COMMUNITY AND POPULATION CHARACTERISTICS OF SOIL ARTHROPODS ALONG A HEAVY METAL GRADIENT IN URBAN BROWNFIELDS

by

SAHIL WADHWA

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Written under the direction of

Claus Holzapfel

and approved by

____

Dr. Frank Gallagher

____

Dr. Cesar Rodriguez-Saona

____

Dr. Jessica Ware

____

Dr. Peddrick Weis

Newark, New Jersey

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Abstract of the Dissertation

Community and population characteristics of soil arthropods along a heavy metal gradient in urban brownfields

By: Sahil Wadhwa

Dissertation Director: Claus Holzapfel, Ph.D.

The rapid urban development over the last several decades is one of the main reasons of declining native biodiversity. With increasing urbanization, it is therefore important to include urban areas in biodiversity conservation efforts. While the contribution of managed urban habitats towards biodiversity conservation is recognized, brownfields, a potentially important urban habitat are often ignored in conservation planning. Brownfields are the unmanaged, often polluted (due to former industrial use), and abandoned habitats with spontaneously grown vegetation. Studies have shown that brownfields can function as reservoirs of biodiversity in urban areas. However, in order to maintain and increase the diversity in these habitats and in urban areas overall, it is crucial to understand how factors such as metal contamination shape biodiversity in brownfields. The objective of this thesis is to examine the community and population characteristics of soil arthropods in relation to metal contamination in urban brownfields. To accomplish this objective, I investigated effects of metal contamination on the: (1) diversity of epigeic invertebrate community in an urban brownfield, (2) abundance patterns and composition of terrestrial isopods assemblages in an urban brownfield, (3) fluctuating asymmetry in the populations of terrestrial isopod and hardwood trees in an urban brownfield, and (4) growth and metal body burdens of a terrestrial isopod, *Philoscia muscorum*. The results showed that the overall diversity of soil invertebrate assemblage was not negatively affected by soil metal contamination. However, negative effects of metal contamination were observed both on the community and on most of the isopod species (3 out of 4) found at the brownfield site. The results suggest that most of the soil invertebrate taxa might have developed adaptive tolerance mechanisms to survive at metal contaminated sites which imply that metal contamination at the brownfield site might not be limiting the food availability for animals on higher trophic levels. On the other hand, these results also highlight the higher sensitivity of isopods to metal contamination which might result from greater accumulation of metals in isopods than other soil invertebrates. Interestingly, differences in sensitivity to metal contamination were also found between different isopod species at the brownfield site. Based on results of this study, it is recommended that isopods at family level (Oniscidae) and species level (*P. muscorum*) can be used as a bio-indicator of metal pollution in terrestrial ecosystems. The results of litter feeding study showed a significant reduction in growth of isopod *P. muscorum* following exposure to metal contaminated food and soil. Moreover, weight change in isopods was found to be more negatively affected by contamination of soil than contamination of the leaf litter. These results are significant for showing the importance of soil as a route of metal exposure in isopods. Overall, results show that despite the metal pollution, brownfields can function as an important habitat for soil arthropods and therefore can contribute to urban biodiversity conservation. The results of this study can be used for designing restoration and management plans for brownfields.

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Dedicated to my brother and parents!

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Chapter One: Introduction

Urbanization has increased notably over the last several decades and it is estimated that more than half of the world's population lives in urban cities as compared to only 30% in 1950 (Desa 2011). This number is even greater for developed countries such as the United States, where 80% of the total populations live in urban areas (Ahmed Obaid 2007). It is expected that by 2030, the global urban population will reach 5 billion, which is about 60% of the projected total global population of 8.3 billion by that time (Ahmed Obaid 2007). The rapid urban expansion and associated human activities pose a massive threat to biodiversity conservation (Antrop 2004; Hansen et al. 2005). Increasing urbanization is considered to be one of the main reasons of declining native species diversity as it alters natural ecosystems resulting in environmental changes, for instance, habitat fragmentation, introduction of invasive species, and increased pollution (Koh and Sodhi 2004; Lessard and Buddle 2005; McKinney 2002, 2006). Biodiversity is an important component of an ecosystem that promotes functional diversity and improves ecological stability by influencing the resistance to environmental change (Chapin Iii et al. 2000) and therefore needs to be conserved in all aspects and scales (Hill 2005). Considering the current urban population growth as a major global trend, preserving and enhancing biodiversity in urban areas is of paramount importance in order to decelerate the rapid rate of biodiversity loss (Alvey 2006).

One of the key strategies to conserve urban biodiversity is to not only develop and protect green spaces within urban areas but also to utilize unused urban habitats (Kattwinkel et al. 2011). While many urban biodiversity conservation approaches tend to focus on managed urban habitats (such as parks, gardens, rooftops), urban brownfields, a potentially important urban habitat, are often ignored in urban conservation planning (Harrison and Davies 2002; Muratet et al. 2007). Urban brownfields are unmanaged green spaces that occur commonly in the vacant urban landscapes as a result of past derelict industrial activities, commercial facilities, rail-yards or railroads, etc. (Gorman 2003). Brownfields are characterized by heterogeneous habitats present at different successional stages and soils that are often contaminated by heavy metals such as zinc, lead, cadmium and arsenic owing to their past industrial or commercial use (Gallagher et al. 2008; Gallagher et al. 2011; Leigh and Coffin 2000; Wood and Pullin 2002). The potential heavy metal contamination of brownfield soils and the associated threat of liability is one of the major deterrents to the redevelopment efforts as private and public investors are reluctant to invest substantial amount of money to conduct extensive soil contamination surveys and to finish the cleanup process. Despite the fact that these sites are left abandoned without any redevelopment work and are being referred to as "urban wastelands" or "urban problem", studies have shown that brownfields can still function as valuable reservoirs of biodiversity (Eyre et al. 2003; Tropek et al. 2008). However, in order to preserve, maintain and increase the diversity in these habitats and in urban areas overall, it is crucial to understand how factors such as heavy metal contamination shape biodiversity in these abandoned areas. Preserving and promoting biodiversity in urban brownfields will not only help to offset the high loss of biodiversity in urban landscapes but it will also provide other services such as improving the quality of life of local residents (Rohde and Kendle 1994) and providing a recreational source for urban residents (Gunnarsson et al. 2009).

Studying the soil arthropod community structure and examining the influence of soil metal contamination on arthropod population in brownfields is important from the perspective of conserving biological diversity as arthropods comprise of the most diverse taxa in an ecosystem, they play a vital role in important ecosystem processes such as degradation of organic matter and nutrient cycling (McIntyre 2000). Soil arthropods are also a logical choice for monitoring the impacts of soil metal contamination in terrestrial ecosystems as they are sensitive to the presence of metal contaminants in the soil environment, are abundant, relatively large sized and therefore are easy to sample, and are present at different trophic levels of food chains (McIntyre 2000; da Silva Souza et al. 2014).

Soil arthropods in metal contaminated environments can acquire metals by different means including ingestion of contaminated soil particles, feeding on metal contaminated diet (leaf litter or prey), and/or through dermal absorption because of their close contact with contaminated soil and litter (Godet et al. 2011; Heikens et al. 2001; Vijver et al. 2005). Although, metals such as zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) are essential for normal growth and several metabolic processes in arthropods, exposure to metal concentrations higher than are required can result in acute or chronic toxicity (Chen et al. 2011; Drobne and Hopkin 1995). On the other hand, metals such as arsenic (As), lead (Pb), and cadmium (Cd) are non-essential and are highly toxic even if the arthropods are exposed to very small concentrations (Chen et al. 2011; Cohen 2015; Žaltauskaitė and Sodienė 2010). Evidence from eco-toxicological studies has shown that short term exposure to elevated metal concentrations in soil invertebrates can result in impaired growth, reduction in reproductive output or energy levels, and structural and behavioral changes (Godet et al. 2011; Vijver et al. 2005; Drobne and Hopkin 1995; Mazzei et al. 2014; Schill and Kohler 2004; Zidar et al. 2004). Most of these risk assessment studies have been conducted in laboratory settings, with experimental designs including exposure to single or only few elements, investigating metal impacts at a species level. Since, soil arthropods in contaminated ecosystems (field conditions) are exposed to different metals at the same time, different taxonomic groups of invertebrates can differ in their susceptibility to metal contamination, it is difficult to extrapolate results of these studies (based on single element exposures in a single species over a short period of time in laboratory settings) on to the field conditions.

Few field studies have investigated effects of heavy metal pollution on soil arthropod communities including a shooting range, old smelter wastes site, and abandoned uranium mining areas (Antunes et al. 2013; Migliorini et al. 2004; Nahmani and Lavelle 2002). In brownfield settings, some of the studies have assessed the role of local and landscape factors including age, soil and vegetation structure on diversity of phytophagous invertebrates (leafhoppers and grasshoppers) in brownfields (Kattwinkel et al. 2011; Strauss and Robert Biedermann 2006). Other studies have surveyed particular insect taxa such as carabid beetles (Schwerk 2000) or studied diversity of leafhopper and grasshoppers (Strauss and Robert Biedermann 2006; Eckert et al. 2017; Sanderson 1992); however, the structure of soil arthropod communities or the influence of factors such as metal contamination on these invertebrate communities has not been studied in urban brownfields.

The objective of this study is to assess the community and population characteristics of soil arthropods in relation to heavy metal contamination in urban brownfields at the Liberty State Park (LSP) in New Jersey, USA.

In chapter 2, changes in the abundance, evenness, and assemblage composition of soil arthropods were studied along a heavy metal gradient at LSP. Particular attention was paid to six major soil invertebrate taxa: Oniscidae (isopods), Diplopoda (millipedes), Haplotaxida (earthworms), Araneae (spiders), Carabidae (ground beetles), and Staphylinidae (rove beetles). I tested the hypothesis that the abundance of different soil invertebrate taxa decreases with increasing soil metal load resulting in changes in the community structure along the soil metal gradient. In addition, I also aimed to find an indicator group(s) of metal pollution at the brownfield site.

In chapter 3, I examined the effects of metal contamination on populations of terrestrial isopods in urban brownfields. Differences in species richness, abundance patterns of different species, and changes in community composition of isopods were studied in relation to soil metal contamination at sites along the metal gradient. I also aimed to find an indicator isopod species of metal pollution at the brownfield site. In addition, abundance of isopod species and community composition of isopods were compared between different urban habitats including managed park areas, unmanaged metal contaminated brownfields, and unmanaged non-polluted areas (reference site) at LSP.

In chapter 4, I studied fluctuating asymmetry (FA) as an indicator of heavy metal stress in the populations of terrestrial isopods and hardwood trees at LSP. For this study, effects of metal contamination on FA of different morphological traits in populations of a terrestrial isopod (*Philoscia muscorum*) and in leaves of hardwood trees (*Betula populifolia* and *Populus deltoides*, dominant trees at LSP) collected from three low and high soil metal load sites were analyzed.

In chapter 5, Impacts of metal contamination on the population dynamics of the terrestrial isopod, *P. muscorum* were studied. To this end, I examined the effects of metal contaminated soil and leaf litter on the growth and metal accumulation in the terrestrial isopod, *P. muscorum* raised in a growth chamber over a period of fourteen days.

References:

Ahmed Obaid T. 2007. State of the World Population 2007: Unleashing the Potential of Urban Growth. New York: UNFPA-United Nations Population Fund.

Alvey AA. 2006. Promoting and preserving biodiversity in the urban forest. Urban Forestry & Urban Greening 5(4): 195-201.

Antrop M. 2004. Landscape change and the urbanization process in Europe. Landscape and Urban Planning 67(1–4): 9-26.

Antunes SC, Castro BB, Moreira C, Gonçalves F, Pereira R. 2013. Community-level effects in edaphic fauna from an abandoned mining area: Integration with chemical and toxicological lines of evidence. Ecotoxicology and Environmental Safety 88: 65-71.

Chapin Iii FS, Zavaleta ES, Eviner VT, Naylor RL, Vitousek PM, Reynolds HL. 2000. Consequences of changing biodiversity. Nature 405(6783): 234-242.

Chen X-q, Zhang Z-t, Liu R, Zhang X-L, Chen J, Peng Y. 2011. Effects of the metals lead and zinc on the growth, development, and reproduction of *Pardosa astrigera* (Araneae: Lycosidae). Bulletin of Environmental Contamination and Toxicology 86(2): 203-207.

Cohen AC. 2015. Insect diets: science and technology: CRC press.

da Silva Souza T, Christofoletti CA, Bozzatto V, Fontanetti CS. 2014. The use of diplopods in soil ecotoxicology–A review. Ecotoxicology and Environmental Safety 103: 68-73.

Desa U. 2011. World urbanization prospects, the 2011 revision. United Nations 505.

Development on Biodiversity: Patterns, Mechanisms, and Research Needs. Ecological Applications 15(6): 1893-1905.

Eckert S, Möller M, Buchholz S. 2017. Grasshopper diversity of urban wastelands is primarily boosted by habitat factors. Insect Conservation and Diversity.

Eyre MD, Luff ML, Woodward JC. 2003. Beetles (Coleoptera) on brownfield sites in England: An important conservation resource? Journal of Insect Conservation 7(4): 223-231.

Gallagher FJ, Pechmann I, Bogden JD, Grabosky J, Weis P. 2008. Soil metal concentrations and vegetative assemblage structure in an urban brownfield. Environmental Pollution 153(2): 351-361.

Gallagher FJ, Pechmann I, Holzapfel C, Grabosky J. 2011. Altered vegetative assemblage trajectories within an urban brownfield. Environmental Pollution 159(5): 1159-1166.

Godet J-P, Demuynck S, Waterlot C, Lemière S, Souty-Grosset C, Scheifler R, et al. 2011. Growth and metal accumulation in Porcellio scaber exposed to poplar litter from Cd-, Pb-, and Zn-contaminated sites. Ecotoxicology and Environmental Safety 74(3): 451-458.

Gorman HS. 2003. Brownfields in historical context. Environmental Practice 5(01): 21-24.

Gunnarsson B, Heyman E, Vowles T. 2009. Bird predation effects on bush canopy arthropods in suburban forests. Forest Ecology and Management 257(2): 619-627.

Hansen, A. J., Knight, R. L., Marzluff, J. M., Powell, S., Brown, K., Gude, P. H., & Jones, K. (2005). Effects of Exurban development on biodiversity: Patterns, mechanisms, and research needs. *Ecological Applications*, *15*(6), 1893–1905.

Harrison C, Davies G. 2002. Conserving biodiversity that matters: practitioners' perspectives on brownfield development and urban nature conservation in London. Journal of Environmental Management 65(1): 95-108.

Heikens A, Peijnenburg WJGM, Hendriks AJ. 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution 113(3): 385-393.

Hill D. 2005. Handbook of biodiversity methods: survey, evaluation and monitoring: Cambridge University Press.

Kattwinkel M, Biedermann R, Kleyer M. 2011. Temporary conservation for urban biodiversity. Biological Conservation 144(9): 2335-2343.

Koh LP, Sodhi NS. 2004. Importance of reserves, fragments, and parks for butterfly conservation in a tropical urban landscape. Ecological Applications 14(6): 1695-1708.

Leigh NG, Coffin SL. 2000. How many brownfields are there? Building an industrial legacy database. Journal of Urban Technology 7(3): 1-18.

Lessard J-P, Buddle CM. 2005. The effects of urbanization on ant assemblages (Hymenoptera: Formicidae) associated with the Molson Nature Reserve, Quebec. Canadian Entomologist 137: 215-225.

McKinny, M. L. (2002). Urbanization, Biodiversity, and Conservation. BioScience, 52(10), 883–890.

McKinney ML. 2006. Urbanization as a major cause of biotic homogenization. Biological Conservation 127(3): 247-260.

McIntyre NE. 2000. Ecology of Urban Arthropods: A Review and a Call to Action. Annals of the Entomological Society of America 93(4): 825-835.

Migliorini M, Pigino G, Bianchi N, Bernini F, Leonzio C. 2004. The effects of heavy metal contamination on the soil arthropod community of a shooting range. Environmental Pollution 129(2): 331-340.

Muratet A, Machon N, Jiguet F, Moret J, Porcher E. 2007. The role of urban structures in the distribution of wasteland flora in the greater Paris area, France. Ecosystems 10(4): 661.

Mazzei V, Longo G, Brundo MV, Sinatra F, Copat C, Oliveri Conti G, et al. 2014. Bioaccumulation of cadmium and lead and its effects on hepatopancreas morphology in three terrestrial isopod crustacean species. Ecotoxicology and Environmental Safety 110: 269-279.

Nahmani J, Lavelle P. 2002. Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. European Journal of Soil Biology 38(3–4): 297-300.

Rohde CLE, Kendle AD. 1994. Human Well-Being, Natural Landscapes and Wildlife in Urban Areas a Review.

Sanderson R. 1992. Hemiptera of naturally vegetated derelict land in north-west England. Entomologist’s Gazette 43(3): 221-226.

Schwerk A. 2000. Ecological aspects of carabid beetle coenoses (Coleoptera: Carabidae) on industrial fallow grounds in the Ruhr Valley Area. Natural history and applied ecology of carabid beetles, Pensoft: 277-287.

Schill RO, Kohler H-R. 2004. Energy reserves and metal-storage granules in the hepatopancreas of Oniscus asellus and Porcellio scaber (Isopoda) from a metal gradient at Avonmouth, UK. Ecotoxicology 13(8): 787-796.

Strauss, Robert Biedermann B. 2006. Urban brownfields as temporary habitats: driving forces for the diversity of phytophagous insects. Ecography 29(6): 928-940.

Tropek R, Spitzer L, Konvicka M. 2008. Two groups of epigeic arthropods differ in colonising of piedmont quarries: the necessity of multi-taxa and life-history traits approaches in the monitoring studies. Community Ecology 9(2): 177-184.

Vijver MG, Wolterbeek HT, Vink JPM, van Gestel CAM. 2005. Surface adsorption of metals onto the earthworm Lumbricus rubellus and the isopod Porcellio scaber is negligible compared to absorption in the body. Science of The Total Environment 340(1–3): 271-280.

Wood BC, Pullin AS. 2002. Persistence of species in a fragmented urban landscape: the importance of dispersal ability and habitat availability for grassland butterflies. Biodiversity and Conservation 11(8): 1451-1468.

Žaltauskaitė J, Sodienė I. 2010. Effects of total cadmium and lead concentrations in soil on the growth, reproduction and survival of earthworm Eisenia fetida. Ekologija 56(1-2): 10-16.

Zidar P, Drobne D, Štrus J, Van Gestel C, Donker M. 2004. Food selection as a means of Cu intake reduction in the terrestrial isopod Porcellio scaber (Crustacea, Isopoda). Applied Soil Ecology 25(3): 257-265.

Chapter Two: Abundance and temporal dynamics of epigeic invertebrate communities in a heavy metal-contaminated urban brownfield

Abstract:

The aim of this study was to examine the effects of metal pollution on the abundance, evenness, and community composition of six major soil invertebrate taxa: Oniscidae (isopods), Haplotaxida (earthworms), Diplopoda (millipedes), Araneae (spiders), Carabidae (ground beetles), and Staphylinidae (rove beetles) along a soil metal gradient in urban brownfields at Liberty State Park (LSP) in New Jersey, USA. Soil invertebrates were sampled using pitfall traps from a reference ("clean"), three low soil metal load (LML), and three high soil metal (HML) sites in 2013 (June-October) and 2014 (May-September). Among all the six invertebrate taxa, Oniscidae (isopods) were by far the most abundant taxa at each study site. The most active period for millipedes, carabids, and isopods was early summer (May-June), whereas, isopods were most abundant during late summer (August). Overall, my results revealed that invertebrate assemblage at the study site was not affected by soil metal contamination; however, negative effects of metal contamination on isopods were seen at the site. During both periods of sampling (in 2013 and 2014), abundance of isopods declined along the metal gradient, whereas, abundance levels of all the invertebrate taxa except Onicidae (isopods) were quite balanced between all the sites. Declining abundance of isopods along the metal gradient might be related to metal toxicity resulting from exposure to elevated concentrations of metals in leaf litter or soils at metal contaminated sites as shown by the results of Canonical correspondence analysis (CCA). Possible reasons for the lack of negative impacts of metal contamination on the invertebrate assemblage including development of

metal resistance by invertebrates inhabiting the metal contaminated sites and/or methodological bias are discussed. The results of this study demonstrate that terrestrial isopods (at a family level: Oniscidae) can be used a bio-indicator of metal pollution in brownfields (or terrestrial ecosystems). Experimental studies in laboratory must be done to assess the toxicity of heavy metals using individual isopod species from this study site.

1. Introduction

During the second half of the twentieth century, most of developed countries around the world experienced a massive wave of de-industrialization (Callis and Cavanaugh 2009; Jameson 1991; Keating 2010). This phenomenon of industrial abandonment was a result of different factors including loss of production/ manufacturing jobs (due to shift towards automation) and relocation of industries to other countries with available cheap labor (Molle 2002; Sleuwaegen and Pennings 2006). This economic decline due to de-industrialization impacted large areas of lands in several countries around the world and has left an extensive of abandoned and derelict landscapes known as brownfields (de Noronha Vaz et al. 2012; Kirkwood 2003; Nassauer and Raskin 2014). For example, it is estimated that there are about 500,000–1,000,000 brownfields in the US (Davis 2002). A survey conducted across 70 major metropolitan areas in United States reported that, on an average, 15% percent of urban landscape is vacant or derelict (Pagano, 2000). Similarly, survey results across the European Union have also shown presence of close to one million abandoned brownfield sites (Oliver et al. 2005). Due to their past industrial use, brownfields soils are often contaminated with metals such as zinc, lead, copper, cadmium, and arsenic (De Sousa 2008; Gallagher et al. 2008a; Leigh and Coffin 2000). Since, metal cleanup process is extremely expensive; brownfields are mostly left abandoned and are not redeveloped (Chang and Sigman 2007; McCarthy 2002). These metals present in the soil are often bound to organic matter or occluded by carbonates, iron-manganese oxides, and primary or secondary minerals (Adriano 1986; Ross 1994). However, when detached from these particles, metals entering soil solution can potentially become bio-available for uptake by plants and other soil-dwelling organisms (Neilson and Rajakaruna 2015). Considering the presence of large number of brownfields in several countries around the world and the potential metal contamination of soils at these sites, brownfields form a major source of metal pollution in terrestrial ecosystems posing a significant threat to humans and other life forms (Gallagher et al. 2008a; Jennings et al. 2002; Ren et al. 2014; Staszewski et al. 2015).

Soils and soil surfaces host highly diverse invertebrate communities which are involved in important ecosystem functions such as decomposition of organic matter, nutrient cycling, and regulation of microbial activity (Cortet et al. 1999; Lee and Foster 1991; Snyder and Hendrix 2008). These soil and epigeic invertebrates are sensitive to metal contamination of soils due to their close proximity with metals in soils and therefore can take up metals through dermal absorption or by ingestion of soil particles (Heikens et al. 2001; Lanno et al. 2004; Vijver et al. 2005a; Vijver et al. 2005b). In addition, in metal contaminated environments, invertebrates can also acquire metals through their diet (e.g. contaminated leaf litter, invertebrates with high metal concentrations) (Heikens et al. 2001; Godet et al. 2011; Hopkin and Martin 1985). Although, invertebrates need essential metals such as zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) in small amounts for various physiological and metabolic functions, exposure to elevated concentrations is harmful (Cohen 2015; Drobne and Hopkin 1995;

Hopkin and Hames 1994; Lukkari et al. 2005). On the other hand, metals such as lead, arsenic, cadmium, and aluminum are not required by invertebrates and are toxic even in very small amounts (Maryanski et al. 2002; Mozdzer et al. 2003; Spurgeon et al. 1994). Studies have shown that metal toxicity can negatively affect abundance, diversity and may alter the distribution of soil invertebrates (Hopkin and Hames 1994; Nahmani and Lavelle 2002; Spurgeon and Hopkin 1996), which in turn may impact important soil and ecosystem functions. Hence, soil invertebrates are considered as bio-indicators for evaluating impacts of metal contamination on ecosystems.

I examined the effects of heavy metal contamination on the composition of the epigeic soil invertebrate community along a metal gradient in an urban brownfield located in Liberty State Park (LSP), Jersey City, New Jersey, USA. The goals of this study were to compare abundance, evenness and assemblage composition of six major soil invertebrate taxa: Oniscidea (isopods), Diplopoda (Millipedes), Haplotaxida (earthworms), Araneae (spiders), Carabidae (ground beetles), and Staphylinidae (rove beetles) among different sites along the soil metal gradient at LSP, New Jersey. Epigeic soil communities were selected since soil invertebrate communities are important for the formation of organic soil layer, and important to the higher levels of the food chain (are chief source for higher predators that hunt on the soil surface e.g. many birds, shrews and some rodents). Earthworms, isopods and millipedes are prominent terrestrial detritivores that perform important ecosystem services such as decomposition of organic matter (Cárcamo et al. 2000; El-Wakeil 2015; Hättenschwiler et al. 2005; Liu and Zou 2002). Spiders are highly abundant and diverse group of predatory invertebrates in natural and urban ecosystems, which play a vital role in food webs both as predators and prey themselves (Jung et al. 2008; Moulder and Reichle 1972). Ground beetles and Rove beetles are the most abundant litter and soil surface dwelling beetle taxa, which comprise different feeding habits but predominantly are generalist predators (Hanski and Hammond 1986; Nitzu et al. 2008). The soil invertebrates were collected using pitfall traps in 2013 (June-October) and 2014 (May-September). We hypothesize that abundance of different soil invertebrate taxa decreases with increasing soil metal load resulting in changes in the community structure along the soil metal gradient.

2. Methods

2.1. Study site

This study was conducted at an urban brownfield study site in Liberty State park (LSP), New Jersey, USA, located on the west bank of Upper New York Bay (centered at 40^{o} 42' 14" N and 74^{o} 03' 14" W). The park used to be an intertidal mudflat before it was filled with construction debris and refuse (between 1860 and 1919) from New York City to use it as a rail yard by Central Rail Road of New Jersey (CRRNJ). After CRRNJ discontinued its operations in 1969, the State of New Jersey acquired this property to develop it as a park (for recreational purposes), which opened in 1976. Before opening to the public, most of the park's area was capped with clean soil. However, approximately 41 ha of the area was fenced off from public use and was left isolated, and undisturbed that served as the study site. Due to the previous use of the area for railroad transportation and coal storage purposes, soils at the site are contaminated with unevenly distributed metals including zinc (Zn), arsenic (As), lead (Pb), copper (Cu), chromium (Cr) and vanadium (V), which occur at concentrations that are higher than ambient concentrations of metals

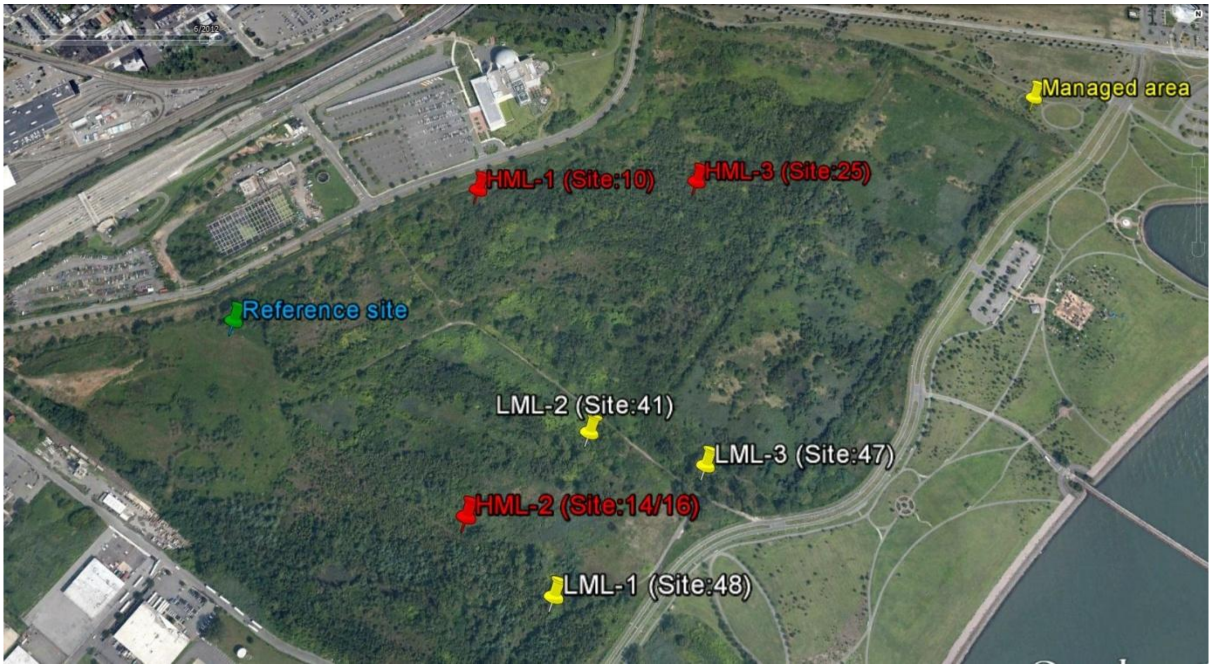


Fig. 1: Location of the reference and metal contaminated site within Liberty State Park, New Jersey, USA. Source: "Liberty State Park, New Jersey." 40°42`16.94`` N 74°03`14.73``W. **Google Earth**. August 11, 2015.

in New Jersey (Gallagher et al. 2008a). Over the last five decades, despite the metal contamination of soils, the brownfield area has been naturally colonized by vegetation succession, which includes dominant hardwood trees (*Betula populifolia*, *Populus deltoides*), shrubs (*Rhus typhina*, *R. glabra*, and *R. copallinum*) and many native and non-native forbs and grasses (*Solidago spp*., *Eupatorium spp*., *Artemisia vulgaris*, etc.) (Gallagher et al. 2008a). The soil metal concentrations at different sites within the brownfield were characterized by Gallagher et al. (2008a). A total soil metal load (TML) was compiled for each site by performing a rank order transformation on different metal species concentrations at a site (Juang et al. 2001). Using a reverse function of linear regression, these results were back transformed between the metal data and the ranks as described by Wu et al. (2006). The rank-ordered values were then summed up to develop a total soil metal load index, with a range between 0 (low) and 5 (high). The TML scale is used as a relative index for comparing soil metal contamination levels between different sites. Previous studies at this site have defined a critical soil metal threshold level of 3.0, above which plant productivity and growth have been significantly impacted by soil metal contamination (Dahle et al. 2014; Gallagher et al. 2008b; Renninger et al. 2013). Moreover, presence of soil metal contaminants has also influenced the development and trajectory of vegetative assembly, with hardwood trees such as *Betula populifolia* and *Populus spp*. dominating the sites with higher total soil metal load (Gallagher et al. 2008a; Gallagher et al. 2011).

For this study, we sampled soil invertebrates from seven sites, which included one reference site, three low soil metal load (LML), and three high soil metal load (HML) sites (Fig. 1). The three LML sites (LML-1 = site 48, LML-2 = site 41, and LML-3 = site 47) have total soil metal load lower than threshold level of 3.0, and HML sites (HML-1 = site 10, HML-2 = site 14/16, and HML-3 = site 25) have TML greater than 3.0 (see Gallagher et al., 2008a). Adjoining the fenced area, there is an 18- acre site that was used as a stockpile area for clean fill during construction of the western section of the park in the 1990's (Fig. 1). There remains several feet of clean fill in this area, which serves as the reference site for the study. The vegetation at this reference site is similar to the metal contaminated sites inside the fenced area. The total soil metal load, concentrations of different metals at the reference, 3 LML and 3 HML sites are listed in Table 1.

Table 1: Soil characteristics including concentrations (μg/g) heavy metals (means with standard deviation in parentheses), soil pH, total soil metal load (TML) for the reference, three low soil metal load (LML-1= site 48, LML-2= site 41, and LML-3 = site 47) and three high soil metal load (HML-1= site 10, HML-2= site 14/16, and HML-3 = site 25) sites at Liberty State Park, New Jersey, USA.

Site	As	Cr	Cu	Pb	Zn	soil pH	TML
Reference[c]	--	14.0 (0.59)	13.9 (3.42)	2.0 (1.22)	39.4 (4.17)	6.84	--
48[a] (LML-1)	13.33 (7.44)	20.87 (24.95)	95.15 (22.07)	244.57 (103.24)	22.08 (15.36)	5.90	1.56
41[a] (LML-2)	14.88 (4.79)	10.90 (5.43)	76.86 (29.64)	96.58 (34.02)	156.75 (116.81)	7.0	1.64
47[b] (LML-3)	4.3	15.9	31.3	46.5	41.8		0.31
10[a] (HML-1)	282.57 (155.43)	91.58 (59.50)	379.18 (32.36)	737.95 (166.50)	192.65 130.53)	5.4	3.59
14-16[a] (HML-2)	68.13 (24.15)	334.56 (141.83)	202.58 (46.42)	857.53 (143.85)	237.97 (42.95)	4.80	3.56

25[a] (HML-3)	384.41 (253.15)	50.5 (39.71)	2200.38 (265.02)	6673.22 (1603.66)	2326.77 (2046.85)	4.6	4.31

a measured in 2005 (Gallagher et al., 2008a)

b measured in 1995 (Gallagher et al., 2008a)

c measured in 2015

2.2. Sampling

Soil invertebrates were sampled from different sites (reference, LML, and HML- Fig. 1) along the metal gradient at LSP during 2013 (June-October) and 2014 (May-September) using pitfall traps. For both periods of sampling, four traps were deployed (15-20 m apart from each other) at each of the study site. The pitfall traps consisted of a plastic cup with a diameter of 10.5 cm and a height of 8 cm. Each trap was placed in soil such that the rim of the cup was at level with the soil surface. In order to minimize the location bias across different sites, trap locations were selected in similar vegetation types. Each trap was placed in a bare area closer to a vegetation patch dominated by mugwort (*Artemisia vulgaris*), a non-native herbaceous perennial plant that is a common plant in urban brownfield vegetation. To capture the soil invertebrates, a mixture of propylene glycol and water (70:30) was placed in the trap (filled halfway). Individual traps were covered with a small wooden board (to prevent from rain and falling leaves) raised 2-3 cm above the trap with nails in each corner. Pitfall traps were maintained for 7 days for every month during sampling periods in 2013 and 2014. Samples collected from the field were preserved in 70% ethanol in the laboratory. The soil invertebrates were identified at the order, class and family level using keys provided in Marshall (2006), Jordal (2015), and Eaton and Kaufman (2007) in the laboratory.

2.3. Data Analysis

The data analysis and statistical tests in this study were performed using SPSS version 21.0 for Windows (SPSS, Chicago, IL). Before the analysis, the data were ln(x+1) transformed to account for the zero values in the data set and to improve the normality for parametric testing. One-way ANOVA analysis (using data from individual traps) with post-hoc Tukey tests was performed to test the differences in overall abundance of different invertebrate taxa between reference, LML, and HML sites, evenness (Shannon equitability index, E_H), and diversity (Shannon diversity index, H) between sampling sites (reference, LML, and HML). Repeated measures ANOVA analysis was performed to compare the temporal variation in abundance levels of different invertebrate taxa at the reference, LML, and HML sites for sampling periods in 2013 (June to October) and 2014 (May to September).

