Ecosystem-wide Transfer of Trace Metal Pollutants from Derelict Metalliferous Mines in the UK

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Abstract

Trace metals are persistent elemental pollutants that can be toxic at relatively low concentrations. While trace metals are present in the Earth's crust at low concentrations, anthropogenic activities, particularly mining, can lead to the accumulation of high concentrations of trace metals. Trace metals can then be inadvertently dispersed throughout the surrounding environments, where they can potentially pose health risks to local fauna. The aim of this project was to assess trace metal contamination across ecosystems, focusing on the distribution of trace metals across landscapes, its transfer into local wildlife, and the potential human health risks.

This project primarily focused on evaluating the ecosystems around two separate derelict metalliferous mine complexes in western Wales. Environmental and animal samples were collected at both sites across three years to assess trace metal contamination. Water, sediment, and soil at the mines and in downstream private properties had elevated Pb, Zn, and, to a lesser extent, Cd and Cu concentrations when compared to nearby control sites. Aquatic invertebrate communities were only slightly less abundant and diverse at the mines than the control sites, but individual invertebrate Pb, Cd, Zn, and Cu body burdens were significantly higher at the mines and private properties than at the control sites. Invertebrate Pb body burdens were particularly high, with approximately one third of the mine invertebrates accumulating Pb to concentrations higher than those found in the surrounding sediments. Rodents at the mines and private properties also had elevated tissue Pb concentrations, potentially indicative of toxicity. Wood mice (*Apodemus sylvaticus*) at the mines and private properties had significantly higher Pb and Cd tissue concentrations than wood mice collected at the control sites, but appeared to be able to regulate their Zn and Cu tissue concentrations through homeostatic mechanisms.

Humans living near these mine complexes could also potentially be exposed to high concentrations of trace metals. To examine exposure routes, the trace metal contents in eggs produced near a metal mine were assessed with respect to human consumption. The eggs contained high concentrations of Pb, and their regular consumption could pose health risks, particularly in young children. Small-scale studies on vegetables grown near mines indicated that they contained similarly elevated, and potentially toxic, concentrations of Pb.

The relationships between environmental and wildlife trace metal concentrations were also explored across a larger, heterogeneously contaminated landscape. The elemental concentrations of livers from European badgers (*Meles meles*) collected across the English Midlands were determined and compared with local soil elemental concentrations, established using datasets from the British Geological Survey. While the badgers appeared to regulate essential elements, such as Cr, Cu, K, Mn, and Zn, the liver Ag, As, Cd, and Pb concentrations correlated with local soil concentrations, suggesting environmental accumulation. The badgers with the highest Pb and Cd liver concentrations were consistently found in Derbyshire, a county with a long Pb-mining legacy.

Overall, trace metal pollution can have significant, ecosystem-wide effects long after the original pollution event. Trace metals can be distributed beyond the original pollution site and accumulate at potentially toxic concentrations in environments that appear pristine. They can also transfer into local wildlife at concentrations indicative of toxicity, and humans could be at risk through the ingestion of locally grown food products. These factors make mine site remediation equally important and difficult, since remediation must extend beyond the mine site itself, and must address the potential exposure and health risks for wildlife and humans. As the demand for metals increases in the future, and as extreme weather events caused by climate change become more frequent, trace metal dispersal will continue. It is therefore increasingly imperative that the effects of trace metal pollution on ecosystems are fully understood in order to effectively mitigate their substantial adverse impacts.

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Declaration

Unless otherwise acknowledged, the work presented in this thesis is original. No part has been submitted for another degree at The University of Nottingham or elsewhere. Any views expressed in the dissertation are those of the author.

Signed: 

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Chapter 1 – Introduction and Literature Review

1.1 Summary

This chapter provides background information on trace metals, including how trace metals are introduced into the environment through anthropogenic activities, and the uptake and effects of trace metals on animals living in contaminated landscapes. The chapter ends with a summary of the aims and research questions of this project, and an overview of the structure of the written thesis.

1.2 Trace Metals

The definition of trace metal varies across literature, depending on the context and study system. While 'metal' directly refers to elements traditionally classified as metals, based on their physical and chemical characteristics (such as high density, malleability, conductivity), the term 'trace' can be used either to refer to the metal's presence at trace amounts within the environment, or within plants or animals (Rainbow, 2018). There is also continued debate on whether to use the term 'trace metal' or 'heavy metal', as both have been historically used in the literature interchangeably. As 'heavy' implies a certain density threshold for these elements, which would include metals not necessarily considered 'heavy metals' (such as radioactive metals like radium), for clarity, this term is not used in this thesis (Rainbow, 2018).

To avoid confusion regarding biological functions of trace metals (which vary greatly depending on the metal in question), in this thesis, trace metals will be defined as natural elements that are present in small quantities in the environment (Rainbow, 2018; Kalisińska, 2019). These metals can be nutritionally essential, serving as key components of several enzymes and participating in oxidation-reduction reactions, or they can be biologically non-essential and serve no known metabolic role (Rainbow, 2002; Nagajyoti, et al., 2010; Tchounwou et al., 2012; Rainbow, 2018; Kalisińska, 2019). Essential trace metals include chromium (Cr), copper (Cu), manganese (Mn), molybdenum (Mo), and zinc (Zn), while non-essential trace metals include aluminium (Al), cadmium (Cd), and lead (Pb) (Rainbow, 2018; Kalisińska, 2019). This thesis will particularly focus on two non-essential elements, lead and cadmium, and two essential elements, zinc and copper, though other trace metals will be discussed when appropriate. Whether essential or non-essential, all trace metals are toxic to plant and animal life if assimilated at high enough concentrations and over a sufficiently long

period of time, and most are toxic at relatively low concentrations (Gall et al., 2015; Rainbow, 2018).

Trace metals are found across the Earth's crust, but are rarely naturally present at high enough concentrations to pose a toxic threat (Tchounwou et al., 2012). Anthropogenic activities, most notably mining, can affect this by unearthing and accumulating trace metals on the land's surface (Tchounwou et al., 2012). Mining processes, such as grinding ores, filtering output, smelting metals, and disposing of the mine waste, can cause trace metals to be dispersed at high concentrations into the surrounding ecosystem (Beavington, 1975; Vivian & Massie, 1970; Jung & Thornton, 1996; Li et al., 2015). Mining has been common across the globe for thousands of years, making trace metal pollution a problem that affects every human-populated continent (Jung & Thornton, 1996; Lottermoser et al., 1999; Aucamp & van Schalkwyk, 2003; Smolders et al., 2003; Reglero et al., 2008; del Pilar Ortega-Larrocea et al., 2010; Schwanck et al., 2016). Trace metals present a serious global health issue and can have drastic effects on ecosystems and, specifically, on animal health (Jung & Thornton, 1996; Li et al., 2014; Bortey-Sam et al., 2015; Gall et al., 2015).

1.3 History of Trace Metal Mining

Mining activities are one of the most common causes of trace metal contamination in surface environments (Tchounwou et al., 2012). During mining, trace metals can be extracted and sorted into mining waste. Over time, the amount of trace metals extracted accumulates to high, and often toxic, concentrations. While many trace metals are inadvertently extracted and treated as waste materials, some metals, most commonly lead, zinc, and copper, were, and still are, intentionally mined (Waldron, 1973; Dudka & Adriano, 1997; Hong et al., 1996; Fashola et al., 2016).

Lead, zinc, and copper have been mined globally for several millennia (Waldron, 1973; Hong et al., 1996; Hernberg, 2000; Rainbow, 2018). Both copper and lead are easily workable metals, capable of being melted and moulded over an open wood fire (Waldron, 1973; Joseph, 1999; Hernberg, 2000). Furthermore, copper can be combined with other metals, such as zinc, to form strong and versatile alloys (Joseph, 1999). Historically, human populations used lead and copper to make a variety of important items, including weaponry, ornamentation, cosmetics, and even medications (Bryan, 1930/1974; Waldron, 1973; Lewis, 1985; Joseph, 1999; Hernberg, 2000). Copper use in particular shaped the development of humanity so thoroughly that one of the ancient historical ages has since been named the "Bronze Age", after the copper/tin alloy (Joseph, 1999; Rainbow, 2018). As more complex

civilizations developed around the world, metal use further increased, peaking with the Romans, who used large amounts of copper for their coinage and lead for their plumbing (Hong et al., 1996; Joseph, 1999). Throughout this period, trace metals were mined and worked so extensively that it led to hemispheric-scale atmospheric copper and lead pollution (Hong et al., 1996). While metal working globally decreased after the fall of the Western Roman Empire, throughout the Common Era new uses were discovered for these metals, such as the use of lead in book printing, shipbuilding, and the manufacturing of guns and ammunition (Lewis, 1985; Dudka & Adriano, 1997; Hong et al., 1996; Hernberg, 2000; Le Roux et al., 2004). This prompted an unprecedented increase in trace metal demand and therefore mining, starting in the eighteenth century and continuing through the industrial age (Shotyk et al., 1998; Hernberg, 2000). More recently, lead was used in lead-based paints and leaded gasoline (as tetraethyl lead), both of which caused serious adverse health impacts and were subsequently globally phased out of use (Lewis, 1985; Wu & Boyle, 1997; Hernberg, 2000; Jacobs et al., 2002). Currently, lead, zinc, and copper are still mined, and are primarily used in electronics and construction (roofing and plumbing) (Hong et al., 1996; Dudka & Adriano, 1997; Rainbow, 2018).

While mining occurs around the world, few areas have been mined as extensively or for as long a period of time as the British Isles. Historically, there was intensive metalliferous mining in Great Britain from the Bronze Age onwards, and the extensive lodes found in modern-day England and Wales were part of what made southern Great Britain a valuable addition to the Roman Empire (Environment Agency Wales, 2002; Mighall et al., 2002; Rainbow, 2018). Mining peaked during the period of 1700-1900, primarily due to the increased demand for metals during the industrial age (Alloway & Davies, 1971a; Davies, 1987; Fuge et al., 1993; Environment Agency Wales, 2002; Rainbow, 2018). The mining efforts at this time were so intensive that, during the mid-1800's, the UK was the world's primary producer of lead, tin, and copper (Davies, 1987; Fuge et al., 1993). Lead and copper mining efforts were primarily based in the Lake District in Cumbria and the Peak District in Derbyshire in England, and in Wales (Rainbow, 2018). Other trace metals, such as arsenic, mercury, and cadmium, were also unintentionally extracted from these mines, though they were generally treated as waste materials and discarded (Waldron, 1973; Davies, 1987). During the early 1900's, it became cheaper to import trace metals from abroad than to continue to work the increasingly depleted UK mines, so metalliferous mines across the UK were gradually closed (Environment Agency Wales, 2002). Now, thousands of abandoned mines can be found across Great Britain, with over 1,300 derelict mines found across Wales

alone (Kitts & Smith, 1996; Environment Agency Wales, 2002). Despite the closures, these mines remain known sources of significant environmental pollution (Environment Agency Wales, 2002). Therefore, these ex-mining regions present excellent opportunities to better understand the impact of trace metal pollution from derelict mines on the surrounding environment and resident animals.

1.4 Introducing Trace Metals into the Environment

During historical mining activities, both waste and targeted materials were deposited into the surrounding environment due to inefficient mining and separation techniques and a limited understanding of the consequences of environmental pollution. Mining involves both extracting raw ore from the earth and separating the ore to isolate the target substance(s). In Wales and the Peak District, ore separation was most frequently done with the aid of nearby streams or rivers (Alloway & Davies, 1971a; Davies, 1987). The ore separating process began by using water wheels to crush the ore, and the resulting material was then placed in "buddles", or a series of shallow pits (Davies, 1987). Water would be allowed to flow through these pits and the lighter portions of the ore (gangue minerals) would be carried in suspension into the neighbouring buddle, leaving behind the heavier target metal ore (e.g. galena, the natural mineral form of lead(II) sulfide) (Davies, 1987). While this method was simple and easy to implement, it was also inefficient, often resulting in the loss of large quantities of the target material. Using buddles also produced waste water that was highly contaminated by both the target substance and other, inadvertently extracted, trace metals. This water was frequently disposed of directly into nearby waterways without consideration or knowledge of the toxic material it contained (Davies, 1987).

Once trace metals have been introduced into waterways, they can disperse throughout the surrounding streams and rivers. While larger, coarse particles drop out of the water column and settle into the sediment relatively quickly, fine particles remain in the water column and can, potentially, travel the full length of the river system, reaching estuarine and marine environments (Ip et al., 2007). Trace metals that have settled into sediments can become re-suspended if the sediment is disturbed; notable disturbance events been known to cause critical pollution events, even many years after the trace metals were originally deposited (Sutherland, 2000). Furthermore, trace metals are not restricted to the waterway in which they were originally introduced. Once in the water column, trace metals can seep through the ground and contaminate groundwater, spreading far beyond the original polluted stream or river (Lee et al., 2007; El Khalil et al., 2008; Hashim et al., 2011). Additionally, if a

polluted waterway floods, trace metals present in the water can be distributed across a larger, previously inaccessible, area (Alloway & Davies, 1971a; Albering et al., 1999; Foulds et al., 2014). Trace metals can then be deposited directly into topsoil, or they can reach other, previously non-contaminated waterways and groundwater (Alloway & Davies, 1971a; Alloway & Davies, 1971b).

Trace metals from mine waste do not need to be deposited into water to have an environmental impact. Mine spoil and other mining waste materials, which often included high concentrations of various trace metals, were commonly stored in large heaps throughout mine sites (Johnson, 2003). Once these spoil heaps were established, they were most frequently abandoned, and only rarely remediated, even after the mine was closed, so many have remained for hundreds of years (Johnson, 2003). Because of the high levels of trace metal contamination and the low amounts of water retention in these heaps, vegetative growth is often inhibited, causing waste heaps to remain bare even decades or centuries after the associated mine has closed (Milton et al., 2003; Alhar et al., 2021). Due to the lack of vegetative cover and the unstable nature of the waste heaps, both air and water erosion can frequently occur, leading to the dispersal of trace metal particulates into the surrounding lands and waterways (Brotons et al., 2010).

Once miners had separated minerals such as sphalerite (ZnS) and galena (PbS) from waste materials, they still needed to extract the target metals. They did this through smelting, which extracts metals from ore through the use of heat and an added agent (e.g. charcoal) (Rainbow, 2018). Smelting releases a variety of substances into the air, including trace metal oxide particulates, such as lead monoxide (Swaine, 2000). Once released through smelting, trace metal particulates generally settle in the soil within 1 to 2 km of the source, though if particulates settle in waterways, they can travel much further (Beavington, 1975; Kachenko & Signh, 2006; Li et al., 2015). At many mines around the world smelting was (or still is) done directly on site. However, in Wales, smelting was generally restricted to specific smelting complexes, the largest of which was located in the Lower Swansea Valley in South Wales (Vivian & Massie, 1970; Davies, 1987).

Because trace metals do not degrade, once they are introduced into the environment through inefficient mine waste disposal, accidental ore loss, or smelting emissions, they remain indefinitely, and are removed only by soil erosion (Pyatt et al., 2000; Wilson & Pyatt, 2007). Even derelict mines can continue to release trace metals into the environment through spoil heap erosion or resuspension of contaminated sediments (Wilson & Pyatt, 2007). This redistribution of trace metals in polluted areas may further increase in the future, as climate

change may cause more flooding and extreme weather events (Jentsch & Beierkuhnlein, 2008; Hirabayashi et al., 2013; Foulds et al., 2014).

1.5 Trace Metals in Vegetation

Most plants inadvertently take up non-essential trace metals through imperfect element selection when attempting to extract essential elements from the soil (Schützendübel & Polle, 2002; Clemens & Ma, 2016; Singh et al., 2016). While plants are generally able to regulate their uptake of essential trace metals, they typically are unable to do so for non-essential trace metals, since these metals are not necessary for life and therefore do not have closely-regulated pathways (van der Ent, 2013). Once inside plants, non-essential trace metals utilize the same pathways as essential elements to move throughout the whole of the plant structure, including shoots, leaves, fruits, and seeds (Andrews et al., 1989; Schützendübel & Polle, 2002; Clemens & Ma, 2016; Singh et al., 2016). Plants exposed to high concentrations of any trace metals, either essential or non-essential, can exhibit stress indicators and experience suppressed root growth and an overall decrease in both growth and yield (Singh et al., 2016). Some plants have defence mechanisms to protect against trace metal toxicity, either by barring trace metals from travelling to aboveground plant parts, or by selectively transporting trace metals to aboveground plant parts, where they are then sequestered and rendered non-reactive (Reglero et al., 2008; Krämer, 2010; Gall et al., 2015; Singh et al., 2016; Reeves et al., 2018). However, these plants are rare, and they cannot grow in soils with extremely high trace metal concentrations, so the likelihood of their growth on spoil heaps is low (Del Río et al., 2002; Ashraf et al., 2011; van der Ent et al., 2013; Mahar et al., 2016). Generally, any vegetation in heavily trace metal polluted areas struggles to grow and thrive (Ashraf et al., 2011).

1.6 Trace Metal Exposure in Fauna

Animals living in metal-contaminated environments generally have relatively high trace metal concentrations within their bodies (Burrows & Whitton, 1983; Shore, 1995; Goodyear & McNeill, 1999; Heikens et al., 2001; Wilkinson et al., 2003; Sánchez-Chardi et al., 2007; Wijnhoven et al., 2007; Gall et al., 2015). Animals are most commonly exposed to trace metals through water, diet, or inadvertent soil ingestion (Thornton & Abrahams, 1983; Posthuma & Van Straalen, 1993; Stewart et al., 1999; Burger, 2002; Swarup & Dwivedi, 2002; Nicholson et al., 2003; Beyer et al., 2007; Millán et al., 2008; Reglero et al., 2008; Reglero et al., 2009; Smith et al., 2009; Berglund et al., 2011; Mahajan et al., 2012; Gall et

al., 2015). The ingestion of contaminated substances, including plants, animals, or human-made items containing trace metals, such as lead based paint chips or lead shot, is a common source of trace metal contamination (Lewis et al., 2001; Swarup & Dwivedi, 2002; Wang, 2002; Battaglia et al., 2005). Some taxa, primarily invertebrates, are also exposed to trace metals through absorption directly through their exoskeleton or other body coverings (Bryan & Uysal, 1978; Posthuma & Van Straalen, 1993; Casey et al., 2005; Gall et al., 2015).

Inadvertent soil ingestion is one of the primary trace metal exposure pathways (Thornton & Abrahams, 1983). It is common for herbivores to ingest soil while grazing (Thornton & Abrahams, 1983; Beyer et al., 2007). For example, soil makes up 18% of dry matter intake for cattle, and up to 30% for sheep (Thornton & Abrahams, 1983). Grazing herbivore soil ingestion percentages can increase further in areas with sparse vegetation cover, as is found in mining-affected areas (Shinde et al., 2005). It has been found that up to 90% of trace metal intake in cattle is due to soil ingestion, likely because contaminated soil generally contains much higher trace metal concentrations than the plants growing on that soil (Thornton & Abrahams, 1983; Chojnacka et al., 2005). Inadvertent soil ingestion is also not limited to herbivores. For example, sandpipers, birds of the Scolopacidae family which peck for invertebrates in mud or sand, have a diet consisting of 7-30% soil, while carnivores that ingest soil organisms, such as the nine banded armadillo (*Dasypus novemcinctus*), American woodcock (*Scolopax minor*), and raccoon (*Procyon lotor*), have soil intakes which account for 9-17% of their diet (Beyer et al., 1994). Animals, such as ungulates, that use natural salt licks to rectify mineral deficiencies or imbalances inadvertently ingest soil while doing so (Beyer et al., 2007; Ayotte et al., 2008). Deposit feeders, such as the bivalve mollusc *Scrobicularia plana*, also ingest large amounts of sediment while consuming organic matter (Bryan & Uysal, 1978). Even though *Scrobicularia plana* is also exposed to trace metals through absorption of minerals directly from water, sediment ingestion accounts for more than 75% of the cadmium, lead, and zinc found in its system (Bryan & Uysal, 1978). Inadvertent soil ingestion can also result from common non-dietary activities, such as burrowing or grooming, causing a large variety of animals who exhibit these behaviours, from invertebrates such as spiders or burrowing mayfly nymphs (*Hexagenia rigida*) to vertebrates such as bats, badgers, or feral pigeons (*Columba livia*), to be further exposed to trace metals (Clausen, 1986; Beyer et al., 1994; Andres et al., 1998; Rogival et al., 2007; Cleary et al., 2009; Ramirez et al., 2011; Gall et al., 2015; Zukal et al., 2015; Frantz et al., 2016).

Whether ingested within food or soil, trace metals most frequently enter the body through the oral route and are primarily absorbed through the gastrointestinal tract (Vázquez et al., 2015). While trace metals can potentially pass through the gastrointestinal tract and be excreted, excretion rates vary depending on the species and metal in question, with some species having no excretion method for certain trace metals (Law, 1996; Rainbow, 2007; Hauser-Davis et al., 2012). However, this does not mean that all ingested trace metals are functionally absorbed into the body (Ledoux & Shannon, 2005). Trace metal bioavailability (the proportion of the ingested amount that is absorbed and converted to a physiologically active form) depends on a large variety of factors, including: species, age, sex, physiological state, nutrition, gut microbiota, interactions with dietary nutrients, and the chemical form and solubility of the trace metal (Jugo, 1977; Roberts & Johnson, 1978; Komarnicki, 2000; Swarup & Dwivedi, 2002; Ledoux & Shannon, 2005; Breton et al., 2013). For example, because of their differing gastrointestinal tracts, ruminants absorb much lower amounts of selenium and copper than non-ruminants, though the exact cause of this difference is not fully understood (Spears, 2003). Bioavailability also varies greatly between different trace metals (Vázquez et al., 2015). For instance, lead has a mean gastrointestinal absorption rate of 21.3% in humans, while the gastrointestinal absorption rate of cadmium in humans is only 1-7% (Vázquez et al., 2015).

Once trace metals are absorbed into the body, they exist either in a detoxified or a metabolically available form (Rainbow, 2002). Both essential and non-essential trace metals are primarily detoxified through binding to high affinity molecules, such as metallothioneins or insoluble metalliferous granules, and sequestered within a ‘storage organ’, most commonly the kidneys, liver, and, in the case of arthropods and molluscs, the hepato-pancreas (Phillips & Rainbow, 1989; Dodds-Smith et al., 1992; Rainbow, 2002; Rainbow, 2007; Berglund et al., 2011). Trace metals can also be sequestered into epidermal growths such as hair, fur, or feathers, and vertebrates can also store metals in calcified tissues, such as bones, otoliths (calcium carbonate structures found in the inner ear of vertebrates), and scales (Hammer et al., 1971; Roberts & Johnson, 1978; Mugiya et al., 1991; Dauwe et al., 2000; Battaglia et al., 2005; Perugini et al., 2014). Because of this, trace metal body burdens generally increase with age as metals progressively accumulate in the storage organs (Komarnicki, 2000). Trace metals that are not sequestered are instead labile and metabolically active. In the case of essential trace metals, a low metabolically active concentration is necessary to meet vital metabolic needs (Rainbow, 2002). However, a higher concentration of essential trace metals, or any concentration of non-essential trace metals, can be toxic (Rainbow, 2002). When

unbound trace metals enter cells, they generally bind to sulphur- and nitrogen-containing ligands, for which they have a high affinity (Rainbow, 2002). Sulphur and nitrogen are key components of amino acids, which make up proteins, so when trace metals bind to sulphur and nitrogen in amino acids, they change the structure of proteins and prevent them from functioning normally (Rainbow, 2002). Once sufficient concentrations of active trace metals are interfering with essential biological processes, the organism will begin to experience adverse health impacts (Rainbow, 2002). Since only metabolically active trace metals cause adverse health impacts, an animal can have high trace metal concentrations but not necessarily exhibit adverse health impacts if the trace metals are effectively sequestered and detoxified in storage organs (Laskowski, 1991; Rainbow, 2002; Rainbow, 2018).

Because animals are able to sequester and detoxify metals, establishing toxicity or lethality thresholds for trace metals in tissues is difficult (Rainbow, 2018). Measurement of total trace metal concentrations in organisms does not allow for differentiation between detoxified and metabolically active metals (Rainbow & Luoma, 2011; Rainbow, 2018). Therefore, a high concentration could be indicative only of large amounts of safely sequestered trace metals, while a low concentration could be indicative of a small amount of metabolically active trace metals. As such, while estimates can be made of concentrations above which adverse health effects can be expected, or have been observed, these are more of an approximate guideline, and can vary widely even between related species (Rainbow, 2018). This also means that trace metal loads or adverse health effects observed in one species may not accurately predict the toxic responses of other species. However, organisms can be used as trace metal 'biomonitors': organisms which accumulate trace metals in their tissues, and can therefore provide a relative measure of trace metal concentrations in their surroundings (Rainbow, 2018). Trace metal concentrations in these biomonitor species can be compared across different sites to assess relative levels of environmental contamination and to provide an indication of the levels of exposure of other local organisms. However, biomonitors can only indicate the possibility of exposure, and their trace metal concentrations cannot necessarily be linked to health risks, either for the biomonitor species or for other local species.

If an organism is preyed upon, any trace metals within their body, active or sequestered, can be transferred to the predator. Concentrations of contaminants can increase with increasing trophic level because individuals in a higher trophic level ingest numerous individuals from a lower trophic level, and therefore assimilate their combined contaminant loads (Borgå et al., 2004). This process is called biomagnification, and is commonly observed

with certain contaminants, including persistent organic pollutants such as dichlorodiphenyltrichloroethanes (DDTs) or polychlorinated biphenyls (PCBs) (Kelly et al., 2007). However, biomagnification is not consistent or straightforward when it comes to trace metals (Laskowski, 1991; Janssen et al., 1993; Rainbow, 2002; Wang, 2002; Rainbow, 2018). While trace metal biomagnification has been observed in some isolated food webs, generally, biomagnification does not account for the various trace metal patterns observed across trophic levels (Laskowski, 1991; Janssen et al., 1993; Goodyear & McNeill, 1999; Gray, 2002; Wijnhoven et al., 2007; Burger, 2008). Some trace metals, such as mercury, generally have higher concentrations at higher trophic levels, as is expected with biomagnification, but other metals, such as lead, can have lower concentrations at higher trophic levels (Roberts & Johnson, 1978; Janssen et al., 1993; Chen et al., 2000; Alleva et al., 2006; Lavoie et al., 2013). To add to this unpredictability, trends are not consistent across all food webs or environments (Wang, 2002; Dehn et al., 2006). For example, while cadmium accumulates at high concentrations in terrestrial insectivorous small mammals, due to their diet of Cd-accumulating earthworms, in classic aquatic planktonic food webs, cadmium concentrations generally decrease as trophic level increases (Cooke, 2011; Wang, 2002).

While trace metal biomagnification is still actively studied, several hypotheses currently exist for why trace metals do not consistently biomagnify. One reason is that trace metals are not distributed evenly throughout the body, but are instead stored in specific organs, such as the kidney, liver, hepato-pancreas, and bones (Rainbow, 2007). If a predator does not consume those specific organs, they will not assimilate the full trace metal load of their prey. Some trace metals, such as lead, accumulate primarily in bone tissue, which not all predators consume, preventing the full lead load from passing from prey to predator (Roberts & Johnson, 1978; Komarnicki, 2000). Specific predator feeding behaviours can also influence trace metal uptake. For example, polar bears selectively feed on seal blubber, which generally contains low cadmium concentrations, therefore preventing cadmium from biomagnifying from seal to polar bear (Dehn et al., 2006). Furthermore, since trace metal bioavailability, accumulation, sequestration, and excretion are all very species-specific processes, the trace metal uptake ratio from prey to predator varies greatly (Laskowski, 1991; Dehn et al., 2006; Rainbow, 2018). This means that, even if a predator takes up the full trace metal load from their prey, if they are more efficient at trace metal excretion, they may have a lower trace metal concentration within their body than their prey, completely negating any potential biomagnification. Furthermore, classic biomagnification relies on prey being the primary source of exposure to the contaminant, but this is not necessarily the case for trace

metals. As discussed previously, inadvertent soil ingestion is the primary exposure route for trace metals for many species (Thornton & Abrahams, 1983). Because of this, species that consume large amounts of soil, including herbivores, omnivores, or insectivores, will generally have relatively higher trace metal concentrations, while animals that prey on these soil consumers will not necessarily consume or retain the full trace metal load of their prey. Overall, the best predictor for trace metal concentrations within an individual is not their trophic level, but rather the local environmental trace metal concentrations, whether the metal is essential or non-essential, their specific diet (including water and soil ingestion), and the bioavailability of each trace metal within their system (Thornton & Abrahams, 1983; Laskowski, 1991; Ma et al., 1991; Janssen et al., 1993; Stewart et al., 1999; Burger, 2002; Beyer et al., 2007; Wijnhoven et al., 2007; Millán et al., 2008; Berglund et al., 2011; Gall et al., 2015; Rainbow, 2018).

1.7 Trace Metal Effects on Fauna

Once trace metals accumulate in a metabolically active form at sufficiently high concentrations within an organism, depending on the metal and species in question, they will begin to cause adverse health impacts. While these effects differ depending on the trace metal(s) in question, generally, metabolically active trace metals negatively affect cell components and enzymes (Wang & Shi, 2001; Tapiero et al., 2003; Beyersmann & Hartwig, 2008; Tchounwou et al., 2012). Trace metals can also bind to and damage DNA, leading to cell cycle modulation or apoptosis, and may even cause chromosomal aberrations (Wang & Shi, 2001; Topashka-Ancheva et al., 2003; Beyersmann & Hartwig, 2008; Tchounwou et al., 2012; Guerrero-Castilla et al., 2014). As a result, exposure to high levels of trace metal contamination has been linked to inhibited growth, reduced fertility, immunosuppression, genetic mutations, and a shortened lifespan in a variety of organisms (Lorenzo et al., 1978; Scheuhammer, 1987; Van Straalen & Donker, 1994; Ma, 1996; Swarup & Dwivedi, 2002; Snoeijs et al., 2004; Swaileh & Sansur, 2006; Devkota, 2006; Kakuschke & Prange, 2007; Sorvari et al., 2007; Fonseca et al., 2009; Mogren & Trumble, 2010; Gall et al., 2015). Furthermore, many trace metals, including cadmium, chromium, lead, and mercury, are also known or probable carcinogens (Tchounwou et al., 2004; Tchounwou et al., 2012).

High trace metal body burdens can have adverse health effects on almost all organ systems. The organs used to sequester trace metals, such as the kidney and liver, can contain relatively high concentrations of inactive trace metals bonded to metallothioneins, but if these bonds are degraded, the trace metals can instead bond with critical amino acids in these

tissues (Johri et al., 2010). This can then cause inflammation, cell damage, tubular necrosis (in kidneys), and complete organ failure (Roberts et al., 1978; Shore & Douben, 1994; Sánchez-Chardi et al., 2009; Johri et al., 2010). While the sequestering organs are generally the most impacted by high trace metal concentrations, active trace metals that are not sequestered can infiltrate any part of the body and interfere with critical cell functions, causing cell damage or apoptosis (El Azzouzi et al., 1994; Sánchez-Chardi et al., 2009). In the brain, exposure to high concentrations of metabolically active trace metals has been linked to the inhibition of dopamine uptake, and can cause permanent brain damage (Järup, 2003; Noureddine et al., 2005; Duruibe et al., 2007; Jaishankar et al., 2014). There is evidence that trace metal exposure can even alter behaviour, causing changes in the expression of aggression (Burger & Gochfeld, 1994; Janssens et al., 2003a; Sloman et al., 2003; Sloman, 2007). The far-reaching impacts of trace metal contamination mean that both acute and chronic exposure can lead to permanent adverse health impacts, if not death. Intensive industrial lead pollution events have been linked to large-scale livestock mortality events, during which entire herds of cattle have died after being exposed to high concentrations of lead (Rajaganapathy et al., 2011).

Metabolically active trace metals impair reproduction through a variety of routes, all acting to inhibit the production of offspring. In invertebrates, trace metal exposure can cause male infertility through sperm abnormalities, impaired sperm mobility, low sperm counts, and reduced fertilization success (Fitzpatrick et al., 2008; Lewis & Ford, 2012). Female invertebrates are also impacted; grasshoppers (*Aiolopus thalassinus*) exposed to lead or mercury exhibit reduced fecundity and oviposition, and increased time between oviposition (Devkota, 2006). Similarly, in snails, the total number of eggs laid has been shown to decrease as trace metal concentrations increase, with high concentrations of copper inhibiting snail egg development entirely (Ansaldo et al., 2009; Das & Khangarot, 2011). In fish, trace metal exposure has been definitively linked to impaired neurotransmission, decreased estrogenic and androgenic secretion, inactivation of gonadotropin-secreting cells, and reductions in gonad size, gamete production, and spawning success, all of which serve to impair fertility (Boyle et al., 2008; Crump & Trudaeu, 2009; Ebrahimi & Taherianfard, 2011). For birds, exposure to high trace metal concentrations can interrupt egg laying patterns and decrease sperm concentrations, clutch size, and hatching success, though this is not uniform across all bird species (Eeva & Lehikoinen, 1995; Janssens et al., 2003b; Dauwe et al., 2004; Albers et al., 2007; Marettová et al., 2015). Trace metals can also affect bird reproduction indirectly; in pre-breeding female eider ducks (*Somateria mollissima*), higher

blood lead concentrations have been correlated with a later arrival at the breeding grounds and, therefore, with lower reproductive success (Provencher et al., 2016). Mammalian reproduction is affected by trace metal exposure through changes in hormone production, damage to reproductive tissues, male and female sterility, impaired foetal development, and foetal death (Ma, 1996; Domingo et al., 2004; Marettová et al., 2015; Henson et al., 2016). For example, female rhesus monkeys (*Macaca mulatta*) exposed to sublethal concentrations of lead exhibited longer, more variable menstrual cycles and shorter menstrual flow, as well as a suppression of luteal function through low progesterone concentrations, all of which can contribute to reduced fertility (Laughlin et al., 1987; Franks et al., 1989).

Developing embryos can also be exposed to, and affected by, trace metal contamination. The risk of trace metal exposure in invertebrate embryos varies across species and depends primarily on the trace metal permeability of the embryonic membrane (Ringwood, 1991; Rayms-Keller et al., 1998). The methods of trace metal transport into the invertebrate embryo are generally poorly understood and appear to depend on the trace metal in question (Radenac et al., 2001). However, internal essential trace metal concentrations are generally regulated by the embryo, with low concentrations actively transported into the embryo, while non-essential trace metals are not regulated, and likely enter the embryo through passive transport (Radenac et al., 2001). In eutherians (placental mammals), pregnant adults exposed to trace metal contamination may inadvertently transfer certain trace metals, such as lead and mercury, through the placental membrane and into foetal tissues (Teigen et al., 1999; Iyengar & Rapp, 2001; Benitez et al., 2009; Gundacker & Hengstschläger, 2012). This transfer is not uniform across all trace metals (for example, cadmium does not transfer as effectively as other trace metals), and the methods of placental transfer are not well understood (Iyengar & Rapp, 2001; Gundacker & Hengstschläger, 2012). Even in eggs, embryos are vulnerable to trace metal contamination. Birds have been known to sequester trace metals in eggs, weakening the shells and exposing the developing embryos to potentially harmful trace metal concentrations (Burger, 2002; Mora, 2003; Zhang et al., 2006). Eggs with high trace metals concentrations are often relatively smaller and have thinner, more porous eggshells, though this again depends on the bird species (Eeva & Lehikoinen, 1995; Dauwe et al., 2004). Egg shells are also not fully impermeable to trace metals, so embryos can be affected if eggs are directly exposed to trace metals during development (Kertész et al., 2006). For example, submersing mallard (*Anas platyrhynchos*) eggs in water contaminated with lead and cadmium increases the rate of embryonic death (Kertész et al., 2006). Regardless of the species, embryonic exposure to trace metals has been

linked to malformation, delayed birth, lowered birth weight, reduced growth, abnormalities in the developing central nervous system, and death (Sierra et al., 1989; Ernhart, 1992; Eeva & Lehikoinen, 1995; Nyholm, 1998; Rayms-Keller et al., 1998; Domingo et al., 2004; Ansaldo et al., 2009; Benitez et al., 2009).

After birth, developing juvenile organisms are at a high risk of acute adverse health impacts from trace metal contamination. Juveniles are generally more likely to ingest trace metals than adults, either through their heightened curiosity, which causes them to lick or chew foreign objects, or, in the case of neonates, through concentrated trace metal exposure from their mother's milk (Bhattacharyya, 1983; Swarup & Dwivedi, 2002). Once trace metals are consumed, juveniles absorb and retain higher trace metal concentrations than adults of the same species. In vertebrates, juveniles generally have higher trace metal intestinal uptake rates and lower trace metal sequestration and excretion rates when compared to adults (Connor, 1972; Jugo, 1977; Kostial et al., 1978; Sullivan et al., 1984; Ringwood, 1991; Swarup & Dwivedi, 2002). The differences in trace metal intestinal uptake rates are hypothesized to be attributable a less selective intestinal barrier and a more variable intestinal pH (which facilitates trace metal absorption) in juvenile organisms compared to adults (Jugo, 1977; Swarup & Dwivedi, 2002). Juvenile trace metal sequestration ratios are also lower than observed in adult kidneys and bones (Jugo, 1977; Swarup & Dwivedi, 2002). In kidneys, this is likely due to juvenile kidneys having lower concentrations of metallothioneins than adult kidneys, therefore severely restricting the kidney's ability to sequester trace metals (Jugo, 1977; Teigen et al., 1999). Juvenile bones are not stable trace metal storage sites because bones are constantly remodelled during growth periods, rereleasing sequestered metals into the bloodstream (Swarup & Dwivedi, 2002). High concentrations of trace metals within juveniles are therefore left to travel throughout the body in metabolically active forms, often accumulating in particularly vulnerable tissues, such as the brain (Jugo, 1977; Lorenzo et al., 1978; Sullivan et al., 1984). This can have especially negative impacts on developing organs, such as the central nervous system, the development of which can be significantly impaired by trace metals (Sierra et al., 1989; Swarup & Dwivedi, 2002). Furthermore, the lower sequestration rates and the greater vulnerability of developing organs means that juvenile organisms generally begin to experience adverse health impacts at lower trace metal concentrations than adults (Connor, 1972; Jugo, 1977; Kostial et al., 1978; Sullivan et al., 1984; Ringwood, 1991; Radenac et al., 2001; Swarup & Dwivedi, 2002).

While research on trace metal health effects are often focused on either individuals or on how the contaminants are passed from parent to offspring, trace metal pollution can also

have population-wide and community-wide impacts. Invertebrate communities can be greatly altered by the presence of trace metal contaminants. Species which are sensitive to trace metal contamination may be extirpated in polluted areas, leaving predominantly metal-tolerant species living in that habitat (Posthuma & Van Straalen, 1993). Other, less metal-tolerant species often migrate away from, or avoid ovipositing in, contaminated areas (Migula & Binkowska, 1993; Behmer et al., 2005; Mogren & Trumble, 2010; Gall et al., 2015). Because of this loss of more metal-sensitive species, invertebrate communities can be less diverse in polluted areas compared to non-polluted areas (Burrows & Whitton, 1983; Smolders et al., 2003). Of the species that remain in polluted sites, some generalists, such as annelids or *Drosophila*, have been known to evolve resistance to trace metals over decades of exposure by increasing either their trace metal excretion or sequestration rates (Migula & Binkowska, 1993; Posthuma & Van Straalen, 1993; Van Straalen & Donker, 1994; Reinecke et al., 1999; Janssens et al., 2009; Gall et al., 2015). While these adaptations allow species to survive in polluted sites, they reduce the genetic variation within the population and they can have unforeseen side effects, such as altering a species' life cycle (Posthuma & Van Straalen, 1993; Van Straalen & Donker, 1994). In vertebrates, although much is still poorly understood about population or community-level trace metal effects, species density, diversity, and community composition appears to be influenced by trace metal pollution (Eeva et al., 2002; Phelps & McBee, 2009; Eeva et al., 2012). In particular, studies have found decreased species density and abundance near trace metal contaminated sites across both bird and small mammal communities (Eeva et al., 2002; Phelps & McBee, 2009; Eeva et al., 2012). This suggests that the impact of trace metal contamination reaches beyond individuals and can affect whole communities, ultimately reshaping ecosystems.

1.8 Humans and Trace Metals

Humans are also susceptible to trace metal contamination. Humans are generally exposed to trace metal contamination either through exposure to manufactured objects containing trace metals, such as lead paint, leaded gasoline, or cigarettes, or by working in or living near mines, smelters, recycling plants, and other sites with high trace metal concentrations (Jacobs et al., 2002; Järup, 2003; Leung et al., 2008; Malekirad et al., 2010; Lin et al., 2011; Tchounwou et al., 2012; Lau et al., 2014). The other common exposure route for humans is consuming metal-contaminated food, such as vegetables grown in trace metal contaminated areas or contaminated animal food products (Islam et al., 2014; Hu et al., 2018; Khan et al., 2018; Atamaleki et al., 2020). The adverse health impacts experienced by

humans exposed to trace metals are similar to those already mentioned in other animals, including significant cellular damage, gastrointestinal diseases, kidney damage, weakened bones (leading to osteoporosis or to multiple bone fractures, known as "Itai-Itai" disease), cancer, and inhibited fertility (Dawson et al., 1998; Benoff et al., 2003; Lin et al., 2011; Louis et al., 2012; Tchounwou et al., 2012; Sharma et al., 2014; Sun et al., 2017; Rainbow, 2018). Furthermore, various human neurodegenerative diseases, such as Alzheimer's disease, have been linked to non-essential trace metal exposure (Duce & Bush, 2010).

Children are particularly vulnerable to the effects of trace metal exposure, due in part to behavioural characteristics, such as their readiness to consume contaminated dust, soil, or human-made items containing lead (such as lead-based paint chips) (Jacobs et al., 2002; Järup, 2003; Tchounwou et al., 2012). Children also absorb more trace metals than adults; a child's gastrointestinal tract absorbs 50% of consumed lead, while an adult's only absorbs 10-15% (Järup, 2003). In addition to the adverse health impacts experienced by adults (described above), children can also exhibit impaired development of both muscular and skeletal systems as a result of trace metal exposure, though the exact cause of this is not fully understood (Lin et al., 2011; Yang et al., 2013). The developing central nervous system is also particularly impacted by toxic trace metal exposure, especially by lead, likely due in part to children having an under-developed lead blood-brain barrier, allowing lead to accumulate in the brain (Järup, 2003; Duce & Bush, 2010). Lead exposure has been linked to lifelong cognitive deficiencies, decreased brain volume, child behavioural disorders, inhibited visual brain development, and slower information processing (Järup, 2003; Cecil et al., 2008; Ethier et al., 2012; Boucher et al., 2014; Liu et al., 2014; Karri et al., 2016). Other trace metals, especially arsenic and cadmium, can also cause brain function impairment and have been linked to intellectual deficits and hearing problems (Bencko, 2010; Kippler et al., 2012; Liu et al., 2014; Karri et al., 2016). Trace metal exposure is therefore a key human health concern, particularly for children.

1.9 Conclusions

Trace metals are a serious ecosystem-wide health issue. All around the globe, trace metals have been extracted and released into the environment through mining activities. This has resulted in landscapes polluted with trace metal contaminants that continue to have profound impacts even hundreds of years after the original pollution event. Animals and plants living in trace metal polluted environments experience myriad adverse health impacts, including inhibited growth and development, reduced reproduction rates, impaired cognitive

function and central nervous system development, and death. Trace metal pollution can therefore have widespread and profound impacts on polluted ecosystems. However, while there are many studies focusing on specific effects of trace metal contamination, fewer have investigated the ecosystem-wide health effects of trace metals. To further understand the overall effects of trace metal contamination on ecosystems, a systematic study is needed which assesses the presence, movement, uptake, and bioaccumulation of both essential and non-essential trace metals, as well as the health implications for organisms living in contaminated areas.

1.10 Aim and Research Questions

The aim of this project was to assess trace metal contamination across ecosystems, primarily through in-depth case studies at two derelict metalliferous mine sites in Wales (Figure 1.1), but also through a broader assessment of elemental transfer from soil to wildlife across the English Midlands. The primary research questions of the project were: (1) how are historical trace metal contaminants distributed across landscapes, and (2) how do historical trace metal contaminants transfer through ecosystems, specifically focusing on local wildlife and on potential human health risks. Ultimately, this project should provide a better understanding of how trace metals pollutants transfer through the surrounding ecosystem, including the potential impacts on both environmental and animal health. Given that there are over 1,300 abandoned metal mines in Wales (Kitts & Smith, 1996), and that 9% of rivers in England and Wales fail WFD standards because of mine waste pollution (Johnston et al., 2008), the results from this project could have substantial implications for the future management of historical trace metal contaminated sites.

