Title

Terrestrial insect defences in the face of metal toxicity

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Author

Antoine Gekière[1]

Affiliation

[1]Laboratory of Zoology, Research Institute for Biosciences, University of Mons, Mons, Belgium

Email address

antoine.gekiere@umons.ac.be

ORCID

0000-0001-5337-1305

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Abstract

Recently, there has been growing concern about the impacts of metal pollutants on insect populations, particularly as human societies increasingly rely on metal-based technologies. Unlike organic pollutants, metals – both essential and non-essential – are non-degradable and readily accumulate in insect tissues, sometimes reaching hazardous levels. While studies have increasingly addressed how insects cope with pesticide pollution in agro-ecosystems, they have shifted their attention away from the way insects confront metal pollution in a metal-dominated world. This paper reviews the routes of entry for metals into insect cells and the molecular damages they trigger. Additionally, it examines the defence mechanisms insects may employ to counteract metal pollution. Firstly, insects may detect and avoid metals in their environment, thereby escaping contaminated food, substrates, and oviposition sites. Secondly, the insect cuticle and gut lining, including the gut microbiota, may serve as physical barriers preventing metal entry into the hemolymph, thereby protecting other organs. Thirdly, insect cells may detoxify metals by sequestering them in metal-scavenging proteins (e.g., metallothioneins) and excreting them via faeces or the cuticle. Fourthly, when metal-related damage occurs, including oxidative stress, protein unfolding, and DNA deformation, insect cells may respond by upregulating antioxidant molecules, chaperone proteins, and DNA repair mechanisms. Enhancing our knowledge of insect-metal interactions sounds crucial for the conservation of insect populations in an increasingly metal-dependent world.

Keywords

Avoidance; Damage; Detoxification; Excretion; Insects; Trace metals and metalloids

1. An increasing threat

Undeniable declines in insect populations have alarmed the global scientific community (Cardoso *et al.* 2020) and these disturbing trends threaten the sustainability of ecosystems and essential ecosystem services (e.g., pollination) (van der Sluijs 2020). Insects face a multifaceted crisis due to the combined impacts of climate change, habitat degradation, and agrochemicals (Raven & Wagner 2021; Wagner *et al.* 2021). For insects, agrochemicals and other pollutants are xenobiotics, namely compounds that are not expected to be present in their bodies. Recently, attention has been directed towards an often overlooked threat, namely the exposure to trace metals and metalloids (hereafter 'metals') which are pervasive environmental pollutants (Gekière *et al.* 2023; Monchanin *et al.* 2021). While metals naturally occur through processes such as rock erosion and volcanic activity, hazardous concentrations are frequently the result of mining, industrial, and agricultural activities (Jayakumar *et al.* 2021). The increasing reliance on metal-based renewable energy technologies heightens the risk posed by metal extraction and related industries to ecosystems (Gregoir & Van Acker 2022; Sonter *et al.* 2020). Metals, whether in soluble (i.e., ions) or solid (i.e., fine particulate) forms, exhibit high environmental mobility, spreading through water runoff, atmospheric dust, and trophic networks (Wuana & Okieimen 2011). Metals could be considered as 'dormant' toxicants, since unlike organic pollutants such as pesticides, metals are nondegradable and bioaccumulate readily in insect tissues, posing a significant risk on the long term (Briffa *et al.* 2020). Despite over 60 metals present in the Earth's crust (Haynes 2017), only a few (e.g., Cu, Fe, Mn, Zn) are essential for biological processes, primarily functioning as enzyme cofactors due to their redox potential (Crichton 2020). Non-essential metals can exert toxic effects even at low doses, and both essential and non-essential metals become toxic at elevated levels (Tchounwou *et al.* 2012), indicating that no metal is inherently 'safe' for insects (Monchanin *et al.* 2021).

Insects have been exposed to metals for millions of years and have evolved behavioural, physiological, and biochemical mechanisms to regulate metal homeostasis and prevent metal-related damage. First, this paper reviews the routes of exposure as well as the transports and mechanisms of action of metals. Then, it explores and illustrates the scientific knowledge addressing the insects' lines of defence to face metal exposure, acknowledging that mechanisms likely differ among metals (e.g., essentials *vs.* non-essentials) and insect species. Briefly, insects may (i) avoid ingesting or interacting with metals in their environment, (ii) prevent metals from entering their bodies, (iii) excrete metals out of their bodies, and (iv) alleviate molecular damage. Finally, it highlights outstanding questions for every line of defence to pave the way for future avenues of research.

2. Routes of exposure

Metals are translocated from the lithosphere, hydrosphere, and atmosphere into plant tissues or onto plant surfaces, representing significant exposure sources for herbivores, including insects (Gall *et al.* 2015; Xun *et al.* 2017). Metals accumulate in all plant tissues (Hladun *et al.* 2015), although such accumulation is dependent on the type of metal, soil conditions, plant species, and specific tissues (Tibbett *et al.* 2021). General trends in metal accumulation could therefore differ among biome-specific soils worldwide, but such meta-analysis is lacking. Metal concentrations in plants increase with higher metal concentrations in the substrate (Xun *et al.* 2017). Hyperaccumulator plant species, or metallophytes, can reach exceptionally high metal concentrations, likely as a defence mechanism against herbivory (i.e., elemental defence hypothesis (Dueli *et al.* 2021)). Most hyperaccumulators accumulate nickel and are endemic to serpentine soils – resulting from ultramafic rock weathering – (Reeves & Baker 2000) found all around the world but such plants are also found in areas polluted by anthropogenic activities. For instance, *Jasione montana* at mining sites accumulates cadmium in its aerial tissues at concentrations 15 times higher than those in the soil (García-Salgado *et* *al.* 2012). Although hyperaccumulators are toxic to most herbivores (Boyd 2007), some specialists are able to circumvent these elemental defences and may accumulate high doses of metals in their bodies (e.g., the mirid bug contains 750 µg of nickel per g of tissue) (Wall & Boyd 2006). While the communities of hyperaccumulator plant species have been increasing studied, even with some cases of speciation reported on serpentine soils, data around metal-tolerant insects are scarce (Brady *et al.* 2005). Atmospheric deposition also plays a crucial role, as metals travel long distances in particulate matter before being deposited on plant surfaces (He *et al.* 2023). For example, along heavily trafficked roads in London, metal concentrations in leaves were negatively correlated with the distance from the road, although deposition was plant- and metal-specific (Peachey *et al.* 2009). Metals in plants affect not only herbivorous insects but also carnivorous insects, as these metals readily pass along the food chain (Butt *et al.* 2018; Tibbett *et al.* 2021). In addition to oral exposure through food, insects are topically exposed to metals through their integument when they come into contact with contaminated soil, nesting material, or water, similar to pesticide exposure (Kopit & Pitts-Singer 2018). For example, a Europe-wide study found more than 30 metals in the nests of three domesticated bee species, indicating that larvae and adults are exposed through contact with their nest environment (Durkalec *et al.* 2024). However, while it is well-established that insects are exposed to metals through both oral and topical routes, the paucity of environmental data hinders the establishment of a threshold that distinguishes 'baseline' from 'abnormal' exposure. As metal pollution continues to intensify, field research should prioritise assessing the extent of insect exposure within their natural habitats.

