. The activities of three antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured in hepatic tissues following the procedures outlined in Jamwal et al. (2016). The enzyme activities were measured using SOD (Catalogue #706002), CAT (Catalogue #707002), and GPx (Catalogue #703102) activity kits as per the manufacturer's (Cayman chemical company, USA) instructions. SOD activity was expressed as % of control. Activities of CAT and GPx were expressed as nmol min^{-1} mg^{-1} protein. One unit of CAT was defined as the amount of enzyme that will cause the formation of 1.0 nmol formaldehyde at 25 °C. One unit of GPx was defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to $NADP^+$ per minute at 25 °C. Estimation of malondialdehyde (MDA) as a marker of lipid peroxidation (LPO) was conducted on frozen livers using thiobarbituric acid reactive substances (TBARS) kit as per the manufacturer's instructions (Catalogue #10009055; Cayman Chemical Company, USA). Briefly, liver samples were homogenized in chilled sample buffer (5 mM Tris-HCl, 5 mM EDTA, pH 7.6) with a tissue weight to buffer volume ratio of 1:10. The homogenate was centrifuged at 1600 ×g for 10 min at 4 °C and the supernatant was frozen at −80 °C until used. The samples were read using a fluorometric assay in a 96-well microplate using a multi-well plate reader (Varioskan Flash, Thermo Electron Corporation, Finland) with excitation wavelength at 530 nm and emission wavelength of 550 nm. The hepatic lipid peroxidation was expressed as μM MDA mg^{-1} tissue protein.

5.6. Statistical analysis

Independent sample *t*-test was used to compare the differences in As concentration and the biochemical parameters in the tissues of snowshoe hares collected from the mine area and the reference location. A two-tailed Pearson correlation was employed to assess the relationships between As levels in the nails and organ tissues, including hepatic antioxidant enzyme activities and LPO levels. The assumptions of normality of distribution, and homogeneity of variances were verified with Shapiro-Wilk and Levene's test, respectively. For the measurement of As concentration and biochemical parameters, at least three replicate samples were analyzed from each animal. The sample size '*n*' indicates the number of independent measurements conducted on tissues collected from different animals. A *p*-value of ≤0.05 was considered to be statistically significant.

6. Results

6.1. As and Cd contamination of surface soil and vegetation

Environmental exposure data for snowshoe hares was obtained through the determination of As and Cd levels in surface soil and willows of the study area. As noted in Table 1 below, relatively higher levels of As and Cd were detected in the soils and willows near the vicinity of the Giant mine compared to the reference locations approximately 20 km away. For example, in the sampling areas between 0.77 km to 2.45 km radius of the mine area, As levels ranged from 35.2 mg/kg to 1636.2 mg/kg in the soil samples and ranged from 1.3 mg/kg to 59.4 mg/kg in the willow samples. In locations between 3.38 km to 9.03 km of the mine area, As levels ranged from 22.5 mg/kg to 157.1 mg/kg in the soil samples and ranged from 0.6 mg/kg to 12 mg/kg in the willow samples. Between 4 km to 12 km radius of the mine area, As levels ranged from 7.9 mg/kg to 55.2 mg/kg in the soil samples and ranged from 0.6 mg/kg to 12.7 mg/kg in the willow samples. In the reference location, between 14 km to 22 km away from the Giant mine, levels of As ranged from 10.1 mg/kg to 75.3 mg/kg in the soil samples and ranged from 1.1 mg/kg to 3 mg/kg in the willow samples. The concentration of As in the surface soils also showed a visual pattern of decreased As concentration with increasing distance from the mine area (Fig. 2). In addition, Cd accumulation was also observed to be relatively higher in the soil and willow samples near the mine area compared to the reference locations.

Table 1
As and Cd concentration (mg/kg) in surface soils and willows from the study area.

Sample ID	Surface soils		Willows		Distance to Giant mine (km)
	As	Cd	As	Cd	
1	29.4	0.2	3	0.05	22
2	10.1	0.05	1.1	0.56	20
3	26.2	0.05	2.9	1.21	18
4	11.4	0.05	3	0.47	16
5	75.3	0.1	2.8	0.04	14
6	8.8	0.05	0.9	0.05	12
7	9.7	0.05	4	0.52	10
8	7.9	0.05	0.6	0.92	8
9	22.3	0.1	6.9	0.18	6
10	55.2	0.2	12.7	0.09	4
11	115.8	0.6	12	0.07	2
12	847.8	0.8	1.7	0.06	0.98
13	321.8	0.2	11	1.55	0.77
14	674.1	0.5	51.4	0.1	1.05
15	353.1	0.1	3.8	0.03	1.34
16	104.2	0.3	3.7	0.49	1.3
17	35.2	0.1	1.3	0.04	1.33
18	1638.2	0.9	59.4	2.39	1.41
19	206.8	0.1	5.1	1.06	1.77
20	156	0.2	2.1	0.02	2.45
21	157.1	0.2	2.9	0.37	5.03
22	34.5	0.05	3.8	0.17	6.5
23	42.8	0.1	3.3	0.05	9.03
24	305.5	0.6	8.8	0.14	6.7
25	89.5	0.3	1	0.03	5.77
26	150.9	0.2	1	0.01	5.33
27	123.1	1.2	12	6.43	5.40
28	37	0.2	1	3.92	5.81
29	22.5	0.05	0.6	0.33	4.60
30	38.6	0.6	1.7	0.83	3.38

