The spatial distribution of total and available heavy metals at areas of historic mining activity in the Peak District and their influence on local vegetation communities

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Declaration

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Declaration Statement of Originality

I declare that, with the exception of any statements to the contrary, the contents of this final year project report are my own work. I declare that data and the solutions presented have been obtained by my own investigation and experimentation and that no part of it has been copied from previous reports/dissertations, books, manuscripts, research papers or the internet. I certify that all material in this project report, which is not my own work, has been identified and properly acknowledged. I certify that it does not contain any material for which a degree had been previously confirmed upon me or anyone else.

I certify that the number of words in this project report does not exceed the maximum of 40,000, excluding appendices. I understand that over length submission without signed authorisation by my final year project supervisor and/or the module leader will be penalized and reported to the Final Year Project and Dissertations Board and the Examinations Board.

Name in block capitals: PAUL DAVID PRESTON

Signed:____

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Glossary

Accumulators -- A plant that is able to accumulate certain elements in their above ground tissues

Bioavailability -- The extent to which elements (such as contaminants) can be absorbed by organisms.

Bioavailable -- The amount of an element or compound that is accessible to an organism for uptake or adsorption across its cellular membrane

Braun-Blanquet Scale -- A method for the description and recording of vegetation communities using six measures of cover/abundance

Calaminarian – Habitats that exist on substrates characterised by elevated levels of heavy metals, such as lead, zinc, and other unusual elements

Calcareous -- A material that contains or consists of calcium carbonate or a species that accumulates calcium carbonate in its tissues

Calcicolous – A species or organism that is limited to or favours habitats rich in calcium carbonate

CCA (Canonical Correspondence Analysis) -- A statistical method for determining the relationships between groups of species and their environments

Cluster Analysis -- A method for identifying variables that are closely associated and arranging them into clusters. Characteristics are determined for each of several objects, such as plant species, and then formed into clusters of those with similar traits and represented on a dendrogram.

Contamination (Environmental Contamination) -- The elevation in the background concentration of a chemical, micro- organism, or radionuclide

Cover/abundance -- A linked scheme for estimating cover visually. It is based on percentages at the top end, but uses abundance estimates for species with low-cover values

DOMIN -- A system for describing the cover/abundance of a species in a vegetation community

Ecotype – A distinct type or species of an organism inhabiting a specific habitat

Excluders – Plant species that exist on metalliferous soils and maintain shoot-metal concentrations at low levels up to a critical point, above which relatively unobstructed transport of metals from root to aerial tissues results

Galena – An ore mineral, lead sulfide (PbS) which is the principal ore of lead

Gangue materials -- Surplus minerals, rock and other waste removed during ore extraction

Hyperaccumulator -- A plant species capable of thriving on soils with excessive concentrations of heavy metals. Certain metals are absorbed through the roots, and accumulated to exceptionally high concentrations in their tissues

ICP-OES (Inductively Coupled Plasma-Optical Emission Spectroscopy) – An analytic technique for identifying and quantifying elements

Indicators – A plant species that can regulate the uptake and transport of metals to the shoot tissues so that plant tissue concentrations mirrors soil concentrations up to a critical point where toxicity can occur

Kriging -- A statistical technique for interpolating between known points spatially. The process was first established for detecting elevated concentrations of metals and minerals for the mining industry

LOI (Loss on Ignition) – An analytic technique for measuring the organic content of soils or sediments. Heating the material to an elevated temperature allows the calculation of the percentage of weight lost after ignition.

Metallophyte -- A plant species that can tolerate high levels of heavy metals

PCA (Principal Component Analysis) -- A multivariate statistical analysis method which amplifies the spread of data by plotting covariance values in a multidimensional or two-dimensional plot allowing the identification of correlations that may have been unseen in the original analysis of data

Pseudo-metallophyte -- A plant species that can thrive in environments with elevated soil heavy metals which can also exist under normal soil conditions

Rake -- Principal ore veins

SOM (Soil Organic Matter) -- The organic matter component of soil, consisting of plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by soil organisms

Sphalerite -- A mineral ore and a primary source of zinc, zinc sulphide (ZnS) also contains quantities of cadmium, iron, and manganese

Xrf (X-Ray Fluorescence) -- A non- destructive method for determining the elemental composition of natural and man- made materials

Abstract

The Peak District in the UK has seen metal ore extraction and other mining activities take place for hundreds of years. As a result, many areas contain elevated soil concentrations of several heavy metals, which can be toxic to many plant species. However, these conditions have favoured several metallophyte plant species which have adapted to the metalliferous soil conditions by developing physiological mechanisms that allow them to tolerate heavy metal toxicity. This research focuses on the spatial distribution of total and available heavy metals and other abiotic variables in relation to the distribution of vegetation species at seven areas of historical mining activity in the Southern Peak District. This study is important as many of these sites have been designated protected areas due to their historical, archaeological, geological or ecological importance, and increased knowledge of these habitats and the many rare and interesting species that inhabit them may identify priority areas for conservation or influence management strategies. The research approach adopted saw the collection of 155 soil samples and the recording of vegetation at 127 points within seven sites. x-ray fluorescence technology was used to evaluate the total heavy metal concentrations, whilst the bioavailable fraction was determined with ICP-OES after a single extraction procedure using $CaCl_2$. Canonical correspondence analysis and principal component analysis were applied to assess relationships between vegetation and soil variables, along with geospatial analysis, using kriging, to allow visualisation of spatial distribution patterns. The findings from this research indicate that several plant species were closely associated with Pb, Zn and Cd and that several similarities in spatial distribution of the metals, vegetation cover and onsite landscape modifications and structures exist. The main conclusions are that landscape modifications and other structures present at the study areas influence abiotic variables, and thus, the vegetation communities that exist on them, demonstrating that multivariate and geostatistical methods are useful for the assessment, characterisation, and monitoring of ecological and mineralogical aspects of historical mining areas.

Chapter 1: Introduction

1.1 Background

The natural geology of the White Peak area of the Peak District, Derbyshire, consists primarily of Carboniferous Limestone, within which major ore fields exist. The ore fields are abundant in metalliferous ores and other minerals which have been mined for hundreds and sometimes thousands of years (Ford & Rieuwerts, 2000). The extraction of these mineral ores left behind areas containing metal rich spoil heaps and other structures, which are a source of elevated levels of lead (Pb), zinc (Zn), cadmium (Cd) and arsenic (As), as well as a variety of other heavy metals and minerals (Baker & Proctor, 1990; Shimwell & Laurie, 1972). The waste materials left behind by the mining activity have eroded over time and contaminated the soils in their proximity (Kossoff, Hudson-Edwards, Howard, & Knight, 2016). Concentrations of Pb and Zn reaching more than 70,000ppm and 40,000ppm respectively have been recorded at some mining areas in the Southern Peak District (Johnson & Bradshaw, 1977; Morrey, Baker, & Cooke, 1988); these are concentrations exceeding those from uncontaminated soils by one hundred times or more (Broadley, White, Hammond, Zelko, & Lux, 2007; Ross, Wood, Copplestone, Warriner, & Crook, 2007). The foremost metalliferous ore in the ore field of the Peak District is Galena (PbS), and this is often found in conjunction with other minerals including the Zn and Cd containing mineral ore Sphalerite (ZnS) as well as, Calcite ($CaCo_3$), Fluorite (CaF_2) and Baryte ($BaSO_4$); these are often termed gangue materials (Ford & Rieuwerts, 2000). These mineral ores are found in rich mineral veins called rakes that form a network which cut across the landscape, sometimes for several kilometres, intersecting with others along their course (Barnatt & Penny, 2004; Ford & Rieuwerts, 2000). To access the much sought-after metals, miners would sink shafts along the lengths of the rakes, leaving waste heaps and hollows as they went or, less commonly, sometimes mine horizontally into smaller veins exposed between strata of limestone in the naturally formed steep-sided dales of Derbyshire (Barnatt & Penny, 2004).

The original extraction and processing of metal ores and the subsequent reworking of the waste heaps containing gangue materials in some areas has left a radically changed landscape and heavily contaminated soils (Penny, 2009), toxic to many plant species (Baker, Ernst, van der Ent, Malaisse, & Ginocchio, 2010). However, over time, the metalliferous spoils and mines have seen specialised, metal tolerant plant species colonise them (Baker *et al.*, 2010; Baker & Proctor, 1990; Shimwell & Laurie, 1972). These unique plants are called metallophytes, and they are able to tolerate toxic levels of heavy metals and other elements, thriving in these contaminated environments without successional pressures from other less tolerant species (Baker *et al.*, 2010; Woch, Kapusta, & Stefanowicz, 2016). Many of these historic mining areas now support a mosaic of grassland vegetation communities, often with calcareous, neutral, calaminarian and acid grassland in close proximity to each other. The calaminarian or metal tolerant communities are often surrounded by these other plant communities, unable to encroach onto the more contaminated areas (Penny, 2009; Woch *et al.*, 2016). Many of these locations, containing rare and internationally important metalliferous vegetation and calaminarian grassland communities, have been designated as protected areas (Barnatt & Penny, 2004). Even with protected status, some of these ecologically, archaeologically and historically important sites are under threat from several sources, including reclamation for agriculture by levelling out or infilling the hillocks and hollows, overgrazing, management decisions, reworking of gangue materials and recreational damage (Penny, 2009; Rodwell, Morgan, Jefferson, & Moss, 2007).

Batty (2005) asserts that a better understanding of the associations between ecological communities and metal enriched areas may enable the improvement and conservation of biodiversity at historic mining sites, while also developing an understanding of how these species can be utilised for biotechnological purposes.

Thus, these areas of historic mining activity which contain rare and interesting plant communities within the Peak District provide a suitable research area for investigating the relationships and spatial distribution of vegetation, heavy metals and other soil properties.

1.2 Study Aim

The aim of this dissertation is to assess the distribution of metal/metalloid contaminants and their bioavailable fractions at sites of historical mining activity situated on limestone geology in the Peak District and to determine their influence on the distribution of calcicolous flora.

This will highlight the spatial distribution of heavy metal contamination at these sites and allow the observation of variables in calcicolous vegetation that could be associated with the contaminated soil and parent substrates. Seven study sites are assessed which contain evidence of several historical mining activities and structures.

1.3 Study Objectives

To fulfil the aim, the following objectives were investigated.

The objectives of this study were:

1. To assess the soil characteristics and calcicolous vegetation at sites of historical mining activity.
2. To analyse the relationship between the soil properties and vegetation species richness, abundance and composition at the sites.
3. To determine the bioavailable fraction of the heavy metals and metalloids in soil samples taken from the sites.
4. To measure the distribution of any heavy metals and other elements found in topsoil samples and to evaluate their influence on vegetation structure.
5. To generate an understanding of any issues concerning the distribution of any soil contamination that may cause harm to or decline in flora and fauna at the study sites and to suggest any remediation procedures.

1.4 Research focus and methods

The areas of study used in this research comprise several sites of historical mining activity. The sites are situated on natural limestone geology and have a long history of mining activity dating back to the Roman era (Barnatt *et al.*, 2013; Barnatt & Penny, 2004). Seven sites that both have evidence of historic mining activity as well as calcicolous vegetation have been selected for this study (Ordnance Survey, 2016).

The study sites are all located in the Southern Peak District and consist of:

- Hay Dale (calcite mine)
- Tansley Dale (disused structure)
- Deep Dale (perceived test pit)
- Dirtlow Rake (opencast mining and grilled shaft)
- Hard Rake (extensive hillocks and hollows)
- Gang Mine (extensive hillocks and hollows)
- High Rake (Mining structures on large ore vein)

A major focus of this research is to examine the patterns of spatial distribution of several heavy metals and other soil properties around these study sites, especially in relation to landscape features and other structures present. Additionally, relationships between individual soil variables are explored, particularly the total and bioavailable fraction of heavy metals and other abiotic properties.

To achieve these goals, soil samples were taken from points placed at intervals of approximately four meters along three transects, originating at the perceived source of mining activity or related structure. Soil samples were meticulously prepared and stored in the laboratory before several analytical procedures took place. X-ray fluorescence analysis was utilised to determine the total heavy metals and other elemental content of soil samples (Thermo Scientific, 2010). A single extraction procedure was used to isolate the bioavailable fraction of heavy metals before analysis with inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Houba, Novozamsky, Huybregts, & Van Der Lee, 1986). The percentage of soil organic matter content was gravimetrically determined by the loss-on-ignition (LOI) procedure (British Standards Institution, 2007), before particle size analysis was undertaken using the resulting ashed samples (Toongsuwan, Chang, Li, Stephens, & Plichta-Mahmoud, 2000). The pH of the samples was determined using the 1:5 soil:water ratio method, recommended by Rayment, Sadler, Craig, Noller, and Chiswell (2003).

Emphasis is also placed on how the abiotic variables relate to the distribution of vegetation species abundance and richness on a relatively small scale. The exploration of vegetation and soil variable relationships is undertaken using several methods of statistical analysis to identify any close associations between plant species and abiotic variables. To realise this focal point, vegetation surveys to determine species abundance, richness and composition of the grasslands were undertaken; it was thought practical to undertake research in August, when many species found in these habitats are in flower, to ease the identification of plant species. The recordings for vegetation were taken using a $1m^2$ quadrat from the same sample points as the soil samples, to ensure that any associations between vegetation and soil characteristics could be identified.

The data presented in this thesis, collected as a result of this field work and laboratory analysis, was analysed using several statistical software packages.

- SPSS statistical software was used to generate descriptive statistics for all abiotic variables, as well as principal component analysis and Spearman's rank order correlation which were used for determining the strength of relationships between soil variables.
- EXCELLSTAT statistical software was used to undertake multivariate analysis, to demonstrate associations between vegetation and soil variables by applying canonical correspondence analysis.

- ArcGIS Geostatistical package was used to produce isopleth maps, allowing visual analysis and comparisons of vegetation and soil variable distribution patterns.

Much of the research into the associations between heavy metal contamination and vegetation has focused on metallophyte plant species and their mechanisms for metal tolerance and accumulation at mining areas (Ent, Baker, Reeves, Pollard, & Schat, 2013; Johnson, McNeilly, & Putwain, 1977; Kapusta, Szarek-Lukaszewska, & Stefanowicz, 2011; Morrey *et al.*, 1988; Shimwell & Laurie, 1972). The ecological aspects of these historic mining areas have also been well explored (Baker *et al.*, 2010; Batty, 2005; Bizoux, Brevers, Meerts, Graitson, & Mahy, 2004; Penny, 2009). Much research on the spatial distribution of heavy metals at historic mining areas has been undertaken, including studies by Acosta *et al.* (2011); Alibrahim, Williams, and Roberts (2017); Holmgren, Meyer, Chaney, and Daniels (1993); Huang, Xie, Cao, Cai, and Zhang (2014); Rodriguez Martin, Arias, and Grau Corbi (2006); Teng *et al.* (2015). The study of the spatial variation of both vegetation and heavy metals in areas of historic mining in Europe has also taken place (Morrey *et al.*, 1988; Rodríguez, Ruiz, Alonso-Azcárate, & Rincón, 2009; Stefanowicz, Woch, & Kapusta, 2014; Woch *et al.*, 2016). Comprehensive studies of the historical mining areas, processes and conservation in the Peak District are comprehensively covered by Barnatt *et al.* (2013); Barnatt and Penny (2004); Ford and Rieuwerts (2000); Kossoff *et al.* (2016) and others. However, negligible literature for the geospatial distribution of both metals and vegetation could be identified for the metal enriched mining areas of the Peak District.

1.5 Value of this research

This research contributes to the knowledge of these important historically and ecologically rich areas in the Peak District. The investigation of these niche vegetation communities and their metalliferous habitats will provide insightful information about how they amalgamate and how their patterns of distribution relate to the environmental factors that exist there.

This investigation of sites of historical mining activity is also an important project, as it may highlight any hot-spots of contamination at the sites that could potentially be harmful or toxic to grazing livestock through possible contaminant transfer to plants and the subsequent steps in the food chain.

Chapter 2: Literature review

2.1 Introduction

This literature review examines key themes in relation to the aim and objectives of the research and focus on the contaminants and vegetation at sites of historical mining activity situated on limestone geology in the Peak District. The study within this review mainly addresses objectives one and two below, whilst objectives three and four are accomplished during the collection and analysis of empirical data. Objective five will be met as a result of the findings from objectives one through four.

1. To assess the soil characteristics and calcicolous vegetation at sites of historical mining activity.
2. To analyse the relationship between the soil properties and vegetation species richness and abundance at the sites.
3. To determine the bioavailable fraction of the heavy metals and metalloids in soil samples taken from the sites.
4. To measure the distribution of any heavy metals and other elements found in topsoil samples and to evaluate their influence on vegetation structure.
5. To generate an understanding of any issues concerning the distribution of any soil contamination that may cause harm to or decline in flora and fauna at the study sites and to suggest any remediation procedures.

The literature encountered during this review forms a significant contribution to the research. The geological structure of the study area and the formation of minerals within are first examined. Next will be the examination of how human activity and interactions with these natural features have influenced and contributed to the development of the conditions and habitats which now exist at many areas of historic mining and mineral extraction in the Peak District. Finally, the availability of the resultant heavy metals into the environments at these sites and their interactions with flora and fauna that inhabit these areas will be explored.

This will help to facilitate a better understanding of the interactions between the vegetation and soil properties that have developed at these unique landscapes.

2.2 Geology and mineral formation

The UK comprises an extremely varied geological assembly contained within a relatively small area and this represents a complicated history of geological events taking place over a vast time period, encompassing many of the major geological eras, including the Cretaceous, Jurassic, Triassic and Carboniferous [*Fig.* 2:1] (British Geological Survey, 2017).

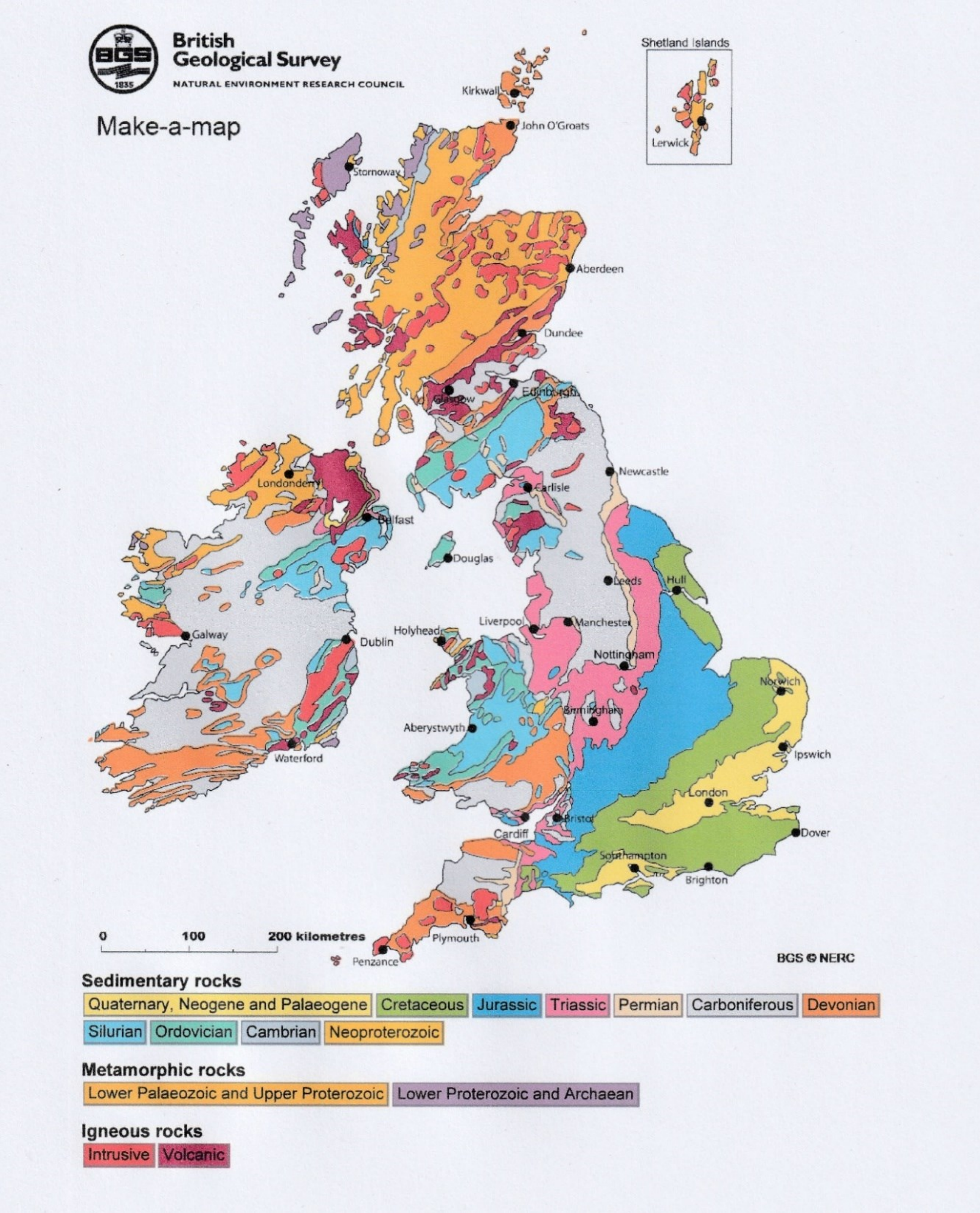


Figure 2:1. The Geology of the UK. Source (British Geological Survey, 2017) 'Based upon [Make-a-map of the geology of the British Isles], with the permission of the British Geological Survey' 'Reproduced with the permission of the British Geological Survey ©NERC. All rights Reserved'

This research is particularly focused on areas that overlie the sedimentary bedrock geology deposited in the carboniferous era and located in the central area of the UK. This area contains the Pennine Hills and the Peak District which extend from Derbyshire in the south to Yorkshire in the north (Aitkenhead, 2002). The bedrock in the Peak District and the Pennines was formed by sedimentary deposits and these can be divided into several distinct strata. The upper strata comprises shales, mudstone and sandstone, examples of which contain, Millstone Grit, Shale Grit and Kinderscout Grit, amongst others; these strata is present beneath much of the Pennines and the Dark Peak area of the Peak District (Wolverson Cope, 1998). The lower lying bedrock strata consists of limestone, generally referred to as Monsal Dale Limestone Formation, Bee Low Limestone Formation and Eyam Limestone Formation in the study area, and these strata are present at the surface in the White Peak area of the Peak District in Derbyshire and parts of the Yorkshire Dales (Aitkenhead, 2002; Wolverson Cope, 1998). These strata were formed when Britain was situated near to the equator, 310-330 million years ago. At this time a shallow, tropical sea was present at the location where the modern-day Peak District exists and this sea was inhabited by an array of shellfish species that existed during this period (Barnatt & Penny, 2004; Ford & Rieuwerts, 2000). The demise of these creatures deposited layers of shell debris onto the seabed, which became hardened and compacted with time to eventually form the organic sedimentary rock limestone (Barnatt & Penny, 2004; Park, 2001). Limestone is predominately composed of calcium carbonate ($CaCO_3$) and can often be of a very high purity, frequently containing more than 95% $CaCO_3$ (Highley & Cameron, 1995; Trudgill, 1985). Other minerals complete the composition of the limestone, including calcite, baryte and fluorite (sometimes referred to as gangue minerals) and other metallic ores (Harrison & Adlam, 1985). These minerals were formed when hydrothermal deposits were precipitated in fissures and faults contained within the limestone with hot mineralising fluids of around 100-150°C, and over time chemical and physical processes forced out any liquids leading to a deposition of minerals (British Geological Survey, 2017; Montgomery, 2003). The chemical elements within these fluids originated in deeper rock formations and created the mineral assemblage during reactions of the liquid to mixing, cooling, heating and also reactions with sulphur compounds and oxygen (Ford & Rieuwerts, 2000).

In Derbyshire, this mineralisation has formed many mineral veins and rakes along vertical faults and near-horizontal veins along bedding planes in the limestone geology. These veins contain lead and zinc sulphide ores, galena (PbS) and sphalerite (ZnS) alongside the calcite, baryte and fluorite (Harrison & Adlam, 1985). In fact, the three gangue minerals together with PbS and ZnS comprise the greater part of the mineral content in more than 2000 mineral veins in the Peak District, although more than 100 trace minerals exist alongside them (Ford & Rieuwerts, 2000). Of these principal minerals, it is the lead and zinc ores that have been exploited for hundreds of years, with lead being extracted since the Roman era (Barnatt & Penny, 2004; Ford & Rieuwerts, 2000). However, more recently, the gangue minerals calcite, baryte and fluorite, often disposed of as waste in the past, have been reworked to extract minerals for use in the chemical and construction industries (Barnatt & Penny, 2004; Ford & Rieuwerts, 2000; Harrison & Adlam, 1985). The following section discusses the extraction methods of these and other minerals detected at the study sites in more detail.

2.3 Mining and extraction of lead and other minerals at the study areas

To understand how these minerals and their extraction have formed the landscape in the study area, it is important to be familiar with the types of mineral deposits and veins and how these common minerals are extracted from them (Ford & Rieuwerts, 2000). The most common minerals are found in a network of various types of mineral veins across the Peak District (Ford & Rieuwerts, 2000) and include:

- Galena (lead sulphide, PbS)
- Sphalerite (zinc sulphide, ZnS)
- Fluorite (calcium fluoride, CaF_2)
- Baryte (barium sulphate, $BaSO_4$)
- Calcite (calcium carbonate, $CaCO_3$)

Mineral information: (Hudson Institute of Mineralogy, 2017)

Many of the applications that made galena, or the resulting lead, so desirable in the past are no longer in use, such as the production of paints and pigments, ammunition, water and plumbing pipes and roofing supplies. Today, although not as widespread, it still has some important uses; it is used for producing leaded fuel, alloys, batteries and as protection against radiation (Ford & Rieuwerts, 2000). The zinc obtained from sphalerite is used for producing alloys and as a zinc coating to corrosive metals, for instance (Montgomery, 2003). Sphalerite, however, can contain some toxic elements such as mercury (Hg) and cadmium (Cd). The other main minerals extracted along with galena and sphalerite were once thought useless; however, these now have several uses and many mine tailings have been reworked to retrieve them (Ford & Rieuwerts, 2000).

Fluorite has many uses in the chemical industry, and other applications include use in anaesthetics, toothpaste, non-stick coatings, and refrigeration and for the fluorination of water supplies. Baryte is mainly used in the oil and chemical industries and in the manufacture of paints, and calcite is utilised mainly in the construction industry (Barnatt & Penny, 2004).

There are four main types of vein where these sought-after minerals are found. There are pipes, flats, scrins and rakes (Barnatt & Penny, 2004). As well as these principal types of vein there are also replacement ore bodies where areas of limestone have irregularly been replaced by mineral deposits, sometimes collapsing, leaving a mixture of limestone rocks and lumps of mineral ore. Areas of gravel ore are also another feature where mineral ores are found, around caves or hollows on the surface of the landscape (Ford & Rieuwerts, 2000). Of the main types of mineral deposits, flats are formed as almost horizontal deposits in between the strata of sedimentary limestone. Pipes form where cavities in the limestone have been mineralised and these can sometimes intrude into the surrounding limestone and replace it, leaving irregular pipe-shaped ore deposits (Barnatt & Penny, 2004).

It is rakes, however, which are the main type of mineral vein, and these provide large deposits of mineral ore. Rakes are vertical fissures that can be several kilometres long (Highley & Cameron, 1995) and often up to fifteen metres wide and 200 metres deep in places (none have been mined to their full depth) (Ford & Rieuwerts, 2000). To mine the mineral ore from the rakes, miners sank shafts into the rakes at regular intervals and then horizontally along the length of the vein (Ford & Rieuwerts, 2000) and this left waste heaps running along the course of the rake, sometimes for great distances (Barnatt & Penny, 2004). Often these shafts were quite shallow due to problems with the drainage of water from the shaft. Scrins are smaller veins (Hunter & Shaw, 2011), usually no more than half a metre wide and less than one kilometre in length, which can sometimes off-shoot from the major rakes in groups (sometimes called swarms) (Ford & Rieuwerts, 2000). The changes in the landscape owing to the extraction of minerals from these types of mineral vein take three main forms; underground and opencast extraction, which leave behind shafts or open pits, or the removal of surface deposits which leaves behind hillocks and hollows (Barnatt & Penny, 2004), sometimes called bell pits (Ford & Rieuwerts, 2000).

It is the latter type of landscape that is found at study sites Hard Rake (Ford, 2002) and Gang Mine (Ford, 2005). Penny (2005) describes this type of landscape as having hillocks and hollows with varying slope and aspect, primarily composed of the waste minerals disposed of whilst mining the more historically expensive metal ores, with a composition of limestone, calcite, baryte and fluorspar. The study area at Deep Dale also has a hollow, although this is possibly a test pit dug with the aim of following the vein from the top down into the dale itself. Robey (1966) describes the desperate search for galena at Field Grove Mine, directly above the Deep Dale sampling area, in order to capitalise on the mine in the 19th century. The landscape at Dirtlow Rake, on the other hand, has undergone vertical opencast excavations along it that date back to the early 17th century. This has left a large open ravine and deep shafts in the landscape (Ford & Rieuwerts, 2000). The present topography at High Rake mine exists on a very large mineral vein up to twelve metres wide (Rieuwerts, 2011), which has a long history of lead mining dating back to the 16th century (Historic England, 2017). This lead mining has left the ruins of several structures associated with the mine that existed here, including ore processing buildings, chimneys and an ore crushing circle. Large mounds and hillocks also exist here a short distance from the ruins, although these are present due to the more recent re-working of the original waste tips for calcite, baryte and fluorspar (Barnatt, 2011; Historic England, 2017) and also due to extensive archaeological excavations and conservation work which took place between the years 2000 and 2008 (Rieuwerts, 2011). Hay Dale contains a small calcite mine which has been worked from an adit, a near horizontal tunnel, on the side of the dale (AditNow Mining History Society (ANMHS), 2017). Adits are sometimes used as a sough to drain a mine set at a higher elevation (Ford & Rieuwerts, 2000). The entrance to this mine has partly collapsed and there are some remains of old equipment scattered around the survey area. These include parts of a compressor shed, an ore chute (which is just above the dale floor) and a wagon (ANMHS, 2017). According to Ford (2002), this is a 20th century mine with little activity having taken place. Tansley Dale is a steep sided dale with several mine workings in and around its margins. Barnatt and Penny (2004, p. 82) describe "a gin circle, a run-in shaft, a coe, a belland yard, a grilled but blocked shaft, a water storage pond, a probable ore-dressing pit and washing ponds" as being present at Tansley Dale. Sampling here was undertaken around a sough or drainage shaft half way up the dale.

The many different structures and landforms found around the study sites are not just a result of the mining and extraction process. Other processes needed to be undertaken to produce an ore of sufficient purity, and many of them took place at the same site where ore was extracted (hence the several structures present at some of the study areas) (Barnatt & Penny, 2004). Only approximately 10% of the minerals found in the vein would consist of galena and of that only 86% was likely to be lead (Ford & Rieuwerts, 2000). Once mined, the mineral ores would need to be washed, fragmented and sorted to separate the lead ore from the other gangue minerals with which it was amalgamated. Only after these processes was the final lead ore sent to the smelters (Barnatt & Penny, 2004).

