Cadmium-induced toxicity to the mite, *Oppia nitens* C.L. Koch, 1836 (Acari: Oribatida): Maternal transfer, bioenergetics, and the influence of habitat quality on the mite's response to cadmium toxicity

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Saskatoon, Saskatchewan

By

Hamzat Oluseun Fajana

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S7N 5A8

Dean
College of Graduate and Postdoctoral Studies
University of Saskatchewan
116 Thorvaldson Building, 110 Science Place
Saskatoon, Saskatchewan S7N 5C9
Canada

Abstract

The mite, *Oppia nitens*, is a true soil dweller, one of many organisms that perform vital functions to support ecosystem services. Environment and Climate Change Canada and International Organization for Standardization recently completed a standardized protocol for *Oppia nitens*. Therefore, *O. nitens* is among the battery of soil invertebrates for toxicity testing, but its applicability is limited by the dearth of information on its responses to and interactions with contaminants in soil, and how soil affects its biology and ecology. The main objective of this study was to assess the responses of *O. nitens* to cadmium (Cd), a model chemical that is potentially toxic to this species, and to understand how habitat quality influences *O. nitens'* reproduction and bioenergetics upon exposure to Cd in soil.

Firstly, a critical review of the literature on the biology and ecology of *O. nitens* with notes on its response to metals and pesticides in soil was done. Also, the possible mechanisms on how *O. nitens* could respond to cadmium was proposed. This study, for the first time, gave detailed information on the bionomics (biology and ecology) of *O. nitens*, thus supporting existing knowledge on the applicability of *O. nitens* as test organisms in soil ecotoxicology.

The toxicity and uptake of cadmium, as cadmium oxide (CdO) in standard soil was assessed on adult *O. nitens* and maternal transfer of Cd from adult to juvenile mites (tritonymphs) was estimated. According to the results, Cd as an oxide caused low toxicity compared to Cd as salts for both survival (LC50 = > 700 mg Cd kg^{-1}) and reproduction (EC50 = 392 mg Cd kg^{-1} and EC25 = 215 mg Cd kg^{-1}). The uptake of Cd by adult and juvenile mites was via the ingestion of total Cd and not via dermal adsorption of dissolved Cd in pore water. Adult *O. nitens* maternally transferred about 39 to 52 % (average of 46 %) of their Cd body burden to juveniles while the maternally acquired Cd in the juveniles accounted for 41 % of their Cd body burden.

Finally, the influence of habitat quality on the reproduction and bioenergetics of *O. nitens* upon exposure to Cd was investigated. Mites raised in high and low habitat quality soils were exposed to Cd in neutral (artificial) soil for 28 days to assess their reproduction and energy reserves, including the activities of glucose metabolism enzymes, glucose-6-phosphate dehydrogenase and pyruvate kinases. Cd was found to alter the carbohydrate reserves of the mites that were exposed to 0–700 mg Cd kg^{-1} and reduced energy production by inhibiting the activities of glucose

metabolism enzymes. Upon exposure of the mites from low and high habitat quality to concentrations equivalent to EC25 and EC50 of Cd, we found habitat quality to directly influence mite's reproduction but not bioenergetics. This study, thus, supports previous knowledge of how habitat quality can modulate metal-induced toxicity on *O. nitens*. The findings from this research thus suggests the incorporation of maternal transfer in setting soil quality guidelines for soil invertebrates and also the inclusion of habitat characterization in procedures for ecological risk assessment of contaminated sites.

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List of Abbreviations

CCME	Canadian Council of Ministers of Environment
EC25 Or EC50	Effective concentration (25 or 50% reduction compared to the control response)
ECx	Effective concentration(s)
LC50	Lethal concentration causing 50% mortality compared to the control response
CEC	Cation exchange capacity
WHAM	Windermere Humic Aqueous Model
DNA	Deoxyribonucleic acid
ISO	International Organization of Standardization
OECD	Organization for Economic Co-operation and Development
SQG_E	Environmental Soil Quality Guideline
USEPA	United States Environmental Protection Agency
SSD	Species sensitivity distribution

1. Introduction

Cadmium (Cd) is a highly toxic heavy metal that is introduced into the environment via mining and smelting activities (Thornton, 1988). The anthropogenic input of cadmium into the soil is the most significant source of cadmium availability to soil biota and humans (Loganathan et al., 2012). Cadmium causes toxicity to organisms in the soil, which include invertebrates, plants, and microbes, thereby affecting soil ecosystem functions (Van Straalen et al., 1989; Das et al., 1997; Vig et al., 2003).

Soil invertebrates significantly accumulate Cd from the soil, and like any other metal, the accumulation from soil depends on toxicokinetics and toxicodynamic processes. Toxicokinetic (uptake and elimination) of Cd depends on both the physicochemical desorption in soil water and the physiology of the invertebrate, such as exposure route, maternal transfer, degree of sclerotization and moulting activities (for soil microarthropods), and Cd compartmentalization in storage sites (Ardestani et al., 2014). The toxicodynamics of Cd involves Cd binding to cellular proteins (e.g., metallothionein and phytochelatin) (Wang et al., 2010a), energy utilization for detoxification, and the induction of metabolic and antioxidant enzymes (Maria et al., 2014). The habitat in which soil organisms live could also influence metal toxicokinetics and toxicodynamics. For example, a high quality habitat could mediate the toxicity caused by metals to soil invertebrates by providing the soil invertebrate with micronutrients or energy to subsidize the toxicity of metal (Jegede et al., 2019b). However, laboratory-based exposure data that are used to set environmental soil quality guidelines (SQG_E) for metals does not usually account for variations that are caused by toxicokinetics and toxicodynamic factors.

Therefore, this research was done to assess some toxicokinetic and toxicodynamic factors (i.e., uptake route, maternal transfer, and bioenergetics), and habitat quality as an ecological factor, that could influence cadmium toxicity in a standard soil invertebrate, *Oppia nitens* (ISO, 2019).

The research objectives and hypotheses are;

- To conduct a critical review of the biology and ecology of the test organism, *O. nitens*, and how it responds to chemicals, especially heavy metals in soil.
- To assess the uptake, toxicity, and maternal transfer of cadmium in *O. nitens* using an artificial OECD soil as test soil.
  H_0: Maternal transfer of cadmium in *O. nitens* is negligible, and cadmium does not cause significant toxicity on life history responses of the mites.
- To assess if habitat quality could mediate the response of *O. nitens* to cadmium at concentrations that are expected to cause 25 and 50 % (i.e., EC25 and EC50 respectively) reduction in reproduction of the mites.
  H_0: Habitat quality does not influence *O. niten*'s response to cadmium toxicity.

The critical review of the literature associated with the first objective is presented as section 2.7 in chapter 2, and was published in the journal, *Environmental Toxicology* and *Chemistry*. The research associated with the second objective is presented as chapter 3, and was published in the journal, *Environmental Pollution*. The research associated with the third objective is presented as chapter 4, and the results will be submitted to the journal, *Soil Biology and Biochemistry*.

2. Literature Review

2.1 Introduction

The soil serves as a habitat to below-ground organisms and plants. Soil also acts as a repository of several contaminants, such as heavy metals. Mining and smelting activities have introduced heavy metals into the soil at concentrations that are toxic to the below-ground soil organisms, such as invertebrates and microbes, as well as plants and humans (Tyler et al., 1989; Godt et al., 2006). Canada is one of the top metal mining hubs in the world, with a production of more than 60 minerals and metals that was valued at $44 billion in 2017 (NRC, 2019). Metal mining in Canada includes gold, zinc, uranium, copper, nickel, and cobalt (Figure 2-1). Despite the considerable contribution of the metal mining industry to the economy of Canada, we cannot deny metal pollution in soil and its associated effect on the environment.

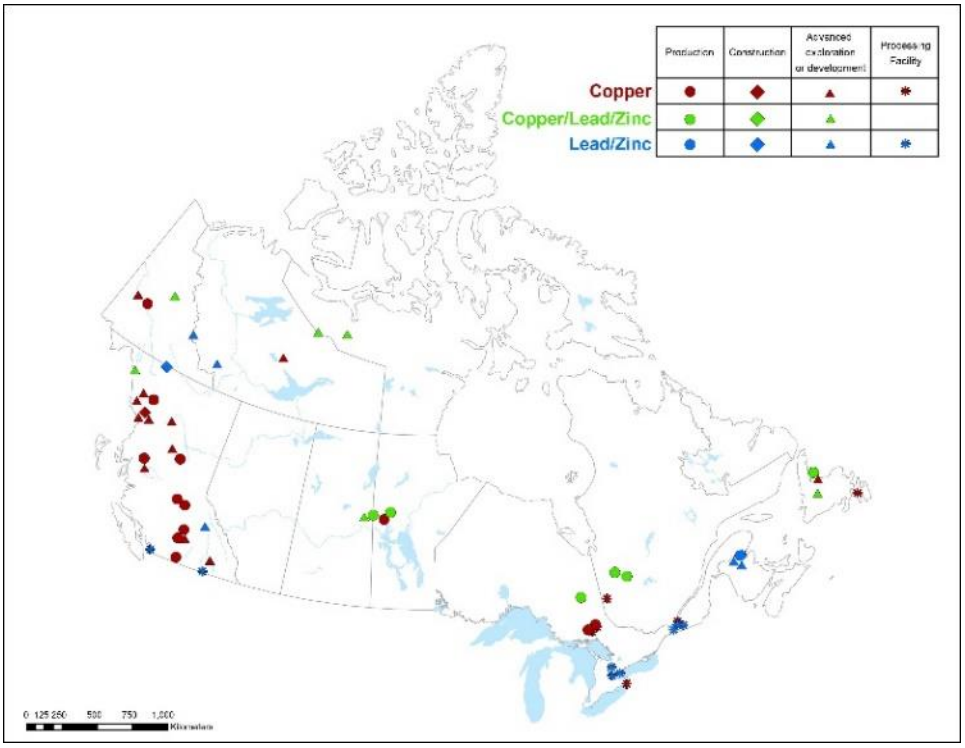

Figure 2-1. A map of Canada showing the geographic location of metal mines, smelters, and refineries that produce copper (Cu), lead (Pb), and zinc (Zn). Modified from NRC (2017).

There has been an increase in global Cd production (Figure 2-2), which is related to the processing of ores that are rich in copper (Cu), lead (Pb), and mostly zinc (Zn). According to Chizhikov (1966) and Nriagu (1980), the Cd associated with the production of zinc ore (sphalerite, ZnS) ranges from 0.2 – 0.7 % by weight, and this estimate of Cd input into the environment is higher than the amount that is realized solely due to the mining of Cd minerals.

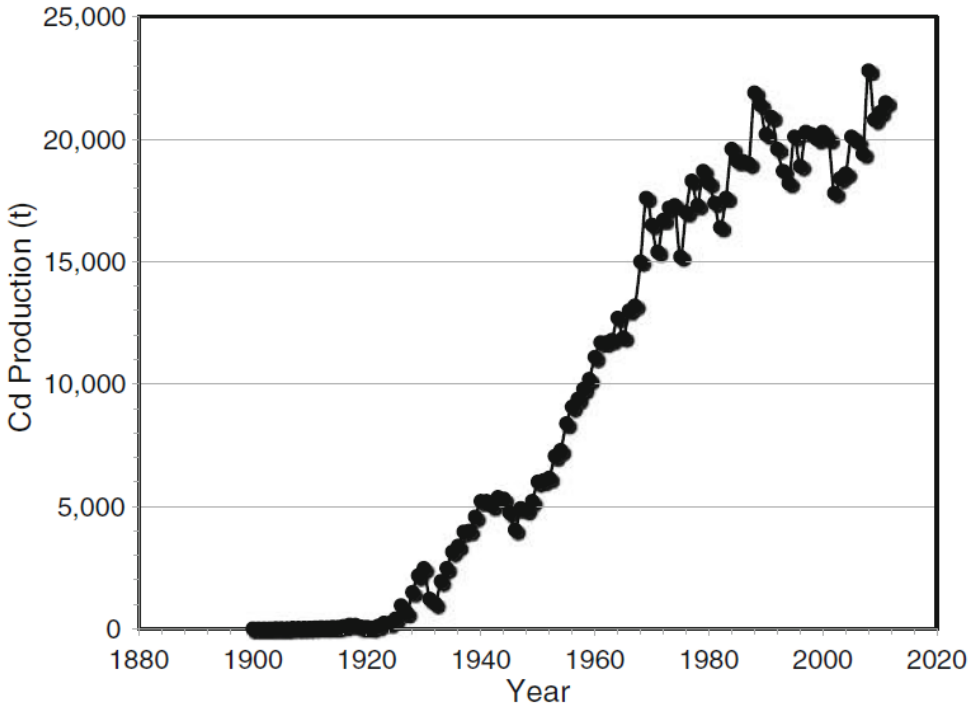

Figure 2-2. A time series on the world production of cadmium as reported by the U.S. Geological Survey (2012). Data before 1937 represent only a subset of countries. Modified from Cullen and Maldonado (2014).

Cadmium is persistent in the soil; as such, it has the potential to become bioavailable to soil invertebrates and plants. For instance, the half-life of cadmium in soil could range from 15 to 1,100 years with a relatively high transfer coefficient between the soil and the soil organisms (Vig et al., 2003). Cadmium mostly occurs naturally in the soil at very low concentrations. However, the anthropogenic input of cadmium into the soil has elevated the level of cadmium to concentrations that are potentially toxic to plants, soil invertebrates, microbes, and humans (Loganathan et al., 2012). Cadmium that results from anthropogenic input predominantly occurs as cadmium oxide (CdO) (Chlopecka et al., 1996). The mode of toxic action of cadmium to soil organisms, including humans, is linked to the generation of reactive oxygen species (ROS) (Lopez et al., 2006). Therefore, the bioaccumulation of cadmium by soil invertebrates and other organisms is of concern because it is a non-essential metal that can cause oxidative damage in cellular compartments and tissues (Zhang and Reynolds, 2019).

Cadmium is highly toxic to soil invertebrates (Van Straalen et al., 1989). The current CCME soil quality guideline (SQG) of Cd to soil invertebrates is 10 mg kg^{-1} in agricultural land use and 22 mg kg^{-1} in commercial and industrial land use (CCME, 1999). Cd toxicity to soil invertebrates includes the inhibition of fecundity, hatching, and growth (Seniczak et al., 2009; Goncalves et al. 2015) as well as other sub-organismal effects such as oxidative damage and inhibition of energy metabolism (Wang et al. 2010b; Gomes et al., 2018). Cd reduces invertebrate reproduction and survival (Van Straalen et al. 1989) and ultimately affects the ecosystem functions and services of soil invertebrates.

2.2 Bioavailability of cadmium and other heavy metals to soil invertebrates

The fraction of a heavy metal that could cause toxicity to soil invertebrates is termed "bioavailability." The bioavailability of metals to soil invertebrates is governed by two factors: (1) the organism's physiological processes relating to the uptake of metal from soil, and (2) the physicochemical processes relating to the properties of soil and the metal in question (McCarthy and Mackay, 1993). Metal uptake from the environment can be termed "environmental bioavailability" because it is the first process that needs to occur before any metal gets into the internal compartment of the organism from the environment. The route of uptake of metal from

the soil is different between soil invertebrates and depends on physiology, microhabitat, trophic guilds, and food specificity (Peijnenburg, 2002).

Metal uptake via food depends on an invertebrate's rate of food consumption and its preference for a particular food in its habitat (Hopkin, 1989). For instance, soil oribatid mites that are grazers accumulate metals at a high rate from fungi (Siepel, 1995; Skubala et al., 2016). Fungi sequester metals from heavy metal contaminated sites (Roth, 1992; Skubala et al., 2016) and soil oribatid mites that are grazers have a preference for fungi because these mites have high activity of the enzyme, chitinase that can digest fungi (Siepel, 1995; Siepel and Ruiter-Dijkman 1993). The quality of food or habitat encourages soil invertebrates to feed (Hope, 2001); thus, high quality habitats lead to an increase in uptake rate of metal contaminated food. Oribatid soil mites, *O. nitens* in a high quality habitat soil have a higher internal zinc concentration than mites in low habitat quality soil do, suggesting a high uptake of zinc for mites in high quality habitat (Jegede et al. 2019b).

Soil invertebrates with hard cuticles, like mites and beetles, predominantly accumulate metals from the soil by ingesting contaminated soil particles or organic matter rather than dermal adsorption via soil solution. For instance, Jegede et al. (2019b) assessed zinc (ZnO) availability in *O. niten*s across 18 soils and found total zinc rather than free zinc (Zn^{2+}) or extractable zinc ($CaCl_2$-extractable zinc) to predict internal zinc concentration in the mites. Also, the uptake of cadmium and lead by *Folsomia candida,* a partially sclerotized soil invertebrate, was strongly explained by the total metal pool in soil, suggesting uptake to be predominantly via ingestion of soil (Vijver et al., 2001). Vijver et al. (2003) also found total Cd pool to predict the uptake of Cd by larvae of the beetle (*Tenebrio molitor*) from OECD and field soils. In soft-bodied soil invertebrates, metal uptake is mostly predicted by water-soluble or pore water metal concentration. The "Pore water hypothesis'' states that exposure to contaminants occurs mainly through the solution phase, or indirectly by phases that are in equilibrium with the pore water (Van Gestel and Ma, 1988). However, the partitioning of metal into solution phase is governed by soil properties such as pH, clay content, organic matter content, and cation exchange capacity (CEC). For example, Lock et al. (2000) investigated the uptake of zinc and cadmium from OECD soil by the enchytraeid, *Enchytraeus albidu*s and found uptake to be via water-soluble Cd

and Zn concentration, with pH, organic matter content and CEC governing the partitioning of the metals between the soil solid phase and pore water. Thus, the result of Lock et al. (2000) suggests "pore water hypothesis" of metal uptake to hold for soft-bodied soil invertebrates. Similarly, in OECD soil that was spiked with cadmium, Crommentuijin et al. (1997) found water-soluble Cd concentration strongly predicted the internal Cd level in *F. candida*. Thus, the authors suggest uptake to be via dermal adsorption of soluble Cd, which was driven by soil pH, and organic matter content.

Soil pH and CEC correlate with equilibrium partitioning of metals between solid soil phase and pore water (Buchter et al., 1989; Jansen et al., 1997). However, for Cd in soils, pH and organic matter content are probably the most critical factors governing the partitioning of Cd between solid soil phase and pore water (Lee et al., 1996). Besides, the speciation of cadmium in soil could influence the equilibrium partitioning of cadmium into the soil solid phase or pore water. For example, the chloride anion (Cl^-) in $CdCl_2$-dosed soil reduces the soil pH to favour desorption into pore water rather than binding to clay, organic matter or forming complexes with metal oxyhydroxides (Fe, Mn). Soil pH could predict the free ion activity [Cd^{2+}] of cadmium but [Cd^{2+}] and likewise [Zn^{2+}], does not often correlate with internal metal concentration in soil invertebrates (Jegede et al. 2019b). It is difficult to measure the biosorption of free metal ion (Me^{2+}) from pore water to soil biota (Plette et al., 1999). However, a biotic ligand model (BLM) for terrestrial system predicts the binding of Me^{2+} to soil invertebrates but soil factors such as pH, dissolved organic matter (DOM), and ionic strength still govern the activity of Me^{2+}in soil solution or pore water (Plette et al., 1999).

2.3 Toxicokinetics of cadmium in soil invertebrates

Most soil invertebrates steadily accumulate but slowly eliminate cadmium (Ardestani et al., 2014; Keshavarz-Jamshidian et al., 2017). However, Cd toxicokinetics in soil invertebrates seem to depend on the biology of the organism, route of exposure (Ardestani et al., 2014), and abiotic factors like temperature (Abdel-Lateif et al., 1998; Donker et al., 1998) including pH and competition between H^+ and Cd^{2+} to biotic ligands (Peijnenburg et al. 1999). Cd toxicokinetics in soil invertebrates can be modelled via either a one- or two-compartment toxicokinetic model (Skip et al., 2014) because Cd storage sites represent a second compartment. Cd is usually

compartmentalized in tissues like the hepatopancreas or bound to metallothionein (MT). For example, soil invertebrates like *Helix pomatia*, the isopod *P. scaber*, and *O. nitens* compartmentalize Cd in their hepatopancreas or proventricular gland (Dallinger and Wieser, 1984; Dallinger and Prosi, 1988; Ludwig et al., 1992). About 85–95 % of Cd in the hepatopancreas of the terrestrial snail, *H. pomatia* was bound to MT (Dallinger et al., 1997). Table 2-1 shows a comparison of Cd toxicokinetic parameters, uptake (k_1) and elimination (k_2) rate constants for some soil invertebrates.

Table 2-1. A review of toxicokinetics of cadmium in some soil invertebrates.

Species	Soil type	Cd (mg kg^{-1})	Uptake (day)	Elimination (day)	k_1 (g_s g_{bw}^{-1} day^{-1})	k_2 (day^{-1})	Model	Soil pH	References
EF	AS	5–56500	1–28	1–100	0.046	0.093	1	5.84	[1],[2],[3]
EF	FS	0.084–325	1–42	1–100	0.86	1.22	1	5.67	[2],[4],[5]
EA	AS	0.2–1000	1–63	1–21	0.89	0.019	1	5.40	[6],[7]
EA	NS	22104	14–224	14–224	0.037	0.0065	1	5.50	[8]
FC	NS	4.1–18.2	1–21	1–21	0.665	0.30	1	5.50	[9]
LR	FS	4.46–63.2	0–21	0–18	0.54	0.67	1,2	6.52	[10],[11],[12]
EC	pw	0.3–92	1–35		0.089	0.23	1	5.10	[15]
PS	f	4.10–9.51	14–63	18	0.0096	0.18	1,2		[13],[14]
ON	NS	15.5–506	49		0.026		1	5.50	[17]
PP	f	16.8–4000	30–63	40–65	0.033	0.015	1,2		[14],[16]

Soil invertebrate species: *EF = Eisenia fetida*, *EA = Eisenia andrei*, *FC = Folsomia candida*, *LR = Lumbricus* r*ubellus*, *EC = Enchytraeus crypticus*, *PS = Porcelio scaber*, *ON = Oppia nitens*, *PP = Platynothrous peltifer*.

AS = Artificial soil, FS = Field soil, NS = Natural soil, pw = pore water, f = food
k_1 = Average uptake rate constant [g_s (gram soil) g_{bw}^{-1} (gram body weight) day^{-1}] unless other units were used, k_2 = Average elimination rate constant (day^{-1}), Model = bioaccumulation kinetics Model (1 = one-compartment model, 2 = two-compartment. model).

[1] Conder et al., 2002. [2] Spurgeon and Hopkins, 1999. [3] Lock and Janssen, 2001. [4] Nahmani et al., 2009. [5] Li et al., 2009. [6] Smith et al., 2010. [7] Peijnenburg et al., 1999a. [8] Yu and Lanno, 2010. [9] Broerse et al., 2012. [10] Vijver et al., 2005. [11] Vijver et al., 2003. [12] Giska et al., 2014. [13] Vijver et al., 2006b. [14] Crommentuijn et al., 1994. [15] Peijnenburg et al., 1999b. [16] Janssen and Bergema, 1991. [17] Keshavarz-Jamshidian et al., 2017.

2.4 Maternal transfer of cadmium in soil invertebrates

Maternal transfer of metals through egg deposition is a toxicokinetic mechanism by which female organisms eliminate metals to reduce their metal body burden (Tsui and Wang, 2004a,b). Cadmium is a non-essential metal that could be transferred from parents to offspring by Cd deposition in the egg. However, no study has reported maternal transfer of Cd in soil invertebrates. Maternal transfer of Cd from adult mayflies, *Centroptilum triangulifer* to eggs was rare as it was only observed in < 3 % of individual adults that transfer Cd to eggs with no discernable Cd burden in eggs (Xie et al., 2009). In *Daphnia magna*, < 10 % of Cd was maternally transferred from adult to offspring (Yu and Wang, 2002; Tsui and Wang, 2007).

2.5 Responses of soil invertebrates to cadmium

Cadmium is highly toxic to soil invertebrates, and the toxicity affects reproduction, growth, and survival (Spurgeon et al., 1994). Cadmium could also cause toxicity to soil invertebrates at sub-cellular levels such as the disruption of calcium (Ca^{2+}) homeostasis, oxidative, mitochondrial and DNA damage, epigenetic alterations, alteration of metal responsive proteins, impairment of gene expression and cell cycle arrest (Wang et al., 2010b; Novais et al., 2012; Maria et al., 2014; Srut et al., 2017; Gomes et al. 2018). Also, Cd can disrupt energy metabolism and allocation for critical physiological processes, thus leading to an adverse outcome in the organism (Novais et al., 2013; Gomes et al., 2018).

2.5.1 Effect on reproduction, growth, and survival

Soil invertebrates are sensitive to cadmium, and the toxicity of Cd on their life history is significant with consequences for population growth (Van Straalen et al. 1989). For example, Cd was 5-, 4- and 11-times more toxic (i.e., the effect on survival) than copper, zinc, and lead respectively to *O. nitens* that were exposed to these metals in artificial soil for 35 days (Owojori and Siciliano, 2012). The authors also reported a reduction in juvenile production of *O. nitens*, having an EC50 of 137 mg kg^{-1} compared to Cu (2,896 mg kg^{-1}), Zn (1562 mg kg^{-1}), and Pb (1678 mg kg^{-1}). Cd lowered cocoon production in the earthworm *Eisenia fetida* after the earthworms were exposed to Cd for 56 days in OECD soil (Spurgeon et al., 1994). The authors reported an EC50 of 46.3 µg Cd/g compared with 53.3, 1940, and 276 µg for Cu, Pb, and Zn, respectively. Besides, cocoon production was a more sensitive

endpoint to Cd toxicity than cocoon viability and mortality (Spurgeon et al., 1994). Similarly, Owojori and Siciliano (2012) reported juvenile production in *O. nitens* to be a more sensitive endpoint to Cd than mortality.

In a study by Crommentuijn et al. (1993), Cd retarded the growth of the collembola, *F. candida* in OECD soil and delayed their reproduction after 9 weeks. According to the authors, the effect on reproduction of *F. candida* was not direct but rather a consequence of the reduced growth that caused the delay in reproduction. However, growth was a more sensitive endpoint to Cd with the EC50 (*F. candida*'s growth) at 256 µg Cd/g lower compared to the EC50 on reproduction (> 326 µg Cd/g) and survival (850 µg Cd/g). The growth efficiency of *Porcellio scaber* (a terrestrial isopod) after ingesting Cd-contaminated food was 27 % compared with 48 % in control isopods (Khalil et al., 1995). Nursita et al. (2005) reported the toxicity of Cd on a species of collembola, *Proisotoma minuta* to have a significant effect on growth and reproduction. The EC50 of Cd (125 µg Cd/g) on reproduction of *P. minuta* indicates Cd as the most toxic of all the metals tested on the collembola followed by Zn (283 µg Zn/g) and Cu (696 µg Cu/g). An embryotoxicity study of Cd to the enchytraeid, *Enchytraeus crypticus* found Cd significantly decreased hatching success across all tested concentrations of Cd (1.6 to 50 mg kg^{-1}) in LUFA 2.2 soil (Goncalves et al. 2015). According to the authors, hatching success of *E. crypticus* (EC50 = 3.1 mg kg^{-1}) was a more sensitive endpoint compared to reproduction (EC50 = 35 mg kg^{-1}). Cd also reduces the fecundity of the oribatid mite, *Archegozetes longisetosus* when exposed to Cd at 130 µg/g of food (Seniczak et al., 2009). Cadmium was observed to cause a decline in the population growth of the oribatid mite, *Platynothrus peltifer* (instantaneous population growth rate, r = 0.38) while the population growth of collembola, *Orchesella cincta* remained stable (r = 0.6) because Cd affected the reproduction of *P. peltifer* with relatively less effect on *O. cincta*'s reproduction (Van Straalen et al., 1989).

2.5.2 Effect on sub-organismal responses

2.5.2.1 Molecular and biochemical responses

Cadmium induces metallothionein (MT) in cellular compartments; thus, much of the internal Cd in soil invertebrates is bound by metallothionein to form Cd-MT complex (Dallinger et al., 1997). This mechanism of Cd interaction with MT is the central role of Cd detoxification in soil invertebrates (Stürzenbaum et al., 2004). However, the internally free Cd^{2+} still causes toxicity upon binding with biological molecules in cells. Cd in LUFA 2.2 soil (29.5 and 40.3 mg kg^{-1}) induced (~500 fold change compared to control) the expression of *mtc* (a metallothionein-like motif-containing protein) gene in *F. candida* (Nota et al. 2011). However, there was no significant change in the expression of *hsp20* (heat shock protein) gene (Nota et al. 2011). Cd (5 and 25 mg kg^{-1}) caused significant induction in the expression of *mt* (metallothionein) gene in *E. fetida* that were exposed to Cd in OECD soil with 6 and 10 % organic matter (OM), but the induction was not significant in OECD soil at a higher (14 %) OM content (Irizar et al., 2015). Also, the transcription of *cat* (catalase) gene, i.e., the enzyme responsible for the breakdown of hydrogen peroxide (H_2O_2) to oxygen and water was inhibited in *E. fetida*, but the inhibition was only significant in OECD soil (10 % OM) (Irizar et al., 2015). An assumption from this study is that soil with high OM content might have provided *E. fetida* with enough food to subsidize stress via Cd detoxification, despite the earthworm in high OM soil accumulating more Cd.

Catalase (CAT) activity in *F. candida* was reduced after days of exposure to 60 mg kg^{-1} of Cd in LUFA 2.2 soil (Maria et al., 2014). In the study (Maria et al., 2014), the activities of other cellular antioxidants like glutathione-s-transferase (GST) and metallothionein (MT) increased, but activities were not high enough to prevent lipid peroxidation (LPO) in the collembola after 4 days of exposure to Cd. The onset of LPO in the collembola at 4 days of exposure to Cd could be attributed to the inhibition of CAT activity. Cd at low concentrations induced the activity of catalase (CAT), and superoxide dismutase (SOD) in the earthworm *Eisenia fetida*; however, high concentrations inhibited the enzymes (Zhang et al., 2009). CAT breaks down H_2O_2 into water and oxygen, thus preventing the buildup of peroxide, which can cause LPO. LPO could occur from the direct effect of Cd on lipid since Cd can generate ROS by indirectly contributing to the stepwise reduction of superoxide

radical (Cuypers et al., 2010). Cd through binding with the cellular antioxidant, glutathione (GSH) can reduce the activity of GST because of substrate depletion. Cd (100mg Cd kg^{-1}) significantly depleted GSH levels and disrupted the activity of GST in the enchytraeid, *E. albidus* (Novais et al., 2011).