6.2. Concentration of As and Cd in nails and femoral bones of snowshoe hares

Evidence of chronic exposure to As and Cd was obtained through the measurement of total As and Cd levels in the nails and the femoral bones of snowshoe hares from the study area (Table 2). Further, an independent sample *t*-test ($\alpha = 0.05$) was utilized to compare As concentration as well as Cd levels in the nails and bones of hares from the mine area and reference location. Total As levels in the nails of snowshoe hares from the reference location ranged from 0.047 μg/g to 0.936 μg/g, while that from the mine area was ranged from1.08 μg/g to 4 μg/g, suggesting that the hares captured near the vicinity of the mine area have been chronically exposed to elevated levels of As. *t*-test further indicated that there was a significant difference between the two groups of snowshoe hares with respect to As accumulation in the nails ($p < 0.001$). Specifically, As content in the nails of the snowshoe hares from the mine area was in the range of 4.2 to 23 times higher than those from the reference site. Cd concentration in the nails of snowshoe hares from the mine area ranged from 0.007 μg/g to 0.476 μg/g, while that from the reference location ranged from 0.003 μg/g to 0.027 μg/g, suggesting that the animals from the mine area were also concomitantly exposed to elevated levels of Cd in their home range. Hares from the mine area showed a 2.3 to 17.6-fold increase in nail Cd levels compared to the samples from the reference site but no significant differences were noted between the two groups with respect to Cd accumulation in the nails ($p = 0.19$). As was generally below detection limit in all the bone samples examined, except for three bone specimens from the mine area which showed detectable levels of the metalloid. Cd tended to be elevated in the bones of snowshoe hares from the mine area compared to the reference locations but no statistically significant differences were noted between the two groups ($p = 0.96$). Correlation matrix (Table 3) revealed a strong relationship between As levels in the nails, with As concentration in liver (0.529) and As stomach content (0.475). The results generally showed that snowshoe hares breeding

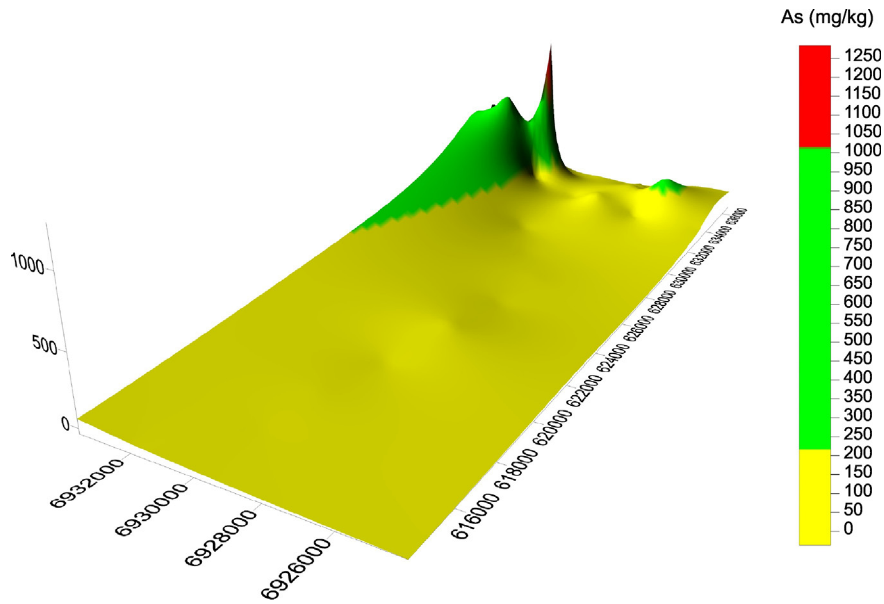

Fig. 2. 3-D interpolation surface of As concentration in soils from the study area.

near the vicinity of the Giant mine area were concomitantly exposed to elevated levels of As and Cd from their habitats compared to the hares from the reference site.