These mineral extraction and other associated processes have had some consequences for the environments in which they took place, and the structures used for these processes can lead to varying levels of contamination in these environments (Kossoff *et al.*, 2016) (*Table.* 2:1).

Table 2:1. Possible contamination levels around mining structures. Source (Kossoff *et al.*, 2016)

Mining heritage structure	Expected level of contamination
Mining and processing sites	High (if not managed)
Underground workings	Moderate (potentially serious)
Adit	High
Spoil heaps	High
Tailing dams	High
Soughs	Low
Remains of smelter chimneys, bellows etc.	Very High
Processing/smelting structures	High
Mining pools or water bodies	High

The next section will discuss the consequences and interactions mining has had on the surrounding environment and further afield.

2.4 Consequences and interactions with environment

In the UK, most soils contaminated with heavy metals are not found in urban or industrialised areas, but in areas dominated by agricultural land that is historically associated with mining activities (the Peak District) (Li & Thornton, 2001; Shotbolt, Rothwell, & Lawlor, 2008). The mining and extraction of mineral ores in these areas can introduce many contaminants into the environment; not just the target metalliferous ore itself, but also the waste gangue materials and associated elements that are also extracted in significant amounts

(Kossoff *et al.*, 2016). The early metal mining and processing operations in the Peak District were often extremely wasteful and consequently large amounts of heavy metals were released into the surrounding environment (Ford & Rieuwerts, 2000; Li & Thornton, 2001). As a consequence of this, it is estimated that an area of approximately 250km^2 is contaminated with Pb in Derbyshire alone (Abrahams & Blackwell, 2013; Li & Thornton, 2001), and other studies show that significant amounts of Zn, As, Cd and other associated metals are also present as substantial contaminants (Li & Thornton, 1993). This heavy metal contamination is present in topsoil (0-15cm depth) at concentrations of up to tens of thousands parts per million for Pb in some areas, especially around old mining works and waste/spoil heaps (Abrahams & Blackwell, 2013). The erosion, leaching and transfer of these top soils can be of concern as some of the heavy metal contaminants can be toxic to plants, animals and humans (Shotbolt *et al.*, 2008). In particular, research on effects of these heavy metal contaminants has revealed negative interactions in aquatic environments and sediments (Macklin, Hudson-Edwards, & Dawson, 1997; Mayes, Potter, & Jarvis, 2010; Shotbolt *et al.*, 2008), agricultural land and livestock (Abrahams & Blackwell, 2013; Geeson, Abrahams, Murphy, & Thornton, 1998), humans (Bridge, 2004; Dudka, Piotrowska, & Terelak, 1996) and other organisms and vegetation (Andrews, Johnson, & Cooke, 1989; Hernandez & Pastor, 2008; Woch *et al.*, 2016).

2.4.1 Aquatic environments

Contamination of water courses from mining areas can impair the water quality in small streams, rivers, entire river basins (Mayes *et al.*, 2010) and coastal areas (Macklin *et al.*, 1997; Trezzi *et al.*, 2016). These contaminants include heavy metals and metalloids such as Pb, As, Zn, Cd, Cu and Ni, which can have a negative impact on aquatic ecosystems, water resources and human health (Mayes, Johnston, Potter, & Jarvis, 2009). Shotbolt *et al.* (2008) state that this contamination has taken place over a long period of time and their study revealed the main routes of transport of these contaminants is via erosion and transport of polluted soils and sediments and also the leaching of dissolved organic matter. Gozzard, Mayes, Potter, and Jarvis (2011); Mayes *et al.* (2010), also, discuss the geochemical mobility of some heavy metals in surface waters and associated runoff as a major contributor to contamination through groundwater inputs. They also cite the resuspension of historically contaminated riverbed sediment as a contributor. Kossoff *et al.* (2016) discuss the wind as a possible contamination route citing deposits of Pb found in Greenland as originating from Roman mining activity in Spain many centuries ago. Certain contaminants, such as Cd and Zn, are also seen to be more easily transported under acidic conditions (Shotbolt *et al.*, 2008).

The most common pollutant to water sources situated in regions of metal mining activity in the UK is Zn (Gozzard *et al.*, 2011); this is due to both the mobility of Zn in marine environments (Mayes *et al.*, 2009) and the waste deposits left over from the extraction of galena (PbS), containing sphalerite (ZnS), which leads to high concentrations of Zn in water discharges from mines (Mayes *et al.*, 2010). Mayes *et al.* (2010) discuss the continuing pollution from disused mines and mining areas in England and Wales and state that Pb, Zn, As and Cd discharges into surface waters from these areas exceed the legally permitted discharges of these contaminants. They also reveal concerns about the Cd levels in the aquatic environment due to its persistence, alongside it being highly toxic and liable to accumulate in biological organisms. Because of this, Cd has been classed as a priority hazardous substance under the European Union's Water Framework Directive (2000/60/EC) (European Parliament, 2009). Other concerns are the deficiency of data concerning Cu, As and Ni releases into surface waters from mining sites (Mayes *et al.*, 2010) and the lack of technologies that are available for the remediation of contaminants originating from metal mining sites (Gozzard *et al.*, 2011; Ryder & Bennett, 2010). Gozzard *et al.* (2011), however, notes that riparian contamination sources can be stabilised with the practices of capping or removal of the source contaminants.

2.4.2 Agriculture and livestock

The historical mining of metalliferous ores and the associated processing have resulted in large areas of agricultural land in the Peak District being contaminated with heavy metals, and much of this land is used for growing crops and the rearing of livestock (Abrahams & Blackwell, 2013). However, these agricultural areas can also be contaminated via the remobilisation of heavy metals if they lie within a floodplain (Kossoff *et al.*, 2016) and Nicholson, Smith, Alloway, Carlton-Smith, and Chambers (2003) also point out that other pathways for contaminants include atmospheric deposition, sewage sludge, fertilisers and compost and agrochemicals.

Dudka *et al.* (1996) investigated the effects of highly elevated concentrations of Pb, Zn and Cd, associated with ore processing, on several crops including barley and potatoes, and reported low concentrations in the crop plants; however, they also noted a low yield for crops in the highly contaminated areas. They concluded that there was no risk of heavy metal transfer up the food chain. Conversely, Gall, Boyd, and Rajakaruna (2015) state that certain crops do accumulate bioavailable metals into their shoots, leaves and other above ground tissues. They state that organisms that feed on these plants could be exposed to heavy metals with the potential of toxicity. This can then be passed along the food chain to primary and secondary consumers, including humans.

The large mammals most likely to consume this vegetation in the Peak District are livestock that are grazing on agricultural grassland areas that are sites of historic mining activity (Abrahams & Blackwell, 2013; Geeson *et al.*, 1998). However, most ingested contaminants appear to come from soil still attached to unwashed vegetation (Abrahams & Blackwell, 2013), and these are involuntarily consumed along with the plants (Geeson *et al.*, 1998). Some problems with the health of livestock grazing on land with high concentrations of heavy metals have occurred (Gall *et al.*, 2015; Geeson *et al.*, 1998) and Gall *et al.* (2015) reports instances of ‘mutagenesis, carcinogenesis, teratogenesis, immunosuppression, growth inhibition, low fertility and problems with reproduction' associated with exposure to high levels of non-essential heavy metals in large mammals. More locally, the Peak District has seen problems such as lambs with bone lesions, stiffness in the back legs, imbalances in trace elements and Cu deficiency in both sheep and cattle (Geeson *et al.*, 1998). Geeson *et al.* (1998) states that these problems have been associated with Pb toxicity and that these can be heightened by high amounts of fluorine in the diet. The gangue materials left behind after the extraction of galena ore contain fluorspar (Ford & Rieuwerts, 2000) and this can lead to an increase in fluorine in the diet of livestock, if soils from the mine tailings are consumed along with vegetation (Geeson *et al.*, 1998). Andrews *et al.* (1989) have also reported increased levels of Pb, Cd and fluorine in vegetation and small mammals on metalliferous grasslands. Abrahams and Blackwell (2013), however, do suggest that a large portion of Pb consumed by livestock is not absorbed in the digestive system and most is excreted along with faeces. The sequential extraction procedure they carried out in their research suggests that the majority of Pb and fluorine in the soil was not soluble in the digestive tract. Any problems that are associated with the consumption of heavy metals and non-essential elements in the diet of livestock can also be controlled by restricting access to areas of contamination, especially between September and March, and in wetter weather where consumption of soil is greater (Smith, Dagleish, & Abrahams, 2010; Thornton, 1996) and an increase in defects in livestock has been noted (Geeson *et al.*, 1998). However, Abrahams and Blackwell (2013) do state that some livestock in certain areas are continually exposed to contamination in their diets all year.

Whilst, Geeson *et al.* (1998), note that every year problems do occur in livestock due to an imbalance of elements, they point out that more research is needed to evaluate the importance of direct soil to animal pathways. Smith *et al.* (2010); Thornton (1996) report that although toxic concentrations of Pb accumulate in the liver and kidneys of livestock, this does not affect the production of meat and dairy products for human consumption with the exception of offal (Thornton, 1996), which Abrahams and Blackwell (2013) recommend should be subjected to specific guidelines to prevent contaminated produce being sold for human consumption.

2.4.3 Human

As previously discussed, heavy metals originating from disused areas of metalliferous mining activities can potentially impact human health through their interactions with aquatic environments (Mayes *et al.*, 2009), certain crops grown in areas of high metal contamination (Gall *et al.*, 2015; Qu *et al.*, 2012), and produce from livestock grazed on contaminated agricultural land (Abrahams & Blackwell, 2013; Gall *et al.*, 2015; Geeson *et al.*, 1998). There are other pathways to human exposure, including inhalation of soil dust and inadvertent transport of contaminant soil dust onto the interior or exterior surfaces of the home (Qu *et al.*, 2012); however, further discussion of these is beyond the scope of this research.

One way in which historical mining and its associated landscapes impact humans is a qualitative one, and this is the way the remnants of this past industry are viewed by the general public (Bridge, 2004). Bridge (2004) discusses the physical changes to the landscape caused by the extraction of minerals, and how these can have contrasting reactions from the public. Some describe these landscapes in a negative way using words such as "unsightly, visually intrusive and repellent" when referring to the pits, waste heaps and built structures left over from mining activities. On the other hand, Bridge (2004) cites an article by Robertson (2000) in which residents close to areas of mining activity see the mining landscape and structures as adding to their community identity and local heritage. These views are repeated in an article by Kossoff *et al.* (2016), where it is noted that these sites are an important part of commercial and industrial heritage, and can be even more valuable to local and regional communities if they form part of conservation and tourism schemes.

Unfortunately, many historic areas of mining activity have been obscured by the removal or levelling out of the waste heaps, and this can have implications not only for the conservation of heritage sites (Barnatt & Penny, 2004; Geeson *et al.*, 1998) but can mean that potentially contaminated agricultural land is not easily identified, and this can lead to potential toxicity to some grazing animals (Geeson *et al.*, 1998). Barnatt and Penny (2004, p. vi) go further and state that "many lead mining remains are of international, national or regional importance for their landscape, cultural, archaeological, ecological and geological interest".

2.4.4 Ecological impacts

Heavy metal contamination has been shown to have several negative effects on plant and animal communities (Erry, Macnair, Meharg, & Shore, 2005; Gall *et al.*, 2015; Mertens, Luyssaert, Verbeeren, Vervaeke, & Lust, 2001). In plants, several heavy metals can have deleterious effects on many species and these can include:

- The eradication of species that are sensitive to contaminants (Woch *et al.*, 2016)
- Decreases in metabolism, plant development and growth and chlorosis (Zn) (Nagajyoti, Lee, & Sreekanth, 2010)
- The browning of the tips of roots, negative effects to photosynthesis and growth and death (Cd) (Nagajyoti *et al.*, 2010)
- The disturbance of metabolic pathways (Cu) (Nagajyoti *et al.*, 2010)
- Adverse effects on morphology, photosynthesis, growth. Inhibition of seed germination and enzyme activities (Sinha, Srinivastava, & Mishra, 1988), disturbances in membrane permeability and mineral nutrition (Pb) (Nagajyoti *et al.*, 2010)
- Chlorosis, necrosis (Pandey & Sharma, 2002), impairment of nutrient balance and cell membrane functions (Ni) (Nagajyoti *et al.*, 2010)

However, the exact nature of any problems caused by historic mining contamination will vary, and may depend on the ores and metals extracted, the location and techniques used to process the material, the geology and the length of time since the activities took place (Batty, 2005). Also, elements in the soil that are freely available for uptake by the plants, referred to as the bioavailable fraction, can depend on several factors, such as pH, cation and anion exchange, the chemical form of the elements present in the soil, soil organic matter, plant species and saturation of the soil (Alloway, 1995; Dubbin, 2001; Geeson *et al.*, 1998). Despite these influencing factors, it is generally accepted that mining has negative consequences for biodiversity at these sites by altering the community structures of living organisms that exist there (Batty, 2005). Nevertheless, many mining sites in the Peak District have a long history and the contaminants at these sites has been present for hundreds and possibly thousands of years (Ford & Rieuwerts, 2000). This situation, has, over time, led to the development of important and rare niche vegetation communities that are able to thrive off the elevated heavy metal concentrations (Batty, 2005). These persistent contaminants have affected these plant communities during this time by a relatively short term elimination of the stress-sensitive plant species and by applying natural selective pressures as the plant communities changed, leading to emerging populations of metal tolerant species and communities (Woch *et al.*, 2016).

2.5 Basic soil principles

There are four principal components of which all soils are composed: a parent material or mineral, water, air and organic material (Ashman & Puri, 2002; Dubbin, 2001). The solid components of these constituents form a complex mix of organic matter and mineral, which means soils can be broadly grouped into either mineral or organic soils depending on their ratio to each other. For instance, organic soils generally contain more than 30% organic matter and are usually found in areas with moist and cool climates (Dubbin, 2001). However, most soils are mineral soils and typically contain around 45% mineral content in the UK (O'Hare, 1992); it is this type of soil that is present at the study sites of this research and hence it is the main focus here.

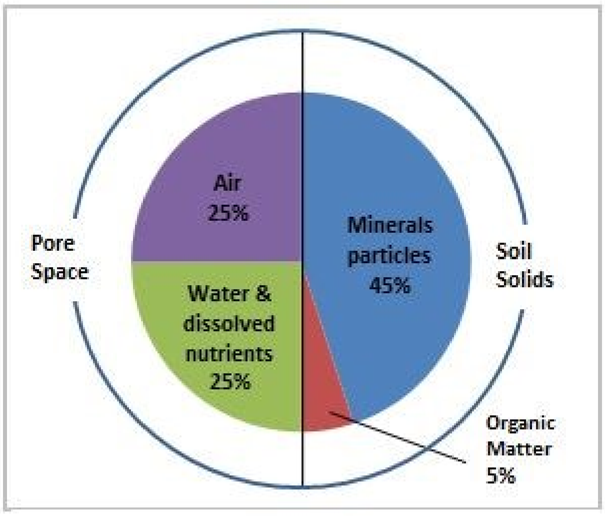


Figure 2:2. Typical constituents contained in UK soil. Source (Zuzek, 2016)

The minerals in soils which form most of its physical framework principally originate from the weathering of its parent material or underlying bedrock and mineral ores (Smithson, Addison, & Atkinson, 2002). In the case of the calcareous soils found in Derbyshire and the Southern Peak District, this bedrock is usually limestone (Wolverson Cope, 1998). The size of the mineral fragments contained within the soils can vary, and this affects the texture of the soils, with mineral particles ranging in size from clay particles, <0.002mm in size, to the larger gravels, 2mm in size (O'Hare, 1992). The organic matter component of soil constitutes decomposing vegetation and other living organisms present in the soil, and when fully decomposed this is termed humus. Together, the varying ratios of parent material and organic matter provide the different textures and colours of the soil (Ashman & Puri, 2002). Within the pore spaces between the solid components of soil exist fluctuating amounts of air and water, dependant on the changing local environmental factors. It is the texture of soil and the ratios of its constituents that influence the retention of nutrients, plant growth and the drainage of the soils (O'Hare, 1992).

The development of soil involves many complex processes taking place over a long period of time, principally the interactions between morphogenesis and weathering processes (Ashman & Puri, 2002). The atmosphere and hydrosphere supply water, O, N and CO_2, which allow the growth and development of plants and other organisms; these in turn provide the organic matter contained in soil. The hydrosphere and atmosphere also cause the weathering of the parent material (CO_2, O and water). This weathering produces residual minerals, quartz, feldspar and other heavier minerals and secondary minerals such as clay minerals, aluminium, iron and hydrous oxides (known as soil colloids along with humus). It is these minerals, along with organic matter residue and a weathering solution which contains silica, cations and anions, which undergo further physical and chemical alterations, along with microbial and morphogenesis processes, to produce soil (Smithson *et al.*, 2002).

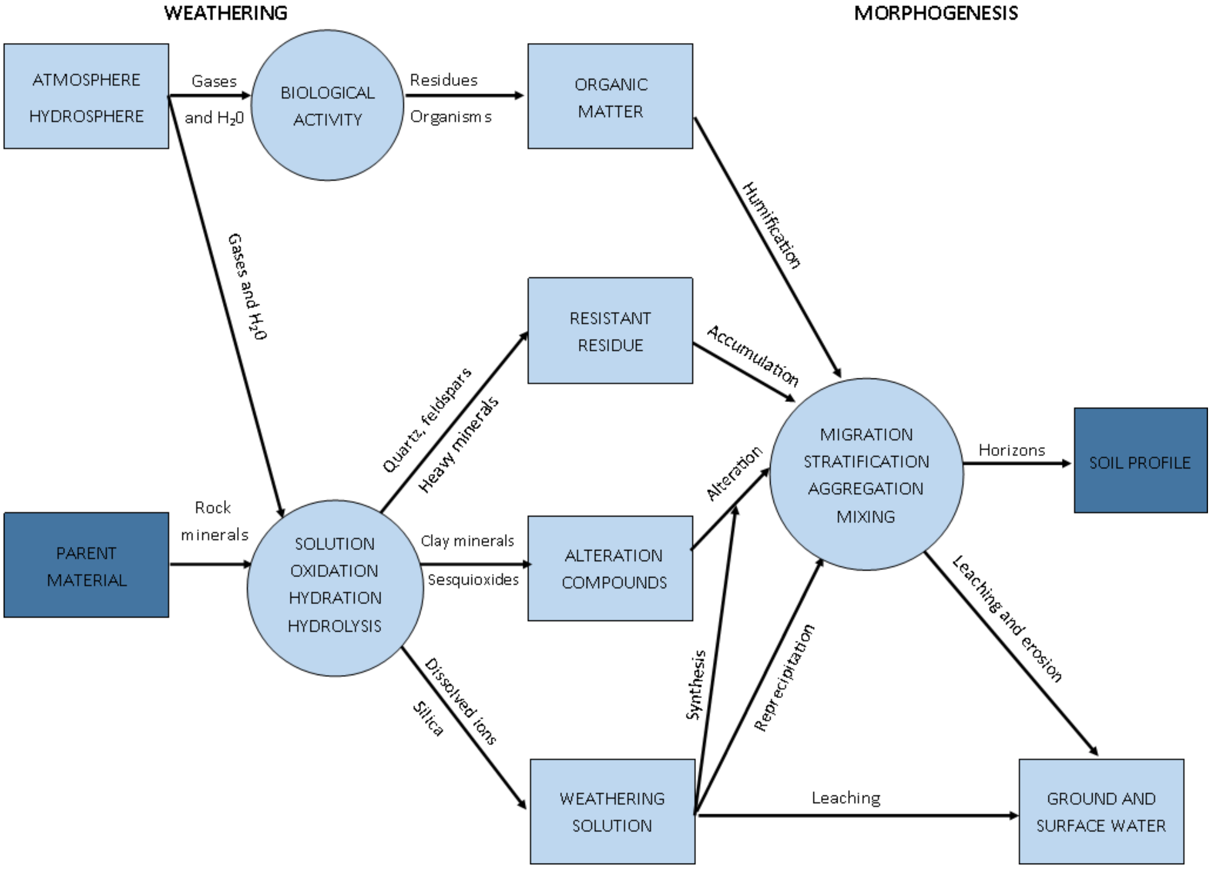


Figure 2:3. Soil production through weathering and morphogenic processes. Source (Park, 2001)

Elements originating from both mineral and organic fractions contained with the soil provide the nutrients required for plant growth, and these are generally grouped into two categories: macronutrients and micronutrients (although these are equally important for plant growth) (Ellis & Mellor, 1995). The primary elements needed for plant growth, such as oxygen (O), carbon (C), nitrogen (N) and hydrogen (H) are required in relatively greater amounts, with secondary elements including phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) required in lesser quantities. Only trace amounts of elements such as cobalt (Co) and molybdenum (Mo) are required for normal plant growth (Ellis & Mellor, 1995; Wild, 1994) Any lack of these essential nutrients or imbalances between them can cause complications for plant growth (Ellis & Mellor, 1995). The nutrient content in soils can vary depending on the ratio and type of mineral and organic constituents, and consequently nutrient rich soils tend to be found on parent materials such as limestone, basalt and some sandstones (Ellis & Mellor, 1995). Fig. X contains examples of some types of mineral and organic materials which provide plentiful amounts of some essential nutrients. Typically, calcareous grassland situated on limestone bedrock will contain high N levels, low P and sufficient K and Mg, which suits the high diversity of plant species that exist there (Ashwood, 2014; Kishchuk, 2000).

Table 2:2. Soils and nutrient content. Source (Ellis & Mellor, 1995)

Soil forms rich in:	**Nutrients occurring**
Feldspars, mica and hornblende	Magnesium, calcium, potassium
Organic matter	Nitrogen
Pyrite, gypsum and organic matter	Sulphur
Apatite	Phosphorous

These elements originating from the eroding of parent materials and the decomposition of organic matter (in addition to those from anthropogenic activities) occur as chemical salts, which supply plants with nutrients in the form of positively charged ions called cations (Ashman & Puri, 2002). However, not all of these nutrients are available for uptake by the plant because of other chemical factors within the soil matrix (Dubbin, 2001). The cations can be leached from soil particles in wetter weather, move between soil particles or be removed from the soil particles by plant roots (O'Hare, 1992). pH can also affect the availability of cations to plants, and it is the positively charged hydrogen ions (H^+) which are also present which account for the acidity or pH of soils. It is the quantity of H^+ ions within the soil which allow the measurement of acidity on the pH scale. The pH scale is a logarithmic scale where pH 0 is extremely acidic, pH 7 is neutral and pH 14 is highly basic (Ashman & Puri, 2002). The availability of essential nutrients and other elements to plants differs depending on the amount of H^+ ions in the soil environment. Many nutrients become less available at the extremities of the pH scale; however, there are exceptions to this rule. For instance, iron (Fe) availability is greater under more acidic conditions, and potassium (K) and sulphur (S) show greater availability for uptake in highly basic soils (Dubbin, 2001). This means that plants more reliant on certain elements which are more available at the upper and lower limits of the pH scale can often be found to be adapted to soils that are more acidic or basic (Dubbin, 2001).

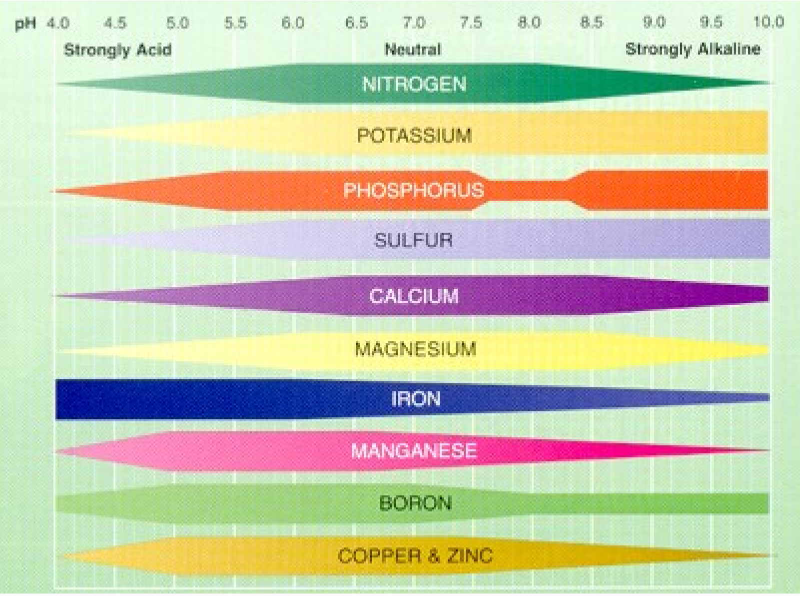


Figure 2:4. Nutrient availability dependant on pH. Source (Zuzek, 2016)

Most fertile soils usually range from a slightly acidic pH 5.5 to a neutral pH 7, which is the optimal range for the availability of most essential plant nutrients (Smithson *et al.*, 2002). However, should the pH of a soil decrease or increase beyond this optimal range, problems can occur (O'Hare, 1992). As well as a decrease in the availability of essential plant nutrients, a pH that is too high or low can also result in the uptake of heavy metals present in the soil due to anthropogenic activities such as mineral extraction (Smithson et al., 2002). Whilst small quantities of certain heavy metals and other elements are important for the normal growth of plants (Alloway, 1995), substantial amounts of these contaminants also possess the capacity to alter the pH of a soil environment more permanently. This in turn increases the potential uptake of heavy metals and can impede essential nutrient availability in extreme cases (Dubbin, 2001; Smithson et al., 2002; O'Hare, 1992).

However, soils have the capacity to resist these variations in pH, and this is termed buffering capacity, which increases with a higher pH, lime content and cation exchange capacity (CEC). The CEC is the total quantity of exchangeable cations that can be adsorbed by the soil. The adsorption process is the adhesion of ions to the surface of clay particles and organic matter present in the soil (Dubbin, 2001). The buffering capacity of a soil can ensure that elevated levels of heavy metals and other harmful elements are not as readily available for uptake by plants (Dubbin, 2001). Calcareous grasslands situated on limestone geology typically have a pH of 6.5 to 8.5 (Jefferson, Smith, & MacKintosh, 2014), and hence a relatively high CEC and buffering capacity. This suggests that grassland situated on limestone geology will offer a low availability of heavy metals and other harmful elements to the vegetation that grows there (Dubbin, 2001). However, with many metalliferous ore veins occurring in these environments situated on limestone geology (Ford & Rieuwerts, 2000), even with the increased buffering capacity of limestone grasslands, Rodwell *et al.* (2007) still consider the input of pollution from many anthropogenic activities to be a key threat to calcareous grassland vegetation.

2.5.1 Geochemical influences on soil variables

After the deposition of ore and gangue material in areas of historical mining activity, which forms the majority of physical features at abandoned mining sites such as mine tailings and pock-marked landscapes (Ford & Rieuwerts, 2000), the heavy metals contained and the surrounding soil properties can be heavily influenced by geochemical processes (Alloway, 1995). Much research has observed the importance of geochemical properties and the influences the can have on pH, clay and organic matter content, the mobility of heavy metals and others (Alloway, 1995; Heikkinen, Räisänen, & Johnson, 2009; Lindsay *et al.*, 2015; Mitchell, 2005).

As previously discussed (Section 2.2), much of the Pb and Zn metal wastes found in the Peak District are sulphides, i.e. galena (Pb) and sphalerite (Zn), and these are often associated with other sulphides for example pyrrhotite and chalcopyrite (Alloway, 1995). Mine tailings containing these sulphides are susceptible to oxidisation through several mechanisms, which are dominated by the presence of water, oxygen and sulphide oxidising bacteria such as *Thiobacillus thiooxidans* or *Thiobacillus ferrooxidans* (Kelly, Shergill, Lu, & Wood, 1997; Suzuki, 2001). This oxidation of sulphides can have an acidifying effect on the soil pore water and the influence the uptake and mobility of metals within the soil matrix (Heikkinen *et al.*, 2009).

Hence, the mineralogical composition of the mine tailings (sulphides in this instance) and the oxidising chemical reactions can directly affect the pore water and groundwater pH (McGregor, Blowes, Jambor, & Robertson, 1998). The intensity of sulphide oxidation and other associated processes can be diverse across the extent of the mine tailings because of variations in the mineralogical composition of the mine waste and gangue materials and the physical properties in the tailings e.g. permeability, water saturation, soil particle sizes. These heterogeneous conditions produce pore water and groundwater with diverse qualities (Heikkinen *et al.*, 2009). For example, decomposition of sulphides in oxidising groundwater can acidify the water, in turn lowering the pH. However, the pH of pore water and groundwater is associated with the balance of acid generating and acid neutralising minerals within the mine tailings, whilst the overall geochemistry is determined by the mineralogical make-up of the ore materials and suspension of acid buffering minerals such as calcite. Therefore, the pH in heavy metal contaminated soils is a consequence of the abundance of sulphide phases in the soil parent material, including lead sulphide, is not solely an action of heavy metal content within the contaminated soils, and is a much more complicated process (Alloway, 1995). Once heavy metal contamination is deposited within the soil matrix, water, pH and redox fluctuations will also affect the bioavailability of heavy metals, and the possibility for the heavy metals to be leached back into soil solution (Alloway, 1995).

2.5.2 Metalliferous soils

Metalliferous soils contain elevated concentrations of heavy metals and other elements originating from the weathering of metal enriched parent materials, environmental factors for example volcanoes and anthropogenic activities such as the extraction and processing of metal ores (Alloway, 1995). In addition to higher concentrations of heavy metals, sometimes to toxic levels, metalliferous soils are usually accompanied by a low nutrient status. Essential nutrients for plants including nitrogen, phosphorous and potassium are sometime decreased as a result of the chemical and physical reactions of heavy metal elements in the soil matrix. These soils can also become acidic and freely draining (Antonovics, Bradshaw, & Turner, 1971; Tordoff, Baker, & Willis, 2000). Although some of these heavy metals are required in small amounts along with other essential nutrients, excessive amounts can result in deleterious symptoms in plants that have not adapted to these, sometimes extreme, conditions and become metal tolerant (Antonovics *et al.*, 1971; Baker *et al.*, 2010; Baker & Proctor, 1990). The availability of heavy metals for uptake by plants is not only due to elevated concentrations, but influenced by abiotic factors including CEC, organic matter and mineral content and the acidity of soils which can all be altered in the presence of increased metal content (Dubbin, 2001). Consequently, for plants to become established and thrive in metalliferous soils necessitates mechanisms of resistance, either through increased tolerance to, or avoidance of, heavy metal uptake or their negative consequences (Briat & Lebrun, 1999; Hall, 2002).

2.5.3 Basic principles of the bioavailability of heavy metals in soils

The bioavailability of heavy metals is a key factor influencing the interactions between the abiotic environment and biotic organisms (Harmsen & Frintrop, 2003; Pietrzykowski, Socha, & van Doorn, 2014). However, the term bioavailability is not easy to define, and users from different scientific backgrounds have differing views on the interpretation of bioavailability (Semple *et al.*, 2004). For instance, Harmsen and Frintrop (2003, p. 199) write that "chemists may define bioavailability as the amount per mass that can desorb from soil or sediment in a particular time and certain conditions", whereas ecologists would define bioavailability as "the amount of heavy metal the organism is exposed to and could potentially uptake to cause an effect". It is the latter definition that is being considered here.