Wang et al. (2010b) also report Cd to cause the up/down-regulation of some proteins that are crucial to the physiological and metabolic processes in the earthworm, *E. fetida*. Some of the regulated proteins include heat shock protein (HSP, involved in protein folding), superoxide dismuthase (SOD, an antioxidant against superoxide radicals), calcium-ion binding protein (involved in regulating intracellular Ca^{2+}) and also proteins that are involved in glucose metabolism and Tricarboxylic Acid Cycle (TCA). Also, Cd disrupts Ca^{2+} homeostasis in the embryo of *Enchytraeus crypticus* by competing intracellularly with Ca^{2+} to reduce Ca^{2+} efflux, thus leading to embryotoxicity (Gomes et al. 2018). In addition to the Ca^{2+} imbalance, Cd affected energy metabolism pathways such as TCA and oxidative phosphorylation (Gomes et al. 2018). Low levels of Cd induced epigenetic alteration on the epigenome of the earthworm, *Lumbricus terrestris,* by increasing DNA methylation in loci that are susceptible to methylation (Srut et al., 2017).

2.5.2.2 Bioenergetics response

Energy is a variable that is conserved across all levels of biological organization. It reflects the health status of organisms and productivity across trophic levels (Forbes *et al*., 2017). The energy acquired by soil invertebrates via feeding is usually stored as carbohydrate, lipid, and protein reserves (Figure 2-3A). These reserves are metabolized and allocated for various physiological processes such as reproduction, growth, and maintenance (Figure 2-3). Exposure to heavy metals alters cellular energy acquisition, metabolism, and allocation in soil invertebrates (Novais et al., 2013). Alteration in the above bioenergetic processes involves a series of molecular mechanisms that cause cascades of metabolic processes leading to reduced fitness such as reproduction and growth of the organism (Ankley *et al*., 2010; Forbes *et al*., 2017). For example, a change in energy allocation might shift energy budgeting to somatic maintenance and detoxification processes, at the cost of reproduction and growth. According to Krebs and Loeschcke (1994), more energy is usually budgeted for

processes like heat-shock protein production during stress at the cost of reproduction. However, organisms have different metabolic strategies for coping with stress.

Cd depleted the cellular energy of *E. albidus* that was exposed to EC50 (6 mg Cd kg^{-1}) and EC90 (150 mg Cd kg^{-1}) of cadmium (Novais et al., 2013). From the study, a significant change in cellular energy was caused by a depletion in carbohydrate reserve and an elevation in electron transport activity, which is a measure of the amount of energy consumed during cellular respiration. Holmstrump et al. (2011) found no correlation between glycogen reserve and Cd body burden in the earthworm, *Dendrobaena octaedra,* at a metal-contaminated site. Despite a high Cd body burden in ground beetles, *Pterostichus oblongopunctatus* that were collected from a metal-polluted site, no change in the beetles' total energy reserves (i.e., carbohydrate, lipid, and protein) was observed (Bednarska et al. 2013). The observations from Holmstrump et al. (2011) and Bednarska et al. (2013) suggest that some soil invertebrates might have a different strategy of coping with high metal concentration without a metabolic cost of energy utilization on metal detoxification.

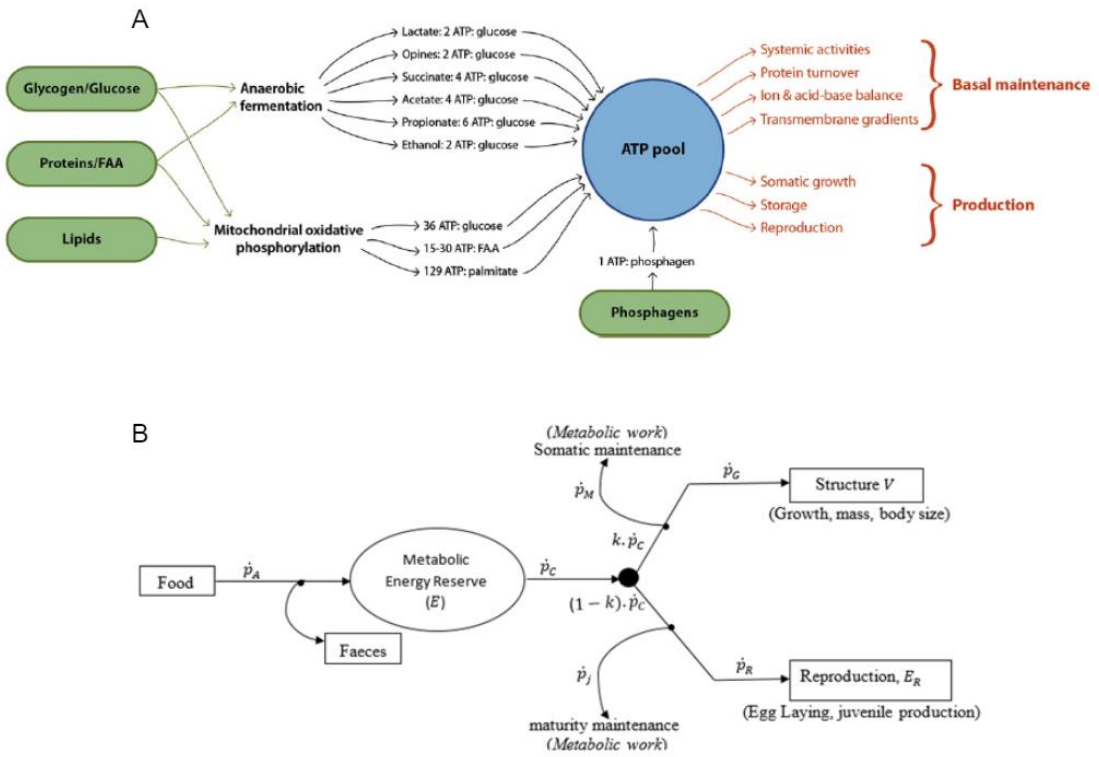

Figure 2-3. (A) A summary of energy acquisition and metabolism to yield ATP (Adenosine triphosphate), designated by the numbers of ATP produced, which is allocated for physiological processes (modified from Sokolova et al., 2012). **(B)** Dynamic energy budgeting in animals (Modified from Kooijman, 1986, 2000). Energy enters the organism as food and is assimilated at a rate of $\dot{p}_A$ into the metabolic reserve, E. The arrows represent energy fluxes. The energy is then mobilized at the rate of $\dot{p}_C$ for allocation to cover somatic maintenance ($\dot{p}_M$), structure ($\dot{p}_G$), reproduction or maturity ($\dot{p}_R$), and maturity maintenance ($\dot{p}_J$). The letter k denotes a constant fraction of the energy reserve being allocated for growth and cost of somatic maintenance with maintenance having priority over growth. The remaining fraction, $1-k$ is allocated for maturity, reproduction and cost of maturity maintenance. $k > 1-k$ because much of the energy reserve in animals are allocated for the cost of somatic maintenance during chemical stress to keep the organism alive.

2.6 Soil Habitat Quality

Toxicants cause stress to soil invertebrates in their habitat, but soils with high quality will provide soil invertebrates with resources to combat the toxicant's stress. The quality of soil as habitat is a function of the soil's intrinsic factors such as pH, organic matter (OM), and soil moisture (Jegede et al., 2019b). Soil habitat where intrinsic factors do not support the biological and physiological fitness of soil invertebrates can be considered as a low habitat quality soil. These factors can either modulate the bioavailability of metals to soil invertebrates (i.e., toxicokinetics) or how soil invertebrates handle metal stress (i.e., toxicodynamics).

Soil organic matter (OM) and pH are considered the most critical soil factors that influence the bioavailability of inorganic and organic chemicals to soil invertebrates (van Gestel, 1992). According to Van Gestel *et al*. (1995), the bioavailability of metals to soil organisms is governed by soil factors that alter the concentration of the metals in soil pore water, and such factors include OM, clay content, iron oxides, and pH. Artificial soil of low pH (4.0) and OM (10 %) increases Zn^{2+} bioavailability to *Eisenia fetida*, thus causing a significant reduction in survival and cocoon production (Spurgeon and Hopkins, 1996). In a study by Bradham *et al*. (2006), natural soils of low pH had higher lead (Pb) toxicity on the earthworm, *E. andrei*. In the study, *E. andrei* in low pH soils had significant internal Pb and Pb bioavailability, which corresponded to a high amount of Pb^{2+} in soil pore water. High moisture content in artificial soil potentiates Zn toxicity on *F. candida* and *O. nitens* by favouring the availability of Zn^{2+} in soil pore water to the organisms (Owojori and Siciliano, 2015).

Soil habitat quality alters *O. nitens*'s toxicodynamic responses to Zn irrespective of the high Zn body burden in the mites. Zn (1500 and 14000 mg kg^{-1}) did not affect the glucose metabolic enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) activities in mites of high quality habitat soil but significantly increased the activities of the enzymes in mites of low habitat quality, indicating cellular stress (Jegede et al., 2019b). There was no significant change in the expression of metallothionein (*mt*) and catalase (*cat*) genes in *E. fetida* that were exposed to Cd at high

OM (14 %). In contrast, a significant induction of *mt* and inhibition of *cat* gene was observed in *E. fetida* at low OM (6 and 10 % OM) (Irizar et al., 2015). The high OM might have provided the earthworm with energy to resist the oxidative damage of Cd.

2.7 *Oppia nitens* C.L. Koch, 1836 (Acari: Oribatida): Current status of its bionomics and relevance as a model invertebrate in soil ecotoxicology[1]

2.7.1 Preface

A critical review of literature on the biology and ecology of *Oppia nitens* was done to provide information on its bionomics and responses to chemicals in soil. The review highlighted its functions and contribution to ecosystem services. A possible molecular mechanism on how the mites could tolerate cadmium in soil was proposed.

[1] Fajana HO, Gainer A, Jegede OO, Awuah KF, Princz JI, Owojori OJ, Siciliano SD. 2019a. *Oppia nitens* C.L. Koch, 1836 (Acari: Oribatida): Current status of its bionomics and relevance as a model invertebrate in soil ecotoxicology. *Environmental Toxicology &* chemistry, 38(12): 2593–2613. https://doi.org/10.1002/etc.4574

Hamzat Fajana: Conceptualization, formal analysis, data curation, writing (sections of the original draft), visualization
Amy Gainer: writing (sections of the original draft, review and editing)
Olukayode Jegede: writing (sections of the original draft, review and editing)
Kobby Awuah: writing (sections of the original draft, review and editing)
Juliska Princz: writing (sections of the original draft, review and editing)
Olugbenga Owojori: writing (sections of the original draft, review and editing)
Steven Siciliano: Conceptualization, resources, writing (review and editing), supervision, project administration, funding acquisition

2.7.2 Abstract

The oribatid soil mite *Oppia nitens* C.L. Koch, 1836, is a model microarthropod in soil ecotoxicity testing. This species has a significant role in supporting soil functions and as a suitable indicator of soil contamination. Despite its significance to the environment and ecotoxicology, however, very little is known of its biology, ecology, and sub-organismal responses to contaminants in the soil. In this review, we give detailed and critical insight into the biology and ecology of *O. nitens* concerning traits that are crucial to its adaptive responses to contaminants in soil. We used a species sensitivity distribution model to rank the species sensitivity to heavy metals (cadmium and zinc) and neonicotinoids (imidacloprid and thiacloprid) compared to other standardized soil invertebrates. Although the International Organization for Standardization (ISO) and Environment and Climate Change Canada (ECCC) are currently standardizing a protocol for the use of *O. nitens* in soil toxicity testing, we believe that *O. nitens* is limited as a model soil invertebrate until its molecular pathways associated with its response to contaminants are better understood. These molecular pathways can only be elucidated with information from the mites' genome or transcriptome, which is currently lacking. Despite this limitation, we propose a possible molecular pathway to metal tolerance and a putative adverse outcome pathway (AOP) to heavy metal toxicity in *O. nitens*.

2.7.3 Introduction

Oribatid mites are diverse and ubiquitous microarthropods in soil. They inhabit all kinds of soils across the globe, irrespective of soil type, nutrient status, and microclimatic conditions (Ivan, 2017). The ubiquity of oribatid mites is due to their ecological position and diverse feeding guilds (Behan-Pelletier, 2003). More than 100,000 species of oribatid mites may exist (Walter and Proctor, 2013). Their presence dominates the upper organic horizon of soil, where densities can reach several hundred thousand individuals per square meter of soil (Norton, 1990). They play a significant role in soil nutrient cycling (e.g., organic matter decomposition) and contribute to the formation and maintenance of soil structure (Wickings and Grandy, 2011). Oribatid mites are a heterogeneous trophic group that are prime bioindicators of soil disturbances due to their abundance and diversity in surface soil

(Behan-Pelletier, 1999). They are ideal indicators of contamination within surface soils because they are primarily found in the organic horizon, where various soil contaminants are sequestered. Despite these advantages, many oribatid mite species are not used in soil ecotoxity studies due to various limitations that prevent them from being model test organisms. Several investigators have evaluated the performance of different species from various geographical ranges (e.g., boreal, temperate, tropical) when used for toxicity testing and bioindication of pollution (e.g., Seniczak et al., 1996; Köhler et al., 2005; Seniczak et al., 2009; Princz et al., 2010; Owojori and Siciliano, 2012; Owojori et al., 2019). With the exploration of the inclusion of different species, limitations were identified that precluded their inclusion as model test organisms. For instance, *Platynothrus peltifer*, which was initially used in lethal and sub-lethal toxicity testing, is limited by its long developmental cycle (>150 days to attain maturity) and by the difficulty in establishing laboratory cultures from samples collected from the field (van Gestel and Doornekamp, 1998). In contrast, other test species, such as *Archegozetes logisetosus* (Aoki, 1965; Seniczak et al., 1996; Heethoff et al., 2013) and *Muliercula inexpectata* (Badejo et al., 2002; Owojori et al., 2019), are useful for laboratory-based toxicity assays because they have a relatively short reproduction cycle and can easily be cultured under laboratory conditions. Moreover, *A. logisetosus* and *M. inexpectata* are respectively Pan- and Afro-tropical in distribution and are thus relevant as test species for tropical ecosystems.

In contrast, *Oppia nitens* C.L. Koch, 1836, is a euryoecious microarthropod (i.e., can live under variable habitat conditions) and is found in boreal and temperate ecozones. It is widely distributed across North America, Europe, and some parts of the Middle East (Subias, 2004), occurring in humus-rich forest and agricultural soils, wetlands, and dry grasslands (see table 1A of Appendix A). Its pattern of distribution and short reproduction cycle (usually 28 days), along with the ease with which researchers can establish synchronized cultures under laboratory conditions, make this species favourable for use in toxicity testing. Furthermore, it is sensitive to heavy metals, pesticides, organic compounds, and salt content in soil (Princz et al., 2010, 2012; Owojori et al., 2012; de Lima e Silva et al., 2017). It also has broad applicability to diverse soil types and pH, with limitations in reproduction observed at soil pH >7.3 (Princz et al., 2010; Owojori and Siciliano, 2012).

Low soil organic matter content may also be a limiting factor for *O. nitens* (Princz et al., 2010), but this is not surprising, as oribatid mite community structure in general is driven by humus form and type (Maraun and Scheu, 2000).

2.7.4 Biogeographical distribution of *O. nitens*

O. nitens is cosmopolitan in distribution, occupying the Holarctic region (i.e., Nearctic and Palaearctic regions). The distribution in the Palaearctic is mostly western (Figure 2-4) but extends beyond the Holarctic region to the sub-Antarctic (Subias, 2004). A subspecies of *O. nitens*, *O. nitens brachytrichinus* Dalenius, 1958, was recorded to be present in the sub-Antarctic archipelagos, the Crozet Islands (Starý and Block, 1998). Their occurrence could be as a result of dispersal from adjacent mainland via phoresy. Lebedeva and Lebedev (2008) found *Oppia* sp. C.L. Koch, 1836, on the plumage of birds in Spitsbergen, an island in the Svalbard archipelago in northern Norway. Records indicate the distribution of *O. nitens* from North America (i.e., Canada and USA) to Europe and some parts of the Western Mediterranean (Figure 2-4; Table 1A of Appendix A). The circumpolar distribution patterns of *O. nitens* (Figure 2-4) are confirmed by its presence within northern temperate regions, spanning boreal and temperate forests, including grasslands. The distribution patterns also suggest occurrence in some parts of Norway and Russia (de Jong et al., 2014), although this has yet to be confirmed. A molecular taxonomic approach, such as metabarcoding of environmental DNA samples (Telfer et al., 2015), should be used to confirm and better understand the distribution pattern of *O. nitens*, especially for regions where its occurrence is uncertain.

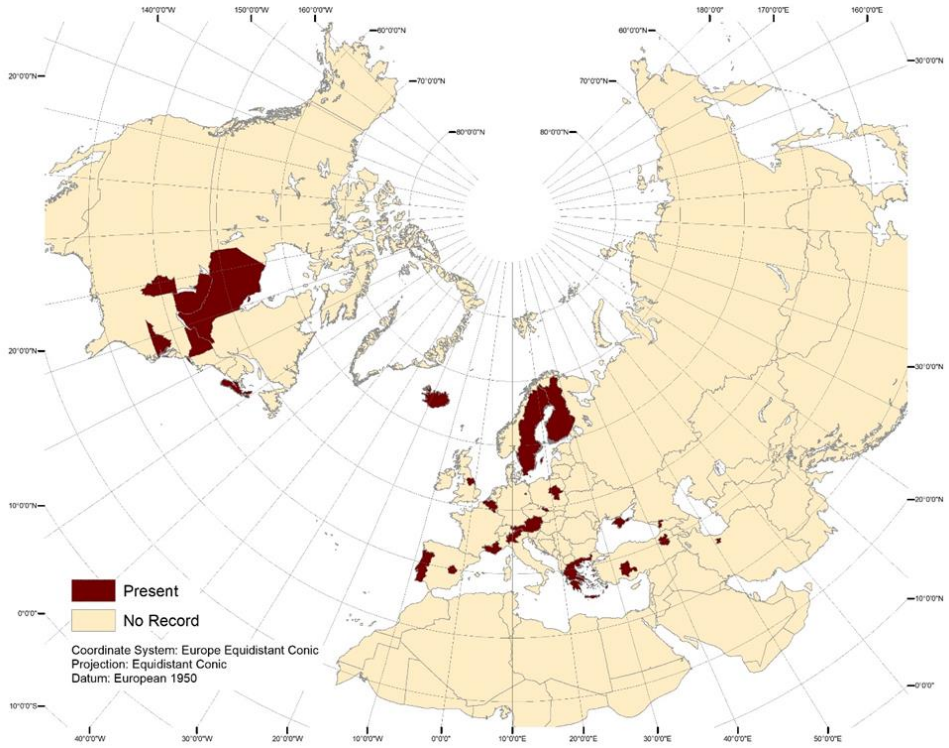

Figure 2-4. Biogeographical distribution of *Oppia nitens*. Highlighted areas are approximate locations from literature where *O. nitens* can be found in the Holarctic region (i.e., Nearctic and Palearctic regions).

2.7.5 Contribution of *O. nitens* to ecosystem services

Oppia nitens is an ecosystem engineer, meaning it modifies resource availability in its habitat, and its activities influence environmental conditions (Jones et al., 1994; Crain and Bertness, 2006). Like other ecosystem engineers, *O. nitens* alters soil functions that support several ecosystem services, benefitting other organisms and humans. Specifically, the activities of *O. nitens* contribute to three of the four categories of ecosystem services: the supporting, provision, and regulating services (Prather et al., 2013).

O. nitens mechanically contributes to the breakdown of plant litter and bioturbation, thus aiding decomposition and stability of soil aggregates (Lavelle et al., 2006). It also fragments detritus and recalcitrant plant residues into finer portions, or frass, which is an energy source for microorganisms. Thus *O. nitens* can selectively activate microbial communities and improve the quality of soil organic carbon (Decaëns et al., 2006; Prather et al., 2013). Furthermore, this decomposition process mediated by *O. nitens* supports

secondary production, forms the basis of the detritus food web (Decaëns et al., 2006; Lavelle et al., 2006), and releases locked nutrients like nitrogen and sulphur into the soil, which are used by other organisms and especially microorganisms. More importantly, *O. nitens* restructures and alters nutrient availability by releasing feces rich in micronutrients. Therefore, *O. nitens* directly influences soil status by feeding on plant residues and releasing nutrients from high-quality litters into the soil.

O. nitens also likely contributes to climate and water regulation services. For example, by contributing to soil bioturbation through its continuous movement, *O. nitens* is helping to form and stabilize soil aggregates, thus aiding carbon storage and climate regulation (Decaëns et al., 2006). Carbon sequestration in stabilized aggregates prevents the release of greenhouse gases like CO_2 and CH_4 (Six et al., 2000; Mangalassery et al., 2013). Aggregate stabilization is also crucial in controlling soil erosion (Barthes and Roose, 2002). Furthermore, although *O. nitens*' contribution to water infiltration is not acknowledged, it should not be discounted as it is a dominant soil microarthropod, especially in the Canadian boreal forests, and as such, it likely contributes to water regulation through decreasing filtration and creating structural porosity. Like other soil biota, *O. nitens* could regulate water infiltration, run-off rate, and storage in soils (USDA, 2001).

Finally, *O. nitens* plays a significant role in maintaining the soil food web. For example, like other soil invertebrates, *O. nitens* augments primary production by constantly releasing nutrients in the rhizosphere, stimulating mutualistic rhizosphere organisms (i.e., bacteria and fungi), and producing plant growth promoters (Lavelle et al., 2006). Also, the fungivorous nature of *O. nitens* and its occasional feeding on potentially pathogenic nematodes protects plants from diseases. Furthermore, *O. nitens* are also prey for several soil invertebrates, including predatory mites. Thus, the supporting and regulating services (Prather et al., 2013) provided by *O. nitens* (Figure 2-5) are essential for maintaining several other ecosystem services.

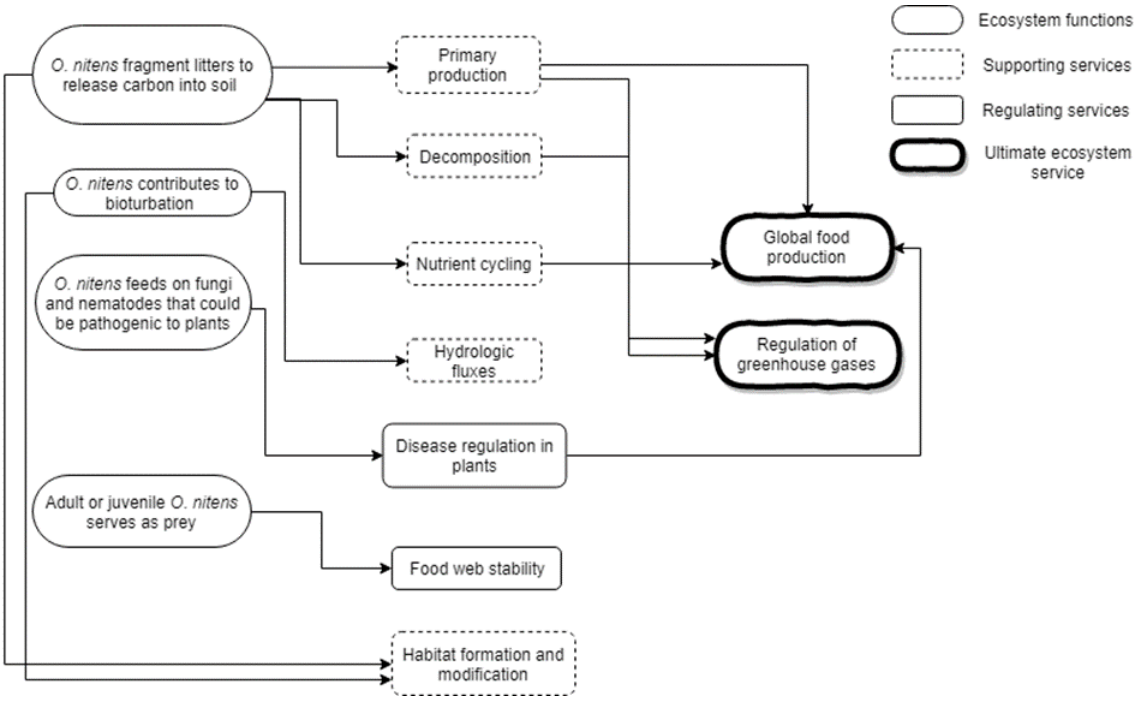

Figure 2-5. The supporting and regulatory contribution of *Oppia nitens* to the provision of ecosystem services.

2.7.6 Biology of *O. nitens*

2.7.6.1 Feeding

Oribatid mites are particulate feeders with diverse feeding guilds that vary across life stages. Their feeding habits can be categorized based on analysis of their gut contents (Schuster, 1956), type of digestive enzymes mainly carbohydrase (Siepel and Ruiter-Dijkman, 1993), and, recently, analysis of the stable isotope ratio (specifically ^{15}N to ^{14}N ratio) of their tissue (Schneider et al., 2004b). Schuster (1956) identified three main categories of feeding guilds in oribatid mites based on their gut contents: macrophytophagous mites, microphytophagous mites, and mites with no specialized feeding habits. Macrophytophagous mites feed on dead plant materials that are either woody or non-vascular in origin; microphytophagous mites feed on soil microflora such as fungi, algae, lichen, and bacteria; and the non-specialized

groups feed on either plant remains or soil microflora. Some groups of oribatid mites, however, are polyphagous: they feed on plant remains, microflora, and also act as predators or scavengers (Walter, 1987).

O. nitens is a polyphagous fungivore, preferentially grazing on organic debris and fungus, although selective feeding of lichen, raw humus, and carrion have been observed (Seniczak and Stefaniak, 1978). This species shows a preference for some types of fungi (Stefaniak and Seniczak, 1981). For example, Singh et al. (1996) reported that *O. nitens* strongly prefers leaf litter mixed with dried mushrooms as opposed to leaf litter or dried mushrooms alone, and it also has very little preference for granulated yeast. Nevertheless, *O. nitens* has been successfully cultured in the laboratory on a single diet of baker's yeast, *Saccharomyces cerevisiae* (Princz et al., 2010).

In the absence of preferred food sources, *O. nitens'* feeding habits vary from herbivory to predation and cannibalism. For example, Stefaniak and Seniczak (1981) observed cannibalism under laboratory conditions when specimens of *O. nitens* were provided with the fungus *Trichothecium roseum* as a food source. Furthermore, stable isotope analysis of tissue found that Oppiidae had high ^{15}N to ^{14}N ratio, indicating they consume animal tissue, either as scavengers or predators, in addition to plants (Schneider et al., 2004a). Gan et al. (2014) reported that *O. nitens* has $\delta^{15}N$ of ~ 4 ‰, which is in a range of the threshold designated for predators or scavengers (Schneider et al., 2004b).

O. nitens also have an efficient microflora in their alimentary canal that can digest recalcitrant polysaccharides (Seniczak and Stefaniak, 1978). However, the nature and quality of the food material ingested can alter the activity of their gut microflora, either by stimulating the microflora to breakdown fungi and plant materials or by inhibiting the activity of the microflora (Stefaniak and Seniczak, 1981). For instance, fungi with antimicrobial potentials, such as *Penicillium sp.*, can reduce gut microbial activities, which in turn may affect the breakdown of fungal materials such as chitin and lectin. *O. nitens* has carbohydrase as its primary digestive enzyme. However, it is unclear whether the mites' enzymes or the enzymes of the symbiotic gut microflora provoke the enzymatic activity in the guts.

2.7.6.2 Life history of O. nitens

O. nitens is a *k*-selected organism with low metabolic rate (Siepel, 1994). Like other *k*-selected organisms, it has a relatively long life span. The life history of microarthropods can be explained by reproduction, development, synchronization, and dispersal (Siepel, 1994).

Although not fully understood, the reproductive mode of *O. nitens* is thought to be through parthenogenesis; however, records of spermatophores are evident in laboratory cultures (Sengbusch and Sengbusch, 1970; Stefaniak and Seniczak, 1981; Kummel, 1982; Alberti et al., 1991). Evidence of males and females has been observed in laboratory cultures (ECCC, 2018), but no evidence of sexual dimorphism. Variants in colouration have been observed in laboratory cultures, although the reasons for this are also uncertain. In some cases, adults are milky-white following eclosion, and they do not mature into the dark brown cuticle typical of adult *O. nitens*. These variants seem less sclerotized, fail to reproduce (ECCC, 2018), and are not to be used for toxicity tests. It is unknown whether this variation is a factor of stress, malnutrition, or genetics.

O. nitens is iteroparous, i.e., members of this species have multiple reproductive cycles, laying eggs more than once throughout their lives. Iteroparity is a common oviposition mechanism in oribatid mites and most soil microinvertebrates (Siepel, 1994). Oviposition pattern is an important life history trait as it influences energy allocation in organisms. The fact that *O. nitens* is iteroparous allows the mites to conserve energy between reproduction and adaptive responses. The gap between successive ovipositions reduces the energy flux that occurs during reproduction, thereby allocating more energy for cellular maintenance and detoxification. Hence, iteroparity confers an advantage to *O. nitens* to cope with stress from either natural or environmental stressors.

In contrast, microinvertebrates that lay all their eggs once in a lifetime (i.e., semelparity) expend greater energy during the one-time oviposition event, leading to less energy allocation for cell maintenance and survival (Siepel, 1994); hence, they are more susceptible to stress from either natural or environmental stressors. The average number of eggs laid by sexually mature *O. nitens* in laboratory culture is 1 egg/female/day (personal observation). These mites prefer to lay eggs around and within crevices and may also hide their eggs deep

inside their food (Sengbusch and Sengbusch, 1970). Specimens in laboratory cultures have also been noted laying eggs in and around the yeast granules provided as a food source (personal observations). The approximate gross reproduction rate (i.e., number of offspring/adult) has been reported at 2.7 juveniles per adult from 0 – 28 days life cycle (Princz et al., 2010). The low fecundity is not surprising, as oribatid mites are characterized by slow metabolism and development, longer life spans, and a limited capacity for population growth (Lebrun and van Straalen, 1995; Norton and Behan-Pelletier, 2009).