6.3. Concentration of As and Cd in stomach content, liver and kidney of snowshoe hares

As and Cd bioaccumulation was determined through measurement of total As levels and Cd concentration in the stomach content, livers and kidneys of snowshoe hares from the mine area and reference location (Table 2). As concentration in the stomach content of hares from the control area ranged from 0.014 μg/g to 0.140 μg/g, while those from the mine area ranged from 0.25 μg/g to 6.85 μg/g. As levels in the stomach content of hares from the mine area was 17.8 to 48.9 times higher than those from the reference site, which was also noted to be statistically different between the two groups ($p = 0.03$). Cd concentration in the stomach content of hares was not significantly different between the two groups ($p = 0.13$), although one specimen from the mine area showed an increased Cd level in the stomach content that was about 5 times higher than those observed from the reference site. Hepatic concentration of As ranged from 0.015 μg/g to 0.766 μg/g in hares from the control locations, while that from the mine area ranged from 0.19 μg/g to 2.30 μg/g. Hepatic concentration of As from the mine area was 3 to 12 times higher compared to the reference hares and the levels were noted to be significantly different between the two groups ($p = 0.01$). Cd concentration in the liver of the reference hares was in the range of 0.09 μg/g to 0.49 μg/g, while those from the mine area was between 0.096 μg/g to 1.092 μg/g and *t*-test revealed that hepatic Cd accumulation tended towards significance between two groups ($p = 0.051$). Renal concentration of As in the hares from the mine area was in the range of 4.2 to 14.5 times more than those from the reference location. In addition, *t*-test showed that As accumulation in the kidneys from the two groups tended towards significance ($p = 0.053$). Renal Cd in hares from the mine area also tended to be relatively higher compared to those from the mine area. Specifically, *t*-test showed that Cd in the kidneys was significantly different between two groups ($p = 0.009$). In general, stomach content, including renal and hepatic tissues of hares from the mine area tended to accumulate a relatively higher concentration of As and Cd compared to those from the reference location.

6.4. Enzyme activities and oxidative stress indicators in Snowshoe hares

The status of antioxidant enzyme activities and oxidative stress indicators were determined in liver of snowshoe hares from the study area (Table 4). Significant differences were noted between the two groups with respect to hepatic enzyme activities of SOD ($p = 0.04$), CAT ($p = 0.018$), GSH ($p = 0.043$), GPx ($p = 0.021$) with the exception of GSSG ($p = 0.282$). The LPO levels was also significantly different between the two groups ($p = 0.003$). Snowshoe hares from the mine area exhibited a relatively higher levels of LPO and depressed activities of SOD, CAT, GSH and GPx compared to the hares from the reference location. A two-tailed Pearson correlation was also computed to further assess the relationships between As exposure data, including As and Cd levels in the nails and organ tissues, including antioxidant enzyme activities and LPO levels (Table 3). Correlation matrix further revealed that As and Cd levels in the soil and willow samples were strongly correlated with hepatic Cd (0.633) and lipid peroxidation levels (0.704) in exposed snowshore hares from the study area. This strong correlation suggested that concomitant environmental exposure to elevated As and Cd may be

Table 2
As and Cd concentration (μg/g wet wt.) in tissues of snowshoe hares from study area.

Locations	Giant mine area ($n = 10$)		Reference area ($n = 10$)	
	Mean (minimum-maximum)/S.D.		Mean (minimum-maximum)/S.D.	
Tissues	As	Cd	As	Cd
Nails	1.93 (1.08–4.0)/0.99	0.07 (0.007–0.476)/0.14	0.31 (0.047–0.936)/0.31	0.01 (0.003–0.027)/0.007
Bones	bdl-0.026	0.023 (0.012–0.053)/0.015	bdl[a]	0.009 (0.006–0.013)/0.002
Stomach content	1.69 (0.25–6.85)/2.04	0.037 (0.015–0.060)/0.016	0.06 (0.014–0.140)/0.04	0.021 (0.003–0.090)/0.027
Liver	0.76 (0.19–2.30)/0.64	0.49 (0.096–1.092)/0.40	0.2 (0.015–0.766)/0.24	0.2 (0.09–0.49)/0.12
Kidney	1.11 (0.334–4.0)/1.15	10.8 (2.544–22.28)/7.39	0.28 (0.023–0.945)/0.31	2.98 (1.76–6.41)/1.5

[a] bdl = below detection limit; S.D. = standard deviation.

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Table 3
Pearson correlation coefficient of analyzed parameters in soils, tissues and biochemical data.

