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Current permissible levels of metal pollutants harm terrestrial invertebrates



Coline Monchanin^{a,b,*}, Jean-Marc Devaud^a, Andrew B. Barron^b, Mathieu Lihoreau^{a,*}

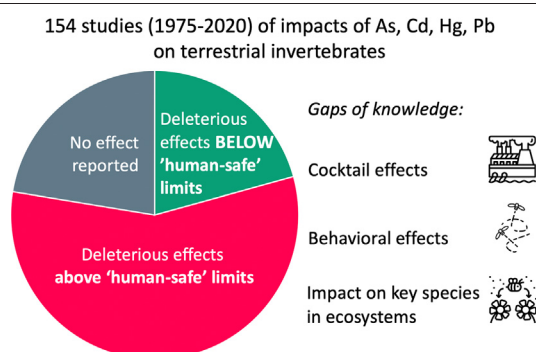
^a Research Center on Animal Cognition (CRCA), Center for Integrative Biology (CBI); CNRS, University Paul Sabatier, Toulouse III, France

^b Department of Biological Sciences, Macquarie University, Sydney, Australia

HIGHLIGHTS

- The current decline of invertebrates worldwide is alarming.
- Major pollutants, like metallic trace elements in the air, soils and water, are a potential cause, so far overlooked.
- We reviewed the scientific literature on the effects of As, Cd, Pb and Hg on terrestrial invertebrates.
- These well-studied pollutants impact invertebrates even at levels below those recommended as 'safe' for humans.
- Our results call for a revision of the regulatory thresholds to protect terrestrial biodiversity.

GRAPHICAL ABSTRACT



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ABSTRACT

The current decline of invertebrates worldwide is alarming. Several potential causes have been proposed but metal pollutants, while being widespread in the air, soils and water, have so far been largely overlooked. Here, we reviewed the results of 527 observations of the effects of arsenic, cadmium, lead and mercury on terrestrial invertebrates. These four well-studied metals are considered as priorities for public health and for which international regulatory guidelines exist. We found that they all significantly impact the physiology and behavior of invertebrates, even at levels below those recommended as 'safe' for humans. Our results call for a revision of the regulatory thresholds to better protect terrestrial invertebrates, which appear to be more sensitive to metal pollution than vertebrates. More fundamental research on a broader range of compounds and species is needed to improve international guidelines for metal pollutants, and to develop conservation plans to protect invertebrates and ecosystem services.

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1. Introduction

Terrestrial invertebrate bioabundance and biodiversity are declining (Wagner, 2020). Since invertebrates are basal to terrestrial food webs and provide key ecosystem services, the short-term ecological consequences of invertebrate decline could be very severe (Goulson, 2019; Sánchez-Bayo and Wyckhuys, 2019). The rate of decline is especially

* Corresponding authors at: CRCA-CBI, Bât 4R4, Univ. Paul Sabatier, 118 route de Narbonne, 31062 Toulouse, France.

E-mail addresses: coline.monchanin@univ-tlse3.fr (C. Monchanin), jean-marc.devaud@univ-tlse3.fr (J.-M. Devaud), andrew.barron@mq.edu.au (A.B. Barron), mathieu.lihoreau@univ-tlse3.fr (M. Lihoreau).

alarming as it has been estimated that land-dwelling insects abundance has been declining at a rate of ca. 1% every year for a century (van Klink et al., 2020). Many factors have been proposed to explain this loss. These include climate change (Wilson et al., 2007), habitat reduction due to intensive agriculture and urbanization (Fattorini, 2011; Dudley and Alexander, 2019), introduced pathogens, predators and competitors (Goulson et al., 2015), as well as chronic exposure to agrochemicals (van Lexmond et al., 2015).

Here we argue that metallic pollution is a major, yet currently overlooked, stressor of insects and other terrestrial invertebrates that needs urgent attention from scientists and stakeholders. At trace levels, metals such as cobalt, copper, iron, manganese, selenium and zinc are essential micronutrients for animals and plants (Phipps, 1981; WHO/FAO/IAEA, 1996). Others, such as cadmium, chromium, mercury, lead and nickel, have no useful biological function and exert toxic effects even at low concentrations (He et al., 2005; Tchounwou et al., 2012). This is also the case for the metalloids arsenic, which we here also refer to as a metal pollutant for the sake of simplicity. While all of them are naturally present in the Earth's crust, their environmental concentrations have considerably increased above natural baselines (Zhou et al., 2018), due to mining and smelting operations, combustion of fossil fuels, industrial production, domestic and agricultural use of metals and metal-containing compounds (Bradl, 2005). This elevated and widespread contamination of air (Suvarapu and Baek, 2017), soils (Wuana and Okieimen, 2011), water (Mance, 1987) and plants (Krämer, 2010) has generated major public health concerns.