Canonical correspondence analysis (CCA) was performed using the CANOCO package for Windows 4.5© (Ter Braak and Smilauer 2002) to investigate the effects of soil metal concentrations, total soil metal load (TML), and site characteristics (soil pH, soil moisture) on the distribution of different soil invertebrate taxa. The two data sets: abundance data for different invertebrate taxa at sampling sites (response variables) and environmental variables (predictors: soil pH, soil moisture, sampling month, total soil metal load, soil metal concentrations, and metal concentrations in leaf litter of *Populus spp.* at all the sampling sites) were first converted into CANOCO 4.0 format with utility program CanoImp. To determine relationships between response and predictor variables, CCA was performed using CANOCO 4.5 for Windows.

3. Results

3.1. Abundance of invertebrate taxa: A total of 12,877 soil invertebrates belonging to six different taxa: Oniscidea (isopods), Diplopoda (millipedes), Haplotaxida (earthworms), Araneae (spiders), Carabidae ground beetles), and Staphylinidae (rove beetles) were collected with pitfall traps during both periods of sampling in 2013 (June-October) and 2014 (May-September). Among the six taxa, Oniscidea was by far the most abundant taxa at the reference, LML and HML sites during 2013 and 2014 (Fig. 2).

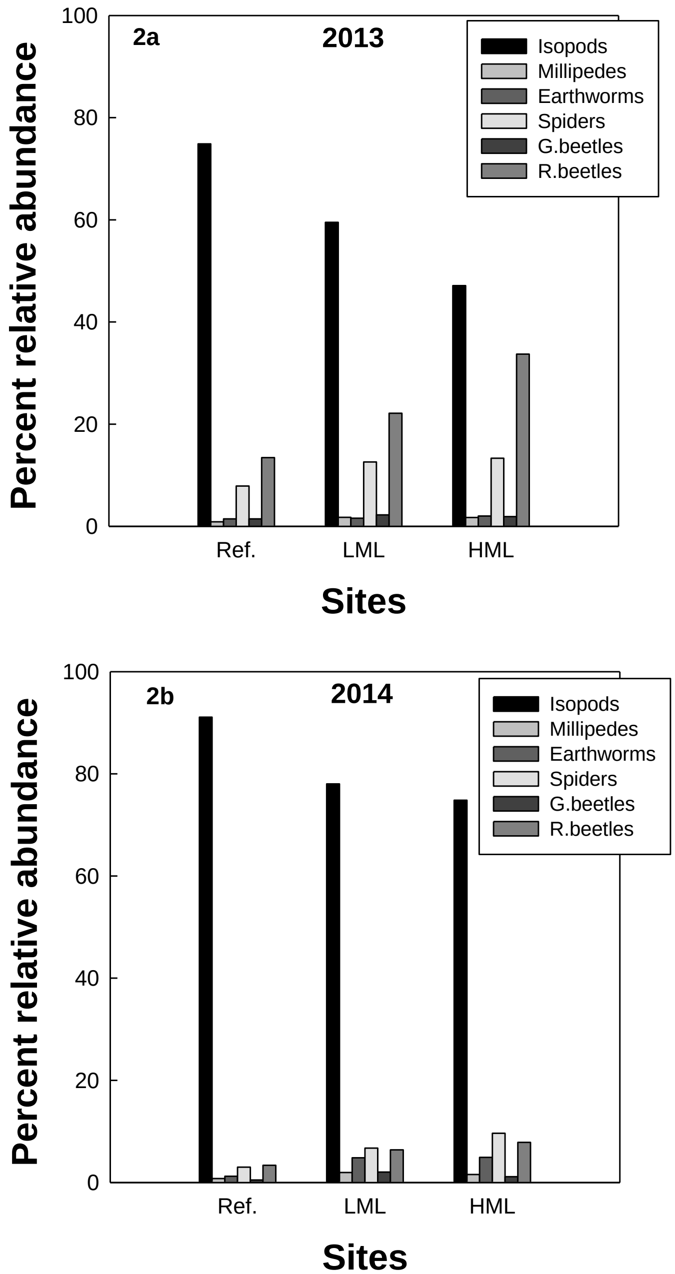
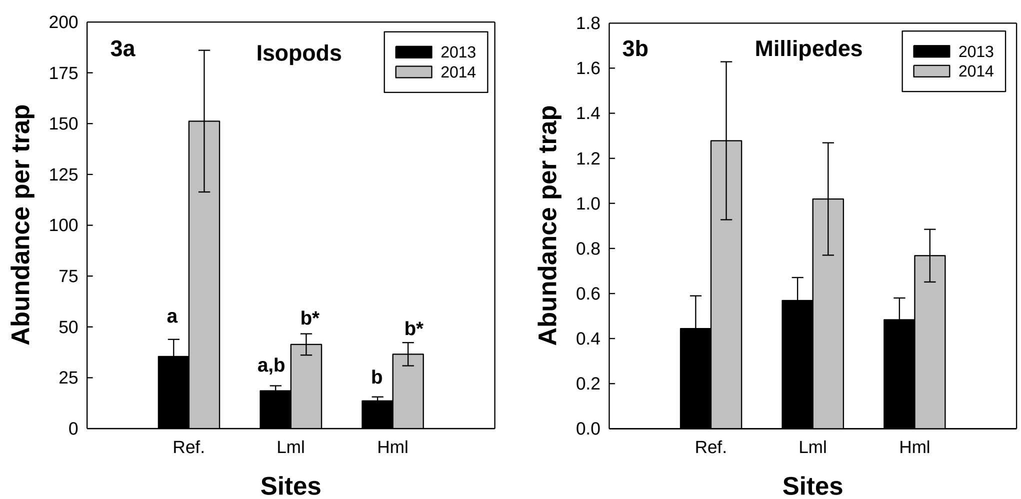


Fig 2: Percent relative abundance of different soil invertebrate groups at the reference, low soil metal load (LML) and high soil metal load (HML) sites for sampling periods in (a) 2013 (June-October) and (b) 2014 (May-September) at Liberty State Park (LSP), New Jersey, USA.

In terms of comparisons of the overall abundance levels for different invertebrate taxa along the metal gradient, significant differences were only observed for isopods, as their abundance showed a significant decline along the metal gradient during both periods of sampling in 2013 ($p= 0.01$, $df= 2$, $F= 4.488$) and 2014 ($p =0.00$, $df= 2$, $F= 21.93$) (Fig. 3a). In 2013, abundance of isopods at the reference site was higher than the HML sites ($p=0.01$, post hoc Tukey tests), however, abundance levels at the LML sites did not differ ($p>0.05$, post hoc Tukey tests) neither with those recorded at the reference nor at the HML sites (Fig. 3a). In 2014, isopod abundance levels sampled at the reference site were higher ($p<0.05$) than those at metal contaminated sites (LML and HML) (Fig. 3a). All the other invertebrate groups did not show significant differences in abundance among the sites with different metal loads (All $p_s<0.05$, Fig. 3b-f).



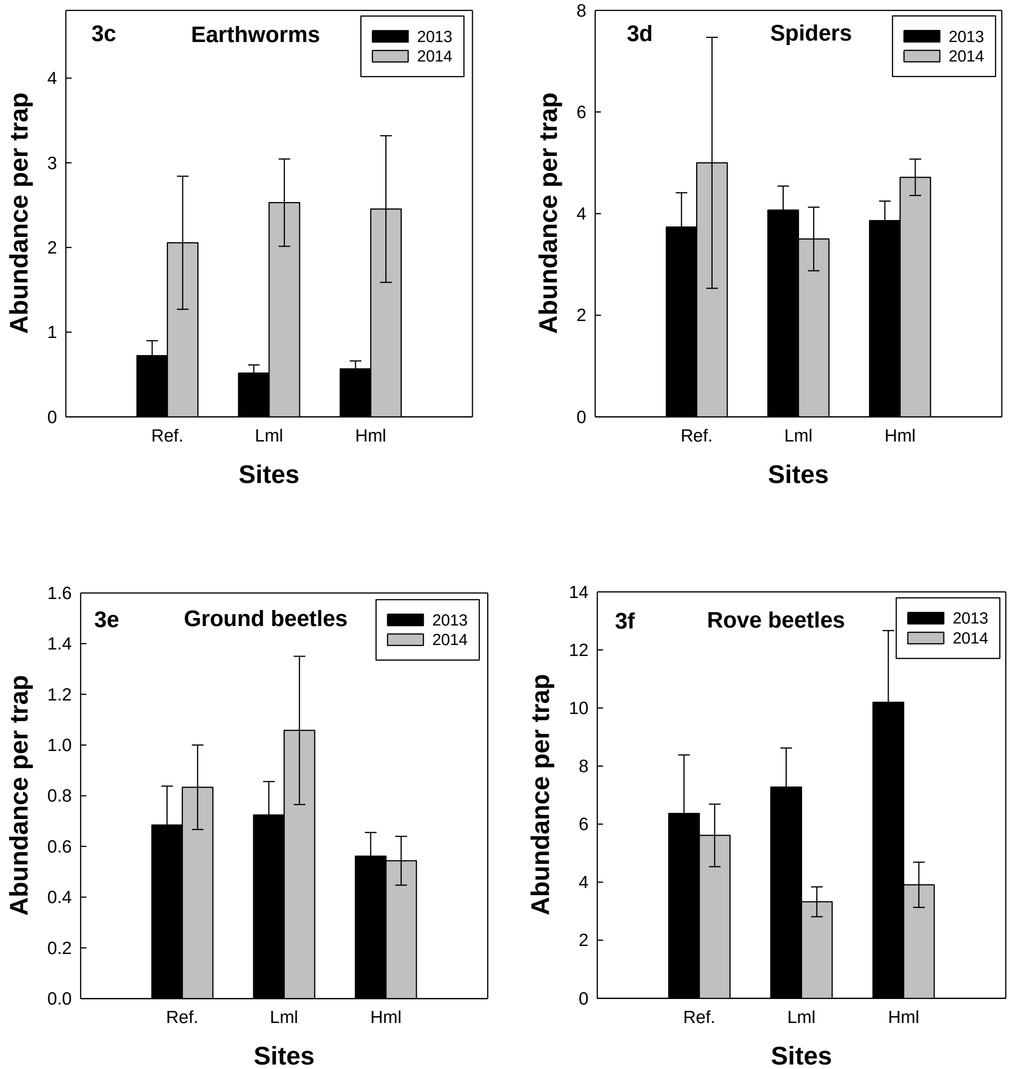


Fig. 3: Average abundance (±1 SE) of (a) isopods, (b) millipedes, (c) earthworms, (d) spiders, (e) ground beetles, and (f) rove beetles sampled with pitfall traps from the reference, low metal load (LML) and high metal load (HML) sites in 2013 (June-October) and 2014 (May- September). Letters (a, b and c) and (a*,b*, and c*) indicate significant ($p \leq 0.05$) abundance differences based on post hoc Tukey multiple comparison tests for data sampled in year 2013 and 2014 respectively. Bars with no letters are not significantly different ($p>0.05$).

3.2. Diversity index and evenness

Regarding the Shannon-Weaver index (H) and Shannon equitability index (E_H), significant differences were not observed for both indexes between the reference, LML, and HML sites in 2013 (Fig. 4a & 4b). However, values of both indexes at the reference site were lower ($p<0.05$) than those observed at the LML sites in 2014 (Fig. 4a & 4b).

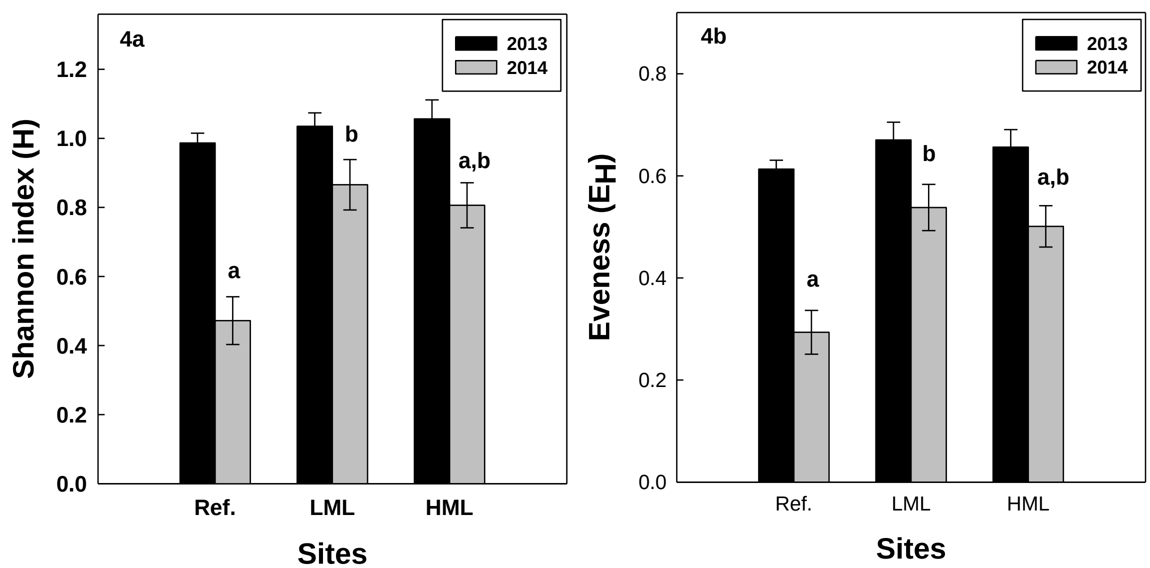


Fig. 4: Soil invertebrate community data metrics**;** Shannon-Weaver index (H) and Shannon's equitability index (E_H) values for reference, low soil metal load (LML), and high soil metal load (HML) sites along the metal gradient at LSP for sampling periods in 2013 and 2014. Different letters indicate significant differences observed in the 2014 sampling data based on the results of post hoc Tukey tests. Differences in the indexes between the sites were not observed for the 2013 data.

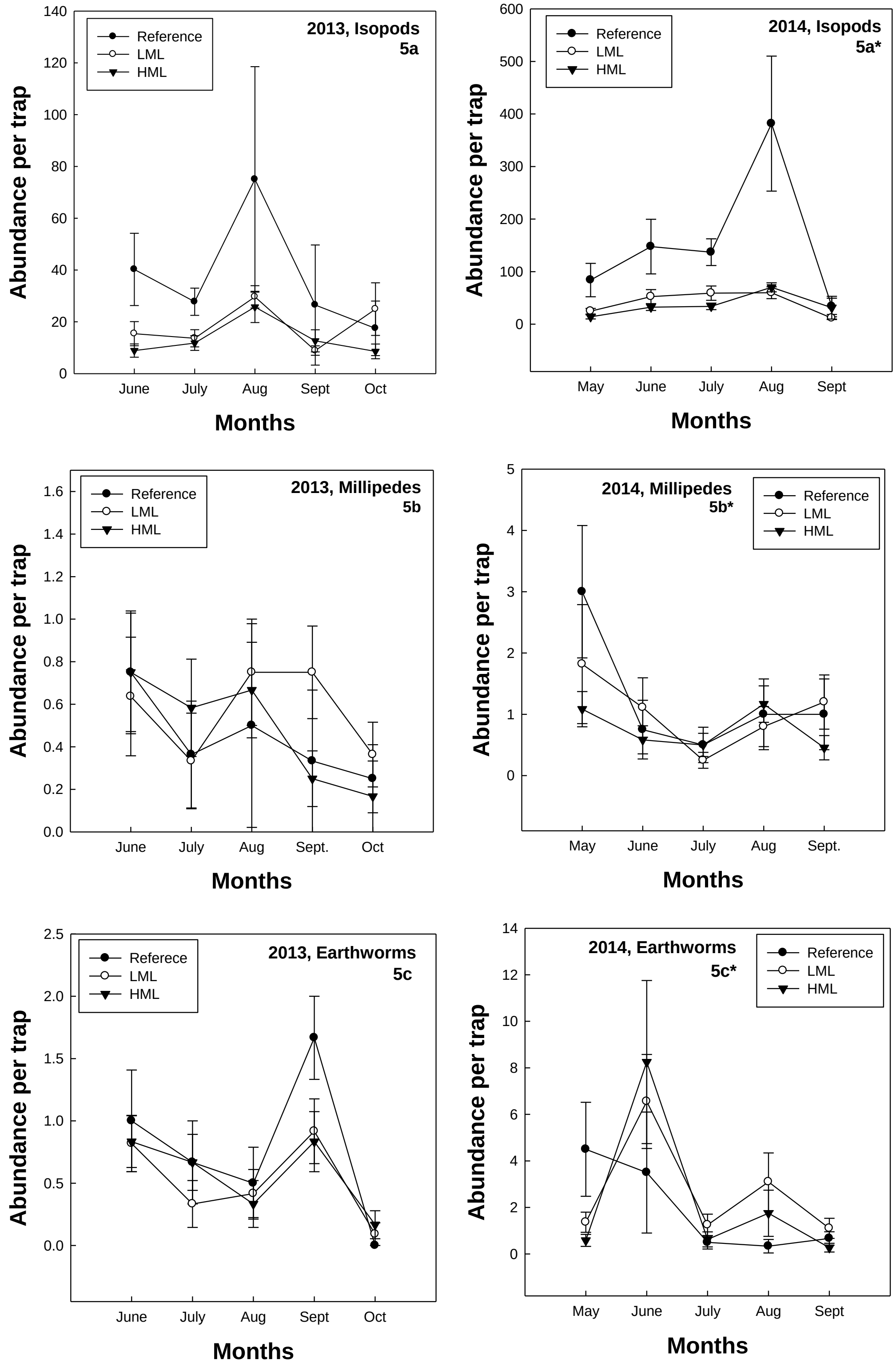
3.3. Temporal dynamics in abundance

Monthly abundance means of different invertebrate taxa sampled at the reference, LML and HML sites in 2013 (June-October) and 2014 (May-September) are shown in Fig. 5.

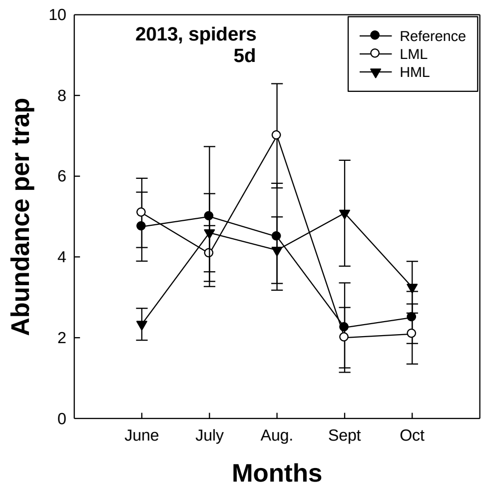
Although, results of the repeated measures ANOVA analysis revealed that abundance levels for most of the invertebrate taxa except Oniscidae (isopods), and Haplotaxida (earthworms) at the LML sites in 2013, and Staphylinidae (rove beetles) at the LML and HML sites in 2013 did not vary significantly over the sampling periods in 2013 and 2014 at the LML and HML sites (Table. 2, due to statistically insufficiency, I could not perform this analysis for data collected from the reference site). Abundance levels for some of the taxa did show single peak activity during the sampling periods, for example, abundance of both Carabidae and Diplopoda peaked (although statistically non-significant) during early summer in 2013 (June) and 2014 (May and June) (Fig. 5b, 5b*, 5e, & 5e*). Similarly, Oniscidae (isopods) also showed a single peak activity (5a & 5a*); for instance, number of isopods trapped in August were higher than the months of June, July, and September ($p<0.05$, post hoc tests, Table. 2) at the LML sites in 2013. Similar peaks (statistically non-significant) in the abundance of isopods for the month of August were also noticed at the reference sites during 2013 and 2014 (Fig. 5a & 5a*).

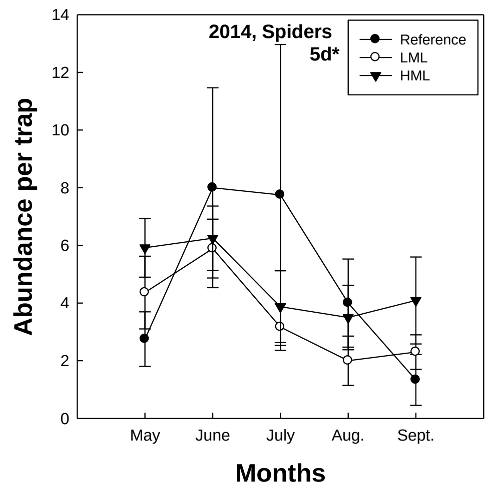
With regards to the third type of detritivores, Haplotaxida (earthworms), abundance levels seemed to peak during early (May and June) and late summer (September). The results of the post hoc analysis showed that earthworm abundance in July, 2013 at the LML sites was lower ($p<0.05$) than September, 2013 (Table. 2). In addition, earthworm abundance levels in October, 2013 at the LML sites were also lower ($p<0.05$) than both June and September of 2013 (Table. 2).

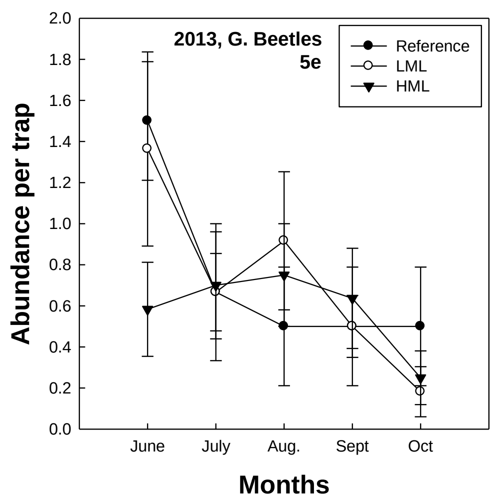
In case of Staphylinidae (rove beetles), the monthly abundance patterns varied during the two study years (Fig. 5e and 5e*). For example, in 2013, highest activity was observed in August at the reference, LML and HML sites. Accordingly, results of the post hoc tests revealed that abundance levels in August were significantly higher ($p<0.05$) than those observed in all other months (June, July, September, and, October) at the LML and HML sites in 2013 (Table. 2). On the contrary, abundance levels of Staphylinidae did not vary between the sampling months in 2014 (Table. 2).

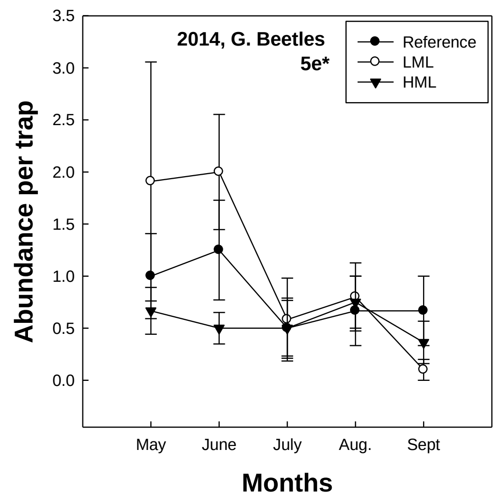


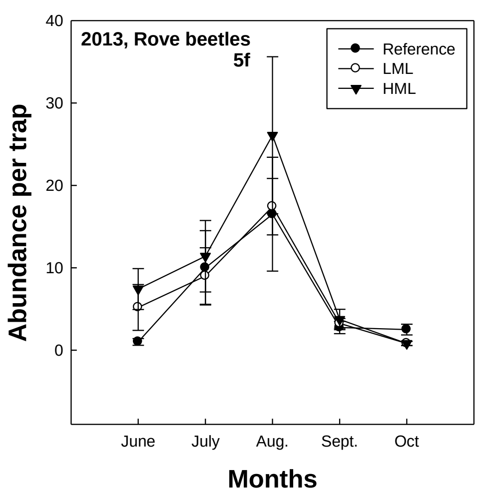
2013, Isopods
5a
Reference
LML
HML
Abundance per trap
0
20
40
60
80
100
120
140
June
July
Aug
Sept
Oct
Months
2014, Isopods
5a*
Reference
LML
HML
Abundance per trap
0
100
200
300
400
500
600
May
June
July
Aug
Sept
Months
2013, Millipedes
5b
Reference
LML
HML
Abundance per trap
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
June
July
Aug
Sept.
Oct
Months
2014, Millipedes
5b*
Reference
LML
HML
Abundance per trap
0
1
2
3
4
5
May
June
July
Aug
Sept.
Months
2013, Earthworms
5c
Referece
LML
HML
Abundance per trap
0.0
0.5
1.0
1.5
2.0
2.5
June
July
Aug
Sept
Oct
Months
2014, Earthworms
5c*
Reference
LML
HML
Abundance per trap
0
2
4
6
8
10
12
14
May
June
July
Aug
Sept
Months


2013, spiders
5d
Reference
LML
HML
Abundance per trap
Months
June
July
Aug.
Sept
Oct


2014, Spiders
5d*
Reference
LML
HML
Abundance per trap
Months
May
June
July
Aug.
Sept.


2013, G. Beetles
5e
Reference
LML
HML
Abundance per trap
Months
June
July
Aug.
Sept
Oct


2014, G. Beetles
5e*
Reference
LML
HML
Abundance per trap
Months
May
June
July
Aug.
Sept


2013, Rove beetles
5f
Reference
LML
HML
Abundance per trap
Months
June
July
Aug.
Sept.
Oct

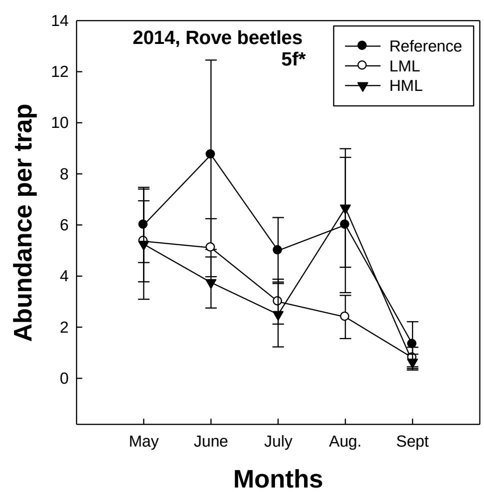

2014, Rove beetles
5f*
Reference
LML
HML
Abundance per trap
Months
May
June
July
Aug.
Sept

Fig. 5: Seasonal variation in abundance (Avg. ± 1 SE) of different invertebrate taxa: (a) Oniscidea (isopods), (b) Diplopoda (millipedes), (c) Haplotaxida (earthworms), (d) Araneae (spiders), (e) Carabidae (ground beetles), and (f) Staphylinidae (rove beetles) sampled with pitfall traps at the reference, low soil metal load (LML), and high soil metal load (HML) sites for sampling periods in 2013 (June-October) and 2014 (5a*-5e*) (May-September).

Table 2: Summary table of the Repeated measures ANOVA analysis, where effects of months (time) on abundance of different invertebrate taxa: Oniscidea (isopods), Diplopoda (millipedes), haplotaxida (earthworms), Araneae (spiders), Carabidae (ground beetles), and Staphylinidae (rove beetles) at the reference, low soil metal load (LML) and high soil metal load (HML) sites were analyzed for sampling periods in 2013 (June-October) and 2014 (May-September).

Invertebrate taxa (Sampling site, sampling year)	Wilk's Lambda (post hoc results)
Oniscidea (Reference, 2013)*	
Oniscidea (Low metal load, 2013)	0. 190, F (4, 8) = 8.51, p =0.00 (June-Aug: 0.04, July-Aug: 0.03, Aug-Aug: 0.00, Aug-Oct=0.01)
Oniscidea (High metal load, 2013)	0.475, F (4, 8) = 2.213, p = 0. 13
Oniscidea (Reference, 2014) *	
Oniscidea (Low metal load, 2014)	0.004, F (4, 4) = 5.857, p = .29
Oniscidea (High metal load, 2014)	0.381, F (4, 4) = 1.623, p = 0.32
Diplopoda (Reference, 2013) *	
Diplopoda (Low metal load, 2013)	0.637, F (4, 7) = 1.097, p = 0.42
Diplopoda (High metal load, 2013)	0.502, F (8, 4) = 2.034, p =0.18
Diplopoda (Reference, 2014) *	

Diplopoda (Low metal load, 2014)	0.621, $F(4, 6) = 0.919$, $p = 0.48$
Diplopoda (High metal load, 2014)	0.200, $F(4, 4) = 4.603$, $p = 0.08$
Haplotaxida (Reference, 2013) *	
Haplotaxida (Low metal load, 2013)	0.205, $F(4, 7) = 6.806$, $p = 0.01$ (July-Sept: 0.01, June-Oct: 0.02, Sept-Oct: 0.01)
Haplotaxida (High metal load, 2013)	0.460, $F(8, 4) = 2.352$, $p = 0.14$
Haplotaxida (Reference, 2014) *	
Haplotaxida (Low metal load, 2014)	0.319, $F(4, 5) = 2.667$, $p = 0. 15$
Haplotaxida (High metal load, 2014)	0.424, $F(4, 4) = 1.360$, $p = 0.38$
Araneae (Reference, 2013) *	
Araneae (Low metal load, 2013)	0.346, $F(4, 7) = 3.301$, $p = 0.08$
Araneae (High metal load, 2013)	0.620, $F(4, 6) = 0.920$, $p = 0.41$
Araneae (Reference, 2014) *	
Araneae (Low metal load, 2014)	0.256, $F(4, 6) = 4.350$, $p = 0.6$
Araneae (High metal load, 2014)	0. 161, $F(4, 4) = 5.230$, $p = 0.08$
Carabidae (Reference, 2013) *	
Carabidae (Low metal load, 2013)	0.329, $F(4, 7) = 3.567$ $p = 0.07$
Carabidae (High metal load, 2013)	0.672, $F(4, 6) = 0.773$, $p = 0.47$
Carabidae (Reference, 2014) *	
Carabidae (Low metal load, 2014)	0.28, $F(4, 5) = 3.156$, $p = 0.12$
Carabidae (High metal load, 2014)	0.74, $F(4, 4) = 0.351$, $p = 0.83$
Staphylinidae (Reference, 2013) *	
Staphylinidae (Low metal load, 2013)	0. 141, $F(4, 7) = 8.276$, $p = 0.00$ (June- Aug: 0.00, July-Aug: 0.01, July-Oct: 0.02, Aug-Sept: 0.00, Aug-Oct: 0.00)

Staphylinidae (High metal load, 2013)	0.127, F (5,4) = 8.612, p = 0.02 (June-Oct: 0.02, Aug-Sept: 0.03, July-Oct: 0.02, Aug-Oct: 0.00)
Staphylinidae (Reference, 2014) *	
Staphylinidae (Low metal load, 2014)	0.22, F (4,5) = 4.266, p = 0.08
Staphylinidae (High metal load, 2014)	0.457, F (4, 4) = 1. 187, p = 0.44

* Multivariate tests could not be performed because of insufficient residual deg. of freedom

3.4. Community composition

The community composition and underlying environmental factors were explored by canonical correspondence analysis (Fig. 6), which was chiefly used to evaluate the distribution pattern of different invertebrate taxa at sites along the metal gradient. The model was significant (F= 6.052, p = 0.002), the sum of all canonical eigenvalues was 0.117 (axis-1: 0.073, axis-2: 0.037, axis-3: 0.005, and axis-4: 0.001), all four canonical axes explained 16.0 % of the variability in invertebrate taxa abundance distribution. Results of CCA showed that soil metal load (TML), concentrations of zinc in *Populus spp.* leaves, and concentrations of zinc, copper and lead in soil have negative correlations with the abundance distribution of isopods and opposing to this positive correlation with the abundance distribution of rove beetles (Staphylinidae) and the other separate taxa (Fig. 6). In addition, abundance distributions for earthworms, ground beetles, and millipedes were affected by the sampling month, as abundances tended to decrease from the beginning of the sampling season towards the end (Fig. 6).

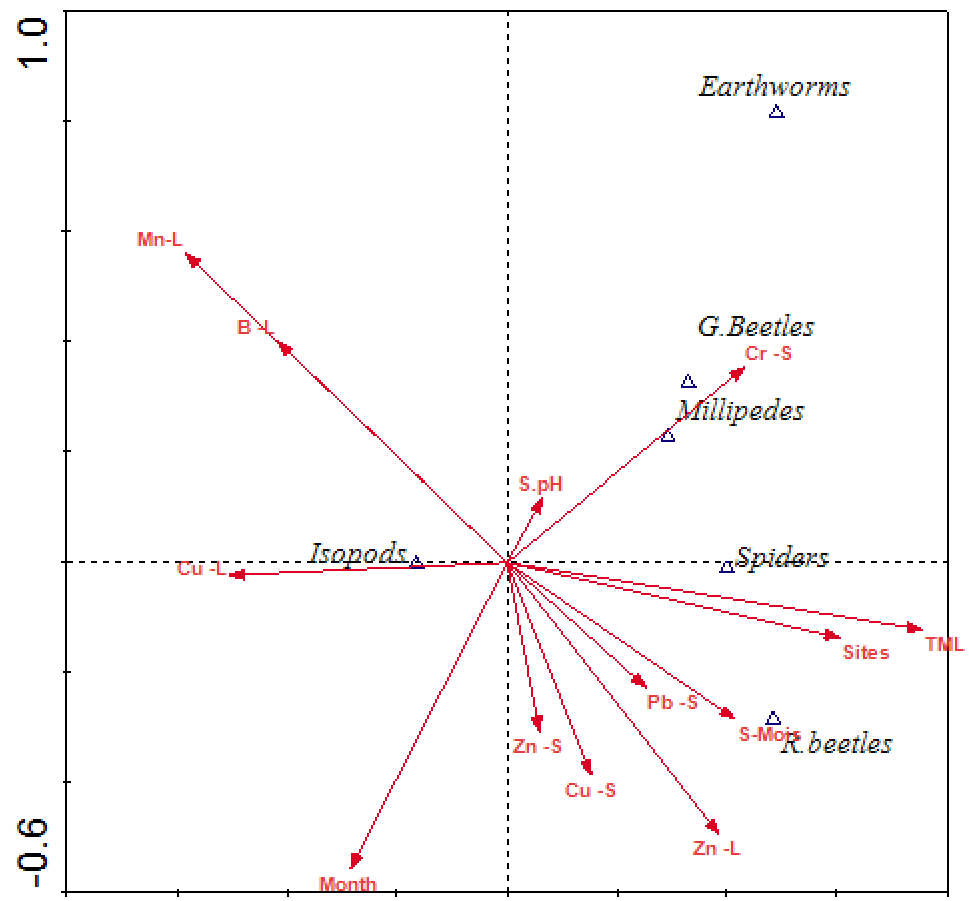


Fig. 6: Canonical correspondence analysis (CCA) plot of distribution of different invertebrate taxa in relation to different variables including soil pH, soil moisture, and total soil metal load, metal concentrations in soil and leaves of *Populus spp.* at various sites along the metal gradient at Liberty State Park.