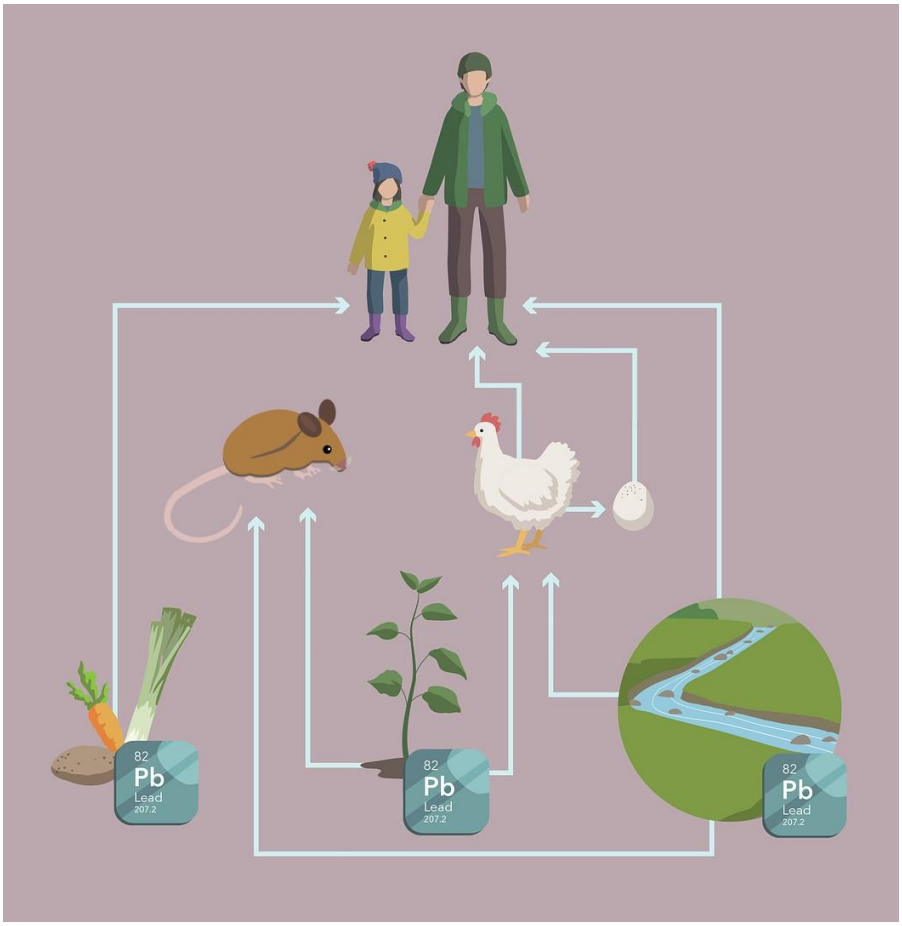

Figure 1.1: Flow chart of Pb transfer throughout ecosystems. Original artwork by Mair Perkins, modified by Andrea Sartorius.

1.11 Thesis Structure

Chapter 1: Introduction and Literature Review. A general introduction to the thesis, including a literature review on trace metals in the environment and their transfer to, and effects on, local fauna.

Chapter 2: Overall Methodologies. A brief overview of the methods used within the study, and a more in-depth explanation of the key study sites.

Chapters 3-6: Studies published or under review in peer-reviewed journals. These chapters are presented in manuscript format, with author contributions outlined. The relationship between the aims of Chapters 3 – 6 and the overall research questions of the thesis are shown in Table 1.1.

Chapter 3: Trace metal accumulation through the environment and wildlife at two derelict lead mines in Wales. An examination of the trace metal contamination in the ecosystems at and around two derelict mine sites in Wales, focusing on contamination in the water, sediment, and soil, and its transfer into key prey species, specifically aquatic invertebrates and wood mice.

Chapter 4: Legacy mines and accumulation of lead in freshwater macroinvertebrates. An investigation of Pb exposure and Pb body burdens in the aquatic invertebrates collected at the mine sites and nearby control sites.

Chapter 5: Human health implications from consuming eggs produced near a derelict metalliferous mine: a case study. An assessment of the Pb concentrations found in eggs produced near a mine site to determine whether they could pose a risk to human health.

Chapter 6: Relationships between soil and badger elemental concentrations across a mosaic landscape. A macroecological study comparing badger liver elemental concentrations across the English Midlands with approximate local soil concentrations.

Chapter 7: Discussions. Discussions of the overall findings of this thesis and implications from this work, as well as the limitations of the study and suggestions for future work.

Individual references are included at the end of each chapter, in APA (American Psychological Association) format.

Table 1.1: The relationship between overall and chapter-specific research aims. The overall thesis aims are in the table header, and the chapter-specific aims are listed under the aim that they pertain to.

Chapter	**Research Question 1:** How are historical trace metal contaminants distributed across landscapes?	**Research Question 2:** How do historical trace metal contaminants transfer through ecosystems, specifically focusing on local wildlife and on potential human health risks?
Chapter 3: *Trace metal accumulation through the environment and wildlife at two derelict lead mines in Wales*	(1) To determine the extent of trace metal contamination at and beyond two derelict metalliferous mines in Wales	(2) To assess the transfer of trace metals into resident wildlife at the bottom of the food web: aquatic invertebrates and rodents.
Chapter 4: *Legacy mines and accumulation of lead in freshwater macroinvertebrates*	(1) Quantify environmental concentrations of Pb across historical mining sites and nearby control sites	(2) Measure body burdens of Pb in invertebrate tissues across all sampled taxonomic groups, at the same sites as environmental measurements (3) Explore the relationships between Pb pollution in the environment and in macroinvertebrates.
Chapter 5: *Human health implications from consuming eggs produced near a derelict metalliferous mine: a case study*		(1) To determine whether chickens living near a derelict lead mine accumulate lead within their tissues (2) Whether they produce eggs with elevated lead concentrations (3) Whether these eggs could be hazardous to human health.
Chapter 6: *Relationships between soil and badger elemental concentrations across a mosaic landscape*	(1) To investigate the relationship between the elemental compositions of topsoil and livers collected from badgers from the English Midlands, examining 25 metals, 3 non-metals, and 2 metalloids	

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Chapter 2 – Overall Methodologies

2.1 Summary

This chapter provides an overview on the methodologies used in Chapters 3, 4, 5, and 6. It also contains descriptive information on the two mine complexes and surrounding areas sampled in Chapters 3, 4, and 5.

2.2 Field Sites

Two abandoned metalliferous mine complexes in western mid-Wales, and their surrounding areas, were sampled during this study. Key findings from these sites are provided in Chapters 3, 4, and 5. To preserve anonymity for local landowners, the exact locations of the sampled sites will not be provided, and the sampled areas will be referred to as "Area 1" and "Area 2", with the mine, stream, private property, and control sites in each area given the corresponding area number. Thus, Area 1 contained Mine Complex 1 (made up of Mine 1a and Mine 1b), Stream 1, which flowed through Mine 1b, Private Property 1, located approximately 4 km downstream of Mine 1b, and Control 1, located approximately 6 km from Mine 1b (Figure 2.1). Area 2 contained Mine 2, Stream 2, which flowed through Mine 2, Private Property 2, located approximately 0.6 km downstream of Mine 2, and Control 2. Located approximately 3 km from Mine 2 (Figure 2.2).

Areas 1 and 2 were sampled in spring 2019 and 2021 and in autumn 2019, 2020, and 2021. A variety of samples were collected at these sites, including environmental samples (water, sediment, and soil), and animal-derived samples (invertebrates, animal tissues, blood, hair, feathers, and eggs).

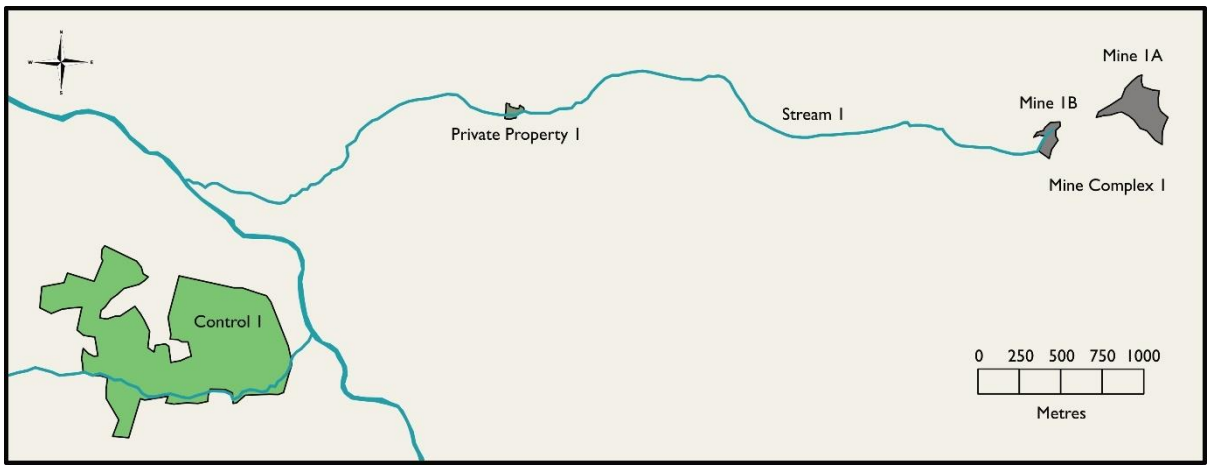

Figure 2.1: Map of Study Area 1. Grey represents the mine sites, grey-green represents the private property, green represents the control site, and blue represents waterways.

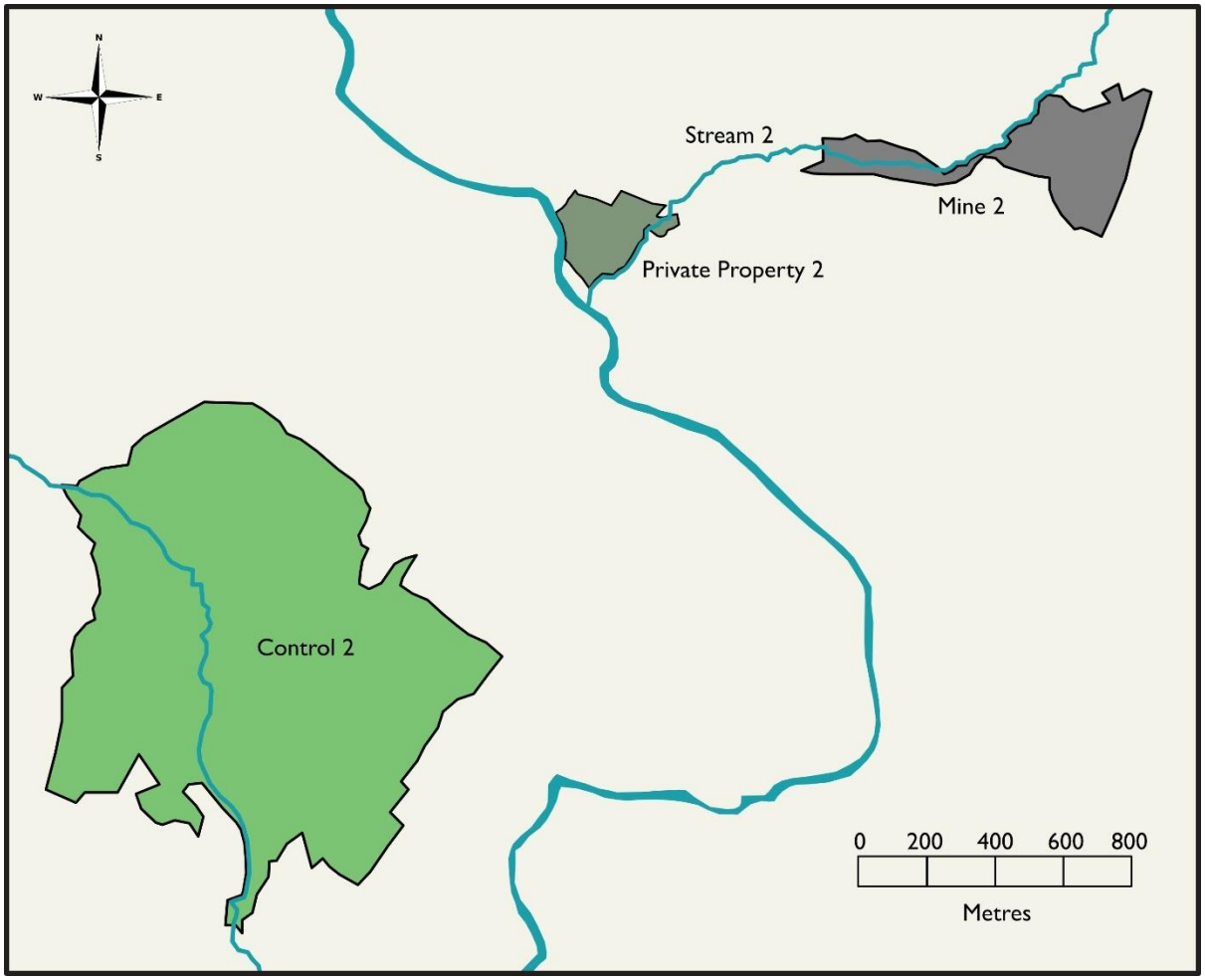

Figure 2.2: Map of Study Area 2. Grey represents the mine sites, grey-green represents the private property, green represents the control site, and blue represents waterways.

The metalliferous mines sampled during this study were abandoned in the early 20th century. These mines are believed to have been intermittently operational since Pre-Roman times, though most of the mining occurred from the late 1700s to the mid/late 1800s (Natural Resources Wales, 2014; Natural Resources Wales, 2016a; Natural Resources Wales, 2016b). Both mines' primary extracts were lead and zinc; copper may have also been extracted from Mine 2. Mines 1a and 1b were grouped together into Mine Complex 1 in this study due to their proximity; historically, these mines were connected and both worked the same mineral vein. Mine 1b and Mine 2 have streams (Stream 1 and Stream 2, respectively) flowing directly through them, with spoil heaps located on their banks (Figures 2.3 – 2.5). Some remediation works (focusing on clay-capping the spoil heaps and diverting water from flowing through mine waste) were done at Mine 1a between 2011 and 2018, but these did not address the full extent of the contamination at this site (Natural Resources Wales, 2016a; Natural Resources Wales, 2016b). At the landowner's request, sampling at Mine 2 was restricted to the downstream section of the mine.

Figure 2.3: Stream 1 through Mine 1b (left) and Stream 2 through Mine 2 (right). Both streams pass directly next to spoil heaps.

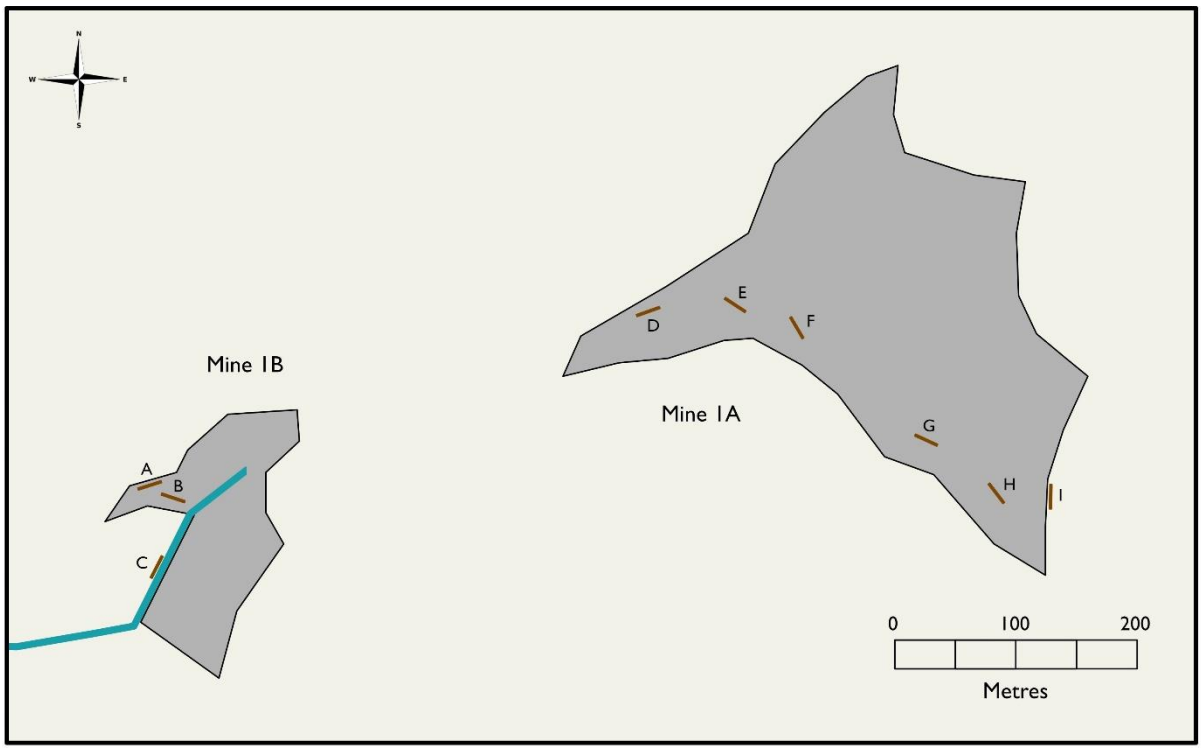

Figure 2.4: Map of Mine Complex 1. Grey represents the extent of the mine site, brown represents the rodent sampling transects, and blue represents waterways. The letters refer to the transect labels.

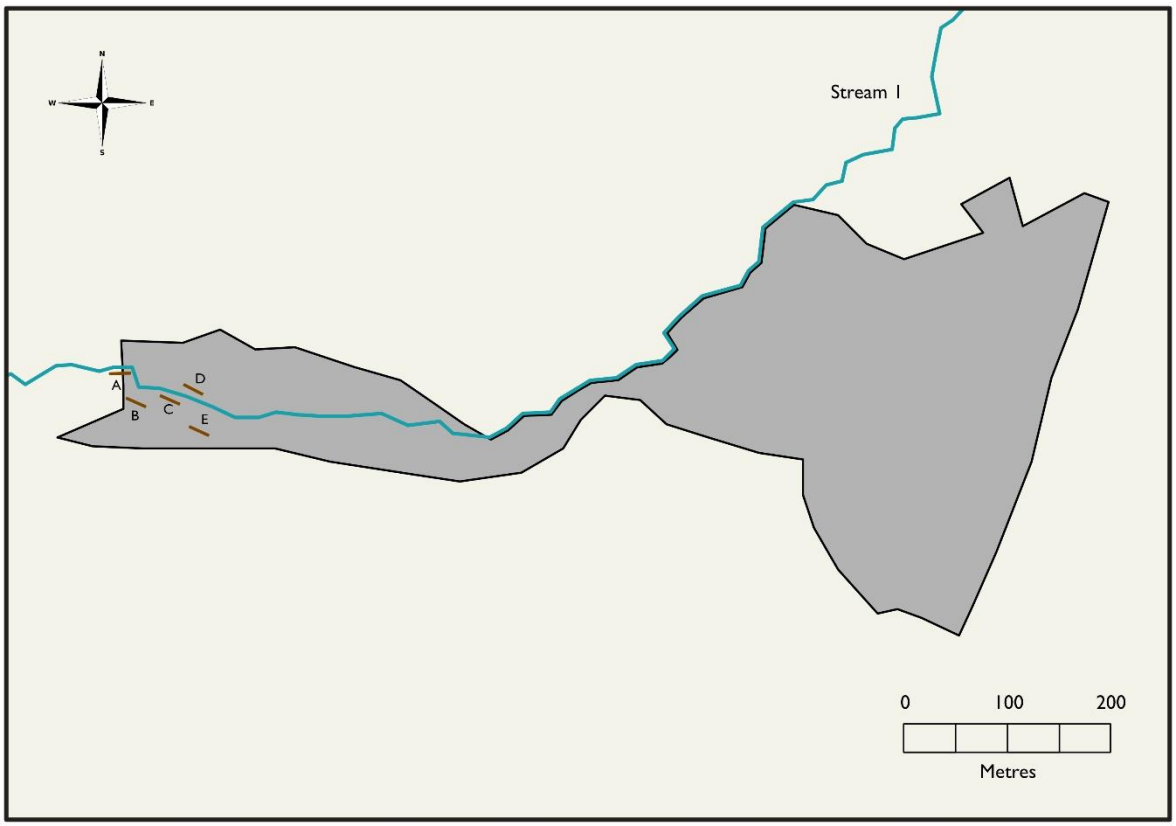

Figure 2.5: Map of Mine 2. Grey represents the extent of the mine site, brown represents the rodent sampling transects, and blue represents waterways. The letters refer to the transect labels.

Private Property 1 consisted of approximately 0.4 ha of land located 4 km downstream of Mine Complex 1 (Figures 2.1, 2.6). It included two buildings, a maintained garden, a vegetable patch, and a non-maintained area of woodland, all adjacent to Stream 1. Domestic geese, ducks, chicken, and cats were present at this site. Private Property 2, a working farm of 8 ha, was located approximately 0.6 km downstream of Mine 2 (Figures 2.2, 2.7). It contained various buildings and outbuildings, seven fields used for livestock grazing, as well as a vegetable patch and a chicken coop and run. There were horses, sheep, chicken, dogs, and cats present at this site.

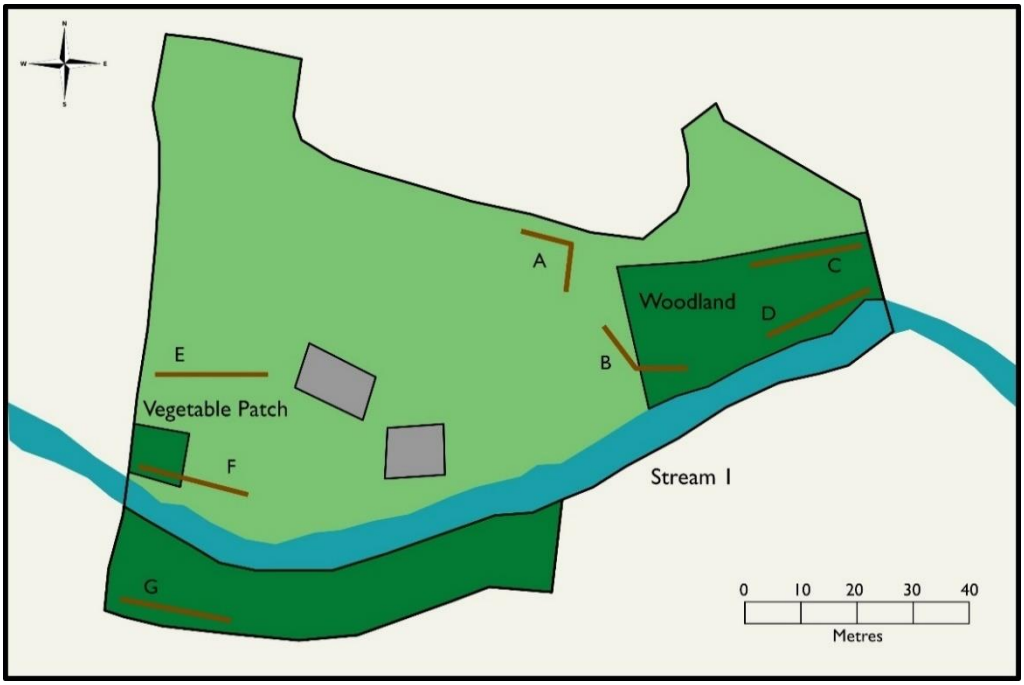

Figure 2.6: Map of Private Property 1. Dark green represents the fields sampled in the property, light green represents areas not sampled in the property, brown represents the rodent sampling transects, grey represents buildings, and blue represents waterways. The letters refer to the transect labels.

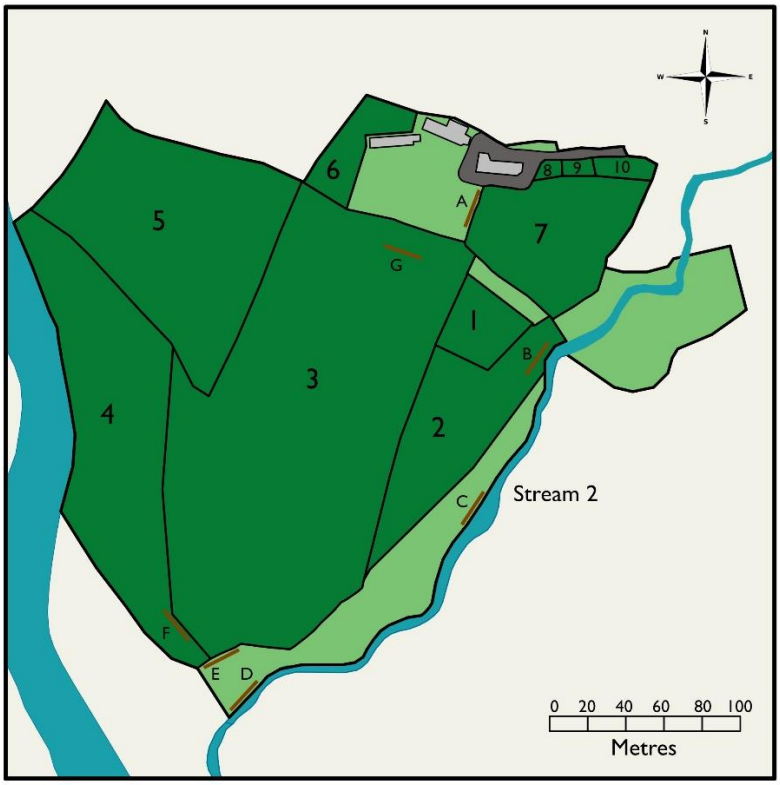

Figure 2.7: Map of Private Property 2. Dark green represents the fields sampled in the property, light green represents fields not sampled in the property, brown represents the rodent sampling transects, dark grey represents the sampled gravel path, light grey represents buildings, and blue represents waterways. The numbers refer to the identification number for each field, and the letters refer to the transect labels.

The control sites sampled during this study were both woodland parks. Control 1 was located approximately 6 km from Mine Complex 1, and 2 km from Property 1 (Figure 2.1). Control 2 was located approximately 3 km from Mine 2, and 2 km from Property 2 (Figure 2.2). Both control sites were primarily forested and minimally managed. For consistency, sampling in the control sites was focused either on areas near streams, or near paths, as these most closely resembled conditions at the mine sites and private properties (Figures 2.8 – 2.9). Water, sediment, soil, and grass samples were collected from possible control sites and tested to confirm that the environmental trace metal concentrations at these sites were below appropriate trace metal thresholds (see section 2.3.3 [‘Threshold Comparisons’] for a description of these thresholds).

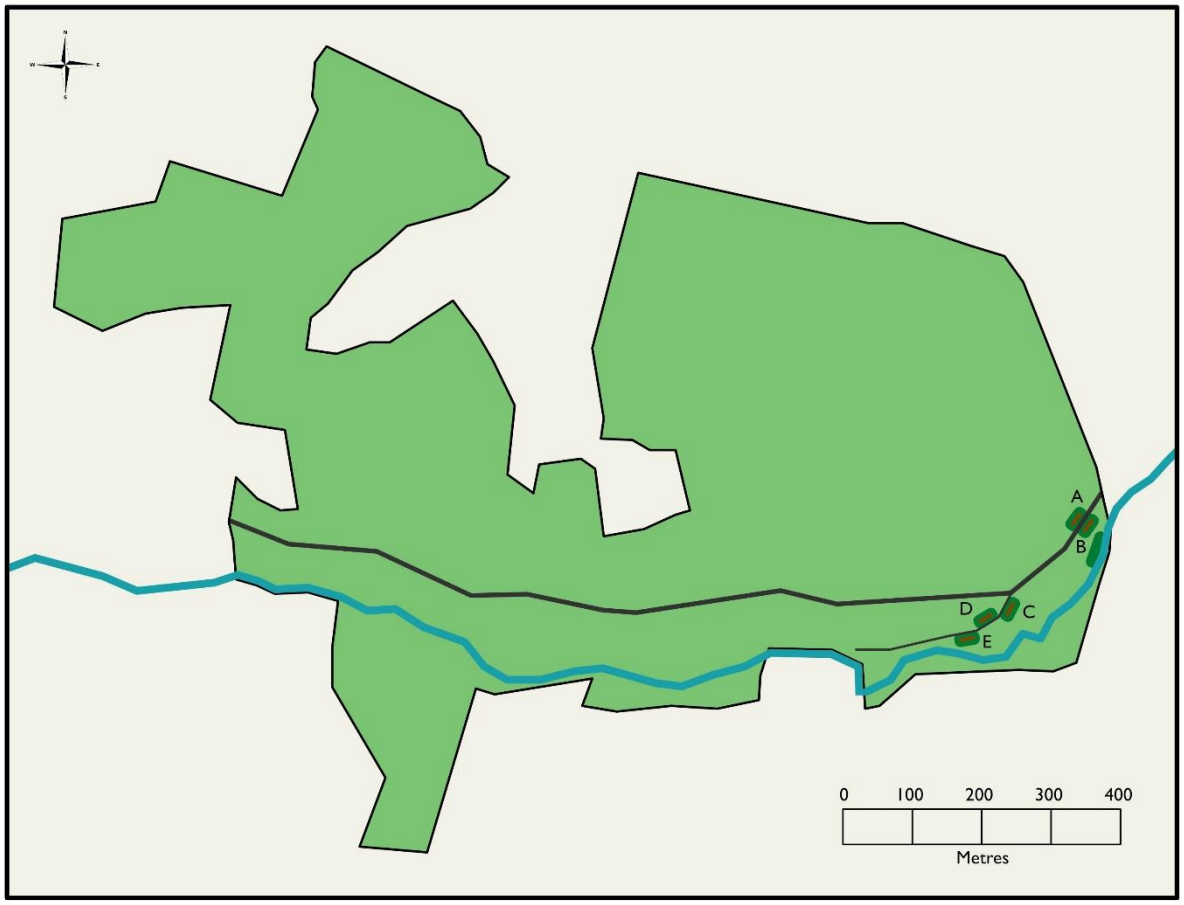

Figure 2.8: Map of Control 1. Dark green represents the areas sampled in the property, light green represents the areas not sampled in the property, brown represents the rodent sampling transects, grey represents paths, and blue represents waterways. The letters refer to the transect labels.

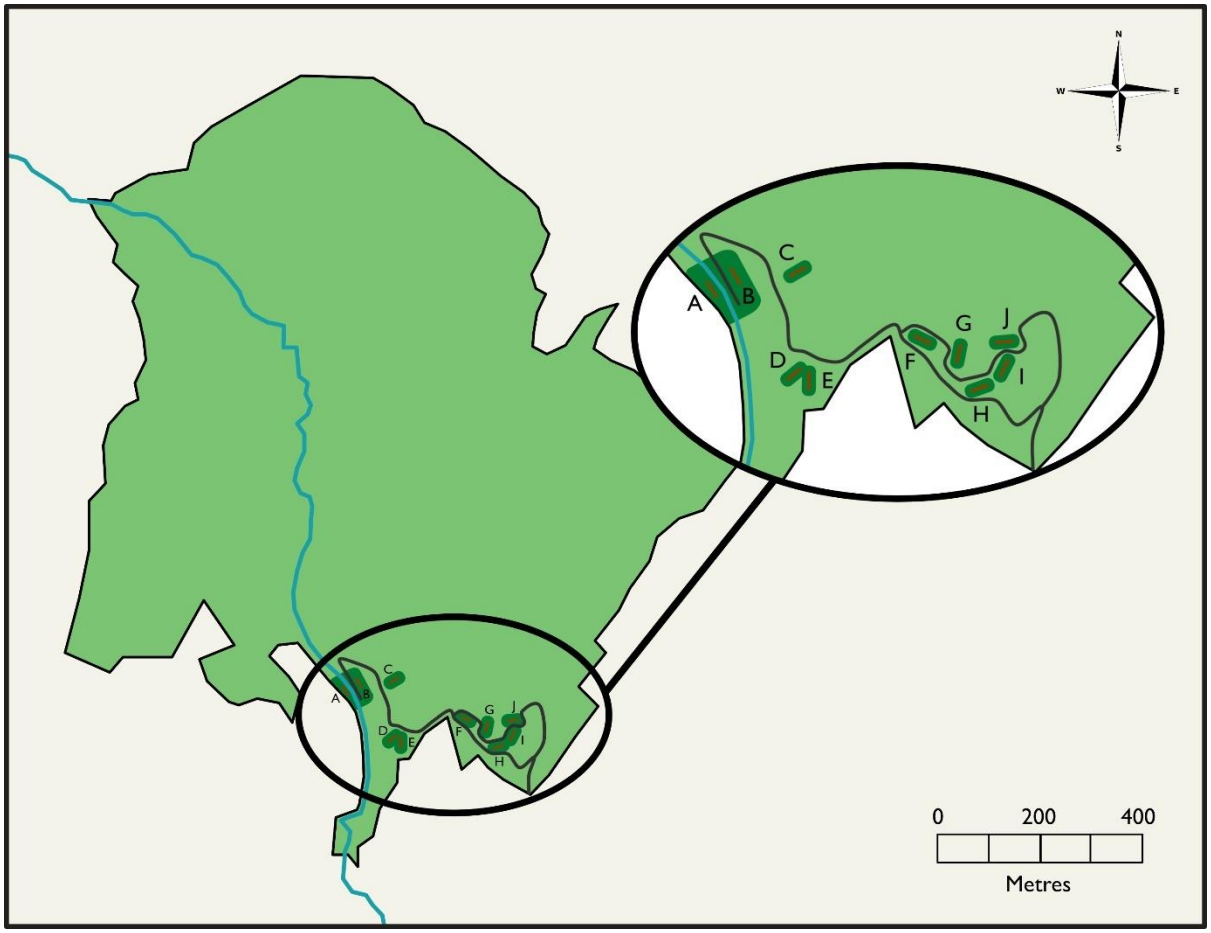

Figure 2.9: Map of Control 2. Dark green represents the areas sampled in the property, light green represents the areas not sampled in the property, brown represents the rodent sampling transects, grey represents paths, and blue represents waterways. The letters refer to the transect labels.

2.3 Methodologies

2.3.1 Sample Collections

Water, sediment, and soil samples were collected at all sites to determine the extent of environmental trace metal contamination. Aquatic invertebrate samples were collected from streams at all six sites. Rodents were collected along the marked transects in Figures 2.4 – 2.9. Full details of the sample collection methodologies in Areas 1 and 2 are given in Chapters 3, 4, and 5.

For Chapter 6, badger carcasses were collected by citizen scientists in the English Midlands prior to the beginning of this project (Swift et al., 2021). The collection methodology is given in Chapter 6, and explained in more detail in Swift et al. (2021).

2.3.2 Sample Processing

All samples were processed in laboratories at the School of Veterinary Medicine and Science at the University of Nottingham. To determine trace metal concentrations, the samples were dried (using either a freeze dryer or an oven) and acid digested (using either a microwave [Model Multiwave Pro; Anton Paar; Graz, Austria] or a teflon-coated graphite hotplate block digester [Model PL-A3; Analab; Bischeim, France]) before elemental analysis by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Model ICAP-Q; Thermo Fisher Scientific, Bremen, Germany). Duplicates of each sample were tested, when possible. A minimum of two blank samples were run with every analysis to confirm the acid digestion and ICP analysis had performed as expected, and to determine the operational limits of detection (calculated as three times the standard deviation of the concentrations measured in 10 blank digestion samples) (Marin et al., 2011). Certified reference materials (sediments and soils: NIST 2711 A, Montana soil; tissues: BRC-185R Bovine Liver [trace elements]) were also run alongside the samples to calculate recovery percentages for the elements of interest (see Chapters 3-6 for relevant recovery percentages). Specific protocols for each sample type are outlined in the methodology sections for Chapters 3-6. All other chapter specific laboratory protocols are outlined in the applicable chapters.

2.3.3 Threshold Comparisons

Trace metal concentrations found in environmental and animal samples were compared to relevant thresholds to indicate expected concentrations, and the potential risk of toxicity, if possible. For water samples, the primary thresholds used were the Department for Environment, Food, and Rural Affairs' (Defra's) 'Predicted No-Effect Concentrations' (PNECs) (Defra, 2014; Water Framework Directive United Kingdom Technical Advisory Group, 2014), which are site-specific trace metal concentrations, calculated using dissolved organic carbon (DOC) and calcium concentrations, below which no adverse effects are expected. Sediment concentrations were compared to the Canadian sediment quality guidelines for the protection of aquatic life, which consist of two thresholds: one above which adverse health effects are possible ('Interim Sediment Quality Guideline'), and one above which adverse health effects are probable ('Probable Effect Level') (Canadian Council of Ministers of the Environment, 2001). Soil concentrations were compared to the British Geological Survey's 'Normal Background Concentrations' (NBCs) for Wales, which indicate the 'normal levels of contaminants' in different domains (areas defined based on soil type and

anthropogenic activity) (Ander et al., 2013). For elements not included in the NBCs, the 'Sludge (Use in Agriculture) Regulations 1989' (Public Health, England and Wales & Public Health, Scotland, 1989) were used instead. For lead in food produced for human consumption, the concentrations were compared to the European Food Safety Authority's benchmark dose levels for daily lead consumption (EFSA, 2010). Animal tissue trace metal concentrations were compared to the Wisconsin Veterinary Diagnostic Laboratory's 'Normal Range Values' (WVDL, 2015), as well as the results from a wide range of applicable field studies, many of which were collated by Rainbow (2018) and Kalisińska (2019). The applied thresholds are discussed in more detail in Chapters 3-6.

2.3.4 Statistical Analyses

Statistical comparisons between samples were performed using R (R Core Team 2018), following advice from Sparks (2000) and Beckerman et al. (2017). Figures illustrating statistical analyses were generated in R using the ggplot2 package (Wickham, 2016). To decrease the chances of type I error, Benjamini - Hochberg corrections were utilized for statistical tests, with the total number of tests reflecting the number of factors included in each test type (Benjamini & Hochberg, 1995). The corrections were implemented by calculating adjusted p-values through multiplying the p-value by the total number of tests and dividing the result by the p-value's rank (when all relevant p-values were ordered smallest to largest), as described in Yekutieli & Benjamini (1999).

2.3.5 Ethical Considerations

The project and methods were ethically reviewed and approved by the University of Nottingham and the School of Veterinary Medicine and Science (Ethics approval: #1893 161103 [Study Area 1] and #2612 181017 [Study Area 2]).

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Chapter 3 – Trace metal accumulation through the environment and wildlife at two derelict lead mines in Wales

Prepared for submission to *Environmental Pollution*.

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Matthew F. Johnson: Conceptualization, Methodology, Resources, Supervision, Investigation, Writing – Review & Editing
Scott Young: Resources, Supervision, Writing – Review & Editing
Malcolm Bennett: Supervision, Methodology, Resources, Investigation, Writing – Review & Editing
Kerstin Baiker: Supervision, Methodology, Investigation
Paul Edwards: Resources, Writing – Review & Editing
Lisa Yon: Conceptualization, Funding Acquisition, Supervision, Investigation, Writing – Review & Editing

Trace metal accumulation through the environment and wildlife at two derelict lead mines in Wales

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Keywords: legacy pollutants, mining, trace metals, wildlife health

3.1 Abstract

Trace metal pollution has become increasingly common across the globe, largely resulting from human activities. Due to the persistence and high toxicity of trace metals, these pollutants can have serious effects across ecosystems. However, few studies have directly linked the presence and impact of trace metal pollution across environments and animal taxa. This study was designed to assess the extent of trace metal pollution and its possible transfer into wildlife in the areas surrounding two abandoned metalliferous mine complexes in Wales. Water, sediment, and soil at the mine sites and in areas downstream had notably elevated concentrations of Pb, Zn, and, to a lesser extent, Cd and Cu, when compared to nearby control sites. These high trace metal concentrations were mirrored in the body burdens of aquatic invertebrates collected in the contaminated streams both at and downstream of the mines. Wood mice collected in contaminated areas appeared to be able to regulate their Zn and Cu tissue concentrations, but, when compared to wood mice from a nearby control site, had significantly elevated concentrations of Cd and, particularly, Pb detected in their kidney, liver, and bone samples. The Pb concentrations found in these tissues correlated strongly with local soil concentrations (kidney: $\rho = 0.690$; liver: $\rho = 0.668$, bone: $\rho = 0.649$), and were potentially indicative of Pb toxicity in between 28% and 83% of the rodents sampled at the mine sites and in areas downstream. The high trace metal concentrations found in the environment and in common prey species (invertebrates and rodents) indicates that trace metal pollution can have far-reaching, ecosystem-wide impacts beyond the origin site of the pollution.

3.2 Introduction

Potentially toxic metals have been mined globally for thousands of years (Hong et al., 1996; Hernberg, 2000). An unintended side effect of these efforts is the production of large amounts of mining waste contaminated with trace metals, elements that are normally found in the Earth's crust at low concentrations (Johnson, 2003; Rainbow, 2018). This waste can pose ecosystem-wide health risks through the exposure of wildlife and human populations to elements that are toxic at relatively low concentrations (Rainbow, 2018).

During mining activities, environmental trace metal contamination commonly occurs through aerial transmission of mining dust or the release of contaminated wastewater (Davies, 1987; Nicholson et al., 2003). Once processed, mining waste materials are frequently deposited in 'spoil heaps', which are typically located at the mine site. These spoil heaps are large, unstable mounds of material with high trace metal concentrations, and are rarely remediated, even after mine closures (Johnson, 2003). Vegetative growth on spoil heaps is often inhibited due to metal toxicity, so wind and water erosion of spoil heaps are common occurrences, as there are no plant roots to help stabilise the structure of these heaps (Alhar et al., 2021). The redistribution of metals may further increase in the future, as flooding and extreme weather events become more common due to climate change (Jentsch & Beierkuhnlein, 2008; Hirabayashi et al., 2013). As trace metals do not degrade, high concentrations of trace metals can continue to contaminate surrounding environments indefinitely after mining activities have ceased (Pyatt et al., 2000; Wilson and Pyatt, 2007).

Animals living in metal-contaminated areas are routinely exposed to these potentially toxic metals, primarily through drinking water, diet, or inadvertent soil ingestion (Hunter & Johnson, 1982; Heikens et al., 2001; Ma, 2011; Gall et al., 2015). There is ample evidence that that animals living in trace metal contaminated sites generally have elevated trace metal body burdens (Kalisińska, 2019). However, few field studies have directly investigated trace metal concentrations across the environment and multiple resident animal taxa to understand the possible ecosystem-wide effects of metal contamination (Hunter & Johnson, 1982; Heikens et al., 2001). Therefore, the aims of this study were (1) to determine the extent of trace metal contamination at and beyond two derelict metalliferous mines in Wales, and (2) to assess the transfer of trace metals into resident wildlife at the bottom of the food web: aquatic invertebrates and rodents. The investigation focused on two 'non-essential' metals (metals that serve no metabolic function), Pb and Cd, and two 'essential' metals (metals that are necessary for life in small concentrations), Cu and Zn, to examine any differences in the

distribution and accumulation of these two classes of trace metals in metal-polluted environments (Tchounwou et al., 2012; Rainbow, 2018).

3.3 Methods

3.3.1 Sites

Sampling efforts were focused on two derelict metalliferous mine sites and the surrounding areas in mid-Wales. Samples were collected from two mine sites, two private properties downstream of the mines, and two control sites (one near each mine) (Figures 2.1 – 2.2) To preserve anonymity, the sampled areas are referred to as Areas 1 and 2, with the mine, stream, private property, and control sites in each area given the corresponding area number. Area 1 contained: (i) Mine Complex 1 (made up of Mine 1a and Mine 1b), (ii) Stream 1, which flowed through Mine 1b and Private Property 1, (iii) Private Property 1, which was approximately 4 km downstream of Mine 1b, and (iv) Control 1, which was approximately 6 km from Mine 1b (Figure 2.1). Area 2 contained: (i) Mine 2, (ii) Stream 2, which flowed through Mine 2 and Private Property 2, (iii) Private Property 2, which was approximately 0.6 km downstream of Mine 2, and (iv) Control 2, which was approximately 3 km from Mine 2 (Figure 2.2). Mine Complex 1 and Mine 2 were primarily Pb mines, though Cu was also infrequently extracted at Mine 2. Some remediation works, focusing on clay-capping some of the spoil heaps and diverting water from flowing through mine waste, were undertaken at Mine 1a between 2011 and 2018, but these did not completely remove or restrict the trace metal pollution at this site (Natural Resources Wales, pers. comm.). Streams 1 and 2 flowed directly through their respective mine sites (passing by large spoil heaps) and the respective downstream private properties before joining larger rivers. The private properties sampled during this study were both small working farms. The control sites were both woodland parks, primarily forested and minimally managed, and contained small streams similar in size to those found at the mine sites. Before being selected as control sites, water, sediment, and soil samples were collected from these sites and tested to confirm that there was minimal trace metal contamination (see 3.3.5. Threshold Comparisons for the threshold concentrations used to judge contamination levels).