The developmental time from egg to adult for most oribatid mites ranges from 23 to 360 days, depending on temperature and food quality (Sengbusch and Sengbusch, 1970). However, the development of *O. nitens* from egg to adult takes about 40 to 45 days at 20℃ (Michael, 1898; Sengbusch and Sengbusch, 1970). Based on an unpublished data, newly emerged adult *O. nitens* start to lay eggs on plaster of Paris substrate within 6 days at 22–23℃ with a few grains of baker's yeast as food (Figure 2-6). Sengbusch and Sengbusch (1970) also reported an average of 8 days before egg laying in cultures maintained at 20℃ and fed with *Protococcus* sp. (a green algae). Eggs are oval, whitish, and translucent (Figure 2-7), with sizes ranging from 90 to 150 μm, and they hatch within about a week after being laid (Sengbusch and Sengbusch, 1970; Seniczak, 1975; Princz, 2014). When cultured in a laboratory between 20-23°C with baker's yeast as a constant food source, *O. nitens* were observed to undergo a larval stage about 8 days after oviposition and then three nymphal stages (i.e., the protonymph, deutonymph, and tritonymph stages), lasting approximately 1 week each (personal observation; ECCC, 2018).

Larvae have three pairs of legs, and the nymphs are whitish in colour (Figure 2-7). Protonymphs are approximately 200 μm long by 105 μm wide, and tritonymphs are approximately 372 μm long by 195 μm wide (Seniczak, 1975). The egg-laying capacity of *O. nitens* is greatest at 12 to 18 days; after that, a greater number of the eggs hatch (Figure 2-6). Each stage of maturation between the instars is characterized by a quiescence period (i.e., the pre-ecdysial stage). At the pre-ecdysial stage, successive immature stages remain dormant without feeding for a few days (usually 2 to 3 days). The pre-ecdysial stage is a way of conserving energy in preparation for moulting, as moulting consumes energy and

nutrients (Murphy and King, 1992). In the final nymphal stage, the tritonymphs undergo a final moulting phase before emerging as adults. The newly emerged adult has a semi-translucent golden-brown or light amber colouration. Post-ecdysis, and within a week (ECCC, 2018), the light amber cuticle becomes melanized and sclerotized into the typical dark red-brown colour of adult *O. nitens*, which usually measures about 510 µm long and 290 µm wide (Michael, 1884) (Figure 2-7).

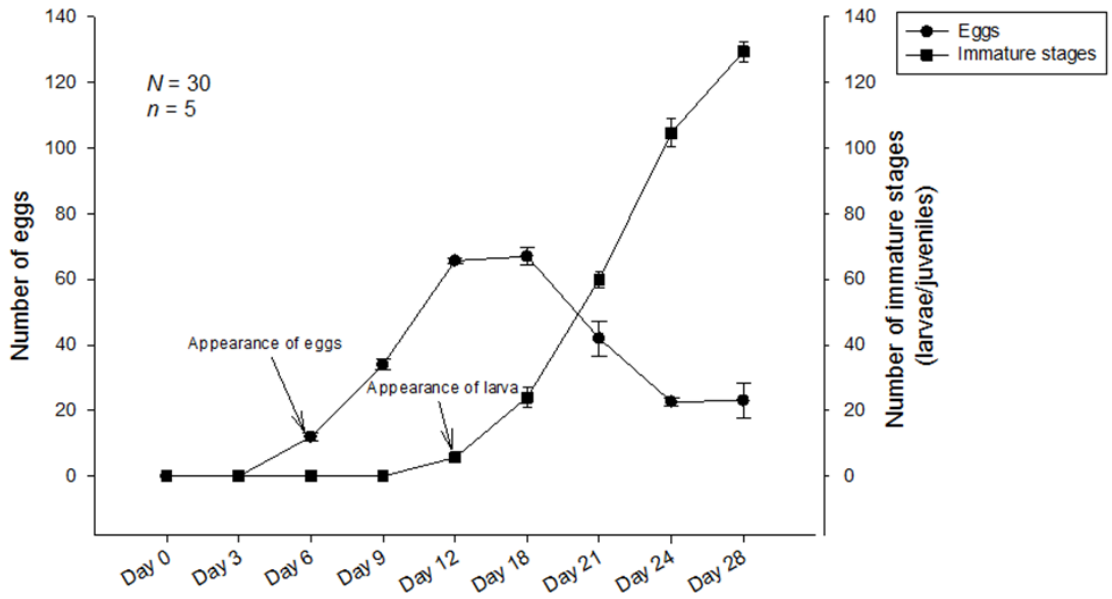

Figure 2-6. Dynamics in egg-laying and hatching capacity of *Oppia nitens* after 28 d in substrate of plaster of Paris and fed with grains of baker's yeast in the laboratory at 21℃ (unpublished data).

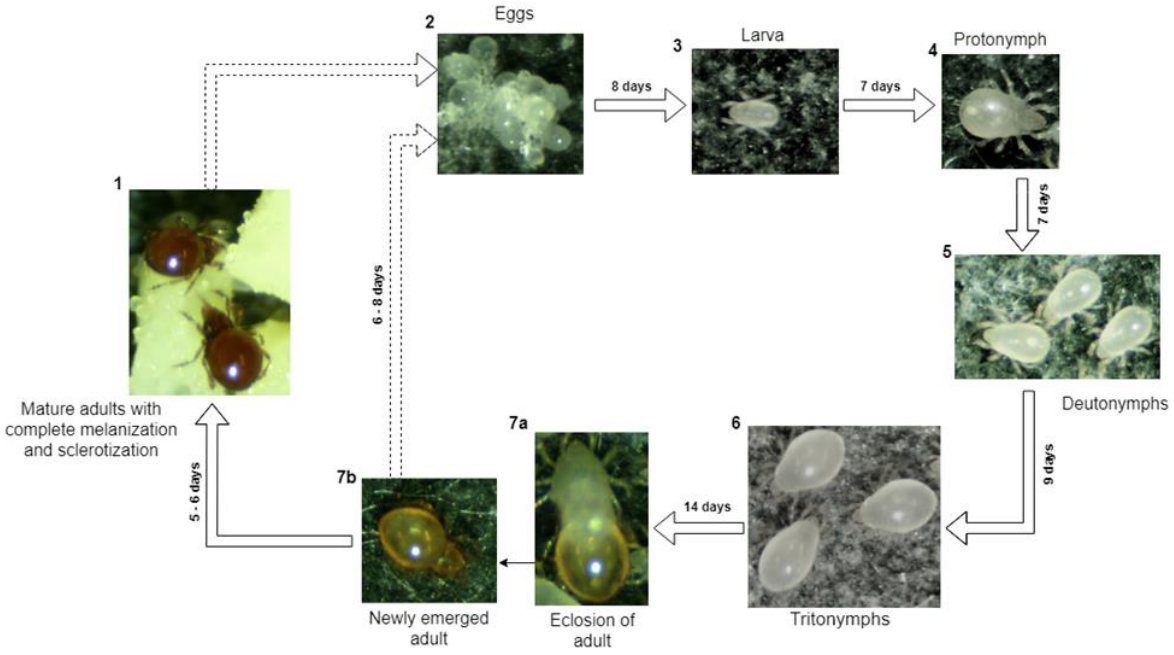

Figure 2-7. Developmental stages of *Oppia nitens* and the period between successive stages. The developmental period (in days), estimates are based on data from Sengbusch and Sengbusch (1970), Princz et al. (2010), and a 2-years observation of laboratory-cultured *Oppia nitens*. The broken arrow indicates egg laying.

Environmental factors such as temperature and diet can influence *O. nitens'* life history. For example, Stefaniak and Seniczak (1981) found that a diet of *Penicillium chrysogenum* and *P. roseo-purpureum* prolongs developmental time and maturation of juveniles by 30 to 32 days, while a diet of *P. spinulosum* and *P. viridatum* reduces it to about 26 days. For cultures at 20°C ± 3°C that are fed baker's yeast, mature adult *O. nitens* take about 26 to 28 days to recruit their first generation through all developmental cycles (Princz et al., 2010). Similarly, Yu et al. (1997) observed a developmental time from adult to juveniles to be within 14 to 21 days at 23°C and a humidity of 85% ± 5%. However, a reproduction cycle of 28 days has been recommended as a full life cycle for soil toxicity tests with *O. nitens* (Princz et al., 2010), as this timeframe enables differentiation between the initial mature adults and their successive generation of progeny.

2.7.6.3 Influence of soil properties on the life history of O. nitens

Intrinsic soil properties, such as pH and the quality and quantity of organic matter, influence the life history of *O. nitens*. Princz et al. (2010) reported that high organic matter (>6%) in soils was associated with increased juvenile production in *O. nitens*, while soil pH (ranging from 3.9 to 6.1) had insignificant effect on juvenile production. Owojori and Siciliano (2012) found low pH soil suitable for juvenile production but that higher pH (>7.3) compromises it. Interestingly, oribatid mites can regulate internal pH in the spherites within their proventricular glands to maintain optimum pH for reproduction (Ludwig et al., 1992). Mites in soils with high organic matter (OM) are likely to ingest contaminants that are bound to the OM. However, the high OM might also serve as food source and provide more energy reserve needed to combat toxicity from the contaminants (Jegede et al., 2019b).

2.7.7 *O. nitens* in soil ecotoxicology

2.7.7.1 Traits of O. nitens influencing toxicokinetics and toxicodynamics

To understand how *O. nitens* interacts with soil contaminants, it is helpful to consider the impact of certain traits of this species; i.e., its physiological, morphological, and ecological attributes (Baird and Van den Brink, 2007). Specifically, three categories of traits are considered key to *O. nitens'* response to soil contamination: its body size and diet, intrinsic sensitivity, and life history characteristics (van Straalen, 1993; Rubach et al., 2012; Gainer et al., 2018).

Exposure is necessary for toxic effects to occur. Therefore, the mites' body size and diet are key because they determine degree of exposure (Rubach et al., 2011; Gainer et al., 2018; Princz et al., 2018). First, small body sizes are associated with high levels of exposure to contaminants due to the larger surface area to volume ratio (de Bello et al., 2010; Hedde et al., 2012; Andriuzzi et al., 2016; Gainer et al., 2018; Princz et al., 2018), and *O. nitens* is one of the smallest of the standardized test invertebrate species in soil ecotoxicology (Table 2-2). Second, the diet of soil invertebrates is key to their oral exposure to contaminants, especially when they ingest soil in addition to their primary food source (Gainer et al. 2018). Although the preferred diet of *O. nitens* is fungi, these mites also feed on dead organic matter (Norton, 1994; Behan-Pelletier, 1999; Schneider et al., 2004b). In field conditions,

they may get exposed to contaminants through ingestion of contaminated algae, other detritus, or carcasses (Seniczak et al., 2017). The degree of exposure through the ingestion of contaminated diet varies with age as only active stages of *O. nitens* feed.

The intrinsic sensitivity of *O. nitens*, that is, its internal toxicokinetic processes, also influences its responses to contaminants. In *O. nitens*, the degree of sclerotization, reflected between the nymphal and adult stages, likely influences the degree of dermal absorption and elimination of chemicals. Immature mites are not fully sclerotized and have soft cuticles, which make them susceptible to both predation and absorption of contaminants via their cuticle (Behan-Pelletier, 1999). In contrast, melanization of the cuticle allows for sequestration of minerals, including metals. Indeed, compartmentalization of lead within spherites of the proventricular glands in oribatid mites has been documented and contributed to detoxification mechanisms (Ludwig et al., 1992). Hugueir et al. (2015) hypothesized that dermal absorption in adult mites primarily occurs through exoskeleton gaps on their legs.

Rubach et al. (2011) identified a link between complete sclerotization and high elimination rates for aquatic arthropods. Thus, we suggest *O. nitens* also may have high elimination rates because of moulting activities, which allow these mites to eliminate contaminants sequestered in their cuticles. Juvenile *O. nitens* frequently undergo moulting throughout their nymphal stages. Thus, the immature stages have greater capacity to eliminate contaminants via ecdysis. Numerous gut enzymes such as cellulase, glucanase, chitinase, trehalase, and amylase have been identified in oribatid mites (Schneider et al., 2004a). However, no literature currently exists on activities of biotransformation enzymes. Gut microflora of *O. nitens* could influence contaminant's biotransformation.

Traits related to toxicodynamics also influence responses to contaminated soils. For example, sclerotization of *O. nitens*, associated with internal toxicokinetics and toxicodynamics, affects the storage of contaminants. Although not currently quantified, *O. nitens* likely sequester contaminants in their exoskeleton, as observed in other terrestrial invertebrates (Schmidt and Ibrahim, 1994) and other sclerotized invertebrates (Alikhan et al., 1990; Keenan and Alikhan, 1991; Alcorlo et al., 2006). Certain organics, like perfluorinated alkyl sulfonates, may accumulate within *O. nitens* exoskeletons, as these

chemicals preferentially bind to proteins (Jones et al., 2003), and the chitinous arthropods exoskeletons contain high levels of proteins. However, this remains to be confirmed (Princz et al., 2018). Storage and compartmentalization of metals in specialized proteins, like metallothionein or midgut electron-dense granules, occur within oribatid mites (*Chamobates borealis*, *Nothrus silvestris* and *Rhyostrititia duplicate*), although the extent to which metals are sequestered is species-specific and likely a mechanism for detoxification (Ludwig et al., 1992; Hugueir et al., 2015; Keshavarz-Jamshidean et al., 2017). Lipid content also influences the toxicity of organic contaminants. Based on findings from Gainer et al. (2018), *O. nitens*, like other oribatid mites, has relatively high lipid contents compared to other standardized soil invertebrates in soil ecotoxicology (Table 2-2), which influences the partitioning of hydrophobic organic chemicals into organisms (Belfroid et al., 1996).

Other morphological, ecological, life history, and physiological traits of *O. nitens* influence the mite's response or susceptibility to chemicals, relative to other standardized soil invertebrates (Table 2-2). For instance, based on evidence from aquatic invertebrate studies, maternal transfer could be another excretion route for *O. nitens*, especially for metals and organics (Keteles and Fleeger, 2001; Conley et al., 2009; Cid et al., 2010). However, the small clutch size of *O. nitens* (Table 2-2) reduces the maternal transfer of contaminants via egg laying. Gaseous exchange via body surface (i.e., cutaneous respiration) is another trait that favours the absorption of volatile organics into the internal compartment of the mites. Furthermore, the active movement of *O. nitens* is a physiological trait that enables the mite to rapidly avoid contaminated soil patches relative to less active or sedentary organisms like earthworms and enchytraeids (Ezzatpanah, 2012).

Another biological mechanism of coping with toxicity or stress is via energy allocation between life history processes and cellular maintenance (Kooijman, 2000; Smolders et al., 2004; Muller et al., 2010). For example, *O. nitens* may delay oviposition, concentrating resources and energy into survival, detoxification, and cellular maintenance upon exposure to contaminants in soil.

Table 2-2. Traits of *Oppia nitens* relative to other standardized soil invertebrates in soil ecotoxicology

Morphological traits			Ecological traits		Life history traits		Physiological traits			
Model soil invertebrates	Average body size (mm)	Degree of sclerotization	Organic matter preference	Average pH preference	Moulting	Average clutch size (eggs/adult/day)	Average lipid content (%)	Mode of respiration	Presence of metal storage sites	Movement
O. n	0.51[1]	Complete (>90 %)	High[2]	6.1[2]	Yes	1	0.13*[3]	Cutaneous[4]	Proventricular gland[5]	Active
H. a	0.85[6] (F)	Good (10 – 90 %)	-	6[6]	Yes	1.93[6]	-	Cutaneous[4]	-	Active
F. c	2.0[6]	Good (10 – 90 %)	High[6]	5.6[6]	Yes	8.1[7]	0.085[8]	Cutaneous[9]	Cells of mid-gut epithelium[7]	Active
E. f	90[11]	0	Very high[6]	6.5[6]	No	0.2[10]	0.015[12,13]	Cutaneous[14]	Chloragogenous glands[15]	Less active
E. c	7.1[16]	0	High[6]	6.2[6]	No	4.6[6]	0.047[17]	Cutaneous[14]		Less active

O. n = Oppia nitens; *H. a = Hypoaspis aculeifer*; *F. c = Folsomia candida*; *E. f = Eisenia fetida*; *E. c = Enchytraeus crypticus*

F = female; *Average lipid content of spp. of Antarctic Oribatid mites. [1]Seniczak (1975b); [2]Princz et al. (2010); [3]Convey (1992); [4]Spieksma (1990); [5] Ludwig et al. (1992); [6]Jansch et al. (2005); [7]Fountain and Hopkin (2005); [8]Holmstrup et al. (2002); [9]Davies (1927); [10]Elvira et al. (1996); [11]Reinecke and Voljoen (1991); [12]Krauss et al. (2000); [13] Wågman et al. (2001); [14]Mendes and Valente (1953); [15]Vijver et al. (2005); [16]Westheide and Graefe (1992); [17]Rodriguez and Verdonschot (2001). Note: Data or traits without reference were based on personal observation.

2.7.7.2 Responses of O. nitens to contaminants in soil

Although more studies have focused on other soil invertebrate species (especially the earthworm, *Eisenia fetida*; springtail, *Folsomia candida*; enchytraeid, *Enchytraeus crypticus*) (Figure 1A of Appendix A), a list of contaminant toxicity to *O. nitens* is gradually building. Yu et al. (1997) likely conducted the first assessment of contaminant toxicity to *O. nitens*; they found that this species was not sensitive to *Bacillus thuringiensis* toxins in transgenic cotton and potato plants. Since this study, many others have been published on the toxicity of metals, pesticides, and organic compounds on *O. nitens*.

2.7.7.2.1 Metals.

The toxic effect of metals to *O. nitens* is predominantly influenced by soil type, speciation, and the amount of metal bound to organic matter (Jegede et al., 2019b). Owojori and Siciliano (2012) assessed *O. nitens*' response to four metals (cadmium, zinc, copper, and lead) in OECD artificial reference soil. Considering the metals' effects on survival and reproduction, Cd was the most toxic metal to the mites, having the lowest median lethal concentration (LC50) value, 603 mg kg^{-1}, and the lowest reproduction median inhibitory concentration (EC50) value, 137 mg kg^{-1}. Keshavarz Jamshidian et al., (2017) reported a higher reproduction EC50 value of 345 mg kg^{-1} Cd in LUFA 2.2, a natural reference soil. Cadmium has been linked to deleterious effects on the community structure of mites in metal-contaminated soils (Khalil et al., 2009). It is the most toxic of the four metals (i.e., cadmium, zinc, copper, and lead) to other soil invertebrates such as the springtails, *F. candida* and *Proisotoma minuta* (Fountain and Hopkin 2001; Nursita et al., 2005), and the earthworm *E. fetida* (Spurgeon et al. 1994).

Zinc (Zn) is an essential metal but is toxic to *O. nitens* at high concentrations. In a metal-contaminated site risk assessment, Owojori and Sciliano (2015) identified Zn as the metal of concern. Owojori and Siciliano (2012) also reported a reproduction EC50 value of 1562 mg kg^{-1} for zinc to *O. nitens* in OECD artificial soil with pH 6 and 10% organic matter. Jegede et al. (2019b) found that in natural agricultural soil with a lower pH of 3.4, the reproduction EC50 values for Zn to *O. nitens* ranged from 103 to 499 mg kg^{-1}. They also found that zinc toxicity to *O. nitens* varied depending on soil properties. For example, comparing sandy and

loamy soils, the authors found the reproduction EC50 values for Zn to *O. nitens* to be 646 and 8,700 mg kg^{-1}, respectively. Most toxicity studies on the effect of Zn to *O. nitens* have been based on single generation exposure. More recently, a multigenerational response of the mite population to Zn (mostly below 800 mg kg^{-1} of zinc in natural soil) was modelled using a population growth rate approach. Population growth rate shows subsequent generations of *O. nitens* to be more sensitive to Zn than their parents (Jegede et al. 2019a).

Like zinc, copper (Cu) is an essential metal that is toxic to *O. nitens* at high concentrations. Owojori and Sciliano (2012) found the reproduction EC50 value of Cu was 2,896 mg kg^{-1} in OECD artificial soil, but (O.O. Jegede et al., in preparation) found it ranged from 1050 to 26,000 mg kg^{-1} in sandy and loamy soils.

Owojori and Siciliano (2012) also found that lead (Pb) has less effect on the survival of *O. nitens* than the other three metals (Cd, Cu, and Zn), with an LC50 value of about 6,700 mg kg^{-1} in OECD artificial soil; however, *O. nitens* reproduction is sensitive to Pb. They also estimated the reproduction EC50 for Pb was 1,678 mg kg^{-1} in artificial soil. However, (O.O. Jegede et al., in preparation) found it ranged from 1,360 to 21,000 mg kg^{-1} in five different natural soils with contrasting physicochemical properties, especially cation exchange capacity (CEC) and organic carbon (OC) content.

The toxicity of nickel and cobalt to *O. nitens* indicates that nickel is more toxic than cobalt and could be as toxic as cadmium, with reproduction EC50 values ranging from 133 mg kg^{-1} to 3,600 mg kg^{-1} in five natural soils of contrasting physicochemical properties (O.O. Jegede et al., in preparation). When assessing the toxic effect of lanthanum (La), a rare-earth metal, on *O. nitens*, Li et al., 2018 reported to having a reproduction EC50 of 1,500 mg kg^{-1} (based on total concentration), 10.2 mg kg^{-1} (pore water concentration), and 15.6 mg kg^{-1} ($CaCl_2$ extractable concentration).

2.7.7.2.2 Pesticides.

Few studies have reported the response of *O. nitens* to pesticides. de Lima e Silva et al., (2017) assessed the toxic effect of two neonicotinoids (thiacloprid and imidacloprid) on *O. nitens*. They found that the LC50 value of thiacloprid was >1,000 mg kg^{-1}, whereas the EC50 value for effect on reproduction was 76 mg kg· However, when the mites were exposed to imidacloprid, the LC50 value was 360 mg kg^{-1}, and reproduction EC50 value was 119 mg kg^{-1}. This indicates that thiacloprid has a more significant reproductive effect than imidacloprid but less effect on mite survival. Results available for other pesticides generally showed low sensitivity of *O. nitens* to pesticides. For example, when *O. nitens* was exposed to the insecticide chlorantraniliprole (a ryanoid), no effect on survival and reproduction at concentrations >1,000 mg kg^{-1} were noted (Lavtizar et al., 2016). Similarly, when *O. nitens* was exposed to the herbicides imazapyr and triclopyr, toxicity could not be determined at concentrations above 4,000 mg a.i.kg/dry weight for imazapyr, while triclopyr only had an effect at EC25 level when the concentration was 1500 mg kg^{-1} (Jimmo et al., 2018).

2.7.7.2.3 Other organic compounds.

The toxicity of organic compounds to *O. nitens* have been assessed in a number of studies. Owojori et al. (2011) reported the toxicity of three organic contaminants (phenanthrene, benzo[a]pyrene, and geraniol) to *O. nitens*. Phenanthrene was very toxic to *O. nitens* with LC50 and reproduction EC50 values of 388 and 95 mg kg^{-1}, respectively. For geraniol, a reproduction EC50 value of 283 mg kg^{-1} was reported (Owojori and Siciliano, 2012). In contrast, benzo[a]pyrene, showed no toxicity to *O. nitens*, even at concentrations above 1,600 mg kg^{-1}.

Princz et al. (2018) assessed the toxicity of a persistent organic pollutant, perfluorooctane sulfonate (PFOS), to *O. nitens*, and they reported reproduction EC50 values of the PFOS in coarse and fine soil were 23 and 95 mg kg^{-1}, respectively. In another study, Hernandez (2014) examined the toxicity of four organic fire retardants (firesorb, fireaide, One Seven Class A, and M51) on *O. nitens* in OECD artificial and LUFA 2.2 soils; they found the toxicities varied among the different chemicals, with their reproduction EC50 values

ranging from 2,462 to >8,600 mg kg^{-1}. Fireaide demonstrated no toxicity to *O. nitens*, even at a concentration above 10,000 mg kg^{-1} in both OECD artificial and LUFA 2.2 soils.

In a study with petroleum hydrocarbon-contaminated soil (about 250,000 mg kg^{-1} TPH [Total petroleum hydrocarbon]), Princz et al. (2012) found no mortality of *O. nitens*; however, they observed reproduction was substantially impacted relative to control. Gainer et al. (2018) assessed the toxicity of lubricating oil, a 50/50 mixture of F2/F3 petroleum hydrocarbons, to *O. nitens* in OECD artificial soil (10% peat) and reported a reproduction EC50 value of 1210 mg kg^{-1}.

2.7.7.3 Avoidance response of O. nitens to soil contaminants

Although a standardized protocol for the avoidance response of *O. nitens* is not available, some studies have assessed the avoidance response of the mites to several contaminants. Most of these studies adapted protocols for earthworms or collembola (ISO, 2006, 2008), and results available show promise of a standard avoidance test for *O. nitens* in the near future. The first-ever information on avoidance of *O. nitens* to contaminants was the work of Owojori et al. (2011), who assessed the influence of soil properties and duration of test on avoidance response of *O. nitens* to eight chemicals (copper, zinc, cadmium, lead, phenanthrene, benzo[a]pyrene, geraniol, and boric acid). The authors found no significant effect of soil properties on avoidance response of *O. nitens* to these chemicals, thus suggesting avoidance response to be a feasible test in screening contaminated soil from large areas of contrasting soil properties. They also reported that significant avoidance could be achieved after only 6 hours of introducing mites into contaminated soil. However, reliable results may require 24 hours to achieve. It should be noted that current guidelines for the avoidance response of earthworms and collembola recommend a test duration of 48 hours and Frankenbach et al. (2014) could even show that 24 h is enough while testing a number of spiked and contaminated soils. Hence, the avoidance response of *O. nitens* is a rapid toxicity test to screen contaminated soils.

Most soil organisms do not avoid all contaminant groups and classes, as already shown for earthworms and collembola (Owojori et al., 2014; Gainer et al., 2019a, b). The results from

Owojori et al. (2011) showed similar response between reproduction and avoidance (i.e., similar values of reproduction and avoidance EC50s) of *O. nitens* to copper, zinc, benzo[a]pyrene, and phenanthrene. A recent study assessed the avoidance behaviour of *O. nitens* to lubricating oil. The mites avoided the lubricating oil at concentrations above 1,000 mg kg^{-1}, although the LC50 value was more than 10,000 mg kg^{-1} TPH (Gainer et al., 2019a). Gainer et al. claim that *O. nitens* avoidance behaviour is as sensitive as plant growth because both avoidance EC50 and plant growth measurements were in the same range of magnitude. Thus, avoidance tests can be used as a rapid screening for hydrocarbon-contaminated soils in place of plant toxicity assessment, which takes a longer time. There is a paucity of literature on the influence of life history on avoidance response of *O. nitens,* but it appears that life history has no discernible influence on avoidance response of *O. nitens* when given a choice between control and NaCl, phenanthrene, or copper-contaminated soil (Gainer et al., 2019a,b).

2.7.7.4 Toxicokinetics of contaminants in O. nitens

Mites generally are known to be high accumulators of metals (Skubala and Kafel, 2004). Few studies have assessed contaminant accumulation in *O. nitens*, partly due to the species' small size, which makes assessing accumulation in traditional survival or reproduction assays challenging. However, a few studies have assessed metal accumulation in this species. Owojori and Siciliano (2012) assessed the biota to soil accumulation factor (BSAF) of Zn, Cu, Cd, and Pb in *O. nitens* after 35 days of mite exposure in OECD artificial soil (10% OM). The BSAF of zinc was the highest (1.07) compared to Cd (0.71), Cu (0.12), and Pb (0.42). The high BSAF of Zn can be attributed to its essentiality in physiological processes. Zn started to cause a toxic effect in *O. nitens* at ~2000 µg Zn/g dry body weight (Owojori and Siciliano, 2012; Jegede et al., 2019b). The toxicokinetics of cadmium to *O. nitens* after a 7-week exposure in LUFA 2.2 soil showed *O. nitens* to be a steady accumulator of Cd with reduced elimination or depuration. Lethal body estimates ranged from 44 to 91 µg Cd/g dry body weight (Keshavarz Jamshidian et al., 2017).

2.7.7.5 Response of O. nitens to contaminants compared to other soil invertebrates

Table 2A of Appendix A presents the response of *O. nitens* to contaminants in comparison with other soil invertebrate species used in soil ecotoxicity testing. Since soil properties could influence the toxicity of chemicals, we only compared data for the same type of soils. The sensitivity of *O. nitens* to metals depends on the type of metal in question, whether essential or non-essential. It appears *O. nitens* is more sensitive to non-essential metals when compared with other soil invertebrate species (i.e., *E. fetida* and *Enchytraeus crypticus/E. albidus*). For example, *O. nitens* is more sensitive to nickel than are *F. candida, E. fetida, and E. albidus/crypticus*. For cobalt, an essential metal, *O. nitens* is less sensitive compared to *E. fetida* and *E. albidus/crypticus* (Table 2A of Appendix A). In the case of Cd and Pb, using reproduction as the endpoint, *O. nitens* appears to be more sensitive than *F. candida* but showed similar sensitivity with the oligochaete species.

The response of *O. nitens* to Zn is interesting because, first, Zn is an essential metal, and second, it is mimicked by Cd. Based on survival as an endpoint, *O. nitens* is more sensitive to Zn than *F. candida*. However, *O. nitens* is less sensitive to Zn based on reproduction when compared with *F. candida, E. fetida,* and *E. crypticus* or *albidus* (Table 2A of Appendix A). A similar trend in the sensitivity of *O. nitens* to Zn was observed for Cu, except for the predatory mite, *Hypoaspis aculeifer*, whose sensitivity to Cu is less compared to *O. nitens* (Table 2A of Appendix A). For the rare-earth metal, lanthanum, *O. nitens* is the least sensitive in term of survival and reproduction compared to *F. candida* and *E. albidus/crypticus* (Table 2A of Appendix A).

All available studies on *O. nitens*' response to pesticides show the mite to be less sensitive to pesticides than other soil invertebrates (de Lima e Silva et al., 2017; Jimmo et al., 2018). In fact, for some neonicotinoid pesticides, *O. nitens* is less sensitive by several orders of magnitude (de Lima e Silva et al., 2017). Greater sensitivity has been observed for *O. nitens* to some organic compounds (Table 2A of appendix A). For example, the reproduction of *O. nitens* was about five times more sensitive to PFOS than was *F. candida* (Princz et al., 2018). *O. nitens* was also observed to be more sensitive to petroleum hydrocarbons than were *F. candida, E. crypticus,* and *H. aculeifer* (Gainer et al., 2018).