There are many detrimental impacts of metal pollutants on vertebrates, which include cellular damage, carcinogenesis and neurotoxicity (Tchounwou et al., 2012; Chen et al., 2016). Many local initiatives exist to reduce their emissions (e.g. lead: (Chadwick et al., 2011), cadmium: (Hayat et al., 2019), mercury: (Pacyna et al., 2009)). Even so, environmental metallic pollution is still high (Järup, 2003), calling for a more systematic assessment on the impact on biodiversity. For example, in 2019 the World Health Organization (WHO) stated that there was no safe level of lead for vertebrates (WHO, 2019), yet the majority of industrial activities are increasing the level of lead in the environment (Järup, 2003; Li et al., 2014). The recent report that bees and flies in densely urbanized areas suffer from exposure to metallic air particles (Thimmegowda et al., 2020) suggests that the consequences of metallic pollution on terrestrial invertebrates could be extremely important and widespread (for a review on aquatic invertebrates see (Rainbow, 2002)).

Here, we assessed the impact of metal pollutants on terrestrial invertebrates through a review of the scientific literature on four well-studied metals over the past 45 years. We found that these metals have detrimental effects on a wide diversity of species at levels below those considered safe for humans. We discuss the need for more fundamental research into the impacts of metal pollutants on insects to improve international guidelines for the regulation of metal pollutants, and better inform conservation plans.

2. Results

2.1. Few studies focus on species delivering important ecological function

The 527 observations extracted from the literature covered 100 species (83% Arthropoda, 15% Annelida, 1.2% Rotifera, 0.4% Tardigrada, 0.2% Mollusca; Fig. 1B). Studies were biased toward pest species with an economic impact (34% of observations; e.g. the gypsy moth *Limantria dispar*, the grasshopper *Aiolopus thalassinus*, the beet armyworm *Spodoptera exigua*) and model species in biology (10%; e.g. fruit fly *Drosophila melanogaster*, large milkweed bug *Oncopeltus fasciatus*). Other groups were comparatively under-represented, including important bioindicator species, such as decomposers (15%; e.g. *Lumbricus terrestris*, *Eisenia fetida* and *E. andre*), predators (10%; e.g. ants *Formica* spp., spiders *Araneus* spp. and *Pardosa* spp.) and pollinators (13%; e.g. the

honey bee *Apis mellifera*). Some taxonomic orders that include large numbers of species involved in nutrient cycling (e.g. proturans, diplurans, earwigs), soil aeration (e.g. centipedes), or pollination (e.g. thrips) were not represented at all. Research is thus needed on these important invertebrate orders with key ecological functions to get a more accurate picture of how metallic pollution disturbs ecosystems (Skaldina and Sorvari, 2019).

2.2. Metal pollutants have detrimental effects below permissible limits

Deleterious effects were reported in 84% of the laboratory observations (N = 263 out of 313) and 49% of the field observations (N = 104 out of 214), thus representing an average of 70% (N = 367 out of a total of 527; Fig. 2A). These negative effects were observed following chronic (69%) or acute (79%) exposure (resp. N = 348 out of 503 and 19 out of 24).

We then compared the doses at which these effects were observed to international permissible limits (i.e. recommended maximum concentrations) based on human toxicity data and determined by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations (see Methods and Table S2). These toxic levels were determined for food, but also water and soils to which arthropods are in direct contact.

When considering only the observations reporting deleterious effects (N = 367), 73% of these effects (N = 269) were measured at concentrations above the maximal estimated permissible limit (see Table 1). Yet, 12% (N = 45) were measured in between the regulatory thresholds and 15% (N = 53) below the minimal estimated limit (Fig. 2A). In addition, a majority (57%, N = 53 observations out of 93) of the observations using at least one concentration below the minimal estimated permissible limit found a negative effect at that low level, irrespective of the metal.

