



Metal transfer in a tri-trophic terrestrial system of naturally metal enriched soils

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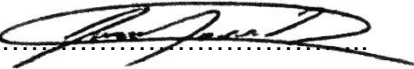
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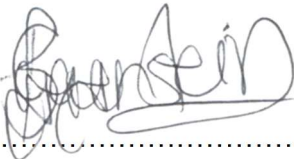
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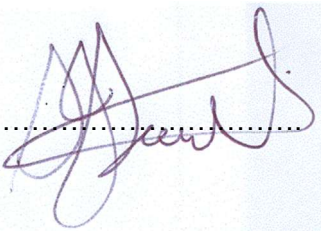
Declaration

I declare that the work presented in this Masters dissertation is my own work, that it has not been submitted for any degree or examination at any other university, and that all the sources I have used or quoted have been acknowledged by complete reference.

Signature of the Student.....

Signature of the Supervisor.....

Signature of the Co-supervisor.....

Signature of the Co-supervisor

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“And God saw everything that He had made, and behold, it was very good.” – Gen. 1:31

Abstract

Potentially toxic metal(loid)s (PTMs) are elements that are toxic to plants and animals at elevated concentrations. In addition to anthropogenically altered environments, PTMs occur naturally in soil derived from ultramafic rocks, often referred to as ultramafic soil. Although some ultramafic ecosystems are well studied, those within South Africa still demand attention, including the ultramafic komatiite outcrops of the Vredefort Dome. There are few reports of ultramafic soil from the dome, and even less on the trophic transfer of PTMs. Therefore, the aim of this study was to assess the transfer of PTMs to three trophic levels within a naturally PTM rich terrestrial ecosystem model, using wild and crop plants as producers. First, a review was conducted on the topic, identifying knowledge gaps and necessary focal points for research. The search engine used was ScienceDirect®. Any article investigating PTM accumulation or trophic transfer within terrestrial ecosystems with a minimum of one living organism was included. Results indicated that most literature (62%) originated from developed regions (USA and Europe), while research from Africa and South America only contributed 2.6% and 6.8%, respectively. Furthermore, only 3.2% of the studies focused on PTMs from natural sources. Therefore, the present study investigated the transfer of PTMs in a tri-trophic food chain using ultramafic (komatiite) and control soil as substrates. The wild plant *Berkheya radula* and the crop plant *Brassica oleracea* var. *acephala* (kale) were selected as primary producers, while the house cricket *Acheta domesticus* and the African twig mantis *Popa spurca* were the primary and secondary consumers, respectively. The concentrations of PTMs in each trophic level were evaluated, as well as the bioaccumulation factors between each link. The effect of PTMs on the development of the African twig mantis was evaluated through the duration of each instar and entire life cycle. Additionally, the effect of PTMs on house cricket feeding behaviour was assessed through olfaction and gustation tests. Although the concentrations of PTMs in the komatiite soil were twice as high as that of the control, the trophic levels did not reflect the PTM contents within the soil. For example, both control and komatiite *Berkheya* bioaccumulated Ba and Sr, while excluding As, Co, Cu, Mo, Se, Pb, and V. Similarly, both control and komatiite kale bioaccumulated Sr, while excluding As, Co, Cu, Mn, Pb, Se, and V. All above mentioned PTMs were higher in komatiite than control soil. Furthermore, crickets in the *Berkheya* trial bioaccumulated the same PTMs regardless of food treatments (i.e. komatiite or control *Berkheya*), and control crickets in the kale trial from komatiite excluded Mo and Rb, which were bioaccumulated by control kale plants. Still, the PTMs that were bioaccumulated by crickets fed on komatiite kale were excluded from these plants. Lastly, mantids in the *Berkheya* trial bioaccumulated Cu, Mo, Ni, Sr, and V regardless of the food treatment (i.e. crickets fed on komatiite or control *Berkheya*), and mantids in the kale trial did the same with As, Cu, and Se. Although some differences were observed regarding mantid developmental duration in both trials, the non-significant differences of PTM concentrations suggested that other factors besides PTM levels contributed to the results. Olfaction and gustation tests revealed that PTM levels within plant leaves were not discriminated by house crickets overall, even though female house crickets showed preference towards *Berkheya* leaves grown in PTM soils during the olfaction test. Ultimately, the concentrations of PTMs in wild plant and crop plant ecosystem models did not reflect soil total PTM concentrations. Thus, PTM bioaccumulation and subsequently availability of PTMs to the next trophic level seems to be a function of the plant species rather than of soil content. The insights gained by this study advanced our understanding of naturally PTM rich ecosystems, especially in the Vredefort Dome, and how organisms within these ecosystems adapted to these conditions. Furthermore, this study adds to the research record from Africa, which is currently underrepresented when compared to the global context.

Keywords: arthropod, *Berkheya*, kale, PTMs, komatiite, serpentine, terrestrial, trophic transfer, Vredefort Dome

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Chapter 1 - Literature review

1.1 Harsh soil ecosystems

Special edaphic ecosystems are habitats driven by unique soil properties, including chemistry, pH, elemental content, and texture (Rajakaruna, 2004; Kay *et al.*, 2011). The challenging conditions created by these soil properties often host specialized communities of plants and animals that have adapted to survive, and even thrive, in these environments (Whiting *et al.*, 2004; Rajakaruna, 2018). There are numerous examples of such special edaphic ecosystems. The harsh soils that characterize these edaphic ecosystems can be categorized as nutrient imbalanced (e.g. gypsum soils) (Cera *et al.*, 2022), high in potentially toxic metal (PTM) content (e.g. laterite soils) (Kumar *et al.*, 2022), and both nutrient imbalance and elevated PTM content (e.g. serpentine soils) (Proctor, 2003).

Gypsum ecosystems, for instance, are characterized by soils rich in calcium sulfate dihydrate ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$) and mostly occur in arid and semi-arid regions around the globe (Escudero *et al.*, 2015). Gypsum ecosystems are unique due to their physical and chemical limiting factors, which include hard biological soil crusts that are difficult for plants to penetrate and establish themselves (Escudero *et al.*, 2000), and are often accompanied by nutrient imbalances such as nitrogen (N), phosphorus (P), and potassium (K) deficiencies, as well as excess calcium (Ca), magnesium (Mg), and sulfur (S) (Merlo *et al.*, 2009).

Another example is that of laterite ecosystems which are characterized by soils rich in iron (Fe) and aluminium (Al) oxides and hydroxides. Typically, they arise in tropical and subtropical areas where high temperatures and rainfall are common (Kumar *et al.*, 2022). These soils are formed through the process of laterization, which involves extensive weathering of rocks, leaching of minerals, and the subsequent accumulation of Fe and Al (Sarkar *et al.*, 2020). Plants tend to struggle on these soils because of their hard and compact structure, poor water retention and low nutrient content, as well as their somewhat high acidity due to leaching (Sarkar *et al.*, 2020, Ettler *et al.*, 2016).

Serpentine ecosystems are derived from the weathering of ultramafic parent rock. This process produces soils that are relatively low in silica (Si) and essential nutrients for plants, such as Ca, K, and P, while high in Fe and Mg (Kierczak *et al.*, 2021). Furthermore, soils in these ecosystems contain high concentrations of certain PTMs, which often include cobalt (Co), chromium (Cr), and nickel (Ni) (Rajakaruna, 2018; Kierczak *et al.*, 2021). While the physical and chemical conditions found in serpentine ecosystems are not ideal for most species, various plants have adapted to this environment and some even depend on these soil types (Rajakaruna *et al.* 2014). Plants that successfully occupy serpentine soils often differ morphologically from closely related species that are not serpentine-tolerant. These differences include xeromorphic leaves, smaller average size, and more developed root systems (Brady *et al.*, 2005; Samojedny *et al.*, 2023).

Serpentine ecosystems are associated with high levels of plant endemism. For instance, California in the USA, and the tropical to sub-tropical islands of Cuba and New Caledonia. California hosts around 250 serpentine endemic plant species across an estimated 6000 km² (Harrison *et al.*, 2006). Cuba has multiple serpentine ecosystems throughout the country, in places such as Moa and Baracoa in the east and Cajalbana in the west (Reeves *et al.*, 1999), which host roughly 854 species. New Caledonia contains around 55 000 km² of ultramafic outcrop across the archipelago (Jaffré, 1992), upon which some 1150 serpentine-endemic plant species occur (Anacker, 2011).

1.2 PTMs in the environment

The increasing food and material demand of the globe's ever-growing population is driving various polluting activities, many of which are enriching areas in PTMs (Zwolak *et al.*, 2019; Adnan *et al.*, 2022). Human activities are thus capable of creating particular edaphic conditions referred to as anthropogenic soils (Capra *et al.*, 2015). Mining activities, for example, often bring metals from deeper within the Earth onto the surface, and mine tailings usually contain high concentrations of PTMs (Demková *et al.*, 2017). These PTMs include Co, Cr, and Ni, as well as others [e.g. arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), mercury (Hg), uranium (U) and zinc (Zn)]. Fashola *et al.* (2020) reported that gold mine tailings in South Africa presented Zn, Ni, Co, As, Pb (lead), and Cd, and all except Cd exceeded the South African recommended standards of PTMs in soils and sediments (Department of Environmental Affairs, 2012). Across mine tailings originating from gold, zinc-lead, and copper mines (Armah *et al.*, 2014; Yang *et al.*, 2016; Kan *et al.*, 2021), the PTMs content is similar to that found in natural edaphic conditions associated with serpentine ecosystems.

Whether from natural or anthropogenic origin, PTMs are incorporated into ecosystems and tend to persist for extremely long periods of time as they are not broken down by biological processes (Sarubbo *et al.*, 2015). Although some metals, such as Cu, Fe, Mn, Ni, and Zn, are essential for organisms to function, generally only small quantities are required and excessive amounts may lead to toxicity (Gall *et al.*, 2015; Shafiq *et al.*, 2020). In addition, there are non-essential metals, such as Al, As, Cd, Pb, and Hg, which quickly become toxic even at low concentrations (Gall *et al.*, 2015; Chmielowska-Bąk *et al.*, 2023). Both groups of metals can be bioaccumulated, posing an ecological risk if transferred through food chains.

Plants are inevitably affected when excessive amounts of metals are present in soil. While there are many metals present in the biosphere, only a small group are considered problematic non-essential metals, which include Cd, Cr, Pb, Hg, and the metalloid As (Rahman & Singh, 2019). These metals become toxic at relatively low concentrations and are often spread through anthropogenic activities, thus making them study subjects.

1.2.1 Cadmium (Cd)

Cadmium is largely distributed into the environment from Ni-Cd batteries, coatings on other materials, plastic stabilizers, burning of fossil fuels and waste, and phosphate-based fertilizers (Rahman & Singh, 2019). Cd toxicity, at a minimum of 5 mg/kg, has a myriad negative effects on plants including, but not limited to, reduction in germination, altered photosynthetic efficiency, retarded growth rate, chlorosis, necrosis, damage to DNA structures, and cell death (Thakur *et al.*, 2022).

1.2.2 Chromium (Cr)

Chromium is primarily used in the metallurgical industry, utilizing around 90% of the total Cr production in activities such as Cr metal and alloy production. The other 10% of Cr production is allocated for the chemical industry and metal-processing industries (Rahman & Singh, 2019). Plant toxic effects may become apparent at as little as 1 mg/kg concentration in soil, and include hampered root development and seed germination, reduction in chlorophyll levels, and reduced biomass production (Thakur *et al.*, 2022).

1.2.3 Lead (Pb)

Lead is mainly distributed to the environment from mining, processing of ores, and recycling of Pb-acid batteries (Rahman & Singh, 2019). Toxic effects of Pb in plants include reduction of photosynthesis and respiration, harm caused by reactive oxygen species (ROS), depletion of soluble protein and glutathione, and reduction of stem development (Thakur *et al.*, 2022).

1.2.4 Mercury (Hg)

Mercury is often used in thermometers, electric switches, dental fillings, and to produce chlorine gas and caustic soda (Rustagi & Singh, 2010). However, its most common use, especially in low- and middle-income countries, is for the extraction of gold (Au) from ores (Pure Earth, 2015). Hg can end up in the environment from a variety of sources, including industrial mining, manufacturing of chemicals and fertilizers, disposal of solid wastes, and metal smelting, but it is through the extraction of Au that it is most commonly released into the environment (Pacyna *et al.*, 2010). The toxic effects of Hg on plants include retardation of growth and photosynthetic efficiency, production of ROS, and damaged biomolecules (Thakur *et al.*, 2022).

1.2.5 Arsenic (As)

Arsenic, although a metalloid, is typically used in the metal industry as an alloying agent, and in processing glass, paper, pigments, preservatives for wood, munitions, and in the industry of hide tanning (World Health Organization, 2022). Environmental exposure of As mainly occurs due to anthropogenic activities, including mining and smelting, burning of fossil fuels, and production of glass, semiconductors, soil fertilizers, and chemotherapy medication (ATSDR, 2007). Plant toxicity effects include stunted growth of roots and shoots, chlorophyll reduction, damage to cell membranes, reduction of genome stability, and production of ROS (Thakur *et al.*, 2022).

1.3 PTM bioavailability and hyperaccumulation

Several soil properties influence the bioavailability of PTMs in soils. Lower soil pH (i.e. acidic soils) generally increases the solubility of PTMs which makes them more bioavailable (Kicińska *et al.*, 2022). Low levels of soil organic matter mean less material for PTMs to bind to, thus increasing PTM bioavailability (Balasoiu *et al.*, 2001). Soil texture also plays a role in PTM bioavailability, as soils rich in clay generally exhibit lower PTM bioavailability compared to sandy soils with less bonding opportunities (Zhang *et al.*, 2014). When soils do exhibit high PTM bioavailability, plants can potentially take up and accumulate the PTMs. Metal cations in the soil are bound to particles that have a negative charge, such as clay particles (Zhang *et al.*, 2014). When metals separate from these particles and enter the soil solution, they become available for plants to be accumulated (Rajakaruna & Boyd, 2008; Neilson & Rajakaruna, 2012).

While bioavailable PTMs are usually detrimental to most plants, some have developed the ability to hyperaccumulate PTMs and seem to use it to their advantage (Rajakaruna, 2018). There are three main hypotheses as to why more than 500 plant species (Cappa & Pilon-Smits, 2014) have developed this trait. The first is the elemental defence hypothesis, which states that hyperaccumulation of PTMs, especially into plant organs that are heavily browsed, deters herbivores (Boyd & Martens, 1992). A second hypothesis is that hyperaccumulator plants excrete accumulated PTMs in an allelopathic manner, resulting in decreased competition from other species (Morris *et al.*, 2009). And thirdly it is possible that these plants accumulate PTMs as a mechanism of protection against drought (Boyd & Martens, 1992). In an example of a

complex relationship involving drought resistance, Zhang *et al.* (2023) reported that trees under both cadmium stress and drought stress potentially experience less osmotic stress due to an increase in abscisic acid accumulation, which promotes stomatal closure and subsequently minimizes water loss.

1.4 Trophic transfer of PTMs

The accumulation of PTMs by plants means that there is a risk that these PTMs will be transferred to higher trophic levels and ultimately affect human health (Pajević *et al.*, 2018; Letshwenyo & Mokokwe, 2020). Herbivores such as cattle, sheep, and poultry can also take up PTMs (Phillips & Tudoreanu, 2011; Roggeman *et al.*, 2013). Naikoo *et al.* (2019) tested the transferability of Pb in a soil-faba bean-aphid-ladybug food chain with amended soils ranging from 0-100 mg/kg Pb. The results indicated that Pb biomagnified from soils to faba bean roots, but biominimized in the plant shoot-aphid-ladybug chain. However, Pb was eliminated by aphids (Hemiptera: Aphididae) through their excretions (honeydew) and by ladybugs (Coleoptera: Coccinellidae) through moulted exoskeletons during metamorphosis. Furthermore, the predation of aphids by ladybugs significantly decreased at 100 mg/kg Pb which could indicate that Pb has a sub-lethal effect on predators.

Exposure to high concentrations of PTMs can influence the different trophic levels in a food chain, and each can react in unique ways. Soil microbes, for example, play a critical role in maintaining soil health by breaking down organic matter, cycling nutrients, and forming soil structure (Shah *et al.*, 2021). The presence of excess metals in the soil, whether essential or non-essential, can have adverse effects on soil microbe functionality and activity, retarding the rate of organic matter decomposition and decreasing overall soil health (Naz *et al.*, 2022). Furthermore, microbes themselves can also accumulate metals, especially bacteria due to their abundance in the soil and large surface area (Haferburg & Kothe, 2007). Their cell walls are negatively charged, which attract metal cations, and their transport systems do not distinguish between helpful and harmful metals (Haferburg & Kothe, 2007; Gadd, 2010). When the negative impacts of metal exposure hamper the ability of soil microbes to carry out their functions, the release of plant-available nutrients is reduced, making those soils more challenging for plant life (Boshoff *et al.*, 2014).

1.5 PTM transfer from plants to arthropods

Plants growing in anthropogenically PTM enriched soils such as agricultural fields, mine tailings, or industrial areas, or in natural ultramafic ecosystems, may transfer PTMs to herbivores (such as herbivorous arthropods) as the next trophic level. Arthropods may unintentionally consume metals from soil or plants or can potentially absorb metals just from prolonged exposure to soils with high metal contents (Hobbelen *et al.*, 2006). Previous research has shown that some arthropod orders are more likely to take in and accumulate metals than others. For instance, Heikens *et al.* (2001) conducted studies on multiple metals (Cd, Cu, Pb, and Zn) between 1993 and 1998 and reported that isopods (Isopoda) accumulated the most metals, followed by the non-arthropod earthworms (Haplotaxida: Lumbricidae), while beetles (Coleoptera) accumulated the least amount of metals. Some arthropods have been known to exclude metal rich plants from their diet after an initial exposure. Both *Schistocerca americana* (Orthoptera: Acrididae), an herbivorous grasshopper, and *Saissetia neglecta* (Hemiptera: Coccidae), the Caribbean black scale insect, indicated an aversion to the Chinese brake fern, *Pteris vittata* (Polypodiales: Pteridaceae), which accumulated approximately 46 mg/kg of As from an As treated hydroponic solution compared to roughly 3 mg/kg in control plants (Rathinasabapathi *et al.*, 2007; Mathews

et al., 2009). Popic *et al.* (2012) studied the transfer of various PTMs to earthworms (Haplotaxida: Lumbricidae) from naturally enriched soils in Norway. The study reported that Cd was transferred to various earthworm species with a transfer factor of around 10, which indicates accumulation of the metal. Similarly, Cd treated Yorkshire fog grass, *Holcus lanatus* (Poales: Poaceae), hosted less grass miners (Diptera: Agromyzidae) than a control, and the effect correlated positively with increasing Cd concentration (Scheirs *et al.*, 2006). Many arthropods can feed on metal rich plant material and tolerate it, while some are even dependent solely on specific hyperaccumulator plants (Wall and Boyd, 2006). Inevitably, these arthropods could potentially be a pathway through which metals reach further trophic levels (Gall *et al.*, 2015). It could also be the case that, through bioaccumulation of metals, arthropods protect themselves from predators – as suggested by the elemental defence hypothesis. Boyd and Wall (2001) reported that feeding *Melanotrichus boydi* (Hemiptera: Miridae), a nickel accumulating insect dependent on the hyperaccumulator plant *Streptanthus polygaloides* (Brassicaceae) (Wall and Boyd, 2006), to crab spiders (Araneae: Thomisidae), resulted in a significant mortality rate of the spiders, while the high nickel concentration of the Mirid insect did not protect it against predation by nematodes or fungus (Boyd, 2002).

There are numerous potentially negative impacts to herbivores when they ingest metals. Some of the most common effects are compromised immune response (Sun *et al.*, 2011), slower developmental rate, and reduction in body mass (Behmer *et al.*, 2005). For instance, Nica *et al.* (2012) reported that snails exposed to cadmium- and lead-contaminated foods had decreased sizes and slower development rates. Finally, in an ecological sense, alteration of development rate could cause desynchronization between males and females, which could drastically affect reproductive efficiency of a population.

1.5.1 Arthropods as bio-indicators

Arthropods are effective bioindicators of metal pollution in ecosystems due to their proximity to, and dependence on, soil and organic detritus (Ghannem *et al.*, 2018). Arthropods are generally easy and inexpensive to sample and can represent multiple trophic levels, from detritivores to predators. Azam *et al.* (2015) stated that various arthropods, but specifically the grasshopper *Oxya hyla hyla* (Orthoptera: Acrididae), contained significantly higher levels of PTMs (Cd, Cu, Cr, Ni, Zn) when collected at industrial sites in Pakistan compared to uncontaminated control sites.

Several arthropods have developed coping mechanisms associated with metal exposure, such as expelling metals in their faeces and through exuvial moulting (Przybylowicz *et al.* 2003), or accumulating metals within organs such as the hepatopancreas (Migula *et al.*, 2011; Nica *et al.*, 2012). The hepatopancreas, although only accounting for around 5% of body weight, can often contain more than 75% of the total PTM concentration accumulated within an arthropod, as evidenced in a study on the woodlouse *Oniscus asellus* (Crustacea: Isopoda) (Hopkin and Martin, 1982; Mazzei *et al.*, 2014). Pollinator arthropods could potentially accumulate PTMs from nectar (Meindl & Ashman, 2013), but there is currently a lack of research on the effect of high nectar PTM content on plant-pollinator relationships.

As with plants, arthropods that do not expel PTMs potentially transfer them to higher trophic levels when consumed by predators, with potential adverse effect to them.

1.6 PTM transfer to vertebrates

Vertebrates are also exposed to excessive amounts of metals due to anthropogenic activities and pollution. These animals will most likely be exposed mainly through ingestion of metals from

food sources, but can also be affected by inhaling metals from the air or when grooming their bodies (Miranda *et al.*, 2009; Van den Brink *et al.*, 2010).

1.6.1 Mammals

Previous PTM accumulation studies on small and large mammals indicated that factors such as the extent of exposure, specific metal, concentration of metal in food sources, species of mammal, age, sex, and metabolism speed all affect bioaccumulation (Wilkinson *et al.* 2003; Miranda *et al.* 2009; van den Brink *et al.*, 2010). For example, higher concentrations of Cd and Cu were found in insectivorous shrews *Sorex araneus* (Soricomorpha: Soricidae) than in granivorous mice *Apodemus sylvaticus* (Rodentia: Muridae) (Hunter and Johnson, 1982). The dietary difference between the two mammals was suggested to have caused the difference, as the shrews' intake of epigeal arthropods exposed them to higher levels of metals when compared to the granivorous mice. The age of mammals influenced metal accumulation in some insectivorous mammals, such as the greater White-toothed shrew *Crocidura russula* (Eulipotyphla: Soricidae). The PTMs Cd and Pb increased as they got older, while Cr decreased with age (Sánchez-Chardi *et al.*, 2007; Sánchez-Chardi & Nadal, 2007). Most likely, in an immobilization process, Cd was bound in the kidneys while Pb was accumulated in the liver and bone-tissue. Cr concentrations, on the other hand, may have decreased due to lower absorption rates within the intestines of adult White-toothed shrews (Sánchez-Chardi *et al.*, 2007; Sánchez-Chardi & Nadal, 2007).

The specific responses to metals are subject to regulatory processes that are more likely to occur with essential metals, meaning that non-essential metals such as As, Cd, and Pb are more readily taken up and accumulated over time (Veltman *et al.*, 2007). Negative PTM effects on mammals include oncogenesis, mutagenesis, pre-birth developmental defects, retarded growth rate, and impaired fertility (Swarup & Dwivedi 2002; Mahajan *et al.* 2012).

1.6.2 Amphibians

Amphibians are prone to metal toxicity, which is among the reasons for the decline of their populations worldwide. Metals, most commonly Cd, negatively impact the metamorphic development, growth, and overall survival of amphibians (Mahmood *et al.*, 2016; Ali & Khan, 2018). Soil co-contamination of Cd and insecticides such as Dichlorodiphenyltrichloroethane (DDT) is a major concern in agricultural areas (Li *et al.*, 2022), however contaminants are not restricted to those areas and often end up in waterways and wetlands where they can affect amphibians. In South Africa, the agricultural use of DDT has been banned since 1985 but it is still being utilized to combat malaria in areas where the disease is present (Bouwman *et al.*, 2011). Consequently, DDT can be found in the bullfrog *Pyxicephalus edulis* (Anura: Pyxicephalidae) at a mean of 4.45 ng/g within Kruger National Park, the largest wildlife conservancy in South Africa (Wolmarans *et al.*, 2021).

1.6.3 Birds

Metal exposure in birds mainly occurs through ingestion of food and water, and sometimes through the intake of soil and soil-like material (Malik & Zeb, 2009). With enough metal exposure over time birds may experience adverse effects, including calcium deficiency when overexposed to cadmium (Luo *et al.*, 2014), hormonal imbalances, reduction in fertility and reproduction, and reduction in overall condition caused by Hg toxicity (Tartu *et al.*, 2013; Whitney & Cristol, 2017).

1.7 PTM transfer to humans

Ultimately, humans are exposed to PTMs through various pathways, including ingestion following trophic transfer, ingestion directly from atmospheric metal deposition on food plants, inhalation from atmospheric metals, and absorption through skin. There often is simultaneous exposure through air- and waterways (i.e. pesticides such as DDT) and occupational exposure, especially in industries such as mining, agriculture, and manufacturing (Amaya *et al.*, 2013; Briffa *et al.*, 2020; Adhikari, 2022).

Overall humans are most likely to be exposed to PTMs through food ingestion (Luo *et al.*, 2009). For instance, humans may ingest Cd from various seafoods, dried seaweed, mushrooms, and animal liver (Briffa *et al.*, 2020); Cu from leafy greens, oysters, dark chocolate, some mushrooms, and animal liver (Briffa *et al.*, 2020); Zn from beef, some cheeses, lamb, herring, and sunflower seeds; As from both fish and shellfish, red meat and poultry, dairy produce, and cereals (Briffa *et al.*, 2020); Hg can be ingested from mushrooms and seafood (Briffa *et al.*, 2020); Pb from older paint, dust inhalation, polluted water, and occupational settings (factories) (Debnath *et al.*, 2019); Cr from contaminated air and water, industrial occupational settings, and cigarette smoke (Hossini *et al.*, 2022); and Ni from dust inhalation, kitchen utensils, various foods, cigarette smoke, and industrial occupational settings (Genchi *et al.*, 2020)

Letshwenyo and Mokokwe (2020) showed that leaves of spinach plants (Caryophyllales: Amaranthaceae) fertilized using secondary wastewater contained levels of all tested metals and exceeded permissible levels after three months of exposure. Concentrations of Cr, Cu, Pb, and Ni in spinach leaves were approximately 9, 28, 2, and 12 mg/kg respectively, whereas permissible metal concentrations in food plants, according to the World Health Organization (WHO, 1996) for Cr, Cu, Pb, and Ni, are 1.30, 10, 2, and 10 mg/kg respectively, while permissible concentration of Cd is 0.2 mg/kg (Pajević *et al.*, 2018). Intake limits for As, Hg, and Zn, according to WHO (1996), are 15 µg/kg weekly, 5 µg/kg weekly, and 45 mg daily, respectively.

Ingestion of PTMs can, however, also occur by atmospheric deposition onto the surface of plant leaves, typically around high-contamination zones such as mine areas. Among other species, Adhikari *et al.* (2022) provided evidence that *Cleome gynandra* (African cabbage, Brassicales: Cleomaceae), a common food plant, contained roughly 28 mg/kg Cr on the surface of its leaves. This was due to atmospheric deposition from mines in the surrounding Sekhukhune District in the Limpopo province of South Africa.

Another probable way by which PTMs could reach dinner tables is with the use of phosphate-based fertilizers on crop plants (Atafar *et al.*, 2010; Jiao *et al.*, 2012). Fertilizer and pesticide residues often disperse high amounts of metals, such as Cd, Co, Cu, Pb, Mn, and Zn (Nziguheba & Smolders, 2008; Eijsackers & Maboeta, 2023). Worse still, the mining and processing of phosphate rock also contributes to environmental metal pollution (Javied *et al.*, 2009).

1.7.1 Cadmium (Cd)

As with other organisms, there are several potential adverse health effects associated with Cd exposure to humans. Cadmium can cause nephrotoxicity/kidney damage, affect the reproductive system (even causing infertility), cause both gastrointestinal and psychological disorders, damage the central nervous system, and even cause/promote cancer (Briffa *et al.*, 2020). Drinking water should not contain more than 0.003 mg/L Cd if ingested over long periods of time (Briffa *et al.*, 2020).

1.7.2 Cobalt (Co)

Cobalt can cause problems to skin and respiratory systems, congestion, and when inhaled for prolonged periods it can cause asthmatic symptoms, pneumonia, fibrosis, liver and kidney problems, nausea, vomiting, and/or diarrhoea (Lauwerys & Lison, 1994; Leyssens *et al.*, 2017). Co air concentration should not exceed 0.1 mg/m^3 over a period of 8 hours (Briffa *et al.*, 2020).