The sensitivity of *O. nitens* relative to other standardized soil invertebrates can also be ranked using a species sensitivity distribution (SSD) (Posthuma et al., 2001). Here, we generated an SSD for Cd and Zn with robust toxicity data from one of the most comprehensive reports on Cd and Zn toxicity to soil invertebrates (Lock and Janssen, 2001a,b) and other available data on Cd and Zn (see Table 3). Also, the sensitivity of *O. nitens* to neonicotinoids (imidacloprid and thiacloprid) relative to other soil invertebrates was generated using the data from de Lima e Silva et al. (2017). The few numbers of species used in generating the SSD might decrease community representativeness (Dowse et al., 2013). However, the SSD shows *O. nitens* to be the least sensitive species (i.e., most tolerant) to Cd relative to other standardized soil invertebrate species, considering reproduction as the endpoint (Figure 2-8A). Considering Cd toxicity on survival, *E. fetida* was the least sensitive species, followed by *F. candida* and *O. nitens* (Figure 2-8B); however, this finding might vary between metals and soil types. A similar pattern, as observed for the sensitivity of *O. nitens* to Cd, was observed for Zn (Figure 2-8C and D). *O. nitens* seems to be the least sensitive species (i.e., most tolerant) to neonicotinoids (imidacloprid and thiacloprid) relative to other standardized soil invertebrates based on reproduction and survival as endpoints (Figure 2-9). *O. nitens* is not affected by the concentration of Cd, Zn, and the neonicotinoids that will potentially affect 25% of the soil invertebrate taxa (i.e., hazard concentration [HC25]) (Figure 2-8 and 9).

Hence, the varying sensitivity of *O. nitens* to soil contaminants is key to capture a wide range of toxicity of chemicals to soil invertebrates, thus allowing a robust risk assessment of chemicals in soil.

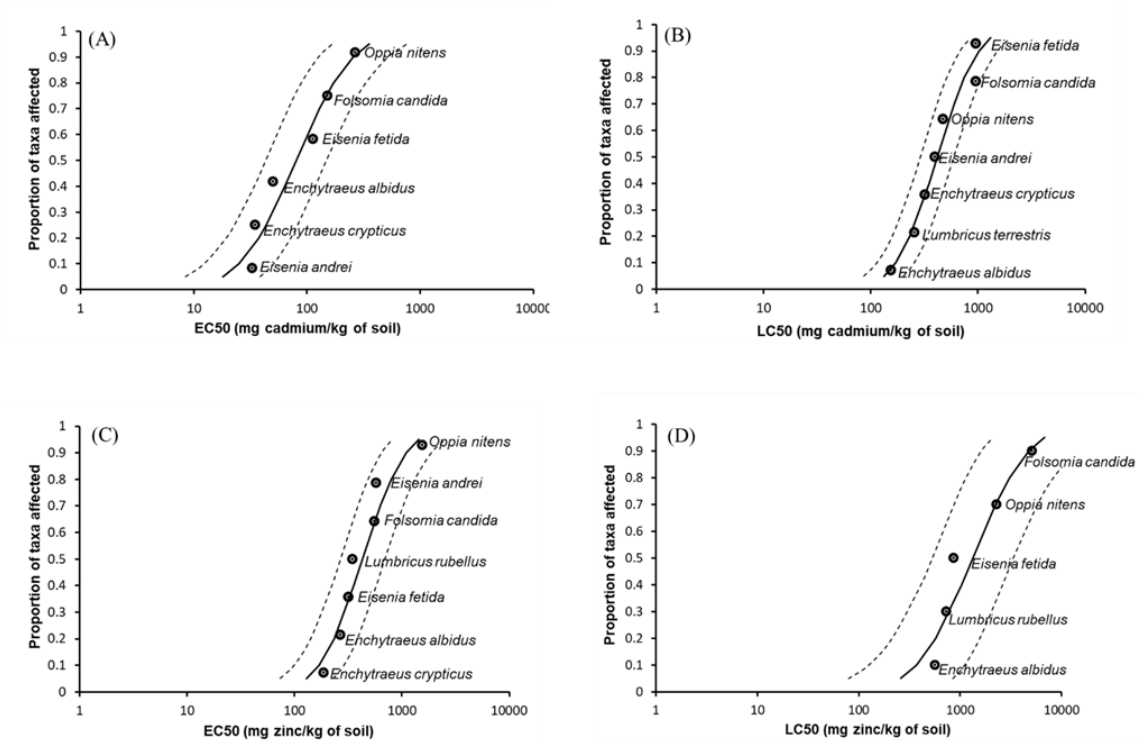

Figure 2-8. Species Sensitivity Distribution (SSD) of model soil invertebrates from (A) reproduction EC50 values of Cd with HC25 = 43.60 (22.97–82.76) mg kg^{-1} (B) survival LC50 values of Cd with HC25 = 260.52 (180.15–376.77) mg kg^{-1} (C) reproduction EC50 values of Zn with HC25 = 263.83 (161.56–430.84) mg kg^{-1} (D) survival LC50 values of Zn with HC25 = 680.54 (258.62–1790.79) mg kg^{-1}. The data used to generate SSD were from Lock and Janssen (2001a,b); Owojori and Siciliano (2012); and *H.O. Fajana et al., in preparation. All the toxicity data were based on standardized soils (OECD and LUFA 2.2) and test duration of 14 – 84 days. *EC50 for Cd to *Oppia nitens* = 392.48 mg kg-1 in OECD soil, pH = 6.2, organic matter = 10%.

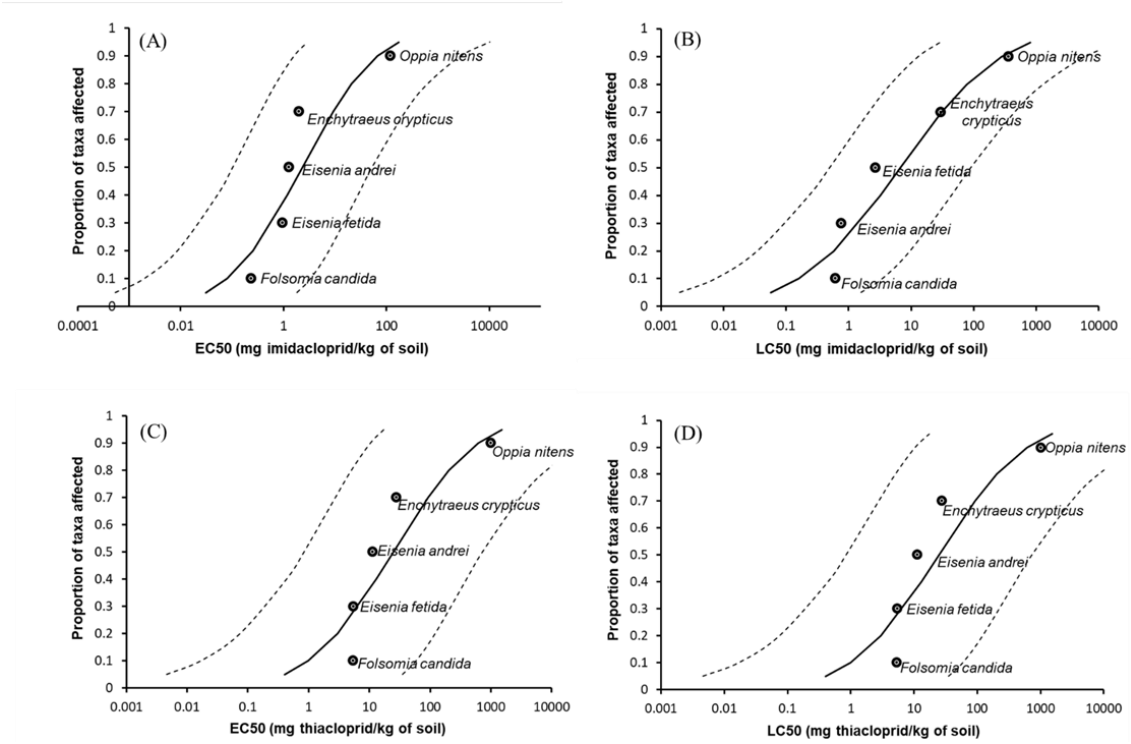

Figure 2-9. Species Sensitivity Distribution (SSD) of model soil invertebrates from (A) reproduction EC50 values of imidacloprid with HC25 = 0.255 (0.009–7.575) mg kg^{-1} (B) survival LC50 values of imidacloprid with HC25 = 0.945 (0.061–14.679) mg kg^{-1} (C) reproduction EC50 values of thiacloprid with HC25 = 0.775 (0.065–9.215) mg kg^{-1} (D) survival LC50 values of thiacloprid with HC25 = 4.548 (0.126–164.289) mg kg^{-1}. The data used to generate SSD were from de Lima e Silva et al. (2017). Note: All the toxicity data were based on standardized soils (OECD and LUFA 2.2).

2.7.8 Standardized test methods for *O. nitens*

Soil ecotoxicology has taken on a central role in global regulatory frameworks for risk assessment of contaminated soils from environmental chemicals of concern, and to regulate the authorization of chemicals such as pesticides. This has in part been accomplished through the development of standardized toxicological tools or tests that can be used to inform risk assessment and management processes. The organizations responsible for standardization efforts primarily include, but are not limited to, the International Organization for Standardization (ISO), the Organization for Economic Co-operation and Development (OECD), Environment and Climate Change Canada (ECCC) (Biological Test Method series), and American Society for Testing and Materials (ASTM) International. When used together, the suite of standardized tests enables a 'test battery' approach that reflects the structural and functional complexity of soils. This approach allows for the consideration of different exposure routes, varied trophic levels, toxicokinetics, and toxicodynamics, while taking into consideration interactions between the exposure medium (soil) and the pollutants (Beck et al., 2005; Princz et al., 2012; van Gestel., 2012; Frankenbach et al. 2014). This, in turn, is meant to support a holistic representation of effects, ultimately allowing for the maintenance and protection of ecosystem structure (e.g., biodiversity), processes (i.e., function), and services.

Standardization efforts ensure comparisons between toxicity tests, providing specific requirements or guidance associated with different aspects of the tests (e.g., species, materials, test parameters, and endpoints). A significant advantage of standardization is that it validates the test method (including requirements or guidance therein). The validation then prompts an in-depth approach involving discussion among scientists within the field, the consideration of practical aspects ensuring broader applicability of the method, and Ring-testing to ensure conformity and comparability of the proposed method (Römbke et al., 2018). This in-depth approach ensures applicability of the test method across numerous chemical substances (e.g., new and existing), and within various field exposure scenarios, to either characterize risk or assist with developing remediation requirements.

The development of a new oribatid mite test method came as a result of a workshop held by Environment Canada in 2003 (EC, 2004). The workshop marked the completion of the first series of soil toxicity test methods suited to agronomic species and habitats, which was held to expand the applicability of soil toxicity methods to other habitats (e.g., boreal and northern regions, wetlands), thus prioritizing the need for a second generation of soil toxicity test methods. Subsequently, a series of potential new plant and soil invertebrate species were recommended, one of which included the use and application of the oribatid mite, *O. nitens* (Princz et al., 2010; Princz et al., 2012; Princz 2014). Although the test method was originally developed for assessing contaminated soils within boreal ecosystems, *O. nitens* has since shown applicability in diverse soil types, reflective of its generalist and ubiquity in the Holarctic region. Moreover, as cited within this review, the species has also been used in the assessment of spiked chemical substances in soils (e.g., metals and pesticides).

O. nitens also meets the criteria for a 'desirable' test species (Stephenson, 2003; Römbke et al., 2006; van Gestel 2012) because of its (1) ecological relevance to temperate and northern ecozones, (2) ability to represent functional and taxonomical diversity (adding to the overall diversity of the available test battery of species), (3) intimate contact with the soil environment and contaminants within, (4) varied contaminant tolerance (as demonstrated through studies to date), (5) varied routes of exposure (e.g., dermal and oral), and its (6) amenability to life cycle tests (e.g., Jegede et al. 2019a).

As part of method research and standardization efforts, numerous studies were conducted with *O. nitens* in various soils (e.g., boreal and agricultural) and horizons to gauge the overall adult survival and reproductive output (ECCC, 2018). Elements of the method research included optimizing culturing techniques, analyzing life cycles to characterize time to maturation and oviposition, and age-synchronization. Although studied, sexual dimorphism in *O. nitens* was not exhibited. However, analyses of sex ratios demonstrated equivalent ratios of both males (49 ± 11%) and females (51 ± 11%) in random reproduction tests (ECCC 2018). The test design was optimized to balance practical aspects with elements of standardization, such as requiring fifteen (15) age-synchronized adults to reduce test variability and minimize the total number of organisms required for a test. Method

validation included an international ring-test, involving laboratories across Canada and Europe, coordinated by Environment and Climate Change Canada (ECCC, 2019). The ring-test assessed the reproductive success of *O. nitens* in a series of control soils (e.g., artificial and field soils), as well as sublethal effects of the reference toxicant, boric acid, in field-collected sandy loam soil and the standard LUFA 2.2 field soil. The results from the international ring-test demonstrated suitable inter-laboratory variability of ≤ 30% for effects on reproduction when *O. nitens* was exposed to the reference toxicant.

The control performance data derived from the international ring-test, together with data accumulated from the method research, provided enough information to derive test validity criteria for adult survival (i.e., > 70% adult survival in control soils) and juvenile production (i.e., > 30 live individuals) (ECCC, 2019). These values reflect the life history traits of the species and enables applicability to different soil exposure scenarios, ranging from standardized soil types (e.g., artificial soil) to natural soils. The standardization efforts resulted in two harmonized standardized test methods: one specific to Canadian environments (ECCC, 2018), and one generalized to global application (ISO, 2019). The standard test method comprises a 28-day test to evaluate effects on *O. nitens* reproduction, although adult mortality may also be evaluated. The test is conducted at a mean temperature of 20 ± 2°C in suitable vials (e.g., 30 mL glass shell vials) containing approximately 20 g of soil at optimum moisture content (ECCC, 2019). The test requires fifteen (15) age-synchronized adults (8-10 days post eclosion) in each replicate test vessel, with a minimum of five replicates per treatment (ECCC, 2018; ISO, 2019). Test endpoints include assessing test validity criteria and the number of live adults and juveniles within each treatment. The test can be conducted as a single- or multiple-concentration test in order to determine the percentage effect concentration estimated for the inhibition of reproduction (e.g., EC*x* or IC*x*) (ECCC, 2018; ISO, 2019).

As demonstrated through the initial method research to the increasing number of available studies cited herein, the test has proven effective for assessing contaminants in soil, whether in contaminated field soils or soils spiked with chemical substances. The standardization of *O. nitens* as a test species contributes to the current test battery available for plants and soil

organisms and fulfills the need for including additional arthropods in standardized soil testing (Römbke et al., 2006; van Gestel, 2012).

2.7.9 Future perspectives on *O. nitens* as a model organism in soil ecotoxicology

2.7.9.1 Sub-organismal responses of O. nitens to xenobiotics in soil

In toxicology, sub-organismal approaches are diagnostic tools to explain how organisms respond to stress from xenobiotics. There are reasonable data on the sub-organismal responses (i.e., cellular, biochemical, or molecular responses) of soil invertebrate models used in soil ecotoxicology (Spurgeon et al., 2004, 2010; Swain et al., 2004; Nota et al., 2009; Novais et al., 2011; Qiao et al., 2015; Gomes et al., 2018). Little is known of *O. nitens'* inherent adaptive mechanisms to xenobiotics. Available toxicity data on survival and reproduction of *O. nitens* has revealed certain underlying mechanisms that govern its response to chemical stress. For instance, *O. nitens* can sequester a substantial amount of heavy metals from contaminated soils with little effect on survival, even across generations (Owojori and Siciliano, 2012; Keshavarz Jamshidian et al., 2017; Jegede et al., 2019a,b). The mites likely have a higher capacity for storage (e.g., internally, but also via redistribution to the cuticle), as well as a high expression of metallothionein (Keshavarz Jamshidian et al., 2017).

The activity of the enzyme lactate dehydrogenase (LDH) in *O. nitens* reveals the possibility of this biomarker as an early warning of zinc toxicity. LDH also shows how mites respond to zinc contamination in soils of varying physicochemical characteristics (Jegede et al., 2019b). High LDH activity was observed at high zinc concentration (14,000 mg kg^{-1}) for mites in soils of low organic carbon and cation exchange capacity (CEC) (Jegede et al., 2019b). This is the first report on a sub-organismal or biochemical response of *O. nitens* to xenobiotics. This finding stresses the need for more detailed studies incorporating sub-organismal endpoints (e.g., gene expression, protein, and metabolite profiling using a high-throughput omics approach) to better understand the molecular responses of *O. nitens* to xenobiotics. The small size of the mites might be a limitation to getting reasonable homogenate or tissues for biochemical or molecular assays, but this could be overcome by optimizing the number of mites required for a toxicity test.

2.7.9.2 Sequencing of the O. nitens genome

The genome provides insight into how genes influence life functions, life history, and maintenance of an organism. Knowing the genome of *Caenorhabditis elegans* and *Drosophila melanogaster* makes these organisms important animal models in both medicine and toxicology. We believe sequencing the genome of *O. nitens* will provide better mechanistic insight into how this organism responds to stress in soil. This will also help us to identify novel and key genes that drive adaptive responses and will possibly solve the mystery behind its efficient metal sequestration. Complete genomes or transcriptomes of other model soil invertebrates (e.g., *F. candida*) are available, with the molecular data revealing vital genes and pathways involved in adaptive responses, xenobiotics-induced apoptosis and epigenetic effects, and possible horizontal gene transfer from their microbiomes (*C. elegans* Sequencing Consortium, 1998; Castro-Ferreira et al., 2014; Zwarycz et al., 2015; Faddeeva-Vakhrusheva et al., 2017). Recently, *O. nitens'* mitochondrial gene, *cytochrome oxidase subunit 1* (*COI*), was sequenced, but for taxonomic purposes (i.e., DNA metabarcoding) (Telfer et al., 2015).

The current trend in ecotoxicology focuses on adverse outcome pathways (AOPs) in response to chemical stressors (Ankley et al., 2010). Therefore, sequencing the complete genome of *O. nitens* will facilitate further studies to establish the AOPs of common soil toxicants for *O. nitens*. For example, heavy metals reduce juvenile production (an adverse outcome at individual level), but no established molecular initiating or key events leading to the adverse outcome in the mites. However, a putative AOP (Figure 2-10) can be established based on known key events for arthropods, such as alteration in bioenergetics, which might lead to reduced egg production (Nisbet et al., 2000; Jager et al., 2005) and adverse outcomes both at the individual and population levels. These adverse outcomes would ultimately affect ecosystem services.

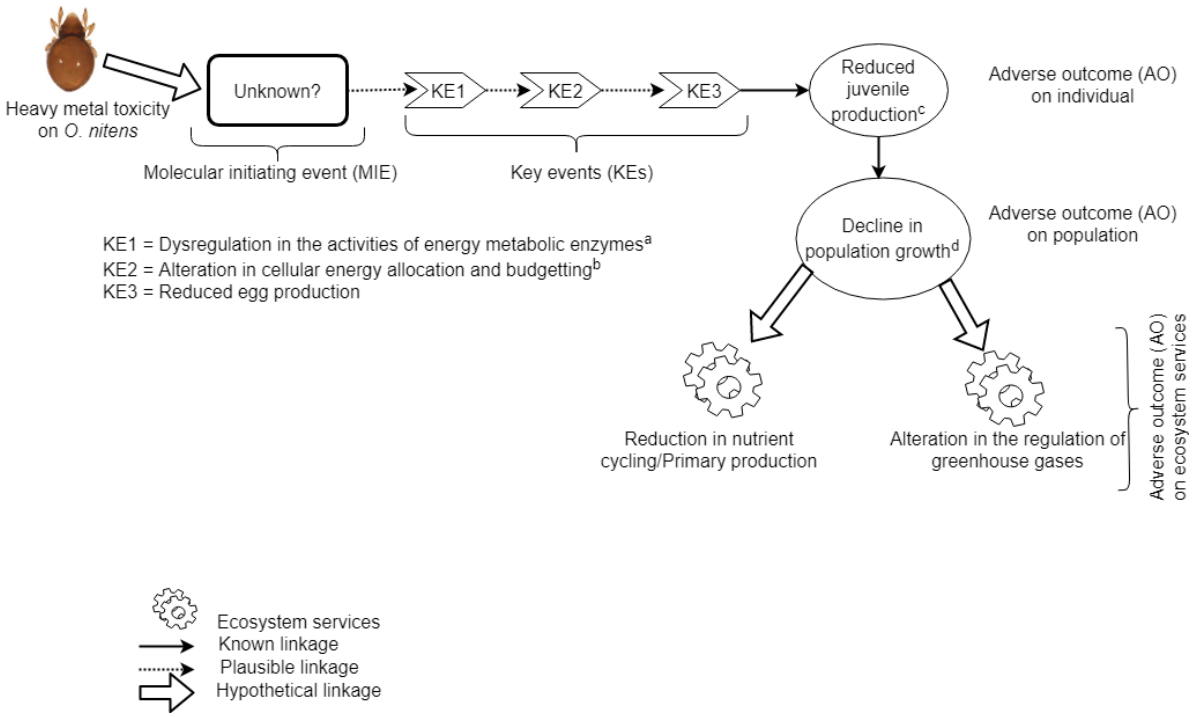

Figure 2-10. A putative adverse outcome pathway (AOP) for heavy metal toxicity on *Oppia nitens* showing adverse outcomes at the individual, population, and ecosystem level. [a]Jegede et al. (2019b); [b]Kramer et al. (2011); [c]Owojori and Siciliano (2012); [d]Jegede et al. (2019a).

Furthermore, understanding *O. nitens'* genome can also clarify underlying molecular mechanisms for xenobiotics and other environmental stressors on *O. nitens*; hence, enable a robust risk assessment of contaminants in the soil environment via omics approaches (i.e., transcriptomics, metabolomics, and proteomics) (Simoes et al., 2018).

Lastly, we envisage a molecular pathway to understanding mechanisms of metal tolerance in *O. nitens*. Information from such a molecular framework will improve our knowledge of metal tolerance in arthropods, and possibly vertebrates, since most metal-responsive genes/regulatory proteins are conserved across organisms (Janssens et al., 2009). Therefore, we propose a potential pathway to metal tolerance in *O. nitens* by considering a possible metal-responsive gene, *AtPCS1* (Clemens et al., 1999; Ha et al., 1999; Vatamaniuk et al., 1999), which encodes phytochelatin synthase (AtPCS1), which is an enzyme that mediates phytochelatin synthesis, which in turn is a peptide needed for metal detoxification in plants and some fungi (Cobbett and Goldsbrough, 2002). Interestingly, a homolog of *AtPCS1*, *ce-pcs-1*, was found and linked to cadmium tolerance in *C. elegans*, as *ce-pcs-1* knockout *C.*

elegans shows high sensitivity to Cd (Vatamaniuk et al., 2001); this was the first study to show the role of phytochelatins in an animal. Other studies have reported the induction of phytochelatins in invertebrates such as the earthworm, *Lumbricus rubellus*, in response to arsenic at both laboratory and field exposures to contaminated soil (Liebeke et al., 2013). The rationale for considering involving phytochelatins-dependent detoxification pathway in metal tolerance of *O. nitens* is that because oribatid mites have evolved to feed on fungi, there might be a horizontal transfer of phytochelatin synthase gene(s) from fungi to the mites. For example, *Tetranychus urticae*, a species of spider mites, was reported to acquire carotenoid biosynthesis genes from fungi via horizontal gene transfer (Bryon et al., 2017). Phytochelatin synthase genes seem not to be transcriptionally regulated in animals, as seen with *C. elegans* and *L. rubellus* (Cui et al., 2007; Liebeke et al., 2013). Hence, a proteomics approach can be used to measure the level of phytochelatins in the mites' tissue to support phytochelatin synthase gene expression. Other intermediate metabolites involved in the stepwise synthesis of phytochelatins from glutathione (e.g., cystathionine) should be measured using a metabolomics approach (Hughes et al., 2009).

We conclude that along with the physiological detoxification mechanism in oribatid mites (Ludwig et al., 1992) and the metallothioneins (MTs)/glutathione (GSH) detoxification pathway, *O. nitens* might also have another metal detoxification mechanism: the phytochelatin-dependent pathway. When the mite is exposed to a high metal concentration, a functional phytochelatin synthase could mediate this pathway (Figure 2-11). Understanding the *O. nitens* genome is thus vital to confirming this pathway to metal tolerance in the mites.

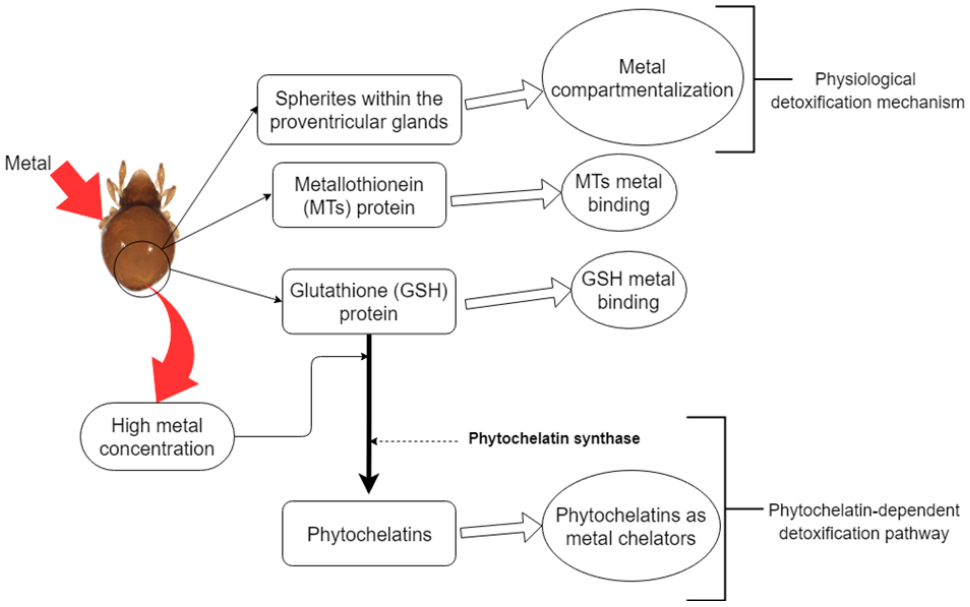

Figure 2-11. Potential mechanisms of metal tolerance in *Oppia nitens*, given an additional detoxification pathway, the phytochelatin-dependent pathway, which is mediated by phytochelatin synthase (PCS) at high metal concentration.

2.7.10 Conclusions

To summarize the results of our review, first of all, detailed knowledge of the biology and ecology of *O. nitens* supports the species' suitability as a model organism in soil ecotoxicology and is the foundation for developing toxicity responses to chemical stressors. *Oppia nitens*' response and sensitivity to different classes of chemicals relative to other standardized soil invertebrates is crucial to developing a robust risk assessment of chemicals in soil. The complete genome and transcriptome of *O. nitens* is needed to understand its molecular responses to xenobiotics in soil and to establish a molecular pathway leading to the mites' metal tolerance and adverse outcome to a contaminant's toxicity. When exposed to high metal concentrations, *O. nitens* might have a functional phytochelatin synthase that mediates a phytochelatin-dependent detoxification pathway.

3. Uptake, toxicity, and maternal transfer of cadmium in the oribatid soil mite, *Oppia nitens*: Implication in the risk assessment of cadmium to soil invertebrates[2]

3.1 Preface

The toxicity of cadmium was investigated on adult *Oppia nitens* to determine the effect of cadmium on reproduction (i.e., juvenile production) and life history (i.e., fecundity and juvenile recruitment) in OECD artificial soil. The maternal transfer of cadmium from adult to juvenile (tritonymphs) mites was also assessed to estimate maternal transfer ratio (τ).

[2] Fajana HO, Jegede OO, James K, Hogan NS, Siciliano SD. (2020). Uptake, toxicity, and maternal transfer of cadmium in the oribatid soil mite, *Oppia nitens*: Implication in the risk assessment of cadmium to soil invertebrates. *Environmental Pollution*, https://doi.org/10.1016/j.envpol.2020.113912

Hamzat Fajana: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing (original draft), visualization
Olukayode Jegede: Investigation, and analysis
Kyle James: Data curation and risk assessment interpretation
Natacha Hogan: Writing (review and editing)
Steven Siciliano: Conceptualization, resources, writing (review and editing), supervision, project administration, funding acquisition

3.2 Abstract

Cadmium (Cd) is a heavy metal of concern in contaminated sites because of its high toxicity to soil biota and humans. Typically, Cd exposure is thought to be dominated by dissolved Cd in soil pore water and, thus, dermal uptake. In this study, we investigated the uptake, toxicity, and maternal transfer of Cd in a standard soil invertebrate, the oribatid mite (*Oppia nitens*), which is common to boreal and temperate ecozones. We found total soil Cd predicted Cd uptake in adult and juvenile *O. nitens* with no significant uptake from pore water by juvenile mites. Cadmium significantly inhibited juvenile production and recruitment as well as reduced adult fecundity. Adult *O. nitens* maternally transferred 39 to 52 % of their Cd body burden to juveniles (tritonymphs) while the maternally-acquired Cd accounted for 41 % of the juvenile internal Cd load. Our results suggest that dermal adsorption of metal ions is not important for *O. nitens* and that maternal transfer of Cd in soil invertebrates has ecological and toxicological implications for populations of soil invertebrates. Maternal transfer should be incorporated as a criterion in setting environmental soil quality guidelines (SQG_E) for cadmium and other non-essential heavy metals.

3.3 Introduction

Cadmium (Cd) is a toxic heavy metal that is introduced into the environment primarily through mining and smelting activities (Thornton, 1988). Although there are pedogenic contributions of Cd from parent materials in the soil, the anthropogenic sources pose a greater environmental threat because they are more accessible to plants, soil biota, and humans (Loganathan et al., 2012). The non-essentiality of Cd makes it internally available because it is not used for any physiological processes, thus favouring binding to cellular receptors, and induce toxicity by generating reactive oxygen species (Lopez et al., 2006). However, much of the internal Cd in soil invertebrates are bound by metallothionein to form Cd-MT complex (Dallinger et al., 1997). This mechanism of Cd interaction with MT is the central role of Cd detoxification in soil invertebrates (Stürzenbaum et al., 2004).