Plants are an essential part of ecosystems due to the role they play in transferring essential and non-essential elements from the abiotic environment i.e. soil, water and air, to living organisms (Hamilton, 1995). Those often considered to be some of the most important elements which are transferred from the soil matrix into plants and then along the food chain are the heavy metals, Pb, As, Zn, Cd, Cu and Ni (Chojnacka, Chojnacki, Gorecka, & Gorecki, 2005). These elements can be toxic to both plant and animal species when transferred up the food chain in high concentrations (McLaughlin, Parker, & Clarke, 1999). The bioavailability of these elements to plants is governed by numerous factors, including soil and environmental conditions, vegetation species, land management, chemical and physical reactions, seasonal responses of plants to elements, reactions between different elements that affect bioavailability and the type of root system a particular species has (Chojnacka *et al.*, 2005). There are several methods currently in use to ascertain the bioavailability of these elements in soils, usually by an extraction process utilising a mild solvent or acid (Bakircioglu, Kurtulus, & İbar, 2011; Houba, Novozamsky, Huybregts, & Van der Lee, 1986; Mocko & Waclawek, 2004; Pueyo, López-Sánchez, & Rauret, 2004). These measurements can used to assess correlation between extracted heavy metal concentrations in soils with concentrations and effects in plants (Chojnacka *et al.*, 2005; Harmsen & Frintrop, 2003). Chojnacka *et al.* (2005) found strong correlations between concentrations of As, Pb, Zn, Cd, Cu in both soil extractant and plant tissues from wheat. However, they found no significant relationship between bioavailable Ni in soil and that in wheat plant tissue. Guala, Vega, and Covelo (2010) correspondingly found that they could predict the heavy metal concentration in plants and plant tissues using a simple model based on the available heavy metal concentrations present in soil samples. An overview of Pb, As, Zn, Cd, Cu and Ni and their bioavailability follows in section 2.5.4 to 2.5.9.

2.5.4 Lead (Pb)

Lead (Pb) is one of the most exploited elements by humans historically, and this has left extensive Pb pollution in some areas. Pb contamination is mainly linked with the mining and smelting of mineral ores, and more recently the use of leaded petrol. The mean Pb concentration present in the Earth's crust is approximately 15ppm and it is not considered an essential or useful element for the function of any living organism (Steinnes, 2013). Environmental and climatic factors and chemical and physical soil properties continually buffer, nullify and obstruct the uptake of Pb by plants (Koeppe, 1977). According to Kerndorff and Schnitzer (1980), the way Pb behaves in soils depends largely on the organic matter fraction of the soil matrix as it is adsorbed strongly to the humus at acidity levels greater than pH 4. Koeppe (1977), states that measuring the exact uptake of Pb by plants is a challenging task, due to its adsorption properties and the fact that most Pb in contact with the plant root remains in an insoluble form. Antonovics *et al.* (1971), argue that the uptake of Pb in plants increases with soil concentrations, until a critical load is surpassed and damage or death of the plant occurs. However, more recent research shows where excessive contamination of lead is present, at a mining area for instance, the species distribution and presence are continually adapted until tolerant species prevail (Steinnes, 2013). Research by Woch *et al.* (2016) and Conesa, Faz, and Arnaldos (2006) also found this to be the case.

2.5.5 Arsenic (As)

Of all the elements that constitute the Earth's crust, arsenic (As) is known to be the 20th most abundant, and is a naturally occurring metalloid present in soils at a mean concentration of 2-3ppm in the Earth's crust (Bhattacharya, 2007). However, elevations in As concentrations due to anthropogenic processes, such as the mining and smelting of mineral ores, can mean the exceedance of the regulatory guidelines put in place to protect human and environmental health from potentially negative impacts (Juhasz, Weber, & Smith, 2011). The bioavailability of As can vary depending on the source of contamination and the physical and chemical properties of soil (Li, Li, Zhu, Juhasz, & Ma, 2015). Arsenic typically exists in soils as anions and subsequently it is less bioavailable under acidic soil conditions when minerals and organic matter have their greatest positive charge (Dubbin, 2001). There are more than two-hundred different forms of As mineral including arsenates, sulphides, sulphosalts and other minor amounts of oxides, silicates, arsenates and arsensides (Bhattacharya, 2007). However, arsenate As^{V} dominates in aerobic soils and arsenite As^{III} dominates in submerged, anaerobic soils (Farooq *et al.*, 2016). As^{V} and As^{III} are the most toxic forms of As and in plants uptake is usually through the roots, although many mechanisms are involved for uptake and metabolism by the plant tissues. As^{III}, for instance can be absorbed through the aquaporin channels, which is where groups of proteins in cell membranes permit the movement of water across the membrane and into the plant tissues. As^{V}, however, is absorbed through the parts of the plant that allow the transport of phosphates (Farooq *et al.*, 2016). The bioavailability of As can be determined by many physical, chemical and environmental factors, as well as the accumulation properties of the plant itself. Plants also have the ability to detoxify As by way of chelation (Farooq *et al.*, 2016) which can reduce the toxicity of certain metals to safe levels by using humic acid and other compounds found in the soil organic matter in the rhizosphere

(Cobbett, 2000). Despite these factors influencing As uptake, several plants are known to accumulate high levels of As; wetland plants are more susceptible to As accumulation, rice being one such plant species (Farooq *et al.*, 2016; Williams *et al.*, 2009). Some plants are also known as hyperaccumulators of As and these belong to the Pteridaceae family, containing fern species and other related species. These plant species can accumulate very high concentrations of As in their plant tissues (Ma *et al.*, 2001).

2.5.6 Zinc (Zn)

Zinc is the twenty-third most abundant element on Earth and is an essential nutrient in plants, particularly as an important constituent in enzymes and proteins (Broadley *et al.*, 2007). Mean soil Zn concentrations are typically between 50-66 ppm, however most agricultural soils are thought to contain between 10 and 300ppm (Alloway, 1995). Most Zn originates from the weathering of parent material (Broadley *et al.*, 2007), although humans have influenced the input of Zn into the environment for many hundreds of years, leaving areas of very high Zn concentrations (Gutierrez, Mickus, & Camacho, 2016; Stefanowicz *et al.*, 2014). These high concentrations of Zn can be toxic to plants (Tsonev & Lidon, 2012), although Zn deficiency in plants can also have negative effects on plants and crops, especially in calcareous soils (Duffner, Hoffland, & Temminghoff, 2012). The Zn in soils produces several soluble and insoluble organic compounds and many soluble salts, and these are typically divided into three principal forms: insoluble Zn, water soluble Zn and Zn that is adsorbed and exchangeable in the soil matrix (Broadley *et al.*, 2007). The distribution of Zn in soil is influenced by several factors including mineral to organic matter ratio, mineral type, moisture and living organisms within the soil, plant uptake and several other chemical, physical and environmental factors. It is pH, however, that is the principal determinant of Zn distribution, with higher pH levels allowing more adsorption to cation exchange sites (Broadley *et al.*, 2007). The uptake of Zn into plants usually takes place with cations attaching to two binding sites or through interactions with organic molecules via the root xylem

(Broadley *et al.*, 2007; Tsonev & Lidon, 2012), and Tsonev and Lidon (2012) report that the phloem tissues are also a possible uptake route. Zn has a very similar chemical structure to that of Cadmium (Cd), a toxic heavy metal that is often associated with Zn and Pb mining sites (Gutierrez *et al.*, 2016). Some plant species have become Zn tolerant on sites with high Zn contamination (Stefanowicz *et al.*, 2014; Woch *et al.*, 2016), none more so than the metallophyte mountain pansy (*Viola lutea*), also known as 'zinc violet', found growing exclusively at sites with extremely high zinc contamination (Kuta, Bohdanowicz, Słomka, Pilarska, & Bothe, 2012; Słomka *et al.*, 2011).

2.5.7 Cadmium (Cd)

Cd is a naturally occurring heavy metal that is often found in association with sphalerite (ZnS) ores, and is frequently found in high concentrations on residual waste left over from Zn or Pb mining activities (Vig, Megharaj, Sethunathan, & Naidu, 2003). It has an unknown essential function in plants (Smolders, 2001) and can be highly toxic in high concentrations, although it is generally found in relatively low concentrations in its parent material and soils (Vig *et al.*, 2003). In soils, Cd occurs is several forms: residual, water soluble, exchangeable, reduceable, oxidisable and acid soluble, each retained by a different soil fraction and varying in availability and solubility (Spence, Hanson, Grant, Hoo Fung, & Rattray, 2014). Like other heavy metals, the bioavailability of Cd is affected by several chemical, physical and environmental properties including CEC, organic matter and mineral type, content and ratio, moisture and soil pH (Smolders, 2001). Bioavailable Cd is easily absorbed by plant roots (Smolders, 2001) through the cortical tissues, where it then enters the xylem through the cell membranes as a result of reactions with organic molecules called ligands (Benavides, Gallego, & Tomaro, 2005). The Cd can then transfer to the other above ground plant tissues, (Smolders, 2001) although the majority of Cd ions reside in the roots and smaller amounts are transferred to the above ground tissues (Benavides *et al.*, 2005). Benavides *et al.* (2005) report that concentrations of Cd in plant tissues decrease in the following order: roots>stems>leaves>fruits>seeds, and that this is also the case for species that hyperaccumulate Cd in their tissues. Smolders (2001) reports that elevated Cd uptake in plants takes place when there is a Zn deficiency in the soils and Benavides *et al.* (2005) goes further, noting a positive correlation in Zn and Cd concentrations in plant tissues, suggesting that they are transported into the plant in a similar way.

2.5.8 Nickel (Ni)

The Earth is composed of approximately 3% Nickel (Ni), and this naturally occurring heavy metal is the twenty-fourth most abundant element (Cempel & Nikel, 2006; Iyaka, 2011). Although widely dispersed in the Earth's crust, Ni is found in greater concentrations in parent material comprised of basic igneous rocks (Echevarria *et al.*, 2006), and in much lower concentrations in sedimentary bedrock such as limestone (Iyaka, 2011). Ni input into the environment is also caused by anthropogenic activities (Cempel & Nikel, 2006). Concentrations of Ni in soils ranges from approximately 3ppm to 1000ppm (Iyaka, 2011), and according to Cempel and Nikel (2006) environmental concerns for Ni are confined to urban areas, although any acidification of soils in agricultural areas may see this become a future rural problem.

Nickel is an essential nutrient for plants; however, highly elevated concentrations in soils can result in toxicity in plants (Nie *et al.*, 2015). There are several factors influencing the availability of Ni to plants, although soil pH is of primary importance, as decreasing pH increases the solubility and mobility of Ni in the soil matrix. Thought to be of secondary significance are soil organic matter, clay and iron-manganese content (Iyaka, 2011). Everhart *et al.* (2006) state that a calcareous soil with a relatively elevated pH and organic matter content would considerably reduce Ni toxicity to plants. Several forms of Ni exist in soils, which include soluble chlorides and nitrates present in the soil solution or insoluble oxides and sulphides which can be adsorbed to organic cation or inorganic cation exchange surfaces or inorganic minerals (Cempel & Nikel, 2006; Iyaka, 2011; Nie *et al.*, 2015). It is the soluble form of Ni that is bioavailable to plants and is taken up into the plant root in a similar way to other essential metals Zn and Cu, with cations attaching to two binding sites (divalent) and being absorbed directly into the root (Chauhan, Thakur, & Sharma, 2008). Once absorbed into the root, Iyaka (2011) states that Ni moves throughout the plant tissues and can accumulate in the leaves and seeds, although Nie *et al.* (2015), report that Ni concentrations are much lower in the leaves and stems and mostly accumulate in roots. Some plants are highly tolerant and are hyperaccumulators of Ni (Chauhan *et al.*, 2008; Echevarria *et al.*, 2006) and Iyaka (2011) notes that legumes of the plant family *Fabaceae* have been identified as Ni hyperaccumulators along with cabbage, turnip and cauliflower.

2.5.9 Copper (Cu)

Copper (Cu) is a naturally occurring metal and is the 25th most abundant element within the crust of the Earth, essential for life as a trace element (Adrees *et al.*, 2015). Cu toxicity can occur at both naturally occurring areas of exposed mineral and through anthropogenic input into the environment through mining activities (Yruela, 2005). Naturally occurring uncontaminated soil concentrations of Cu vary between 3 and 100ppm; however, agricultural soils contain less than 30ppm and vineyard soils may contain up to 500ppm (Adrees *et al.*, 2015). The availability of Cu to plants is determined by several factors including organic matter and clay content, metal oxides, CEC and, principally, pH (Chaignon, Sanchez-Neira, Herrmann, Jaillard, & Hinsinger, 2003). Most Cu in soils is bound to organic matter, but with a pH of less than six, Cu becomes readily available for uptake (Adrees *et al.*, 2015). Cu can be adsorbed to other soil components and Chaignon *et al.* (2003) state that the distribution between these different fractions can also have a strong influence on its availability.

For plant growth, Cu is an essential element and is needed in small amounts for the formation of enzymes which are required for several important processes, such as respiration and photosynthesis, to allow normal plant growth and development (Pirrone, Žaltauskaitė, &

Šliumpaitė, 2013). Plants can develop both deficiency and toxicity symptoms in response to slightly increased or decreased Cu availability (Yruela, 2005). Cu is absorbed through the roots of the plant and then transported through the plant root system to the xylem. The transport into the xylem primarily uses two routes called apo-plastic and symplastic pathways. The Cu is then transported to the shoots of the plant by way of the xylem and phloem vessels (Adrees *et al.*, 2015). Both Adrees *et al.* (2015) and Yruela (2005) state that more research into the transport and absorption of Cu in plants is needed. Some plants can accumulate or tolerate high levels of Cu by way of reduction in uptake, accumulating Cu in less responsive cells, chelation and binding Cu into cell walls (Adrees *et al.*, 2015). One example of this was noted in a review by Adrees *et al.* (2015) where soybean (a legume from the family *Fabaceae)* accumulates concentrations of Cu by binding it to its vacuoles and cell walls.

2.6 The habitats of historic lead mining sites

The historic lead mining industry in the Peak District has left a scarred landscape strewn with many waste hillocks, hollows and other man-made structures (Barnatt & Penny, 2004). The excavation of mineral ores from the limestone bedrock has resulted in areas of high heavy metal contamination, in particular Pb, Zn and Cd (Anderson & Shimwell, 1981; Richards & Waite, 2017). These areas of contamination are present across the spoil-heaps and hollows, and the topsoil often contains a mixture of limestone, fluorspar, baryte and calcite (gangue materials) (Penny, 2009). These hillocks and derelict mining structures not only survive as the legacy of a once thriving industry in the Peak District (Barnatt & Penny, 2004), but also act as a habitat for a thriving community of plants that have adapted to the harsh conditions (Penny, 2009). The conditions at these sites are often poisonous to many plants (Anderson & Shimwell, 1981; Richards & Waite, 2017); however, since the cessation of mining activity, many complex factors have allowed the colonisation of flourishing communities of locally rare and important plant species. The factors that have allowed these ecological changes include the age of the site or time since activity stopped, the chemical and physical properties of the substrate, the slope and aspect of the spoil-heaps, the overall topography of the area, proximity to local or regional seed mix and management (Barnatt & Penny, 2004).

The plant communities that grow on the spoil heaps along lead rakes and mine entrances need time to develop. The reworking of the gangue materials in the 19th century due to the development of new processing technologies meant that many of these heaps and hollows have been undisturbed for up to 200 years (Barnatt & Penny, 2004). Smith (1979) goes further, stating with some certainty that most plant communities that exist on metalliferous spoil heaps on calcareous geology have developed to their current condition over the last 150 years, although this article was published almost 40 years ago. Penny (2009) suggests that when these sites were abandoned almost 200 years ago, that natural succession allowed the regeneration of plant species communities that were present in the 19th century, and today these species are no longer common in other locations. Penny (2009) also states that surveys undertaken on the remains of lead mines have shown that the older the mining area is, the more diverse the vegetation community present is.

Szarek-Lukaszewska (2009) and Woch *et al.* (2016) both found this to be the case in more recent research in Poland. They state that the rate that areas of mining activity are colonised and developed by plants depends upon the plant communities that exist close to the disturbed areas. Also in areas of calcareous grassland situated on metalliferous waste heaps, Woch *et al.* (2016) reports that not only do persistent heavy metal contaminants alter the plant species populations in the short term by the eradication of less tolerant species, but also over long periods of time by influencing the evolution of local plant species, leading to new heavy metal tolerant and genetically distinct varieties of local plant species. The species that are present in these areas of highly contaminated soil can comprise of plants termed metallophytes (Bates 2007) and pseudo-metallophytes (Baker & Proctor, 1990). Metallophytes are rare and ecologically important plants which can hyperaccumulate heavy metals far in excess of other species (Alford, Pilon-Smits, & Paschke, 2010) and in the UK there are four key species present on lead rakes. These include mountain pansy (*Viola lutea)*, pyrenean scurvy grass (*Cochlearia pyrenaica)*, alpine penny-cress (*Thlaspi caerulescens),* and spring sandwort (*Minuartia verna)*. Pseudo-metallophytes are species that are common locally to the metalliferous wastes and occur on both uncontaminated soils and as heavy metal tolerant varieties which can grow on highly contaminated land (Baker & Proctor, 1990).

Calcareous grasslands are typically ecosystems consisting of a very species rich sward which are largely constrained to dry, shallow soils situated on a calcareous parent material, often growing on the south facing slope of steep sided dale (like those at Hay Dale, Tansley Dale and Deep Dale) (Carroll, Caporn, Johnson, Morecroft, & Lee, 2003). However, the topography present at lead rake sites, where waste hillocks and hollows have been left, offer a unique and complex environment where mosaics of grassland communities can thrive. The hillocks provide variable slopes and aspects which can provide diverse microclimates and contrasting exposure to sunlight and weather conditions within a relatively small area (Penny, 2009). Couple this with the spatial variation of heavy metal contamination across relatively small areas (Stefanowicz *et al.*, 2014), typical of metalliferous substrates, and it can result in considerably diverse and interesting vegetation communities (Smith, 1979).

The issues regarding management, which are the main threat to these habitats, originate from recreational damage, farming practices and applications to further extract minerals from the waste heaps (Barnatt & Penny, 2004). Historically, threats from farming have included the removal and levelling of waste heaps to capitalise on useable agricultural land (Geeson *et al.*, 1998), the disposal of farming waste and equipment onto the lead rakes (waste regulations now inhibit this practice) and grazing methods (Penny, 2009). The type of livestock grazed on these areas can have an impact, as the eating habits of cattle tend to cause damage to the waste heaps, leaving bare patches where they have torn up vegetation down to the soil. This can also have a negative impact on the cattle feeding in these areas (Geeson *et* *al.*, 1998; Penny, 2009). Recreational damage can occur through the use of motorbikes and by larger motor vehicles gaining access to the sites, as well as applications to extract further gangue material from the lead rakes, although this is now in decline (Penny, 2009). It is because of these threats to the specialised habitats, with rare plant species, geological areas of interest and the heritage and archaeology of the Peak District's lead mining sites, that they are now a conservation concern (Barnatt & Penny, 2004). The Lead Legacy Project was established in 2004 by English Heritage, English Nature and the Peak District National Park Authority (PDNPA) to ensure that the Peak District's lead mining heritage, and its many qualities, was available for future generations to explore and enjoy. The aim was to undertake a comprehensive survey of the lead rakes to identify areas of conservation concern, raise awareness amongst the general public, negotiate with farmers, establish partnerships with conservation organisations and protect important sites (Barnatt & Penny, 2004).

2.7 Metal tolerant plant species

The contaminated soils that are present on metalliferous mine waste and lead rakes have developed distinctive floral swards that are most often only found at these historically abandoned mining sites (Penny, 2009). The plant species (also sub-species or ecotypes) that have adapted to these contaminated conditions include metallophytes and pseudo-metallophytes, more recently termed obligatory and facultative metallophytes (Kuta *et al.*, 2012; Pollard, Reeves, & Baker, 2014), that have developed a resistance to the heavy metal concentrations over decades or hundreds of years. In the UK, these sites are being designated as protected areas to protect the distinctive biota that exist here (Baker & Proctor, 1990; Batty, 2005).

Plant species that survive on metalliferous soils develop mechanisms that enable them to tolerate the ordinarily harsh conditions. Several strategies have been identified and plants may be seen as metal indicators (Hernandez & Pastor, 2008), excluders, accumulators and hyperaccumulators (Gall *et al.*, 2015).

Indicators control the uptake of heavy metals to shoot tissues so that the concentrations present in the plant are at a constant with metal concentrations within the soil (Baker, 1981). Certain species of these plants can be used as an indicator for the detection of metalliferous parent materials (Brooks, Lee, Reeves, & Jaffré, 1977).

Excluders develop mechanisms which avoid the accumulation of toxic levels of heavy metals in tissues which are more susceptible to damage. This is achieved by several mechanisms at the cellular level which restrict uptake in the roots and impede translocation to shoot tissues (Baker, 1981; Rascio & Navari-Izzo, 2011). These mechanisms differ from species to species and also depend on the type of metals and adaptations the species has undergone (Hall, 2002).

Accumulators actively accumulate heavy metals in their above-ground tissues despite the amount present in the soil. Whilst the roots can still contain high amounts of accumulated metals, the shoots will contain larger quantities (Baker, 1981; Baker & Walker, 1989). The quantity of metal accumulation and the mechanisms of transport used to uptake metals is determined by the plant species or ecotype, the metals present and the interactions between them (Baker & Walker, 1989; Rascio & Navari-Izzo, 2011).

Hyperaccumulators are plant species that can accumulate vast quantities of heavy metals compared to other species found on metal contaminated soils without any adverse effects (Pollard *et al.*, 2014). These specially adapted species are able to uptake heavy metals from soils proficiently and have efficient mechanisms for translocation from root to shoot tissues. Once in the aerial tissues the hyperaccumulator is capable of detoxification and sequestration into leaf tissues (Ent *et al.*, 2013; Rascio & Navari-Izzo, 2011).

Baker and Walker (1989) observe that the majority of metallophytes are usually accumulators, whilst the pseudo-metallophytes are frequently excluders and these are discussed further below.

Pseudo-metallophytes are species whose seed mix originates in the same area (Batty, 2005); however, over time selective reproduction processes allow certain species to thrive in areas of high contamination, whilst a short distance away the same species may be less tolerant to heavy metals (Stefanowicz *et al.*, 2014; Woch *et al.*, 2016). Tordoff *et al.* (2000) state that this natural selection process develops due to a low occurrence of metal tolerant genetics in populations existing at nearby, less contaminated or uncontaminated sites. They explain that as a species encroaches into an area of contaminated waste, high selection pressures coupled with the highly inheritable gene can mean rapid colonisation and an area of very tolerant species. It is this ability of plant communities to develop metal tolerances that determine the sward development, structure and density in such species rich metalliferous areas (Simon, 1978).

Some examples of pseudo-metallophytes that thrive on heavy metal contaminated waste in the limestone grasslands of the Peak District and other calcareous mine spoils are:

- Wild thyme, *Thymus polytrichus* (Penny, 2009; Smith, 1979)
- Salad burnet, *Sanguisorba minor* (Penny, 2009)
- Harebell, *Campanula rotundifolia* (Smith, 1979)
- Common birds foot trefoil, *Lotus corniculatus* (Bradshaw, 1997; Shimwell & Laurie, 1972)
- White clover, *Trifolium repens* (Bradshaw, 1997; Shimwell & Laurie, 1972)
- Eyebright, *Euphrasia sp.* (Shimwell & Laurie, 1972; Simon, 1978)
- Red clover, *Trifolium pratense* (Bradshaw, 1997)
- Meadow buttercup, *Ranunculus acris* (Cooke, Johnson, Davidson, & Bradshaw, 1976)

There are several hundred other pseudo-metallophyte species that can adapt to living on metalliferous waste (Tordoff *et al.*, 2000) and these species are a selection that are also commonly found on calcareous grassland and meadows (Streeter, Hart-Davies, Hardcastle, Cole, & Harper, 2009) such as those found in the Southern Peak District. In particular, the legumes (white and red clover and common birds-foot trefoil) amongst these species highlighted above have an important role in the establishment of the species rich plant communities found at mining sites in the Peak District (Bidar *et al.*, 2007; Bradshaw, 1997; Johnson *et al.*, 1977). On heavy metal contaminated areas where there may be a low nutrient status, these legumes play an important role in nitrogen cycling and bioremediation over time (Hao *et al.*, 2014).

Metallophytes are plant species that are able to accumulate metals and metalloids several times the concentration of other species, and this is sometimes called hyperaccumulation (Alford *et al.*, 2010; Reeves & Brooks, 1983). The concept of hyperaccumulation according to Pollard *et al.* (2014) is that concentrations of heavy metals and other trace elements should be two or three times higher than those usually found in most other plant species growing on uncontaminated soils. The thresholds for heavy metals contained in dry leaf tissue are as follows: 100ppm for Cd, 300ppm for Cu, 1000ppm for As, Ni and Pb and 3000ppm for Zn (Gall *et al.*, 2015; Pollard *et al.*, 2014). There are currently approximately 500 plant species that hyperaccumulate heavy metals, and most of these are limited to growing on metalliferous wastes and soils (Pollard *et al.*, 2014). Ufimtseva (2015) reports that some plant families, especially Caryophyllaceae, Violaceae and Fabaceae, are specialists at accumulating certain metals and, in the case of Violaceae, this is Zn. However, they also note that metallophytes can either accumulate several heavy metals or be selective of individual elements. There are presently known hyperaccumulators that can tolerate and accumulate Pb. As, Zn, Cd, Cu, Ni, Co, Se and Mn, although 75% of these are specialist Ni accumulators (Ufimtseva, 2015). Metallophytes can accumulate bioavailable heavy metals in their shoots, leaves and other above ground tissues (Gall *et al.*, 2015), although Pollard *et al.* (2014) state that the majority of species that thrive on metalliferous soils prohibit the accumulation of heavy metals in the shoots. Alford *et al.* (2010), however, do note that much research is needed to ascertain the transport of heavy metals between uptake at the roots and the accumulation in other parts of the plant in metallophytes.

In the Peak District, the metalliferous wastes and mining areas support four key metallophytes (Baker *et al.*, 2010):

- Spring sandwort (*minuartia verna*), or Leadwort as it is sometimes referred to, is in flower May-September, and in the North of England favours Carboniferous Limestone geology, almost exclusively on lead mining spoil heaps (Barnatt & Penny, 2004; Streeter *et al.*, 2009). This species has the capability to accumulate large amounts of the heavy metals Pb and Zn. Penny (2009) reports that this species has been known to accumulate up to 500ppm of Pb and 7500 ppm of Zn. This is a species that is scarce in the UK and the majority of this species is found in the Peak District (Penny, 2009).
- Alpine penny-cress (*thlaspi caerulescens*) flowers April to August and is found on limestone outcrops and mining spoil, especially those rich in Pb and Zn (Streeter *et al.*, 2009). It is tolerant of these heavy metals and accumulates them in its leaves (Penny, 2009). Those found in the UK are at its north-westerly limit and it too is a nationally scarce species (Barnatt & Penny, 2004).
- Mountain pansy (*viola lutea*) flowers from May to August and is limited to the uplands of the UK (Streeter *et al.*, 2009). The species is also known by the name ‘zinc violet’ due to its tolerance and accumulation of the heavy metal Zn (Kuta *et al.*, 2012). The plant family Violaceae contains many metallophyte species (such as *viola lutea*) which occur exclusively on contaminated soils and are capable of accumulating very large amounts of heavy metals in their above ground tissues (Słomka *et al.*, 2011). This species occurs in two colours in the UK (yellow and blue/violet) and the Peak District contains a significant portion of the yellow population (Barnatt & Penny, 2004; Penny, 2009).
- Pyrenean scurvy grass (*cochlearia pyrenaica*) from the family Brassicaceae is usually confined to open and wet locations and flowers mid-summer (Streeter *et al.*, 2009). Like other members of its plant family, it is a metallophyte that can accumulate large

amounts of heavy metals (Nawaz, Iqbal, Bliek, & Schat, 2017) and is also found on spoil heaps of lead and zinc mines. Although it infrequently found in the Peak district, there are good examples near Castleton (Penny, 2009).

2.8 Conclusion

This literature review has examined the how the geological formation of the Peak District more than 300 million years ago led to the sedimentary deposition of Carboniferous Limestone in the White Peak area (Wolverson Cope, 1998). This sedimentary limestone underwent physical and chemical processes which lead to the deposition of metalliferous mineral ores into faults and fissures within the bedrock geology (Montgomery, 2003). These mineral ores were fervently sought after from the Roman era right up to the 20th century for their monetary value and industrial uses (Ford & Rieuwerts, 2000). This mineral extraction and other associated processes changed not only the landscape but also the ecology of many places in the Peak District (Barnatt & Penny, 2004). The limestone geology in the Peak District has had a substantial role in influencing the species richness, diversity and composition of calcareous grasslands which have developed (Anderson & Shimwell, 1981). However, areas of grassland within this calcareous landscape have been radically changed by the historic mining processes, leaving behind a terrain with a changed topography and contaminated substrates (Penny, 2009). Changes to the soil properties have seen extremely high concentrations of heavy metal contamination in soils in some areas, so much so that some local vegetation communities are unable to tolerate them (Baker & Proctor, 1990; Shimwell & Laurie, 1972). It is the bioavailable fraction of these heavy metals that are available for uptake by plant species, and several factors can affect this within the soil and the plant itself (Dubbin, 2001; Gall *et al.*, 2015). This heavy metal contamination, the chemical and physical properties of the soil (Stefanowicz *et al.*, 2014), a changed topography, management regimes, conservation efforts (Penny, 2009), the selective pressures and the genetic propensity of some species (Tordoff *et al.*, 2000) have allowed the establishment of metal tolerant plant communities which thrive without competition from less stress tolerant species (Woch *et al.*, 2016).

Chapter 3: Methodology

3.1 Introduction

This research has several inter-related objectives associated with the distribution and relationships of numerous soil properties and plant species located in areas known to have a history of mining activity and the presence of limestone grasslands.

The inter-related objectives of this study were:

1. To assess the soil characteristics and calcicolous vegetation at sites of historical mining activity.
2. To analyse the relationship between the soil properties and vegetation species richness and diversity at the sites.
3. To determine the bioavailable fraction of the heavy metals and metalloids in soil samples taken from the sites.
4. To measure the distribution of any heavy metals and other elements found in topsoil samples and to evaluate their influence on vegetation structure.

It is the aim of this chapter to describe and discuss the study sites and their selection criteria, sampling strategies, laboratory techniques, data analysis and the justification for their selection. The methods and techniques outlined here were used to achieve the aims and objectives of this research. Sections 3.2 to 3.10 of this chapter discuss both the location and the relevant site history. This is followed by sections on sample collection, survey methods and soil sample preparation in sections 3.11 to 3.13. Finally, the methods for sample analysis and data analysis are discussed in sections 3.14 through 3.20.