1.7.3 Copper (Cu)

Copper exposure side effects include insomnia, anxiety, hyperactivity, and kidney disease (Briffa *et al.*, 2020). It can cause metal fever, which includes diarrhoea, vomiting, dizziness, and irritation of the mouth and eyes (Briffa *et al.*, 2020) as well as Wilson's disease, with accompanying side effects such as appetite loss, tiredness, jaundice, struggling speech, difficulty to swallow, and brain damage (Briffa *et al.*, 2020). Extreme copper toxicity could even be fatal (Briffa *et al.*, 2020). Furthermore, drinking water should contain less than 1.3 mg/L Cu (Briffa *et al.*, 2020).

1.7.4 Lead (Pb)

Lead toxicity in humans may cause many adverse effects, including hypertension, birthing complications such as premature and stillbirths, problems with the urinary tract, brain injury and loss of cognitive function, nerve damage, and impairment of sperm cells (Abadin *et al.*, 2019; Briffa *et al.*, 2020). In children, lead toxicity could result in altered development of the brain and central nervous system (World Health Organisation, 2023). Concentrations exceeding 0.01 mg/L in drinking water could prove dangerous to human health (Briffa *et al.*, 2020).

1.7.5 Manganese (Mn)

Manganese exposure could cause a condition known as manganism, presenting side effects such as damage to the nervous system (lethargy, frailty, and changes in behaviour) and Parkinson's disease-like symptoms (anxiety, involuntary movement, loss of coordination, and lack of facial expressions) (Briffa *et al.*, 2020). Further side effects include hypotension, sperm cell impairment, and pneumonia (Dobson *et al.*, 2004; Coles *et al.*, 2012). The drinking water suggested limit is 0.4 mg/L (Briffa *et al.*, 2020).

1.7.6 Zinc (Zn)

Zinc overexposure in humans can have a myriad of adverse effects, which include anaemia, copper deficiency, tiredness, nausea and vomiting, stomach aches, problems with the pancreas, and lowered immune system efficiency (Fosmire, 1990; USEPA, 2005). Over an 8-hour period, the concentration of zinc chloride and zinc oxide fumes and dust inhaled should be less than 1 mg/m^3 and 5 mg/m^3 , respectively (Briffa *et al.*, 2020).

1.8 Ultramafic areas as model ecosystems

Considering the increase in PTM pollution accompanying global population growth rates and urbanization, and the abovementioned human health risks these PTMs have, it is important to broaden our knowledge on the transfer of PTMs through trophic levels. It is also imperative that we build a better understanding of the mechanisms and strategies of organisms accustomed to naturally PTM rich ecosystems, such as ultramafic ecosystems. This will generate a baseline knowledge of PTM toxicity and subsequent coping mechanisms of organisms exposed to PTM

toxicity, so as to more accurately predict future consequences and reactions to increases in soil PTM levels globally (Joubert *et al.*, 2024).

Research on trophic transfer in ultramafic ecosystems has been dominated by countries in Europe and North America, with South America, Asia (excluding China), and Africa lagging behind (Joubert *et al.*, 2024). For example, in Asia, India and Sri Lanka are mostly conducting preliminary ultramafic ecological research (Garnica-Díaz *et al.*, 2022) and knowledge of the flora of the Philippines' ultramafic areas is still very limited (Proctor, 2003).

African ultramafic research has mostly been conducted in South Africa and Eswatini (Siebert *et al.*, 2002; Williamson & Balkwill, 2015; Scoon & Viljoen, 2019). Serpentine outcrops mainly occur in the mountainous areas of the Barberton Greenstone Belt, Mpumalanga, South Africa (Williamson & Balkwill, 2015). Previous studies in this area evidenced that the herbivorous grasshopper *Stenoscepa* sp. (Orthoptera: Pyrgomorphidae) (Boyd *et al.*, 2007) and beetle *Chrysolina clathrata* (Coleoptera: Chrysomelidae) (Boyd *et al.*, 2009) considered the Ni-hyperaccumulator plant *Berkheya coddii* (Asterales: Asteraceae) a suitable food source, with *C. clathrata* possibly being host-specific on *B. coddii*. While both insect species indicated exclusion (transfer factor of below 1) of Ni from consumed *B. coddii* leaves (containing around 15 000 µg Ni/g), *Stenoscepa* sp. did contain somewhat high levels of Ni in body tissue (around 950 mg/kg) which could act as a source of Ni to higher trophic levels (Boyd *et al.*, 2007).

Studies of other ultramafic areas, such as the Vredefort Dome, have highlighted the effect of sharp changes in soil properties on floristic composition (Boneschans *et al.*, 2015). The collar zone of the Vredefort Dome contains ultramafic rocks that give rise to naturally PTM rich soils (Reimold & Koeberl, 2014). These include komatiite, an ultramafic rock found in the southeastern part of the dome, which are associated with the PTMs Ba, Co, Sr, and Zn (Lana *et al.*, 2003). The combination of various geological strata that create abrupt variations in soil properties, and the presence of ultramafic/serpentine ecosystems, makes the Vredefort Dome a suitable model for a comparative study between naturally PTM enriched ecosystems and non-PTM enriched/control ecosystems. *Berkheya radula* (Asterales: Asteraceae), although not considered a hyperaccumulator, shares some traits with other species within the genus that are, such as the previously mentioned Ni-hyperaccumulator, *B. coddii*. These traits include improved photosynthetic efficiency and developmental rate when grown in serpentine soil, as well as the capacity to take up and tolerate PTMs (Roebuck *et al.*, 2022).

There is a need to broaden our knowledge on the biota that inhabits ultramafic ecosystems. Most likely there are still a large number of PTM hyperaccumulating plants to be discovered, which could promote agromining and phytoremediation of PTM-polluted ecosystems, especially those created by the mining industry (Whiting *et al.*, 2004; Gall & Rajakaruna, 2013). Other taxa, including bryophytes and lichens, also require further investigation as they potentially contain many undiscovered PTM-tolerating species (Boyd *et al.*, 2009). With its impressive biological diversity (Hoveka *et al.*, 2020), natural ultramafic ecosystems, widespread mining activity (Sorensen, 2011), and relatively little PTM-related research (Joubert *et al.*, 2024), South Africa would be a fitting model country for such studies.

1.9 Main finding

Despite the research that has been done on ultramafic ecology and trophic transfer of PTMs in terrestrial ecosystems, there is still much to be learned to gain a better understanding of this topic (Boyd *et al.*, 2009). This is especially true for developing regions, including Southern Africa, which has huge potential in both natural and anthropogenically enriched PTM ecosystems. Future research is vital and should focus on identifying new hyperaccumulator

plants and PTM accumulating animals, understanding the translocation of PTMs through multiple trophic levels, documenting the adverse effects of PTMs on organisms within terrestrial ecosystems, determining the mechanisms behind abilities of some arthropods to excrete PTM's, and ultimately learn how PTMs potentially affect human health.

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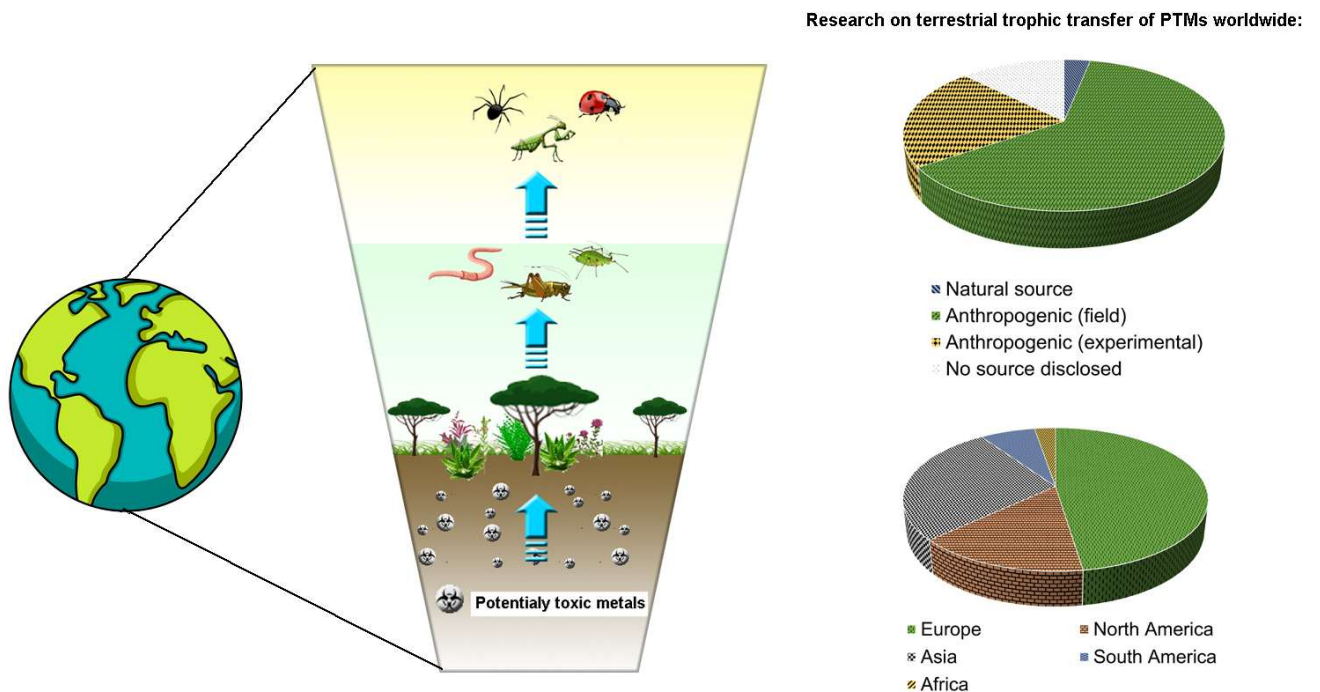
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Chapter 2 - Transfer of potentially toxic metals and metalloids from terrestrial plants to arthropods - A mini review.

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SPECIAL FEATURE REVIEW

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Ultramafic Ecology: Proceedings of the 10th International Conference on Serpentine Ecology

Transfer of potentially toxic metals and metalloids from terrestrial plants to arthropods—A mini review

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Abstract

Potentially toxic metals and metalloids (PTMs) may become a concern to plant and animal health when soil concentrations exceed toxicity limits. These limits are often exceeded in polluted environments (anthropogenic sources) and may transfer through the trophic system, from contaminated soil to plants, and thereafter to herbivores and predators. The aim of this mini review was to consider trophic transfer of PTMs in terrestrial ecosystems, focusing on arthropods as the endpoint. ScienceDirect® was used as search engine with a set of keywords. The most assessed PTMs were Cd, Pb, and Zn, and 63% of all PTMs considered in research were attributed to anthropogenic sources. Industrial or mining sites were most frequently studied, and agricultural sites the least. *Brassica* was the most studied plant genus as the main extractor of PTMs from the soil as they are easy growing food plants known to take up PTMs. Research mostly focused on primary consumers, with Coleoptera being the most investigated arthropod order, probably because of the diversity of the order and its common occurrence worldwide. Most research was conducted in Europe, while South America and Africa only contributed 6.8% and 2.6%, respectively. The majority of the investigated articles (61%) assessed trophic transfer. From those, only 24% directly measured PTM transfer with experimental trials and included only one trophic level, that is, primary consumers, while 7% of research directly measured PTM transfer across multiple trophic levels (primary and secondary consumers).

KEYWORDS

arthropod, metal, pollution, terrestrial, trophic transfer

1 | INTRODUCTION

Potentially toxic metals and metalloids (PTMs) are abundant worldwide (Shi et al., 2021). The potential of PTMs to become toxic is realized when their concentrations

exceed specific limits, resulting in toxicity to living organisms (Chaffai & Koyama, 2011). Toxicity, in the context of this review, refers to the adverse effects on an organism (plant, animal, or human) by the exposure to a compound (in this case PTMs). This exposure leads to

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deterioration of the health of the organism and potentially, but not necessarily, death. The toxicity thresholds of each organism will differ and are reliant on many factors. For example, PTM toxicity in plants will vary between species, specific PTM and its concentration, and soil physical and chemical properties (Gill, 2014).

However, in animals such as rats, toxic effects of PTM exposure were reported by Aragão et al. (2018) and included damage to the hippocampus as well as degradation in cognitive ability if the rats were orally exposed to inorganic mercury (Hg) at 0.375 mg/kg/day for 45 days. Struzynska et al. (2007) administered 15 mg/kg lead (Pb) acetate to pregnant rats for 2 weeks, leading to probable central nervous system inflammation in the brains of unborn rats. Furthermore, disruption of the metabolizing pathways of copper (Cu) and zinc (Zn) were observed in rats that were exposed to cadmium (Cd) at concentrations of 1 and 5 mg/kg (Borowska et al., 2017).

Even though the toxic or potentially detrimental effects of PTMs on laboratory animals have been well documented, mainly for human health research, other animals such as arthropods, as well as various plant species, have not been investigated as thoroughly. PTM toxicity and its effects on organisms, especially when referring to trophic transfer of PTMs, cannot be realistically studied without also considering soil as a crucial contributor thereof. Soils are the source from which plants acquire elements, and if soils have elevated levels of PTMs (whether from natural or anthropogenic sources), plant potential to accumulate PTMs increases.

Soil toxicity, due to high concentrations of PTMs emanating from activities to exploit mineral resources, is an increasing concern. For instance, anthropogenic activities such as mining, smelting, agriculture, and manufacturing are major contributors of excess PTMs in the environment, despite efforts to regulate these industries (Fayiga et al., 2018; Gall et al., 2015). The mining process to extract metals from the Earth's crust is, generally, a destructive and polluting activity, but it is not the only source of direct PTM pollution. For example, Lu et al. (2019) reported that around 71% of anthropogenic Cd pollution in China is attributed to the nonferrous metal smelting industry, while Pb is used in the manufacturing of many products like lead-acid batteries, protection against x-rays, and munitions (Tchounwou et al., 2012). Furthermore, arsenic (As) is incorporated into agricultural products such as algicides, herbicides, insecticides, and sheep dips (Tchounwou et al., 2012).

Most PTMs are readily taken up by certain plants which is of importance to ecological and human health.

For example, many species in the genus *Brassica* are consumed by both animals and humans (Mourato et al., 2015), despite these plants potentially growing in metal polluted soils (Gautam et al., 2023). When PTM concentrations in plant tissue exceed tolerable levels, which again will differ depending on the PTM and plant species in question, it becomes a threat to ecological safety, by potentially changing the biochemical characteristics of an ecosystem, and ultimately to human health (Gautam et al., 2023; Intawongse & Dean, 2006). Cd effects on plants include reduction of germination rate, cell death, and DNA damage, but its critical level varies for wheat and soybean in the ranges of 5–7 and 18–45 mg/kg, respectively (Thakur et al., 2022). Similarly, chromium (Cr) inhibits root development and seed germination, and reduces chlorophyll and plant biomass in ryegrass at 1 mg/kg, but only at 10 times more (10 mg/kg) does it affect barley (Thakur et al., 2022). Terrestrial arthropods that regularly feed on plants that accumulate PTMs, are dietary necessities for countless animals, as well as humans.

Malnutrition and food insecurity are common in poor rural areas, which are prominent in developing countries (Praharaj et al., 2021). In these countries, arthropods form a vital portion of people's diets because of their high calories, adequate protein and fat content, while being readily available (Mariod, 2020). More than 2 billion humans regularly include arthropods in their diet, with over 1600 species that are known to be edible (Pal & Roy, 2014). Countries in which human consumption of arthropods are practiced include Nigeria, where Mopane worms are eaten (Ekpo, 2011), Papua New Guinea where black palm weevils are utilized as an extra source of protein (Bukkens, 1997), and Thailand where weaver ant larvae are sold for consumption (van Huis, 2013). This highlights the importance of understanding how PTMs are transferred to different trophic levels in terrestrial ecosystems, and how this affects the organisms (Soliman et al., 2022). An early study by Winder et al. (1999) found that application of Zn contaminated sewage sludge on wheat fields resulted in the transfer of Zn from wheat to aphids to predatory beetles. There was a significant relationship between the number of contaminated aphids fed to beetles and subsequent concentration of Zn in beetles. A more recent study by Naikoo et al. (2021), on a soil-faba plant-aphid-ladybug chain, also exhibited the potential of PTM transfer across multiple trophic levels. Treatments ranged from control soil (0 mg/kg added Cd) to a maximum of 30 mg/kg Cd treated soils, with results indicating a significant difference in Cd concentration in faba plants, aphids, and ladybugs. Ladybugs (the highest tropic

level) feeding on Cd treated plant trials contained 124 times more Cd than the control treatment.

Arthropods also serve as effective indicators of ecological disturbances, which makes them a good choice as indicators in studies focusing on the effects of PTMs on the food chain (Maleque et al., 2006; Solascasas et al., 2022). In a review by Solascasas et al. (2022), it was reported that spiders, ants, and grasshoppers, specifically in grasslands, are effective indicators of the effects of management practices (i.e., prescribed fire, fertilization, and mowing) because of their significant responses to different practices. Deutsch et al. (2005) indicated that green lacewings, important pest control predators, are also good indicators of biodiversity loss in agricultural settings. In the case of PTMs, Simon et al. (2016) concluded that the beetle *Pterostichus oblongopunctatus* is an effective indicator of pollution as it accumulated significantly higher concentrations of Cu and Zn along an urbanization gradient.

This mini review aims to identify gaps in the research of trophic transfer of PTMs and stimulate future studies in those areas lacking attention. This review identifies gaps in our understanding of PTMs in trophic transfer by evaluating what is currently focused on within the topic, and which study sites, organisms, species, and elements are deemed relevant. Therefore, the objectives of the review were to address the (1) quantity of research over the years and per continent, (2) specific PTMs targeted by previous research, (3) PTMs sources and types of study sites, and (4) plants and animals (with specific emphasis on arthropods) considered.

2 | METHODOLOGY

ScienceDirect® was employed as the search engine for this mini review. The following search string, containing a specific set of keywords, was used to obtain an adequate data set of past research: “metal AND (insect OR arthropod) AND (trophic OR ‘food web’) AND (transfer OR relation) AND terrestrial.” The articles secured with these keywords were assessed to determine whether they were on topic, that is, any study on accumulation of PTMs in terrestrial organisms and/or trophic transfer, while mentioning at least one living organism involved in the study. The reason for including studies containing one living organism was to accommodate studies from soil science that include leaf litter arthropods, such as spring tails (Collembola), and earthworms. Only original research articles were assessed. All herbivorous arthropods were seen as primary consumers, while predatory and parasitic arthropods were considered as secondary consumers.

Data recorded included research from 1998 to most recent (2023), and the following information was obtained: (1) year, continent and country; (2) targeted PTMs; (3) PTM sources: anthropogenic (field), anthropogenic (lab), natural or unavailable; study site: agricultural, experimental, industrial/mining, urban, pristine, and unavailable; and (4) arthropod trophic level and accompanying fauna: primary, secondary consumers or both, amphibians, reptiles, birds, and mammals. All arthropods were grouped according to order as studies including arthropods were rarely identified to family or genus level. Furthermore, plants (genus level); and presence and type of trophic transfer assessment (i.e., directly, from organisms known to have consumed food exposed to PTMs, or indirectly, from organisms sampled at PTM enriched sites) were obtained from the articles within this study. The continent in which research was conducted was considered. In the case of laboratory studies, the location of either the laboratory, or where samples were collected, or both, were considered. Classification of the developed status of countries was based on the United Nations document “World Economic Situation and Prospects” (United Nations, 2024).

3 | RESULTS AND DISCUSSION

The search on ScienceDirect® produced 2832 results, of which 190 articles met the criteria of this review, that is, research articles that included PTMs that were transferred between one or more trophic levels, namely from soil to a plant species or a primary consumer to a secondary consumer. An upward trend was observed in the number of articles dealing with the trophic transfer of PTMs (Figure 1) from 1998 (3 publications) to 2023 (22 publications). The 190 articles used for the review were divided into several topics for discussion.

3.1 | Potentially toxic metals and metalloids

Most studies were conducted in agricultural, industrial/mining, natural, and urban sites which did not necessarily focus on specific elements, but reported a set of PTMs, whether natural or a contaminant. This provided valuable information, as it suggested how common these PTMs are in these ecosystems. Throughout, among others, Cd (96 articles), Cu (79 articles), Hg (49 articles), Pb (83 articles), and Zn (82 articles) were recorded the most (Figure 2). There were, however, research papers that focused on single PTMs only. For instance, Cd

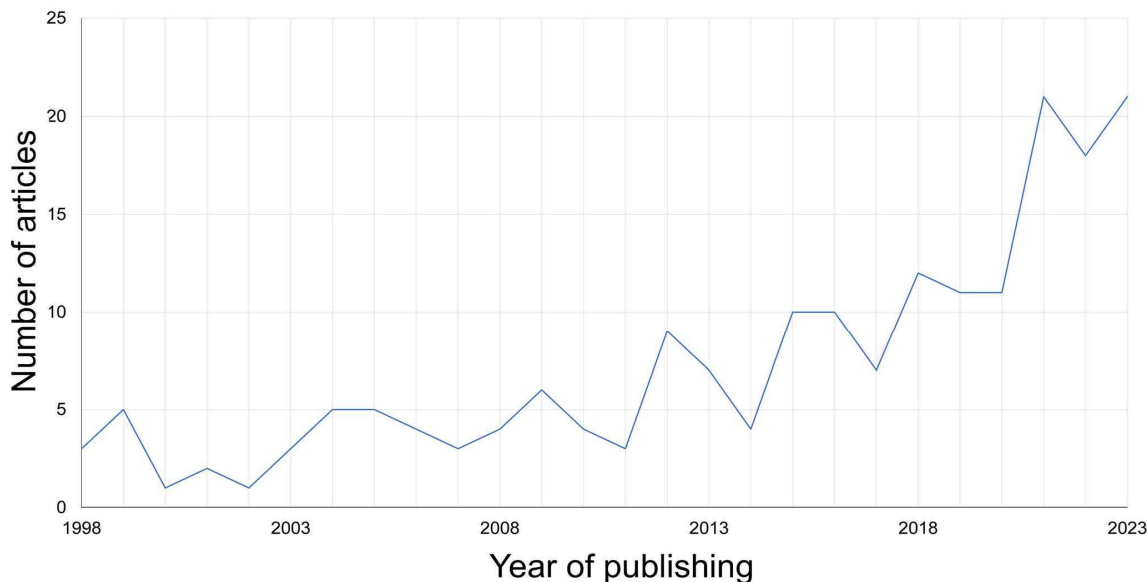


FIGURE 1 Number of articles dealing with trophic transfer within terrestrial ecosystems per year from 1998 to 2023.

(Vlahović et al., 2017; Zhu et al., 2016), because of its common occurrence, non-essential status, low toxic concentration, and Zn (Kızılkaya, 2005; Shu et al., 2012) because it is an essential metal that has the potential for toxicity (Table S1).

Despite the temporal increase in publications, there is still a need to create awareness on the potential negative impacts of PTMs on organisms (Chen & Deng, 2020; Zhou et al., 2022). This is especially important for the public and industries directly affected, such as the mining and agricultural sectors.

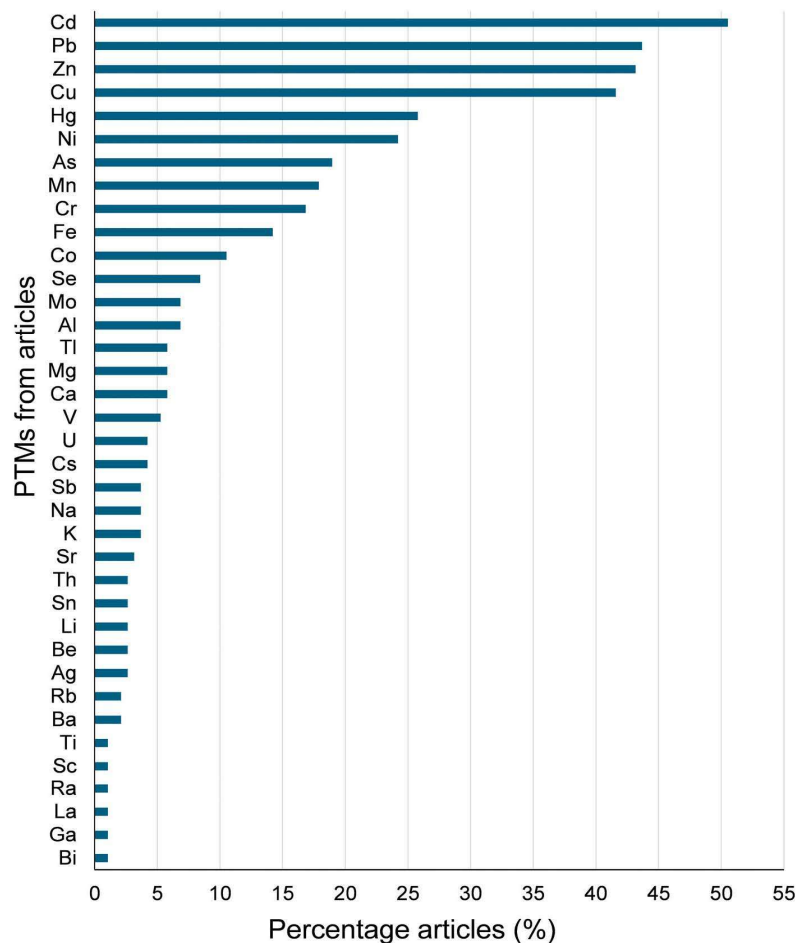
3.2 | Global contributions to the topic

Developed countries generated most of the research on the topic of trophic transfer of PTMs, with 62% originating from Europe and North America, while Africa and South America, comprising developing countries, only delivering 9% of the overall research considered in this study (Figure 3). Although PTM transfer across trophic levels is equally relevant worldwide, developed countries have additional resources and ability to impose environmental legislation, create awareness, and conduct research, while developing countries might lack the resources, political ability and/or willingness to handle these scenarios similarly (Saha et al., 2017; Ukaogo et al., 2020). Research focusing on arthropods in trophic transfer was more frequent in developed countries. The

ecology of arthropods in these regions is better understood, which provides sufficient data to enable research on the topic (Cock, 2003). Therefore, the gap in research on trophic transfer of PTMs is predominant in developing countries, even though PTMs could have detrimental effects on ecological interactions, irrespective of the country or region they occur in.

For humans, crops grown in soils containing bio-available PTMs could potentially have health risks (Alengebawy et al., 2021; Ali & Khan, 2019; Atta et al., 2023). However, many species of *Brassica* (cabbage, cauliflowers, and broccoli) and *Triticum* (wheat, spelt, and durum), which are known to readily take up PTMs (Jamali et al., 2009; Morales-Silva et al., 2022), are produced in lesser researched developing countries (Food and Agriculture Organization of the United Nations, 2023). This includes Argentina and Brazil which are both among the top 20 largest producers of wheat, and Kenya which is among the top 10 largest producers of cabbage globally (Food and Agriculture Organization of the United Nations, 2023), and yet Africa and South America combined delivered less than 10% of the total research articles produced on PTMs in trophic transfer. Exacerbating the risk to human health is that human populations of developing countries are growing faster than developed countries, inevitably leading to rapid urbanization that is ultimately the root cause of many PTM polluting activities (Ahmad et al., 2022). This means people in developing

FIGURE 2 The prominence of potentially toxic metals and metalloids (PTMs) in articles/dealing with elemental uptake and trophic transfer within terrestrial ecosystems. Each element is shown here as a percentage of total papers, with inclusion in the table dependent on a lower threshold of at least 1% of total articles.



countries could be more affected by the transfer of PTMs through trophic levels than developed countries, stressing the urgent need for research.

Asia is suffering from severe overpopulation, as more than half the global population live on this continent (Rahman et al., 2024). This has resulted in rapid urbanization and associated negative effects, including pollution (Sheykhi, 2021). The health risks associated with elevated environmental PTM levels are enhanced by Asian countries that are also major producers of food crops, especially China and India, which are the largest producers of cabbage and wheat crops globally (Food and Agriculture Organization of the United Nations, 2023). Within this review, publications originating from Asian countries suggest their awareness of PTM trophic transfer, given the large proportion of articles (26.7%) originating from the continent. In addition, articles from Asia were largely focused on human health risks associated with trophic

transfer of PTMs. The bulk of research papers (59%; Table S1) originated from China, with remaining papers published by researchers working in India, Kazakhstan, Pakistan, and Taiwan (Table S1).

3.3 | Source of PTMs and type of study site

The global research focus on PTMs from anthropogenic field sources (Figure 4) is likely explained by a concern for the harmful effects of PTMs on the environment, like the deterioration of physiological processes (e.g., photosynthesis) in plants (Khalef et al., 2022) or impeding growth in arthropods (Vlahović et al., 2017).

Agricultural sites feature relatively infrequently compared to others (Figure 5), despite the health threats associated with accumulated PTMs in crop plants (Adhikari

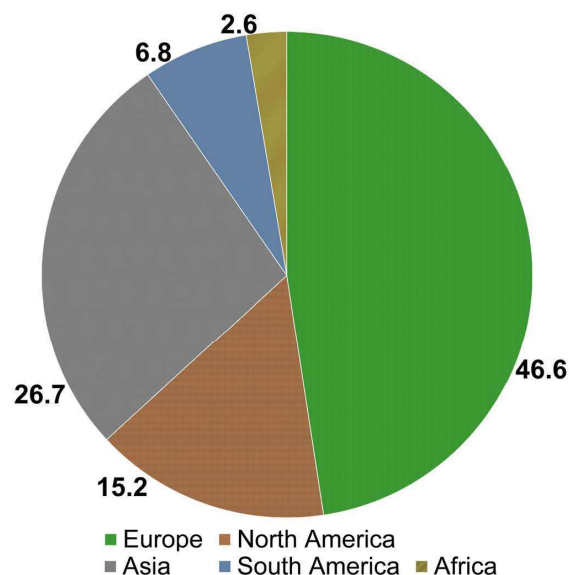


FIGURE 3 Percentage of research articles per continent considering trophic transfer of potentially toxic metals and metalloids.

