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Research paper

# Skeletal pathology and bone mineral density changes in wild muskrats (*Ondatra zibethicus*) and red squirrels (*Tamiasciurus hudsonicus*) inhabiting arsenic polluted areas of Yellowknife, Northwest Territories (Canada): A radiographic densitometry study

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ABSTRACT

The City of Yellowknife is a known hotspot of arsenic contamination and there is a growing body of evidence suggesting that local wildlife in the vicinity of the abandoned Giant Mine site may be at risk of decreased bone mineralization and various bone disorders. The purpose of this study was to preliminarily measure bone mineral density (BMD) changes and investigate the incidence, pattern, and severity of bone lesions in wild muskrats and red squirrels breeding in three (3) catchment areas at different distances from the Giant Mine Site in Yellowknife, Northwest Territories (Canada): ~2 km (location 1), ~18 km (location 2), and ~40–100 km (location 3). Full femoral bones of 15 muskrats and 15 red squirrels were collected from the three sampling locations (5 from each location) and subjected to radiographic analysis and densitometric measurements. The patterns and severities of bone lesions, including changes in bone mineral density, were evaluated and compared between groups. As levels were significantly higher in the bones of muskrats caught from location 1 and 2, relative to location 3. Further, As and Cd levels were significantly higher in the bones of squirrels caught from locations 1 and 2 relative to squirrels caught from location 3. The preliminary results from bones revealed that radiographic abnormalities such as bone rarefaction, osteopenia, and thinning of the femoral shafts with significant ossific cystic lesions and bowing were the most common skeletal pathologies found in bones of red squirrels from the three locations. Radiographic appearances of massive sclerosis and dysplasia, including severe osteocondensation and osteopathia striata-like abnormalities, were found in the bones of muskrats from all the sampling locations. Densitometric evaluation showed no significant differences between the three locations in the bone parameters measured. However, there was a statistically significant correlation between As content in the bones of muskrats and percent fat content in the femur samples, which suggests that accumulation of As could have been a causal factor for a change in percent fat in femurs of muskrats.

## 1. Introduction

Arsenic (As) is a toxic metalloid that is widely present in the environment as a pollutant. In many parts of the world, As contamination is typically associated with geogenic contamination or industrial gold mining and processing of arsenopyrite ores (Kinimo et al., 2018; Mensah et al., 2020; Miller et al., 2019; Naila et al., 2019). Human and animal populations worldwide are exposed to As by low-level uptake through their food or by ingesting of groundwater contaminated with As (Mondal et al., 2010; Podgorski and Berg, 2020). Whereas acute exposures result in As accumulating primarily in the kidney and liver (Lewchalermvong et al., 2018), chronic exposures additionally target the integumentary, nervous, respiratory, reproductive and skeletal systems (Abdul et al., 2015). While some laboratory accumulation data is

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available, field research on the accumulation of As in the bone tissues and associated skeletal effects in wild animals residing in heavily contaminated areas is very scarce.

As poisoning causes a wide variety of deleterious effects in human and animal populations, including melanosis, neuropathy, Alzheimer disease, impaired respiratory and cardiovascular function, and a variety of bone disorders (reviewed in Abdul et al., 2015). The skeletal system is thought to be especially susceptibile to chronic As toxicity, as it is known to interfere with osteoblastic and osteoclastic activities, disrupting the proper regulation of bone metabolism (Hu et al., 2012; Wu et al., 2014). Short-term As exposure of a high dose of inorganic As (applied via intraperitoneal injection) affected bone remodeling in rats (Hu et al., 2012). Several investigations have shown that extra cellular signal-regulated kinase (ERK) activation plays a key role in osteoblast differentiation and osteoclast formation (Lai et. al, 2001; Matsushita et. al, 2009), and some studies have confirmed that ERK signaling can be disturbed due to As exposure resulting in bone loss. Nuclear factor E2-related factor 2 (Nrf2) has been implicated as a key component of the regulatory mechanism counteracting As-induced osteoclastogenesis by upregulating cytoprotective enzymes (Liu et al., 2019). Thus, by disrupting bone metabolism, chronic As exposure may increase the risk of bone disorders. The factors that influence As absorption from the digestive tracts are the As dosage and valency of its compounds. As accumulates slowly in the nails, hairs and skin over time while As compounds are readily expelled from body fluids (Amuno et al., 2020a, 2020c, 2020b; Suzuki et al., 2002).

In Yellowknife, a vast area particularly within a 30 km radius from the abandoned Giant Mine site, were found to contain markedly elevated As levels in soil, water and vegetation. Since many small mammals inhabit these locations and forage in these areas, they are at risk of As induced toxicity with bone complications. Some of our recent studies showed that there were significant changes in retina layers and a higher antioxidant response in the brain, with increased As and Cd levels in tissues such as the brain and nails of muskrats and red squirrels caught from As endemic areas as compared to reference sampling sites, suggesting the vulnerability of small mammalian species living near the mining site (Amuno et al., 2020a, 2020c, 2020b). Moreover, we also detected higher As and Cd levels in the soil and vegetation in the sites near the mining sites relative to the reference site (Amuno et al., 2020a). Overall, the exposure data for As and Cd along with their known toxicological effects on bone tissues in various species suggests that the skeletal system in species living in the As endemic sites near the Yellowknife mining regions might be vulnerable.