3.2 Site selection

All study sites were chosen as areas situated on the Carboniferous Limestone in the Peak District, UK which contain evidence of historic mining activity and examples of typical limestone grassland vegetation (in particular, calcareous and calaminarian grasslands communities). Examples were sought using the Natural England Designated Sites View Website (Natural England, 2017), DEFRA website Multi-Agency Geographic Information for the Countryside (MAGIC) (Ordnance Survey, 2016) and with the expert knowledge of land managers and advisors at Natural England, The Peak District National Park Authority, Plantlife and Derbyshire Wildlife Trust. Initial site investigations of eleven sites narrowed down the number of study sites to seven safe and accessible areas meeting the study inclusion criteria. Lathkill Dale, Winnatts Pass, Rose End Meadows and Pindale were dismissed due to safety and access issues. The area encompassing the seven selected study areas (The Peak District) and the individual sites are described in the following sections.

3.3 Study area -Peak District

The Peak District National Park became Britain's first National Park in 1951 (McMorrow, 2005) and covers an area of 555 square miles (1,438 square kilometres), incorporating areas of four main counties; Derbyshire, Yorkshire, Staffordshire, and Cheshire (PDNPA, 2017) as well as parts of Greater Manchester to the north-west (McMorrow, 2005). It sits at the southern tip of the Pennines (Anderson & Shimwell, 1981) and is a region of contrasting landscapes containing three distinct areas; the White Peak, the Dark Peak and the South-west Peak [*Fig*. 3:1] (PDNPA, 2017). These areas are defined by their underlying geology.

The Dark Peak geology consists of gritstones and shales (Anderson & Shimwell, 1981), giving it a landscape containing deep valleys and cloughs, dramatic, vast plateaus and long, rocky ridges which support upland habitats such as blanket bog, large moorland areas (McMorrow, 2005) and associated vegetation such as heather, cotton-grass and bracken (Anderson & Shimwell, 1981).

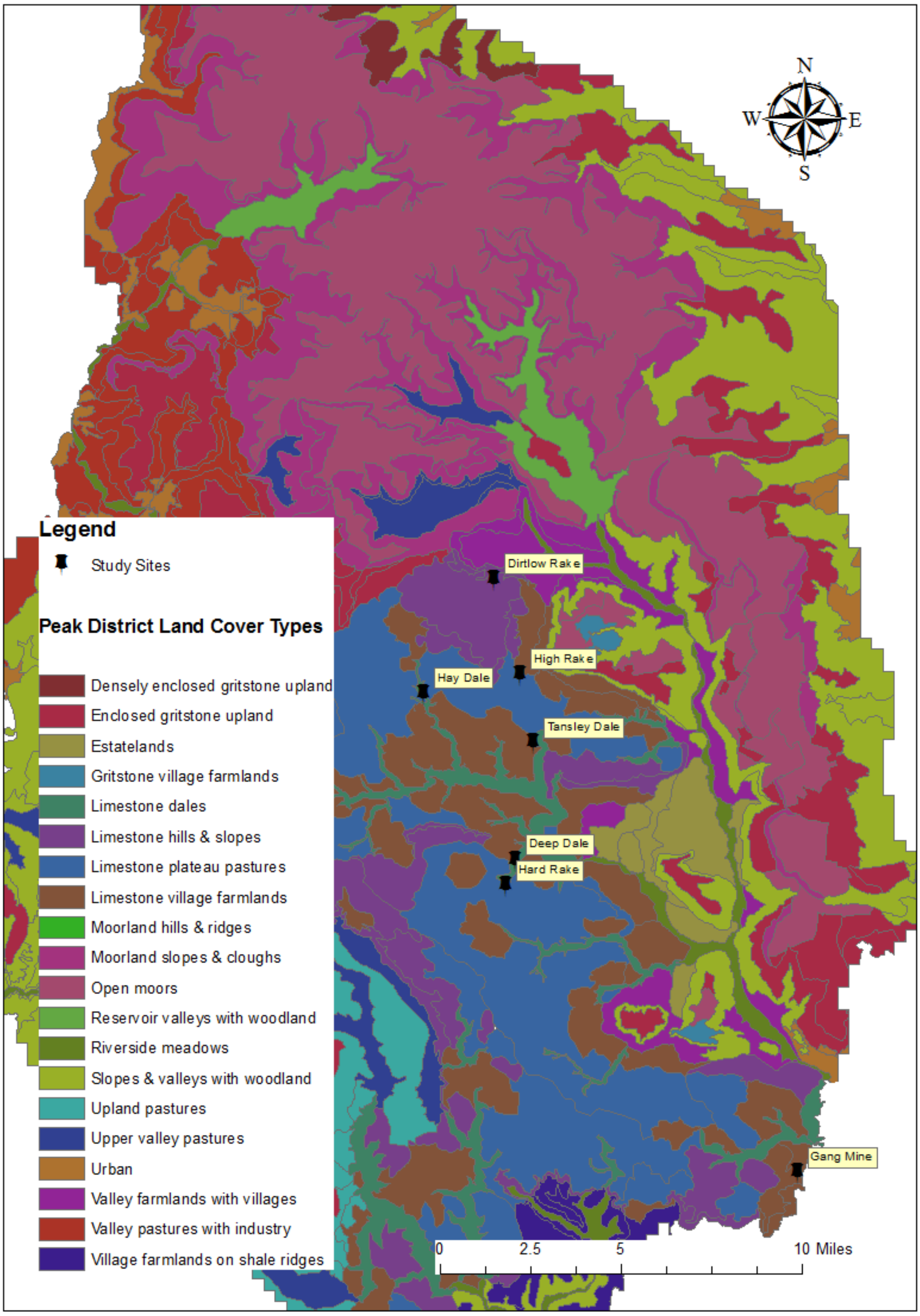


Figure 3:1. The contrasting landscapes of the Peak District. Source (PDNPA, 2017)
GIS Open data Landscape character type map kindly supplied by the Peak District National Park GIS office

It is this sombre-looking and dark-coloured vegetation and these habitats that give it the name Dark Peak. The South-west Peak is smaller in area than the Dark Peak and it too has gritstone geology and contains a mixture of large moorland areas in the uplands and pastures and hay meadows within its lower valley areas, which eventually give way to the lower hills within the counties of Cheshire, Staffordshire and Shropshire (PDNPA, 2017). However, the research undertaken here focuses on locations within the White Peak of the National Park [*Fig*. 3:2].

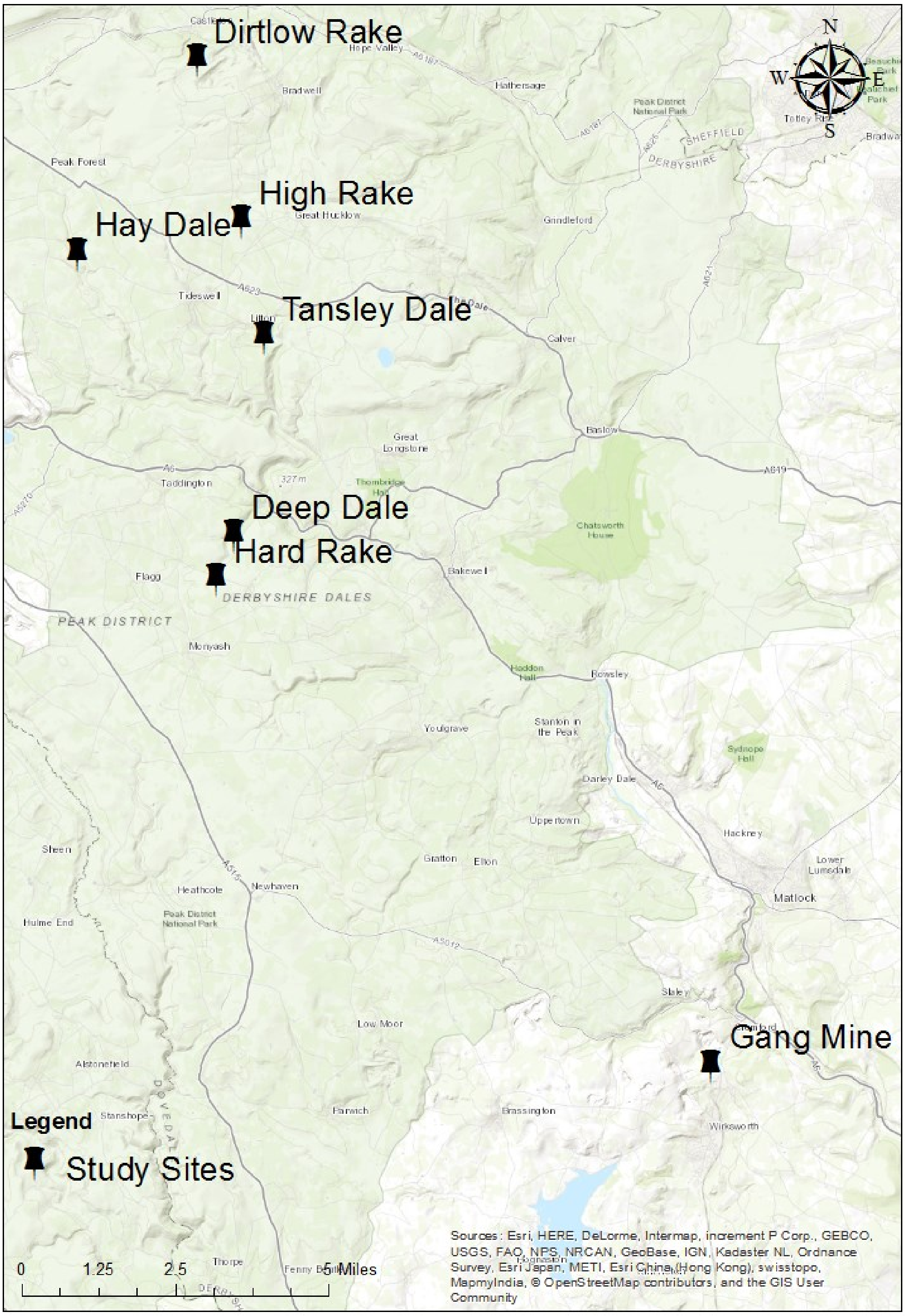


Figure 3:2. Study site locations in the White Peak. Source (ESRI, 2018; Google Earth Pro, 2017)

The geology of the White Peak is primarily made up of a major outcrop of sedimentary Carboniferous Limestone (Monsal Dale Limestone Formation, Bee Low Limestone Formation and Eyam Limestone Formation and others), formed over 300 million years ago (Anderson & Shimwell, 1981). In places, it is twenty kilometres wide and thirty kilometres in length, and this limestone outcrop of the White Peak is mostly bounded by the younger millstone grit and shale geology (Millstone Grit, Shale Grit and Kinderscout Grit and others) of the Dark Peak. The outcrop of limestone in the White Peak is sometimes referred to as the Derbyshire Dome (Banks *et al.*, 2012). It contrasts in character with the Dark Peak, and this is evident in the landscape forms and associated vegetation that have developed in its environment (Anderson & Shimwell, 1981). Other geological and chemical processes associated with the Carboniferous Limestone also allowed the formation of many mineral veins in the White Peak area (Ford & Rieuwerts, 2000). It is these minerals veins (that were eventually mined and extracted) and the underlying limestone geology that allow the exploration of the unique vegetation and historic mining aspects of this thesis. The following sections provide descriptions of the chosen study sites and, where possible, the location, geology, mining activity and vegetation communities that occur in these areas are presented.

3.4 Deep Dale

Deep Dale is situated in the Carboniferous Limestone area of the Peak District and is considered one of the area's most scenic dales. It is located near Sheldon, Derbyshire (Grid Ref: SK 165 698) (Plantlife, 2010) and runs roughly north-south, starting in the Wye Valley to the north and terminating one and a half kilometres west of the village of Chelmorton. The larger town of Buxton sits approximately three kilometres to its north-west of Deep Dale. The orientation of the dale ensures that there are both shaded areas and parts that are exposed to abundant solar radiation, and this is reflected in the diversity of plant species found in Deep Dale (Derbyshire Wildlife Trust, n.d.). The principal grassland vegetation communities present at the study area are CG2, MG5 and U4 (Costley, 2013) and the dale has been designated a Site of Special Scientific Interest (SSSI) (Plantlife, 2010). This study area on the western facing side of the dale has been owned and managed by Plantlife since 1999, and covers an area of 36 hectares. The opposite dale side is under private ownership (Costley, 2013) and consists mainly of wooded areas. It is a typical limestone dale containing steep-sided scree slopes and high limestone cliffs. A footpath runs almost the entire length of the dale, as does a seasonal stream which resurges from the dale floor in wetter weather. The central area of the dale is intersected by several mineral veins, containing calcite and galena, which have a long history of mining activity (Heathcote, 2004). A prominent hollow in Deep Dale [*Fig.* 3:3] was perceived to be a test pit of some kind. According to Ford (2000), chert, a silica rock containing quartz, was mined in Deep Dale for the pottery industry and extensive lead mining also took place directly above on the dale-tops (Heathcote, 2004).

Figure 3:3. The perceived test pit in Deep Dale. Source (Author, 2017)

3.5 Dirtlow Rake

Dirtlow Rake is located on Carboniferous Limestone in the Peak District to the south of the town of Castleton (Grid Ref: SK 156 822) (Natural England, 2008). It has a characteristic mineral vein system (Waltham, 2006) and one of the main fissures in the ore fields of the Southern Pennines (Natural England, 2008). The site contains both historic open cast mining in the form of a trench or chasm, and deep, grilled shafts [*Fig*. 3:4] (Ford & Rieuwerts, 2000; Waltham, 2006) and is five hectares in size (Newman, 2016). The typical mineral assembly found at Dirtlow Rake comprise calcite, fluorite, barite and galena (Natural England, 2008; Waltham, 2006) and the site has a long history of mining activity (Waltham, 2006). Because of the divergent geological formations, both here and at Pindale, a short distance to the east the site it has been designated a SSSI (Natural England, 2008). Since the cessation of activity at Dirtlow, conservation work in the area endeavoured to leave a landscape with mostly tolerably low lead concentrations interspersed with areas of galena-contaminated soil in the hope that it would encourage metallophyte plant communities to develop to improve local biodiversity (Waltham, 2006). Presently, the site supports a complex mosaic of grassland communities including calcareous, calaminarian and semi-improved grassland, with the areas of thin mineral substrate dominated by rich and diverse herb vegetation (Newman, 2016).

Figure 3:4. The open cast mining area at Dirtlow Rake. Source (Dace, 2011)

3.6 Gang Mine

Gang Mine is situated on Carboniferous Limestone in the Southern Peak District and lies two kilometres north of Wirksworth, and approximately four and a half kilometres south-west of Matlock (Grid Ref: SK 287 558) and covers an area of 8.26 hectares (JNCC, 2010). The mine has a long history of mining activity and was reported to be an ancient lead mine even in the 17th Century (Natural England, 2012). The main mineral deposits lie at the outcrop of the Gang Vein that runs through the Gang Mine site. The area now comprises of rough ground made up of heaps of lead miners' waste spoil, now covered in vegetation to leave grassy hillocks and pits (Ford, 2005). The key minerals found at the site are galena, barite and calcite (Ford, 2005; Natural England, 2012) and smaller amounts of sphalerite, pyrite and fluorspar (Ford, 2005). The site has been designated a Site of Special Scientific Interest (SSSI) and a Special Area of Conservation (SAC) due to the abundance of species rich and diverse plant communities found on these calaminarian and calcareous grasslands (JNCC, 2010; Natural England, 2012). The high levels of lead and cadmium present in the soils support a unique assembly of plant species that are tolerant to the metalliferous soil conditions and reduced competition from other species allows them to thrive. Such communities of metallophyte (metal tolerant) species are confined to contaminated, mineralised limestone areas like those found here (Natural England, 2012).

3.7 Hard Rake

The study site at Hard Rake is situated on Carboniferous Limestone in the Peak District (British Geological Survey, 2017). It is one and a half kilometres to the west of the village of Sheldon and approximately six kilometres west of Bakewell, Derbyshire (Grid Ref: SK 1578 6833) (UK Grid Reference Finder, 2011). The site contains the remnants of mining activity in the form of deep hollows surrounded by spoil heaps creating a pock-marked landscape [*Fig*. 3:5 & 3:6]. It is close to the well-known Magpie Mine and there is a long history of lead mining in the area dating back to at least the seventeenth century due to the many ore veins that intersect the area (Ford & Rieuwerts, 2000). Little information can be found listing the key mineral deposits found at Hard Rake; however, galena, fluorspar, calcite and barite form more than ninety percent of the minerals present in veins in this area (Ford & Rieuwerts, 2000). Ford (2002) does state, however, that calc-spar was being produced at Hard Rake in the nineteenth century as a by-product of the previous lead mining spoil. The grassland at Hard Rake contains a mosaic of calcareous vegetation communities (Newman, 2016) and as such the land was purchased by the Peak District National Park Authority (with funding from the Heritage Lottery Fund) with the aim of conserving the species rich vegetation present at the site (PDNPA, 2016).

Figure 3:5. Extensive landscape modification at Hard Rake. Source (Author, 2017)

Figure 3:6. Large hollow at Hard Rake. Source (Author, 2017)

3.8 Hay Dale

Hay Dale (Grid Ref: SK 120 770) is one of the five dales that make up Derbyshire Dales National Nature Reserve which in total covers 385 hectares, the majority of which is designated as a SSSI. The dale is located at the northern most end of Monks Dale SSSI, and lies between the villages of Peak Forest and Wheston, 6.8 kilometres north-east of Buxton and 12.7 kilometres north-west of Bakewell (Natural England, 2014). The dale is situated on Carboniferous Limestone bedrock which is exposed along its length with areas of scree also present. The dale is part of the Limestone Way and has a grass-lined track running its complete length. It has an area of approximately eight hectares, and it runs north-west to south-east permitting species rich and diverse flora to thrive on its south-west facing slope (Natural England, 2014). The grassland present in the dale contains a florally rich calcicolous sward (Natural England, 2014). Mine workings are present midway along the dale which are reported to have been worked for calcite as recently as the 1960s after being originally worked in the 1930s (Ford, 2002). Ford (2002) reports it as being a local development which mined calcite from an isolated lead vein present in the Hay Dale geology [*Fig*. 3:7]. He also notes that no records from the working life of the mine exist, so it assumed here that minerals present are calcite, fluorspar, barite and galena consistent with other mineral deposits in the area.

Figure 3:7. Access ramp leading away from Hay Dale calcite mine. Source (Author, 2017)

3.9 High Rake

High Rake is located west of Great Hucklow, close to the hamlet of Windmill, Derbyshire (Grid Ref: SK 1636 7775) (Historic England, 2017). The mine at High Rake is a rare and well-preserved monument of lead mining history in Derbyshire and the UK, and as such is scheduled under the Ancient Monuments and Archaeological Areas Act 1979 (Historic England, 2017). It contains the remnants of ore processing buildings, an ore crushing circle, chimneys and other structures, along with large mounds and hillocks [*Fig.* 3:8] (Historic England, 2017). The site is situated on a large mineral vein up to twelve metres wide in places (Rieuwerts, 2011), and has been mined for lead since at least the sixteenth century. Evidence of excavations on the same vein, close by, date back to the thirteenth century (PDNPA, 2011). As well as the extraction of lead, waste mounds in the local area have also been re-worked for calcite (Barnatt, 2011), fluorspar and barite, valuable gangue materials associated with lead ore (Historic England, 2017). Between 2000 and 2008, the High Rake site was the focus of extensive archaeological excavations and conservation work (Rieuwerts, 2011). Today, the lead rake is owned by the Peak District National Park (Newman, 2016; PDNPA, 2004) and includes planted trees and areas of calcareous grassland (Newman, 2016).

Figure 3:8. Mining structures at High Rake mine. Source (Author, 2017)

3.10 Tansley Dale

Tansley Dale (Grid Ref: SK 1702 7474) is situated at the northern end of Cressbrook Dale SSSI, Derbyshire and branches eastward in the direction of the village of Litton, with the smaller village of Wardlow lying to the east. The dale sits on the Carboniferous Limestone of the White Peak and Tansley Dale runs east to west with steep sided dale sides facing to the south and north (Natural England, 1986). Cressbrook Dale, as a whole, is described by Natural England (1986) as one of the most important geological sites in Derbyshire. The area within Tansley Dale [*Fig*. 3:9] and along its tops contains a large mining complex (Barnatt *et* *al.*, 2013) and has an area of approximately 21 hectares (Historic England, 2017). The area holds several historic mining features including Hading Vein, Seedlow Rake, Nay Green Mine and the Arbourseats Vein and activity long predates the 18th and 19th century features that are present (Barnatt & Penny, 2004). Because of the complex nature of mining features at Tansley Dale it is scheduled under the Ancient Monuments and Archaeological Areas Act 1979 (Historic England, 2017). Little documentation exists of the activity at Tansley Dale, although 19th century maps show features in the dale as ‘old lead mines’ and these are known to have been reworked in the 20th century for gangue minerals which accompany the metallic ores in veins and deposits (Historic England, 2017). Cressbrook Dale, including Tansley Dale, contain some of the best examples of limestone grasslands in the White Peak and the steep sided dale sides at Tansley dale comprise short sward of calcareous vegetation (Natural England, 1986).

Figure 3:9. Looking up Tansley Dale from dis-used structure. Source (Author, 2017)

3.11 Vegetation surveys and sampling strategy

Vegetation surveys were undertaken to determine the species richness, diversity and composition of the grassland communities present at each site. It was thought practical to undertake this aspect of research between May and September, when many species found on limestone grassland habitats are in flower (Streeter *et al.*, 2009), as this allowed for ease of identification of plant species. In order to supply an evaluation of the species richness, composition and cover/abundance of vegetation at the study areas, it was proposed that recordings should be made using 1m x 1m quadrats. Numerous statutory and volunteer organisations record and survey vegetation cover/abundance and presence using quadrats (Emmett *et al.*, 2011). The quadrats were placed along three transects, emanating in three directions from a perceived mining source at intervals of approximately four metres and originating two metres from the apparent centre of the pit, test pit, mine entrance or area of mining activity. This method was chosen in order to identify zones of heavy metal mobilisation and highlight apparent influence on vegetation within these zones. Sampling two metres from the perceived source would also avoid the sampling of any raw materials existing at the mining source. Vegetation was recorded at sixteen points in total along three transects, each transect reaching approximately twenty metres from the transect origin point (two metres from the perceived mining source) [*Fig.* 3:10].

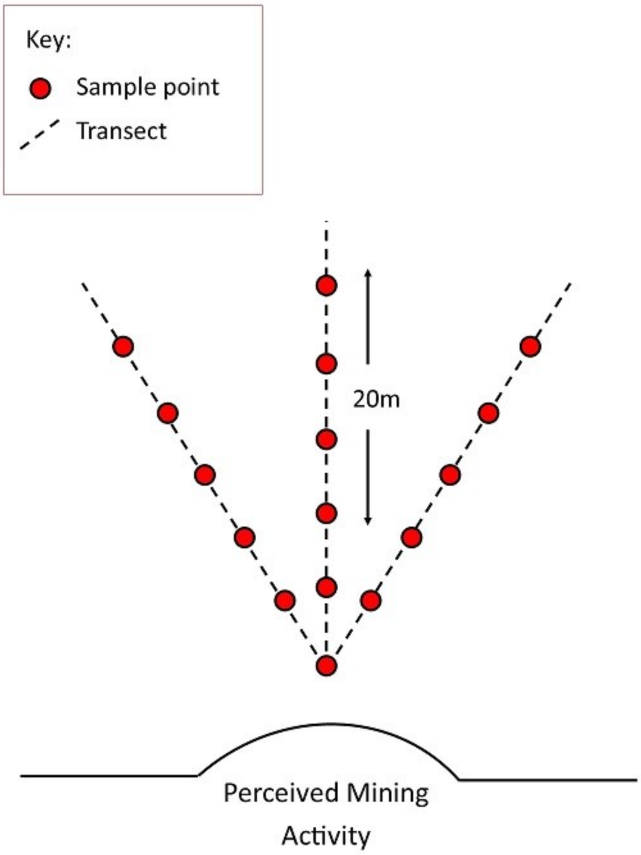


Figure 3:10. Sampling transects origin point close to perceived source of mining activity. Source (Author, 2016)

The presence and cover/abundance of calcicolous and other species commonly found on limestone and metalliferous grasslands were recorded to define composition and quantity of flora present to enable calculations of species richness and species distribution (Emmett *et al.*, 2011).

The percentage cover/abundance of each species found on these areas of grassland was visually estimated using two methods, the standard DOMIN scale [*Table.* 3:1] and the Braun-Blanquet scale [*Table.* 3:2]. Both the Braun-Blanquet and DOMIN scales of cover/abundance measure the extent of the living parts of a plant species from a vertical perspective of the ground cover. The Oxford dictionary of plant sciences describes cover/abundance as a linked system, whereby these scales report percentages of cover where vegetation is plentiful and abundance where fewer individuals exist (Allaby, 2017). The DOMIN scale was also chosen as a method to aid in data analysis because of the simple grading system it employs. The Braun-Blanquet scoring system was selected as it utilises comparatively few cover percentage categories (six) and so was an effective way of recording the vegetation cover/abundance (Lepš & Hadincová, 1992). It must be noted that these scales of cover/abundance can exceed 100% cover due to the structure and overlap of plant species because of growth height and spread (Rodwell, 2006).

Table 3:1. Domin scale of cover/abundance Source (Rodwell, 2006)

Cover	Domin
91-100%	10
76-90%	9
51-75%	8
34-50%	7
26-33%	6
11-25%	5
4-10%	4
<4%(many individuals)	3
<4% (several individuals)	2
<4% (few individuals)	1

Table 3:2. Braun-Blanquet scale of cover/abundance. Source (Rodwell, 2006)

Cover	Braun-Blanquet Scale
76-100%	5
51-75%	4
26-50%	3
6-25%	2
1-5%	1
<1%	0

The identification of plant species incorporated the use of the Collins: Flower Identification Guide Book (Streeter *et al.*, 2009) and grassland plant identification guides published by the Field Studies Council (Bebbington, 2005; Gulliver & Gulliver, 2002). The identification of flowering plants species was selected as species not in flower are potentially difficult to classify for the non-botanist. Each site was visited over the course of four days in August 2016 to ensure that species were at the same stages of their growth cycles.

Global Positioning Satellite (GPS) recording technology was utilised to record the exact location (+/- 2m) and elevation of each quadrat to enable GIS analysis and ensure precision when collecting soil samples.

3.12 Soil sampling

The soil sampling strategy employed was to take samples along the same transects as the recording of vegetation, using the same coordinates taken from the GPS data in addition to using compass bearings and a retractable tape measure. Three or four other random samples outside the transect zones were also collected at each site. Due to the typically shallow nature of calcareous soils overlying limestone geology (Carroll *et al.*, 2003; Hermy, Honnay, & Adriaens, 2008), samples were taken from 0-15cm below surface vegetation. Firstly, any vegetation at the surface of the sample point was carefully prised free to permit access to the soil below and allow replantation of the turf. Each sample was collected using a measuring trowel for ease of sample collection from the correct depth, and placed in a polythene bag. After placing the sample in the bag, much of the air was expelled and the bag sealed. Each sample was then labelled with the date, GPS coordinates, site name and plot number and transported to the laboratory for storage (Rowell, 1994). A total of nineteen or twenty samples were taken from each site, including the extra random samples, except for the Hard Rake site. Due to the size of the site and extent of mining activity present at Hard Rake, two areas were sampled approximately 250 metres apart, producing 38 samples in total from two sources of mining activity. In total, 155 samples were taken from all sites.

3.13 Sample preparation

Samples were first dried in preparation for further analysis. Each sample was placed into a paper bag and positioned upright in an oven at 40^{o} C for forty-eight hours. Once dried, a pestle and mortar were used to disaggregate the samples into smaller fragments in a fume cupboard to safeguard against inhalation of harmful particles. Any stones, roots or other vegetative matter were ejected. After disaggregation an automated sieving machine was utilised to pass each sample through a 2mm sieve for 5 minutes. Sieving through a 2mm sieve is a widely used method for several reasons, it excludes larger soil aggregates and vegetative matter, gives a fine earth sample which includes all primary soil particles and is a particle size which allows the determination of many soil variables using standard analytical procedures (Rowell, 1994). Once sieved and dried, a sub-sample was retained and labelled for identification using the sample location, plot number, particle size and date. All samples were then stored pending further analysis (Rodwell, 2006). The drying, disaggregation, sieving and homogenising of soil samples has been shown to significantly improve the quality of data recorded from analytical procedures (Shefsky, 1999).

3.14 Determination of total heavy metal content

The determination of total heavy metal concentration in each soil sample was undertaken using a Thermo Scientific NITON XL3t 600 series X-ray fluorescence (Xrf) instrument. Xrf analysis is widely used in the mining exploration and production industries (Thermo Scientific, 2010) as it allows an efficient, in-depth and fast analysis of soil samples and ores with relatively little preparation and handling (Gazulla, Rodrigo, Vicente, & Orduña, 2010; Thermo Scientific, 2010).

Firstly, a homogenised sub-sample was separated from each bulk, pre-sieved sample using the cone and quarter method. Soil from the bulk sample was spread on a tray, this was divided into four quadrants using a spatula and soil was taken from each of these quadrants and mixed. This was repeated until a sub-sample of the required size was obtained (Rowell, 1994; Shefsky, 1999). Each bulk sample had three sub-samples prepared using the cone and quarter method and these were placed in a plastic sample pot lined with Mylar 6-micron thin film. Each sample was then compacted in the sample pot to ensure no air pockets that could affect analysis were present, and then sealed with a lid.

Secondly, the Xrf analyser was initialised and the optimum analysis running time was determined by analysing a sample at increasing intervals of one to five minutes. The optimal Xrf operational duration was determined to be four minutes, as this was the analysis time at which little or no fluctuation of metal concentration in the sample was noted. A certified reference material (CRM) was analysed at the commencement of each sample analysis session and repeated after every forty samples to ensure that no erroneous readings were recorded in case of operational errors. The CRM used during analysis was CRM 73308 for stream sediments. Limits of detection in soil (ppm) were as follows: Pb/10ppm, Zn/16ppm, As/13ppm, Cd/25ppm, Ni/65ppm, Cu/25ppm.

Lastly, each sample was analysed by the Xrf instrument for 4 minutes and the total heavy metal concentration in samples was recorded and stored on a spreadsheet for further analysis.

3.15 Determination of the available fraction of heavy metal contaminants

It is well established, that the measurement of the total heavy metal concentrations in soils is inadequate to fully explain the environmental impacts of these contaminants (Harmsen & Frintrop, 2003; Kim *et al.*, 2015; Maiz, Esnaola, & Millfin, 1997). Fractions of heavy metals are sometimes bound to soil particles so strongly that it is unlikely they will have any notable effects on the surrounding environment, and so it is commonplace today to try and assess the portion of heavy metal contaminants that are available for uptake by biota, often referred to as the bioavailable fraction (Harmsen & Frintrop, 2003). There are several definitions of bioavailability dependent on the organism that is exposed to the contaminant (Kim *et al.*, 2015). Harmsen and Frintrop (2003) describe available fraction as that loosely bound to soil particles and in soil pore water and therefore freely and easily available for uptake by plants. They also discuss the potential bioavailability, that is the portion of contaminants that can become available within a specific time dependent on chemical and physical conditions within the soil. Because of these different forms of available contaminants, several methods of extraction have been developed to measure them (Harmsen & Frintrop, 2003; Kim *et al.*, 2015; Maiz *et al.*, 1997).