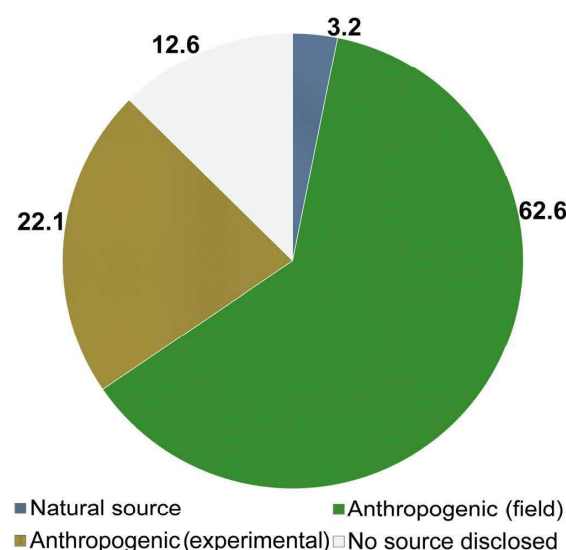


FIGURE 4 Sources of the potentially toxic metals and metalloids considered in papers dealing with trophic transfer within terrestrial ecosystems (in percentage).

et al., 2022; Mtemi et al., 2023). Ultimately, PTMs can be ingested by humans through foods, potentially leading to side effects including neurotoxicity and gastrointestinal toxicity (Karri et al., 2016; Tchounwou et al., 2003). Therefore, elevated PTM levels in soils (Department of

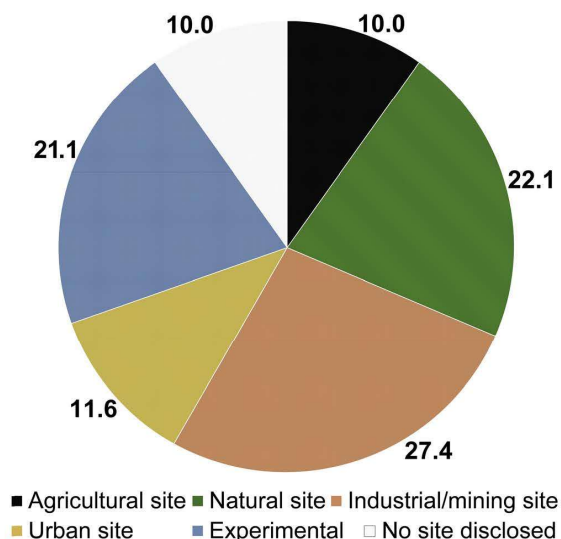


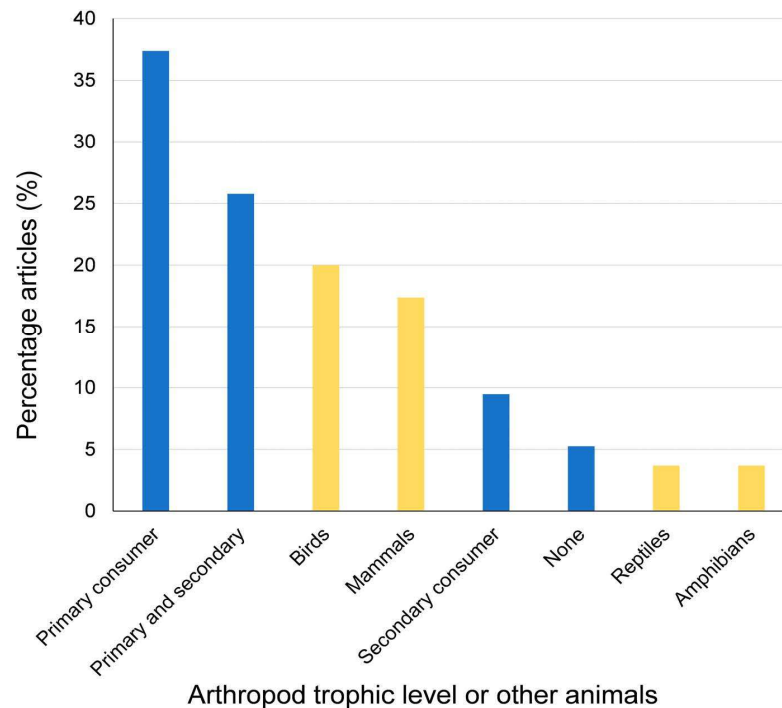
FIGURE 5 Proportions of local study sites in papers dealing with trophic transfer of potentially toxic metals and metalloids within terrestrial ecosystems (in percentage).

Environmental Affairs, 2012) are a threat to food security when it affects the growth of crop plants (Gwenzi, 2020; Rodriguez-Eugenio et al., 2018). These effects are steadily becoming more relevant in relation to current rapid population growth, increasing human food consumption demands and urbanization.

While PTM levels in anthropogenic environments are useful for assessing the pollution levels of an area, spiked soils in experimental situations provide opportunities to conduct tests of PTM transferability across trophic levels. The research employing this technique focuses directly on the concentration of PTMs being transferred to higher trophic levels and the effects thereof (Table S1). These studies typically focused on the PTMs Cd, Co, Hg, Ni, and Zn. While it is important to extensively research polluted sites, in situ and ex situ, the lack of literature from naturally PTM rich areas (Figure 4), such as ultramafic ecosystems (Adhikari et al., 2022), leaves a knowledge gap on how metals are transferred through naturally enriched ecosystems and the associated organisms.

Only five studies on trophic transfer (of any level) of PTMs in ultramafic or natural systems were recorded in this mini review. One of the studies (i.e., Popic et al., 2012) investigated the accumulation and transfer factor of ^{232}Th and ^{238}U and associated metals (As, Cd, Pb, and Cr) in carbonatite rocks of magmatic origin in Norway on earthworm species. Burrowing earthworms had higher transfer factors of Cd, Pb, and As than surface and leaf litter living earthworm species.

FIGURE 6 Percentage of articles featuring each of the arthropod trophic levels (blue) and other animals (yellow) in studies dealing with the uptake/trophic transfer of potentially toxic metals and metalloids within terrestrial ecosystems.



Such evidence highlights the importance of conducting research on naturally PTMs-enriched soils in trophic transfer processes.

3.4 | Arthropod trophic level and accompanying fauna

A third of articles (37%) dealt with primary consumers only and a quarter of the articles studied both primary and secondary consumer arthropods (Figure 6). Generally, articles considering both primary and secondary consumers, were based on samples from the field and did not necessarily directly measure PTM transfer between these trophic levels in controlled settings. Trophic transfer was assessed by 61% of articles included in this review, but only 24% directly measured PTM transfer, typically in experimental trials, and a mere 7% directly measured PTM transfer (Table S1) while also assessing primary and secondary consumers (thus across multiple trophic levels). This is despite the fact that 76% of the articles contained arthropods. This indicated a lack of studies investigating trophic transfer through different trophic levels.

An example of a multiple trophic level study, conducted by Naikoo et al. (2021), clearly demonstrated trophic transfer. Since arthropods are a major part of the

diet of many animals and humans (Cross et al., 2020; Nyffeler et al., 2018), they are an important group of organisms to consider in the food chain when studying trophic transfer of PTMs. Furthermore, some arthropods have developed mechanisms to avoid the toxicity of PTMs, such as excretion through feces or in molted exoskeletons (Borowska et al., 2004; Przybyłowicz et al., 2003). Therefore, studying trophic transfer of PTMs from plants to herbivorous and predatory arthropods can provide insights into mechanisms underlying the adaptations and survival strategies of this diverse phylum to environmental changes. Such investigations could provide important outcomes regarding the fate and impacts of dangerous PTMs in ecological communities.

3.5 | Arthropod orders and plant genera

Arthropods are good indicators of the health of an ecosystem because they are generally sensitive to change. Many taxa are widespread and easily identified at least up to order level, and thus can be used as a tool to compare the state of different ecosystems (Azam et al., 2015). The arthropod orders Araneae (Conti et al., 2018), Coleoptera (Ghannem et al., 2018), Haplotaenidia (Pérès et al., 2011), Hemiptera (Saleem & Afsheen, 2022), and Lepidoptera (Pallottini et al., 2023) have been popularly

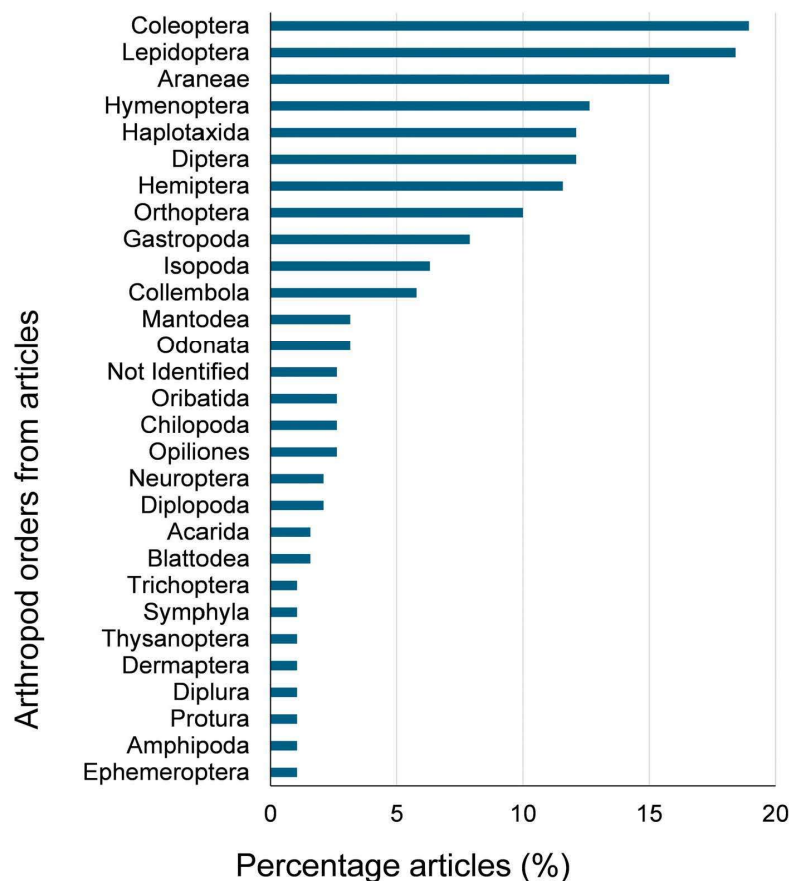


FIGURE 7 Arthropod orders contributing focal taxa to research articles dealing with trophic transfer of potentially toxic metals and metalloids in terrestrial ecosystems. Each arthropod order is shown here as a percentage of total papers, with inclusion in the table dependent on a lower threshold of at least 1% of total articles.

used as bioindicators and were also the most common orders encountered in this review (Figure 7). Furthermore, Coleoptera is one of the most diverse arthropod orders (Roger et al., 2022) and is present in most terrestrial ecosystems (Butovsky, 2011; Guéi & Tondoh, 2012), which is probably why it occurred in many field-focused studies (Table S1). Many Lepidopteran insects are widespread and common, their adults and larvae are an important source of food for higher trophic animals (Ghazanfar et al., 2016), and they readily eat leaf material (Krenn, 2010). These factors make Lepidopterans a suitable insect order for PTM transfer studies, including experimental setups where arthropods are exposed to foods containing certain concentrations of PTMs and thus elemental transfer can be directly tested. Araneae (spiders) species are well-known predators (Samiayyan, 2014) that are excellent models for secondary consumers in a food web. Soil dwelling arthropods, such as Haplotaenidia (earthworms), are decomposers within the soil, thus they are considered good bioindicators for soil PTM content as they are restricted to the medium (Tischer, 2009).

A total of 86 plant genera were considered in trophic transfer studies (Figure 8; Table S1). *Brassica*, *Oryza*, and *Triticum* were the most frequently studied, as they are widely cultivated crop plants (Golovnina et al., 2007; Rakow, 2004). These plants were used in studies with experimental setups requiring easily accessible seeds, relatively fast-growing individuals and, most importantly, utilized by humans as food crops. Alonso et al. (2009) found that, on average, *Triticum* shoots accumulated around a hundred times more Cd in spiked soils. Furthermore, studies considering PTM uptake by plants gravitate toward plant species known to accumulate/hyperaccumulate PTMs. As mentioned, Brassicaceae is one of the plant families that is known for its hyperaccumulators of PTMs (Manara et al., 2020), thus this plant family is readily used in PTM uptake studies. Morales-Silva et al. (2023) observed that kale plants (*Brassica*) accumulated approximately 22 times more Pb from contaminated soil.

The only other genera that occurred in more than 2% of studies, *Betula* and *Pinus*, are trees commonly found in

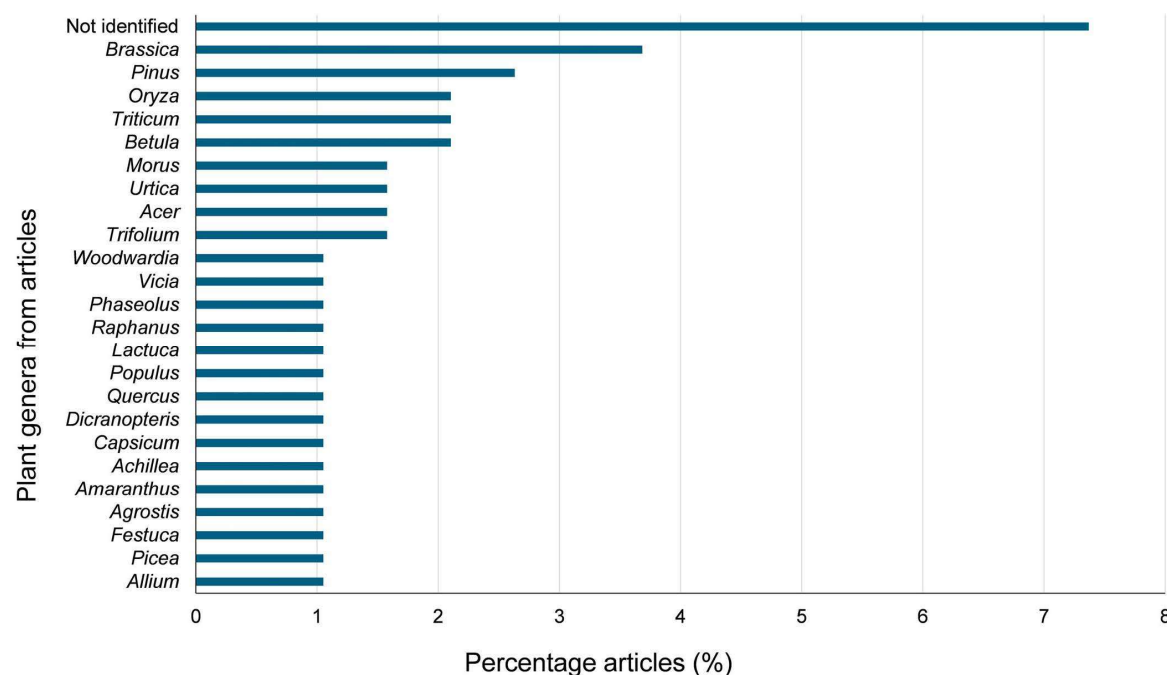


FIGURE 8 Percentage of plant genera considered in research articles dealing with trophic transfer of potentially toxic metals and metalloids within terrestrial ecosystems, with an inclusion threshold of at least 1% of total articles.

woodlands across Europe and Asia (Trees for Life, 2023). The articles featuring these genera came from field-focused studies (on both natural and anthropogenically polluted sites) where they were dominant tree species.

4 | CONCLUSIONS AND FUTURE RECOMMENDATIONS

This review documented that research on trophic transfer of PTMs in terrestrial ecosystems was conducted mostly in developed countries, especially in Europe and North America. Developing countries, in particular, Africa and South America, did not contribute significantly to this topic, even though they are among the largest crop producers globally of plants that take up PTMs and their population growth rate exacerbates related health risks. Future research in these regions would aid in bridging the current knowledge gap within these specific ecosystems under different climatic conditions. Agricultural sites were the least researched study site. This is concerning as this provides for the primary food source of humans. Therefore, future research should be focused on agricultural sites to better understand the risk potential to human health of PTM ingestion through food plants and livestock.

Few studies were conducted on natural PTM sources. A better understanding of terrestrial ecosystems naturally rich in PTMs could highlight bases of comparison for possible toxic effects within polluted areas as well as metal defense strategies of organisms within naturally enriched environments. This could aid in determining whether organisms in polluted areas have similar capabilities to withstand PTMs and if they will indeed be able to adapt to these changed environments. It is important to establish trophic movement of PTMs from lower to upper levels to make substantiated conclusions on environmental risks (Marcelo-Silva & Christofolletti, 2019). More investigations with experimental setups that include known PTM concentrations or artificially manipulated concentrations are also important, as directly measuring the trophic transfer of PTMs in a controlled study could contribute to understanding the effects and mechanisms involved of all organisms within such environments. This will also fill the knowledge gap regarding safe and potentially threatening levels of PTMs that are not readily available, especially for arthropods.

Finally, incorporating both primary and secondary consumers in future research, especially within one trophic chain or experimental setup, could further improve knowledge related to health risks regarding PTM ingestion, especially if prominent crop species are investigated.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The full datasheet for this review can be found attached as Table S1. All data referred to within the review are represented in the datasheet.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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Supplementary Table S1 – List of publications included within the mini-review

35

[illegible]

[illegible]

List of articles (literature) used within the mini- review and their associated identification numbers (ID).

- 1 Rosten, L.S., Kaalaas, J.A., Mankovska, B. and Steinnes, E. (1998). Mercury exposure to passerine birds in areas close to local emission sources in Slovakia and Norway. *Science of the Total Environment*, 213, 291-298.
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Chapter 3 - Tri-trophic transfer of PTMs in a naturally metal rich terrestrial ecosystem

Abstract

Potentially toxic metal(oid)s (PTMs) are elements which have the potential to become toxic to plants and animals at elevated concentrations. PTMs are a growing global concern due to various industries releasing PTM rich pollutants into the environment as wastes, especially in soils. PTMs also occur naturally and in high concentrations worldwide, typically in soils that are derived from ultramafic rocks. These are commonly referred to as ultramafic soils/ecosystems. Well studied examples include serpentine ultramafic areas found in California and New Caledonia. Among South Africa's natural ultramafic ecosystems is the relatively well studied serpentine in the Barberton Greenstone belt. However, other lesser studied ultramafic areas exist within the country, including the Vredefort Dome. Ultramafic komatiite rocks are found in the collar zone of the dome, but no literature exists on the trophic transfer of PTMs from this area. This study evaluated the transfer of PTMs along three trophic levels located on ultramafic "komatiite soil" on which grew wild and crop plants. After analysis, the komatiite soil did not fit the description of serpentine soil but did have elevated levels of multiple PTMs. Interestingly, more instances of bioaccumulation occurred from control treatment than from komatiite. In general, PTM uptake by plants (*Berkheya radula* & *Brassica oleracea* var. *acephala*), primary consumers (*Acheta domesticus*), and secondary consumers (*Popa spurca*) were not influenced by soil treatment but rather by species requirements. There were, however, differences in development rates of *P. spurca* between treatments, but no specific PTM could be pinpointed as responsible due to non-toxic concentrations. Future research comparing both natural and anthropogenic PTM enriched environments, as well as with the use of hyperaccumulators, are recommended. Utilising plant and animal species that are known to accumulate PTMs would aid in achieving a deeper understanding of the full transferability potential of PTMs in terrestrial environments.

3.1 Introduction

Potentially toxic metal(oid)s (PTMs), also referred to as "heavy metals" (Gall *et al.*, 2015), are elements which, whether considered essential or non-essential, can be toxic to plants and animals. PTMs are a growing global concern due to ever increasing human need for resources (Zwolak *et al.*, 2019; Adnan *et al.*, 2022). Industries such as mining, agriculture, and manufacturing are releasing PTM rich pollutants into the environment as wastes, especially in soils (Fayiga *et al.*, 2018; Gall *et al.*, 2015).

However, PTMs also occur naturally and in high concentrations in specific environments worldwide, typically in soils that are derived from the weathering of naturally PTM rich parent rocks, such as ultramafic rocks (Brady *et al.*, 2005; O'Dell *et al.*, 2006; Kierczak *et al.*, 2021). An example of such ultramafic weathering is serpentine soil (Safford & Miller, 2020). There are many known serpentine soil rich ecosystems (serpentine ecosystems) globally. For instance, California serpentine soils cover almost 1.5% of the land surface and host many endemic species (Harrison *et al.*, 2000; Safford, 2011; Safford & Miller, 2020). New Caledonia contains around 55 000 km² of ultramafic outcrop across its archipelago (Jaffré, 1992), upon which some 1150 serpentine-endemic plant species occur (Anacker, 2011). Africa also hosts ultramafic rich areas with soils containing elevated PTM levels (including serpentine ecosystems), with most research being conducted in Southern Africa (Scoon & Viljoen, 2019; Williamson & Balkwill, 2015).

Among South Africa's natural serpentine ecosystems is the Barberton greenstone belt in Mpumalanga Province, associated with the Barberton Centre of Endemism (± 80 plant species or 3.6%; Williamson & Balkwill, 2015). Within this roughly 4000 km² are 30 sizeable outcrops of serpentine soil, with the largest being ± 19 km² (Balkwill *et al.*, 1997; Ward, 2000; Lötter *et al.*, 2002; Williamson & Balkwill, 2015). The Vredefort Dome, situated in the North-West Province of South Africa, also contains ultramafic rocks within its collar that give rise to naturally PTM rich soils (Reimold & Koeberl, 2014). These include komatiite, an ultramafic rock found in the southeastern part of the dome, which is associated with the PTMs barium (Ba), cobalt (Co), strontium (Sr), and zinc (Zn) (Lana *et al.*, 2003). Nevertheless, relatively few PTM-related trophic studies have been conducted in both areas. The challenging conditions for plants on ultramafic soil include chemical stress due to high levels of PTMs and nutrient deficiency attributed to poor soil nutrient content (Jenny, 1980; Brady *et al.*, 2005). Plants that successfully occupy serpentine soils, for example, often differ morphologically from closely related species that are not serpentine-tolerant. These differences include xeromorphic leaves, smaller average size, and more developed root systems (Brady *et al.*, 2005; Samojedny *et al.*, 2023). Generally, plants that are adapted to ultramafic soils are metallophytes that have either evolved the ability to accumulate and store (hyperaccumulators), or exclude, PTMs from these soils (Hossain *et al.*, 2012; Gall & Rajakaruna, 2013; Siebert *et al.*, 2018; Manara *et al.*, 2020; Rue *et al.*, 2020).

Other groups of organisms, such as arthropods, have also adapted to such conditions. These adaptations also include exclusion or accumulation of PTMs (Gall *et al.*, 2015). For example, scale insects *Saissetia neglecta* (Hemiptera: Coccidae) reduced their ingestion of *Pteris vittata* (Polypodiales: Pteridaceae) with high arsenic (As) concentration (Mathews *et al.*, 2009), suggesting exclusion as the coping mechanism. The insect *Melanotrichus boydi* (Hemiptera: Miridae) however was reported to be monophagous on the nickel (Ni) hyperaccumulator plant *Streptanthus polygaloides* (Brassicales: Brassicaceae), the first to be discovered in Northern America (Wall & Boyd, 2006). *M. boydi* collected from *S. polygaloides* accumulated around 770 mg/kg Ni in its body (Schwartz & Wall, 2001). Lastly, in South Africa the beetle *Chrysolina pardalina* (Coleoptera: Chrysomelidae), which is monophagous on *Berkheya coddii* (Asterales: Asteraceae), has been reported to accumulate up to 2 650 $\mu\text{g/g}$ Ni (Mesjasz-Przybylowicz & Przybylowicz, 2001). The mechanism by which arthropods adapt to elevated PTM levels is complex and has individual and ecosystem consequences, through bioaccumulation and biomagnification, respectively (Mann *et al.*, 2011; Bourioug *et al.*, 2015; Gall *et al.*, 2015).

PTMs from either natural or anthropogenic origin can be incorporated into ecosystems and will persist within soils, as biological processes do not break them down (Sarubbo *et al.*, 2015). Some metals, for instance copper (Cu), iron (Fe), manganese (Mn), Ni, and Zn, are necessary for biological processes within organisms (i.e. essential nutrients), however only in small quantities and excessive concentrations may cause toxicity (Chaffai & Koyama, 2011). On the other hand, non-essential nutrients such as aluminium (Al), cadmium (Cd), lead (Pb), and mercury (Hg), and the metalloid As, will become toxic at much lower concentrations (Gall *et al.*, 2015). Therefore, they may become an ecological risk to food chains if bioaccumulated.

Plants can act as pathways of PTMs to higher trophic levels, as some plants have the ability to accumulate PTMs in their leaves (Kumar *et al.*, 2019). A study by Pajević *et al.* (2018) reported that spinach (*Spinacia oleracea*), grown in an agricultural area in Serbia, contained Cd in much higher concentrations than the permissible limits. Herbivore arthropods subsequently ingest and accumulate these PTMs through grazing. Devkota and Schmidt (2000) reported bioaccumulation of Cd and Hg in several species of wild grasshoppers (Orthoptera: Acrididae). Similarly, Soliman and El-Shazly (2017) found that grasshoppers (Orthoptera: Acrididae) bioaccumulated Cd, Cu, Pb, and Zn at a polluted industrial site in Cairo, Egypt. Mwelwa *et al.* (2023) collected wild moths of *Cirina forda* (Lepidoptera: Saturniidae), an insect that is consumed by humans, from a polluted

area within Zambia's Copperbelt Province, and reported elevated concentrations of Cd, Ni, and Pb. In addition, Boyd *et al.* (2006) reported that honey bees *Apis mellifera* (Hymenoptera: Apidae) that visited flowers of a Ni hyperaccumulator plant contained about eight times more Ni in their bodies than those visiting non-hyperaccumulators' flowers, suggesting that PTMs can also be transported to, and further mobilized from, the flowers of hyperaccumulator plants.

PTMs can subsequently be transferred to arthropods in the third trophic level (predatory arthropods). Mogren *et al.* (2013) observed significant As accumulation of the mantid *Tenodera aridifolia sinensis* (Mantodea: Mantidae) when fed on As contaminated mosquitos (Diptera: Culicidae). Similarly, Soliman and El-Shazly (2017) recorded higher levels of Cd, Cu, Pb, and Zn in the mantid *Miomantis paykullii* (Mantodea: Miomantidae) nearer to a pollution site than a control site. Although some literature assessed PTM accumulation in mantids, within field and controlled studies, there are still questions on how mantid development is affected under exposure to elevated PTM levels. PTM accumulation within arthropods in the third trophic level may lead to toxicity and affect their biology. Naikoo *et al.* (2019) studied a soil-plant-aphid-ladybug food chain and reported that, although the ladybugs excluded Pb, they reduced their predation rate, and lower ladybug biomass was associated with higher Pb concentrations. Akhtar *et al.* (2021) evidenced increased mortality of dragonfly nymphs (Odonata: Libellulidae) fed on mosquito larvae with high Cd, Cu, Hg, and Pb levels. A study by Ortel *et al.* (1993) indicated significantly reduced emergence of adults of *Glyptapanteles liparidis* (Hymenoptera: Braconidae) when its host was fed Pb contaminated food. There are also reports suggesting no adverse effects to accumulation of PTMs, as in Mogren *et al.* (2013), which evidenced bioaccumulation of As in *Tenodera aridifolia* with no harm to this mantid's developmental parameters. However, as suggested earlier, effects of PTMs on the biology of arthropods can differ between arthropod species. Nonetheless, if PTMs are transferred through trophic levels, they can reach humans and could have various health implications. Excess ingestion of PTMs can cause damage to the skin, the respiratory, nervous, and immune systems, organs such as the kidney and liver, infertility, gastrointestinal and psychological disorders, and ultimately cancer (Fosmire, 1990; Lauwerys & Lison, 1994; WHO, 2003; Dobson *et al.*, 2004; USEPA, 2005; Coles *et al.*, 2012; Leyssens *et al.*, 2017; Abadin *et al.*, 2019; Briffa *et al.*, 2020). The ever-increasing PTM concentrations in ecosystems due to anthropogenic activity, the adverse effects PTMs have on organisms within ecosystems, and their health risks to humans warrants a better understanding of the transfer of PTMs through trophic levels. Hence it is imperative that we improve our current knowledge of the mechanisms and strategies of organisms adapted to naturally PTM-rich ecosystems. This will generate baseline knowledge of PTM toxicity and subsequent coping mechanisms of the organisms exposed, which could more accurately predict future consequences to increases in soil PTM levels globally (Joubert *et al.*, 2024). Furthermore, it is important to study the transfer of PTMs to and from food plants as they are a major potential exposure pathway to humans, including that caused by use of PTM-rich fertilizers (Atafar *et al.*, 2010; Jiao *et al.*, 2012).