3.3.2 Sample Collection

Water samples were collected across all sites by rinsing a 20 mL plastic Discardi II syringe (BD, Franklin Lakes, New Jersey, United States) with water at the site, then filtering (< 0.22 µm) an 18 mL aliquot of the water through a polyethersulfone 0.22 µm filter (Chromatography Direct, Runcorn, United Kingdom) and into a 20 mL plastic universal tube containing 2 mL 10% Primar grade HNO_3. To determine the total organic carbon, water samples were collected using the same method, but without the addition of acid. Two water samples were collected using each method, and all samples were double bagged and stored at 4˚C pending analysis.

Sediment samples were collected at all water collection sites. Sediment was manually scooped from the riverbed substrate using a 250 mL plastic jug, targeting patches of visibly fine sediment (< 5 mm). Any excess water was poured off at the time of collection, though care was taken to retain as much fine sediment as possible. Each sediment sample was stored in a 50 mL conical centrifuge tube, at ambient temperature, pending analysis.

Soil samples were collected along rodent trapping transects (described below). Sub-samples were collected at each trap site along the transect, as well as approximately five metres to the left and right of the traps (when walking the transect). All sub-samples were deposited and mixed within the same bag. The soil samples were then double bagged and stored at ambient temperature pending transport to the laboratory.

Aquatic invertebrates were sampled in streams at all sites following the Environment Agency's standard protocol (Her Majesty's Stationary Office, 1985; Environment Agency, 2009) consisting of a three minute 'kick sample', plus a one minute hand search, using a 1 mm^2 mesh net with an opening of 0.25 m wide and 0.22 m deep. The operator moved systematically upstream, ensuring that different habitats were proportionally sampled. The collected invertebrate communities were stored in alcohol, double bagged, and kept at ambient temperature until analysis.

Rodents were collected in May and October 2019 and in September 2021. In May 2019, rodents were trapped at Private Property 1, Private Property 2, and Mine Complex 1; in October 2019, rodents were trapped at all six sites; in September 2021, rodents were trapped at Mine Complex 1, Mine 2, and Control 2. Rodents were trapped using Longworth small mammal traps with shrew holes (Penlon Ltd., Oxford, UK) set along transects in each site. The transects were approximately 20 m long, with two traps set every five metres. At Private Property 1 and 2, Stream 1 and Stream 2, respectively, were suspected to be the primary sources of the trace metal contamination from the mine sites, so trapping transects were

preferentially placed along these stream banks. For consistency, transects at the mine and control sites were also primarily situated along streams whenever possible. Transects located away from streams were placed in areas that were identified as containing suitable habitat for rodents, such as field boundaries (Montgomery & Dowie, 1993; M. Bennett, pers. comm.). As the traps were strategically placed in order to maximize capture rates, no population estimates can be made from the trapping frequency data.

Seven transects (six at Property 1) were set at each sampled site in May 2019, and five transects were set at each sampled site in October 2019 and September 2021. The number of transects were increased in spring to account for lower rodent populations in spring when compared to autumn (Gurnell, 1978; Green, 1979). Each Longworth trap was prepared with rodent bedding and was baited with bird seed and a small portion of a fresh vegetable (cucumber or carrot). All traps set in the field were checked in both the morning and evening in case of rodent capture, and bait and bedding were replaced as necessary. When checking the traps, all closed traps were collected and replaced with fresh traps in the same location. Trapping took place for between two to five days, depending on site capture rates. Generally, a target was set of collecting between 10 to 20 rodents per site and sampling trip; as the overall rodent population sizes at these sites were unknown, power calculations could not be used to set more precise collection targets. If a closed trap contained a rodent, the species, sex, and age group (juvenile or adult) were recorded. The animal was euthanised using cervical dislocation, with death confirmed by exsanguination, and the kidney (only in 2019), liver, and femur were removed and stored at -20˚C.

3.3.3 Sample Processing

Water samples were stored at 4˚C prior to analysis. Trace metal concentrations in the filtered, acidified water samples were determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Model ICAP-Q; Thermo Fisher Scientific, Bremen, Germany). Dissolved organic carbon (DOC) in the filtered, non-acidified water samples was determined using a TOC-VCPH instrument (Shimadzu Corporation, Kyoto, Japan).

The sediment and soil samples were air-dried, sieved to <2 mm with a stainless steel sieve, and ground into fine powder using a Retsch PM 400 planetary ball mill (Retsch, Haan, Germany). Duplicates of 0.4 g of each sample were then digested in Aqua Regia (1 mL HNO_3 and 3 mL HCl) at 95˚C for two hours using a teflon-coated graphite hotplate block digester (Analab, Bischeim, France). The samples were then cooled and dispensed into

volumetric flasks; the volume was then made up to 50 mL with MilliQ water (18.2 MΩ cm; Millipore Corporation, Darmstadt, Germany). The solutions were diluted 1:10 with MilliQ water prior to elemental analysis by ICP-MS. A certified soil reference material (NIST 2711A, Montana soil) was run for quality assurance purposes; recovery values were: Pb (93.1%), Cd (104.5%), Zn (99.9%), and Cu (91.8%). For each element, the operational limit of detection (LOD) was calculated as three times the standard deviation of the concentrations measured in 10 blank digestion samples run alongside the samples (Marin et al., 2011). A value of 0.5*LOD was ascribed to samples where the elemental concentration was lower than the LOD (Kushner, 1976).

The Unified BARGE (the BioAccessibility Research Group of Europe) Method (UBM) was used to determine the bioaccessibility of trace metals in the soil samples collected along the rodent transects (Wragg et al., 2009; BARGE & INERIS, 2010). The rodent transect soil samples were run in triplicate, using 0.47 g of the sieved soil each time. All solution volumes in the original method were scaled down to match the amount of soil used; otherwise, no alterations were made to the UBM (BARGE & INERIS, 2010). The amended protocol can be found in Appendix C. The final supernatant was diluted 1-in-10 with 2% HNO_3 prior to elemental analysis by ICP-MS. After obtaining the final concentrations, the bioaccessibility percentage was calculated for each soil sample as [concentration of bioaccessible metal (mg kg^{-1}) / concentration of total metal in sample (mg kg^{-1})] * 100, following BARGE & INERIS (2010).

The invertebrates were sorted in the laboratory, with each individual identified to family, with the exception of Oligochaeta, which were identified to sub-class. After identification, the invertebrates were freeze dried to a constant mass and prepared for acid digestion. Any invertebrate weighing over 0.01 g (dry weight) was acid digested individually. For invertebrates weighing under 0.01 g, multiple individuals of the same family and collected from the same site and at the same time were pooled together to reach a cumulative weight greater than 0.01 g. Samples were acid digested and prepared for ICP-MS analysis following a similar protocol to the sediment and soil samples, but with 4 mL 70% HNO_3 and 1 mL H_2O_2. A certified reference material for trace elements in biological samples (BRC-185R Bovine Liver [trace elements]) was run for quality assurance purposes; recovery values were: Pb (94.2%), Cd (103.9%), Zn (101.2%), Cu (94.9%). As with the soil and sediment samples, the LOD was calculated as three times the standard deviation of the concentrations measured in 10 blank digestion samples (Marin et al., 2011), and any samples where the

elemental concentration was lower than the LOD was ascribed the concentration 0.5*LOD (Kushner, 1976).

Rodent kidney, liver, and bone samples were freeze dried to a constant mass prior to acid digestion. After freeze drying, bone samples were manually cleaned of soft tissue. Rodent tissues were acid digested through hotplate digestion and prepared for ICP-MS elemental analysis, following the same protocol used for the invertebrate samples. All concentrations were calculated as mg kg^{-1} dry weight; comparisons with published fresh weight concentrations and thresholds were performed assuming 24% dry matter in kidneys and 26% dry matter in livers, as reported for wood mice by Ma (1989).

3.3.4 Statistical Analysis

Relationships between trace metal concentrations were examined through statistical comparisons in R (R Core Team, 2018). Due to a lack of normality in total and bioaccessible soil concentration distributions (determined using Shapiro-Wilk tests), Spearman's correlation coefficients were calculated to determine the possible relationships between the soil trace metal concentrations. The soil concentrations were log-transformed for figures to highlight possible relationships more readily. Invertebrate metal concentrations were compared across site type and family using Kruskal-Wallis tests (and, when necessary, post-hoc pairwise Wilcox tests), because the concentrations were not normally distributed. Overall rodent metal concentrations were also not normally distributed, so concentrations between species were compared using Kruskal-Wallis tests. However, wood mouse trace metal concentrations were normally distributed when log-transformed, allowing the dataset to fit ANOVA assumptions (Sparks, 2000). The wood mouse log_{10}-transformed trace metal concentrations were modelled against the type of site (mine, private property, or control), sex (female or male), age class (juvenile or adult), collection month and year (May 2019, October 2019, September 2021), location (Area 1 or Area 2), and individual site (Mine Complex 1, Mine 2, Private Property 1, Private Property 2, Control 1, Control 2). Tukey post-hoc tests were also run comparing metal concentrations across site types. The correlations between wood mouse tissue and soil trace metal concentrations were determined using Spearman's correlation coefficients, once again chosen due to the non-normal distribution of the soil concentrations. Figures illustrating the statistical tests were generated in R using the ggplot2 package (Wickham, 2016).

To decrease the chance of a type I error, Benjamini - Hochberg corrections were utilized for each statistical test, with the total number of sub-tests reflecting the number of factors included in each test type (Benjamini & Hochberg, 1995). Adjusted p-values were calculated by multiplying the p-value by the total number of tests, and then dividing by the p-value's rank (when all relevant p-values were ordered smallest to largest), as described in Yekutieli & Benjamini (1999).

3.3.5 Threshold Comparisons

Water trace metal concentrations were compared to the "Predicted No-Effect Concentrations" (PNECs) defined by the Department for Environment, Food, and Rural Affairs (Defra) (Defra, 2014). PNECs take into account a number of factors, including dissolved organic carbon (DOC) and calcium concentrations, to estimate trace metal bioavailability and generate threshold trace metal concentrations below which no adverse effects are expected. PNECs are therefore site-specific, even within the same water system. DOC was not measured for every water sample, so, for samples without DOC measurements, DOC values previously measured by Natural Resources Wales in the same locations in the streams were used to generate approximate PNECs (Natural Resources Wales, pers. comm.). PNECs were calculated using the Metal Bioavailability Assessment Tool (M-BAT) Microsoft Excel calculators, generated by the Water Framework Directive United Kingdom Technical Advisory Group (2014). PNECs could only be calculated for Pb, Zn, and Cu, as no PNEC calculator currently exists for Cd.

The sediment sample trace metal concentrations were compared to the Canadian sediment quality guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 2001). These guidelines are composed of two values for each pollutant: the Interim Sediment Quality Guideline (ISQG), above which adverse health effects for aquatic life are possible, and the Probable Effect Level (PEL), above which adverse health effects for aquatic life are probable.

The soil sample trace metal concentrations were compared to the Normal Background Concentrations (NBCs), which were generated by the British Geological Survey (BGS) to indicate 'normal levels of contaminants' in different 'domains' (areas defined based on soil type and anthropogenic activity) within the UK (Ander et al., 2013). In this report, the NBC values for the 'Principal' (background) and 'Mineralization' (mining areas) domains resolved for Wales by the BGS in 2013 are referenced for comparison purposes (Ander et al., 2013).

These can be used to determine if the sampled soil trace metal concentrations are elevated compared to expected soil concentrations in Wales, particularly in mining areas (Ander et al., 2013). The soil trace metal concentrations were also compared to the 'Sludge (Use in Agriculture) Regulations 1989', which specify the maximum soil trace metal concentrations below which it is permitted to apply sewage sludge (biosolids) to agricultural land (Public Health, England and Wales & Public Health, Scotland, 1989). These thresholds were designed to protect against the contamination of agricultural produce, and are broadly similar to previous limits relating to the re-development of contaminated land, so they can provide an approximate indication of contamination levels.

3.4 Results

3.4.1 Environmental Samples

The water samples at the mines and private properties had consistently higher Pb, Cd, Zn, and Cu concentrations than those measured at the control sites (Figure 3.1; Table 3.1; Supplementary Figure 3.1). The metal concentrations at the private properties generally resembled those found in the mine sites, indicating that these metals remain at high concentrations in the water at least 4 km downstream of the assumed point of entry (Table 3.1). Beyond 4 km in Area 1, and 2 km in Area 2, the streams from the mine sites joined larger waterways contaminated with metals from other sources (not shown in Figure 2.1), so determining the full spatial extent of metal contamination in the waterways from the sampled mines was not possible. All sampled sites at the mines and private properties greatly exceeded their site-specific PNECs for Pb and Zn water concentrations (Table 3.1), indicating that the concentrations of those metals in the streams could cause adverse health effects in resident aquatic organisms.

Table 3.1: Water trace metal concentrations across the mine, private property, and control sites. Means are reported as ± standard deviation, ranges are provided in italics. Concentrations are in µg L^{-1}. PNECs are reported for Pb, Zn, and Cu; no PNECs can be generated for Cd.

Site		Pb	Cd	Zn	Cu
Mine	Mean	369 ± 186	4.13 ± 4.69	1370 ± 1690	3.32 ± 2.17
	Range	*23.1 – 1210*	*0.765 – 13.0*	*384 – 4570*	*0.905 – 6.51*
	PNEC[a,1]	4.93 ± 2.35	–	16.8 ± 5.09	7.81 ± 3.69
Private Property	Mean	230 ± 186	2.77 ± 1.07	1040 ± 890	2.89 ± 1.60
	Range	*26.5 – 524*	*2.05 – 4.15*	*448 – 2190*	*0.885 – 4.70*
	PNEC	3.32 ± 0.202	–	12.5 ± 0.754	5.26 ± 0.321
Control	Mean	0.360 ± 0.0785	0.0245 ± 0.0170	2.81 ± 0.352	1.06 ± 0.540
	Range	*0.280 – 0.493*	*0.00270 – 0.0394*	*2.45 – 3.29*	*0.390 – 1.58*
	PNEC	7.24 ± 3.70	–	22.2 ± 8.99	11.6 ± 5.91

[a]Probable No-Effect Concentration.
[1]Defra, 2014.

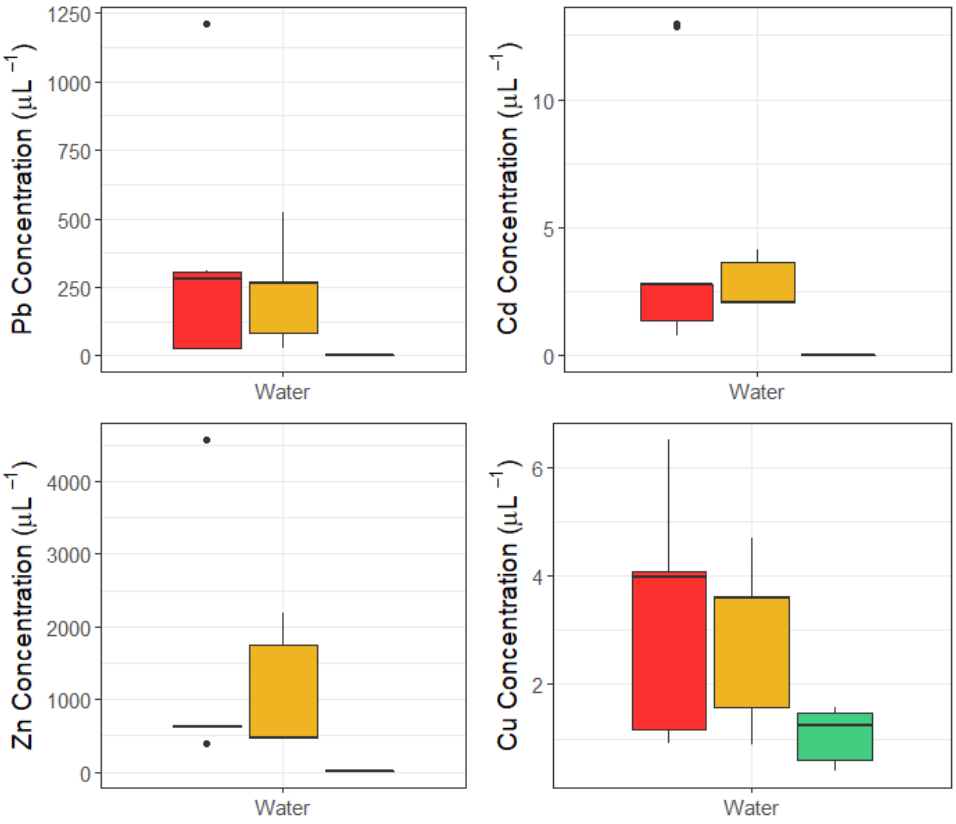

Figure 3.1: Water trace metal concentrations across mine, private property, and control sites. Red represents the mine sites, yellow represents the private properties, and green represents the control sites.

Sediment samples collected at the mine and private property sites had consistently higher trace metal concentrations than sediment samples collected at the control sites (Figure 3.2; Table 3.2; Supplementary Figure 3.2). All the sediments collected from the mines and private properties exceeded the PEL for Pb and Zn, indicating that adverse health effects were probable, and exceeded the ISQG for Cd, indicating that adverse health effects from Cd exposure were possible (Table 3.2) (Canadian Council of Ministers of the Environment, 2001). The Cu concentrations also exceeded the ISQG for all mine and private property sampling locations except for two (one at Mine 1b and one at Private Property 1) (Table 3.2) (Canadian Council of Ministers of the Environment, 2001).

Table 3.2: Sediment trace metal concentrations across the mine, private property, and control sites. Means are reported as ± standard deviation, ranges are provided in italics. Concentrations are in mg kg^{-1}.

Site	Pb	Cd	Zn	Cu
Mine	4080 ± 1160	4.03 ± 5.17	2030 ± 2850	46.0 ± 11.3
	1990 – 5960	*1.41 – 16.3*	*703 – 8820*	*14.7 – 25.7*
Private Property	3270 ± 1590	1.63 ± 0.170	775 ± 187	48.7 ± 19.7
	1190 – 4480	*1.47 – 1.88*	*619 – 1050*	*22.8 – 66.8*
Control	64.4 ± 37.3	0.319 ± 0.152	178 ± 55.7	18.8 ± 5.29
	28.4 – 126	*0.134 – 0.491*	*131 – 252*	*14.7 – 25.7*
ISQG[a,1]	35.0	0.6	123	35.7
PEL[b,1]	91.3	3.5	315	197

[a]Interim Sediment Quality Guideline.
[b]Probable Effect Level.
[1]Canadian Council of Ministers of the Environment, 2001.

Soil samples were similarly highly contaminated at the mines and private properties (Figure 3.2; Table 3.3; Supplementary Figure 3.2). The soil Pb concentrations exceeded the Welsh mineralization domain NBC (280 mg kg^{-1}) at all sites except for Control 2, indicating that these sites contained soil Pb concentrations higher than even those expected in mining areas in Wales (Table 3.3) (Ander et al., 2013). The Cd and Cu soil concentrations found at Mine 2 also exceeded the mineralization domain (Cd: 2.2 mg kg^{-1}; Cu: 96 mg kg^{-1}), as did the Cd concentrations at Mine Complex 1. While there are currently no NBCs for Zn, the Zn soil concentrations were above the 'Sludge (Use in Agriculture) Regulations 1989' concentration (200 mg kg^{-1}) at all sites, with the exception of Control 2 (Table 3.3) (Public Health, England

and Wales, & Public Health, Scotland, 1989). While the trace metal concentrations found at the control sites were lower than those found in the mine or private properties (Figure 3.2; Supplementary Figure 3.2), the soil samples from Control 1 had consistently higher trace metal concentrations than the soil samples from Control 2, particularly when sampling in locations around a dirt path.

Table 3.3: Mean total and bioaccessible soil trace metal concentrations across the mine, private property, and control sites. Means are reported as ± standard deviation. Concentrations are in mg kg^{-1}.

Site		Pb	Cd	Zn	Cu
Mine	Total	6100 ± 4950	5.58 ± 10.2	3550 ± 8570	80.5 ± 59.8
	Bioaccessible	451 ± 506	1.17 ± 2.99	549 ± 1890	14.1 ± 9.76
	Bioaccessible %	7.41 ± 4.04	13.2 ± 9.81	4.31 ± 5.62	19.5 ± 7.73
Private Property	Total	4115 ± 3880	1.34 ± 1.06	596 ± 486	67.6 ± 54.5
	Bioaccessible	400 ± 396	0.277 ± 0.262	43.3 ± 55.0	17.2 ± 11.8
	Bioaccessible %	12.3 ± 7.40	20.7 ± 7.03	7.13 ± 6.44	30.7 ± 18.9
Control	Total	999 ± 1887	1.27 ± 2.00	520 ± 828	20.6 ± 7.54
	Bioaccessible	156 ± 306	0.300 ± 0.438	63.8 ± 111	5.84 ± 0.00
	Bioaccessible %	16.6 ± 8.64	25.5 ± 8.85	8.30 ± 6.54	30.7 ± 11.0

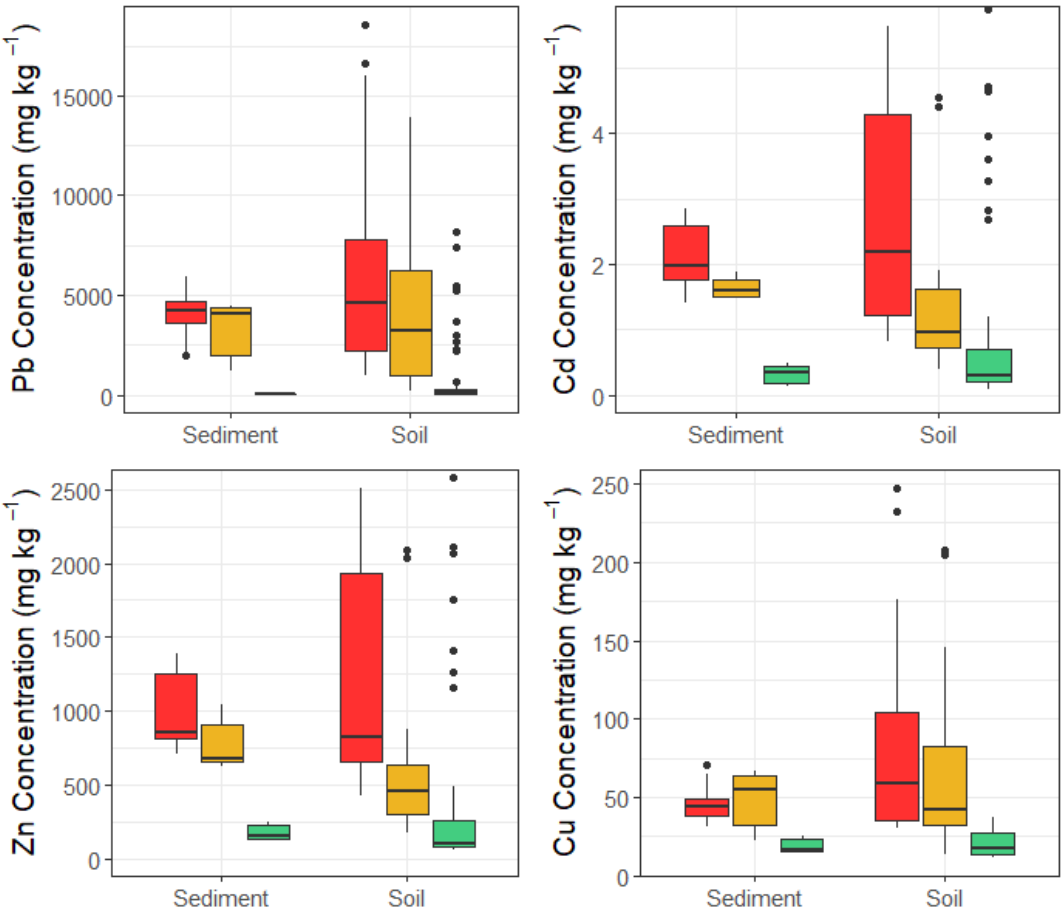

Figure 3.2: Sediment and soil trace metal concentrations across mine, private property, and control sites. Red represents the mine sites, yellow represents the private properties, and green represents the control sites.

3.4.2 Soil Bioaccessibility

Following the BARGE extraction protocol to assess bioaccessible concentrations of the trace metals, the soil trace metal concentrations decreased by between 68.2% and 96.1% (Table 3.3) (Wragg et al., 2009; BARGE & INERIS, 2010). Despite this decrease, the intestinal bioaccessible soil metal concentrations remained strongly correlated with the corresponding total soil metal concentrations for all four metals examined (Supplementary Figure 3.3). Bioaccessibility percentages were generally very low, especially for Zn and Pb, and particularly at the mine sites (where the mean percentages were 4.31% and 7.41%, respectively). Despite this, the mean Zn and Pb bioaccessibility concentrations at the mines (549 mg kg^{-1} and 451 mg kg^{-1}, respectively) were still elevated above those found at the private properties and control sites, due to the high total metal concentrations found at the mine sites (Table 3.3).

3.4.3 Invertebrates

Invertebrate full-body trace metal concentrations were highly variable across the sites (Table 3.4). This was likely due to variations in inter-species trace metal accumulation, which significantly differed across families (Pb: df = 11, χ^2 = 30.125, p = 0.00202; Cd: df = 11, χ^2 = 36.9, p = 0.000478; Zn: df = 11, χ^2 = 34.8, p = 0.000544; Cu: df = 11, χ^2 = 20.4, p = 0.0404; Supplementary Figure 3.4). When aggregated across families, invertebrate body burdens significantly varied across the mine, private property, and control sites for Pb (df = 2, χ^2 = 114, p < 0.0001), Cd (df = 2, χ^2 = 85.1, p < 0.0001), and Zn (df = 2, χ^2 = 79.6, p < 0.0001) (Figure 3.3). There were clear patterns of higher Pb, Cd, and Zn concentrations at the mine and private properties than at the control sites (Figure 3.3; Table 3.4). Lead, Cd, and Zn invertebrate body burdens were also strongly positively correlated with water (Pb: S [Spearman's Correlation Coefficient] = 163000, p < 0.0001, r_s = 0.770; Cd: S = 225000, p < 0.0001, r_s = 0.682; Zn: S = 209000, p < 0.0001, r_s = 0.705) and sediment (Pb: S = 175000, p < 0.0001, r_s = 0.743, Cd: S = 279000, p < 0.0001, r_s = 0.591; Zn: S = 258000, p < 0.0001, r_s = 0.622) concentrations.

While Cu concentrations did not vary significantly across the three sites (df = 2, χ^2 = 4.20, p = 0.122), the pattern of Cu invertebrate body burdens resembled that of Cu sediment concentrations, with the highest concentrations in the private properties, followed by the mine sites, and then the control sites (Figures 3.2 – 3.3). Invertebrate Cu body burdens were significantly correlated with sediment Cu concentrations (S = 493000, p = 0.000364, r_s = 0.278), though not with water concentrations (S = 612000, p = 0.0832).

Table 3.4: Invertebrate trace metal concentrations across the mine, private property, and control sites. Means are reported as ± standard deviation, ranges are provided in italics. Concentrations are in mg kg^{-1}.

Site	Pb	Cd	Zn	Cu
Mine	1830 ± 2520 *79.5 – 15900*	7.20 ± 6.07 *0.52 – 38.7*	2020 ± 1460 *217 – 6650*	32.0 ± 18.2 *1.92 – 106*
Private Property	3050 ± 2980 *141 – 10200*	9.52 ± 5.97 *0.87 – 28.8*	2380 ± 1890 *107 – 8890*	47.3 ± 33.3 *12.6 – 130*
Control	52.9 ± 140 *2.24 – 921*	1.81 ± 1.11 *0.23 – 5.18*	543 ± 296 *118 – 1620*	32.3 ± 29.4 *10.2 – 254*

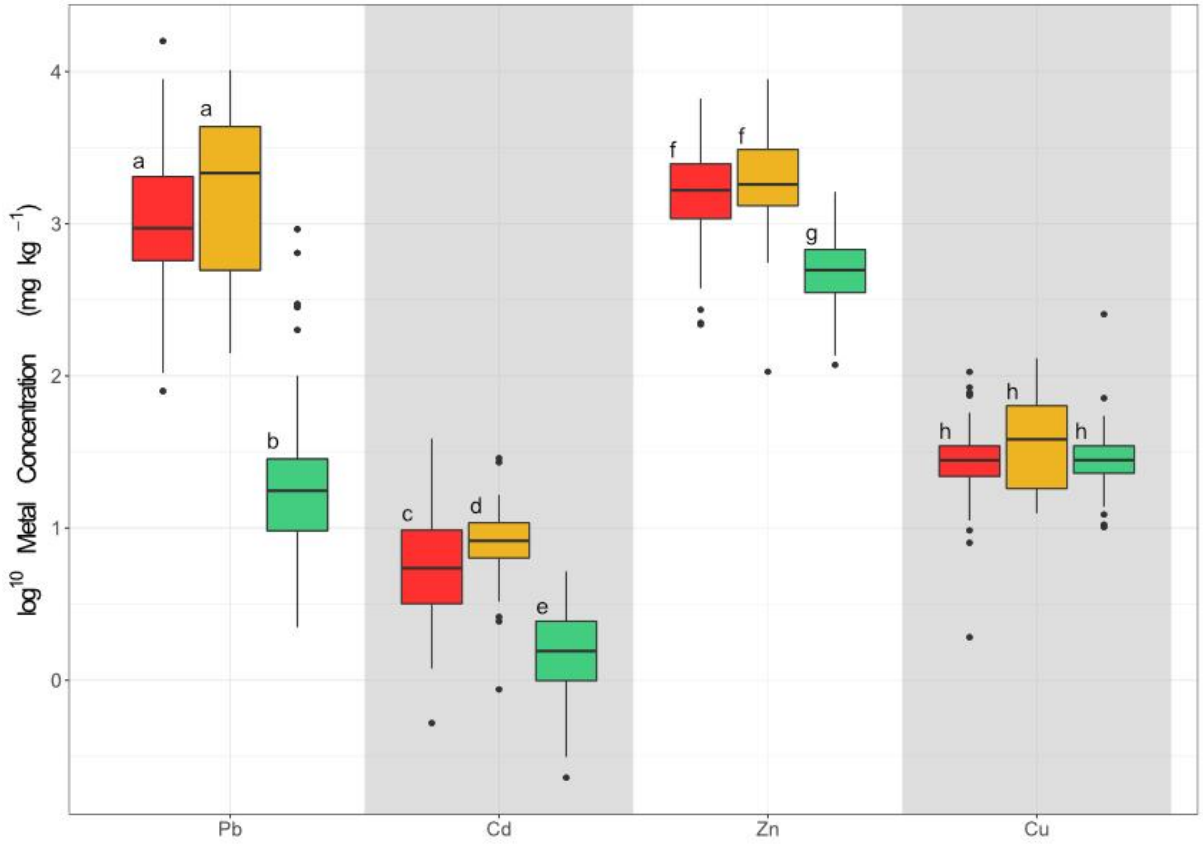

Figure 3.3: Invertebrate log^{10} transformed trace metal body burdens across mine, private property, and control sites. Red represents the mine sites, yellow represents the private properties, and green represents the control sites. For each metal, site types with no letter in common are significantly different (pairwise Wilcox test; α = 5% with Benjamini - Hochberg corrections).

3.4.4 Rodents

Four rodent species were collected during this study. Most of the rodents collected were wood mice (*Apodemus sylvaticus*, 111, 67.3% of total), but yellow-necked mice (*Apodemus flavicollis*, 22, 13.3% of total), field voles (*Microtus agrestis*, 18, 10.9% of total), and bank voles (*Myodes glareolus*, 14, 8.48%) were also found at the sites. Yellow-necked mice were only collected in Area 2, as Area 1 was outside their natural range (Marsh et al., 2001). When trace metal concentrations were compared across the species, only Zn concentrations in bone varied significantly across the four species (df = 3, $\chi 2$ = 19.2, p = 0.00305); otherwise, metal concentrations were broadly similar across tissues for the species. Despite this, to avoid species-specific variations in metal concentrations, which have been well documented in small mammals (Talmage & Walton, 1991; Shore & Douben, 1994a; Wijnhoven et al. 2007; Cooke, 2011; Van den Brink et al., 2011), and may have more profound effects across individual sites, further investigations on metal concentrations were done focusing on the most frequently collected species, the wood mouse.

The concentrations of the non-essential metals examined during this study (Pb and Cd) varied significantly between the mine, private property, and control sites in wood mouse kidney (Pb: df = 2, F = 103, p < 0.0001; Cd: df = 2, F = 6.25, p = 0.0329), liver (Pb: df = 2, F = 71.1, p < 0.0001; Cd: df = 2, F = 5.13, p = 0.0181), and, for Pb, in bone (Pb: df = 2, F = 112, p < 0.0001) (Table 3.6). Across all three tissues, Pb concentrations tended to be greatest at the mine sites, followed by the private property, and finally by the control sites (Figure 3.4; Table 3.5). Cadmium in bone followed the same pattern, but mean Cd concentrations in kidney and liver were highest at the private properties, followed by the mine sites and then the control sites (Figure 3.4; Table 3.5). Wood mouse tissue Pb concentrations were strongly and significantly correlated with soil concentrations at the collection transects (kidney: df = 94, S= 45600, p > 0.0001, ρ = 0.690; liver: df = 107, S = 71700, p > 0.0001, ρ = 0.668, bone: df = 103, S = 67700, p > 0.0001, ρ = 0.649). Despite the significant differences in Cd across the site types, Cd concentrations did not significantly correlate with transect soil concentrations (kidney: df = 94, S = 133000, p = 0.449; liver: df =107, S = 182000, p = 0.151; bone: df = 103, S = 152000, p = 0.0712).

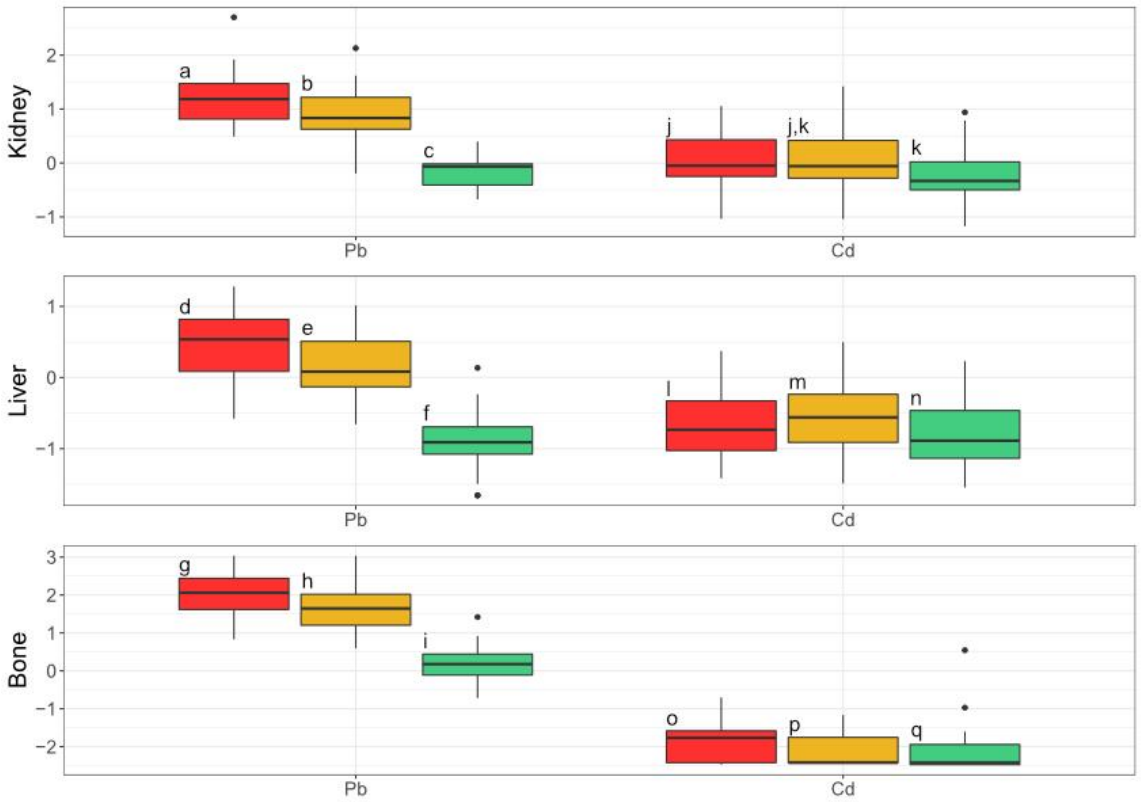

Figure 3.4: Wood mouse log^{10}transformed kidney, liver, and bone non-essential trace metal concentrations. Red represents the mine sites, yellow represents the private properties, and green represents the control sites. For each metal and tissue, groups with no letter in common are significantly different (Tukey posthoc test; α = 5% with Benjamini - Hochberg corrections).

The concentrations of the essential metals examined during this study, Zn and Cu, also varied significantly across the mine, private property, and control sites in wood mice kidneys (Zn: df = 2, F = 7.60, p = 0.00324; Cu: df = 2, F = 6.28, p = 0.00796) and livers (Zn: df = 2, F = 11.0, p = 0.000294; Cu: df = 2, F = 6.05, p = 0.00846), as well as in bone for Cu df = 2, F = 8.86, p = 0.00137) (Table 3.6). However, the trends across the site types were completely different to those observed for Pb and Cd. The Zn and Cu kidney concentrations were highest in the mine sites, followed by the control sites, and then the private properties, while the liver Zn and Cu concentrations were highest in the control sites, then private properties, and then mine sites, and the bone Zn and Cu concentrations were highest in the control sites, followed by the mine sites, and then the private properties (Figure 3.5). The only significant correlation detected between the essential elements across soil transects and wood mouse tissues was an inverse relationship between Cu concentrations in soil and wood mouse bones (df = 103, S = 251000, p = 0.00503, ρ = -0.303).

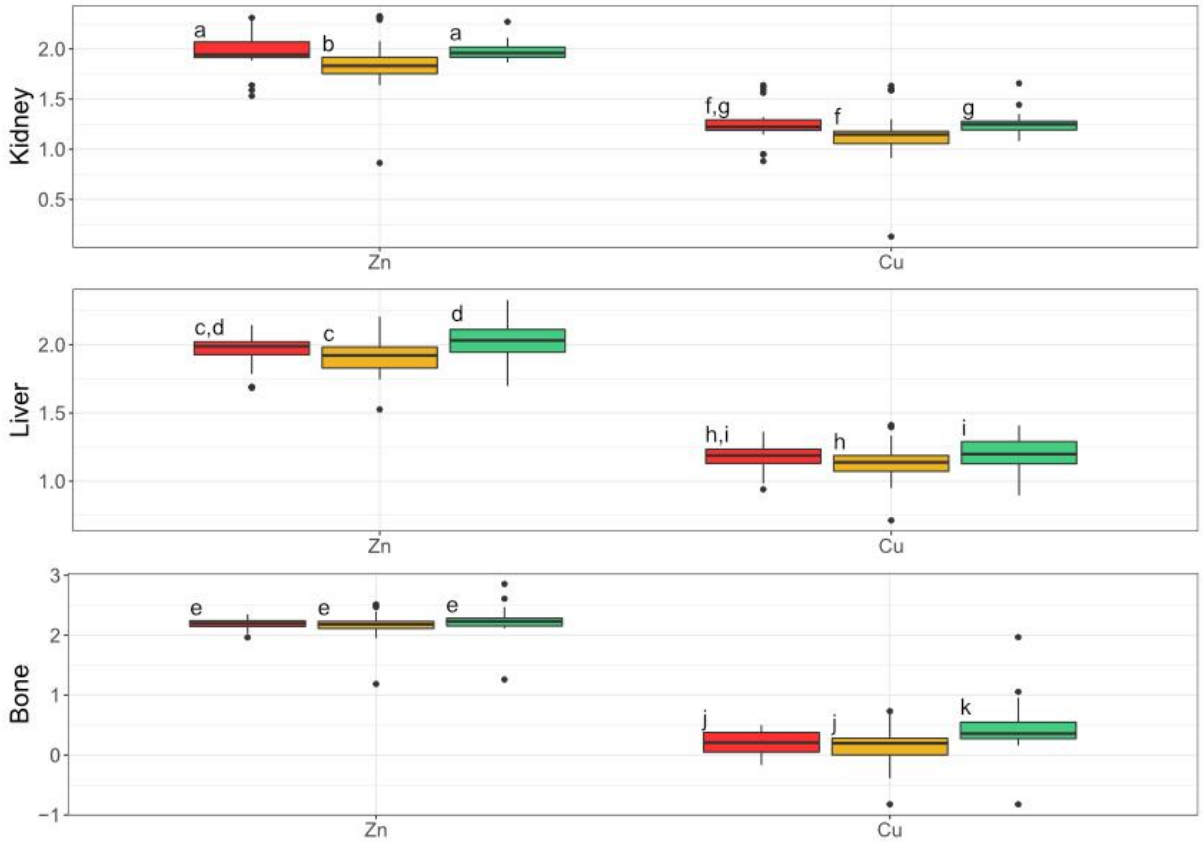

Figure 3.5: Wood mouse log[10]transformed kidney, liver, and bone essential trace metal concentrations. Red represents the mine sites, yellow represents the private properties, and green represents the control sites. For each metal and tissue, groups with no letter in common are significantly different (Tukey posthoc test; α = 5% with Benjamini - Hochberg corrections).

Beyond site type, trace metal concentrations varied depending on a range of factors (Table 3.6). In particular, the collection time (month and year) significantly affected concentrations of every trace metal and tissue, with the exception of Cu in bones and Cd in kidneys (Table 3.6; Supplementary Table 3.1). The mean concentrations of Pb and Cd in kidney and liver tissues were similar across the collection months, though with greater variation in May 2019 for Cd and October 2019 for Pb, while mean Pb and Cd bone concentrations were highest in September 2021 (Supplementary Figure 3.5; Supplementary Table 3.1). For the essential metals, concentrations were consistently lower in May 2019 than in October 2019 across all three tissues, though concentrations in September 2021 varied depending on the metal and tissue (Supplementary Figure 3.5; Supplementary Table 3.1). Other factors that influenced trace metal concentrations were whether the wood mouse was collected in Area 1 or Area 2 (there were significant differences across all metals and tissues, except for Cd in bones and Zn in kidneys and bones) and whether the wood mouse was a juvenile or adult (there were significant differences across all metals in kidneys, Zn and Cu in livers, and Pb in bones) (Table 3.6; Supplementary Table 3.1). Factors that did not appear to greatly affect trace metal concentrations included the sex of the wood mouse (only significant differences were between Cd concentrations in kidneys and in livers) and the individual site in which the wood mouse was collected (only significant differences were between Cd concentrations in kidneys and in bones) (Table 3.6; Supplementary Table 3.1).

Table 3.5: Wood mouse trace metal concentrations across the mine, private property, and control sites. Means are reported as ± standard deviation, ranges are provided in italics. Concentrations are in mg kg^{-1} dry weight.

Site	Tissue	n	Pb	Cd	Zn	Cu
Mine	Kidney	21	42.8 ± 107 *3.09 – 503*	2.16 ± 2.68 *0.0925 – 11.4*	107 ± 52.4 *34.0 – 205*	20.0 ± 10.6 *7.59 – 43.6*
	Liver	30	3.47 ± 4.36 *0.156 – 19.0*	0.337 ± 0.510 *0.0293 – 2.36*	77.2 ± 34.3 *24.2 – 140*	12.9 ± 4.99 *2.94 – 23.0*
	Bone	28	220 ± 265 *6.77 – 1090*	0.0244 ± 0.0369 *0.00336 – 0.199*	158 ± 32.0 *91.7 – 223*	1.74 ± 0.737 *0.680 – 3.16*
Private Property	Kidney	50	12.5 ± 19.7 *0.642 – 134*	2.24 ± 3.96 *0.0914 – 26.3*	80.8 ± 45.8 *7.28 – 212*	15.2 ± 7.94 *1.35 – 42.8*
	Liver	50	2.31 ± 2.48 *0.221 – 10.3*	0.485 ± 0.606 *0.0324 – 3.14*	83.8 ± 21.3 *33.6 – 161*	14.0 ± 3.49 *5.16 – 25.8*
	Bone	50	109 ± 198 *3.97 – 1080*	0.0113 ± 0.0124 *0.00377 – 0.0686*	153 ± 51.0 *15.4 – 327*	1.63 ± 0.970 *0.151 – 5.40*
Control	Kidney	27	0.887 ± 0.618 *0.211 – 2.50*	1.25 ± 2.01 *0.0672 – 8.70*	98.0 ± 24.0 *73.1 – 186*	18.6 ± 6.35 *12.0 – 45.4*
	Liver	31	0.220 ± 0.270 *0.0220 – 1.37*	0.259 ± 0.379 *0.0284 – 1.70*	105 ± 45.5 *22.2 – 213*	15.8 ± 5.12 *4.28 – 25.7*
	Bone	30	2.92 ± 4.85 *0.191 – 26.2*	0.0109 ± 0.0194 *0.00336 – 0.107*	174 ± 63.4 *18.2 – 406*	3.05 ± 2.32 *0.151 – 11.4*

Table 3.6: Significance table of wood mouse tissue trace metal concentrations across factors. All relationships were calculated using ANOVAs modelling metal concentration across site type (mine, private property, or control), sex (female or male), age class (juvenile or adult), collection time (May 2019, October 2019, September 2021), location (Area 1 or Area 2), and individual site (Mine Complex 1, Mine 2, Private Property 1, Private Property 2, Control 1, Control 2). The reported p-values are adjusted following Yekutieli & Benjamini (1999). A star signifies a significant p-value.

