Title: Influence of Heavy Metal Contamination on Urban Natural Enemies and Biological Control

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Abstract: Urban agriculture is increasing worldwide. Many urban agroecosystems are constructed on former residential or industrial land. A history of contamination within urban landscapes may negatively impact the biota necessary for sustainable crop production, including arthropod natural enemies. This investigation revealed that heavy metal contamination can influence the composition of natural enemy communities and exposure can have reproductive, developmental, immunological and behavioral impacts on predators and parasitoids. Natural enemies exposed to heavy metals typically live shorter lives, take longer to develop and exhibit a reduced reproductive potential. Further, they may incur significant energy costs through the production of detoxification enzymes. How these factors influence biological control services is virtually unknown. With increasing agricultural activity as well as greater focus on the conservation potential of urban greenspaces it is critical to understand how heavy metal exposure influences natural enemy-prey interactions in cities. Natural enemies are likely to forage within a diversity of greenspaces that vary in soil heavy metal composition and concentration. Thus, even if contamination on-farm is low, predators and parasitoids could be susceptible to heavy metal uptake via their larger environment. This is a new and relatively unexplored area for biological control research, with important implications for our understanding of urban agricultural food web interactions.

Introduction

Agriculture has long been part of the urban landscape, from home gardens to small scale farms [1] but in recent decades, interest in producing food in cities has grown dramatically [2]. It is estimated that more than 30% of the global urban population is engaged in some form of urban agriculture (UA) [3]. Furthermore, households with limited access to fresh produce are more likely to engage in UA [4], resulting in greater acquisition of fresh fruits, vegetables, eggs, and other agricultural products within low income communities. Urban greenspaces, including community gardens and farms, have also been demonstrated to reduce human health risks via provision of additional biophysical ecosystem services [5] including filtration of pollutants from the air, reduction of the heat island effect, supplying space for physical activity, and improved neighborhood aesthetics [1,2].

UA often occupies vacant land that formerly supported industrial, commercial, or residential land use (Figure 1). These habitats frequently have a history of contamination [6] by heavy metal (HM) pollutants, with soils serving as the major sink [6,7]. HM's include both nonessential elements (arsenic, cadmium, chromium, lead, mercury, and nickel) and elements essential to life that become toxic at higher concentrations (cobalt, copper, manganese, selenium, and zinc) [8]. There are a multitude of routes to contamination that have facilitated HM pollution including vehicle exhaust, coal combustion, interior and exterior paint, smelting and waste disposal [6]. HM thresholds for UA sites has focused on lead (Pb) due to its ubiquity as an urban soil contaminant, correlation with the presence of other HM contaminants, and the documented exposure risks to human health [2]. Worldwide Pb thresholds vary widely from 85-500 ppm [2], with differences found between countries, and in some instances even among regions of a country. These thresholds are aimed at limiting human exposure to Pb via the consumption of contaminated produce or accidental soil ingestion or inhalation.

Research and regulations to ensure UA produces food safe for human consumption is paramount, but these contaminants also have important environmental impacts which are less clearly understood or regulated. Key among these is how HM contamination influences the beneficial arthropod fauna that support the ecosystem services necessary for sustainable UA. HMs can impact UA by influencing both top-down and bottom-up processes [9]. In this article, we examine the impacts of HM contamination on crop plant – pest – natural enemy food webs. In particular, we focus on how HM contamination influences the composition and fitness of natural enemies foraging in contaminated landscapes and identify the potential impacts of HM exposure on biological control within UA.

Heavy Metals, Crop Plants, and Herbivores

Production of crops in HM contaminated soils can result in decreased seed germination, reduced growth and development, abnormalities in morphology, altered enzyme activity, disruptions in metabolic pathways, reduced ability to uptake essential nutrients and water, chlorosis, early senescence and phytotoxicity [10]. Given the potential human and plant health consequences, urban growers employ several strategies to limit HM uptake by crop plants. These actions can be extensive, including upper level soil removal or “capping” wherein a barrier is applied to a site and soil for plant production is added above it, although the latter approach is typically cost

prohibitive [2]. More typical recommendations involve the use of raised beds, frequently with HM free potting medium and compost added [11], or amending existing soil to reduce the bioavailability of HMs. Soil properties including pH, cation exchange capacity, oxides and organic matter can affect plant uptake of HMs [6]. For example, soils with high organic matter content and neutral or alkaline pH generally have a lowered bioavailability of HM to flora and fauna [6]. Thus, the application of phosphorus-based fertilizers or organic amendments such as biosolids or compost are recommended to reduce the likelihood for uptake of Pb [2]. Management practices such as soil tillage to mix surface and subsoil can reduce bioavailable HM [6,12] as can the presence of earthworms and mycorrhizal fungi [13]. Establishing turf or mulching areas between UA plantings can also limit recontamination of managed areas with HM dust from unmanaged areas of an urban farm.