The collar zone of the Vredefort Dome is a suitable model ecosystem for the study of trophic transfer of PTMs, as it contains naturally PTM rich soil from komatiite rocks and this topic has never been investigated there. In addition, the use of a wild local plant will give insights into the strategies used by organisms adapted to elevated PTM levels. *Berkheya radula* (Harv.) De Wild (Asterales: Asteraceae), for example, naturally occurs throughout the Vredefort Dome area and has been reported to bioaccumulate PTMs from enriched soils (Roebuck *et al.*, 2022). Investigating a food chain with a crop plant grown in ultramafic soil can give insights into the effects of PTMs on crop ecosystems. For example, *Brassica oleracea* var. *acephala* (Brassicaceae) is a leafy vegetable widely consumed by humans, which also has the ability to accumulate PTMs at elevated levels (Morales-Silva *et al.*, 2022). Moving up the food chain, *Acheta domesticus* (Orthoptera: Gryllidae) is a generalist herbivore with a wide range of potential predators and is abundant globally (Amorello *et al.*, 2024), making it a relevant herbivore species

for studies. Lastly, the African stick mantid, *Popa spurca* (Mantodea: Deroplatyidae) is a fitting predatory species as they are widespread within South Africa including the Vredefort Dome area. Moreover, mantids are generalist predators and utilize a wide array of prey (Reitze & Nentwig, 1991; Mebs *et al.*, 2016), and their developmental parameters under South African conditions has only recently been described (Greyvenstein *et al.*, 2021b).

This study describes the transfer of PTMs in a tri-trophic food chain set on ultramafic soil with wild and crop plants. This was accomplished by evaluating the concentrations of PTMs in each trophic level, as well as the bioaccumulation factors between each link. Additionally, the study investigated the effect of naturally elevated PTMs on the development of a secondary consumer (predator). PTM accumulation in mantids was assessed in exuviae, feces, and whole bodies of sub-adult and adults and mantid life history was examined to determine if developmental parameters, such as duration of instars and overall life cycle duration, were affected by PTM exposure.

3.2 Materials and methods

3.2.1 Soil collection

PTM enriched soil was collected from an ultramafic komatiite outcrop (as classified by Lana *et al.*, 2003) in the southeastern part of the Vredefort Dome, in the North-West Province, South Africa (27°08'31.3"S 27°37'24.8"E). The area is part of the oldest and largest meteor impact site on Earth (Whitfield, 2015). The ridges that form the collar of the dome contain ultramafic rocks (Reimold & Koeberl, 2014) that give rise to ultramafic soil. Control soil was collected from the Witpoort Farm (26°28'35"S 26°58'22"E) on the outskirts of Potchefstroom, also in the North-West Province, South Africa. Both soil types were sampled by hand. Vegetation and topsoil was removed by use of a shovel and subsequent loosening of soil with a pickaxe facilitated sampling of only sub-surface soils at a minimum depth of 30 cm. Sampling for each soil type consisted of compositing three subsamples from within a 20m² area. All soils were passed through a 2mm sieve to eliminate plant and animal debris and create some uniformity within soil texture. Soil was then chemically analyzed and thoroughly mixed before being utilized in the study trials.

3.2.2 Model species

3.2.2.1 Producer model species

Berkheya radula and *Brassica oleracea* subsp. *acephala* (DC.) Metz (kale) were chosen for this study as primary producers. The first, also known as diphate or African sun daisy, is a naturally occurring species in the Vredefort Dome area, and has been characterised by Roebuck *et al.* (2022) as a metallophyte capable of bioaccumulating metals. Kale is widely grown as an agricultural crop and consumed globally, and also is known to accumulate PTMs in its leaves (Morales-Silva *et al.*, 2022, 2024).

3.2.2.1 Herbivore model species

The house cricket *Acheta domestica* was selected as the herbivore model in this trophic system. This insect species is widespread throughout the world (Magara *et al.*, 2021), and in recent years has become easy to acquire from local pet shops as they are used for feeding exotic pets. This insect is a detritivore and thus feeds on a wide variety of foods (Inouye *et al.*, 2007). Furthermore, this species has been successfully utilized as prey in previous studies (Greyvenstein *et al.*, 2020, 2021a). House crickets of early nymphal instars [i.e. pinheads (1st instar), extra small (2nd instar)] were acquired from a local pet shop.

3.2.2.2 Predator model species

The African twig mantis *Popa spurca* (Mantodea: Deroplatyidae) served as the third trophic level (predator model). There is virtually no baseline information regarding the species within the South African context. The genus *Popa* is widespread across South Africa, has been successfully reared for research purposes (Greyvenstein *et al.*, 2021b), and is a bioaccumulator of PTMs (Soliman & El-Shazly, 2017).

3.2.3 Experimental procedure

Forty *B. radula* plants were planted in 20 cm deep pots, twenty of which were filled with komatiite soil (henceforth referred to as 'komatiite *Berkheya*' or KB) and the other twenty filled with control soil (henceforth referred to as 'control *Berkheya*' or CB). Forty kale plants were also planted in 20 cm deep pots, twenty of which were filled with komatiite soil (henceforth referred to as 'komatiite Kale' or KK) and the other twenty filled with control soil (henceforth referred to as 'control Kale' or CK) ($\sum n_{\text{plants}} = 20 \text{ KB} + 20 \text{ CB} + 20 \text{ KK} + 20 \text{ CK} = 80$). All plants were grown during springtime for 60 days in pots in a greenhouse, given distilled water three times weekly, kept under controlled temperature of 26 °C, and exposed to natural daylight.

House crickets were starved for two days (to standardise hunger levels) before providing them with the appropriate plant material as food source. The crickets were split in four groups, each fed with plants from the four different treatments: crickets fed on Berkheyas ('crickets *Berkheya*' or CB) grown in the komatiite ('Komatiite crickets *Berkheya*' or KCB), and control soil ('Control Crickets *Berkheya*' or CCB); crickets fed on Kale ('Crickets-Kale' or CK) grown in the komatiite ('Komatiite Crickets Kale' or KCK), and control soil ('Control crickets Kale' or CCK). Each treatment received a fresh 250 mL container of house crickets (around 150 individuals) every two weeks. They were housed and fed in identical 5 L plastic containers (20 cm x 20 cm x 17 cm). *B. radula* leaves were dried at 75°C for 7 days, ground to a powder using a tungsten ring mill, and were fed to house crickets as a paste made from leaf powder and distilled water in the ratio of (1:1). This was done due to the house crickets' refusal to consume raw *B. radula* leaf material in pilot trials, presumably due to high amounts of leaf trichomes. The house crickets did, however, feed on raw kale leaves, haphazardly harvested from each treatment and placed in the respective containers. Hydrogel was given to crickets in the kale treatments as a source of extra hydration. Plant material and hydrogel were replenished weekly for the duration of the life cycle of the mantids, to which house crickets were being fed, to sustain the crickets while mantids fed upon them.

Praying mantids (*Popa spurca*) used for this study were originally bred from wild mantids caught on the outskirts of Potchefstroom. Stock colony females were mated with wild males to produce oothecae that were used within this trial. After an ootheca hatched, the 1st instar mantid nymphs were split in half and assigned to a specific treatment. One half was divided into two groups that fed on crickets fed on Berkheyas ('*Berkheya* mantid' or MB) grown in komatiite ('Komatiite-Mantids-Crickets-Berkheya' or KMB) or control soil ('Control-Mantids-Crickets-Berkheya' or CMB); and the other half was also divided into two groups which fed on crickets fed on Kale ('kale mantids' or MK) grown in komatiite ('Komatiite-Mantids-Crickets-Kale' or KMK) or control soil ('Control-Mantids-Crickets-Kale' or CMK). The total of mantids at the start of the study were as follows: KB mantids = 176, CB mantids = 173, KK mantids = 162, CK mantids = 169.

Mantid individuals were housed in plastic containers (pill holders) of approximately 150 mL until they reached the fifth instar, after which they were moved to a larger 500 mL plastic jar. All jars had a hole in the top with a gauze cover to facilitate airflow, and contained a toothpick (pill holders) or round wooden skewer (jars) to allow for mantids to climb and hang, which is essential during molts. Mantids were housed in a temperature controlled rearing room at 27 ± 1°C and a

photoperiod cycle of 14 L: 10D (Greyvenstein *et al.*, 2021b). Each mantid was fed one cricket and received distilled water mist three times per week for the duration of their life cycle (maximum of 62 weeks). Mantid molted exoskeletons (exuviae) and feces were collected as they developed through instars for chemical analysis. In the case of mortalities, mantid bodies were frozen for the same purpose. Cricket and mantid bodies, as well as mantid exuviae and feces, were dried at 75°C for 48 hours before being ground to a powder for PTM analysis. To evaluate the effects of komatiite PTMs on mantid lifecycles, the number of days between instar changes (shedding of exuviae) were recorded for each mantis, as well as if/when a mantis reached maturity, to compare the developmental cycles between komatiite and control treatments for both *Berkheya* and kale food sources. All mantids in the *Berkheya radula* groups (MB and CB) naturally completed their whole cycle. However, due to time constraints, mantids in the kale trial were euthanized after 210 days. Effective survival rate for this treatment was, therefore, based on the number of mantids that reached the time they were euthanized. The maximum lifespan of a mantis in this study was 432 days. The total duration of the trials were: *Berkheya* trial = 492 days; Kale trial = 270 days.



Figure 1: Schematics of the mantid trials. First to fourth instar *P. spurca* were kept in 150 mL plastic containers with a toothpick (left). Fifth instar onwards were moved to 500 mL jars with a wooden skewer (right). All containers/jars had a mesh top to facilitate airflow.

3.2.4 PTMs detection

Soil samples utilized for ICP-MS analysis consisted of three smaller samples harvested from the total bulk. This was done for both komatiite and control soils alike ($\sum n_{\text{soil}} = 3 \text{ komatiite} + 3 \text{ control} = 6$). Six leaves from one individual were sampled from each species per treatment ($\sum n_{\text{leaves}} = 6 \text{ KB} + 6 \text{ CB} + 6 \text{ KK} + 6 \text{ CK} = 24$). House crickets were sampled in composite, and three samples per treatment were analysed ($\sum n_{\text{crickets}} = 3 \text{ composite CCB} + 3 \text{ composite KCB} + 3 \text{ composite CCK} + 3 \text{ composite KCK} = 12$). Mantids were divided into exuviae, feces, semi-adult body and adult body groups before sampling, also in composite ($\sum n_{\text{mantids}} = 3 \text{ composite exuviae CCB} + 3 \text{ composite feces CCB} + 3 \text{ composite semi-adult whole body CCB} + 3 \text{ composite adult whole body CCB} + 3 \text{ composite exuviae KCB} + 3 \text{ composite feces KCB} + 3 \text{ composite semi-adult whole body KCB} + 3 \text{ composite adult whole body KCB} + 3 \text{ composite exuviae CCK} + 3 \text{ composite feces CCK} + 3 \text{ composite semi-adult whole body CCK} + 3 \text{ composite adult whole body CCK} + 3 \text{ composite exuviae KCK} + 3 \text{ composite feces KCK} + 3 \text{ composite semi-adult whole body KCK} + 3 \text{ composite adult whole body KCK} = 48 \text{ total samples}$).

Samples of 200 mg of each material, from each treatment, were analysed through the 3051A microwave acid digestion method (EPA, 2007). First, the dried samples were blended with 3 ml 32 % hydrochloric acid (HCl) and 9 ml 65 % nitric acid (HNO₃), and the mixture inserted in a Teflon tube. Digestion was achieved by placing the tubes in a Milestone, Ethos UP, Maxi 44 rotor-based microwave digestion system until it reached 1800 MW and 200 °C. Samples were kept under these conditions for another 15 minutes and were then allowed to cool. Sample volumes were adjusted to 50 mL before analyses in an Agilent 7500 series inductively coupled plasma mass spectroscope (ICP-MS), calibrated with standard multi-element solution provided by the manufacturer. The evaluated elements for soil, plants, crickets, and mantids were As, Ba, Co, Cu, Mn, molybdenum (Mo), Ni, Pb, rubidium (Rb), selenium (Se), Sr, vanadium (V), and Zn. Additionally, soil was evaluated for Ca, Mg, and P. Total PTM concentrations were measured for all samples.

Table 1. List of acronyms referring to different trophic levels and their respective treatments

Plants (First trophic level)	
KB	Komatiite <i>Berkheya</i>
CB	Control <i>Berkheya</i>
KK	Komatiite Kale
CK	Control Kale
Crickets (Second trophic level)	
KCB	Komatiite crickets <i>Berkheya</i>
CCB	Control crickets <i>Berkheya</i>
KCK	Komatiite crickets Kale
CCK	Control crickets Kale
Mantids (Third trophic level)	
KMB	Komatiite-mantids-crickets- <i>Berkheya</i>
CMB	Control-mantids-crickets- <i>Berkheya</i>
KMK	Komatiite-mantids-crickets-Kale
CMK	Control-mantids-crickets-Kale

3.2.5 Soil pH

Forty grams of soil was sieved using a 2 mm sieve and added to 100 ml de-ionized water to create a soil/water suspension in the ratio 1:2,5. The suspension was stirred, rested for 50 min, stirred again and rested for 10 min. Then pH readings were carried out using a calibrated XS PC5 digital pH meter. Each sample was analyzed thrice.

3.2.6 Bioaccumulation factor

A bioaccumulation factor (BAF) was calculated for each PTM for every study species within the different treatments, in relation to its previous trophic level. The following equation was used (Infante *et al.*, 2021):

$$\text{BAF} = \frac{\text{Average PTM concentration in organism}}{\text{Average PTM concentration in source}}$$

A BAF value greater than one (1) was considered as bioaccumulation, and below one was considered exclusion. Mantids were divided into sub-adult and adult samples, to assess if bioaccumulation patterns differed in those life stages.

3.2.7 Statistical analysis

Data from PTM analyses were Log (x+1)-transformed to lessen effects of non-normal distributions (Shapiro-Wilk, 5 % significance). Principal component analyses (PCAs) were used for ordination of multivariate data from each trophic level. The statistical designs were applied for each PTM (as a dependent variable) as follows: (1) soil samples: Student's t-test (groups: control, komatiite); (2) plant leaves: Student's t-test (groups: control, komatiite) for *Berkheya* and kale; (3) control crickets (CB) and komatiite crickets (CK): Student's t-test (food treatments: CB, KB; and CK, KK, respectively); (4) *Berkheya* mantids (MB): two-way ANOVA followed by Tukey HSD (factor 1: food treatment, levels: CMB, KMB; factor 2: body parts, levels: exuviae, semi-adult body (SA body), adult body (A body), feces); (5) kale mantids (MK): two-way ANOVA followed by Tukey HSD (factor 1: food treatment, levels: CMK, KMK; factor 2: body parts, levels: exuviae, semi-adult body (SA body), adult body (A body), feces); (6) mantid life cycle (MB and MK): Student's t-test (groups: control, komatiite). The significance level for all tests was set at 5%.

3.3 Results and Discussion

3.3.1 Soil PTM content and pH

The characteristics of the komatiite soil from the Vredefort dome do not allow for classification as typical "serpentine soil" (e.g. Venter *et al.*, 2018). This was mainly due to the ratio of calcium (Ca) and magnesium (Mg). The Ca:Mg ratio of the komatiite soil was approximately two (Appendix A, Table A.1), whereas serpentine soils are known for having a ratio of less than one (Rajakaruna & Boyd, 2014)

Nevertheless, the mafic/ultramafic komatiite study soil still contained more than double the concentration of 11 out of 13 evaluated PTMs, warranting continuation of the study. The mean pH of the control soil was $6,02 \pm 0,14$, while that of komatiite soil was $5,42 \pm 0,41$. PTMs are generally more mobile and available for plant uptake in soils with lower pH (Sintorini *et al.*, 2021; Kicińska *et al.*, 2022). Only Cu within the komatiite soil exceeded the South African guideline threshold of 16 mg/kg (DEA, 2012). It is also worth noting that Mn was relatively close to the guidelines limit (average concentration of 693 mg/kg, whereas the limit is 740 mg/kg). When considering all PTMs simultaneously within both soil treatments, komatiite and control soil samples grouped clearly (Figure 3A). The following PTMs were significantly more abundant in komatiite soil than control soil: As, Ba, Co, Cu, Mn, Mo, Ni, Pb, Rb, Se, Sr, V, and Zn (Appendix A, Table A.1).

3.3.2 *Berkheya* trial

3.3.2.1 Plant material

Berkheya leaves data grouped irrespective of soil treatments, when all PTMs were evaluated together in a PCA (Figure 3B). In terms of individual PTMs, leaves from the KB treatment had significantly higher concentrations of Mn and Sr when compared to CB by Student's t-tests (Appendix A, Table A.2). However, when considering *Berkheya*'s BAFs, CB plants

bioaccumulated five PTMs (Ba, Mn, Rb, Sr, and Zn), while KB bioaccumulated three (Ba, Sr, and Zn) (Figure 2; Appendix A, Table A.5). Therefore, Ba, Sr, and Zn were bioaccumulated regardless of the soil treatments. While Zn is an essential element, it is possible that Ba and Sr were inadvertently taken up by *B. radula* given their similar physicochemical properties to Ca (Waterhouse, 1951; Jovanović *et al.*, 2021). *B. radula* has been observed to perform better in soils with elevated Sr compared to soils with lower Sr (Roebuck *et al.*, 2022). This has also found for the crop plant *Sorghum bicolor* (Wang *et al.*, 2017). Adhikari *et al.* (2022) reported that wild plants sampled from disturbed PTM-elevated sites bioaccumulated less PTMs in their leaves than plants from less disturbed sites, similar to *B. radula* in this study. Overall, the concentrations of PTMs in *B. radula* leaves did not reflect soil total PTM concentrations. Thus, PTM bioaccumulation and subsequently availability of PTMs to the next trophic level seems to be a function of species requirement rather than of soil content.

3.3.2.2 House crickets

House cricket data grouped according to the *Berkheya* treatment they were fed in the PCA (Figure 3D). House crickets from the KB treatment had higher concentrations of Ba, whereas crickets from the CB treatment had higher Pb and V, according to Student's t-tests (Appendix A, Tables A.3). The BAF calculation revealed that KB and CB house crickets bioaccumulated the same six PTMs: Co, Cu, Mo, Pb, Se, and Zn (Figure 2; Appendix A, Table A.5). Thus, CCB and KCB crickets only bioaccumulated one PTM that was also bioaccumulated in their food source, which was Zn. Bioaccumulation of the same PTMs between the treatments might, as with *Berkheya*, reflect biological requirements toward these specific PTMs rather than a passive transfer. Most of the bioaccumulated PTMs are in fact essential elements (Co, Cu, Mo, Se, and Zn) (Bánfalvi, 2011), while Pb is non-essential (So *et al.*, 2023). It is intriguing that Pb, being a non-essential and highly toxic PTM, was not bioaccumulated by *Berkheya*, but still was bioaccumulated by house crickets (even though in small concentrations). Morales-Silva *et al.* (2024) and Ali *et al.* (2019) also reported Pb bioaccumulation in insects, with no apparent cause, despite the concentrations in their food sources. Hence for the time being this remains unresolved.

3.3.2.3 Praying mantids

3.3.2.3.1 PTM concentrations

Berkheya mantid data grouped according to their body parts rather than feeding treatments (Figure 3F). Co, Se, and Zn, all essential elements, were significantly influenced by the interaction between body parts and food treatments in the ANOVA tests, while Cu, Mo, Mn, Ni, Pb, Rb, Sr, and V were only a function of mantis body parts (regardless of the soil treatment; Figure 4; Appendix A, Table A.7, A.8). Cu, Mo, Mn, and Ni are essential elements, while Pb, Rb, Sr, and V are non-essential, suggesting that most PTM concentrations were dependent on the biology of *P. spurca* rather than their availability in food. However, when the concentrations were dependent on different body parts under the food treatments, Co, Se, and Zn were generally higher in feces and lower in exuviae, with MKB feces having higher Co than MCB, and MCB exuviae having higher Se than MKB. Previous studies have indicated that insects can excrete PTMs within both feces and exuviae when exposed to elevated PTM levels. Przybylowicz *et al.* (2003) observed excretion of Ni in both feces and exuviae of *C. pardalina*, Wu *et al.* (2020) found Cu and Cd excretion in feces of Black Soldier Fly larvae (Diptera: Stratiomyidae), and Lindqvist and Block (1994) reported Cd excretion with exuviae of the grasshopper *Omocestus viridulus* (Orthoptera: Acrididae). Mantids have also been observed to excrete toxic substances in feces, like tetrodotoxin (Mebs *et al.*, 2016), while no PTM excretion has been reported until this study.

When PTMs were only influenced by the “body part” factor, Mn, Rb, and V were generally higher in feces, while Mo and Pb were higher in both exuviae and feces. Sr was higher in adult bodies, Cu was lower in exuviae, and Ni was lower in adult bodies. Cu is an essential element within

insects that plays an important role in insects' defense reactions (Hrdina & Iatsenko, 2022). Previous studies with Sr absorption are scarce, however, it has been found in the eye material of *Copium teucarii* (Hemiptera: Tingidae), which was attributed to uptake in placestead of Ca (Garcia-Guinea *et al.*, 2011). The interactions for Ni could simply be attributed to excretion at non-significant levels due to low concentration.

3.3.2.3.2 PTMs bioaccumulation

3.3.2.3.2.1 Sub-adult and adult bodies

CMB sub-adult mantids bioaccumulated the most PTMs (i.e. As, Ba, Cu, Mo, Ni, Rb, Se, Sr, and V) of any species within this study, while KMB sub-adult mantids bioaccumulated seven PTMs, which were the same as those from CMB with the exception of As and Rb. The PTMs As, Ba, Ni, Rb, Sr, and V were bioaccumulated by CMB sub-adult mantids but not by their food source (CCB house crickets). Similarly, KMB sub-adults bioaccumulated Ni, Rb, Sr, and V – which also were not bioaccumulated by KCB house crickets. In both mantid treatments, Co, Pb, and Zn were generally excluded through feces (Figure 4, Appendix A, Table A.4, A.7, A.8).

CMB adult mantids bioaccumulated Ba, Cu, Mo, Ni, Se, Sr, and V, and KB adult mantids bioaccumulated Cu, Mo, Ni, Sr, and V. Thus, CMB mantids excluded Rb and As. The former was excreted by all mantids within their feces, and the latter were already present in negligible concentrations in sub-adults (Figure 4, Appendix A, Table A.4, A.7, A.8). KMB mantids excluded Rb and Se from sub-adults to adults. The former, as previously shown, was higher within feces, and the latter, which was lower in exuviae of KMB mantids than in CMB mantids, was most probably excreted at low concentrations within feces. This suggests that the life stage of mantids reflected distinctive demands and/or tolerance toward different PTMs. In addition, sex of the mantids could potentially also have had an influence (Neupane *et al.*, 2023; Teder & Kaasik, 2023) although it was not assessed within this study.

3.3.2.3.2.2 Mantid developmental parameters

The KMB treatment started with 176 *P. spurca* individuals and 23 reached adulthood (13.07% survival rate), of which three individuals reached adulthood at the eighth instar (13%), eleven individuals at the ninth instar (47.8%), and nine at the tenth instar (39.1%). The CB treatment started with 173 individuals and 13 reached adulthood (7.51% survival rate), of which one individual reached adulthood at the eighth instar (7.7%), eight individuals at the ninth instar (61.5%), and four individuals at the tenth instar (30.8%). Overall, KMB mantids required more time between instar changes than CMB, with several significant differences the average period spent in each instar phase were compared: first ($p=0.02$), second ($p=0.0001$), fourth ($p=0.005$) and seventh ($p=0.01$) (Appendix A, Table A.9.). Comparing the mean duration of each life stage of mantids from this trial to that of *P. spurca* from Greyvenstein *et al.* (2020) revealed that no one treatment followed a regular life cycle more closely than another. Greyvenstein *et al.* (2020) reported an average lifespan of 332 days, which is considerably longer than both KMB and CMB mantids. This could possibly be attributed to genetic differences rather than our treatments, which is evident when considering differences in total eggs per ootheca, hatch rate, and sex ratio compared to what was reported in their study. Three instar changes were significantly longer and one significantly shorter in KMB mantids compared to CMB mantids. The only one shorter in KMB mantids was the 7th instar, which is the instar leading up to maturation and generally has the longest duration (Greyvenstein *et al.*, 2020). Although CMB mantids followed this norm, KMB mantids did not, potentially pointing to further effects on development. CMB mantids bioaccumulated more PTMs when compared to KMB mantids (Figure 4; Appendix A, Table A.7, A.8). However, the small differences in the developmental rates between KMB and CMB mantids can hardly be attributed to the PTMs, especially at the relatively low PTM concentrations recorded in this study. Thus, if one aims to look further into

the influence of PTMs on mantid development, it must be done with higher and artificial exposure to PTMs.

3.3.2.3.3 Trophic transfer

By evaluating the food chain for the *Berkheya* trial (i.e. soil, *B. radula*, house crickets, and mantids) we observed that, in total, more PTMs were bioaccumulated in the control *Berkheya* treatment (28) than in komatiite treatment (20). None of the PTMs were bioaccumulated at all trophic levels. Ba and Sr, which are not known as essential elements, were bioaccumulated only within CB plants, sub-adult mantids, and adult mantids, and Se (essential element) in CCB house crickets, CMB sub-adult mantids, and CMB adult mantids. Cu and Mo (essential elements) were bioaccumulated in house crickets, sub-adult mantids, and adult mantids of both treatments. As an important note, Mn (which was close to the DEA soil limit) and As, the former essential and the latter non-essential elements – and both higher in komatiite soil, were completely excluded from all organisms in the KB treatment.

One of the potential explanation of these findings is the saturation effect, in which plants (and primary and secondary consumers in this case) have higher efficiency in taking up PTMs when exposed to low PTM concentrations compared to exposure to higher levels (Dudka and Miller, 1999). Adhikari *et al.* (2022) discovered a similar pattern, in which plants from highly contaminated sites bioaccumulated less PTMs than those from little/no contamination. Similarly, along a pollution gradient, Nummelin *et al.* (2007) observed lower Cd levels in *Gerris argentatus* (Hemiptera: Gerridae) insects close to the point of pollution compared to a reference site. Regarding exclusion PTMs, Naikoo *et al.* (2019) and Naikoo *et al.* (2021) also noted Pb and Cd, respectively, were excluded along a tri-trophic food chain and reported excretion of PTMs through feces and/or exuviae. Although the majority of literature is focused on polluted soils or foods (Joubert *et al.*, 2024), Soliman and El-Shazly (2017) focused on PTM concentration in wild sampled plants, grasshoppers, and praying mantids along a pollution gradient. Their plants did not bioaccumulate PTMs from soil, but grasshoppers and praying mantids did bioaccumulate more PTMs as pollution increased. This shows that, as with our study, bioaccumulation may skip a trophic level and reappear in the next, depending on the biological requirements of the species involved.

3.3.2 Kale trial

3.3.2.1 Plant material

Kale data grouped according to their respective soil treatments in the PCA (Figure 3C). Student's t-tests evidenced that Mn, Se and Sr, were higher within KK samples when compared to CK, whereas Cu, Mo, and V showed the opposite result (Appendix A, Table A.2). The BAF calculations for kale plants revealed that CK plants bioaccumulated a total of four PTMs (Ba, Mo, Rb, and Sr), while KK plants only bioaccumulated one (Sr) (Figure 2; Appendix A, Table A.5). Thus, kale exposed to elevated PTM soil excluded all with the exception of Sr, which may have been inadvertently bioaccumulated (Jovanović *et al.*, 2021). *Brassica* species (including *B. oleracea*) have previously been reported to effectively exclude PTMs, keeping the concentration of PTMs virtually the same in exposed and non-exposed plants (Gisbert *et al.*, 2006; Marchiol *et al.*, 2004). This suggests that, as with the *Berkheya* trail, the uptake of PTMs was defined by the plant species, rather than the total concentration in the soils.

3.3.2.2 House crickets

House cricket samples also grouped according to food treatment in the PCA (Figure 3E). CCK house crickets had higher concentrations of As, Co, Mo, Mn, Se, and Zn, according to the Student's t-tests. Sr was the only PTM higher in KCK house crickets (Appendix A, Table A.3, A.6).

BAF calculations revealed that CCK house crickets bioaccumulated six PTMs (As, Cu, Pb, Se, V, and Zn), and KCK house crickets bioaccumulated seven PTMs (As, Cu, Mo, Pb, Rb, Se, and Zn) (Figure 2; Appendix A, Table A.5). PTMs bioaccumulated by both CCK and KCK house crickets were As, Cu, Pb, Se, and Zn. Both KCK and CCK house crickets excluded all of PTMs that were bioaccumulated by their food sources from their respective treatments. Interestingly, Sr was among the PTMs excluded by house crickets in both *Berkheya* and kale trials, indicating a possible exclusion mechanism, instead of taking up Sr inadvertently (Waterhouse, 1951; Jovanović *et al.*, 2021). PTMs bioaccumulated by house crickets (without being bioaccumulated by their food source) included non-essential PTMs, which is similar to the results of the *Berkheya* trial. These were V in CCK, and As and Pb in both CCK and KCK house crickets. However, PTMs could have accumulated in exoskeletons to be later excreted with exuviae, although it was not assessed within this study. Inouye *et al.* (2007) reported that more than half of the total Pb body burden within *A. domesticus* was accounted for within its exoskeleton.

