Science of the Total Environment 704 (2020) 135426

Contents lists available at ScienceDirect

Science of the Total Environment

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

Neuropathological changes in wild muskrats (*Ondatra zibethicus*) and red squirrels (*Tamiasciurus hudsonicus*) breeding in arsenic endemic areas of Yellowknife, Northwest Territories (Canada): Arsenic and cadmium accumulation in the brain and biomarkers of oxidative stress

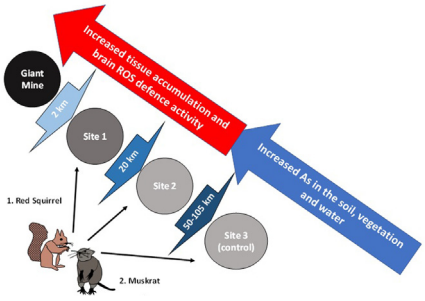
S. Amuno [a,*], K. Shekh [b,c], V. Kodzhahinchev [b], S. Niyogi [b,c]

[a] School of Environment and Sustainability, University of Saskatchewan, Saskatoon, Canada
[b] Department of Biology, University of Saskatchewan, Saskatoon, Canada
[c] Toxicology Centre, University of Saskatchewan, Saskatoon, Canada

HIGHLIGHTS

- As concentration in the nails of both species from arsenic endemic areas was higher.
- As was undetected in all the brains of muskrat from the study area.
- Cd was detected in brains of some muskrat brains close to the vicinity of the mine.
- As in the brains of squirrel from the arsenic endemic areas but not in reference site.
- Higher antioxidant activities in the brain of animals from the arsenic endemic areas.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 22 August 2019
Received in revised form 3 November 2019
Accepted 6 November 2019
Available online 26 November 2019

Editor: Filip M.G. Tack

Keywords:
Arsenic poisoning
Metal exposure
Brain damage
Neurological effect
Muskrats
Squirrels

ABSTRACT

The brain is one of the critical organs particularly susceptible to the damaging effects of chronic arsenic poisoning and there is a growing body of evidence that suggest that oxidative stress plays a key role in the pathogenesis of neurodegenerative disorders. The aim of this present work was to comparatively assess biomarkers of oxidative stress and status of antioxidant enzyme activities in the brains of muskrats and squirrels breeding in arsenic endemic areas, specifically near the vicinity of the abandoned Giant mine site (~2 km radius), and an intermediate location approximately 20 km from the mine area and in reference locations spanning 52–105 km from the city of Yellowknife, Northwest Territories (Canada). Analysis included measurement of total arsenic and cadmium concentration in the nails, brain, and stomach content of muskrats and squirrels, in addition to biochemical evaluation of lipid peroxidation levels and antioxidant enzymes defense: catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) in the brain tissues. The results revealed that arsenic concentration in the nails of muskrats collected closest to the vicinity of the mine area was in the range of 11 to 35.1 times higher than those from the reference site. The maximum concentration of arsenic in the nails of muskrats from the intermediate location was 47.6 times higher than the maximum concentration observed in the reference muskrats. Cadmium was generally undetected in the nails of muskrats and squirrels from the three sampling locations. Arsenic in the gut contents of muskrats from the arsenic affected area was 4.5 to 49.1 times higher than those from the reference site. Cadmium levels in the guts of muskrats from the mine area almost doubled those from the reference site. Arsenic accumulated in the nails of squirrels

* Corresponding author.
E-mail address: soa882@usask.ca (S. Amuno).

https://doi.org/10.1016/j.scitotenv.2019.135426

from the areas closest to the mine but was undetected in the squirrel nails from the reference location. The maximum arsenic levels in the stomach content of squirrels from the mine area was ~40 times higher than those from the reference site. Arsenic did not accumulate in the brains of muskrats, but cadmium was detected in a few brains of muskrats. Brains of squirrels from the mine area and intermediate locations accumulated both arsenic and cadmium. The brains of squirrels and muskrats from the arsenic affected area showed no evidence of increased lipid peroxidation compared to the animals from the reference site. However, SOD, CAT and GPx activities in the brains of animals from the arsenic endemic areas tended to be higher compared to the control sites. This is the first study documenting evidence of oxidative stress and altered antioxidant enzyme activities in brains of wild rodent population in arsenic endemic areas of Canada.

1. Introduction

Arsenic (As) is a non-essential element that is widely distributed in the natural environment (Strawn, 2018). Its occurrence is closely linked with geologic sources and can be exacerbated by anthropogenic influences such as industrial actvities, particularly mining (Bhattacharya et al., 2007; Pothier et al., 2018). Arsenic contamination is often associated with historic gold mining activity since arsenic commonly occurs in the ore of gold bearing metal sulfide deposits (Cott et al., 2016). Several studies have reported elevated concentrations of arsenic in groundwaters, soils and vegetation in environments associated with gold mining activities (Ko et al., 2003; Kim et al., 2005; Huang et al., 2019). Chronic exposure of human and animal populations to high concentrations of arsenic have been reported in arsenic endemic areas of Iran (Mosaferi et al., 2003), Bangladesh (Mori et al., 2018) and Yellowknife (Canada) (Amuno et al., 2018; Clark and Raven, 2004). Such chronic exposures have been associated with multiorgan damage, including skin lesions, peripheral vascular disease (PVD), blackfoot disease, cataracts, cancers and metabolic syndrome including bone abnormalities and osteoporosis (Tseng et al., 2005; Chen and Chiou, 2011; Yoshida et al., 2019; Amuno et al., 2018).

Epidemiological research and experimental studies have also linked chronic arsenic exposure with damage to Central Nervous System (CNS) with concomitant symptoms including olfactory dysfunction, parkinsonian-like symptoms, slower vasomotor functioning, peripheral neuropathy and cognitive impairment (Tyler and Allan, 2014). In addition, cognitive dysfunction and severe brain damage including many neurodegenerative diseases and psychiatric disorders have been linked with prolonged arsenic ingestion (O'Bryant et al., 2011; Tyler and Allan, 2014). There is also growing evidence that suggests that arsenic exerts its neurotoxic effects on the brain through the generation of reactive oxygen species (ROS) and free radicals (Rao and Avani, 2004) inducing perturbation of the enzymes involved in the antioxidant defense system and causing lipid peroxidation in the brain (Talukdar, 2013). Studies have shown that lipid peroxidation is one of the major sources of free radical mediated injury that can directly damage neuronal membranes and yields a number of secondary products responsible for extensive cellular damage of brain tissues and CNS (Lobo et al., 2010). Increased lipid peroxidation in brain tissues and body fluids have been linked to many neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Down's syndrome (DS) and Parkinson's disease (PD) (Barrera et al., 2018). Some studies have also suggested that alterations in antioxidant enzymes activities may play a role in the etiopathogenesis of CNS disorders (Scarisbrick, 2008). Similarly, a deficit in the brain antioxidant defense systems have been associated with Alzheimer's disease (AD) (Ruszkiewicz and Albrecht, 2015).

Given that Arsenic is ubiquitous in the natural environment there are concerns that wildlife, particularly rodent species foraging in arsenic contaminated areas may be at risk of arsenic-induced neurological effects due to increased levels of oxidative stress caused by chronic arsenic exposure (Chung et al., 2014; Eisler, 2004). Terrestrial and aquatic species may accumulate arsenic and other metals directly from the food and surrounding water and vegetation, and may bioconcentrate large amounts of such contaminants in their tissues (Ishii et al., 2017). While most scientific investigations on arsenic-induced neurotoxicity has focused on humans and laboratory rodents (Islam and Khanna, 2009; Yadav et al., 2009), investigations focused on wild populations are relatively scarce. To the best of our knowledge, no study has investigated the status of brain oxidative stress in wild rodent populations breeding in arsenic endemic areas of Canada.

The aim of this present work was to comparatively assess the status of Arsenic (As) and Cadmium (Cd) exposure in wild rodent populations (i.e. muskrats and squirrels) breeding in arsenic endemic areas of Yellowknife, near the vicinity of the abandoned Giant mine site, and to compare them with the background locations. Another focus of this study was to further compare and determine the markers of oxidative stress and antioxidant enzyme activities in the brains of the animals from the arsenic affected areas and the background locations. Our rationale for measuring concentrations of As and Cd including markers of oxidative stress in the brain tissues was because As is known to cause increased production of free radicals, like reactive oxygen species (ROS), and the brain is one of the critical organs particularly susceptible to the damaging effects of ROS, which can result in cognitive deficit and neurodegenerative diseases. The present investigation was built on the results of our previous study which found evidence of chronic arsenicosis as well as increased hepatic oxidative stress levels and prevalence of bone abnormalities in wild population of snowshoe hares breeding near the vicinity of Yellowknife (Amuno et al., 2018). Despite these biochemical changes and osteopathological findings, our understanding of the impact of chronic arsenicosis on brains of wildlife species in arsenic endemic areas is still very limited.

