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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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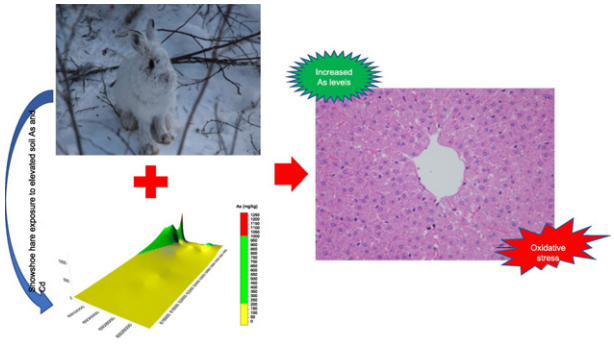
HIGHLIGHTS

- 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

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