3.3.2.3 Praying mantids

3.3.2.3.1 PTM concentrations

MK samples grouped according to body parts rather than the feeding treatment in the PCA (Figure 3G). The ANOVA showed that As, Mo, Sr, and Pb (all non-essential elements) were significantly influenced by the interaction between food treatment and body parts, while Ba, Co, Cu, Mn, Ni, Rb, Se, V, and Zn concentrations were only affected by mantid body parts (Figure 5; Appendix A, Table A.7, A.8). This suggests that most PTM bioaccumulation was again dependent on the biology of *P. spurca*, while some PTMs were also influenced by the concentration available in the food. As, Mo, Sr, and Pb were generally higher in feces than all body parts. Specifically, As was higher in CMK feces than KMK, while Sr was generally higher in all KMK body parts than CMK. As for Mo, exuviae also presented higher levels, although with no difference between food treatments. Finally, Pb was also higher in exuviae and sub-adult body parts from those in CMK. For PTMs significantly influenced by body parts alone, Ba, Co, Ni, Rb, V, and Zn were all higher in feces than other body parts.

Different individual PTMs had significant “food treatment x body parts” interactions in the *Berkheya* trial compared to the kale trial. Also, these PTMs within *Berkheya* mantids were all essential elements, while those within the kale trial were all non-essential. This indicates that the transfer of essential elements were hampered along higher levels of the kale food chain model.

3.3.2.3.2 Sub-adult and adult bodies

Sub-adult CMK bioaccumulated five PTMs (Ba, Co, Cu, Pb, and Se) while KMK sub-adults bioaccumulated four (As, Cu, Se, and Sr). Co, Cu, and Se are essential elements, while Ba and Pb are not (Freeman *et al.*, 2007; Bánfalvi, 2011). Previous literature, although scarce, reported a positive relationship between increased Pb exposure and Pb mantid body content (Zhang *et al.*, 2009, Soliman & El-Shazly, 2017), contradicting results of this experiment. Co and Se were bioaccumulated even though CMK crickets did not bioaccumulate these PTMs, while As, V, and Zn bioaccumulated by the crickets were excluded. KMK sub-adult mantids bioaccumulated As and Sr, both non-essential PTMs. This might indicate that, different from the house crickets, these mantids tolerate Sr as this PTM was bioaccumulated by them but not by their food source. The PTMs Mo, Pb, Rb, and Zn were excluded from KMK mantids’ sub-adult bodies.

There was a decrease in the number of bioaccumulated PTMs from sub-adults to adult mantids. CMK mantid adults excluded Co, Ba, and Pb, whereas KMK adult mantids excluded Sr. Previous studies have also reported differences in PTM concentration at various life stages of insects. Diener *et al.* (2015) observed generally lower bioaccumulation of Cd, Pb, and Zn in adult Black Soldier Flies (Diptera: Stratiomyidae) than in both larval and pupal stages, which was found for low, medium, and high exposures. Lindqvist and Block (1995) evidenced that the beetle, *Tenebrio*

molitor (Coleoptera: Tenebrionidae), excluded the highest levels of Cd during the process of metamorphosis. It is interesting that an insect that develops through complete metamorphosis (beetle) behaves similarly to one with an incomplete metamorphosis (mantid). Although most excluded PTMs were non-essential ones, the reasons why specific PTMs were excluded by KMK and CMK mantids while others were accumulated remain to be clarified.

3.3.2.4 Mantid developmental parameters

The KMK treatment started with 162 *P. spurca* individuals and CMK with 169 individuals. Of these, 89 KMK mantids survived until euthanasia (54.9% survival rate), while 86 CMK mantids reached this stage (50.9% survival rate). In contrast to the *Berkheya* trial, CMK mantids required more time between instar changes than KMK mantids on average, especially at first instar ($p=0.04$), third ($p=0.0001$), fourth ($p=0.0006$), and fifth instar ($p=0.02$) (Appendix A, Table A.9). There was no significant difference between the lifespan of KMK and CMK mantids ($p=0.3$). Comparing the mean duration of each mantid life stage from this trial to that of *P. spurca* from Greyvenstein *et al.* (2020) suggests again that no one treatment closely followed a regular life cycle. The decreased lifespan could rather be a product of genetic variability, but it is important to consider that these mantids were not allowed to complete their full, natural life cycle.

As already mentioned, Sr was bioaccumulated by both sub-adult and adult KMK. This element is not considered essential but has been suggested to have a biological beneficial effect on some species (Wang *et al.*, 2017). Pb is a non-essential PTM shown to have adverse effects on insect biology (Xu *et al.*, 2009; Mogren *et al.*, 2010; Lidman *et al.*, 2020; Morales-Silva *et al.*, 2022). Pb levels bioaccumulated in sub-adult bodies of CMK are comparatively much lower than toxic levels cited by previous literature (Xu *et al.*, 2009; Mogren *et al.*, 2010; Lidman *et al.*, 2020; Morales-Silva *et al.*, 2022). Once again, based on the relatively low PTM concentrations in this study's mantids, it should be unlikely to find major developmental differences between CMK and KMK. However, some significant differences were observed regarding specific instar changes between the two mantis treatments. PTMs accumulated in this trial could be related to such differences, but for definitive results further investigation would need to be done with much higher PTM concentrations.

3.3.2.5 Trophic transfer

Looking at the kale model food chain, instances of bioaccumulation were generally smaller than in the *Berkheya* trials, although the komatiite treatment (15 bioaccumulation instances) also presented less bioaccumulation than the control (17 bioaccumulation instances). Like with the *Berkheya* trial, none of the PTMs were bioaccumulated at all trophic levels. However, some PTMs were bioaccumulated at all but one trophic level. Cu and Se (both essential elements) were bioaccumulated in house crickets and sub-adult and adult mantids of both treatments. However, As was bioaccumulated in these same compartments but only in the komatiite treatment. Since As is a non-essential PTM it is important to note its potential to be bioaccumulated by both primary and secondary consumers (house crickets and mantids) within this study, even at relatively low concentrations. Similarly, van der Fels-Klerx *et al.* (2016) indicated that *Tenebrio molitor* (Coleoptera: Tenebrionidae) bioaccumulated As at multiple exposure levels, including their control exposure.

Both *Berkheya* and kale trials presented a pattern of facultative bioaccumulation along the trophic levels in this study, showing that trophic transfer of PTMs in ultramafic environments is not a direct function of their concentrations in soil.

3.4 Conclusion

This study evaluated the transfer of natural concentrations of PTMs from ultramafic (komatiite) soil to a three-trophic level food chain model starting from the wild plant (*B. radula*) and the crop plant kale as producers, to a primary consumer (house cricket *A. domesticus*), and to a secondary consumer (African stick mantid, *P. spurca*). It unveiled patterns of trophic transfer and developmental influences on secondary consumers. Both *B. radula* and kale grown in komatiite soil had generally higher PTM concentrations in their leaves than in control soil, especially Mn and Sr. Within the trophic chain with *B. radula* as producer, Cu, Se, Sr, and Mo showed the potential to be bioaccumulated through plants and house crickets to mantids. This was consistent in both komatiite and control treatments. These elements are essential elements, whose concentrations throughout this trophic system were non-toxic. Sr is not known as an essential element; however, in limited literature it has been suggested to promote the health of plants (Wang *et al.*, 2017).

In the trophic chain with a crop plant (kale) as food source, As from the komatiite treatment showed higher potential to be bioaccumulated through plants and house crickets to mantids than in the control treatment. As is a non-essential element which is toxic to plants, animals, and humans. This is an important result to note because, although As concentrations were below permissible limits within this study, its bioaccumulation through a food chain from crop plants could point to potential risk for humans.

PTM concentrations of the different treatments were not portrayed in house crickets. Within the *Berkheya* trial house crickets from both control and komatiite soils bioaccumulated the same PTMs (Co, Cu, Mo, Pb, Se, and Zn). In the kale trial, most of the PTMs bioaccumulated by control and komatiite house crickets remained constant between the two treatments (As, Cu, Pb, Se, and Zn). Additionally, all of the PTMs bioaccumulated by the previous trophic level (kale plants) were excluded from both control and komatiite house crickets.

Generally, the PTM concentrations within mantids were not influenced by food treatment but rather by their “body parts” (exuviae, sub-adult body, feces, or adult body). In cases where food treatment did influence PTM concentrations, mantids excreted these either via exuviae, feces, and/or both. This was the case for mantids from both *Berkheya* and kale treatments.

In conclusion, the sub-lethal yet elevated PTM levels present within the komatiite soil in this study generally did not impact the tri-trophic transfer of PTMs through either a wild or crop plant food chain. Overall, both bioaccumulation and transfer of PTMs were instead dependent on the species’ biological particularities rather than soil PTM concentration. However, some key results warrant future research, especially including comparison with anthropogenically PTM-enriched soils (e.g. mine tailings) and the use of accumulator species in one or more trophic levels.

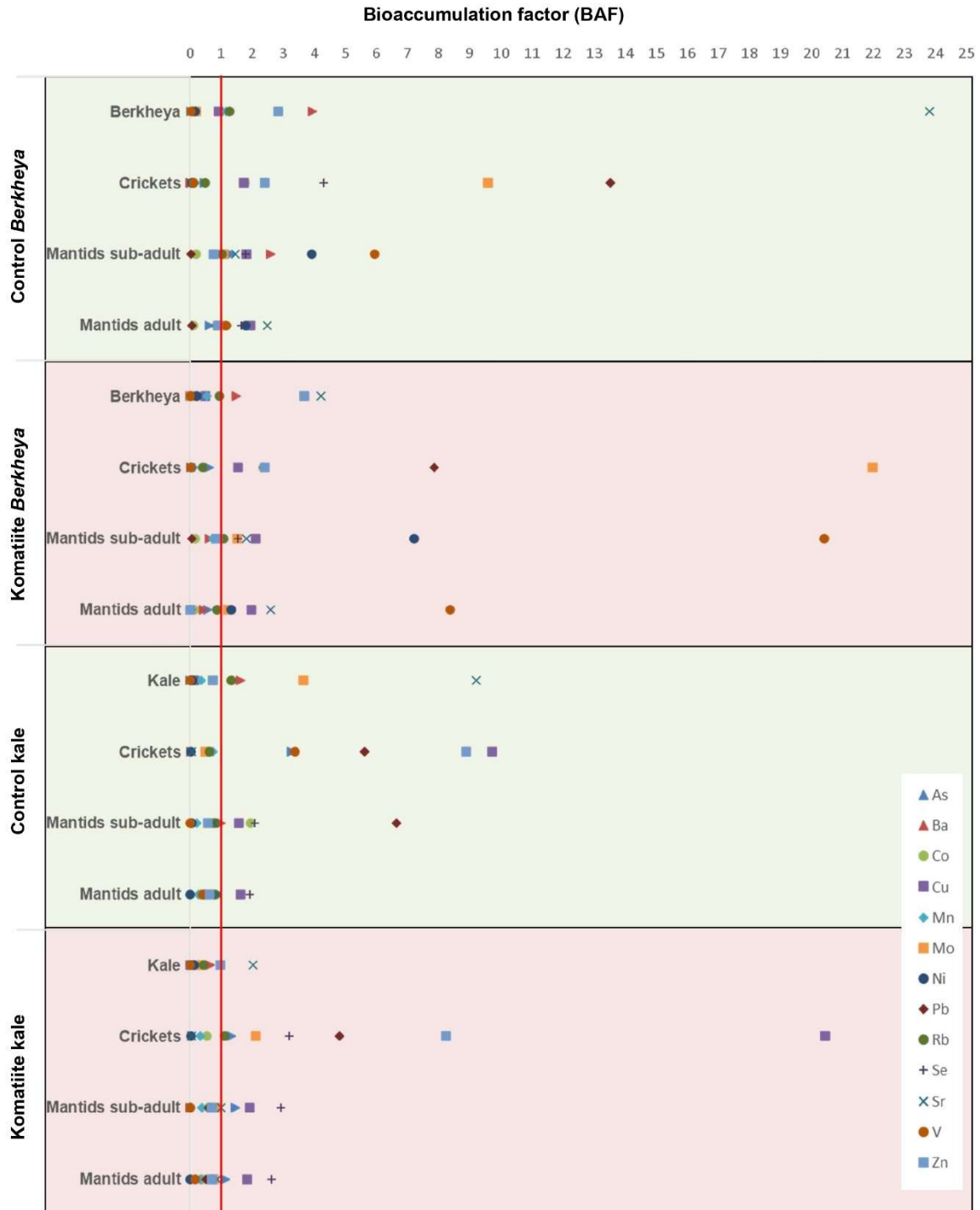


Figure 2. Bioaccumulation factors of each PTM within every organism in their respective treatments. BAF > 1 (1 is indicated by red line) was considered bioaccumulation of the specific PTM.

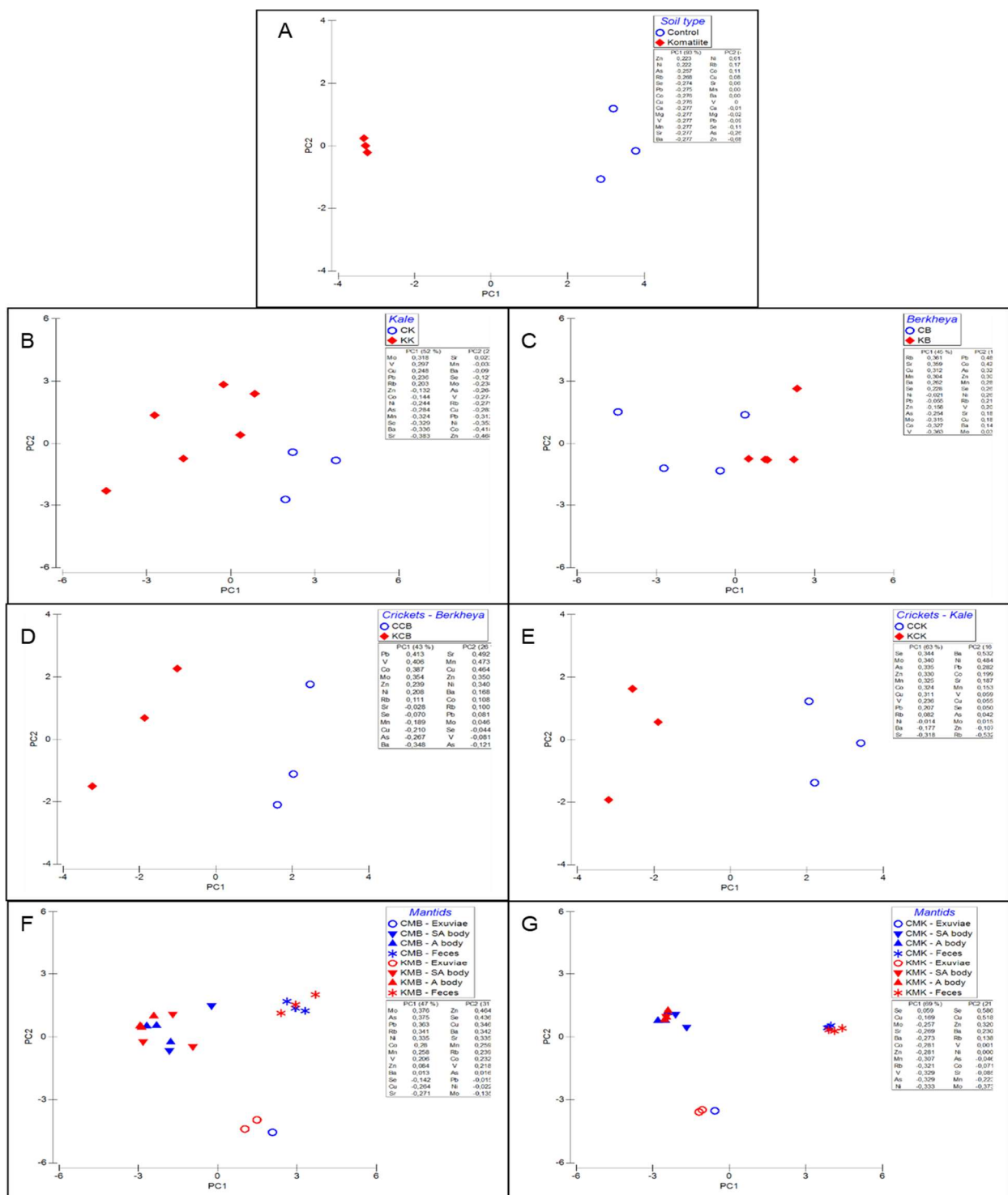
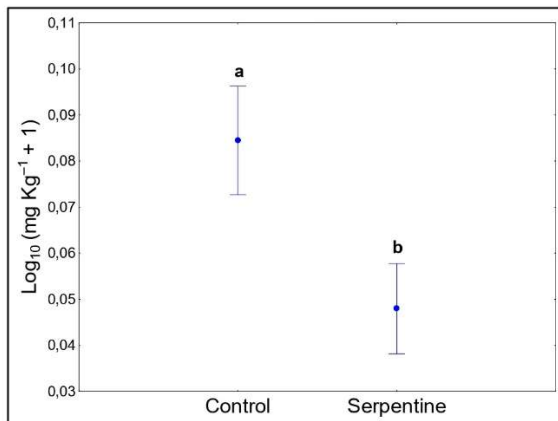
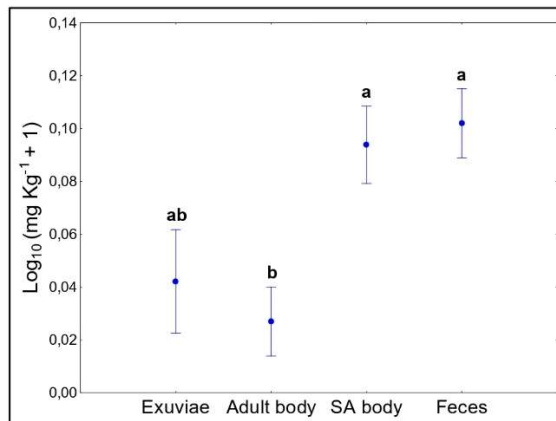


Figure 3. Data biplot ordination from each study compartment. A: Control/Komatiite (soil type), B: CB/SB = control/Komatiite *Berkheya*, C: CK/KK = control/Komatiite kale, D: CCB/KCB = control cricket *Berkheya*/Komatiite cricket *Berkheya*, E: CCK/KCK = control cricket Kale/Komatiite cricket Kale, F: CMB/KMB = control mantids *Berkheya*/Komatiite mantids *Berkheya*, G: CMK/KMK = control mantids Kale/Komatiite mantids Kale.

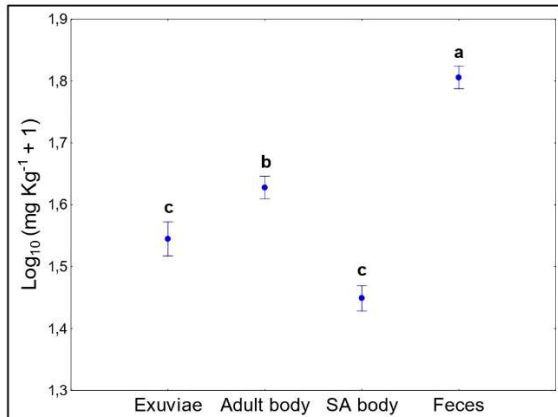
V (soil types)



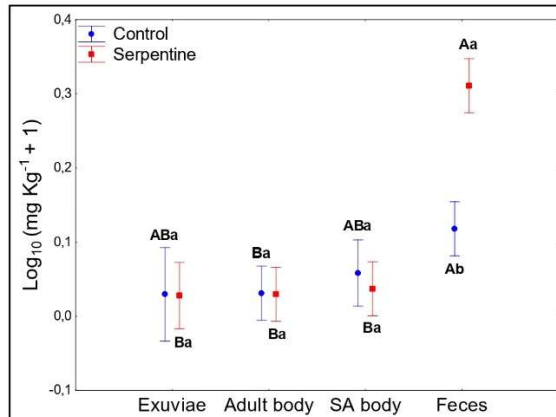
V (body parts)



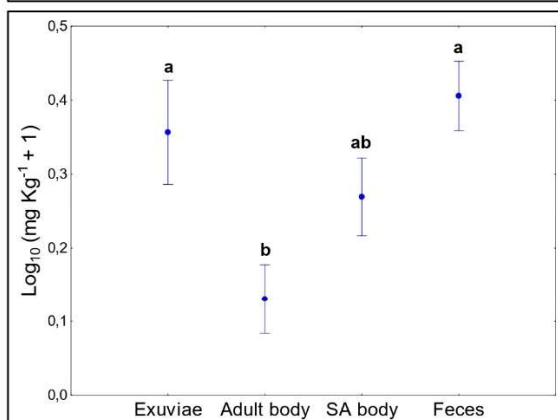
Mn (body parts)



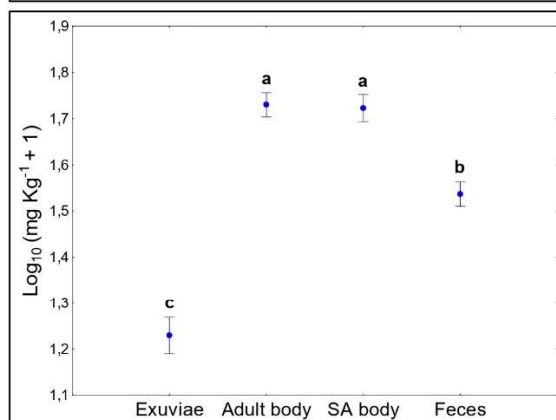
Co (soil type x body parts)



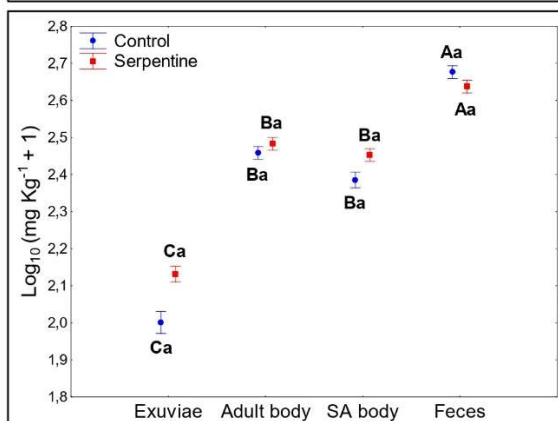
Ni (body parts)



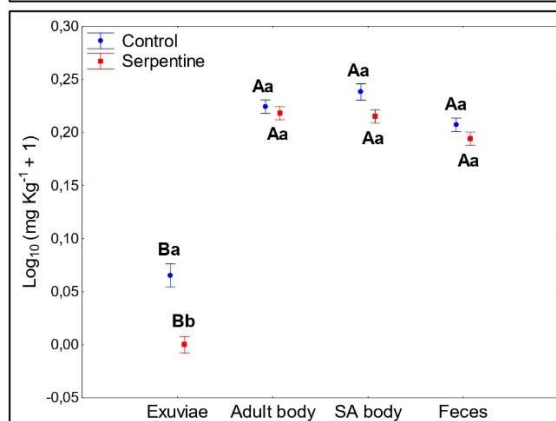
Cu (body parts)



Zn (soil types x body parts)



Se (soil types x body parts)



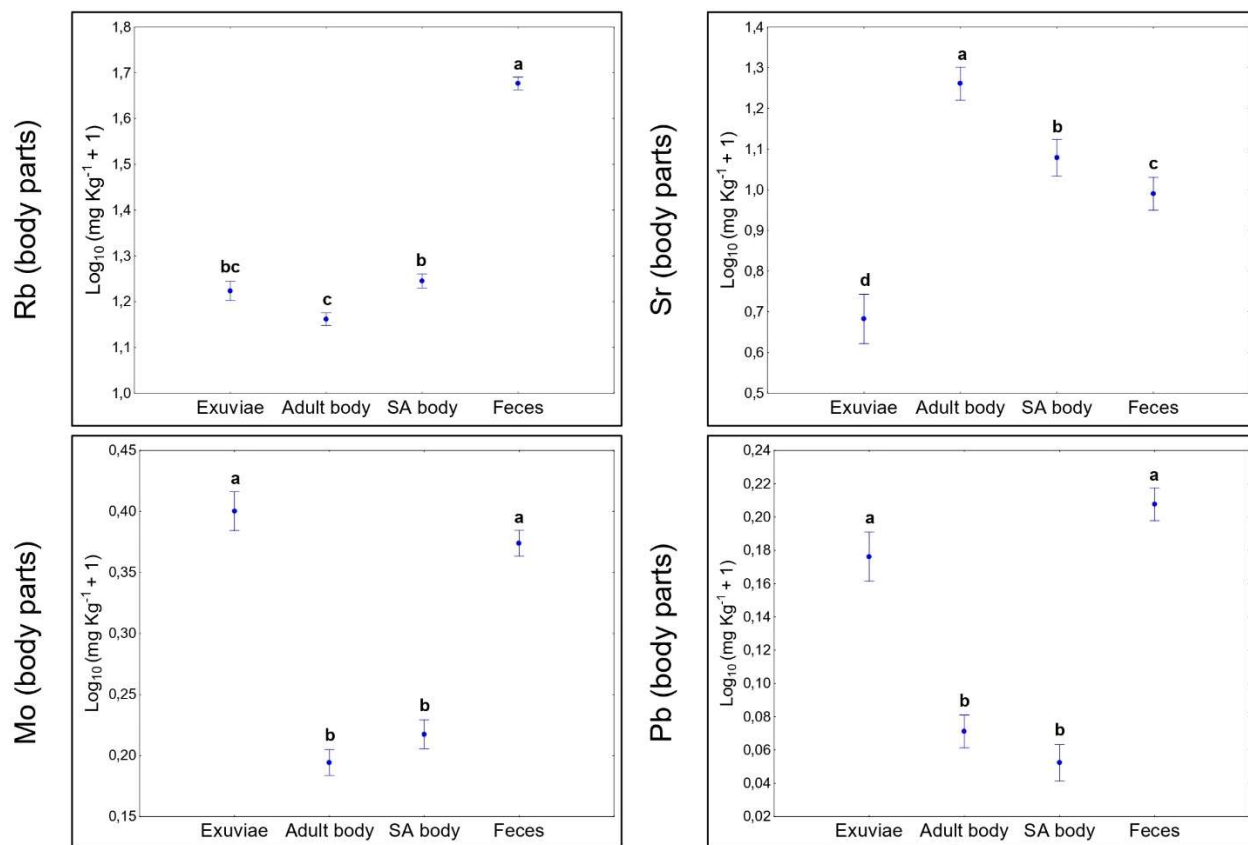
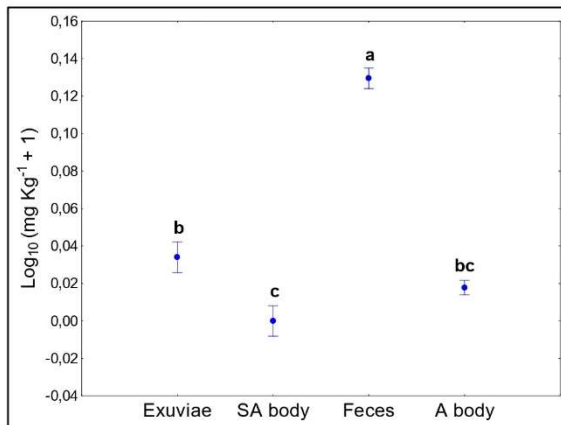
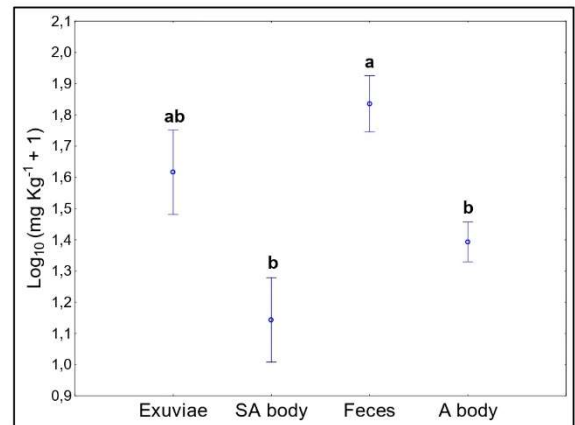


Figure 4. Mean $\pm$ SD of PTMs' transformed concentrations from mantids fed on crickets fed on *Berkheya* under significant influence of the factors "food treatment," "body parts," and "soil type x body parts". Different uppercase letters: significant differences ($p < 0.05$) among body parts for the same soil type. Different lowercase letters (for "soil type x body parts"): significant differences between soil treatments for the same body part. Different lowercase letters (for "soil type" and "body parts"): significant differences between soil type and body parts.