We have also previously described and compared the bone pathology of snowshoe hares inhabiting As affected areas within 2 km and 20 km radius of the Giant Mine Site in 2017 (Amuno et al., 2018) and reported various bone abnormalities in the species. However, there is no information about the bone pathology of other small mammals in the study area, such as red squirrels and muskrats, which are also known to accumulate high As levels in their nails and other tissues (Amuno et al., 2020b). The purpose of this study was to measure bone mineral density (BMD) and investigate the incidence, pattern, and severity of bone lesions in wild muskrats and red squirrels breeding in three (3) catchment areas at different distances (~2 km, ~18 km, and ~40–100 km) from the Giant Mine Site in Yellowknife, Northwest Territories (Canada).

## 2. Material and methods

A wildlife research permit (WL500561) and ethical clearance for wildlife sampling was obtained from the Department of Environment and Natural Resources, Government of the Northwest Territories and 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 sampling. A local furbearer hunter was employed to assist in determining habitat locations of muskrats and squirrels prior to trapping them in the study area. All the animals used for this study were sampled between March and April 2018. All trapped animals were euthanized and dissected. Target organs such as the brain, liver, kidney, nails, gut content, and bones were separated in individual falcon tubes and frozen. Animals were not separated into male and female categories due to the unequal numbers of male and female animals captured during the field work. The ages of the squirrels and muskrats were estimated based on their overall body weight and length. We considered a squirrel to be an adult if its body length was between 10 and 15 in. (25.4–38.1 cm) and weighed between 260 and 350 g (Pennsylvania State University 2002). A muskrat was considered adult if it weighed between 2.5 and 4 pounds with total body length ranging from 23 to 26 in. (58.42–66.04 cm) and with a tail length of 8–11 in. (20.32–27.94 cm) (New York Department of Environmental Conservation). All animal samples utilized for this study were generally within the adult range.

### 2.1. Sampling locations

The study aimed to assess bone mineral density changes and bone pathology of animals near the vicinity of the Giant Mine Site, a closed gold mine located near the city of Yellowknife, Northwest Territories, Canada. The femurs of adult muskrats (*O. zibethicus*) and red squirrels (*T. hudsonicus*)collected at the immediate vicinity of the mine (within ~2 km radius), and at an intermediate location (20 km away from the mine) were compared with animals from a reference site (muskrats and squirrels trapped between 53.4 and 62 km and 95–105 km away from Yellowknife, respectively: Fig. 1).

### 2.2. Laboratory analysis

(i) As and Cd levels in Bones and other Biological Tissues

The results for the measurements of As and Cd in the nails and other biological tissues of the muskrats and squirrels from the study area have been published in our most recent studies and not repeated in this paper. Please refer to (Amuno et. al, 2020a, 2020b, 2020c) for full metal(loid) data in the tissues of the animals. This study only focused on the measurements of those parameters that were not previously measured, such as As and Cd in bone tissues of muskrats and squirrels from the study area. Prior to digestion, all bone samples were rinsed in deionized water and blotted dry. The bone samples were then digested at room temperature in 15 mL polyethylene vials containing concentrated nitric acid (16 N; Ultrapure, Merck, Canada) over 72 h. The concentrations of metals in digested bones were measured in a graphite furnace atomic absorption spectrometer (Analyst 800, Perkin Elmer Ltd., USA) after making appropriate dilutions with 0.2% nitric acid. The quality control of the analytical method was maintained by using certified standards for both Cd and As, appropriate method blanks, and by estimating the recovery of elements in a certified reference material (DOLT-4; National Research Council of Canada). The reference material was digested and analyzed concurrently with the tissues samples and it showed a recovery of both elements between 96% and 104%. The detection limits for both As and Cd in animal tissue samples were 0.005 μg/g.

(ii) Radiographic analysis and densitometric measurements

Radiographic assessment of the femurs of red squirrels and muskrats was conducted at the Centre for Bone and Periodontal Research, McGill University, Quebec Canada. A single X-ray of femur samples was taken using a Kubtec Xpert 80 radiography system (KUB Technologies Inc.) with automatic calibration. Densitometric measurements were also obtained using an animal densitometer (Luna Piximus II Densitometer) (GE Medical Systems, Madison, WI) to assess bone mineral density. A quality control was run and passed by using the phantom provided with the instrument (BMD = 0.055 g/cm; %Fat = 10.2%) prior to

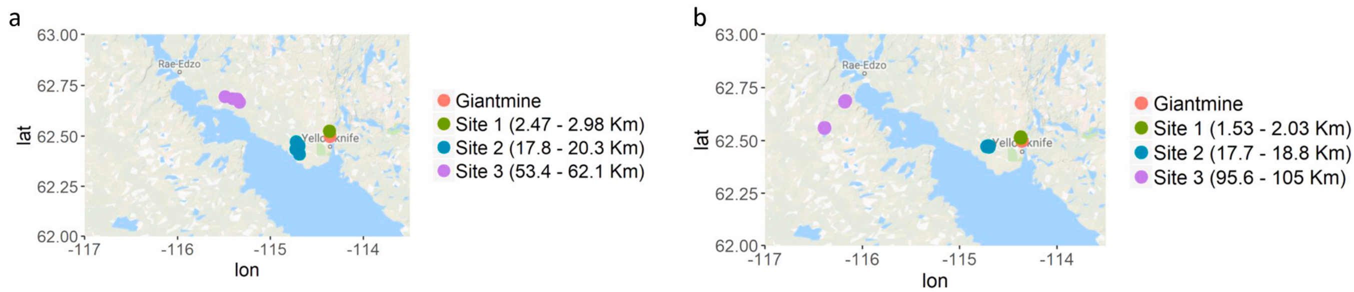


**Fig. 1.** (a)/(b) Map of muskrat and squirrel sampling areas.

scanning the samples. Bone samples were scanned individually (one femur per scan). Any extra liquid was absorbed with KIM-TECH paper from the sample's surface and the sample was positioned in the center of the tray before being scanned. The parameters of bone mineral density (BMD) ($g/cm^2$), bone mineral content (BMC) (g), bone area (BA) ($cm^2$) and tissue area (TA) ($cm^2$) were then analyzed by using custom-defined ROI function built in the software.