3. Transport and mechanisms of action

Both essential and non-essential metals are readily absorbed by the gut epithelium through protein transporters originally designed for essential ions, as charged molecules cannot diffuse freely through cell membranes. Conversely, the mechanisms by which metals penetrate the insect integument are less understood (*vide infra*). To enter gut cells, essential metals such as iron, copper, and zinc utilise specific membrane transporters. Non-essential metals, such as cadmium, lead, and mercury, exploit these transporters to gain entry into the cytosol (Martinez-Finley *et al.* 2012) (**Figure 1**). For instance, in fruit flies, the Malvolio transporter, which imports ferrous ions, also facilitates the uptake of cadmium and lead ions. Similarly, the Ctr1B transporter, which imports cupric ions, serves as a pathway for cadmium and mercury ions (Slobodian *et al.* 2021). Metals may also enter through calcium channels or aquaglyceroporins, as shown for arsenic, and through amino acid transporters, as demonstrated for mercury (Martinez-Finley *et al.* 2012). However, metal uptake in the gut varies among different metals, with some being more tightly regulated than others. For instance, ground beetles exhibit the ability to regulate essential zinc uptake effectively, whereas they lack a similar regulatory mechanism for non-essential cadmium uptake (Kramarz 1999). Once inside the cells, metals may be released into the intercellular space or hemolymph through efflux systems designed for essential metals, though data on non-essential metals in insects is lacking (Gorman 2023). Metals likely reach the hemolymph in a protein-bound state, although metal transport in insect hemolymph remains largely uncharted, except for iron (Gorman 2023). Due to insects' open circulatory system (Hillyer & Pass 2020), metals can easily reach various organs, including neural ganglia (Monchanin *et al.* 2023), fat bodies (Ballan-Dufrançais 2002; Zhang *et al.* 2001), Malpighian tubules (Maroni & Watson 1985), and gonads (Ballan-Dufrançais 2002; Zhang *et al.* 2001), where they cause severe damage. It is important to note, however, that metal distribution after ingestion varies depending on the specific metal. For example, in a ground beetle, cadmium was found to accumulate primarily in the integument, whereas mercury and zinc were not. Also, hydrophobic methylated mercury showed higher accumulation than its non-methylated counterpart, as hydrophobic compounds more readily penetrate tissues (Lindqvist *et al.* 1995).

Once inside cells, the mechanisms of metal toxicity are threefold (Valko *et al.* 2005) (**Figure 1**). First, metals readily bind to the electron-dense sites of DNA, such as phosphate backbones or nucleobases, causing structural deformation through the perturbation of hydrogen bonds or inducing strand breaks (Kanellis & dos Remedios 2018). Second, due to their high affinity for thiol (-SH) groups, metals bind to cysteine residues in proteins, altering their conformation and function. This binding can displace essential metals from metalloproteins, impeding their catalytic activity, including DNA repair functions (Oliveira *et al.* 2022). Metals also bind to nonenzymatic antioxidant molecules, such as glutathione, reducing antioxidant defences and leading to oxidative stress via increased concentrations of reactive oxygen species (ROS) in the cytosol, such as superoxide anions produced by the mitochondria (Coffman & Su 2019). Third, some metals, such as iron and copper, act as catalysts in the Haber-Weiss reaction, converting hydrogen peroxide to the highly reactive hydroxyl radical (Halliwell & Gutteridge 2007). Reactive oxygen species rapidly oxidise proteins, DNA, and lipids, causing significant functional and cellular damage. The increased concentration of ROS also disturbs redox signalling and activates apoptosis pathways (Mittler 2017). Therefore, toxic concentrations of metals in the cytosol ultimately prevent proper enzymatic activities, damage macromolecules including DNA, and lead to cell death. Toxic concentrations of metals could also prevent proper enzymatic function of extracellular membrane-bound proteins (e.g., (Sumida *et al.* 1994)).

4. Insect defence mechanisms

4.1. First line: Avoidance

The most efficient way to prevent metal-related toxicity is to avoid ingesting or coming into contact with toxic concentrations of metals. It has been proposed that insects can detect and avoid metals in their food resources and substrates (**Figure 2**), but the results are controversial (Mogren & Trumble 2010). In bees, solutions laced with various concentrations of different metals elicited varied rejection responses through antennal and proboscis stimulations, highlighting metal-, concentration-, and body part-specific detection abilities. However, these studies utilised simple sucrose solutions to solubilise metals, raising the question of whether bees would still be capable of avoiding metals present within complex pollen blends (Burden *et al.* 2019; Monchanin *et al.* 2022). Likewise, semi-field studies showed that bees had reduced visit durations on metal-contaminated flowers, either using contaminated artificial nectar or plants grown in contaminated soil (Meindl & Ashman 2013; Phillips *et al.* 2021; Sivakoff & Gardiner 2017; Xun *et al.* 2017). In fruit flies, both adults and larvae preferred control diets over metal-rich diets (Bahadorani & Hilliker 2009), and receptors located on the labellum and pharynx were shown to be modulated by the presence of metals in food (Xiao *et al.* 2022). Intriguingly, the findings of this study highlighted two distinct mechanisms through which metals may drive avoidance behaviour: (i) zinc was found to activate bitter-sensing neurons, and (ii) copper was shown to suppress the response of sugar-sensing neurons to sucrose, thereby reducing the solution's palatability (Xiao *et al.* 2022). However, avoidance may vary among life stages, as seen in Colorado potato beetle larvae, which showed greater aversion than adults (Kwartirnikov *et al.* 1999). Discrepancies may also occur among larval instars, as observed in beet armyworm larvae, which avoided metals during their fourth instar but not during their third instar (Vickerman & Trumble 1999). Avoidance behaviours not only occur via sensory neurons but also due to post-digestive physiological responses. For instance, locusts exhibited a reduced feeding rate on zinc-contaminated diets, but no difference was observed during the first meal, suggesting they could not directly detect zinc contamination and rather relied on physiological feedback after zinc poisoning (Behmer *et al.* 2005). Interestingly, by using food flavoured with coumarin, this study showed that locusts learnt to associate specific tastes with zinc contamination, demonstrating that zinc avoidance was not simply due to behavioural malaise (Behmer *et al.* 2005).