2. Description of the study area

Yellowknife has a long history of gold mining activities with three mines that operated within the city between 1938 and 2004: Con Mine (1938–2003) and Negus Mine (1939 –1952) in the south and Giant Mine (1948–2004) in the north (Thienpont et al., 2016). These periods of gold mining particularly during the early operations of the Giant mine caused historical release of As and other toxic metals into the terrestrial and aquatic ecosystems due to milling and ore-roasting of arsenopyrite (Thienpont et al., 2016). The introduction of pollution control equipment in the 1950's for the collection of arsenic fumes and dust from the roaster exhaust reduced As air emissions dramatically from Giant mine

site; however, this also resulted in the collection of approximately 237,000 tonnes of by-product predominantly arsenic trioxide dust from gold ore extraction and roasting processes throughout the operations of the mine (GMRP 2018). After the cessation of mining activities at the Giant mine site in 2004, efforts were made to collect and store this large amount of arsenic trioxide in several frozen underground chambers in the mine, making this one of the worst toxic legacies in Canada. The potential for this huge amount of As to be discharged into the wider environment poses serious risks to biodiversity and is a major public health concern for many Yellowknife residents. While the potential health effects of chronic arsenic exposure have been reported in various parts of the world, As contamination in the terrestrial and aquatic ecosystems of Yellowknife have received a great amount of attention (Jamieson, 2014), and there are ongoing studies now focusing on wildlife responses to chronic As exposure in the environment (Amuno et al., 2018). In this study, we chose wild muskrats (*Ondatra zibethicus*) and red squirrels (*Tamiasciurus hudsonicus*) as model species because both of these species are widely distributed across Northern Canada; hence, they are an appropriate choice for examining the As-induced toxicity associated with proximity to the Giant Mine site. Moreover, muskrats are semi-aquatic organisms, whereas, squirrels are fully terrestrial. In other words, these two species represent different ecological niches; hence, studying both species could provide a better perspective on the importance of different routes of environmental As exposure to mammalian communities in the Northwest Territories. Finally, both species have been reported to exhibit narrow home-ranges, which allowed us to assume that the organisms captured in this study were actually representative of the sites where they were captured (Ahlers et al., 2010; Gurnell, 1984).

3. Assessment of chronic arsenicosis in muskrats and red squirrels

The diagnosis of chronic arsenicosis in the wild muskrats and squirrels followed the definition and criteria described in previous studies (Amuno et al., 2018; Saha, 2003; Bertin et al., 2013; Mandal, 2017; Datta et al., 2012; Nain and Smits, 2012) and included the following assessment:

(1) Arsenic exposure data: Given that muskrats are semi-aquatic, and squirrels are herbivores animals, environmental exposure data mainly considered the measurements of As and Cd concentrations in surface soil, surface water and vegetation samples from the study area. Our previous investigation reported elevated concentrations of As in the soils and vegetation samples from ~2 to 20 km radius of the vicinity of the Giant mine site (Amuno et al., 2018). In addition, a previous investigation of up to 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 As exposure: Evidence of prolonged As exposure was obtained through the measurement of total As levels in the nail samples of muskrats and squirrels from As endemic areas and the reference location. Cd was also measured in the nails of all the animals from the study area similar to our previous study (Amuno et al., 2018).
(3) Biomarker of current exposure: The current exposure status of the animals was determined through measurement of total As and Cd levels in the stomach content of muskrats and squirrels from the study area similar to our previous study (Amuno et al., 2018).

Other key biochemical parameters relevant to arsenic toxicity such as lipid peroxidation levels and antioxidant enzyme capacity were also determined in tissues of the animals.

4. Materials and methods

4.1. Sampling

A wildlife research permit (WLWL500561) and ethical clearance for wildlife handling was obtained from the Department of Environment and Natural Resources, Government of the Northwest Territories as well from the University of Saskatchewan. A general research licence (No. 16190) was also obtained from the Aurora Research Institute prior to commencement of the field study. We sampled a total of 30 muskrats and 30 squirrels from three locations across the project area (Fig. 1a, b). Only adult muskrats and squirrels were utilized for this study. The criteria for assessment of the age of individual organisms were based on body weight and length. The criteria for adult squirrel was an overall body length between 10 and 15 in. and weight between 260 and 350 g (Pennsylvania State University, 2002), whereas the criteria for adult muskrat was the weight between 2.5 and 4 lb with total body length ranging from 23 to 26 in. and with a tail length of 8–11 in. (New York Department of Environmental Conservation). Animals were generally trapped from the vicinity of the abandoned mine (within ~2 km radius; referred to as site 1) as well as in an intermediate location approximately 20 km from the Giant mine site (referred to as site 2) and from a background/reference area (referred to as site 3). The muskrats used for reference were trapped in areas between ~53.4 km to 62 km from Yellowknife. The squirrels used for reference were trapped from areas between 95 km and 105 km away from the city of Yellowknife. Trapping of animals took place between March and April 2018. Similar to our previous study, since access to the Giant mine site was not possible at the time of the fieldwork, a certain proxy location near the mine area was used as a central point for estimating the distances of the sampling points to the Giant mine area (Amuno et al., 2018).

Various tissue samples from squirrels and muskrat were obtained through the legal harvest of the animals from a local furbearer hunter in Yellowknife. Samples kits for proper tissue storage and sampling instructions were sent to the hunter prior to the hunting season. All trapped animals were euthanized and dissected, with target organs being collected and separated in individual falcon tubes and frozen. Samples were then shipped back to the lab at the University of Saskatchewan for biochemical and metal analysis. All animal waste and carcasses were stored in MS biobags (100% biodegradable) and transported to the landfill in Yellowknife for final disposal. In order to establish the naturally occurring background levels of trace elements and to assess the extent of contamination, additional soil and vegetation samples (i.e. willows, grass plants and cattails) including water samples were collected from the arsenic affected areas and reference location and subsequently analyzed. The GPS locations of where each soil, vegetation and animal samples were collected were recorded in relation to distance from the Giant mine area (Amuno et al., 2018).

4.2. Analysis of arsenic and cadmium in brain, nails, gut content, soil and vegetation samples

Brain and gut contents of muskrats and squirrels were digested in polyethylene conical centrifuge tubes for 48 h in 5 volumes of 1 N trace metal grade Nitric acid (EMD Millipore, Billerica, MA, USA) at 60 °C. The nail samples were digested similarly in concentrated nitric acid (16 N). After digestion, tissue digests were centrifuged at 2500*g* for 10 min and supernatants were collected.

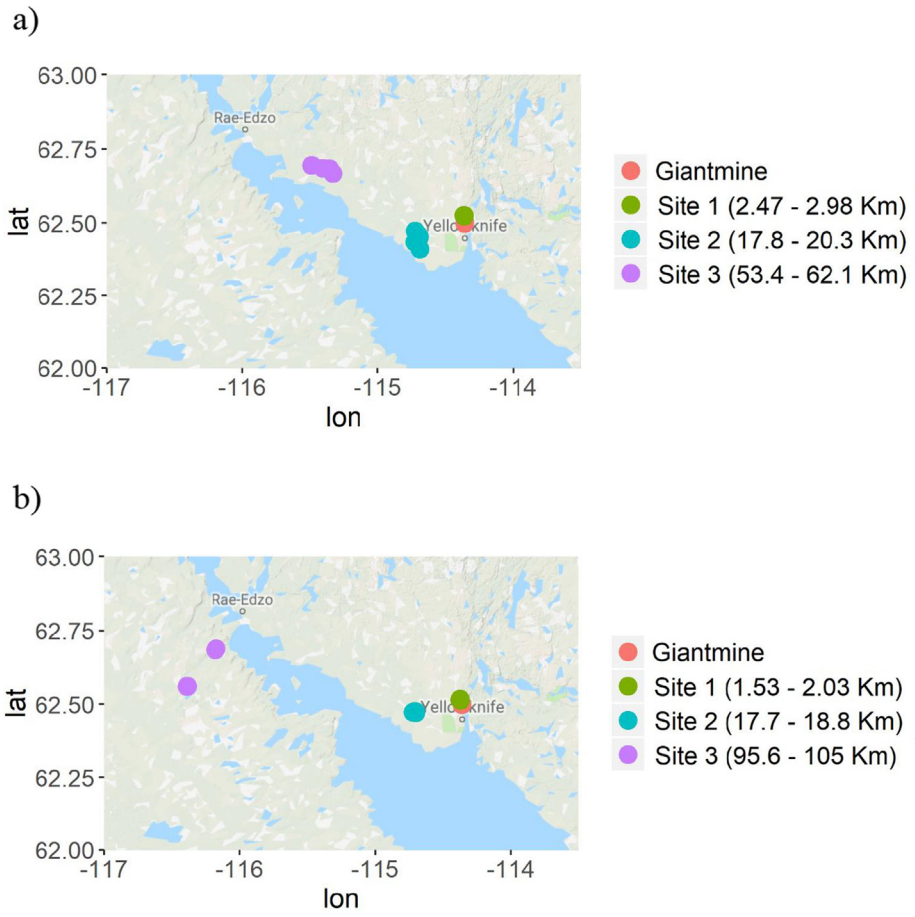


Fig. 1. Map of study area showing specimen collection and mining area. a) Muskrat b) squirel.

The concentrations of As and Cd in supernatants were measured using a graphite furnace atomic absorption spectrometer (AAnalyst 800, Perkin Elmer, USA) after appropriate dilution of samples in 0.2% nitric acid. For quality control and assurance purposes, appropriate method blanks, certified Cd and As standards (Fisher Scientific, Canada), and a reference material (DOLT-4; National Research Council of Canada) were included in the measurements. The recovery percentage of Cd and As in the reference material was 96% and 92%, respectively. The trace metal concentrations in animal tissues were normalised on the basis of wet tissue weight.