### 2.3. Statistical analysis

The assumptions of normality of distribution and homogeneity of variances were confirmed with a Shapiro-Wilk and Levene's test, respectively. Statistical comparisons were made by independent *t*-test (SPSS software, version 16.0). A p-value of $\leq 0.05$ was considered to be statistically significant. A two-tailed Pearson correlation was employed to assess the relationships between As and Cd levels in the bones and densitometric parameters.

## 3. Results

(i) Status of As and Cd accumulation in bones of red squirrels and muskrats

Cd was not detected in the bones of muskrats. However, As levels were significantly higher in the bones of muskrats caught from locations 1 and 2compared to location 3 (1.56 and 1.66 times higher, respectively as compared to location 1; both $p < 0.001$). In contrast, Cd was detected in the bones of squirrel; moreover, both As and Cd levels in the bones of squirrels caught from location 1 and 2 were significantly higher compared to squirrels caught from location 3 (Fig. 2 and 3). As levels in squirrel bones caught from locations 1 and 2 were 3.02 and 2.3 times higher than As levels in the bones of squirrel caught from location 3 (both $p < 0.001$). Similarly, Cd content in the bones of squirrels caught from locations 1 and 2 were 4.4 and 2.7 times higher than location 3 (both $p < 0.001$) (Tables 1 and 2).

(ii) Bone densitometric Results (descriptive/*t*-test/correlation)

In squirrels, none of the studied parameters showed any significant differences based on the location of sample collection. Hence, it appears that the skeletal system of squirrels is resistant to densitometric changes due to As and Cd contamination (Fig. 5 and Table 4). In muskrats, percent fat in the femur bone showed significant difference across locations (Fig. 4 and Table 3). While no difference was detected between sample locations 1 and 2 ($p = 0.34$); there were statistically significant differences between locations 1 and 3 ($p = 0.02$) as well as locations 2 and 3 ($p = 0.02$). For both bone mineral content (BMC) and bone area (BA), there was a significant difference between muskrats collected from sites 1 and 2 but there was no difference between sites 1 and 3; hence the difference between sites 1 and 2 does not appear to be related to As and Cd contamination levels. There was a statistically significant correlation between As content in the bones of muskrats and percent fat content in the femur, which suggest that accumulation of As could have been a causal factor for a change in percent fat in femurs of muskrats (Fig. 6a).

(iii) Radiographic assessment of animals based on proximity from the Giant Mine area

A radiographic analysis of 15 femurs of red squirrels and 15 muskrats was conducted. For each location, 5 femurs were randomly selected and examined for bone abnormalities. Radiographic assessment of bones from the three catchment areas in proximity to the Giant Mine area is described below:

(~2 km)

Red squirrels:

In this area, all the bones of squirrels showed evidence of osteopenia with ossific like cystic changes over the superior metaphyses. Such abnormal ossific lesions signify progressive weakness of the femoral necks with susceptibility to fracture. Specifically, the greater trochanters in (7a) look ill-defined and

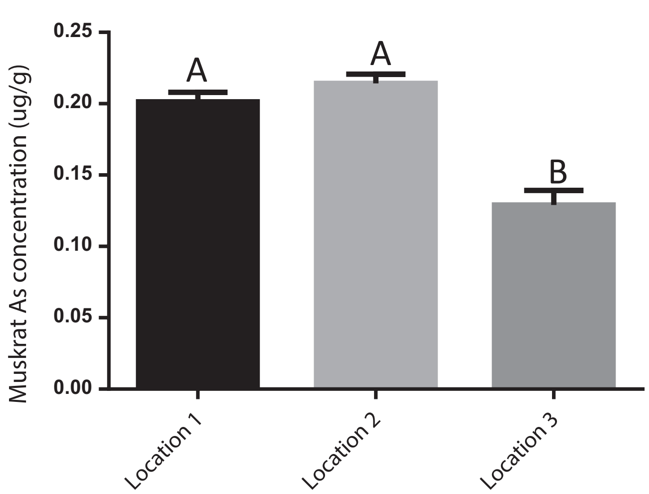


**Fig. 2.** Boxplots on As levels in bones of muskrats collected from three different sites. Different letters above bars signify significant difference (*t*-test; $p < 0.05$) (figures).

**Table 1**
Statistical analysis of As accumulation in bones of muskrats with comparative *t*-test results between locations 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). Bolded p values signify significance significant difference.

| Muskrat | | | |
|---|---|---|---|
| | 1vs2 | 1vs3 | 2vs3 |
| As | t (14) = 1.373; p = 0.1913 | **t(14) = 5.911; p ≤ 0.0001** | **t(14) = 6.992; p ≤ 0.0001** |
| Cd | All groups below detection | | |

**Table 2**
Statistical analysis of As and Cd accumulation in bones of squirrel with comparative *t*-test results between locations 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). Bolded p values signify significance significant difference.

| **Squirrel** | 1vs2 | 1vs3 | 2vs3 |
|---|---|---|---|
| As | t(14) = 1.820; p = 0.0903 | **t(14) = 5.492; p ≤ 0.0001** | **t(14) = 6.122; p ≤ 0.0001** |
| Cd | t(14) = 1.645; p = 0.1223 | **t(14) = 3.407; p = 0.0043** | **t(14) = 5.334; p = 0.0001** |