Cadmium can be 10-fold more toxic to soil invertebrates than other heavy metals like zinc, copper, and lead (Spurgeon et al., 1994; Owojori and Siciliano, 2012). Cadmium reduces the reproduction and survival of populations of soil invertebrates even at low concentration (Crommentuijn et al.,

1994). For instance, Cd inhibits hatching success of the enchytraeids, *Enchytraeus crypticus*, at a concentration as low as 3.1 mg kg^{-1} (i.e., EC50 of Cd for hatching) (Goncalves et al., 2015).

Cadmium toxicokinetics varies among soil invertebrates and does not necessarily reach a plateau in some soil invertebrates. The oribatid mite, *Platynothrus peltifer*, slowly accumulates Cd and reaches steady state within 80 days; thus, indicating equilibrium between uptake and elimination (Janssen et al., 1991; Crommentuijn et al., 1994). In contrast, *Oppia nitens* steadily accumulates Cd with reduced elimination and after seven weeks, steady state was not reached (Keshavarz-Jamshidian et al., 2017). Despite this continuous accumulation, it took longer for significant toxicity to be reached in *O. nitens* (Keshavarz-Jamshidian et al., 2017).

Maternal transfer is a mechanism of eliminating metals and contaminants in female adult organisms (Tsui and Wang, 2004a,b). Currently, there is no literature regarding the maternal transfer of metals, including Cd in *O. nitens*. Furthermore, maternal transfer of Cd has previously not been investigated in any soil invertebrate species. However, maternal transfer accounts for 11–15 % of the total loss of elemental mercury and 32–41 % for methylmercury in the aquatic invertebrate *Daphnia magna* (Tsui and Wang, 2004b). As maternal transfer reduces metal burden in adult animals, it, in turn, increases the metal load in offspring across generations (Nagle et al., 2001). Hence, maternal transfer is important in multigenerational exposure of organisms to contaminants such as metals (Tsui and Wang, 2004a; Lam and Wang, 2006). For example, populations of *O. nitens* that were continuously exposed to zinc for generations were more impacted (i.e., reduced biological fitness) than the population that was exposed to zinc just for one time. The high impact of zinc on the continuously exposed population of *O. nitens* might be caused by maternal transfer of Zn which increase the zinc burden in the mites; thus, causing more toxicity and reduce their tolerance niche for metals across generations (Jegede et al., 2019a). Maternal transfer in organisms is predominantly influenced by contaminant concentration in adults (i.e., maternal body burden) (Lam and Wang, 2006). Additional factors influencing maternal transfer include clutch size, number of neonates/juveniles produced, and season, especially in invertebrates (Lam and Wang, 2006; Hamilton et al., 2006).

Soil invertebrates accumulate metal by either ingesting contaminated food (algae or fungi), soil, and organic matter, or dermally adsorbing metal ions in soil solution through their cuticle.

Accumulation depends on the uptake or bioavailability of the metal in soil (Vijver et al., 2001) and the uptake of Cd from soil by soil invertebrates is mostly related to the total soil Cd concentration. For example, the total Cd pool in soil strongly predict Cd uptake in the collembola *Folsomia candida* (Vijver et al., 2001). However, in soft bodied soil invertebrates like earthworms and enchytraeids, Cd uptake is relative and depends on Cd partitioning between pore water and soil. According to Oste et al. (2001), Cd uptake by earthworms is not solely predicted by the total Cd pool, i.e., there are other factors such as pH that could influence the dissolution of Cd in pore water as $[Cd^{2+}]$ to become available for dermal adsorption. Zinc uptake by *O. nitens* is speculated to relate to total soil zinc pool rather than free zinc ion activity $[Zn^{2+}]$ in soil solution (Jegede et al., 2019b). According to the authors, *O. nitens* may have consumed zinc from the total zinc pool in soil and organic matter via ingestion.

Cadmium could exists in various forms or species in the environment such as sulfides, oxides, carbonates or complexes with minerals and particulate organic matter (Roberts et al., 2005; Sparks, 2005). Anthropogenic input of Cd in soil is predominantly in the oxide form (Chlopecka et al., 1996). Thus, it is logical to assess the toxicity of Cd to mites using the predominant environmental form in contaminated sites, i.e., cadmium oxide (CdO).

In Canada, the Canadian Council of Ministers of the Environment (CCME) determines federal soil quality guidelines. The CCME Cd soil quality guideline for environmental health is set at 10 mg kg^{-1} (residential land use) based on the soil contact pathway, which derives the guideline value by combining soil invertebrate and plant datasets (CCME, 1999). The datasets were combined due to a limited number of literature values for soil invertebrates meeting minimum data requirements. Notably, the soil invertebrate dataset was limited to two soft-bodied organisms, earthworm and collembola with five (5) species of earthworms (*Dendrobaena rubida*, *Lumbricus rubellus*, *E. fetida*, and *E. andrei*) and one collembolan, *F. candida* species (Environment Canada 1999). In addition, the United States Environment Protection Agency (USEPA) Ecological Soil Screening Level (Eco-SSL) for Cd derives a soil invertebrate screening value from an earthworm species, *E. andrei*, a collembolan species *F. candida*, and a nematode (*Plectus acuminatus*) (USEPA, 2005). In light of this, it is important to include toxicity data from other species of soil invertebrates such as

the oribatid mite (*O. nitens*), which is a completely sclerotized invertebrate, in deriving soil quality guidelines for cadmium and other heavy metals.

Oppia nitens is a standard soil invertebrate in toxicity testing (Princz et al., 2010; ISO, 2019; ECCC, 2018). *O. nitens* is completely sclerotized and found in soils that are rich in organic matter. Despite their small size, approximately 510 µm in length and 290 µm in breath (Michael, 1884), *O. nitens* bioaccumulate soil metals (Owojori and Siciliano, 2012; Keshavarz-Jamshidian et al., 2017) and as such, they are good indicators of metal pollution at contaminated sites.

The goal of this study was to examine Cd toxicity, as CdO, to the soil invertebrate, *O. nitens*, for use in environmental protection at contaminated sites. Also, for the first time, we assessed maternal transfer of a non-essential metal, Cd in a soil invertebrate.

3.4 Materials and Methods

3.4.1 Test chemical and soil

Cadmium oxide (CdO) ≥ 99.99 % trace metal basis (Sigma-Aldrich, Canada) was used as the test chemical. The test soil used was an artificial OECD (Organization for Economic Co-operation and Development) soil that is made up of 10 % sphagnum peat, 20 % kaolinite clay, 70 % sand (a mixture of 45 % fine sand and 25 % coarse sand), and 0.5 % powdered $CaCO_3$ to stabilize pH. The pH (in 0.01 M $CaCl_2$) of the OECD soil was stabilized from 5.84 to 6.50 before use. The OECD soil has an optimal water holding capacity (% WHC) of 68.5 %. The WHC of the OECD soil was determined following the method described in Annex C of the ISO 11268-1 (ISO, 2012).

3.4.2 Toxicity testing of cadmium on *O. nitens* in OECD soil

Specific quantities of air-dried OECD soil were spiked with different concentrations of CdO to give 22, 44, 88, 175, 350, and 700 mg of Cd kg^{-1} of dry soil. The dosed soils were stirred using a wooden spatula, and the soil moistened to 60 % of its WHC with ultra-pure water. About 25 g of the Cd-dosed soil was added to a glass vial, and equilibrated for 24 hours before mites were introduced into the dosed soil. Fifteen (15) age synchronized adult *O. nitens* (8-d post eclosion) were introduced into each treatment in replicates of four. The synchronized adult mites were progeny of a mite culture that was established in the laboratory for 2 years. The vial containing the dosed soil and adult mites were kept in a temperature-controlled chamber at 20–21 ℃ and %

relative humidity of 60 %. The mites were fed grains of baker's yeast and water *ad libitum* at an interval of 7 days for 28 days test duration. We ended the test after 28 days, and the mites were extracted from the Cd-dosed soil using a modified Berlese-Tullgren heat extractor set at 32℃. The extracted adult and juvenile mites were counted on a dissecting microscope to estimate adult survival and juvenile production. The reproduction data (refer to Table S2 of supplementary data) was used to estimate effective concentrations (ECx) of Cd that will inhibit reproduction by 25 % (EC25) and 50 % (EC50). The adult and juvenile mites that survived were stored in Eppendorf tubes at -80 ℃ for metal analysis.

3.4.3 Validation of ECx and determination of maternal transfer

Two experimental setups for toxicity testing, each consisting of control, EC25, and EC50 of Cd in OECD soil were conducted. Age synchronized adult mites were introduced into each set-up. After 14 days, adult mites (six replicates per treatment) were extracted from the first set-up and transferred to an uncontaminated Plaster of Paris (POP) substrate. These mites were then fed grains of baker's yeast and water *ad libitum* at an interval of seven days for another 14 days in the POP to account for egg-laying (total number of eggs laid) and juvenile recruitment (number of juveniles per total number of eggs laid). After 28 days, adult and juvenile mites were extracted from the second set-up to account for survival and juvenile production. The extracted adult and juvenile mites from both set-ups were stored in Eppendorf tubes at -80 ℃ for metal analysis to estimate maternal transfer.

The maternal transfer was determined from adult mites that were exposed to 0, EC25, and EC50 of Cd for 14 days and left for another 14 days to complete the 28-days reproduction cycle (i.e., mites from the first set-up). At the end of the 28-days reproduction cycle, Cd body burden in adults and tritonymphs [last nymphal stage of juveniles (refer to Fajana et al., 2019)] was then analyzed. Since the concentration of Cd in tritonymphs is wholly due to maternal transfer, then the maternal transfer ratio (τ) was estimated from equation 1 – 3. These equations assumed that depuration via other routes is negligible; hence, this equation can be modified in future studies to correct for depuration and excretion via other routes. From the measured Cd body burden in adult mites, we can estimate maternally acquired Cd in juveniles using equation 3.

$$C_m = \tau(\bar{C}) \quad (1)$$

$$\bar{C} = C + C_m \quad (2)$$

$$C_m = \frac{\tau C}{1-\tau} \quad (3)$$

Where C represent measured Cd body burden in adult mites (µg g^{-1} body weight), C_m represent maternally acquired Cd in juvenile mites (µg g^{-1} body weight), $\bar{C}$ stands for absolute internal Cd in adult mites before maternal transfer (µg g^{-1} body weight), and τ is the maternal transfer ratio (dimensionless). When $\tau = 0$ (No maternal transfer); $\tau = 1$ (Adult mite transfer all of its body burden to offspring). Note that $\tau = 1$ is not realistic, therefore, the parameter, τ is only realistic for the interval: $0 < \tau < 1$.

3.4.4 Processing of soil samples

After ending the toxicity test, pore water samples were extracted from the soil by saturating the soil to 100 % of its WHC and allowed to attain equilibrium for 7 days at room temperature. The pore water was then extracted following the method of Zang et al. (2019). $CaCl_2$ and water-extractable Cd were extracted using 0.01 M $CaCl_2$ and ultrapure water respectively, in a ratio of 1:5 (soil: solvent), according to Zang et al. (2019). The light fraction organic matter (OM) was extracted from the soil by agitating 5 g of the soil sample in 25 ml of ultra-pure water. The OM settled for 24 hours after which OM on the surface of the supernatant was separated by decanting the supernatant over a 0.45 µm filter paper. The OM, as residue on the filter paper, was air-dried and stored for metal analysis.

3.4.5 Metal analysis in soil samples and mite's tissue

Total soil Cd, 0.01 M $CaCl_2$-extractable Cd, and water-extractable Cd were analyzed using the procedures and methods described in Jegede et al. (2019b). Total soil Cd was analyzed by XRF (X-ray Fluorescence) in a Thermofisher ARL Optim-X X-ray analyzer, and Montana 2710a soil was used as the certified reference material having a recovery > 85 % for Cd. The concentration of Cd in pore water was analyzed using a similar method that was described for 0.01 M $CaCl_2$-extractable Cd in Jegede et al. (2019b). Mite samples were digested in HNO_3/H_2O_2, and the resulting digest in 2 % HNO_3 was analyzed for Cd in an ICP-MS (Inductively Coupled Plasma Mass Spectrometer) as

described by Jegede et al. (2019b). The recovery of Cd from the QA/QC (i.e., lobster hepatopancreas from National Research Council, Canada) was 83 %.

3.4.6 Speciation analysis

Anions (Cl^-, NO_3^-, SO_4^{2-}, CO_3^{2-}, PO_4^{3-}) and cations (Na^+, Ca^{2+}, K^+, Mg^{2+}) were extracted from the soil using ultrapure water in a ratio of 1:5 (soil: solvent) according to the procedures and methods described in Jegede et al. (2019). The pH of the extract was determined in a Mettler Toledo pH meter. The concentration of the ions was analyzed in an IC (ion chromatography) with a Dionex ICS-2000 system. DOC (dissolved organic carbon) was measured in a Mandel Total Organic Carbon (TOC) analyzer following the method described by Jegede et al. (2019b). The Cd^{2+} in water extract that was used for speciation analysis was analyzed using an Agilent microwave plasma atomic emission spectrometer (MP-AES) based on the description in Jegede et al. (2019b). The speciation calculation was done using the software, Windermere Humic Aqueous Model version 7 (WHAM 7) (Tipping et al., 2011).

3.4.7 Statistics

The reproduction ECx (EC50 and EC25) for internal Cd concentration in adults was predicted using 3-parameter Weibull regression with the drc package in R (Ritz et al., 2015). The ECx for other measures of Cd in soil were predicted using a simple linear regression model ($ECx_{Measured\ Cd\ in\ soil} = \beta_0 + \beta_1 ECx_{Internal\ Cd\ in\ adult\ mites}$) in Sigmaplot 12.0. The dose-response curves were plotted using a global curve fitting for 4-parameter logistic regression in Sigmaplot 12.0. The uptake (k) or bioavailability of Cd from soil was also predicted from a simple linear regression model ($C_{internal\ Cd\ in\ mites} = C_0 + C_{Measured\ Cd\ in\ soil} \times k$) where $C_{internal\ Cd\ in\ mites}$ represent internal Cd concentration in adult and juvenile mites (µg Cd g body weight^{-1}), C_0 stands for predicted background Cd in mites (µg g^{-1} body weight), $C_{Measured\ Cd\ in\ soil}$ represents measured Cd concentration in soil, and k is predicted uptake or bioavailability (g soil/g body weight).

The linear regression models were cross-validated using PRESS (predicted residual error sum of squares) statistics to estimate predicted coefficient of determination (r^2_{Pred}) based on the total sum of square (SSTO);

$$r^2_{Pred} = 1 - \frac{PRESS}{SSTO}$$

One-way analysis of variance (ANOVA) with Tukey post-hoc test was used to determine significant differences in toxicity endpoints across treatments. All graphs were plotted using Sigmaplot 12.0. Errors associated with Cd body burden in adult and juvenile mites were propagated from the uncertainty associated with the certified reference material, lobster hepatopancreas, which is ± 0.13 μg g^{-1} body weight for $n = 3$.

3.5 Results

3.5.1 Toxicity of cadmium to *O. nitens*

Cadmium had no significant effect on the survival of *O. nitens* even at the highest Cd concentration (LC50 > 700 mg kg^{-1}, based on spiked concentration) in the OECD soil (Figure 1B of Appendix B).

Judging from the pattern of dose-response curves, mite reproduction as a function of the internal Cd concentration in adults seems to give a better dose-response compared to reproduction as a function of other Cd concentration in soil (Figure 3-1a-d). Therefore, we estimated the ECx for nominal Cd, total Cd, and 0.01 M $CaCl_2$-extractable Cd from a linear relationship between the above measures as a function of the internal Cd concentration in adults (refer to table 3B of Appendix B).

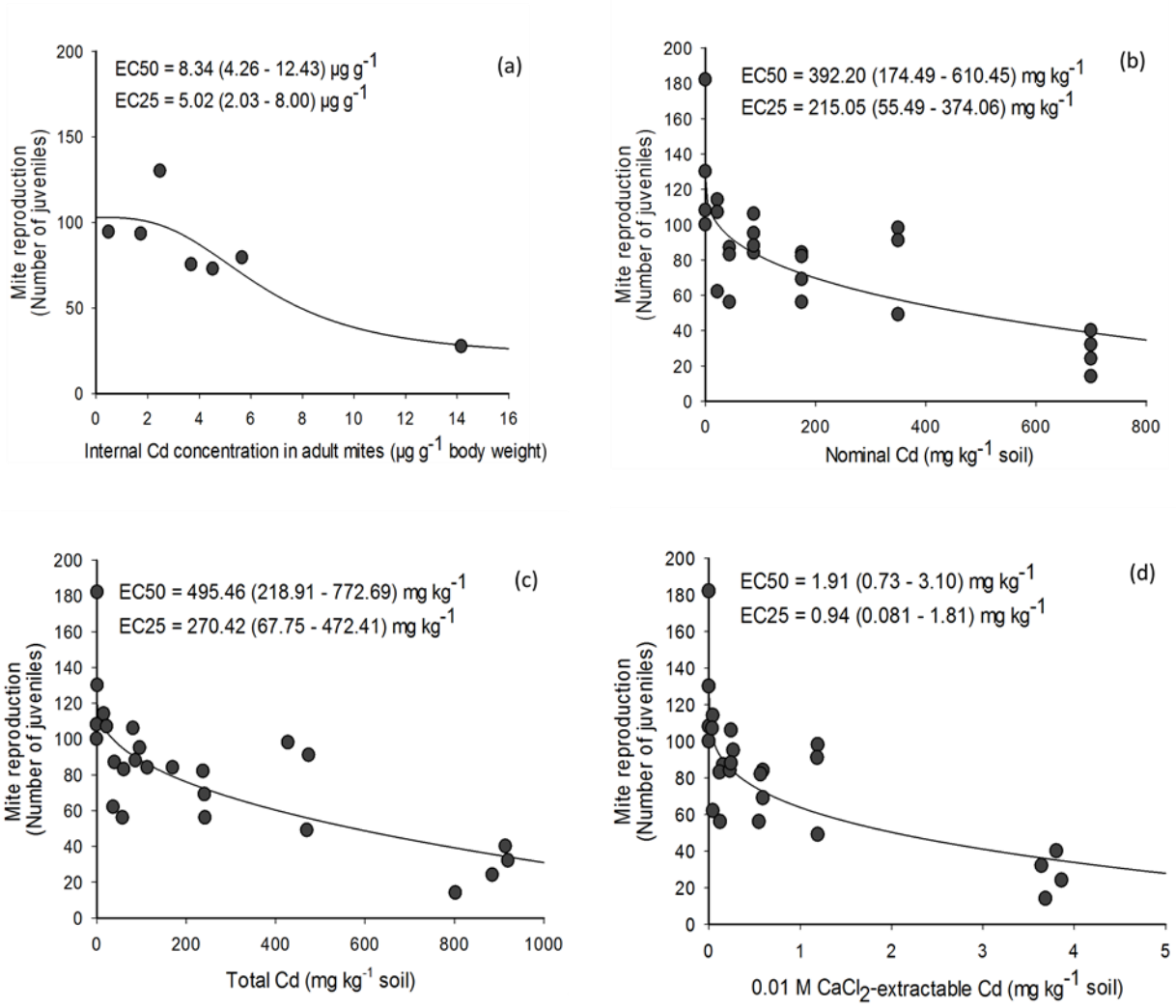

Figure 3-1. Dose-response relationship from four-parameter logistic regression for *Oppia nitens* reproduction (number of juveniles) as a function of (a) internal Cd concentration (b) nominal Cd concentration (c) total Cd concentration in soil (d) measured extractable Cd concentration in soil. EC50 and EC25 = Effective concentration causing 50 % and 25 % inhibition respectively in juvenile production of the mites after 28 days of exposure to Cd in OECD soil. Data in parentheses are the 95 % lower and upper confident interval.

3.5.2 Maternal transfer of cadmium from adult to juvenile mites

The maternal transfer ratio (τ) of cadmium was 52 (± 6) % at EC25 and 39 (± 3) % at EC50 (Table 3-1). These estimates assume that Cd excretion or depuration via other routes is negligible. Approximately 41.0 (± 1.2) % of Cd in juvenile mites (tritonymphs) was maternally acquired from adults (Table 3-2).

Table 3-1. Maternal transfer ratio (τ) of cadmium (Cd) from adults to juveniles (tritonymphs) after 14 days exposure of adult *Oppia nitens* to Cd in OECD soil and 14 days depuration period in POP substrate

	Measured Cd in adult ($\boldsymbol{C}$)	Maternally acquired Cd in juveniles ($\boldsymbol{C_m}$)	Absolute Cd in adults before maternal transfer ($\boldsymbol{\bar{C}}$)	Maternal transfer ratio ($\boldsymbol{\tau}$)
0	0	0	0	n.a
EC25	1.28 (0.13)	1.41 (0.13)	2.69 (0.18)	0.52 (0.06)
EC50	2.71 (0.13)	1.76 (0.13)	4.47 (0.18)	0.39 (0.03)

Data in parentheses are propagated uncertainty from the error associated with standard = 0.13 µg g^{-1} body weight.
Assumption: Excretion via other mechanism is negligible.
Cd concentration in adult and juvenile mites are in µg g^{-1} body weight.
n.a = not applicable.
Note: Maternal transfer ratio (τ) was derived from equation 1; $C_m = \tau(\bar{C})$ while absolute Cd in adults before the maternal transfer was derived from equation 2; $\bar{C} = C + C_m$

Table 3-2. Maternally acquired cadmium (Cd), juvenile accumulation, and absolute Cd after 28 days of *Oppia nitens* exposure to Cd in OECD soil

	Measured Cd in adults ($\boldsymbol{C}$)†	Measured Cd in juveniles†	Maternally acquired Cd in juveniles ($\boldsymbol{C_m}$)	Estimated Cd in juvenile via uptake from the soil	Absolute Cd in adults before maternal transfer ($\boldsymbol{\bar{C}}$)	The proportion of Cd in juveniles due to maternal transfer
0	0	0	0	0	0	n.a
EC25	4.85 (0.13)	14.48 (0.13)	5.25 (0.14)	9.23 (0.19)	10.10 (0.19)	0.36 (0.01)
EC50	11.60 (0.13)	16.43 (0.13)	7.42 (0.08)	9.01 (0.15)	19.02 (0.15)	0.45 (0.006)
					Average	0.41 (0.012)

Data in parentheses are propagated uncertainty from the error associated with standard = 0.13 µg g^{-1} body weight.

†Values were corrected for background Cd = 2.22 µg g^{-1} in adult and 1.32 µg g^{-1} in juvenile mites of control soil. Cd concentration in adult and juvenile mites are in µg g^{-1} body weight.

n.a = not applicable

Note: Maternally acquired Cd in juveniles (tritonymph) (C_m) was derived from equation 5; $C_m = \frac{\tau C}{1-\tau}$ while absolute Cd in adults before maternal transfer ($\bar{C}$) was derived from equation 2;
$\bar{C} = C + C_m$

3.5.3 Validation of predicted ECx

We validated the effects of Cd exposure at nominal EC25 and EC50 of Cd on mite reproduction, survival, fecundity, and juvenile recruitment (Figure 3-2). We found reproduction at the nominal EC50 and EC25 to be significantly lower than the reproduction in control mites after 28 days of exposure (Tukey: $F_{(2,20)} = 13.04$, $p_{(EC50)} = < 0.001$ and $p_{(EC25)} = 0.003$) (Figure 3-2b). The reproduction at EC50 was 50 % lower than control (Figure 3-2b). There was no effect on the survival of mites at both EC25 and EC50 (Figure 3-2a).

Adult mites that were exposed to nominal EC25 and EC50 for 14 days had reduced fecundity (eggs/mite) (Figure 3-2c) and a significant reduction in juvenile recruitment (number of juvenile/total number of eggs per mites) at EC50 compared to control (Tukey: $F_{(2,13)} = 4.45$, $p = 0.048$) (Figure 3-2d).

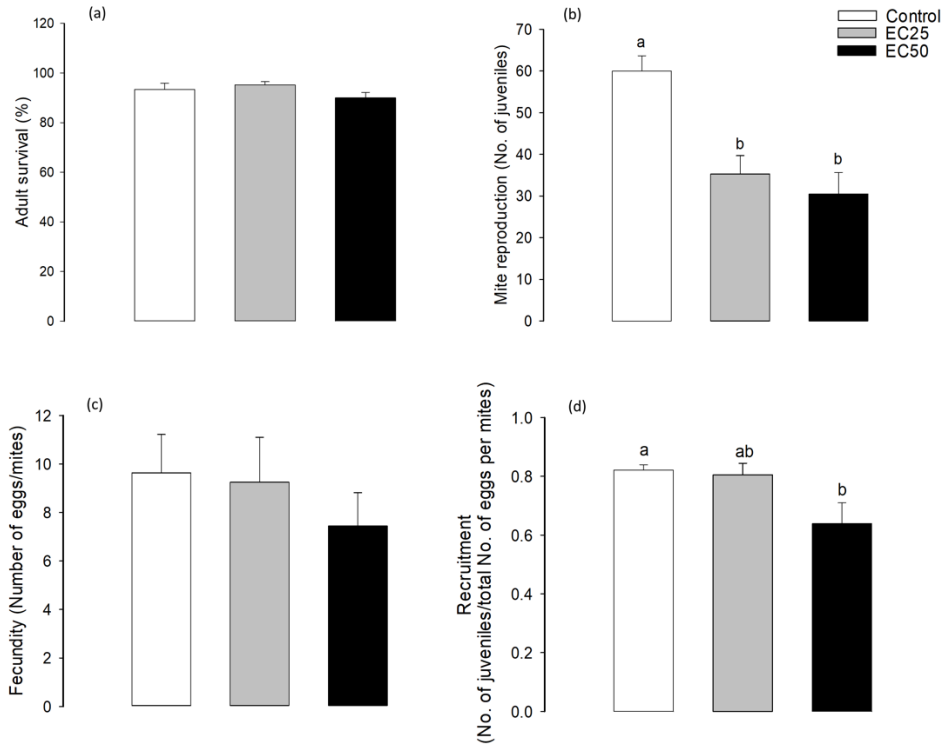

Figure 3-2. Toxicity of cadmium at EC50 = 392 mg Cd kg^{-1} and EC25 = 215 mg Cd kg^{-1} on *Oppia nitens* (a) survival (b) mite reproduction (number of juvenile) after 28 days of exposure, and (c) fecundity (number of eggs per mites) (d) juvenile recruitment (number of juveniles per total number of eggs laid) after 14 days of exposure in OECD soil.

3.5.4 Uptake of Cd by adult and juvenile mites

Adult and juvenile mites accumulated available Cd via the ingestion of either contaminated soil particles or organic matter. Pore water Cd concentration, water-soluble Cd, free Cd ion activity [Cd^{2+}], and fulvic acid-bound Cd are probably not a source of Cd uptake from the soil by mites. For example, total soil Cd was the best predictor of Cd uptake, followed by 0.01 M $CaCl_2$-extractable Cd, which is a measure of exchangeable Cd, and lastly, Cd bound to light fraction OM (Table 3-3). Absolute Cd concentration in adults before maternal transfer gave a better model for uptake than measured internal Cd concentration (Table 3-3).

Table 3-3. Cadmium uptake by adult and juvenile *Oppia nitens* from OECD soil after 28 days

		k	C_0	p	r^2_{Adj}	r^2_{Pred}
Total soil Cd (mg kg^{-1})	Adult†	0.014 ± 0.0019	1.35 ± 0.72	< 0.001	0.89	0.70
	Adult‡	0.018 ± 0.0015	2.45 ± 0.59	< 0.001	0.96	0.94
	Juvenile#	0.0071 ± 0.0012	1.59 ± 0.50	0.003	0.83	0.27
0.01 M $CaCl_2$-extractable Cd (mg kg^{-1})	Adult†	3.28 ± 0.34	1.897 ± 0.505	< 0.001	0.94	0.92
	Adult‡	4.34 ± 0.45	3.31 ± 0.68	< 0.001	0.94	0.64
	Juvenile#	1.52 ± 0.44	2.04 ± 0.67	0.018	0.64	-2.61
Pore water Cd (mg l^{-1})	Adult†	2.30 ± 0.36	2.73 ± 0.69	0.001	0.87	-4.78
	Adult‡	2.93 ± 0.60	4.51 ± 1.16	0.005	0.78	-14.04
	Juvenile#	0.91 ± 0.43	2.56 ± 0.82	0.086×	0.37	-52.28
Water soluble Cd (mg l^{-1})	Adult†	6.16 ± 0.91	2.65 ± 0.66	0.001	0.90	-3.41
	Adult‡	7.90 ± 1.52	4.40 ± 1.10	0.003	0.81	-12.22
	Juvenile#	2.50 ± 1.10	2.51 ± 0.80	0.073×	0.41	-51.74
Cd bound to light fraction OM (mg kg^{-1})	Adult†	0.050 ± 0.005	1.87 ± 0.46	< 0.001	0.95	0.77
	Adult‡	0.064 ± 0.0082	3.33 ± 0.82	< 0.001	0.91	0.067
	Juvenile#	0.022 ± 0.0076	2.11 ± 0.76	0.036	0.54	-3.93
Free Cd activity (µM)	Adult†	1.59 ± 0.26	2.90 ± 0.72	0.002	0.85	-26.23
	Adult‡	2.02 ± 0.44	4.73 ± 1.19	0.006	0.77	-67.97
	Juvenile#	0.62 ± 0.30	2.64 ± 0.82	0.096×	0.35	-236.3
Fulvic acid-bound Cd (µM)	Adult†	2.78 ± 0.43	2.77 ± 0.69	0.001	0.87	-8.78
	Adult‡	3.55 ± 0.72	4.56 ± 1.14	0.004	0.79	-25.36
	Juvenile#	1.11 ± 0.51	2.57 ± 0.81	0.083×	0.40	-95.0

C_0 = Predicted background Cd in animal (µg g^{-1} body weight); k = Predicted uptake or bioavailability (g soil g^{-1} body weight).
Measured background Cd = 2.22 µg g^{-1} in adult and 1.32 µg g^{-1} in juvenile mites of control soil.
†Based on measured internal Cd concentration (C) in adults
‡Based on absolute Cd concentration before maternal transfer ($\bar{C}$).
#Measured Cd in juvenile was corrected for maternal transfer.
×Model not significant at $p > 0.05$. r^2_{Adj} and r^2_{Pred} represent adjusted and predicted coefficient of determination, respectively.