4. Discussion

The results of this study did not support my hypothesis that diversity of soil invertebrate assemblage decreases with increasing soil metal load as determined by the Shannon-Wiener index (Fig. 4a). Although, the negative effects of metal contamination were not seen on the invertebrate assemblage, distribution of isopod communities was negatively affected along the metal gradient at the brownfield site (Fig. 6). The abundance of isopods declined with increasing soil metal load (Fig. 3a & 3a*) both in 2013 and 2014, whereas, abundance of other five taxa (Diplopoda, Haplotaxida, Araneae, Carabidae, and Staphylinidae) did not vary significantly ($p>0.05$) along the metal gradient both in 2013 and 2014 (Fig. 3a-3e & 3a*-3e*). Possible explanations for the limited effect of metal pollutants on abundances of most arthropod groups could be the use of adaptive defense mechanisms to minimize the harmful effects of metals by soil invertebrates inhabiting the metal contaminated sites (Vijver et al. 2005a; Hopkin 1990; Janssen et al. 1991). The lack of sensitivity of most of the invertebrates groups except isopods to metal contamination might also be related to the taxonomical rank (methodological bias) selected in this study (class, order and family) that might not have provided adequate resolution for detecting species level differences between the study sites.

4.1. Shannon-Weaver diversity index (H)

Based on the findings of previous eco-toxicological studies showing negative effects of metals (e.g. increased mortality, growth inhibition) in different soil invertebrates (Drobne and Hopkin 1995; Godet et al. 2011; Lukkari et al. 2005; Mozdzer et al. 2003), it was expected that diversity of invertebrate assemblage decreases in response to the soil metal contamination. However, the Shannon-Weiner diversity index values in this study did not vary along the metal gradient in 2013 (June-October) (Fig. 4a). Moreover, the Shannon-Weiner diversity index values estimated for the metal contaminated sites (LML and HML) were even higher than the reference ("clean") site in 2014 (May-September) (Fig. 4a). These strong effects on the soil invertebrate community were largely driven by higher evenness values (Shannon's equitability index, (E_H)) at the metal contaminated sites (LML and HML) as compared to the reference site (Fig. 4b). The higher evenness at the metal contaminated sites was a result of a significant decline in the abundance of isopods at these sites (compared to reference site, Fig. 3a) leading to relatively reduced dominance of isopods at the LML (78%) and HML (74%) sites than the reference site (91%) (Fig. 2), as abundance levels of all the other taxa appeared to be fairly similar between all the sites in 2014 (Fig. 3b*-3e*).

4.2. Abundance patterns of terrestrial isopods (Oniscidae) along the metal gradient

In comparison to other groups of soil invertebrates, isopods are characterized by higher accumulation of metals, for instance, Heikens et al. (2001) compared metal concentrations accumulated in isopods, earthworms, and beetles sampled from sites contaminated with Cd, Cu, Pb and Zn and found highest metal concentrations in isopods with earthworms accumulating intermediate metal concentrations among the three invertebrate groups. Higher accumulation of metals in isopods as compared to other invertebrates might be due to differences in the metal exposure routes, and/or differences in their metal regulation capabilities. For instance, isopods mostly feed on the organic matter and therefore have more intensive contact with contaminated litter or soil, whereas, beetles which are herbivores or second-order consumers acquire metals mainly through their food and are not in such direct contact with the soil (Butovsky 2011). In another study, Hunter et al. (1987) also found highest concentrations of metals (Cu and Cd) in isopods as compared to earthworms, spiders, ground beetles and rove beetles sampled from contaminated and semi-contaminated grasslands close to a copper refinery. In a laboratory study, Graf et al. (1997) exposed a terrestrial isopod (*Porcellio scaber*) and millipedes (*Julus scandinavius*) to metal contaminated food (leaf litter) and soil for three weeks and found higher metal concentrations in isopods than millipedes. The authors explained that lower metal concentrations in millipedes were due to the efficient elimination of excess metals via exfoliation of the midgut epithelium. In comparison, isopods store metals in the hepatopancreas, which are not molted, thus, resulting in relatively greater accumulation of metals (Hubert 1979). In fact, metal sequestration in hepatopancreas is used as a detoxification mechanism by isopods as it provides a barrier for metals, protecting other organs from toxic effects of metals (Hopkin 1990; Hopkin and Martin 1982). However, studies have shown that metal accumulation at extremely higher concentrations (greater than the storage capacity) can lead to metal poisoning as a result of metals interfering with important biochemical reactions (Hopkins 1990). In this study, it is likely that exposure to higher concentrations of metals at HML sites, especially Zn, Cu, and Pb in soils and Zn in leaves of hardwood trees (*Betula populifolia* and *Populus spp.*, dominant at HML sites at LSP) might have contributed to an accumulation of metals at extremely higher concentrations (in hepatopancreas), leading to reduced abundance of isopods along the metal gradient (Fig 3a & 6).

4.3. Abundance patterns of non-isopod invertebrate taxon along the metal gradient

In the present study, non-isopod invertebrate taxon (Diplopoda, Haplotaxida, Araneae, Carabidae, and Staphylinidae) does not seem to be affected by metal contamination, as their abundance levels were quite similar between the investigated sites (Fig. 3b-3e). The lack of negative effects of metal contamination on distribution of these taxa might be related to the development of efficient detoxification and/or excretion mechanisms to neutralize the lethal metal effects by soil invertebrates at the metal contaminated sites. Studies have shown that soil invertebrates inhabiting metal contaminated areas can develop adaptive mechanisms (detoxification and/or excretion) enabling them to cope up with adverse effects of metals. One of the common strategies is internal compartmentalization of metals (in inert form) in specific organs. For example, earthworms and millipedes are known to sequester metals in the posterior alimentary canal and in epithelial cells of the midgut gland respectively, thereby, preventing the passage of large concentrations of metals into other tissues (Davis 2002; Kania and Lechowski 2014; Köhler and Alberti 1992; Köhler et al. 1995; Morgan and Morgan 1990). Similarly, predatory invertebrates such as spiders have been shown to store metals in midgut glands (Brown 1982; Hopkin 1989; Wilczek and Babczynska 2000). In addition, spiders are also able to remove excess metal concentrations by intensifying metal excretion with feces (Wilczek and Babczynska 2000). Among soil invertebrates, beetles are known to have the lowest concentrations of accumulated metals (Butovsky 2011; Heikens et al. 2001), which may be due to their ability to store metals in midgut epithelium, which gets renewed with molting, allowing them to get rid of excess of metals (Hopkin 1989).

In addition to efficient detoxification and excretion mechanisms, the apparent insensitivity of some of the predatory taxa to metal contamination than isopods might also be related to a lesser direct contact with metals resulting in relatively lower accumulations of metals in the predatory invertebrates than isopods (Heikens et al. 2001). As discussed previously, these differences in metal exposures might occur due to different feeding preferences of the invertebrates. For instance, isopods inhabit the contaminated leaf litter and mostly feed on the soil organic matter and therefore are exposed to higher concentrations of metals than predatory invertebrates such as ground beetles, which have a less intensive contact with metals.

Another explanation for lack of sensitivity of non-isopod invertebrate taxa to metal contamination could be the taxonomical rank used in this study (class, order and family) which might not have provided adequate resolution for detecting the impacts of metal contamination. Studies have shown that different species present at the same site can differ from each other in terms of their sensitivity to metal pollution because of the differences in physiological processes employed for metal detoxification and excretion (Dallinger 1993), or due to behavioral and ecological differences that might influence the exposure to heavy metals (Van Gestel et al. 1992). Such inter-specific differences can affect distribution of species such that sensitive species are eliminated from metal contaminated sites and available empty niche(s) are occupied by the metal tolerant species (Spurgeon and Hopkin 1996; Wilczek and Babczynska 2000; Beyrem et al. 2007; Read et al. 1998). In such a case, even if metal sensitive species belonging to a particular family or order is/are absent from a metal contaminated site, overall abundance examined at family or order level might not be significantly affected. Therefore, it is possible that effects of metal contamination on soil invertebrates might be more detectable at a lower taxonomical rank (e.g. species level) than the ones used in this study (family, order or class). Although, it might be better to study the effects of metal contamination on the invertebrate communities at a lower taxonomical level, it is important to note that previous studies have actually found significant effects of metal contamination on soil invertebrate communities using the same taxonomic level (family, order, or class) as used in this study (Nahmani and Lavelle 2002; Migliorini et al. 2004). Moreover, one also needs to consider that for most of the soil invertebrates, identification at species level (lower taxonomic rank) is extremely time consuming making this approach less suitable for risk assessment studies (Nahmani et al. 2006).

4.4. Monthly abundance patterns of different invertebrate taxa

The three detritivores taxa (Isopoda, Diplopoda, and Haplotaxida) examined in this study showed relatively different patterns of seasonal activity (Fig. 5). For instance, the most active period for isopods was late summer (around August), whereas, millipedes were most active during early summer (May and June) (Fig. 5b & 5b*). In comparison, abundance levels of earthworms peaked both in late (September) as well as during early summer (May and June) (Fig. 5c & 5c*). The increase in numbers for isopods in August during both years of sampling (Fig. 3a & 3a*) might be explained by their breeding patterns. Terrestrial isopods, *Philoscia muscorum* and *Armadillidium vulgare* (commonly found isopods species at LSP, Chapter 3), breed in late spring or early summer and the gestation periods generally last from eight to twelve weeks (Beauché and Richard 2013; Zimmer 2004). It is possible that the abundance peaks observed during late summer or early fall in this study might be due to recruitment of young juveniles in the population (juveniles were also noticed in the pitfall traps during the month of August; personal observation).

The monthly abundance pattern of earthworms observed in this study (peak activity during early and late summer) in 2013 and 2014 (Fig. 5c & 5c*) might be explained by considering the life cycle of an epi-endogeic (inhabits litter and top layers of the soil) earthworm species: *Amynthas agrestis*. Biological surveys conducted at LSP in

2014 showed that *A. agrestis* was the only earthworm species found at this study site (Henshue, 2015). Originally from Asia, *A. agrestis* is an invasive species in North America and is commonly found in disturbed as well as relatively disturbed areas (Gates 1982; Reynolds and Wetzel 2004). *A. agrestis* follows an annual life cycle where coccons are produced in late summer or early fall, overwintering occurs in the coccon stage, and adults emerge in the spring and mature during the summer (Uchida et al. 2004). It is likely that abundance peak levels observed during late summer (in August or September) at LSP could be due to increased activity of matured adults. The early summer abundance peak observed during 2014 (Fig. 5c*) can be attributed to the presence of large numbers of juveniles in the pitfall traps sampled in the months of May and June, 2014 (personal observation).

The abundance patterns for millipedes observed in this study are in general agreement with findings of Grelle et al. (2000) who sampled millipedes using pitfall traps (February to November, 1995) from a metal contaminated area close to a zinc and lead smelting site. Grelle et al. (2000) observed that millipedes were most active during the months of April and May, followed by a significant decline during the summer. Other studies have also reported similar results, for example, Riutta et al. (2012) sampled millipedes from a temperate deciduous forest and found higher abundance in the month of May as compared to September. Increased activity during spring as compared to summer in millipedes might be related to their sensitivity to higher temperatures (David and Handa 2010; Hassall et al. 2010), which can have a regulatory influence on their seasonal patterns in temperate regions (Crawford 1992).

Although the monthly abundance levels of Carabidae did not differ significantly over the sampling periods in 2013 and 2014 (Table. 2), the average trap catch was greatest during May and June (initial months of sampling) as compared to the other months of the sampling period (also evident from the results of CCA, Fig. 6). It is known that seasonal activity of ground beetles can be influenced by their breeding patterns, for instance, spring breeding carabids are active during spring or early summer and overwinter as adults, whereas, fall breeders are active during autumn and overwinter as larvae (Wang et al. 2014; Yu et al. 2006). Considering these lifestyles, my results suggest that most of the Carabidae species obtained in the pitfall traps from LSP might be spring breeders. It has been suggested that northern regions of the world tend to have more spring breeders as this life style provides offspring's with sufficient time to develop before the winter (Anderson, 1984; Niemela et al., 1992).

Studies have found that found that overall abundance of Staphylinidae in temperate eastern hardwood forests generally peak during early and mid-summer (Levesque and Levesque 1986a, b). Abundance levels of Staphylinidae (rove beetles) observed in this study exhibited peak activity during late summer (August) in 2013. Moreover, no differences in the abundance levels between different sampling months were observed in 2014. These differences might be related to seasonal phenology of individual species, which can influence local abundance levels, for example, some species are more active during early summer as compared to others being more active during late summer (Pohl et al. 2008). In addition, changes in ephemeral microhabitats can also result in year to year variation in their overall abundance levels. Based on the data collected from this study, it is difficult to predict how much of the seasonal or yearly variation in the abundance can be attributed to individual species phenology or to changes in the microhabitat conditions (Pohl et al. 2008). Future studies (using higher taxonomical resolution, e.g. species) evaluating the role of microhabitat conditions on specific species abundance might be helpful to understand these differences in monthly or yearly abundance patterns of Staphylinidae at this study site.

Abundance patterns of Araneae (spiders) did not vary over the sampling period during both years (2013 and 2014) (Table. 2). The lack of a significant variation in the monthly abundance levels of spiders might be indicative of temporal partition of resources by different spider species at this study site, thereby reducing interspecific competition (Berry 1971; Breymeyer 1966).

In summary, significant negative effects of soil metal contamination on the soil invertebrate assemblage were not observed for the majority of sampled taxa, and only one Oniscidae (isopods) were adversely impacted by the metal contamination of soils at the brownfield site. Based on these results, it is recommended that terrestrial isopods (at the family level: Oniscidae) can be used as a bio-indicator of heavy metal pollution in terrestrial ecosystems. Since, isopods play important role in the decomposition of organic matter, changes in isopod abundance as a result of metal contamination might have negative impacts on the overall recycling of nutrients in brownfields. The apparent insensitivity of all the invertebrate taxa (examined in this study except Oniscidae) to metal pollution might be indicative of effective tolerance mechanisms developed by invertebrates inhabiting the metal contaminated sites. Although these results were not expected, the overall lack of the negative effects of metal contamination on most of the soil invertebrate taxa suggests that metal contamination might not be affecting the availability of food for animals belonging to the higher trophic levels of the food chain. The results of the present study provide evidence that brownfields can function as important habitats for different soil invertebrates groups. The present study also provides the groundwork for further assessments of the soil arthropod communities in the brownfields at LSP.

References:

Adriano D. 1986. Trace elements in the terrestrial environment. Spring-Verlag, New York.
Ross SM. 1994. Toxic metals in soil-plant systems: JSTOR.

Anderson, J. 1984. A re-analysis of the relationship between life cycle patterns and the geographic distributions of Fennoscandian carabid beetles. J. Biogeography 11:479-489

Berry J. 1971. Seasonal distribution of common spiders in the North Carolina Piedmont. American Midland Naturalist: 526-531.

Beyrem H, Mahmoudi E, Essid N, Hedfi A, Boufahja F, Aissa P. 2007. Individual and combined effects of cadmium and diesel on a nematode community in a laboratory microcosm experiment. Ecotoxicology and environmental safety 68(3): 412-418.

Beauché F, Richard F-J. 2013. The best timing of mate search in *Armadillidium vulgare* (Isopoda, Oniscidea). Plos one 8(3): e57737.

Breymeyer A. 1966. Relations Between Wandering Spiders and the Other Epigeic Predatory Arthropoda: PaĹ „stwowe Wydawnictwo Naukowe.

Brown BE. 1982. The form and function of metal containing granules in invrtebratetissues. Biological Reviews 57(4): 621-667.

Butovsky RO. 2011. Heavy metals in carabids (Coleoptera, Carabidae). ZooKeys(100): 215-222.

Callis R, Cavanaugh L. 2009. Census bureau reports on residential vacancies and home ownership. Housing vacancies and home ownership. US Census Bureau.

Cárcamo H, Abe T, Prescott C, Holl F, Chanway C. 2000. Influence of millipedes on litter decomposition, N mineralization, and microbial communities in a coastal forest in British Columbia, Canada. Canadian Journal of Forest Research 30(5): 817-826.

Chang HF, Sigman H. 2007. The effect of joint and several liability under superfund on brownfields. International Review of Law and Economics 27(4): 363-384.

Cohen AC. 2015. Insect diets: science and technology: CRC press.

Cortet J, Gomot-De Vauflery A, Poinsot-Balaguer N, Gomot L, Texier C, Cluzeau D. 1999. The use of invertebrate soil fauna in monitoring pollutant effects. European Journal of soil biology 35(3): 115-134.

Crawford CS. 1992. Millipedes as model detritivores. Berichte des Naturwissenschaftlich-Medizinischen Verein Innsbruck 10: 277-288.

Dahle GA, Gallagher FJ, Gershensond D, Schäfer KV, Grabosky JC. 2014. Allometric and mass relationships of *Betula populifolia* in a naturally assembled urban brownfield: implications for carbon modeling. Urban ecosystems 17(4): 1147-1160.

Dallinger R. 1993. Strategies of metal detoxification in terrestrial invertebrates. Ecotoxicology of metals in invertebrates 245.

David JF, Handa IT. 2010. The ecology of saprophagous macroarthropods (millipedes, woodlice) in the context of global change. Biological Reviews 85(4): 881-895.

Davis TS. Brownfields: A comprehensive guide to redeveloping contaminated property. 2002. American Bar Association.

de Noronha Vaz E, Cabral P, Caetano M, Nijkamp P, Painho M. 2012. Urban heritage endangerment at the interface of future cities and past heritage: A spatial vulnerability assessment. Habitat international 36(2): 287-294.

De Sousa CA. 2008. Brownfields redevelopment and the quest for sustainability: Emerald Group Publishing.

Drobne D, Hopkin S. 1995. The Toxicity of Zinc to Terrestrial Isopods in a. Ecotoxicology and environmental safety 31(1): 1-6.

Eaton ER, Kaufman K. 2007. Kaufman Field Guide to Insects of North America: Houghton Mifflin Company.

El-Wakeil KFA. 2015. Effects of terrestrial isopods (Crustacea: Oniscidea) on leaf litter decomposition processes. The Journal of Basic & Applied Zoology 69: 10-16.

Gallagher FJ, Pechmann I, Holzapfel C, Grabosky J. 2011. Altered vegetative assemblage trajectories within an urban brownfield. Environmental Pollution 159(5): 1159-1166.

Gallagher FJ, Pechmann I, Bogden JD, Grabosky J, Weis P. 2008a. Soil metal concentrations and vegetative assemblage structure in an urban brownfield. Environmental Pollution 153(2): 351-361.

Gallagher FJ, Pechmann I, Bogden JD, Grabosky J, Weis P. 2008b. Soil metal concentrations and productivity of *Betula populifolia* (gray birch) as measured by field spectrometry and incremental annual growth in an abandoned urban Brownfield in New Jersey. Environmental Pollution 156(3): 699-706.

Gates G. 1982. Farewell to North American megadriles. Megadrilogica 4(1-2): 12-77.

Godet J-P, Demuynck S, Waterlot C, Lemière S, Souty-Grosset C, Scheifler R, et al. 2011. Growth and metal accumulation in *Porcellio scaber* exposed to poplar litter from Cd-, Pb-, and Zn-contaminated sites. Ecotoxicology and environmental safety 74(3): 451-458.

Grelle C, Fabre MC, Leprêtre A, Descamps M. 2000. Myriapod and isopod communities in soils contaminated by heavy metals in northern France. European Journal of Soil Science 51(3): 425-433.

Hanski I, Hammond P. 1986. Assemblages of carrion and dung Staphylinidae in tropical rain forests in Sarawak, Borneo. In: Proceedings of the Annales Entomologici Fennici, 1986.

Hassall M, Edwards DP, Carmenta R, Derhé MA, Moss A. 2010. Predicting the effect of climate change on aggregation behaviour in four species of terrestrial isopods. Behaviour 147(2): 151-164.

Hättenschwiler S, Tiunov AV, Scheu S. 2005. Biodiversity and litter decomposition in terrestrial ecosystems. Annu Rev Ecol Evol Syst 36: 191-218.

Heikens A, Peijnenburg W, Hendriks A. 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution 113(3): 385-393.

Henshue N. 2015. Summary of summer research. http://henshue.org.

Hopkin S, Hames C. 1994. Zinc, among a 'cocktail'of metal pollutants, is responsible for the absence of the terrestrial isopod *Porcellio scaber* from the vicinity of a primary smelting works. Ecotoxicology 3(1): 68-78.

Hopkin S. 1990. Species-specific differences in the net assimilation of zinc, cadmium, lead, copper and iron by the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. Journal of Applied Ecology: 460-474.

Hopkin SP. 1989. Ecophysiology of metals in terrestrial invertebrates: Elsevier Applied Science Publishers.

Hopkin S, Martin M. 1985. Assimilation of zinc, cadmium, lead, copper, and iron by the spider *Dysdera crocata*, a predator of woodlice. Bulletin of Environmental Contamination and Toxicology 34(1): 183-187.

Hopkin S, Martin M. 1982. The distribution of zinc, cadmium, lead and copper within the woodlouse *Oniscus asellus* (Crustacea, Isopoda). Oecologia 54(2): 227-232.

Hubert M. 1979. Localization and identification of mineral elements and nitrogenous waste in diplopoda. Myriapod Biology Academic Press Inc, New York: 127-133.

Hunter B, Johnson M, Thompson D. 1987. Ecotoxicology of copper and cadmium in a contaminated grassland ecosystem. II. Invertebrates. Journal of Applied Ecology: 587-599.

Jameson F. 1991. Postmodernism, or, the cultural logic of late capitalism: Duke University Press.

Janssen M, Bruins A, De Vries T, Van Straalen N. 1991. Comparison of cadmium kinetics in four soil arthropod species. Archives of Environmental Contamination and Toxicology 20(3): 305-312.

Jordal B. 2015. Beetles of Eastern North America by Arthur V. Evans. The Quarterly Review of Biology 90(2): 233-234.

Juang K-W, Lee D-Y, Ellsworth TR. 2001. Using rank-order geostatistics for spatial interpolation of highly skewed data in a heavy-metal contaminated site. Journal of Environmental Quality 30(3): 894-903.

Jennings AA, Cox AN, Hise SJ, Petersen EJ. 2002. Heavy Metal Contamination in the Brownfield Soils of Cleveland. Soil and Sediment Contamination: An International Journal 11(5): 719-750.

Jung M-P, Kim S-T, Kim H, Lee J-H. 2008. Species diversity and community structure of ground-dwelling spiders in unpolluted and moderately heavy metal-polluted habitats. Water, air, and soil pollution 195(1-4): 15.

Kania G, Lechowski J. 2014. Bioaccumulation of some elements in the millipede *Glomeris hexasticha* (Brandt, 1833)(Diplopoda, Glomerida). Journal of Elementology 19(1).

Keating WD. 2010. Redevelopment of vacant land in the blighted neighbourhoods of Cleveland, Ohio, resulting from the housing foreclosure crisis. Journal of Urban Regeneration & Renewal 4(1): 39-52.

Kirkwood N. 2003. Manufactured sites: rethinking the post-industrial landscape: Taylor & Francis.

Köhler H-R, Körtje K-H, Alberti G. 1995. Content, absorption quantities and intracellular storage sites of heavy metals in Diplopoda (Arthropoda). BioMetals 8(1): 37-46.

Köhler H-R, Alberti G. 1992. The effect of heavy metal stress on the intestine of diplopods. Advances in Myriapodology Ber Nat-med Ver Innsbruck, Suppl 10: 257-267.

Lanno R, Wells J, Conder J, Bradham K, Basta N. 2004. The bioavailability of chemicals in soil for earthworms. Ecotoxicology and environmental safety 57(1): 39-47.

Lee K, Foster R. 1991. Soil fauna and soil structure. Soil Research 29(6): 745-775.

Leigh NG, Coffin SL. 2000. How many brownfields are there? Building an industrial legacy database. Journal of Urban Technology 7(3): 1-18.

Levesque C, Levesque G. 1986a. Activité et succession saisonnière de Coléoptères épigés d'une forêt décidue du sud du Québec. Nat Can 113: 39-46.

Levesque C, Levesque G. 1986b. Abondance relative et activité saisonnière de Leiodidae et Staphylinidae (Coleoptera, Staphylinoidea) du biotopes forestiers decidus des Laurentides (Québec). Le Naturaliste Canadien 111: 229-233.

Liu Z, Zou X. 2002. Exotic earthworms accelerate plant litter decomposition in a Puerto Rican pasture and a wet forest. Ecological Applications 12(5): 1406-1417.

Lukkari T, Aatsinki M, Väisänen A, Haimi J. 2005. Toxicity of copper and zinc assessed with three different earthworm tests. Applied Soil Ecology 30(2): 133-146.

Marshall SA. 2006. Insects: Their Natural History and Diversity : with a Photographic Guide to Insects of Eastern North America: Firefly Books.

Maryanski M, Kramarz P, Laskowski R, Niklinska M. 2002. Decreased energetic reserves, morphological changes and accumulation of metals in carabid beetles (*Poecilus cupreus* L.) exposed to zinc-or cadmium-contaminated food. Ecotoxicology 11(2): 127-139.

McCarthy L. 2002. The brownfield dual land-use policy challenge: reducing barriers to private redevelopment while connecting reuse to broader community goals. Land Use Policy 19(4): 287-296.

Migliorini M, Pigino G, Bianchi N, Bernini F, Leonzio C. 2004. The effects of heavy metal contamination on the soil arthropod community of a shooting range. Environmental Pollution 129(2): 331-340.

Molle W. 2002. Globalization, regionalism and labour markets: Should we recast the foundations of the EU regime in matters of regional (rural and urban) development? Regional studies 36(2): 161-172.

Morgan JE, Morgan A. 1990. The distribution of cadmium, copper, lead, zinc and calcium in the tissues of the earthworm *Lumbricus rubellus* sampled from one uncontaminated and four polluted soils. Oecologia 84(4): 559-566.

Moulder BC, Reichle D. 1972. Significance of Spider Predation in the Energy Dynamics of Forest- Floor Arthropod Communities. Ecological Monographs 42(4): 473-498

Mozdzer TJ, Kramarz P, Piśkiewicz A, Niklińska M. 2003. Effects of cadmium and zinc on larval growth and survival in the ground beetle, *Pterostichus oblongopunctatus*. Environment international 28(8): 737-742.

Nahmani J, Lavelle P, Rossi J-P. 2006. Does changing the taxonomical resolution alter the value of soil macroinvertebrates as bioindicators of metal pollution? Soil Biology and Biochemistry 38(2): 385-396.

Nahmani J, Lavelle P. 2002. Effects of heavy metal pollution on soil macrofauna in a grassland of Northern France. European Journal of soil biology 38(3–4): 297-300.

Nassauer JI, Raskin J. 2014. Urban vacancy and land use legacies: A frontier for urban ecological research, design, and planning. Landscape and Urban Planning 125: 245-253.

Neilson S, Rajakaruna N. 2015. Phytoremediation of agricultural soils: using plants to clean metal-contaminated arable land. In: Phytoremediation:Springer, 159-168.

Niemela J., Spence J.R., Spence D.H., 1992. Habitat associations and seasonal activity of ground-beetles (Coleoptera, Carabidae) in Central Alberta. The Canadian Entomologist 124: 521–540.

Nitzu E, Nae A, Popa I. 2008. The fauna of soil beetles (Edaphic Coleoptera) as a sensitive indicator of evolution and conservation of ecosystems. A study on the altitudinal gradient in the Rodnei Mountains Biosphere Reserve (the Carpathians). Advances in Arachnology and Developmental Biology, Vienna-Belgrade-Sofia Monogr 12: 405-417.

Oliver L, Ferber U, Grimski D, Millar K, Nathanail P. The scale and nature of European brownfields. In: Proceedings of the CABERNET 2005-International Conference on Managing Urban Land LQM Ltd, Nottingham, UK, Belfast, Northern Ireland, UK, 2005.

Paoletti MG, Hassall M. 1999. Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agriculture, Ecosystems & Environment 74(1): 157-165.

Quadros AF, Araujo PB. 2007. Ecological traits of two neotropical oniscideans (Crustacea: Isopoda).

Pohl G, Langor D, Klimaszewski J, Work T, Paquin P. 2008. Rove beetles (Coleoptera: Staphylinidae) in northern Nearctic forests1. The Canadian Entomologist 140(4): 415-436.

Read HJ, Martin M, Rayner J. 1998. Invertebrates in woodlands polluted by heavy metals—an evaluation using canonical correspondence analysis. Water, Air, & Soil Pollution 106(1): 17-42.

Ren W, Xue B, Geng Y, Sun L, Ma Z, Zhang Y, et al. 2014. Inventorying heavy metal pollution in redeveloped brownfield and its policy contribution: Case study from Tiexi District, Shenyang, China. Land Use Policy 38: 138-146.

Renninger HJ, Wadhwa S, Gallagher FJ, Vanderklein D, Schäfer KV. 2013. Allometry and photosynthetic capacity of poplar (*Populus deltoides*) along a metal contamination gradient in an urban brownfield. Urban ecosystems 16(2): 247-263.

Reynolds JW, Wetzel MJ. 2004. Terrestrial Oligochaeta (Annelida: Clitellata) in North America north of Mexico. Megadrilogica 9(11): 71-98.

Snyder BA, Hendrix PF. 2008. Current and potential roles of soil macroinvertebrates (earthworms, millipedes, and isopods) in ecological restoration. Restoration Ecology 16(4): 629-636.

Spurgeon DJ, Hopkin SP. 1996. The effects of metal contamination on earthworm populations around a smelting works: quantifying species effects. Applied Soil Ecology 4(2): 147-160.

Spurgeon D, Hopkin S, Jones D. 1994. Effects of cadmium, copper, lead and zinc on growth, reproduction and survival of the earthworm *Eisenia fetida* (Savigny): assessing the environmental impact of point-source metal contamination in terrestrial ecosystems. Environmental Pollution 84(2): 123-130.

Sleuwaegen L, Pennings E. 2006. International relocation of production: Where do firms go? Scottish Journal of Political Economy 53(4): 430-446.

Staszewski T, Malawska M, Studnik-Wójcikowska B, Galera H, Wiłkomirski B. 2015. Soil and plants contamination with selected heavy metals in the area of a railway junction/Zanieczyszczenie gleby i roślin wybranymi metalami ciężkimi na terenie węzła kolejowego. Archives of Environmental Protection 41(1): 35-42.

Ter Braak CJ, Smilauer P. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5).www. canoco. com.

Uchida T, Kaneko N, Ito M, Futagami K, Sasaki T, Sugimoto A. 2004. Analysis of the feeding ecology of earthworms (Megascolecidae) in Japanese forests using gut content fractionation and δ15N and δ13C stable isotope natural abundances. Applied Soil Ecology 27(2): 153-163.

Van Gestel C, DIRVEN VAN BREEMEN E, Baerselman R. 1992. Influence of environmental conditions on the growth and reproduction of the earthworm *Eisenia andrei* in an artificial soil substrate. Pedobiologia 36(2): 109-120.

Vijver MG, Vink JP, Jager T, Wolterbeek HT, van Straalen NM, van Gestel CA. 2005a. Biphasic elimination and uptake kinetics of Zn and Cd in the earthworm *Lumbricus rubellus* exposed to contaminated floodplain soil. Soil Biology and Biochemistry 37(10): 1843-1851.

Vijver MG, Wolterbeek HT, Vink JP, van Gestel CA. 2005b. Surface adsorption of metals onto the earthworm *Lumbricus rubellus* and the isopod *Porcellio scaber* is negligible compared to absorption in the body. Science of the Total Environment 340(1): 271-280.

Wang X, Müller J, An L, Ji L, Liu Y, Wang X, et al. 2014. Intra-annual variations in abundance and species composition of carabid beetles in a temperate forest in Northeast China. Journal of insect conservation 18(1): 85-98.

Wilczek G, Babczynska A. 2000. Heavy metals in the gonads and hepatopancreas of spiders(Araneae) from variously polluted areas. Ekologia(Bratislava)/Ecology(Bratislava) 19: 283-292.

Wu J, Norvell W, Welch R. 2006. Kriging on highly skewed data for DTPA-extractable soil Zn with auxiliary information for pH and organic carbon. Geoderma 134(1): 187-199.

Yu X, Luo T, Zhou H. 2006. Habitat associations and seasonal activity of carabid beetles (Coleoptera: Carabidae) in Dongling Mountain, North China. Entomologica Fennica 17(2): 174.

Zimmer M. 2004. Effects of temperature and precipitation on a flood plain isopod community: a field study. European Journal of soil biology 40(3–4): 139-146.

Chapter Three: Abundance patterns and composition of terrestrial isopod assemblages along a heavy metal gradient in urban brownfields

Abstract

Abundance patterns and composition of terrestrial isopods assemblages (Oniscidea) were examined along a heavy metal contamination gradient in urban brownfields at Liberty State Park (LSP), Jersey City, New Jersey, USA. Isopods were sampled using pitfall traps from a reference site, three low soil metal load (LML), and three high soil metal load sites (HML) at LSP during 2013 (June-October) and 2014 (May-September). In addition to unmanaged sites along the metal gradient, isopods were also sampled from the managed areas of the park in 2014 (July-September). I hypothesized that: (1) abundance of isopods decreases with increasing soil metal contamination, (2) species richness decreases with increasing soil metal load resulting in changes the community composition along the metal gradient, and (3) unmanaged polluted sites such as brownfields can provide better habitats for isopods than urban managed habitats (e.g., formal parks, formal gardens, etc.). A total of 10,417 individuals belonging to four different isopod species: *Philoscia muscorum, Armadillidium vulgare, Armadillidium nasatum,* and *Trachelipus rathkii* were captured from all sites (reference, LML, HML, and managed) during all sampling periods in 2013 and 2014. Among the four species, *P. muscorum* dominated the pitfall traps at all sites (reference, LML and HML) regardless of metal concentration. Interestingly, one of the species, *A. nasatum* was completely absent at metal contaminated sites (LML and HML), hence, a decrease in species richness and a change in community composition was observed at sites along the metal gradient.

Overall, results of this study partly supported my first hypothesis as effects of metal pollution on isopod abundance were not the same for all four species. Considering the abundance comparisons (per trap) for each of the four species captured along the metal gradient and results of canonical correspondence analysis (CCA), *A. nasatum* and *P. muscorum* were most affected by metal contamination whereas *A. vulgare* was the most tolerant species. Various mechanistic hypotheses including metal poisoning resulting from accumulation of metals, differences in critical concentrations of hepatopancreas among different isopod species, effects of metal exposure on processes such as growth and reproduction in isopods are discussed to explain the observed inter-species variation in sensitivity to metal contamination. Regarding the managed areas, my third hypothesis was not supported as abundance of isopods did not differ between urban managed areas and unmanaged metal contaminated (LML and HML) brownfield sites. However, management practices had an effect on relative abundance of isopods, as *A.vulgare* was the dominant species at managed areas. A change in relative abundance pattern of isopod species observed at managed sites might be reflective of the isopods ability to adapt to temperature changes and moisture losses at managed areas which could be related to habitat structure (frequent open areas). Overall, lower abundance levels of isopods at metal contaminated brownfield (unmanaged sites) and managed areas in comparison to unmanaged and non-polluted reference site indicate that isopods are affected by different forms of stress at these two habitats, for example, soil metal contamination at brownfields and management practices in the managed areas.