**Table 3**
Statistical analysis of bone densitometry parameters in bones of muskrat with comparative *t*-test results between locations 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). *Bolded p values signify significant difference. Legends: BMD (bone mineral density), BMC (bone mineral content), B Area (bone area), T Area (tissue area), % Fat (percent fat).

| **Muskrat N = 15** | Parameters | 1vs2 | 1vs3 | 2vs3 |
|---|---|---|---|---|
| | BMD | t(8) = 2.2; p = 0.0590 | t(8) = 1.701; p = 0.1273 | t(8) = 0.4011; p = 0.6988 |
| | BMC | **t(8) = 2.630; p = 0.0302** | t(8) = 1.871; p = 0.0983 | t(8) = 0.7341; p = 0.4838 |
| | B Area | **t(8) = 2.732; p = 0.0258** | t(8) = 1.679; p = 0.1317 | t(8) = 0.7823; p = 0.4565 |
| | T Area | t(8) = 2.078 p = 0.0713 | t(8) = 1.101; p = 0.3029 | t(8) = 0.8127; p = 0.4399 |
| | % Fat | t(8) = 1.1018; p = 0.3386 | **t(8) = 2.905; p = 0.0197** | **t(8) = 2.766; p = 0.0244** |

**Table 4**
Statistical analysis of bone densitometry parameters in bones of squirrel with comparative *t*-test results between locations 1, 2 and 3. Analysis is reported as t(df) and p = p-value. Bolded p values signify significance significant difference.

| **Squirrel** N = 15 | Parameters | 1vs2 | 1vs3 | 2vs3 |
|---|---|---|---|---|
| | BMD | t(8) = 0.1933; p = 0.8515 | t(8) = 0.1441; p = 0.8890 | t(8) = 0.06495; p = 0.06495 |
| | BMC | t(8) = 0.04845; p = 0.6410 | t(8) = 0.09787; p = 0.9244 | t(8) = 0.5429; p = 0.602 |
| | B Area | t(8) = 1.331; p = 0.2199 | t(8) = 0.08234; p = 0.9364 | t(8) = 1.340; p = 0.2170 |
| | T Area | t(8) = 1.476; p = 0.1783 | t(8) = 0.5662; p = 0.5868 | t(8) = 2.180; p = 0.0608 |
| | % Fat | t(8) = 0.1701; p = 0.8692 | t(8) = 0.6041; p = 0.5625 | t(8) = 0.7650; p = 0.4663 |

sloping (this signify poor muscular attachments to the glutei muscles, namely gluteus minimus, subgluteus medius, and the subgluteus maximus), which may leads to greater trochanteric pain syndrome (previously known as trochanteric bursitis). The metaphyses of the superior femora in all images look osteopenic with unusual strands of ossification. The long bone of (7a) is mildly bowing, while (7e) showed thinning of the femoral shaft. Overall, all radiographic images show variable width of the femoral shafts with varying degrees of bowing.

Muskrats:

The radiographs of the femurs show sclerosing bone disorder. Sclerosing disorders often have characteristic radiological features as shown in the images (Fig. 10). All images manifest osteocondensation which can result from decreased bone resorption. Principally, massive sclerosis as seen in these images are basically caused by a disturbance of the balance between bone formation and bone resorption that has had an especially high impact on the overall structure of the bones. The diffuse sclerosis of the long bones can lead to simultaneous ossification of the medullary cavities of the long bones which lead to anemia with extramedullary hemopoiesis. These animals can suffer cranial bones involvement of osteosclerosis with adverse effect on vision, hearing and neurological deficits. Sclerosis of the foramen magnum can lead to foramen magnum syndrome. The value of studying these bones is to understand the overall involvement of the whole skeletal and the extra-skeletal organs.

(~20 km)

Red squirrels

Radiograph of the femurs showed (Fig. 8a, b) evidence of rarefaction and osteopenia along the femoral necks and the whole metaphyses (balck arrowhead) in (Fig. 8a, b) associated with islands of ectopic calcifications. The femoral shafts in both images are widened (though more marked in (Fig. 8a) and both showed signs of pathological changes. In all these images, the lesser trochanters are more pronounced (arrowhead). Impingement of a pronounced *lesser trochanter* (i.e overgrowth of the lesser trochanter) on ischium is a potential *cause* for hip pain and disability, through the pathological development of ischio-femoral impingement (defined as narrowing of the space between the lateral aspect of os ischium and the lesser trochanter). The metaphyses of the superior femora look osteopenic with unusual strands of ossification. In all images (Fig. 8c,d,e) there is ossific-cystic like changes over the superior metaphyses (heterotopic *ossification*). Such abnormal ossific lesions signify progressive weakness of the femoral necks with susceptibility to fracture.

Muskrats

All images (Fig. 11) manifest osteocondensation associated with osteopathia-striata-like abnormality (abnormal lines of calcification mostly seen along the superior metaphyses of the femora and inferior metaphyses, respectively).

(~40–100 km)

Red squirrels

Radiographs of the femurs show (Fig. 9a, b) evidence of rarefaction and osteopenia along the femoral necks and the whole metaphyses, and the inter-trochanteric crest looks distorted and is replaced by ectopic ossifications. The superior metaphyses manifest diffuse islands of ectopic calcifications (black arrow). All images (Fig. 9a-e) showed ill-defined gluteal tuberosities (signify poor muscular attachment) (arrows).

Muskrats

The radiographs (Fig. 12) showed increased bone mass resembling bone sclerosis and dysplasia in humans. It is highly likely that the primary defect in these animals occurred as a result of increased bone resorption that lead to the development of osteolytic lesions. In accordance with the physiological coupling between bone resorption and formation, the increased number and size of osteoclasts present in these animals which were pathological are compensated by increased osteoblastic bone formation. The final picture (Fig. 12e) is increased bone turnover resulting in the formation of bone tissue that is disorganized and of poor quality, resulting in deformities, and the long bones are losing their physiological flexibility and replaced by rigid bones which are fragile.