For terrestrial insects, avoidance behaviours also include averting contaminated substrates. Many insect groups tend to avoid chernozem soils heavily contaminated with metals (Gongalsky *et al.* 2009). In contrast, fruit fly larvae do not avoid contaminated soils for pupation (Bahadorani & Hilliker 2009). Gravid females may avoid ovipositing on contaminated soils as a strategy to protect their offspring from hazardous exposure (**Figure 2**), as demonstrated in fruit flies (Bahadorani & Hilliker 2009). Intriguingly, both fruit flies and beet armyworms prefer to oviposit on substrates with low concentrations of essential metals over control, likely because reasonable amounts of essential metals can enhance offspring fitness (Bahadorani & Hilliker 2009; Vickerman *et al.* 2002). However, beet armyworm females cannot discriminate toxic concentrations of metals in their oviposition sites, which could be detrimental to their larvae (Vickerman *et al.* 2002).

4.2. Second line: Barrier

If insects ingest or come into contact with toxic concentrations of metals, they could be protected by endogenous or exogenous barriers. Insects possess a cuticle, also known as an exoskeleton, composed of tightly bound proteins, lipids, hydrocarbons, and chitin, forming a tough, impermeable layer that provides protection against desiccation and parasites. Additionally, the insect cuticle prevents the entry of xenobiotics, such as pesticides, into the epidermis and hemolymph. Cuticle thickness and composition have been associated with pesticide resistance (Balabanidou *et al.* 2018), highlighting its significance as a physical and chemical barrier. This barrier is particularly relevant for insects due to their elevated surface-to-volume ratios, which increase their vulnerability to contact poisons (Lewis 1980). Nonpolar, hydrophobic substances readily penetrate the cuticle, making the determination of the lipophilicity/hydrophilicity of pesticides a crucial step in risk assessments (Duchowicz 2020). Conversely, charged molecules, including metal ions, penetrate the hydrophobic cuticle less easily, suggesting that the insect cuticle offers resistance to metals. However, the cuticle contains numerous pores from gland and trichogen cells, as well as spiracles for gas exchange, which could potentially allow metal entry into the epidermis and hemolymph, though strong evidence for these routes is lacking (**Figure 3A**) (Scheie & Smyth 1967). Additionally, mechanical damage to the cuticle could facilitate metal entry into the hemolymph, as the protective barrier would be compromised at the damaged sites (Parle *et al.* 2017). Much work remains to be done at the cuticle-metal interface, particularly in determining metal absorption through the cuticle.

In insects, the majority of enzymatic digestion and nutrient absorption takes place in the midgut, as both the foregut and hindgut have an impermeable cuticular inner lining (Holtof *et al.* 2019). Along the midgut, most insects possess a semipermeable peritrophic membrane composed of chitin, proteins, and glycoproteins, which protects the midgut epithelium from mechanical and chemical damage, as well as from pathogens and xenobiotics (Erlandson *et al.* 2019; Huang *et al.* 2015). The peritrophic membrane is riddled with pores, and factors such as pH, thickness, and pore size determine the permeability of various molecules (Merzendorfer *et al.* 2016). As large molecules like amino acids diffuse through the pores, it is likely that metals also easily cross the peritrophic membrane, but accurate descriptions of metal diffusion are lacking (**Figure 3B**). Metal ions might interact with the chitin and proteins in the peritrophic membrane, potentially altering its structure but also reducing the amount of metal ions reaching the epithelium (Rayms-Keller *et al.* 1998). While the peritrophic membrane has been shown to play crucial roles in defence against xenobiotics such as pesticides or phytochemicals (Barbehenn 2001), its role in facing metal exposure is yet to be determined. Since carbohydrate digestion and water osmosis occur in the foregut and hindgut, respectively, it is possible that metals could also enter the gut epithelium in these regions despite the cuticular inner lining (Holtof *et al.* 2019).

In addition to endogenous barriers, exogenous barriers such as microbial gut symbionts may protect insects against metal exposure. The gut microbial communities of insects vary significantly in size, composition, and function among species (Engel & Moran 2013). For instance, honey bees contain 10^4 times more bacterial cells than fruit flies, with symbionts restricted to the hindgut in honey bees, whereas fruit flies harbour their symbionts mainly in the midgut (Broderick & Lemaitre 2012; Martinson *et al.* 2012). Gut symbionts may protect insects against metals as some strains utilise metals for metabolic functions or possess detoxification mechanisms, thereby preventing metals from reaching the gut epithelium (**Figure 3B**). For example, in housefly larvae, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* adsorbed and aggregated copper, preventing gut microbiota dysbiosis and gut damage (Wang *et al.* 2023; Yin *et al.* 2023). In silkworm larvae, exposure to cadmium increased the abundance of *Thermus*, *Glutamicibacter*, and *Sphingomonas* bacteria, which can reduce and precipitate metals, facilitating their excretion in the faeces (Chen *et al.* 2023). In bees, genes associated with metal resistance have been identified in *Snodgrassella alvi* and *Gilliamella apicola*, although it remains unclear whether bees exploit these resistance mechanisms (Rothman *et al.* 2020). Importantly, some insects harbour few or no bacteria, or have bacteria only in their hindgut, meaning metals encounter few or no bacteria when accessing the midgut. Much research remains to be done to assess the importance of insect gut symbionts in mitigating metal exposure.