	As-soil	Cd-soil	As-willow	Cd-willow	LPO	GPx	Cd-stomach content	As-stomach content	Cd-kidney	As-kidney	Cd-liver	As-liver	SOD	CAT	GSH	GSSG	GSH:GSSG	Cd-nails	As-nails
As-soil	1	0.582**	0.796**	0.133	0.704**	−0.323	0.081	0.220	0.562**	0.280	0.633**	0.320	−0.502	−0.507	−0.407	0.074	−0.392	−0.040	0.346
Cd-soil	0.582**	1	0.502**	0.535**	0.398	−0.363	0.212	0.138	0.312	0.353	0.348	0.501*	−0.288	−0.586*	−0.305	0.270	−0.465	0.371	0.554*
As-willow	0.796**	0.502**	1	0.191	0.455	−0.278	0.154	−0.065	0.651**	0.522*	0.690**	0.497*	−0.497	−0.282	−0.260	−0.125	−0.141	0.040	0.067
Cd-willow	0.133	0.535**	0.191	1	−0.234	0.042	0.052	0.097	0.399	−0.110	0.528*	−0.200	−0.315	−0.286	0.053	−0.135	0.095	−0.126	−0.036
LPO	0.704**	0.398	0.455	−0.234	1	−0.370	0.179	0.626*	0.713**	0.461	0.601*	0.527	−0.523	−0.255	−0.346	0.259	−0.484	0.011	0.342
GPx	−0.323	−0.363	−0.278	0.042	−0.370	1	−0.350	−0.286	−0.337	−0.335	−0.221	−0.384	0.279	0.457	0.386	0.255	0.119	−0.261	−0.398
Cd-stomach content	0.081	0.212	0.154	0.052	0.179	−0.350	1	0.060	−0.002	0.150	0.019	0.220	−0.303	−0.547	−0.426	0.187	−0.501	0.301	0.281
As-stomach content	0.220	0.138	−0.065	0.097	0.626*	−0.286	0.060	1	0.412	0.128	0.496*	0.208	−0.253	−0.372	−0.457	0.188	−0.509	−0.029	0.475*
Cd-kidney	0.562**	0.312	0.651**	0.399	0.713**	−0.337	−0.002	0.412	1	0.529*	0.899**	0.459*	−0.614*	−0.182	−0.292	−0.007	−0.286	−0.102	0.165
As-kidney	0.280	0.353	0.522*	−0.110	0.461	−0.335	0.150	0.128	0.529*	1	0.392	0.956**	−0.446	−0.380	−0.396	0.035	−0.361	−0.009	0.440
Cd-liver	0.633**	0.348	0.690**	0.528*	0.601*	−0.221	0.019	0.496*	0.899**	0.392	1	0.337	−0.433	0.039	−0.196	−0.026	−0.200	−0.160	0.115
As-liver	0.320	0.501*	0.497*	−0.200	0.527	−0.384	0.220	0.208	0.459*	0.956**	0.337	1	−0.432	−0.447	−0.389	0.152	−0.455	0.178	0.529*
SOD	−0.502	−0.288	−0.497	−0.315	−0.523	0.279	−0.303	−0.253	−0.614*	−0.446	−0.433	−0.432	1	0.595*	0.533	−0.077	0.546	−0.051	−0.284
CAT	−0.507	−0.586*	−0.282	−0.286	−0.255	0.457	−0.547	−0.372	−0.182	−0.380	0.039	−0.447	0.595*	1	0.676*	0.191	0.249	−0.351	−0.651*
GSH	−0.407	−0.305	−0.260	0.053	−0.346	0.386	−0.426	−0.457	−0.292	−0.396	−0.196	−0.389	0.533	0.676*	1	0.374	0.442	0.104	−0.404
GSSG	0.074	0.270	−0.125	−0.135	0.259	0.255	0.187	0.188	−0.007	0.035	−0.026	0.152	−0.077	0.191	0.374	1	−0.638*	0.334	0.339
GSH:GSSG	−0.392	−0.465	−0.141	0.095	−0.484	0.119	−0.501	−0.509	−0.286	−0.361	−0.200	−0.455	0.546	0.249	0.442	−0.638*	1	−0.191	−0.556*
Cd-nails	−0.040	0.371	0.040	−0.126	0.011	−0.261	0.301	−0.029	−0.102	−0.009	−0.160	0.178	−0.051	−0.351	0.104	0.334	−0.191	1	0.359
As-nails	0.346	0.554*	0.067	−0.036	0.342	−0.398	0.281	0.475*	0.165	0.440	0.115	0.529*	−0.284	−0.651*	−0.404	0.339	−0.556*	0.359	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 4
Antioxidant enzyme activities and lipid peroxidation levels in snowshoe hares from study area.

Biochemical responses	Giant mine area	Reference site
	Mean (minimum-maximum)/S.D.	Mean (minimum-maximum)/S.D.
CAT	2.3 (0.74–5.39)/1.62	5.6 (2.63–9.16)/2.47
SOD	4.7 (2.7–6.65)/1.64	7.8 (5.66–13.4)/2.88
GSH	527.7 (275.6–730.9)/153.2	709.3 (504.2–980.1)/158.6
GSSG	29.3 (20.1–36.8)/6.8	24.2 (15.3–40.9)/10.1
GSH:GSSG	17.9 (12.4–22.4)/3.48	32.2 (19.7–52.4)/10.8
GPx	1678.9 (141.4–3498.7)/1293.5	8155.9 (3207.2–22010)/6335
LPO	15 (9.08–25.7)/6.3	5.7 (3.6–7.08)/1.09

S.D. = standard deviation.