## 4. Discussions

BMD, BMC, BA, and percent fat have all been used as indicators of the bone weakening (Brzóska and Moniuszko-Jakoniuk, 2005; Orssatto et al., 2018; Schellinger et al., 2004). Therefore, the incidence, pattern, and severity of bone lesions were investigated in wild muskrats and red squirrels breeding in three separate catchment areas at different distances (~2 km, ~18 km, and ~40–100 km) from the Giant Mine Site in Yellowknife, Northwest Territories (Canada) (Fig. 1). The animals selected for this study have relatively short home ranges and are assumed to have been confined within their respective sampling sites (100 m and 1–2.4 ha for wild muskrats (*O. zibethicus*) and red squirrels (*T. hudsonicus*), respectively) (Wilson and Ruff, 1999). Thus, the differences observed for Cd and As levels in the bone across three sites are likely a result of their life history and interaction with the environment at each of those sites.

In our recent studies, we showed that the muskrats and squirrels captured from the As endemic areas as in the present study had significant pathologies in retinal layers in the eye and higher antioxidant responses in brain, and higher As and Cd levels in tissues such as brain and nails relative to the reference sampling site, suggesting the vulnerability of species living near the mining site. Moreover, we also detected considerably higher As and Cd levels in the soil and vegetation in the sites near the mining sites than the reference site (Amuno et al., 2020a, 2020b, 2020c). Overall, these prior exposure data for As and Cd along with their known toxicological effects on bone in various species suggest that skeletal system in the species living in the As endemic sites near the Yellowknife mining regions might be susceptible to structural and/or functional damage.

The radiographic data from our investigation indicated several abnormalities (Figs. 7–12) such as evidence of osteopenia with ossific like cystic changes over metaphyses in squirrels and sclerosing bone disorders in the muskrats caught from within 2 km range of mining region; however, none of the animals from the As affected areas exhibited any distinctive and significant pathological bone changes that were different from the reference location. Therefore, the radiographic bone abnormalities observed in this study cannot be fully attributed to As and Cd contamination in the study area. Levels of arsenic in the bones of both squirrels and muskrats and the levels of cadmium in the bones of squirrel caught from locations 1 and 2 were higher than those from location 3.

We found a significant increase in the percentage of fat content in femur bones in muskrat which correlated with bone arsenic content, strongly suggesting that the contamination of As could be partly responsible for inducing pathological changes in bones of muskrats (Table 3). Several studies have scrutinized bone marrow fat for its potential diagnostic value (Martin et al., 1990; Meunier et al., 1971; Nuttall et al., 1998; Wang et al., 1977; Wronski et al., 1986). In fact, an etiological connection between increased bone marrow fat and osteopenia has been suggested (Nuttall and Gimble, 2000). Hence, our study suggests that the bones of muskrats in the location close to the Giant Mine site might have been affected to a certain degree by chronic As exposure. However, none of the other bone parameters in muskrats seemed to have been affected by As accumulation, the reasons of which are unknown. It is probable that fat content of the femur might be a more sensitive marker of bone strength in comparison to other parameters such as BMD, BA, BMC, and TA. However, more studies are needed to understand the relative sensitivity of these markers to chronic arsenic poisoning. Interestingly, there was no significant impact of both As and Cd on any of the bone densitometry parameters measured in the squirrels caught near the vicinity of the Giant Mine area (location 1), despite having significantly higher As and Cd accumulation in the femur compared to squirrels caught in the location 3.

Lack of changes in densitometry parameters in squirrels despite a significantly higher Cd and As was surprising because several studies have shown that long-term Cd and As exposure can cause bone abnormalities. For example, cadmium has been shown to be associated with an increased risk of osteoporosis in humans (Alfvén et al., 2000; Staessen et al., 1999). Cd has also been shown to disrupt bone metabolism in small rodents (Brzóska and Moniuszko-Jakoniuk, 2005). A chronic exposure to Cd in young female rats inhibited the process of bone formation and caused osteopenia (Brzóska and Moniuszko-Jakoniuk, 2005). Furthermore, in the same study, a continuation of Cd exposure up to the skeletal maturity resulted in high bone turnover with increased resorption, which in turn enhanced the prevalence of osteopenia. The ability of Cd to disrupt the functions of calcium transporters and channels in both the plasma membrane and intracellular cites leads to the disruption of the calcemic regulation of osteoclasts and osteoblasts, ultimately resulting to mineral leach and lower bone quality (Bridges and Zalups, 2005; Engström et al., 2012; Öhrvik et al., 2011). These observations indicate that a long-term Cd exposure can cause a disturbance in bone metabolism during skeletal development as well as after maturity. Long term exposure to Cd has also been reported to cause toxicity in bones of other species such as mice, monkeys and humans (Bhattacharyya, 2009; Umemura, 2000).

An illustration of this mechanism is the *itai-itai* disease, characterized by soft, weak and ultimately brittle bones in humans poisoned by Cd (Ufelle and Barchowsky, 2019). Similarly, Epidemiological studies have shown a link between As poisoning and Paget's disease, characterized by an imbalance in bone remodeling, which in turn leads to bone deformities, increased fracture rates, and pain (Lever, 2002). Effects of As on the bones of small animals have also been demonstrated in some previous studies. For example, long bone-endochondral ossification was inhibited in rats even after a low dose intoxication with As (Aybar Odstrcil et al., 2010). A mechanism of As-induced bone damage has also been demonstrated previously, with exposure to inorganic As decreasing the maturation of osteoclast precursors and osteoclastic activity by decreasing RANKL expression and TRAP activity, respectively, which in turn alters the bone resorption process (Hu et al., 2012).