Surface soils and vegetation samples (e.g. cattail, mushrooms, and blade grass) were collected from the study area and in proximity to the three (3) sampling locations for the animals. The soil and plant samples were all shipped to a commercial laboratory (ACME laboratory, Vancouver) for trace element analysis as outlined in Amuno et al. (2018). Water samples were also collected from the three (3) muskrat sampling stations and analyzed for total As and Cd using graphite furnace atomic absorption spectrometer (AAnalyst 800, Perkin Elmer, USA)

4.3. Lipid hydroperoxide (LPO) assay

Lipid peroxidation in both species were analysed directly by measuring lipid hydroperoxide (LPO). Muskrat and squirrel brain tissues were homogenised in HPLC- grade water on ice and treated with the Lipid Hydroperoxide Assay kit (ab133085, Abcam Inc, ON, Canada) according to the manufacturer's specifications. Samples were then loaded onto clear 96 well plates and measured for their 500 nm absorbance using a multimode plate reader (Verioskan Flash, Thermo Scientific, ON, Canada). All samples were run in duplicates, taking the average of the two values as the final concentration. LPO concentrations were then normalised against wet tissue weight.

4.4. Cellular thiol redox balance (GSH:GSSG ratio)

GSH and GSSG levels were measured in brain tissues of muskrats and squirrels using a previously described method (Jamwal et al., 2016). Briefly, brain tissue homogenates were centrifuged at 10,000*g* at 4 °C for 10 min and the supernatants were collected and separated into two parts, labelled as GSSG fraction (200 μL) and GSH fraction (remaining supernatant). N-ethylmaleimide (0.04 M) was added to the GSSG fraction and stored at room temperature for 15 min. GSH content was measured in 96 well plates and the final reaction mixture included 180 μL of phosphate–EDTA buffer, 10 μL of ophthalaldehyde (OPT, 100μg per 100 μL of methanol), and 10 μL of sample. The final reaction mixture for GSSG measurement was 140 μL of 0.1 N NaOH 20 μL of OPT, and 40 μL of sample. The measurement of GSH and GSSG was based on fluorometric method with the excitation and emission wavelengths of 350 and 450 nm, respectively. GSH and GSSG concentrations were normalized to protein content for respective brain sample. Protein concentrations in the brain tissues were measured by Bradford reagent (Sigma, Canada, catalogue # B6916).

4.5. Antioxidant enzyme activity measurements

Antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities were measured

in brain tissues of muskrats and squirrels using commercially available kits for each enzyme (Cayman chemical company, USA). Briefly, brain homogenates were centrifuged at 25,000*g* for 20 min at 4 °C and supernatants were collected and separated into three fractions (SOD, CAT, GPx) and the activities of each enzyme was measured according to the protocols provided by the manufacturer (Catalogue #s 706002, 707002 and 703102, respectively). Activities for each enzyme were normalized to protein content of the brain tissue and the protein concentration in each tissue was measured by Bradford reagent.

4.6. Statistical analysis

The concentrations of As and Cd in the soil, water, vegetation samples as well as animal tissues including the brain biochemical analysis endpoints (LPO content, SOD, CAT and GPx activities) were compared between the reference site (site 3) and As endemic sites (site 1 and site 2; 2 km and 20 km away from Giant mine, respectively) using T-tests. If the assumptions of equal variance and normal distribution for any of the analysed data sets was not matched, a non-parametric Mann-Whittney *U* test was used instead. Analysis was carried out using SigmaPlot11 (SystatSoftware Inc., San Jose, California, USA). The relationship between all the parameters analysed in this study was assessed by applying two tailed Pearson correlation analysis in R software (version 3.5.1) (R Core Team, 2018). Correlation tables were created by using 'corrplot' package in R. In all statistical procedures, a p-value of $\leq$0.05 was considered to be statistically significant.

5. Results

5.1. As and Cd contamination of soil and vegetation

Information regarding the contamination status of the study area was obtained through measurement of As and Cd in the soils, including vegetation and surface water samples, which were collected from three (3) different locations relative to the Giant mine site in Yellowknife (Table 1). The soil samples collected closest to the vicinity of Yellowknife, (site 1; 0.8–2 km radius of the Giant Mine) showed elevated soil concentration of As ranging from 74.6 mg/kg to 400.2 mg/kg. All the vegetation samples collected from the area similarly showed elevated concentrations of As that ranged from 21.9 mg/kg to 727.2 mg/kg except for three samples that showed relatively low concentration of As between 0.2 mg/kg to 1.8 mg/kg. Cd concentration in the soils near the vicinity of the mine site ranged from 0.2 mg/kg to 0.7 mg/kg, while in vegetation samples Cd ranged from 0.04 mg/kg to 1.13 mg/kg. In the intermediate location (site 2; approximately 20 km from Giant mine), As levels ranged from 0.6 mg/kg to 54.3 mg/kg in soils and 0.2 mg/kg to 7.8 mg/kg in vegetation samples. Cd concentration in the soils was generally below detection limit in some samples but up to a maximum of 0.8 mg/kg in a few samples, whereas in the vegetation Cd ranged from below the detection limit (<0.05 μg/L) to1.13 mg/kg. For the reference site (site 3; approximately 50–105 km from Giant mine), As levels in the soils ranged from 0.9 mg/kg to 10.3 mg/kg, whereas in the vegetation As was below detection limit (<0.1 μg/L) in many of the samples with the exception of 8 samples where As concentration ranged from 0.2 mg/kg to 0.6 mg/kg. In the reference area, Cd concentration in the soils ranged from below detection limit to 0.7 mg/kg, and in vegetation it ranged from 0.34 mg/kg to 3.15 mg/kg.

5.2. As and Cd levels in surface water

The water samples collected from the vicinity of the Giant mine area contained elevated levels of As ranging from 110.3 μg/L to 213.6 μg/L, which was more than 10–20 times the permitted levels (10 μg/L). At the intermediate location, approximately 20 km from the mine site, As concentration in the water samples ranged from 10.14 μg/L to 23.58 μg/L. As was not detected in any of the water samples collected from the background area. Cd was generally below detection limit of 0.05 μg/L in the water samples from the three locations.

5.3. Accumulation of as and Cd in the nail samples of muskrats and squirrels

Evidence of chronic exposure to As was obtained through detection of total As levels in the nails of muskrats and squirrels from the study area (Fig. 2 a-b). Total As levels in the nails of muskrats from the reference location (site 3) ranged from below detection to the maximum of 0.063 μg/g. As concentration in the nails of muskrat from the intermediate location (site 2) ranged from below detection to 3.02 μg/g, whereas those from closest to the mine area ranged from 0.66 μg/g to 2.1 μg/g. As concentration in the nails of muskrats from the mine area (site 1) was in the range of 11 to 35.1 times higher than those from the reference site. The maximum concentration of As in nails in the muskrats from the intermediate location was 47.6 times higher than the maximum concentration observed in reference muskrats. Cd was not detected in the nails of muskrats and squirrels from the all study sites. For the muskrats, statistical differences were observed in relation to arsenic accumulation as evidenced by nail As levels in the muskrats from the mine area and the reference location ($p = 0.003$), as well as between the muskrats from the intermediate location and reference area ($p = 0.001$) (Table 2). For the squirrels, *t*-test results indicate a significant difference in relation to arsenic accumulation (nail arsenic levels) between the squirrels from the mine area (site 1) and the intermediate location ($p = 0.004$) as well as between the squirrels from the mine area and the reference area ($p = 0.01$) (Table 3).

5.4. Levels of As and Cd in gut content of muskrats and squirrels

The levels of As in the stomach content of muskrats from the mine area ranged from 0.15 μg/g to 24.09 μg/g, while that from the intermediate location was between 0.2 μg/g to 2.53 μg/g and those from the reference site ranged from 0.033 μg/g to 0.49 μg/g (Fig. 3a). As in gut contents of muskrats from the mine area

Table 1
Measured concentrations of As and Cd in surface soils, vegetation, and water from three different sites near mining area. Site 3 represents reference site, site 2 represents intermediate location (~20 KM from mine), and site 1 represents locations in the vicinity of mine (within ~2 KM radius). The levels that were below detection limit are indicated as "Bdl".

Site	Surface soils (mg/kg)		Vegetation (mg/kg)		Water (μg/L)	
	As	Cd	As	Cd	As	Cd
1	74.6–400.2	0.2–0.7	21.9–727.2	0.04–1.13	110.3–213.6	Bdl
2	0.6–54.3	Bdl – 0.8	0.2–7.8	Bdl – 1.13	10.14–23.56	Bdl
3	0.9–10.3	Bdl – 0.7	Bdl – 0.6	0.34–3.15	bdl	bdl

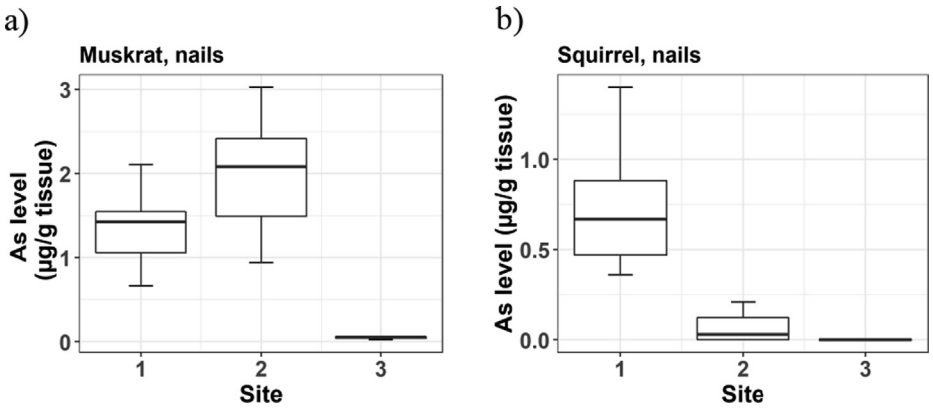


Fig. 2. Boxplots on the complete data for metal accumulation in the nails of muskrats and squirrels collected from three different sites, where site 3 represents reference site, site 2 represents intermediate location (~20 KM from mine), and site 1 represents locations in the vicinity of mine (within ~2 KM radius). The sample size (*n*) was 8 for each group. The closed circles represent outliers. As was below detection limit in nails of most squirrel samples from site 3. For both species, Cd was not detectable in nails.