involved in the generation of increased levels of oxidative stress in exposed hares from the study area. It was also observed that hepatic LPO levels strongly correlated with As levels in the stomach content (0.626) and hepatic Cd concentration (0.601), suggesting the potential for oral exposure to contaminated forage and soil to induce increased reactive oxygen species and free-radical reactions, which may initiate lipid peroxidation in exposed hares from the study area. As and Cd levels in the nails also strongly correlated with the concentrations of Cd in the soils (0.554), As in the stomach content (0.475) and As in the liver (0.529), suggesting the role of chronic environmental exposure in increasing contaminant body burdens in the animals. In addition, a negative strong correlation was observed between the levels of As in the nails, CAT activity (−0.651) and GSH/GSSG ratio (−0.556), suggesting the potential for prolonged As exposure to decrease activities of antioxidant enzymes and induce oxidative stress in exposed hares from the study area.

6.5. Ocular and hepatic histopathology

The liver sections of hares from the mine area and reference locations showed similar histopathological changes. The major liver lesion noted in both group was steatosis (fatty liver change) (Fig. 5a and b). Overall, there was no evidence of fibrosis, necrosis or edema in any of the hepatic tissues examined. With respect to the eye globes, no evidence of light microscopic or gross lesions was detected in either group (Figs. 3 and 4). Furthermore, histologic examination revealed no discernable ocular differences or abnormalities in either group. No evidence of corneal or lenticular lesions, including cataracts and neurocrest disorders was noted in either group of hares.

7. Discussion

7.1. As and Cd contamination of the Yellowknife environment

Studies have shown that animals inhabiting As contaminated areas are at a higher risk of chronic arsenicosis through ingestion of contaminated forage, soils and drinking water (Mandal, 2017; Ashrafihelan et al., 2013). Chronic arsenicosis have been reported in farm and domestic animals in As endemic areas of Asia and the Middle East (Rana et al., 2012; Bera et al., 2010; Ashrafihelan et al., 2013), but no evidence of chronic As toxicity and Cd exposure has been reported in terrestrial wildlife species from the study area despite the relatively higher concentration of As and Cd in the soils and vegetation in the Yellowknife environment (Jamieson, 2014; Bromstad, 2011; Bright et al., 1994). The results of our present study showed that the soil and willow samples from the Giant mine area were significantly enriched with elevated levels of As and Cd compared to the reference location. Both As and Cd in the soil and vegetation samples tended to decrease in concentration away from the Giant mine area. Except for As, Cd concentration in the soil samples was generally within the safe limit. Only one sample showed a Cd concentration that was above 1 mg/kg. The maximum

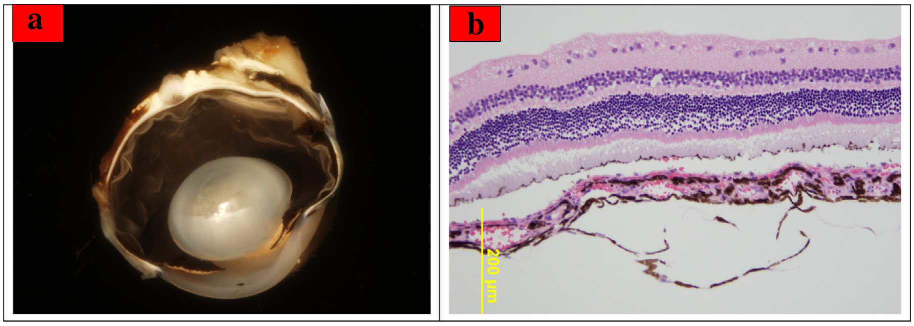

Fig. 3. Ocular pathology of snowshoe hares from the contaminated region. This gross ocular section (3a) revealed no abnormalities. The formalin fixation created an artefactual retinal separation (3b).

concentration of soil As levels in our study area was 1636.2 mg/kg around the mine area. Previous studies have shown that the natural levels of As in soils underlying the Yellowknife Greenstone belt can exceed 1500 mg/kg (Richardson, 2002). Specifically in the Yellowknife area, soil As concentration of 150 mg/kg has been regarded as the average concentration in soils with an upper limit of 300 mg/kg (Richardson, 2002). The likely reasons for the elevated levels of As and Cd in the soil and vegetation samples from the study area may be due to the influence of previous mining activities from the Giant mine and Con mine sites. In addition, the natural geology of the area, which is within the greenbelt zone is typically characterized with rich mineral deposits, of which As and other metals are present in elevated concentration as natural contaminants (Richardson, 2002). The elevated concentration of As and Cd noted in the soil and vegetation samples may likely increase the susceptibility of terrestrial wildlife species to chronic arsenicosis and increased Cd exposure due to ingestion of contaminated forage within the animal home range.