1. Introduction

The economic decline and de-industrialisation in several urban areas throughout the world has resulted in an increase in abandoned post-industrial sites referred to as "brownfields". Due to former industrial activities, brownfield soils are often contaminated with trace metals such as zinc, chromium, arsenic, lead, and copper (Dudka et al. 1996; Gallagher et al. 2008a). Unlike organic matter, metals are not biodegradable and therefore persist in soil and trophic chains for a long time (Noll 2003). Exposure of trace metals to soil organisms at concentrations greater than required can negatively affect their abundance and diversity ultimately posing threat to ecosystem health (Caussy et al. 2003; Creamer et al. 2008; Pedersen et al. 1999).

Within the vast diverse group of soil organisms, terrestrial isopods (Isopoda: *Oniscidea*) are one of the dominant soil dwelling arthropods (David and Handa 2010) and are commonly used in eco-toxicological risk assessments studies. They are prominent terrestrial detritivores and play a significant role in the decomposition process (El-Wakeil 2015) through fragmentation and digestion of the leaf litter and by enhancing microbial activity (Jia et al. 2015; Zimmer 2002). Isopods are considered as bio-indicators of metal pollution in terrestrial environments (Drobne 1997; Köhler et al. 1996) due to their ability to accumulate large amount of metals (in hepatopancreas in form of intracellular granules) (Schill and Kohler 2004), which reflect the concentrations of metal of the soils/habitat (Köhler et al. 1996; Pouyat et al. 2015). The published literature on effects of metal contamination in isopods covers a wide range of topics including influence on body size and weight, energy levels in hepatopancreas, molting cycle frequency, survival rates, and body processes such as reproduction and growth (Calhôa et al. 2012; Drobne and Štrus 1996; Godet et al. 2011; Hopkin 1990; Jones and Hopkin 1996, 1998; Mazzei et al. 2013; Odendaal and Reinecke 2004; Witzel 1998). Structural, morphological and behavioral changes, for example, alterations in structure of hepatopancreas, fluctuating asymmetry levels of bilateral traits, feeding activity, discrimination between contaminated and non-contaminated food/soil have also been examined in isopods in response to metal contamination (Schill and Kohler 2004; Loureiro et al. 2006; Mazzei et al. 2014; Peters et al. 2001; Zidar et al. 2005; Zidar et al. 2003; Zidar et al. 2004; Žnidaršič et al. 2003). Other studies have documented the role of detoxification mechanisms such as expression of metallothioneins and heat shock proteins in relation to metal contamination (Donker et al. 1990; Mazzei et al. 2015; Žnidarŝiĉ et al. 2005). Interestingly, the majority of studies examining the effects of metal pollution on isopods have been conducted under laboratory conditions, which generally involves exposing isopods to artificially contaminated (usually with a single metal element) substrate and/or food (Godet et al. 2011; Loureiro et al. 2006; Zidar et al. 2003). In comparison, organisms are exposed to a mixture of contaminants in natural field conditions. Therefore, it is difficult to extrapolate results of experimental studies to expected field effects and studies on field populations are important and necessary. However, despite the suitability of isopods as indicators of metal contamination, very few field studies have examined the impact of metal pollution on isopods in field sites (Jones and Hopkin 1998; Hussein et al. 2006). Specifically information on diversity within isopod populations and communities in abandoned urban habitats such as brownfields is still scarce.

I examined species richness, abundance patterns and community composition of terrestrial isopods along a heavy metal gradient in urban brownfields located at the

Liberty State Park (LSP), Jersey City, New Jersey, USA. Metals such as zinc, copper, iron, and manganese are required by isopods for various physiological and metabolic functions; however, they are potentially harmful (for growth or reproduction) if present at concentrations higher than optimal (Calhôa et al. 2012; Godet et al. 2011). Isopod species can also differ in their susceptibility to metal stress (Hopkin 1990). Such differences can possibly eliminate the sensitive species at sites with higher metal load, thereby reducing species richness. Therefore, I hypothesized that: (1) abundance of isopods should decrease with increasing soil metal load, and (2) species richness should decrease with increasing soil metal load resulting in changes in community composition of isopods along the heavy metal gradient. In addition, I also compared the abundance of isopods between polluted "wild" urban habitats (brownfields) with "clean" managed garden sites. It is expected that polluted sites such as brownfields can provide better habitats for isopods than urban managed habitats such as formal parks, formal gardens, etc. (Bonthoux et al. 2014; Gardiner et al. 2013). Considering the ecological importance of isopods, studying their abundance patterns in managed as well as in unmanaged urban habitats such as brownfields will help us to assess these areas for biodiversity conservation and to formulate better redevelopment strategies for these habitats.

2. Materials and methods

2.1. Study Site

Terrestrial isopods in this study were sampled from urban brownfields located in LSP (Fig. 1). Located on the west bank of Upper New York Bay (centered at 40^{o} 42’ 14” N and 74^{o} 03’ 14” W), LSP used to be an intertidal mudflat and a salt marsh. Starting in the late 1800’s, the Central Rail Road of New Jersey (CRRNJ) developed it to use as a railyard and for transportation and storage of commodities including coal. Prior to its development, the area was filled with construction material, and debris from New York City and the surface was stabilized with cinder and ash materials. LSP was developed in

Fig. 1: Location of the reference and metal contaminated sites (Low soil metal load (LML) and High soil metal load (HML)) within Liberty State Park, New Jersey, USA. Source: "Liberty State Park, New Jersey." $40^{o}42`16.94``$ N $74^{o}03`14.73``$W. **Google Earth**. August 11, 2015.

1970's after CRRNJ discontinued its operations in 1967; however, a 251-acre area located in the center of the park was left isolated and unmanaged. This area is classified as an urban brownfield as soil metal concentrations in this abandoned area are higher than ambient levels resulting from its past industrial use (Gallagher et al. 2008a). This brownfield area with restricted public access served as one of the field sites in this study. In addition there is an adjoining 18-acre site that was used as a stockpile area for clean fill during construction of the western section of the park in the 1990's (Fig. 1). There remains several feet of clean fill in this area which served as the reference site for the study. Over the last five decades, the entire study site has been reclaimed by spontaneous vegetation succession, which includes dominant hardwood trees (*Betula populifolia, Populus spp.*), shrubs (*Rhus typhina, R. glabra,* and *R. copallinum*) and many native and non-native forbs and grasses (*Solidago spp*., *Eupatorium* spp., *Artemisia vulgaris,* etc.) (Gallagher et al. 2011).

Based on a sampling conducted in 2005, Gallagher et al. (2008a) examined the distribution of different metals (arsenic, chromium, copper, mercury, lead, vanadium and zinc) in soils at LSP (Table 1). A cumulative index of soil metal contamination was generated by performing logarithmic and rank order transformations on soil metal data sampled from different sites as described in Mc Grath et al. (2004) and Juang et al. (2001). Individual rank transformed metal concentrations were then summed (Juang et al. 2001) to develop the total soil metal load (TML) index, scaled between 0 and 5. Using the reverse function of the linear regression, the results were back transformed between the original data and the ranks (Wu et al. 2006). Finally, the data were krigged and a total soil metal load (TML) distribution map for the study area was generated (Gallagher et al. 2008a). The TML map represents a well-defined gradient from comparatively lower soil metal concentrations areas to areas of higher concentrations. Previous studies at LSP have reported a critical total soil metal load threshold value of 3.0 above which plant productivity and seed viability have been significantly influenced (Gallagher et al. 2008a;

Gallagher et al. 2011; Gallagher et al. 2008b). In addition, significantly slower growth rates of *Betula popufolia* and *Populus deltoides* (dominant tree species at LSP) have been shown at sites with TML greater than the critical threshold value of 3.0 (Dahle et al. 2014; Heidi et al. 2012). Furthermore, development of vegetative assemblage at LSP is also influenced by soil metal load as the sites with higher TML (greater than 3.0 or above) are dominated by metal tolerant hardwood trees such as *Betula populifolia* and *Populus spp.*

For studying the abundance patterns of isopods along the soil metal gradient, I sampled isopods from a reference area, three low soil metal load (LML) sites (LML-1 = site 48, LML-2 = site 41, and LML-3 = site 47), and three high soil metal load (HML) sites (HML-1 = site 10, HML-2 = site 14/16, and HML-3 = site 25) (Fig. 1). The metal contaminated sites (LML and HML) at LSP were selected based on the their respective TML values, for example, sites with total soil metal load greater than 3.0 were selected as high total metal load sites and sites with total soil metal load less than 3.0 were selected as low total metal load sites.

In order to compare isopod abundance between managed and unmanaged urban habitats (such as metal contaminated brownfields and unmanaged reference site), isopods were also sampled from managed areas of LSP. The managed area is sandwiched between the fence bordering brownfield sites and the lawn area, which is commonly used by public for recreational purposes (Fig. 1). The lawn area is maintained (involved cutting grass every 2-3 weeks) by the LSP staff. The ground surface in the managed areas is mostly covered with woody mulch and vegetation in this area includes planted trees such as *Ilex spp.*, *Juniperus spp.*, *Acer rubrum*, *Liquidambar spp.*, *Quercus bicolor*, and

Quercus palustris, shrubs (*Cornus amomum* , *Myrica pensylvanic*) and herbaceous plants (*Achillea millefolium*). The vegetation and mulch at the site are maintained by the generosity of the New Jersey Tree foundation.

Table 1: Soil characteristics including concentrations (µg/g) heavy metals (means with standard deviation in parentheses), soil pH, total soil metal load (TML) for the reference, three low soil metal load (LML-1= site 48, LML-2 = site 41, and LML-3 = site 47), and three high soil metal load (HML-1 = site 10, HML-2= site 14/16, and HML-3 = site 25) sites at Liberty State Park, New Jersey, USA.

Site	As	Cr	Cu	Pb	Zn	soil pH	TML
Reference[c]	--	14.0 (0.59)	13.9 (3.42)	2.0 (1.22)	39.4 (4.17)	6.84	--
48[a] (LML-1)	13.33 (7.44)	20.87 (24.95)	95.15 (22.07)	244.57 (103.24)	22.08 (15.36)	5.90	1.56
41[a] (LML-2)	14.88 (4.79)	10.90 (5.43)	76.86 (29.64)	96.58 (34.02)	156.75 (116.81)	7.0	1.64
47[b] (LML-3)	4.3	15.9	31.3	46.5	41.8		0.31

10[a] (HML-1)	282.57 (155.43)	91.58 (59.50)	379.18 (32.36)	737.95 (166.50)	192.65 130.53)	5.4	3.59
14-16[a] (HML-2)	68.13 (24.15)	334.56 (141.83)	202.58 (46.42)	857.53 (143.85)	237.97 (42.95)	4.80	3.56
25[a] (HML-3)	384.41 (253.15)	50.5 (39.71)	2200.38 (265.02)	6673.22 (1603.66)	2326.77 (2046.85)	4.6	4.31

a measured in 2005 (Gallagher et al., 2008a)

b measured in 1995 (Gallagher et al., 2008a)

c measured in 2015

2.2. Sampling

Isopods were sampled from all field sites (reference, LML, and HML) during June-October in 2013 and from May-September in 2014. Sampling at the managed sites was conducted during July-September, 2014. In this study, pitfall traps were used to sample terrestrial isopods. Pitfall traps is one of the most frequently used methods for sampling ground dwelling arthropods (Phillips and Cobb 2005; Santos et al. 2007; Standen 2000) and is widely used in ecological research to address a number of questions such as evaluating differences in population size of soil arthropods in time or space, estimating relative abundance of species within a given habitat or studying the effect of a disturbance on biodiversity of an ecosystem (Noemí Mazía et al. 2006; Pekár 2002). The advantages of using pitfall traps are that they are simple, inexpensive, and work even in the absence of an observer (Pekár 2002), which means data are not influenced by individual knowledge or fatigue. Furthermore, these traps are easy to install and, once placed, sampling can be continued for a short or long period of time with minimal disturbance to the habitat. At each site, four pitfall traps (15-20 m apart from each other) made of plastic cups (10.5 cm x 8 cm), were placed in the soil such that the rim of an individual cup was at level with the soil surface. To minimize variation in the location of pitfall traps across different sites, each trap was placed in a bare area closer to a vegetation patch composed of the herbaceous mugwort (*Artemisia vulgaris*). Since the managed area is continuous along the fence, six (6) pitfall traps were placed in the soil (15-20 m apart from each other) along a transect (2.5-3.0 m away from the fence). Each trap at all the sites was filled halfway with a solution of propylene glycol and water (70:30) and was covered with a small wooden board (to prevent from rain and falling leaves) raised 2-3 cm above the trap with nails in each corner. The traps were placed in the field for one week once per month. Samples collected from individual pitfall traps were preserved in 70% ethanol in the laboratory. All individuals were identified to species level using an identification key. (http://udel.edu/~mcdonald/genetics_isopod_key.pdf).

2.3. Soil moisture measurements

Soil moisture measurements were taken in August and September 2013. In August, soil samples were collected from 3 LML and 3 HML sites. At each of the 6 sites, four soil samples were taken randomly from each site using a soil auger (13 cm x 6 cm). In September of 2013, we collected soil samples from the reference, 3 LML and 3 HML sites. At each of the 7 sites, four soil samples were collected with each sample taken at a distance of 1 m from the locations of pitfall traps at the site using a soil core. Both in August and September, soil samples taken in the field were placed immediately in zip-lock bags and were later weighed in the laboratory and then were dried in an oven at $60^{o}C$ for 48 hours. Dried soil samples were weighed to estimate the total soil moisture content in each sample. Small stones and plant material from individual dried soil sample were separated using a 2 mm sieve to estimate the net amount of soil per sample. Finally, net dried soil was weighed again to estimate the percentage soil moisture content present per net amount of soil in each sample.

2.4. Data analysis

Data analysis and statistical tests in this study were performed using SPSS version 21.0 for Windows (SPSS, Chicago, IL). Before the analysis, the data were ln (x+1) transformed to account for the zero values and to improve normality for performing parametric tests. One-way ANOVA analysis with post-hoc Tukey test was performed to compare (a) differences in abundance per trap for each isopod species among reference, LML and HML sites, (b) differences in number of individuals collected per trap among reference, LML and HML sites and managed areas, and (c) percentage soil moisture differences between reference, LML and HML sites. Bivariate correlation analysis was done to analyze correlations between percentage soil moisture for each pitfall trap (estimated within 1 m distance of the trap) and number of individuals of isopod species collected from each trap at all the sites in September 2013.

Canonical correspondence analysis (CCA) was performed using CANOCO package for Windows 4.5© (Ter Braak and Šmilauer, 2002) to relate the distribution of different isopod species with different environmental factors including soil metal concentrations, metal concentrations in leaf litter (for *Populus spp.*), soil pH, soil moisture, and month of sampling.

3. Results

3.1 Isopod species sampled

A total of 10,417 individuals belonging to four different isopod species: (1) *Philoscia muscorum* (Scopoli, 1763), (2) *Armadillidium vulgare* (Latreille, 1804), (3) *Armadillidium nasatum* (Budde-Lund, 1885), and (4) *Trachelipus rathkii* (Brandt, 1833) were collected from all sites (reference, LML, HML, and managed) during all sampling periods in 2013 and 2014 (Fig. 2). Out of these four species, *A. nasatum* was only captured from the reference site and not even a single individual was collected from all of the metal contaminated sites (LML and HML sites) or in the managed areas during both periods of sampling in 2013 and 2014 (Fig. 3). Among the other three species, *P. muscorum* was the dominant species at the reference and metal contaminated sites, whereas *A. vulgare* dominated at the managed areas (Fig. 3).

a **b** **c** **d**

Fig. 2: Four isopod species: (a) *Philoscia muscorum*, (b) *Armadillidium vulgare*, (c) *Armadillidium nasatum*, and (d) *Trachelipus rathkii* collected with pitfall sampling from different sites in Liberty State Park, New Jersey.

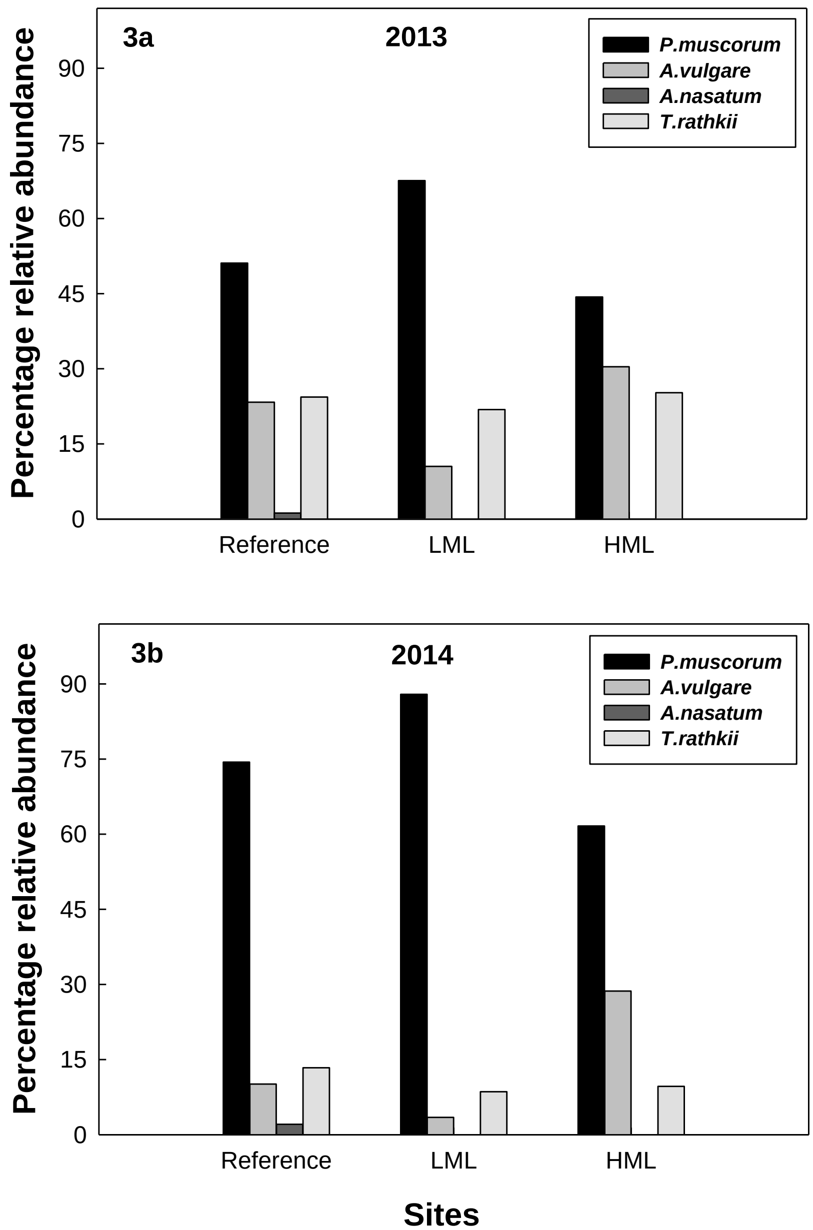


Fig. 3: Percentage relative abundance of different isopod species sampled from reference low metal load, high metal load, and managed sites during (a) 2013 and (b) 2014 (Sampling at LML, HML and reference sites was done from June to October in 2013 and from May to September in 2014. Sampling at the managed sites was done from July to September in 2014.

3.2 Abundance along metal gradient

Overall, abundance per trap of three isopod species: *P .muscorum* (in 2013: $p=0.03$, $df=2$, $F= 3.50$; in 2014: $p=0.00$, $df=2$, $F=17.290$); *A. vulgare* (in 2013: $p=0.01$, $df=2$, $F=6.496$; in 2014: $p=0.01$, $df=2$, $F=4.285$), and *T. rathkii* (in 2013: $p=0.02$, $df=2$, $F=3.910$, in 2014: $p=0.00$, $df=2$, $F=18.540$) varied significantly along the heavy metal gradient during both periods of sampling in 2013 and 2014 (Fig. 4). Abundance of *P. muscorum* showed a declining trend along the gradient (Fig. 4). The results of post hoc Tukey tests revealed that abundance levels of *P. muscorum* at the reference site were significantly higher than in the HML sites in 2013 and metal contaminated sites (LML and HML) in 2014 (Fig. 4). In case of *T. rathkii*, significantly higher ($p<0.05$) abundance levels were observed at the reference site as compared to metal contaminated sites (LML and HML) in 2013 and 2014 (Fig**.** 4).

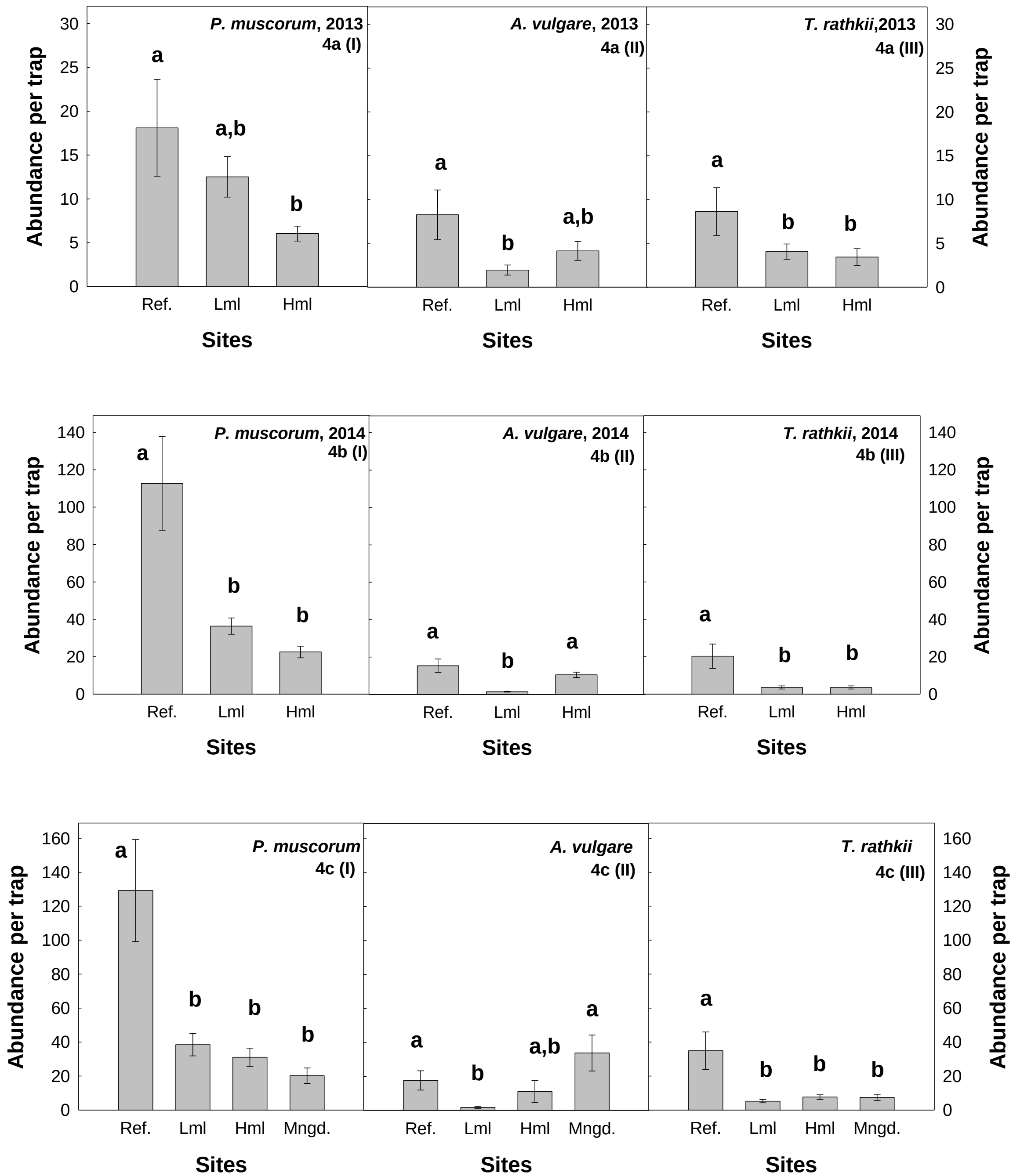


Fig 4: Average abundance (±1 SE) of *P. muscorum*, *A. vulgare*, and *T. rathkii*, sampled with pitfall traps from reference (Ref.), low metal load (Lml) and high metal load (Hml) sites and managed areas (Mngd.) during (a) 2013 (June-October), (b) 2014 (May-September), and (c) 2014 (July-September). Sampling at managed areas was done only in

2014 from July to September. Letters a, b and c indicate significant ($p \leq 0.05$) differences based on post hoc Tukey multiple comparison test.

A comparison of *A. vulgare* abundance between different sites along the metal gradient showed a different abundance pattern as compared to the other two species. During 2013 and 2014, abundance levels of *A. vulgare* at the reference site and the HML sites did not differ (Fig**.** 4). Abundance levels recorded for *A. vulgare* were not different between LML and HML sites in 2013, however, significantly higher abundance were observed at HML sites than LML sites in 2014 (Fig**.** 4).

3.3. Abundance at managed areas

Overall, abundance levels of *P. muscorum* and *T. rathkii* observed at managed areas were significantly lower than the reference site and did not differ significantly from metal contaminated sites (LML and HML). However, this was not the case for *A. vulgare* (species dominant at managed areas); its abundance levels at the managed areas were higher than the LML sites but were not significantly different from those observed at the reference site and HML sites (Fig**.** 4).

3.4. Canonical correspondence analysis

Isopod species distribution at sites along the metal gradient was evaluated with Canonical correspondence analysis (CCA plot, Fig. 5). The model was significant ($F = 19.215$, $p = 0.002$), the sum of all canonical eigenvalues was 0.217 (axis-1: 0.147, axis-2: 0.059, and axis-3: 0.011), all three canonical axes explain 31.5% of the variability in species abundance distribution. Results of CCA showed that concentrations of zinc in *Populus* *spp.* leaves, and concentrations of zinc, copper and lead in soil have positive correlations with the abundance distribution of *A. vulgare*, but negative correlations with abundance distribution of *A. nasatum* and *P. muscorum* (Fig**.** 5).

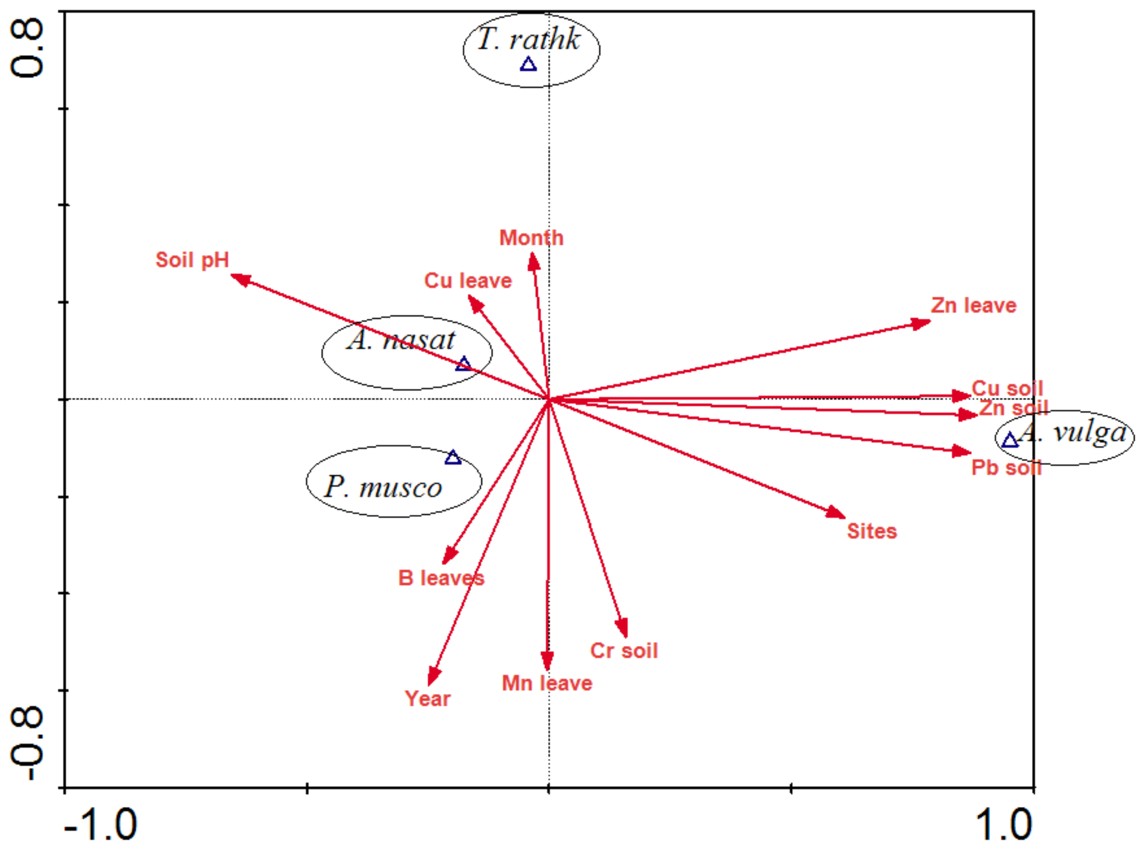


Fig. 5 Canonical correspondence analysis (CCA) plot of distribution of terrestrial isopods in relation to different variables including soil pH and metal concentrations in soil and leaves of *Populus spp.* at various sites along the metal gradient at Liberty State Park.

3.6. Percentage soil moisture

Soil moisture estimates for August 2013 showed that percentage soil moisture at HML sites was significantly higher than LML sites ($p<0.05$, two-tail t-test) sites (Fig. 6a). Soil moisture values for September varied significantly (p= 0.00, df=2, F=12.095) along the heavy metal gradient. Results of post hoc Tukey tests revealed that percentage soil moisture per net amount of soil estimated for HML sites was higher as compared to LML and reference site (N=11, p=0.00). However, soil moisture values at LML sites did not differ with the reference site (Fig. 6b).

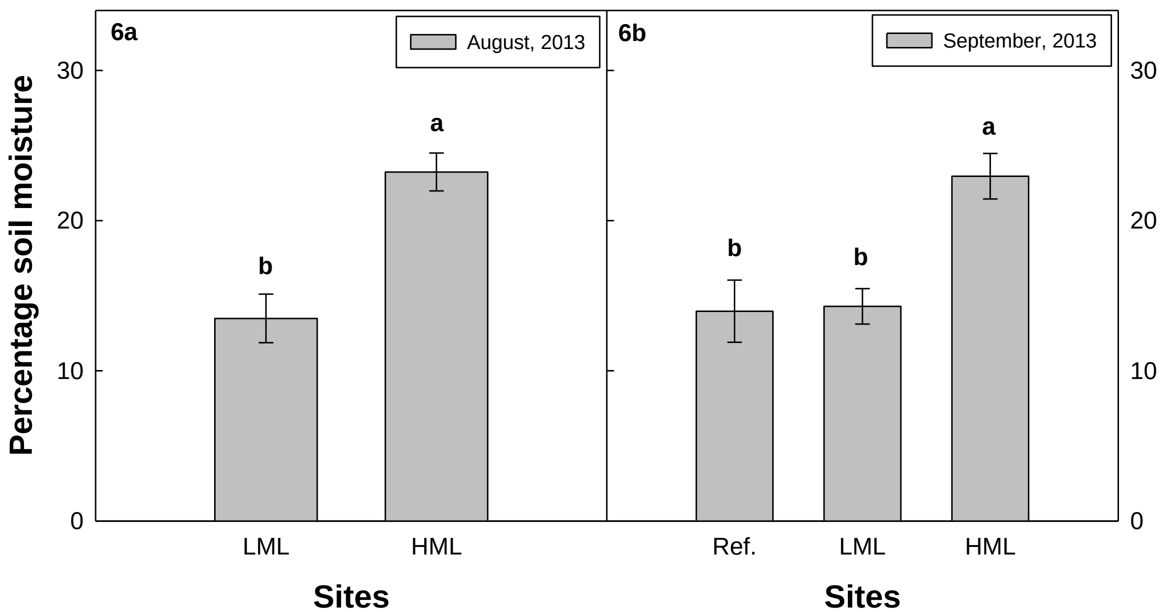


Fig. 6 (a): Average percentage soil moisture (August, 2013) estimated per net amount of soil at low metal load and high metal load sites, (b): average percentage soil moisture (September, 2013) estimated per net amount of soil at the reference low metal load (LML) and high metal load sites (HML) at Liberty State Park.

3.7. Correlation with soil moisture

No significant correlation was found between percent soil moisture (estimated by taking soil sample within 1 m distance of each trap) and the number of *P. muscorum* (p=0.34, Pearson correlation coefficient= -0.17), *A. vulgare* (p=0.47, Pearson correlation coefficient= 0.13), and *T. rathkii* (p=0.64, Pearson correlation coefficient= -0.08) individuals collected in each trap (total of 26 traps) at all the seven sites (1 reference, 3 LML and 3 HML) in September 2013.

4. Discussion

Using the pitfall traps, four terrestrial isopod species: *P. muscorum*, *A. vulgare*, *A. nasatum*, and *T. rathkii* were identified in the urban brownfield sites at LSP. All four isopod species are non-native and have been introduced to the USA from Europe and are fairly common in the Northeastern United States (Gruner 1966; Hornung and Szlávecz 2003; Jass and Klausmeier 2000; Schultz 1982; Schultz 1961). Abundance of some isopod species: *A. nasatum*, *P. muscorum*, and *T. rathkii* decreased with increasing soil metal load, whereas abundance levels for *A. vulgare* did not vary between the "clean" reference and high soil metal load sites (Fig.4). Since the effects of metal pollution on isopod abundance were not the same for the four species, results of this study partially support our first hypothesis that abundance of isopod species decreases with increasing soil metal load (Fig .4). Isopod community composition at the study site also changed along the soil metal gradient, as reference site supported relatively greater number of isopod species (all four) than the metal contaminated (LML and HML) sites (three) (Fig. 3). The results of this study indicate an inter-species variation in sensitivity to metal contamination in isopods. This species-specific response to metal contamination might be explained by: (a) differences in the physiological processes involved in metal accumulation and detoxification (Schill and Kohler 2004; Hopkin 1990; Jones and Hopkin 1996), and (b) effects of metal exposure on processes such as growth and reproduction.