When considering only the laboratory studies, in which exposure concentrations were controlled (Fig. 2B–C), only 32% of the studies (N = 98 out of 313) used at least one concentration below or in between permissible limits. 57% of the studies that examined levels below the maximal permissible limits (N = 56 observations out of 98) reported deleterious effects on invertebrates below the permissible limits. Of the laboratory studies investigating acute exposure below the maximal permissible limits (N = 16), ten found deleterious effects (Fig. 2B). Hence, acute exposure, while presumably rare in nature, can have deleterious effects on invertebrates below current permissible exposure levels. This suggests that the permissible limits designed for humans are not appropriate for terrestrial invertebrates, who seem to be more sensitive to metal pollutants.

2.3. Few studies address the behavioral effects of metal pollutants

79% of the 154 studies we found were published after 2007 (Fig. 3A). About half of the observations focused on physiology (52%), followed by studies on development (17%), survival (13%), population dynamics (6%), reproduction (6%) and behavior (6%) (Fig. 3B). It has become increasingly clear that understanding the sublethal behavioral effects of a stressor (e.g. mobility, navigation, feeding behavior, learning, memory) is crucial to assess the long-term impact of that stressor on invertebrate populations (Mogren and Trumble, 2010). This has become evident for bees, for instance, for which any impairment of the cognitive functions involved in foraging can result in a disruption in food supply to the colony compromising larval growth (Klein et al., 2017). In our review, 33 experiments reported behavioral effects (Fig. 3B), but only two explored cognitive effects (Philips et al., 2017; Piccoli et al., 2020). This is a very low number considering the well-known neurotoxic effects of the four metals on humans (Chen et al., 2016; Wright and Baccarelli, 2007) and other animals, including aquatic invertebrates (Salanki, 2000).