7.2. As and Cd bioaccumulation in snowshoe hares

There are no published data regarding the normal range of As and Cd levels in organ tissues of snowshoe hares from the Yellowknife area. However, in the Yukon Territory, Northern Quebec and Wabush mine in Labrador, baseline levels for As and Cd in snowshoe hares have been reported (Gamberg and Whitehorse, 2000; Bordeleau et al., 2016; Intrinsik, 2015). In addition, baseline concentration of As and Cd levels in organ tissues of other hare species, such as the European hares and Arctic hares have also been reported in similar studies (Amuno et al., 2016; Bukovjan et al., 2016; Škrivanko et al., 2008). Prior to our investigation, an earlier study conducted by Dr. Reimer[2] preliminarily investigated the speciation and concentration of total As in snowshoe hares at both the Giant mine and Con mine sites, and a background site next to the Ingraham trail, about 10 km northeast of Yellowknife. The results revealed that the concentration of total As in the muscle tissue of hares from contaminated sites (430–1720 μg/kg wet weight) ranged from 6.2–25 times the concentration of total As of the hares captured at the background site (69 μg/kg wet weight). The study also noted that the major As species identified in the hare tissue analyzed (muscle, kidney and liver) was the relatively non-toxic arsenical dimethylarsinous acid, and the minor As species identified in the hare tissues was monomethylarsonous acid, arsenite, arsenocholine and trimethylarsine oxide (Aurora Research Institute, 2012). We clarify here that the determination of arsenic species was outside the scope of our preliminary study. In our view, one of the major gaps that the results of our study has addressed was to provide baseline data regarding the biochemical responses associated with chronic As and Cd exposure, including measurements of As and Cd levels in the stomach content, nails and organ tissues of snowshoe hares in the Yellowknife area. As has a high affinity to sulfhydryl groups, and therefore it tends to accumulate in high amounts in nails, which is enriched in keratin (a cysteine containing protein) (Shen et al., 2013). As accumulates in the hair and fingernails more than in any other tissue, and thus both can be used as an effective marker of chronic As exposure (National Research Council, 1999). We specifically note here that our data regarding As and Cd levels in the nails is novel and can provide circumstantial evidence of chronic As and Cd exposure in snowshoe hares from the study area. The concentration of total As observed in renal and hepatic tissues of snowshoe hares from the Giant mine area was relatively higher than those reported in other Canadian locations and European cities (Table 5). The mean Cd concentration in renal and hepatic tissues of hares from the Giant mine area was noted to be lower compared to the values observed in hares from the Yukon Territory, but was also noted to be relatively higher than those reported in hares from the Wabush mine site in Labrador, including Czech and Croatia. In addition, the maximum concentration of hepatic Cd noted in the specimens from the Giant mine area was noted to be lower than those observed in Arctic hares inhabiting the vicinity of the former Nanisivik lead-zinc mine in Nunavut, Canada.[3]

In addition, it also important to note that sodium deficiency has been identified as major driver of soil-eating behaviour in herbivores, and because terrestrial plants tend not to accumulate sodium at high concentration, some small mammals, such as snowshoe hares may seek sodium directly from soil licks during seasons of high physiological activities, thus increasing oral intake of contaminated soils (Worker, 2013). Several studies have documented the detoxifying benefits of geophagy in animals (Finkelman, 2006; Mills and Milewski, 2007), but in recent years, other scholarly investigations have noted that geophagy may increase the risk of metal toxicity, including parasitic infections in exposed animals (Kutalek et al., 2010; Gilardi et al., 1999; Al-Rmalli et al., 2010). While this current study detected elevated levels of As and Cd in the nails of the hares from the mine area, which is reflective of chronic environmental exposure, it should be noted that the higher As and Cd accumulation observed in the stomach content was indicative of recent exposure due to increased ingestion of As and Cd contaminated forage or drinking water from the natural environment. We also clarify that the level of metals or metalloid in the stomach content of animals is typically used to estimate recent exposure, whereas levels

[2] The As data was not published in a scientific journal, but the raw laboratory results was submitted directly to the Aurora Research Institute, which was subsequently made public in the 2009–2010 compendia of research undertaken in the NWT (http://nwtresearch.com/sites/default/files/2009-2010-compendium-of-research-in-the-nwt.pdf).

[3] Please note that arsenic data on page 256 of the original paper stated the concentration of total arsenic in μg/g instead of ng/g. As a result, the authors of the paper have submitted clarification in form of a corrigendum to the editorial office indicating that none of the tissue samples analyzed in the study contained elevated concentration of arsenic. A corrigendum to Amuno et al. (2016) is currently being processed at the time of writing this publication.