4.1. Isopod abundance along the heavy metal gradient

Among the four species, *P. muscorum* was the most dominant species at all the sites along the metal gradient (Fig. 4). Abundance levels of *P. muscorum* tended to decrease along the metal gradient although these differences were not statistically significant (Fig. 4) between the metal contaminated sites (LML and HML). One explanation for this declining trend in abundance along the metal gradient could be increased mortality, especially of the older members of the population at the HML sites. This might occur due to metal toxicity resulting from exposure to higher concentrations of metals at HML sites. Many studies have demonstrated the ability of isopods to accumulate metals such as zinc, lead, and copper when exposed to contaminated litter and soils (Godet et al. 2011; Zidar et al. 2003; Donker et al. 1996; Udovic et al. 2009; Vijver et al. 2006). Metals are assimilated over the life time and are mainly stored in hepatopancreas in insoluble intracellular granules (Hopkin 1990; Hopkin and Martin 1982). Hepatopancreas expand in their volume with each molting process, which occurs more frequently in early life of isopods and is sufficient to store accumulated metals. However, the rate at which molting occurs decreases with increasing age and therefore, the capacity of hepatopancreas to store metals also becomes limited. Individuals can begin to die of metal poisoning if accumulated concentrations exceed the storage capacity (critical concentration), resulting in metals interfering with important biochemical reactions. Hopkins (1990) reported evidence for this suggestion in a laboratory study which showed presence of excessive amounts of zinc in different body organs of moribund isopods (*Oniscus asellus* and *Porcellio scaber*) individuals as compared to the healthy ones. In addition, Hopkins (1990) also reported that *P. scaber* and *O. asellus* individuals (especially older members) in populations closer to a smelting area died due to zinc poisoning as assimilated metal concentrations in hepatopancreas exceeded the storage capacity. In our study, it is possible that exposure to higher concentrations of metals at HML sites especially Zn, Cu, and Pb in soils and Zn in leaves (Fig. 5) of hardwood trees (*Betula populifolia* and *Populus spp*.; dominant at HML sites at LSP) might have adverse effects on populations of *P. muscorum*, resulting in a declining abundance trend along the metal gradient.

Another probable reason for the observed declining trend in abundance of *P. muscorum* along the metal gradient may lie in the physiological mechanism of metal detoxification. To cope up with metal stress, organisms inhabiting metal contaminated areas can evolve physiological adaptations such as intensifying detoxification mechanisms that could limit the energy allocation for processes such as reproduction or growth (Sibly and Calow 1989). Many studies have reported negative effects of metal contamination on growth and reproduction in isopods including reduced rate of juvenile production in relation to metal contamination (Calhôa et al. 2012; Godet et al. 2011; Odendaal and Reinecke 2004; Beyer and Anderson 1985; Farkas et al. 1996). In one of our studies, I also observed reduction in the growth rate of *P. muscorum* exposed to soil and poplar leaf litter collected from one of the HML sites (site 25) (Chapter 4). Growth inhibition, particularly in females can negatively affect population abundance levels due to a positive association between fecundity/natality and size of the female (Alikhan 1995; Sutton et al. 1984). In this study, it is likely that a negative impact on reproduction and/or growth is a reason for the declining abundance trend of *P. muscorum* along the metal gradient.

One of the main findings of this study was the decrease in the number of species at metal contaminated sites (LML and HML) as compared to the "clean" reference sites (Fig. 3). I observed that *A. nasatum* was not present at the metal contaminated sites (LML and HML), indicating that this species might be extremely sensitive to metal contamination as compared to the other three species. Considering the lower abundance levels of *T. rathkii* at metal contaminated sites (LML and HML) as compared to the reference site, it is also reasonable to assume that *T. rathkii* might be relatively more sensitive to metal contamination than *P. muscorum.* Increased sensitivity of *A. nasatum* and *T. rathkii* relative to *P. muscorum* might be indicative of lower critical concentration levels (concentration at or above which metal poisoning can occur) of metals accumulated in hepatopancreas in these species. Evidence for species-specific differences in sensitivity to metal contamination has been reported by Hopkins (1990b) who analyzed zinc concentrations in hepatopancreas of isopods *P. scaber* and *O. asellus* fed with metal contaminated litter collected from a smelter site. Based on the comparison in concentrations of zinc between body tissues of healthy and dead individuals of both species, Hopkins (1990b) reported that isopods died of zinc toxicity resulting from zinc concentrations exceeding the storage capacity of hepatopancreas (critical concentration). In addition, Hopkins (1990b) noted that *O. asellus* was more sensitive to zinc poisoning than *P. scaber* as critical concentrations of zinc in hepatopancreas of *O. asellus* were significantly lower than *P. scaber*. Similar results were found in field populations of both species, where concentrations levels of zinc observed in moribund individuals were higher than their respective critical concentration levels.

My results showed that abundance levels of *A. vulgare* at HML sites did not differ significantly with reference site in 2013 and 2014, and were higher as compared to LML sites in 2013 (not statistically significant) and 2014 ($p<0.05$) (Fig. 4). Clearly, *A. vulgare* appears to be the most tolerant of all four isopod species to the metal contamination (Fig. 4 and 5). Higher abundance levels of *A. vulgare* at HML sites could be related to a change in the reproductive characteristics in response to metal exposure at these sites. In contaminated environments, some species under the selection pressures for increased resistance to metal stress have been shown to evolve altered reproduction strategies (Jones and Hopkin 1996; Sibly and Calow 1989). For example, Donker et al. (1996) reported evidence of early reproduction and increased reproductive effort in populations of a terrestrial isopod *P. scaber* from a zinc smelter and a lead mine site as compared to reference sites. In an example of stress factors other than heavy metals, higher abundance of isopods was observed at sites contaminated with petrochemical wastes than reference sites which could have occurred due to an increased reproductive allocation caused by the exposure to toxicants (Faulkner and Lochmiller 2000). Studies conducted in laboratory have also reported increased reproductive stimulation in population of isopods (*O. asellus*) following the exposure to polycyclic aromatic hydrocarbons (Van Brummelen et al. 1996)**.**

4.2. Isopod abundance and other soil parameters (moisture and pH)

The average percent soil moisture levels recorded at our study sites (13-23%, Fig. 6a and 6b) lie within the preferred range and are not considered unfavorable for growth of isopods (Little 1983). Therefore, it seems unlikely that soil moisture could have accounted for the differences in isopod species abundance observed along the metal gradient. This was also evident from the results of the correlation analysis, which showed that abundance of isopods species per trap was not correlated with soil moisture.

Terrestrial isopods are also sensitive to variations in soil pH (Soejono Sastrodihardjo and Van Straalen 1993; Van Straalen and Verhoef 1997). Among the isopod species sampled in this study from different sites along the metal gradient, *P. muscorum* has been shown to have a broadly dispersed preference for pH (5.6-6.1), whereas, *A. vulgare* is a weakly alkalophilous species with preference for pH 7.0 (Van Straalen and Verhoef 1997). Since, in this study we observed lower abundance levels of *P. muscorum* at LML sites (soil pH=5.9-7.0) than reference site in 2013 (not statistically significant) and 2014 (Fig. 4), and similar abundance levels of *A. vulgare* at reference (soil pH= 6.84) and HML sites (soil pH= 4.6-5.4) in both 2013 and 2014 (Fig. 4), it is not wrong to assume that the abundance patterns for isopods observed at sites along the metal gradient cannot be explained by differences of soil pH between the study sites.

4.3. Isopod abundance at the managed areas

The pitfall trap samples in the managed area were dominated by *A. vulgare* as compared to *P. muscorum* which dominated at all the sites along the metal gradient (Fig. 3). My results indicate that management practices have a clear impact on the relative abundance of the isopods, consequently changing the dominant species in this area of the park. Moreover, managed sites also supported lower abundance levels of *P. muscorum* and *T. rathkii* than reference sites (Fig. 4), however, their abundance did not vary between managed and metal contaminated sites (Fig. 4). In contrast, abundance of *A. vulgare* did not differ significantly between “clean” reference site, HML, and managed sites (Fig. 4). The observed abundance patterns of isopod species at managed sites and the differences in their relative abundance between managed sites and sites along the metal gradient might be related to conditions of increased exposure to sunlight (due to habitat structure) at managed areas and also to the degree to which isopods species are specialized in adapting to these conditions. The managed areas sampled in this study have frequent open spaces between vegetation that might allow for direct exposure to direct sunlight and possibly lesser shaded micro-sites as compared to vegetated areas at reference and metal contaminated sites. Among the different species captured from managed areas, *A. vulgare* is better suited to deal with high temperatures than *P. musrocum* (a runner) and *T.rathkii* as it is a rolling species and has the ability to burrow through several centimeters in the soil in comparison to *P .muscorum* and *T. rathkii* whose bodies are not well adapted to burrowing (Sutton 1968). Furthermore, *A. vulgare* is also better adapted to reduce moisture losses because of well-developed pleopodal lungs as compared to the other two species *P. muscorum* and *T. rathkii* (Dias et al. 2013). Since isopods are sensitive to temperature and moisture changes, abundance patterns of different species observed at managed areas might be reflective of the physiological differences among isopods, allowing them to cope up with the different soil moisture and temperature conditions at these sites.

In summary, results of this showed that isopod assemblages at the urban brownfield site are strongly affected by metal pollution. Although, abundance patterns of isopods showed inter-species variability in relation to metal contamination, abundance of the most dominant isopod species *P. muscorum* at the brownfield site declined significantly along the metal gradient. Based on these results, it is recommended that *P. muscorum* be used as a suitable indicator for monitoring metal pollution in terrestrial ecosystems. The negative effects of metal contamination in isopods (possibly due to increased accumulation of metals) present a greater risk of metal transfer across the food chain (particularly for carnivorous invertebrates e.g. spiders and centipedes or vertebrates whose diet includes isopods) at the brownfield site. This could be particularly significant at highly polluted sites where isopods are exposed to greater concentrations of metals in the soil as well as leaf litter. In this context, studying metal accumulations by different isopod species is important to understand the risks of trophic metal transfer at the brownfield site. Compared to urban unmanaged "clean" site, management practices at the managed areas of the park seem to have a negative effect on the abundance levels of isopods (for most species). In addition, habitat modifications as a result of management practices also affected the relative abundance of different isopods in managed areas. The abundance of *A. vulgare*, which is better suited to deal with changes in temperature and moisture losses increased while abundance of *P. muscorum* and *T. rathkii* which are comparatively more sensitive to temperature and moisture changes decreased at the managed areas (Dias et al. 2013; Sutton 1968). Based on these results, it is recommended that extensive modification of the managed areas at LSP be avoided.

References

Abd El-Wakeil KF. 2015. Effects of terrestrial isopods (Crustacea: Oniscidea) on leaf litter decomposition processes. The Journal of Basic & Applied Zoology 69(0): 10-16.

Alikhan A. 1995. Terrestrial isopod biology: CRC Press.

Beyer WN, Anderson A. 1985. Toxicity to woodlice of zinc and lead oxides added to soil litter. Ambio 14(3): 173-174.

Calhôa CF, Soares AM, Loureiro S. 2012. Effects on survival and reproduction of *Porcellio dilatatus* exposed to different Cd species. Ecotoxicology 21(1): 48-55.

Caussy D, Gochfeld M, Gurzau E, Neagu C, Ruedel H. 2003. Lessons from case studies of metals: investigating exposure, bioavailability, and risk. Ecotoxicology and Environmental Safety 56(1): 45-51.

Creamer R, Rimmer D, Black H. 2008. Do elevated soil concentrations of metals affect the diversity and activity of soil invertebrates in the long-term? Soil Use and Management 24(1): 37-46.

David J-F, Handa IT. 2010. The ecology of saprophagous macroarthropods (millipedes, woodlice) in the context of global change. Biological Reviews 85(4): 881-895.

Dias AT, Krab EJ, Mariën J, Zimmer M, Cornelissen JH, Ellers J, et al. 2013. Traits underpinning desiccation resistance explain distribution patterns of terrestrial isopods. Oecologia 172(3): 667-677.

Donker MH, Raedecker MH, Van Straalen NM. 1996. The role of zinc regulation in the zinc tolerance mechanism of the terrestrial isopod *Porcellio scaber*. Journal of Applied Ecology: 955-964.

Drobne D. 1997. Terrestrial isopods—a good choice for toxicity testing of pollutants in the terrestrial environment. Environmental Toxicology and Chemistry 16(6): 1159-1164.

Drobne D, Štrus J. 1996. Moult frequency of the isopod *Porcellio scaber*, as a measure of zinc-contaminated food. Environmental Toxicology and Chemistry 15(2): 126-130

Dudka S, Ponce-Hernandez R, Tate G, Hutchinson T. 1996. Forms of Cu, Ni, and Zn in soils of Sudbury, Ontario and the metal concentrations in plants. Water, Air, and Soil Pollution 90(3-4): 531-542.

Edney E. 1951. The evaporation of water from woodlice and the millipede *Glomeris*. Journal of Experimental Biology 28(1): 91-115.

Farkas S, Hornung E, Fischer E. 1996. Toxicity of copper to *Porcellio scaber* Latr.(Isopoda) under different nutritional status. Bulletin of Environmental Contamination and Toxicology 57(4): 582-588.

Faulkner B, Lochmiller R. 2000. Increased abundance of terrestrial isopod populations in terrestrial ecosystems contaminated with petrochemical wastes. Archives of Environmental Contamination and Toxicology 39(1): 86-90.

Gallagher FJ, Pechmann I, Holzapfel C, Grabosky J. 2011. Altered vegetative assemblage trajectories within an urban brownfield. Environmental Pollution 159(5): 1159-1166.

Gallagher FJ, Pechmann I, Bogden JD, Grabosky J, Weis P. 2008a. Soil metal concentrations and vegetative assemblage structure in an urban brownfield. Environmental Pollution 153(2): 351-361.

Gallagher FJ, Pechmann I, Bogden JD, Grabosky J, Weis P. 2008b. Soil metal concentrations and productivity of *Betula populifolia* (gray birch) as measured by field spectrometry and incremental annual growth in an abandoned urban Brownfield in New Jersey. Environmental Pollution 156(3): 699-706.

Godet J-P, Demuynck S, Waterlot C, Lemière S, Souty-Grosset C, Scheifler R, et al. 2011. Growth and metal accumulation in *Porcellio scaber* exposed to poplar litter from Cd-, Pb-, and Zn-contaminated sites. Ecotoxicology and Environmental Safety 74(3): 451-458.

Gruner H. 1966. Die Tierwelt Deutschlands und der angrenzenden Meeresteile nach ihren Merkmalen und nach ihrer Lebensweise. 53. Teil. Krebstiere oder Crustacea, V. Isopoda.-2. Lieferung. Jena: Fischer Verl.

Hassall M, Edwards DP, Carmenta R, Derhé MA, Moss A. 2010. Predicting the effect of climate change on aggregation behaviour in four species of terrestrial isopods. Behaviour 147(2): 151-164.

Hopkin S. 1990. Species-specific differences in the net assimilation of zinc, cadmium, lead, copper and iron by the terrestrial isopods *Oniscus asellus* and *Porcellio scaber*. Journal of Applied Ecology 27(2): 460-474.

Hopkin S, Martin M. 1982. The distribution of zinc, cadmium, lead and copper within the woodlouse Oniscus asellus (Crustacea, Isopoda). Oecologia 54(2): 227-232

Hornung E, Szlávecz K. 2003. Establishment of a Mediterranean isopod (Chaetophiloscia sicula Verhoeff, 1908) in a North American temperate forest. Crustaceana Monographs 2: 181-189.

Jass J, Klausmeier B. 2000. Endemic and Immigrants: North American terrestrial isopods (Isopoda, Oniscidae) North of Mexico. Crustaceana 73(7): 771-799.

Jia Y, Lv Y, Kong X, Jia X, Tian K, Du J, et al. 2015. Insight into the indirect function of isopods in litter decomposition in mixed subtropical forests in China. Applied Soil Ecology 86(0): 174-181.

Jones D, Hopkin S. 1998. Reduced survival and body size in the terrestrial isopod *Porcellio scaber* from a metal-polluted environment. Environmental Pollution 99(2): 215-223.

Jones D, Hopkin S. 1996. Reproductive allocation in the terrestrial isopods *Porcellio scaber* and *Oniscus asellus* in a metal-polluted environment. Functional Ecology: 741-750.

Juang K-W, Lee D-Y, Ellsworth TR. 2001. Using rank-order geostatistics for spatial interpolation of highly skewed data in a heavy-metal contaminated site. Journal of Environmental Quality 30(3): 894-903

Köhler H-R, Hiittenrauch K, Berkus M, Gräff S, Alberti G. 1996. Cellular hepatopancreatic reactions in *Porcellio scaber* (Isopoda) as biomarkers for the evaluation of heavy metal toxicity in soils. Applied Soil Ecology 3(1): 1-15.

Little C. 1983. The Colonisation of Land: Origins and Adaptations of Terrestrial Animals. Cambridge University Press

Loureiro S, Sampaio A, Brandão A, Nogueira AJ, Soares AM. 2006. Feeding behaviour of the terrestrial isopod *Porcellionides pruinosus* Brandt, 1833 (Crustacea, Isopoda) in response to changes in food quality and contamination. Science of the Total Environment 369(1): 119-128.

Mazzei V, Longo G, Brundo M, Copat C, Conti GO, Ferrante M. 2013. Effects of heavy metal accumulation on some reproductive characters in Armadillidium granulatum Brandt (Crustacea, Isopoda, Oniscidea). Ecotoxicology and Environmental Safety 98: 66-73.

Mazzei V, Longo G, Brundo MV, Sinatra F, Copat C, Oliveri Conti G, et al. 2014. Bioaccumulation of cadmium and lead and its effects on hepatopancreas morphology in three terrestrial isopod crustacean species. Ecotoxicology and Environmental Safety 110: 269-279.

McGrath D, Zhang C, Carton OT. 2004. Geostatistical analyses and hazard assessment on soil lead in Silvermines area, Ireland. Environmental Pollution 127(2): 239-248.

Noemí Mazía C, Chaneton EJ, Kitzberger T. 2006. Small-scale habitat use and assemblage structure of ground-dwelling beetles in a Patagonian shrub steppe. Journal of Arid Environments 67(2): 177-194.

Noll MR. 2003. Trace elements in terrestrial environments: biogeochemistry, bioavailability, and risks of metals. Journal of Environmental Quality 32(1): 374.

Odendaal J, Reinecke A. 2004. Effect of metal mixtures (Cd and Zn) on body weight in terrestrial isopods. Archives of Environmental Contamination and Toxicology 46(3): 377-384.

Pedersen MB, Strandberg B, Jensen J, Attrill MJ. 1999. The impact of a copper gradient on a microarthropod field community. Ecotoxicology 8(6): 467-483.

Pekár S. 2002. Differential effects of formaldehyde concentration and detergent on the catching efficiency of surface active arthropods by pitfall traps. Pedobiologia 46(6): 539-547.

Peters EL, Kaufmann-Daszczuk B, Brant HA, Jagoe CH, Richter R. 2001. Element concentrations and fluctuating asymmetry in a terrestrial isopod (*Armadillidium vulgare*) in metropolitan Chicago Parklands. Department of Biological Sciences, Chicago State University: 1p.

Phillips ID, Cobb TP. 2005. Effects of Habitat Structure and Lid Transparency on Pitfall Catches.

Pouyat RV, Szlavecz K, Yesilonis ID, Wong CP, Murawski L, Marra P, et al. 2015. Multi-scale assessment of metal contamination in residential soil and soil fauna: A case study in the Baltimore–Washington metropolitan region, USA. Landscape and Urban Planning 142: 7-17.

Santos SAP, Cabanas JE, Pereira JA. 2007. Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type. European Journal of Soil Biology 43(2): 77-83.

Santos SAP, Cabanas JE, Pereira JA. 2007. Abundance and diversity of soil arthropods in olive grove ecosystem (Portugal): Effect of pitfall trap type. European Journal of Soil Biology 43(2): 77-83.

Schill RO, Kohler H-R. 2004. Energy reserves and metal-storage granules in the hepatopancreas of *Oniscus asellus* and *Porcellio scaber* (Isopoda) from a metal gradient at Avonmouth, UK. Ecotoxicology 13(8): 787-796.

Schultz G. 1982. Terrestrial isopods (Crustacea: Isopoda: Oniscoidea) from North Carolina. Brimleyana 8: 1-26.

Schultz GA. 1961. Distribution and establishment of a land isopod in North America. Systematic Zoology 10(4): 193-196.

Sibly R, Calow P. 1989. A life cycle theory of responses to stress. Biological Journal of the Linnean Society 37(1-2): 101-116.

Soejono Sastrodihardjo F, Van Straalen N. 1993. Behaviour of five isopod species in standardized tests for pH preference. European Journal of Soil Biology 29(3-4): 127-131.

Standen V. 2000. The adequacy of collecting techniques for estimating species richness of grassland invertebrates. Journal of Applied Ecology 37(5): 884-893.

Sutton SL. 1968. The Population Dynamics of *Trichoniscus pusillus* and *Philoscia muscorum* (Crustacea, Oniscoidea) in Limestone Grassland. Journal of Animal Ecology 37(2): 425-444.

Sutton SL, Hassal MW, R., David RC, Grund A, Sunderland KD. 1984. Life histories of terrestrial isopods: a study of intra- and interspecific variation. Symp Zool Soc Lond 53: 269–294.

Ter Braak CJ, Smilauer P. 2002. CANOCO reference manual and CanoDraw for Windows user's guide: software for canonical community ordination (version 4.5).www. canoco. com.

Thums CR, Farago ME, Thornton I. 2008. Bioavailability of trace metals in brownfield soils in an urban area in the UK. Environmental Geochemistry and Health 30(6): 549-563.

Udovic M, Drobne D, Lestan D. 2009. Bioaccumulation in *Porcellio scaber* (Crustacea, Isopoda) as a measure of the EDTA remediation efficiency of metal-polluted soil. Environmental Pollution 157(10): 2822-2829.

Van Brummelen TC, Van Gestel CA, Verweij RA. 1996. Long-term toxicity of five polycyclic aromatic hydrocarbons for the terrestrial isopods *Oniscus Asellus* and *Porcellio Scaber*. Environmental Toxicology and Chemistry 15(7): 1199-1210.

Van Straalen NM, Verhoef HA. 1997. The development of a bioindicator system for soil acidity based on arthropod pH preferences. Journal of Applied Ecology: 217-232.

Vijver MG, Vink JP, Jager T, Van Straalen NM, Wolterbeek HT, Van Gestel CA. 2006. Kinetics of Zn and Cd accumulation in the isopod Porcellio scaber exposed to contaminated soil and/or food. Soil Biology and Biochemistry 38(7): 1554-1563.

Vilisics F, Elek Z, Lövei GL, Hornung E. 2007. Composition of terrestrial isopod assemblages along an urbanisation gradient in Denmark. Pedobiologia 51(1): 45-53.

Waloff N. 1941. The mechanisms of humidity reactions of terrestrial isopods. Jour Exp Biol 18: 8-135.

Witzel B. 1998. Uptake, storage and loss of cadmium and lead in the woodlouse *Porcellio scaber* (Crustacea, Isopoda). Water, Air, and Soil Pollution 108(1-2): 51-68.

Wu J, Norvell W, Welch R. 2006. Kriging on highly skewed data for DTPA-extractable soil Zn with auxiliary information for pH and organic carbon. Geoderma 134(1): 187-199.

Zidar P, Božič J, Štrus J. 2005. Behavioral response in the terrestrial isopod Porcellio scaber (Crustacea) offered a choice of uncontaminated and cadmium-contaminated food. Ecotoxicology 14(5): 493-502.

Zidar P, Drobne D, Štrus J, Van Gestel C, Donker M. 2004. Food selection as a means of Cu intake reduction in the terrestrial isopod *Porcellio scaber* (Crustacea, Isopoda). Applied Soil Ecology 25(3): 257-265.

Zidar P, Drobne D, Štrus J, Blejec A. 2003. Intake and assimilation of zinc, copper, and cadmium in the terrestrial isopod *Porcellio scaber* Latr.(Crustacea, Isopoda). Bulletin of Environmental Contamination and Toxicology 70(5): 1028-1035.

Zimmer M. 2002. Nutrition in terrestrial isopods (Isopoda: Oniscidea): An evolutionary-ecological approach. Biological Reviews of the Cambridge Philosophical Society 77(4): 455-493.

Žnidaršič N, Štrus J, Drobne D. 2003. Ultrastructural alterations of the hepatopancreas in *Porcellio scaber* under stress. Environmental Toxicology and Pharmacology 13(3): 161-174

Chapter Four: Exposure to heavy metal stress does not increase fluctuating asymmetry in population of isopod and hardwood trees[4]

Abstract

Fluctuating asymmetry (FA) refers to random, small and non-directional deviations from ideal bilateral symmetry is proposed as a bio-indicator of abiotic stress in both animals and plants. We investigated the effect of heavy metal stress on FA levels of morphological traits in a terrestrial isopod (*Philoscia muscorum*) as well as in the leaves of two hardwood tree species: Gray Birch (*Betula populifolia*) and Eastern Cottonwood (*Populus deltoides*), in an urban brownfield in New Jersey. FA levels measured for five traits (length of two segments of antennae and three segments of the seventh legs) were compared in male and female populations of *P. muscorum* sampled from three low and three high soil metal load sites within the brownfield. FA levels measured for leaf width (perpendicular distance from a midpoint on midrib to the widest point of the lamina in right and left half in a leaf) were compared for both Gray Birch and Eastern Cottonwood leaves collected from the same low and high soil metal load sites. Contrary to the hypothesis that FA increases with higher heavy metal stress in isopods and trees, our results revealed that true asymmetry in Gray Birch and for some isopod traits (2nd antenna article, 3rd antenna article and merus of males and females, carpus and prodopus

[4]Wadhwa, S., Gallagher, F. J., Rodriguez-Saona, C., and Holzapfel, C. 2017. Exposure to heavy metal stress does not increase fluctuating asymmetry in populations of isopod and hardwood trees. Ecological Indicators, 76, 42-51.

in females) did not differ between low and high metal contaminated sites. Furthermore, FAs measured in Eastern Cottonwood leaves and other isopod traits (carpus and prodopus in males) were found to be even lower at high metal contaminated sites than the low metal load sites. The overall effect of metal stress was shown as reduction in growth (measured as body size for a given head width in an individual) of isopods at high metal load sites as compared to the low metal load sites. Various hypotheses including induction of detoxification mechanisms in response to metal stress, selection against individuals with presumably lower fitness (high FA), difference in sensitivity of traits to stress, and plasticity are discussed to explain the observed lack of a significant association between FA and heavy metal stress in isopods and trees.

Keywords : *Philoscia muscorum*, *Betula populifolia, Populus deltoides*, urban brownfield, metal contamination, fluctuating asymmetry

1. Introduction

Soil contamination with metals is a widespread environmental problem in urban and post-industrial regions throughout the world (Alloway 1995; Mireles et al. 2012; Yaylalı-Abanuz 2011). Metals such as zinc, lead, copper, arsenic, are often present in urban soils at concentrations greater than background and regulatory levels (Ge et al. 2000). Industrial activities, energy production, construction activities, traffic and vehicular emissions, and municipal wastes are main sources of heavy metals in urban soils (Wei and Yang 2010; Wong et al. 2006). Most of these metals persist in the soils for long time as they are not biodegradable. Therefore they have the potential to alter the physical, chemical and biological properties of the soils (Friedlova 2010; Pouyat et al. 2010) ultimately having adverse effects on urban ecosystems. Considering the growth of urban landscapes, their complexity and input of metals from a variety of sources; monitoring the impacts of metal induced stress on urban ecological health is an increasing challenge for biologists and environmental scientists (Li et al. 2013).

To assess the effect of metal induced stress on ecosystem health, previous studies have focused on quantifying metal concentrations in soils or in tissues of various plants and animals (Ge et al. 2000; Gallagher et al. 2008a; Manta et al. 2002; Sawidis et al. 2011; Tomašević et al. 2004), and on examining changes in species richness or diversity of different taxa of plants and animals (Fountain and Hopkin 2004; Murray et al. 2000). Most of the methods employed in these studies are often quite expensive, invasive, and time consuming. Over recent years, examining fluctuating asymmetry (FA) in populations of plants and animals has been proposed as an attractive, inexpensive, yet efficient, non-invasive and reliable method for monitoring heavy metal stress (Beasley et al. 2013; Lazić et al. 2013). FA refers to the small, non-directional deviations from ideal bilateral symmetry of morphological traits in organisms (Valen 1962). High levels of FA in a population is considered as an indication of increased developmental instability (DI); the inability of an individual to buffer development against environmental or genetic disturbances to produce a symmetric form (Lerner 1954; Waddington 1942). The underlying premise is that under increased levels of stress, organisms struggle to maintain necessary levels of developmental precision resulting in a more reliable relationship between FA and DI (Sommer 1996). Numerous studies have shown that abiotic stress such as increased heavy metal contamination, chemical pollution, increased human disturbances (e.g., habitat fragmentation), and changes in microclimate (Lazić et al. 2013; Chang et al. 2007; Helle et al. 2011; Kozlov et al. 1996; Mal et al. 2002; Vilisics et al.

2005) can disrupt developmental processes that in turn may result in increase of FA in both plants and animals.

The objective of this study is to quantify effects of heavy metal soil contamination on FA in populations at two distinct trophic levels, that of a terrestrial isopod: *Philoscia muscorum* (common striped woodlice) and two hardwood trees species: *Betula populifolia* (Gray Birch) and *Populus deltoides* (Eastern Cottonwood). In metal contaminated environments, plants take up metals from the soils and store it in various tissues including leaves (Castiglione et al. 2009; Gallagher et al. 2008b). Eventually, metals can be transferred to invertebrates directly through feeding on contaminated leaf litter (e.g. isopods) or indirectly via different tropic interactions resulting in accumulation of heavy metals in their body (Jelaska et al. 2007; Peterson et al. 2003). Studies have shown that metal accumulation at higher concentrations than required can negatively impact growth, reproduction, physiology, or body symmetry (Mal et al. 2002; Ambo-Rappe 2011; Arena et al. 2013; Di Baccio et al. 2003; Todeschini et al. 2011) in both plants and invertebrates. Since metal stress can have detrimental effects on different components of an ecosystem including producers and decomposers, in this study we expect to find a positive relationship between FA and metal stress in case of both hardwood trees (primary producers) and isopods (decomposers). In this study, we examined FA: (a) in five different traits (length of two segments of antennae and three segments of seventh leg) in males and female isopod populations, and (b) for leaf width in Gray Birch and Eastern Cottonwood collected from low and high soil metal load sites at post-industrial landscapes preserved in Liberty State Park (LSP), Jersey City, New Jersey (USA). In addition to FA, we also compared the growth rate (ratio of body size to head width) of isopods between low and high metal load sites. We tested the hypothesis that symmetry of: (a) morphological traits in isopods and (b) leaves of Gray Birch and Eastern Cottonwood increases with heavy metal pollution.

2. Material and methods

2.1. Study site

The study was conducted in Liberty State Park (LSP), Jersey City, New Jersey on the west bank of Upper New York Bay (centered at 40^{o} 42' 14" N and 74^{o} 03' 14" W). The area originally was an intertidal mudflat before it was filled in the 1800's with debris from construction projects and refuse from New York City region. Developed by Central Rail Road of New Jersey (CRRNJ), the area was used as a rail yard and for the transport and storage of coal and other goods. After CRRNJ discontinued its operations in 1967, the State of New Jersey purchased this area to develop it as a state park. While most of the area was capped with clean soil, the central area of the park, approximately 41 ha was fenced off and left isolated and undisturbed that serves as our study site. Over the last five decades, the site has been reclaimed by spontaneous vegetation succession which includes dominant hardwood trees (*Betula populifolia, Populus deltoides*), shrubs (*Rhus typhina, R. glabra*, and *R. copallinum*) and many native and non-native forbs and grasses (*Solidago spp*., Eupatorium spp., *Artemisia vulgaris, etc.*) (Gallagher et al. 2011). Due to its industrial history, soils at the site are unevenly polluted with metal contaminants including Arsenic (As), Chromium (Cr), Copper (Cu), Lead (Pb), Vanadium (V), and Zinc (Zn) (Gallagher et al. 2008a). Based on the concentrations of these metals in soil, Gallagher et al. (2008a) developed a total metal load index (TML) index. The TML represented a rank order transformation of the normalized soil metal concentration and has a range from 0 (low) to 5 (high). The scale is used as a relative index to compare soil contamination levels between different sites (see Fig. 1 in Gallagher et al. 2008b). Previous work at LSP has shown that plant productivity and growth are negatively impacted by the heavy metal stress at sites with a threshold TML level of 3.0 and above (Gallagher et al. 2008a; Dahle et al. 2014; Renninger et al. 2013). It has been shown that the metal loads also had an influence on the assemblage and trajectory of the vegetation, with hardwood trees (Gray Birch and Eastern Cottonwood) being more dominant at sites with higher soil metal load (Gallagher et al. 2008b; Gallagher et al. 2011) (see Fig.1 in Gallagher et al. 2008b). For the purpose of this study, we selected three low metal load (LML) sites (with TML <3.0, LML-1: site 48, LML-2: site 41, LML-3: site 47) and three high metal load (HML) sites (TML> 3.0, HML-1: site 10, HML-2: site 14/16, HML-3: site 25) (Fig.1). The soil metal load levels and concentrations of heavy metals at 3 LML and 3 HML sites are shown in Fig. 2.

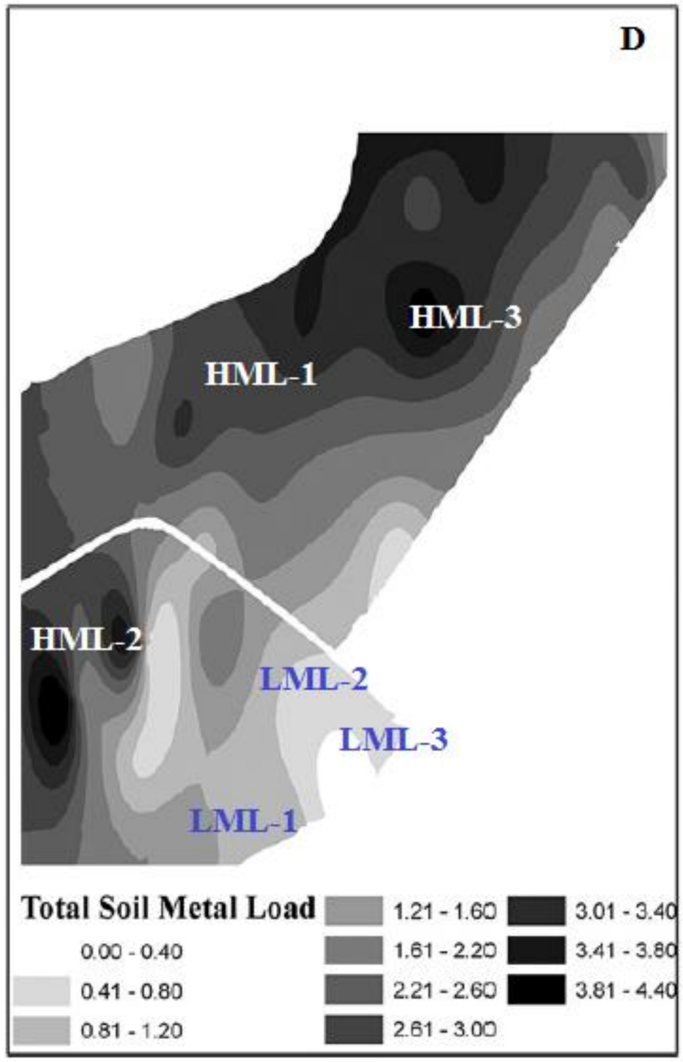


Fig. 1: The study site (A) in Liberty State Park on the west bank of Upper New York Bay (centered at 40°42'14"N; 74°03'14"W), (B) The Statue of Liberty, (C) Ellis Island, and (D) location and Total soil metal load (TML) of all the six sites (3 low meal load [blue] and 3 high metal load sites [red]) within Liberty State Park (from Gallagher et al. 2011).