4.3. Third line: Detoxification

In insects, xenobiotics such as pesticides or phytochemicals activate transcription factors upon entering the cells, initiating detoxification pathways (Amezian *et al.* 2021). Detoxification generally involves converting active, lipid-soluble substances into inactive, water-soluble metabolites that can be excreted. This transformation typically occurs in three phases. In phase I, enzymes such as cytochrome P450 monooxygenases reduce the biological activity of the substance through oxidation-reduction reactions. Phase II involves enzymes like glutathione S-transferases, which conjugate the modified substance with endogenous molecules to enhance its solubility. In phase III, membrane transporters, including ATP-binding cassettes, translocate the conjugated compounds out of the cell for excretion (Berenbaum & Johnson 2015; Gao *et al.* 2022). However, metals, being inorganic and non-degradable, cannot be inactivated and excreted via this three-step detoxification pathway. To prevent cellular damage, insects must sequester and excrete metals before they can interact with vital macromolecules.

In a general cellular response to metal entry, both essential and non-essential metals activate Metal Transcription Factor 1 – through zinc binding. This factor binds to a Metal Response Element in the nucleus, promoting the transcription of metal response genes (Janssens *et al.* 2009). Key among these genes are those coding for non-enzymatic metal ion-binding proteins known as metallothioneins. These proteins bind both essential and non-essential metal ions and facilitate their transport out of the cell for excretion (**Figure 4**). Metallothioneins are low molecular weight proteins, approximately 60 amino acids long and 6–10 kDa, characterised by high cysteine content and the absence of aromatic amino acids. The thiol groups of cysteine residues, typically found in two clusters along the protein structure, bind a wide range of metal ions – with specific affinities – with up to 12 monovalent ions per cluster (Hamer 1986; Kägi 1991). Over 300 metallothionein genes have been identified across 13 insect orders, including six genes in fruit flies (Luo *et al.* 2020), one gene in honey bees, and two putative genes in bumble bees (Purać *et al.* 2019). These findings in bees remain ambiguous, as no metallothionein gene has been identified in the newly annotated genome of the bumblebee *Bombus impatiens* (Toth *et al.* 2024), and the metallothionein gene previously annotated in the honey bee genome has been removed from the reference genome (A. Toth, pers. comm.). While metallothioneins form a crucial part of the general metal response system, more specific responses also occur. For instance, proteins specifically transporting copper, iron, or zinc to their respective target proteins as co-factors have been identified in fruit flies (Slobodian *et al.* 2021).

Metallothioneins accumulate in lysosomes and are primarily produced in the midgut, hemocytes, salivary glands, fat bodies, and Malpighian tubules (Badiou-Bénéteau *et al.* 2013; Polykretis *et al.* 2016; Yiwen *et al.* 2022). Metallothionein-bound metals are likely sequestered in the fat bodies and eliminated through the Malpighian tubules (Borsuk *et al.* 2021; Farina *et al.* 2022; Li *et al.* 2019). Besides, gut cells directly accumulating metals undergo apoptosis to be desquamated into the gut lumen for excretion before metals enter the hemolymph (Rost-Roszkowska *et al.* 2008). However, the physiological pathway through which metals are excreted from the insect body via faeces remains uncharted (**Figure 4**). For instance, it is unclear whether metals are excreted in the gut in a metallothionein-bound state. Besides faecal excretion, metals are also expelled in the exoskeleton during moulting or in the pupal exuviae in insect larvae, as observed in mealworm beetles (Lindqvist & Block 1995) and leaf beetles (Przybyłowicz *et al.* 2003) (**Figure 4**). In adult fruit flies, the total body content of copper correlates with the darkness of the cuticle, while in ground beetles cadmium accumulated in the integument, suggesting that metals could be sequestered in the cuticle in adults as well (Lindqvist *et al.* 1995; Vásquez-Procopio *et al.* 2020). Nevertheless, the precise mechanism underlying metal accumulation in the exoskeleton during larval development or in adult stages remains unknown.

4.4. Fourth line: Alleviation

Metal ions that are not sequestered and excreted interfere with crucial macromolecules, increasing ROS production, inhibiting proper enzymatic functions, damaging DNA and membranes, and ultimately leading to cell death. At this stage, cellular mechanisms are activated to mitigate such damage. Harmful ROS, including hydroxyl radicals and superoxide anions, are neutralised by antioxidant metabolic enzymes such as superoxide dismutases and catalases, which convert them into harmless water and oxygen (**Figure 5**) (Merritt & Bewick 2017). For instance, these two enzymes were more highly expressed in honey bees from urban, metal-polluted areas than in honey bees from mountainous, metal-poor areas (Gizaw *et al.* 2020). Non-enzymatic ROS-scavenging molecules also protect insects from high ROS levels (**Figure 5**). Black soldier fly larvae pretreated with exogenous tocopherol (vitamin E) had higher body masses and fewer oxidative damages than untreated larvae after cadmium exposure (Shi *et al.* 2024). Interestingly, it has been suggested that insects might ingest exogenous antioxidants to prevent or treat oxidative stress, a phenomenon known as prophylactic and therapeutic self-medication, respectively (Beaulieu & Schaefer 2013). For instance, butterflies were found to increase their polyphenol intake when exposed to hot, ROS-producing conditions, which alleviated oxidative damage in their tissues (Beaulieu *et al.* 2015). Yet, whether insects preferentially consume antioxidant-rich food resources when exposed to xenobiotics, including metals, remains to be investigated.