Both single and sequential extraction methods have been developed to assess either target elements or elements bound to a particular phase of soils, sediments or compounds. These methods use extractants to free elements from exchange sites or those attached to or linked with soil organic matter. Both single and sequential extraction procedures have advantages and disadvantages when assessing the phytoavailable fraction of heavy metals (Rao, Sahuquillo, & Sanchez, 2008).

Sequential extraction methods utilise a combination of reagents of differing strengths such as HCl, EDTA, DTPA, $CaCl_2$, and H_2O, usually requiring three to seven steps. These reagents extract metal fractions from specific soil phases, with stronger reagents used to target and extract metals strongly bound to their soil phase. These reagents are used in stages with the soil filtrate remaining from weaker extraction reagents used as the sample matter for further stronger extractants (Rao *et al.*, 2008).

The majority of SEPs are variations of the approaches recommended by Ure, Quevauviller, Muntau, and Griepink (1993)(often referred to as the BCR method) and Tessier, Campbell, and Bisson (1979). These SEP methods differ in the amount of metal fractions extracted and the type of reagents used. The Tessier technique extracts and partitions metals into five fractions which include exchangeable, carbonate, Fe and Mn oxides, those bound to organic matter and residual fractions. The BCR method however, encompasses only three steps and extracts metals bound to organic matter, Fe oxides and carbonates. European experts have adapted the BCR method in order to create a widely accepted and standard protocol that can be easily reproduced in laboratories.

SEPs can provide valuable information in environmental research as they can extract specific metals bound to certain exchange sites or fixed to or linked with the mineral or organic matter phases of soil samples and can, under certain environmental conditions, measure the mobile or potentially exchangeable fractions of elements (Rao *et al.*, 2008). This is especially true for the relatively less mobile metals such as Pb and Cu especially under certain pH ranges where a strong acid reagent is required (Kim *et al.*, 2015). Bakircioglu *et al.* (2011) also state that the phase specificity offered by sequential extraction procedures is its principal advantage over single stage extraction procedures.

However, Shan and Chen (1993) and Qiang, Xiao-Quan, Jin, and Zhe-Ming (1994) observe a problem with sequential extraction procedures, stating a non-selectivity of the reagents during the extraction process. This may alter the surface characteristics of the materials being analysed and cause certain metals to redistribute and adsorb to other phases or exchange sites on the newly exposed surfaces. Another drawback to sequential extraction procedures was noted in a review by D'amore, Al-Abed, Scheckel, and Ryan (2005)who notes that many sequential extraction procedures were developed for the extraction of trace metals in finer sediment materials. He also states that heavily contaminated soils (like those found at mining areas) where many trace elements are found in highly elevated concentrations, may alter the metal chemistry in the procedure. Furthermore, McLaughlin, Hamon, McLaren, Speir, and Rogers (2000) state that the use of sequential extraction data is not usually successful at predicting the phytoavailability or toxicity of metals. Rao *et al.* (2008) express that this can be due to the complexity of heavy metal and biotic interactions and that SEPs are best undertaken alongside a vegetation bioassay. However, this can be costly (Neel, Soubrand-Colin, Piquet-Pissaloux, & Bril, 2007), and should only be applied with a full understanding of their constraints (Soriano-Disla *et al.*, 2010; Sutherland & Tack, 2003) and other factors such as soil type and contamination level (Soriano-Disla *et al.*, 2010).

Heavy metal extraction procedures that make use of a single extractant are also widely used in environmental science. A single extractant procedure is commonly used to dissolve a phase whose metal content available for uptake by plants and is well established for major elements (Rauret, 1998). Single extraction procedures are methodologically appealing and can generally be separated in to three types of reagents, neutral salts (e.g. $CaCl_2$, $NaNO_3$, NH_4NO_3, $AlCl_3$, $MgCl_2$), dilute acids (e.g. Nitric acid, HCl) and chelating agents (e.g. EDTA, DTPA) (Laing, 2010). Moreover, different methods tend to differ depending on the extraction time used, soil: solution ratio, concentration of extractants, shaking speed and temperature (Laing, 2010).

Of the reagents commonly used for single extractant procedures, neutral salt solutions of either Ca, Ba or Mg are usually used to extract metal fractions that are assumed to be loosely bound to soil exchange sites. These neutral salts have been stated to be very effective and highly selective reagents (Laing, 2010). The mobile and loosely bound fractions are thought to be the bioavailable or phyto-available metals (Novozamsky, Lexmond, & Houba, 1993) and the fraction that governs metal uptake by other soil organisms (Peijnenburg *et al.*, 1999). Several studies have evaluated the use of a single extraction procedure using neutral salts to assess bioavailable fraction of heavy metals in soils (Houba, Lexmond, Novozamsky, & Van der Lee, 1996; Kim *et al.*, 2015; Novozamsky *et al.*, 1993; Rauret, 1998; Ure *et al.*, 1993). Some drawbacks to extraction with neutral salts can be a poor correlation between bioavailable and total metal content in soils especially more strongly bound metals Pb and Cu (Bakircioglu *et al.*, 2011). Salts can also suffer background interferences whilst analysing by spectroscopic techniques such as atomic emission spectroscopy (Laing, 2010). Despite these negative aspects to single extraction techniques using neutral salt solutions, many studies have found an extraction using $CaCl_2$ to be a simple and rapid method to measure heavy metal bioavailability (Bakircioglu *et al.*, 2011; Houba *et al.*, 1996; Kim *et al.*, 2015; Novozamsky *et al.*, 1993; Ure *et al.*, 1993). Rao *et al.* (2008) suggest that SEPs using $NaNO_3$, $CaCl_2$,

NH_4NO_3 should be standardised in a similar way to the BCR sequential procedure to enable widely accurate and reproducible procedures to improve research comparisons.

As previously mentioned for the more freely available and mobile contaminants, a mild solution is usually used, and for the less mobile and strongly adsorbed metals, a stronger acid extraction is preferred (Harmsen & Frintrop, 2003; Kim *et al.*, 2015; Pueyo *et al.*, 2004). The focus of this research is that of the freely available fraction of heavy metals available to plants and therefore a mild extractant was chosen. More specifically a solution 0.01 M of Calcium Chloride ($CaCl_2$), which has been widely cited for its overall simplicity and ease of operation for measuring bioavailability (Bakircioglu *et al.*, 2011; Houba *et al.*, 1996; Kim *et al.*, 2015; Novozamsky *et al.*, 1993; Ure *et al.*, 1993). This solution has been found to provide important information of the available heavy metal fraction in soils, especially when combined with XRF analysis (Houba *et al.*, 1996). Moreover, this extracting solution compliments the soil solution regarding the soil pH, concentration and composition (Houba *et al.*, 1996).

For the extraction procedure, a solution of 0.01 M of Calcium Chloride ($CaCl_2$) was mixed. 1.1099g of $CaCl_2$ granules were mixed with distilled water in a beaker until the granules were dissolved. The solution was poured into a 1 litre volumetric flask through a glass funnel and then carefully filled to 1 litre with distilled water and shaken. A 0.01M $CaCl_2$-soil solution was mixed to a ratio of 10:1. Three grams of oven-dried soil was weighed into a crucible and transferred to 50ml centrifuge tube. Next, 30ml of the $CaCl_2$ solution was added to the centrifuge tube and shaken. Batches of thirty-two samples were prepared in this manner and then shaken vigorously for two hours in an automated shaker. The samples were then centrifuged for fifteen minutes at 3000rpm. The supernatant from each sample was then filtered through a 25mm syringe filter, with a pore size of 0.2µm, into a 15ml centrifuge tube, labeled and stored in a fridge at 2°C until analysis using an inductively coupled plasma optical emission spectrometer (ICP-OES). This method and slight variations of it are recommended by Houba *et al.* (1986), Pueyo *et al.* (2004), Maiz *et al.* (1997), Kim et al. (2015) and others. The process was thought to be simple (Houba, et al.,1986), timesaving (Maiz *et al.*, 1997), cheap and environmentally friendly (Houba, Temminghoff, Gaikhorst, & Van Vark, 2000).

The analysis of extracted heavy metals was undertaken using inductively coupled plasma-optical emission spectroscopy (ICP-OES). The filtered solution resulting from the extraction procedure was placed into an auto sampler attached to the ICP-OES apparatus. The ICP-OES nebulizes the samples into the centre of an inductively coupled argon plasma where extremely high temperatures vaporise the sample solution and the elements contained within it are atomised. The atomised elements are then detected and measured using the intensity of the radiation that is created. They are detected by the optical emission spectrometer (OES) part of the apparatus. The wavelengths of the detected elements are then converted into concentrations using known standards (EAG Laboratories, 2017; Thomas, 2003). The resulting data was then stored awaiting data analysis. A blank using the same $CaCl_2$ and distilled water solution was used to calibrate the apparatus. For ICP there is a tendency to not use a CRM but to run all samples in triplicate. The instrument is set up using a range of standards at different concentrations. Trained laboratory technicians at the University of Salford ran the samples through the ICP apparatus and typical limits of detection for ICP-OES are as follows: Pb/1µg/L, Zn/0.2µg/L, Cu/0.4µg/L, Ni/0.5µg/L, Cd/0.1µg/L (Elmer, 2008).

3.16 Determination of soil organic matter content

Any material produced by plant or animal organisms in soil is termed soil organic matter (SOM). As this mixture of plant and animal matter decomposes within the soil matrix, it becomes a stable portion of the soil and is known as humus. It is this matter at various stages of decomposition and the stable humus that make up SOM. The SOM serves two main purposes: it contains all of the nutrients considered essential for plant growth and the humus absorbs and stores these nutrients in a form that is readily available for plant uptake (Bot &

Benites, 2005). The measurement of SOM through the loss on ignition method approximates the total content of organic matter within the soil sample and is a useful guide to highlight the differences between soils (Rowell, 1994).

Empty crucibles were placed in an oven at 105°C for 24 hours to ensure no moisture was present. The oven-dried crucibles were placed in a desiccator and then weighed on calibrated precision scales and placed back in the desiccator. Soil samples were then placed in the crucibles and again oven dried at 105°C for another 24 hours. These samples and crucibles were then placed in a desiccator and weighed individually to give a pre-ignition weight. The samples were then transported to a muffle furnace, pre-heated to 550°C, and placed in trays inside the muffle furnace. Once the furnace had returned back to the desired 550°C, after opening, the samples were heated for four hours. When sufficient time had passed, the samples were carefully removed using tongs and heat resistant gloves and allowed to cool slightly before being placed in the desiccator again with the aid of tweezers. The post-furnace samples and crucibles were then weighed individually to give a post-ignition weight.

The weight of the empty crucible was subtracted before calculating the percentage of SOM using the following equation.

$$\%SOM = \frac{preignition\ weight\ (g) - postignition\ weight\ (g)}{\text{preignition weight (g)}}\ X\ 100\%$$

3.17 Particle size distribution

Whilst particle size span is used as a single measurement in the interpretation of results here, it must be noted that it is standard practice to report several measurements to represent particle size distribution. The span is a frequently used calculation to quantify the particle distribution width: (D90 – D10) / D50. However, reporting both a central measurement of the range of distribution (e.g. mean, median, and mode) and a variable that describe the width of particle size distribution (e.g. span, standard deviation) lead to a superior interpretation of the particle size distribution (Horiba Scientific, 2012). Including the median is the most stable calculation produced by particle size analysis and is usually the value most commonly used for a single point interpretation of particle size. Using span alone gives no explanation of the distribution of the differing particle sizes measured during laser diffraction e.g. the amounts of coarse, mid-range and fine particles and only the width of distribution (Horiba Scientific, 2012).

Whilst the Horiba LA-950 Particle Size Analyser used for this research was set-up to the University of Salford's laboratory standards by qualified laboratory technicians, it is important to note several factors during sample preparation and analysis that may influence particle size distribution results. In a White Paper on Guided, Automated Method Development for the Horiba LA-950 Particle Size Analyser, Horiba Instruments (2014) discuss several aspects of instrument set-up and sample preparation including:

- The sub-sample introduced to the analytical instrument should be properly homogenised to minimise error.
- The system should be tested using a temporal check (minutes) using the same sample to ensure that the soil sample is not settling or swelling and shifting the results of the particle size distribution over time.
- The refractive index should be predetermined to the appropriate refractive index (RI) value for the sample (in this case soil) to prevent errors when the instrument converts the scattered light data into a particle size distribution.
- The circulation pump speed is important. Low circulation speeds may not adequately allow suspension of larger or denser soil particles. Additionally circulation pump speeds that are too high may cause air bubbles to form or break more fragile particles.
- The amount of energy used for ultrasound used for soil particle dispersion can have a substantial effect on the reported particle size distribution as a higher energy ultrasound can alter the properties of the soil particle.

- The measurement duration of samples introduced to the instrument i.e. the longer the measurement time the larger proportion of particles will be detected and measured. Samples with wide-ranging distribution such as soils are generally analysed for longer periods to inspect a satisfactory amount of particles across all size ranges.

In the case of instrument set-up for the particle size analysis here, the ultrasonic level was set to 6, circulation speed and agitation speed were set to 7 and the refractive index test reported in the results was (1.510 - 0.000i). No liquid dispersion medium was used and samples were homogenised only using the cone and quarter method. Samples were measured at a transmittance level of between 70-90% at 2 minutes, 4 minutes and after ultrasound at 5 minutes (x3).

The determination of particle size distribution for each sample was undertaken with the use of the Horiba LA-960 Laser Particle Size Analyzer. This equipment uses laser diffraction instruments and measures the angle and intensity of light scattered by the introduced soil particles. Different sized particles scatter light in different directions and these patterns and the intensity of the measured light are used to calculate the particle size distribution of samples (Horiba Scientific, 2014). Particle size analysis was undertaken to determine the particle size range within each soil sample. Abouelnasr (2010) reports that many studies have revealed that samples with greater quantities of smaller-sized particles have been shown to contain higher concentrations of heavy metals, and that this can be associated with the larger surface area present with smaller particles. Particle size analysis is applied in numerous industries including ore extraction, the manufacture of ceramics and the steel industry, as well as for soil science. In soil science, particle size analysis can determine the textural class and particle size distribution of soils (Andrenelli, Fiori, & Pellegrini, 2013).

Once initialised, the particle size analyser was first rinsed by feeding, circulating and agitating without adding a sample. The alignment and blanking of the instrument was undertaken to ensure background correction. A small amount of dry ash (produced as a by-product of the loss on ignition method) was placed into the instrument's mixing chamber and next, stirred and circulated in the chamber until a stable laser transmittance was established (Toongsuwan *et al.*, 2000). Measurements were then taken at two minutes and four minutes. After four minutes, ultrasound was applied to the sample for one minute to break down any aggregates still remaining in the sample, and a final measurement was recorded. The results were displayed on the particle size analyser software and then stored pending further analysis.

3.18 pH

Soil pH was measured as it is widely considered one of the most important measurable variables relating to the function of soils. It can influence the availability of many nutrients for plants, the function of important microbes within the soil matrix and the potential outcome of any contaminants that are present in soils (Dubbin, 2001; Rayment *et al.*, 2003).

A soil-water solution was mixed in a ratio of 1:5. Twenty grams of soil was weighed using calibrated precision scales, then placed into a 150ml beaker, before 100ml of distilled water was carefully added. The soil-water mixture was vigorously stirred using an automated electric stirrer and a magnetic stirring rod for five minutes, and left to settle for one hour (Rayment & Lyons, 2011). A calibrated pH meter was used to take readings from the supernatant of the settled mixture to prevent any obstruction of instrument sensors. The meter was rinsed with distilled water and dried after each reading to ensure no cross-contamination of fluids. This was repeated for all soil samples. The readings were recorded for future analysis.

3.19 Data analysis

The analysis of the large amount of data collected during the sampling strategies and analytical procedures explained here was of prime importance for achieving the aim and objectives of this research. Several statistical software packages were utilised to aid in presentation of results, determine the distribution of measured soil properties and; plant species identified, and to explore the relationships between soil and vegetation variables.

IBM SPSS (Statistical Package for the Social Sciences) 23 for Windows was used to generate all descriptive statistics and charts. General descriptive statistics are displayed in table form and include number of samples, mean, standard deviation and minimum and maximum values as these are widely used and considered important for exploring variation within the data and also identifying trends and patterns (Kent & Coker, 1994). Box and whisker plots are utilised to compare and display variables across all sites as these are a useful way of presenting the spread of data and highlighting outliers contained within the data (Dytham, 2011; Kent & Coker, 1994). The boxes also display upper and lower quartiles and median.

Using SPSS, Spearman’s rank order correlation was used to find the associations and correlations (Dytham, 2011) between the measured total metal concentrations and the extracted available fraction of heavy metals and also to investigate the validity of the bioavailable metal extraction method used. The data was tested for normal distribution firstly by plotting histograms to test for skewness. Secondly the Kolmogorov-Smirnov test was carried out to determine the probability that the data set was normally distributed as this test is considered useful for large sets of continuous data (Dytham, 2011). Both histograms and Kolmogorov-Smirnov test show the data set to be highly skewed. Spearman’s rank order correlation was utilised as this non-parametric test does not assume normally distributed data and is commonly used for skewed data (Kent & Coker, 1994). The resulting Spearman’s correlations are presented as a matrix for ease of interpretation of results from the multiple variables tested (Dytham, 2011).

Correlation analysis was undertaken using XLSTAT 2017 software to highlight relationships between the total and available heavy metal variables. Prior to correlation analysis outliers were identified using the Dixons test for outliers and removed (Dytham, 2011).

Principal component analysis (PCA) was also carried out using SPSS 23. PCA enables the analysis of large quantities of correlated multivariate data to find structure, patterns and clusters of relationships amongst the variables (Dytham, 2011; Webster, 2001). PCA is a mathematical technique which synthesises large amounts of data and rotates it onto new compound axes, explaining variance and reducing the data sets into principal components (Webster, 2001; Zhiyuan, Dengfeng, Huiping, & Zhiping, 2011). This analysis technique is popular among soil scientists, biologists (Webster, 2001) and ecologists and it allows ease of visualisation of the results (Kent & Coker, 1994). PCA was used to explore relationships between total and available metal content, particle size, pH and several other soil variables.

Canonical correspondence analysis (CCA) was undertaken, using XLSTAT 2017 software, to discover relationships between vegetation data and total and available heavy metal data. CCA is an ordination technique used to examine relationships between the distribution of species and environmental factors (Kent & Coker, 1994). CCA works in a similar way to PCA, however the variables are placed into groups, i.e. identified vegetation species and measured soil variables and quadrats, before analysis (Dytham, 2011). The process undertaken by the software used is a complicated procedure and is fully explained by the developer of CCA, Ter Braak (1986). However, to summarise, it uses multiple regression to find associations between combinations of the environmental variables and combinations of the species variables to produce weightings which allow the calculation of the combinations that have the most variance and these are then plotted onto an ordination diagram (Kent & Coker, 1994; Ter Braak, 1986). To compliment the CCA, cluster analysis was also utilised. The vegetation variables were standardized using z-scores after which Euclidean distances were calculated to find similarities (Li & Feng, 2012). The Ward linkage method was used to carry out hierarchical clustering on the standardised data set and then displayed on a dendrogram. Cluster analysis can be used to generate dendrograms which group individuals into clusters which may be taxonomically similar. This method is commonly used in ecology and vegetation classification (Dytham, 2011).

Arc GIS 10.5 software was utilised to investigate the spatial distribution of all measured variables. Simple sample point data were plotted in ArcMap to show the zones of sample collection and vegetation survey quadrats at each site. The distribution and mobilisation of soil variables were predicted using the Geostatistical Analyst tool in ArcMap. Universal Kriging was used to predict soil and vegetation variable values between sample collection points of each study area (Childs, 2004; Martín, Arias, & Corbí, 2006). Kriging is a linear interpolation method that estimates spatial variable values between known points. These estimates are derived from measuring the relationship of the known sample values using a complicated weighted average procedure (Childs, 2004; Johnston, Ver Hoef, Krivoruchko, & Lucas, 2001; Martín *et al.*, 2006). Kriging is a statistical technique that calculates the correlation of known variables using variography. It weights the surrounding known values to predict the spatial variable values for unknown locations. The weights assigned to the known variables not only take into account the distance between measured points, but also the spatial arrangement between the known points and their values (Childs, 2004; Johnston *et al.*, 2001; Martín *et al.*, 2006). Kriging is a two-step procedure: firstly, the spatial covariance assembly of the known measured points is established by fitting a variogram. In the second step, the weights resulting from the covariance assembly are used to interpolate values for unknown points across the sample area. A variogram or semivariogram is a visual representation of the covariance revealed between each pair of known values (Childs, 2004). The calculation of the semivariogram is undertaken for each sample point's separation from every other sample in the dataset, and is calculated as the mean squared difference in variable value between pairs of variables with equal spatial separation (Childs, 2004). In universal kriging, it is assumed that there is a trend in the data and that it can be modelled by a polynomial, which is subtracted from the known values, and the correlation is modelled from the random errors. The polynomial is then added back to these predictions to give the final results (Childs, 2004). Kriging has been extensively used in soil science research and the spatial mapping of contamination and pollution (Amini, Afyuni, Khademi, Abbaspour, & Schulin, 2005; Lee, Li, Shi, Cheung, & Thornton, 2006; Xie *et al.*, 2011).

As well as kriging method used here, several alternative methods are available for the interpolation of spatial data such as soil contamination distribution. These include inverse distance weighting (IDW), nearest neighbour interpolation and spatial autocorrelation (ESRI, 2016). Following is an overview of some of these methods and their possible merits for this research.

The IDW (Inverse Distance Weighting) method of interpolation estimates a weighted value inversely proportional to its distance from the target variable being interpolated (ESRI, 2016). The principle of using inverse distances are based on Tobler’s first law of geography, “everything is related to everything else, but near things are more related than distant things’’ (Tobler, 1970). This method and accentuates the spatial similarity of nearby variables (ESRI, 2016).

As IDW uses a weighted distance average, the value generated cannot be greater than the maximum known value or lower than the lowest known value, thus lie in the range of known variables (Philip & Watson, 1985). This would limit the chances of an over or under representation of heavy metal contamination values at a site. The IDW method can functions most effectively when samples are relatively dense within the sampling areas as If the sample points are sparse or irregular, the results may not satisfactorily represent the anticipated surface contamination (Philip & Watson, 1985).

Nearest neighbour interpolation allocates an interpolated evaluation to an undetermined position, which is assigned a value equal to the nearest known variable. The method creates Thiessen polygons around the sampled value (Li & Heap, 2011). It scrutinises the spatial variance of all sample points and its nearest neighbour, and then makes a comparison to predicted values for a randomised sample of variables from a comprehensive spatial randomness pattern. Nearest neighbour interpolation aims to preserve localised value differences and can be a relatively straightforward form of interpolation where no additional parameters need to be allocated to the nearest neighbour (Li & Heap, 2011).

Whilst the kriging method utilised for this research, and the associated isopleth maps generated, offer a visual comparison of the spatial distribution of vegetation and heavy metal attributes across the study sites, there are more rigorous spatial correlation methods available in GIS.

Spatial autocorrelation allows the assessment of how well variables correlate with other nearby measurements across a spatial area (Getis, 2007). This allows the definition of whether spatial characteristics of one variable are affecting other variables in the surrounding environment and if there is a clear relationship or dependency. One such type of spatial autocorrelation available in ArcGIS is termed Moran's I (Getis, 2007). This allows the measurement of how well variables correlate across a defined target area. Another type of autocorrelation available is Geary's ratio, which allows a more sensitive approach to measure whether local variations can be associated with other local patterns within a dataset (Getis, 2007). These methods have been used to find strong spatial associations between species diversity and language use (Turvey & Pettorelli, 2014), land use and land cover (Fan & Myint, 2014) and health care and survival rates (Prudhomme O'Meara, Platt, Naanyu, Cole, & Ndege, 2013).

3.20 Consent

Permission to carry out this research was sought due to the study sites consisting of several protected areas including, SSSI, SAC, NNR or scheduled under the Ancient Monuments and Archaeological Areas Act. The Peak District National Park Authority, Natural England, Derbyshire Wildlife Trust and Plantlife all consented to this research being undertaken on the premise that the results of it be shared with them upon completion.

Chapter 4: Descriptive results

4.1 Introduction

This chapter presents the findings of this research resulting from the sampling strategy, analytical methods and data analysis set out in Chapter 3: Methodology. This research focuses on the analysis of soil and vegetation variables resulting from the sampling strategy and fieldwork at seven study sites. Relationships between these variables and the examination of their spatial distribution was explored to highlight any relationships between abiotic variables and how these relate to vegetation communities that exist at these sites of historical mining activity.

The presentation of results is structured to display simple descriptive statistics for each measured soil variable in sections 4.2 through 4.6.6. These will include box and whisker plots and tables presenting minimum, maximum, mean and standard deviation values for each variable and survey site. The box plots also display upper and lower quartiles and median. Where necessary, box plots are presented across two charts due to some sites, especially the naturally occurring dales, having comparatively low values when compared to other more extensively worked rakes with significantly elevated levels of contamination. Vegetation statistics are presented in section 4.7.

Due to the large amount of data and results generated during this research some data have been omitted from this results chapter. Where this is the case, they are contained within the appendices and signposted where relevant in this chapter.

4.2 Alkalinity

Results for pH measurements range from slightly acidic pH 5.07 and 5.26 at Hard Rake to moderately high basic soils of > pH 8 at High Rake and Dirtlow Rake [*Table.* 4:1]. Mostly samples have a pH ranging between pH 6 and > pH 7 which is characteristic of calcareous grasslands (Carroll *et al.*, 2003). Both sample plots at Hard Rake display the highest standard deviation with samples ranging from low pH 5 to above pH 7 [*Table.* 4:1].

Deep Dale is the only site to contain outliers and Hard Rake shows the largest spread of data in the pH results [*Fig.* 4:1] Kriging prediction isopleth maps of pH levels across each site of mining activity are included in Appendix A. These display some relationship with the spatial distribution of heavy metal bioavailability and are discussed further in Chapter 6: Discussion.

Table 4:1. pH descriptive statistics

Site Name	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	6.00	7.54	6.88	.36
Dirtlow Rake	20	7.32	8.36	7.92	.27
Gang Mine	20	5.85	7.10	6.45	.29
Hard Rake 1	19	5.07	7.46	6.37	.81
Hard Rake 2	19	5.26	7.26	6.53	.69
Hay Dale	19	6.30	7.49	6.98	.40
High Rake	19	7.35	8.78	8.16	.42
Tansley Dale	19	7.01	7.82	7.38	.27
All Sites	155	5.07	8.78	7.08	.79

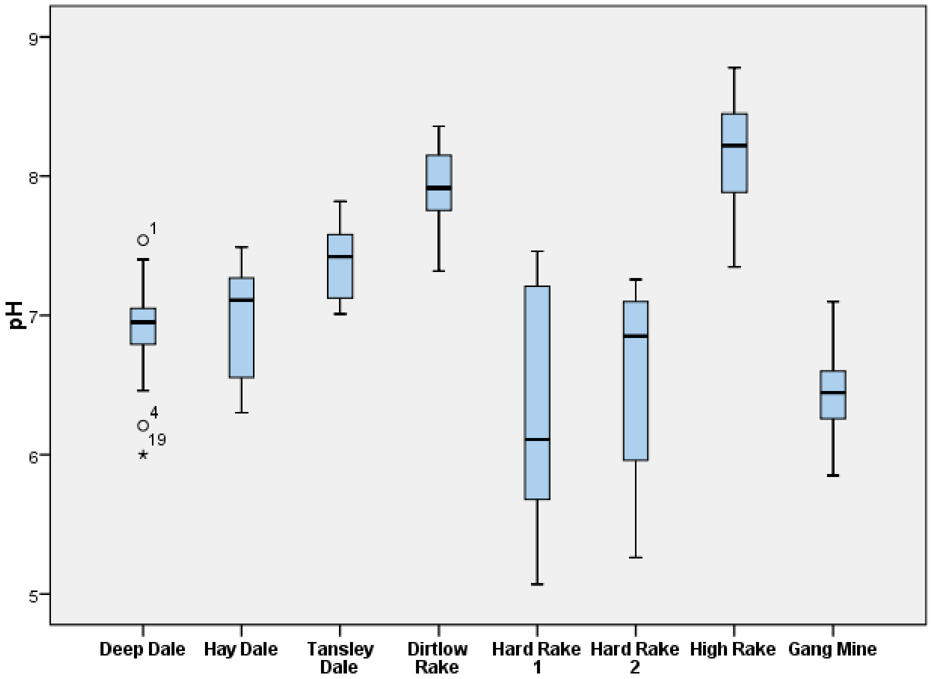


Figure 4:1. pH box and whisker plot **(* =Extreme outlier ○=Minor outlier)**

4.3 Soil organic matter

High Rake and Dirtlow Rake display the lowest SOM content with < 5%. Both Hard Rake 1 & 2 and Gang Mine exhibit the highest recorded SOM content in samples, with all three sites showing samples containing above 50% organic matter [*Table.*4:2]. Those samples with very high SOM were re-analysed to ensure accuracy of results. With the exception of Dirtlow Rake, Gang Mine and High Rake, most samples had a mean SOM content of approximately 20-30% [*Table.* 4:2]. Hard Rake 1 and Gang Mine show the greatest spread in SOM% results and the Deep Dale, Dirtlow Rake, Hard Rake 2 and Gang Mine data sets each contained outliers [*Fig.* 4:2]. Kriging prediction isopleth maps of the study sites are included in Appendix B.