Table 2
Statistical analysis of muskrat metal accumulation in the brain, nail and gut content compared with t-tests between sites 1, 2 and 3. Analysis is reported as t(df)=; p = for parametric tests, with only the p value reported if a non-parametric Mann-Whitney *U* test was used (i.e. if the assumptions of normality or equal variance between sites were not met). * indicates significant difference.

	1vs2	1vs3	2vs3
Cd (brain)	p = 0.31	p = 0.31	p = 1
As (brain)	p = 1	p = 1	p = 1
As (nail)	t(14) = 1.1019; p = 0.326	p = 0.003*	p = 0.001*
Cd (gut)	p = 0.015*	t(14) = 2.038; p = 0.61	p = 0.161
As (gut)	p = 0.021*	p = 0.001*	p = 0.007*

Table 3
Statistical analysis of squirrel metal accumulation in the brain, nail and gut content compared with t-tests between sites 1, 2 and 3. Analysis is reported as t(df)=; p = for parametric tests, with only the p value reported if a non-parametric Mann Whitney *U* test was used (i.e. if the assumptions of normality or equal variance between sites were not met). * indicates significant difference.

	site 1 vs site 2	site 1 vs site 3	site 2 vs site 3
Cd (brain)	t(8) = 0.657; p = 0.530	t(8) = 3.838; p = 0.005*	t(8) = 2.951; p = 0.018*
As (brain)	t(8) = 1.421; p = 0.193	t(8) = 1.812; p = 0.108	p = 0.008*
As (nail)	t(15) = 3.405; p = 0.004*	p = 0.01*	p = 0.096
Cd (gut)	t(15) = 1.906; p = 0.076	t(14) = 2.381; p = 0.032*	p = 0.266
As (gut)	p = 0.663	p = 0.065	p = 0.11

was 4.5 to 49.1 times higher than those from the reference site and was also noted to be statistically different between the two groups (Table 2). Concentration of As in the gut content was also significantly different between the muskrats from the mine area and intermediate location (p = 0.021) (Table 2). The results suggest that muskrats closest to the mine area accumulated higher levels of As in their gut contents compared to the other two locations. Cd levels in the guts of muskrats from the mine area ranged from 0.003 µg/g to 0.029 µg/g, while that from the intermediate location was between 0.0018 µg/g to 0.017 µg/g and those from the reference site ranged from 0.003 µg/g to 0.012 µg/g (Fig. 3c). Cd levels in the guts of muskrats from the mine area almost doubled those from the reference site and was significantly differred between the two groups (p = 0.015) (Table 2). The maximum As concentration in the gut content of squirrels from the reference area was 0.44 µg/g, while those from intermediate site (site 2) was 9.81 µg/g; and those from site 1 was 17.66 µg/g (Fig. 3b). The maximum As levels in the gut content of squirrels from the mine area was ~40 times higher than those from the reference site, but was not statistically different between the three groups (p greater than 0.05) (Table 3). Cd levels in the guts of squirrels did not differ between the groups except for the squirrels from the mine area (site 1) and the reference location (p = 0.032) (Fig. 3d and Table 3).

5.5. Accumulation of As and Cd in the brains of muskrats and squirrels

As was undetected in all the brains of muskrats from the study area; however, Cd was detected in two brain samples of muskrats closest to the mine area (0.0018 µg/g–0.0024 µg/g). As was detected in the brains of squirrels from the mine area (site 1; 0.06–4.18 µg/g) and intermediate location (site 2; 0.072–0.95 µg/g) and was undetected in the squirrel brain samples from the reference site (site 3) (Fig. 4a). A significant difference was observed in As accumulation in the brain samples of squirrels from the intermediate and reference location (p = 0.008) (Table 3). Cd accumulated in the brains of squirrels from the three locations, but statistical differences were only noted between samples from the mine site area versus reference site (p = 0.005) and intermediate versus reference site (p = 0.018) (Table 3; Fig. 4b).

5.6. Brain antioxidant enzyme activities and oxidative stress indicators in muskrats and squirrels

The status of antioxidant enzyme activities and oxidative stress indicators were determined in the brains of muskrats and squirrels from all the three study sites. The general trend in LPO data indicated a slight increase at the reference site as compared to the mining site in case of both muskrat and squirrel (Fig. 5a and 6a), which was unexpected based on metal (loid) accumulation data which was more pronounced near the vicinity of the mine area. Nonetheless, the increase in LPO at reference site was not statistically significant as compared to any other study site (Tables 4 and 5). In general, an increase in the activities of enzymes such as SOD, CAT, and GPx was noted at mining site (site 1) as compared to site 2 and site 3; however, the statistically significant results were only observed in the case of GPx in squirrel brain (Figs. 5 and 6; Tables 4 and 5).

5.7. Correlation results

A two-tailed Pearson correlation was also presented to further show the relationships between environmental exposure data, including As and Cd levels in the nails and gut contents, antioxidant enzyme activities and LPO levels in the brains of the animals

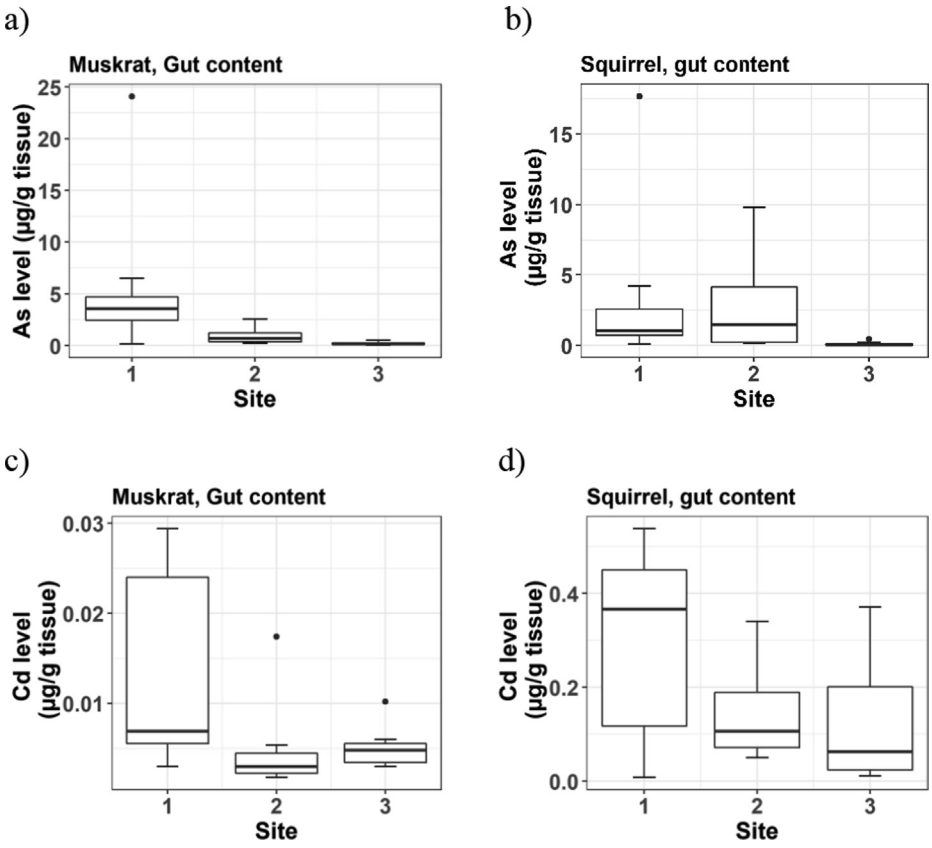


Fig. 3. Boxplots on the complete data for metal accumulation in the gut contents of muskrats and squirrels collected from three different sites, where site 3 represents reference site, site 2 represents intermediate location (~20 KM from mine), and site 1 represents locations in the vicinity of mine (within ~2 KM radius). The sample size (n) was 8 for each group. The closed circles represent outliers.