The exposure of herbivores to HM contaminated host plants can alter weight gain, growth, survival, fecundity and eclosion success [9]. Interestingly, the strength and direction of these relationships are influenced by the concentration of HM contamination. In some cases, predictable negative impacts of HM exposure on herbivore population growth have been documented. For example, the net reproductive rate of the English grain aphid, *Sitobion avenae* (Hemiptera: Aphididae), and the number of offspring per female decreased with increasing cadmium (Cd) concentration [14]. However, at low concentrations, which may be more typical of managed beds within UA, HM contamination can lead to increased growth rates of herbivores. For example, low concentrations of Cd promoted population increase of the beet armyworm, *Spodoptera exigua* (Lepidoptera: Noctuidae), whereas high doses within their diet inhibited growth in population [15]. Exposure to HMs is also highly dependent on host plant selection and feeding strategy. In general, HM concentrations decrease from roots > leaves > stem > inflorescences > seeds, however this can vary by plant species [16]. Furthermore, some plants are considered hyperaccumulators, able to sequester high concentrations of metals in their tissues [17], thus consumption of these plants could result in an increased likelihood of an herbivore ingesting a significant concentration of HM contaminants. Feeding strategy can also influence impacts of HM; for example, feeding on host plants containing nickel (Ni) led to reduced survival of leaf and root chewing herbivores but not phloem feeders [18].

Natural Enemies in a Contaminated Landscape

The composition, configuration, and contamination legacy of the urban landscape is likely to have a significant impact the levels of contamination present within natural enemies supplied to UA [19]. Many of the natural enemies important in the suppression of UA pests immigrate into crop production beds from surrounding greenspaces such as vacant lots [20-23], unlikely to be managed for HM. For example, in Cleveland Ohio, U.S.A. soil Pb concentrations were four times higher in vacant lots than nearby urban farms (Figure 2A-B). Factors, such as neighborhood housing age, concentration of major roadways and proximity to historic sources of HM pollutions such as smelters, could influence the contamination present within these source habitats. Therefore, even if urban farm soils are free of HM contamination, populations of natural enemies may encounter HM via their diet, water consumption and soil contact within source habitats. For example, no difference in Pb concentrations were found within sheet web spiders (Araneae: Linyphiidae) collected from vacant lots and nearby farm sites (Figure 2C). Thus it is important to consider that the biological control function of natural enemies found

within UA could be altered by contamination, even if soil testing and management practices are employed to reduce bioavailable HM on-farm.

At the community level, foraging in a contaminated landscape often results in reduced natural enemy richness, abundance and altered community composition [23–26]. However, these community properties can be unaffected, or even positively correlated, with HM contamination [27,28]. Bioindication research demonstrates that HM contamination in natural enemies often correlates with habitat contamination but can vary greatly within and between species. Variables such as life stage, sex and seasonal diet changes influence HM concentrations within a species collected from a contaminated habitat [29,30]. Among species, several traits including capacity for dispersal, prey choice, ability to store or excrete HMs and seasonal occurrence can influence concentrations of HMs found within natural enemies [31–37]. For example, seasonality was found to influence Pb contamination of sheet web spiders (Araneae: Linyphiidae) in vacant lots and urban farms; those collected in early June contained significantly greater concentrations of Pb than those present in July or August (Figure 2D). Although its unknown why this variation occurred, seasonal food web changes could alter exposure risk. Diet variation is known to influence predator contamination, for example, ground beetles (Coleoptera: Carabidae) that fed on larger snails and earthworms accumulated higher concentrations of Cd than species that consumed small snails or a diversity of invertebrates and carrion [37].

Impacts of HM Contamination on Natural Enemies and Biological Control

To fully understand the influence of contamination on biological control, it is necessary document how HM exposure alters the physiology and behavior of natural enemies. Studies documenting these impacts have focused predominantly on predatory taxa, with significant focus on a small number of ground beetle and spider species. These studies have revealed reproductive, developmental, immunological and behavioral impacts of contamination on the studied natural enemy fauna (Table 1).