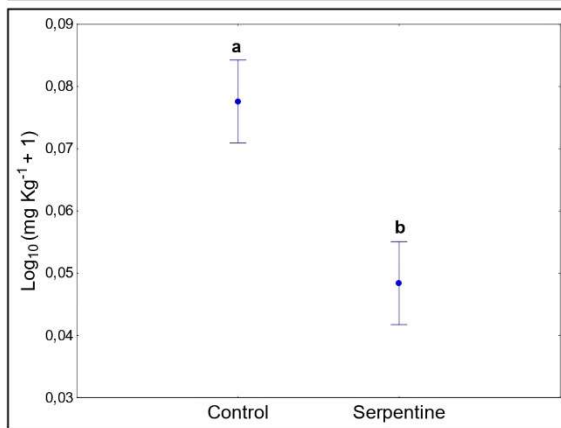
V (body parts)



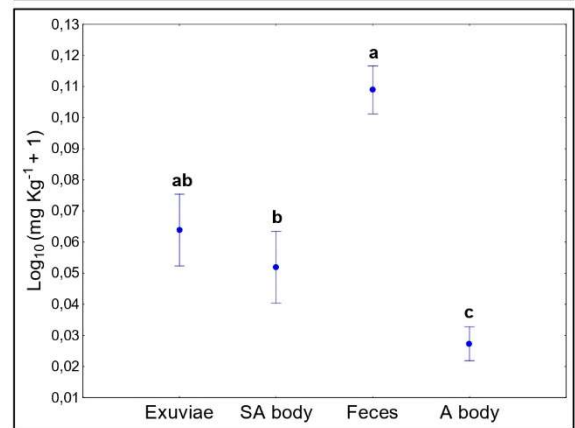
Mn (body parts)



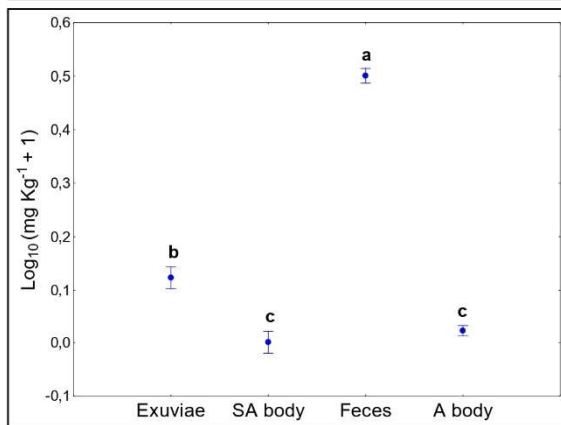
Co (soil type)



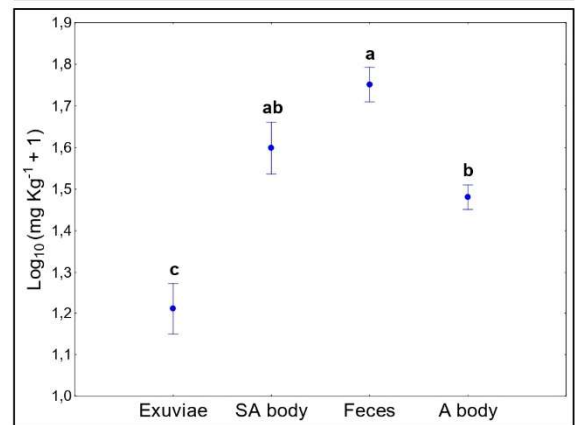
Co (body parts)



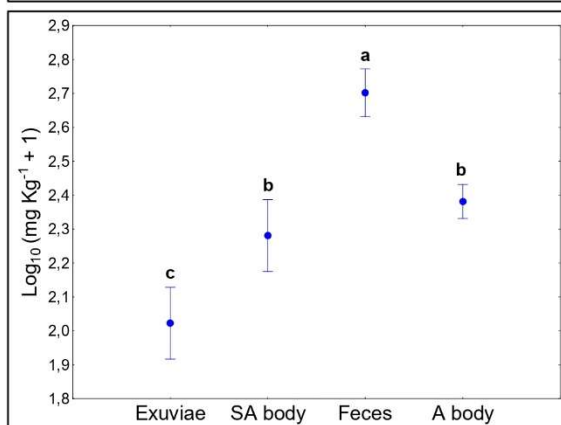
Ni (body parts)



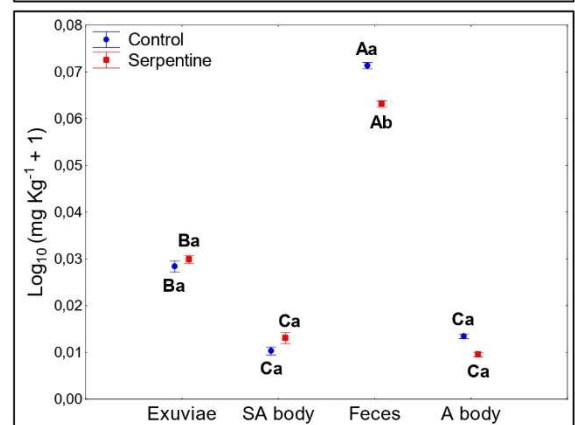
Cu (body parts)



Zn (body parts)



As (soil types x body parts)



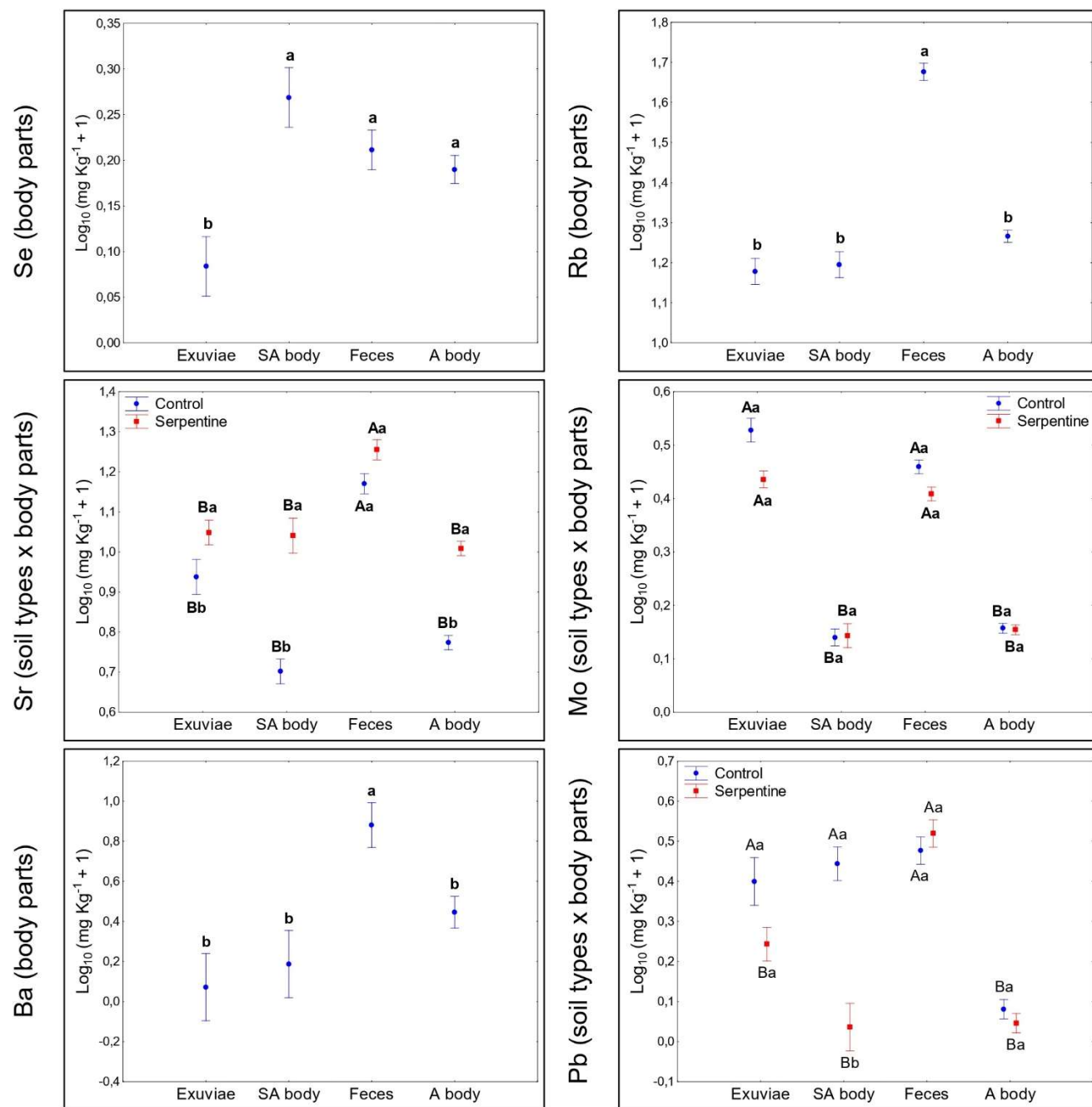


Figure 5. Mean $\pm$ SD of PTMs' transformed concentrations from mantids fed on crickets fed on kale under significant influence of the factors "food treatment," "body parts," and the interaction "soil type x body parts". Different uppercase letters: significant differences ($p < 0.05$) among body parts for the same soil type. Difference lowercase letters (for "soil type x body parts"): significant differences between soil treatments for the same body part. Different lowercase letters (for "soil type" and "body parts"): significant differences between soil type and body parts.

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Appendix A:

Table A.1. Mean $\pm$ SD of elements concentration (mg kg⁻¹) from the study's soil samples. Different letters (a and b) indicate significant differences between soil types (p < 0.05).

Soil type	Ca	Mg	P	V	Mn	Co	Ni	Cu
Control	638,064 $\pm$ 108,31 a	647,16 $\pm$ 42,91 b	238,605 $\pm$ 11,30 a	31,714 $\pm$ 1,83 b	181,397 $\pm$ 31,57 a	6,791 $\pm$ 0,72 b	28,908 $\pm$ 1,97 a	16,047 $\pm$ 1,67 b
Komatiite	5113,304 $\pm$ 258,68 b	2582,834 $\pm$ 43,69 a	260,295 $\pm$ 47,79 a	70,771 $\pm$ 0,79 a	693,058 $\pm$ 4,80 b	15,011 $\pm$ 0,25 a	25,859 $\pm$ 0,32 a	37,602 $\pm$ 0,54 a
	Zn	As	Se	Rb	Sr	Mo	Ba	Pb
Control	47,387 $\pm$ 7,04 a	1,893 $\pm$ 0,28 b	0,843 $\pm$ 0,09 b	24,498 $\pm$ 3,30 b	8,374 $\pm$ 1,33 b	0,302 $\pm$ 0,10 b	42,141 $\pm$ 4,19 b	4,044 $\pm$ 0,23 b
Komatiite	35,593 $\pm$ 2,77 a	2,613 $\pm$ 0,01 a	2,386 $\pm$ 0,23 a	40,095 $\pm$ 1,76 a	94,472 $\pm$ 6,60 a	0,644 $\pm$ 0,09 a	161,155 $\pm$ 0,28 a	8,643 $\pm$ 0,34 a

Table A.2. Mean $\pm$ SD of elements concentration (mg kg⁻¹) from the study's plant samples. Different letters (a and b) indicate significant differences between soil types (p < 0.05).

<i>Berkheya</i> treatment	V	Mn	Co	Ni	Cu	Zn	As
Control	0,558 $\pm$ 0,21 a	213,424 $\pm$ 55,21 b	0,419 $\pm$ 0,39 a	4,868 $\pm$ 1,90 a	14,728 $\pm$ 4,51 a	135,169 $\pm$ 20,32 a	0,051 $\pm$ 0,01 a
Komatiite	0,242 $\pm$ 0,08 a	377,991 $\pm$ 136,37 a	0,215 $\pm$ 0,11 a	5,853 $\pm$ 3,51 a	18,381 $\pm$ 43,81 a	130,546 $\pm$ 50,76 a	0,042 $\pm$ 0,01 a
	Se	Rb	Sr	Mo	Ba	Pb	
Control	0,094 $\pm$ 0,04 a	31,113 $\pm$ 15,45 a	199,380 $\pm$ 123,57 b	0,057 $\pm$ 0,06 a	165,887 $\pm$ 32,21 a	0,331 $\pm$ 0,07 a	
Komatiite	0,177 $\pm$ 0,10 a	38,236 $\pm$ 14,25 a	398,912 $\pm$ 48,84 a	0,022 $\pm$ 0,03 a	240,745 $\pm$ 66,35 a	0,341 $\pm$ 0,08 a	
<i>Kale</i> treatment	V	Mn	Co	Ni	Cu	Zn	As
Control	0,027 $\pm$ 0,01 a	64,363 $\pm$ 24,35 b	0,181 $\pm$ 0,05 a	2,325 $\pm$ 0,97 a	2,392 $\pm$ 0,39 a	34,937 $\pm$ 9,22 a	0,010 $\pm$ 0,01 a
Komatiite	0,000 $\pm$ 0,00 b	118,988 $\pm$ 39,78 a	0,149 $\pm$ 0,07 a	3,265 $\pm$ 2,20 a	1,037 $\pm$ 0,54 b	34,401 $\pm$ 23,30 a	0,016 $\pm$ 0,01 a
	Se	Rb	Sr	Mo	Ba	Pb	
Control	0,000 $\pm$ 0,00 b	32,422 $\pm$ 6,11 a	77,065 $\pm$ 14,64 b	1,100 $\pm$ 0,36 a	68,761 $\pm$ 12,72 a	0,051 $\pm$ 0,01 a	
Komatiite	0,098 $\pm$ 0,08 a	17,685 $\pm$ 16,68 a	191,018 $\pm$ 77,87 a	0,231 $\pm$ 0,15 b	107,060 $\pm$ 53,04 a	0,031 $\pm$ 0,01 a	

Table A.3. Mean $\pm$ SD of PTM concentration (mg/kg) from the cricket samples. Different letters (a and b) indicate significant differences between soil types ($p < 0.05$).

Treatment	V	Mn	Co	Ni	Cu	Zn	As
<i>Berkheya</i>							
Control	0,063 $\pm$ 0,01a	34,746 $\pm$ 2,32a	0,734 $\pm$ 0,12a	0,2546 $\pm$ 0,27a	25,675 $\pm$ 1,33a	323,848 $\pm$ 7,64a	0,023 $\pm$ 0,00a
Komatiite	0,006 $\pm$ 0,01b	37,836 $\pm$ 1,76a	0,508 $\pm$ 0,06a	0,123 $\pm$ 0,20a	28,202 $\pm$ 1,58a	315,584 $\pm$ 21,66a	0,026 $\pm$ 0,00a
	Se	Rb	Sr	Mo	Ba	Pb	
Control	0,407 $\pm$ 0,02a	15,619 $\pm$ 0,75a	6,616 $\pm$ 1,32a	0,543 $\pm$ 0,04a	1,548 $\pm$ 0,81a	4,478 $\pm$ 0,61a	
Komatiite	0,415 $\pm$ 0,02a	15,514 $\pm$ 0,55a	7,05 $\pm$ 0,56a	0,475 $\pm$ 0,03a	4,206 $\pm$ 0,61b	2,682 $\pm$ 0,56b	
	V	Mn	Co	Ni	Cu	Zn	As
<i>Kale</i>							
Control	0,09 $\pm$ 0,01a	47,726 $\pm$ 1,57a	0,111 $\pm$ 0,01a	0,096 $\pm$ 0,06a	23,261 $\pm$ 1,36a	310,481 $\pm$ 7,20a	0,036 $\pm$ 0,00a
Komatiite	0,038 $\pm$ 0,07a	39,255 $\pm$ 2,89b	0,08 $\pm$ 0,01b	0,129 $\pm$ 0,14a	21,186 $\pm$ 0,68a	282,997 $\pm$ 9,17b	0,021 $\pm$ 0,00b
	Se	Rb	Sr	Mo	Ba	Pb	
Control	0,385 $\pm$ 0,01a	20,065 $\pm$ 2,02a	5,248 $\pm$ 0,51a	0,54 $\pm$ 0,01a	1,766 $\pm$ 0,94a	0,285 $\pm$ 0,14a	
Komatiite	0,312 $\pm$ 0,01b	19,491 $\pm$ 0,60a	9,924 $\pm$ 1,19b	0,487 $\pm$ 0,01b	7,066 $\pm$ 5,24a	0,148 $\pm$ 0,01a	

Table A.4. Mean $\pm$ SD of PTM concentrations (mg kg⁻¹) from *Popa spurca* evaluated in the study.

<i>Berkheya</i> treatment							
Control	V	Mn	Co	Ni	Cu	Zn	As
Exuviae	0,116	37,696	0,07	1,566	15,43	99,250	0,041
SA body	0,378 $\pm$ 0,23	26,986 $\pm$ 6,11	0,145 $\pm$ 0,07	0,996 $\pm$ 0,350	46,575 $\pm$ 4,29	242,906 $\pm$ 31,15	0,031 $\pm$ 0,01
A body	0,075 $\pm$ 0,01	41,641 $\pm$ 1,40	0,074 $\pm$ 0,02	0,452 $\pm$ 0,53	49,967 $\pm$ 2,79	286,394 $\pm$ 9,40	0,014 $\pm$ 0,00
Feces	0,329 $\pm$ 0,09	60,807 $\pm$ 1,06	0,312 $\pm$ 0,01	1,686 $\pm$ 0,54	37,935 $\pm$ 6,00	473,598 $\pm$ 15,45	0,039 $\pm$ 0,01
	Se	Rb	Sr	Mo	Ba	P	
Exuviae	0,162	17,188	3,754	1,566	0,000	0,494	
S body	0,732 $\pm$ 0,12	16,225 $\pm$ 0,11	9,62 $\pm$ 2,50	0,586 $\pm$ 0,05	4,035 $\pm$ 2,75	0,109 $\pm$ 0,05	
body	0,676 $\pm$ 0,03	13,416 $\pm$ 0,36	16,47 $\pm$ 4,40	0,551 $\pm$ 0,08	1,775 $\pm$ 1,05	0,278 $\pm$ 0,04	
Feces	0,612 $\pm$ 0,04	46,869 $\pm$ 4,40	1,44 $\pm$ 0,06	1,439 $\pm$ 0,06	3,240 $\pm$ 0,67	0,686 $\pm$ 0,01	
Komatiite	V	Mn	Co	Ni	Cu	Zn	As
Exuviae	0,088 $\pm$ 0,01	30,799 $\pm$ 1,22	0,066 $\pm$ 0,02	1,013 $\pm$ 0,07	16,495 $\pm$ 1,17	135,252 $\pm$ 21,59	0,029 $\pm$ 0,00
SA body	0,131 $\pm$ 0,13	27,698 $\pm$ 3,15	0,089 $\pm$ 0,03	0,885 $\pm$ 0,95	59,668 $\pm$ 19,19	282,775 $\pm$ 18,94	0,0218 $\pm$ 0,01
A body	0,054 $\pm$ 0,01	41,272 $\pm$ 0,48	0,071 $\pm$ 0,01	0,315 $\pm$ 0,16	55,830 $\pm$ 6,16	303,084 $\pm$ 0,36	0,015 $\pm$ 0,00
Feces	0,206 $\pm$ 0,05	65,814 $\pm$ 11,03	1,063 $\pm$ 0,33	0,146 $\pm$ 0,34	26,667 $\pm$ 0,95	424,904 $\pm$ 10,09	0,040 $\pm$ 0,00
	Se	Rb	Sr	Mo	Ba	P	
Exuviae	0,000	14,417 $\pm$ 1,76	3,880 $\pm$ 0,28	1,46 $\pm$ 0,14	1,820 $\pm$ 1,91	0,507 $\pm$ 0,02	
SA body	0,641 $\pm$ 0,03	17,006 $\pm$ 1,70	12,770 $\pm$ 1,89	0,724 $\pm$ 0,19	2,572 $\pm$ 1,52	0,152 $\pm$ 0,12	
A body	0,652 $\pm$ 0,01	13,640 $\pm$ 1,21	18,109 $\pm$ 1,55	0,579 $\pm$ 0,05	3,010 $\pm$ 1,84	0,086 $\pm$ 0,02	
Feces	0,564 $\pm$ 0,03	46,35 $\pm$ 3,19	9,959 $\pm$ 0,52	1,297 $\pm$ 0,10	3,052 $\pm$ 0,57	0,547 $\pm$ 0,12	

Kale treatment							
Control	V	Mn	Co	Ni	Cu	Zn	As
Exuviae	0,086	39,52	0,202	0,415	16,530	101,18	0,067
SA body	0,000	11,10 ± 0,64	0,218 ± 0,17	0,004 ± 0,01	36,700 ± 1,98	178,57 ± 3,97	0,024 ± 0,00
A body	0,037 ± 0,02	12,85 ± 0,46	0,039 ± 0,01	0,000	37,679 ± 1,01	190,364 ± 6,08	0,029 ± 0,00
Feces	0,313 ± 0,01	63,17 ± 0,82	0,305 ± 0,00	2,085 ± 0,080	58,253 ± 0,32	488,354 ± 8,60	0,178 ± 0,01
	Se	Rb	Sr	Mo	Ba	P	
Exuviae	0,222	13,72	7,668	2,372	0,392	1,51	
SA body	0,807 ± 0,03	16,007 ± 1,71	4,052 ± 0,60	0,380 ± 0,05	1,780 ± 2,06	1,892 ± 1,13	
A body	0,744 ± 0,04	16,486 ± 0,19	4,695 ± 0,72	0,400 ± 0,01	1,295 ± 1,75	0,134 ± 0,02	
Feces	0,674 ± 0,03	48,635 ± 3,11	13,829 ± 1,19	1,881 ± 0,04	4,850 ± 0,78	2,00 ± 0,23	
Komatiite	V	Mn	Co	Ni	Cu	Zn	As
Exuviae	0,076 ± 0,01	41,079 ± 0,24	0,116 ± 0,02	0,253 ± 0,17	14,093 ± 0,61	107,593 ± 0,03	0,071 ± 0,00
SA body	0,000	14,997	0,048	0,000	40,820	202,250	0,031
A body	0,006 ± 0,01	12,761 ± 0,71	0,031 ± 0,00	0,000	39,265 ± 1,35	197,209 ± 11,67	0,024 ± 0,00
Feces	0,383 ± 0,03	71,989 ± 1,19	0,265 ± 0,01	2,257 ± 0,19	52,727 ± 0,73	516,880 ± 11,21	0,157 ± 0,00
	Se	Rb	Sr	Mo	Ba	P	
Exuviae	0,203 ± 0,02	14,470 ± 1,24	10,187 ± 0,06	1,728 ± 0,01	0,000	0,752 ± 0,08	
SA body	0,909	13,480	9,976	0,391	0,000	0,087	
A body	0,815 ± 0,06	14,437 ± 1,56	8,613 ± 1,04	0,370 ± 0,02	0,904 ± 0,07	0,077 ± 0,01	
Feces	0,581 ± 0,018	44,414 ± 0,36	17,004 ± 0,67	1,562 ± 0,05	9,073 ± 2,21	2,347 ± 0,65	

Table A.5. Bioaccumulation factors of each PTM within every organism in their respective treatments. BAF > 1 was considered bioaccumulation of the specific PTM (indicated by colored values).

		Compartment	V	Mn	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Mo	Ba	Pb	Number of bio-accumulation instances	
																Per compartment	Treatment total
Berkheya treatment	Control	Berkheya	0,02	1,18	0,06	0,17	0,92	2,85	0,03	0,11	1,27	23,81	0,19	3,94	0,08	5	28
		Crickets	0,11	0,16	1,75	0,05	1,74	2,40	0,46	4,31	0,50	0,03	9,58	0,01	13,52	6	
		Mantids sub-adult	5,96	0,78	0,20	3,91	1,81	0,75	1,33	1,80	1,04	1,45	1,08	2,61	0,02	9	
		Mantids adult	1,18	1,20	0,10	1,78	1,95	0,88	0,62	1,66	0,86	2,49	1,02	1,15	0,06	8	
	Komatiite	Berkheya	0,00	0,55	0,01	0,23	0,49	3,67	0,02	0,07	0,95	4,22	0,03	1,49	0,04	3	21
		Crickets	0,03	0,10	2,36	0,02	1,53	2,42	0,61	2,34	0,41	0,02	21,98	0,02	7,86	6	
		Mantids sub-adult	20,40	0,73	0,18	7,20	2,12	0,90	0,85	1,55	1,10	1,81	1,53	0,61	0,06	7	
		Mantids adult	8,36	0,01	0,14	1,34	1,98	0,00	0,57	0,03	0,88	2,60	1,22	0,44	0,03	5	
		V	Mn	Co	Ni	Cu	Zn	As	Se	Rb	Sr	Mo	Ba	Pb			
Kale treatment	Control	Kale	0,00	0,35	0,03	0,08	0,15	0,74	0,01	0,00	1,32	9,20	3,64	1,63	0,01	4	17
		Crickets	3,38	0,74	0,62	0,04	9,72	8,89	3,27	-	0,62	0,07	0,49	0,03	5,61	6	
		Mantids sub-adult	0,00	0,23	1,96	0,05	1,58	0,58	0,72	2,09	0,80	0,77	0,71	1,01	6,64	5	
		Mantids adult	0,41	0,27	0,35	0,00	1,62	0,61	0,87	1,93	0,82	0,89	0,63	0,73	0,47	2	
	Komatiite	Kale	0,00	0,17	0,01	0,13	0,03	0,97	0,01	0,04	0,44	2,02	0,36	0,66	0,00	1	15
		Crickets	-	0,33	0,54	0,04	20,44	8,23	1,32	3,20	1,10	0,05	2,10	0,07	4,80	7	
		Mantids sub-adult	0,00	0,38	0,60	0,00	1,93	0,71	1,47	2,91	0,69	1,01	0,80	0,00	0,58	4	
		Mantids adult	0,15	0,33	0,39	0,00	1,85	0,70	1,15	2,61	0,74	0,87	0,76	0,13	0,52	3	

Table A.6. Pairwise comparisons between cricket groups fed on *Berkheya* and kale grown in control and komatiite soils. Bold: significant difference ($p < 0.05$).

PTM	Kale house crickets		<i>Berkheya</i> house crickets	
	Control x Komatiite		Control x Komatiite	
	t-value	p-value	t-value	p-value
V	-1,34	0,251	-6,65	0,003
Mn	-4,27	0,013	1,82	0,143
Co	-3,35	0,029	-3,02	0,039
Ni	0,32	0,764	-0,66	0,544
Cu	-2,43	0,072	2,13	0,100
Zn	-4,05	0,016	-0,66	0,546
As	-11,12	0,000	1,09	0,339
Se	-10,17	0,001	0,48	0,653
Rb	-0,42	0,697	-0,19	0,861
Sr	7,13	0,002	0,59	0,586
Mo	-5,41	0,006	-2,43	0,072
Ba	1,44	0,224	3,50	0,025
Pb	-1,77	0,151	-3,62	0,022

Table A.7. ANOVA table from the 2-way analysis performed with mantid PTMs. *Berkheya* mantids = mantids fed with crickets fed with *B. radula* leaves, Kale mantids = mantids fed with crickets fed with kale leaves. Bold: significant ($p < 0.05$).

<i>Berkheya</i> mantids	Treatment				Body part				Treatment x body part			
	df	MS	F	p	D f	MS	F	p	df	MS	F	p
V	1	0,006	5,68	0,0346	3	0,007	7,17	0,0052	3	0,001	1,42	0,2842
Mn	1	0,001	0,27	0,6140	3	0,121	59,86	0,0000	3	0,002	1,07	0,3976
Co	1	0,008	9,23	0,0103	3	0,044	52,71	0,0000	3	0,014	16,84	0,0001
Ni	1	0,014	1,04	0,3285	3	0,082	6,18	0,0088	3	0,001	0,08	0,9703
Cu	1	0,001	0,28	0,6060	3	0,188	45,32	0,0000	3	0,010	2,31	0,1285
Zn	1	0,009	10,34	0,0074	3	0,218	246,42	0,0000	3	0,005	5,85	0,0106
As	1	0,000	3,33	0,0930	3	0,000	22,18	0,0000	3	0,000	1,33	0,3097
Se	1	0,003	26,72	0,0002	3	0,026	222,58	0,0000	3	0,001	4,90	0,0190
Rb	1	0,001	0,68	0,4267	3	0,316	273,85	0,0000	3	0,001	1,13	0,3769
Sr	1	0,019	9,27	0,0102	3	0,217	105,38	0,0000	3	0,003	1,22	0,3461
Mo	1	0,000	0,00	0,9741	3	0,051	76,70	0,0000	3	0,001	1,37	0,2978
Ba	1	0,040	1,16	0,3023	3	0,120	3,46	0,0512	3	0,050	1,43	0,2834
Pb	1	0,002	3,79	0,0754	3	0,030	51,89	0,0000	3	0,002	3,31	0,0573

Kale mantids	Treatment				Body part				Treatment x body part			
	df	MS	F	p	df	MS	F	p	df	MS	F	p
V	1	0,000	0,00	0,9726	3	0,019	107,39	0,0000	3	0,001	3,00	0,0617
Mn	1	0,006	0,12	0,7372	3	0,396	8,14	0,0016	3	0,007	0,14	0,9366
Co	1	0,003	9,61	0,0069	3	0,009	25,27	0,0000	3	0,001	1,72	0,2034
Ni	1	0,000	0,17	0,6827	3	0,331	294,66	0,0000	3	0,001	0,85	0,4868
Cu	1	0,001	0,13	0,7242	3	0,202	19,57	0,0000	3	0,002	0,17	0,9161
Zn	1	0,002	0,32	0,5805	3	0,322	48,50	0,0000	3	0,001	0,17	0,9161
As	1	0,000	10,89	0,0045	3	0,004	3202,12	0,0000	3	0,000	18,46	0,0000
Se	1	0,000	0,01	0,9192	3	0,016	5,78	0,0071	3	0,000	0,14	0,9368
Rb	1	0,004	1,25	0,2796	3	0,289	102,13	0,0000	3	0,001	0,33	0,8022
Sr	1	0,148	77,46	0,0000	3	0,150	78,66	0,0000	3	0,014	7,23	0,0028
Mo	1	0,005	10,16	0,0057	3	0,160	319,84	0,0000	3	0,002	4,02	0,0262
Ba	1	0,002	0,03	0,8584	3	0,550	7,33	0,0026	3	0,079	1,06	0,3939

Pb	1	0,077	21,84	0,0003	3	0,262	74,01	0,0000	3	0,035	9,79	0,0007
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Table A.8. Tukey tests table showing significant interactions for “Treatments” x “Body parts” of mantid PTMs. Bold values: significant difference ($p < 0.05$). *Berkheya* mantids = mantids fed with crickets fed with *B. radula* leaves, Kale mantids = mantids fed with crickets fed with kale leaves.