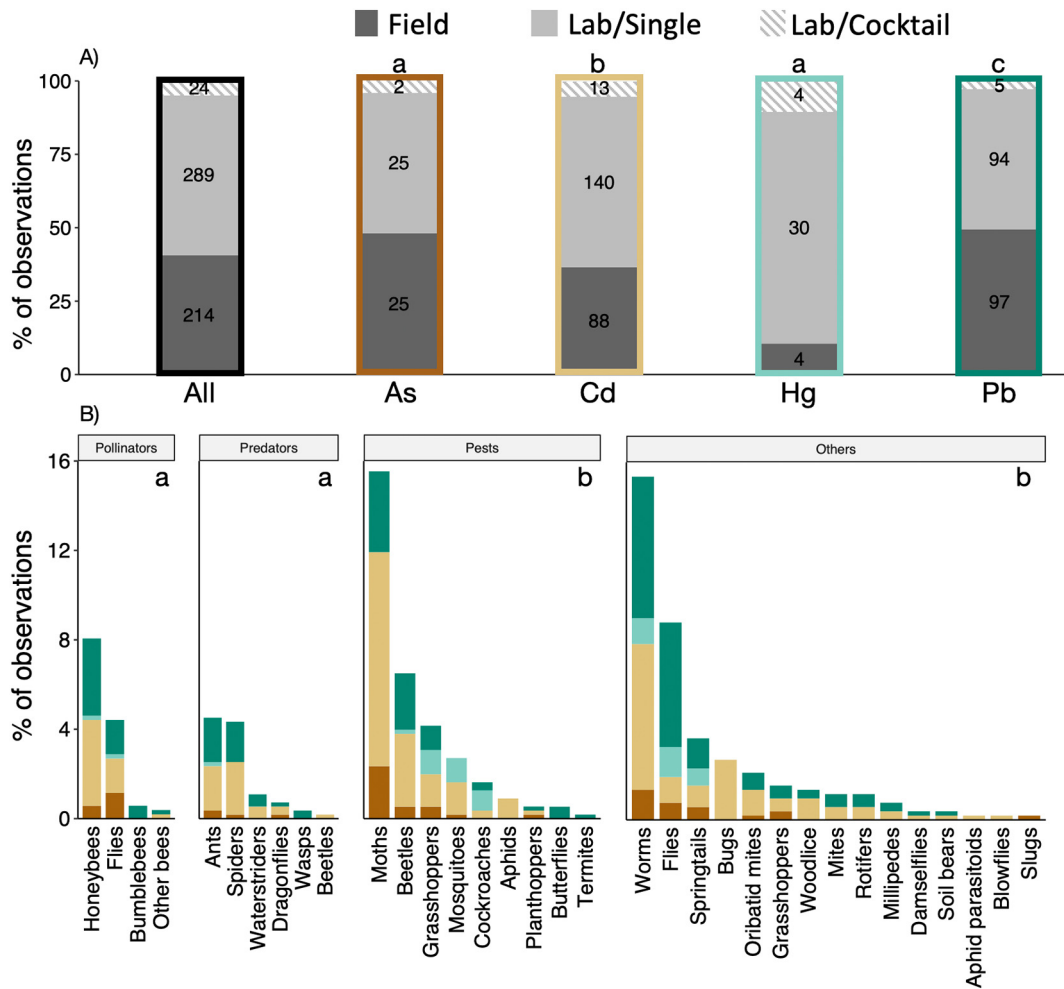


Fig. 1. Summary of invertebrate and experimental diversity in the surveyed literature. A) Percentage of observations conducted in the field (dark grey) or in the lab (light grey) per metal pollutant. Observations with mixtures of pollutants in the lab are displayed in textured light grey. Numbers of observations are shown in bars. Letters show statistical significance from chi-square test of homogeneity of proportions of observations per metal pollutant ($\chi^2 = 315.88$, $df = 3$, $p < 0.001$). B) Diversity of invertebrate groups classified by broad categories according to their ecological function and economic importance (based on (Skaldina and Sorvari, 2019)). Observations with different metal pollutants are marked using the same color code as Table 1 (As: brown, Cd: beige, Hg: light green, Pb: dark green). Letters show statistical significance from chi-square test of homogeneity of proportions of observations per functional group ($\chi^2 = 180.83$, $df = 3$, $p < 0.001$).

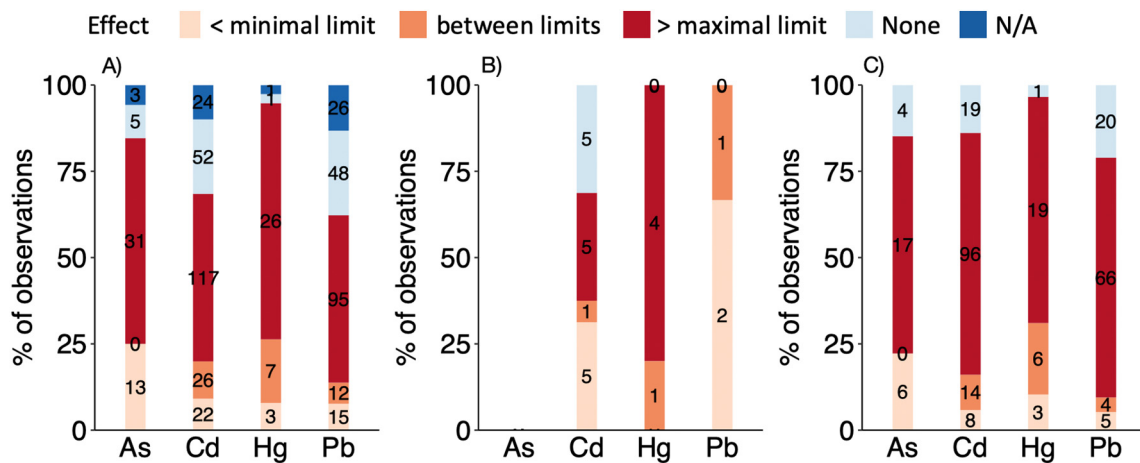


Fig. 2. Effects observed according to permissible limits. We defined the following ranges below the minimal estimated limit, between the minimal and the maximal estimated limits, or above the maximal estimated limit. A) All studies (N = 527). B) Laboratory studies with acute exposure (N = 24) and C) chronic exposure (N = 288). None: no observable effect, N/A: no conclusion available. Sample sizes are in black. Concentration ranges were marked using the same color code as Table 1.

Table 1

Permissible limits (ppm) for metal pollutants in food, water and soil. For each metal, we defined three concentration ranges: below the minimal estimated permissible limit (beige), between the minimal and maximal estimated permissible limits (orange), and above the maximal estimated permissible limit (red).