Our data showed the increased burden of arsenic and/or cadmium in squirrels and muskrats from the vicinity of the Giant Mine site but with no unique pathological and densitometric changes compared to the reference site apart from changes in percentage of fat content which suggest that threshold for bone related toxic effects in the studied species may be above the measured levels in the bones of muskrats and red squirrels. Arsenic is well studied metalloid in common laboratory animals such as rats, rabbits and mice. In short term studies, the only bone related effect to arsenic exposure was reported in mice at a relative higher exposure level of 6000 μg/kg/day, where decreased polychromatic erythrocytes in the bone marrow was observed. At 3000 μg/kg/day dose within the same study, there was no effect in mice (ATSDR, 2007). On the other hand, long term 12-week exposure of rats to arsenic trioxide (0.05 or 0.5 ppm) in drinking water altered BMD and microstructure of bones (Wu et al., 2014). The levels of As reported in water in location 1 near the Giant Mine site are 0.1 – 0.2 ppm (Amuno et al., 2020a); hence, adverse effect on bone was expected due to reported effect of these levels on BMD in other species. The lack of effect on BMD in our study suggest potential species-specific difference in the response to arsenic. It is also interesting to note that there was no significant increase in the accumulation of cadmium in the bones of muskrats captured from any of sites, which is in contrast to squirrel for which significantly higher cadmium was found in animals captured from locations 1 and 2 compared to location 3. Species specific differences in the metal accumulation has been observed in other small mammalian species captured from field (Wijnhoven et al., 2007). The species-specific differences in the accumulation of metal is very common in living organisms and the most obvious reason is the differences in absorption, distribution, metabolism and excretion of metals in various species.

Finally, our results suggest a species-specific difference in the response towards metal(loids) between squirrel and muskrats as there was a statistically significant correlation between As content in the bones and percent fat content in femur of muskrats but not in squirrels. These species-specific differences in the response to As and Cd exposure could be either due to inherent tolerance of the squirrel bones or due to the toxicokinetic differences between squirrels and muskrats. In our previous studies also, marked differences in parameters such accumulation of As in brain and oxidative stress response were recorded between these two species (Amuno et al., 2020c). Further studies are required to understand the basis of these differences in the response to chronic As and Cd exposure between muskrats and squirrels.

## 5. Conclusions

Chronic exposures to As and Cd have been indicated to be associated with bone disorders in several studies. Our previous monitoring study in Yellowknife revealed that the bones of snowshoe hares from 2 km and 20 km radius of the vicinity of the Giant Mine exhibited similar skeletal abnormalities along the axial and appendicular bones, respectively. Specifically, we noted that hares from 2 km and 20 km radius of the Giant Mine site both showed evidence of growth defects, osteoporosis, cortical fractures, sclerosis, and cyst like changes. The results from this present study provide baseline densitometric and radiographic information about the bones of red squirrels and muskrats from arsenic hotspot areas of Yellowknife and background areas. The radiographic data from our investigation indicate that none of the animals from the As affected areas exhibited any distinctive and significant pathological bone changes that is different from the reference location. Overall, the radiographic bone changes observed are not distinct enough between locations to link the incidence, pattern, and severity of bone abnormalities to chronic arsenic poisoning in the study area. But nevertheless, to draw a clearer picture and reach conclusive results, we need to further expand our studies via including the skulls, spine, long and short bones of these animals. In addition, for a better etiology understanding, it is crucial to link the current animal bone and other visceral disorders with the causations of significant skeletal and extra-skeletal abnormalities in humans in and around the vicinity of Yellowknife. The only parameter from densitometric measurements that showed a significant difference in the muskrats collected from different sites was the percentage of fat content in the femur, which indicates the possibility of disturbance in bone physiology. Moreover the effect on percentage of fat content was inversely correlated with As accumulation in bone, further suggesting that As contamination near Yellowknife mining region may have caused bone damage in muskrats at biochemical level; however those effects were not manifested at the morphological levels in the radiological assessments.

## CRediT authorship contribution statement

**Solomon Amuno**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation. **Som Niyogi:** Conceptualization**,** Data curation, Investigation, Methodology, Project administration, Resources, Supervision, Validation. **Al Kaissi:** Conceptualization, Formal analysis, Investigation, Methodology, Validation. **Kamran Shekh:** Data curation, Formal analysis, Investigation. **Vladmir Kodzhahinchev:** Data curation, Formal analysis, Investigation.

## 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.

## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111721.

## References

Alfvén, T., Elinder, C.G., Carlsson, M.D., Grubb, A., Hellström, L., Persson, B., Pettersson, C., Spång, G., Schütz, A., Järup, L., 2000. Low-level cadmium exposure and osteoporosis. J. Bone Miner. Res. 15, 1579–1586. https://doi.org/10.1359/jbmr.2000.15.8.1579.

Amuno, S., Al Kaissi, A., Jamwal, A., Niyogi, S., Quenneville, C.E., 2018. Chronic arsenicosis and cadmium exposure in wild snowshoe hares (Lepus americanus) breeding near Yellowknife, Northwest Territories (Canada), part 2: manifestation of bone abnormalities and osteoporosis. Sci. Total Environ. 612, 1559–1567. https://doi.org/10.1016/J.SCITOTENV.2017.08.280.