Table 4:2. Descriptive statistics for soil organic matter

Soil Organic Matter (%)

Site Name	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	18.84	37.60	26.60	4.18
Dirtlow Rake	20	4.74	22.97	8.75	4.43
Gang Mine	20	7.30	58.81	36.70	11.46
Hard Rake 1	19	14.22	50.41	24.05	9.42
Hard Rake 2	19	18.06	62.18	27.63	9.86
Hay Dale	19	14.86	30.19	21.69	4.58
High Rake	19	1.30	13.64	5.79	3.92
Tansley Dale	19	10.61	34.22	19.52	7.86
All Sites	155	1.30	62.18	21.39	12.03

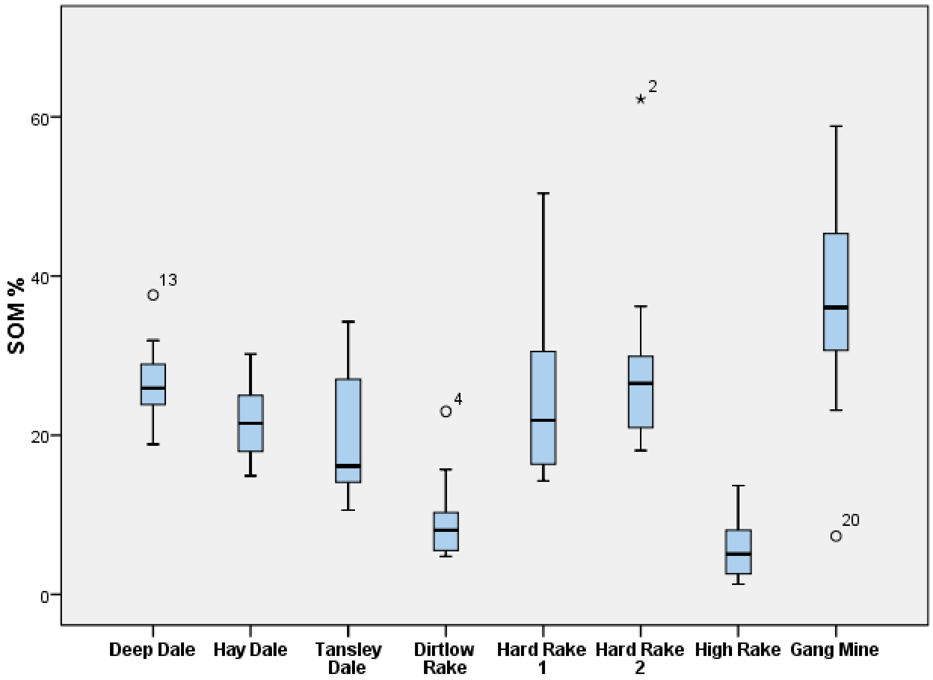


Figure 4:2. Box and whisker plot of SOM % (* =Extreme outlier ○=Minor outlier)

4.4 Particle size analysis

Particle size analysis of samples is presented here as both a particle size span and median particle size, as these are thought to give a good interpretation of the particle size distribution of the samples (Horiba Scientific, 2014). Particle size span was calculated using the following simple equation: Span = (Dv0.9 – Dv0.1) / Dv0.5 (see *Fig*. 4:3). The following link provides access to all raw data of PSA:

https://www.dropbox.com/sh/bdbxz67kjgw6vay/AADY6rj5JxDpe_oSiP0wXs93a?dl=0

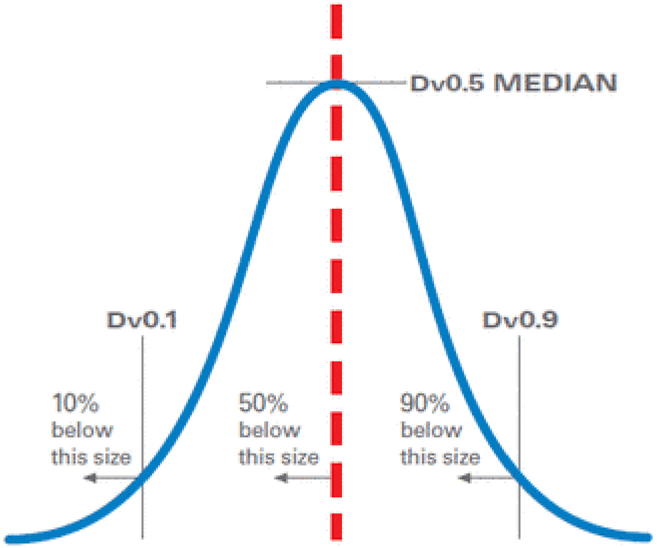


Figure 4:3. Terms relating to spread of particle size (Horiba Scientific, 2014)

A particle size span of between 1.50 to 16.42 is calculated for the soil samples from all sites. Hard Rake, Hay Dale and Deep Dale exhibit the lowest particle size span, with Deep Dale and Hay Dale also showing the lowest maximum particle size span. The mean particle size span ranges from < 3 at Hay Dale to 7.50 at Gang Mine [*Table.* 4:3]. Hard Rake 2 and Gang Mine show the greatest range in particle size span [*Fig.* 4:4].

Table 4:3. Particle size span descriptive statistics

Particle Size Span

Site Name	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	1.67	6.81	3.80	1.48
Dirtlow Rake	20	2.38	9.33	6.47	2.02
Gang Mine	20	2.46	14.34	7.50	3.59
Hard Rake 1	19	1.94	14.92	4.51	3.40
Hard Rake 2	19	1.50	16.42	6.21	4.12
Hay Dale	19	1.99	4.48	2.86	.63
High Rake	19	2.38	15.17	4.80	3.38
Tansley Dale	19	2.05	12.78	4.37	2.88
All Sites	155	1.50	16.42	5.08	3.19

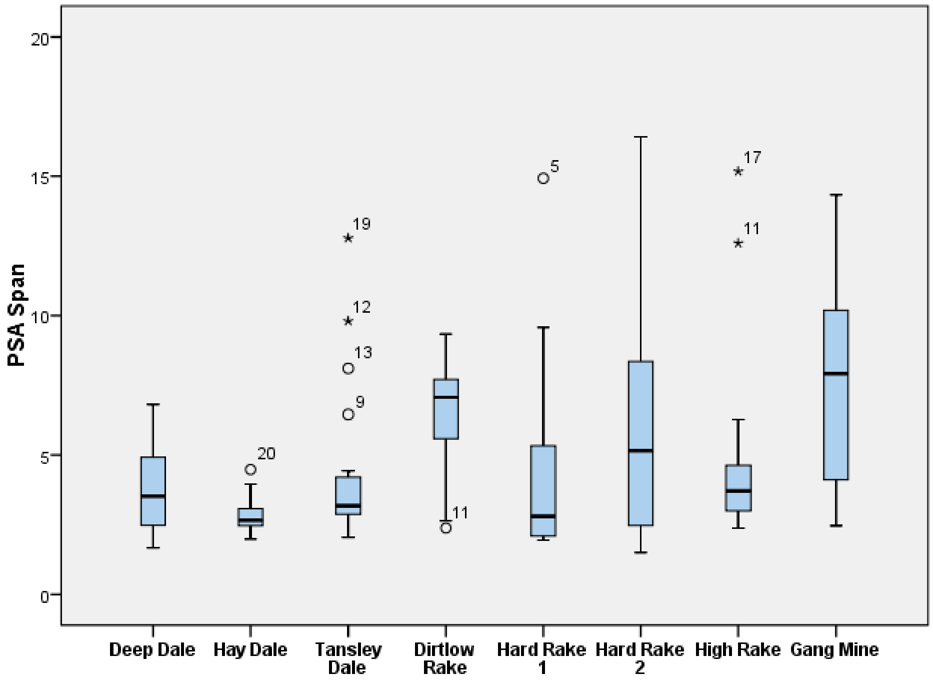


Figure 4:4. Box and whisker plot for particle size span (* =Extreme outlier ○=Minor outlier)

Median particle size shown in *Table* 4:4 below reveals the three dales sites as having the largest minimum median particle size, being approximately double the median particle size of the rakes sites. Hay Dale exhibits the largest median particle size at almost 500µm and Dirtlow Rake displays the lowest median particle size of 22.81µm [*Table*.4:4]. *Figure* 4:5 illustrates that High Rake displays the greatest median particle size range.

Table 4:4. Median particle size descriptive statistics

Particle Size Median (µm)

Site Name	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	58.62	101.65	77.06	11.39
Dirtlow Rake	20	22.81	104.31	65.36	22.97
Gang Mine	20	29.10	119.00	54.32	19.87
Hard Rake 1	19	28.38	79.93	48.42	16.51
Hard Rake 2	19	27.16	87.74	55.70	14.01
Hay Dale	19	59.87	494.95	124.51	97.74
High Rake	19	34.10	326.36	205.53	88.06
Tansley Dale	19	61.26	242.58	114.15	46.30
All Sites	155	22.81	494.95	92.60	70.29

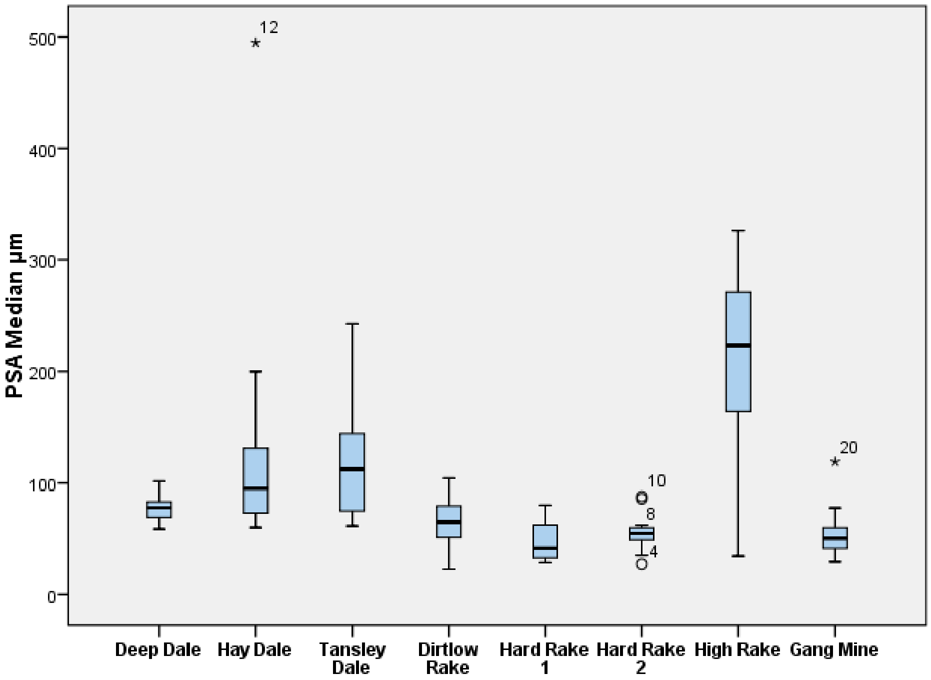


Figure 4:5. Box and whisker plot displaying particle size median (* =Extreme outlier ○=Minor outlier)

4.5 Total metal concentrations

Results from Xrf analysis recorded an extensive range of elements within soil samples. However, this research focuses on the concentrations of heavy metals and metalloids present at the study sites. Arsenic is often referred to as a metalloid, which has properties between a metal and a non-metal. Accordingly, this results chapter will present the findings for the following heavy metals and metalloids:

- Lead (Pb)
- Arsenic (As)
- Zinc (Zn)
- Cadmium (Cd)
- Nickel (Ni)
- Copper (Cu)

These elements were chosen for this study as they are frequently present as a result of mining activity (Ford & Rieuwerts, 2000), are widely considered an environmental hazard

(Anju & Banerjee, 2011) and have been known to offer niche habitats for certain plant species (Richards & Waite, 2017). The term heavy metal denotes any element which has a density greater than 5-6g/cm^3 and the six metals/metalloid presented here are some of the most common pollutants in soil (Ashman & Puri, 2002).

4.5.1 Lead (Pb)

Total Pb concentrations displayed in *Table* 4:5 demonstrate the relatively low concentrations of Pb at the Hay Dale and Deep Dale sites with minimum, maximum and mean concentrations being considerably less than Tansley Dale and the rakes sites. Total Pb measurements across all of the sites show some very high concentrations, with samples from Hard Rake and Gang Mine containing extremely high levels (above 6.5% Pb). Dirtlow Rake contained no samples with less than 8000ppm, displaying high concentrations across all sample points. *Figures* 4:6 & 4:7 display outliers from several of the sites occurring at sample points 17, 18, 19 and 20. These consist of samples taken from extra points outside of the transects shown in *Figure* 3:9 in Chapter 3: Methodology, and are almost exclusively taken from a lower elevation than samples 1 through 16. Hard Rake 2 and Dirtlow Rake in *Figure* 4:7 have several outliers from within the transect points, and this may be explained by the extensive mining activity across the sites that intersect the sample transects.

Table 4:5. Descriptive statistics for total Pb concentrations

Total Pb (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	309.56	989.24	392.80	153.69
Dirtlow Rake	20	8162.01	34,504.84	19,752.30	6416.93
Gang Mine	20	2849.44	73,057.47	19,434.11	15,934.37
Hard Rake 1	19	339.21	22,079.37	11,252.64	7913.59
Hard Rake 2	19	1639.08	66,960.00	18,560.89	17,541.84
Hay Dale	19	189.82	4724.56	570.61	1010.57
High Rake	19	948.94	19,353.33	11,219.43	5732.83
Tansley Dale	19	2170.57	25,050.55	6047.24	5199.11
All Sites	155	189.82	73,057.47	10,948.06	12,005.21

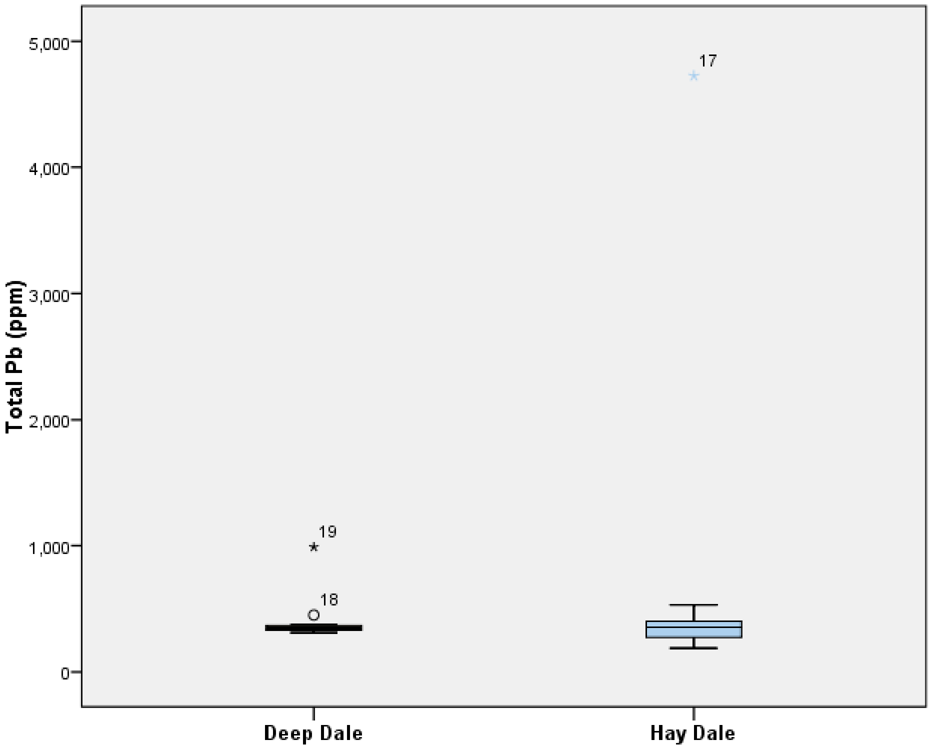


Figure 4:6. Box and whisker plot displaying total Pb concentrations (* =Extreme outlier ○=Minor outlier)

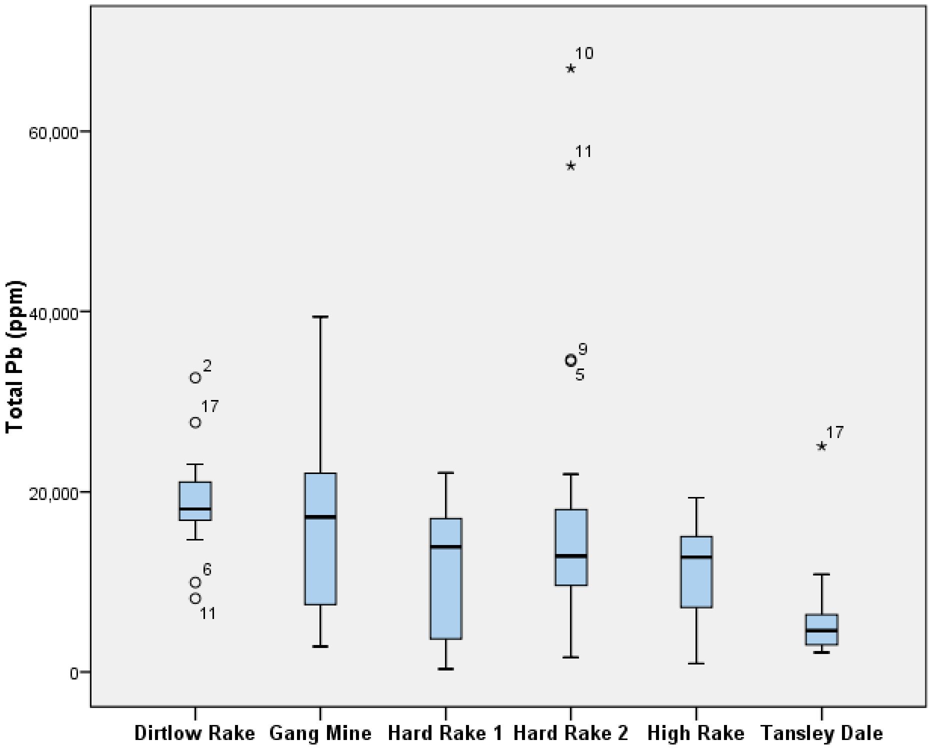


Figure 4:7. Box and whisker plot displaying total Pb concentrations (* =Extreme outlier ○=Minor outlier)

4.5.2 Arsenic (As)

Total arsenic concentration statistics shown in *Table* 4:6 reveal Dirtlow Rake to have the lowest concentrations. Deep Dale and Hay Dale also have relatively low levels of arsenic.

Hard Rake and Gang Mine exhibit the greatest total concentrations of arsenic, with Hard Rake 2 presenting very high arsenic levels. *Figure*s 4:8 & 4:9 once more present outliers at sites from sample points 17 to 20, with the exception of Hard Rake 2, which again displays elevations in concentration across its transect sample points (1-16).

Table 4:6. Descriptive statistics for total As

Total As (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	25.11	34.54	29.39	3.02
Dirtlow Rake	20	8.06	24.72	11.78	3.67
Gang Mine	20	153.99	2012.54	748.62	449.81
Hard Rake 1	19	15.13	1235.38	587.82	469.86
Hard Rake 2	19	43.27	6166.46	1059.57	1531.18
Hay Dale	19	20.55	74.43	32.00	11.92
High Rake	19	19.17	1187.25	532.23	315.70
Tansley Dale	19	16.22	1128.75	197.71	255.61
All Sites	155	8.06	6166.46	397.25	692.27

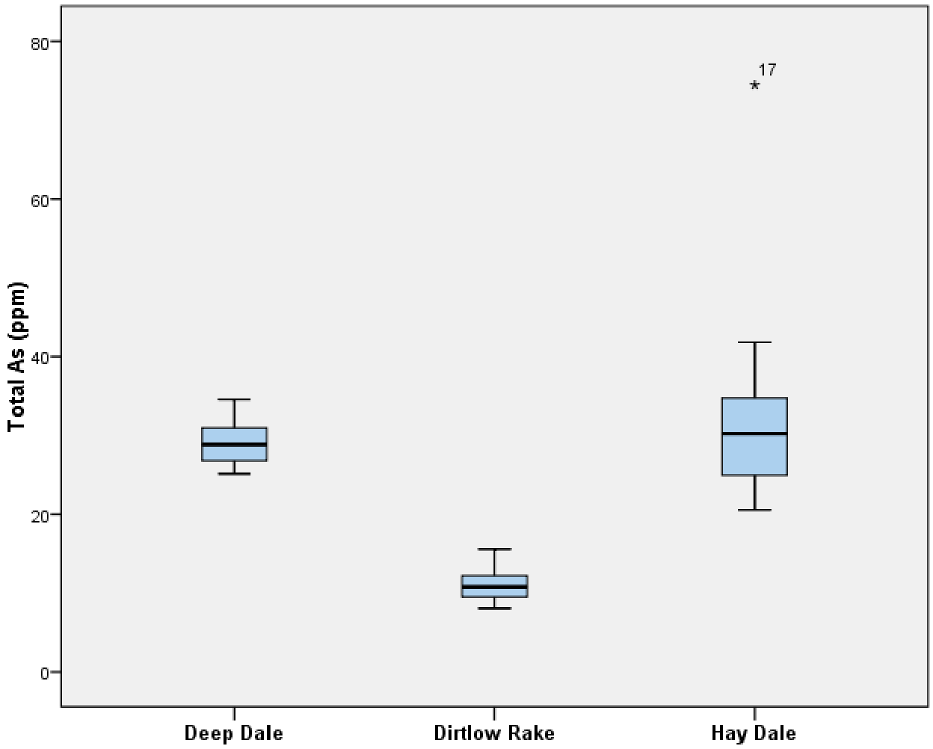


Figure 4:8. Box and whisker plot displaying total As concentrations (* =Extreme outlier ○=Minor outlier)

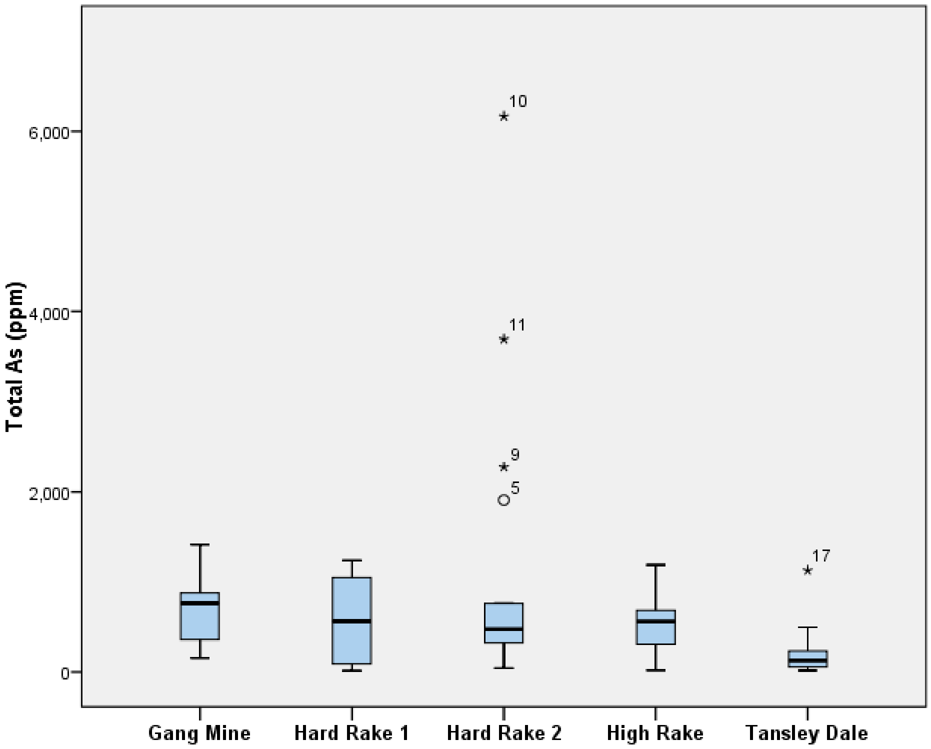


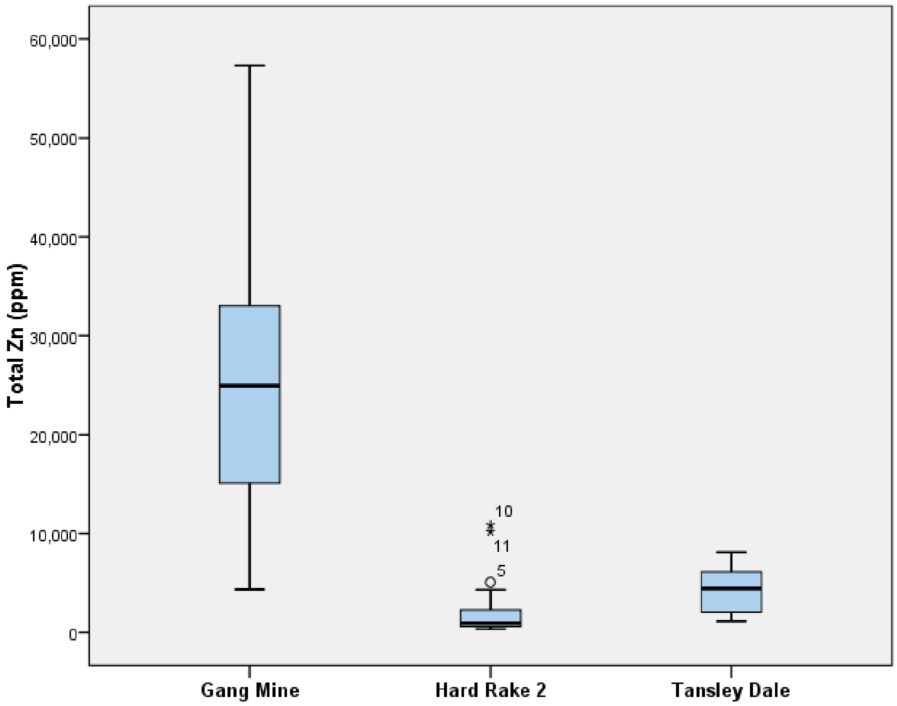
Figure 4:9. Box and whisker plot displaying total As concentrations (* =Extreme outlier ○=Minor outlier)

4.5.3 Zinc (Zn)

Zinc concentrations at Gang Mine are extremely high, reaching over 140,000ppm and a high minimum value of 4347.19ppm [*Table.*4:7]. Hard Rake 2 and Tansley Dale also display high levels of Zn contamination. Outliers for Hard Rake 2 are shown across the site [*Fig.* 4:10] with Deep Dale and Dirtlow Rake having their highest concentrations at the sample points outside the area of the transects (17-20) [*Fig.* 4:11]. Lowest Zn concentrations are present at Deep Dale, Hay Dale and Hard Rake 1 [*Fig.*4:11].

Table 4:7. Descriptive statistics for total Zn

Total Zn (ppm)	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	382.90	1294.31	544.43	186.11
Dirtlow Rake	20	766.08	4079.13	1879.42	698.97
Gang Mine	20	4347.19	140,226.58	32,275.90	29,557.92
Hard Rake 1	19	84.03	1364.57	725.42	474.47
Hard Rake 2	19	343.50	10,859.59	2355.75	3166.31
Hay Dale	19	212.34	1604.18	728.52	400.83
High Rake	19	380.61	4323.43	1488.37	927.01
Tansley Dale	19	1132.78	8125.53	4359.03	2283.01
All Sites	155	84.03	140,226.58	5661.16	14,722.26



*Please note sample point 20 removed from plot (140,226.58 ppm)

Figure 4:10. Box and whisker plot displaying total Zn concentrations (* =Extreme outlier ○=Minor outlier)

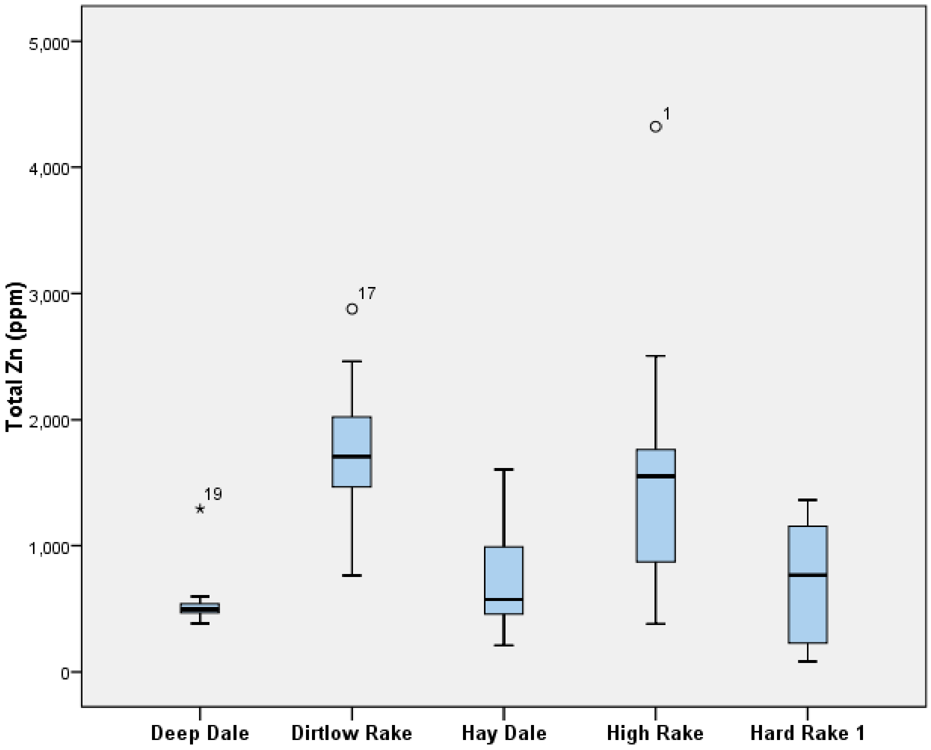


Figure 4:11. Box and whisker plot displaying total Zn concentrations (* =Extreme outlier ○=Minor outlier)

4.5.4 Copper (Cu)

Table 4:8 displays total Cu concentration for all sites. Hard Rake 2 and Gang Mine display the greatest Cu concentrations (above 400 ppm) [*Fig.* 4:13]. The lowest levels were found at Hard Rake 1 and High Rake, which had some samples below the limit of detection. Hard Rake 2 exhibits the greatest range of concentrations across its sample locations. Relatively low levels of Cu are also found at Deep Dale, Dirtlow Rake and Hay Dale [*Fig.* 4:12].

Table 4:8. Descriptive statistics for total Cu

Total Cu (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	30.26	44.63	36.22	4.30
Dirtlow Rake	20	101.05	266.23	148.78	33.86
Gang Mine	20	128.19	437.64	244.43	91.69
Hard Rake 1	19	14.96	204.17	111.45	70.84
Hard Rake 2	19	40.62	552.25	154.17	155.73
Hay Dale	19	29.79	140.88	66.81	31.33
High Rake	19	.00[d]	94.72	49.02	24.83
Tansley Dale	19	47.95	143.28	97.11	27.19
All Sites	155	.00[d]	552.25	114.07	95.18

d. Readings of .00 do not indicate absence but readings may be below the limit of detection for Xrf analysis.