Matrices	Arsenic (As)			Cadmium (Cd)			Mercury (Hg)			Lead (Pb)		
	<0.1	0.1-0.2	>0.2	>0.05	0.05-2	>2	<0.5	0.5-1	>1	<0.01	0.01-3	>3
Food	<0.1	0.1-0.2	>0.2	>0.05	0.05-2	>2	<0.5	0.5-1	>1	<0.01	0.01-3	>3
Water	<0.01	0.01-0.1	>0.1	<0.003	0.003-0.01	>0.01	<0.001	NA	>0.001	<0.01	0.01-5	>5
Soil	<20	NA	>20	<0.9	0.9-3	>3	<0.03	0.03-2	>2	<30	30-50	>50

2.4. Few studies investigated co-occurrences despite clear synergistic effects

Only 7 out of the 154 studies addressed the question of combined effects of metal pollutants in laboratory conditions (Fig. 1A). Nonetheless the effects are clear: 55% of the observations (N = 10) reported synergistic detrimental consequences. For instance, ants (*Formica aquilonia*) chronically exposed to both cadmium and mercury failed to develop compensatory mechanisms to maintain energetic balance, causing colony collapse, while being able to cope when exposed to each metal alone (Migula et al., 1997). Similarly, the lethal effects of cadmium and zinc on aphids (*Myzus persicae*) were potentiated when the two metals were combined, which led to accelerated extinction of the treated population (Stolpe and Müller, 2016). These two metals were reported to be either synergistic or antagonist on earthworms (*E. fetida*) depending on their concentrations (Wu et al., 2012). Finally, the joint exposure of honey bees (*A. mellifera*) to cadmium and copper caused an increased development duration, elevated mortality, and decreased food intake and sucrose response (Di et al., 2020). Thus, the effects of metal co-exposure are complex and variable. The paucity of studies may be because they require more sophisticated experimental designs, larger sample sizes (factorial designs) and may yield results

that are more difficult to interpret. Yet, these studies are crucial if we are to revise the current regulations which presently only consider permissible limits for metals in isolation (Tables 1 and S2).

3. Discussion

Our review of the literature on lead, arsenic, cadmium and mercury shows many negative effects of these metal pollutants on terrestrial invertebrates. Excessive exposure to these compounds lead to a plethora of consequences, such as cytotoxicity (Braeckman, 1997), carcinogenic and/or mutagenic effects (Kheirallah et al., 2019), and disruption of metabolic processes (Ortel, 1995). Particularly worrisome are the reports of negative effects observed at doses below permissible limits in most of the studied taxa. There are reported lethal effects on grasshoppers (Schmidt et al., 1991), moths (Andrahennadi and Pickering, 2008), flies (Massadeh et al., 2008) and other groups (Osman et al., 2015; Polykretis et al., 2016; Stolpe et al., 2017). Metal exposure causes a number of sub-lethal effects, sometimes difficult to assess, such as impaired fertility (grasshoppers: (Schmidt et al., 1991); springtail: (Crouau and Pinelli, 2008); earthworm: (Konečný et al., 2014)), developmental defects (blowfly: (Nascarella et al., 2003); moth: (van Ooik et al., 2007); ant:

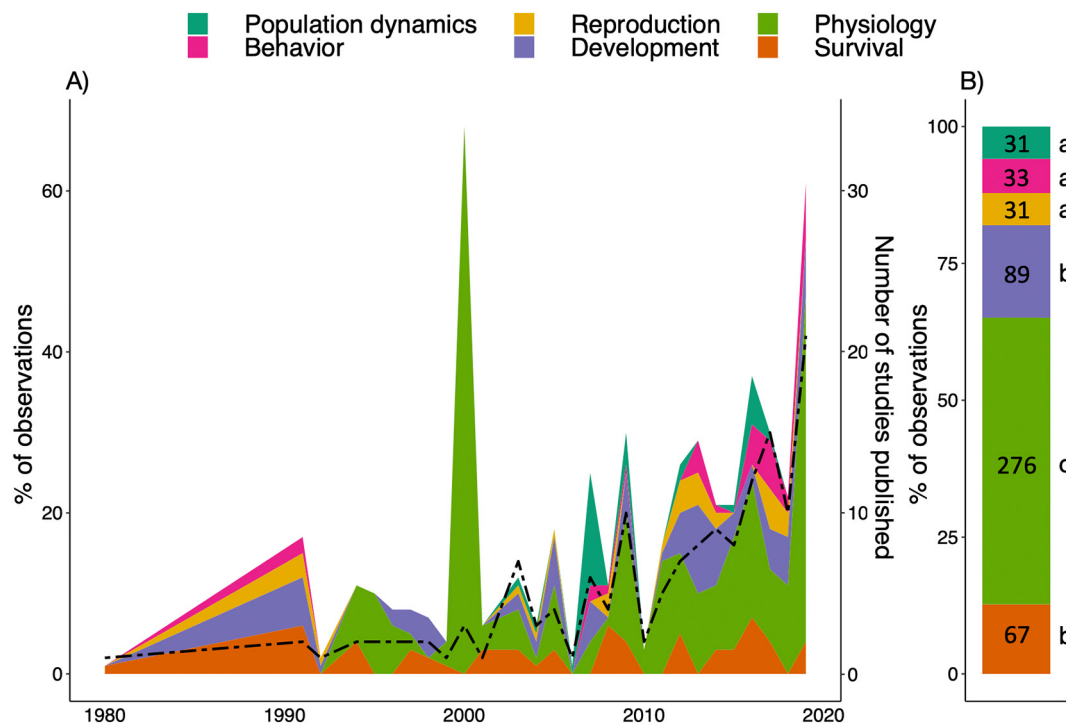


Fig. 3. Biological variables measured. A) Area chart of the number of observations per biological variable (year 2020 was omitted). The peak in 2000 is due to three large studies of physiological effects in the field (38 observations). The black dashed line represents the number of studies published yearly. B) Overall proportions of observations per biological variable (numbers of observations in black). Letters show statistical significance from chi-square test of homogeneity of proportions ($\chi^2 = 619.02$, $df = 5$, $p < 0.001$).

(Skaldina et al., 2018)), resistance to pathogens (ant: (Sorvari et al., 2007); honey bee: (Polykretis et al., 2016)) and also altered feeding behavior (aphid: (Stolpe et al., 2017); honey bee: (Burden et al., 2019)).

3.1. The impact of metal pollutants is poorly understood

At present, it is likely that the severity of these effects is underestimated. Many laboratory experiments gave animals rather limited exposure times, rarely reaching the duration of a complete life cycle. Besides, most studies overlooked any consequences of exposure to multiple metal contaminants, which would be a common occurrence in nature. There is now growing interest in assessing the sublethal impacts of metals. This trend echoes the recent shift seen in pesticide research on beneficial insects, especially pollinators, which has moved from decades of standard survival assays to experimental designs aiming at characterizing the effects on behavior and cognition (Klein et al., 2017; Desneux et al., 2007). Just like pesticides, metal pollutants have subtle, but potentially serious, effects on pollinators' behavior by disturbing foraging activity (Sivakoff and Gardiner, 2017; Xun et al., 2018), food perception (Burden et al., 2019) and the learning and memory abilities required for efficient foraging (Burden et al., 2016; Monchanin et al., 2021). Through all of these mechanisms, exposure to metal pollutants can compromise food supply to the offspring, and hence the viability of a colony or population.

There are potentially complex interactions between behavior and pollutant exposure. Since an animal's behavior can influence how much metal pollution it is exposed to (Mogren and Trumble, 2010; Gall et al., 2015), behavioral disturbances may affect exposure and sensitivity to metals. For example, impaired locomotion may reduce the capacity of individuals to avoid contaminated sites (Hirsch et al., 2003) and indiscriminate oviposition may jeopardize the survival of offspring if they are deposited on an unfavorable food plant (Cervera et al., 2004; Tollett et al., 2009). It is thus likely that we are currently underestimating the impact of metal pollution on invertebrates, due to a lack of understanding of their sublethal effects on most species.

In nature, pollutants rarely occur alone. Metals are no exception since they share common emission sources (Varela et al., 2019). For instance, cadmium, copper, zinc and lead frequently co-occur due to the output from smelters, or the application of sewage sludge as fertilizer (Bradl, 2005). High positive correlations between chromium, cadmium and arsenic amounts have been found in soil samples (Chen et al., 1999; Navas and Machín, 2002), and many studies have shown the co-accumulation of several trace metals in insects (Wilczek and Babczyk, 2000; Nummelin et al., 2007; Goretti et al., 2020). As such, co-occurring metals could have additive, antagonistic or synergistic effects (Jensen and Trumble, 2003). These interactive effects may also be influenced by the presence of other environmental stressors, such as pesticides or parasites (Alaux et al., 2010).