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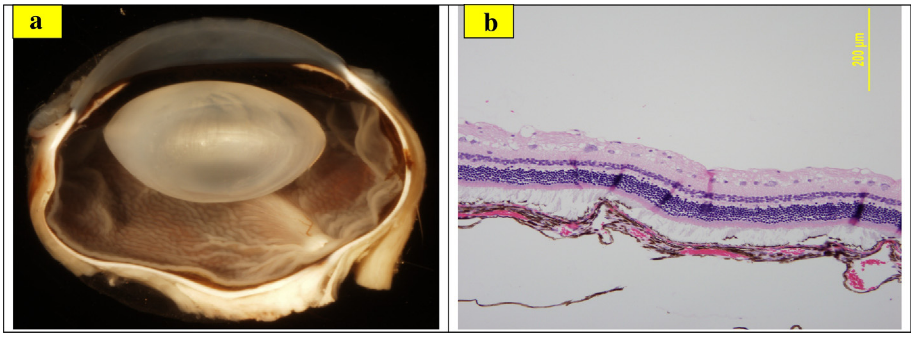

Fig. 4. Ocular pathology of snowshoe hares from the reference location. Gross evaluation (4a) and histological assessment (4b) revealed no abnormalities.

in keratin rich matrices, such as nails, skin and hair are considered reliable measures of chronic poisoning (Karagas et al., 2000). Therefore, our observation of elevated As and Cd concentration in stomach content of hares from the mine area is suggestive of a higher level of dietary intake of As and Cd (including incidental ingestion of soil, contaminated vegetation and water) overtime, which resulted in higher As and Cd concentration in the stomach content and different organ tissues (Worker, 2013; Rea et al., 2013; Hui, 2004).

7.3. As and Cd-induced biochemical changes

The SOD–CAT system provides the first line of defense against reactive oxygen toxicity with SOD catalyzing the dismutation of the superoxide anion radical to hydrogen peroxide, which is reduced to water and molecular oxygen by CAT activity (Radovanović et al., 2010). Simultaneous induction of SOD and CAT activities has been observed in biological organisms exposed to pollutants (Dimitrova et al., 1994). In the present study, hepatic activities of SOD and CAT were observed to be reduced in hares from the mine area in comparison to the reference location. The reduced activity of CAT may be attributed to the high production of superoxide anion radical, which can overwhelm and inhibit CAT activity (Dimitrova et al., 1994). GSH in addition to being a necessary cofactor for GPx and GST activities is an effective scavenger of oxyradicals (Rahman, 2007). The relatively higher GSH concentration observed in the hares from the reference site suggest an adaptive and protective role against oxidative stress induced by metals or metalloids. The lower hepatic concentration of GSH in the hares from the mine area may be indicative of the inability of the liver to successfully scavenge oxyradicals. GPx is important for protecting cells against oxidative damage by catalyzing the reduction of both organic and hydrogen peroxides, using GSH as a reducing agent (Mohamed et al., 2005). GPx converts peroxides and hydroxyl radicals into nontoxic forms often with the concomitant oxidation of reduced glutathione (GSH) into the oxidized form glutathione disulfide (GSSG) (Raffa et al., 2011). The results of our study showed that chronic arsenicosis and environmental exposure to low levels of Cd induces increased lipid peroxidation and impairs the antioxidant capacity of snowshoe hares from the study area. It is also likely that the decreased antioxidant enzymes including GPx activity in the liver may be responsible for the marked lipid peroxidation levels noted in snowshoe hares from the mine area (Polavarapu et al., 1998).

7.4. As and Cd-induced histopathological changes

The liver and eye tissues are target organs for As and Cd toxicity. Several hepatic and ocular abnormalities have been associated with chronic As and Cd exposure in both human and animal studies (Santra et al., 2000; Mazumder et al., 1997; See et al., 2011; See et al., 2007). In addition, liver is the primary site of As metabolism (Vahter, 2002), and also both eye and liver tissues have high lipid depositions, and thus are more susceptible to oxidative damage (Anderson et al., 1984; Ibrahim et al., 1997). The experimental study conducted by Nain and Smits (2012) for example showed that mice exposed to As contaminated water for 5 months exhibited both hepatocellular steatosis and depletion of hepatic antioxidant system. While no evidence of ocular abnormalities, such as cataracts or conjunctivitis was detected in the hares from our study area, it was interesting to note that our study only detected hepatic steatosis (fatty liver change) in the hares from both the mine area and the reference location. Steatosis is the accumulation of lipids within the liver cells and is likely that this pathology may have been influenced by oxidative stress and disturbance in antioxidants enzymes due to the increased accumulation of As and Cd in hepatic tissues (Liu and Waalkes, 2008; Santra et al., 2000). We also observed that despite the higher accumulation levels of As and Cd in tissues of hares from the mine area compared to the reference site, no distinct pathological changes were specifically observed in the livers of hares from the mine site as both groups exhibited fatty liver change. Given that fatty steatosis was