Reproduction

HM exposure can influence the probability of mating, fecundity, and egg hatch among natural enemies [38–43]. For example, females of the wolf spider, *Pirata piraticus* (Araneae: Lycosidae), from uncontaminated sites exhibited a decreased probability of copulation when food deprived, whereas females collected from polluted sites did not [43]. In general, HM contaminated predators produced fewer, larger eggs, with reduced hatching success. One exception was reported by Babczyńska et al. [41] who found that funnel web spiders, *Agelena labyrinthica* (Araneae: Agelinidae), collected from contaminated habitats produced a larger number of eggs, albeit smaller ones, and these eggs exhibited reduced hatching success relative to uncontaminated females.

Development Time and Body Size

HM exposure can alter natural enemy development time, adult weight, longevity and starvation tolerance. HM contaminated prey or water sources have been revealed to lengthen development time [44–48]. For example, when feeding on hosts contaminated with copper (Cu), *Nasonia*

vitripennis (Hymenoptera: Pteromalidae), exhibited lengthened developmental time and reduced emergence of adult parasitoids from the pupal stage [45]. The adult wasps also had a shortened adult life span and females exhibited reduced fecundity [45]. Interestingly, exposure to HMs results in reduced body mass in some predators [48] and greater body mass in others [49]. For example, the ground beetle, *Pterostichus oblongopunctatus* (Coleoptera: Carabidae), collected from HM contaminated sites had a greater body mass [49]; the authors speculated this pattern may be due to reduced competition with other predators.

Detoxification, Immunity and Energy Costs

HM exposure can influence the production of metallothioneins and enzymes involved in detoxification, including carboxyesterases (CarE), catalases (CAT), glutathiones (GSH), glutathione-S-transferases (GST), glutathione reductase (GR), superoxide dismutases (SOD), and selenium-dependent (GPOX) and selenium-independent (GSTPX) glutathione peroxidases [36, 50, 57-59, 63-64, 69]. HM exposure frequently leads to greater detoxification enzyme activity; however, variation exists both within and between species, often varying by sex [36,50]. Induction of detoxification enzymes above normal levels may have fitness consequences for arthropods as energetic demands shift from maintenance and reproduction. In ants, for example, encapsulation rates (a measure of immunity) actually increased with moderate HM contamination but decreased in heavily contaminated ants [51]. Whole-organism respiration rates have also been shown to increase with HM concentration, suggesting that natural enemies can incur energy costs with toxicity [52].

Biocontrol Services

Few studies have focused on the impact of HM contamination on predator-prey interactions and biological control. However, we can infer some potential impacts from studies examining how predator fitness and behavior are altered by HM contamination. For example, feeding on contaminated prey is likely to reduce the longevity of biocontrol services provided by an individual natural enemy. Although the predation rate of the ground beetle, *Carabus splendens* (Coleoptera: Carabidae), was not affected by consuming Cd contaminated snails, the beetles experienced significantly greater mortality [53]. In some cases, HM contamination has been demonstrated to reduce the aggressiveness of natural enemies, which could alter their efficiency as a predator [54]. For example, ants exposed to contamination from a Cu smelter were less likely to demonstrate aggressiveness to foreign colonies, a behavior hypothesized to be either a result of direct toxicity or changes in resource availability [54]. Exposure to HM may also make natural enemies less able to tolerate cyclic prey populations common in UA. The ground beetle, *Pterostichus oblongopunctatus* (Coleoptera: Carabidae), collected from HM polluted sites exhibited increased susceptibility to food deprivation [55]. HM exposure may also alter outcomes of integrated pest management programs by causing greater sensitivity of natural enemies to pesticides [55,56]. For example, the parasitoid, *Aphidius ervi* (Hymenoptera: Braconidae), exposed to aphids feeding on plants grown in soil contaminated with Cd exhibited reduced adult emergence, an effect that was magnified when imidacloprid was applied to the soil [56].

Future Research Directions

Herein, we identify HM as a factor that could influence biological control, a key ecosystem service provided to UA. The data presented here illustrate reduced survivorship, altered developmental time and reproductive output, and energetic investment into the production of detoxification enzymes. However, will these impacts translate into measureable reductions in biological control? Testing this hypothesis is complex, as the likelihood and extent of natural enemy contamination by HM is dependent on identifying contamination risk within multiple dimensions of niche space. For example, factors such as the extent of surface soil contact, use of alternative prey and non-prey foods, overwintering strategies, and capacity for dispersal vary extensively among common natural enemies. Such factors could all influence HM contamination and the biological control services afforded by different species, and thus should be investigated. Further, the field studies reviewed here are predominately gradient analyses wherein natural enemies were collected within semi-natural habitats at a successive distance from a point source of contamination such as a former mining site or smelter. Within an UA context, however, focal habitats are embedded in a landscape with varying sources and levels of contamination, not necessarily one identifiable source of HM pollution. Therefore, identifying how a species niche influences contamination risk and its potential to provide biological control services should we studied within the content of the surrounding urban landscape matrix.