3.2. Multiple possible causes of invertebrates' high sensitivity to metal pollution

Our survey of the literature suggests that invertebrates may be more sensitive to the damaging effects of metal pollutants than the mammals (e.g. humans, rodents) typically used to determine "safe" environmental levels. This may be explained by differences in sensitivity to pollutants that can vary between species and with different metals (Malaj et al., 2016). Some species can discriminate metal contaminated food from uncontaminated food (Mogren and Trumble, 2010), but other species seem unable to (Stolpe et al., 2017; Burden et al., 2019). This is particularly critical for animals feeding on resources that can accumulate metals, such as leaves (Krämer, 2010) or nectar (Gutiérrez et al., 2015). Perhaps more importantly, there is emerging evidence that invertebrates may have higher levels of exposure to metal pollutants in the field than large mammals. Surveys of terrestrial biotopes show that non-essential metals tend to be accumulate at higher levels in

invertebrates than in vertebrates (Hsu et al., 2006). This seems to also be the case for aquatic taxa (Xin et al., 2015). Due to their small size, their relatively high surface area/volume ratio and the niches they occupy, invertebrates are frequently in intimate contact with soils and vegetation, or could get contaminated by specific feeding modes such as filter-feeding or deposit-feeding (De Lange et al., 2009). Their limited dispersal capacities may reduce their ability to move away from polluted areas, even if they can detect harmful levels of trace elements. As a result, metals accumulate in the bodies of individuals (Goretti et al., 2020; Nannoni et al., 2011; Mukhtorova et al., 2019; Schrögel and Wätjen, 2019) and in the nests of social species (Skaldina et al., 2018; Veleminsky et al., 1990). Some terrestrial invertebrates (e.g. ants, earthworms, bees, Isopoda) could therefore be relevant and sensitive bioindicators of metal pollution due to their particular vulnerability to metal contamination.

Invertebrates do have mechanisms to process metal pollutants. Excessive metals can be eliminated through feces (Przybyłowicz et al., 2003), accumulated in insect exoskeleton before molting (Borowska et al., 2004), or stored in specific organs (Nica et al., 2012) like the Malpighian tubules (the excretory system of invertebrates) (Rabitsch, 1997). They can also induce expression of proteins involved in metal excretion and/or detoxification, like metallothioneins (for reviews, see (Janssens et al., 2009; Merritt and Bewick, 2017)). Yet, while these detoxification mechanisms may protect species to a point, they are unlikely to spare them from the sublethal effects of metal pollutants. This can impair brain or organ function, especially since invertebrates nervous systems are size constrained with brains containing relatively few neurons (Niven and Farris, 2012). Cellular damage or death in the insect brain can result in severe consequences for the individual (Klein et al., 2017). We clearly need a better characterization of the physiological and molecular mechanisms underlying metal transfer, toxicity and tolerance in invertebrates in order to better understand their sensitivity to metal pollutants.

3.3. A need to revise guidelines of safe environmental levels of metal pollutants

Since metals are such widespread and persistent pollutants in the environment, it is a priority to develop a better assessment of their impacts on invertebrates. Our most concerning finding is the evidence that terrestrial invertebrates are highly sensitive to metal pollutants. In particular, a high percentage of studies of arsenic reported toxic effects below international permissible limits, thus pointing toward the need for more research on this specific metal (Ng et al., 2003). Our review of the literature also highlights important gaps in our knowledge. We need to study a larger diversity of species, and have more systematic investigation of doses below permissible limits. We should consider potential cocktail effects, and extend studies beyond the four metals addressed here. Although our study focuses on four metal pollutants that are well studied and considered as priority for public health concerns, other metallic compounds have been reported to negatively impact terrestrial invertebrate populations at low doses, such as selenium (deBruyn and Chapman, 2007), zinc (Cheruiyot et al., 2013), copper (Di et al., 2016), cobalt (Cheruiyot et al., 2013), nickel (Cheruiyot et al., 2013), manganese (Ben-Shahar, 2018) and chromium (Sgolastra et al., 2018). Characterizing the impacts of metal pollutants on insect fitness is going to demand an integrative and interdisciplinary research agenda, just like what has been established to assess pesticide impacts on beneficial insects. For example, focusing awareness on the sublethal effects of neonicotinoids on pollinators (Henry et al., 2012; Crall et al., 2018), triggered a revision of the risk assessments scheme and their ban in the European Union in 2018.