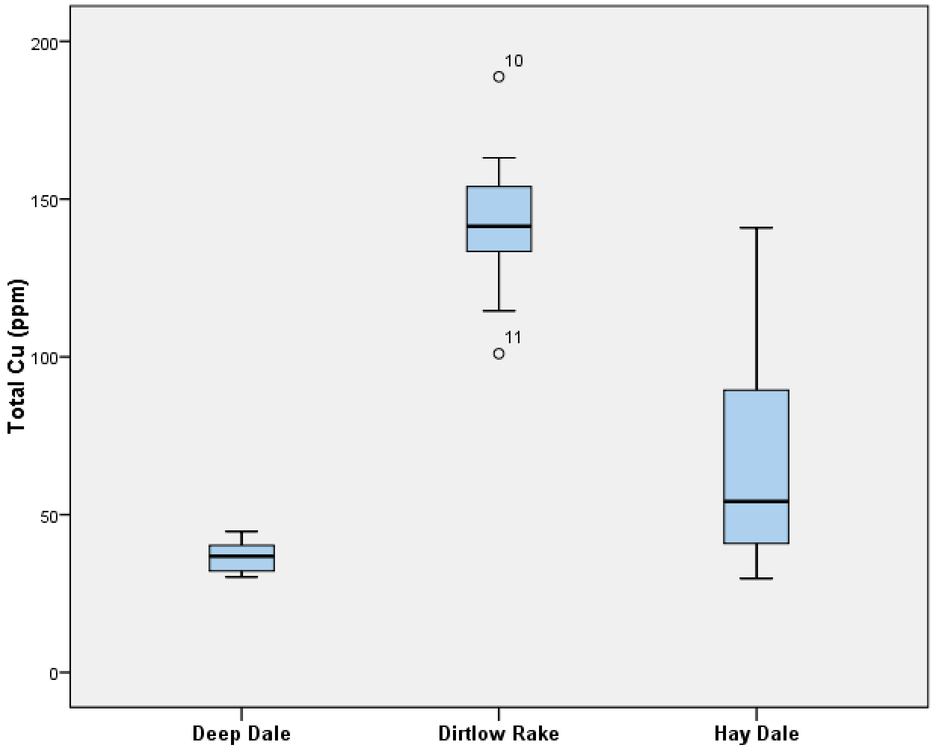


Figure 4:12. Box and whisker plot displaying total Cu concentrations (* =Extreme outlier ○=Minor outlier)

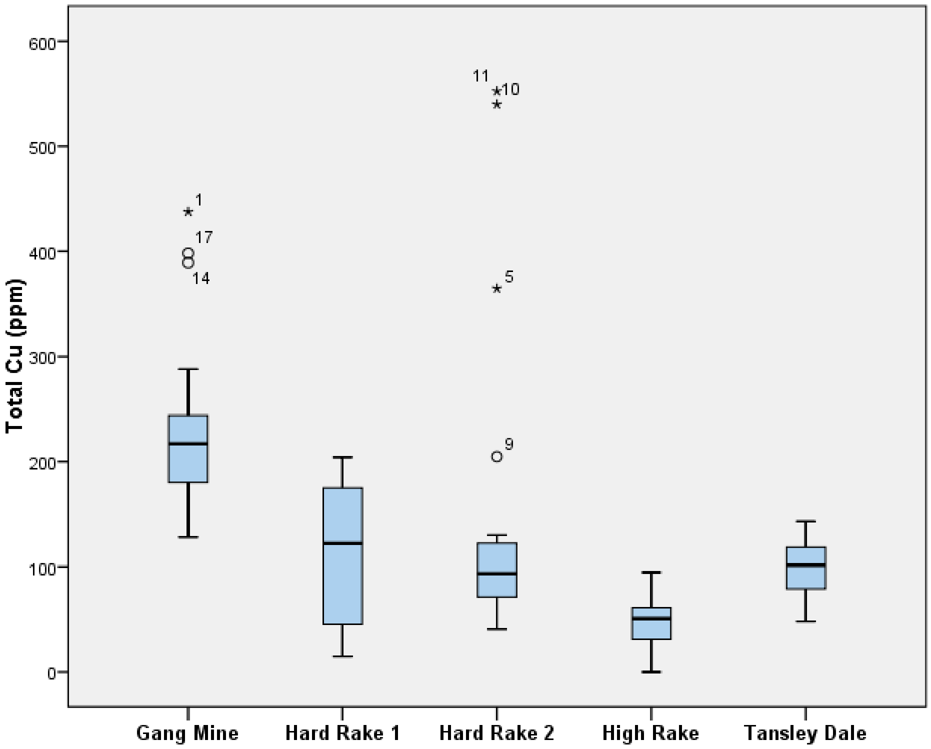


Figure 4:13. Box and whisker plot displaying total Cu concentrations (* =Extreme outlier ○=Minor outlier)

4.5.5 Cadmium (Cd)

Cadmium concentrations were higher overall at Gang Mine with maximum readings of over 1000 ppm [*Fig.* 4:14]. This is much higher than all other sites and samples were reanalysed to ensure validity. High Rake displayed the highest concentration of all other sites. Deep Dale had no Cd present that was within the limits of detection (LOD) for the Xrf analyser and Hay Dale, Hard Rake and High Rake had several soil samples that contained amounts of Cd below the LOD [*Table.*4:9]. For an analysis time of 60 seconds the LOD for Cd is 25ppm, the LOD decreases with analysis time (Thermo Fisher Scientific Inc, 2009) and analysis time for soil samples here was 240 seconds. Several sites had peaks in concentration at a range of sample points along their transects [*Fig.* 4:15].

Table 4:9. Descriptive statistics for total Cd

Total Cd (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	.00[a]	.00[a]	.00[a]	.00[a]
Dirtlow Rake	20	6.64	16.81	9.56	2.39
Gang Mine	20	10.24	1032.03	260.58	236.45
Hard Rake 1	19	.00[a]	3.64	.73	1.09
Hard Rake 2	19	.00[a]	32.19	6.35	9.41
Hay Dale	19	.00[a]	1.13	.16	.39
High Rake	19	.00[a]	67.97	41.94	17.75
Tansley Dale	19	2.07	10.30	6.35	2.40
All Sites	155	.00[a]	1032.03	41.66	119.40

a. Readings of .00 do not indicate absence but readings may be below limits of detection for Xrf analysis.

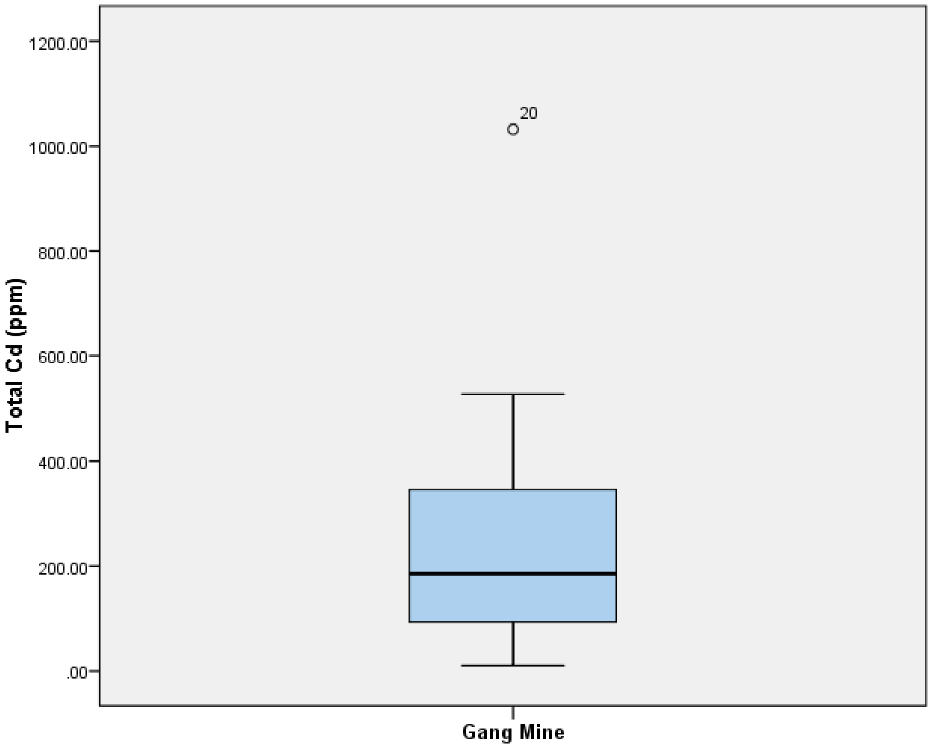


Figure 4:14. Box and whisker plot for total Cd at Gang Mine **(* =Extreme outlier ○=Minor outlier)**

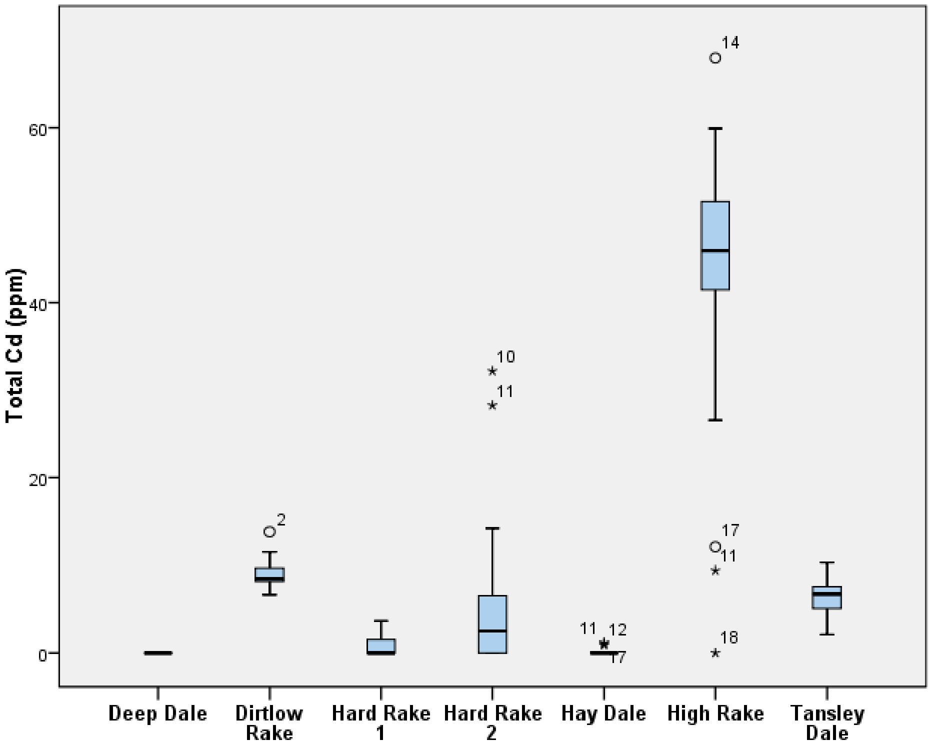


Figure 4:15. Box and whisker plot for total Cd at all sites excluding Gang Mine **(* =Extreme outlier ○=Minor outlier)**

4.5.6 Nickel (Ni)

Table 4:10 presents statistics relating to total Ni across the study sites. Several sites had samples with Ni below the level of detection during analysis, with Deep Dale having the lowest number of samples with Ni detectable [*Table.*4:10]. Dirtlow Rake displays the highest mean Ni concentrations, and Hard Rake 2 had the greatest levels present (>1500.00ppm) at sample points 9 and 10 [*Fig.* 4:17]. Deep Dale, High Rake, Gang Mine and Hay Dale samples contained the lowest concentrations of Ni [*Fig.* 4:16 & 4:17].

Table 4:10. Descriptive statistics for total Ni

Total Ni (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	.00[a]	61.19	4.13	13.62
Dirtlow Rake	20	779.26	1641.76	1074.28	189.46
Gang Mine	20	.00[a]	192.98	68.62	61.48
Hard Rake 1	19	.00[a]	1295.86	653.81	495.31
Hard Rake 2	19	.00[a]	1767.50	561.54	474.67
Hay Dale	19	.00[a]	449.21	96.99	100.00
High Rake	19	50.16	222.61	117.86	51.38
Tansley Dale	19	147.81	528.44	299.80	106.41
All Sites	155	.00[a]	1767.50	360.07	433.98

a. Readings of .00 do not indicate absence but readings may be below limits of detection for Xrf Analysis.

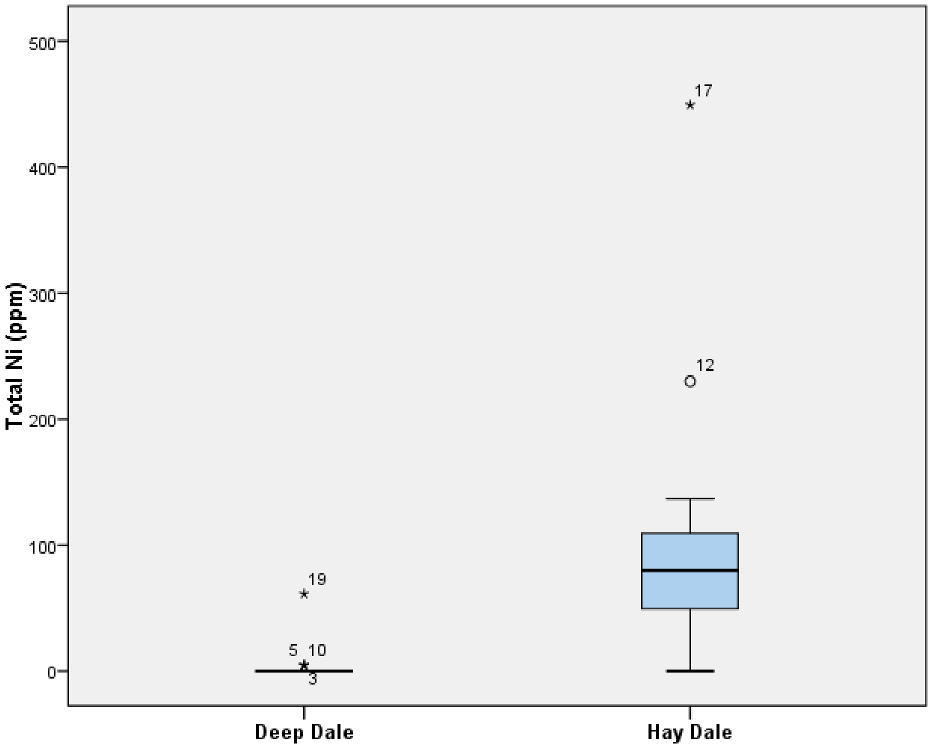


Figure 4:16. Box and whisker plot displaying total Ni concentrations **(* =Extreme outlier ○=Minor outlier)**

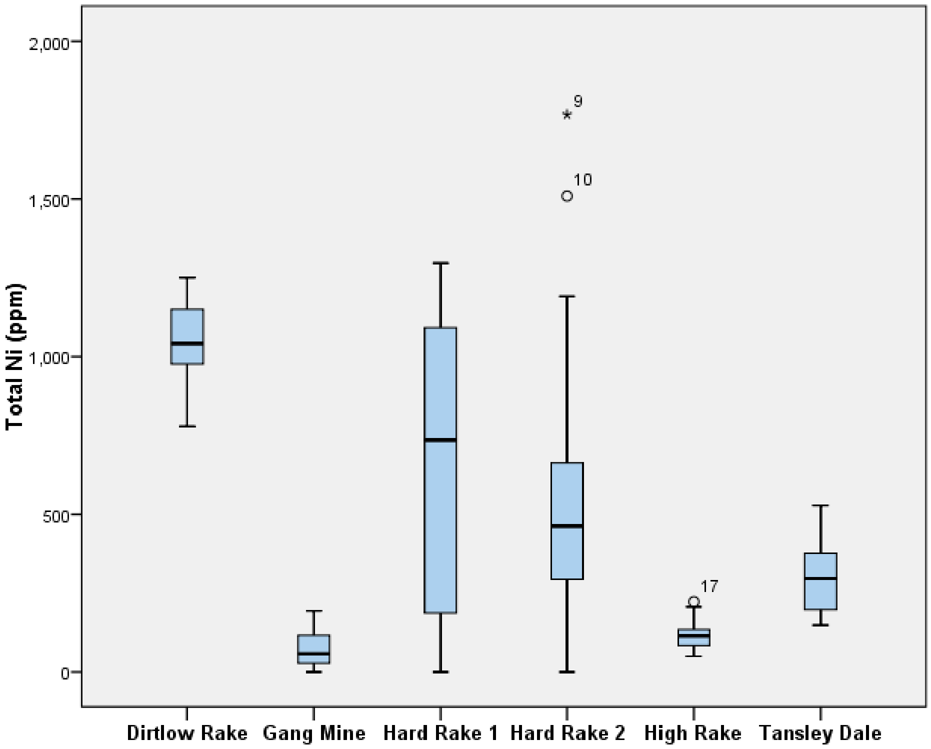


Figure 4:17. Box and whisker plot displaying total Ni concentrations **(* =Extreme outlier ○=Minor outlier)**

4.6 Bioavailable metal concentrations

The analysis of extracted heavy metals was undertaken using an ICP-OES, and the resulting data is presented here using tables of descriptive statistics and box and whisker plots. The concentrations of extracted metals are low compared to total metal concentrations, and indeed several samples from all sites contained limits of As and Pb that were below the levels of detection using the analytic methods described in Chapter 3: Methodology. Arsenic measurements in particular exhibited many instances of no detection, especially at Dirtlow Rake, Hard Rake 1 and High Rake. Concentrations of the extracted available heavy metals at Hard Rake and Gang Mine display considerably higher readings than the other sites for several metals and this corresponds with the results for total heavy metals in the previous section.

4.6.1 Bioavailable Lead (Pb)

Hard Rake and Gang Mine contain high levels of available Pb in comparison to other sites, with samples at Hard Rakes 2 reaching above 7.00ppm [*Table.*4:11]. Hay Dale samples contain minimal available Pb in comparison to other sites. Several sample points contain peaks in concentrations at Hard Rake, whilst Deep Dale and Hay Dale included many samples with available Pb below the limits of detection [*Fig.* 4:18 & 4:19].

Table 4:11. Descriptive statistics for bioavailable Pb

Extracted Bioavailable Pb (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	.000[a]	.193	.038	.045
Dirtlow Rake	20	.102	.799	.243	.153
Gang Mine	20	.086	1.451	.480	.405
Hard Rake 1	19	.123	5.056	.803	1.134
Hard Rake 2	19	.109	7.003	1.383	1.989
Hay Dale	19	.000[a]	.033	.011	.012
High Rake	19	.049	.439	.202	.116
Tansley Dale	19	.011	.146	.051	.039
All Sites	155	.000[a]	7.003	.399	.915

a. Readings of .000 do not indicate an absence but readings may be below limits of detection for ICP analysis.

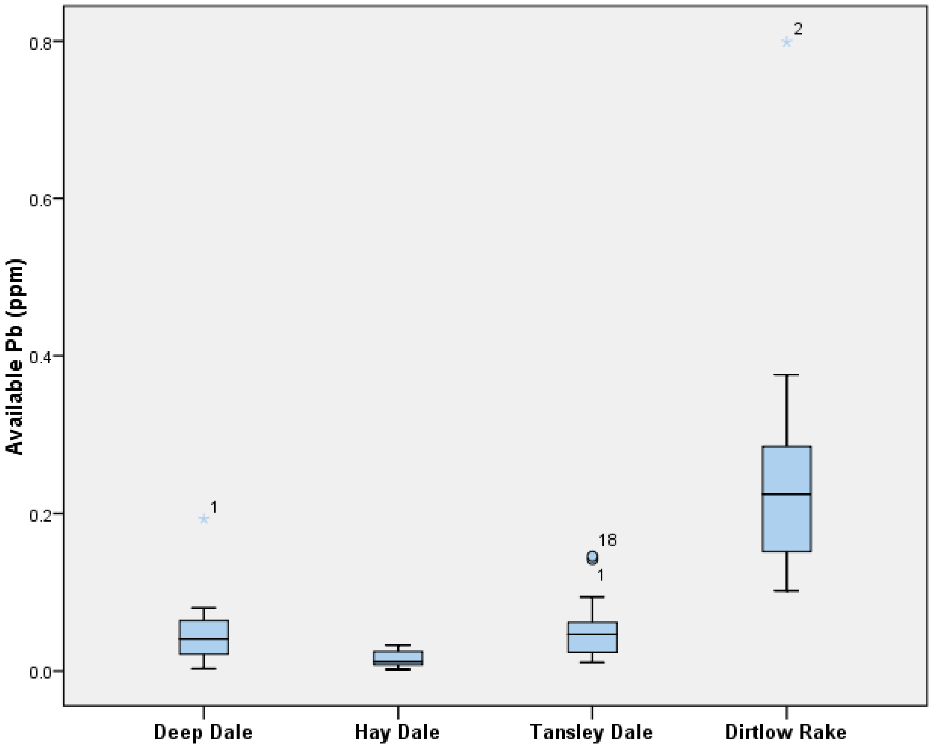


Figure 4:18. Box and whisker plot displaying available Pb concentrations **(* =Extreme outlier ○=Minor outlier)**

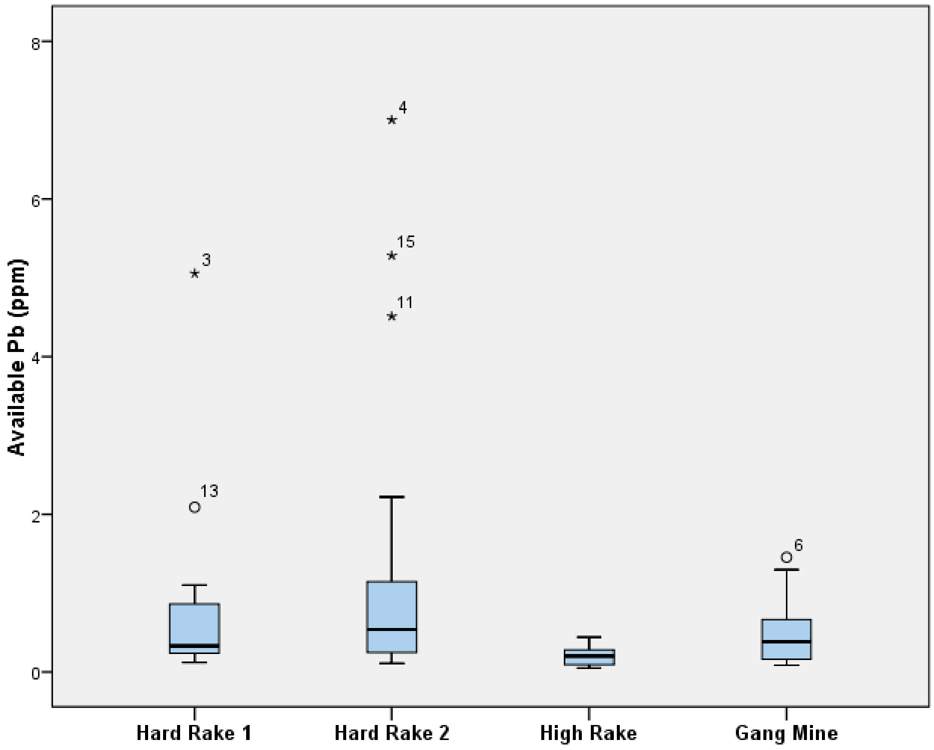


Figure 4:19. Box and whisker plot displaying available Pb concentrations **(* =Extreme outlier ○=Minor outlier)**

4.6.2 Bioavailable Arsenic (As)

All sites had samples that contained available arsenic levels below detection limits, and High Rake had no arsenic detected in any samples analysed [*Table.*4:12]. Dirtlow Rake and Hay Dale display the lowest maximum concentrations of arsenic [*Table.*4:12]. Gang Mine exhibits arsenic concentrations in excess of all other sites, reaching concentrations of more than 3.00ppm [*Fig.* 4:20 & 4:21]. Gang Mine also displays the widest range of arsenic values in samples [*Table.*4:12].

Table 4:12. Descriptive statistics for available As

Extracted Bioavailable As (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	.000	.093	.012	.026
Dirtlow Rake	20	.000	.017	.003	.005
Gang Mine	20	.000	3.098	1.186	.938
Hard Rake 1	19	.000	.046	.007	.014
Hard Rake 2	19	.000	.435	.046	.100
Hay Dale	19	.000	.020	.006	.008
High Rake	19	.000	.000	.000	.000
Tansley Dale	19	.000	.034	.009	.011
	155	.000	3.098	0.460	0.788

a. Readings of .0000 do not indicate absence but readings may be below the limits of detection for ICP analysis.

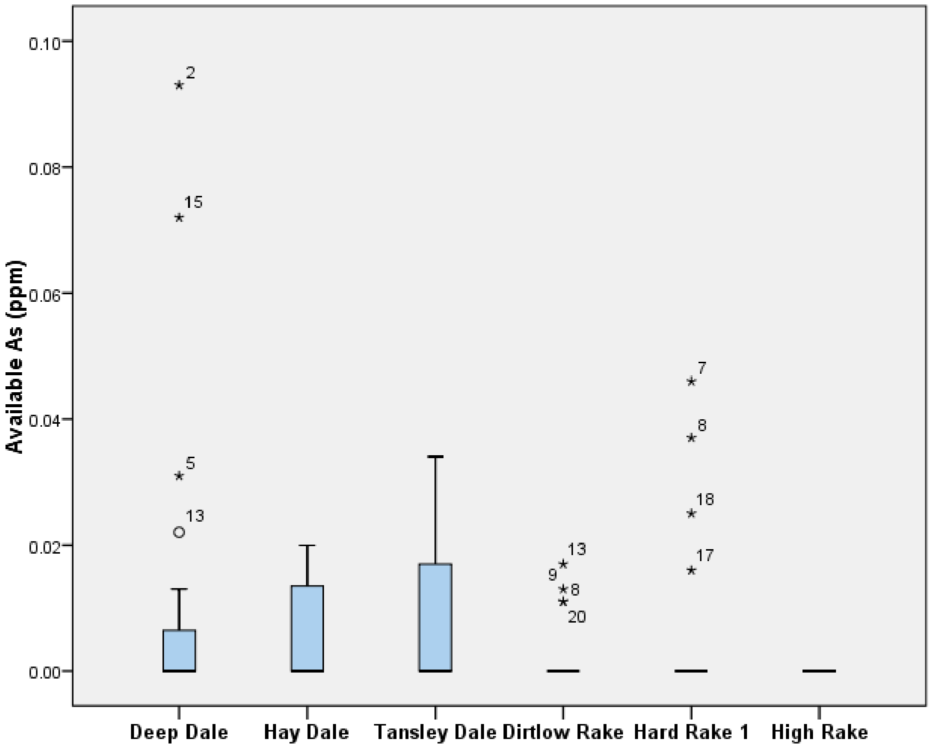


Figure 4:20. Box and whisker plot displaying available As concentrations **(* =Extreme outlier ○=Minor outlier)**

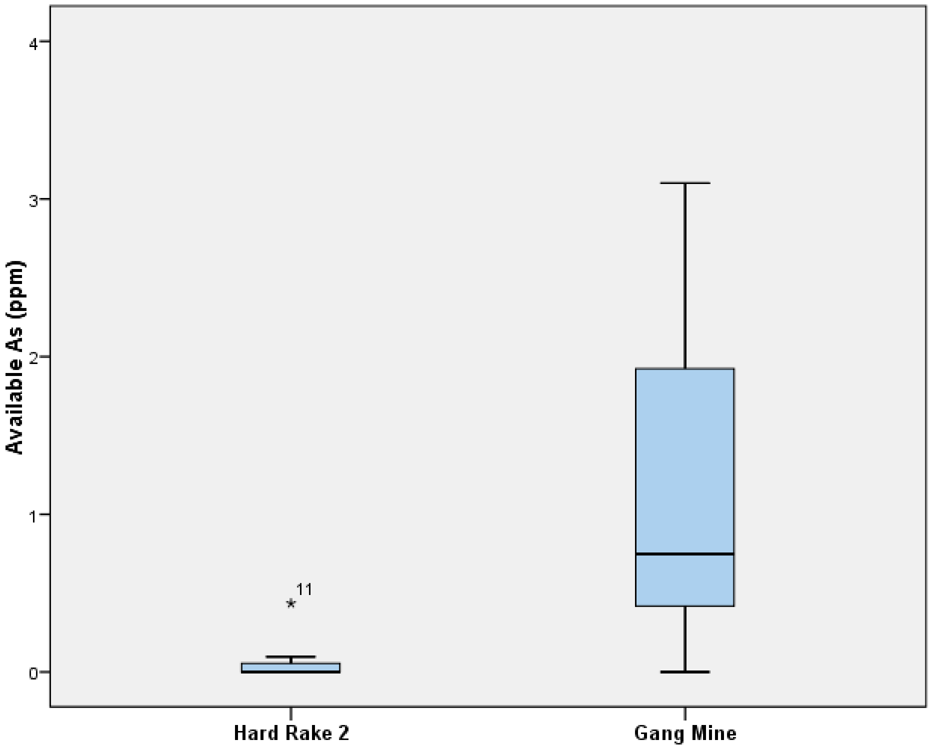


Figure 4:21. Box and whisker plot for available As concentrations **(* =Extreme outlier ○=Minor outlier)**

4.6.3 Bioavailable Zinc (Zn)

Bioavailable Zn presents the greatest concentrations of all extracted heavy metal results, reaching in excess of 43.00ppm in Gang Mine samples [*Table.*4:13]. Both Gang Mine and Hard Rake samples again display higher concentrations than other sites [*Fig.* 4:23]. Hay Dale contains the lowest Zn levels with a maximum reading of 0.064ppm [*Table.*4:13]. Most sites have spikes in Zn concentration at a number of sample points across their survey area [*Fig.* 4:22].