3.6 Discussion

3.6.1 Toxicity of cadmium to *O. nitens*

We expected Cd to affect the survival of the mite at the tested concentrations due to Cd's high toxicity to soil invertebrates (Lock and Janssen, 2001). A study by Owojori and Siciliano (2012) reported an LC50 of Cd to *O. nitens* in OECD soil (10 % OM) to be 603 mg kg^{-1} and it was the most toxic of the metals tested in the study having a toxicity of 5-, 4-, and 11-fold higher than copper, zinc, and lead, respectively. However, Owojori and Siciliano (2012) used $CdCl_2$ as test metal (as compared to CdO in the present study), and the $CdCl_2$ form may have increased the availability of metal in the soil solution to the mites, thereby increasing the toxicity of Cd. The higher toxicity of $CdCl_2$ may also be caused by a synergistic effect of salinity and parent metal. For example, the LC50 of $ZnCl_2$ on the survival of earthworm (*Eisenia fetida*) and enchytraeid (*Enchytraeus albidus*) is 2- and 5-fold respectively less than the LC50 of ZnO to these invertebrates (Lock and Janssen, 2003). Similarly, Kool et al. (2011) reported the toxicity of $ZnCl_2$ on the survival (LC50) of *Folsomia candida* to be 6-fold more toxic than ZnO. Our findings depict cadmium oxide as less toxic than cadmium salts in terms of the effects on survival of *O. nitens* and suggest that current estimates of Cd toxicity may not represent toxicity that occurs from CdO deposition.

The derived EC50 for nominal Cd on reproduction (392 mg kg^{-1}) in this study (Figure 3-1b) is approximately 3-fold of the value (137 mg kg^{-1}) reported by Owojori and Siciliano (2012) after a 35-day exposure of mites to Cd in OECD soil. The fact that we used a similar OECD soil suggests that CdO is likely less toxic than $CdCl_2$ in terms of reproduction in *O. nitens*. It is important to note, however, that the duration of exposure varies between our study (28 days) and that of Owojori and Siciliano (2012) (35 days) and that this difference of one week in test duration might contribute to the difference in EC50s. Kool et al. (2011) also reported the EC50 of ZnO (i.e., 1591 mg kg^{-1}) to be five times higher than the EC50 of $ZnCl_2$ (i.e., 298 mg kg^{-1}) on the reproduction of *F. candida* in LUFA 2.2 soil. As earlier stated, a probable reason for why Cd salt (e.g., $CdCl_2$) might be more toxic than CdO is that the chloride ($CdCl_2$) increases the dissolution of Cd in soil solution (Backstrom et al. 2004), which is more bioavailable to mites. However, several studies have reported that Cd reduces the reproduction of soil invertebrate, resulting in a decline in their population in soil (McGrath, 1999; van Straalen et al., 1989; Lock and Janssen, 2001).

Though internal metal concentration in invertebrates often relates more to toxicity (Van Straalen, 1996). Also, for a non-essential metal like Cd, it accumulates and becomes internally free to ultimately cause toxicity in a dose-dependent response because no fraction of the metal is utilized for physiological processes, except for portions that might bound to proteins such as metallothionein (Dallinger et al., 1997).

3.6.2 Maternal transfer of cadmium in *O. nitens*

Adult mayfly (*Centroptilum triangulifer*) transfers about 46.5 (± 8.8) % of their selenium body burden to eggs (Conley at al., 2009). Lam and Wang (2006) also reported adult *D. magna* maternally transferred approximately 19 to 24 % of selenium they accumulated from diet to neonates of the F_1 generation. Maternal transfer of Cd from adult mayfly, *C. triangulifer* to eggs was rare as it was only observed in < 3 % of individual adults that transferred Cd to eggs with no discernable Cd burden in eggs (Xie et al., 2009). In *D. magna*, < 10 % of Cd was maternally transferred from adult to offspring (Yu and Wang; 2002; Tsui and Wang, 2007). We, therefore, conclude that the high maternal transfer ratio of Cd in *O. nitens* is influenced by the toxicokinetics of Cd in the mites; *O. nitens* excrete Cd slowly (Keshavarz-Jamshidian et al., 2017); thus, the best route for the mite to depurate their internal Cd is via maternal transfer.

The maternal transfer is an important route of excretion of contaminants, and while it reduces contaminant burden in adult organisms, it could drive transgenerational toxicity of metals (Tsui and Wang, 2004a). For instance, continuous multigenerational exposure of *O. nitens* population to zinc inhibits reproduction in mites of successive generations, due to an increase in sensitivity to zinc (Jegede et al. 2019a). However, we suggest that maternally acquired zinc in offspring increases the internal zinc load of mites in successive generations, and thus, inhibits their reproduction capacity or possibly induce teratogenicity in offspring/juveniles. For instance, Matta et al. (2001) demonstrated that maternally acquired methylmercury could reduce the reproduction capacity of fish offspring. The maternal transfer could also reduce the "metal niche width" (i.e., the metal tolerance limit) of subsequent mite populations exposed to metals in contaminated sites (Jegede et al. 2019a), thus causing a population decline.

3.6.3 Validation of ECx

The 50 % reduction in the reproduction of mites at EC50 confirms that we accurately predicted our EC50, and most ecotoxicity studies do not validate their predicted ECx. Since we derived our nominal ECx from the internal Cd ECx (refer to table 3B of Appendix B), it further confirms internal Cd concentration in mites to be the best predictor of toxicity. Hence, we suggest internal metal concentration in test organisms to be the ideal index for toxicity, especially for non-essential metals such as Cd.

At the estimated EC50 (i.e., 392 mg Cd kg^{-1}), Cd reduced the fecundity of *O. nitens* and disrupted embryological processes leading to hatching, which in turn impacted recruitment and ultimately reduced the number of juveniles produced. Cadmium reduced the fecundity of a species of the oribatid mite, *Archegozetes longisetosus* when exposed to 130 µg Cd g of $food^{-1}$ (Seniczak et al., 2009). Also, Adult blowflies (*Lucilia sericata*) exposed to Cd via diet had reduced fecundity (Moe et al., 2001). Though we did not account for hatching success, recruitment is an outcome of hatching, and a few studies have shown Cd to reduce hatching success in other soil invertebrates. For example, Cd reduced hatching in the enchytraeid, *E. crypticus*, after exposure to Cd in LUFA 2.2 soil with an estimated EC50 of 3.1 mg Cd kg^{-1} on hatching success of the enchytraeid (Goncalves et al. 2015). Gomes et al. (2018) linked the reduction in hatching success of *E. crypticus* to the disruption of calcium homeostasis during embryogenesis.

3.6.4 Uptake of Cd from soil by *O. nitens*

The result shows absolute Cd concentration in adults before maternal transfer gave a better model for uptake than measured internal Cd concentration (Table 3). Hence, we suggest the incorporation of maternal transfer to correct for metal bioaccumulation to help predict uptake or bioavailability of metals in adult *O. nitens*. Ideally, pore water Cd concentration should be a source of Cd for juveniles because of the soft cuticle of juvenile mites, which might facilitate dermal adsorption. However, pore water Cd was insignificant for Cd uptake by juvenile mites (Table 3-3). Total soil Cd predicted uptake for the earthworm (*Eisenia fetida*) in natural soil (Janseen et al., 1997) and the authors suggest the rejection of pore water-mediated uptake hypothesis (Van Gestel and Ma, 1988) for Cd uptake. Vijver et al. (2003) also found total Cd pool to predict the uptake of Cd by larvae of the beetle (*Tenebrio molitor*) from OECD and field soils. Like the larvae of *T. molitor, O. nitens*

feed on organic matter in soil (Ramos-Elorduy et al., 2002; Fajana et al., 2019). Juvenile *O. nitens* also feed on organic matter at certain stages of their life cycle to store energy as reserve for moulting and other developmental processes (Fajana et al., 2019). Across 18 natural soils, total zinc strongly predicted Zn uptake by adult *O. nitens* (Jegede et al. 2019b). Therefore, the contribution of dermal adsorption to the uptake or accumulation of Cd from soil is insignificant for juvenile and adult *O. nitens*, and ingestion of Cd via soil water is likely minimal. It is difficult to measure the biosorption of free metal ion from pore water to soil biota; however, a biotic ligand model (BLM) for terrestrial system (Pelette et al., 1999) can be used to predict the binding of Cd^{2+} from pore water to the mites.

3.7 The implication of this study in risk assessment of cadmium

In a typical ecological scenario, cadmium and most other heavy metals predominantly occur as oxides in contaminated sites (Chlopecka et al., 1996; Hamilton et al., 2016). Cadmium oxide (CdO) causes less toxicity than cadmium chloride to *Oppia nitens* and possibly to other soil invertebrates. Current CCME environmental soil quality guidelines (SQG_E) and USEPA Eco-SSL for Cd and most metals are derived from literature that was based on metal salt toxicity to soil invertebrates, microbes, and plants (CCME, 1999; EC, 1999; USEPA, 2005). Therefore, the threshold for the SQG_E of Cd to soil invertebrates might not be ecologically relevant and may be overprotective. Using metal oxide in soil exposure tests likely provides a more accurate estimate of metal toxicity to soil organisms including microbes (Awuah et al., 2019).

Additionally, if cadmium oxides are less bioavailable than cadmium salts, for both soil invertebrates and plants, then SQG_E for Cd may need to be revised to account for the discrepancy. A limitation of the feasibility of this revision is the lack of data on CdO toxicity to soil invertebrates. More research is needed to generate robust toxicity data on metal oxide toxicity to soil invertebrates, microbes, and plants.

To calculate the threshold effects concentration for the soil contact pathway, Environment Canada (1999) applies an uncertainty factor of two to account for the potential for Cd to bioaccumulate. This uncertainty factor was derived from the geometric mean of bioconcentration factors (BCFs) from leaves, shoots, and roots of plants; however, the BCFs for soil invertebrates are 8.5 (EC,

1999). The potential for Cd to be maternally transferred across generations means that a population of F_1 offspring receives 39 to 52 % of their parents' Cd body burden in addition to Cd uptake from soil. Therefore, the SQG_E for Cd that will protect the populations of *O. nitens* needs to be adjusted for maternal transfer. Our suggestion is to incorporate maternal transfer into ESSD25 or ESSD50 values, and one would take the average maternal transfer rate and multiply that by the EC25 or EC50 from a single generation study. For example, an EC50 of 392 mg Cd kg^{-1} soil for *O. nitens* would be multiplied by the average maternal transfer ratio (0.46, this study), to derive a multigenerational EC50 of 180 mg Cd kg^{-1} soil which would then be used in the derivation of ESSD50 (Figure 3-3). The bioconcentration correction step would no longer be necessary, and we would suggest that when possible, maternal transfer be used rather than bioconcentration factors. Alternatively, one can generate toxicity data for metals such as ECx to soil invertebrate over multiple generations (Jegede et al., 2019a).

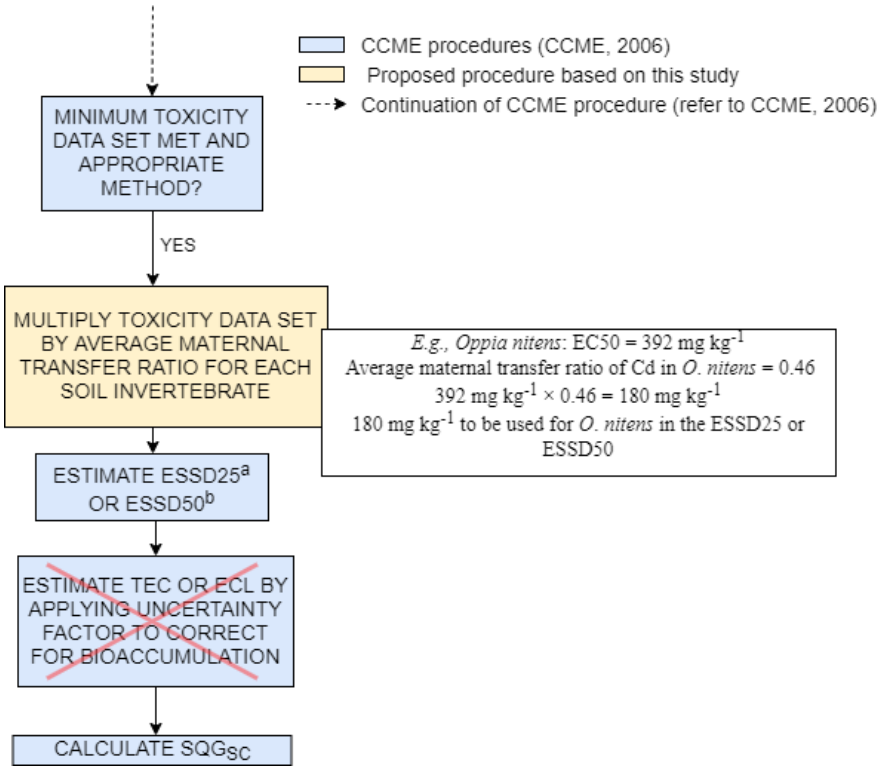

Figure 3-3. Incorporating maternal transfer ratio (τ) as a safety factor into CCME procedures (CCME, 2006) to account for the multigenerational impact of heavy metal to soil invertebrates in deriving environmental soil quality guidelines for soil contact in either agricultural, residential/parkland, commercial and industrial uses. [a]Agricultural and Residential/Parkland Land Use. [b]Commercial and Industrial Land Use. SQG_{SC} represents soil quality guidelines for soil contact.

4. Does habitat quality influence bioenergetics and reproduction of *Oppia nitens* in response to cadmium-induced toxicity?[3]

4.1 Preface

The influence of soil habitat quality on the reproduction and bioenergetics of *Oppia nitens* was investigated after exposure to cadmium in OECD soil. Mite reproduction (i.e., juvenile production) was determined. The amount of energy (protein, lipid, and carbohydrate) reserves and the activities of glucose metabolism enzymes (glucose-6-phosphate dehydrogenase [G6PDH] and pyruvate kinase [PK]) were estimated in the mites.

[3] Fajana HO, Hogan NS, Siciliano SD. Does habitat quality influence bioenergetics and reproduction of *Oppia nitens* in response to cadmium-induced toxicity? (To be submitted to *Soil Biology and Biochemistry*).
Hamzat Fajana: Conceptualization, methodology, validation, formal analysis, investigation, data curation, writing (original draft), visualization
Natacha Hogan: Writing (review and editing)
Steven Siciliano: Conceptualization, resources, writing (review and editing), supervision, project administration, funding acquisition

4.2 Abstract

Soil invertebrates interact with their habitat to provide services that are vital for the soil ecosystem. In this study, we evaluated the influence of habitat quality on the reproduction and bioenergetics of the oribatid mite, *Oppia nitens* in response to cadmium-induced toxicity. Adult mites that were exposed to cadmium (0–700 mg kg^{-1}) had high carbohydrate reserve at intermediate Cd concentrations but without a change in lipid and protein reserve. Cadmium at high concentration (700 mg Cd kg^{-1}) inhibits the activities of glucose metabolism enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and pyruvate kinase (PK) with estimated EC50 of 50.7 and 21.7 mg Cd kg^{-1} for PK and G6PDH respectively. Habitat quality influenced mites' reproduction (i.e., juvenile production), but did not directly influence bioenergetics. Our results suggest that cadmium reduces energy production in *O. nitens*, and habitat quality affect the reproduction of *O. nitens*. We conclude that the effect of habitat quality could be more significant than metal concentration on the reproduction (i.e., juvenile production) of *O. nitens*. Hence, habitat characterization of contaminated sites could improve the relevance of ecological risk assessment, since the quality of the habitat can affect the reproduction of soil invertebrates.

4.3 Introduction

Habitat affects the biological and physiological fitness of animals through variation in resources and environmental conditions (Bernstein et al., 1991; Pullian, 2000). In the soil, intrinsic factors define the quality of the habitat, and these include particle size distribution, clay content, organic matter and mineral contents, liquid limit and presence of water stable aggregates. These factors influence other soil properties such as porosity, bulk density, water holding capacity (WHC), pH, and cation exchange capacity (CEC) (Larney et al., 1988; Miralles et al., 2009). Soil habitats differ in their particle size distribution, amount and flow of water and gases, amount of organic matter and chemical properties that interact with soil organisms (Wall and Moore, 1999). Soil particle sizes and soil bulk density determine the habitable pore spaces or interstices of the soil habitat (Larsen *et al*., 2004). The abundance and activities of soil invertebrates that are euedaphic (i.e., true soil dwellers) are attributed to the channels or the networks of their habitable pore spaces or interstices (Larsen *et al*., 2004). Mites are euedaphic soil invertebrates that live in moist interstices and therefore need water and other essential resources to thrive. The acquisition of resources such as

water, oxygen, and food is only possible if soil invertebrates can move across connected interstices in the soil.

According to Koenning *et al*., (1996) and Xavier *et al*. (2014), soils of clay content > 20% cause a reduction in the egg-laying capacity of the soil nematode, *Rotylenchulus reniformis*. The high clay content reduces the motility of the soil nematode population and provides low aeration (oxygen) to the organism due to the compaction of the soil (Xavier *et al*., 2014). In a study by Princz *et al*. (2010), natural soils of > 6 % organic matter content increased the reproduction of *Oppia nitens*. However, the cumulative variation in the other soil factors (particle size distribution, NO_3^-, P, C: N, pH and sodium adsorption ratio) contribute 68% of the reduction that was observed in the reproduction of *O*. *nitens* in the natural soils. The intrinsic soil properties (i.e., organic carbon, pH, and CEC) contributed more to zinc toxicity on the reproduction of *O*. *nitens* (Jegede et al., 2019b).

The variation in intrinsic factors can either modulate the bioavailability of toxicants to soil invertebrates or provide soil invertebrates with resources (i.e., micronutrients and energy) to combat chemical stress. For instance, soil organic matter (OM) and pH are critical soil factors that influence the bioavailability of inorganic and organic chemicals to soil invertebrates (van Gestel, 1992; Fountain and Hopkins, 2005). Soils of low pH (i.e., acidic soils) favour the availability of metals to soil invertebrates. Natural soils of low pH induce higher lead toxicity on the earthworm, *E*. *andrei*, with internal lead concentrations that correspond to high Pb in pore water (Bradham *et al*. 2006). Also, soil moisture potentiates zinc toxicity to the collembola, *Folsomia candida* and mite, *O*. *nitens* (Owojori and Siciliano, 2015). CEC and organic carbon (OC) were also suggested as important factors that could determine habitat quality of soils because CEC provides micronutrients, while OM provides food as an energy source (Jegede et al. 2019).

In this study, we viewed habitat quality (HQ) in terms of providing resources for soil invertebrates to combat chemical stress in soil. Habitat quality often correlates with the availability of food resources (Hope, 2001). In the case of soil, food resources are usually measured as OM, which varies in quality and quantity. The oribatid soil mite *O*. *nitens* is a euedaphic soil invertebrate that interacts with the soil as an ecosystem engineer (Fajana et al., 2019). The quality of habitat influences mite's activities in soil. Therefore, it is important to understand habitat pressure, either natural or anthropogenic, that might affect the fitness of the mites in soil.

In a recent study by Jegede et al. (2019b), *O. nitens* in a high quality soil showed resilience to Zn-induced toxicity by maintaining the activities of glucose metabolism enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and lactate dehydrogenase (LDH) even at high zinc concentration (14000 mg Zn kg^{-1}) compared to the mites with disrupted enzyme activities in low HQ soil. The mites in high HQ soil might have passively acquired more energy reserves from the surrounding habitat to modulate Zn toxicity without a change in bioenergetics. Here, we hypothesize that mites, *O. nitens* that are reared in high HQ soil, will show more resilience to Cd stress because the mites might have passively acquired nutrients or energy, which could be used in adaptive responses against Cd toxicity. To investigate the "carry over effect" of habitat quality (O'Connor et al., 2014) or separate habitat quality influences on toxicokinetics from toxicodynamics, we exposed mites that are reared in low and high HQ soils to Cd on a neutral soil (OECD artificial soil).

4.4 Materials and Methods

4.4.1 Test chemical and soils

Cadmium oxide (CdO) ≥ 99.99 % trace metal basis (Sigma-Aldrich, Canada) was used as the test chemical. The test soil used was an artificial OECD (Organization for Economic Co-operation and Development) soil that is made up of 10 % sphagnum peat, 20 % kaolinite clay, 70 % sand (a mixture of 45 % fine sand and 25 % coarse sand), and 0.5 % powdered $CaCO_3$ to stabilize pH. The pH (in $CaCl_2$) of the OECD soil was stabilized from 5.84 to 6.50 before use. The OECD soil has an optimal water holding capacity (% WHC) of 68.5 %.

Six habitat quality soils (S1 to S6) were selected from a group of natural soils that were collected from western Canada as described in Jegede et al. (2019b). Briefly, the soils were grouped into high and low HQ based on the performance of three standard test organisms that are used in soil ecotoxicity testing. The three organisms were Northern Wheatgrass *(Elymus lanceolatus*), collembola (*Folsomia candida*), and enchytraeids (*Enchytraeus crypticus*). As a measure of the HQ level of the soils, the reproduction (number of juveniles) of collembola and enchytraeids in the soils was assessed, while for the Northern Wheat grass, plant biomass was assessed. Habitat quality was calculated by combining plant biomass, enchytraeid, and springtail reproduction tests into a single index, as described in Jegede et al. (2019b). Soils that support the performance of these organisms

above average were categorized as high HQ soils while those below average were the low HQ soils (Table 4-1).

4.4.2 Toxicity testing on *O. nitens*

Adult mites were exposed to Cd (CdO) in OECD soil according to the procedure that was described in Jegede et al., 2019a,b. Specific quantities of air-dried OECD soil were spiked with different concentrations of CdO to give 22, 44, 88, 175, 350, and 700 mg of Cd kg^{-1} of dry soil. The dosed soil was moistened to 60 % of its WHC with ultra-pure water. About 25 g of the Cd-dosed soil was added to a glass vial, and fifteen (15) age synchronized adult *O. nitens* (45 days old from the larvae stage) were introduced into each treatment in replicates of four. The vial containing the dosed soil and adult mites were kept in a temperature-controlled chamber at 20–21 ℃ and % relative humidity of 60 %. The mites were fed grains of baker's yeast and water *ad libitum* at an interval of 7 days for 28 days test duration. We ended the test after 28 days, and the mites were extracted from the Cd-dosed soil using a modified Berlese-Tullgren heat extractor set at 32℃. The extracted adult and juvenile mites were counted on a dissecting microscope to estimate juvenile production. The adult mites that survived were stored in Eppendorf tubes at -80 ℃ for biochemical analysis.

Table 4-1. Normalized biological index and physical properties of the habitat quality soils

Habitat quality level	Soils	Normalized biological index				Physical properties			
		Plant biomass	Enchytraeid reproduction	Collembola reproduction	Cumulative score	% sand	% silt	% clay	WHC
Low	S1	8	94	45	147	89.2	2.1	8.6	29.05
	S2	8	65	103	176	90.2	7.5	2.3	20.07
	S3	8	16	88	112	71.8	13.1	15.1	19.83
High	S4	167	219	58	444	25.9	55.3	18.8	38.26
	S5	91	67	45	203	47.9	30.1	22.0	26.78
	S6	221	195	35	451	42.8	32.0	25.2	33.12

WHC = Water holding capacity

$Normalized\ index = \frac{Plant\ biomass\ or\ animal\ reproduction\ in\ soil}{Average\ number\ of\ plant\ or\ animal} \times 100\ \%$ (See Jegede et al., 2019b)

4.4.3 Experimental design for habitat quality

Newly emerged adult mites (38 days from the larvae stage, i.e., 1-day post eclosion) were reared for 28 days in the six (6) uncontaminated HQ soils (S1-S6) in a temperature-regulated chamber at a temperature of 22±2℃. The mites were fed grains of baker's yeast and water *ad libitum* during rearing. After 28 days of rearing the mites in the high and low HQ soils, the mites were extracted from the soils by hand sorting to avoid thermal stress. The extracted mites (65 days old, i.e., 28-d post eclosion) were then exposed to EC25 (215 mg Cd kg^{-1}) and EC50 (392 mg Cd kg^{-1}) of cadmium in an OECD artificial soil (Figure 4-1). Before mites were introduced into the artificial soil for toxicity tests, their energy reserve (protein, lipid, and carbohydrate) was estimated.

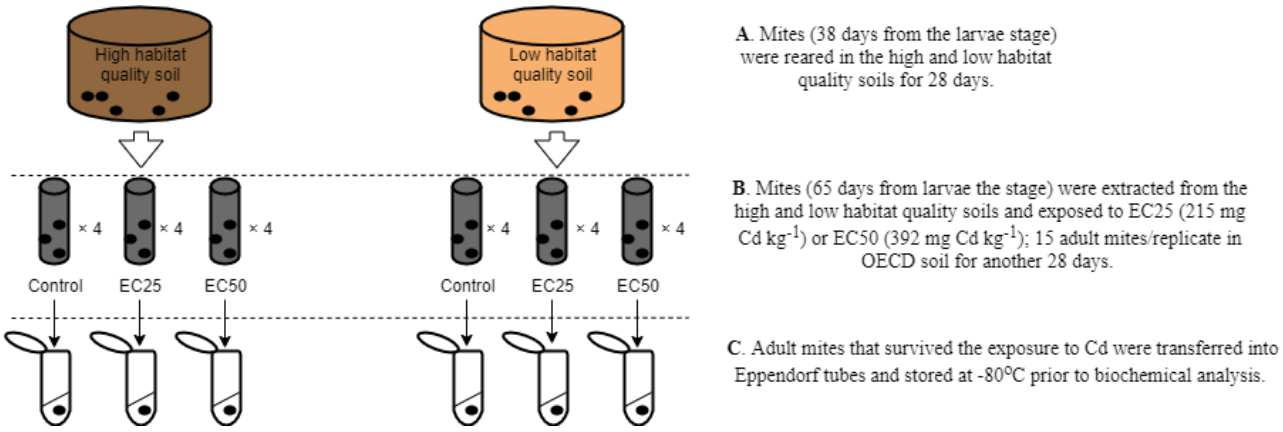

Figure 4-1. A set-up showing the steps involved in the design of the habitat quality experiment. **Note:** Step B was in a neutral soil, OECD artificial soil to ensure mites are exposed to same condition.

4.4.4 Biochemical analysis

4.4.4.1 Estimation of energy reserves

A sequential quantification of protein, total carbohydrate, and lipid from mite's tissue was done following the method described in Forey et al. (2012). Individual adult mites from each concentration of Cd was pooled together per replicate (n = 4). Due to the small body size of the mites, two replicates were further merged from the n = 4 to get enough mites tissue for homogenization; thus making a population size of n = 2 replicates for the enzyme assay. Adult mites were placed into a 2-mL Eppendorf tube, and 150 µL of aqueous lysis buffer solution [100mM KH_2PO_4, 1 mM dithiothreitol (DTT) and 1 mM ethylenediaminetetraacetic acid (EDTA),

pH 7.4] was added and homogenized with a Teflon pestle. The resulting homogenate was centrifuged for 15 mins at 1000 × g at 4℃ to get a supernatant. Protein was quantified by Bradford assay (Bradford, 1976) from a portion of the supernatant Bovine Serum Albumin (BSA) was used as the standard, and the protein quantification based on absorbance of 595 nm. We added 20 µl of 20 % sodium sulphate solution (Na_2SO_4) to the remaining portion of the supernatant to dissolve the total carbohydrate in the supernatant. The total lipid in the supernatant was also solubilized by adding a chloroform-methanol solution (1: 2 v/v). The total carbohydrate was estimated using the colorimetric method based on anthrone reagent (van Handel, 1965). The procedure was adapted for microplate assay in a 96-well borosilicate microplate because the organic solvents and high incubation temperature for the bioassay are not compatible with the standard polystyrene microplate. The total carbohydrate was determined by measuring the absorbance of the sample and standard (glucose) at 625 nm.

The solubilized total lipid content in the supernatant was determined following the vanillin assay procedure (van Handel, 1985) using triolein (Cat No. 92860, Sigma) as the standard. The supernatant was added into a 96-well borosilicate microplate and heated at 90℃ until the complete solvent evaporates. Then, 10 µL of 98 % sulphuric acid was added to each well and incubated at 90℃ for 2 min in a water bath. We cooled the microplate on ice and add 190 µL of vanillin reagent to each well. The microplate was incubated at room temperature for 15 min, and absorbance was measured at 525 nm to determine the total lipid content. The protein, carbohydrate, and lipid concentration (µg) were transformed into energetic equivalents using the energy of combustion (Gnaiger, 1983): 17,500 mJ mg^{-1} carbohydrate, 24,000 mJ mg^{-1} protein, and 39,500 mJ mg^{-1} lipid.

4.4.4.2 Enzyme assay

Individual adult mites from each concentration of Cd was pooled together per replicate (n = 4). Due to the small body size of the mites, two replicates were further merged from the n = 4 to get enough mites tissue for homogenization; thus making a population size of n = 2 replicates for the enzyme assay. The adult mites were homogenized with a Teflon pestle in 50 µL of aqueous lysis buffer solution [100mM KH_2PO_4, 1 mM dithiothreitol (DTT) and 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.4]. The resulting homogenate was centrifuged for 15 mins at 1000 × g at 4℃ to get a supernatant. The activities of glucose metabolism enzymes, glucose-6-phosphate dehydrogenase

(G6PDH) and pyruvate kinase (PK) were measured as described by De Coen et al. (2001) but adapted for a 96-well microplate using commercial kits (G6PDH assay kit, Cat. No. MAK015; PK assay kit, Cat. No. MAK072) from Sigma-Aldrich, Canada. The activity of the enzymes was measured at an absorbance of 450 nm in a spectrophotometer and the relative activity (mU/mL) was corrected by the protein concentration for each sample to obtain the specific activity (mU/mg of protein) of the enzymes.

4.4.5 Estimation of growth parameters

The length (L) and width (l) of the mites was measured using a compound microscope with an eyepiece graticle. Randomly selected mites at day 0 (i.e., before exposure to Cd) and after 28 days of exposure to Cd in OECD artificial soil were mounted on a lens coated with wax to immobilize the mites for few seconds. The recorded length from the eyepiece graticle was converted to actual length of the mites based on the calibration from a stage micrometer (0.48 µm/division). The wet weight (W_w) of individual mites was estimated from the length and width using the linear equation from Lebrun (1971) :

$$logW_w = 1.53 \times logL + 1.53 \times logl - 6.67$$

The wet weight (W_w) of the mites was transformed to physical volume (V_w) using the formula;

$$V_W = \frac{W_w}{d_{Vw}}$$

Where d_{Vw} is a fixed specific density ≈ 1 g cm^{-3} (Kooijman, 2000).