3.4. Concluding remarks

This survey of the existing literature clearly indicates that terrestrial invertebrates appear particularly vulnerable to arsenic, cadmium, lead

and mercury, and that most existing standards are not suited to protect them. We now need more integrative toxicological studies, on a broader range of metal pollutants and invertebrate species to better assess their impact on fitness, and to update the current environmental regulation. Only by addressing these important challenges will we be able to mitigate consequences on ecosystems and food safety, in a context of rapid and widespread decline of invertebrate biodiversity.

4. Methods

4.1. Literature review and data extraction

We focused on the four most hazardous metals documented for humans (ATSDR, 2019), for which international regulatory implementations exist (Table 1): arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb). We searched articles in the ISI Web of Knowledge database (search performed on 25/03/2020) using keywords combined with Boolean operators: *Topic = (heavy metal* OR metalloid* AND (insect* OR invertebrate* AND (cadmium OR lead OR arsenic OR mercury))*. The search was restricted to articles published between 1975 and 2020 (maximum available year range on ISI Web of Knowledge). Among the 460 hits, we selected those studies focusing on terrestrial invertebrates (i.e. protostomes) from the abstracts, and excluded review articles. This filtering yielded a subset of 154 articles from which we extracted 527 observations investigating effects of metal pollutants on terrestrial invertebrates (see raw data in S1 Table).

From each observation, we extracted: (1) the name of targeted invertebrate species, (2) the metal(s) used, (3) the experimental conditions (field, laboratory), (4) the mode of exposure to the metal (food, water, soil), (5) the type of exposure (acute: <24 h, chronic: >24 h), (6) the range of metal concentrations tested (min- max in ppm), (7) the biological responses measured (e.g. survival, reproduction, behavior), and (8) the lowest metal concentration for which an effect was observed. Heterogeneity of proportions was assessed using chi-square test.

Briefly, the vast majority of the observations focused on cadmium (46%) and lead (37%), while less information was available on arsenic (10%) and mercury (7%) (Fig. 1A). 59% of the observations were obtained in field surveys and 41% in laboratory experiments with controlled exposure. Since the effects can greatly vary depending on the duration of exposure and time of assessment, here we considered as acute exposure any case where individuals were exposed to a single dose and assessed within 24 h. Despite the diversity of protocols, most studies used chronic exposure (95%), through the diet (49%) or the soil (43%).

4.2. Concentration ranges

All permissible limits are based on human toxicity data. Levels were determined from the international standards set by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations. The permissible limits are recommended values for: 'food and drinking water', as defined in the Codex Alimentarius (Codex Alimentarius, 2015), to deal with 'contaminants and toxins in food and feed' and to be 'applied to commodities moving in international trades' (Codex Alimentarius, 2015); guidelines for water quality in irrigation (Ayers and Westcot, 1994); critical values in soil based on the Organization for Economic Co-operation and Development (OECD) risk assessment studies (de Vries et al., 2003) and FAO standards (WHO/FAO, 2001). These limits vary across types of food, water (i.e. drinking, irrigation) and soils (i.e. allotment, commercial, residential, agricultural). Local guidelines (see S2 Table), when they exist, can vary across countries and are less conservative (higher thresholds) than the international standards, especially for soils and water. For each of these matrices, we thus considered the minimal and the maximal estimates of permissible limits. We defined three concentration ranges:

below the minimal estimated limit, between the minimal and maximal estimated limits, and above the maximal estimated limit (Table 1). Whenever only one threshold value was defined, no intermediate range could be defined (NA: not applicable). Note that for water, whenever possible, we considered the minimal value for drinking water and the maximal value for irrigation water.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.146398>.

Data accessibility statement

Raw data used of the analyses are available in S1 Table.

CRediT authorship contribution statement

CM and ML designed the study. CM collected the data, analyzed the data, and wrote the first draft of the manuscript. All authors contributed substantially to revisions.

Declaration of competing interest

The authors declare no conflict of interest.

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