Amuno, S., Bedos, L., Kodzhahinchev, V., Shekh, K., Niyogi, S., Grahn, B., 2020a. Comparative study of arsenic toxicosis and ocular pathology in wild muskrats (Ondatra zibethicus) and red squirrels (Tamiasciurus hudsonicus) breeding in arsenic contaminated areas of Yellowknife, Northwest Territories (Canada). Chemosphere 248, 126011. https://doi.org/10.1016/J.CHEMOSPHERE.2020.126011.

Amuno, S., Rudko, D.A., Gallino, D., Tuznik, M., Shekh, K., Kodzhahinchev, V., Niyogi, S., Chakravarty, M.M., Devenyi, G.A., 2020b. Altered neurotransmission and neuroimaging biomarkers of chronic arsenic poisoning in wild muskrats (Ondatra zibethicus) and red squirrels (Tamiasciurus hudsonicus) breeding near the City of Yellowknife, Northwest Territories (Canada). Sci. Total Environ. 707, 135556 https://doi.org/10.1016/J.SCITOTENV.2019.135556.

Amuno, S., Shekh, K., Kodzhahinchev, V., Niyogi, S., 2020c. 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. Sci. Total Environ. 704, 135426 https://doi.org/10.1016/J.SCITOTENV.2019.135426.

ATSDR, Agency for Toxic Substances and Disease Registry: Toxicological Profile for Arsenic. 2007. U.S. Department of Health and Human Services. 〈https://www.atsdr.cdc.gov/〉.

Aybar Odstrcil, A., del, C., Carino, S.N., Diaz Ricci, J.C., Mandalunis, P.M., 2010. Effect of arsenic in endochondral ossification of experimental animals. Exp. Toxicol. Pathol. 62, 243–249. https://doi.org/10.1016/J.ETP.2009.04.001.

Bhattacharyya, M.H., 2009. Cadmium osteotoxicity in experimental animals: mechanisms and relationship to human exposures. Toxicol. Appl. Pharmacol. 238, 258–265. https://doi.org/10.1016/j.taap.2009.05.015.

Bridges, C.C., Zalups, R.K., 2005. Molecular and ionic mimicry and the transport of toxic metals. Toxicol. Appl. Pharmacol. 204, 274–308. https://doi.org/10.1016/j.taap.2004.09.007.

Brzóska, M.M., Moniuszko-Jakoniuk, J., 2005. Disorders in bone metabolism of female rats chronically exposed to cadmium. Toxicol. Appl. Pharmacol. 202, 68–83. https://doi.org/10.1016/j.taap.2004.06.007.

Engström, A., Michaëlsson, K., Vahter, M., Julin, B., Wolk, A., Åkesson, A., 2012. Associations between dietary cadmium exposure and bone mineral density and risk of osteoporosis and fractures among women. Bone 50, 1372–1378. https://doi.org/10.1016/J.BONE.2012.03.018.

Hu, Y.-C., Cheng, H.-L., Hsieh, B.-S., Huang, L.-W., Huang, T.-C., Chang, K.-L., 2012. Arsenic trioxide affects bone remodeling by effects on osteoblast differentiation and function. Bone 50, 1406–1415. https://doi.org/10.1016/j.bone.2012.03.012.

Kinimo, K.C., Yao, K.M., Marcotte, S., Kouassi, N.L.B., Trokourey, A., 2018. Distribution trends and ecological risks of arsenic and trace metals in wetland sediments around gold mining activities in central-southern and southeastern Côte d'Ivoire. J. Geochem. Explor. 190, 265–280. https://doi.org/10.1016/j.gexplo.2018.03.013.

Lever, J.H., 2002. Paget's disease of bone in Lancashire and arsenic pesticide in cotton mill wastewater: a speculative hypothesis. Bone 31, 434–436. https://doi.org/10.1016/s8756-3282(02)00833-5.

Lewchalermvong, K., Rangkadilok, N., Nookabkaew, S., Suriyo, T., Satayavivad, J., 2018. Arsenic Speciation and accumulation in selected organs after oral administration of rice extracts in Wistar rats. J. Agric. Food Chem. 66, 3199–3209. https://doi.org/10.1021/acs.jafc.7b05746.

Liu, Z., Hou, Y., Li, L., Yang, Y., Jia, J., Hong, Z., Li, T., Xu, Y., Fu, J., Sun, Y., Yamamoto, M., Wang, H., Pi, J., 2019. Nrf2 deficiency aggravates the increase in osteoclastogenesis and bone loss induced by inorganic arsenic. Toxicol. Appl. Pharmacol. 367, 62–70. https://doi.org/10.1016/j.taap.2019.02.003.

Martin, R.B., Chow, B.D., Lucas, P.A., 1990. Bone marrow fat content in relation to bone remodeling and serum chemistry in intact and ovariectomized dogs. Calcif. Tissue Int. 46, 189–194. https://doi.org/10.1007/BF02555043.

Mensah, A.K., Marschner, B., Shaheen, S.M., Wang, J., Wang, S.-L., Rinklebe, J., 2020. Arsenic contamination in abandoned and active gold mine spoils in Ghana:

geochemical fractionation, speciation, and assessment of the potential human health risk. Environ. Pollut. 261, 114116 https://doi.org/10.1016/j.envpol.2020.114116.

Meunier, P., Aaron, J., Edouard, C., Vignon, G., 1971. Osteoporosis and the replacement of cell populations of the marrow by adipose tissue. A quantitative study of 84 iliac bone biopsies. Clin. Orthop. Relat. Res. 80, 147–154. https://doi.org/10.1097/00003086-197110000-00021.