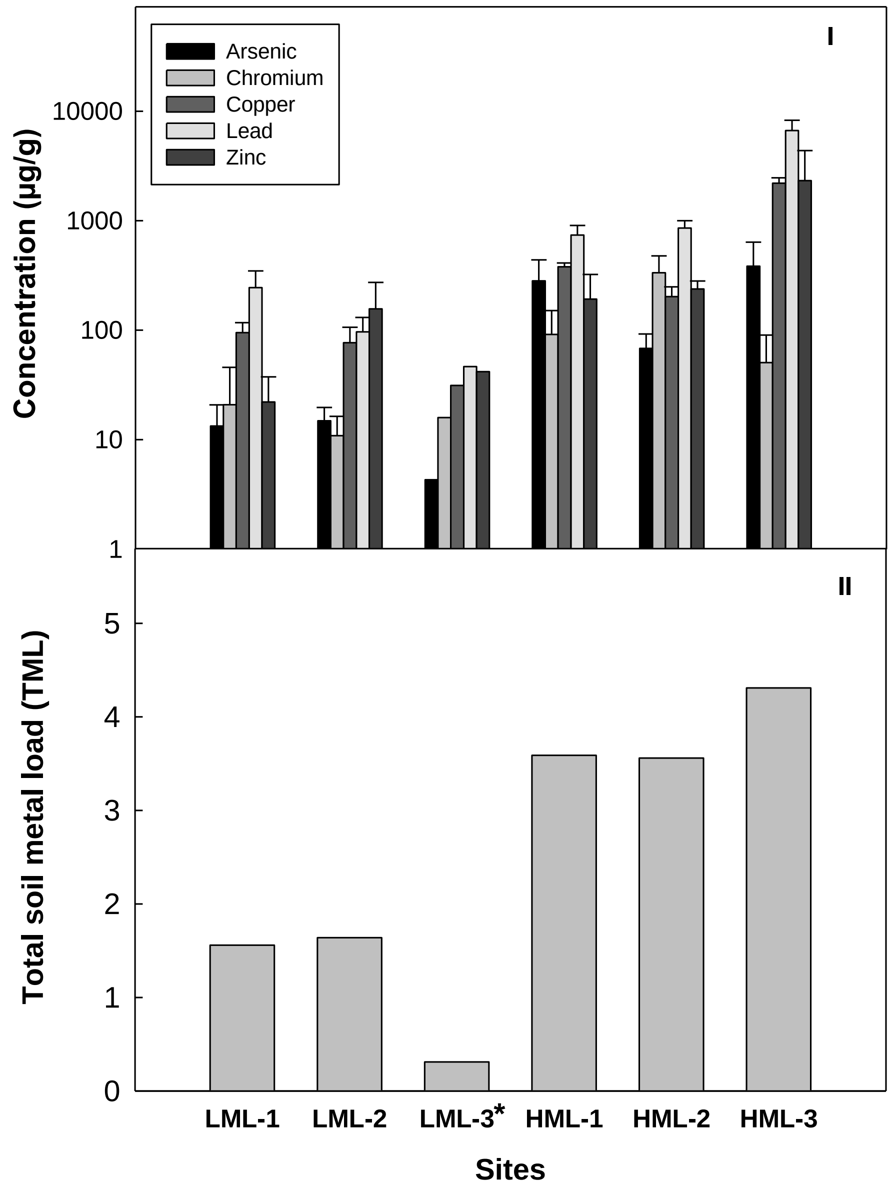


Fig. 2: (I) Soil metal concentrations (average value with standard deviation, x-axis is in log scale), and (II) total soil metal load for low metal load (LML) and high metal load sites (HML) study sites

Soil metals µg/g (2005)

* Sampled in 1995, one sample was analyzed from this site.

2.2. Choice of organisms and data acquisition

Terrestrial isopods (Isopoda: *Oniscidea* sp.) were selected for the FA analysis as they are one of the most abundant soil dwelling arthropods (David and Handa 2010). They have remarkable ability to accumulate large amounts of heavy metals in their body (in hepatopancreas) (Gál et al. 2008) and play an important role in the litter decomposition process. Based on pitfall sampling, we found that *Philoscia muscorum* was the most dominant of all the isopods species at our study sites. The species is common in the Mid-Atlantic region of the United States and primarily feeds on the decaying leaf litter (Hassall and Jennings 1975). Isopods were sampled using pitfall traps during June-July of 2013 and May of 2014. For FA analysis, 18-25 male and 19-26 female individuals were selected from each of the six study sites (for a total of 279 individuals, 141 male and 138 female individuals from six sites). Sampled isopods were individually stored in polypropylene vials with 3 ml of 70% ethanol in the laboratory. For FA analysis, five traits were measured: (1) length of two antennae segments (Fig. 3a): article 2 and article 3, and (2) length of three segments of seventh leg (pereiopod) merus, carpus and prodopus from the collected specimens (Fig. 3b). As reported by previous studies (Villisics et al. 2005), these traits were selected as they are not difficult to measure and also the measurements are repeatable. In addition, to the two antennae and three pereipodal segments, head width of each individual was also measured to correct for the size dependence. To examine the impact of metal contamination of soils on the growth rate of isopods, maximum body length (length from middle point on the head to pleotelson) of randomly selected isopods from LML (N=54) and HML (N=62) sites was recorded. To compare the growth rate of isopods between LML and HML sites, growth rate of each individual was estimated as the ratio of body size to the head width (or body size for a given age, using head width as a proxy for age (Donker et al 1993; Jones and Hopkin, 1998). For all the *P. muscorum* individuals, images of the selected antennae and pereopodal traits (right and left sides) were taken with Infinity-1 digital camera connected with a NIKON - SMZ745T stereomicroscope (Nikon Europe, Düsseldorf, Germany). Selected traits were measured using Image J morphometrical software (http://imagej.nih.gov/ij/).

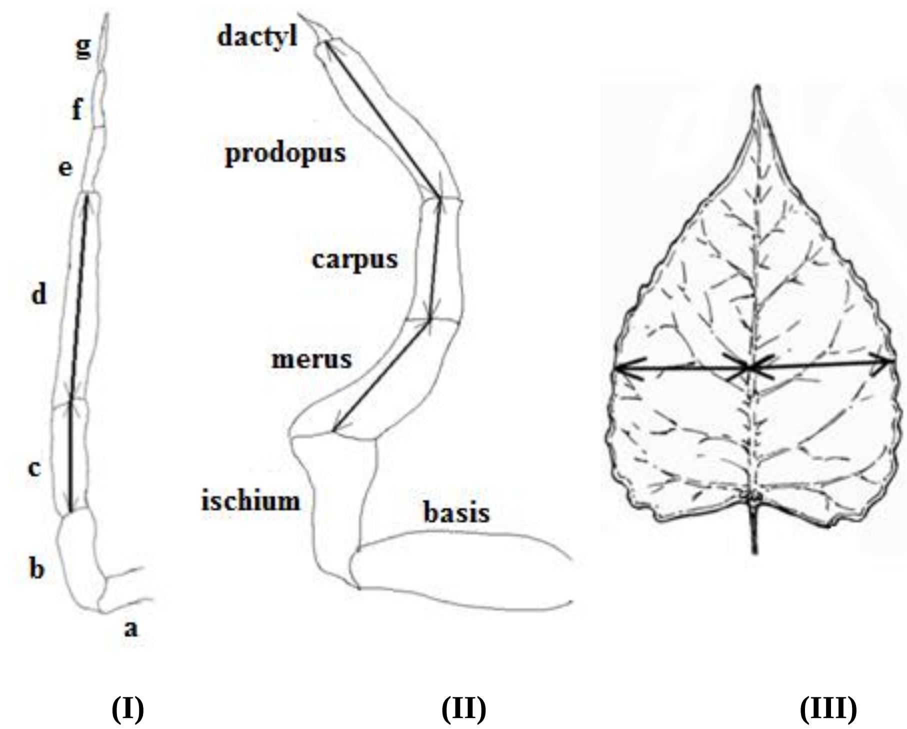


Fig. 3 (I) *Philoscia muscorum* antenna segments (a: basis; b: article 1; c: article 2; d: article 3; e: flagellum 1; f: flagellum 2; g: flagellum 3), (II) *P.m.* pereiopod segments (, and (III) leaf-width in right and left half of an Eastern Cottonwood leaf. Lines in the antennal segments (c and d) and pereipodal segments and in the leaf indicate the measured lengths of selected segments**.**

Leaves of Gray Birch (*Betula populifolia*) and Eastern Cottonwood (*Populus deltoides)*, the dominant hardwood trees at the study sites, were selected for FA analysis. Previous work at these sites has shown that these two trees tend to accumulate heavy metals in plant tissues, specifically zinc in leaves (Gallagher et al. 2008b). In addition, long term growth rate of both Gray Birch and Eastern Cottonwood is negatively impacted by heavy metal stress at HML sites as compared to LML sites (Dahle et al. 2014; Renninger et al. 2013). For the FA analysis, we collected 92-105 leaves of Gray Birch and Eastern Cottonwood respectively from each of three LML and three HML sites at LSP in May 2014. At each site, leaves were collected randomly from six-seven trees (Gray Birch was absent at 2 sites: site 10, HML1 and site 47, LML3). In the laboratory, leaves were pressed in a herbarium press and then scanned at 800 dpi with a scanner (EPSON perfection V30, Seiko Epson Inc., Long Beach, CA, USA). For FA analysis, leaf width of the right (R) and left sides (L) was measured for each sampled leaf using Image J morphometrical software (http://imagej.nih.gov/ij/). Leaf width on either side (right or left) of a leaf is defined as the perpendicular distance from a midpoint on midrib (mid-point between the base and the tip) to the leaf margin on that side (right or left) (Fig. 3c).

2.3. Data analysis

For all the traits examined in isopods and trees, asymmetry index (A.I) was calculated as the right minus left side value of the trait. In order to assess if variation between sides is greater than differences due to measurement error (ME), two-way ANOVA (as recommended by Palmer and Strobeck, 1986) was performed for each trait, gender and site. This analysis also tests the presence of directional asymmetry (DA), non-directional asymmetry (refers to FA or antisymmetry), and differences due to size/shape within the individuals. In addition to two-way ANOVA, DA was also evaluated using one sample T-test against a mean of zero. Results of two-way ANOVA tests showed significantly higher variation for MS_{SI} (mean squares of the side X individuals) than MS_{ME} (mean squares of the measurement error) ($p < 0.05$) for all tests performed (isopods and leaves). In addition, no significant differences between sides were observed indicating absence of DA ($p>0.05$). Results of the one-sample t-test performed for each trait, gender and site also revealed absence of DA ($p> 0.05$) in almost all the tests realized (for isopods and leaves) except for only 2 out of 60 tests (2nd antenna article and carpus in females from LML-1) and 2 out of 10 tests (Eastern Cottonwood from HML-2 and Gray Birch from LML-2) in isopods and tree species respectively.

With regards to antisymmetry, we found some of the FA indices (3 out of 60 ANOVA tests performed for each trait, site and gender) were influenced by antisymmetry. It is important to consider that if a trait shows presence of antisymmetry, some part of this between side variation might have a genetic component, therefore, these between side differences might not be necessarily attributable to developmental noise (Palmer and Strobeck, 1992). As a result, deviations of A.I values from normality were also checked with Kolmogorov–Smirnov test for each trait, gender and site**.** Normal distribution of right minus left (R-L) value differences ($p> 0.05$) were observed for all traits in both the data sets (isopods and tree leaves).

To test for the size dependence of FA in the data, regression analysis was performed between |R−L| (absolute value of right minus left trait value) and width of the head capsule (a measure of body size) for each trait, gender and site (method following

Palmer 1996). Similarly, regression was tested between leaf width and |R−L| (absolute value of right side width minus left side width value). Since significant positive relationships were found in 18 out of the 60 regression tests performed for the isopod data and 7 out of 10 tests performed for leaves data, FA indexes were calculated with trait size correction as: $FA = |R-L| / ((R + L)/2)$ following Palmer and Strobeck (1986).

FA values measured for all the traits were analyzed in two different ways. Firstly, FA values estimated for each trait were compared separately for male and female isopods between all the six sites using one-way ANOVA with post-hoc Tukey test. Similar analysis was performed to compare FA levels for leaf width in Gray Birch and Eastern Cottonwood among all sites. For the second approach, trait specific FA values were grouped together from LML and HML sites for male and female isopod populations and Gray Birch and Eastern Cottonwood leaves. One-way-ANOVA with post-hoc Tukey tests was performed to compare the differences among male and female populations from LML and HML sites. To determine differences in growth rate (ratio of body size to head width) and head width of isopods between LML and HML sites (combined data) two tail t- tests were performed.

Finally, to test the differences in FA in *P. muscorum* populations, a general linear model analysis (GLM) was performed between genders (male and female) and total soil metal loads (HML and LML) with gender and total soil metal load as fixed factors and traits as random factors. Similar GLM analysis was performed for leaf width between tree species and soil metal loads. Tests for normality, two tail and one sample t-tests, analysis of variance (ANOVA) with Tukey tests, and GLM analysis were performed on the data sets using SPSS version 21.0 for Windows (SPSS, Chicago, IL).

3. Results

3.1. Fluctuating asymmetry in *P. muscorum* populations in relation to metal load

Overall there was no significant effect of metal load on the magnitude of FA when all traits are considered (Table 1), however as indicated by the significant three-way interaction revealed in the GLM analysis, FA for some traits varied in relationship to both metal load and gender. Accordingly, when individual traits were compared for male and female populations, only few FA values differed between metal loads (Fig. 5). These included FA measurements for the pereiopodal segment, carpus of males from HML sites that were significantly lower than both males and females from LML sites (Fig. 5). FA levels measured for prodopus in males from LML sites was also significantly greater than males from HML sites (Fig. 5) ($p<0.05$).

Comparing the individual traits in male populations across all six sites, the differences that were found for males were the FA values estimated for carpus in individuals from site LML-1 that were higher than individuals from sites HML-1, HML-2 and HML-3, respectively ($p<0.05$, Fig. 4). In addition, FA values observed for prodopus were significantly higher in individuals from site LML-2 than individuals from sites HML-1, HML-2 and HML-3, respectively ($p<0.05$, Fig. 4).

For females in the populations of *P. muscorum*, FA measured for the 2nd article of antenna, merus, carpus, and prodopus did not differ ($p>0.05$, Fig. 4) between all of the six sites (3 LML and 3 HML sites). In case of the 3rd antenna article, FA values estimated in females from site LML-1 were significantly greater than individuals from site HML-2 ($p<0.05$, Fig. 4).

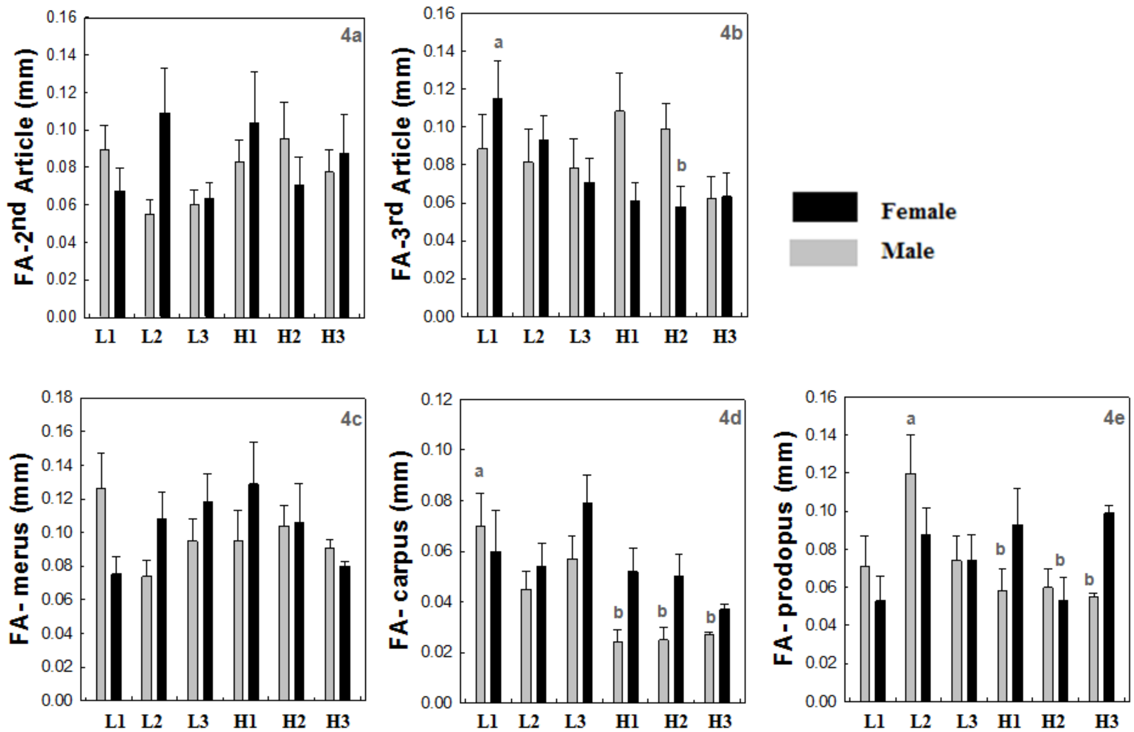


Fig. 4: Fluctuating asymmetry values (mean ± SE) observed for different traits: (a) 2nd and the (b) 3rd articles of antenna and merus (c), carpus (d) and propodus (e) of the 7th pereiopods in *P. muscorum* collected from the three low metal (L1, L2, and L3) and the three high metal load (H1, H2 and H3) sites in brownfields at Liberty State Park. Different letters indicate significant differences ($P < 0.05$) between sites tested by one-way ANOVA with post hoc Tukey for each trait among males and females respectively from all six sites

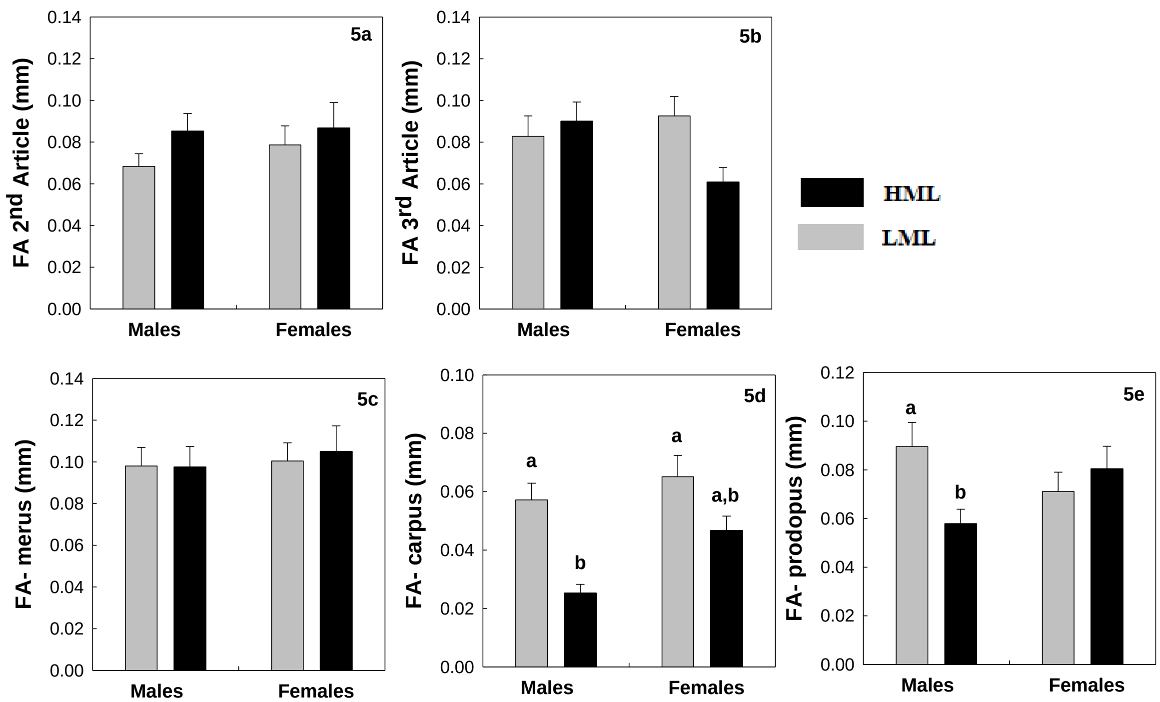
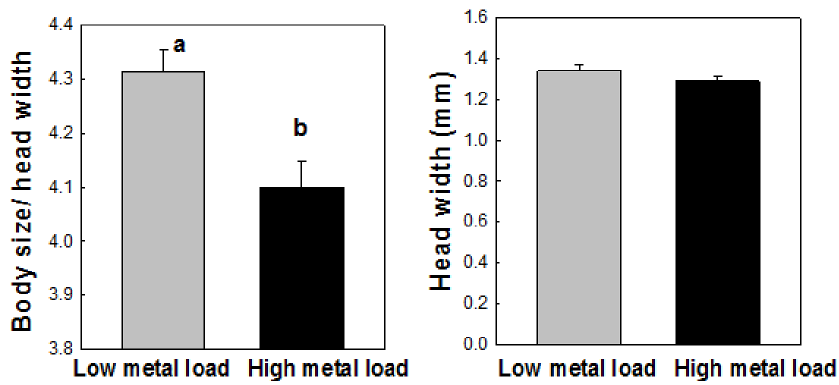


Fig. 5 Fluctuating asymmetry values (mean ±SE;) values observed for different traits: (a) 2nd and the (b) 3rd articles of antenna, and (c) merus, (d) carpus and (e) propodus of the 7th pereiopods in *P. muscorum* collected from brownfield sites at Liberty State Park and grouped by habitat type (low metal load and high metal load) and by gender (male and female). Different letters indicate significant differences (P < 0.05, one-way ANOVA, post-hoc Tukey tests) between different groups.

3.2. Head width to body length ratio analysis in population of *P.muscorum*

The ratio of body size to head width was significantly higher in isopod populations collected from LML sites than from HML sites (Fig. 6a). The results showed that growth (measured as ratio of body size and head width in an individual) of isopods is significantly reduced at HML sites as compared to LML sites (p<0.05, two-tail t-test). However, the head-width in the isopod populations from low metal load and high metal load sites were not significantly different from each other (p >0.05 two tail t-test) (Fig. 6b).



(a) (b)

Fig. 6 (a) Head width to body length ratio in *P. muscorum* individuals collected from low metal load (N=54) and high metal load sites (N=62) (b) Head width of *P. muscorum* individuals collected from low metal load (N=54) and high metal load sites (N=62). Different letters indicate the significant differences (P < 0.05, two-tailed t-test) between isopod populations from low metal and high metal load sites.

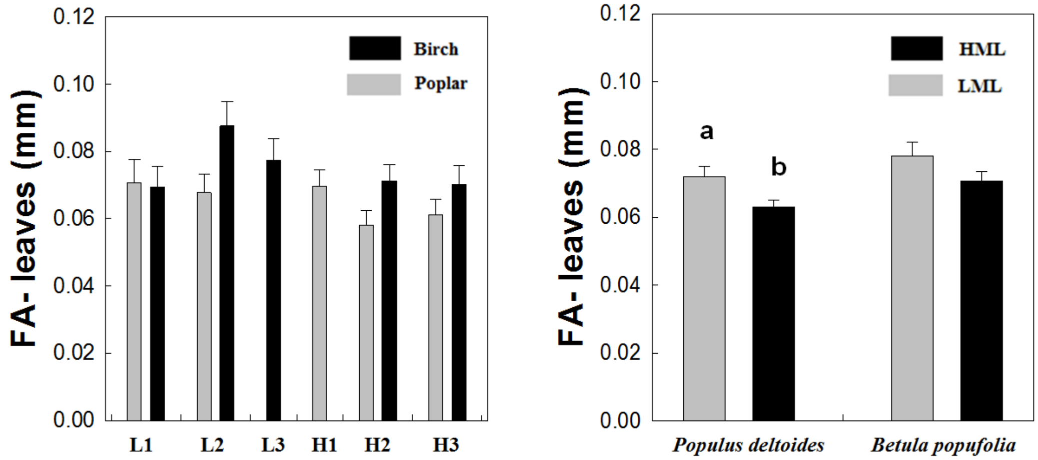
Table 1: Results of the GLM (general linear model) analysis of fluctuating asymmetry among 5 traits (article c and article d of antenna and merus, carpus and prodopus of the 7th pairs of pereipods) observed for *P.muscorum* between genders (male and female) and between two habitats (low metal load and high metal load).

Source of Variation	Df	MS	F-test
Intercept	1	7.995	85.825*
Habitats (H, fixed)	1	0.016	1.103
Gender (G, fixed)	1	0.004	0.829
Traits (T, random)	4	0.093	18.464
G x H	1	0.001	0.350
G x T	4	0.005	0.364
H x T	4	0.014	0.984
H x G x T	4	0.014	2.922*
Error	1330	0.005	

*****Significant differences: $p < 0.05$

3.3. Fluctuating asymmetry in leaves of Gray Birch and Eastern Cottonwood

Results of the one way ANOVA analysis with Tukey post hoc tests revealed no significant differences between FA values calculated in leaves of Gray Birch and Eastern Cottonwood between at the six sites (Fig. 7a) ($p > 0.05$). However, for the data combined on basis of soil metal load (HML and LML), FA was higher in Eastern Cottonwood leaves from HML than LML sites ($p < 0.05$, two-tail t-test) (Fig. 7b). No differences were found between Gray Birch leaves from LML and HML sites ($p > 0.05$, two-tail t-test) (Fig. 6b). GLM analysis performed on FA measured in Gray Birch and Eastern Cottonwood leaves for lumped LML versus HML sites showed that differences in FA values were dependent on the total soil metal load ($p < 0.05$) but not on species alone ($p > 0.05$, Table 2).



(a) (b)

Fig. 7: (a) Fluctuating asymmetry values (mean ±SE;) for leaf width of *Populus deltoides* and *Betula populifolia* collected from three low metal load (L1, L2 and L3) and three high metal load (H1, H2 and H3) sites and grouped on the basis of habitat type (low metal load and high metal load) and according to tree species. 6(b) fluctuating asymmetry values (mean ±SE;) values observed for leaf width observed in *Populus deltoides* and *Betula populifolia* and grouped on the basis of habitat type (low metal load and high metal load) and according to tree species. Different letters indicate the significant differences ($P < 0.05$, two-tailed t-test) between *Populus deltoides* leaves sampled from low metal and high metal load sites.

Table 2: GLM (general linear model) analysis for fluctuating asymmetry in leaves of Gray Birch and Eastern Cottonwood from two habitats (low metal load and high metal load)

Source of Variation	Df	MS	F-test
Intercept	1	4.810	1500.937*
Habitats (H, fixed)	1	0.016	5.126*
Species (S, fixed)	1	0.012	3.729
H x S	1	0.000	0.033
Error	994	0.003	

*****Significant differences: $p < 0.05$

4. Discussion

The magnitude of asymmetry and in particular fluctuating asymmetry (FA) has been proposed as a reliable indicator of abiotic stress in different organisms including plants (Kozlov et al. 1996; Mal et al. 2002; Jari and Mikhail V 2001) and invertebrates (Chang et al. 2007; Vilisics et al. 2005; Peters et al. 2001). Therefore, we had hypothesized that FA levels for antenna and pereiopod segments in isopods and for leaf width in trees increase with higher metal stress. In contrast, the results of this study showed that FA in isopods and trees either did not increase or even decreased with soil metal induced stress. That this is not simply due to the lack of overall pollution effects as indicated by the expected reduction of growth associated with higher metal levels. As expected, isopods in sites with higher metal loads are relatively smaller in size (indicated by lower body size to head width ratio) compared to sites with lower metal stress, this even though their ages as indicated by head width (Donker et al 1993; Jones and Hopkin, 1998) were similar. Likewise, previous work at Liberty State Park has demonstrated that growth rates of the two tree species (Gray Birch and Eastern Cottonwood) are also impaired by soil metal contamination (Renninger et al. 2013; Dahle et al. 2014). Similar to our results, the lack of any relationship, or even negative relationship between FA and stress across different traits and species have also been reported by others researchers (Ambo-Rappe et al. 2008; Floate and Fox 2000; Godet et al. 2012; Hódar 2002; Rabitsch 1997). For instance, Godet et al. (2012) observed that FA measured for antenna and periopod segments in male population of terrestrial isopod, *Porcellio scaber* were lower at heavy metal contaminated sites as compared to uncontaminated ones. In another study, Rabitsch (1997) also found no relation between FA of four morphological traits in the ant *Formica pratensis* and levels of heavy metals. Similarly, Ambo-Rappe et al. (2008) found no increase in FA of the seagrass *Halophila ovalis* sampled from a heavy metal polluted site and a control site. Overall, the inconsistency in literature concerning the relation of FA and stress is demonstrated by the fact that some studies have reported increased FA in response to stress (Kozlov et al. 1996; Mal et al. 2002; Jari and Mikhail V 2001), whereas some including ours have failed to detect a clear link between FA and stress.

4.1. Fluctuating asymmetry in isopods

The observed lower FA levels in the isopod population at highly contaminated sites may be related to synthesis of heat shock protiens (HSPs) known to be stress-defense proteins, and the metal-binding proteins metallothioneins (MTs). These specific set of proteins play an important role in defense against metal toxicity and in maintaining cellular homeostasis (Ackerman and Iwama 2001; Beckmann et al. 1990; Viarengo et al. 1999). Previous studies have demonstrated metal induced expression of HSPs and MTs in several soil invertebrates (Arts et al. 1999; Morgan et al. 2004; Nadeau et al. 2001; Spurgeon et al. 2004; Sterenborg and Roelofs 2003). In terrestrial isopods, Mazzei et al. (2015) reported induction of Hsp-70 and MTs by metals in hepatopancreas, which is the primary organ for metal storage and detoxification in isopods. It is possible that activation of detoxification mechanisms based on the expression of HSPs or MTs protects isopod development from damage due to metal stress leading to a decrease in asymmetry. These protective mechanisms, however, are energetically expensive and may shift resources away from energy demanding process such as growth (Feder and Hofmann 1999; Krebs and Feder 1997; Schill and Kohler 2004; Sibly and Calow 1989). This could explain why *P. muscorum* in the heavy metal load site tended to be smaller in size compared to sites with lower metal stress even though their ages as indicated by head width were similar.

Exposure to abiotic stress can act also as a form of selection against developmentally unstable phenotypes if stress induced asymmetry is negatively correlated with natural and/or sexual components of fitness (Moller 1997). Higher FA in antennae and pereiopods might have serious fitness cost for individuals and therefore natural selection may remove asymmetric phenotypes from isopod population at highly contaminated sites. In addition, selection pressures by mate choice may also mask a relationship between FA and metal stress if asymmetry is associated with reduced mating success in isopods. These hypotheses are better understood when considering the function of antennae and pereiopods in isopods.

Antennae are involved in important sensory functions such as hygroreception and chemoreception (Haug and Altner 1984; Warburg 1993; Zimmer 1996). It has been suggested for some isopods that the olfactory or chemoreceptors present on the antenna are responsible for their avoidance behavior towards contaminated food or soil (Weißenburg and Zimmer 2003; Zidar et al. 2005). Additionally, in some species antennae are used by courting males to locate a receptive female (Johnson 1985) and to assess female quality (Mead 1973). Furthermore, males also use antennae in aggressive behavior against other contesting males during mating (Lefebvre et al. 2000). Pereiopods are the walking legs in isopods (Webb and Sillem 1906) and in addition to locomotion, pereiopods, especially the 7th pair, are also used by males to grasp females during the mating process (Forest 1999). Assuming that presence of asymmetry in these traits might: (i) be associated with loss or impairment of functions that are critical for survival (a fitness component), and/or (ii) influence the process of mate choice in favor of symmetric individuals. It is therefore plausible that selection might explain the absence of a relationship between FA and metal stress at highly polluted sites. A number of studies have determined negative impacts of asymmetry on fitness components in different invertebrates (Allen and Simmons 1996; Møller and Zamora-Munoz 1997; Naugler and Leech 1994; Thornhill 1992; Ueno 1994), however, these relationships have not been tested in the case of terrestrial isopods.

4.2. Fluctuating asymmetry in trees

We also hypothesized that FA in leaves of Gray Birch and Eastern Cottonwood increase with metal stress. However, despite the evidence for metal induced growth stress of Gray Birch and Eastern Cottonwood at highly contaminated sites (Dahle et al. 2014; Renninger et al. 2013), results of the FA analysis in this study do not support our hypothesis (Fig. 7a). One plausible explanation of our results could be activation of stress protection enzymes such as phytochelatin synthase upon the exposure to metal contamination. These enzymes catalyze the synthesis of metal binding proteins termed phytochelatins which play a key role in detoxification in plants. In fact, phytochelatin synthesis has been shown to be involved in maintenance of Zn homeostasis in Eastern Cottonwood (Adams et al. 2011). It is also known that Zn was the only metal to translocate to the leaves of these species at or above concentrations found in the soil. The other metals were sequestered within the root or excluded at the root soil interface (Gallagher et. al. 2008). It is possible that activation of phytochelatin synthase functions to protect plant development from high soil metal load, however the energy cost for such protection could impair long term growth (Dahle et al. 2014).

Some authors have argued that different traits vary in their sensitivity to stress and therefore examining FA levels of a sensitive trait can increase the likelihood of detecting FA and stress relations. For example, Ivanov et al. (2015) suggested that leaf width is not the most sensitive trait for FA analysis in Birch due to metal contamination as compared to other traits such as distance between the bases of the first and second lateral veins. Other researchers also recommend examining FA for multiple traits as relations between FA and stress are usually weak, hence, studying multiple traits can maximize the odds of detecting such a relationship (Leung et al. 2000). On the other hand, some comparative studies have reported positive correlations between metal stress and FA measured for leaf width in plants (Mal et al. 2002; Franiel 2008). Therefore, it is difficult to argue whether selection of a single and/or a relatively insensitive trait (leaf width) for FA analysis in trees was one of the reasons why we did not observe an increase in FA with metal stress.

Plasticity could be another factor that might facilitate resistance to metal stress in trees at high metal load sites. Unlike most animals, sessile organisms such as plants which demonstrate indeterminate and modular growth (Fenster and Galloway 1997; Schmid 1992) can often respond to stressful environmental conditions through plasticity involving changes in their growth patterns (Bradshaw 1965; Schmid 1992). Trees have been shown to develop resistance in form of phenotypic plasticity to cope up with metal stress, for example, Watmough and Hutchinson (1997) showed that callus cell lines derived from mature maple trees growing in metal contaminated soils possessed the ability to tolerate elevated levels of metals. Studies have also reported evidence of phenotypic plasticity in plants in response to other factors such as herbivory, light gradient, salt, or water stress (Abbruzzese et al. 2009; Chazdon and Kaufmann 1993; Goulet and Bellefleur 1986; Pedrol et al. 2000; Rautio et al. 2002). Since Gray Birch and Eastern Cottonwood trees have been growing on high metal load sites for many years, it is possible that ability to acclimatize or plasticity might be a contributing factor to metal tolerance in tree populations resulting in absence of a relation between FA and metal stress.