Under oxidative stress, numerous proteins lose their conformation and function due to ROS-induced carbonylation of various amino acids and subsequent protein aggregation. Consequently, the production of chaperone proteins, such as heat shock proteins (HSPs), is upregulated to assist in the refolding of misfolded proteins or guide them through proteolysis (**Figure 5**) (Hayes & Dice 1996). In fruit flies and bumble bees, exposure to cadmium and mercury, respectively, led to increased expression of *hsp70*, a gene coding for a protein known for its chaperoning functions (Hu *et al.* 2019; Nogueira *et al.* 2019). Additionally, metal exposure and the associated ROS production can cause DNA damage (Ceschi-Bertoli *et al.* 2020), necessitating the activation of DNA repair mechanisms (**Figure 5**). In fruit flies, cerium exposure increased the expression levels of *Mlh1* and *Brca2* genes, that code for proteins involved in double-strand break repairs (Yalçın *et al.* 2022). As a final response to substantial and irreparable damage from metal exposure, cells initiate apoptosis (i.e., programmed cell death) to prevent excessive inflammation, malignant mutations, and tumour formation (**Figure 5**). For instance, stingless bees exposed to copper showed increased levels of caspase-3 proteins in their midgut epithelium, indicating activation of the apoptotic pathway (Bernardes *et al.* 2021). Similarly, fruit fly larvae exposed to silver exhibited higher activity of caspase-3 as well as caspase-9, another protease involved in the apoptotic pathway (Ahamed *et al.* 2010).

5. Autocatalytic toxicity

Metal intoxication can escalate rapidly when the defence mechanisms of insects are compromised by the metals themselves, thereby increasing subsequent exposure and damage. In honey bees, lead exposure has been shown to alter sucrose sensitivity upon antennal stimulation (Burden *et al.* 2019), a phenomenon also observed with biopesticides (Carlesso *et al.* 2020). If insects initially detect and avoid metals in their environment but are subsequently exposed, metal-induced cognitive impairment may hinder their ability to avoid future exposure. Further research is necessary to determine whether metal exposure can impede subsequent metal detection in insects.

Additionally, metals can damage the cuticle or gut of insects, weakening these critical barriers against future exposure. For example, in beetles, charged nanoparticles of alumina bind to the cuticle via triboelectric forces, disrupting the wax layer and causing dehydration (Stadler *et al.* 2017). Similarly, copper exposure in stingless bees compromised the integrity of the peritrophic matrix (Bernardes *et al.* 2021), a disruption also observed with cadmium and lead nanoparticles in honey bees (Dabour *et al.* 2019). The rupture of these barriers can facilitate increased metal entry upon subsequent exposures.

Metals may also interfere with their own detoxification pathways, though evidence on this is limited. Metals induce the production of ROS, which can alter protein conformation. It is plausible that ROS may denature metal-scavenging metallothioneins or proteins involved in the transcription of metallothionein genes. Investigating whether metals can ultimately impair the proteins essential for their detoxification is crucial. Also, metals have been shown to deplete energy reserves in carabid beetles (Maryanski *et al.* 2002) and cotton bollworms (Baghban *et al.* 2014), potentially reducing the energy available for detoxification processes during subsequent exposures.

Furthermore, metals can disrupt the cellular repair processes for metal-induced damage. Cadmium, for instance, is known to interfere with DNA repair mechanisms, hindering the repair of metal- and ROS-induced DNA damage (Bertin & Averbeck 2006). Metals may also alter the structure of antioxidant and chaperone proteins, preventing ROS degradation and proper protein folding, although data in this area are sparse.

Surprisingly, these 'autocatalytic' toxicity processes are rarely mentioned in ecotoxicological studies, regardless of the pollutant in question. Understanding these self-amplifying mechanisms is essential for a comprehensive assessment of metal toxicity in insects.

6. Perspectives

In the last decade, research has increasingly highlighted the importance of elemental stoichiometry in insect ecology, a field previously dominated by studies focusing on macromolecule composition (Filipiak & Filipiak 2022; Filipiak & Weiner 2017). Despite the necessity for insects to ingest and regulate essential metals for their metabolic activities, knowledge about metal homeostasis and insect-metal interactions remains limited (**Box 1**). While it is known that insects evaluate their food resources to regulate macronutrient intake (Vaudo *et al.* 2016), further investigations are needed to understand how insects regulate their intake of essential metals. Such research is crucial for elucidating the ability of insects to detect and avoid toxic, non-essential metals in their environment, as well as toxic concentrations of essential metals. Furthermore, although research has examined the passage of pesticides through the insect cuticle and gut epithelium (Balabanidou *et al.* 2018; Barbehenn 2001), there is a paucity of information regarding the passage of metals through these barriers. Similarly, while the role of insect gut microbiota in degrading pesticides has been explored (Siddiqui *et al.* 2022), its role in mitigating metal exposure remains overlooked. There is an increasing body of research addressing cellular detoxification of metals, including studies on metal-scavenging metallothioneins, but the physiological processes underlying metal excretion remain unclear. For instance, it is unknown if metals accumulating in organs (e.g., ovaries) may eventually be excreted in insect faeces. In contrast, there is substantial data on the cellular pathways activated to manage cellular damage following metal exposure, as these pathways are common in response to various stressors (e.g., temperature, parasites). Given the increasing reliance on metal-based technologies and the associated rise in metal harvesting, it is imperative to consider metal pollution as an emerging threat to insect populations. Laboratory and field studies are necessary to elucidate the defence mechanisms insects employ against metal exposure, which is crucial for preventing metal-driven declines in insect populations. Besides the conservation of insect populations, developing research on insect-metal interactions could also benefit the understanding of metal-related diseases in humans. Laboratory insects such as *Drosophila melanogaster* have proven to be excellent models for understanding such diseases (Calap-Quintana *et al.* 2017). As humans are affected by several genetic diseases related to metal homeostasis (e.g., Wilson's disease characterised by excess copper), research on insect-metal interactions could then provide unexpected contributions to human health, making it a matter of both ecological and medical importance.

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Figure 1. Cellular imports and toxicity mechanisms of metals. Essential and non-essential metals cross the cell membrane through legitimate and non-legitimate influxes. Protein transporters are either metal-specific (e.g., Malvolio for ferric ion) or unspecific (e.g., Ca^{2+} channel). The toxicity mechanisms are threefold: (i) deforming DNA structure by binding to phosphates and nucleobases, (ii) altering protein conformation and function by binding to thiol (-SH) groups, and (iii) producing ROS via Haber-Weiss reactions. ROS are originally byproducts of vital metabolic activities (e.g., mitochondrial respiration). ROS damage DNA, proteins and lipids through oxidation. As metals impair protein function, they also disturb DNA repair and antioxidant activities, thereby indirectly increasing DNA damages and ROS production.