4.4.6 Metal analysis in mite's tissue

Mite samples from the habitat quality experiment were digested in HNO_3/H_2O_2, and the resulting digest in 2 % HNO_3 was analyzed for cadmium in an ICP-MS (Inductively Coupled Plasma Mass Spectrometer) as described by Jegede et al. (2019b). The Cd uptake rate or bioavailability (k) in the mites from low and high HQ soils after exposure to nominal EC25 (215 mg Cd kg^{-1}) or EC50 (392 mg Cd kg^{-1}) of Cd in OECD soil was estimated following Keshavarz Jamshidian et al. (2017). To

estimate k, the measured internal Cd concentration ($C_{int.}$) in mites at each effective concentrations, was divided by the total Cd EC25 (270 mg Cd kg^{-1}) or EC50 (495 mg Cd kg^{-1}):

$$k = \frac{C_{int.}}{Total\ ECx} = \frac{\mu g\ Cd\ g^{-1} body\ weight\ of\ mites}{\mu g\ Cd\ g^{-1} soil}$$

Where k is measured in g soil g^{-1} body weight of mites.

4.4.7 Statistics

A one-way analysis of variance (ANOVA) was used to determine significant difference in energy reserves of adult mites between control and treated groups. The EC50 from enzyme activities was predicted using 3-parameter logistic regression via dynamic curve fit in SigmaPlot 12.0. Pearson moment correlation coefficient was used to determine the relationship between mite reproduction or change in physical volume, and energy reserves parameters (protein, carbohydrate, and lipid reserves) and enzyme activities. Two-way ANOVA was used to determine the influence of habitat quality and Cd concentration on mite reproduction, carbohydrate reserve, or enzyme activities. All graphs were also plotted in SigmaPlot 12.0.

4.5 Results

4.5.1 Bioenergetics responses of mites to cadmium

There was no significant change in protein and lipid reserves (Figure 4-2a and b). Carbohydrate reserve significantly increased at 88 and 175 mg Cd kg^{-1}. However, carbohydrate reserve in mites at the highest concentrations of Cd (i.e., 350 and 700 mg Cd kg^{-1}) reduced to a level that was not significantly different from control mites (Figure 4-2c). The change in physical volume of mites was not significantly different ($p > 0.05$) between Cd concentrations in soil (Figure 1C of Appendix C).

There was no significant correlation between mite's carbohydrate reserve and reproduction as well as the percentage change in physical volume (V_W) which is a measure of growth in the mites (Table 4-2).

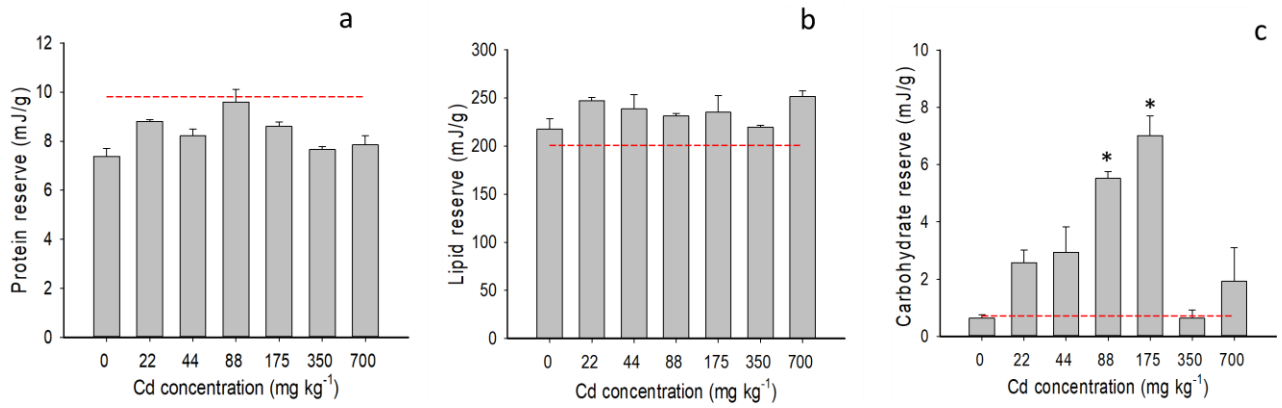

Figure 4-2. (a) Protein (b) lipid (c) carbohydrate reserves in adult *Oppia nitens* that are exposed to cadmium in OECD artificial soil for 28 days. Asterisk indicate statistical difference at $p < 0.05$ using a one-way ANOVA. The bar shows the mean ± S.E ($n = 2$) of the energy reserves while the red line marks the level of the energy reserves at day 0 (protein reserve: 9.81 ± 0.43 mJ/g; lipid reserve: 200.84 ± 0.15 mJ/g; carbohydrate: 0.72 ± 0.04 mJ/g).

Cadmium also inhibits the activities of glucose metabolism enzymes, pyruvate kinase (PK) and glucose-6-phosphate dehydrogenase (G6PDH), and the inhibition was dose-dependent with an estimated EC50 of 50.7 and 21.8 mg Cd kg^{-1} respectively for PK and G6PDH (Figure 4-3a and b). We found a significant relationship between mite reproduction and the activities of PK (p = 0.002), and G6PDH in the mites (p = 0.03) (Table 4-2).

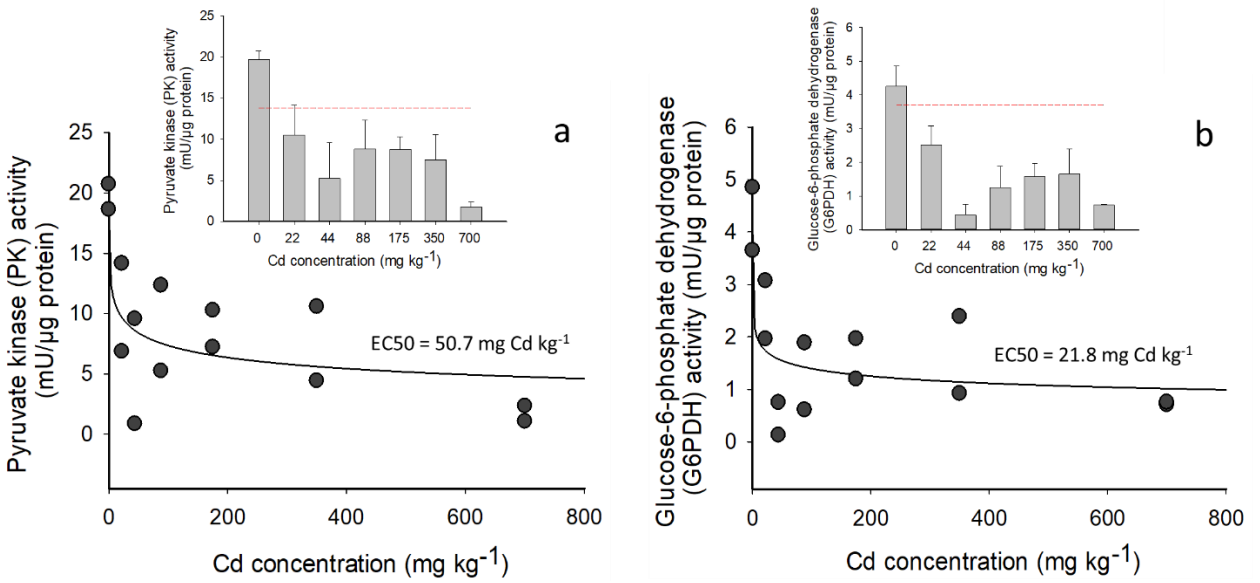

Figure 4-3. Dose-response relationship from four parameter logistic regression for the activities of pyruvate kinase (PK) and glucose metabolism enzymes, glucose-6-phosphate dehydrogenase (G6PDH) in *Oppia nitens*, and Cd concentration in OECD soil. Upper and lower 95 % confident intervals could not be estimated (refer to table 2C and 3C of Appendix C for fitting parameters of the logistic regression). The bar graphs show the mean ± S.E (n = 2) of the enzyme activities while the red line marks the level of activities on day 0 (PK: 13.79 ± 3.88 mU/µg protein; G6PDH: 3.69 ± 0.57 mU/µg protein).

Table 4-2 Pearson moment correlation showing the relationship between mite reproduction or physical volme and bioenergetics parameters in adult mites after 28 days of mite's exposure to cadmium (0–700 mg Cd kg-1) in OECD artificial soil.

		Protein reserve (mJ/g)	Lipid reserve (mJ/g)	Carbohydrate reserve (mJ/g)	Total Energy reserve (mJ/g)	PK (mU/µg protein)	G6PDH (mU/ µg protein)
Mite reproduction (No. of juvenile)	*r*	0.0030	-0.68	-0.14	-0.65	0.93	0.80
	p	0.995	0.0913	0.765	0.114	0.00234**	0.0304*
% Δ in $\boldsymbol{V_W}$	*r*	0.52	-0.33	0.24	-0.24	0.70	0.52
	p	0.228	0.464	0.6	0.611	0.0823	0.228

V_W = Physical volume; *r* = correlation coefficient
* $p < 0.05$; ** $p < 0.01$ significance.

4.5.2 Influence of habitat quality on mite's reproduction and bioenergetics

Bioavailability of Cd in mites from low and high HQ soil was not significantly different (Table 4-3). Juvenile production in mites from low and high HQ soil was not significantly different between control, EC25, and EC50. However, HQ significantly ($p = 0.024$) caused the difference in mite reproduction (Figure 4-4b) without a significant effect of Cd concentration ($p = 0.45$) or the interaction of HQ × Cd concentration ($p = 0.65$) on the reproduction of mites (Table 4C of Appendix C). The average number of juveniles in mites from high HQ soil was also above the cut-off (i.e., > 70 juveniles) at all concentrations of Cd while the average number of juveniles for mites from low HQ soils was only above cut-off at EC50 (Figure 4-4a). There was no significant effect of HQ or Cd concentration on carbohydrate reserves in mites (Table 4C of Appendix C).

Table 4-3. Chemical properties of the different habitat quality soils and cadmium bioavailability in *Oppia nitens* from high and low habitat quality soils

		Chemical properties			***k*** (g soil g^{-1} body weight)	
Habitat quality level	Soils	OC	pH	CEC	EC25	EC50
Low	S1	1.2	5.6	13.6	0.12	0.072
	S2	0.4	4.6	9.9	0.064	0.15
	S3	1.0	6.8	18.2	0.050	0.10
					0.078 ± 0.021[a]	*0.11 ± 0.023*[b]
High	S4	6.8	7.5	29.7	0.034	0.0061
	S5	3.0	6.9	21.6	0.027	0.039
	S6	2.7	7.1	27.4	0.053	0.14
					0.038 ± 0.0078[a]	*0.062 ± 0.040*[b]

OC = Organic Carbon (%); CEC = Cation Exchange Capacity (mmol/100 g soil). S1–S6 represents the habitat quality soils. Data in italics are the average uptake rate or bioavailability of Cd in mites at EC25 and EC50. Data with the same alphabet are not significantly different (t-test: $p = 0.126^{a}$ and $p = 0.355^{b}$).
Note: CEC was determined using the colorimetric method based on methylene blue adsorption (Soon, 1988).

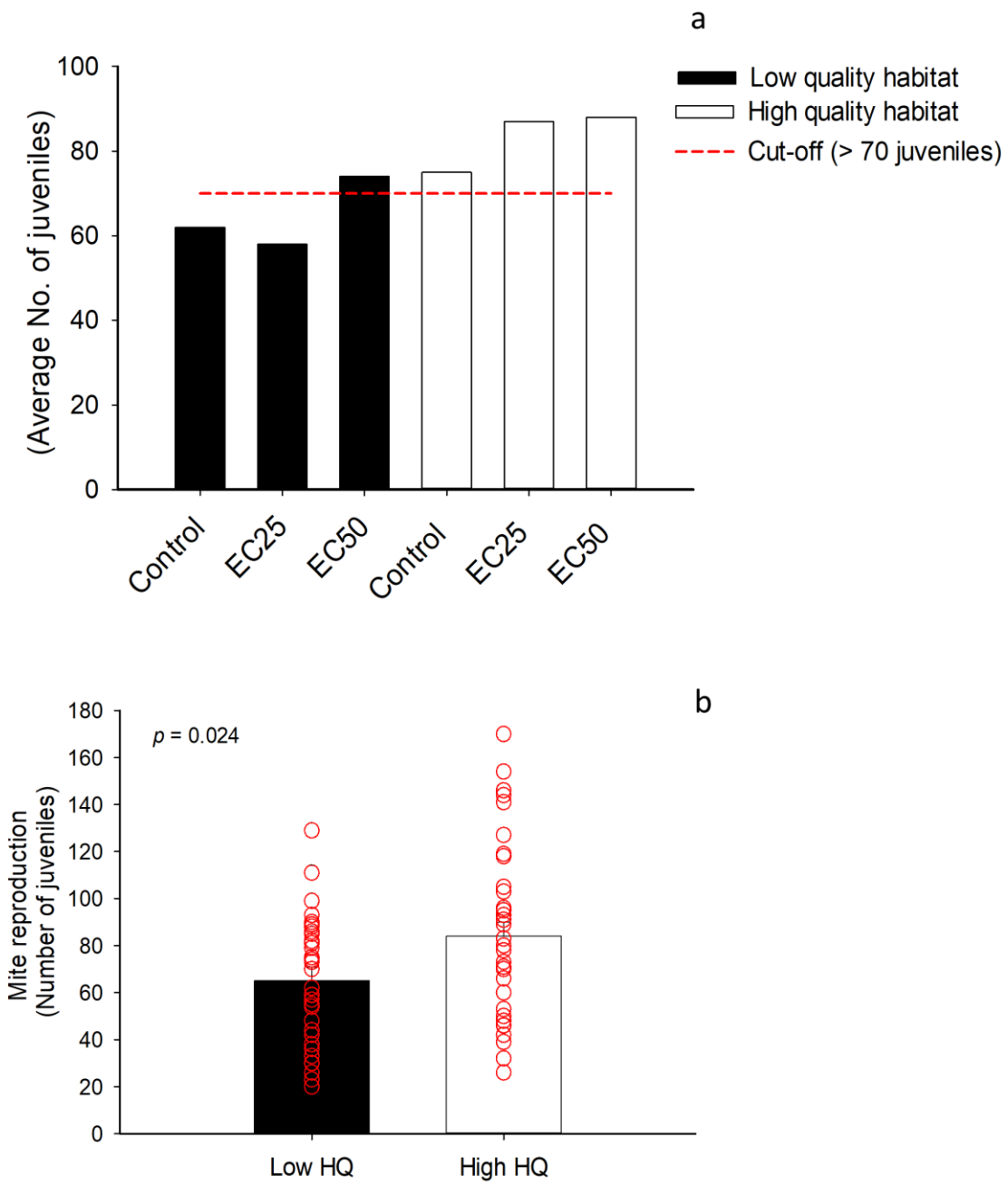

Figure 4-4. (a) Average number of juveniles at control, EC25, and EC50 for mites from both low and high habitat quality soil. (b) The influence of habitat quality on mite reproduction. The open circles represent data points for the number of juveniles. The red line represents the cut-off, > 70 juveniles (i.e., average number of juveniles for high and low HQ mites in the control). $p < 0.05$ indicates significant difference in mite reproduction between high and low habitat quality soil.

Cadmium concentration significantly influenced the activities of the glucose metabolism enzymes, PK (p = 0.044) and G6PDH (p = 0.035) in mites irrespective of habitat quality. The highest activity of both enzymes was observed at EC50 (392 mg Cd kg^{-1}) compared to EC25 (215 mg Cd kg^{-1}) and control (Figure 4-6a and b). There was no significant effect of HQ or the interaction of HQ × Cd concentration on the enzymatic activities (Table 4C of Appendix C).

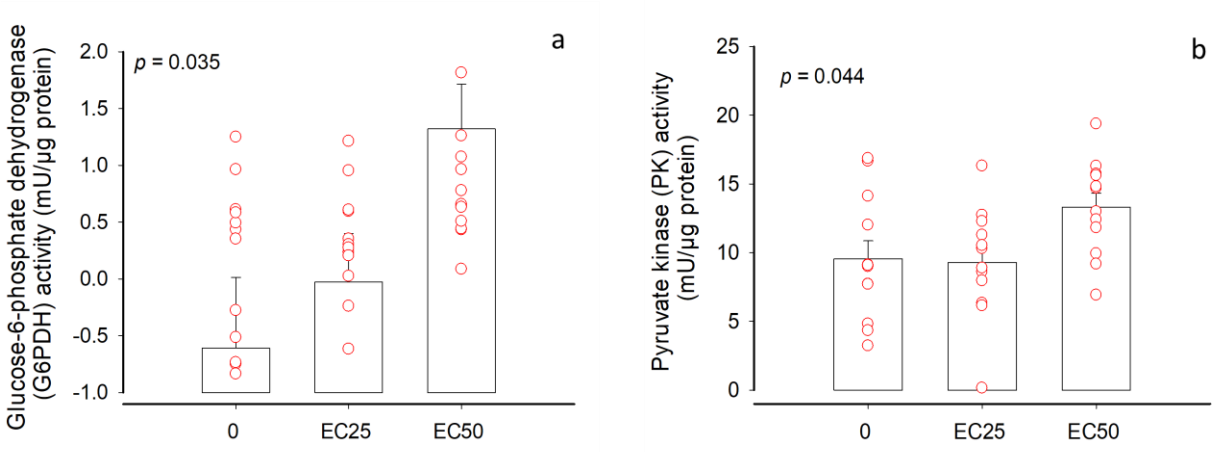

Figure 4-5. Influence of cadmium concentration (EC25 = 215 mg Cd kg^{-1} or EC50 = 392 mg Cd kg^{-1}) on the (a) activities of glucose metabolism enzymes, glucose-6-phosphate dehydrogenase (G6PDH) and (b) pyruvate kinase (PK) of *Oppia nitens* from low and high habitat quality soils after exposure to cadmium in OECD soil for 28 days. $p < 0.05$ indicates a significant difference in enzyme activities between Cd concentrations. The open circles represent data points for the activities of enzymes.

4.6 Discussion

4.6.1 Effect of cadmium on bioenergetics of *Oppia nitens*

In our study, cadmium altered the carbohydrate reserve of *O. nitens* without any discerning effect on protein and lipid reserves. However, Cd increased carbohydrate reserve of *O. nitens* at intermediate Cd concentrations, but decreased the reserve at a high concentration (350 and 700 mg Cd kg^{-1}). The high carbohydrate reserve at the intermediate Cd concentrations indicates that Cd might have triggered the storage of soluble carbohydrate for refuelling during Cd toxicity. The decrease in the level of carbohydrate reserve at high Cd concentration is probably due to the rapid depletion of carbohydrate to provide energy for metal detoxification. Similarly, cadmium (6 and 150 mg Cd kg^{-1}) increased the carbohydrate reserve in *Enchytraeus albidus* (Novais et al., 2013). Zinc oxide nanoparticles (1000 mg kg^{-1}) lowered the carbohydrate reserve of the earthworm *Eisenia andrei* without any effect on lipid and protein reserve (Swiatek and Bednarska, 2019). Carbohydrate reserve is the first energy source that is rapidly mobilized to combat toxicity in animals (Moolman et al. 2007) which is likely the reason why our study and the study of Swiatek and Bednarska (2019) only observed a change in carbohydrate reserve during metal stress in soil invertebrates. In addition, the exposure time, 28 days for our study, might not be enough to trigger the use of protein or lipid reserves by the mites.

It is also interesting to know that the carbohydrate reserve did not correlate with mite's reproduction or change in physical volume (V_W, a measure of growth). The mites might have chanelled their carbohydrate reserve to support other physiological processes such as cellular maintenance and Cd detoxification, besides growth and reproduction. However, a fundamental principle is that reproduction often pays the cost for survival, and when there are limited resources, organisms are faced with a decision on whether to allocate energy for maintenance or growth (English and Bonsall, 2019).

Our estimates of EC50 for Cd from the activities of the enzymes, glucose-6-phosphate dehydrogenase (G6PDH) [EC50 = 21.7 mg Cd kg^{-1}] and pyruvate kinase (PK) [EC50 = 50.7 mg Cd kg^{-1}] shows that the activities of the enzymes were inhibited at concentrations less than the EC50 on reproduction (i.e., 392 mg Cd kg^{-1}) (Fajana et al., 2020). The inhibition in the activities of G6PDH suggests less available reducing energy to cells via the pentose phosphate pathway (PPP). Similarly,

the inhibition of PK activity indicates less production of pyruvate, a substrate for the tricarboxylic acid cycle (TCA) in the mitochondria to produce ATP. Hence, we could say that Cd reduces energy production in the mites by inhibiting the activities of important energy metabolism enzymes. For example, very low concentration of cadmium (5 mg Cd kg^{-1}) down-regulated genes involved in glucose metabolism, TCA cycle, and oxidative phosphorylation in the enchytraeid, *E. crypticus* (Gomes et al., 2018). Lead (Pb) inhibits PK activity in the brain cortex of rats (Lepper et al., 2010). In contrast to our study, De Coen et al. (2001) reported that mercury (Hg) elevates the activities of G6PDH and PK in *Daphnia magna*. Increased activity of G6PDH and PK in *D. magna* could reflect the production of more energy as an adaptive mechanism to Hg toxicity.

In this study, the activities of PK and G6PDH significantly correlate with mite reproduction, as reproduction increases with an increase in the enzymatic activities of the mites that were exposed to Cd (Table 4-3). However, others found that increased activity of PK negatively correlated with population performance, such as reproduction, growth, and survival of *D. magna* that were exposed to Cd for 48 and 96 hours (De Coen and Janssen, 2003). The authors attributed the negative relationship to the demand for a high-energy producing pathway, which PK cannot offer since it is involved in anaerobic glycolysis where only two ATP molecules are produced. Also, De Coen et al. (2001) did not found any relationship between the intrinsic growth rates of *D. magna* population and PK and G6PDH activities, respectively, after 48 and 96 h.

4.6.2 Influence of habitat quality on mite reproduction and bioenergetics

There was a hormetic-like response in mite reproduction from low and high HQ soils after exposure to cadmium's EC25 and EC50 (Figure 4-4a). We suggest that future studies, following similar experimental design should include a much higher toxicity value such as EC90 to test for habitat quality. However, this hormetic-like effect can be attributed to the age of the mites. ECx values for *O. nitens* are usually based on mites that are 45–47 days old (i.e., 8–10 days post eclosion) (Fajana et al., 2019). However, the experimental design for this study does not allow the mites to be exposed at such age, but instead, the mites were exposed at 65 days old. Age can influence the sensitivity of mites to metal because older adult mites are more sclerotized and could efficiently compartmentalize metals in their cuticle; therefore increases their resilience to metals than young adults. Mortality increased, and reproduction decreased with age respectively in *D. magna* that was

exposed to selenium (Se), arsenic (As), lead (Pb), or copper (Cu) (Hoang and Klaine, 2007). However, mortality decreased and cumulative reproduction increased when *D. magna* became older adults.

Irrespective of the hormetic-like response in mite reproduction, mites from low HQ soils had reduced juvenile production compared to mites from high HQ soil; thus habitat quality alone has a much stronger influence than Cd toxicity on the reproduction of *O. nitens*. In another study, *O. nitens* that were exposed to zinc in low HQ soils had reduced juvenile production compared to mites in high HQ soils (Jegede et al., 2019b). The intrinsic soil properties that constitute habitat quality, i.e., pH and the quality and quantity of OM, can influence life history, such as juvenile production and recruitment of *O. nitens*. For example, *O. nitens* that were reared in soil of > 6 % OM had high juvenile production compared to mites in low OM soil (Princz et al., 2010).

Interestingly, the estimated bioavailability of Cd at EC25 and EC50 was not significantly different between mites from high or low HQ soils (Table 4-3). Thus, habitat quality does not alter mite's Cd uptake from the soil. There is a possibility of mites in a high HQ soil to accumulate more Cd via ingestion because of the high % OM that might have bound Cd. For example, Jegede et al. (2019b) showed that there was no difference in Zn bioavailability between mites in high and low HQ soils; yet, the mites in the high HQ soils accumulated more Zn than mites in the low HQ soils.

The mite's G6PDH and PK activities was not influenced by habitat quality; instead, as a function of Cd concentration in soil. The induction in G6PDH and PK activity with increasing Cd concentration suggest that the mites increase their energy production to combat Cd toxicity, which favours reproduction even at high Cd concentration, i.e., EC50 = 392 mg kg^{-1} (Figure 4-4a). These pattern of bioenergetics response for G6PDH and PK activities could be because of the mite's age at exposure (65 days old from the larvae stage). Cd causes a dose-dependent inhibition of G6PDH and PK activities in young adult mites (45 days old from the larvae stage) (Figure 4-3a and b). The difference in the pattern of bioenergetic response to Cd toxicity between young and older mites could be caused by differences in food consumption rate and growth conversion efficiency, as observed for the benthic forage fish, *Fundulus heteroclitus* in degraded and polluted habitat (Goto and Wallace, 2010).

This study has shown that the effect of habitat quality could be more significant than metal concentration on the reproduction of *O. nitens*. Although, the effect of metal concentration on mite's bioenergetics could indirectly influence reproduction. We, therefore, conclude that soil habitat characterization should be done for contaminated sites to improve the relevance of ecological risk assessment since the quality of the habitat can affect the reproduction of soil invertebrates. Alternatively, the quality of contaminated habitats could be improved via ecological restorative processes that will target critical intrinsic soil factors such as CEC, OC, and pH (Jegede et al., 2019b).

5. Synthesis, conclusions and future directions

Soil contamination with heavy metals is on the rise because of increased industrial activities, especially for countries like Canada, where a significant portion of the economy revolves around metal mining. Therefore, data are needed to set soil quality guidelines for metals in contaminated sites. So far, the available data for soil contact is based on earthworms, springtails and plants, which might not be representative of some ecoregions. *Oppia nitens* (a true soil-dwelling microarthropod) is abundant in the boreal ecozones, and the boreal forest soil covers about 51 % of Canada's landmass. Hence, it is essential to include *O. nitens* as test organisms to generate toxicity data for Canadian soil. There is, however, a data gap on how *O. nitens* respond to metals in soil and habitat changes.

The primary goal of this research was to provide information on *O. niten*s response to cadmium and how habitat quality influences their fitness in cadmium-contaminated soil. The findings from this study were based on;

1. The dearth of information on the responses of *O. nitens* to chemicals in soil, and how they interact with the soil ecosystem as addressed in chapter 2.
2. *O. nitens* usually accumulate metals from the soil by ingesting contaminated soil rather than dermal adsorption of dissolved metal, as addressed in chapter 3.
3. There is a possibility of maternal transfer of metals, which could lead to transgenerational toxicity in *O. nitens,* as addressed in chapter 3.
4. The habitat where *O. nitens* live can modulate their reproduction and bioenergetic responses to metal-induced toxicity, as addressed in chapter 4.

The principal goals of this research were to;

1. Provide information and data that could be used to improve risk assessment of cadmium but can also be adapted to other metals in contaminated sites.
2. Assess the role of habitat quality on the fitness of soil organisms to improve the ecological relevance of risk assessment of metals in contaminated sites.

The fundamental research questions addressing these objectives were;

1. How do we understand *O. niten*s response to metals in contaminated sites, including possible molecular responses of the mites to metal stress?

2. What role could maternal transfer of metals play in transgenerational toxicity, and how can data from maternal transfer be used to improve ecological soil quality guidelines of metals for soil invertebrates.
3. Does the quality of habitat for soil invertebrates affect how they respond to metals in soil, and how can habitat quality be incorporated into the ecological risk assessment of metal-contaminated sites?

5.1 Biology and Ecology of *Oppia nitens* (Chapter 2)

5.1.1 Synthesis and conclusions

In chapter 2, we provided detailed information on the biology and ecology of *O. nitens* in soil with emphasis on their functions and contribution to ecosystem services. Despite the applicability of the mites in soil toxicity testing, this chapter stresses the need for knowledge on the genome of *O. nitens*. Sequencing of *Oppia nitens*'s genome will provide information on their mechanism of response to stress in the soil, which could be used to develop an adverse outcome pathway (AOP) for existing and emerging chemicals that pose a threat to soil invertebrates.

5.1.2 Future directions

This study opened many opportunities for future research because it is the first-ever review on the bionomics of *Oppia nitens*. This chapter will provide researchers in the area of soil toxicology with first-hand information on the biology of *O. nitens* concerning their responses to chemicals in soil. The proposed adverse outcome pathway (AOP) in this chapter should be further investigated for *O. nitens*, considering the impact of the adverse outcome on ecosystem services. Besides, this chapter has proposed a potential mechanism for metal tolerance in the mite. The proposed mechanism could be investigated in the future, thus opening a new approach to how soil microarthropods tolerate heavy metals in contaminated soils.

5.2 Toxicity and maternal transfer of cadmium in *O. nitens* (Chapter 3)

5.2.1 Synthesis and conclusions

This chapter provides data on cadmium toxicity to soil invertebrates with a novel finding on the maternal transfer of cadmium in *O. nitens*. Contrary to the dogma of pore water-mediated toxicity in soil invertebrates, this chapter concluded the accumulation of metal by *O. nitens* in the soil is not via

dermal adsorption of metal from pore water but ingestion of contaminated soil. The profound finding from chapter 3 is that adult mites maternally transfer a significant amount of cadmium to juveniles. The information on the maternal transfer of Cd in *O. nitens* is crucial because it supports existing data on the multigenerational effect of other metals such as zinc in *O. nitens* (Jegede et al., 2019a).

5.2.2 Future directions

This study, for the first time, has shown the possibility of maternal transfer of heavy metal in soil invertebrates. Thus, it will foster more research to investigate the maternal transfer of Cd and other heavy metals in other soil invertebrates such as earthworms, collembola and enchytraeids. Since this chapter proposed how maternal transfer data could be included as a criterion for setting soil quality guidelines, we recommend that regulatory bodies such as CCME and U.S EPA should look in the direction of incorporating maternal transfer data for other soil invertebrates into setting environmental soil quality guidelines for heavy metals in soil.