Metal	Tissue	Site Type	Collection Month	Location	Age	Sex	Individual Site
Pb	Kidney	F = 103 *p > 0.0001**	F = 9.48 *p = 0.00796**	F = 23.3 *p > 0.0001**	F = 11.1 *p = 0.00438**	F = 0.352 *p = 0.624*	F = 0.964 *p = 0.455*
	Liver	F = 71.1 *p > 0.0001**	F = 8.47 *p = 0.00159**	F = 20.7 *p =0.000119**	F = 2.11 *p = 0.210*	F = 4.293 *p = 0.0668*	F = 1.17 *p = 0.390*
	Bone	F = 112 *p > 0.0001**	F = 4.59 *p = 0.0228**	F = 14.6 *p = 0.00120**	F = 6.52 *p = 0.0238**	F = 3.80 *p = 0.140*	F = 1.50 *p = 0.309*
Cd	Kidney	F = 4.27 *p = 0.0320**	F = 4.43 *p = 0.0638*	F = 13.8 *p = 0.00151**	F = 5.43 *p = 0.0387**	F = 7.19 *p = 0.0185**	F = 4.77 *p = 0.0222**
	Liver	F = 5.13 *p = 0.0170**	F = 4.06 *p = 0.0371**	F = 8.95 *p = 0.00866**	F = 3.93 *p =0.0801*	F = 7.94 *p = 0.0137**	F = 3.06 *p = 0.0805*
	Bone	F = 6.94 *p = 0.00484**	F = 8.89 *p = 0.00137**	F = 0.138 *p = 0.776*	F = 0.800 *p = 0.448*	F = 1.37 *p = 0.327*	F = 11.9 *p = 0.000167**
Zn	Kidney	F = 7.60 *p = 0.00324**	F = 30.7 *p > 0.0001**	F = 3.179 *p = 0.117*	F = 15.9 *p = 0.000748**	F = 0.0480 *p = 0.862*	F = 1.06 *p = 0.426*
	Liver	F = 11.0 *p = 0.000294**	F = 78.8 *p > 0.0001**	F = 5.54 *p = 0.0371**	F = 24.3 *p > 0.0001**	F = 3.76 *p = 0.0846*	F = 1.23 *p = 0.374*
	Bone	F = 0.918 *p = 0.468*	F = 8.21 *p = 0.00193**	F = 0 *p = 0.992*	F = 0.0390 *p = 0.869*	F = 0.218 *p = 0.661*	F = 1.41 *p = 0.327*
Cu	Kidney	F = 6.28 *p = 0.00796**	F = 19.3 *p = 0.000200**	F = 7.47 *p = 0.0135**	F = 10.5 *p = 0.00510**	F = 1.97 *p = 0.227*	F = 0.224 *p = 0.856*
	Liver	F = 6.05 *p = 0.00846**	F = 34.3 *p > 0.0001**	F = 9.31 *p = 0.00796**	F = 10.7 *p = 0.00484**	F = 0.644 *p = 0.485*	F = 2.46 *p = 0.133*
	Bone	F = 8.86 *p = 0.00137**	F = 3.43 *p = 0.0621*	F = 7.99 *p = 0.0137**	F = 1.33 *p = 0.327*	F = 0.0190 *p = 0.902*	F = 0.228 *p = 0.856*

3.5 Discussion

3.5.1 Trace Metal Contamination Distribution

Notably high and potentially toxic concentrations of Pb, Cd, Zn, and Cu were found in the water, sediment, and soils of the two derelict mine sites, as well as the two private properties located 1 km and 4 km, respectively, downstream of the mine sites. Despite this distance, the private properties had substantially elevated trace metal concentrations in the water, sediments, and soils; these concentrations were sometimes even higher than those detected at the mine site. As the mine sites had likely been operational on and off since pre-Roman times, areas downstream of the mines were consistently exposed to high concentrations of metals over this time, leading to large-scale trace metal environmental pollution (Rainbow, 2018).

High trace metal concentrations in stream water and sediments, as were detected at both the mine sites and downstream private properties, can act as a secondary source of trace metal pollution. Floods, which are frequent in this region, can distribute contaminated water and sediment onto nearby soils (Middelkoop, 2000; Foulds et al., 2014; Marrugo-Negrete et al., 2017). Furthermore, if a contaminated stream's channel changes, either through natural or anthropogenic means, it will leave behind a contaminated streambed (Martín-Crespo et al., 2020). For example, in Private Property 2, the stream was diverted in the past to work a water-powered mill system. While the stream's channel has since been diverted again, the dry streambed likely contained significant amounts of trace metal pollution (Martín-Crespo et al., 2020), and could partially explain why the whole of Private Property 2 appeared to be contaminated with trace metals, despite the stream from Mine 2 only running along the side of the 8 ha property (Figure 2.7).

Soils at both the mine sites and private properties had notably elevated trace metal concentrations. While abandoned spoil heaps provided a clear and ongoing source of trace metals at the mine sites, the private properties appeared pristine, without any obvious signs of trace metal contamination. The high metal concentrations found at the private properties may be partly due to a combination of wind (causing aerial deposition of particulates from the uncovered spoil heaps at the mine sites) (Moreno et al., 2007; Corriveau et al., 2011; Martín-Crespo et al., 2020) and past flooding events (Middelkoop, 2000; Foulds et al., 2014; Marrugo-Negrete et al., 2017). Additional anthropogenic activities can further distribute trace metal particulates; for example, both property owners reported the frequent use of

recreational off-road vehicles at the mine sites, which can generate airborne dust and mobile tailing particulates (Corriveau et al., 2011).

Anthropogenic activities can also more directly cause the spread of trace metals beyond the mine sites. In the surveyed region, it was common in the past to utilize mine waste as gravel for roads or tracks, due to its easy availability. In Private Property 2, a gravel track was found to have notably high Pb concentrations (23100 ± 2070 mg kg^{-1}), resembling those found at the mine site. Chickens living adjacent to this track and consuming the gravel as grit had substantially elevated Pb concentrations in their blood, feathers, and bones, and consistently laid eggs with high, potentially toxic Pb concentrations (Sartorius et al., 2022). Mine waste was also found further afield, in areas where metal contamination would otherwise not be present. For example, Control 1 was upstream of all nearby mine sites, however, rodent transects located around a dirt path had notably elevated Pb soil concentrations (2410 ± 1790 mg kg^{-1}) relative to other sampled areas in Control 1, which had minimal Pb pollution. When a portion of this dirt path was excavated, it was found to have originally been made with gravel, and its surface Pb concentration (7750 ± 545 mg kg^{-1}) was similar to those found in the mine spoil in Mine 1b, suggesting that the path had been made with mining waste gravel. However, despite the high Pb soil concentrations, the rodent Pb tissue concentrations sampled at these sites did not significantly differ with those found at Control 2 (kidney: $t = -1.80$, $p = 0.276$; liver: $t = 0.0704$, $p = 0.945$; bone: $t = -0.508$, $p = 0.935$). In this instance, due to the artificial nature of the path, its small width (approximately 1 m), and the fact that the gravel was covered with soil, it appears that the high Pb soil concentrations were localized to the path and its immediate surroundings. This, along with the lack of vegetative cover and, therefore, poor foraging opportunities on the path, likely explains why rodents trapped adjacent to the path did not have significantly elevated Pb concentrations. However, it is still important to note that the widespread use of mine spoil as gravel can spread mine waste beyond the original mine site and potentially result in elevated trace metal concentrations in animals (Sartorius et al., 2022).

3.5.2 Trace Metal Transfer into Wildlife

Aquatic invertebrates collected at the mine sites and downstream private properties had significantly higher Pb, Cd, and Zn body burdens than invertebrates from the control sites. Trace metal accumulation patterns varied across families, as expected, as metal tolerance in invertebrates is well known to vary greatly across taxonomic groups (Rainbow, 2018), but these differences did not mask the effects of exposure to high trace metal

concentrations. Other studies have similarly observed elevated concentrations of these trace metals in aquatic invertebrates in metal-contaminated environments (Spehar et al., 1978; Eisler, 1988; Goodyear and McNeill, 1999; Solà & Prat, 2006; Santoro et al., 2009; De Jonge et al., 2013; Rainbow, 2018; Arnold et al., 2021). The invertebrate Pb body burdens were particularly high, representing some of the highest concentrations recorded in literature (Goodyear & McNeill, 1999; Solà & Prat, 2006; Santoro et al., 2009; De Jonge et al., 2013; Rainbow, 2018; Arnold et al., 2021). While the reported concentrations may be elevated above the true invertebrate tissue concentrations, as the gut contents of the invertebrates were not purged (to allow for a better estimate of predator trace metal exposure), many of the invertebrate concentrations were higher than their corresponding sediment concentrations, suggesting metal accumulation. The invertebrate Pb, Cd, and Zn body burdens were also strongly correlated with the water and sediment concentrations, as previously observed for freshwater macroinvertebrates (Goodyear & McNeill, 1999). Unlike the other metals, invertebrate Cu concentrations did not significantly differ across site types or correlate with water concentrations, though they did correlate with Cu sediment concentrations. This is consistent with the relatively low Cu contamination at all sampled sites.

Similar to the macroinvertebrates, wood mice collected from contaminated sites accumulated significantly more Pb and Cd than wood mice from the control sites, as has been observed in previous studies (Johnson et al., 1978; Milton et al., 2002; Rogival et al., 2007; Sánchez-Chardi et al., 2007, Tête et al., 2014). While other factors, such as location, season, and age, affected non-essential metal exposure, as expected (Hunter et al., 1987; Milton et al., 2002; Milton et al., 2004; Sánchez-Chardi et al., 2007; Tomza-Marciniak et al., 2019), the concentrations of non-essential trace metals in the environment did have an effect on trace metal tissue concentrations in wood mice.

Pb concentrations in wood mouse tissues were strongly positively correlated with Pb soil concentrations. Lead bioaccessibility in the sampled soils, particularly at the mine sites, was generally low, which is consistent with prior studies on Pb-contaminated soils (Bosso & Enzweiler, 2007; Rogival et al., 2007), especially at Pb mine sites (Rieuwerts et al., 2000; Hettiarachchi & Pierzynski, 2004; Intawongse & Dean, 2006). While bioaccessibility was assessed during this study using a simulated human gut system, murine gut systems are broadly similar to those of humans, with some exceptions (Nguyen et al., 2015), so this model should provide a reasonable approximation of bioaccessibility to rodents. Despite the low bioaccessibility of Pb in the soil, the high total concentrations of Pb in the mines and private properties meant that rodents were still exposed to high bioaccessible concentrations

of Pb (Table 3.3), resulting in accumulation within their tissues (Baranowska-Bosiacka et al., 2019).

Toxicity thresholds for trace metals are generally difficult to develop, as concentration measurements do not differentiate between detoxified and metabolically active (toxic) trace metals (Rainbow, 2018). However, there have been a few suggested toxicity thresholds for Pb in rodents, primarily linked to observations of potentially Pb-caused health effects in the field. Roberts et al. (1978) suggested Pb kidney, liver, and bone thresholds for wood mice based on kidney damage observed during field studies; these thresholds were 47 mg kg^{-1} d.w. for kidney, 11.7 mg kg^{-1} d.w. for liver, and 352 mg kg^{-1} d.w. for bone. Between two (based on liver Pb concentrations) and eight (based on bone concentrations) wood mice sampled during the current study exceeded these thresholds (five from Mine 2 and three from Private Property 2, all collected in Area 2). Based on Shore and Douben's (1994b) proposed Pb toxic threshold for small mammals (25 mg kg^{-1} d.w. in kidneys), ten individuals (nine wood mice and one bank vole) were at risk of Pb toxicity (seven from Mine 2 and three from Private Property 2, again all collected in Area 2) (Fritsch et al., 2010). In a review of the existing literature, Ma (2011) suggested that mammalian kidney Pb concentrations greater than 15 mg kg^{-1} d.w., and bone Pb concentrations greater than 25 mg kg^{-1} d.w., can be associated with kidney damage. Based on Ma's (2011) kidney threshold, 28 individuals (26 wood mice, one yellownecked mouse, and one bank vole), 14 each at the mines and private properties, were at potential risk of kidney damage. Ma's (2011) proposed bone Pb threshold was exceeded by 39 individuals (93% of the collected rodents) at the mine sites, and 52 individuals (76% of the collected rodents) at the private properties.

As was found for Pb, Cd kidney and liver concentrations were elevated in wood mouse from the mine sites and private properties when compared to the control sites. Bone Cd concentrations did not significantly differ across the site types, likely due to Cd not commonly accumulating in bones (Johnson et al., 1978). Wood mice living in Cd-polluted sites have been known to accumulate Cd in their kidneys and livers, as was observed in the current study (Johnson et al., 1978; Rogival et al., 2007), though most studies in Cd contaminated areas reported Cd tissue concentrations much higher than those observed in the current study (Talmage & Walton, 1991; Shore, 1995; Rogival et al., 2007; Tête et al., 2014; Tomza-Marciniak et al., 2019). These previous studies were focused on areas with higher environmental Cd contamination than found during the current study (Talmage & Walton, 1991; Shore, 1995), as mine sites in west Wales are known to have relatively low soil Cd concentrations compared to other metal-contaminated areas (Milton et al., 2004).

The concentrations of Cd in the kidneys and livers of wood mice sampled during the current study did not directly correlate with the soil Cd concentrations from their respective rodent transects. Prior studies have found that Cd concentrations in wood mice are affected primarily by dietary composition, as opposed to local soil concentrations (Van den Brink et al., 2011). As Cd does not normally bioaccumulate in the dietary components of wood mice (primarily seeds, acorns, and fruits) (Hunter & Johnson, 1982; Montgomery & Montgomery, 1990; Talmage & Walton, 1991; Tomza-Marciniak et al., 2019), wood mouse Cd tissue concentrations are normally low compared to other rodents, even in contaminated sites (Cooke, 2011; Van den Brink et al., 2011). As such, the wood mouse Cd concentrations were uniformly below Shore & Douben's (1994a) proposed threshold for kidney damage in small mammals of 105 mg kg^{-1} d.w..

In contrast to the accumulation patterns observed with the non-essential elements, the concentrations of essential elements (Cu and Zn) in wood mice examined during the current study did not appear to be affected by local contamination. This has been found in previous studies on rodents, and researchers have suggested that the lack of Cu and Zn accumulation was due to the precise biological regulation of essential elements in the body (Talmage & Walton, 1991; Milton & Johnson, 2002; Fritsch et al., 2003; Świergosz-Kowalewska et al., 2005; Rogival et al., 2007; Martiniaková et al., 2012). While some studies have found elevated Cu or Zn rodent tissue concentrations in highly contaminated sites (Talmage & Walton, 1991; Rogival et al., 2007), generally, wood mice do not bioconcentrate Cu or Zn in kidneys or livers, even when fed high-Zn diets (Hunter et al., 1987; Talmage & Walton, 1991). In fact, inverse relationships between environmental contamination and Cu or Zn concentrations, observed during the current study between Cu in soil and wood mouse bones, have previously been found for Zn in wood mice (Milton & Johnson, 2002) and bank voles (Milton et al., 2003) collected near mines in west Wales, and for Cu in shrews in Poland (Świergosz-Kowalewska et al. 2005). One possible explanation could be that rodents in contaminated areas modify their diets to avoid high concentrations of Cu and Zn, which has been observed in both laboratory experiments (Beernaert et al., 2008) and field studies (Ozaki et al., 2018). Another potential explanation is that rodents exposed to high concentrations of dietary Zn may reduce Zn absorption (through synthesis of metallothionein proteins, triggered by the high Zn concentrations) and increase their Zn excretion rate, therefore decreasing overall Zn accumulation (Milton & Johnson, 2002).

The concentrations of Cu and Zn in wood mouse tissues appeared to be more influenced by season than by the contamination gradient, based on the consistent increases in

Cu and Zn concentrations from May to October 2019 (Supplementary Figure 3.5). Prior studies also found indications that rodent Cu and Zn tissue concentrations vary at least partially based on season (Wijnhoven et al. 2007; Demir & Yavuz, 2020). These differences are most likely attributable to seasonal changes in diet (Eisler, 1993), which are well-recorded in wood mice (Montgomery & Montgomery, 1990) and have been known to affect seasonal Cu and Zn exposure in rodents (Włostowski et al., 1988). Prior studies on bank voles found changes in Cu and Zn tissue concentrations corresponding with seasonal changes in Cu and Zn exposure (Włostowski et al., 1988).

3.5.3 Individual and Ecosystem Health Implications

Chronic exposure to high trace metal concentrations can have a variety of adverse health effects. In aquatic invertebrates, trace metal exposure is well known to result in increased mortality, affecting community structures and often causing full extirpation of species from metal-contaminated environments (Dickman et al., 1990; Hare, 1992; Clements, 1994; Maret et al., 2003; Rainbow, 2018). Trace metals can also cause a variety of sublethal health effects, impacting invertebrate oviposition, hatching, development, emergence, growth, locomotion, and feeding (Hare, 1992; Di Veroli et al., 2014; Rainbow, 2018).

Rodents can be similarly impacted by living in environments contaminated by trace metals. In mammals, chronic exposure to high Pb concentrations can have negative health effects across multiple physiological systems, including the central nervous system, renal system, hematopoietic system, the cardiovascular system, and the gastrointestinal system (Ma, 2011). Measuring chronic negative health effects in short-lived species, such as rodents, is difficult, but a few studies have successfully linked environmental trace metal exposure with histological abnormalities (Roberts et al., 1978; Ma, 2011; Tête et al., 2014). In the current study, rodents collected at mines and in downstream areas had high Pb concentrations in their kidneys and bones that could be indicative of kidney damage (Ma, 2011), but future work is necessary to directly assess whether kidney damage can be clearly detected in rodents exposed to environments with high trace metal concentrations.

Determining the exposure to and accumulation of trace metals in common prey species, such as aquatic invertebrates and rodents, is the first step in understanding trace metal accumulation and impacts across all resident fauna. While trace metals do not traditionally biomagnify, they can transfer directly from prey to predator. For example, earthworms can accumulate high concentrations of Cd, which can then transfer to

insectivorous small mammals, who consequently develop higher Cd tissue concentrations than is found in their surrounding environment (Cooke, 2011). Furthermore, even if trace metal biomagnification is limited, invertebrates and rodents can be used as trace metal biomonitors to assess relative environmental contamination and determine the potential exposure risks for other resident animals (Hare et al., 1992; Al Sayegh Petkovšek et al., 2014; Rainbow, 2018). If aquatic invertebrates and rodents are accumulating high, potentially toxic concentrations of trace metals in their tissues, it is reasonable to suspect that other local animals are doing so as well. In this way, these prey species can act as 'canaries in the coal mine', signalling the ecosystem-wide effects of trace metal pollution (Sánchez-Chardi et al., 2007; Cooke, 2011; Al Sayegh Petkovšek et al., 2014; Rainbow, 2018).

3.6 Conclusions

Trace metal contamination can have wide-reaching impacts across ecosystems, even centuries after the original pollution event. While the former metalliferous mines examined during the current study have been closed for over a hundred years, the trace metal pollution remaining at these sites continues to have effects at, and beyond, the original mine sites. High concentrations of Pb, Zn, and, to a lesser extent, Cd and Cu, were found in the environments of both the mine sites and at properties several km downstream of the mines, in areas that appeared visually pristine. The trace metal concentrations found in the water and sediments of the streams flowing through the mine sites were sufficiently elevated that they could pose a health risk to local aquatic animals. High concentrations of Pb, Cd, Zn, and Cu were also found in aquatic invertebrates living in these streams. These elevated invertebrate trace metal body burdens were directly correlated with the concentrations of these metals in the water (for all but Cu) and in the sediment at their collection sites. On land, although wood mice appeared able to regulate the concentrations of the essential elements (Zn and Cu) that they were exposed to, they accumulated high concentrations of Pb, positively related to their exposure to Pb in soil. While there are currently no clear Pb health thresholds for rodents, the Pb concentrations may be indicative of kidney damage in between 28% and 83% of the rodents collected at both the mine sites and areas downstream. The wood mice at the mine sites and downstream private properties also had elevated Cd concentrations when compared to wood mice from control sites, though the Cd concentrations were not elevated above current proposed health thresholds. Overall, when assessing metal pollution, full ecosystem surveys, encompassing areas beyond the pollution's origin point, are necessary to assess potential ecosystem damage. Globally, there are hundreds of thousands, if not millions, of

abandoned or currently active metal mines, and the continued high demand for metals will encourage further metal mining in the future (Watari et al., 2021). Furthermore, more frequent flooding and storms as a result of climate change (Jentsch & Beierkuhnlein, 2008; Hirabayashi et al., 2013; Foulds et al., 2014) will mobilise and re-distribute trace metal pollutants already present in the environment, exacerbating their reach and impact. A better understanding of the environmental distribution of trace metal pollution, its transfer to local fauna, and the resultant health effects is necessary to more effectively detect, manage, and mitigate the impacts of this global pollutant.

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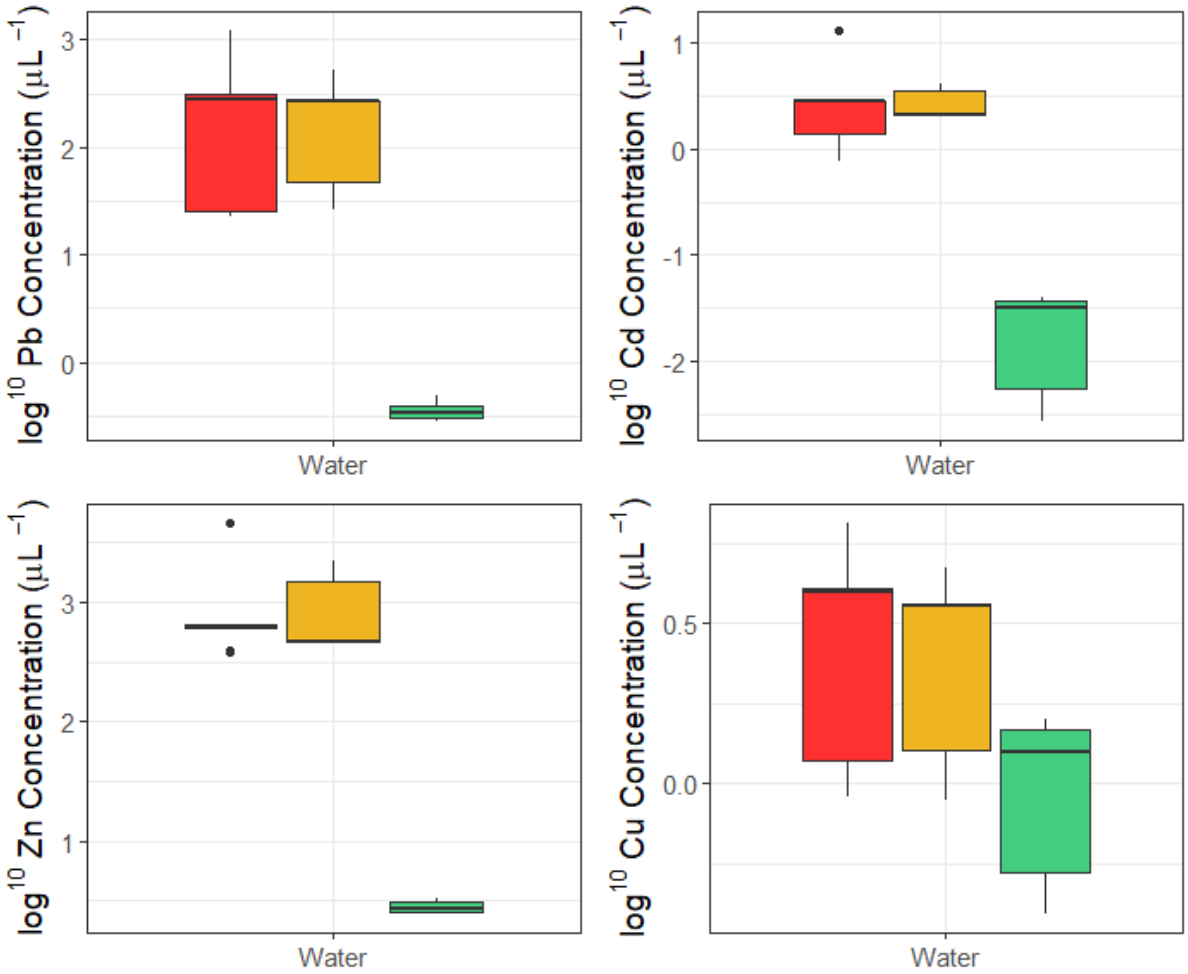
Data Availability

The data that support the findings of this study are available upon request.

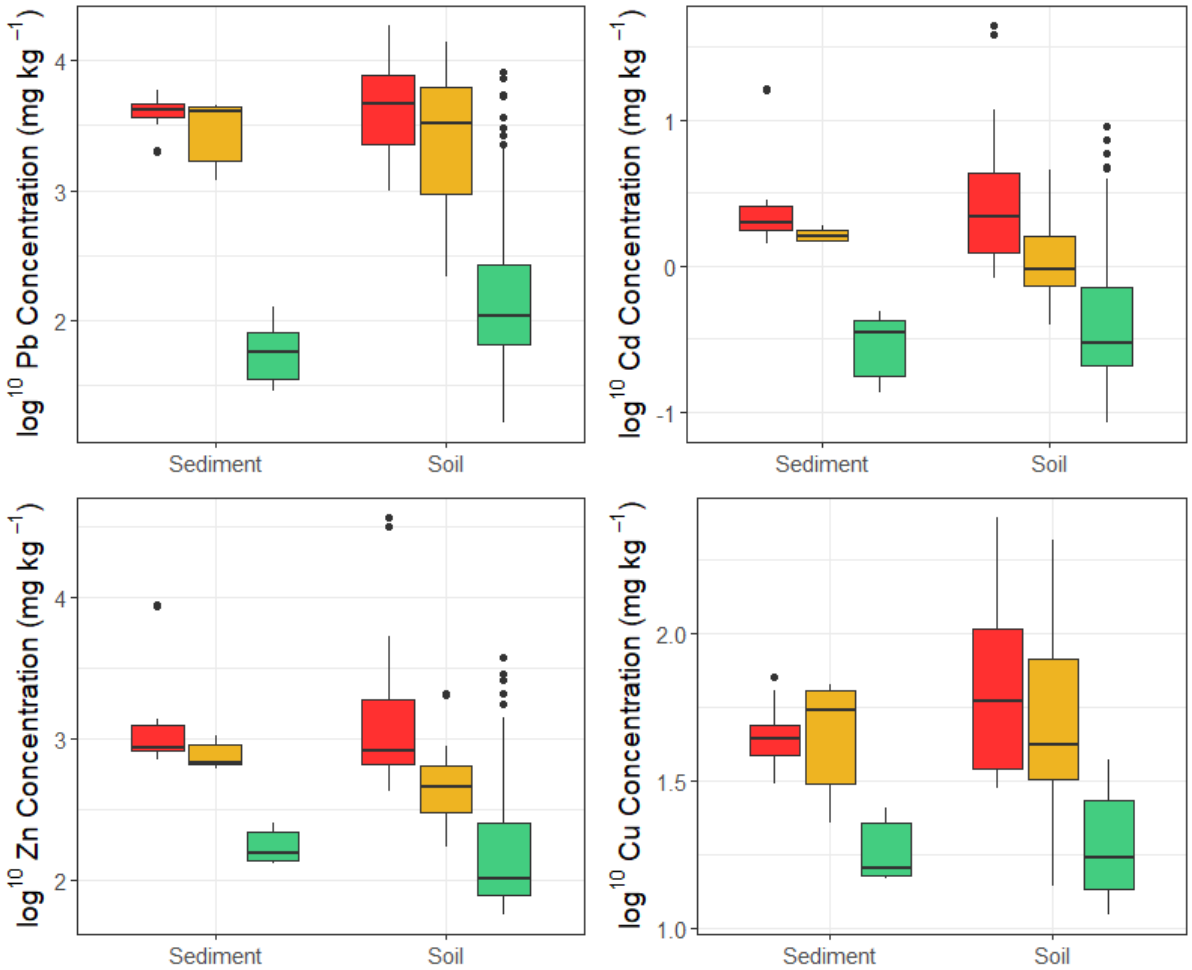
The Natural Resources Wales data used for dissolved organic carbon concentrations is publicly available and contains public sector information licensed under the Open Government Licence v3.0.

Appendices

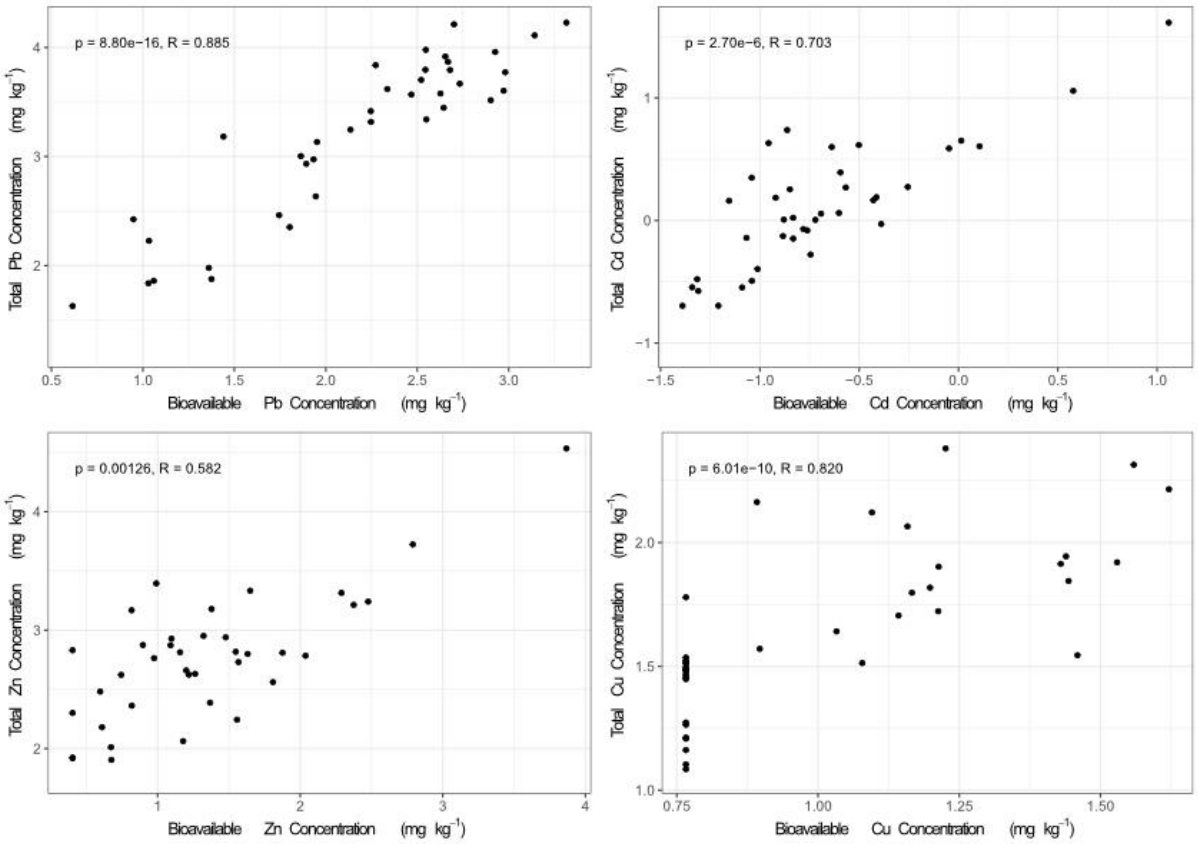
Appendix A: Supplementary Figures



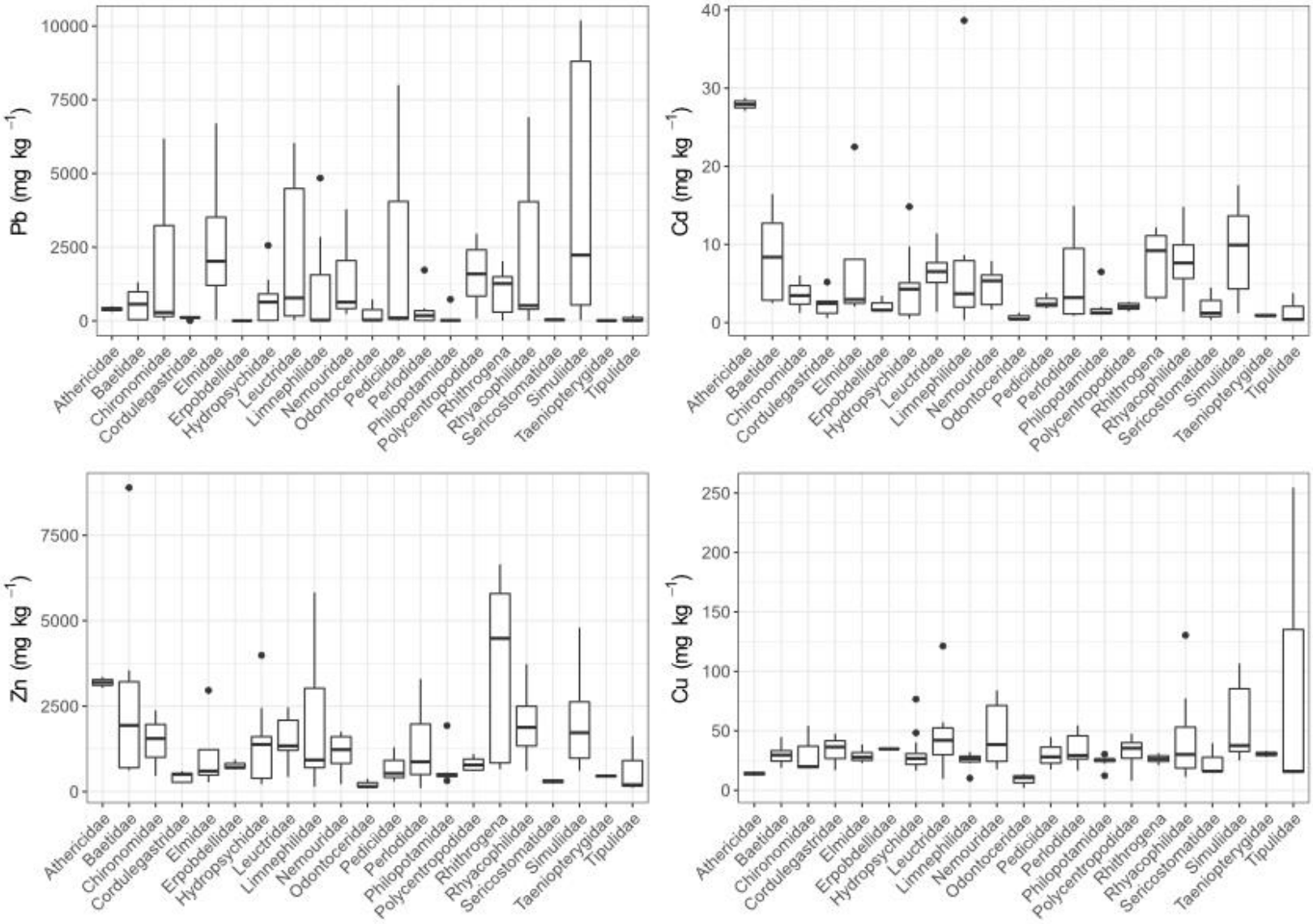
Supplementary Figure 3.1: Water log^{10} trace metal concentrations across mine, private property, and control sites. Red represents the mine sites, yellow represents the private properties, and green represents the control sites.



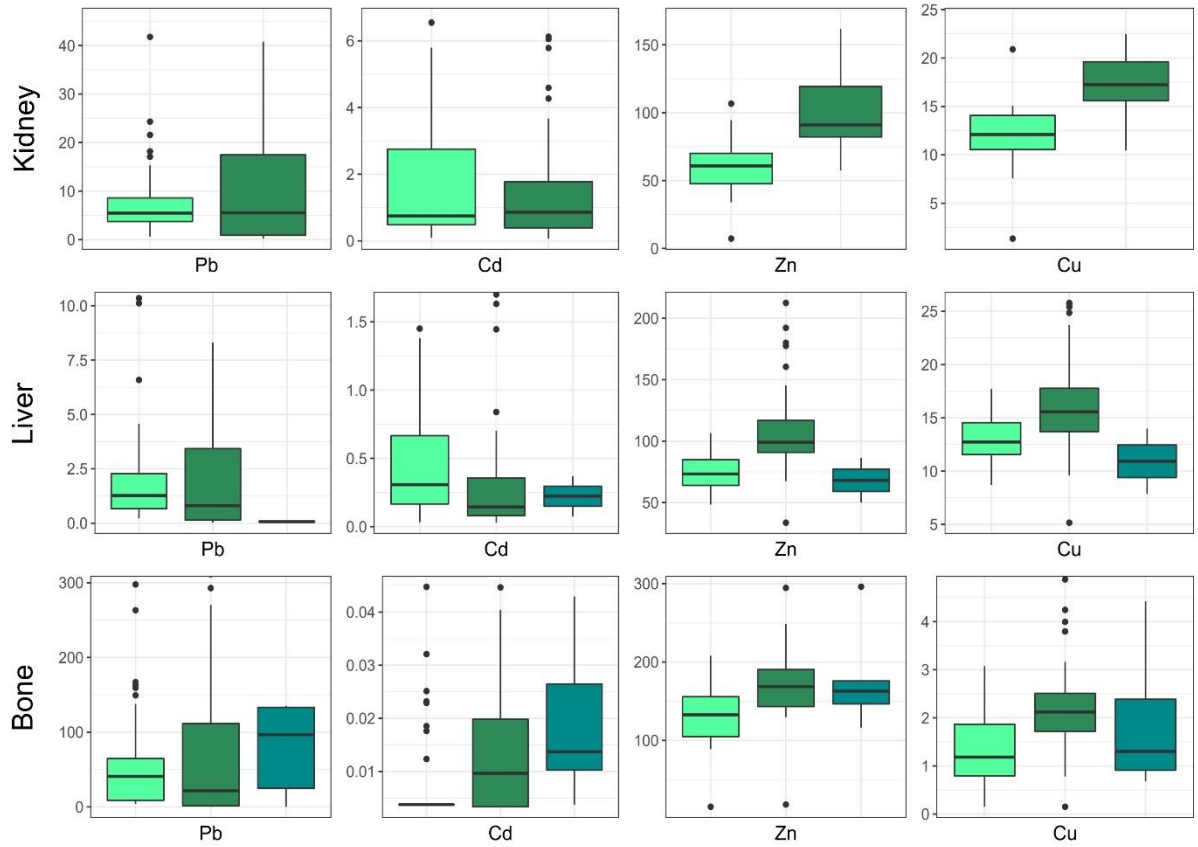
Supplementary Figure 3.2: Sediment and soil log[10] trace metal concentrations across mine, private property, and control sites. Red represents the mine sites, yellow represents the private properties, and green represents the control sites.



Supplementary Figure 3.3: Total versus bioaccessible soil trace metal concentrations. Spearman's correlation coefficients are shown in the top right; the p-values are adjusted following Yekutieli & Benjamini (1999).



Supplementary Figure 3.4: Pb, Cd, Zn, and Cu concentrations across collected invertebrate families at all sampled sites.



Supplementary Figure 3.5: Wood mouse kidney, liver, and bone trace metal concentrations across collection month. Rodents collected in May 2019 are on the left, in light green, rodents collected in October 2019 are on the middle, in dark green, and rodents collected in September 2021 are on the right in blue.

Appendix B: Supplementary Tables

Supplementary Table 3.1: Wood mouse trace metal concentrations across analysed factors. Means are reported as ± standard deviation, ranges are provided in italics. Concentrations are in mg kg^{-1} dry weight.