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Figure Captions

Figure 1. A) Urban agricultural production is growing worldwide, particularly in cities where protracted economic decline and home foreclosure have resulted in a significant amount of vacant land [22]. A) When foreclosed or abandoned homes hold little to no property value they are eventually torn down by municipalities. B) Demolition involves removal of debris from the site, but this process could be a source of additional HM soil contamination from materials such as lead-based paints. C) Following demolition, a vacant lot plant community establishes that may contain seeded grasses and/or grasses and forbs from the existing seed bank. Vacant lots are maintained as early-successional habitats with periodic mowing. D). Communities are reimagining a portion of available vacant land as a resource for agricultural production.

Figure 2. A) The city of Cleveland, Ohio U.S.A. has over 100 UA operations occupying formerly-vacant land. These sites vary in distance from historic smelting sites and are embedded within landscapes where factors such as housing age and roadway density can influence HM soil contamination. Within four UA sites and four nearby vacant lots we measured the Pb concentration of soils and sheet-web spiders (Linyphiidae). B) Pb concentrations within vacant lot soils were significantly higher than nearby urban farms ($F=35.94$, $p<0.001$). Relative to the Cuyahoga County background soil Pb level of 51.7 ppm [74], vacant lot soils contained nearly four times the Pb, whereas urban farms averaged close to the background Pb concentrations. C) Interestingly, despite the difference in soil contamination we found no difference in Pb concentrations within sheet-web spiders (Araneae: Linyphiidae) collected from urban farm and vacant lot habitats ($F=1.42$, $p=0.23$). D) However, variation in linyphiid contamination was detected by month, with greater Pb levels found in spiders collected in June than July or August ($F=9.65$, $P<0.001$).

A **B** **D** **C**

Table 1. Summary of studies examining the reproductive, developmental, behavioral and physiological impacts of HM exposure on predator and parasitoid arthropods. Both laboratory bioassays and analyses of field-contaminated arthropods are included.

	HM ¹	Effects of HM Exposure	Ref.
Araneae: Agelenidae			
<i>Agelena labyrinthica</i>	M	Increased detoxifying enzyme (CarE) activity	[57]
<i>Agelena labyrinthica</i>	M	Increased activity of detoxifying enzymes (GPOX and GSTPX) in females	[58]
<i>Agelena labyrinthica</i>	M	Increased metallothionein protein concentrations, reduced mitochondrial potential, higher ADP/ATP ratio	[59]
<i>Agelena labyrinthica</i>	M	Increased metallothionein protein concentrations correlated with concentrations of Zn, but not Cd, Cu, or Pb	[36]
<i>Agelena labyrinthica</i>	M	Enhanced egg production, reduced hatching success	[41]
<i>Agelena labyrinthica</i>	Mg	Lengthened developmental time, reduced survivorship, similar web construction but greater variation in placement	[60]
Araneae: Araneidae			
<i>Araneus diadematus</i>	M	Increased metallothionein protein concentrations correlated with concentrations of Cd, Pb, and Zn but not Cu	[36]
Araneae: Linyphiidae			
<i>Linyphia triangularis</i>	M	Increased metallothionein protein concentrations correlated with concentrations of Pb and Zn, but not Cd or Cu	[36]
Araneae: Lycosidae			
<i>Pardosa astrigera</i>	Pb, Zn	Increased developmental time, reduction in body weight at high metal concentrations, production of fewer eggs	[65]
<i>Pardosa lugubris</i>	M	Increased detoxifying enzyme (CarE) activity	[57]
<i>Pardosa saltans</i>		Reduced body size, delayed reproductive period for females, production of fewer, larger eggs	[66]
<i>Pirata piraticus</i>	M	Negative relationship between fluctuating asymmetry and clutch size	[61]
<i>Pirata piraticus</i>	M	Reduced fecundity and clutch size, production of larger eggs	[39]
<i>Pirata piraticus</i>	M	Production of larger eggs, decreased growth rate	[62]
<i>Pirata piraticus</i>	M	More likely to reproduce under food stress than females collected from uncontaminated habitat	[43]
<i>Pirata subpiraticus</i>	Cd	Lengthened developmental time and reduced starvation tolerance	[46]