Conclusions

The results of this study did not support our hypothesis that FA increases in isopods and trees with higher heavy metal stress even though a growth reduction was shown at the site. Our results support the growing body of literature demonstrating the failure to detect a direct link between stress and FA. This need not imply that FA should no longer be used as a marker for environmental stress as argued by some skeptics (Bjorksten et al. 2000), however, it does point to the need for identifying the reasons for these observed inconsistencies and for a better understanding of the underlying developmental mechanisms related to the origin of FA. Natural processes such as selection have been suggested in studies as a possible mechanism that can mask the direct relationship between FA and stress (Bergstrom and Reimchen 2003; Hendrickx et al. 2003). Experiments under controlled settings using multiple generations of isopods will be helpful to verify the negative effects of increased FA on fitness. In addition, one could ask whether traits that contribute less to fitness and therefore are less selected on, are better candidates to detect FA. Results of these studies may provide an insight into understanding the influence of selection on this relationship between FA and stress. Given the growing interest of researchers in studying FA as an indicator of stress, examining the role of detoxification mechanisms (e.g. induction of MTs, HSPs, or phylochelatins) in conjunction with FA measurements might also help to better explain FA and stress relations.

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References

Abbruzzese G, Beritognolo I, Muleo R, Piazzai M, Sabatti M, Mugnozza GS, et al. 2009. Leaf morphological plasticity and stomatal conductance in three Populus alba L. genotypes subjected to salt stress. Environmental and experimental Botany 66(3): 381-388.

Ackerman PA, Iwama GK. 2001. Physiological and cellular stress responses of juvenile rainbow trout to vibriosis. Journal of Aquatic Animal Health 13(2): 173-180.

Adams JP, Adeli A, Hsu CY, Harkess RL, Page GP., Poplar maintains zinc homeostasis with heavy metal genes HMA4 and PCS1. *J Exp Bot* 62, 2011, 3737–3752.

Allen GR, Simmons LW. 1996. Coercive mating, fluctuating asymmetry and male mating success in the dung flySepsis cynipsea. Animal Behaviour 52(4): 737-741.

Alloway B. 1995. Heavy metals in soils: Springer Science & Business Media.

Ambo-Rappe R, Lajus, D. L., & Schreider, M. J, et al. 2011. Heavy metal impact on growth and leaf asymmetry of seagrass, Halophila ovalis. Journal of Environmental Chemistry and Ecotoxicology 3: 149–159.

Ambo-Rappe R, Lajus DL, Schreider MJ. 2008. Increased heavy metal and nutrient contamination does not increase fluctuating asymmetry in the seagrass Halophila ovalis. Ecological Indicators 8(1): 100-103.

Anders Pape Møller. 1997. Developmental Stability and Fitness: A Review. The American Naturalist 149(5): 916-932.

Arena C, De Maio A, De Nicola F, Santorufo L, Vitale L, Maisto G. 2013. Assessment of Eco-Physiological Performance of Quercus ilex L. Leaves in Urban Area by an Integrated Approach. Water Air Soil Pollut 225(1): 1-12.

Arts M-JSJ, Schill RO, Knigge T, Eckwert H, Kammenga JE, KÖhler H-R. Stress Proteins (hsp70, hsp60) Induced in Isopods and Nematodes by Field Exposure to Metals in a Gradient near Avonmouth, UK. Ecotoxicology 13(8): 739-755.

Beasley DAE, Bonisoli-Alquati A, Mousseau TA. 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis. Ecological Indicators 30: 218-226.

Beckmann RP, Mizzen L, Welch WJ. 1990. Interaction of Hsp 70 with newly synthesized proteins: implications for protein folding and assembly. Science 248(4957): 850-854.

Bergstrom C, Reimchen T. 2003. Asymmetry in structural defenses: insights into selective predation in the wild. Evolution 57(9): 2128-2138.

Bjorksten TA, Fowler K, Pomiankowski A. 2000. What does sexual trait FA tell us about stress? Trends in Ecology & Evolution 15(4): 163-166.

Bradshaw AD. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in genetics 13: 115-155.

Castiglione S, Todeschini V, Franchin C, Torrigiani P, Gastaldi D, Cicatelli A, et al. 2009. Clonal differences in survival capacity, copper and zinc accumulation, and correlation with leaf polyamine levels in poplar: A large-scale field trial on heavily polluted soil. Environmental Pollution 157(7): 2108-2117.

Chang X, Zhai B, Liu X, Wang M. 2007. Effects of temperature stress and pesticide exposure on fluctuating asymmetry and mortality of Copera annulata (selys) (Odonata: Zygoptera) larvae. Ecotoxicology and Environmental Safety 67(1): 120-127.

Chazdon RL, Kaufmann S. 1993. Plasticity of leaf anatomy of two rain forest shrubs in relation to photosynthetic light acclimation. Functional Ecology: 385-394.

David JF, Handa IT. 2010. The ecology of saprophagous macroarthropods (millipedes, woodlice) in the context of global change. Biological Reviews 85(4): 881-895.

Dahle G, Gallagher F, Gershensond D, Schäfer KR, Grabosky J. 2014. Allometric and mass relationships of Betula populifolia in a naturally assembled urban brownfield: implications for carbon modeling. Urban Ecosyst: 1-14.

De Anna EB, Bonisoli-Alquati A, Mousseau TA. 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: A meta-analysis. Ecological Indicators 30: 218-226.

Di Baccio D, Tognetti R, Sebastiani L, Vitagliano C. 2003. Responses of Populus deltoides × Populus nigra (Populus × euramericana) clone I-214 to high zinc concentrations. New Phytologist 159(2): 443-452.

Donker MH, Capelleveen HEv, Straalen. aNMv. 1993. Metal contamination affects size-structure and life-history dynamics in isopod field populations In RDallinger and PSRainbow, eds, Ecotoxicology of Metals in Invertebrates Lewis, Boca Raton, FL, USA: 383–399.

Feder ME, Hofmann GE. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual review of physiology 61(1): 243-282.

Fenster CB, Galloway LF. 1997. Developmental homeostasis and floral form: evolutionary consequences and genetic basis. International Journal of Plant Sciences: S121-S130.

Floate KD, Fox AS. 2000. Flies under stress: A test if fluctuating asymmetry as a biomonitor of environmental quality. Ecological Applications 10(5): 1541-1550.

Forest J. 1999. Traité de zoologie : anatomie, systématique, biologie: Crustacés péracarides. Monaco: Musée océanographique.

Fountain M, Hopkin S. 2004. Biodiversity of Collembola in urban soils and the use of Folsomia candida to assess soil 'quality'. Ecotoxicology 13(6): 555-572.

Franiel I. 2008. Fluctuating asymmetry of *Betula pendula* Roth leaves - an index of environment quality. Biodiv Res Conserv 9(10): 7-10

Friedlova M. 2010. The influence of heavy metals on soil biological and chemical properties. Soil Water Res 5(1): 21-27.

Gál J, Markiewicz-Patkowska J, Hursthouse A, Tatner P. 2008. Metal uptake by woodlice in urban soils. Ecotoxicology and Environmental Safety 69(1): 139-149.

Gallagher FJ, Pechmann I, Bogden JD, Grabosky J, Weis P. 2008a. Soil metal concentrations and productivity of Betula populifolia (Gray birch) as measured by field spectrometry and incremental annual growth in an abandoned urban Brownfield in New Jersey. Environmental Pollution 156(3): 699-706.

Gallagher FJ, Pechmann I, Bogden JD, Grabosky J, Weis P. 2008b. Soil metal concentrations and vegetative assemblage structure in an urban brownfield. Environmental Pollution 153(2): 351-361.

Gallagher FJ, Pechmann I, Holzapfel C, Grabosky J. 2011. Altered vegetative assemblage trajectories within an urban brownfield. Environmental Pollution 159(5): 1159-1166.

Ge Y, Murray P, Hendershot WH. 2000. Trace metal speciation and bioavailability in urban soils. Environmental Pollution 107(1): 137-144.

Ge Y, Murray P, Hendershot WH. 2000. Trace metal speciation and bioavailability in urban soils. Environmental Pollution 107(1): 137-144

Godet J-P, Demuynck S, Waterlot C, Lemière S, Souty-Grosset C, Douay F, et al. 2012. Fluctuating asymmetry analysis on Porcellio scaber (Crustacea, Isopoda) populations living under metals-contaminated woody habitats. Ecological Indicators 23(0): 130-139.

Godet J-P, Demuynck S, Waterlot C, Lemière S, Souty-Grosset C, Scheifler R, et al. 2011. Growth and metal accumulation in Porcellio scaber exposed to poplar litter from Cd-, Pb-, and Zn-contaminated sites. Ecotoxicology and Environmental Safety 74(3): 451-458.

Goulet F, Bellefleur P. 1986. Leaf morphology plasticity in response to light environment in deciduous tree species and its implication on forest succession. Canadian journal of forest research 16(6): 1192-1195.

Hassall M, Jennings JB. 1975. Adaptive Features of Gut Structure and Digestive Physiology in the Terrestrial Isopod Philoscia muscorum (Scopoli) 1763. Biological Bulletin 149(2): 348-364.

Haug T, Altner H. 1984. A cryofixation study of presumptive hygroreceptors on the antennule of a terrestrial isopod. Tissue and Cell 16(3): 377-391.

Helle S, Huhta E, Suorsa P, Hakkarainen H. 2011. Fluctuating asymmetry as a biomarker of habitat fragmentation in an area-sensitive passerine, the Eurasian treecreeper (Certhia familiaris). Ecological Indicators 11(3): 861-867.

Hendrickx F, Maelfait JP, Lens L. 2003. Relationship between fluctuating asymmetry and fitness within and between stressed and unstressed populations of the wolf spider Pirata piraticus. Journal of Evolutionary Biology 16(6): 1270-1279.

Hódar JA. 2002. Leaf fluctuating asymmetry of Holm oak in response to drought under contrasting climatic conditions. Journal of Arid Environments 52(2): 233-243.

Hovorka MD, Robertson RJ. 2000. Food stress, nestling growth, and fluctuating asymmetry. Canadian journal of zoology 78(1): 28-35.

Ivanov VP, Ivanov YV, Marchenko SI, Kuznetsov VV. 2015. Application of fluctuating asymmetry indexes of silver birch leaves for diagnostics of plant communities under technogenic pollution. Russ J Plant Physiol 62(3): 340-348.

Jari V, Mikhail V K. 2001. Impact of climatic factors on the developmental stability of mountain birch growing in a contaminated area. Journal of Applied Ecology 38(3): 665-673.

Jelaska LŠ, Blanuša M, Durbešić P, Jelaska SD. 2007. Heavy metal concentrations in ground beetles, leaf litter, and soil of a forest ecosystem. Ecotoxicology and Environmental Safety 66(1): 74-81.

Johnson C. 1985. Mating Behavior of the Terrestrial Isopod, Venezillo evergladensis (Oniscoidea, Armadillidae). American Midland Naturalist 114(2): 216-224.

Jones DT, Hopkin SP. 1998. Reduced survival and body size in the terrestrial isopod Porcellio scaber from a metal-polluted environment. Environmental Pollution 99(2): 215-223.

Köhler H-R, Knödler C, Zanger M. 1999. Divergent kinetics of hsp70 induction in Oniscus asellus (Isopoda) in response to four environmentally relevant organic chemicals

(B [a] P, PCB52, γ-HCH, PCP): Suitability and limits of a biomarker. Arch Environ Contam Toxicol 36(2): 179-185.

Kozlov MV, Wilsey BJ, Koricheva J, Haukioja E. 1996. Fluctuating Asymmetry of Birch Leaves Increases Under Pollution Impact. Journal of Applied Ecology 33(6): 1489-1495.

Krebs RA, Feder ME. 1997. Deleterious consequences of Hsp70 overexpression in Drosphilla melanogaster larvae. Cell stress & chaperones 2(1): 60.

Lazić MM, Kaliontzopoulou A, Carretero MA, Crnobrnja-Isailović J. 2013. Lizards from urban areas are more asymmetric: using fluctuating asymmetry to evaluate environmental disturbance. PloS one 8(12): e84190.

Lefebvre F, Limousin M, Caubet Y. 2000. Sexual dimorphism in the antennae of terrestrial isopods: a result of male contests or scramble competition? Canadian journal of zoology 78(11): 1987-1993.

Lerner IM. 1954. Genetic homeostasis. Genetic homeostasis.

Leung B, Forbes MR, Houle D. 2000. Fluctuating asymmetry as a bioindicator of stress: comparing efficacy of analyses involving multiple traits. The American Naturalist 155(1): 101-115.

Li X, Liu L, Wang Y, Luo G, Chen X, Yang X, et al. 2013. Heavy metal contamination of urban soil in an old industrial city (Shenyang) in Northeast China. Geoderma 192: 50-58.

Mal TK, Uveges JL, Turk KW. 2002. Fluctuating asymmetry as an ecological indicator of heavy metal stress in Lythrum salicaria. Ecological Indicators 1(3): 189-195.

Manta DS, Angelone M, Bellanca A, Neri R, Sprovieri M. 2002. Heavy metals in urban soils: a case study from the city of Palermo (Sicily), Italy. Science of The Total Environment 300(1–3): 229-243.

Markow TA, Ricker JP. 1992. Male size, developmental stability, and mating success in natural populations of three Drosophila species. Heredity 69(2): 122-127.

Mazzei V, Giannetto A, Brundo MV, Maisano M, Ferrante M, Copat C, et al. 2015. Metallothioneins and heat shock proteins 70 in Armadillidium vulgare (Isopoda, Oniscidea) exposed to cadmium and lead. Ecotoxicology and Environmental Safety 116: 99-106.

Mead F. 1973. Recherches sur la reproduction et le comportement sexuel des Isopodes terrestres. PhDthesis Universit de Provence Marseille, France

Mireles F, Davila J, Pinedo J, Reyes E, Speakman R, Glascock M. 2012. Assessing urban soil pollution in the cities of Zacatecas and Guadalupe, Mexico by instrumental neutron activation analysis. Microchemical Journal 103: 158-164.

Møller AP. 1997. Developmental stability and fitness: a review. The American Naturalist 149(5): 916-932.

Møller AP, ZAMORA-MUÑOZ C. 1997. Antennal asymmetry and sexual selection in a cerambycid beetle. Animal Behaviour 54(6): 1509-1515.

Morgan AJ, Stürzenbaum SR, Winters C, Grime GW, Aziz NAA, Kille P. 2004. Differential metallothionein expression in earthworm (Lumbricus rubellus) tissues. Ecotoxicology and Environmental Safety 57(1): 11-19.

Murray P, Ge Y, Hendershot WH. 2000. Evaluating three trace metal contaminated sites: a field and laboratory investigation. Environmental Pollution 107(1): 127-135.

Nadeau D, Corneau S, Plante I, Morrow G, Tanguay RM. 2001. Evaluation for Hsp70 as a biomarker of effect of pollutants on the earthworm Lumbricus terrestris. Cell stress & chaperones 6(2): 153-163.

Naugler CT, Leech SM. 1994. Fluctuating asymmetry and survival ability in the forest tent caterpillar moth Malacosoma disstria: implications for pest management. Entomologia Experimentalis et Applicata 70(3): 295-298.

Odendaal JP, Reinecke AJ. 2004. Effect of Metal Mixtures (Cd and Zn) on Body Weight in Terrestrial Isopods. Archives of Environmental Contamination and Toxicology 46(3): 377-384.

Palmer AR, Strobeck C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual review of Ecology and Systematics: 391-421.

Palmer AR, Strobeck C. 1992. Fluctuating asymmetry as a measure of developmental stability: implications of non-normal distributions and power of statistical tests. Acta Zoologica Fennica, 191, 57-72.

Palmer AR. 1996. Waltzing with asymmetry. Bioscience: 518-532.

Pedrol N, Ramos P, Reigosa MJ. 2000. Phenotypic plasticity and acclimation to water deficits in velvet-grass: a long-term greenhouse experiment. Changes in leaf morphology, photosynthesis and stress-induced metabolites. Journal of Plant Physiology 157(4): 383-393.

Peters EL, Kaufmann-Daszczuk B, Brant HA, Jagoe CH, Richter R. 2001. Element concentrations and fluctuating asymmetry in a terrestrial isopod (Armadillidium vulgare) in metropolitan Chicago Parklands. Department of Biological Sciences, Chicago State University: 1p.

Peterson LR, Trivett V, Baker AJ, Aguiar C, Pollard AJ. 2003. Spread of metals through an invertebrate food chain as influenced by a plant that hyperaccumulates nickel. Chemoecology 13(2): 103-108.

Pouyat RV, Szlavecz K, Yesilonis ID, Groffman PM, Schwarz K. 2010. Chemical, physical, and biological characteristics of urban soils. Urban ecosystem ecology(urbanecosysteme): 119-152.

Rabitsch WB. 1997. Levels of asymmetry in Formica pratensis Retz. (Hymenoptera, insecta) from a chronic metal-contaminated site. Environmental Toxicology and Chemistry 16(7): 1433-1440.

Rautio P, Markkola A, Martel J, Tuomi J, Härmä E, Kuikka K, et al. 2002. Developmental plasticity in birch leaves: defoliation causes a shift from glandular to nonglandular trichomes. Oikos 98(3): 437-446.

Renninger H, Wadhwa S, Gallagher F, Vanderklein D, Schäfer KR. 2013. Allometry and photosynthetic capacity of poplar (Populus deltoides) along a metal contamination gradient in an urban brownfield. Urban Ecosyst 16(2): 247-263.

Rothwell GW, Wyatt SE, Tomescu AM. 2014. Plant evolution at the interface of paleontology and developmental biology: An organism-centered paradigm. American journal of botany 101(6): 899-913.

Sawidis T, Breuste J, Mitrovic M, Pavlovic P, Tsigaridas K. 2011. Trees as bioindicator of heavy metal pollution in three European cities. Environmental Pollution 159(12): 3560-3570.

Schill R, KÖhler H-R. 2004. Energy Reserves and Metal-storage Granules in the Hepatopancreas of Oniscus asellus and Porcellio scaber (Isopoda) from a Metal Gradient at Avonmouth, UK. Ecotoxicology 13(8): 787-796.

Schmid B. 1992. Phenotypic variation in plants. Evolutionary Trends in Plants 6(1): 45-60.

Sibly RM, Calow P. 1989. A life-cycle theory of responses to stress. Biological Journal of the Linnean Society 37(1-2): 101-116.

Silva JJd, Mendes J, Lomônaco C. 2004. Developmental stress by diflubenzuron in Haematobia irritans (L.) (Diptera: Muscidae). Neotropical Entomology 33: 249-253.

Simmons LW. 1995. Correlates of male quality in the field cricket, Gryllus campestris L.: age, size, and symmetry determine pairing success in field populations. Behavioral Ecology 6(4): 376-381

Sommer C. 1996. Ecotoxicology and developmental stability as an in situ monitor of adaptation. Ambio: 374-376.

Spurgeon DJ, Stürzenbaum SR, Svendsen C, Hankard PK, Morgan AJ, Weeks JM, et al. 2004. Toxicological, cellular and gene expression responses in earthworms exposed to copper and cadmium. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 138(1): 11-21.

Sterenborg I, Roelofs D. 2003. Field-selected cadmium tolerance in the springtail Orchesella cincta is correlated with increased metallothionein mRNA expression. Insect Biochemistry and Molecular Biology 33(7): 741-747.

Thornhill R. 1992. Female preference for the pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (Panorpa japonica: Mecoptera). Behavioral Ecology 3(3): 277-283.

Todeschini V, Lingua G, D'Agostino G, Carniato F, Roccotiello E, Berta G. 2011. Effects of high zinc concentration on poplar leaves: A morphological and biochemical study. Environmental and Experimental Botany 71(1): 50-56.

Tomašević M, Rajšić S, Đorđević D, Tasić M, Krstić J, Novaković V. 2004. Heavy metals accumulation in tree leaves from urban areas. Environ Chem Lett 2(3): 151-154.

Ueno H. 1994. Fluctuating asymmetry in relation to two fitness components, adult longevity and male mating success in a ladybird beetle, Harmonia axyridis (Coleoptera: Coccinellidae). Ecological Entomology 19(1): 87-88.

Valen LV. 1962. A Study of Fluctuating Asymmetry. Evolution 16(2): 125-142.

Vilisics F, Sólymos P, Hornung E. 2005. Measuring fluctuating asymmetry of the terrestrial isopod Trachelipus rathkii (Crustacea: Isopoda, Oniscidea). European Journal of Soil Biology 41(3–4): 85-90.

Viarengo A, Burlando B, Cavaletto M, Marchi B, Ponzano E, Blasco J. 1999. Role of metallothionein against oxidative stress in the mussel Mytilus galloprovincialis. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 277(6): R1612-R1619.

Waddington CH. 1942. Canalization of development and the inheritance of acquired characters. Nature 150(3811): 563-565.

Warburg MR. 1993. Evolutionary biology of land isopods: Springer Science & Business Media.

Watmough SA, Hutchinson TC. 1997. Metal resistance in red maple (Acer rubrum) callus cultures from mine and smelter sites in Canada. Canadian journal of forest research 27(5): 693-700.

Webb WM, Sillem C. 1906. The British Woodlice: Being a Monograph of the Terrestrial Isopod Crustacea Occurring in the British Islands: Duckworth & Company.

Wei B, Yang L. 2010. A review of heavy metal contaminations in urban soils, urban road dusts and agricultural soils from China. Microchemical Journal 94(2): 99-107.

Weißenburg M, Zimmer M. 2003. Balancing nutritional requirements for copper in the common woodlouse, Porcellio scaber (Isopoda: Oniscidea). Applied Soil Ecology 23(1): 1-11.

Witzel B. 2000. The influence of zinc on the uptake and loss of cadmium and lead in the woodlouse, Porcellio scaber (Isopoda, Oniscidea). Ecotoxicology and Environmental Safety 47(1): 43-53.

Wong CSC, Li X, Thornton I. 2006. Urban environmental geochemistry of trace metals. Environmental Pollution 142(1): 1-16.

Yaylalı-Abanuz G. 2011. Heavy metal contamination of surface soil around Gebze industrial area, Turkey. Microchemical Journal 99(1): 82-92

Zidar P, BoŽIČ J, ŠTrus J. 2005. Behavioral Response in the Terrestrial Isopod Porcellio scaber (Crustacea) Offered a Choice of Uncontaminated and Cadmium-Contaminated Food. Ecotoxicology 14(5): 493-502.

Zimmer M, Topp, W.,. 1996. Olfaction in terrestrial isopods (Crustacea: Oniscidea): responses of Porcellio scaber to the odour of litter. Eur J Soil Biol 32: 141-147.

Chapter 5: Effects of soil and litter metal exposures on growth and metal accumulation in the terrestrial isopod *Philoscia muscorum*.

Abstract

This study investigated the effects of metal contaminated soil and poplar litter on growth and metal accumulation in the terrestrial isopod *Philoscia muscorum*. In order to evaluate the relative importance of different metal exposure routes, e.g. soil and leaf litter, experiments were conducted using soil and poplar litter collected from a metal contaminated brownfield and a reference site located in Liberty State Park, in New Jersey. Isopods were exposed to six different treatments under controlled conditions, where each treatment was a combination of one of the three poplar leaf litter types (contaminated, non-contaminated and mixed) and one of the two soil types (contaminated and non-contaminated). The weight of isopods was recorded initially at the start and after 7 and 14 days of exposure. In addition, at the end of the experiment, zinc, copper and lead body burdens of isopods were determined using Atomic absorption spectroscopy. Results of the GLM analysis revealed that weight of isopods was significantly affected by exposure to soil and litter treatments after 7 days, however, after 14 days of exposure, weight change in isopods was significantly more negatively affected by soil as compared to litter**.** Exposure to contaminated soil and litter resulted in higher body concentrations of zinc and copper in isopods as compared to non-contaminated soil and litter treatment, however, lead body burdens were not significantly different between the two treatments. Finally, the mortality levels observed for isopods among all the treatments did not differ significantly after 14 days of exposure, however, seem to be approaching significance. In addition, we found that mortality in isopods after 14 days of exposure was more affected by soil contamination as compared to litter. Our results demonstrate the importance of different exposure routes (soil and litter) in toxicity studies aimed at determining the effects of metals in isopods. In conclusion, weight change (growth) of terrestrial isopods i.e., *P. muscorum* can be used as a potential tool for assessment of metal contamination in litter and soil with ecological significance.

Keywords: Terrestrial isopods, Metal exposure, Growth, Bio-accumulation, Eco-toxicology.

1. Introduction

Terrestrial isopods (commonly known as woodlice), included in the suborder Oniscidea, are a group of crustaceans that have successfully colonized many terrestrial habitats. They form an important component of the soil macro-fauna community in terrestrial ecosystems and play a crucial role in litter decomposition (Paoletti and Hassall 1999; Špaldoňová and Frouz 2014). Commonly found in the litter layers covering the soil, isopods enhance the process of nutrient cycling in different ways. For example, directly by break down of leaf litter into smaller fragments and via digestive degradation of compounds such as cellulose or phenols present in the leaf litter (AF Quadros and PB Araujo 2007; Zimmer et al. 2005). The indirect influence is through return of consumed litter in form of feces that provides a suitable substrate for colonizing microbial populations (Anderson et al. 1983; Hanlon and Anderson 1980; Hassall et al. 1987). As abundant litter detritivores in terrestrial ecosystems, isopods have been shown to consume an estimated 10% of annual litter input, highlighting their importance in regulation of organic matter and nutrients (Dias and Sprung 2003; Hassall and Sutton 1978).

Among soil invertebrates, terrestrial isopods are the choice of organisms for heavy metal pollution monitoring studies. This is because they are quite abundant, have adequate body size, are easy to collect, and can tolerate and accumulate elevated concentrations of heavy metals as compared to other soil organisms (Paoletti and Hassall 1999; Godet et al. 2011; Heikens et al. 2001; Köhler et al. 1996). Previous laboratory studies have used several toxicological endpoints with isopods to determine the potential toxic effects of metals in terrestrial environments. The commonly studied toxicological endpoints are growth, reproduction, feeding rates, accumulated metal concentrations, mortality rates, behavioral changes, molting frequency, and structural and cellular changes in hepatopancreas (Godet et al. 2011; Drobne and Štrus 1996; Loureiro et al. 2005; Odendaal and Reinecke 1999; Zidar et al. 2005; Žnidaršič et al. 2003). Among these parameters, change in weight over time or responses such as avoidance behaviors are considered as sensitive endpoints in eco-toxicological studies with invertebrates (Godet et al. 2011; Zidar et al. 2005).

Most of the experimental studies examining a parameter(s) in response to metal contamination are conducted by exposing the organisms to toxicant(s) using contaminated food or litter (Godet et al. 2011; Loureiro et al. 2005). Generally, metal concentrations in food are manipulated by applying prepared solutions (Odendaal and Reinecke 2004; Zidar et al. 2003). Furthermore, the laboratory studies often evaluate toxic effects of a single element (Zidar et al. 2005; Calhôa et al. 2012; Farkas et al. 1996) whereas, in polluted environments, organisms are exposed to elevated concentrations of different metals. Therefore, this approach makes it difficult to extrapolate the results of these experiments to actual field conditions, thus compromising the relevance and usefulness of these studies. Another common shortcoming in these experimental studies is that the effects of metal exposure via soil in isopods are not tested as isopods are often placed on an artificial substrate (e.g. plaster of Paris or uncontaminated soil) (Godet et al. 2011; Calhôa et al. 2012; Kampe and Schlechtriem 2016; Loureiro et al. 2006). In contaminated habitats, the food source and microhabitats of isopods coincide with the upper layers of the soil where metal accumulation occurs, therefore, metal exposure can occur via both food and soil (Donker et al. 1996; Martin et al. 1982; Vijver 2005). Indeed, researchers have stressed upon the importance of considering different exposure routes while assessing the effects of contaminants in isopods in eco-toxicological studies aimed at understanding the larger impact of metal contaminants in terrestrial ecosystems (Vink et al. 1995).

The objectives of this experimental study were to evaluate the effects of metal exposure through soil and food (separately and simultaneously) on (a) growth of the terrestrial isopod, *P. muscorum* after 7 and 14 days, and (b) zinc, copper and lead body burdens of isopods at the end of the exposure period. The results of this study will help to understand the physiology of *P.muscorum* in relation to different pathways of metal contamination and to establish its role as a suitable candidate for future bio-monitoring studies.

2. Material and Methods

2.1. Study organism and culture

Terrestrial isopods, (*Philoscia muscorum*) were collected in September 2013 from a reference site (40°42'11.61"N, 74° 3'34.72"W) located at Liberty State Park (LSP), New Jersey, USA. This reference site encompasses an area of 18 acres within LSP and is filled with several feet of clean soil. It is an unmanaged site which has been naturally colonized by various plant assemblages including hardwood trees (*Populus deltoides* (Eastern Cottonwood), *Populus tremululoides* (Quaking Aspen), and *Betula popufolia* (Gray Birch)), shrubs (*Rhus copallina* (Winged Sumac), and *Rhus typhina* (Staghorn Sumac)) and herbs (*Artemisa vulgaris* (Mugwort), and Solidago spp. (Goldenrod)). The concentrations of metals in soil and poplar leaf litter at this site are listed in Table 1. *P. muscorum* was selected as the study organism because it is the most dominant of all the isopod species found in the park (Chapter 3). Prior to this study, isopod cultures were maintained in laboratory for eleven months. In laboratory rearing, isopods were kept in plastic culture boxes (each filled with 2-3 cm layers of moistened sand) and were fed with poplar leaf litter collected from the reference site. Before litter was offered to isopods, leaves were moistened in a jar with de-mineralized water for one week. In the laboratory, isopods in culture boxes were at maintained at 20 ± 1^{o}C with a photoperiod of 16:8 (light: dark).

2.2. Leaf litter and soil collection

For this study, poplar leaf litter was used as the food source for isopods as it is one of the dominant hardwood trees present at LSP (Gallagher et al. 2008). Freshly fallen leaf litter was collected in October 2013 from the reference site and one heavy metal contaminated site (denoted as TP-25 in Gallagher et al. 2008) located at LSP. The metal contaminated site (TP-25) is located in an abandoned area (about 251 acre) of the park that was used as a railyard by Central rail road of New Jersey (CRRNJ) from the late 1800's to the mid 1900's. The area is closed for public access due to presence of metals (arsenic, lead, zinc, chromium and vanadium) in soils at concentrations higher than the ambient levels (Gallagher et al. 2008). Despite the contamination, the area has been spontaneously colonized by many native and non-native species, with dominant trees being *B. populifolia*, *P. deltoides* and *P. tremuloides* (Gallagher et al. 2008). Previous work at LSP has shown that the structure and development of vegetative assembly has been impacted by soil metal contamination (Gallagher et al. 2008; Gallagher et al. 2011). Among the metals, zinc has been shown to accumulate at extremely high concentrations in the leaf tissue of trees (*B. populifolia* and *P. deltoides*), whereas chromium is primarily assimilated in root tissue of the trees. The leaf litter collected from the two sites (reference site and metal contaminated site (TP-25)) was air dried in the laboratory, and stored in brown paper bags. The soils used for the non-contaminated and contaminated treatments were also collected from the same reference and contaminated site (TP-25) respectively. Soil collected from both reference and contaminated sites was air dried in the laboratory and sieved with a 2 mm sieve to remove any undesirable components. Heavy metal concentrations of soil and poplar leaf litter collected from the metal contaminated site (TP-25) and reference site in LSP are listed in Tables 1 and 2, respectively.

Table 1: Soil characteristics including concentrations heavy metals (means with standard errors in parentheses), soil pH, organic matter (OM), and cation exchange capacity (CEC) for the reference and metal contaminated (TP-25) sites at Liberty State Park, New Jersey, USA

Site	**Cu (μg/g)**	**Zn (μg/g)**	**Cr (μg/g)**	**pH**	**OM**	**CEC**
Reference, LSP	67.1 (30.99)[a]	128.8 (59.18)[a]	14 (0.34)[a]	6.84	3.78	13.82
TP-25, LSP	2200 (160)[b]	2330 (1200)[b]	50.5 (23.0)[b]	4.6	23.1	8.4

a measured in 2015

b measured in 2005 by Gallagher et al. (2008)

Table 2: Chemical composition of poplar leaf litter collected from reference and metal contaminated (TP-25) sites at Liberty State Park, New Jersey, USA.

Site	**%P**	**%K**	**%Ca**	**%Mg**	**Cu (ppm)**	**Zn (ppm)**
Reference	0.25[a]	1.24[a]	2.09[a]	0.31[a]	21.49[a]	455.14[a]
TP-25, LSP	0.16[b]	0.93[b]	1.76[b]	0.16[b]	9.0[b]	1883[b]

a measured in 2015

b measured in 2011

2.3. Experimental design

Experiments were conducted using *P. muscorum* individuals (4-5 months old and weighing 20-25 mg) obtained from the laboratory culture. Isopods were exposed to six different treatments, where each treatment was a combination of one of the three poplar leaf litter types and one of the two soil types (Table 3). For each treatment, eight replicates were used with 4 individual isopods per replicate. The four individuals were placed in a mesocosm (plastic box: 10.5 cm diameter and 8 cm high) filled with 2-3 cm of soil depending on the treatment (Table 3). In each of these boxes, isopods received prepared poplar litter (approx. 450 mg dry weight) depending on the treatment (Table 3). Small holes around 1 mm in size were made in the lids of the plastic boxes to allow for air to ventilate through the boxes. Isopods were kept at 20 ± 1^{o}C, with a photoperiod of 16:8 (light: dark) in a growth chamber.

Combined weight of all the isopods in each box was recorded at the start of the experiment and subsequently after 7 and 14 days of the exposure. After 7 days, dead individuals were counted and removed from each of the box and litter was also replaced. Mortality levels in all the treatments were also recorded after 14 days of exposure. The present study was conducted over a period of 14 days as previous studies examining the lethal and/or sub-lethal effects of toxicants in isopods have shown that isopods do respond to changes in the food or soil quality over a short period of time (e.g. 14 days) (Zidar et al. 2003; Loureiro et al. 2006; Lapanje et al. 2008).

Table 3: Six different treatments based on the combination of different soil and leaf litter types.