<i>Berkheya</i> mantids						
PTM	Factor	Interaction		Factor	Interaction	
Co	Treatment		p	Body part		p
	Control	Exuviae x SA body	0,9890	Exuviae	Control x Komatiite	1,0000
	Control	Exuviae x A body	1,0000	SA body	Control x Komatiite	0,9896
	Control	Exuviae x Feces	0,2295	A body	Control x Komatiite	1,0000
	Control	SA body x A body	0,9607	Feces	Control x Komatiite	0,0002
	Control	SA body x Feces	0,3863			
	Control	A body x Feces	0,0461			
	Komatiite	Exuviae x SA body	1,0000			
	Komatiite	Exuviae x A body	1,0000			
	Komatiite	Exuviae x Feces	0,0002			
	Komatiite	SA body x A body	1,0000			
	Komatiite	SA body x Feces	0,0002			
	Komatiite	A body x Feces	0,0002			
Zn	Treatment		p	Body part		p
	Control	Exuviae x SA body	0,0002	Exuviae	Control x Komatiite	0,0532
	Control	Exuviae x A body	0,0002	SA body	Control x Komatiite	0,2952
	Control	Exuviae x Feces	0,0002	A body	Control x Komatiite	0,9629
	Control	SA body x A body	0,2165	Feces	Control x Komatiite	0,7429
	Control	SA body x Feces	0,0002			

	Control	A body x Feces	0,0002			
	Komatiite	Exuviae x SA body	0,0002			
	Komatiite	Exuviae x A body	0,0002			
	Komatiite	Exuviae x Feces	0,0002			
	Komatiite	SA body x A body	0,8962			
	Komatiite	SA body x Feces	0,0003			
	Komatiite	A body x Feces	0,0008			
Se	Treatment		p	Body part		p
	Control	Exuviae x SA body	0,0002	Exuviae	Control x Komatiite	0,0062
	Control	Exuviae x A body	0,0002	SA body	Control x Komatiite	0,3543
	Control	Exuviae x Feces	0,0002	A body	Control x Komatiite	0,9955
	Control	SA body x A body	0,8444	Feces	Control x Komatiite	0,8078
	Control	SA body x Feces	0,1119			
	Control	A body x Feces	0,5578			
	Komatiite	Exuviae x SA body	0,0002			
	Komatiite	Exuviae x A body	0,0002			
	Komatiite	Exuviae x Feces	0,0002			
	Komatiite	SA body x A body	1,0000			
	Komatiite	SA body x Feces	0,3373			
	Komatiite	A body x Feces	0,2094			
Kale mantids						
PTM	Factor	Interaction		Factor	Interaction	
As	Treatment		p		Treatment	p
	Control	Exuviae x SA body	0,0002	Exuviae	Control x Komatiite	0,9552
	Control	Exuviae x A body	0,0002	SA body	Control x Komatiite	0,5530

	Control	Exuviae x Feces	0,0002	A body	Control x Komatiite	0,0008
	Control	SA body x A body	0,0715	Feces	Control x Komatiite	0,0002
	Control	SA body x Feces	0,0002			
	Control	A body x Feces	0,0002			
	Komatiite	Exuviae x SA body	0,0002			
	Komatiite	Exuviae x A body	0,0002			
	Komatiite	Exuviae x Feces	0,0002			
	Komatiite	SA body x A body	0,1710			
	Komatiite	SA body x Feces	0,0002			
	Komatiite	A body x Feces	0,0002			
Sr	Treatment		p	Body part		p
	Control	Exuviae x SA body	0,0081	Exuviae	Control x Komatiite	0,4711
	Control	Exuviae x A body	0,0500	SA body	Control x Komatiite	0,0004
	Control	Exuviae x Feces	0,0056	A body	Control x Komatiite	0,0002
	Control	SA body x A body	0,5010	Feces	Control x Komatiite	0,3124
	Control	SA body x Feces	0,0002			
	Control	A body x Feces	0,0002			
	Komatiite	Exuviae x SA body	1,0000			
	Komatiite	Exuviae x A body	0,9442			
	Komatiite	Exuviae x Feces	0,0019			
	Komatiite	SA body x A body	1,0000			
	Komatiite	SA body x Feces	0,0110			
	Komatiite	A body x Feces	0,0002			
Mo	Treatment		p	Body part		p
	Control	Exuviae x SA body	0,0002	Exuviae	Control x Komatiite	0,0605

	Control	Exuviae x A body	0,0002	SA body	Control x Komatiite	1,0000
	Control	Exuviae x Feces	0,2083	A body	Control x Komatiite	1,0000
	Control	SA body x A body	0,9767	Feces	Control x Komatiite	0,1642
	Control	SA body x Feces	0,0002			
	Control	A body x Feces	0,0002			
	Komatiite	Exuviae x SA body	0,0002			
	Komatiite	Exuviae x A body	0,0002			
	Komatiite	Exuviae x Feces	0,8694			
	Komatiite	SA body x A body	0,9997			
	Komatiite	SA body x Feces	0,0002			
	Komatiite	A body x Feces	0,0002			
Pb	Treatment	Body part	p	Body part	Treatment	p
	Control	Exuviae x SA body	0,9983	Exuviae	Control x Komatiite	0,4285
	Control	Exuviae x A body	0,0029	SA body	Control x Komatiite	0,0009
	Control	Exuviae x Feces	0,9433	A body	Control x Komatiite	1,0000
	Control	SA body x A body	0,0002	Feces	Control x Komatiite	0,9837
	Control	SA body x Feces	0,9984			
	Control	A body x Feces	0,0002			
	Komatiite	Exuviae x SA body	0,1512			
	Komatiite	Exuviae x A body	0,0161			
	Komatiite	Exuviae x Feces	0,0023			
	Komatiite	SA body x A body	1,0000			
	Komatiite	SA body x Feces	0,0002			
	Komatiite	A body x Feces	0,0002			

Table A.9. Duration (mean $\pm$ sd, in days) of each life stage of *Popa spurca* in the *Berkheya* (KB and CB) and kale (KK and CK) trials under laboratory conditions. Bold: significant difference ($p < 0.05$).

Life stage	Mean duration (days)		Pairwise tests		Mean duration (days)		Pairwise tests	
	KB	CB	t-value	p-value	KK	CK	t-value	p-value
1 st Instar	20,57 $\pm$ 8,19	18,03 $\pm$ 5,17	2,43	0,02	18,12 $\pm$ 7,8	21,63 $\pm$ 15,44	-2,05	0,04
2 nd Instar	35,33 $\pm$ 9,5	28,9 $\pm$ 8,06	4	0,00	24,14 $\pm$ 9,58	25,48 $\pm$ 14,08	-0,78	0,44
3 rd Instar	23,35 $\pm$ 9,7	21,71 $\pm$ 11,99	0,71	0,48	34,28 $\pm$ 9,6	40,05 $\pm$ 10,32	-3,92	0,00
4 th Instar	30,79 $\pm$ 12,52	24,17 $\pm$ 9,43	2,93	0,00	39,34 $\pm$ 11,96	45,27 $\pm$ 10,07	-3,49	0,00
5 th Instar	38,42 $\pm$ 21,33	37,54 $\pm$ 14,49	0,22	0,83	42,28 $\pm$ 9,29	47,02 $\pm$ 12,3	-2,38	0,02
6 th Instar	52,4 $\pm$ 17,8	55,55 $\pm$ 15,88	-0,75	0,46	37,73 $\pm$ 7,94	40,63 $\pm$ 8,33	-0,87	0,40
7 th Instar	48,75 $\pm$ 19,78	67,28 $\pm$ 22,82	-2,98	0,01	43,67 $\pm$ 8,02	-	-	-
8 th Instar	47,12 $\pm$ 18,37	49,4 $\pm$ 18,57	-0,38	0,71	-	-	-	-
9 th Instar	34,11 $\pm$ 15,38	32,5 $\pm$ 13,67	0,19	0,86	-	-	-	-
Life cycle duration	207,03 $\pm$ 123,2	182,52 $\pm$ 137,64	1,18	0,24	156,43 $\pm$ 69,14	164,29 $\pm$ 73,05	0,54	0,35

Chapter 4 - Effect of sub-lethal metal concentrations in leaves on house cricket olfaction and gustation.

Abstract

Potentially toxic metals and metalloids (PTMs) are globally abundant and, whether from natural or anthropogenic origin, will persist within ecosystems for long periods. Both essential metals/elements (such as Cu, Fe, Mn, Ni, and Zn) and non-essential ones (such as Al, As, Cd, Pb, and Hg) can be toxic to organisms at certain concentrations. PTMs in soils of terrestrial ecosystems often result in their uptake by plants, potentially posing the risk that these PTMs will be transferred to higher trophic levels, and ultimately affect human health through the food plants in our diet. There is a lack of research on the movement of PTMs across multiple trophic levels (e.g. soils to plants to primary consumer to secondary consumer) and their effects on consumers, which is important to fully understand the consequences of elevated soil PTM levels. The ability of insects to distinguish between PTM-contaminated and non-contaminated food is a starting point to understand PTM uptake and transfer to primary and secondary consumers. This study tested the ability of house crickets (*Acheta domesticus*) to distinguish between PTM-contaminated and non-contaminated food plants in diphate (*Berkheya radula*), a native grassland species, and kale (*Brassica oleracea* var. *acephala*), a food plant, and whether plant metabolites play a role in this relationship. Olfaction tests revealed no preference between kale treatments, while female crickets preferred the “smell” of PTM-contaminated *B. radula* leaves and males non-contaminated leaves. Gustation tests revealed no preference by house crickets to any treatment’s plant leaves, and no qualitative differences in plant metabolite profiles were observed. The results suggest PTM levels within plant leaves in this study were under detection limits by house crickets.

4.1 Introduction

Potentially toxic metals and metalloids (PTMs) are found in abundance globally (Shi *et al.*, 2021). Whether from natural or anthropogenic origin, PTMs are incorporated into ecosystems and tend to persist for long periods of time as they cannot be broken down by biological processes (Sarubbo *et al.*, 2015). Although some metals, such as Cu, Fe, Mn, Ni, and Zn, are essential for organisms to function, generally only small quantities are required and excessive amounts may lead to toxicity (Gall *et al.*, 2015; Shafiq *et al.*, 2020). There are also non-essential metals, such as Al, As, Cd, Pb, and Hg, which quickly become toxic when present within ecosystems, even at low concentrations (Gall *et al.*, 2015; Chmielowska-Bąk *et al.*, 2023). Presence and persistence of PTMs in soils of terrestrial ecosystems often result in their uptake by plants, potentially posing the risk that these PTMs will be transferred to higher trophic levels, and ultimately affect human health through the food plants in our diet (Pajević *et al.*, 2018; Letshwenyo & Mokokwe, 2020). Herbivores that form part of humans’ diet, such as cattle, sheep, and poultry, could also take up PTMs, leading to adverse health effects in humans (Phillips & Tudoreanu, 2011; Roggeman *et al.*, 2013). While transfer of PTMs between two trophic levels is relatively well studied, there is a lack of research on the movement of PTMs across multiple trophic levels (e.g. soils to plants to primary consumer to secondary consumer), which is important to fully understand the consequences of elevated soil PTM levels (Joubert *et al.*, 2024). The ability of insects to distinguish between PTM-contaminated and non-contaminated food is a starting point to understand PTM uptake and transfer to primary and secondary consumers. Distinguishing between PTM-contaminated and non-contaminated food by insects (primary consumers) includes

their ability to “smell” (olfaction) and/or “taste” (gustation) PTMs in food, and whether that has an impact on their feeding choices.

Insects rely on olfactory information to, among other functions, locate potential sexual partners, avoid predation, and find suitable sources of food (Renou, 2014; Basu *et al.*, 2021). Odorants bind to olfactory receptor neurons (ORNs), transforming the chemical signals to neural signals which are sent to the insects’ brains to be processed. These ORNs are located on olfactory sensilla, which are found most abundantly on insects’ antennae but can also occur on the labial palps and/or maxillary palps (Renou, 2014), which are protruding appendages near the mouthparts. In short, olfaction is the process of detecting volatile chemical signals typically in motion in the air (i.e., smell). Gustation, on the other hand, involves detecting chemical signals that are non-volatile, thus in solid or liquid state (i.e. taste) (King & Gunathunga, 2023). The gustatory sensilla are also mostly located on mouthparts, antennae, labial palps and/or maxillary palps, and contain gustatory receptor neurons (GRNs) that transform chemical signals to neural signals which are processed by the insect (King & Gunathunga, 2023). Gustation can also help insects identify and avoid toxic substances (Masagué *et al.*, 2020). For instance, when given the choice between plain sucrose solution and solution with lipopolysaccharides (LPS), the fly *Drosophila melanogaster* (Diptera: Drosophilidae) presented significant aversion toward the LPS solution, indicating gustatory awareness of the chemical difference (Soldano *et al.*, 2016). There has also been evidence that PTMs within plants can deter herbivorous insects through gustation. For instance, *Schistocerca americana* (Orthoptera: Acrididae) feeding tempo on *Pteris vittata* (Polypodiales: Pteridaceae) grown in 1.0 mM sodium arsenate was significantly slower compared to *P. vittata* grown in 0.1 mM sodium arsenate (Rathinasabapathi *et al.*, 2007). Also, *Melanotrichus boydi* (Hemiptera: Miridae) feeds on the nickel (Ni) hyperaccumulator *Streptanthus polygaloides* (Brassicales: Brassicaceae), and preferred this food item during a host-choice test, while *Lygus hesperus* (Hemiptera: Miridae) showed aversion to the same food item (Wall & Boyd, 2006). Although some literature suggests preferences for PTMs by certain species (Boyd *et al.*, 2007; Stolpe & Müller, 2016; Glavan *et al.*, 2017), most studied arthropods have shown an aversion toward food containing high levels of PTMs (Behmer *et al.* 2005; Scheirs *et al.* 2006; Gonçalves *et al.*, 2007; Rathinasabapathi *et al.* 2007; Vesik & Reichman, 2009; Kazemi-Dinan *et al.*, 2014; Zidar *et al.*, 2019; Xiao *et al.*, 2022). The topic is, however, still not well explored, as many insect orders remain to be tested.

The house cricket *Acheta domesticus* (Orthoptera: Gryllidae) is an omnivorous detritivore that feeds on an array of materials, including food wastes, paper and cardboard, cloth, plant detritus, other small arthropods, and fresh plant material (Hazelton *et al.*, 2002). Thought to originate from Southwest Asia, house crickets now occur worldwide (Amorello *et al.*, 2024). Very little literature exists on the relationship between house crickets and PTMs. Freeman *et al.* (2007) tested the impact of selenium (Se) on Orthopteran (including *A. domesticus* house crickets) herbivory and subsequent Se concentration in the insects. Their results suggested that house crickets avoid Se treated plant material when given a choice, and those forced to feed on Se treated leaves contained around 20 times more Se in their bodies than those fed non-Se treated plants. Being widespread globally, abundantly available through their sale from pet stores, a generalist omnivore, and a food source for various animals and humans (Siddiqui *et al.*, 2024), house crickets are part of an important link in the food chain of many terrestrial ecosystems. In the event that PTMs are taken up by house crickets, there is potential of transferring those PTMs to the next trophic level.

Plants produce and emit a multitude of volatiles and metabolites which play important roles in plant communication and mediation of stresses, among others (Schuman, 2023). Under normal conditions the volatiles released by plants are relatively low in both concentration and quantity (Aartsma *et al.*, 2017). Under attack by herbivores, however, plants often emit herbivore-induced

plant volatiles (HIPVs), most likely in a direct or indirect protective reaction (War *et al.*, 2011). These HIPVs include green leaf volatiles (GLVs), phenylpropanoids, and terpenoids (Aartsma *et al.*, 2017). Also, under abiotic stress, including PTM related stress, plants often produce excessive amounts of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), singlet oxygen (O_2) or superoxide (O_2^-), which could damage plant cells (Winter *et al.*, 2012). In reaction to excess ROS production, plants emit isoprenoid volatile organic compounds (VOCs) to prevent cell damage (Bibbiani *et al.*, 2018; Berni *et al.*, 2019). VOCs produced by plants are primarily represented by isoprene, methanol, and monoterpenes but the specific compounds emitted are dependent on various factors, including the plant species and growth conditions (Dudareva *et al.*, 2013; Loreto *et al.*, 2014). The Ni hyperaccumulator *Berkheya coddii* (Asterales: Asteraceae) has been reported to contain increased levels of chelidonic acid as ligands associated with Ni uptake in the plant (Naicker *et al.*, 2016). VOCs can indirectly help protect plants against herbivory, for instance, the change in metabolite profile could make plants less desirable to herbivore insects, or VOCs can be used by predators of herbivores to locate the plants and subsequently the herbivores themselves (Paré & Tumlinson, 1999; Turlings & Wäckers, 2004; Adams *et al.*, 2024). Such relationships are complex and remain understudied.

Ultramafic ecosystems are a suitable model for studying insects' behaviour toward PTM rich plant foods. Ultramafic ecosystems contain soils that are derived from the weathering of ultramafic parent rock. This process produces soils that are relatively low in silica (Si) and essential nutrients for plants, such as calcium (Ca), potassium (K), and phosphorous (P), while high in PTMs such as iron (Fe), magnesium (Mg), cobalt (Co), chromium (Cr), and nickel (Ni) (Rajakaruna, 2018; Kierczak *et al.*, 2021). Many plant species growing in these soils, including *Berkheya radula* (Asterales: Asteraceae), have developed the ability to take up PTMs and store them within their leaves, thus creating a potential pathway for PTMs to reach the herbivores of these wild plants (Roebuck *et al.*, 2022). However, studying food crops is also relevant to understand the risks humans are facing regarding PTM ingestion. Many food crops, including *Brassica oleracea* var. *acephala* (Brassicales: Brassicaceae), also have the ability to accumulate PTMs from the soil, thus creating a potential pathway for PTMs to reach humans (Morales-Silva *et al.*, 2022).

The objective of this study was, as a novel approach to understand trophic transfer of PTMs, to (1) investigate whether the house cricket, *A. domesticus*, discriminates, both through olfaction and gustation, between *B. radula* and *B. oleracea* var. *acephala* grown in naturally PTM rich soil (komatiite) or control soil; and (2) to screen for general patterns in metabolite profiles from both plant species from both soil treatments. The insights gained from this study will aid in understanding the interactions between primary producers and consumers under different levels of PTM exposure.

4.2 Materials and methods

4.2.1 Soil collection

PTM enriched soil was collected from a mafic-ultramafic komatiite outcrop (as classified by Lana *et al.*, 2003) in the southeastern part of the Vredefort Dome, in the North-West Province, South Africa (27°08'31.3"S 27°37'24.8"E). The area is part of the oldest and largest meteor impact site on Earth (Whitfield, 2015). The ridges that form the collar of the dome contain some ultramafic rocks (Reimold & Koeberl, 2014), creating naturally PTM rich soils. Control soil was collected on Witpoort farm (26°28'35"S 26°58'22"E) on the outskirts of the town of Potchefstroom, North-West Province, South Africa. Both soil types had vegetation and topsoil removed by use of a shovel, and subsequently loosening soil with a pickaxe to sample sub-surface soils at a minimum depth

of 30 cm. Soils were then sieved through a 2 mm sieve and thoroughly mixed before being utilised in 20 L pots, as well as in the chemical analysis.

4.2.2 Sample preparation

4.2.2.1 Soil

Soil samples were dried in an oven at 105° C for 24 hours, then crushed and sieved using a 2 mm sieve to remove organic material and soil aggregates. Inductively coupled plasma mass spectroscopy (ICP-MS) was used to determine bulk chemical composition, focusing on PTMs (Appendix A, Table A.1).

Soil pH was also measured. Forty grams (40 g) of prepared soil was added to 100 ml de-ionized water to create a soil:water suspension in the ratio 1:2.5. The suspension was stirred, rested for 50 min, stirred again, and rested for 10 min. pH readings were carried out using a calibrated XS PC5 digital pH meter. Three samples for each soil type were analysed thrice, for a total of nine readings.

4.2.2.2 Plant material

Six haphazardly selected *B. radula* and *B. oleracea* var. *acephala* leaves were dried within a plant press at 75°C for 48 hours before ICP-MS was implemented to determine PTM concentration within leaves (Appendix A, Table A.2). For this procedure the leaves were powdered using a tungsten ring mill.

4.2.2.3 PTM detection

Soil and plant samples, 200 mg of each, underwent microwave acid digestion (EPA3051A). The dried samples were blended with 3 ml 32% hydrochloric acid (HCl) and 9 ml 65% nitric acid (HNO₃), and the mixture inserted in a Teflon tube. Digestion was achieved by placing the tubes in a Milestone, Ethos UP, Maxi 44 rotor-based microwave digestion system until it reached 1800 MW and 200°C, which was after 20 minutes. Samples were kept under these conditions for another 15 minutes and were then allowed to cool. Sample volumes were adjusted to 50 mL to be analysed for PTMs in an Agilent 7500 series ICP-MS.

4.2.3 Study species

4.2.3.1 Insect herbivore

The house cricket, *Acheta domesticus* (Orthoptera: Gryllidae), was used as herbivore in this study. This insect species is widespread throughout the world (Amorello *et al.*, 2024) and in recent years has become easy to acquire from local pet shops as they are used for feeding exotic pets. As a detritivore, house crickets feed on a wide variety of foods. House crickets have been confirmed to feed on kale plants and performed well (Oloo *et al.*, 2020). Due to its diet this species has the potential to take up and transfer PTMs to the following trophic level.

4.2.3.2 Plant producers

Berkheya radula (diphate; African sun daisy) and *Brassica oleracea* var. *acephala* (kale) plants were grown from seed in komatiite soil from the Vredefort Dome, South Africa, as well as control soil from the outskirts of the town of Potchefstroom. The ridges that form the collar of the dome contain some ultramafic rocks (Reimold & Koeberl, 2014), creating localised serpentine ecosystems. *Berkheya radula* was chosen for this study as a naturally occurring species in the Vredefort Dome area, while it has also been shown by Roebuck *et al.* (2022) to be a metallophyte capable of bioaccumulating metals. To establish *B. radula*'s status as a potential food source for

herbivores, thirty individuals were haphazardly selected in a natural field on the outskirts of the town Potchefstroom, and three leaves were harvested from each of these (total of 90 leaves). The leaves were analysed for herbivory by using the BioLeaf – Foliar Analysis™ phone application (Machado *et al.*, 2016) to calculate the percentage of leaf consumption for each leaf. The result was a mean of 4.2% and a maximum of 23.6%, leading to the conclusion that *B. radula* indeed has natural herbivores. Kale has been reported to accumulate PTMs in its leaves (Morales-Silva *et al.*, 2022) and is widely grown and consumed globally and in South Africa. Plants were grown in the following soil conditions (treatments): *Berkheya* grown in komatiite (KB) and control soil (CB), and kale grown in komatiite (KK) and control soil (CK).

4.2.4 Olfaction tests

Two-way olfactometer (Y-tube) tests were conducted for both species grown in all treatments (KB, CB, KK, and CK) to establish whether *A. domesticus* would, through olfaction, be able to distinguish and prefer a certain leaf type. Leaves from diphate and kale plants grown in komatiite and control soils, and therefore with different PTM tissue concentrations, were used in the olfactory tests.

An electric air compressor was connected to a glass Y-tube (34 cm in length, 2.5 cm in diameter) (Fig. 1). At both top ends of the Y-tube were plastic containers that allowed for air to move through them and into the pipe back to the Y-tube. The air pump was implemented in such a manner that suction would move air from the plastic containers (with the leaves of the specified treatment) back through the Y-tube towards the pump and the live cricket. A flow regulator was connected between the pump and the Y-tube and set to 1500 mL/min. Lastly a box containing a 60 W light bulb was placed over the setup to ensure that crickets could not see objects or movement outside of its immediate vicinity, this was done to eliminate startling or distracting the cricket and thus influencing its behaviour. The Y-tube was washed with hexane before each run to ensure the removal of any scent from previous trials. Acclimation of crickets was achieved by tilting the Y-tube upwards for five minutes after a cricket was placed inside, ensuring that the cricket could not advance within the apparatus. The Y-tube would then be gently lowered flat and the direction of movement of the cricket recorded. A choice was considered made when the cricket passed one of the marks designated as “points of decision” in Fig. 1, 10 cm from the split of the Y-tube.

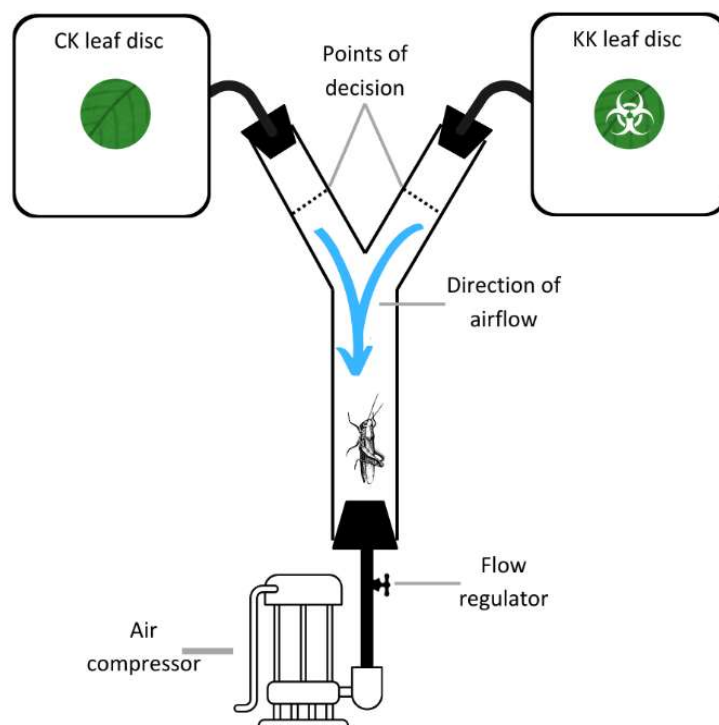


Figure 1. Schematics of the experimental setup of the two-way olfaction (Y-tube) test.

As means of calibration and certification, the first set of crickets, consisting of six individuals (three male and three female), were given the choice between a freshly sliced carrot, a common food item in *A. domesticus*' diet, and an empty container. Carrots were preferred in a proportion of 5:1 times in comparison with the empty container.

Sixteen house crickets, of which eight were male and eight female (total 16 repetitions), were put through the Y-tube olfaction test with diphate and kale leaves. House crickets in the size "large" were used to equalize any hormonal effects potentially present in adult crickets. House crickets were starved for 48 hours before individually entering the olfaction test, to increase hunger effect. Leaves were freshly cut from live plants into a 2 cm² square, each from a new haphazardly selected leaf. A leaf square was cut out of *Berkheya* leaves from each treatment, and placed into the plastic containers on the ends of the Y-tube, whereas kale leaf samples were discs punched with a 1.5 cm diameter hole punch. Disc-shaped samples were used for kale due to its smaller leaf size and rounded shape compared to diphate leaves.

4.2.3 Gustation tests

Gustation trials were conducted to determine whether food preference (in terms of taste) of house crickets is affected by elevated PTM concentrations in leaves. These trials were, however, conducted only with kale leaves as house crickets did not feed on diphate leaves from any treatment in preliminary tests.

House crickets were individually housed in 20 L buckets during gustation trials. Large house crickets were starved for 48 hours to clear their digestive tracts, after which they were each given a frozen pea, maize kernel, and two small pieces of carrot to feed on for 24 hours to eliminate the hunger effect (Chen & Ye, 2008). Kale leaves (62 leaf samples from each kale treatment KK and CK) were punched into 5 mm diameter discs. Leaf discs were dried in a plant press, at 75° C for three hours, to keep them flat but not eliminate all moisture and suppleness.

Dried leaf discs were offered to house crickets on a circular white moulded resin receptacle (70 x 4 mm) with downward slanting edges for easy access (Fig. 2). A 150 mL plastic container with a mesh top was filled with 80 mL distilled water and placed in the middle of the bucket to increase humidity of the system (Fig. 2). Each house cricket received one leaf disc from each treatment for 48 hours, after which leaf discs were removed and percentage foliar consumption analysed utilising the BioLeaf – Foliar Analysis™ phone application (Machado *et al.*, 2016). The trial took place in a temperature controlled rearing room at 27°C in constant darkness. A total of 62 house crickets (31 male and 31 female) were included in separate gustation tests.