Factor		Tissue	n	Pb	Cd	Zn	Cu
Collection Month	May 2019	Kidney	37	8.01 ± 7.98 *0.642 – 41.8*	2.46 ± 4.52 *0.0914 – 26.3*	60.2 ± 17.8 *7.28 – 107*	12.1 ± 3.20 *1.35 – 20.9*
		Liver	37	2.12 ± 2.42 *0.221 – 10.3*	0.537 ± 0.650 *0.0324 – 3.14*	74.4 ± 14.9 *48.3 – 107*	13.0 ± 2.08 *8.71 – 17.7*
		Bone	37	60.6 ± 71.9 *3.97 – 298*	0.0100 ± 0.0139 *0.00377 – 0.0686*	132 ± 36.8 *15.4 – 208*	1.45 ± 0.960 *0.151 – 5.40*
	October 2019	Kidney	61	20.5 ± 66.4 *0.211 – 503*	1.64 ± 2.18 *0.0672 – 11.4*	110 ± 44.0 *57.4 – 212*	20.3 ± 9.00 *10.5 – 45.4*
		Liver	60	2.34 ± 3.61 *0.0220 – 19.1*	0.340 ± 0.481 *0.0284 – 2.36*	108 ± 30.7 *33.6 – 213*	16.3 ± 4.23 *5.16 – 25.8*
		Bone	62	136 ± 257 *0.191 – 1090*	0.0172 ± 0.0284 *0.00336 – 0.199*	175 ± 51.6 *18.2 – 406*	2.44 ± 1.74 *0.151 – 11.4*
	September 2021	Kidney	0	– –	– –	– –	– –
		Liver	14	0.530 ± 0.743 *0.0319 – 2.48*	0.147 ± 0.156 *0.0293 – 0.582*	38.8 ± 17.0 *22.2 – 86.4*	8.34 ± 4.05 *2.94 – 15.8*
		Bone	8	125 ± 141 *1.51 – 451*	0.0166 ± 0.0131 *0.00377 – 0.0429*	170 ± 54.5 *116 – 296*	1.68 ± 1.23 *0.680 – 4.43*

Factor		Tissue	n	Pb	Cd	Zn	Cu
Location	Area 1	Kidney	41	5.61 ± 5.26 *0.211 – 22.5*	1.26 ± 2.03 *0.0672 – 11.4*	78.3 ± 33.9 *34.0 – 212*	13.6 ± 3.15 *7.59 – 20.9*
		Liver	45	1.27 ± 1.71 *0.0319 – 7.94*	0.279 ± 0.415 *0.0284 – 2.36*	81.9 ± 33.3 *28.4 – 213*	13.0 ± 4.16 *4.00 – 24.9*
		Bone	44	50.6 ± 69.4 *0.191 – 332*	0.0167 ± 0.0306 *0.00377 – 0.199*	161 ± 64.7 *18.2 – 406*	1.78 ± 1.90 *0.151 – 11.4*
	Area 2	Kidney	57	23.1 ± 68.4 *0.254 – 503*	2.45 ± 3.86 *0.154 – 26.3*	100 ± 47.7 *7.28 – 209*	19.8 ± 9.91 *1.35 – 45.4*
		Liver	66	2.56 ± 3.61 *0.0220 – 19.1*	0.452 ± 0.589 *0.0337 – 3.14*	92.2 ± 35.2 *22.2 – 192*	15.1 ± 4.57 *2.94 – 25.8*
		Bone	63	150 ± 255 *0.596 – 1090*	0.0132 ± 0.0170 *0.0336 – 0.107*	159 ± 39.3 *15.4 – 296*	2.23 ± 1.22 *0.151 – 9.06*
Age	Juvenile	Kidney	42	26.3 ± 79.4 *0.211 – 503*	*1.27* ± 2.02 *0.0672 – 11.4*	*113* ± 49.8 *34.0 – 212*	*21.1* ± 10.2 *7.59 – 45.4*
		Liver	47	2.41 ± 3.86 *0.0220 – 19.1*	0.261 ± 0.426 *0.0284 – 2.36*	96.8 ± 34.1 *29.1 – 192*	15.2 ± 4.59 *4.00 – 25.7*
		Bone	46	154 ± 270 *0.191 – 1090*	0.0152 ± 0.0296 *0.00336 – 0.199*	164 ± 41.4 *18.2 – 327*	2.19 ± 1.02 *0.151 – 6.65*

Factor		Tissue	n	Pb	Cd	Zn	Cu
Age	Adult	Kidney	56	7.91 ± 7.95 *0.226 – 41.8*	2.46 ± 3.89 *0.0914 – 26.3*	74.6 ± 29.4 *7.28 – 209*	14.2 ± 5.04 *1.35 – 39.3*
		Liver	64	1.76 ± 2.27 *0.0319 – 10.3*	0.470 ± 0.582 *0.0324 – 3.14*	81.6 ± 33.8 *22.2 – 213*	13.5 ± 4.33 *2.94 – 25.8*
		Bone	61	75.9 ± 132 *0.416 – 829*	0.0143 ± 0.0178 *0.00336 – 0.107*	157 ± 57.3 *15.4 – 406*	1.93 ± 1.84 *0.151 – 11.4*
Sex	Female	Kidney	40	23.9 ± 79.3 *0.227 – 503*	2.78 ± 4.55 *0.151 – 26.3*	92.3 ± 46.7 *7.28 – 212*	16.3 ± 8.18 *1.35 – 43.6*
		Liver	45	2.79 ± 3.92 *0.0220 – 19.1*	0.507 ± 0.638 *0.0293 – 3.14*	92.9 ± 39.4 *24.2 – 213*	14.8 ± 5.54 *2.94 – 25.8*
		Bone	43	156 ± 258 *0.416 - 1090*	0.0187 ± 0.0338 *0.00336 – 0.199*	157 ± 54.1 *15.4 – 406*	2.12 ± 1.96 *0.151 – 11.4*
	Male	Kidney	58	10.2 ± 18.9 *0.211 – 135*	1.38 ± 1.79 *0.0672 – 7.70*	90.5 ± 41.8 *34.0 – 207*	17.8 ± 8.51 *7.59 – 43.4*
		Liver	66	1.53 ± 2.16 *0.0220 – 10.3*	0.296 ± 0.426 *0.0284 – 2.29*	84.6 ± 30.8 *22.2 – 192*	13.8 ± 3.62 *4.28 – 25.7*
		Bone	64	78.3 ± 157 *0.191 - 1080*	0.0119 ± 0.0121 *0.00336 – 0.0448*	162 ± 49.1 *18.2 – 327*	1.99 ± 1.21 *0.151 – 6.65*

Factor		Tissue	n	Pb	Cd	Zn	Cu
Individual Sites	Mine Complex 1	Kidney	8	7.88 ± 4.83 *3.09 – 15.3*	2.46 ± 3.86 *0.0925 – 11.4*	75.8 ± 33.5 *34.0 – 117*	13.5 ± 4.67 *7.59 – 20.9*
		Liver	12	1.49 ± 1.99 *0.156 – 6.58*	0.347 ± 0.647 *0.0293 – 2.36*	58.9 ± 25.0 *28.4 – 97.8*	9.76 ± 3.73 *4.00 – 13.8*
		Bone	11	104 ± 109 *6.77 – 332*	0.0408 ± 0.0547 *0.00377 – 0.195*	139 ± 34.7 *91.7 – 223*	1.28 ± 0.796 *0.680 – 3.16*
	Private Property 1	Kidney	25	6.49 ± 5.32 *0.759 – 22.7*	0.838 ± 1.14 *0.0914 – 5.79*	72.3 ± 35.4 *43.6 – 212*	13.2 ± 2.55 *9.17 – 19.9*
		Liver	25	1.50 ± 1.76 *0.221 – 7.94*	0.246 ± 0.332 *0.0324 – 1.63*	84.0 ± 24.9 *33.6 – 161*	13.7 ± 3.40 *5.16 – 24.9*
		Bone	25	42.6 ± 39.8 *4.68 – 143*	0.00721 ± 0.00695 *0.00377 – 0.0282*	162 ± 55.1 *101 – 327*	1.41 ± 1.01 *0.412 – 5.40*
	Control 1	Kidney	8	0.587 ± 0.336 *0.211 – 1.08*	1.39 ± 1.38 *0.0672 – 3.67*	99.8 ± 22.1 *73.1 – 130*	15.0 ± 3.14 *12.0 – 20.8*
		Liver	8	0.201 ± 0.182 *0.0319 – 0.485*	0.282 ± 0.191 *0.0284 – 0.540*	110 ± 45.2 *75.0 – 213*	15.7 ± 4.42 *11.7 – 23.7*
		Bone	8	2.70 ± 3.04 *0.191 – 8.30*	0.0134 ± 0.00768 *0.00377 – 0.0250*	189 ± 109 *18.2 – 406*	3.63 ± 3.62 *0.151 – 11.2*

Factor		Tissue	n	Pb	Cd	Zn	Cu
Individual Sites	Mine 2	Kidney	13	64.3 ± 133 *5.85 – 503*	1.97 ± 1.77 *0.319 – 6.06*	126 ± 53.6 *77.7 – 205*	24.1 ± 11.3 *14.5 – 43.6*
		Liver	18	4.78 ± 5.03 *0.244 – 19.1*	0.330 ± 0.416 *0.0373 – 1.75*	89.3 ± 34.8 *24.2 – 140*	15.0 ± 4.65 *2.94 – 23.0*
		Bone	17	296 ± 309 *17.1 – 1090*	0.0138 ± 0.0110 *0.00336 – 0.0447*	171 ± 23.5 *139 – 217*	2.04 ± 0.625 *1.27 – 2.87*
	Private Property 2	Kidney	25	18.5 ± 26.3 *0.641 – 135*	3.65 ± 5.16 *0.276 – 26.3*	89.4 ± 53.7 *7.28 – 209*	17.2 ± 10.7 *1.35 – 42.8*
		Liver	25	3.12 ± 2.84 *0.737 – 10.3*	0.724 ± 0.721 *0.0690 – 3.14*	83.6 ± 17.3 *55.8 – 126*	14.4 ± 3.61 *3.61 – 8.90*
		Bone	25	175 ± 263 *3.97 – 1080*	0.0155 ± 0.0152 *0.00377 – 0.0686*	144 ± 45.8 *15.4 – 249*	1.85 ± 0.898 *0.151 – 4.88*
	Control 2	Kidney	19	1.01 ± 0.671 *0.254 – 2.50*	1.19 ± 2.25 *0.154 – 8.70*	97.2 ± 25.3 *77.5 – 186*	20.1 ± 6.80 *13.7 – 45.4*
		Liver	23	0.227 ± 0.298 *0.0220 – 1.37*	0.251 ± 0.430 *0.0337 – 1.70*	104 ± 46.5 *22.2 – 192*	15.8 ± 5.43 *4.28 – 25.7*
		Bone	21	3.00 ± 5.44 *0.596 – 26.2*	0.0100 ± 0.0224 *0.00336 – 0.107*	168 ± 36.6 *130 – 296*	2.84 ± 1.67 *1.67 – 1.44*

Appendix C: Unified BARGE Method Protocol

Protocol modified from BARGE & INERIS (2010).

A) Digestive Fluids Preparation

1) Label eight 500 mL volumetric flasks, two for each digestive fluid (Saliva, Gastric, Duodenal, or Bile).
2) For each digestive fluid, label one flask with "inorganic" and the other flask with "organic".
3) Add the necessary components to the corresponding bottles, as shown in Supplementary Table 3.2.
4) Mix each corresponding organic and inorganic solution together in 1 L bottles.
5) Store at room temperature until the day before analysis.
6) Label new bottles for each digestive fluid.
7) Add enzymes to each bottle as specified in Supplementary Table 3.3, followed by the indicated amount of each digestive fluid.
8) Mix the bottles on spinner plates for 1 hour.
9) Adjust the pH of the digestive fluids using 1M NaOH and 1M HCl solutions until they reach the following pHs:
    a. Saliva: 6.5 ± 0.5
    b. Gastric: 1.1 ± 0.1
    c. Duodenal: 7.4 ± 0.2
    d. Bile: 8 ± 0.2
10) Store overnight at 4˚C.

B) Unified BARGE Method Test

1) Weigh out 0.47 g of soil and place in a 50 mL polycarbonate tube.
    a. Run each soil sample in triplicate to account for potential pH variations during the UBM.
2) Add 7.0 mL of Saliva fluid by pipette.
3) Shake by hand for approximately 10 seconds.
4) Add 10.5 mL of Gastric fluid by pipette.
5) Check pH = 1.20 ± 0.05. Adjust with NaOH 1M and/or HCl 37% if necessary.
6) Shake by hand for approximately 10 seconds.
7) Check pH = 1.20 ± 0.05. Adjust with NaOH 1M and/or HCl 37% if necessary.
8) Place tubes into an incubator set to 37˚C and 200 shakes per minute.
9) Leave the samples in the incubator for 1 hour.
10) Check if pH < 1.50 for each sample. If pH < 1.50, note the pH.
11) Add 21 mL of Duodenal fluid by pipette.
12) Add 7.0 mL of Bile fluid by pipette.
13) Check pH = 6.30 ± 0.5. Adjust with NaOH 1M and/or HCl 37% if necessary.
14) Place tubes into an incubator set to 37˚C and 200 shakes per minute.
15) Leave the samples in the incubator for 4 hours.
16) Note the final pH of the extracts.
17) Centrifuge the samples for 15 minutes at 4500 g.
18) Collect the top 20 mL of the supernatant.
19) Store supernatant at -20˚C until ICP-MS analysis.

Supplementary Table 3.2: Digestive fluid reagents for the UBM. Numbers in italics are in mL, while numbers not in italics are in g.

Reagent		Saliva	Gastric	Duodenal	Bile
INORGANIC					
KCl	Potassium chloride	0.896	0.824	0.564	0.376
NaH_2PO_4	Sodium dihydrogen phosphate dihydrate	0.888	0.266		
KSCN	Potassium thiocynate	0.200			
Na_2SO_4	Sodium sulfate	0.570			
NaCl	Sodium chloride	0.298	2.752	7.012	5.26
$CaCl_2$	Calcium chloride dehydrate		400		
NH_4Cl	Ammonium chloride		306		
$NaHCO_3$	Sodium hydrogen carbonate			5.607	5.786
KH_2PO_4	Potassium dihydrogen orthophoshate			0.080	
$MgCl_2$	Magnesium chloride			0.050	
1 M NaOH	Sodium hydroxide	*1.8*			
37% HCl	Hydrochloric acid		*8.3*	*0.180*	*0.180*
Pure H_2O	Milli-Q Water	*500*	*500*	*500*	*500*
ORGANIC					
	Urea	0.200	0.085	0.100	0.250
	Glucose		0.650		
	Glucuronic acid		0.020		
	Glucosamine hydrochloride		0.330		
Pure H_2O	Milli-Q Water	*500*	*500*	*500*	*500*

Supplementary Table 3.3: Digestive fluid enzymes for the UBM. Numbers in italics are in mL, while numbers not in italics are in g.

Enzyme	Saliva	Gastric	Duodenal	Bile
Alpha amylase	0.029			
Mucin	0.010	0.900		
Uric acid	0.003			
Bovine Serum Albumin		0.300	0.600	0.360
Pepsin		0.300		
$CaCl_2$			0.120	0.044
Pancreatin			1.800	
Lipase			0.300	
Bile				1.200
Digestive Fluid	*200*	*300*	*600*	*200*

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Chapter 4 – Legacy mines and accumulation of lead in freshwater macroinvertebrates

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Legacy mines and accumulation of lead in freshwater macroinvertebrates

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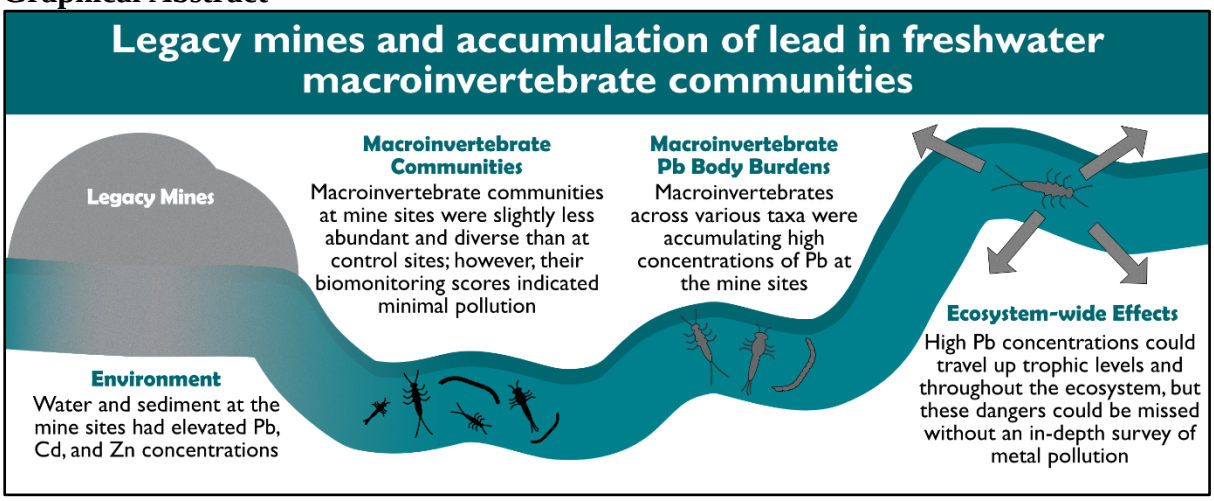
Highlights

- Potentially toxic Pb concentrations were found in streams at abandoned Pb mines
- Aquatic invertebrates from mine sites had strongly elevated Pb body burdens
- Mine communities were slightly less abundant and diverse than control communities
- One third of mine invertebrates accumulated more Pb than was in the sediment

Graphical Abstract



4.1 Abstract

Many freshwater bodies around the world are polluted with trace metals, often associated with current or past mining activities. These metals can have negative health effects on local flora and fauna, including aquatic macroinvertebrates. As aquatic macroinvertebrate communities are a vital trophic rung, it is critically important to understand how trace metal pollution can affect invertebrates on both the individual and community level. Water, sediment, and macroinvertebrate samples were collected at abandoned Pb mine sites in Wales and at nearby, uncontaminated control sites to explore the impacts of Pb on macroinvertebrates. The invertebrate communities at the mine sites had a slightly lower abundance and species richness when compared to the control sites, and the community structure differed between the sites across taxonomic order, functional feeding group, and locomotion type. The Pb body burdens of invertebrates from the mine sites were notably high across families, with between 50% and 98% of the sampled individuals exceeding proposed Pb hyperaccumulation thresholds. This has implications for the bioaccumulation of Pb from abandoned and contemporary mine sites, as the relatively abundant and diverse communities that persevere in such areas could be an important food source, and thus a source of contamination, for other aquatic and terrestrial animals. Metal pollution should therefore be considered when evaluating freshwater ecosystems and water quality. The findings also raise important questions about the acclimation of multiple taxa to notably high Pb concentrations in freshwater environments.

4.2 Introduction

Trace metals are common pollutants of freshwater bodies around the world (Zhou et al., 2020). Anthropogenic activities, such as mining, manufacturing, and fertilizer and pesticide usage, are the most common sources of trace metal pollution (Zhou et al., 2020). Even in areas where this pollution is now heavily regulated, or where polluting activities have ceased, historical pollution continues to have detrimental effects across ecosystems (Coulthard & Macklin, 2003). In particular, derelict metal mines remain a key source of water and sediment metal pollution, even after being abandoned for centuries (Coulthard & Macklin, 2003; Mayes et al., 2010). In the U.K. alone, metal pollution from thousands of abandoned metalliferous mines affects the water and sediments of at least 1,500 km of waterways (Hudson-Edwards et al., 2008; Coal Authority, 2016). This pollution will likely worsen in the future, as climate change is expected to cause more frequent and intense flooding events, which will re-suspend and distribute trace metal contaminated sediments across landscapes (Hudson-Edwards et al., 2008; Foulds et al., 2014).

Trace metals, such as Pb, Cd, Cu, and Zn, are known for their toxicity at relatively low concentrations, so pollution events involving these metals can have large-scale health consequences (Rainbow, 2018). Trace metals pose a major health risk to all biological systems and have a wide range of negative effects, including inhibiting or impairing reproduction, kidney function, and nervous system development, and well as potentially acting as a carcinogen (Tchounwou et al., 2012). Animals living in contaminated waterways can be profoundly impacted by their exposure to trace metals. Many animals are extirpated when their habitat becomes polluted, thus greatly affecting community structures (Hare, 1992; Clements, 1994; Rainbow, 2018). Animals that are able to persist in these environments can accumulate high concentrations of trace metals, and frequently experience adverse health effects (Hare, 1992). Trace metals can also potentially travel up the trophic levels and into predators that consume prey with high trace metal body burdens, thereby affecting the whole ecosystem (Rainbow, 2002; Rainbow et al., 2004).

Due to their ubiquity, aquatic macroinvertebrates are commonly used as biological indicators of pollution in waterways (Arnold et al., 2021). However, relatively little is known of aquatic invertebrate patterns of metal accumulation or expected trace metal body burdens, particularly beyond a few key taxa (primarily Ephemeroptera, Plecoptera, Trichoptera, and Chironomidae) (Rainbow, 2018). Furthermore, while most studies focus primarily on large waterways with multiple diffuse metal pollution sources, fewer examine macroinvertebrates collected at the pollution's point of origin (Solà & Prat, 2006; De Jonge et al., 2013;

Rainbow, 2018). To investigate this, the current study was developed to directly compare invertebrate trace metal body burdens to environmental trace metal concentrations at former Pb mine sites. Therefore, the aim of this study was to: (1) quantify environmental concentrations of Pb across historical mining sites and nearby control sites, (2) measure body burdens of Pb in invertebrate tissues across all sampled taxonomic groups, at the same sites as environmental measurements, and (3) explore the relationships between Pb pollution in the environment and in macroinvertebrates. Lead was the principal metal of concern in this study due to its high toxicity, biologically non-essential nature, and likelihood of high concentrations at former Pb mining sites (Dudka & Adriano, 1997; Tchounwou et al., 2012).

4.3 Methods

4.3.1 Sites

This study was part of a larger project on the effects of trace metal contamination from historical mines on surrounding ecosystems. Sampling efforts were focused on two abandoned Pb mine sites located in central Wales (Mine Complex 1 and Mine 2) and two control sites (Control 1 and 2) near each mine; for anonymization purposes to protect local residents, the exact locations of these sites are not provided. While these mines were worked most intensively during the industrial revolution, there is evidence of mining activities at these sites for at least 2,000 years (Natural Resources Wales, 2014; Natural Resources Wales, 2016a; Natural Resources Wales, 2016b). Both sites had streams that flowed directly through the mine sites, with spoil heaps on their banks (Figure 4.1). Some remediation work, focusing on clay-capping spoil heaps and diverting water from flowing through mine shafts, was done at Mine Complex 1 between 2011 and 2018; however, the remediation works did not fully address the metal contamination in the sampled streams (Natural Resources Wales, 2016a; Natural Resources Wales, 2016b). The control sites were both woodland parks, and were primarily forested and minimally managed. Both sites contained small streams with similar sediment type, width, depth, and slope to the streams found at the mine sites. The control sites were located approximately 3 – 6 km from the mine sites (Figure 4.1). Prior to the start of this study, water, sediment, and soil samples were collected from the control sites and tested to confirm that the sites contained minimal trace metal contamination. Water, sediment, and invertebrate samples were collected in autumn 2020 at three locations in Mine Complex 1 (two in two separate streams running along spoil heaps, and one directly after their confluence), one location in Mine 2, one location in Control 1, and one location in

Control 2, and in spring 2021 from two locations in Mine Complex 1 (again in the two separate streams running along spoil heaps) and one location in Control 1 (Figure 4.1).

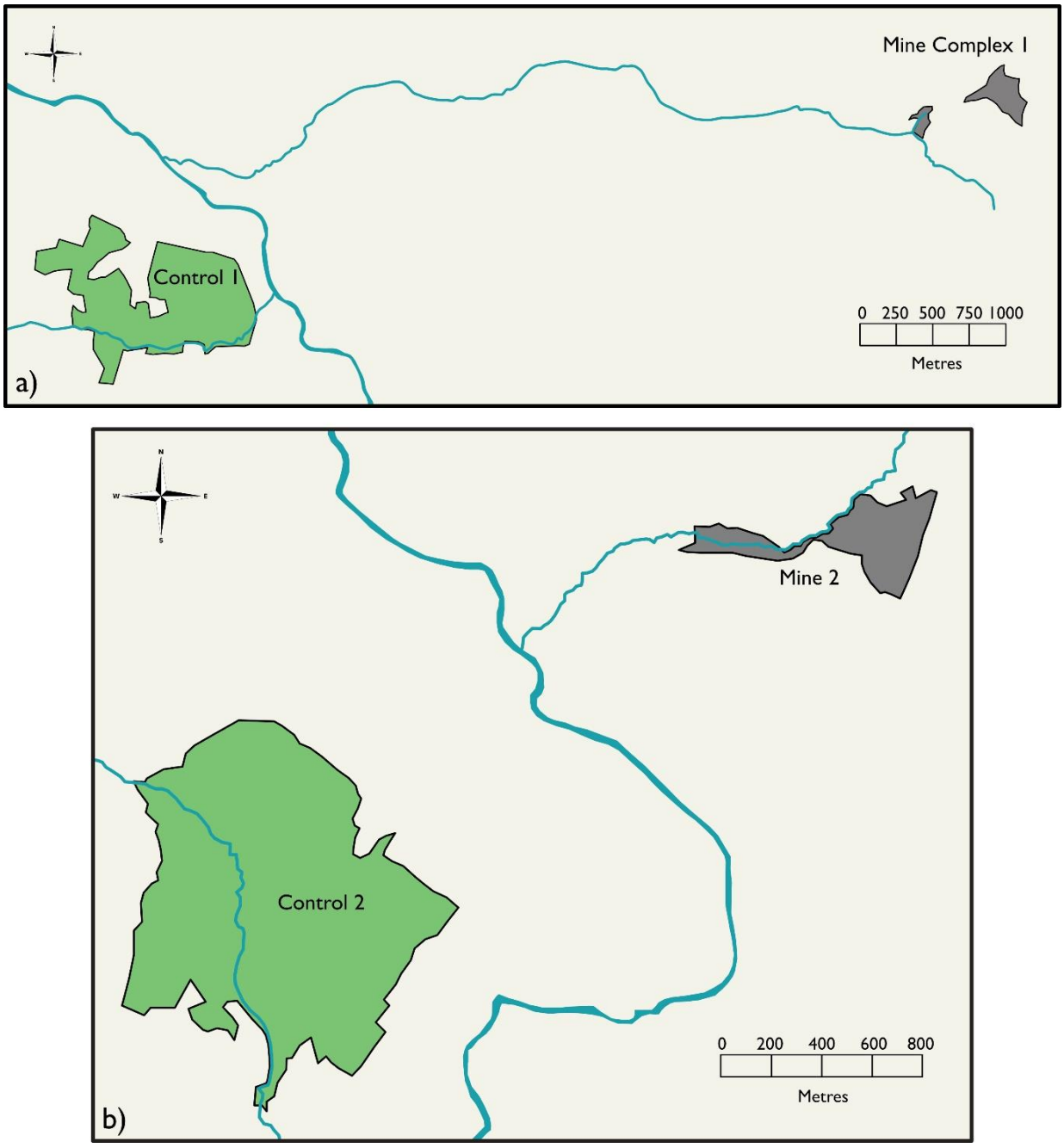


Figure 4.1: Map of a) Mine Complex 1 and Control 1 and b) Mine 2 and Control 2. Grey represents the mine sites, green represents the control sites, and blue represents waterways.

4.3.2 Environmental Samples

Water and sediment samples were collected at all sites in conjunction with the invertebrate sampling to determine the extent of the environmental trace metal contamination. Water samples (20 mL) were collected by fully submerging a 20 mL plastic Discardi II syringe (BD, Franklin Lakes, New Jersey, United States) triple-rinsed with the water sample and filtering the water through a polyethersulfone 0.22 µm filter (Chromatography Direct, Runcorn, United Kingdom) into a 20 mL plastic universal tube containing 2 mL 10% Primar grade HNO_3. All water sample tubes were double bagged and stored at 4 ˚C prior to analysis. In the laboratory, 10 mL of the filtered, acidified water sample was analysed for multiple elements using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Model ICAP-Q; Thermo Fisher Scientific, Bremen, Germany). To determine the total organic carbon of the water samples, 20 mL of filtered, non-acidified water samples were run through a TOC-VCPH (Shimadzu Corporation, Kyoto, Japan). The total organic carbon concentrations and metal concentrations were used to calculate the "Predicted No-Effect Concentrations" (Defra, 2014), Pb, Cd, Zn, and Cu concentrations below which no adverse effects are expected, for each sampled site using the Metal Bioavailability Assessment Tool and Pb Screening Tool (Water Framework Directive & United Kingdom Technical Advisory Group, 2014).

Sediment samples were collected by manually scooping sediment from the river-bed substrate using a 250 mL jug, targeting patches of visibly fine sediment (< 5 mm). Any excess water was poured off at the time of collection, though care was taken to retain as much fine sediment as possible. All sediment samples were stored in 50 mL conical centrifuge tubes, double bagged, and kept at ambient temperature. The sediment samples were air-dried before being sieved to <2 mm and subsequently ground into fine powder using a Retsch planetary ball mill PM 400 (Retsch, Haan, Germany). Approximately 0.4 g of each sample were then digested in Aqua Regia (1 mL HNO_3 and 3 mL HCl) at 95˚C using a teflon-coated graphite hotplate block digester (Analab, Bischeim, France). After two hours, the samples were allowed to cool before being dispensed into plastic volumetric flasks and made up to 50 mL with MilliQ water (18.2 MΩ cm; Millipore Corporation, Darmstadt, Germany). After the volumetric flasks were inverted, the samples were left for at least ten minutes to allow heavier material to settle to the bottom of the tube, and 10 mL of the supernatant solutions were decanted and stored at ambient temperature. The solutions were diluted 1-in-10 with MilliQ water prior to elemental analysis by ICP-MS. A certified reference material (NIST 2711A, Montana soil) was run for quality assurance purposes (Pb recovery was 93.1% of the certified value).

4.3.3 Invertebrates

Aquatic invertebrate communities were sampled following the Environment Agency's standard protocol (Murray-Bligh, 1999) of a three-minute kick sample plus a one-minute hand search, using a 1 mm^2 mesh net with an opening of 0.25 m wide and 0.22 m deep. The operator moved systematically upstream, ensuring, to the best of their ability, that different habitats were proportionally sampled through time based on spatial coverage. The collected sample was stored in alcohol to preserve the invertebrates. The invertebrates were sorted in the laboratory, with each individual identified to genus level, where possible, and counted. Those taxa that were not identified to genus level were identified instead to taxonomic family, such as Chironomidae and Simuliidae, with the exception of Oligochaeta, which were identified to sub-class. A consistent taxonomic resolution was used across all samples for comparative purposes.

Sampled invertebrates were freeze dried to a constant mass and prepared for acid digestion. Any invertebrate weighing over 0.01 g (dry weight) was acid digested individually. For invertebrates weighing under 0.01 g, multiple individuals of the same taxonomic family (chosen to allow for the pooling of a larger number of individuals) and collected in the same kick sample were pooled together to reach a cumulative weight greater than 0.01 g. If a cumulative weight greater than 0.01 g could not be reached, the invertebrates were not analysed. Invertebrate samples were acid digested and prepared for ICP-MS analysis following a similar protocol to the sediment samples, except the invertebrates were acid digested with 4 mL 70% HNO_3 and 1 mL H_2O_2. Lead concentrations were quantified in 114 invertebrate samples. The limit of detection (LOD) was calculated as three times the standard deviation of the concentrations measured in 10 blank digestion samples (Marin et al., 2011). Any sample with an elemental concentration lower than the LOD was ascribed the concentration 0.5*LOD (Kushner, 1976). A certified reference material for Pb in biological samples (BRC-185R Bovine Liver [trace elements]) was run for quality assurance purposes (Pb recovery was 94.2% of the certified values).

4.3.4 Invertebrate Traits

Invertebrate traits, specifically functional feeding groups and locomotion type, were evaluated as potential factors affecting Pb exposure. The trait categories and defining characteristics were obtained from the website www.freshwaterecology.info (Schmidt-Kloiber & Hering, 2015), based on Moog (1995) for functional feeding group, and Schmedtje & Colling (1996) for locomotion type, and were applied at the genus level. As the traits were

recorded using a ten-point assignment system, the highest ranking trait type was classified as the primary trait type for analytical purposes. If two trait types were both ranked at the same level, the trait type for the genera was recorded as "trait type 1" + "trait type 2". Definitions of the trait types used in this study are listed in Supplementary Table 4.1.

4.3.5 Statistical Analyses

Statistical tests were used to compare invertebrate community composition and Pb body burdens in R (R Core Team, 2018). Statistical tests were only performed on groups with more than five samples (sites, individuals, or Pb concentrations); any groups with fewer samples were removed prior to analysis. The six invertebrate community samples from the mine sites and the three samples from the control sites were grouped based on site type for community composition and Pb concentration comparisons.

To compare Pb body burdens between invertebrates from the mine and control sites, a Wilcoxon test was used (due to a lack of normality in Pb concentrations, as determined using a Shapiro-Wilk test). To compare invertebrate and environmental Pb concentrations, Spearman rank correlation coefficient tests were used (chosen to account for multiple invertebrates collected at the same site). Kruskal-Wallis tests (again, due to lack of normality in Pb concentrations) were used to compare the Pb body burdens of invertebrates collected at the mine sites across taxonomic order, functional feeding group, and locomotion type. For these comparisons, to decrease the chance of a type I error, Benjamini - Hochberg corrections were utilized (Benjamini & Hochberg, 1995). Adjusted p-values were calculated by multiplying the p-value by the total number of tests, and then dividing by the p-value's rank (when all relevant p-values were ordered smallest to largest), as described in Yekutieli & Benjamini (1999). Figures visualizing these statistical tests were generated in R using the ggplot2 package (Wickham, 2016).

4.4 Results

4.4.1 Environmental Contamination

The waterways flowing through the mine sites were heavily contaminated with trace metals. The Pb, Cd, Zn, and Cu concentrations in both the water and sediment were notably elevated when compared to the concentrations found in the streams in the nearby control sites (Table 4.1). Sediment trace metal concentrations were between 2 and 63 times higher at the mine sites than the control sites, while the water concentrations were between 3 and 1025 times higher. The Pb and Zn water concentrations at the mine sites were 75 and 82 times higher, respectively, than the site-specific Predicted No-Effect Concentrations (PNECs) defined by Defra (Defra, 2014), indicating that adverse health effects in resident organisms were possible (Table 4.1). Similarly, the mean Pb, Cd, and Zn sediment concentrations were elevated above the Probable Effect Levels (PEL) from the Canadian sediment quality guidelines for the protection of aquatic life (Canadian Council of Ministers of the Environment, 2001), indicating that adverse health effects for aquatic life were probable in the waterways at the mine sites (Table 4.1). This is anecdotally reflected *in situ*, as fish were not present in the sampled waterways at the mine sites, but were present in nearby, similar, but less contaminated, streams.

Table 4.1: Mean environmental trace metal concentrations found across the two site types. Results are represented as mean ± standard deviation. Concentrations are in μg L^{-1} for water, and dry weight mg kg^{-1} for sediment. For the mine sites, n=5, and for the control sites, n=3. PNECs are reported for Pb, Zn, and Cu; no PNECs can be generated for Cd.

	Site Type	Pb	Cd	Zn	Cu
Water	Mine	369 ± 462	4.13 ± 4.69	1370 ± 1690	3.32 ± 2.17
	Control	0.360 ± 0.0784	0.0245 ± 0.0170	2.81 ± 0.352	1.06 ± 0.540
PNEC[a]	Mine	*4.93 ± 2.35*	-	*16.8 ± 5.09*	*7.81 ± 3.68*
	Control	*7.24 ± 3.70*	-	*22.2 ± 8.99*	*11.6 ± 5.91*
Sediment	Mine	4080 ± 1159	4.03 ± 5.17	2030 ± 2850	46.0 ± 11.3
	Control	64.4 ± 37.3	0.319 ± 0.152	178 ± 55.7	18.8 ± 5.29
PEL[b]		*91.3*	*3.5*	*315*	*197*

[a]Probable No-Effect Concentration (Defra, 2014).
[b]Probable Effect Level (Canadian Council of Ministers of the Environment, 2001).

4.4.2 Invertebrate Communities

The invertebrate communities collected at the mine and control sites differed across a number of key metrics. The mine sites had a lower mean total abundance and community richness (125 ± 62 individuals and 11 ± 4 species, respectively) when compared to the control sites (141 ± 177 individuals and 16 ± 6 species, respectively), though the differences were relatively small, and the diversity and abundance values were not indicative of a significantly polluted site (Figure 4.2). The distribution of taxonomic orders also varied between the mine and control sites (Figure 4.3). Three orders (Arhynchobdellida, Hemiptera, and Hygrophila; leeches, true bugs, and molluscs) were not present at the mine sites, and only one oligochaete individual was found across all mine site sampling occasions, compared to 30 at the control sites (Figure 4.3). Similarly, the mine and control site communities also had different distributions of key life traits, specifically functional feeding groups and locomotion types. Across functional feeding groups, there was a notably higher percentage of passive filter feeders at the control sites (37.0%) than the mine sites (21.8%) (Figure 4.4). Furthermore, invertebrates whose primary locomotion type included burrowing/boring were underrepresented in the mine sites compared to the control sites (0.643% versus 5.02% for only burrowing/boring, 12.2% versus 21.5% for burrowing/boring and sprawling/walking; Figure 4.4).

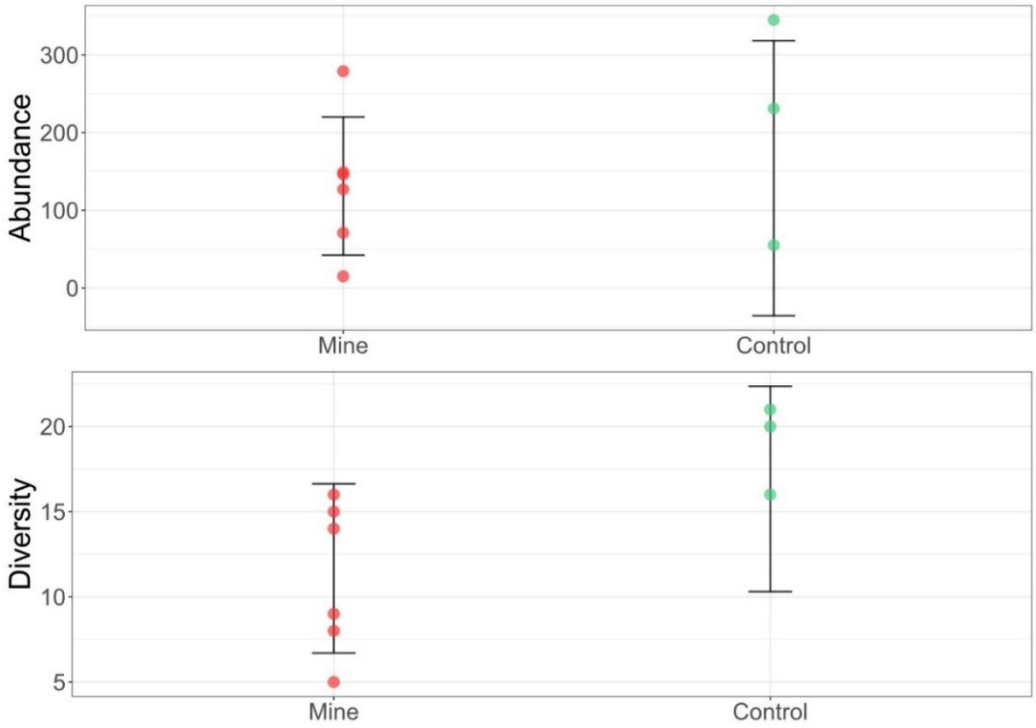


Figure 4.2: Abundance and community diversity across mine and control sites. For the mine sites, n=6, and for the control sites, n=3.

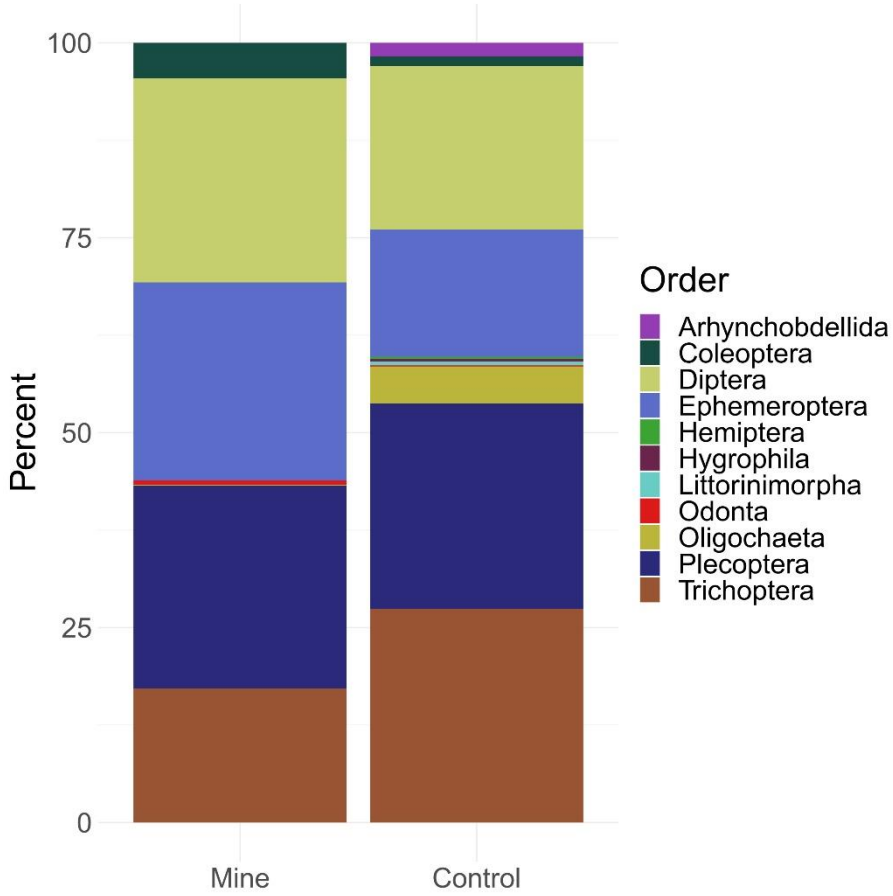


Figure 4.3: Percentage breakdowns of overall mine and control site communities by taxonomic order.

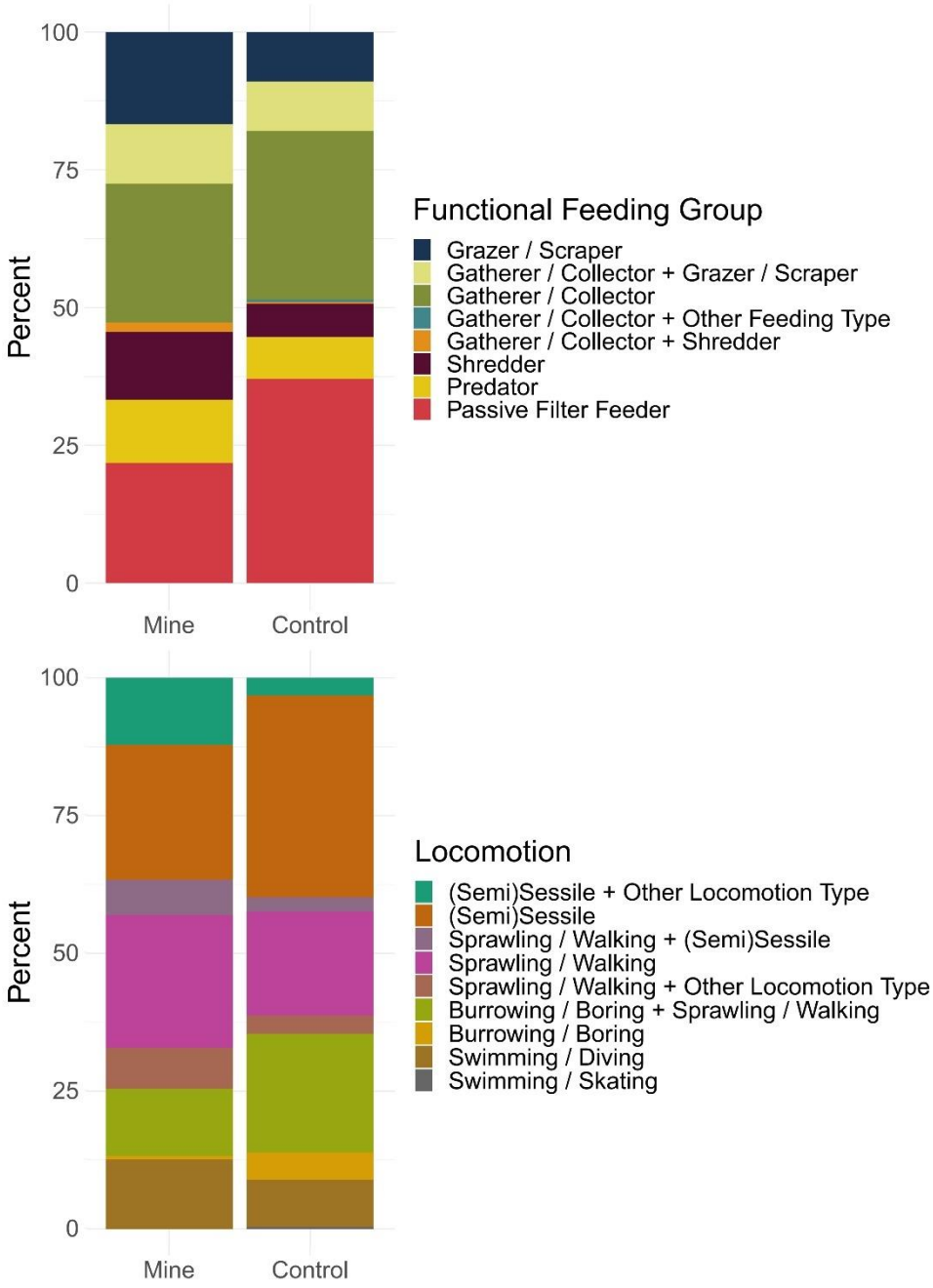


Figure 4.4: Percentage breakdowns of mine and control site communities by functional feeding group (top) and locomotion type (bottom).

4.4.3 Invertebrate Pb Body Burdens

Aquatic invertebrates collected at the mine sites had significantly higher Pb body burdens than invertebrates from the control sites (W [Kruskal-Wallis test statistic]= 29, p-value <0.001). The invertebrate Pb body burdens overall had a significant strong positive correlation with the water (r_s = 0.698, $p < 0.001$; Figure 4.5) and sediment Pb concentrations (r_s = 0.701, $p < 0.001$; Figure 4.5). Over one third (38.5%) of the sampled invertebrates at the mine sites had Pb concentrations that exceeded the concentrations found in the sediment.

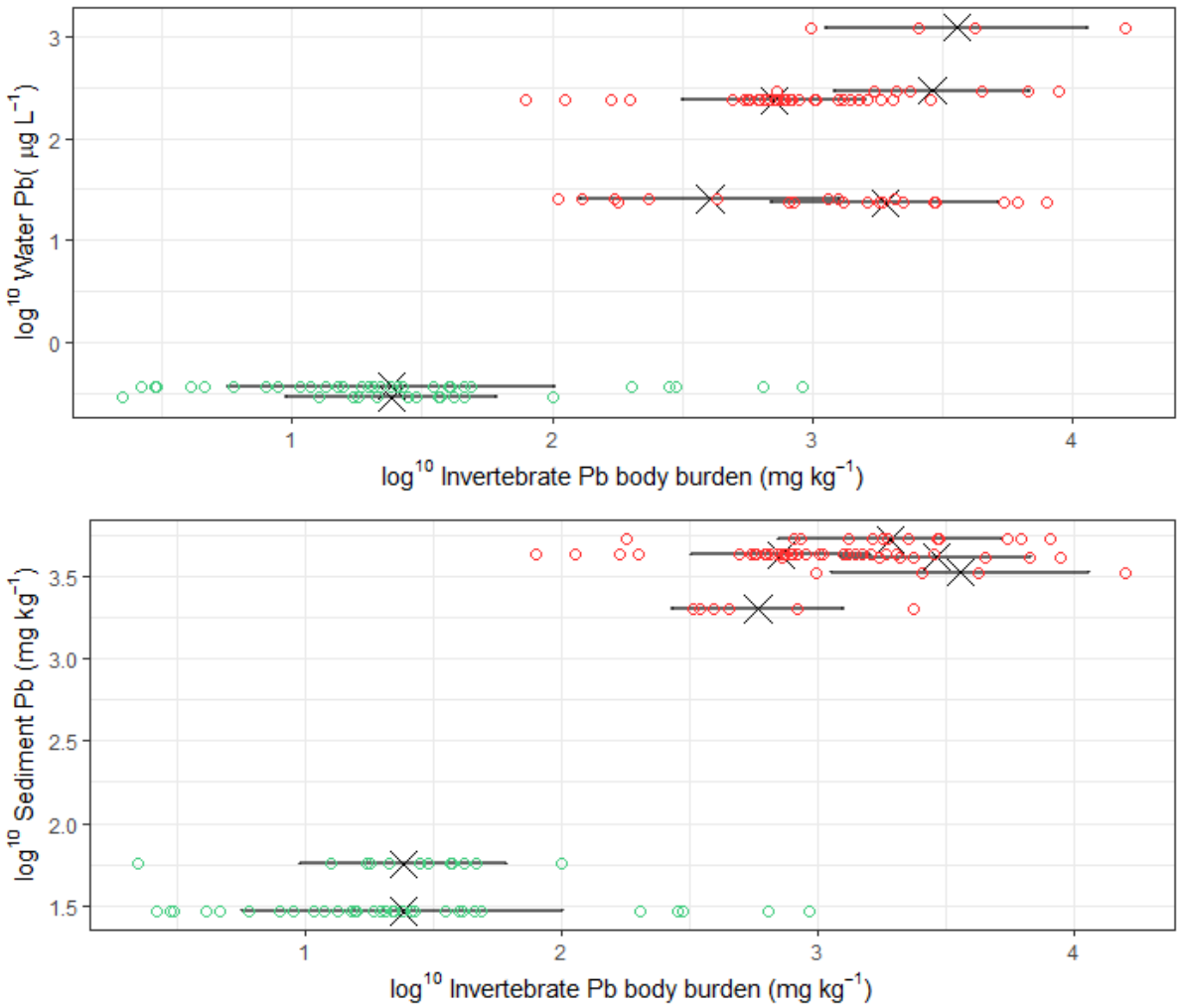


Figure 4.5: Invertebrate Pb concentrations compared to water (top) and sediment (bottom) Pb concentrations. The "x" and line represent the mean and standard deviation, respectively, of the $\log^{10}$ invertebrate Pb body burden at each sampled site. Invertebrates from the mine sites are in red (and are found in the top right of the graph), while invertebrates from the control sites are in green (and are found in the bottom left of the graph).