5.3 Soil habitat quality's influence on cadmium-induced toxicity (Chapter 4)

5.3.1 Synthesis and conclusions

Chapter 4 reported a novel finding on how habitat quality could influence reproduction and energy metabolism in *O. nitens*, and we found habitat quality influences the reproduction of *O. nitens* in response to cadmium-induced toxicity. There are speculations that habitat quality could improve the adaptive responses of *O.* nitens to metals, e.g., zinc (Jegede et al., 2019b). This chapter, therefore, supports such assumptions and concludes that habitat quality directly influences mite's reproduction irrespective of the concentration of Cd in soil. The approach used in chapter 4 was novel because it captured how the mites could passively acquire resources such as energy and micronutrients from their habitat, which could be used to combat metal toxicity.

5.3.2 Future directions

Chapter 4 has shown that habitat quality does matter for the soil mite, *O. nitens*, in the context of acquiring resources from a high habitat quality soils. Therefore, this finding should prompt a similar investigation for other soil invertebrates. Also, the intrinsic soil factors that influence the quality of the habitat should be investigated. For instance, the contribution of quality and quantity of organic matter (OM) to habitat quality should be critically assessed. Furthermore, our findings from this

chapter suggest habitat characterization of contaminated sites is needed, which will help to improve the ecological relevance of environmental risk assessment.

5.4 Overall synthesis of findings from this study

First, knowledge of the ecology and biology of *O. nitens* is essential to soil ecotoxicologist because of the ecological relevance of the mites, and its use as a standard test organism in toxicity testing. The mite's cadmium uptake from the soil, which we concluded to be via the ingestion of contaminated soil or Cd bound to the soil, is a piece of valuable information that is vital to terrestrial ecotoxicology. We often believed that soil invertebrates significantly take up metals via dermal adsorption of metal ions from soil solution or pore water. Hence, we can begin to view metal uptake from a different perspective with particular interest to total metal pool in soil rather than free metal ion activity. Secondly, our data on the maternal transfer of cadmium from adults to juveniles could change the procedures for setting soil quality guidelines by incorporating maternal transfer ratio as a safety factor. Maternal transfer could also improve our understanding of transgenerational toxicity of metals in the mites. Third, we also found habitat quality to be a significant factor that affects the reproduction of the mites. The findings on the influence of habitat quality to the mite's response could change the landscape of soil ecotoxicology from the perspective of ecological stress and metal tolerance in soil invertebrates. The overall contribution of this thesis to soil ecotoxicology is highlighted in figure 5-1.

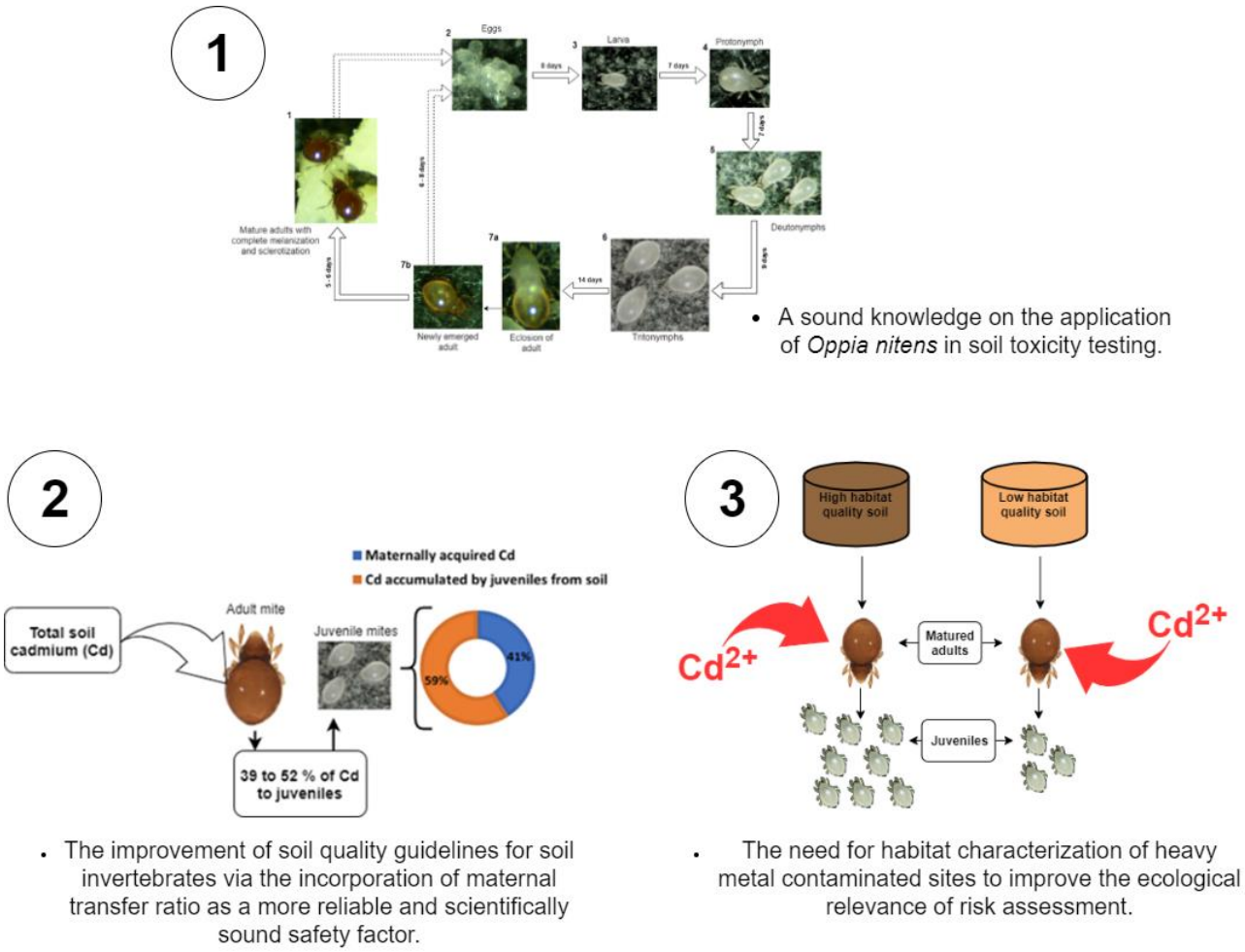

Figure 5-1. A graphical summary of the contribution of this thesis to the advancement of soil ecotoxicological research using *Oppia nitens* as test organism.

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7. Appendix A: Supplementary information for chapter 2

Table 1A. Location and habitat preferences of *Oppia nitens*, by country

Country	Location	Habitat preference	Reference
Canada	Ontario Nova Scotia	Forest soil, ~7 % organic matter	Godfrey et al. 2013; Telfer et al. 2015
USA	Illinois, New York, Virginia, Michigan	Northern hardwood forest, which consists of Sandy soil overlaid with >80 % sugar maple (*Acer Saccharum*) litter; pH (4.41-4.70)	Ewing, 1909; Sengbusch, 1951, 1957; Gan et al. 2014
Czech Republic	Moravia		Stary, 2000
Georgia	Caucasus (Kolkheti) Caucasus (Colchic lowland)	Humus-rich soil of flooded alder forest; Wetland composed of deposit from dead Juncus plant material	Murvanidze et al. 2011; Murvanidze and Kvavadze, 2010
Italy	South Tyrol Bergamo	Hypolite pond (wetland) of a boggy forest with reed swamp; dry bushland and rocky steppe; Woodland (agricultural area)	Fischer and Schatz, 2010; Schatz, 2018; Migliorini et al. 2003
Germany	Berlin-Buch -	Decomposed litter of *Agropyron repens;* Mesophilic deciduous broadleaf forest litter;	Pieper, 2004 Weigmann and Kratz, 1982
Poland	Mazovia	Compost heap	Gryziak, 2009
Turkey	Erzurum Konya	Soil and litter under Oak tree -	Baran and Ayyildiz, 2004 Dik et al. 1995
United Kingdom	England (Yorkshire)	Mull-like soil that contains non-calcareous drift;	Wood, 1967
Sweden	-	Moss and dead wood.	Michael, 1898
The Netherlands	Leeuwarden -	Fossilized soil deposit; Litters of cultivated wood	Schelvis, 2015 Hammen, 1952
Switzerland	Liestal		Schweizer, 1957

Spain	Province of Cuenca	Decomposed wood of poplar slump	Subías and Arillo, 2000
Iceland	-	-	Hammer, 1946
France	Massane, Trevaresse, Provence	-	Trave, 1956; Lions, 1966a, 1966b
Ukraine	Crimea	Forest soil	Kurcheva, 1973
Finland	-	-	Niemi et al. 1997
Greece, Austria, Belgium, Portugal			de Jong et al. 2014
Iran	Alborz province (Taleghan)	Soil and litter under cherry trees, *Prunus avium* L.	Jamshidian et al. 2015
Antarctica	Sub-Antarctic archipelagos (Crozet Islands)		Starý and Block, 1998

Table 2A. Comparison of median lethal (LC50-survival) and inhibitory (EC50-reproduction and avoidance) concentrations for the effect of metals, pesticides, organic compounds, and reference substance between *Oppia nitens* and other standardized soil invertebrates (the collembola, *Folsomia candida*; earthworm, *Eisenia fetida*; pot worms, *Enchytraeus albidus/crypticus*, and predatory mite, *Hypoaspis aculeifer*) in soil ecotoxicity testing

Class of chemicals	Chemicals	Species									
		O. nitens		*F. candida*		*E. fetida*		*E. albidus/crypticus*		*H. aculeifer*	
		LC50	EC50	LC50	EC50	LC50	EC50	LC50	EC50	LC50	EC50
Metals	Copper	3311[1]	2896[1]	1810[10]	700[10]	764[11]	316[11]	799[23]	305[15]	4482[30]	2459[30]
		-				836[12]	715[12]			>100[31]	647[31]
		-	4265*[2]								944*[30]
	Zinc	2291[1]	1562[1]	5150[14]	900[10]	1340[14]	705[14]	566[14]	267[14]		
		-			750[10]	745[13]	462[13]		188[22]		
	Lead	6761[1]	1678[1]		2970[10]	>10000[12]	1629[12]	11400[23]	320[15]		
	Cadmium	603[1]	137[1]	854[16]	>326[16]	>300[12]	295[12]	476[18]	158[18]		
						1260[18]	108[18]				
	Cobalt	-	1213[24A]		>6840[33A]		300[19]	-	200[28]		
			14921[24B]		>17515[33B]				>17515[33B]		
	Nickel	-	133[24A]		5238[33A]		362[15]		275[29]		
			3606[24B]		>4766[33B]				4433[33B]		
	Lanthanum	>5820[9]	1500[9]	1690[9]	1220[9]	1850[9]	529[9]	1650[9]	1010[9]		

Organics	Lubricating oil	11293[5]	1210[5]	6172[5]	3160[5]	2860[5]		>186000[5]	31736[5]		
	Perfluorooctane sulfonate (PFOS)	65[4]	23[4]	130[4]	94[4]	540[27]	-	-	-		
		>180[4]	95[4]	>350[4]	233[4]						
	Benzo[a]pyrene	>1600[2]	>1600[2]	>4345[25]	>4345[25]			2000[25]	559[25]	>947[30]	>947[30]
	Phenanthrene	388[2]	95[2] 83*[2]	366[25]	257[25]	41[26]	-	>3690[25]	>3690[25]	684[30]	49[30] 26*[30]
Pesticides	Triclopyr	2000[7]	1500[7]	1000–3000[7]	500[7]	-	-	75.5–2800[7]	75.5–2600[7]		
	Imazapyr	-	-	3500–4000[7]	500[7]	-	-	200 - 1800[7]	12–3000[7]		
	Imidacloprid	360[8]	119[8]	0.47[8]	0.26[8]	0.77[8]	0.39[8]	>30[8]	2[8]		
	Thiacloprid	>1000[8]	76[8]	3.9[8]	1.7[8]	7.1[8]	0.44[8]	>30[8]	12[8]		
Reference chemical	Boric acid	1468[2]	314[2]	125.7[18]	54.5[18]		484[21]	357[17]	104[17]	668[30]	332[30]
		530[3]	96[3] 2454*[2]		183*[17]			302[17]	105[17] >2000*[20]		296[32] 1234*[30]

*avoidance

[1]Owojori and Siciliano (2012); [2]Owojori et al. (2011); [3]Princz et al. (2010); [4]Princz et al. (2018); [5]Gainer et al. (2018); [6]Gainer et al. (2019a); [7]Jimmo et al. (2018); [8]de Lima e Silva et al. (2017); [9]Li, Verweij, and van Gestel (2018); [10]Sandifer and Hopkin (1996); [11]Owojori et al. (2009); [12]Spurgeon and Hopkin (1995); [13]Spurgeon and Hopkin (1994); [14]Lock and Janssen (2001b); [15]Lock and Janssen (2002a); [16]Crommentuijn et al. (1995); [17]Amorim et al. (2012); [18]Lock and Janssen (2001a) [19]Hartenstein et al. (1981); [20]Amorim et al. (2008); [21]Becker et al. (2011); [22]Posthuma et al. (1997); [23]Lock and Janssen (2001c); [24]Jegede et al., in preparation; [25]Bleeker et al. (2003); [26]Wu et al. (2011); [27]Yuan et al. (2017); [28]Ribeiro et al. (2018); [29]Lock and Janssen (2002b); [30]Owojori et al. (2014); [31]Krogh and Axelsen (1998); [32]Smit et al. (2012); [33]M. Renaud, unpublished data.

A = Natural soil (pH = 3.4, % OC = 1.7, % Clay = 4.5, CEC = 8 mmol/100g); B = Natural soil (pH = 5.6, % OC = 1.2, % Clay = 2.4, CEC = 28 mmol/100g).

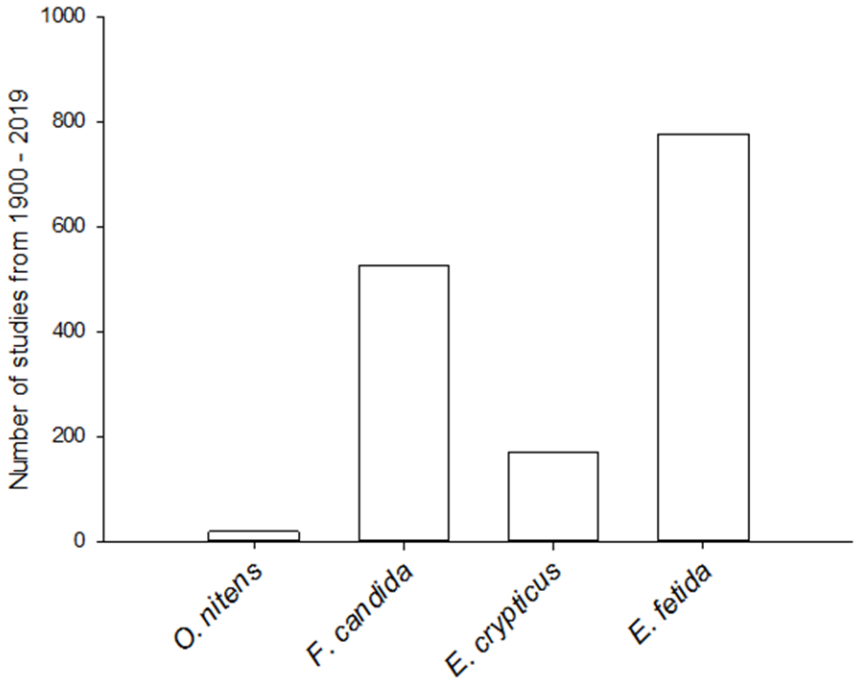

Figure 1A. Number of studies from 1900-2019, estimated using "test organisms + toxicity" as key words on Web of Science.

8. Appendix B: Supplementary information for chapter 3

Table 1B. Average values of measured and estimated cadmium data in soil and mites

Nominal Cd (mg kg^{-1})	Pore water Cd (mg l^{-1})	0.01 M CaCl2-extractable Cd (mg kg^{-1})	Total soil Cd (mg kg^{-1})	Measured Cd in adult mites (µg g^{-1})	Measured Cd in juvenile mites (µg g^{-1})	Estimated Cd in Juvenile via uptake from soil (µg g^{-1})	Cd bound to OM (mg kg^{-1})	Free Cd (µM)	Fulvic acid bound Cd (µM)	Water soluble Cd (mg l^{-1})
700	5.05	3.75	880.45	14.17	11.64	6.87	253.05	7.18	4.16	1.90
350	0.4	1.20	459.90	5.66	11.53	6.80	60.14	0.40	0.35	0.21
175	0.3	0.58	222.97	4.53	4.75	2.80	48.35	0.14	0.14	0.087
88	0.2	0.25	94.55	1.74	3.47	2.05	13.58	0.09	0.093	0.060
44	0	0.13	48.73	3.70	3.53	2.08	18.59	0.036	0.038	0.027
22	0	0.04	25.20	0.49	2.63	1.55	3.04	0.012	0.015	0.013
0	0	0	0.68	2.48	1.96	1.16	0.50	0	0	0

Table 2B. Mean ± standard error (n = 4) of reproduction and survival data for mites, *Oppia nitens* after 28 days exposure to cadmium in OECD artificial soil

Nominal cadmium doses (mg kg^{-1})	Mite reproduction (number of juveniles)	Survival (% adult survival)
0	130.00 ± 18.46^{a}	81.67 ± 1.67
22	94.33 ± 16.29^{a}	75.55 ± 4.44
44	75.33 ± 9.74^{ab}	80.00 ± 3.85
88	93.25 ± 4.82^{a}	85.00 ± 1.67
175	72.75 ± 6.50^{b}	63.33 ± 4.30
350	79.33 ± 15.30^{ab}	73.33 ± 10.18
700	27.50 ± 5.56^{b}	66.66 ± 6.67

Mite reproduction data with the same superscripts are not significantly different at 5 % probability from a one-way Analysis of variance using Bonferroni t-test for multiple comparison.

Table 3B. Linear regression model for deriving ECx (EC25 and EC50) of nominal, total soil, and 0.01 M $CaCl_2$-extractable cadmium to *Oppia nitens* in OECD artificial soil after 28 days toxicity test

Model	$r^2_{Adj.}$	*p*
$\boldsymbol{ECx_{Nominal\ Cd} = -52.83 + 53.36 \times ECx_{Internal\ Cd\ in\ adult\ mites}}$	0.90	< 0.001
$\boldsymbol{ECx_{Total\ Cd} = -69.84 + 67.78 \times ECx_{Internal\ Cd\ in\ adult\ mites}}$	0.89	< 0.001
$\boldsymbol{ECx_{0.01\ M\ CaCl2\ ext.Cd} = -0.51 + 0.29 \times ECx_{Internal\ Cd\ in\ adult\ mites}}$	0.94	< 0.001

EC25 and EC50 for internal Cd in adult mites are 5.02 (2.03 – 8.0) and 8.34 (4.26 – 12.43) µg g^{-1} respectively. The EC25 and EC50 for internal Cd in adult mites were predicted using 3-parameter Weibull regression with the drc package in R[4].

Note: r^2_{Adj} = Adjusted coefficient of determination; *p* = p-values at 5 % probability

[4] Ritz, C., Baty, F., Streibig, J. C., & Gerhard, D. (2015). Dose-response analysis using R. *PloS one*, *10*(12), e0146021.

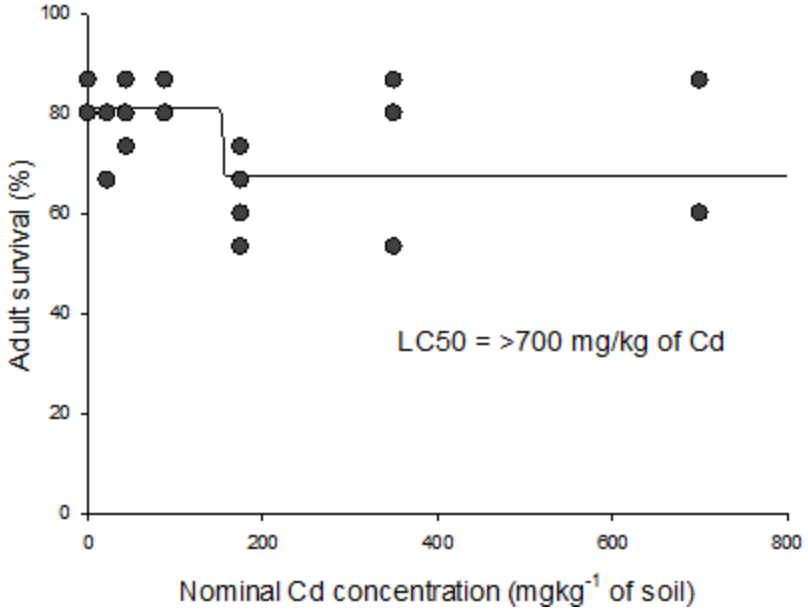

Figure 1B. Dose-response curve of cadmium (Cd) on the survival (% adult survival) of *Oppia nitens* after 28 days of exposure to Cd in OECD artificial soil.

9. Appendix C: Supplementary information for chapter 4

Table 1C. Raw reproduction (i.e., juvenile production) and enzyme activity (glucose-6-phosphate dehydrogenase [G6PDH] and pyruvate kinase [PK]) data

Habitat quality level	Cd dose	Reproduction
Low	Control	30
Low	Control	57
Low	Control	90
Low	Control	59
Low	Control	82
Low	Control	48
Low	Control	73
Low	Control	70
Low	Control	59
Low	Control	88
Low	Control	54
Low	Control	36
Low	EC25	44
Low	EC25	79
Low	EC25	75
Low	EC25	59
Low	EC25	23
Low	EC25	33
Low	EC25	38
Low	EC25	74
Low	EC25	62
Low	EC25	89
Low	EC25	42
Low	EC25	81
Low	EC50	20
Low	EC50	30
Low	EC50	26
Low	EC50	99
Low	EC50	85
Low	EC50	55
Low	EC50	86
Low	EC50	111
Low	EC50	129
Low	EC50	89
Low	EC50	93
High	Control	46
High	Control	50
High	Control	66
High	Control	127

Habitat quality	Cd dose	G6PDH	PK
Low	Control	-0.27792	4.813254
Low	Control	-0.51548	3.225021
Low	Control	0.962073	14.1094
Low	Control	-0.75009	8.993635
Low	Control	0.433323	12.00949
Low	Control	0.492404	9.112645
Low	EC25	-0.24027	0.158453
Low	EC25	0.350586	6.325229
Low	EC25	0.59478	16.31197
Low	EC25	0.23366	8.607311
Low	EC25	1.210946	11.28475
Low	EC25	0.301694	10.30742
Low	EC50	0.642678	14.69004
Low	EC50	0.433388	6.91673
Low	EC50	0.77723	16.2986
Low	EC50	0.962073	12.98835
Low	EC50	1.813698	19.36303
Low	EC50	0.440977	14.80569
High	Control	0.6104	7.711459
High	Control	0.350711	7.696593
High	Control	0.582175	16.66007
High	Control	1.248871	16.85655
High	Control	-0.83594	4.330519
High	Control	-0.73607	9.08478
High	EC25	0.952215	8.881978
High	EC25	0.274104	7.957502
High	EC25	0.606615	12.73279
High	EC25	0.024459	12.27783
High	EC25	0.204641	10.52479
High	EC25	-0.61693	6.14491
High	EC50	0.656575	9.932681
High	EC50	0.630037	9.173145
High	EC50	0.507537	15.72053
High	EC50	0.086024	15.61616
High	EC50	1.259199	12.40271
High	EC50	1.073468	11.81562

High	Control	80
High	Control	53
High	Control	91
High	Control	71
High	Control	78
High	Control	95
High	EC25	26
High	EC25	42
High	EC25	39
High	EC25	46
High	EC25	93
High	EC25	32
High	EC25	141
High	EC25	118
High	EC25	89
High	EC25	105
High	EC25	154
High	EC25	170
High	EC50	119
High	EC50	48
High	EC50	103
High	EC50	73
High	EC50	146
High	EC50	144
High	EC50	83
High	EC50	32
High	EC50	60
High	EC50	70
High	EC50	96

Table 2C. Fitting parameters for the dose-response of pyruvate kinase (PK) activity as a function of cadmium concentration in soil

Pyruvate Kinase (PK)
Equation: Standard Curves, Four Parameter Logistic Curve
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.7970	0.6352	0.5257	4.2213

	Coefficient	Std. Error	t	P
min	-3.3313	104.2858	-0.0319	0.9751
max	19.6985	2.9849	6.5994	<0.0001***
EC50	50.6585	1832.0049	0.0277	0.9785
Hillslope	-0.2530	1.4761	-0.1714	0.8673

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	310.2447	103.4149	5.8036	0.0146
Residual	10	178.1910	17.8191		
Total	13	488.4357	37.5720		

Statistical Tests:

Normality Test (Shapiro-Wilk): Passed (P = 0.4141)

W Statistic= 0.9397 Significance Level = 0.0500

Constant Variance Test: Passed (P = 0.7499)

Table 3C. Fitting parameters for the dose-response of glucose-6-phosphate dehydrogenase (G6PDH) activity as a function of cadmium concentration in soil

Glucose-6-phosphate dehydrogenase (G6PDH)
Equation: Standard Curves, Four Parameter Logistic Curve
f1 = min + (max-min)/(1 + (x/EC50)^(-Hillslope))
f = if(x<=0, if(Hillslope>0,min,max), f1)

R	Rsqr	Adj Rsqr	Standard Error of Estimate
0.9411	0.8857	0.7714	0.6134

	Coefficient	Std. Error	t	P
min	1.1332	0.3066	3.6960	0.0344**
max	4.2521	0.6134	6.9316	0.0062***
EC50	21.8391	3724753.0631	5.8632E-006	1.0000
Hillslope	-30.6306	711526065.6070	-4.3049E-008	1.0000

Analysis of Variance:

	DF	SS	MS
Regression	4	30.8414	7.7103
Residual	3	1.1289	0.3763
Total	7	31.9703	4.5672

Corrected for the mean of the observations:

	DF	SS	MS	F	P
Regression	3	8.7474	2.9158	7.7487	0.0633
Residual	3	1.1289	0.3763		
Total	6	9.8762	1.6460		

Statistical Tests:

Normality Test (Shapiro-Wilk): Passed (P = 0.6605)

W Statistic= 0.9424 Significance Level = 0.0500

Constant Variance Test: Passed (P = 0.3884)

Table 4C. Two-way Analysis of variance (ANOVA) for the effect of habitat quality and cadmium concentration on the reproduction (juvenile production) of *Oppia nitens* after exposing mites from low and high habitat quality (HQ) to cadmium (EC25 = 215 mg Cd kg^{-1} or EC50 = 392 mg Cd kg^{-1}) for 28 days in an OECD artificial soil

	Source of variation	df	SS	MS	*F*	*p*
Mite reproduction (Number of juveniles)	HQ	1	6093.7	6093. 1	5.4	0.024*
	Cd	2	1858.9	929. 8	0.82	0.45
	HQ x Cd	2	997.0	498.5	0.44	0.65
	Residual	62	70263.3	1133.3		
	Total	67	79600.9	1188.1		
Carbohydrate reserve (mJ/g)	HQ	1	0.76	0.76	1.38	0.25
	Cd	2	3.28	1.64	3.0	0.067
	HQ x Cd	2	0.37	0.19	0.34	0.72
	Residual	30	16.61	0.55		
	Total	35	21.01	0.60		
G6PDH activity (mU/µg protein)	HQ	1	0.027	0.03	0.078	0.78
	Cd	2	2.61	1.31	3.8	0.035*
	HQ x Cd	2	0.18	0.091	0.26	0.77
	Residual	30	10.40	0.35		
	Total	35	13.22	0.38		
PK activity (mU/µg protein)	HQ	1	0.75	0.75	0.043	0.84
	Cd	2	121.37	60.69	3.5	0.044*
	HQ x Cd	2	19.27	9.64	0.55	0.58
	Residual	30	522.47	17.42		
	Total	35	663.86	18.96		

HQ = Habitat quality; Cd = Cd concentration
*significant difference at $p < 0.05$

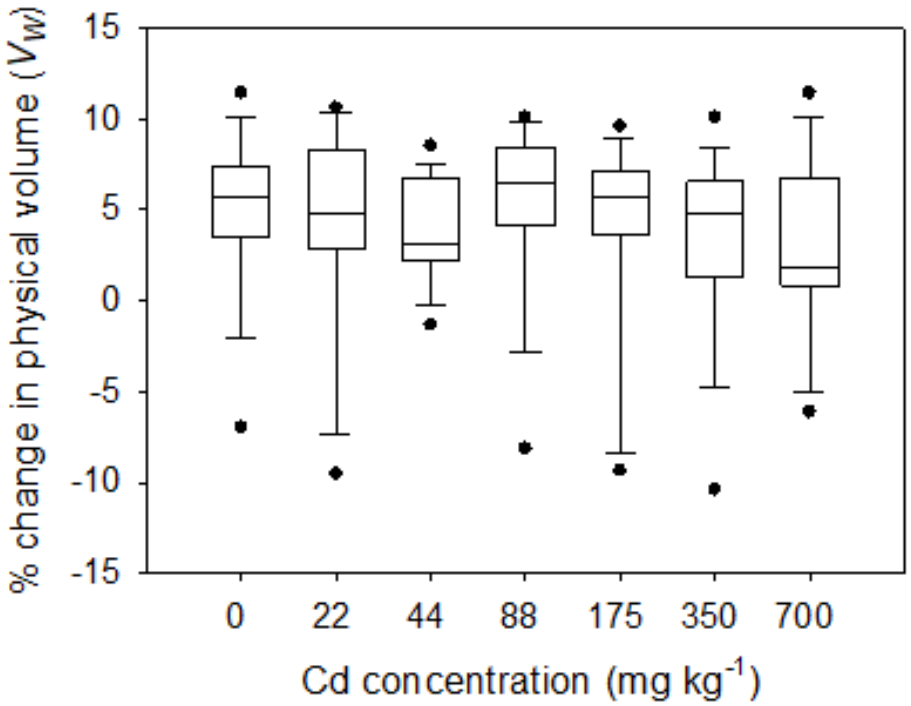

Figure 1C. Percentage change in physical volume (V_W) of *Oppia nitens* after exposing the mites to cadmium for 28 days in OECD artificial soil. **Note**: Percentage change in V_W was calculated relative to V_W at day 0 (i.e., before mites were exposure to cadmium).