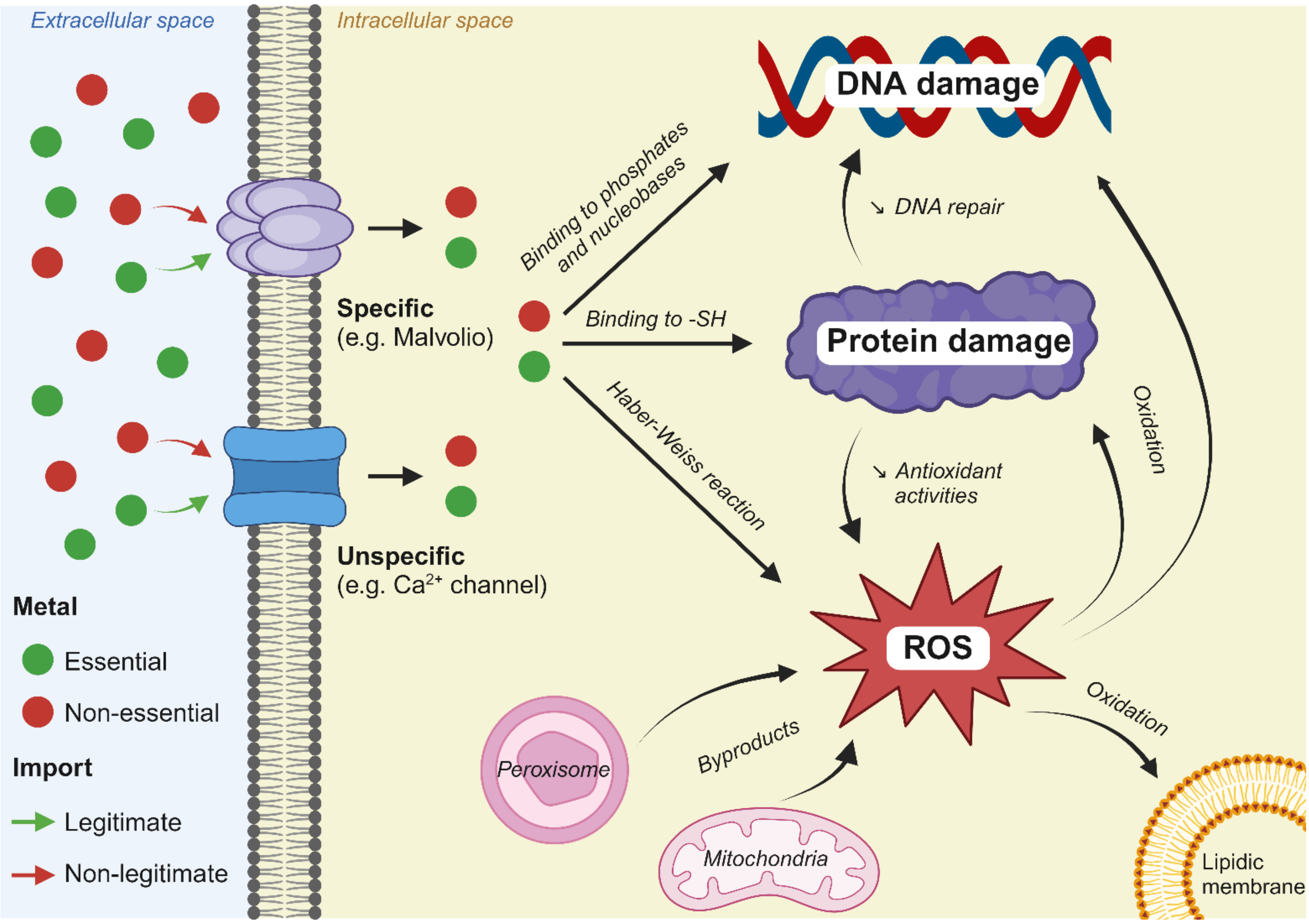


Figure 2. First line of defence: avoidance. Insects may detect and avoid toxic concentrations of metals in their food (i.e., no oral exposure), in their substrate (i.e., no topical exposure) or in their oviposition sites (i.e., no exposure for offspring).