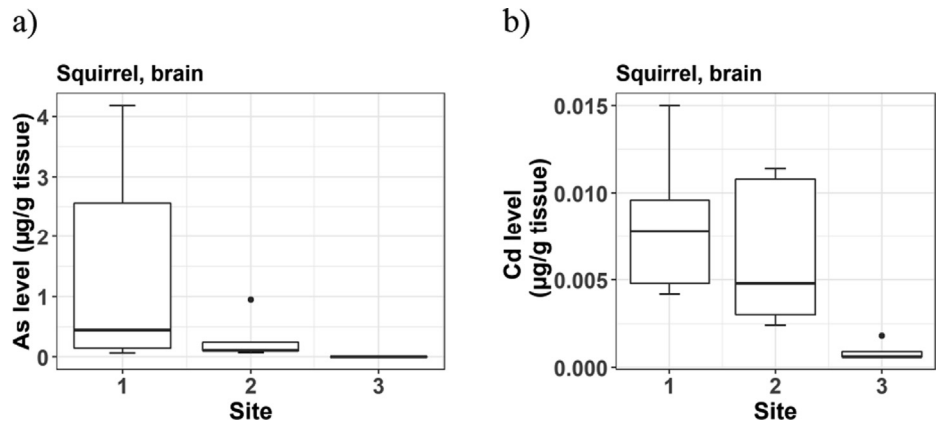


Fig. 4. Boxplots on the complete data for metal accumulation in the brains of squirrels collected from three different sites, where site 3 represents reference site, site 2 represents intermediate location (~20 KM from mine), and site 1 represents locations in the vicinity of mine (within ~2 KM radius). The sample size (n) was 5 for each group. The closed circles represent outliers. As was below detection limit in brain of squirrel from site 3. Metals were undetectable in muskrat brains.

(Figs. 7 and 8). For muskrats, distance showed a significant negative correlation with As concentration in the nails (−0.673), as well as concentrations of As (−0.74) and Cd (−0.75) levels in the soils, which suggest that proximity of the muskrats to the arsenic endemic areas may have strongly contributed to the incidence of chronic arsenicosis (Fig. 7). In addition, a high positive and significant correlation was observed between distance from the mine site and lipid peroxidation (0.52) which suggest that proximity and exposure to As and Cd contaminated areas might have played a key role in enhancing the rate of lipid peroxidation in exposed muskrats. For squirrels, correlation analysis showed a significant negative correlation between brain LPO levels and As levels in soils (−0.55), Cd concentration in vegetation (−0.64) and Cd levels in the gut content (−0.56). These negative correlations observed between these parameters may be indicative of the influence of environmental contamination and the role of oral exposure as a pathway for increasing brain lipid peroxidation. Some antioxidant enzyme activities such as CAT showed a strong correlation with Cd levels in the brain (0.66), which suggest that Cd exposure through the environment might also be affecting the activity of this

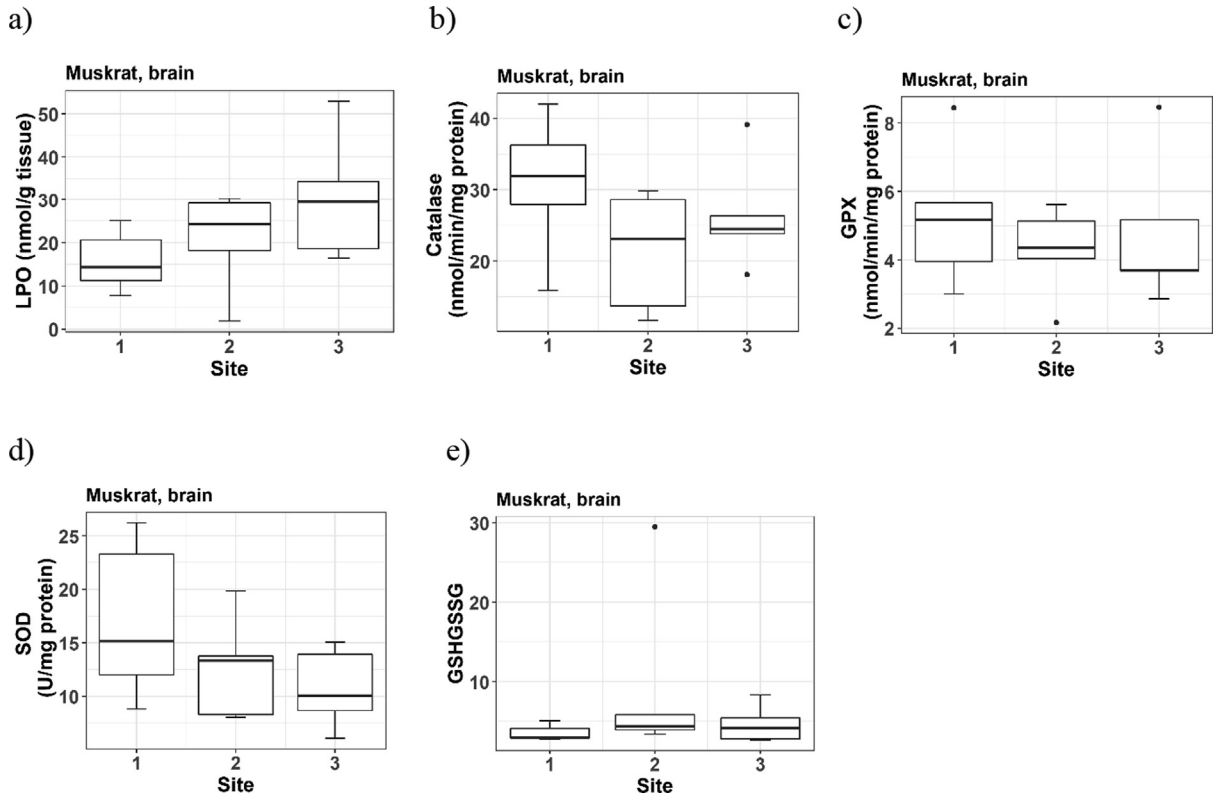


Fig. 5. Boxplots on the complete data for oxidative stress parameters in the brains of muskrats collected from three different sites, where site 3 represents reference site, site 2 represents intermediate location (~20 KM from mine), and site 1 represents locations in the vicinity of mine (within ~2 KM radius). The sample size (*n*) was 5 for each group. The closed circles represent outliers. (a) LPO – lipid hydroperoxides; (b) catalase; (c) GPX – glutathione peroxidase; (d) SOD – superoxide dismutase; (e) GSH:GSSG – reduced to oxidized glutathione ratio.

Table 4
Statistical analysis oxidative stress parameters in the muskrat brains compared with t-tests between sites 1, 2 and 3. Analysis is reported as t(df)=; p = for parametric tests, with only the p value reported if a non-parametric Mann-Whitney *U* test was used (i.e. if the assumptions of normality or equal variance between sites were not met). * indicates significant difference.

Parameter	Sites	Test statistics	Statistical significance
LPO	Site 1 vs Site 2	t = −0.815, P = 0.439	No
	Site 1 vs Site 3	t = −2.001, P = 0.080	No
	Site 2 vs Site 3	t = −1.148, P = 0.284	No
GSH:GSSG	Site 1 vs Site 2	P = 0.151	No
	Site 1 vs Site 3	t = −0.973, P = 0.359	No
	Site 2 vs Site 3	P = 0.548	No
CAT	Site 1 vs Site 2	t = 1.628, P = 0.142	No
	Site 1 vs Site 3	t = 0.788, P = 0.453	No
	Site 2 vs Site 3	t = −0.978, P = 0.357	No
SOD	Site 1 vs Site 2	t = 1.117, P = 0.296	No
	Site 1 vs Site 3	t = 1.707, P = 0.126	No
	Site 2 vs Site 3	t = 0.702, P = 0.503	No
GPX	Site 1 vs Site 2	t = 0.897, P = 0.396	No
	Site 1 vs Site 3	t = 0.350, P = 0.735	No
	Site 2 vs Site 3	t = −0.441, P = 0.671	No

Table 5
Statistical analysis oxidative stress parameters in the squirrel brains compared with t-tests between sites 1, 2 and 3. Analysis is reported as t(df) and p = p-value. * indicates significant difference.

Parameter	Sites	Test statistics	Statistical significance
LPO	Site 1 vs Site 2	t = −2.48, P = 0.042	Yes
	Site 1 vs Site 3	t = −1.76, P = 0.115	No
	Site 2 vs Site 3	t = −0.28, P = 0.783	No
GSH:GSSG	Site 1 vs Site 2	t = −0.43, P = 0.674	No
	Site 1 vs Site 3	t = −0.61, P = 0.553	No
	Site 2 vs Site 3	t = −0.27, P = 0.789	No
CAT	Site 1 vs Site 2	t = 0.503, P = 0.629	No
	Site 1 vs Site 3	t = 1.267, P = 0.241	No
	Site 2 vs Site 3	t = 1.089, P = 0.308	No
SOD	Site 1 vs Site 2	t = 0.229, P = 0.825	No
	Site 1 vs Site 3	t = 0.965, P = 0.363	No
	Site 2 vs Site 3	t = 1.106, P = 0.301	No
GPX	Site 1 vs Site 2	t = 0.681, P = 0.522	No
	Site 1 vs Site 3	t = 2.460, P = 0.049	Yes
	Site 2 vs Site 3	t = 0.475, P = 0.651	No

enzyme. SOD and GPx activities in the brains of squirrels showed strong correlation with Cd accumulation in the brain (0.59) and As in nails (0.62), which suggest the combine roles of As and Cd in altering brain antioxidant capacity and inducing oxidative stress (Fig. 8). Distance also showed a significant correlation with As levels in soil (−0.67) and vegetation (0.64), respectively, and also correlated negatively to Cd concentrations in vegetation (−0.76) and squirrels brains (−0.70) from the project area. The significant correlations observed between these variables suggest that contamination of the environment may have played a key role in contributing strongly to Cd accumulation in the brain.