The Pb concentrations found in invertebrates from the mine sites were compared across taxonomic order and traits, to determine whether these factors influenced Pb accumulation. Lead concentrations varied significantly across taxonomic order in the mine site invertebrates (χ^2 = 12.2, df = 2, p-value = 0.00681; Figure 4.6), with Diptera having the highest mean Pb concentration (5500 ± 2820 mg kg^{-1}). However, Pb concentrations did not vary significantly across either functional feeding groups (χ^2 = 4.18, df = 3, p = 0.365; Figure 7) or locomotion types (χ^2 = 0.803, df = 1, p=0.370; Figure 4.7) in mine site invertebrates.

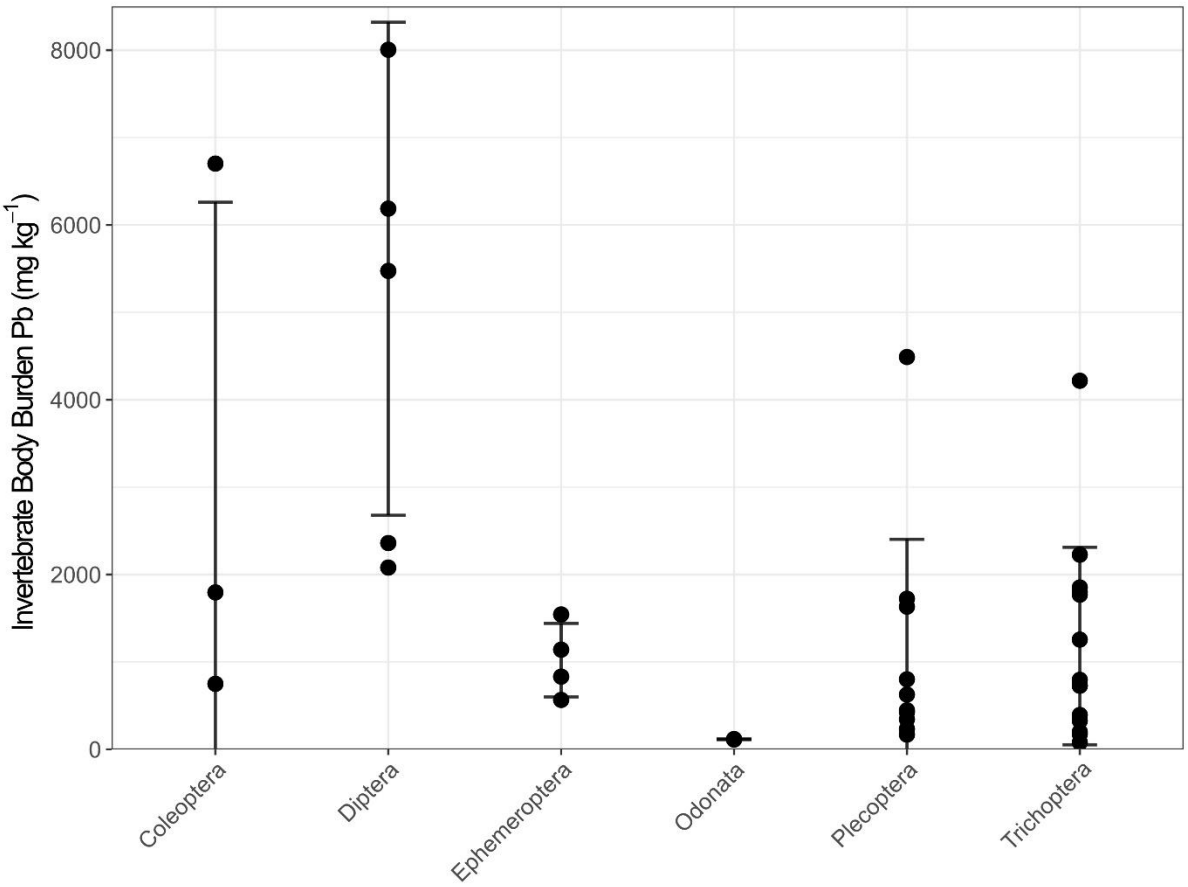


Figure 4.6: Lead concentrations of invertebrates from the mine sites across taxonomic orders. The error bars represent the Pb concentration standard deviation for each order.

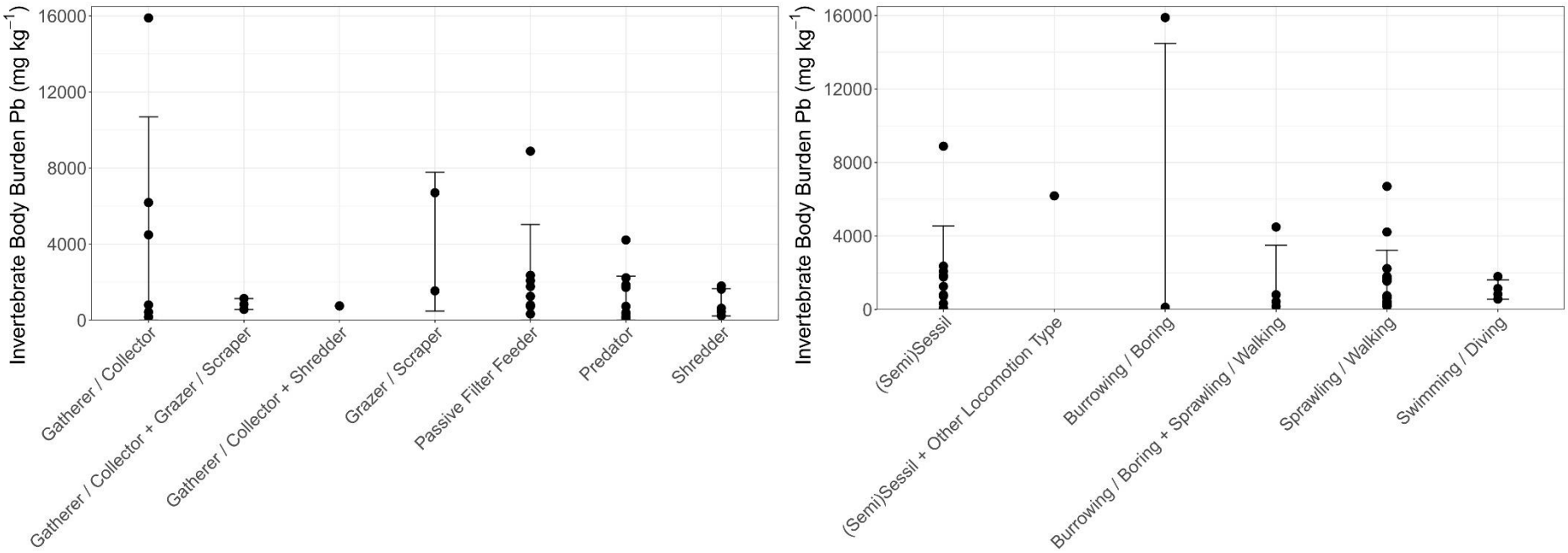


Figure 4.7: Lead concentrations of invertebrates from the mine sites across functional feeding groups (left) and locomotion types (right). The error bars represent the Pb concentration standard deviation for each order.

4.5 Discussion

4.5.1 Invertebrate Communities in Trace Metal Contaminated Environments

The aquatic invertebrates collected at the mine sites were living in an environment that had been highly contaminated by trace metals for hundreds of years. It is therefore unsurprising that these high metal concentrations appear to have impacted the resident invertebrates. As observed in prior studies, the aquatic invertebrate communities at the mine sites had both lower total abundance and community richness than the control site communities (Dickman et al., 1990; Hare, 1992; Clements, 1994; Maret et al., 2003; Rainbow, 2018). While the differences detected between the mine and control communities should be treated with caution due to potential confounding factors (including environmental variations, most notably tree coverage), the high Pb concentrations at the mine sites and in the invertebrates collected at these sites could have at least partially influenced the observed community structures.

The invertebrate community composition varied between the mine and control sites, with the mine communities containing proportionally more Diptera (26.2% at mine, 20.9% at control) and Ephemeroptera (25.4% at mine, 16.3% at control), and less Oligochaeta (0.127% at mine, 4.75% at control) and Trichoptera (17.2% at mine, 27.4% at control). While Diptera (specifically, Chironomidae) are generally well documented as metal tolerant (Dickman et al., 1990; Canfield et al., 1994; Clements, 1994), the distribution of the other orders differed from prior studies. Generally, Trichoptera are considered more metal tolerant than Ephemeroptera (Clements, 1994; Hickey & Clements, 1998; Rainbow, 2018), and Oligochaeta have been found in metal polluted sites (Canfield et al., 1994; Santoro et al., 2009). However, metal tolerance is known to vary depending on species-specific physiological traits, such as body size, and on the metal(s) in question (Clements, 1994; Kiffney & Clements, 1996; Hickey & Clements, 1998; Cortelezzi et al., 2011; Rainbow, 2018). Location-based environmental factors (weather, sediment type, and flow rates, for example) will also influence invertebrate responses to metal pollution. Previous studies in mid-Wales from more than sixty years ago also found that oligochaetes, leeches, crustaceans, and molluscs were extirpated from metal contaminated streams, mirroring the results of the current study (Jones, 1958; Rainbow, 2018).

The invertebrate mine and control site communities also differed across both locomotion type and functional feeding group. This could be linked to behavioural traits, which may affect the level of pollution to which an animal is exposed. For example,

burrowers live within the sediment itself, and therefore could be exposed to notably high concentrations of trace metal (Van Griethuysen et al., 2004; Peter, 2018). This may explain why there were proportionally fewer burrowers at the mine sites than at the control sites, and, in particular, may account for the almost complete absence of oligochaetes at the mines. Functional feeding groups may also affect trace metal exposure; for example, passive filter feeders can be directly exposed to trace metals in the water during feeding (Pastorino et al., 2020). As the water from the mine sites contained notably elevated Pb, Cd, and Zn concentrations, it is unsurprising that there were proportionately fewer passive filter feeders at the mines compared to the control sites.

4.5.2 Invertebrate Pb Body Burdens

The invertebrates collected at the mine sites had significantly elevated Pb concentrations when compared to invertebrates from nearby control sites, with the mean invertebrate Pb concentration at the mine sites (2190 mg kg^{-1}) more than 42 times higher than the mean invertebrate Pb concentration at the control sites (51.0 mg kg^{-1}). High Pb concentrations at polluted sites have been previously observed in invertebrates (Goodyear & McNeill, 1999; Solà & Prat, 2006; Santoro et al., 2009; De Jonge et al., 2013; Rainbow, 2018; Arnold et al., 2021), though the concentrations found in the current study represent some of the highest concentrations recorded in the literature. This is likely because most prior studies did not sample directly at mine sites, focusing instead on larger, polluted waterways further downstream from mines (Solà & Prat, 2006; De Jonge et al., 2013; Rainbow, 2018).

Lead concentrations varied significantly across taxonomic orders, but did not vary across either functional feeding group or locomotion type. This is likely due to the low overall sample size at the mine sites, as the collected invertebrates had to be pooled together to reach a sufficient cumulative weight (0.01g) for elemental analysis. Because of this, there was generally poor coverage across the locomotion types and functional feeding groups. Previous studies have found that behavioural traits, such as functional feeding groups, are good predictors of metal accumulation, since they can influence the levels of metal exposure (Farag et al., 1998; Loureiro et al., 2018*;* Peter et al., 2018; Pastorino et al., 2020), though these findings have not been consistent across all studies (Santoro et al., 2009).

Metal hyperaccumulation, the accumulation by an organism of an atypically high concentration of a trace metal (Rainbow, 2018), has been well researched in plants, but has been more rarely studied or observed in animals (Thompson et al., 2018). There are two

common proposed thresholds of hyperaccumulation in animals: Gifford et al. (2007) proposed using hyperaccumulation thresholds generated for plants (>100 mg kg^{-1} for Pb), while Thompson et al. (2018) suggested using 1,000 mg kg^{-1} as a threshold across all elements. Based on the Gifford et al. (2007) threshold, 97.7% of the sampled invertebrates (all but one sample) from the mine sites were hyperaccumulating Pb, while based on the Thompson et al. (2018) threshold, 50.0% of the sampled invertebrates in the mine sites were hyperaccumulating Pb. While the true percentage of hyperaccumulators in the mine invertebrates is likely lower, as the gut contents of the invertebrates were not purged (to allow for a better estimate of predator Pb exposure when consuming these species) (Thompson et al., 2018), the Pb concentrations were notably elevated, and over a third of the invertebrates from the mine sites had Pb concentrations that exceeded those found in the sediment. These invertebrates, which included coleopterans, dipterans, plecopterans, trichopterans, and one oligochaete, were likely storing and accumulating Pb within their bodies over time to reach concentrations higher than found in their surroundings. Dipterans in particular accumulated lead at concentrations higher than those found in their surrounding sediment, even in the control sites, where they were exposed to relatively low environmental Pb concentrations. This is in agreement with prior studies, which have found that Diptera (specifically, Chironomidae) accumulate higher trace metal concentrations than other invertebrate orders in the same environments (Arslan et al., 2010; Rainbow, 2018). The highest accumulator tested during this study was the single oligochaete found at a mine site, which had a Pb concentration of 15,900 mg kg^{-1}, almost 5 times more than the sediment Pb concentration (3340 mg kg^{-1}), and well above either of the proposed Pb hyperaccumulation thresholds (Gifford et al., 2007; Thompson et al., 2018). This Pb concentration greatly exceeded both the Pb concentrations found in oligochaetes at the control sites (30.3 ± 18.2 mg kg^{-1}) and previously established baseline oligochaete Pb concentrations in uncontaminated sites (Méndez-Fernández et al., 2017). Oligochaetes are well known as hyperaccumulators of Pb and other trace metals, and are also considered metal tolerant, along with Diptera (Arslan et al., 2010; Protano et al., 2014).

Hyperaccumulation in macroinvertebrates is still not well understood, with the underlying physiological mechanisms and biological costs still under investigation (Thompson et al., 2018). These hyperaccumulators may only be able to tolerate a certain level of specific metals, above which they are not able to survive. Metal hyperaccumulators are also known to experience sublethal effects, such as developmental defects, after exposure to metals, so metal pollution can still negatively affect these species (Di Veroli et al., 2014;

Rainbow, 2018). While the ability of macroinvertebrate metal hyperaccumulators to endure in sites where other species would be extirpated ensures their survival, it also allows for high concentrations of trace metals to be transferred into invertebrate predators. Even if these metals are stored in a non-bioavailable form, are present primarily in the individual's digestive tract, or are sequestered in a carapace, predators consuming these invertebrates could still be fully exposed to their elevated trace metal load, putting them at risk of a wide range of substantial negative health effects.

4.6 Conclusions

Trace metal pollution can substantially affect aquatic invertebrates, particularly at an individual level. High, potentially toxic concentrations of Pb were found in the water and sediment of streams in derelict mine sites. These high Pb concentrations were mirrored in the local macroinvertebrates, which had significantly higher Pb body burdens than macroinvertebrates living in nearby, uncontaminated streams. Of the invertebrates collected at the mine sites, over a third had accumulated Pb above local Pb sediment concentrations. Despite this, the invertebrate communities at the mine sites had only slightly lower abundance and species richness than the control site communities, though a couple of orders were completely absent from the mine sites. Overall, it appears that chronic exposure to high Pb concentrations can cause certain aquatic invertebrate species to accumulate high concentrations of Pb, while extirpating others. However, there needs to be a better understanding of the significant effects metal pollution can have on invertebrates and on their predators. Future work should investigate the strategies macroinvertebrate taxa employ to acclimate to metal contamination and explore why some taxa have not been able to adapt. Metal pollution is common globally, and will likely become more widespread in the future, due to more frequent extreme weather events, caused by climate change, redistributing existing metal contaminants across landscapes (Hudson-Edwards et al., 2008). When assessing waterways, understanding the presence and effects of trace metals on invertebrates will be key to more fully characterise and understand the ecosystem level impacts of metal pollution.

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Data Availability

The data that support the findings of this study are openly available in the Nottingham Research Data Management Repository at https://doi.org/10.17639/nott.7217.

Appendices

Appendix A: Supplementary Tables

Supplementary Table 4.1: Definitions of traits used during this study. Definitions were obtained from Moog (1995) and Schmedtje & Colling (1996).

Trait	Category	Explanation
Functional Feeding Group[1]	Grazer / Scraper	Feed on endolithic and epilithic algal tissues, biofilm, partially particulate organic matter, partially tissues of living plants
	Miner	Feed on leaves of aquatic plants, algae and cells of aquatic plants
	Xylophagous taxa	Feed on woody debris
	Shredder	Feed on fallen leaves, plant tissue, and coarse particulate organic matter
	Gatherer / Collector	Feed on sedimented fine particulate organic matter
	Active Filter Feeder	Feed on suspended fine and coarse particulate matter; micro prey is whirled; food is actively filtered from the water column
	Passive Filter Feeder	Feed on suspended fine and coarse particulate matter or prey; food is filtered from running water, e.g., by nets or specialised mouthparts
	Predator	Feed on prey
	Parasite	Feed on host
	Other Feeding Types	Use other food sources not meeting the above categories
Locomotion Type[2]	Swimming / Skating	Floating in lakes or drifting in rivers passively
	Swimming / Diving	Swimming or active diving
	Burrowing / Boring	Burrowing in soft substrates or boring in hard substrates
	Sprawling / Walking	Sprawling or walking actively with legs, pseudopods, or on a mucus
	(Semi)Sessile	Tightening to hard substrates, plants, or other animals
	Other Locomotion Types	Other locomotion type, like flying or jumping (mainly outside the water)

[1]Moog, 1995.
[2]Schmedtje & Colling, 1996.

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Chapter 5 – Human health implications from consuming eggs produced near a derelict metalliferous mine: a case study

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Author Contributions

Andrea Sartorius contributed to designing the study, collected samples, processed samples in the laboratory, did the analyses, and wrote the manuscript draft.
Matthew Johnson contributed to designing the study, helped collect samples, and revised the manuscript.
Scott Young provided technical advice for the laboratory methods and revised the manuscript.
Malcolm Bennett helped collect samples and revised the manuscript.
Kerstin Baiker helped collect samples, provided technical advice for the laboratory methods, and revised the manuscript.
Paul Edwards isolated the study site, liaised with the landowner, and revised the manuscript.
Lisa Yon contributed to designing the study, helped collect samples, and revised the manuscript.

Human health implications from consuming eggs produced near a derelict metalliferous mine: a case study

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5.1 Abstract

Lead pollution from metalliferous mines can have major environmental and health effects long after the mines have closed. Animals living near derelict mine sites can inadvertently ingest lead-contaminated soils, causing them to accumulate lead and potentially experience significant adverse health effects. Human food products, such as eggs, produced near metalliferous mines may also be contaminated with lead. The focus of this case study was to determine whether free-range chickens living near a derelict lead mine had high lead body burdens, whether they were producing eggs with elevated lead concentrations, and whether these eggs could be hazardous to human health. Soil samples and chicken egg, feather, blood, and bone samples were collected from a small farm near an abandoned metalliferous mine. The soil in and around the chicken pens contained lead concentrations that were elevated above established soil lead baseline concentrations. The lead concentrations in the chicken feather, blood, and bone samples were consistent with lead toxicity, and indicated long-term, continuous exposure. Finally, the lead concentrations in the eggs were significantly greater than those found in commercial eggs. Based on previously established lead benchmark dose levels, humans, and in particular, children, could experience adverse health impacts if they routinely consumed these eggs. Environmental lead contamination continues to pose a major health risk for humans, and further research, understanding, and awareness are required to safeguard the public from the risks of consuming food produced near derelict mines.

5.2 Introduction

Lead is a toxic metal that has significant adverse impacts on human health throughout the world. In 2017, lead exposure was estimated to have caused more than one million deaths and over 24 million disability-adjusted life years (years of life lost due to premature mortality or living with a disability) worldwide (Mathers et al., 2011; Stanaway et al., 2018). The effects of lead exposure are profound, as it can affect multiple organs, including the kidneys, liver, central nervous system, and reproductive system (Tchounwou et al., 2012). Therefore, over the past 40 years, there have been concerted efforts to decrease human lead exposure, primarily through banning or limiting the use of products containing lead, such as lead-based paint, leaded petrol, and lead pipes (Tchounwou et al., 2012). However, since lead is an elemental toxin and does not degrade, historical contamination can continue to pose a threat to human health.

A key route of lead exposure for humans is through the consumption of lead-contaminated food (Tong et al., 2000; Tchounwou et al., 2012). Fruit and vegetables grown in lead-contaminated environments have been found to have correspondingly high lead concentrations (Finster et al., 2003; Feleafel & Mirdad, 2012). Similarly, animals reared in lead-polluted environments can ingest lead by drinking contaminated water, consuming contaminated food products, or inadvertently ingesting contaminated soil (Franson & Pain, 2011). In birds and mammals, lead accumulates primarily in the kidney, liver, and bones; high lead exposure, however, can result in lead accumulating across multiple systems, including tissues used for human food products, such as milk or eggs (Trampel et al., 2003; Franson & Pain, 2011).

Human exposure to lead through the consumption of domestic chicken (*Gallus gallus domesticus*) eggs is of particular concern, as lead has been known to specifically accumulate in the eggs of lead-exposed chickens (Trampel et al., 2003). A number of studies have examined eggs produced by chickens in urban or semi-urban areas, and found elevated lead concentrations, reflecting the elevated local environmental lead levels (Roegner et al., 2013; Bautista et al., 2014; Spliethoff et al., 2014; Grace & MacFarlane, 2016; Leibler et al., 2018; Cowie & Gartrell, 2019). However, the few studies that have investigated eggs produced in highly lead polluted areas have focused on chickens living near currently active sources of pollution, such as working lead smelters (Martins et al., 2010; Zariff et al., 2019). Abandoned lead workings can also serve as significant sources of contamination, as lead concentrations around derelict mines or smelters may remain high for hundreds of years after operation has ceased (Pyatt et al., 2000; Venkateswarlu et al., 2016). These areas consistently have higher

lead concentrations than urban areas, though local residents are not always aware of the contamination or the associated risks (Palmer, 2006; Dogaru et al., 2009; Johnson et al., 2012). The aim of this study was therefore to determine whether chickens living near a derelict lead mine accumulate lead within their tissues, whether they produce eggs with elevated lead concentrations, and whether these eggs could be hazardous to human health. This case study focused on eggs produced over a 16-month period by a flock of chickens from a small, private farm, located directly downstream from a derelict lead mine.

5.3 Materials and Methods

5.3.1 Site

All samples were collected from an 8 ha farm in Wales located approximately 1 km downstream from an abandoned lead mine (Fig. 1). Due to the proximity of the mine, the owner of the farm requested that their farm be investigated for environmental lead contamination. Preliminary survey of vegetables produced at this property (and a similar small Welsh farm also located near an abandoned lead mine) found high, potentially toxic levels of lead (Supplementary Table 5.1).

Eight chickens lived on the farm in October 2018, and were managed under free-range conditions. Two died during the course of this study (one in November 2018 due to predation, another in May 2019 due to unknown causes). Apart from these deaths, all other chickens appeared clinically healthy throughout the duration of the study. The chickens were kept within two adjacent pens (Pens 1-2), one of which contained a chicken coop (Pen 2), and they also had regular access to a third, larger pen (Pen 3; Figure 5.1). All three pens were elevated above a stream flowing from the mine site, so the chickens had no direct access to the stream.

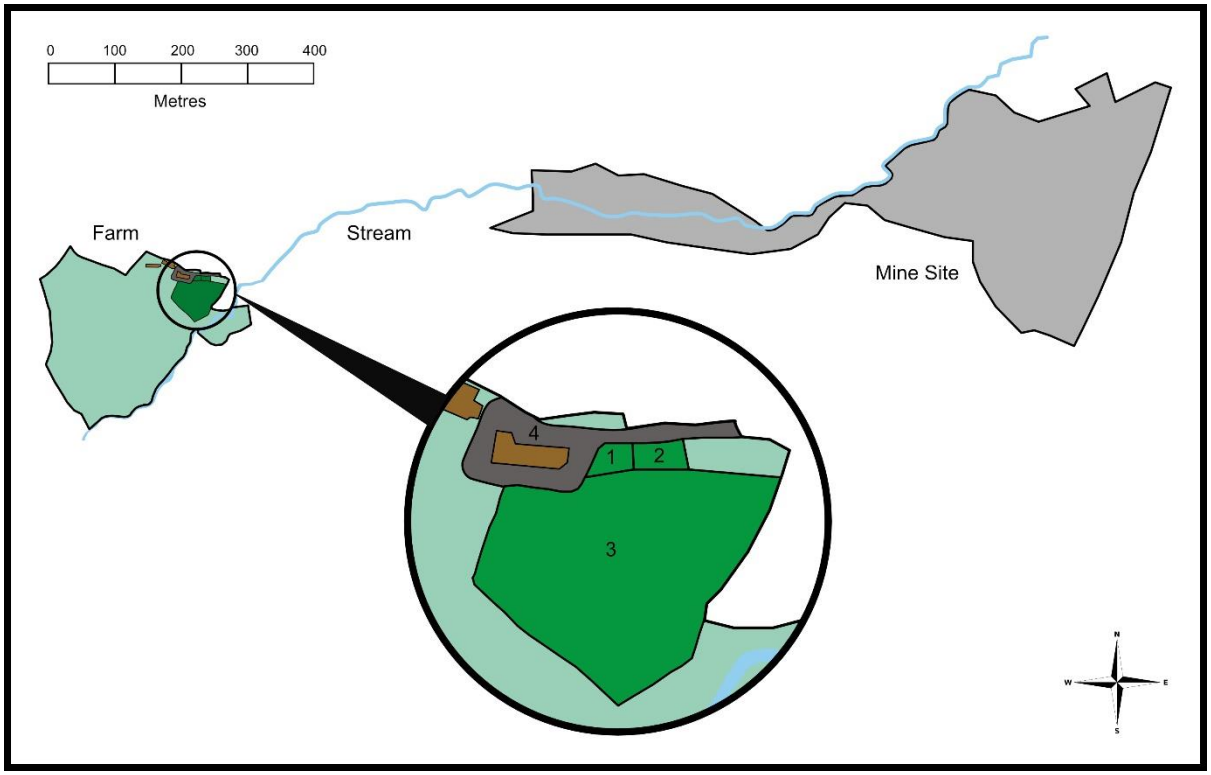

Figure 5.1. Map of sampling locations. Dark green represents the fields sampled for this study, light green represents fields not sampled for this study, brown represents buildings, dark grey represents the sampled gravel path, light grey represents the mine site, and blue represents waterways. The numbers represent the sampled sites at the farm: Pens 1, 2, and 3, and the gravel track (4).

5.3.2 Sample Collection

Soil samples were collected from the chickens' three pens, as well as a gravel track adjacent to the pens. The samples were collected using the 'W-transect' method: approximately 10 samples were collected using a stainless steel trowel at roughly equivalent distances within a 'W' shaped pattern across the sampling area. The samples were deposited into a single plastic bag and mixed by hand. Each pen was treated as an individual sampling area. Sampling from the track was restricted to the areas that were directly adjacent to the chicken pens.

Soil samples were also collected from the mine site and from a nearby control site (a protected woodland area) located 3 km from the mine. The control site was chosen to represent a relatively uncontaminated baseline for soil lead concentrations in the immediate region. These samples were collected at approximately 5 m intervals across five 20 m transects distributed across the sampling site. All the samples from a single transect were placed into a plastic bag and mixed by hand. Two distinct areas within the mine site (a mine

spoil heap and an adjacent area of flattened mine spoil) were each sampled using the W-transect method.

Six eggs were collected from the farm on four occasions (in October 2018, May 2019, October 2019, and January 2020), and ten eggs were collected in February 2019. The farm owner gathered fresh eggs on the day of collection, and the eggs were kept at 4 ˚C prior to analysis.

A total of 12 commercially available eggs, obtained from two different sources, were analysed for comparison purposes. In January 2020, six eggs were purchased from a privately run, free-range farm near Sutton Bonington, Leicestershire, UK. In January 2021, six further eggs were purchased from a major supermarket chain; these were commercially produced, free-range eggs from Wales. All eggs were kept at 4 ˚C prior to analysis.

Blood and feather samples were collected from the chickens in October 2018. Qualified veterinarians collected blood samples via venous sampling, which they dispensed into 4 mL lithium-heparinised tubes, mixed thoroughly, and stored at 4 ˚C prior to analysis. One feather from each chicken was carefully clipped close to the skin using stainless steel scissors. Due to sampling difficulties, blood samples were collected from four of the chickens, while feather samples were collected from all eight chickens.

The carcasses of the two chickens that died during the study period were opportunistically collected. One carcass was frozen and thawed before dissection (to avoid decay), while the other was stored at 4 ˚C until dissection. During post mortem dissection, a femur was removed from each chicken for elemental analysis. The bone samples were stored at -20 ˚C.

5.3.3 Sample Processing

The soil samples were air-dried before being sieved to <2 mm with a stainless steel sieve and subsequently ground into fine powder using a Retsch PM 400 planetary ball mill (Retsch, Haan, Germany). Duplicates of each sample were then acid digested using a teflon-coated graphite hotplate block digester (Analab, Bischeim, France). Approximately 0.4 g of sample, along with 1 mL HNO_3 and 3 mL HCl, were heated on a hotplate block digester at 95 ˚C. After two hours, the samples were allowed to cool to ambient temperature before being dispensed into plastic volumetric flasks. The volume was then made up to 50 mL with MilliQ water (18.2 MΩ cm; Millipore Corporation, Darmstadt, Germany). The solutions were diluted 1:10 with MilliQ water prior to elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS) (Model ICAP-Q; Thermo Fisher Scientific, Bremen, Germany).

A certified reference material for lead (NIST 2711A, Montana soil) was run for quality assurance purposes (Pb recovery was 93.1% of the certified value).

The egg samples were separated into shell, yolk, and albumen and weighed before being freeze dried to a constant mass. The original wet weight and the final dry weight for each egg constituent was recorded. While most yolk and albumen samples were successfully separated upon extraction from the egg, if the samples became mixed, after freeze drying the yolk and albumen were separated based on their different consistency and colour. Approximately 0.1 - 0.2 g of each sample, along with 3 mL 70% HNO_3 (Primar Plus™ grade, Thermo Fisher Scientific), 2 mL H_2O_2, and 3 mL MilliQ water, were digested in a microwave oven (Model Multiwave Pro, Anton Paar) at 140 ˚C for 30 minutes, following a protocol established by Anton Paar, based on U.S. EPA Method 3051A (U.S. EPA, 2007). After digestion, 7 mL of MilliQ water was added to the samples. The digestants were diluted 1:10 with MilliQ water prior to elemental analysis by ICP-MS. A certified reference material for lead in biological samples (BRC-185R Bovine Liver [trace elements]) was run for quality assurance purposes (Pb recovery was 98.8% of the certified values).

The blood samples were homogenised using a roller mixer for 10 minutes. The blood was diluted 1:20 with 0.1% HNO_3 prior to elemental analysis by ICP-MS. A sample of certified reference material for lead (Seronorm Trace Elements in Whole Blood L-2, Lot 1702825) was also included for quality assurance (Pb concentration = 303 µg L^{-1}).

The feather samples were washed following a protocol modified from Ishak et al. (2015). Briefly, the feathers were first rinsed with deionized water to remove as much external contamination as possible. Afterwards, they were rinsed with the following solvents in sequence for 15 minutes at a time: deionized water, 0.5% Triton-X, deionized water, acetone, and deionized water. Before each new solvent was added, the previous solvent was poured off and the samples and tubes were briefly rinsed with deionized water. The samples were stirred using a roller mixer during the washing process. After washing was complete, the samples were dried at 60 ˚C in an oven overnight. The samples were then cut into smaller pieces (~2 cm) using scissors, and were acid digested and analysed using ICP-MS, as previously described for the egg samples.

The chicken bone samples were freeze dried to a constant mass. After freeze drying, bone samples were manually cleaned of extraneous soft tissue. The bone samples were then fully acid digested following the same method as the egg samples (microwave assisted) prior to elemental analysis by ICP-MS.

5.3.4 Statistical Analyses

Lead concentrations were compared between the edible portions (yolk and albumen) of the eggs (wet weight). The edible lead concentration was determined by combining the yolk and albumen concentrations (with adjustment for relative mass).

The limit of detection (LOD) was calculated as 3 times the standard deviation of the lead concentrations of 19 blank samples run alongside the egg samples. The LOD was 0.262 µg L^{-1}, which converted to 0.0227 mg kg^{-1} dry weight. Unique LODs for the amount of lead per egg and the wet weight lead concentration were then calculated for the shell, yolk, and albumen portions of the egg using the average wet and dry weights of the relevant component across all eggs sampled. The amount of lead LOD was 0.000140 mg for shell, 0.000190 mg for yolk, and 0.000102 mg for albumen, while the wet weight lead concentration LOD was 0.0160 mg kg^{-1} for shell, 0.0113 mg kg^{-1} for yolk, and 0.00276 mg kg^{-1} for albumen. Any sample concentrations or amounts below the LOD were reported as <LOD. For statistical and graphical comparisons, samples with a concentration or amount below the LOD had their concentration changed to half of the LOD. The limit of quantitation (LOQ) was calculated as 10 times the standard deviation of the 19 blank samples run alongside the egg samples. The LOQ was 0.874 µg L^{-1}, which converted to 0.0757 mg kg^{-1} dry weight.

The lead concentrations found in the edible portions of the eggs were compared between the farm and the commercially available eggs, as well as between the farm eggs across the different collection months, using analysis of variance (ANOVA) in R (R Core Team, 2018). Two ANOVAs were used: one comparing the lead concentrations in the farm eggs from each collection month to the two sets of commercially available eggs, and one comparing the lead concentrations in the farm eggs across the collection months. A graph displaying the lead concentrations in the farm and the commercially available eggs across collection months was made in R using the ggplot2 package (Wickham, 2016).

5.3.5 Threshold Comparisons

The soil lead concentrations were compared to the Normal Background Concentrations (NBCs), which were generated by the British Geological Survey (BGS) to indicate the 'normal levels of contaminants' in different domains (areas defined based on geology and anthropogenic activity) of the UK (Ander et al., 2013). Specifically, the NBC dry weight mg kg^{-1} concentrations for the 'Principal' (background) and 'Mineralization' (mining areas) domains resolved for Wales by the BGS in 2013 were referenced for comparison (Ander et al., 2013).

The amount of lead in the edible portions of the eggs was compared to lead consumption thresholds for known impacts on human health. The European Food Safety Authority (EFSA) determined benchmark dose levels (BMDLs; the lowest 95% confidence limit below the benchmark dose, or dose that is associated with a specific response) for daily lead consumption (EFSA, 2010). These BMDLs focused on the risks of developmental neurotoxicity (intellectual deficits) in children, and nephrotoxicity (specifically, chronic kidney disease) and cardiovascular effects (specifically, higher systolic blood pressure) in adults (EFSA, 2010; Hardy et al., 2017). These BMDLs indicate threshold dietary intake values in μg kg^{-1} body weight per day, so the average weight of an adult in England in 2019 (78.6 kg) was used to determine how much lead an adult could consume in a day before exceeding the BMDL (Moody, 2019). Due to the large differences in weight across age during childhood, the threshold for children was calculated for six age brackets (0-1, 2-4, 5-7, 8-10, 11-12, and 13-15 years old), following those determined in the Health Survey for England 2019 (Moody, 2019). To compare the egg lead concentrations to the BMDLs, the amount of lead in the edible portions of the eggs was calculated using the measured lead concentrations and the mass of each egg component.

5.4 Results

5.4.1 Soil

The three chicken pens (Pens 1-3) and the adjacent gravel track all had elevated soil lead concentrations when compared to the soils from the nearby control site (Table 5.1). The soil lead concentrations from the farm also exceeded the NBCs for Wales for both the Principal (230 mg kg^{-1}) and Mineralization (280 mg kg^{-1}) domains, further indicating that the lead concentrations were elevated above ‘normal’ levels (Ander et al., 2013). Of the soils sampled at the farm, the highest lead concentration was found in the gravel track, which was similar to the lead concentration in spoil heap at the nearby mine site (Table 5.1).

Table 5.1: Lead concentrations in soil samples. Results are represented as mean ± standard deviation.

Site	Lead (mg kg^{-1} dry weight)
Mine Site	
Spoil Heap	23100 ± 2070
Flattened Spoil	11900 ± 3250
Five transects across the site[1]	6380 ± 1320
Farm	
Pen 1	2030 ± 232
Pen 2	1830 ± 131
Pen 3	885 ± 54.6
Track	23000 ± 5160
Control Site	
Five transects across the site[1]	66.7 ± 16.8

[1]n=5

5.4.2 Feathers, Blood, and Bones

The lead concentrations found in the feathers, blood, and bones of the chickens were consistently elevated above expected, non-contaminated levels. In fact, the feather lead concentrations exceeded not just those of control chickens from previous studies, but were more than double those found in prior experimental studies where chickens were fed metal-enriched feed (13.8 mg kg^{-1} in Zhuang et al., 2014; 11.2 mg kg^{-1} in Kim et al., 2020; Table 5.2). The blood lead concentrations were similarly elevated above the standard background concentration of lead in bird blood (200 µg L^{-1}; Franson & Pain, 2011) and those found in an experimental study (990 µg L^{-1} in Zhuang et al., 2014; Table 5.2). Indeed, one of the four chickens sampled had a blood lead concentration that was more than double the acute lead poisoning threshold in chickens (1500 µg L^{-1}; Trampel et al., 2003). Similarly, the lead concentrations found in the bones of the two chickens that died during the course of this study were indicative of 'excessive lead exposure' (above 20 mg kg^{-1}), as found by Franson and Pain (2011), and were higher than those recorded during an experimental study where chicks were fed a metal-enriched diet (21.4 mg kg^{-1} in Baykov et al., 1996; Table 5.2).

Table 5.2: Lead concentrations within chicken feathers, blood, and bone samples. Mean is reported as ± standard deviation.

Chicken	Feather (mg kg^{-1} dw^{1})	Blood (ug L^{-1})	Bone (mg kg^{-1} dw)
1	35.1		492
2	38.0	4670	42.1
3	19.0		
4	26.0	1020	
5	47.1		
6	27.6	1070	
7	48.7	394	
8	33.6		
Mean	**34.4 ± 10.2**	**1790 ± 1950**	**267 ± 318**

[1]dry weight

5.4.3 Eggs

Lead concentrations in the eggs were generally highest in the shells, followed by the yolk, and then the albumen (Table 5.3). Due to concerns about human health risks from consuming the eggs, this study primarily focused on the lead concentration in the normal dietary components, specifically, the yolk and albumen (the 'edible portion'). The lead concentration in the edible portion of the eggs differed significantly across all collection months and between both commercially available egg groups, with the lead concentrations in the farm eggs noticeably elevated ($F = 16.7$, $df = 6$, $p < 0.001$; Figure 5.2). The lead concentrations in the edible portions of the eggs from the farm also varied significantly across the different collection months ($F = 7.73$, $df = 4$, $p < 0.001$; Figure 5.2).

To estimate the human health risks associated with consuming these eggs, the amount of lead within the yolk and albumen of the eggs was compared to the EFSA BMDLs for developmental neurotoxicity in children, and nephrotoxicity and cardiovascular effects in adults (EFSA, 2010). Children under eight years old could exceed the developmental neurotoxicity BMDL by eating one to two of the farm eggs per day (Table 5.4). By comparison, children under eight years old would have to eat between 9 and 38 of the commercially available eggs tested during this study per day to exceed the BMDL (Table 5.4). The average adult could exceed the nephrotoxicity BMDL by consuming between two and six of the farm eggs (dependent on the collection month); to exceed the same threshold through eating the sampled commercially available eggs, an adult would have to eat almost a hundred eggs per day (Table 5.4). The cardiovascular BMDL threshold is more than double

the nephrotoxicity BMDL, but it could still potentially be exceeded by the daily consumption of more than five of the farm eggs collected in May 2019 (Table 5.4).

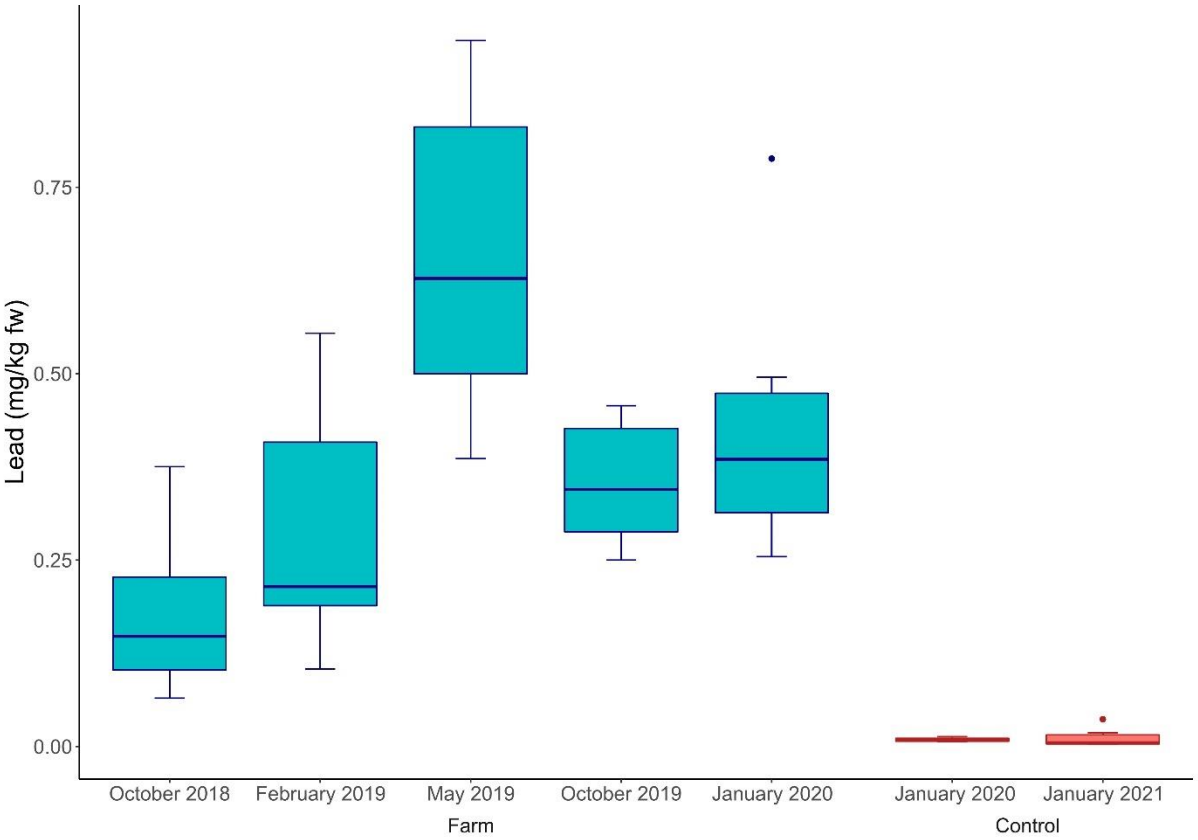

Figure 5.2. Pb concentrations in edible portions (yolk and albumen) of eggs from the farm and the commercially available eggs across collection months. Blue represents eggs sampled from the farm located near a mine, while red represents the control eggs.