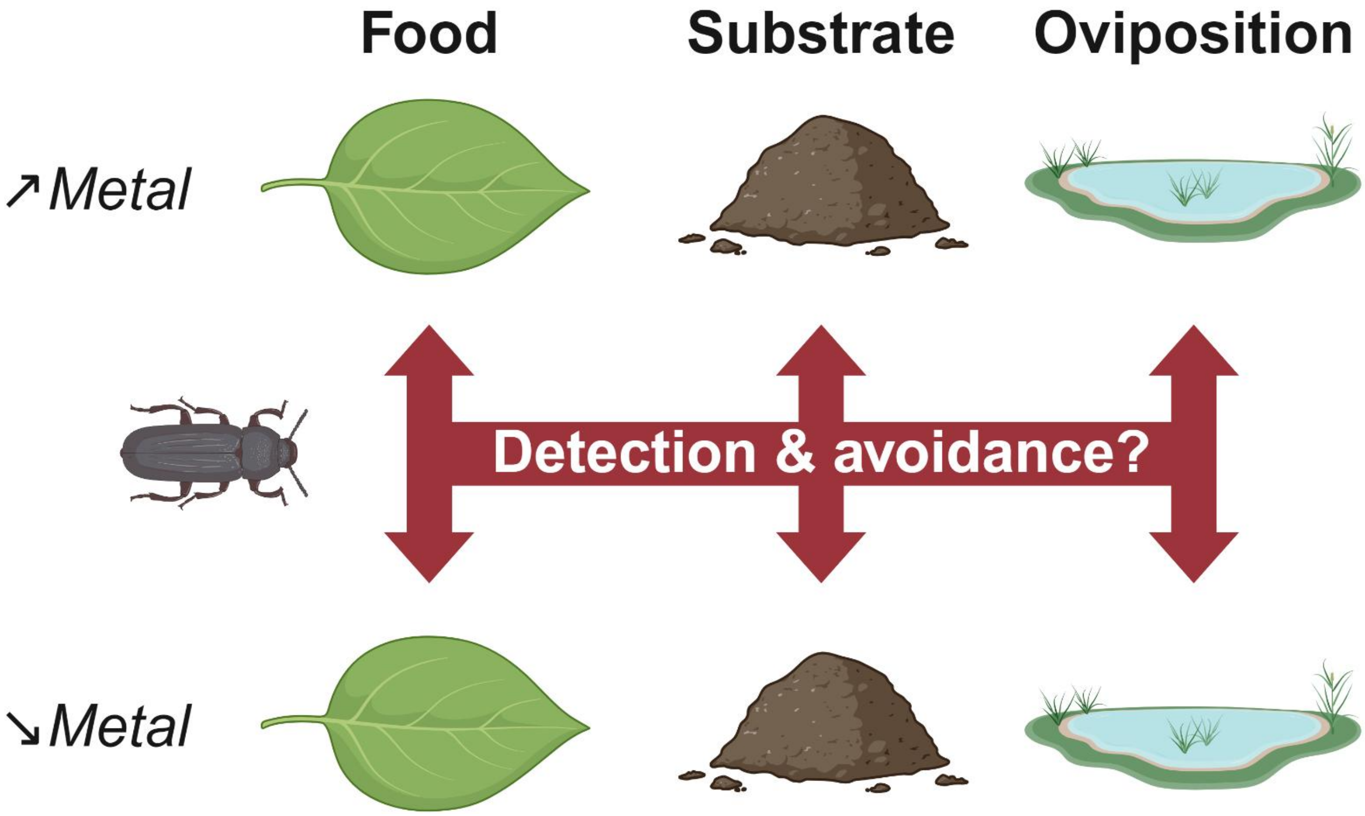


Figure 3. Second line of defence: Barrier. A. Insects harbour a cuticle that may prevent metals from accessing the epidermis, although openings are present due to trichogen cells, gland cells and spiracles (not represented). **B.** In the midgut, the peritrophic membrane as well as the microbial symbionts may prevent metals from accessing the gut epithelium.

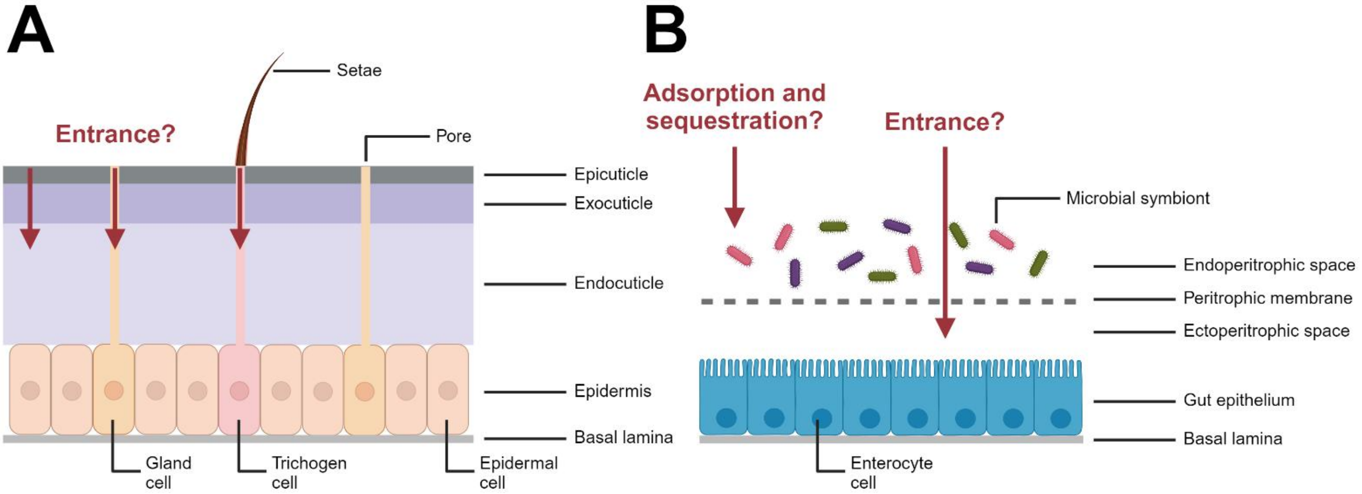


Figure 4. Third line of defence: Detoxification. To prevent metals from causing damages, insect cells may sequester them in metal-scavenging proteins (e.g., metallothioneins). Metals may accumulate in the fat bodies and may be excreted in the faeces or in the exoskeleton.