6. Discussion

As is a potential neurotoxic agent and involved in the etiology of a variety of neurodegenerative disorders in human and animal studies (Vahidnia et al., 2007). In the present work, we investigated whether chronic arsenicosis and metal exposure caused increased accumulation of As and Cd accumulation and exerted specific biochemical changes (i.e. degree of oxidative stress levels and antioxidant enzyme activities) in brains of muskrats and squirrels collected from the study area. Despite the elevated levels of As detected in the nails of the wild rodents from the As endemic areas, As tended to only accumulate in the brains of the squirrels and not

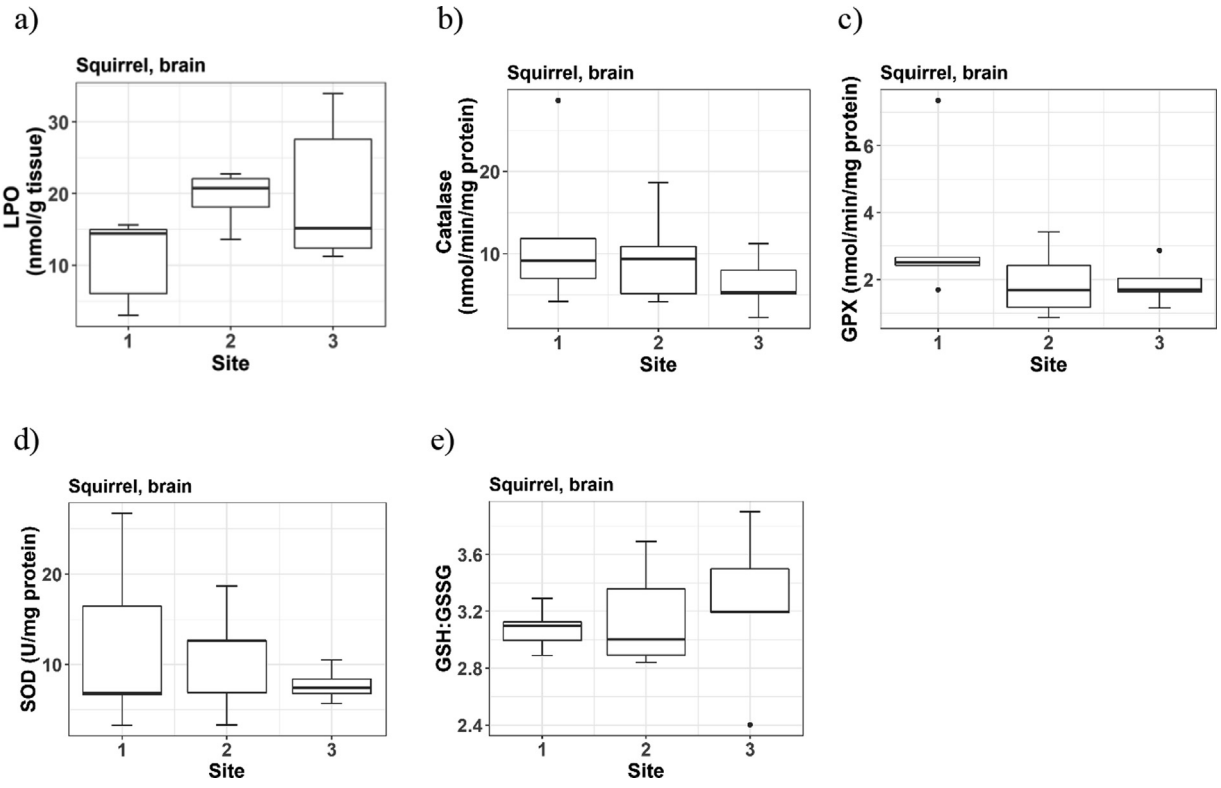


Fig. 6. Boxplots on the complete data for oxidative stress parameters in the brains of squirrels collected from three different sites, where site 3 represents reference site, site 2 represents intermediate location (~20 KM from mine), and site 1 represents locations in the vicinity of mine (within ~2 KM radius). The sample size (*n*) was 5 for each group. The closed circles represent outliers. (a) LPO – lipid hydroperoxides; (b) catalase; (c) GPX – glutathione peroxidase; (d) SOD – superoxide dismutase; (e) GSH:GSSG – reduced to oxidized glutathione ratio.

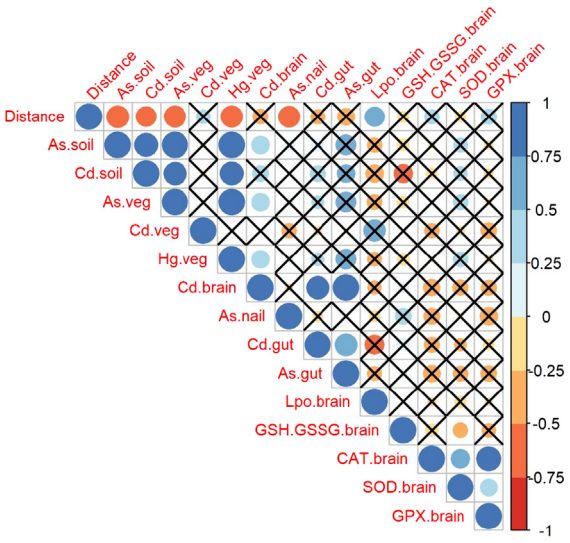


Fig. 7. Correlation between different parameters in muskrats. Positive correlation is depicted with blue colour and negative correlation is depicted with red colour. Correlations which are not significant at the 0.05 level are denoted by a cross. In the figure, accumulation of arsenic (As) and cadmium (Cd) are shown in different compartments analysed in this study (soil; vegetation (veg); brain; gut; nail). Parameters of oxidative are also included as: LPO – lipid hydroperoxides; CAT - catalase; GPX – glutathione peroxidase; SOD – superoxide dismutase; GSH.GSSG – reduced to oxidized glutathione ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

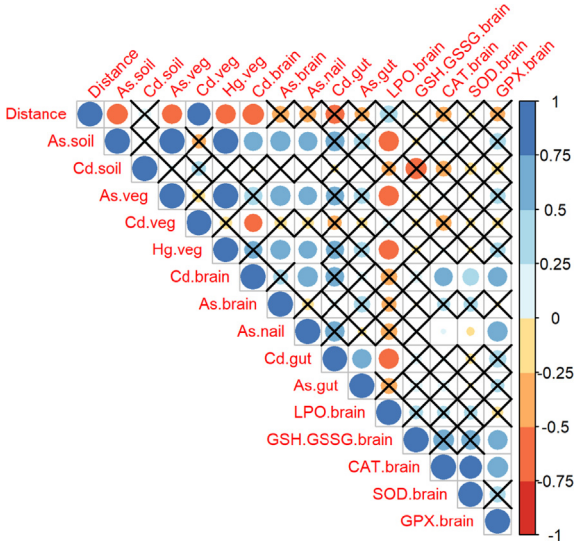


Fig. 8. Correlation between different parameters in squirrels. Positive correlation is depicted with blue colour and negative correlation is depicted with red colour. Correlations which are not significant at the 0.05 level are denoted by a cross. In the figure, accumulation of arsenic (As) and cadmium (Cd) are shown in different compartments analysed in this study (soil; vegetation (veg); brain; gut; nail). Parameters of oxidative are also included as: LPO – lipid hydroperoxides; CAT - catalase; GPX – glutathione peroxidase; SOD – superoxide dismutase; GSH.GSSG – reduced to oxidized glutathione ratio.

in muskrats from the study area. The increased accumulation of As and Cd in the brains of the squirrels may be indicative of a compromised blood-brain barrier causing these metal(loids) to penetrate and accumulate in the brain, which is likely to induce long-term cognitive and behavioural consequences. Despite the longer exposure history of the animals from the arsenic endemic areas, a lower level of LPO was observed in the brains of the animals from As affected areas compared to the reference area, which was not

expected. It has been shown previously that peroxidation of membrane lipids by free radicals could results in loss of membrane integrity and function which may have long-term effects on memory and cognitive function by damaging the cholinergic pathways of the brain (Itri et al., 2014). Lipid peroxidation is one of the major causes of free radical mediated injury that directly damages neuronal membranes and yield a number of secondary products responsible for extensive cellular damage of brain tissues (Kumaret al., 2012). In fact, markers of lipid peroxidation have been found to be elevated in brain tissues and body fluids of subjects with amyotrophic lateral sclerosis, Huntington's disease, Down's syndrome, Parkinson's disease and Alzheimer's disease (Barone et al., 2017).