Table 5.3: Chicken egg lead concentrations (wet weight). All values are in mg kg^{-1} wet weight. "ND" indicates that a concentration was below the detection limit, while <LOD indicates that the overall mean fell under the detection limit.

Part of Egg		Farm Eggs					Commercially Available Eggs	
		October 2018 (n=6)	**February 2019** (n=10)	**May 2019** (n=6)	**October 2019** (n=6)	**January 2020** (n=6)	**January 2020** (n=6)	**January 2021** (n=6)
Shell	Mean	0.470	0.874	1.15	0.516	0.340	<LOD	0.0361
	SD	*0.421*	*0.395*	*0.813*	*0.168*	*0.0686*	*NA*	*0.0689*
	Range	*0.0884 – 1.29*	*0.405 – 1.74*	*0.230 – 2.63*	*0.391 – 0.846*	*0.275 – 0.452*	*NA – 0.0235*	*NA – 0.177*
Yolk	Mean	0.330	0.565	1.30	0.705	0.866	0.0161	0.0207
	SD	*0.190*	*0.314*	*0.452*	*0.174*	*0.386*	*0.00686*	*0.0268*
	Range	*0.128 – 0.656*	*0.207 – 1.11*	*0.771 – 1.89*	*0.499 – 0.913*	*0.508 – 1.58*	*ND – 0.0250*	*0.00563 – 0.0717*
Albumen	Mean	0.0289	0.00220	<LOD	<LOD	<LOD	<LOD	0.00333
	SD	*0.0432*	*0.00258*	*NA*	*NA*	*NA*	*NA*	*0.00302*
	Range	*ND – 0.0948*	*ND – 0.00953*	*ND – 0.0629*	*ND – ND*	*ND – 0.00727*	*ND – 0.00732*	*ND – 0.00745*
Yolk and Albumen	Mean	0.179	0.284	0.657	0.353	0.434	0.00922	0.0120
	SD	*0.115*	*0.157*	*0.224*	*0.0871*	*0.193*	*0.00265*	*0.0134*
	Range	*0.0648 – 0.375*	*0.104 – 0.554*	*0.386 – 0.947*	*0.250 – 0.457*	*0.255 – 0.788*	*0.00647 – 0.0132*	*0.00351 – 0.0366*
Overall	Mean	0.276	0.480	0.820	0.408	0.403	0.0102	0.0201
	SD	*0.169*	*0.192*	*0.278*	*0.0967*	*0.121*	*0.00330*	*0.0265*
	Range	*0.0727 – 0.539*	*0.208 – 0.889*	*0.475 – 1.19*	*0.297 – 0.574*	*0.290 – 0.623*	*0.00699 – 0.0153*	*0.00501 – 0.0712*

Table 5.4: Number of eggs that can be consumed in one day before exceeding EFSA thresholds. The number of eggs was rounded down to the nearest whole number. '0' means that eating one egg would exceed the respective EFSA threshold.

EFSA Threshold Type	Threshold (mg/kg b.w. per day)	Age	Weight[a] (kg)	Threshold (mg of lead per day)	Farm Eggs					Commercially Available Eggs	
					October 2018 (n=6)	February 2019 (n=10)	May 2019 (n=6)	October 2019 (n=6)	January 2020 (n=6)	January 2020 (n=6)	January 2021 (n=6)
Developmental neurotoxicity BMDL[b,1]	0.00050	0-1 years	9.5	0.00475	0	0	0	0	0	15	9
		2-4 years	16.2	0.00810	1	0	0	0	0	27	16
		5-7 years	22.8	0.0114	1	1	0	0	0	38	22
		8-10 years	34.1	0.0171	2	1	0	1	1	57	33
		11-12 years	47.0	0.0235	3	2	1	1	1	78	46
		13-15 years	59.7	0.0299	4	3	1	2	2	100	59
Nephrotoxicity BMDL[1]	0.00063	Adult	78.6	0.0472	6	5	2	3	3	166	98
Cardiovascular BMDL[1]	0.00150	Adult	78.6	0.118	16	12	5	9	8	395	234

[a]Average weights based on those recorded in the Health Survey for England 2019 (Moody, 2019).
[b]Benchmark dose level (the lowest 95% confidence limit below the benchmark dose, or dose that is associated with a specific response).
[1]EFSA, 2010.

5.5 Discussion

5.5.1 Chickens' Lead Exposure

The chickens in this study were exposed to high concentrations of lead via the lead-contaminated soil in and around their pens. This contaminated soil is likely their primary route of lead exposure, as the chickens were provided with store-bought feed, were given water directly from the mains, and did not have any access to the nearby stream. While mains water can be contaminated with lead, even notably high water lead concentrations (7.8 mg/L) associated with contaminated drinking water have relatively low lead concentrations compared to those found in the soil during this study (Table 5.1) (Paranthaman & Harrison, 2010). The three chicken pens all contained soil with elevated lead concentrations (compared to the soil NBCs), but the track adjacent to the chicken pens had a particularly high lead concentration. The landowner indicated that it was likely that the track had been built using mine spoil, as they stated that this was a common practice in the region. This seems probable, as the lead concentration found in the track strongly resembles the lead concentration found in the mine spoil itself.

When in their pens, the chickens could peck at gravel from the track, part of which ran along the pens' boundaries. The landowner stated that the chickens favoured using gravel from the track as grit, even preferentially selecting it over oyster shells provided by the landowner. Prior studies investigating the effects of chickens consuming lead-based grit have found high lead concentrations in the birds, and have observed significant negative health effects, including mass mortality in some cases (Salisbury et al., 1958). Lead-contaminated grit is particularly dangerous, as the bird will be continuously exposed to lead as the grit slowly degrades in the gizzard (Salisbury et al., 1958). If the chickens were ingesting gravel from the track to use as grit, this could explain the high lead concentrations found within their feathers, blood, and bones.

The lead concentrations in the chickens' feather, blood, and bone samples were suggestive of severe lead toxicity, and indicated long-term, continuous lead exposure. While lead remains in blood for a few weeks after an exposure incident, lead is stored in bones and remains sequestered there for years after exposure (Franson & Pain, 2011). High lead concentrations in both the blood and bone samples therefore suggests that the chickens had been historically exposed to lead, and were still being exposed to lead when the blood samples were collected (in October 2018). This is consistent with the chickens being continuously exposed to lead over years, likely through the soil in their pens and the adjacent

gravel track. Despite the high lead concentrations in the feather, blood, and bone samples, symptoms of overt lead toxicity were not observed in the chickens during the course of this study. However, this is common in cases where chickens are exposed to lead, even at high concentrations (Salisbury et al., 1958; Roegner et al., 2013; Bautista et al., 2014; Kim et al., 2020).

5.5.2 Environmental Exposure and Lead Concentrations in Eggs

The lead concentrations in the edible portions of the farm eggs varied significantly between sampling events. This could be due to variations in the chickens' lead exposure as a result of management changes throughout the study period. Prior to the beginning of the study, the chickens' owner fed them by placing food directly in a trough. After the first site visit in October 2018, the chickens were instead encouraged to forage for food, with the owner spreading supplementary feed onto the grass and soil within their pens. It is possible that the more foraged diet led to the chickens inadvertently ingesting more lead-contaminated soil and taking up more grit from the nearby contaminated track to aid with digestion (van der Meulen et al., 2007; Grace & MacFarlane, 2016; Takasaki & Kobayashi 2020). This could explain the observed increase in egg lead concentrations in February and May 2019 (Table 5.3). The owner then decided to move the chickens to an indoor barn with concrete flooring and no outdoor access. After this move, the chickens' egg lead concentrations decreased slightly, though they remained higher than the lead concentrations found in the eggs at the beginning of the study, even after the chickens had been kept in the barn for more than six months (Table 5.3). It is possible that the chickens were mobilizing lead stored in their bones while transferring calcium from bones for eggshell production, and therefore were still producing lead-contaminated eggs, even after their exposure to environmental lead was limited (Bar, 2009; Bautista et al., 2014). After January 2020, and the end of this study, the owner returned the chickens to the original pens, so there is no indication as to whether the lead concentrations in the eggs would have continued to decline if the chickens remained in the barn.

5.5.3 Eggs and Human Health Risks

Based on the amount of lead detected in the edible portions of the farm eggs, human adults consuming between three and seven of these eggs in a day would be at risk of developing chronic kidney disease (EFSA, 2010). This level of egg consumption is rare for egg-eating adults in the UK, who eat around 5-6 eggs per week (Gibson & Gray, 2020). Furthermore, lead exposure would greatly vary depending on which egg components are consumed; a person preferentially eating the albumen from these eggs would be exposed to less lead that someone regularly eating either the yolk or yolk and albumen. However, while following the EFSA thresholds would likely minimize the risk of developing severe adverse health impacts, even low levels of chronic lead exposure have been known to affect adults. Low lead exposure has been linked to various symptoms, including cognitive impairments, mood disorders, and a higher risk of cardiovascular disease (Vorvolakos et al., 2016; Obeng-Gyasi, 2020). Because of the extensive effects of exposure to lead at low concentrations, the World Health Organization has stated that 'there is no level of exposure to lead that is known to be without harmful effects' (World Health Organization, 2019).

Children are particularly vulnerable to lead's toxic effects. This is partly due to the active development of their organs/systems (in particular, their developing central nervous system), and also due to their higher lead uptake rates: a child's gastrointestinal tract absorbs 50% of consumed lead in food, while an adult's only absorbs 10-15% (Tong et al., 2000; Järup, 2003). Lead exposure in children has been linked to inhibited growth and impaired physical and neurobehavioral development (Yang et al., 2013; Vorvolakos et al., 2016). In particular, lead can cause lifelong cognitive and behavioural problems, including decreased brain volume, lower IQ scores, inhibited visual brain development, and slower information processing (Tong et al., 2000; Järup, 2003; Cecil et al., 2008; Ethier et al., 2012; Boucher et al., 2014; Liu et al., 2014; Karri et al., 2016; Vorvolakos et al., 2016). Based on the EFSA BMDL for children, if a young child regularly ate one to two of the eggs tested in this study, they could become cognitively impaired (measured by a reduction in Full Scale IQ scores) (EFSA, 2010). Even low levels of lead exposure in children (indicated by blood lead concentrations from 15 μg dL^{-1} down to <5 μg dL^{-1}) could lead to a variety of neurocognitive and behavioural impairments, including a lack of attention, increased anxiety, and reduced executive function performance (the ability to plan and adapt) (Chiodo et al., 2004; Roy et al., 2007; Vorvolakos et al., 2016). Lead exposure in children should therefore be minimized as much as possible to avoid lifelong repercussions (Vorvolakos et al., 2016).

5.6 Conclusions

Chicken eggs can be a key source of lead exposure for humans. This case study demonstrates that a flock of chickens living near a derelict metal mine can accumulate lead within their bodies and produce lead-contaminated eggs. The presence of lead in chicken eggs is of particular concern, because, despite the potentially severe adverse health impacts, lead contamination in eggs is difficult for owners or consumers to detect, as chickens rarely exhibit symptoms of lead toxicity, and lead-contaminated eggs appear normal. Consuming lead-contaminated eggs can have profound negative health effects, especially if the eggs are eaten on a regular basis, and/or if they are eaten by children. As there are potentially hundreds of thousands, if not millions, of abandoned metalliferous mine sites worldwide (United Nations Environment Programme, 2001; Venkateswarlu et al., 2016), and over 1,300 in Wales alone (Environment Agency Wales, 2002), further studies examining the lead concentrations in eggs produced in similar environments to those in this case study are clearly necessary. Better public awareness, additional research, and increased regulations on eggs produced in lead-contaminated areas are necessary to reduce the risk of lead toxicity from the consumption of lead-contaminated eggs.

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Data Availability

The data that support the findings of this study are openly available in the Nottingham Research Data Management Repository at https://doi.org/10.17639/nott.7117.

Appendices

Appendix A: Supplementary Tables

Supplementary Table 5.1: The number of each vegetable that can be consumed in one day before exceeding EFSA thresholds. The number of vegetables was rounded down to the nearest whole number. '0' means that eating one vegetable would exceed the respective EFSA threshold. The vegetables were washed, and the carrot and potatoes were peeled, prior to analysis to reduce external soil contamination. Rhubarb was collected from Pen 1; all other vegetables were collected from another small Welsh farm, also located near a derelict lead mine.

					Marrow (n=1)	**Potato** (n=2)	**Rhubarb** (n=2)	**Leek** (n=1)	**Carrot** (n=1)
Mean Pb (mg per vegetable)					0.0801	0.0417 ± 0.0527	0.0255 ± 0.0160	0.00311	0.00151
Overall Weight (mg wet weight)					329	102 ± 8.14	46.4 ± 2.69	148	32.0
EFSA Threshold Type	**Threshold** (mg/kg b.w. per day)	**Age**	**Weight**[a] (kg)	**Threshold** (mg of lead per day)					
Developmental neurotoxicity BMDL[b,1]	0.00050	0-1 years	9.5	0.00475	0	0	0	1	3
		2-4 years	16.2	0.00810	0	0	0	2	5
		5-7 years	22.8	0.0114	0	0	0	3	7
		8-10 years	34.1	0.0171	0	0	0	5	11
		11-12 years	47.0	0.0235	0	0	0	7	15
		13-15 years	59.7	0.0299	0	0	1	9	19
Nephrotoxicity BMDL[1]	0.00063	Adult	78.6	0.0472	0	1	1	15	32
Cardiovascular BMDL[1]	0.00150	Adult	78.6	0.118	1	2	4	37	78

[a]Average weights based on those recorded in the Health Survey for England 2019 (Moody, 2019).
[b]Benchmark dose level (the lowest 95% confidence limit below the benchmark dose, or dose that is associated with a specific response).
[1]EFSA, 2010.

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Chapter 6 – Relationships between soil and badger elemental concentrations across a mosaic landscape

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Relationships between soil and badger elemental concentrations across a mosaic landscape

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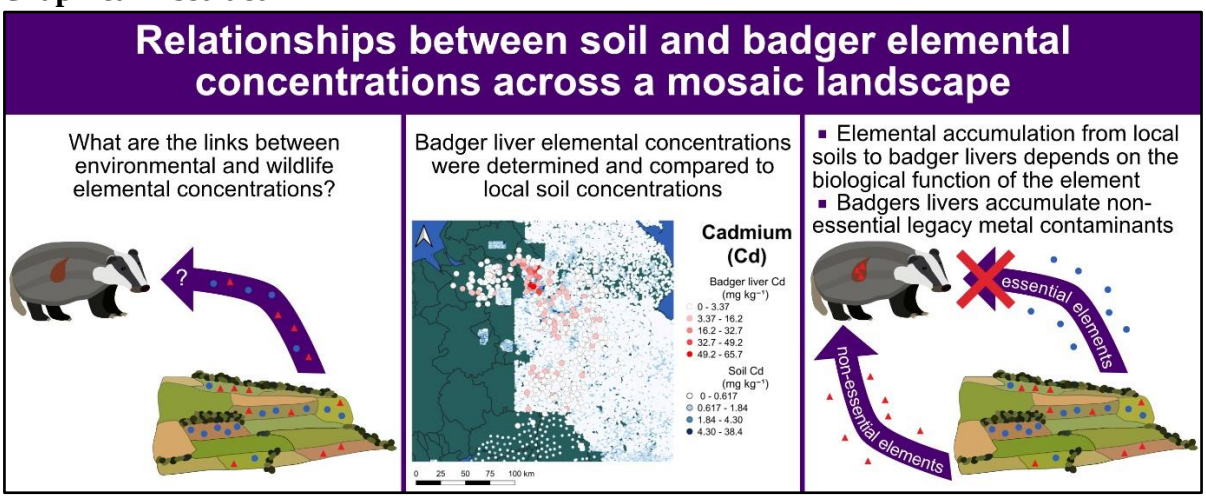
Keywords: environmental elemental concentrations, badgers, legacy pollutants, human-modified environments

Highlights

- Element concentrations in liver and soil were determined for 448 badgers in England
- No relationships were seen between liver and soil essential element concentrations
- Non-essential element liver and soil concentrations were positively correlated
- Pb and Cd liver concentrations were notably elevated in ex-mining areas
- Badgers can be used to highlight local non-essential elemental contamination

Graphical Abstract



6.1 Abstract

Understanding the links between environmental and wildlife elemental concentrations is key to help assess ecosystem functions and the potential effects of legacy pollutants. In this study, livers from 448 European badgers (*Meles meles*) collected across the English Midlands were used to investigate the relationship between elemental concentrations in topsoils and wildlife. Local soil concentrations, calculated using data from the British Geological Survey's 'Geochemical Baseline Survey of the Environment', were compared to badger liver elemental concentrations, focusing primarily on Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn. Generally, the badgers appeared to have elemental concentrations within normal levels, though Cu concentrations tended to be lower than expected. While there was no relationship between soil and badger liver concentrations for most biologically essential elements, biologically non-essential elements, specifically Pb, Cd, As, and Ag, were positively correlated between soil and badger livers. Lead and Cd, the elements with the strongest relationships between soils and badger livers, were primarily elevated in badgers collected in Derbyshire, a county with a millennia-long history of Pb mining and significant Pb and Cd soil pollution. Cadmium concentrations in badgers were also, on average, almost nine times higher than the local soil concentrations, likely due to Cd biomagnification in earthworms, a dietary staple of badgers. While badgers are good models for studying associations between soil and wildlife elemental concentrations, due to their diet, high levels of soil contact, and site fidelity, all flora and fauna local to human-modified environments could be exposed to and impacted by legacy pollutants.

6.2 Introduction

The elemental composition of soils varies due to both geological factors (Garrett, 2013) and anthropogenic pollution, stemming from activities such as mining, smelting, industrial processes and combustion of fossil fuels (Wuana & Okieimen, 2011; Kabir et al, 2012; Tchounwou et al., 2012; Khan et al., 2021). For example, centuries of intensive Pb and Zn mining in Derbyshire in the UK has resulted in high metal concentrations in soils across the region (Rawlins et al., 2012). Elemental pollution can remain in the environment for hundreds of years after the responsible processes have ceased operation, so soil elemental compositions can reflect historical, as well as current, anthropogenic activities (Merrington & Alloway, 1994; Alibrahim et al., 2017).

Both the composition and concentration of elements in the soil can affect their uptake by resident animals (Hettiarachchi & Pierzynski, 2004; Scheckel et al., 2009). Transfer into animals depends on the bioavailability of the contaminant, which is affected by soil conditions and the chemical form of the contaminant (Tchounwou et al., 2012). Other factors influencing elemental transfer include the nutritional and metabolic function of the contaminant and the animal's level of exposure to the contaminated soil (Hettiarachchi & Pierzynski, 2004; Scheckel et al., 2009; Tchounwou et al., 2012). Many studies have examined elemental (primarily trace metal) concentrations in wild terrestrial mammals and have attempted to correlate these with environmental concentrations (Wren, 1986; Gall et al., 2015; Rainbow, 2018). These studies indicate that mammals can take up elements, particularly non-biologically essential elements, from environmental sources, and that they can potentially accumulate them to toxic levels (Rainbow, 2018). However, few studies have focused on multiple elemental compositions, and few have been able to correlate soil elemental concentrations directly with levels in individual animals.

The European badger (*Meles meles*) is a medium-sized mammal commonly found across Europe. Badgers are generalist feeders, and primarily consume earthworms, as well as insects and plant matter, which they obtain mainly by foraging through topsoil (Shepherdson et al., 1990; Cleary et al., 2009; Balestrieri et al., 2019). Badgers also have a high site fidelity, as they live in family-based groups with relatively small territories, meaning that they are unlikely to move much further than 4 – 5 km from their place of birth (Kruuk & Parish, 1982; Gaughran et al., 2018). This combination of high soil exposure and strong site fidelity make badgers an ideal study species when investigating elemental transference from the environment to animals.

While a few studies have examined elemental compositions in badgers, they have mainly focused on a relatively small number of individuals and elements (Van den Brink & Ma, 1998; Bilandžić et al., 2012; Ozimec et al., 2015; Mullineaux et al., 2021). A multi-elemental analysis of hundreds of badgers collected across a continuous area with diverse geology and varied historical and current human activities may enable a better understanding of the relationships between soil and animal elemental compositions. The aim of this study was therefore to investigate the relationship between the elemental compositions of topsoil and livers collected from badgers from the English Midlands, examining 25 metals, 3 non-metals, and 2 metalloids. Of these, 10 elements (eight metals: Ag, Cd, Cr, Cu, K, Mn, Pb, Zn; one metalloid: As; and one non-metal: Se) were explored in more detail to determine possible accumulation variations across different elements and locations within the region.

6.3 Methods

6.3.1 Sample Collection and Processing

Carcasses of found-dead badgers were collected from an approximately 1.4 million hectare area of England (including the counties of Cheshire, Derbyshire, Nottinghamshire, Leicestershire, Warwickshire, and Northamptonshire) in 2016 – 2017, as part of a study of bovine tuberculosis (Figure 6.1; Swift et al., 2021). These badger carcasses were primarily collected on roadsides by citizen scientists. Liver samples, chosen because of their key role in element regulation and accumulation (Kalisińska, 2019), were extracted from 448 of these badgers and freeze dried before being acid digested.

Between 0.1 – 0.2 g of liver, along with 4 mL 70% HNO_3 and 1 mL H_2O_2, were incubated on a hotplate block digester at 95 ˚C. After two hours, the samples were allowed to cool before being dispensed into plastic volumetric flasks, made up to 50 mL with MilliQ water (18.2 MΩ cm; Millipore Corporation, Darmstadt, Germany), and gently mixed. The samples were then left for at least ten minutes to allow for heavier material to settle to the bottom of the tube, and 10 mL of the supernatant solutions were decanted and stored at ambient temperature. The solutions were diluted 1-in-10 with MilliQ water prior to elemental analysis using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) (Model ICAP-Q; Thermo Fisher Scientific, Bremen, Germany). A certified reference material for trace elements in biological samples (BRC-185R Bovine Liver [trace elements]) was run for quality assurance purposes (recovery values for specific elements were: As [74.9%], Cd [103.9%], Cu [94.9%], Mn [99.1%], Pb [94.2%], Se [90.1%], and Zn [101.2%]). For each element, the operational limit of detection (LOD) was calculated as three times the standard

deviation of the concentrations measured in 10 blank digestion samples run alongside the badger livers (Marin et al., 2011) (Table 6.1). A value of 0.5*LOD was used in instances where the elemental concentration was lower than the LOD (Kushner, 1976). All concentrations were calculated as mg kg^{-1} dry weight; comparisons with published fresh weight concentrations were performed assuming a liver water content of 72.1%, as described for European badgers by Kalisińska et al. (2009).

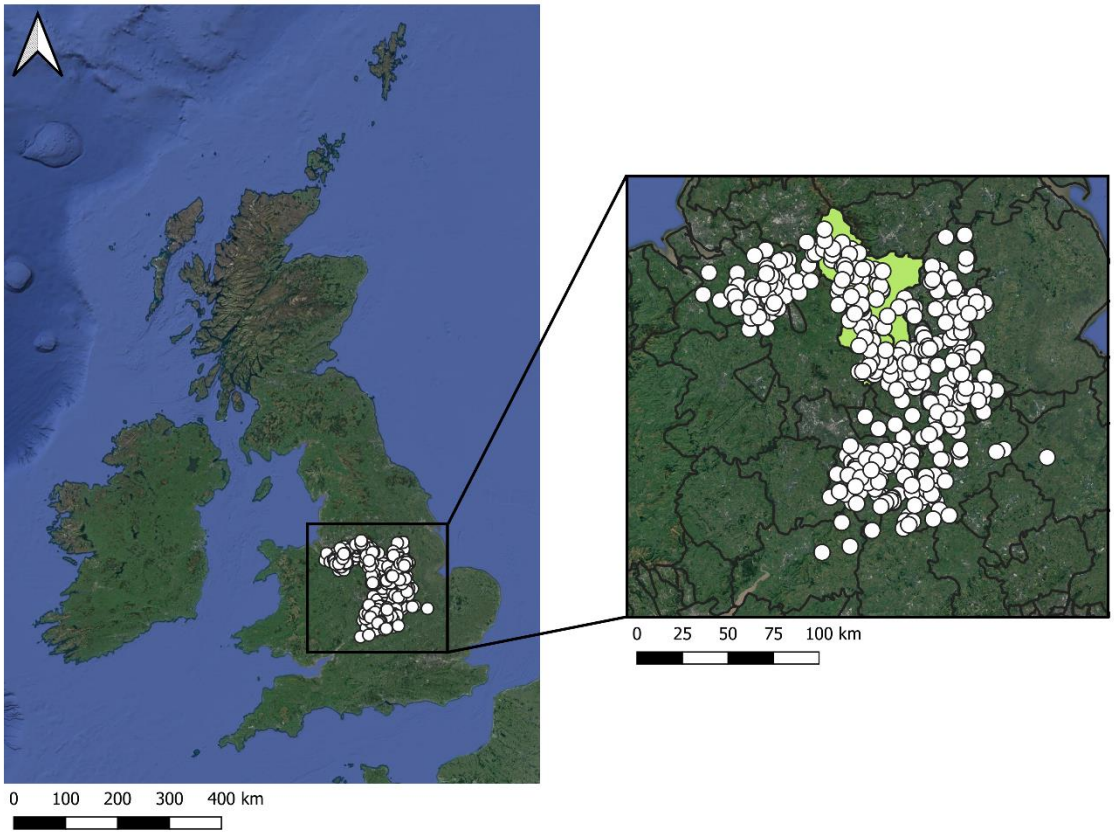


Figure 6.1: Map of badger collection locations. Derbyshire is highlighted in green. Satellite imagery map data ©2022 Google.

6.3.2 Data Analyses

Soil and sediment elemental concentrations were obtained from the British Geological Survey's *Geochemical BAseline Survey of the Environment* (G-BASE) project (Johnson et al., 2005). These datasets consists of over 40,000 topsoil and 54,000 sediment sampling points across England and Wales where elemental concentrations were measured using X-Ray Fluorescence (XRF) (Johnson et al., 2005). For each element examined, maps were generated in QGIS (QGIS.org, 2022), with soil (or sediment, in the absence of soil) concentrations categorized into four classes: 1) 0 to mean, 2) mean to mean + one standard deviation, 3) mean + one standard deviation to mean + two times the standard deviation, and

4) mean + two times the standard deviation to maximum. This scale was chosen to provide an overall image of soil (or sediment) concentrations in the area, while also highlighting locations with elevated concentrations. All calculations were based on the soil (or sediment) concentrations within the mapped area surrounding the badger collection locations (-3.508178, 51.522730: 0.427985, 53.700807). Any G-BASE soil concentrations above the BGS's published maximum concentration of the element in soils in England and Wales (Rawlins et al., 2012) were removed to avoid outliers from skewing the scale. If no soil concentration data was available for a particular element, comparisons were instead based on the sediment concentrations, if available.

Elemental concentrations in badger livers were mapped alongside the BGS soil and sediment maps using QGIS (QGIS.org, 2022). The badger elemental concentrations were categorized into five classes, designed to highlight notably elevated concentrations: 1) 0 to mean, 2) mean to two times the standard deviation, and 3-5) three equal intervals between two times the standard deviation and the maximum concentration of the element found in the badger livers.

To compare soil and badger liver elemental concentrations, mean soil elemental concentrations were determined at the badger collection locations for some selected elements (Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn). Soil concentrations at the three nearest sampling points, within two km, to each badger collection location were averaged. If there were fewer than three soil collection points within two km of the badger, no overall soil concentration was determined. Two km was chosen based on badger territory diameters, which are usually between 1 – 2 km, dependent on badger population density and food availability (Kruuk & Parish, 1982). While badgers can disperse over long distances (Byrne et al., 2014), most movements are within territories, so elemental concentrations within a two km radius of the collection site should represent likely exposure levels for the badgers.

Relationships between elemental concentrations in the liver and soil were examined through correlations in R (R Core Team, 2018). Due to a lack of normality in the data, identified by Shapiro-Wilk tests, Spearman's correlation coefficients were calculated, and the concentrations were log-transformed for any corresponding graphs to show relationships more clearly. To decrease the chance of a type I error, Benjamini - Hochberg corrections were utilized for each statistical test type, with the total number of tests reflecting the number of elements included in each test type (Benjamini & Hochberg, 1995). This was implemented by calculating adjusted p-values by multiplying the p-value by the total number of tests and dividing by the p-value's rank (when all relevant p-values were ordered smallest to largest),

as described in Yekutieli & Benjamini (1999). Graphs visualizing these statistical tests were generated in R using the ggplot2 package (Wickham, 2016).

6.4 Results

6.4.1 Elemental Concentrations in Badger Livers

The elemental concentrations in badger livers varied greatly across the 30 elements investigated (Table 6.1). Most elemental concentrations, however, appeared to have an approximately normal distribution, but with a long positive tail that prevented the concentrations from being truly normally distributed (Figure 6.2; Table 6.1; Supplementary Figure 6.1).

Table 6.1: Summary of badger liver elemental concentrations. All concentrations are in mg kg^{-1} dry weight. If the minimum value was below the limit of detection, it is reported as "LOD" in this table, and treated as 0.5*LOD for all summary statistic calculations.

Element	Mean	Median	Minimum	Maximum	Standard Deviation	Limit of Detection
Ag	0.0381	0.0255	0.00154	0.580	0.0424	0.000425
Al	8.37	3.82	LOD	279	22.8	3.22
As	0.0906	0.0737	0.0185	0.683	0.0648	0.00636
B	1.63	0.695	LOD	27.2	3.20	0.00217
Ba	0.487	LOD	LOD	50.3	2.43	0.250
Be	0.0257	LOD	LOD	0.127	0.0173	0.0372
Ca	286	224	65.0	1970	193	0.0500
Cd	3.37	1.74	0.0450	65.7	6.39	0.00317
Co	0.149	0.118	0.0327	1.03	0.105	0.00228
Cr	0.894	0.0640	LOD	12.5	2.14	0.0229
Cs	0.00707	0.00435	LOD	0.111	0.0104	0.00111
Cu	32.0	26.2	2.38	143	19.8	0.115
Fe	1160	1110	298	4990	488	0.442
K	8140	8250	2810	13400	1670	0.00334
Mg	596	605	201	898	111	0.00176
Mn	16.0	8.59	0.823	122	20.1	0.0517
Mo	2.54	2.57	0.176	4.13	0.609	0.00697
Na	3930	3830	1020	9620	1100	0.0184
Ni	0.145	0.0795	LOD	1.07	0.172	0.0325
P	9020	9210	2800	13200	1690	0.00718
Pb	1.38	0.486	0.0744	32.6	3.56	0.0269
Rb	6.45	5.05	0.898	38.7	4.70	0.00596
S	8590	8990	1880	12900	2000	0.665
Se	3.05	2.92	1.06	15.4	1.13	0.00299
Sr	0.328	0.245	LOD	3.57	0.312	0.0927
Ti	105	76.3	30.7	1550	112	9.28×10^{-6}
Tl	0.0143	0.0112	0.000636	0.117	0.0113	7.39×10^{-5}
U	0.00235	LOD	LOD	0.0526	0.00545	0.000684
V	0.282	0.232	0.0278	1.70	0.208	0.00357
Zn	127	117	25.7	674	57.0	2.55

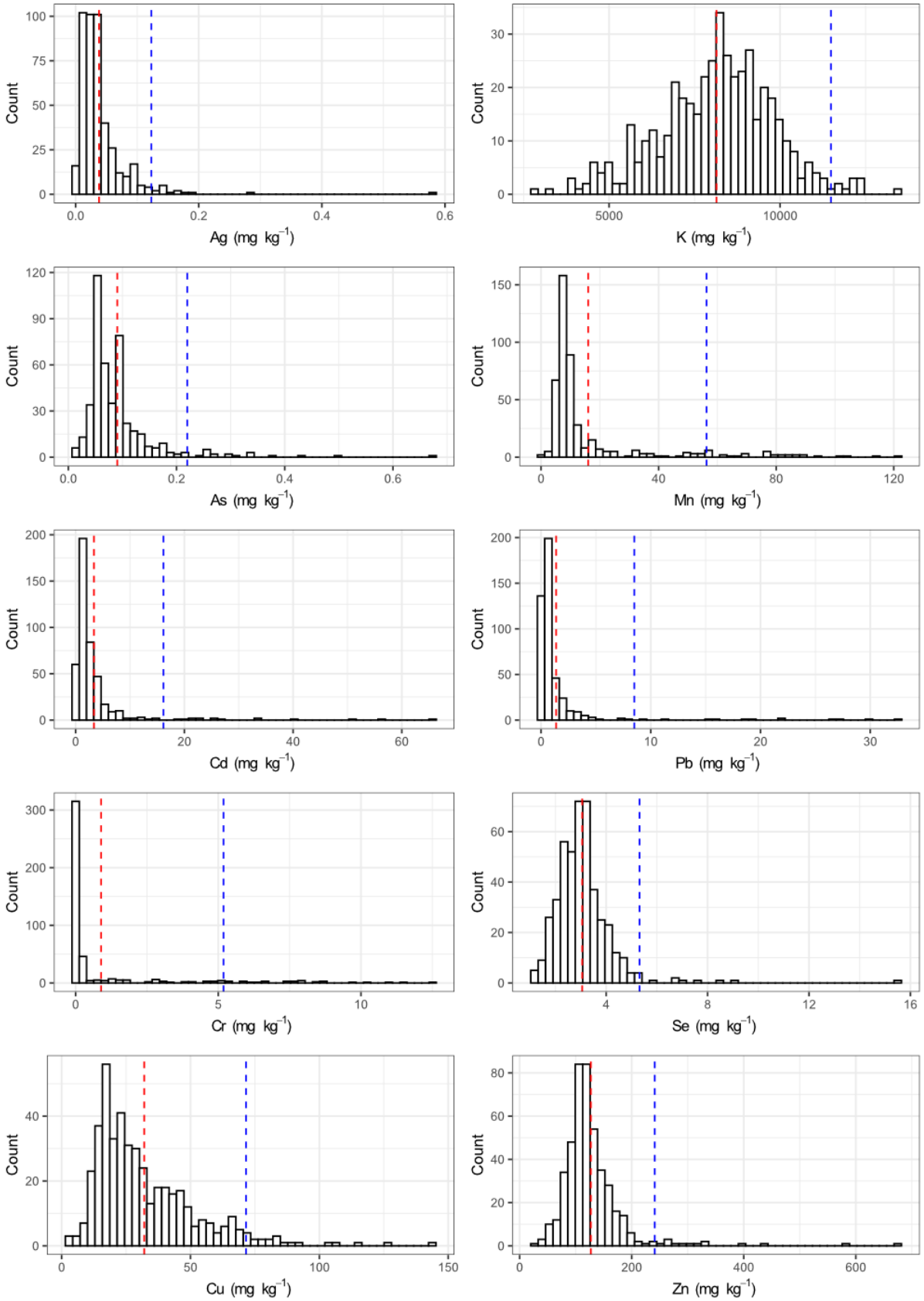


Figure 6.2: Histograms of badger liver concentrations of 10 key elements (Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn). The red line represents the mean, while the blue line represents two standard deviations from the mean.

6.4.2 Relationship Between Soil and Liver Concentrations

Elemental concentrations in both soil and badger livers varied across the study area (Figure 6.3; Supplementary Figure 6.2). The soil elemental concentrations in the study area are known to vary, due to both natural geochemical properties and anthropogenic pollution (Rawlins et al., 2012). Badger liver concentrations across the study area appeared to reflect patterns in local soil concentrations for some of the examined elements (such as As and Pb), although not for all (Figure 6.3).

The relationships between soil and badger elemental concentrations were explored in greater depth for ten elements: eight metals (Ag, Cd, Cr, Cu, K, Mn, Pb, Zn), one metalloid (As), and one non-metal (Se). These elements were chosen because of relatively complete G-BASE sampling for these elements in the soils in the study area, and because of the high degree of variability in both the soil and badger liver concentrations. Furthermore, the group of elements was specifically chosen to include both biologically essential and non-essential elements, to assess whether this factor affects accumulation in badgers. Of these ten elements, there were significant correlations between concentrations in badger livers and local soils for five of the elements: Ag, As, Cd, Pb, and Se (Figure 6.4). These correlations varied from slight (As: $\rho = 0.155$, Se: $\rho = 0.165$) to moderate (Ag: $\rho = 0.309$, Cd: $\rho = 0.368$), with the strongest relationship being between Pb in soils and livers ($\rho = 0.459$). The concentrations of the other five elements (Cr, Cu, K, Mn, and Zn) were not significantly correlated between livers and soils.

All five elements that were correlated between badger livers and soils were also significantly correlated with each other in badger livers (Table 6.2). In particular, Se concentrations were moderately correlated with all other metal concentrations (Ag: $\rho = 0.350$; As: $\rho = 0.334$; Cd: $\rho = 0.339$; Pb: $\rho = 0.277$), while Pb was strongly correlated with Cd ($\rho = 0.560$), and moderately correlated with Ag ($\rho = 0.292$) (Table 6.2). Cd and Se liver concentrations also significantly varied between juveniles and adults (Cd: $z = -4.00$, $p = 0.000324$; Se: $z = 2.74$, $p = 0.00616$), with Cd higher in adults than juveniles, and Se higher in juvenile than adults. However, Ag ($z = 1.99$, $p = 0.0775$), As ($z = -0.355$, $p = 0.723$), and Pb ($z = 0.483$, $p = 0.723$) liver concentrations did not significantly vary between juveniles and adults.

All five elements were also correlated with each other in the soils from around the badger collection points, with the exception of As and Cd, which were not significantly correlated to each other (Table 6.2). Selenium was strongly correlated with Pb ($\rho = 0.622$)

and As (ρ = 0.543), and Pb, Cd, and Ag were all moderately correlated with each other (Table 6.2). Despite being positively correlated in the badger livers, Ag and As had a slight negative correlation in soils at badger collection points (ρ = -0.176) (Table 6.2).

Table 6.2: Significant correlations between Ag, As, Cd, Pb, and Se. On the bottom left of the table, shown in grey, are correlations between the soil concentrations found at the badger collection sites, while on the top right of the table, shown in white, are the correlations between the badger liver concentrations. Non-significant correlation ρ values are not reported, and are replaced by "–". The reported p-values were adjusted following Yekutieli & Benjamini (1999). All correlations were calculated using the Spearman's correlation coefficient.

		Ag	**As**	**Cd**	**Pb**	**Se**
Ag	ρ		0.213	0.139	0.292	0.350
	p		*< 0.0001*	*0.00353*	*< 0.0001*	*< 0.0001*
As	ρ	-0.176		0.0995	0.157	0.334
	p	*0.00111*		*0.0353*	*0.00108*	*< 0.0001*
Cd	ρ	0.424	–		0.560	0.339
	p	*< 0.0001*	*0.577*		*< 0.0001*	*< 0.0001*
Pb	ρ	0.336	0.267	0.430		0.277
	p	*< 0.0001*	*< 0.0001*	*< 0.0001*		*< 0.0001*
Se	ρ	0.138	0.534	0.360	0.622	
	p	*0.0104*	*< 0.0001*	*< 0.0001*	*< 0.0001*	

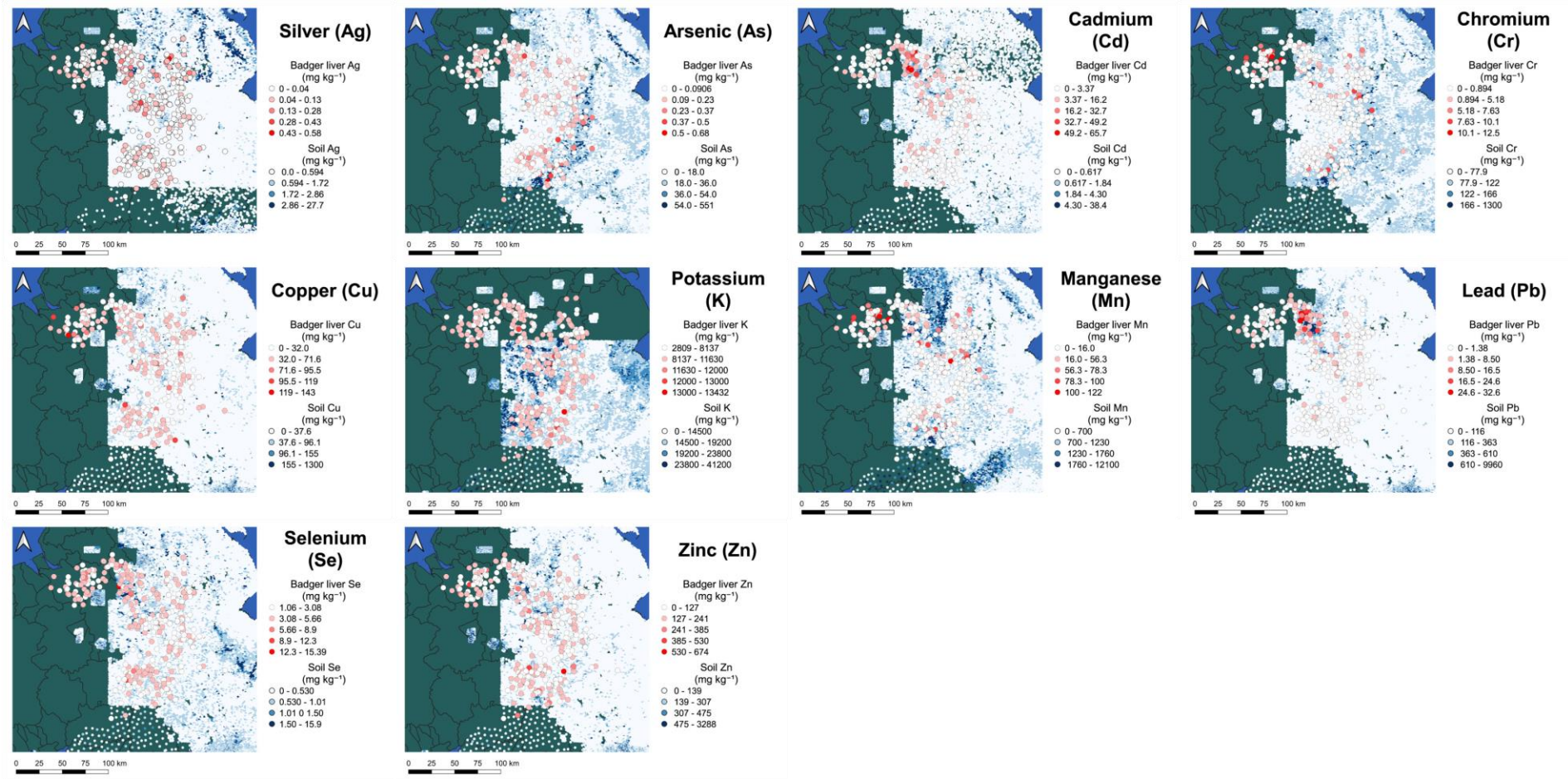


Figure 6.3: Maps of badger collection sites, showing badger liver concentration and soil concentrations of 10 key elements (Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn). Soil concentrations are derived from 2,300,000 scale BGS Digital Data under Licence No. 2021/086 British Geological Survey © and Database Right UKRI. All rights reserved. Soil concentrations are grouped into four classes: 1) 0 – mean, 2) mean – mean + standard deviation, 3) mean + standard deviation – mean + two times the standard deviation, and 4) mean + two times the standard deviation – maximum. Badger liver concentrations are grouped into five classes: 1) 0 – mean, 2) mean – two times the standard deviation, and 3-5) three equal intervals between two times the standard deviation and the maximum concentration of the element found in the livers.