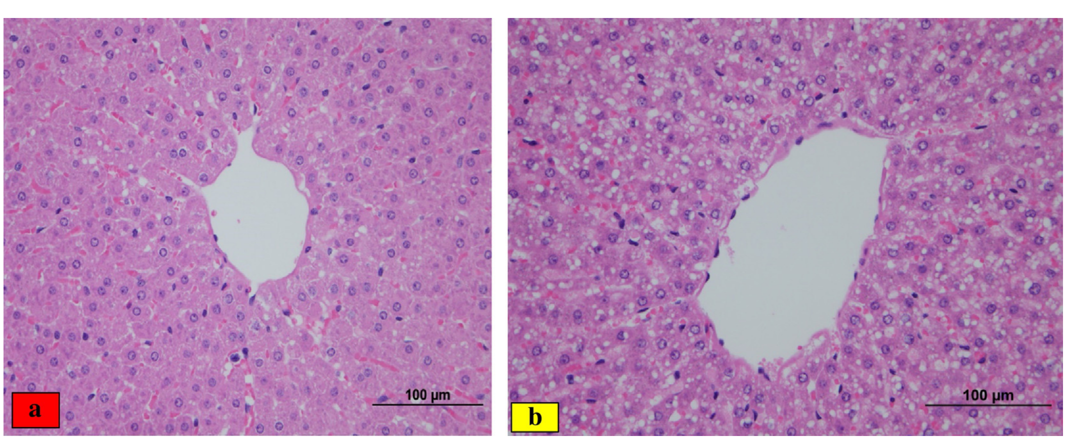

Fig. 5. Histological evaluation of hepatic tissues of snowshoe hares from the mine (5a) and reference areas (5b) revealed steatosis (fatty liver change).

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Table 5
Comparative levels of As concentration (μg/g) in hares species from Canadian locations and European countries.

Hare species	Locations	As concentration		Cd concentration		References
		As liver	As kidney	Cd liver	Cd kidney	
Lepus arcticus	Nanisivik mine, Nunavut, Canada ($n = 11$)	bdl-0.007	bdl-0.00378	0.018–3.44[d]	0.01[e]	Amuno et al. (2016)
Lepus americanus	Yukon[a], Canada					Gamberg and Whitehorse (2000)
	Haines Junction ($n = 5$)	0.08[b]	0.2	0.54	12.94	
	Old Crow ($n = 1$)	< 0.01	0.05	4.22	67.4	
	Ross River ($n = 6$)	< 1.71	< 1.02	2.6	19.55	
	Watson Lake ($n = 12$)	0.64	0.41	2.24	31.79	
	Whitehorse ($n = 4$)	0.56	0.33	0.12	8.77	
Lepus americanus	Wabush Mine, Labrador, Canada ($n = 10$)	<0.01–0.015 (0.0105)[f]	<0.01–0.015 (0.0105)[f]	0.0366–0.562 (0.174)	0.448–11.7 (4.55)	Intrinsik, 2015
Lepus americanus	Quebec, Canada	bdl	–	3.79[a]	–	Bordeleau et al. (2016)
Lepus europaeus	Croatia (winter; $n = 41$)	–	<0.002	–	0.938	Škrivanko et al. (2008)
Lepus europaeus	Croatia (spring; $n = 30$)	–	0.025	–	0.7	Škrivanko et al. (2008)
Lepus europaeus	Czech ($n = 105$)	0.0189	0.026	–	–	Bukovjan et al. (2016)
Lepus europaeus[c]	Czech	0.01–0.04	0.02–0.05	–	–	Bukovjan and Karpenko (1996)

bdl = below detection limit.
[a] (Data from Yukon and Quebec are in dry weight).
[b] ($n = 4$).
[c] Hares with malignant neoplastic disease.
[d] Number of livers analyzed ($n = 7$).
[e] Number of kidneys ($n = 3$).
[f] Mean.

common in hares from both locations it is likely that the pathology represented a background lesion in sub-adult snowshoe hares from the study area. In recognizing that our sample size was relatively small and limited to sub-adult hares, it is important to clarify that our histopathological findings, which mainly consisted of fatty liver change should not be considered representative of the entire pathology of snowshoe hares under chronic environmental exposure to As and Cd, especially for the study area.

8. Conclusions

The results of this present study confirmed that the snowshoe hares captured around the vicinity of the Giant mine area carried a relatively higher concentration of As and Cd in the organ tissues compared to the hares from the reference location. The relatively higher concentration of total As and Cd noted in the nails provided circumstantial evidence that the hares from the Giant mine area were chronically exposed to elevated levels of As over a long period of time with concomitant environmental exposure to Cd . Furthermore, increased levels of oxidative stress, and significant depletion of antioxidant enzyme activities were also noted in the hares from the mine area compared to the reference site. Despite the differences in the levels of As and Cd exposure between the two groups, the only pathology observed in the liver of hares from the study area was steatosis. Given that the sample size utilized for this biomonitoring study was relatively small, the data presented in this paper should be considered as a baseline information for future biomonitoring studies, in order to inform the public and residents regarding the status of As and Cd exposure in terrestrial wildlife species harvested from the Yellowknife area. There is also need for collection more samples from other wild furbearer species, in order to generate increased understanding of the prevalence of chronic arsenicosis and Cd exposure in terrestrial wildlife species from the Yellowknife area.

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