<i>Pirata subpiraticus</i>	Cd	Increased metallothionein protein concentrations, decreased growth rate, decreased survival	[63]
<i>Pirata subpiraticus</i>	Mg	Increased activity of detoxification enzyme GSH, reduced activity of SOD, CAT, and GST	[64]
<i>Xerolycosa nemoralis</i>	M	Reduced egg production, similar hatching success compared with females from uncontaminated sites	[41]
Coleoptera: Carabidae			
<i>Carabus splendens</i>	Cd	No effect on prey consumption, increased mortality	[53]
<i>Poecilus cupreus</i>	Zn	No difference in body mass at pupal emergence, decreased body mass following overwintering	[67]
<i>Pterostichus melanarius</i>	M	Reduced fat content in adults	[68]
<i>Pterostichus oblongopunctatus</i>	M	Reduced tolerance to food deprivation, increased susceptibility to pesticide exposure	[55]
<i>Pterostichus oblongopunctatus</i>	Zn	Reduced egg production, increased development time and body size of laboratory-reared F1 generation	[40]
<i>Pterostichus oblongopunctatus</i>	M	Higher detoxification enzyme activity (GST and CarE) in female beetles within some contaminated sites relative to reference site, no difference in enzyme activity among male beetles	[50]
<i>Pterostichus oblongopunctatus</i>	M	Decreased detoxification enzyme activity when exposed to Cd (GPOX), Pb (SOD), or Zn (GST), increased activity when exposed to Pb (GSTPX, GR) Cu (GPOX), and Cd (GSTP, GR)	[69]
<i>Pterostichus oblongopunctatus</i>	M	Larger body mass	[49]
<i>Pterostichus oblongopunctatus</i>	M	No difference in laboratory-reared F2 generation in susceptibility to starvation or insecticide exposure	[40]
<i>Pterostichus oblongopunctatus</i>	Zn	Reduced elytra length	[70]
<i>Pterostichus oblongopunctatus</i>	Zn	Greater egg production, reduced egg hatch, no effect on laboratory-reared F1 generation survivorship or developmental rate	[40]
<i>Pterostichus oblongopunctatus</i>	Cd, Zn	Increased whole-organism respiration rate	[52]

Coleoptera: Curculionidae			
<i>Phyllobius betulae</i>	M	Reduced detoxification enzyme activity when exposed to Cu (GPOX), Pb	[69]

		(GSTP), or Zn (GSTP)	
Coleoptera: Scarabaeidae			
<i>Geotrupes stercorosus</i>	M	Increased detoxification enzyme activity when exposed to Cd (GSTP, GR), Cu (GSTP, GST), Pb (SOD, GSTP, GPOX, GR), and Zn (GPOX, GR), reduced activity when exposed to Cd (GST)	[69]
Coleoptera: Staphylinidae			
<i>Staphylinus caesareus</i>	M	Increased detoxification enzyme activity when exposed to Cd (SOD, GSTP, GPOX), Cu (SOD, GPOX, GST), Pb (SOD, GPOX), Zn (GST), reduced activity when exposed to Pb (GST, GR), and Cd (GST, GR)	[69]
Hemiptera: Pentatomidae			
<i>Podisus maculiventris</i>	Se	Longer developmental time, reduced adult weight, increased mortality	[48]
<i>Podisus maculiventris</i>	M	Longer developmental time, reduced adult weight, no effect on survivorship	[47]
Hymenoptera: Formicidae			
<i>Formica aquilonia</i>	M	Increased encapsulation at moderate and decreased encapsulation at high levels of HM exposure	[51]
<i>Formica aquilonia</i>	M	Reduced mound volume (indicative of reduced colony size), no difference in worker morphological characteristics	[71]
<i>Formica aquilonia</i>	Cu	Reduced intraspecific aggression	[54]
Parasitoids			
Hymenoptera: Braconidae			
<i>Aphidius ervi</i>	Cd	Cd alone and in combination with imidacloprid insecticide reduced adult emergence from host	[56]
<i>Glyptapanteles liparidis</i>	M	No effect on parasitization success	[72]
Hymenoptera: Diapriidae			
<i>Coptera occidentalis</i>	M	No effect on parasitoid fecundity or percentage of females produced in laboratory-reared F1 generation	[38]
<i>Coptera occidentalis</i>	Cd, Cu	Equal parasitism of control and HM contaminated hosts, reduced emergence of females from contaminated hosts, no effect on developmental rate, life span, or fecundity	[44]
Hymenoptera: Ichneumonidae			

<i>Pimpla turionellae</i>	Cd, Pb	Reduced life span	[73]
Hymenoptera: Pteromalidae			
<i>Nasonia vitripennis</i>	Cu	Reduced growth and increased developmental time, reduced emergence of adults, shortened adult life span, reduced fecundity in females	[45]

¹M=multiple heavy metals (more than 2) studied.