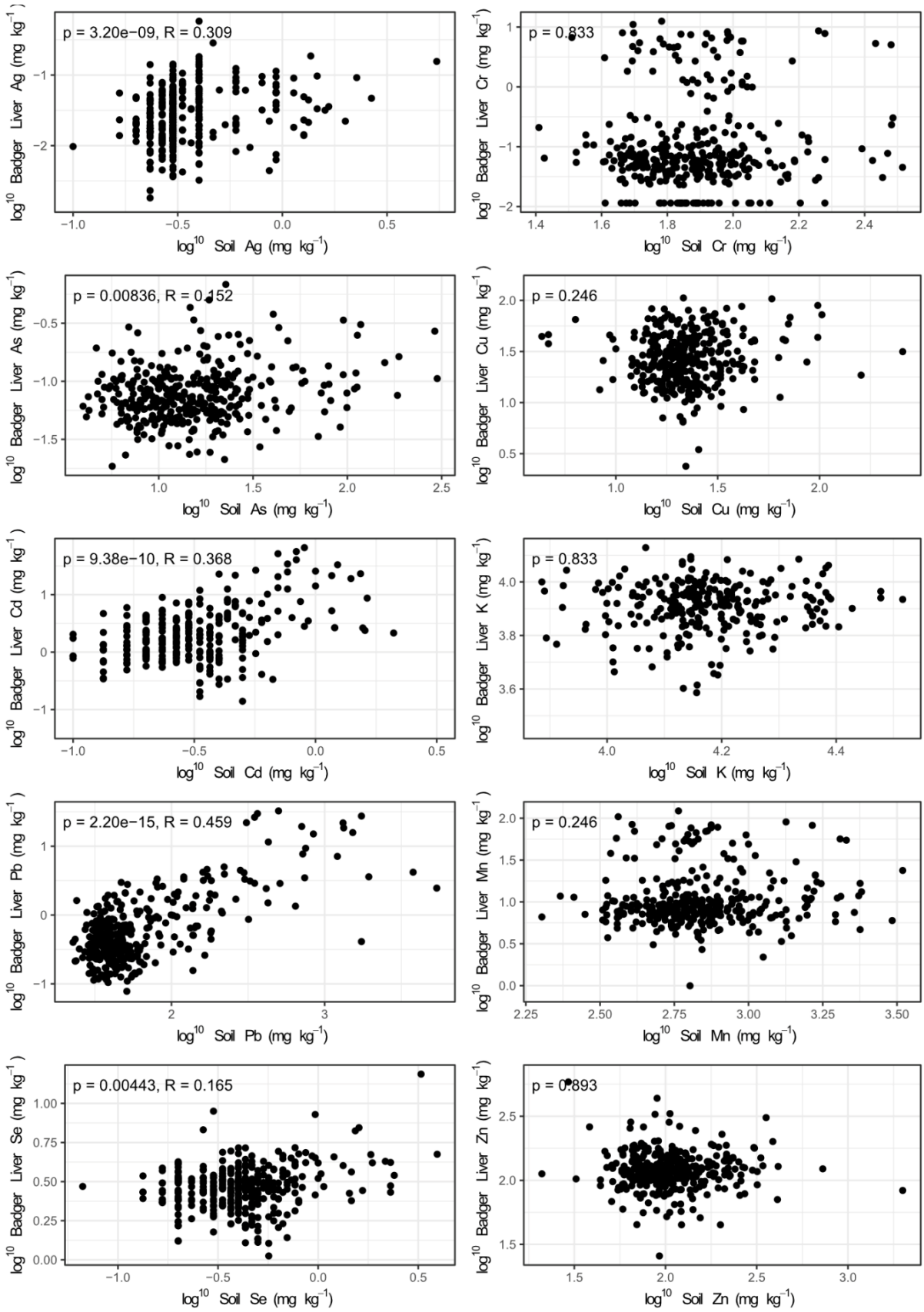


Figure 6.4: Badger liver metal $\log_{10}$ concentration versus soil metal $\log_{10}$ concentration of 10 key elements (Ag, As, Cd, Cr, Cu, K, Mn, Pb, Se, Zn). Metals that correlate between liver and soil concentrations are on the left, while metals with no correlation between liver and soil are on the right. The p-values are adjusted following Yekutieli & Benjamini (1999).

6.5 Discussion

6.5.1 Implications of Badger Liver Elemental Concentrations

Overall, most of the badgers collected during this study appeared to have hepatic elemental concentrations broadly similar to those found in prior studies examining wild European badgers or other wild mustelids (Bilandžić et al., 2012; Ozimec et al., 2015; Kosik-Bogacka et al., 2019; Pilarczyk et al., 2019; Mullineaux et al., 2021) and to established 'normal ranges values' for domestic species (primarily bovine, porcine, and ovine) (WVDL, 2015). However, the mean Cd concentration (3.37 ± 6.39 mg kg^{-1} dry weight) was elevated above concentrations found in wild badgers in Croatia in prior studies (geometric mean of 1.92 mg kg^{-1} dry weight [converted] across three badgers, Bilandžić et al., 2012; mean of 1.42 mg kg^{-1} dry weight [converted] across 29 badgers, Ozimec et al., 2015). Furthermore, four individuals had Cd liver concentrations indicative of possible toxicity (35.8 mg kg^{-1} dry weight [converted] in vertebrate livers, Eisler, 1985), while eight individuals had Pb concentrations indicative of possible toxicity (17.9 mg kg^{-1} dry weight [converted] in mammal livers, WVDL, 2015).

The Cu concentrations detected in the badger livers were relatively low compared to prior studies. A quarter of the sampled badgers had liver Cu concentrations that were indicative of Cu deficiency in mustelids (2.86 – 17.9 mg kg^{-1} dry weight [converted]; WVDL, 2015). Copper deficiency can have long-term health effects, including slow growth, emaciation, impaired immune response, and reduced survival in mammals (Eisler, 1998). However, there have been few studies investigating Cu concentrations and deficiency thresholds for mustelids, so it is unclear whether the badgers were truly Cu deficient, or just had naturally relatively low Cu concentrations. Copper concentrations in livers are known to vary widely, even across similar species and areas (Łanocha-Arendarczyk & Kosik-Bogacka, 2019). When compared to previous studies on wild badgers, the mean liver Cu concentration (32.0 ± 19.8 mg kg^{-1} dry weight) was lower than those found in Northern Ireland (mean of 77.6 mg kg^{-1} dry weight across 57 badgers; Mullineaux et al., 2021) and Croatia (geometric mean of 54.5 mg kg^{-1} dry weight [converted] across three badgers; Bilandžić et al., 2012). As the liver Cu concentrations did not correlate with local soil concentrations, and as the badgers with low liver Cu concentrations were distributed throughout the whole of the study area, the significance and possible causes of these low concentrations are unclear, though Cu deficiencies are usually linked to insufficient Cu in diets (Fisher, 1975).

6.5.2 Essential Element Accumulation in Badgers

Of the 10 elements examined in more detail, Cr, Cu, K, Mn, Se, and Zn are considered biologically essential elements, as they perform key biochemical and physiological functions necessary for life (Tchounwou et al., 2012). Their concentrations are normally highly regulated in the body, and excretion methods are in place to remove excessive concentrations (though these mechanisms can be overwhelmed) (Tchounwou et al., 2012; Kalisińska & Budis, 2019; Łanocha-Arendarczyk & Kosik-Bogacka, 2019). The concentrations of the essential elements in the soil at the badger collection locations varied from the bottom 20% to the top 10% of the respective elemental concentrations found overall in English and Welsh soils (Rawlins et al., 2012). Despite this wide environmental variation, Cr, Cu, K, Mn, and Zn concentrations in the badger livers remained relatively consistent across the study area, and there was no relationship between their concentrations in the soil and the badger livers.

Essential elements are not usually accumulated from the environment and sequestered into bodies, primarily due to active regulation of these elements. Previous studies on Cr, Cu, and Mn have found little evidence of accumulation in terrestrial mammals related to environmental exposure (Kalisińska & Budis, 2019; Kośla et al., 2019; Łanocha-Arendarczyk & Kosik-Bogacka, 2019). Potassium accumulation has not been studied in depth, as few studies examine K in wildlife (Vengušt & Vengušt, 2004), but, as a key essential element, its lack of accumulation in badger livers is unsurprising. Accumulation of Zn has been reported, but depends on the species in question, as well as the tissue examined (Kosik-Bogacka & Łanocha-Arendarczyk, 2019). Generally, while Zn seems to accumulate in ruminants living in Zn-polluted environments, this trend has not been strongly detected in carnivorous mammals (Dip et al, 2001; Kosik-Bogacka & Łanocha-Arendarczyk, 2019; Ziętara et al., 2019). In some studies, Cu and Zn concentrations have been found to vary between mustelids in control and Cu-Zn contaminated sites (Van den Brink & Ma, 1998; Sanders et al., 2020). However, these variations could be attributed to dietary differences, which are known to affect Cu and Zn concentrations in mustelids (Brzeziński et al., 2014), or to sampling in discrete areas or areas with a clear contamination gradient, as opposed to a heterogeneously contaminated landscape, such as the area sampled during this study.

Of the essential elements explored in more detail during this study, only Se soil and liver concentrations were positively significantly correlated, albeit only slightly ($\rho = 0.165$). This agrees with prior studies, as Se has been found to accumulate in terrestrial mammals based on their environment (Pilarczyk et al., 2019). Selenium accumulation has been

observed in rodents exposed to contaminated sediments, food, or water (Clark, 1987; Stoewsand et al., 1990; Levengood & Heske, 2008). One study, focusing on a floodplain contaminated with a number of elements, including Se, Zn, and Cu, found that Se in particular accumulated to potentially toxic concentrations in white-footed mouse (*Peromyscus leucopus*) livers (Levengood & Heske, 2008). In mustelids, higher Se concentrations in river otters (*Lontra canadensis*) have been linked to local environmental Se pollution (Sanders et al., 2020).

Selenium has also been known to vary across juveniles and adults, normally accumulating to higher concentrations with age (Pilarczyk et al., 2019). This is the opposite pattern to that observed in this study, where adults had significantly, but only slightly, lower liver Se concentrations than juveniles. It is possible that badgers accumulate Se only until they are sexually mature, as has been hypothesized for raccoons (Clark et al., 1989), or that the differences observed in this study are affected by season (Pilarczyk et al., 2019) and when the juveniles were born (Malzahn & Lang, 1980). It is also possible that comparing Se concentrations across only two age classes, as opposed to exact ages, affected the observed pattern.

6.5.3 Non-Essential Element Accumulation in Badgers

The other four elements examined more closely during this study (Ag, As, Cd, and Pb) are non-essential and potentially toxic at relatively low concentrations (Tchounwou et al., 2012), so their presence in badgers could potentially adversely affect badger health and population dynamics. The concentrations of all four of these non-essential elements were correlated between soils and badger livers, indicating that the badgers accumulated these elements at least partly based on their environmental exposure.

Lead, the element with the strongest soil-liver correlation ($\rho = 0.459$) in this study, is known to accumulate in mammalian livers when animals are exposed to lead pollution from human mining or industrial activities (Baranowska-Bosiacka et al., 2019). Cd, which also had a notable soil-liver correlation ($\rho = 0.368$), has similarly been known to accumulate in the tissues of terrestrial animals living in Cd-contaminated environments (Tomza-Marciniak et al., 2019). Elevated Pb and Cd concentrations have been found in badgers and other mustelids in previous studies on wildlife living in human-modified landscapes (Harding et al., 1998; Alleva et al., 2006; Ozimec et al., 2015; Goretti et al., 2018; Sanders et al., 2020). Cadmium in particular is highly bioavailable to earthworms when present in the soil, so

insectivores focusing on earthworms, such as badgers, are particularly at risk of Cd accumulation (Spurgeon & Hopkin, 1996; Van den Brink & Ma, 1998; Cleary et al., 2009; Tomza-Marciniak et al., 2019). Badgers in Great Britain are known to have a diet rich in earthworms, focused mainly on *Lumbricus terrestris* (Kruuk & Parish, 1982; Shepherdson et al., 1990; Goszczyński et al., 2000; Cleary et al., 2009), so it seems likely that this was the principal Cd exposure route for the sampled badgers. Cadmium concentrations have also been known to increase with age in wild mustelids (Harding et al., 1998; Sanders et al., 2020), as was observed in this study, suggesting that the badgers are accumulating higher concentrations of Cd in their livers over time.

Both Ag and As concentrations were also correlated between soils and badger livers, though the relationships (Ag: $\rho = 0.309$, As: $\rho = 0.155$) were weaker than those observed with Pb and Cd. Furthermore, unlike Pb and Cd, Ag and As accumulation due to environmental exposure has not been thoroughly studied in terrestrial mammals. Few studies have examined Ag in the livers of wild terrestrial mammals (Eisler, 1996), but those that have found evidence of Ag accumulation, dependent on its chemical structure (Strużyńska, 2019). Studies on As accumulation have mainly focused on small mammals, and have found indications that As accumulates in rodents living in contaminated areas, albeit at low ratios (Erry et al., 2000; Drouhot et al., 2014; Binkowski, 2019). Like Cd, As has also been found to accumulate in earthworms living in contaminated sites (Yang et al., 2018), making badgers particularly vulnerable to As accumulation.

6.5.4 Anthropogenic Pollution

The UK has a long-standing, extensive legacy of elemental pollution, primarily from mining and smelting, but also from a wide range of other industrial activities (Rainbow, 2018). In the study area, the most notable metal-polluted region is Derbyshire, which has a more than two thousand year long history of Pb and Zn mining and smelting (Figure 6.1) (Rawlins et al., 2012; Rainbow, 2018). While all metal mines in the county have now been shut down, the soils in areas surrounding historical metal mines and smelters in Derbyshire still contain elevated levels of Pb, Zn, Cd, As, and Ag (Li and Thornton, 1993).

Though most of the badgers used in this study were collected from locations with soil concentrations below the British Geological Survey's Normal Background Concentration (NBC) for 'Principal Domains' (areas not affected by urbanisation or mining activities) (Johnson et al., 2012), 12.7% of the badgers were collected from sites with soil Pb

concentrations in the 90th percentile of the soil Pb concentrations found in England and Wales (Rawlins et al., 2012). The vast majority (89.5%) of these high Pb badger collection sites were in Derbyshire, with the others collected in a neighbouring county (Nottinghamshire). A similar trend was observed for Cd, though only 16 badgers were collected from sites with soil Cd concentrations in the 90th percentile of the soil Cd concentrations found in England and Wales (Rawlins et al., 2012), of which 14 were found in Derbyshire.

Lead, Cd, and Ag soil concentrations from around the badger collection sites were correlated with one other, mirroring the anthropogenic pollution associations between these metals (Table 6.2) (Li and Thornton, 1993; Rawlins et al., 2012). High concentrations of As are also commonly found near metal mines or smelters (Li and Thornton, 1993), but, in this study, while As was correlated with Pb, it was not correlated with Cd, and had a negative correlation with Ag (Table 6.2). This is likely due to natural high As concentrations found along an ironstone band in the south-eastern portion of the study area, a region without significant Pb, Cd, Ag, or Zn pollution (Figure 6.3) (Rawlins et al., 2012). Soil Zn concentrations near the badger collections sites were also correlated with soil Pb ($p < 0.0001$, $\rho = 0.237$), Cd ($p = 0.0125$, $\rho = 0.164$), and As ($p = 0.00887$, $\rho = 0.156$), though not Ag ($p = 0.570$).

Lead, Cd, Ag, and As concentrations in badger livers were all correlated with concentrations in soils, indicating that the badgers were accumulating these elements within their livers based at least in part on local environmental concentrations. Despite the presence of Zn in the soil, and its correlations with the other elements within the soil, Zn concentrations did not correlate between soil and badger livers, likely due to active regulation of this essential element. The mean bioaccumulation factors (BAFs) for Ag, As, and Pb (calculated as BAF = concentration of element in liver / concentration of metal in soil, following Ali & Khan [2018]) were low (Ag: BAF = 0.111; As: BAF = 0.00688; Pb: BAF = 0.0129), suggesting that only relatively small amounts of these elements were transferred from the soil into the badger livers. However, since this transfer is proportional, if the soil element concentration is sufficiently elevated, badgers could theoretically accumulate high, potentially toxic amounts of the element.

Unlike the other three non-essential elements, Cd had a notably high mean BAF (8.80), indicating that the badgers were accumulating cadmium in their livers at a higher concentration than was found in the environment. This is likely due to the badgers' diet rich in earthworms (Shepherdson et al., 1990), which are known Cd accumulators (Spurgeon & Hopkin, 1996; Van den Brink & Ma, 1998; Cleary et al., 2009; Tomza-Marciniak et al.,

2019). High Cd concentrations have frequently been observed in insectivorous small mammals, even in non-contaminated areas, and attributed to the consumption of earthworms and other surface-soil dwelling invertebrates (Cooke, 2011). In contaminated landscapes, small insectivorous mammals have been known to accumulate over twenty times more Cd in their livers than herbivorous or omnivorous species, with a BAF value of over 15 (Sánchez-Chardi & Nadal, 2007; Sánchez-Chardi et al., 2009; Cooke, 2011). In badgers, high kidney Cd concentrations have been recorded, in particular near contaminated floodplains where earthworms were known to have high Cd concentrations (Van den Brink & Ma, 1998). While these high Cd concentrations may pose a health risk, small insectivorous mammals are able to tolerate high Cd concentrations, and it may be that badgers are similarly adapted (Cooke, 2011).

6.5.5 Badgers as Bioindicators

Based on the results of this study, it appears that non-essential elements accumulate in badger livers, depending at least partially on concentrations in local soil. While these local soil concentrations were estimated, based on a radius of two km from the badger collection location, since badgers have high site fidelity and strongly defined territories, these estimations should roughly reflect the soil concentrations to which the badgers are exposed. However, there are potentially other routes of elemental exposure for badgers that are more localized and were therefore not fully investigated during this study. Dietary composition is known to affect metal concentrations in mustelids, and, due to the badgers' exploitation of human-provided food resources, the elemental composition of their diets could vary greatly, even within areas with similar elemental concentrations in soil (Cleary et al., 2009; Brzeziński et al., 2014; Balestrieri et al., 2019). Badgers could also be affected by more localized industrial pollution, agricultural activities, or the use of cattle mineral licks (Ward et al., 2010), all of which would not necessarily be reflected in soil elemental concentrations. Furthermore, as most of the badgers were collected at roadsides, they could have been exposed to legacy Cd, Cu, Pb, Zn, and other contaminants associated with fossil fuels and vehicle pollutants (Akbar et al., 2006).

As long as possible local exposure routes are kept in mind, the results of this study indicate that badgers can be used as bioindicators for environmental non-essential element contamination, particularly for Pb and Cd. Badgers are ideal mammalian bioindicator species, due to their high site fidelity, soil-based foraging, and their frequent road traffic mortality

events, which results in a potentially large supply of badger carcasses (Clarke et al., 1998). As was demonstrated in this study, citizen scientists can aid in the collection of badger carcasses, allowing for elemental concentrations, as well as other environmental pollutants, to be compared across a wide geographic area. Establishing further badger baseline elemental concentrations would be helpful for comparison in future studies to determine whether badger elemental concentrations are deficient, normal, or elevated.

6.6 Conclusions

The links between environmental and wildlife elemental concentrations need to be further understood to both allow for a better assessment of ecosystems, and to determine whether raised environmental concentrations could pose a risk to wildlife. In this study, the relationships between elemental concentrations in topsoils and badger livers were explored using one of the largest collections of wildlife for which elemental concentrations were determined. While there are few clear thresholds for expected badger liver concentrations, the badgers generally appeared to have elemental concentrations within normal levels, though Cu concentrations were lower than expected in mustelids. While the concentrations of most essential elements in badger livers did not appear to be affected by local soil concentrations, non-essential elements, specifically Pb, Cd, Ag, and As, did accumulate in the badger livers at least partially depending on soil concentrations. These elements are common legacy pollutants, found at particularly high concentrations near metal mines or industrial areas, and can be toxic at low concentrations, so their accumulation in badger livers can pose a potential health risk. Cadmium in particular accumulated in badger livers to concentrations on average almost nine times higher than the concentrations found in local soils, likely due to the badgers' consumption of Cd-bioaccumulating earthworms. While badgers in particular can be heavily exposed to these elements, due to their diet rich in earthworms and their high levels of soil contact and ingestion, other animals living in the same areas, including domestic animals and humans, could also be exposed to similarly high concentrations of these elements. As local soil elemental concentrations are expected to change over time, due both to increases in anthropogenic pollution (Van den Brink and Ma, 1998) and element re-distributions due to more frequent flooding as a result of climate change (Jentsch & Beierkuhnlein, 2008), continued monitoring of environmental and corresponding wildlife elemental concentrations is pivotal to both the further understanding of ecosystems and the monitoring of wildlife health risks.

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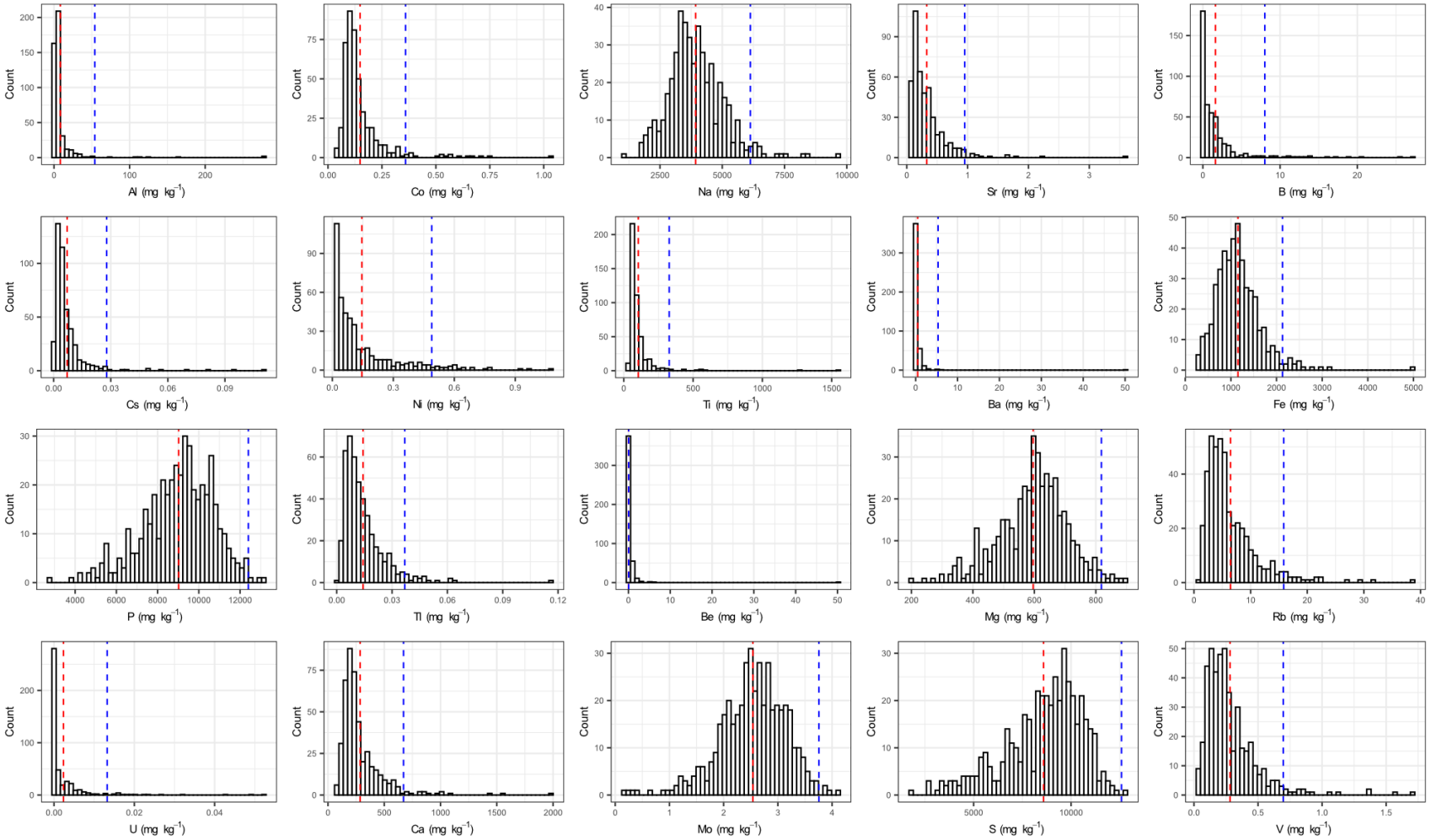
Data Availability

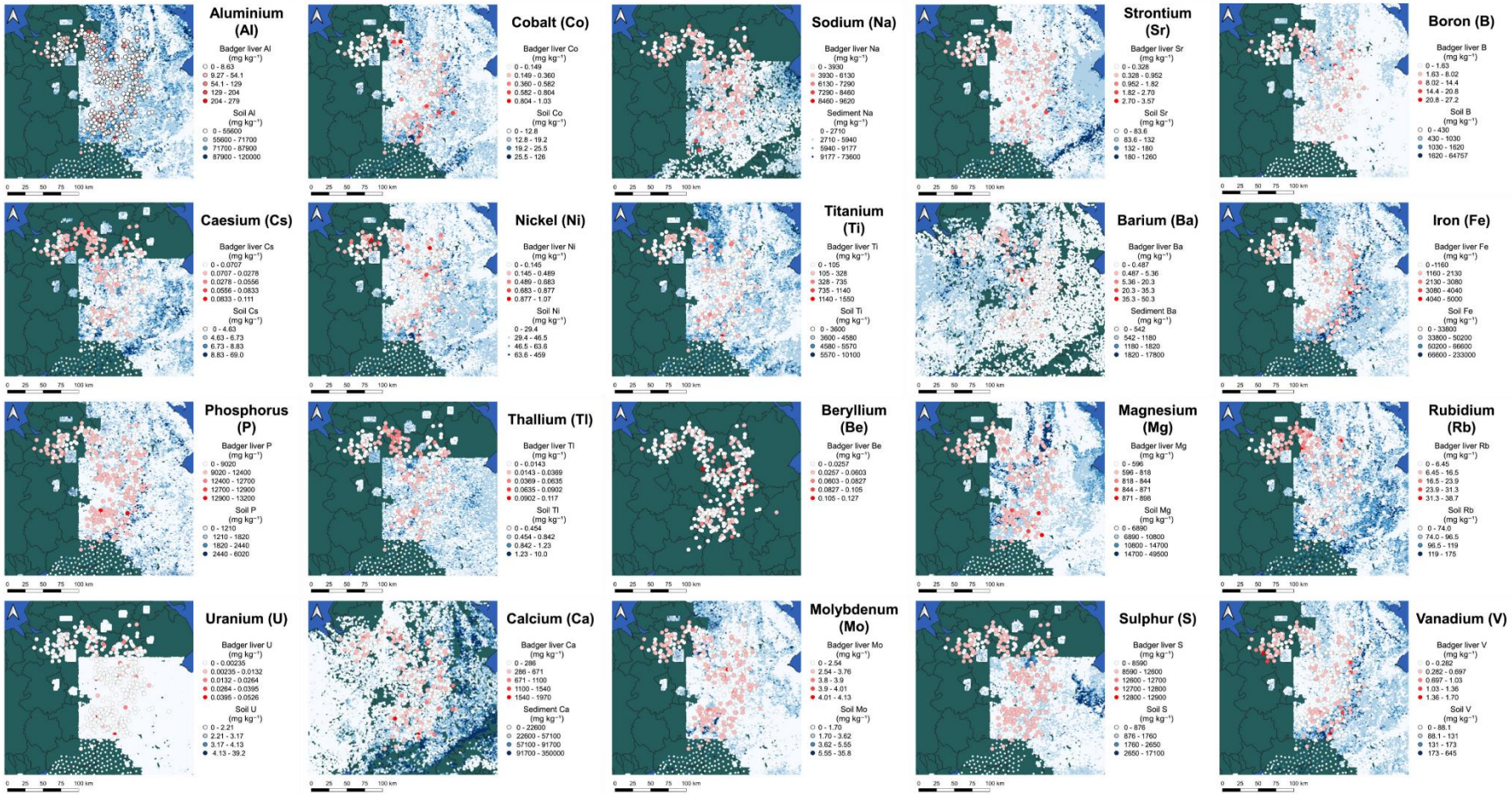
The data that support the findings of this study are openly available in the Nottingham Research Data Management Repository at http://doi.org/10.17639/nott.7237.

Appendices

Appendix A: Supplementary Figures



Supplementary Figure 6.1: Histograms of badger liver concentrations of 20 further elements (Al, B, Ba, Be, Ca, Co, Cs, Fe, Mg, Mo, Na, Ni, P, Rb, S, Sr, Ti, Tl, U, V). The red line represents the mean, while the blue line represents two standard deviations from the mean.



Supplementary Figure 6.2: Maps of badger collection sites, showing badger liver concentration and soil or sediment concentrations of 21 further elements (Al, B, Ba, Be, Ca, Co, Cs, Fe, Li, Mg, Mo, Na, Ni, P, Rb, S, Sr, Ti, Tl, U, V). Soil and sediment concentration are derived from 2,300,000 scale BGS Digital Data under Licence No. 2021/086 British Geological Survey © and Database Right UKRI. All rights reserved. Soil or sediment concentrations are grouped into four classes: 1) 0 – mean, 2) mean – mean + standard deviation, 3) mean + standard deviation – mean + two times the standard deviation, and 4) mean + two times the standard deviation – maximum. Badger liver concentrations are grouped into five classes: 1) 0 – mean, 2) mean – two times the standard deviation, and 3-5) three approximately equal intervals between two times the standard deviation and the maximum concentration of the element found in the livers.

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Chapter 7 – Discussions

7.1 Project Overview

The overarching aim of this project was to assess the transfer of trace metal contamination through ecosystems. Trace metals, natural elements that are present in small quantities in the environment (Rainbow, 2018), are toxic at low concentrations and remain centuries after a pollution event, causing long-lasting, large-scale impacts on ecosystems. However, while metal mining is globally on the rise (Watari et al., 2021), trace metal pollution is often perceived as a solely historical issue, particularly in Europe. Studies that assess trace metal contamination typically focus on individual species, as opposed to ecosystems, and many do not directly link environmental contamination with transfer to animals. This project was therefore developed to take a more holistic approach to trace metal pollution. The two primary research questions of this project were: (1) how are historical trace metal contaminants distributed across landscapes, and (2) how do historical trace metal contaminants transfer through ecosystems, specifically focusing on local wildlife and on potential human health risks. This project primarily focused on the areas in and around two derelict metalliferous mines in Wales, but also examined trace metal transfer into wildlife across the English Midlands, a larger, heterogeneously contaminated landscape.

To address the first research question, environmental trace metal pollution was evaluated across landscapes containing abandoned metal mines. In the studies focused on two abandoned Pb mines in Wales, high concentrations of Pb, Cd, Zn, and Cu were found in water, sediment, and soil at the mine sites and in surrounding areas. In particular, water and sediment concentrations at the mine sites, and up to 4 km downstream, had trace metal concentrations that were indicative of probable health issues in aquatic animals. By contrast, control sites within 3 – 6 km of the mine sites, but not directly downstream or downwind of the mines, had much lower water, sediment, and soil trace metal concentrations. In the larger-scale project across the Midlands, soil elemental concentrations varied greatly, depending on local geography and both past and current anthropogenic pollution (Johnson et al., 2005; Rawlins et al., 2012). Notably, Derbyshire, a county with a long metal mining history (Rainbow, 2018), had high Ag, Cd, Pb, and Zn soil concentrations compared to the rest of the Midlands.

Multiple animal taxa were used to assess the transfer of trace metal pollution into wildlife. At the mine sites and areas downstream, aquatic invertebrates and rodents were

collected to act as biomonitors, organisms that provide a relative measure of environmental pollution, and to indicate the potential for metal accumulation in prey species. Invertebrate communities at the mines were slightly less abundant and diverse than the communities at the control sites, and were missing a few key taxa, but were not notably impacted, despite the high trace metal contamination at the mine sites. However, invertebrates at, and downstream of, the mine sites were found to have significantly higher concentrations of Pb, Cd, Zn, and Cu than invertebrates at the control sites. Moreover, approximately one third of the invertebrates at the mines had accumulated Pb to concentrations higher than those found in the sediment. Like the invertebrates, wood mice from the mine sites and downstream private properties accumulated significantly higher Pb and Cd tissues concentrations than wood mice from the nearby control sites. In particular, Pb concentrations in rodents from the mine sites and downstream areas exceeded thresholds at which adverse health effects (such as renal disease) are possible. However, while non-essential elements, such Pb and Cd, did accumulate in wood mice, the essential metals, Zn and Cu, appeared to be strictly regulated by homeostatic mechanisms, and their concentrations did not vary based on local environmental concentrations.

The positive relationships between environmental contamination and aquatic invertebrate and rodent tissue concentrations indicate that these animals are effective biomonitors of non-essential, and, in the case of invertebrates, essential trace metals. The invertebrates and rodents at these sites had far lower trace metal concentrations than those collected at the mine sites or in areas downstream, despite some minor trace metal contamination in the control sites (particularly adjacent to a path made of a material with high Pb concentrations, likely mine spoil). In fact, the sampled species appeared to be somewhat tolerant of metals at the mine sites. The aquatic invertebrate communities at the mine sites were notably diverse, despite living in waterways so polluted that fish had been completely extirpated. While the ability of the invertebrates to survive in these polluted environments has allowed their communities to endure, this could have knock-on effects on their predators. Although trace metals do not uniformly biomagnify, studies have suggested that high trace metal body burdens in prey species (such as aquatic invertebrates or rodents) can lead to high trace metal body burdens in predators (Cooke, 2011), potentially causing adverse health effects.

Elemental accumulation in wildlife was also measured by studying badgers collected across a larger landscape in the Midlands. As was found in the Welsh rodent studies, environmental concentrations of essential elements (Cr, Cu, K, Mn, Se, and Zn) did not

appear to affect badger liver elemental concentrations, with only one element, Se, having a slight ($\rho = 0.165$) correlation between local soil and liver concentrations. However, non-essential elemental liver concentrations (Ag, As, Cd, and Pb) did correlate with local elemental soil concentrations (Ag: $\rho = 0.309$, As: $\rho = 0.155$, Cd: $\rho = 0.368$, Pb: $\rho = 0.459$), suggesting some level of accumulation linked with environmental contamination. Lead and Cd were particularly elevated in the livers of badgers collected in Derbyshire, correlating with high trace metal concentrations found in soils within the county, associated with past metal mining activities. Badger livers were also found to have Cd concentrations, on average, eight times higher than the concentrations found in local soils, regardless of whether they were collected in Cd-contaminated environments. This may be due to accumulation from their diet, which consists primarily of earthworms (Shepherdson et al., 1990), known Cd accumulators (Spurgeon & Hopkin, 1996), as similarly elevated Cd concentrations have been found in the tissues of other primarily earthworm-consuming mammals (Spurgeon & Hopkin, 1996; Cooke, 2011). This possible relationship between earthworm and badger Cd concentrations provides a key example of how trace metals can accumulate within animals to concentrations higher than found in the environment and then transfer from prey to predator. Understanding animal metal body burdens and food chain dynamics is therefore critical to better assess trace metal impacts in metal-contaminated areas.

A preliminary investigation was conducted exploring potential human health risks from living in a trace metal-contaminated environment. The trace metal content in selected foodstuff intended for human consumption and produced near metalliferous mines was determined and compared to established thresholds. While the Cd, Zn, and Cu concentrations were all generally low and below food safety thresholds, Pb concentrations in these food items could potentially pose a risk to human health. Eggs produced at a private property approximately 1 km downstream from a mine had particularly elevated Pb concentrations when compared to commercially available eggs. Based on the European Food Safety Authority's daily lead consumption thresholds, adults eating between three and seven of these Pb-contaminated eggs would be at risk of developing chronic kidney disease, while a young child regularly eating one to two of these eggs could become cognitively impaired (Table 5.4) (European Food Safety Authority, 2010). Vegetables grown directly downstream of mine sites were also opportunistically collected from landowners' gardens to assess possible routes of trace metal exposure. Previous studies have found that vegetables grown in trace metal contaminated environments can have elevated, or even potentially toxic, trace metal concentrations (Xu et al., 2013; Islam et al., 2014; Khan et al., 2018). While it was not

possible to collect enough vegetables to definitively report overall trace metal concentrations, the amount of Pb in the sampled vegetables could potentially pose health risks to humans, and particularly children, if consumed (Supplementary Table 5.1).

7.2 Limitations to the Study

7.2.1 Overall Study Limitations

The greatest limitation in this project was the number of mines and surrounding areas surveyed for the studies detailed in Chapters 3 – 5. While the studies were thorough, and the findings were supported by prior research, particularly research focusing on west Wales, surveying further mine sites would have allowed for more powerful comparisons and would have ensured that the results found were consistent across metalliferous mines in the sampled area. Similarly, larger numbers of all sampled items (environmental, animal, and human food product samples) would have allowed for more rigorous statistical comparisons, particularly across multiple factors or when examining subsets of datasets. For example, in Chapter 4, comparisons in aquatic invertebrate body burdens across key traits were restricted due to low numbers of invertebrates in each trait class. Low samples numbers were particularly of concern with samples that were only able to be collected opportunistically, either due to their limited nature (i.e. deceased domestic animals) or to specific landowner requests. Further collection of human food items, including eggs from sites beyond Private Property 2, and more vegetables from both sampled farms, would have allowed for a more rigorous assessment of Pb in human food items. Lastly, another key limitation in this project was the lack of in-depth exploration into the health effects of trace metal exposure. This work was originally planned as part of the project, but was not able to be completed due to the COVID-19 pandemic, as discussed in the following section.

7.2.2 Impacts of the COVID-19 Pandemic

The COVID-19 pandemic had a profound impact on all aspects of this project. Firstly, all further field visits beyond 2019 had to either be cancelled or greatly reduced in scope. Originally, further rodent samples were scheduled to be collected in spring and autumn 2020, to directly compare with the rodent samples from spring and autumn 2019, but this was not possible. There were also plans to collect aquatic invertebrate samples during spring and autumn 2020; these sampling trips had to be postponed to autumn 2020 and spring 2021, and modified to day trips as opposed to week-long visits, reducing the number of sites that could

be sampled, and the time spent at each site. Otherwise, plans to investigate trace metal contamination as a function of soil depth and to sample aerial trace metal deposition at the private properties had to be cancelled due to the reduction in frequency and duration of field visits.

The COVID-19 pandemic also significantly limited laboratory access from early 2020 to the middle of 2021, and restricted group working. This reduced the ability to do routine sample processing, especially with volunteers, as originally intended, and greatly hampered any further planned laboratory work. In particular, the health effects of trace metal exposure were going to be evaluated using samples collected from rodents in 2019. This was planned as a multi-level health assessment, examining splenic immune function markers, histological abnormalities in kidney, liver, and gonad samples, and gut microbiome communities across the contamination gradient. However, the lack of laboratory access for most of 2020, and the social distancing regulations restricting in-laboratory training significantly limited the possibility to undertake these analyses during the pandemic, preventing their inclusion in this project.

Another effect of the COVID-19 pandemic was that it greatly hampered collaborations with other researchers. While there were a considerable number of collaborators involved in this project, focusing on various offshoot ideas, from examining rodent viral load to assessing soil microbiome communities, the pandemic set back their work and made it harder to directly coordinate with collaborators. While collaboration projects have now restarted, the pandemic set these projects back by at least two years, making it impossible to complete them before the end of the PhD period.

7.3 Future Work

This project highlighted the need for ecosystem-wide assessments of trace metal pollution, both through in-depth studies of metal-polluted sites, and through studies focusing more broadly on larger, heterogeneously polluted landscapes. In particular, trace metal pollution needs to be more routinely assessed across multiple ecosystem components, and the spatial coverage of these assessments needs to be more exhaustive. Current surveys of trace metal pollution focus primarily on water, despite water routinely containing lower trace metal concentrations than sediments, as seen in the metal-contaminated streams sampled during this study (Table 3.1 – 3.2). The coverage of sediment and soil elemental samples within the UK is uneven, generally favouring urban and semi-urban areas within the south-west, and varying greatly across elements. Furthermore, as shown in this study, local fauna can take up high

concentrations of trace metals, yet their elemental concentrations are only rarely surveyed. Further studies, whether examining specific polluted sites or larger landscapes, must therefore take a more holistic approach to assessing trace metal pollution by sampling across ecosystems, including multiple environmental and animal components. Future studies focused on polluted sites could examine the spatial extent of trace metal pollutants in both the environment and local wildlife, or they could investigate the trophic transference of trace metals by sampling from predatory species, such as bats, owls, or raptors. Future studies evaluating trace metal pollutants across larger landscapes could focus on increasing the spatial coverage of samples across the whole of an area, and could further examine multiple animals to compare elemental accumulation patterns across species.

Other future work could focus on assessing the health effects experienced by animals living in trace metal contaminated areas. Trace metals can have negative health effects across almost every organ system, so health measures can be explored across a variety of systems. In particular, rodent health effects could be explored throughout the body using a variety of tissues. Testes could be examined for histological abnormalities, including chemically induced lesions (abnormally large and dense residual bodies) (Creasy, 2012) and calcification (large Ca deposits), which is indicative of Cd exposure and has been linked to testicular tubular obliteration, germ cell calcification, and death (do Carmo Cupertino et al., 2017; Shojaeepour et al., 2021). Kidneys could be examined for lead inclusion bodies, characteristic histologic lesions associated with Pb exposure and toxicity (Ceruti et al., 2002). While these lesions are rarely seen in wild rodents, likely due to their short lifespans not providing enough time for the lesions to develop, they have been detected before in rats living in urban areas (Ceruti et al., 2002). Other studies could compare gut microbiome communities across trace metal contaminated gradients using rodent faecal samples. Exposure to trace metals in diet or soil has been linked to co-selection of antimicrobial resistance genes (Yazdankhah et al., 2014; Yazdankhah et al., 2018), so it is hypothesized that the rodents living in contaminated areas will have an elevated presence of antimicrobial resistance genes in the microbes present in their gastrointestinal tract. Rodent spleens could also be used to evaluate rodent immune function by assessing gene expression of immune function markers from splenic RNA (Kasten-Jolly et al., 2010). Previous studies in laboratory mice found that Pb exposure increased apoptosis, B-cell differentiation, and Th2 development (Kasten-Jolly et al., 2010), so it is expected that rodents from contaminated areas could exhibit similar changes in immune function. Immune function in rodents could also be assessed more indirectly by determining the viral load in rodent liver and GI tract tissues. Due to the immunosuppressive

nature of trace metals (Lehmann et al., 2011), it is suspected that rodents from contaminated areas would have, on average, a higher viral load than rodents from uncontaminated areas. Together, the results from these analyses would indicate some of the effects animals exposed to high concentrations of trace metals within their environment could experience. This would greatly aid in our understanding of the continuing effects of these legacy pollutants on animal, and, potentially, human health.

Lastly, the findings of this project can be used to identify and reduce risks for people and domestic animals living in metal contaminated environments. Remediation efforts are necessary to help reduce current metal pollution and mitigate further pollution events. Natural Resources Wales are currently working on reducing metal pollution from mines, including some remediation work done at Mine 1a (Natural Resources Wales, 2016), and are planning to continue this work at other mine sites. In the UK, remediation efforts are primarily focused on containing trace metals within the mine sites and limiting their spread through strategies such as capping spoil heaps with clay or diverting waterways so they no longer flow directly through mine sites (Natural Resources Wales, 2013). While these efforts are necessary, as they can reduce future pollution events, this current study has shown that pollution from historical mines can have already spread into the surrounding environment at high, potentially toxic concentrations. As such, to fully tackle trace metal pollution, remediation efforts must be implemented beyond the origin point of pollutants, and particular focus should be paid to areas downstream of mines. In addition, local residents must be better informed of the potential risks of living near abandoned metalliferous mines, and how they can mitigate these risks. The landowners who participated in this study were mostly unaware of the potential impacts of trace metal pollution, and struggled to find resources providing concrete advice on reducing their exposure risk while living near metal mines. Simple actions, such as providing animal feed in troughs, growing vegetables in raised beds, and avoiding tracking outdoor soil or dust into homes, can potentially reduce trace metal exposure in domestic animals and humans. Despite the simplicity of these measures, there is little publicly available advice on living in trace metal contaminated areas, and most public-facing information primarily focuses on acute exposure, or on the risks faced when living in urban areas. There is a clear need for easily accessible information about potential risks and mitigation strategies for people living in historically metal-contaminated areas, as well as outreach programmes to directly inform and aid affected people.

7.4 Conclusions

The findings of this project indicate that trace metal pollution can have ecosystem-wide effects. Trace metals are not localized to their point of origin, and they can transfer into a wide range of local wildlife, and, potentially, into humans through foodstuff. These effects can be observed both at a fine scale, focusing on a particular mine site and its surroundings, and at a larger scale, across landscapes with a heterogenous distribution of historical pollution. While the general public in Europe considers trace metal pollution to be a largely historical issue, these 'past' pollutants will continue to be present in local ecosystems, and impact wildlife, domestic animal, and human health. This presence will only continue in the future, as more frequent flooding events, resulting from climate change, will mobilize and redistribute trace metals, further contaminating landscapes (Foulds, 2014). Almost all metal mines in the UK closed down before there were any legal requirements for remediation (Coal Authority, 2016), resulting in a legacy of metal pollution outlasting the industry from which it stemmed, and with no identifiable parties responsible for remediation efforts. Globally, the demand for metals only continues to grow (Watari et al., 2021), and metal mining is currently common across Africa, the Americas, Asia, and Oceania. Studies on the effects of these legacy pollutants on the environment, and on wildlife, domestic animal, and human health can serve as cautionary tales about the current and potential future impacts of these working mines. Findings from these studies can be used to inform mine site mitigation and remediation plans, in the hopes that future mine closures may not leave such a devastating and ongoing impact on surrounding ecosystems.

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