Treatment	Soil type (S)	Leaf litter type (L)	Acronyms
1	Contaminated ($\mathbf{S_C}$)	Contaminated ($\mathbf{L_C}$)	($\mathbf{S_C L_C}$)
2	Contaminated ($\mathbf{S_C}$)	Non-contaminated ($\mathbf{L_{NC}}$)	($\mathbf{S_C L_{NC}}$)
3	Contaminated ($\mathbf{S_C}$)	Mixed (half contaminated & half non-contaminated, $\mathbf{L_M}$)	($\mathbf{S_C L_M}$)
4	Non-contaminated ($\mathbf{S_{NC}}$)	Contaminated ($\mathbf{L_C}$)	($\mathbf{S_{NC} L_C}$)
5	Non-contaminated ($\mathbf{S_{NC}}$)	Non-contaminated ($\mathbf{L_{NC}}$)	($\mathbf{S_{NC} L_{NC}}$)
6	Non-contaminated ($\mathbf{S_{NC}}$)	Mixed (half contaminated & half non-contaminated, $\mathbf{L_M}$)	($\mathbf{S_{NC} L_M}$)

2.4. Heavy metal analysis

Metal body burdens were analyzed for isopods exposed to two of the six treatments: (a) non-contaminated soil and non-contaminated litter (most clean), and (b) contaminated soil and contaminated litter (most contaminated) treatments. To estimate metal body burdens, isopod samples (N=5 per treatment) were washed with deionized water (3 times). The cleaned samples were then dried to weight constance in an oven at 60^{o}C for 48 hours. The oven dried samples were then individually digested on hot-plates using 10 ml of 69% HNO_3. The digested samples were diluted with 10 ml solution of 1% HNO_3 and metal concentrations (zinc, copper and lead) were estimated using flame atomic absorption spectroscopy (Perking-Elmer 1100-B, Norvale, USA and Varian AA240FS). A DOLT-4 standard (dogfish liver [Institute for National Measurement Standards, Certified Reference Materials for trace metals, Ottawa, Canada]) was used as reference material. Replicates of standard material and blank reagents were also included in the digestion and metal analysis to ensure accuracy and precision of the process. All of the metal analysis work was done at the Environmental Toxicology lab at Rutgers New Jersey Medical School, Newark, New Jersey.

2.5. Statistical analysis

One-way ANOVA analysis with post-hoc Tukey test was performed to compare the initial weight of isopods between all six treatments. A general linear model (GLM) analysis was performed to test the dependence of weight change and mortality (after 7 and 14 days) in isopods with soil and litter. Deviations from normality in weight change data for each treatment (after 7 and 14 days) were tested with Kolmogorov Smirnov test. Normal distributions were observed for all the treatments ($p > 0.05$) except for the contaminated soil and contaminated litter ($S_C L_C$) treatment after 14 days. Two tail T-tests were performed to compare the zinc, copper and lead body burdens between isopods exposed to contaminated (soil and litter) and non-contaminated (soil and litter) treatments. All statistical tests and analysis performed in this study were done using SPSS version 21.0 for Windows (SPSS, Chicago, IL).

3. Results

The initial weight of isopod populations did not differ across all six treatments at the start of the experiment ($p=0.86$, $F=0.369$, $df=5$) (Fig. 1). Results of the GLM analysis revealed that weight change in isopods showed a significant change after 7 ($p=0.00$, $df=5$, $F=6.554$, $MSS=1.554$) and 14 ($p=0.04$, $df=5$, $F=2.456$, $MSS=42.608$) days of exposure.

The weight change in isopods was significantly impacted by both soil and litter after 7 days, and only by soil after 14 days of exposure ($p<0.05$) (Table. 3).

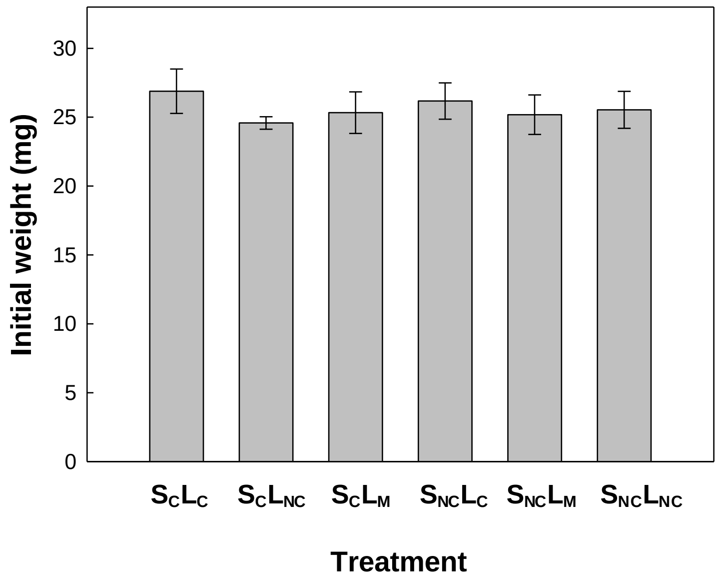

Fig. 1: Initial weight (mean± 1 Std. error) of *P. muscorum* for the six different treatments.

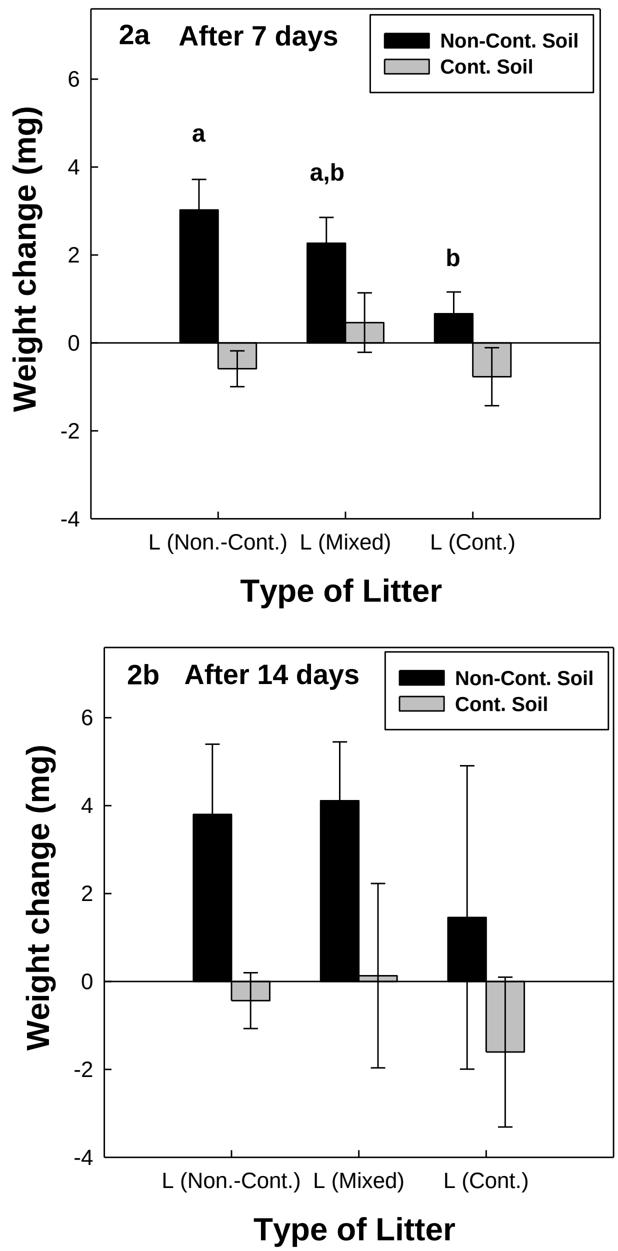

Fig. 2: Weight change (mean± 1 Std. error) of *P. muscorum* across six different treatments after (a) 7 and (b) 14 days of exposure. Letters (a, b, and c) indicate significant differences ($p<0.05$, post hoc Tukey) between different litter treatments with Non-Contaminated soil (($S_{NC}L_C$), ($S_{NC}L_{NC}$), and ($S_{NC}L_M$)) after 7 days of exposure. Bars with no letters are not significantly different.

On average the lowest number of dead individuals was observed for isopods exposed to the non-contaminated soil and non-contaminated litter treatment. Results of GLM analysis showed that mortality of isopods did not vary between different treatments after 7 days of exposure (p=0.53, df=5, F=0.829, MSS=0.521). However, mortality after 14 days appeared to be approaching significance (p=0.054, df=5, F=2.383, MSS=2.283). Moreover, the mortality in isopods after 14 days of exposure was significantly affected by contamination of soils (p=0.05, Table. 4) as compare to contamination of leaf litter (p>0.05, Table. 4)

Table 3: Results of GLM analysis (general linear model) for weight change in isopods observed after 7 and 14 days of exposure to soil (contaminated and non-contaminated) and poplar litter (non-contaminated, contaminated and mixed)

	Mean square		**Df**		**F-test**		**p-value**	
Source of Variation	after 7 days	after 14 days	after 7 days	after 14 days	after 7 days	after 14 days	after 7 days	after 14 days
Model	18.584	42.608	5	5	6.554	2.456	0.00	0.045
Intercept	34.172	72.438	1	1	12.050	4.176	0.01	0.047
Soil	62.563	165.690	1	1	22.063	9.552	0.00	0.004
Litter	9.739	21.514	2	2	3.434	1.240	0.04	0.30
Soil x Litter	5.441	1.493	2	2	1.919	0.086	0.16	0.91
Error	2.836	17.345	42	41				
Total			48	47				

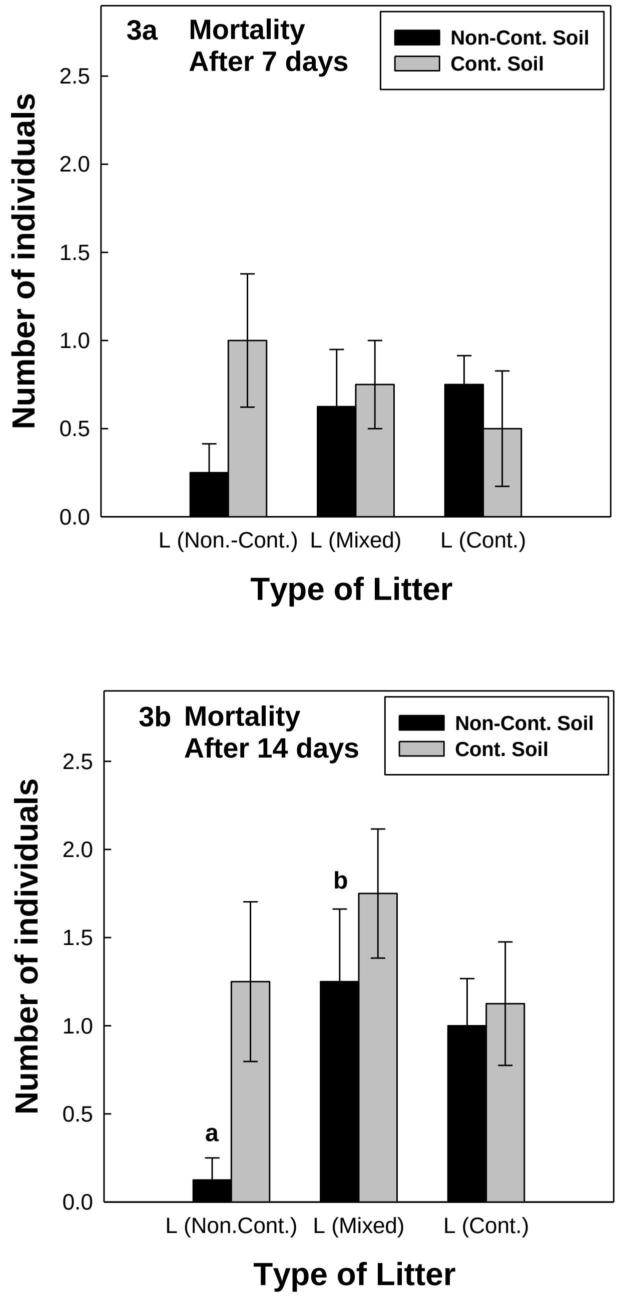

Fig. 3: Mortality (mean ± 1 Std. error) observed in six different treatments after 7 and 14 days of exposure. Letters (a, b, and c) indicate significant mortality differences ($p<0.05$, post hoc Tukey) between different litter treatments with Non-Contaminated soil (($S_{NC}L_C$), ($S_{NC}L_{NC}$), and ($S_{NC}L_M$)) after 14 days of exposure. Bars with no letters are not significantly different. No differences in mortality rates were observed among different treatments after 7 days.

Table 4: Results of GLM analysis (general linear model) for mortality rates observed in isopods after 7 and 14 days of exposure to soil (contaminated and non-contaminated) and poplar litter (non-contaminated, contaminated and mixed)

Source of Variation	Mean square		df		F-test		p-value	
	after 7 days	after 14 days	after 7 days	after 14 days	after 7 days	after 14 days	after 7 days	after 14 days
Model	0.521	2.283	5	5	0.829	2.383	0.53	0.054
Intercept	20.021	56.333	1	1	31.882	58.783	0.00	0.00
Soil	0.521	4.083	1	1	0.829	4.261	0.37	0.05
Litter	0.021	2.646	2	2	0.033	2.761	0.97	0.08
Soil x Litter	1.021	1.021	2	2	1.626	1.065	0.21	0.35
Error	0.628	0.958	42	42				
Total			48	48				

Metal body burdens for zinc, copper, and lead were analyzed using Atomic absorption spectroscopy for isopods exposed to two different treatments: (a) contaminated soil and contaminated litter, and (b) non-contaminated soil and non-contaminated litter. Results of metal analysis showed that zinc and copper body burdens for isopods in contaminated treatment (soil and litter) were significantly higher than the ones exposed to the non-contaminated (soil and litter) treatment ($p< 0.05$, two-tail T-test, Fig. 4). However, I did not find any significant difference between body burdens measured for lead in isopods exposed to these two treatments ($p>0.05$, two-tail T-test, Fig. 4).

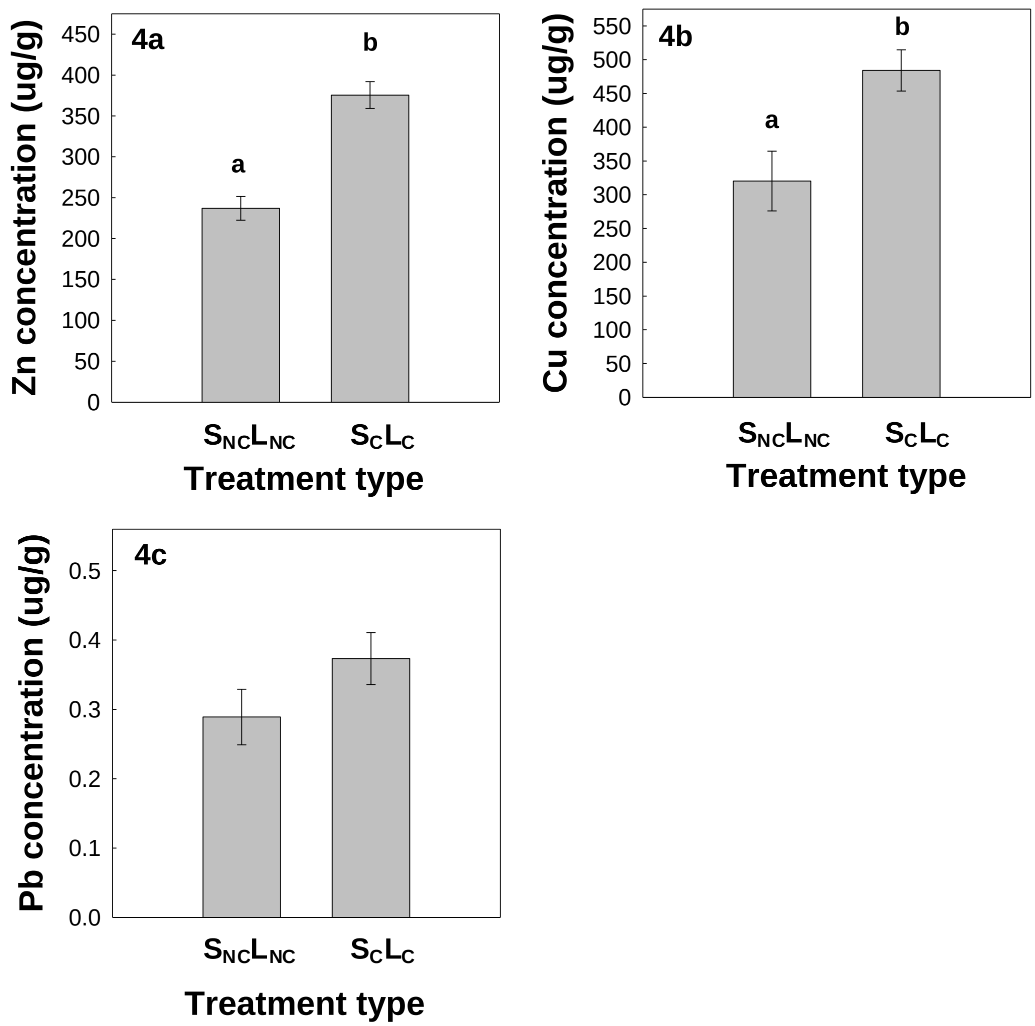

Fig. 4: (a) Zinc, (b) copper, and (c) lead body concentrations (mean ± 1 Std. error) of *P. muscorum* after 14 days of exposure to non-contaminated (soil and litter) and contaminated (soil and litter) treatments. Letters indicate significant differences between metal concentrations ($p<0.05$, two tailed T-test). Bars with no letters are not significantly different.

4. Discussion

The present study examined the growth and metal body burdens in the terrestrial isopod, *P. muscorum* exposed to soil and poplar litter contaminated with heavy metals. Overall, results of GLM analysis revealed that weight change in isopods was negatively affected by metal contamination of both litter and soil after 7 days of exposure. However, after 14 days, contaminated soil had a significant negative impact on weight change in isopods as compared to contamination of the leaf litter (Table. 3). One possible explanation for the growth reduction due to metal contamination might be related to a shift in energy budget, which means that energy is spent for physiological repair and to tolerate the contaminant rather than on growth (Donker 1992; Sibly and Calow 1989). Another explanation for the decrease in growth could be avoidance behavior resulting in reduced intake of food due to metal contamination (Zidar et al. 2005; Zidar et al. 2003).

Previous studies have reported negative impacts on the growth of isopods exposed to elevated concentrations of metals in food (over a short period of time, e.g. 7, 14 days). For instance, Godet et al. (2011) observed a significant decrease in growth of the terrestrial isopod *Porcellio scaber* (from 7th day and after 14th, 21st and 28 days of exposure) fed with poplar leaf litter contaminated with Zn (1861 mg kg^{-1}), Pb (258 mg kg^{-1}), and Cd (40.9 mg kg^{-1}). Similarly, Odendaal and Reinecke (2004) reported reduced growth in *P. leavis* after two weeks of exposure to oak litter spiked up with zinc (1000 mg kg^{-1}). However, only few studies have assessed the effects of metal contaminants in isopods using soil as route of exposure. Løkke and Van Gestel (1998) studied the effects on growth of *P. scaber* juveniles exposed to soil contaminated with copper chloride and reported a fifty percent inhibition in growth at a concentration of 1858 mg kg^{-1} (EC50 value). Tourinho et al. (2015) evaluated toxic effects of AgNPs and ionic Ag exposures via soil in *P. pruninosus* and found significant biomass loss and reduced food consumption after 14 days of exposure. Similar effects on body weight of isopods have been reported in studies after exposure to chemical toxicants (e.g. benomyl, abamectin) through soil (Vink et al. 1995; Kolar et al. 2010). In addition to reduced body weight, higher assimilation rates of contaminants in isopods have been reported from soil as compared to dietary exposures (Vink et al. 1995; Sousa et al. 2000; Tourinho et al. 2015). The difference in the assimilation rates might be due to lower bioavailability of contaminants via dietary exposure than soil because of higher organic content of food resulting in stronger binding of contaminants (Vink et al. 1995; Sousa et al. 2000). In the present study as well, weight change of isopods after 14 days was negatively impacted by contaminated soil as compared to litter (Table. 3), which might be due to greater exposure of metals via soil than litter because of the higher metal assimilation rates from soil as compared to litter.

Overall mortality in isopods after two weeks of exposure to metal contaminated soil and litter did not vary significantly between all the six treatments; however it did seem to be approaching significance (GLM analysis, p=0.054, Table 4). In addition, soil contamination significantly impacted mortality as compared to contamination of poplar litter (Table 4). The negative impact of soil contamination might be due to reduced consumption rates as found by Tourinho et al. (2013 & 2015) who observed a significant decline in food consumption of isopods after two weeks of exposure to ZnO and ionic Ag NP via soil. Result of the present study are also in agreement with findings of Godet et al. (2003) showing that exposure to metal contaminated poplar (for 28 days) litter did not have a significant impact on the mortality of isopods (*P. scaber*). Similarly, Zidar et al. (2005) reported that exposure to cadmium contaminated food (hazel leaves, gelatin and fish food) over a period of three weeks did not result in an increase in mortality of *P. scaber*.

In addition to the negative effects of metals on mortality, significant growth impairment in isopods was also observed (Table 3). Since, in isopods, the number of offspring's born per female is proportional to female body size (Sutton et al. 1984), a negative impact on growth in females can eventually affect population size and dynamics at metal contaminated sites.

In contaminated environments, metal intake in isopods can occur through different routes including orally by ingestion of contaminated soil and food particles (Koster et al. 2005; Udovic et al. 2009; Vijver et al. 2006) and via pleopods which draw soil pore water through capillary action (Sutton 2013). The results of this study showed higher zinc and copper body burdens ($p<0.05$) in isopods exposed to contaminated soil and litter as compared to ones exposed to non-contaminated soil and litter, whereas lead body burdens did not differ between the two treatments. For a similar exposure time, Zidar et al. (2003) also reported a comparable increase in zinc body burdens of isopods fed with contaminated litter. In case of metal exposure through soil, an increase in accumulated concentration of zinc in isopods (*P. scaber* after 14 days of exposure) has been shown with increasing soil metal concentrations (Udovic et al. 2009). Clearly, internal concentrations of zinc in isopods can be affected by both exposure routes (soil and litter). Furthermore, studies analyzing accumulation kinetics of zinc in isopods showed that intake of zinc from soil or food is generally dependent on its concentration, and accumulation might be a net result of uptake from different exposure routes (Vijver et al. 2006). In this study, I did not record the feeding or consumption rates of litter, therefore, I could not distinguish the influence of individual exposure routes (soil or litter) on the zinc body concentrations of isopods. Regarding copper body burdens, I found that copper body burdens of isopods (320 µg/g and 466 µg/g in isopods exposed to $S_{NC}L_{NC}$ and $S_{C}L_{C}$ treatments respectively) were several times higher than concentrations of litter (21.49 µg/g and 9.0 µg/g in non-contaminated and contaminated litter respectively). The findings of this study are supported by Hopkin and Martin (1982) who reported that internal copper concentrations of isopods in metal contaminated environments are generally several times higher than concentrations in food. Copper is an essential element and is required for the production of hemocyanine; the respiratory pigment in isopods (Wieser 1965). Terrestrial isopods tend to accumulate excessive amounts of copper which is stored for a life time in the S-cells of hepatopancreas (Martin et al. 1982; Dallinger and Rainbow 1993). I did not account for the amount of litter consumed by isopods, but based on the findings of this study, it appears that the increase in body burdens for copper in isopods might be affected more by contamination of soil as compared to litter as concentrations of copper were higher in contaminated soil than non-contaminated soil, and were lower in contaminated litter as compared to non-contaminated litter (Tables 1&2).

Isopods have been shown to have lower concentration factor (CF, measured as ratio between accumulated concentration in body and concentration of metal in substrate) for lead than other metals such as zinc and copper, and therefore are considered as de-concentrators of lead (Dallinger 1993; Dallinger et al. 1992; Hopkin and Martin 1982). In this study, lead body burdens measured for isopods exposed to the contaminated metal and contaminated litter treatment did not vary with the ones exposed to non-contaminated soil and non-contaminated litter treatment. This result may be explained by effective excretion mechanisms (Witzel 1998). In isopods, borderline metals such as lead and zinc are stored in copper granules of S-cells as well as in the granules of B-cells of hepatopancreas. In comparison to S-cells, which serve the purpose of long term storage, metal storage in B-cells is temporary as cell contents are voided daily via aprocrine secretion (Hopkin and Martin 1982; Dallinger and Prosi 1988). Studies have shown that a difference in relative proportions of metals assimilated in B and S-cells of hepatopancreas can influence the net accumulated concentration of metals in the body. For example, Hopkin (1990) showed that zinc body burdens in isopods *O. asellus* were significantly lower than *P. scaber* sampled from the same metal contaminated site. The author explained that this was possibly due to *O. asellus* storing most of zinc in B-cells than S-cells, thereby, excreting zinc deposited in these cells as compared to *P. scaber* which assimilated zinc predominantly in the S-cells of hepatopancreas. The lack of increase in lead body burdens in isopods observed in this study may be related to isopods assimilating lead mostly in B-cells in the hepatopancreas, which might be lost readily because of the rapid turnover of B cell cytoplasm (Hames 1989).

To summarize, results of the present study demonstrate that litter and soil contamination can affect growth of the terrestrial isopod, *P. muscorum*. Overall, soil metal contamination had a significant negative effect on growth of isopods than contamination of leaf litter. Mortality in isopods seemed to be approaching significance after 14 days. Since, this study was conducted for a relatively short time (14 days) as compared to the life span (2-3 years) of isopods, it is likely that long term exposures might result in significantly higher mortality rates. Exposure to contaminated litter and soil for a short period of time (14 days) resulted in a significant increase of zinc and copper body burdens of isopods. Based on results of this study, it is concluded that weight change of *P. muscorum* can be used as a potential tool to evaluate soil and litter contamination. The results of this study also showed the importance of soil as a route of metal exposure in isopods, which is significant because most of the toxicity studies tend to focus only on evaluating effects of contaminants through food. However, in this study, isopods were exposed to combined treatments of soil and litter. To further our understanding on the influence of different metal exposure routes in isopods, it is recommended that future toxicity studies should also consider testing the effects of metals via individual exposure routes (soil or litter).

References:

Anderson J, Ineson P, Huish S. 1983. Nitrogen and cation mobilization by soil fauna feeding on leaf litter and soil organic matter from deciduous woodlands. Soil Biology and Biochemistry 15(4): 463-467.

Calhôa CF, Soares AM, Loureiro S. 2012. Effects on survival and reproduction of Porcellio dilatatus exposed to different Cd species. Ecotoxicology 21(1): 48-55.

Dallinger R, Rainbow PS. 1993. Ecotoxicology of metals in invertebrates: CRC Press.

Dallinger R. 1993. Strategies of metal detoxification in terrestrial invertebrates. Ecotoxicology of metals in invertebrates 245.

Dallinger R, Berger B, Birkel S. 1992. Terrestrial isopods: useful biological indicators of urban metal pollution. Oecologia 89(1): 32-41.

Dallinger R, Prosi F. 1988. Heavy metals in the terrestrial isopod *Porcellio scaber* Latreille. II. Subcellular fractionation of metal-accumulating lysosomes from hepatopancreas. Cell Biology and Toxicology 4(1): 97-109.

Dias N, Sprung M. 2003. Population dynamics and production of the isopod, *Tylos ponticus* in a Ria Formosa saltmarsh (south Portugal). Crustaceana Monographs 2: 133-149.

Donker M. 1992. Energy reserves and distribution of metals in populations of the isopod Porcellio scaber from metal-contaminated sites. Functional ecology: 445-454.

Donker MH, Raedecker MH, Van Straalen NM. 1996. The role of zinc regulation in the zinc tolerance mechanism of the terrestrial isopod *Porcellio scaber*. Journal of Applied Ecology: 955-964.

Drobne D, Štrus J. 1996. Moult frequency of the isopod *Porcellio scaber*, as a measure of zinc- contaminated food. Environmental Toxicology and Chemistry 15(2): 126-130.

Farkas S, Hornung E, Fischer E. 1996. Toxicity of copper to Porcellio scaber Latr.(Isopoda) under different nutritional status. Bulletin of environmental contamination and toxicology 57(4): 582-588.

Gallagher FJ, Pechmann I, Bogden JD, Grabosky J, Weis P. 2008. Soil metal concentrations and vegetative assemblage structure in an urban brownfield. Environmental Pollution 153(2): 351-361.

Gallagher FJ, Pechmann I, Holzapfel C, Grabosky J. 2011. Altered vegetative assemblage trajectories within an urban brownfield. Environmental Pollution 159(5): 1159-1166.

Godet J-P, Demuynck S, Waterlot C, Lemière S, Souty-Grosset C, Scheifler R, et al. 2011. Growth and metal accumulation in *Porcellio scaber* exposed to poplar litter from Cd-, Pb-, and Zn-contaminated sites. Ecotoxicology and Environmental Safety 74(3): 451-458.

Hames CA. 1989. The digestive system and metals in terrestrial isopods: University of Reading.

Hanlon R, Anderson J. 1980. Influence of macroarthropod feeding activities on microflora in decomposing oak leaves. Soil Biology and Biochemistry 12(3): 255-261.

Hassall M, Sutton S. 1978. The role of isopods as decomposers in a dune grassland ecosystem. In: Proceedings of the Scientific Proceedings of the Royal Dublin Society A, Vol. 6. 235-245.

Hassall M, Turner J, Rands M. 1987. Effects of terrestrial isopods on the decomposition of woodland leaf litter. Oecologia 72(4): 597-604.

Heikens A, Peijnenburg W, Hendriks A. 2001. Bioaccumulation of heavy metals in terrestrial invertebrates. Environmental Pollution 113(3): 385-393.

Hopkin S, Martin M. 1982. The distribution of zinc, cadmium, lead and copper within the woodlouse *Oniscus asellus* (Crustacea, Isopoda). Oecologia 54(2): 227-232.

Kampe S, Schlechtriem C. 2016. Bioaccumulation of hexachlorobenzene in the terrestrial isopod Porcellio scaber. Environmental Toxicology and Chemistry 35(11): 2867-2873.

Köhler H-R, Hiittenrauch K, Berkus M, Gräff S, Alberti G. 1996. Cellular hepatopancreatic reactions in *Porcellio scaber* (Isopoda) as biomarkers for the evaluation of heavy metal toxicity in soils. Applied Soil Ecology 3(1): 1-15.

Koster M, Reijnders L, van Oost NR, Peijnenburg WJ. 2005. Comparison of the method of diffusive gels in thin films with conventional extraction techniques for evaluating zinc accumulation in plants and isopods. Environmental Pollution 133(1): 103-116.

Lapanje A, Drobne D, Nolde N, Valant J, Muscet B, Leser V, et al. 2008. Long-term Hg pollution induced Hg tolerance in the terrestrial isopod Porcellio scaber (Isopoda, Crustacea). Environmental Pollution 153(3): 537-547.

Løkke H, van Gestel CAM. 1998. Handbook of Soil Invertebrate Toxicity Tests: Wiley.

Loureiro S, Sampaio A, Brandão A, Nogueira AJ, Soares AM. 2006. Feeding behaviour of the terrestrial isopod Porcellionides pruinosus Brandt, 1833 (Crustacea, Isopoda) in response to changes in food quality and contamination. Science of the Total Environment 369(1): 119-128.

Loureiro S, Soares AM, Nogueira AJ. 2005. Terrestrial avoidance behaviour tests as screening tool to assess soil contamination. Environmental Pollution 138(1): 121-131.

Martin MH, Duncan EM, Coughtrey PJ. 1982. The distribution of heavy metals in a contaminated woodland ecosystem. Environmental Pollution Series B, Chemical and Physical 3(2): 147-157.

Odendaal J, Reinecke A. 1999. The toxicity of sublethal lead concentrations for the woodlouse, *Porcellio laevis* (Crustacea, Isopoda). Biology and fertility of soils 29(2): 146-151.

Odendaal PJ, Reinecke JA. 2004. Effect of metal mixtures (Cd and Zn) on body weight in terrestrial isopods. Archives of Environmental Contamination and Toxicology 46(3): 377-384

Paoletti MG, Hassall M. 1999. Woodlice (Isopoda: Oniscidea): their potential for assessing sustainability and use as bioindicators. Agriculture, Ecosystems & Environment 74(1): 157-165.

Quadros AF, Araujo PB. 2007. Ecological traits of two neotropical oniscideans (Crustacea: Isopoda).

Sibly R, Calow P. 1989. A life‐ cycle theory of responses to stress. Biological Journal of the Linnean Society 37(1‐ 2): 101-116.

Smit C, Van Gestel C. 1996. Comparison of the toxicity of zinc for the springtail *Folsomia candida* in artificially contaminated and polluted field soils. Applied Soil Ecology 3(2): 127-136.

Špaldoňová A, Frouz J. 2014. The role of *Armadillidium vulgare* (Isopoda: Oniscidea) in litter decomposition and soil organic matter stabilization. Applied Soil Ecology 83: 186-192.

Sutton S, Hassall M, Willows R, Davis R, Grundy A, Sunderland K. 1984. Life histories of terrestrial isopods: a study of intra-and interspecific variation. In: Proceedings of the Symposia of the Zoological Society of London, Cambridge University Press, 269-294

Sutton S. 2013. Woodlice: Elsevier.

Tourinho PS, van Gestel CAM, Lofts S, Soares AMVM, Loureiro S. 2013. Influence of soil pH on the toxicity of zinc oxide nanoparticles to the terrestrial isopod *Porcellionides pruinosus*. Environmental Toxicology and Chemistry 32(12): 2808-2815.

Tourinho PS, van Gestel CAM, Jurkschat K, Soares AMVM, Loureiro S. 2015. Effects of soil and dietary exposures to Ag nanoparticles and AgNO3 in the terrestrial isopod *Porcellionides pruinosus*. Environmental Pollution 205: 170-177.

Udovic M, Drobne D, Lestan D. 2009. Bioaccumulation in *Porcellio scaber* (Crustacea, Isopoda) as a measure of the EDTA remediation efficiency of metal-polluted soil. Environmental Pollution 157(10): 2822-2829.

Vijver MG. 2005. The ins and outs of bioaccumulation: metal bioaccumulation kinetics in soil invertebrates in relation to availability and physiology.

Vijver MG, Vink JP, Jager T, Van Straalen NM, Wolterbeek HT, Van Gestel CA. 2006. Kinetics of Zn and Cd accumulation in the isopod Porcellio scaber exposed to contaminated soil and/or food. Soil Biology and Biochemistry 38(7): 1554-1563.

Vink K, Tompot A, Hermans M, van Straalen NM, Dewi I, Bedaux J. 1995. The importance of the exposure route when testing the toxicity of pesticides to saprotrophic isopods. Environmental Toxicology and Chemistry 14(7): 1225-1232.

Wieser W. 1965. Electrophoretic studies on blood proteins in an ecological series of isopod and amphipod species. Journal of the Marine Biological Association of the United Kingdom 45(02): 507-523.

Zidar P, Drobne D, Štrus J, Blejec A. 2003. Intake and assimilation of zinc, copper, and cadmium in the terrestrial isopod *Porcellio scaber* Latr.(Crustacea, Isopoda). Bulletin of Environmental Contamination and Toxicology 70(5): 1028-1035.

Zidar P, Božič J, Štrus J. 2005. Behavioral response in the terrestrial isopod *Porcellio scaber* (Crustacea) offered a choice of uncontaminated and cadmium-contaminated food. Ecotoxicology 14(5): 493-502.

Zimmer M, Oliveira R, Rodrigues E, Graça MA. 2005. Degradation of leaf litter phenolics by aquatic and terrestrial isopods. Journal of Chemical Ecology 31(8): 1933-1952.

Žnidaršič N, Štrus J, Drobne D. 2003. Ultrastructural alterations of the hepatopancreas in *Porcellio scaber* under stress. Environmental Toxicology and Pharmacology 13(3): 161-174.