Miller, C.B., Parsons, M.B., Jamieson, H.E., Swindles, G.T., Nasser, N.A., Galloway, J.M., 2019. Lake-specific controls on the long-term stability of mining-related, legacy arsenic contamination and geochemical baselines in a changing northern environment, Tundra Mine, Northwest Territories, Canada. Appl. Geochem. 109, 104403 https://doi.org/10.1016/j.apgeochem.2019.104403.

Mohammed Abdul, K.S., Jayasinghe, S.S., Chandana, E.P.S., Jayasumana, C., De Silva, P. M.C.S., 2015. Arsenic and human health effects: a review. Environ. Toxicol. Pharmacol. 40, 828–846. https://doi.org/10.1016/j.etap.2015.09.016.

Mondal, D., Banerjee, M., Kundu, M., Banerjee, N., Bhattacharya, U., Giri, A.K., Ganguli, B., Roy, S., Sen, Polya, D.A., 2010. Comparison of drinking water, raw rice and cooking of rice as arsenic exposure routes in three contrasting areas of West Bengal, India. Environ. Geochem. Health 32, 463–477. https://doi.org/10.1007/s10653-010-9319-5.

Naila, A., Meerdink, G., Jayasena, V., Sulaiman, A.Z., Ajit, A.B., Berta, G., 2019. A review on global metal accumulators—mechanism, enhancement, commercial application, and research trend. Environ. Sci. Pollut. Res. 26, 26449–26471. https://doi.org/10.1007/s11356-019-05992-4.

Nuttall, M.E., Gimble, J.M., 2000. Is there a therapeutic opportunity to either prevent or treat osteopenic disorders by inhibiting marrow adipogenesis? Bone 27, 177–184. https://doi.org/10.1016/s8756-3282(00)00317-3.

Nuttall, M.E., Patton, A.J., Olivera, D.L., Nadeau, D.P., Gowen, M., 1998. Human trabecular bone cells are able to express both osteoblastic and adipocytic phenotype: implications for osteopenic disorders. J. Bone Miner. Res. 13, 371–382. https://doi.org/10.1359/jbmr.1998.13.3.371.

Öhrvik, H., Ullerås, E., Oskarsson, A., Tallkvist, J., 2011. Effects of cadmium on calcium transporter SPCA, calcium homeostasis and β-casein expression in the murine mammary epithelium. Toxicol. Lett. 201, 80–85. https://doi.org/10.1016/J.TOXLET.2010.12.008.

Orssatto, L.B. da R., Bezerra, E. de S., Moura, B.M. de, de Castro, J.A.C., Silva, D.A.S., Moro, A.R.P., Diefenthaeler, F., 2018. Estimating bone mineral content based on different types of muscle strength tests. J. Bodyw. Mov. Ther. 22, 586–591. https://doi.org/10.1016/j.jbmt.2017.09.020.

Podgorski, J., Berg, M., 2020. Global threat of arsenic in groundwater. Science 368, 845–850. https://doi.org/10.1126/science.aba1510.

Schellinger, D., Lin, C.S., Lim, J., Hatipoglu, H.G., Pezzullo, J.C., Singer, A.J., 2004. Bone marrow fat and bone mineral density on proton MR spectroscopy and dual-energy X-ray absorptiometry: their ratio as a new indicator of bone weakening. AJR Am. J. Roentgenol. 183, 1761–1765. https://doi.org/10.2214/ajr.183.6.01831761.

Staessen, J.A., Roels, H.A., Emelianov, D., Kuznetsova, T., Thijs, L., Vangronsveld, J., Fagard, R., 1999. Environmental exposure to cadmium, forearm bone density, and risk of fractures: prospective population study. Public Health and Environmental Exposure to Cadmium (PheeCad) Study Group. Lancet 353, 1140–1144. https://doi.org/10.1016/s0140-6736(98)09356-8.

Suzuki, Kazuo T., Mandal, Badal K., Ogra, Yasumitsu, 2002. Speciation of arsenic in body fluids. Talanta 58 (1), 111–119.

Ufelle, A.C., Barchowsky, A., 2019. Toxic effects of metals. In: Klaassen, C.D. (Ed.), Casarett & Doulls Toxicology: The Basic Science of Poisons. McGraw-Hill Education, pp. 1107–1161.

Umemura, T., 2000. Experimental reproduction of itai-itai disease, a chronic cadmium poisoning of humans, in rats and monkeys. Jpn. J. Vet. Res. 48, 15–28.

Wang, G.J., Sweet, D.E., Reger, S.I., Thompson, R.C., 1977. Fat-cell changes as a mechanism of avascular necrosis of the femoral head in cortisone-treated rabbits. J. Bone Jt. Surg. Am. 59, 729–735.

Wijnhoven, S., Leuven, R.S.E.W., van der Velde, G., Jungheim, G., Koelemij, E.I., De Vries, F.T., Eijsackers, H.J.P., Smits, A.J.M., 2007. Heavy-metal concentrations in small mammals from a diffusely polluted floodplain: importance of species-and location-specific characteristics. Arch. Environ. Contam. Toxicol. 52 (4), 603–613.

Wilson, D.E., Ruff, S., 1999. The Smithsonian book of North American mammals. American Society of Mammalogists.

Wronski, T.J., Walsh, C.C., Ignaszewski, L.A., 1986. Histologic evidence for osteopenia and increased bone turnover in ovariectomized rats. Bone 7, 119–123. https://doi.org/10.1016/8756-3282(86)90683-6.

Wu, C.T., Lu, T.Y., Chan, D.C., Tsai, K.S., Yang, R., Sen, Liu, S.H., 2014. Effects of arsenic on osteoblast differentiation in vitro and on bone mineral density and microstructure in rats. Environ. Health Perspect. 122, 559–565. https://doi.org/10.1289/ehp.1307832.