A recent study also revealed increased lipid peroxidation biomarkers in post-mortem brains of subjects with mild cognitive impairment, the earliest clinically detectable phase of dementia and preclinical AD (Bradley-Whitman and Lovell, 2015). Previous studies have also provided substantial empirical evidence implicating oxidative stress in the etiology of various neurodegenerative diseases. Oxidative stress can be defined as the imbalance among the formations of reactive oxygen species (ROS) and antioxidant system, causing oxidative damage to important structural component of cells (Luque-Contreras et al., 2014, Bhattacharya, 2015). Oxidative stress generally precedes amyloid deposition and perturbs the antioxidant defense systems causing exacerbation of the amyloid pathology, which is one of the most significant features of AD and many other neurodegenerative diseases (Nunomura et al., 2000). There is also increasing evidence that ROS is involved in amyloid fibrilization and neurofibrillary tangles formation in AD (Chauhan and Chauhan, 2006). The results of the present study have confirmed the presence of oxidative stress in the brains of exposed muskrats and squirrels from the study area, which may cause brain damage and trigger cognitive impairment. Based on the observations of higher accumulation of As and/or Cd in the soils, vegetation, including nails, gut content and brains of the animals, higher oxidative damage was certainly expected in the muskrats and squirrels from the project area. However, the results of LPO measurement did not produce the expected results partly because there was relatively higher LPO levels in the animal brains from the reference site, and partly because there was high variation in some data points. It is possible that other unknown stressors apart from toxin exposures may have been responsible for increasing LPO levels in brains of animals from the reference site. Nonetheless, partial increase in the activities of antioxidant enzymes in the brains of the animals collected near the mining site (site 1) suggest increased levels of oxidative stress as compared to the animals from reference site (Figs. 5 and 6).

Superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) are three (3) main enzymes involved in cellular protection against damage caused by free radicals (Ighodaro and Akinloye, 2018). The SOD–CAT system is considered as the first line of defense against the toxicity caused by reactive oxygen species, with SOD catalyzing the dismutation of the superoxide anion radical to hydrogen peroxide, which is further reduced to water and molecular oxygen by CAT (Fukai and Ushio-Fukai, 2011). In the present study, brain activities of SOD and CAT were observed to be elevated in the muskrats and squirrels from the arsenic endemic areas compared to the reference location, although the results were not statistically significant (Fig. 5b, d and Fig. 6b, d). The increased activity of SOD in animals from the As endemic area may be as a result of enhanced generation of superoxide anion, which compensates for the overproduction of reactive oxygen compounds. The increased SOD may have been effective to a certain extent in combating oxidative damage and reducing LPO levels in the brains of animals from the mine area. Some epidemiological studies have postulated that SOD is induced by oxidative stress in the early stages of AD and is suppressed in the later stages of disease (Luca et al., 2015). Several studies have also found no difference in SOD levels between AD and control subjects, while others have found significant higher SOD levels in AD subjects (Ceballos-Picot et al., 1996, Fernandes et al., 1999, Bourdel-Marchasson et al., 2001). CAT has dual functions: it has a catalytic role in the decomposition of H_2O_2 to water and oxygen, and a peroxidic role in which the peroxide is used to oxidize a range of hydrogen donors (Jaydari et al., 2011). The present study showed an increase in CAT activities in the brains of muskrats and squirrels from As endemic areas compared to the reference area (Figs. 5b and 6b). The increased levels of CAT activities in brains of animals from the As endemic area may be indicative of a compensatory mechanism that upregulate the enzyme and its activity against the damaging effects of As and Cd exposures. The result from this present study showed increase in GPx activity in the brains of animals from As endemic areas, which is indicative of a protective mechanism to neutralize the formation of hydrogen peroxide produced by SOD, whose activity was observed to be increased in the brains of the animals closest to the vicinity of the mine area. Many studies have shown that increased oxidative stress and reduced antioxidants activities are involved in the development of AD and other neurodegenerative diseases (Persson et al., 2014). During our field sampling program during the winter, we observed that the reference lakes where muskrats were harvested, had overflowed causing water from below the ice to seep up through the cracks and muskrat push-ups. We suspect that the event may have caused some sort of habitat alterations, which may have caused some additional stress response to the muskrats and subsequently affected their access to food. Some muskrats were also found dead and trapped frozen in some of the push-ups on the frozen lake. One of the most common cause of lake overflow during is the winter is weight of snow load pressing down on the ice causing ice to break-up and force water up through the cracks/push-ups and may cause stress to the musktrats. It is unknown why the squirrels from the reference area showed relatively higher levels of LPO compared to the arsenic endemic areas. Finally, it is worth noticing here that the Cd and As brain accumulation in the squirrels and muskrats was different. Previous studies have shown that metal(loid) may alter the permeability of blood brain barrier (BBB) (reviewed by Kim et al., 2013). Effects on the permeability of BBB may amplify the accumulation capacity of both As and Cd. Our results showed that the level of accumulation of both Cd and As in muskrats from all sites were below detection limit, whereas the level of both Cd and As were measurable in the brain of squirrels captured from all three locations. There could be several factors responsible for this species specific difference in the accumulation of metal(loids) in the brains. One possibility is that the BBB of muskrats might have remained intact despite the exposure which could have prevented the access of both Cd and As to the brain of muskrats. More efficient detoxification of metal(loids) and differences in the habitats as well as food intake habits are other possible factors for differences in metal(loid) accumulation. However, these factors were not evaluated in this study. Similarly, slight differences in biochemical responses were observed between species and these differences in the response could be attributed to differences in food intake habits, differences in the level of metal(loid) accumulation, and/or inherent genetic differences between species. Nonetheless, as discussed above, characterizing these differences between species was not the objective of this study.

7. Conclusion

The data presented in this paper has revealed the current status of As and Cd exposure in wild muskrats and red squirrels breeding

in As endemic areas of Yellowknife. The results provide preliminary evidence that chronic arsenicosis and Cd exposure may be associated with altered biochemical parameters in the brains of exposed wildlife species. It is likely that differences in feeding behaviours, habitat conditions and metabolic status may have been responsible for the differential pattern of contaminant accumulation and exposure-related effects in the muskrats and squirrels. Despite the differences in the exposure history of the animals to arsenic, our study observed lipid peroxidation and altered antioxidant enzyme activities in the brains of animals from the As endemic and non-endemic areas. Specifically, the brains of the animals from the As endemic area showed increased levels of SOD, CAT and GPx enzyme activities compared to the reference site likely due to the influence of environmental exposure to As and Cd. While it is understood that oxidative stress responses, particularly LPO and antioxidant enzyme activities cannot be explained through a simple model, the potential role of other external factors including habitat alterations during the winter sampling season may also be a contributing factor to biological effects. The presence of oxidative stress markers in the brains of the wild muskrats and squirrels suggest that exposed wildlife species in the study area may be prone to oxidative injury, which may trigger neurodegenerative diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

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.

References

Ahlers, A.A., Heske, E.J., Schooley, R.L., Mitchell, M.A., 2010. Home ranges and space use of muskrats *Ondatra zibethicus* in restricted linear habitats. Wildlife Biol. 16 (4), 400–408.

Amuno, S., Jamwal, A., Grahn, B., Niyogi, S., 2018. 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. Sci. Total Environ. 618, 916–926.

Barone, E., Head, E., Butterfield, D.A., Perluigi, M., 2017. HNE-modified proteins in Down syndrome: involvement in development of Alzheimer disease neuropathology. Free Radical Biol. Med. 111, 262–269.

Barrera, G., Pizzimenti, S., Daga, M., Dianzani, C., Arcaro, A., Cetrangolo, G., Giordano, G., Cucci, M., Graf, M., Gentile, F., 2018. Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. Antioxidants 7 (8), 102.

Bertin, F.R., Baseler, L.J., Wilson, C.R., Kritchevsky, J.E., Taylor, S.D., 2013. Arsenic Toxicosis in Cattle: Meta-Analysis of 156 Cases. J Vet Intern Med 27 (4), 977–981.

Bhattacharya, P., Welch, A.H., Stollenwerk, K.G., McLaughlin, M.J., Bundschuh, J., Panaullah, G., 2007. Arsenic in the Environment: Biology and Chemistry. Elsevier.

Bhattacharya, S., 2015. Reactive Oxygen Species and Cellular Defense System. Free Radicals in Human Health and Disease. Springer, pp. 17–29.

Bourdel-Marchasson, I., Delmas-Beauvieux, M.C., Peuchant, E., Richard-Harston, S., Decamps, A., Reignier, B., Emeriau, J.P., Rainfray, M., 2001. Antioxidant defences and oxidative stress markers in erythrocytes and plasma from normally nourished elderly Alzheimer patients. Age Ageing 30 (3), 235–241.

Bradley-Whitman, M.A., Lovell, M.A., 2015. Biomarkers of lipid peroxidation in Alzheimer disease (AD): an update. Arch. Toxicol. 89 (7), 1035–1044.

Ceballos-Picot, I., Merad-Boudia, M., Nicole, A., Thevenin, M., Hellier, G., Legrain, S., Berr, C., 1996. Peripheral antioxidant enzyme activities and selenium in elderly subjects and in dementia of Alzheimer's type—place of the extracellular glutathione peroxidase. Free Radical Biol. Med. 20 (4), 579–587.

Chauhan, V., Chauhan, A., 2006. Oxidative stress in Alzheimer's disease. Pathophysiology 13 (3), 195–208.

Chen, C.-J., Chiou, H.-Y., 2011. Health Hazards of Environmental Arsenic Poisoning: From Epidemic to Pandemic. World Scientific.

Chung, J.-Y., Yu, S.-D., Hong, Y.-S., 2014. Environmental source of arsenic exposure. J. Preventive Med. Public Health 47 (5), 253.

Clark, I.D., Raven, K.G., 2004. Sources and circulation of water and arsenic in the Giant Mine, Yellowknife, NWT, Canada. Isot. Environ. Health Stud. 40 (2), 115–128.