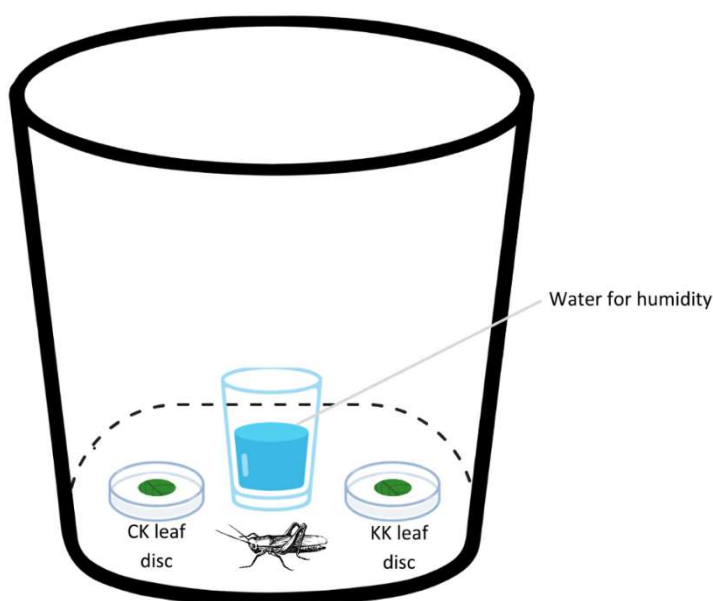


Figure 2: Schematic of the experimental setup of gustation tests.

4.2.4 Non-targeted High Performance Liquid Chromatography

General metabolite profiles (non-targeted analysis) were rendered for leaves from all treatments to depict potential metabolic changes. Three leaves from each treatment were dried and powdered, making composite samples of 100 mg each. The extraction was performed with 5 ml methanol followed by sonication for 10 minutes. The solution was filtered through a 22 µm mesh prior to analysis. A HPLC Shimadzu iNexera LC-2040C with a photodiode array (PDA) detector system was used with a chromatographic column, Agela Venusil® XBP C18, 2.1 X 100 mm, 3 µm. The mobile phase was of chromatography grade acetonitrile, ultrapure water (1 %, v/v) and acetic acid (0,1 %, v/v), and the total run time was 10 minutes. Detection of chemical components was made at 200 and 254 nm wavelength.

4.2.5 Statistical analysis

4.2.5.1 Olfaction tests:

Chi-squared tests were applied to data from the Y-tube olfaction trials in Microsoft Excel, using data from the control treatments as the expected range and komatiite treatments as the observed range (5 % significance).

4.2.5.2 Gustation test:

Data from the gustation test, consisting of the percentages of leaf samples eaten by crickets, were Log (X+1)-transformed to attenuate non-normal distribution. A chi-squared test was run in Microsoft Excel to test for significant differences between control (expected) and komatiite (observed) treatments (5 % significance).

4.3 Results

4.3.1 Soil pH and PTM content

The mean pH of control soil was 6.02 ± 0.14 , while that of komatiite soil was 5.42 ± 0.41 .

Soil ICP-MS results revealed that the following PTMs were significantly more abundant in komatiite soil than control soil: As, Ba, Ca, Co, Cu, Mn, Mo, Mg, Pb, Rb, Se, Sr, and V (Appendix A, Table A.1).

4.3.2 Plant PTM concentration

Berkheya plant material used in olfaction tests with house crickets had significantly different concentrations of manganese (Mn) and strontium (Sr), these being higher for plants from KB samples compared to CB (Appendix A, Table A.2).

Kale plant material used in olfaction and gustation tests with house crickets had significantly different concentrations of Mn, selenium (Se), Sr, copper (Cu), molybdenum (Mo), and vanadium (V). The first three PTMs were found to be higher within KK samples when compared to CK, and the last three significantly higher in CK leaves compared to KK leaves (Appendix A, Table A.2). Both Se and V were present within samples at low concentrations.

4.3.3 Olfaction and gustation tests

The Y-tube olfaction tests indicated differences in house cricket choice when offered diphenyl leaves but no difference was observed between kale leaves. Female house crickets significantly approached KK leaves over CK leaves (Table 1), while males performed conversely, evidencing the role of the sex in choice via olfaction. Regarding gustation, the Chi-squared tests revealed no significant differences in consumption between house crickets from the two treatments (Table 1).

Table 1. Proportion of leaves selected (olfaction test) and consumed (gustation test) by the house cricket *A. domesticus* in the study trials. Values in bold text indicate significant differences.

	Kale (olfaction test)		<i>B. radula</i> (olfaction test)		Kale (gustation test)	
	Male (%)	Female (%)	Male (%)	Female (%)	Male log (x+1 %)	Female log (x+1 %)
Control (expected)	50	50	62,5	25	1,44	2
Komatiite (observed)	50	50	37,5	75	1,38	2
Degrees of freedom	1		1		1	
Chi ²	0	0	10	100	0,003	0
p-value	1	1	0,002	<0.001	0,959	1

4.3.4 Non-targeted High Performance Liquid Chromatography

The non-targeted HPLC analyses provided plant metabolite profiles of each plant species grown in the treatments, giving broad insight into their metabolic response to elevated PTM exposure. The general qualitative profiles were very similar for CB and KB, and CK and KK, treatments at wavelengths of 200 nm and 254 nm, as evidenced by their retention times (Figs. 3 and 4). Quantitatively, however, there was overall a higher number of metabolites in control leaves of both species. Hence, the differences lay in the quantity of metabolites rather than quality for both plant species.

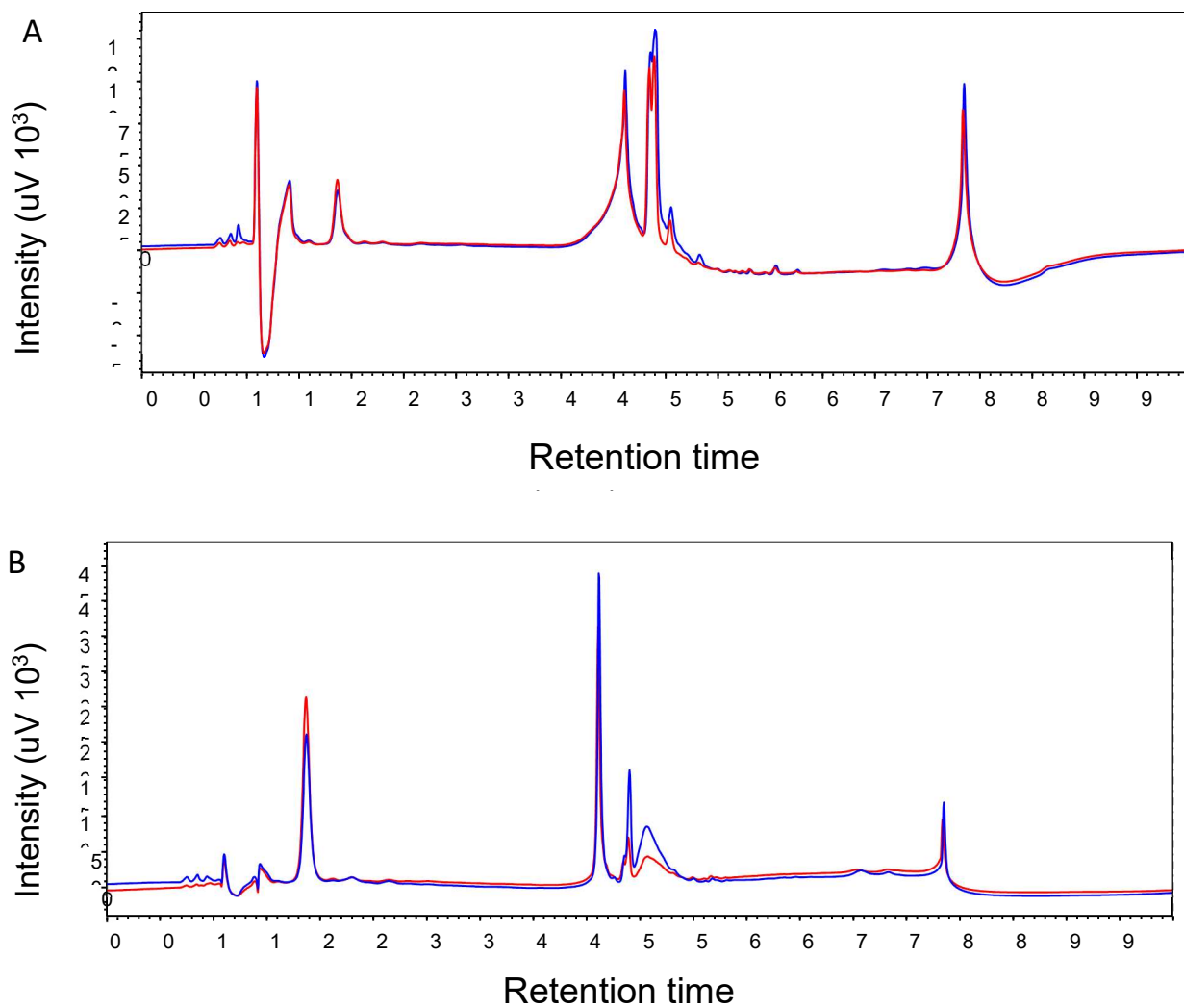


Figure 3. Non-targeted High Performance Liquid Chromatography (HPLC) profile at (A) 200nm and (B) 254nm of *Berkheya radula* leaves from individuals grown in control soil (blue line) and komatiite soil (red line).

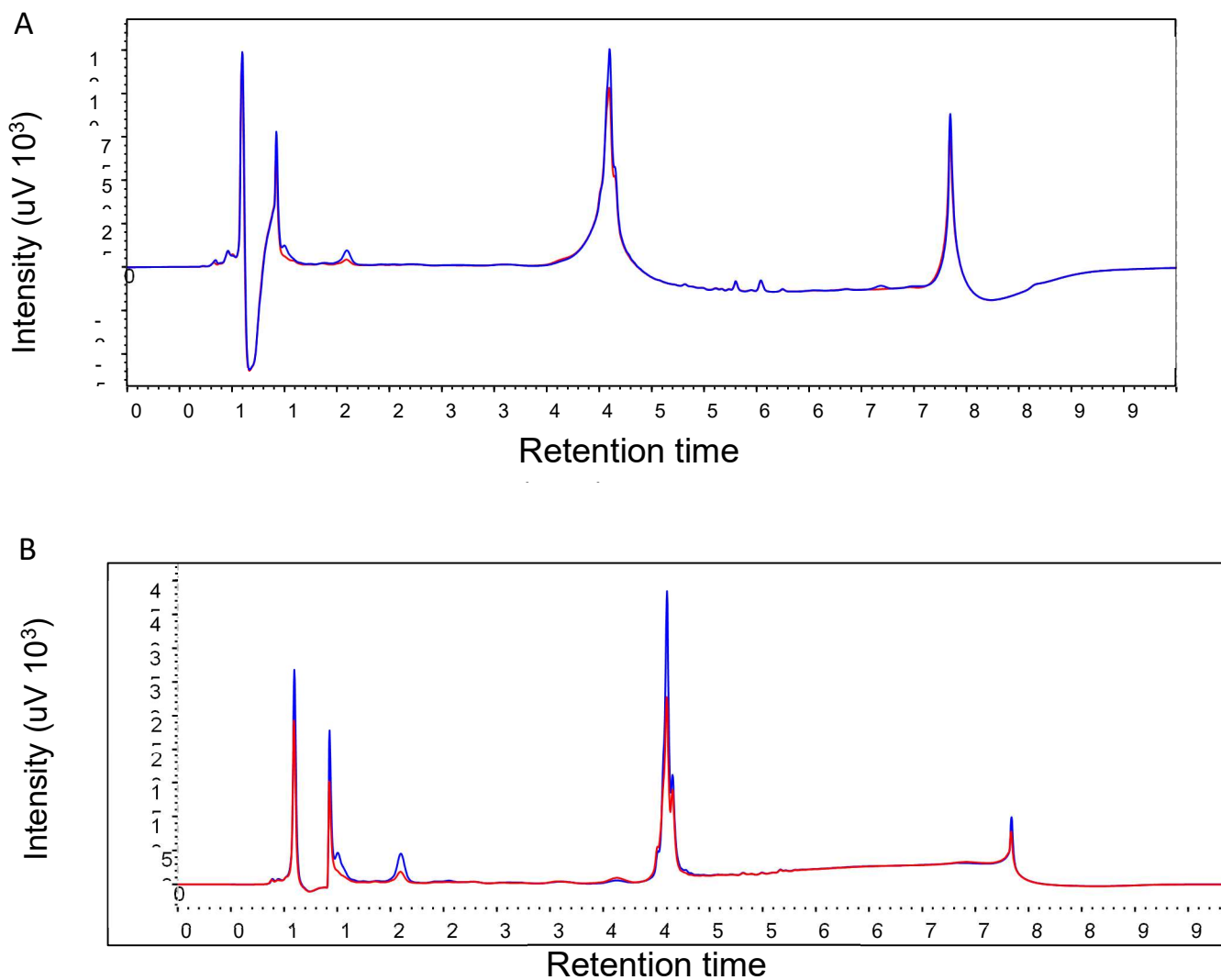


Figure 4. Non-targeted High Performance Liquid Chromatography (HPLC) profile at (A) 200 nm and (B) 254 nm of kale leaves from individuals grown in control soil (blue line) and komatiite soil (red line).

4.4 Discussion

Female house crickets chose KB diphtate leaves significantly more times than CB diphtate leaves when given the choice within a two-way olfactometer (Y-tube). Conversely, male house crickets chose CB diphtates significantly more than KB diphtates, suggesting deterrence toward leaves with slightly elevated PTMs. A study by Lin *et al.* (2022) reported differences in insect olfaction choices when given the choice between Cd-stressed and non-stressed *Populus yunnanensis* (Malpighiales: Salicaceae) plants. However, in contrast with the female house crickets in our study, *Stauronematus compressicornis* (Hymenoptera: Tenthredinidae) and *Plagiodera versicolora* (Coleoptera: Chrysomelidae) females used by Lin *et al.* (2022) preferred the control leaves over PTM tainted leaves (no males were used).

Other literature has also reported that the sex of an insect can affect feeding behaviour and sensitivity to abiotic stress (Neupane *et al.*, 2023; Teder & Kaasik, 2023), possibly due to differing nutritional and/or reproductive needs of males and females.

House crickets displayed neither preference nor deterrence toward the two kale treatments. Most literature suggests insect deterrence toward PTM tainted food. Martinek *et al.* (2018) reported a negative relationship between Mn concentration in food and consumption by adult *Melolontha hippocastani* (Coleoptera: Scarabaeidae). While some literature found indifference in feeding behaviour of insects when given the choice of PTM tainted and untainted food (Brill *et al.*, 1987; Boyd & Martens, 1999), it is more likely that PTM levels in our kale leaves were under detection limits for house crickets.

The plant metabolites profiled in this study were generally decreased in komatiite treatments compared to control ones, except for one instance at 254 nm where the higher peak suggests KB plants produced more of that specific metabolite than CB plants (Figure 4a). Most studies state increased plant metabolite production by plants under PTM stress. del Carman Sosa *et al.* (2016) found that increasing lead (Pb) contamination of soils resulted in increased VOC (terpenoid) emission by *Tagetes minuta* (Asterales: Asteraceae) and Bibbiani *et al.* (2018) reported a positive relationship between Zn contents of growing substrate and subsequent plant VOCs emitted by *Tetradenia riparia* (Lamiales: Lamiaceae). In line with our KB result, at least to some extent, Naicker *et al.* (2016) found that *Berkheya coddii*, a Ni-hyperaccumulator plant, produced increasing amounts of a metabolite (chelidonic acid) when exposed to higher concentrations of Ni. Furthermore, *Brassica oleracea* have been reported to contain significantly decreased amounts of secondary metabolites (glucosinolates) in its leaves under cadmium (Cd) stress (Jakovljević *et al.*, 2013)

Previous literature has suggested that plant metabolites can affect the feeding behaviour of insects. Lin *et al.* (2022) found a negative relationship between VOC emission and insect preference. It is possible that the plant metabolite higher in KB leaves was responsible for the deterrence of male house crickets in olfaction trials, however, this would have to be further studied.

4.5 Conclusions

This study tested the ability of house crickets to distinguish between PTM-contaminated and non-contaminated food plants in diphate, a native grassland species, and kale, a food plant, and whether plant metabolites play a role in this relationship.

Female house crickets preferred KB diphate leaves when given the choice within a two-way olfactometer (Y-tube), while male house crickets showed deterrence toward KB leaves. This difference might be related to small changes in plant metabolite concentrations between treatments, however, further in-depth studies focusing on the identification of such metabolites will be needed to achieve a clearer picture. House crickets indicated neither preference nor deterrence toward the two kale treatments in both olfaction or gustation tests. It is suggested that, even considering preference/deterrence results in the diphate olfaction tests, the PTM levels within plant leaves in this study were under the detection limits of house crickets. This is in part due to the small number of repetitions in the olfaction tests. However, these results warrant future studies with this experimental procedure, with a larger number of repetitions to improve result accuracy.

Although some of this study's plant metabolite results agree with findings in previous literature, limited conclusions can be drawn on the relationship between plant VOCs and insect olfaction without identification of specific metabolites. However, the results add to the evidence regarding

sensitivity and behaviour of primary consumers. Future research should build on this through more in-depth studies.

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Appendix A

Table A.1. Mean $\pm$ SD of elements concentration (mg kg⁻¹) from the study's soil samples. Different letters (a, b) indicate significant differences between soil treatments ($p < 0.05$).

	Ca	Mg	P	V	Mn	Co
Control	638,064 $\pm$ 108,31 a	647,16 $\pm$ 42,91 b	238,605 $\pm$ 11,30 a	31,714 $\pm$ 1,83 b	181,397 $\pm$ 31,57 b	6,791 $\pm$ 0,72 b
Komatiite	5113,304 $\pm$ 258,68 b	2582,834 $\pm$ 43,69 a	260,295 $\pm$ 47,79 a	70,771 $\pm$ 0,79 a	693,058 $\pm$ 4,80 b	15,011 $\pm$ 0,25 a
	Ni	Cu	Zn	As	Se	Rb
Control	28,908 $\pm$ 1,97 a	16,047 $\pm$ 1,67 b	47,387 $\pm$ 7,04 a	1,893 $\pm$ 0,28 b	0,843 $\pm$ 0,09 b	24,498 $\pm$ 3,30 b
Komatiite	25,859 $\pm$ 0,32 a	37,602 $\pm$ 0,54 a	35,593 $\pm$ 2,77 a	2,613 $\pm$ 0,01 a	2,386 $\pm$ 0,23 a	40,095 $\pm$ 1,76 a
	Sr	Mo	Ba	Pb		
Control	8,374 $\pm$ 1,33 b	0,302 $\pm$ 0,10 b	42,141 $\pm$ 4,19 b	4,044 $\pm$ 0,23 b		
Komatiite	94,472 $\pm$ 6,60 a	0,644 $\pm$ 0,09 a	161,155 $\pm$ 0,28 a	8,643 $\pm$ 0,34 a		

Table A.2. Mean $\pm$ SD of elements concentration (mg kg⁻¹) from the study's plant samples. Different letters (a, b) indicate significant differences between soil treatments (p < 0.05).

<i>Berkheya</i>	V	Mn	Co	Ni	Cu	Zn	As
Control	0,558 $\pm$ 0,21 a	213,424 $\pm$ 55,21 b	0,419 $\pm$ 0,39 a	4,868 $\pm$ 1,90 a	14,728 $\pm$ 4,51 a	135,169 $\pm$ 20,32 a	0,051 $\pm$ 0,01 a
Komatiite	0,242 $\pm$ 0,08 a	377,991 $\pm$ 136,37 a	0,215 $\pm$ 0,11 a	5,853 $\pm$ 3,51 a	18,381 $\pm$ 43,81 a	130,546 $\pm$ 50,76 a	0,042 $\pm$ 0,01 a
	Se	Rb	Sr	Mo	Ba	Pb	
Control	0,094 $\pm$ 0,04 a	31,113 $\pm$ 15,45 a	199,380 $\pm$ 123,57 b	0,057 $\pm$ 0,06 a	165,887 $\pm$ 32,21 a	0,331 $\pm$ 0,07 a	
Komatiite	0,177 $\pm$ 0,10 a	38,236 $\pm$ 14,25 a	398,912 $\pm$ 48,84 a	0,022 $\pm$ 0,03 a	240,745 $\pm$ 66,35 a	0,341 $\pm$ 0,08 a	
<i>Kale</i>	V	Mn	Co	Ni	Cu	Zn	As
Control	0,027 $\pm$ 0,01 a	64,363 $\pm$ 24,35 b	0,181 $\pm$ 0,05 a	2,325 $\pm$ 0,97 a	2,392 $\pm$ 0,39 a	34,937 $\pm$ 9,22 a	0,010 $\pm$ 0,01 a
Komatiite	0,000 $\pm$ 0,00 b	118,988 $\pm$ 39,78 a	0,149 $\pm$ 0,07 a	3,265 $\pm$ 2,20 a	1,037 $\pm$ 0,54 b	34,401 $\pm$ 23,30 a	0,016 $\pm$ 0,01 a
	Se	Rb	Sr	Mo	Ba	Pb	
Control	0,000 $\pm$ 0,00 b	32,422 $\pm$ 6,11 a	77,065 $\pm$ 14,64 b	1,100 $\pm$ 0,36 a	68,761 $\pm$ 12,72 a	0,051 $\pm$ 0,01 a	
Komatiite	0,098 $\pm$ 0,08 a	17,685 $\pm$ 16,68 a	191,018 $\pm$ 77,87 a	0,231 $\pm$ 0,15 b	107,060 $\pm$ 53,04 a	0,031 $\pm$ 0,01 a	

Chapter 5 - Conclusions and recommendations

5.1 Conclusions

The transfer of PTMs in a three trophic levels model based on a terrestrial ecosystem with naturally PTM rich soil (i.e. the komatiite soil from the Vredefort Dome area, in South Africa) was investigated in this study. The first step of this investigation was a detailed literature review on the topic of trophic transfer of PTMs in terrestrial systems, with focus on the data published from previous studies to define the state of art and identify current research gaps. The review (Chapter 2) concluded that previous studies on the topic were mainly performed in developed countries within North America and Europe, while research from developing regions, particularly South America and Africa, is lagging behind. This is despite the fact that the latter continents include the most important crop producers in the world. Many crops are known to take up PTMs, and these regions generally have higher population growth trends which intensify related health risks. Moreover, agricultural sites received the least focus, which is concerning since humans are dependent on these ecosystems for food. Natural sites with naturally PTM rich soils were also neglected: these areas should form part of the baseline understanding of how organisms cope with exposure to elevated PTM levels. This could prove invaluable information and aid in predicting the effects of PTM pollution on terrestrial ecosystems.

In light of the findings within the review, an experiment was designed regarding transfer of naturally occurring PTMs across three trophic levels (Chapter 3) in a PTM rich simulated environment. This study was based on soil collected from an area within the collar zone of the Vredefort Dome consisting of mafic/ultramafic komatiite rocks. Although serpentine soil has been described in this general area before, upon testing our soil with ICP-MS it became clear that this was not serpentine soil, due to an incompatible Mg:Ca ratio, a pH less acidic than ideal, and relatively low PTM levels compared to previous accounts of serpentine soil (Rajakaruna & Boyd, 2014; Venter *et al.*, 2018). However, this soil (now referred to as “komatiite soil”) still had significantly elevated concentrations of 13 PTMs. In addition, very little is known about komatiite soil and subsequent PTM transfer, and thus this study will aid in generating baseline data on PTM transfer from this soil type and possible effects within these types of ecosystems.

From the model system created using komatiite soil, *B. radula* (a wild plant) and kale (a crop plant) as producers, house crickets as primary consumer, and *P. spurca* as secondary consumer, the following conclusions were reached:

Considering leaf PTM concentration and bioaccumulation factors (BAFs), the concentrations of PTMs in *B. radula* and kale leaves did not reflect soil PTM levels. Thus, PTM bioaccumulation and subsequent availability of PTMs to the next trophic level was a function of species requirement rather than soil PTM content.

Most of the PTMs bioaccumulated by house crickets from both komatiite and control treatments, in both *Berkheya* and kale trials, were essential elements for these organisms. This possibly suggests, as with producers, a species-specific uptake of these PTMs rather than a passive transfer.

The majority of PTM concentrations in mantids were influenced by their biology (“body parts”) regardless of their food treatment, also for both *Berkheya* and kale trials. The PTMs that depended on concentration in the previous trophic level for accumulation seemed to generally be excreted within either feces or exuviae.

Some differences were observed regarding mantid development. KMB generally took longer throughout their developmental stages than CMB (with lower survival rates in the latter), and the opposite was observed between KMK and CMK (with no differences regarding survival rates).

Although some bioaccumulated PTMs differed between komatiite and control mantids in both *Berkheya* and kale trials, non-significant differences of the PTM concentrations made it difficult to associate such mild alterations to PTM levels. The differences reported in the study do, however, warrant further investigation.

When the food chain from the *Berkheya* and the kale trials so considered for generalities, only Cu, Mo, and As were bioaccumulated in more than two trophic levels from the komatiite soil. However, from the control soil, more PTMs were bioaccumulated in more than two trophic levels. As is a non-essential PTM and thus it is important to note that it has the potential to be bioaccumulated by both primary and secondary consumers as evidenced within this study. However, in the end, the most trophic levels were relatively uninfluenced by soil treatments.

When given a choice between KB and CB leaves, female house crickets chose significantly more of those grown in komatiite soil than those grown in control soil, while males reacted conversely. When kale leaves were tested on house cricket, no treatment was preferred in either olfaction or gustation tests. This is despite the difference of PTM concentration in MK and CK leaves.

Non-target metabolite profiles of komatiite *Berkheya* and komatiite kale were only quantitatively distinct from the control treatment, as the chromatogram peaks were present in the same retention times, but with somewhat different heights. However, these differences were small and not present in all peaks. Therefore, PTM concentrations in soils did not sufficiently change the general metabolite profiles and thus had no consequences for preference by the crickets.

The insights gained by the present study aid in understanding naturally PTM rich ecosystems, especially those found within the Vredefort Dome collar zone, and how plants, herbivore insects, and predator insects react to these conditions. It also adds to the literature within Africa regarding PTM transfer within natural ecosystems, which is currently underrepresented when compared to the global context.

5.2 Recommendations

Some key questions were raised from this study, and should be addressed by future research:

- If not on komatiites, where are the hypothesized serpentine outcrops within the Vredefort Dome?
- Do ultramafic systems present the same patterns of trophic transfer when compared to “extreme ultramafic,” including mine tailings derived from ultramafic rocks?
- Is there trophic transfer when PTM accumulator species occupy one or more trophic level(s) in the above mentioned ecosystems?
- Are the requirements of male and female crickets different in terms of nutrients, and subsequently PTMs, and thus influence their choice for food with different PTM concentrations?
- Which factor(s) drove the difference in mantid development? Would such differences be more pronounced under higher PTM concentrations?

Future studies should be conducted within the African continent, as there is a lack of research originating in this region compared to, for example, North America or Europe (Joubert *et al.*, 2024). Continuing research within South Africa would be an ideal starting point because this country has several natural and man-made PTM-rich ecosystems. Reports of serpentine outcrops have been made from within the Vredefort Dome (Lana *et al.*, 2003), but little attention has been given since. This study was also an initial contribution to serpentine research within the dome, and already made it clear that further exploration is needed to identify suitable

serpentine outcrops. It is important to discover more serpentine outcrops and the species associated with them, especially plants, as polluting activities are only increasing and these adapted species can provide valuable insights to PTM tolerance, as well as offer potential solutions through phytoremediation (Gall *et al.*, 2015).

Our results on the trophic transfer of PTMs somewhat contradicted previous literature, which frequently showed PTMs being transferred and impacting different trophic levels (Xu *et al.*, 2009; Mogren *et al.*, 2013; Lidman *et al.*, 2020; Morales-Silva *et al.*, 2022). Nevertheless, most studies lack the use of naturally enriched soil and more than two trophic levels. Thus, the results of this study are solid and clear regarding the observed patterns, and where the approach was not deep enough, there are suggestions for future studies. As a side note, if the same experimental procedure is followed, use of *B. radula* as food for house crickets is not recommended as this plant was quite unpalatable. Leaves in the field, however, showed signs of herbivory, suggesting the presence of natural herbivores. These should be identified and possibly integrated into a future study of potentially PTM-adapted herbivores. Furthermore, bioavailability of soil PTMs (in the extractable phase) should also be incorporated into future studies, as this will provide more accurate insight into PTM uptake and bioaccumulation.

The developmental differences of mantids between komatiite and control treatments pointed only weakly toward possible effects attributed to PTM exposure. Future studies should investigate this trend further and do so at higher PTM exposure to possibly amplify and finally clarify the small differences observed in this study. Additionally, primary consumers should also be evaluated for developmental effects. Finally, olfaction trials with house crickets and *B. radula* proved promising, and more repetitions are suggested. The experimental procedure proved itself quick, of low cost, and versatile enough to be done with various combinations of insects and metallophyte plants.

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