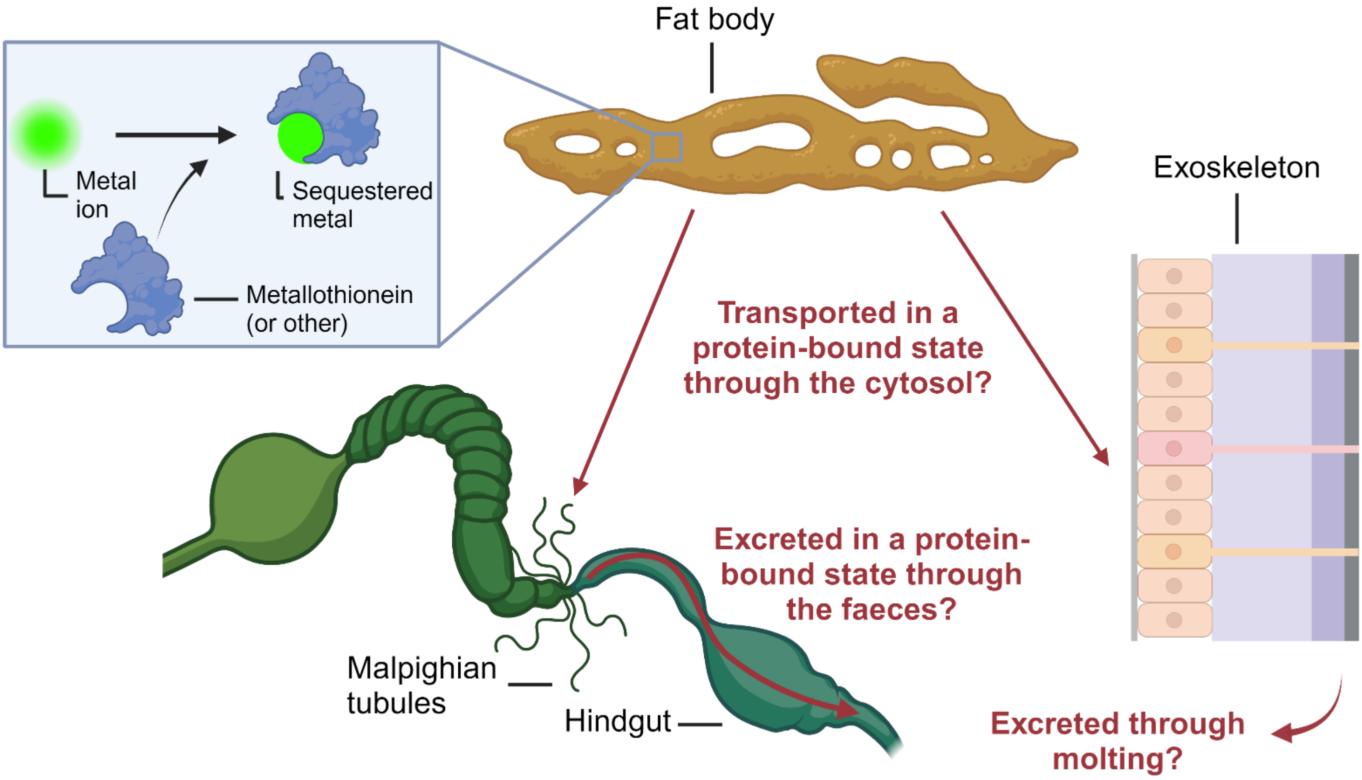
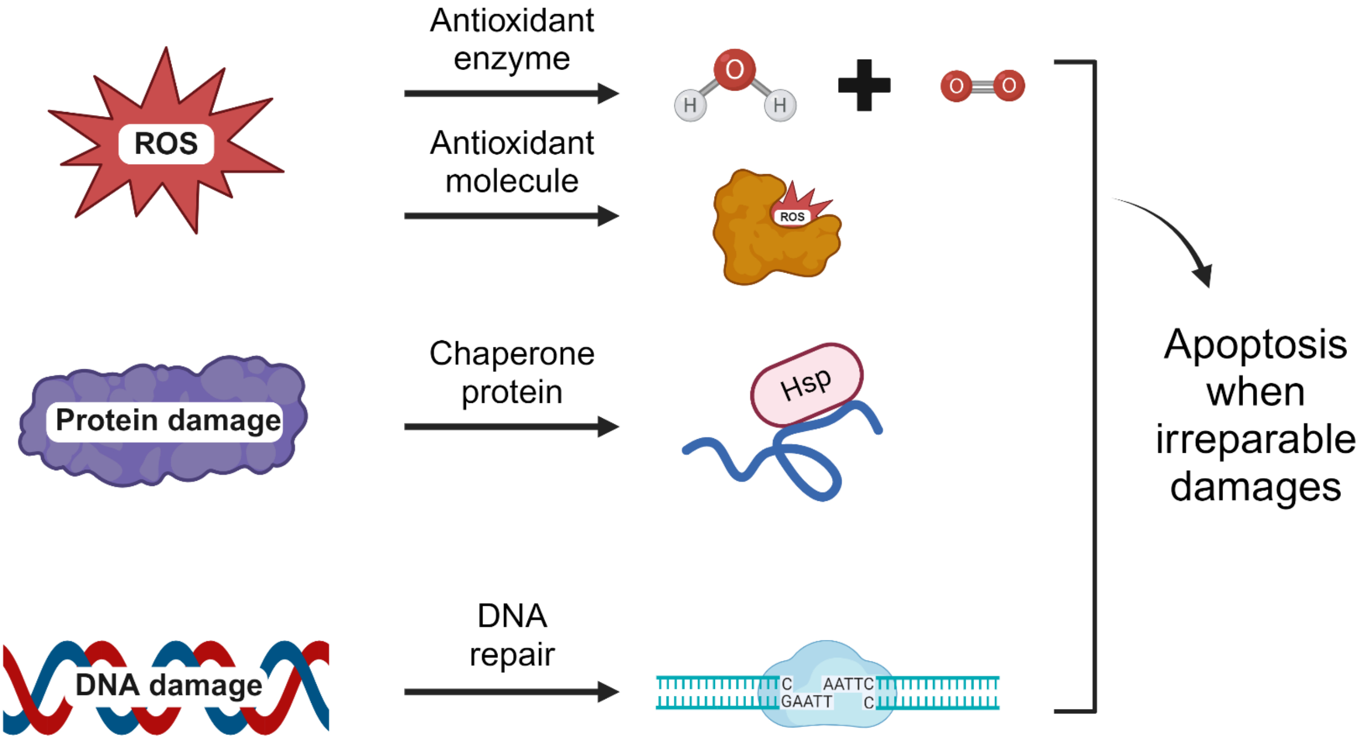


Figure 5. Fourth line of defence: Alleviation. Once metals enter the cells and interact with macromolecules, cellular mechanisms take place to mitigate damages. Enzymatic proteins transform reactive oxygen species (ROS) into harmless molecules, while antioxidants sequester ROS. Chaperone proteins help for protein refolding or guide misfolded proteins through proteolysis. DNA repair mechanisms are also activated. When cellular damages are too substantial, the cells enter apoptosis.



Box 1 – Outstanding questions at the insect-metal interface

Mechanism 1 – Avoidance

- Are metal-activated bitter-sensing receptors conserved across insect species, and how do they vary in sensitivity to different metals?
- Does the nutritional complexity of food influence the detection and avoidance of metals by insects?

Mechanism 2 – Barrier

- What role does the peritrophic membrane play in regulating metal absorption into the gut epithelium?
- How do gut microbes with genes for metal detoxification enhance the host insect's resistance to metals?

Mechanism 3 – Detoxification

- What are the cellular and molecular pathways involved in the transfer of metal-bound metallothioneins from storage organs to the gut lumen for excretion?
- Is the sequestration of metals into the cuticle a widespread detoxification mechanism across insects, and how does this strategy vary with metal type and insect developmental stage?

Mechanism 4 – Alleviation

- Do insects preferentially consume antioxidant-rich food when exposed to metals?
- Are insects more resilient to oxidative damage caused by essential metals compared to non-essential metals, and what mechanisms underlie these differences?

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