Cott, P.A., Zajdlik, B.A., Palmer, M.J., McPherson, M.D., 2016. Arsenic and mercury in lake whitefish and burbot near the abandoned Giant Mine on Great Slave Lake. J. Great Lakes Res. 42 (2), 223–232.

Datta, B.K., Bhar, M.K., Patra, P.H., Majumdar, D., Dey, R.R., Sarkar, S....Chakraborty, A.K., 2012. Effect of environmental exposure of arsenic on cattle and poultry in Nadia district, West Bengal, India. Toxicol. Int. 19 (1), 59.

Eisler, R., 2004. Arsenic Hazards to Humans, Plants, and Animals from Gold Mining. Reviews of Environmental Contamination and Toxicology. Springer, pp. 133–165.

Fernandes, M., Proenca, M., Nogueira, A., Grazina, M., Oliveira, L., Fernandes, A., Santiago, B., Santana, I., Oliveira, C., 1999. Influence of apolipoprotein E genotype on blood redox status of Alzheimer's disease patients. Int. J. Mol. Med. 4 (2), 179–265.

Fukai, T., Ushio-Fukai, M., 2011. Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid. Redox Signal. 15 (6), 1583–1606.

Gurnell, J., 1984. Home range, territoriality, caching behavior and food supply of the red squirrel (*Tamiasciurus hudsonicus fremonti*) in a subalpine lodgepole pine forest. Animal Behavior. 32 (4), 1119–1131.

Huang, G., Luo, X., Li, D., Li, Z., Chen, Z., Zhu, M., 2019. A multidisciplinary investigation on bio-oxidation gold mine tailings from Dandong, China as a potential arsenic pollution source. J. Geochem. Explor. 196, 33–41.

Ighodaro, O., Akinloye, O., 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. Alexandria J. Med. 54 (4), 287–293.

Ishii, C., Ikenaka, Y., Nakayama, S.M., Mizukawa, H., Yohannes, Y.B., Watanuki, Y., Fukuwaka, M., Ishizuka, M., 2017. Contamination status and accumulation characteristics of heavy metals and arsenic in five seabird species from the central Bering Sea. J. Vet. Med. Sci. 79 (4), 807–814.

Islam, F., Khanna, V.K., 2009. Attenuation of arsenic neurotoxicity by curcumin in rats. Toxicol. Appl. Pharmacol. 240, 367–376.

Itri, R., Junqueira, H.C., Mertins, O., Baptista, M.S., 2014. Membrane changes under oxidative stress: the impact of oxidized lipids. Biophys. Rev. 6 (1), 47–61.

Jamieson, H.E., 2014. The legacy of arsenic contamination from mining and processing refractory gold ore at Giant Mine, Yellowknife, Northwest Territories, Canada. Rev. Mineral. Geochem. 79 (1), 533–551.

Jaydari, F., Johari, H., Taati, M., Asadian, P., Alirezaei, M., Sheikhzadeh, F., 2011. The effects of fruit extract on catalase activity and lipid peroxidation in the heart and erythrocytes of rats following chronic ethanol consumption. J. Vet. Res. 3, 179–183.

Kim, S.-O., Kim, W.-S., Kim, K.-W., 2005. Evaluation of electrokinetic remediation of arsenic-contaminated soils. Environ. Geochem. Health 27 (5–6), 443–453.

Kim, J.H., Byun, H.M., Chung, E.C., Chung, H.Y., Bae, O.N., 2013. Loss of integrity: impairment of the blood-brain barrier in heavy metal-associated ischemic stroke. Toxicol. Res. 29 (3), 157–164.

Ko, I., Sung Ahn, J., Seog Park, Y., Kim, K.-W., 2003. Arsenic contamination of soils and sediments from tailings in the vicinity of Myungbong Au mine, Korea. Chem. Speciat. Bioavailab. 15 (3), 67–74.

Kumar, H., Lim, H.-W., More, S.V., Kim, B.-W., Koppula, S., Kim, I.S., Choi, D.-K., 2012. The role of free radicals in the aging brain and Parkinson's disease: convergence and parallelism. Int. J. Mol. Sci. 13 (8), 10478–10504.

Lobo, V., Patil, A., Phatak, A., Chandra, N., 2010. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn. Rev. 4 (8), 118.

Luca, M., Luca, A., Calandra, C., 2015. The role of oxidative damage in the pathogenesis and progression of Alzheimer's disease and vascular dementia. Oxid. Med. Cell. Longevity 2015, 1–8. https://doi.org/10.1155/2015/504678.

Luque-Contreras, D., Carvajal, K., Toral-Rios, D., Franco-Bocanegra, D., Campos-Peña, V. 2014. Oxidative stress and metabolic syndrome: cause or consequence of Alzheimer's disease? Oxidative medicine and cellular longevity 2014.

Mandal, P., 2017. An insight of environmental contamination of arsenic on animal health. Emerg. Contam. 3 (1), 17–22.

Mori, S., Lowenstein, E., Steffen, C., 2018. The largest mass poisoning in history: arsenic contamination of well water in Bangladesh. Skinmed 16 (4), 265–267.

Mosaferi, M., Yunesion, M., Mesdaghinia, A., Naidu, A., Nasseri, S., & Mahvi, A. H. (2003, February). Arsenic occurrence in drinking water of IR of Iran: the case of Kurdistan Province. In Fate of arsenic in the environment. Dhaka: BUET-UNU International Symposium, International Training Network Centre, Bangladesh University of Engineering and Technology, United Nations University, Tokyo (pp. 1–6).

Nain, S., Smits, J.E., 2012. Pathological, immunological and biochemical markers of subchronic arsenic toxicity in rats. Environ. Toxicol. 27 (4), 244–254.

Nunomura, A., Perry, G., Pappolla, M.A., Friedland, R.P., Hirai, K., Chiba, S., Smith, M. A. 2000. Neuronal oxidative stress precedes amyloid-β deposition in Down syndrome. J. Neuropathol. Exp. Neurol. 59(11): 1011–1017.

O'Bryant, S.E., Edwards, M., Menon, C.V., Gong, G., Barber, R., 2011. Long-term low-level arsenic exposure is associated with poorer neuropsychological functioning: a Project FRONTIER study. Int. J. Environ. Res. Public Health 8 (3), 861–874.

Persson, T., Popescu, B.O., Cedazo-Minguez, A. 2014. Oxidative stress in Alzheimer's disease: why did antioxidant therapy fail? oxidative medicine and cellular longevity 2014.

Pothier, M.P., Hinz, A.J., 2018. Insights into arsenite and arsenate uptake pathways using a whole cell biosensor. Front. Microbiol. Poulain, 9.

Rao, M., Avani, G. 2004. Arsenic induced free radical toxicity in brain of mice.

Ruszkiewicz, J., Albrecht, J., 2015. Changes in the mitochondrial antioxidant systems in neurodegenerative diseases and acute brain disorders. Neurochem. Int. 88, 66–72.

Saha, K.C., 2003. Diagnosis of arsenicosis. Journal of Environmental Science and Health. J Environ Sci Heal A 38 (1), 255–272.

Scarisbrick, I., 2008. The Multiple Sclerosis Degradome: Enzymatic Cascades in Development and Progression of Central Nervous System Inflammatory Disease. Advances in Multiple Sclerosis and Experimental Demyelinating Diseases. Springer:, pp. 133–175.

Strawn, D.G., 2018. Review of interactions between phosphorus and arsenic in soils from four case studies. Geochem. Trans. 19 (1), 10.

Talukdar, D., 2013. Arsenic-induced oxidative stress in the common bean legume, Phaseolus vulgaris L. seedlings and its amelioration by exogenous nitric oxide. Physiol. Mol. Biol. Plants 19 (1), 69–79.

Thienpont, J.R., Korosi, J.B., Hargan, K.E., Williams, T., Eickmeyer, D.C., Kimpe, L.E., Palmer, M.J., Smol, J.P., Blais, J.M., 2016. Multi-trophic level response to extreme metal contamination from gold mining in a subarctic lake. Proc. R. Soc. B 283 (1836), 20161125.

Tseng, C.-H., Huang, Y.-K., Huang, Y.-L., Chung, C.-J., Yang, M.-H., Chen, C.-J., Hsueh, Y.-M., 2005. Arsenic exposure, urinary arsenic speciation, and peripheral vascular disease in blackfoot disease-hyperendemic villages in Taiwan. Toxicol. Appl. Pharmacol. 206 (3), 299–308.

Tyler, C.R., Allan, A.M., 2014. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: a review. Curr. Environ. Health Reports 1 (2), 132–147.

Vahidnia, A., Van Der Voet, G.B., de Wolff, F.A., 2007. Arsenic neurotoxicity-a review. Hum. Exp. Toxicol. 26 (10), 823–832.

Yadav, R.S., Sankhwar, M.L., Shukla, R.K., Chandra, R., Pant, A.B., Islam, F., Khanna, V. K., 2009. Attenuation of arsenic neurotoxicity by curcumin in rats. Toxicol. Appl. Pharmacol. 240 (3), 367–376.

Yoshida, T., Sun, G., Pi, J., Li, X., Li, B., Yamauchi, H., 2019. Field Researches on Chronical Arsenic Poisoning in Inner Mongolia, China. Arsenic Contamination in Asia. Springer, pp. 61–81.