Table 4:13. Descriptive statistics for available Zn

Extracted Bioavailable Zn (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	.080	.872	.182	.169
Dirtlow Rake	20	.017	.342	.120	.070
Gang Mine	20	1.464	43.591	19.791	13.951
Hard Rake 1	19	.089	.986	.338	.268
Hard Rake 2	19	.104	18.574	2.056	4.360
Hay Dale	19	.011	.064	.036	.011
High Rake	19	.123	.789	.297	.212
Tansley Dale	19	.001	.139	.041	.033
All Sites	155	.001	43.591	2.932	8.308

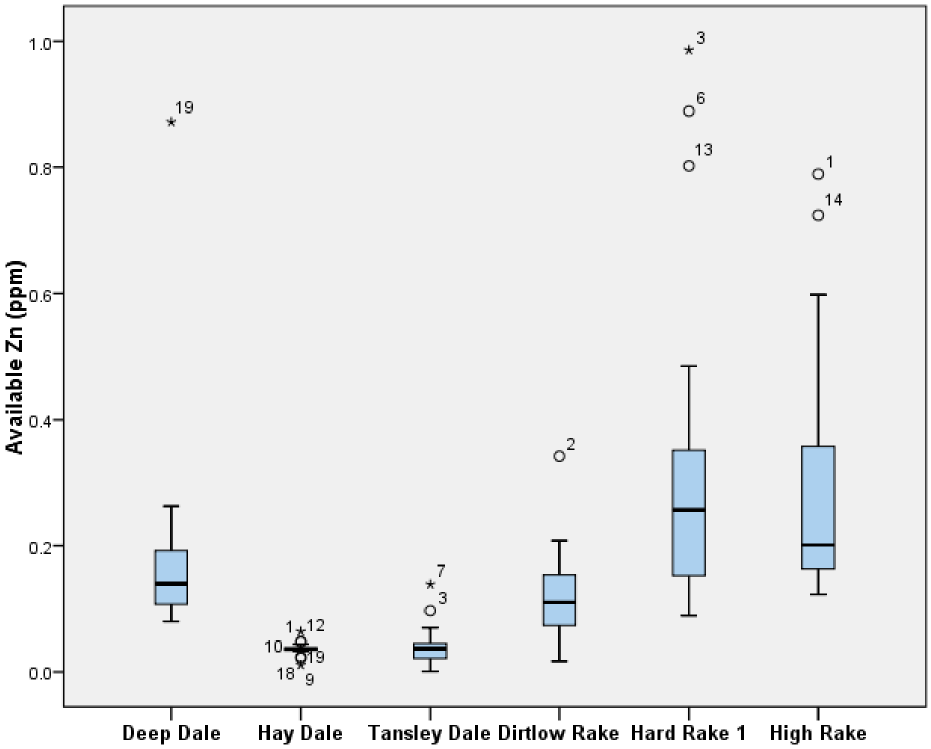


Figure 4:22. Box and whisker plot displaying available Zn concentrations **(* =Extreme outlier ○=Minor outlier)**

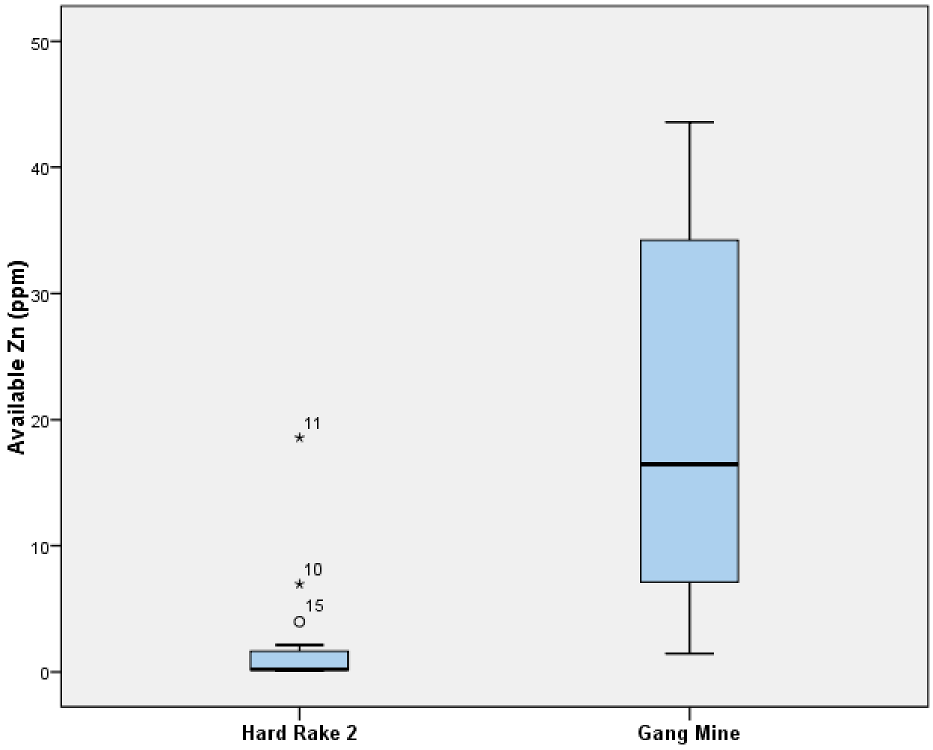


Figure 4:23 Box and whisker plot displaying available Zn concentrations (*** =Extreme outlier ○=Minor outlier)**

4.6.4 Bioavailable Copper (Cu)

Available Cu concentrations display the most consistent results of all the extracted metals across all sites [*Fig.* 4:24]. Hard Rake 1 samples contained both the lowest and greatest concentrations of Cu displaying the widest range in values [*Table.*4:14]. Hay Dale samples present the highest mean Cu concentrations [*Table.*4:14].

Table 4:14. Descriptive statistics for available Cu

Extracted Bioavailable Cu (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	.103	.121	.110	.006
Dirtlow Rake	20	.167	.194	.173	.006
Gang Mine	20	.106	.134	.114	.007
Hard Rake 1	19	.095	.354	.127	.060
Hard Rake 2	19	.098	.118	.108	.005
Hay Dale	19	.201	.242	.217	.011
High Rake	19	.099	.113	.105	.005
Tansley Dale	19	.162	.208	.184	.017
All Sites	155	.095	.354	.142	.046

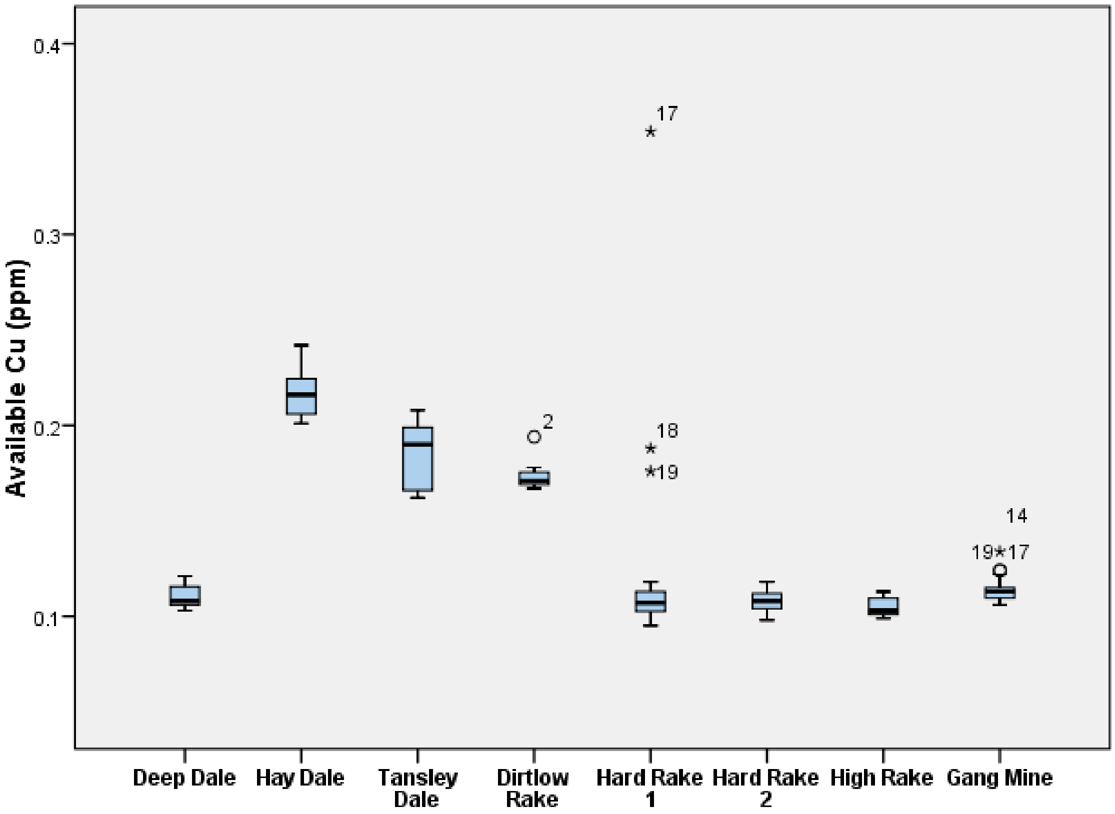


Figure 4:24. Box and whisker plot for available Cu measurements at all sites **(* =Extreme outlier ○=Minor outlier)**

4.6.5 Bioavailable Cadmium (Cd)

Cd concentrations reach a maximum of 0.951ppm in Gang Mine samples, and the highest mean levels of Cd are also found at Gang Mine [*Table.*4:15 & Fig.4:26]. The lowest minimum concentrations are found in Hard Rake 1 samples. Hard Rake 2 displays spikes in concentration at sample point 10 and 11 [*Fig.* 4:25]. Generally, Cd concentrations are evenly distributed across all sites with the exception of Gang Mine [*Fig.* 4:25].

Table 4:15. Descriptive statistics for available Cd

Extracted Bioavailable Cd (ppm)

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	.050	.117	.088	.020
Dirtlow Rake	20	.045	.073	.058	.008
Gang Mine	20	.068	.951	.461	.292
Hard Rake 1	19	.042	.116	.086	.023
Hard Rake 2	19	.060	.374	.122	.064
Hay Dale	19	.057	.077	.068	.005
High Rake	19	.081	.116	.097	.009
Tansley Dale	19	.046	.088	.065	.011
All Sites	155	.042	.951	.132	.166

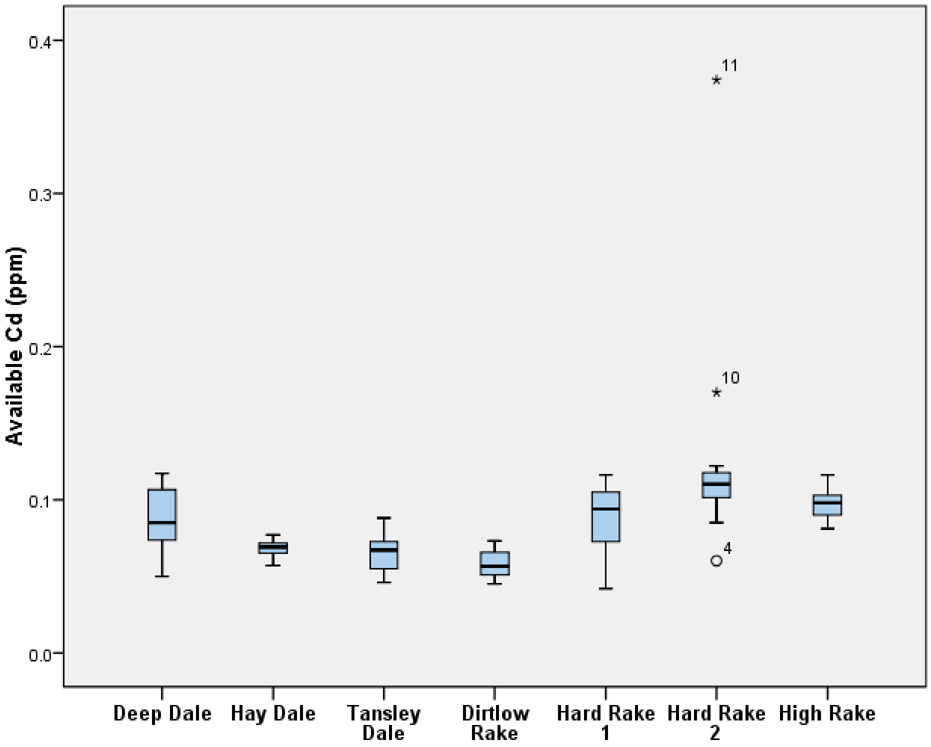


Figure 4:25. Box and whisker plot for available Cd measurements at all site except Gang Mine **(* =Extreme outlier ○=Minor outlier)**

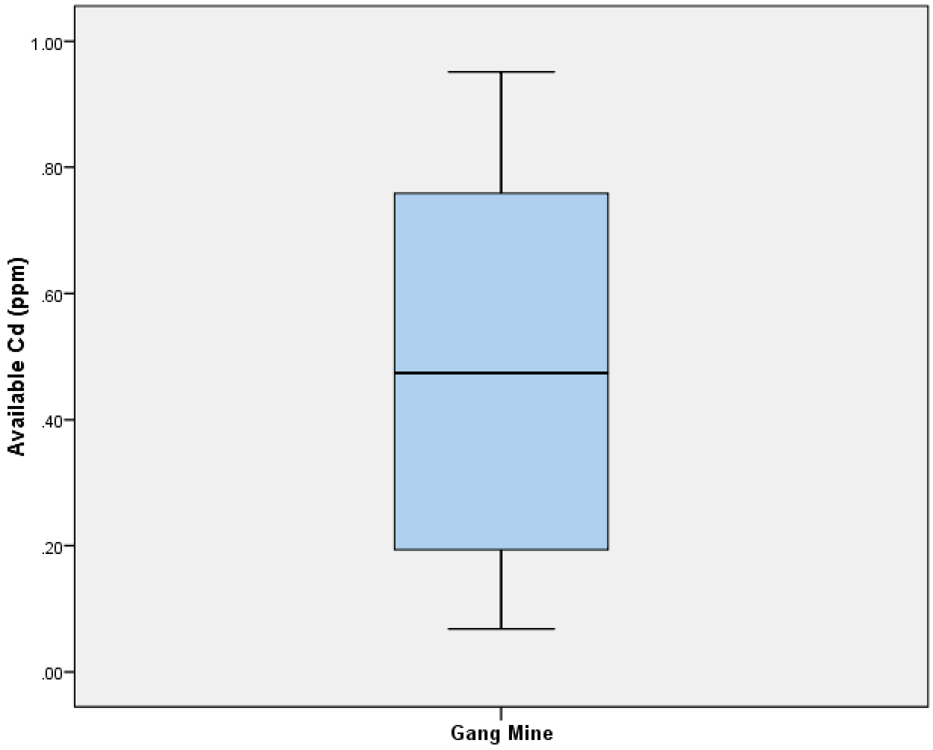


Figure 4:26. Box and whisker plot for available Cd measurements at Gang Mine **(* =Extreme outlier ○=Minor outlier)**

4.6.6 Bioavailable Nickel (Ni)

Available Ni concentrations are fairly evenly distributed across all sites, ranging from a minimum of 0.055ppm at Hard Rake 1 to a maximum concentration of 0.230ppm at both Deep Dale and Hard Rake 1[*Fig.* 4:27 & *Table.*4:16]. Hay Dale displays the highest mean concentration of available Ni with 0.178ppm [*Table.*4:16].

Table 4:16. Descriptive statistics for available Ni

Extracted Bioavailable Ni (ppm)

Descriptive Statistics

	N	Minimum	Maximum	Mean	Std. Deviation
Deep Dale	20	.071	.230	.105	.038
Dirtlow Rake	20	.133	.159	.141	.006
Gang Mine	20	.063	.150	.098	.021
Hard Rake 1	19	.055	.230	.109	.039
Hard Rake 2	19	.061	.188	.093	.030
Hay Dale	19	.163	.191	.178	.008
High Rake	19	.073	.100	.089	.007
Tansley Dale	19	.136	.194	.163	.020
All Sites	155	.055	.230	.122	.040

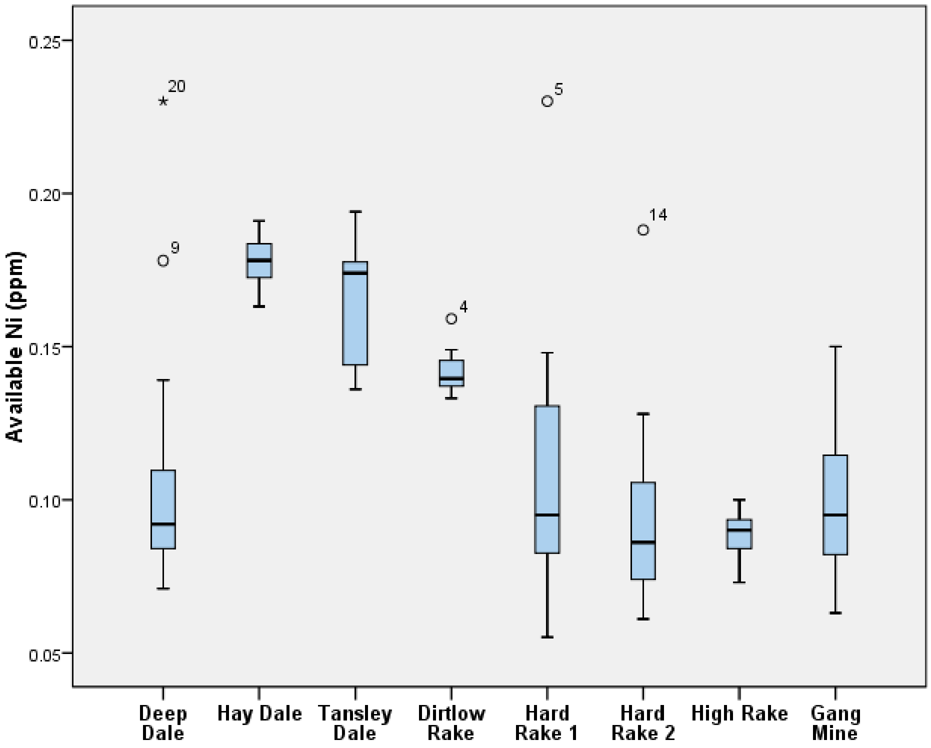


Figure 4:27. Box and whisker plot for available Ni measurements at all sites **(* =Extreme outlier ○=Minor outlier)**

4.7 Vegetation survey results

Vegetation identification and recording at the study sites was undertaken using both the DOMIN and Braun-Blanquet scales of cover/abundance. Systematic sampling was used along fixed interval sampling quadrats using the transect design presented in Fig.3:9 in Chapter 3: Methodology. This method was utilised in order to explore relationships between the vegetation recorded and environmental variables measured at the same spatial position. The transects include sixteen sample points: one two metres from the perceived point of mining activity, and three transects each with five quadrats placed four metres apart.

Twenty-six flowering species were identified across all sites [*Table.*4:17], all of which are commonly found on calcareous grasslands and meadows (Streeter *et al.*, 2009): twenty-three perennials, two biennials and one annual flowering species in total. Although vegetation surveys for this research were carried out using both the DOMIN and Braun-Blanquet scale of cover/abundance to ensure a thorough collection of vegetation data, from here on only the DOMIN scale data will be reported in these results to exclude any repetition of similar data.

The DOMIN scale of cover/abundance offers more precise subdivisions in its percentage cover/abundance scoring system, offering a ten-point scale as opposed to the six-point Braun-Blanquet scale. It is also a more widely used method in Britain and is used by the Joint Nature Conservation Committee for the National Vegetation Classification of Britain (Rodwell, 2006). All Braun-Blanquet data results can be found in Appendix K.

Table 4:17. Species identified at all sites

	Common Name	**Latin Name**	**Family**	**Life Cycle**
1	BURNET-SAXIFRAGE	(Pimpinella saxifraga)	Apiaceae	Perennial
2	CARLINE THISTLE	Carlina vulgaris	Asteraceae (Compositae)	Biennial
3	COMMON BIRDSFOOT TREFOIL	Lotus corniculatus	Fabaceae (Legumes)	Perennial
4	COMMON KNAPWEED	*Centaurea nigra*	Asteraceae (Compositae)	Perennial
5	COMMON MOUSE EAR	*Cerastium fontanum*	Caryophyllaceae	Perennial
6	DEVILS BIT SCABIOUS	*Succisa pratensis*	Dipsacaceae	Perennial
7	DROPWORT	*Filipendula vulgaris*	Saxifragaceae	Perennial
8	DWARF THISTLE	*Cirsium acaule*	Asteraceae (Compositae)	Perennial
9	EYEBRIGHT	*Euphrasia officinalis*	Scrophulariaceae	Annual
10	HAREBELL	*Campanula rotundifolia*	Campanulaceae	Perennial
11	LADYS BEDSTRAW	*(Galium verum)*	Rubiaceae	Perennial
12	LESSER HAWKBIT	*Leontodon saxatilis*	Asteraceae (Compositae)	Perennial
13	MEADOW BUTTERCUP	*Ranunculus acris*	Ranunculaceae	Perennial
14	MILKWORT	*Polygala vulgaris*	Polygalaceae	Perennial
15	MOUNTAIN PANSY	*Viola lutea*	Violaceae	Perennial
16	MOUSE EAR HAWKWEED	*Pilosella officinarum*	Asteraceae (Compositae)	Perennial
17	RED CLOVER	*Trifolium pratense*	Fabaceae (Legumes)	Perennial
18	ROCK ROSE	*Helianthemum nummularium*	Cistaceae	Perennial
19	ROUGH HAWKBIT	*Leontodon hispidus*	Asteraceae (Compositae)	Perennial
20	SALAD BURNET	*Sanguisorba minor*	Rosaceae	Perennial
21	SMALL SCABIOUS	*Scabiosa columbaria*	Dipsacaceae	Perennial
22	SPEAR THISTLE	*Cirsium vulgare*	Asteraceae (Compositae)	Biennial
23	TORMENTIL	*Potentilla erecta*	Rosaceae	Perennial
24	WHITE CLOVER	*Trifolium repens*	Fabaceae (Legumes)	Perennial
25	WILD THYME	*Thymus polytrichus*	Lamiaceae	Perennial
26	YARROW	*Achillea millefolium*	Asteraceae (Compositae)	Perennial

4.7.1 Species richness and cover/abundance

Species cover/abundance revealed Dirtlow Rake to be the most floristic site with a total DOMIN score of 290 for all sixteen quadrats. Tansley Dale contained the least flowering calcicolous vegetation, scoring 92. Hay Dale and Deep Dale scored very similarly, as did Gang Mine, High Rake and Hard Rake. Dirtlow Rake also had the quadrat with the most species cover/abundance. Several sites included quadrats where no flowering vegetation was present [*Table.*4:18].

Table 4:18. Total DOMIN score for each quadrat

DOMIN Score	**Deep Dale**	**Dirtlow Rake**	**Gang Mine**	**Hard Rake1**	**Hard Rake 2**	**Hay Dale**	**High Rake**	**Tansley Dale**
Quadrat 1	18	4	10	15	24	26	11	8
Quadrat 2	21	22	14	13	10	16	10	5
Quadrat 3	12	0	2	13	17	10	12	5
Quadrat 4	13	0	8	12	9	20	12	8
Quadrat 5	14	0	12	17	16	16	22	9
Quadrat 6	16	23	6	0	8	14	4	8
Quadrat 7	17	28	10	19	19	21	13	12
Quadrat 8	21	35	9	18	13	12	8	15
Quadrat 9	14	22	12	12	8	17	13	7
Quadrat 10	17	10	2	2	6	22	14	7
Quadrat 11	19	10	4	9	6	24	12	8
Quadrat 12	19	37	23	29	12	20	0	0
Quadrat 13	11	34	7	8	14	9	3	0
Quadrat 14	10	39	3	16	8	0	20	0
Quadrat 15	12	26	15	4	6	0	0	0
Quadrat 16	6	0	11	7	11	6	4	0
Total	**240**	**290**	**148**	**194**	**187**	**233**	**158**	**92**
Mean	**15**	**18.13**	**9.25**	**12.13**	**11.69**	**14.56**	**9.88**	**5.75**
Max	**21**	**39**	**23**	**29**	**24**	**26**	**22**	**15**
Min	**6**	**0**	**2**	**0**	**6**	**0**	**0**	**0**
Std Dev	**4.23**	**14.58**	**5.5**	**7.23**	**5.21**	**7.92**	**6.41**	**4.65**

Table 4:19 presents the DOMIN score for each species recorded at each site. The most frequently occurring species was eyebright (*Euphrasia officinalis*); other prominent species include red clover (*Trifolium pratense),* yarrow (*Achillea millefolium)* and common birds-foot trefoil (*Lotus corniculatus*). The least frequently occurring species identified were carline thistle (*Carlina vulgaris*), dwarf thistle (*Cirsium acaule*) and rock rose (*Helianthemum nummularium*). Some species only occurred at one location and these include common knapweed (*Centaurea nigra*), devils-bit scabious (*Succisa pratensis*) and lesser hawk-bit (*Leontodon saxatilis*) at Deep Dale, dwarf thistle (*Cirsium acaule*) at Hard Rake and rock rose (*Helianthemum nummularium*) at Tansley Dale. Of particular interest is the occurrence of mountain pansy (*Viola lutea*), a well-studied metallophyte (Hermann *et al.*, 2013; Kuta *et al.*, 2012; Słomka *et al.*, 2011), at both Gang Mine and Hard Rake 2.

Table 4:19. DOMIN score of cover/abundance for each species

Common Name	Latin Name	Deep Dale	Dirtlow Rake	Gang Mine	Hard Rake 1	Hard Rake 2	Hay Dale	High Rake	Tansley Dale
BURNET-SAXIFRAGE	*Pimpinella saxifraga*	0	0	18	0	0	0	2	0
CARLINE THISTLE	*Carlina vulgaris*	0	0	0	0	0	4	6	0
COMMON BIRDSFOOT TREFOIL	*Lotus corniculatus*	6	6	33	24	20	0	5	6
COMMON KNAPWEED	*Centaurea nigra*	41	0	0	0	0	0	0	0
COMMON MOUSE EAR	*Cerastium fontanum*	0	0	0	15	0	0	0	0
DEVILS BIT SCABIOUS	*Succisa pratensis*	79	0	0	0	0	0	0	0
DROPWORT	*Filipendula vulgaris*	4	0	0	0	0	15	0	2
DWARF THISTLE	*Cirsium acaule*	0	0	0	3	0	0	0	0
EYEBRIGHT	*Euphrasia officinalis*	2	44	32	19	45	52	0	25
HAREBELL	*Campanula rotundifolia*	4	4	3	10	2	27	17	20
LADYS BEDSTRAW	*Galium verum*	0	41	0	4	4	10	3	0
LESSER HAWKBIT	*Leontodon saxatilis*	55	0	0	0	0	0	0	0
MEADOW BUTTERCUP	*Ranunculus acris*	0	0	11	22	5	0	0	0
MILKWORT	*Polygala vulgaris*	0	4	0	6	6	0	0	0
MOUNTAIN PANSY	*Viola lutea*	0	0	8	0	11	0	0	0
MOUSE EAR HAWKWEED	*Pilosella officinarum*	0	0	0	4	13	11	0	0
RED CLOVER	*Trifolium pratense*	4	42	2	25	17	39	59	0
ROCK ROSE	*Helianthemum nummularium*	0	0	0	0	0	0	0	4
ROUGH HAWKBIT	*Leontodon hispidus*	0	44	0	0	0	0	4	0
SALAD BURNET	*Sanguisorba minor*	45	0	0	3	0	10	0	7
SMALL SCABIOUS	*Scabiosa columbaria*	0	30	14	0	0	14	24	5
TORMENTIL	*Potentilla erecta*	0	0	0	0	14	12	0	0
WHITE CLOVER	*Trifolium repens*	0	0	12	27	24	0	8	0
WILD THYME	*Thymus polytrichus*	0	21	15	0	6	18	0	28
YARROW	*Achillea millefolium*	0	54	0	14	20	12	32	0
Total Species Recorded at Site		**10**	**10**	**10**	**15**	**12**	**13**	**11**	**9**

Chapter 5: Analysis and interpretation of results

5.1 Introduction

The associations between soil variables and floristic distribution at the study areas is explored in section 5.1 with the use of canonical correspondence analysis (CCA) and cluster analysis.

Sections 5.2 and 5.3 focus on the relationships between soil variables, particularly the total and available heavy metal variables, and the examination of particle size analysis (PSA) results, along with how these relate to heavy metal concentrations. Principal component analysis (PCA) and scatterplots are utilised to display these results.

The use of GIS isopleth maps is employed in section 5.4 to explore the spatial distribution of total Pb and vegetation cover/abundance at the study sites and any patterns of similarity that exist between them, however a more thorough analysis of metal and vegetation distribution patterns are presented in Chapter 6: Discussion.

Due to the large amount of data and results generated during this research, tables and isopleth maps have been omitted from this results chapter. Where this is the case, they are contained within the appendices and signposted where relevant in this chapter.

5.1.1 Relationships between vegetation and soil variables

Canonical correspondence analysis (CCA) was utilised as a method to simplify the large number of environmental variables and the species abundance data and to investigate their relationships. Because of gaps in the vegetation data due to the absence of certain species or their poor distribution across the study sites, subjective partitioning was undertaken on initial CCA plots (Kent & Coker, 1994) to reduce the twenty-six plant species to fifteen plant families. This process reduced crowding on the CCA plots, thus reducing the requirement to disregard some of the rarer species in favour of using only the most abundant species during analysis (Chahouki, 2013). This decision was also influenced by the fact that species from the same family have similar biological traits and evolutionary relationships

(Purves, Orians, Heller, & Sadava, 2004). Only the rarest species, rock-rose, *Helianthemum nummularium,* of the family Cistaceae (representing less than 0.26% of floristic cover/abundance) was disregarded from the CCA using heavy metal variables. The family of each species is presented in *Table* 4:17.

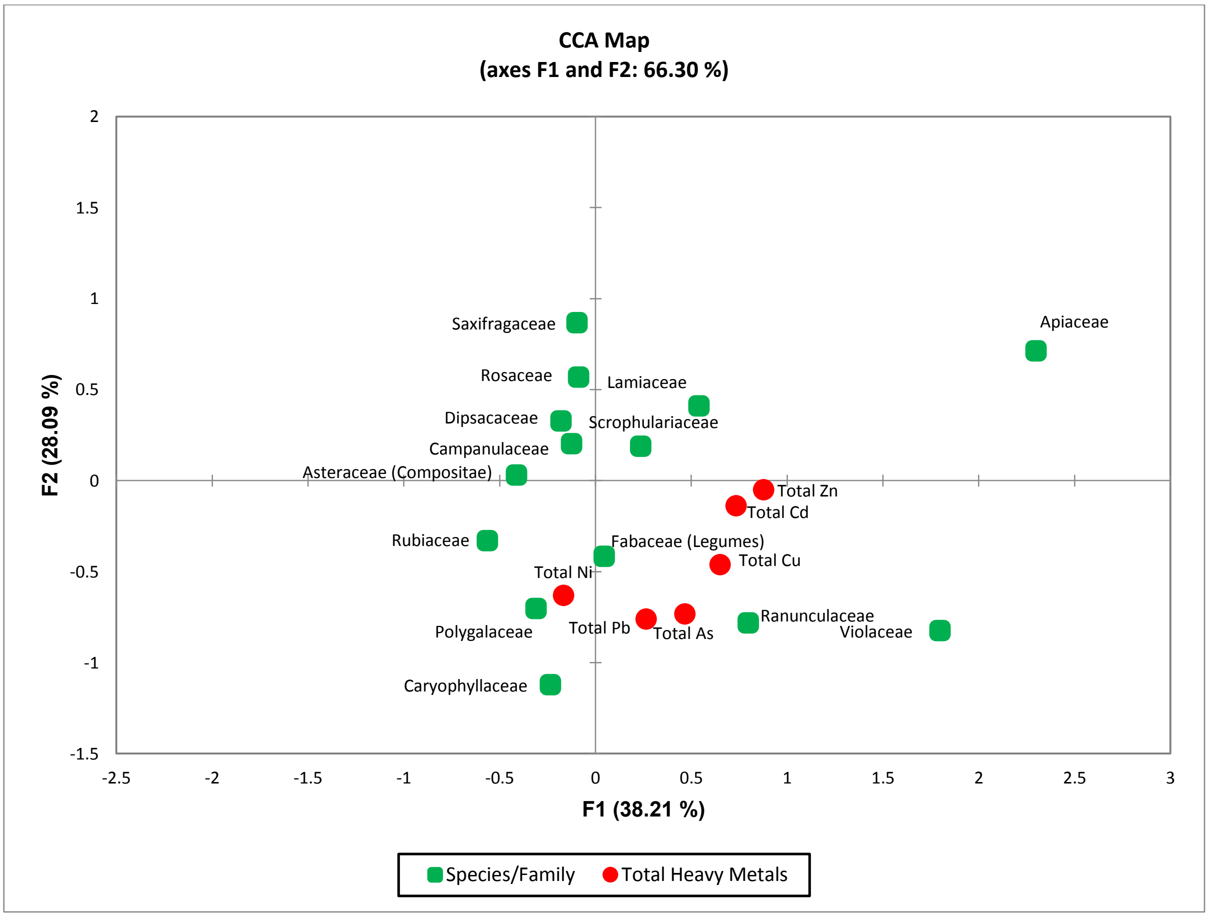


Figure 5:1. CCA of plant families and total heavy metal concentrations from all sites

The CCA plot in *Figure* 5:1 displays the thirteen plant families (green) alongside the six total heavy metal variables (red) and axes F1 and F2 represent 66.30% of the variance in the data-set. A clear division runs diagonally in the central area of the biplot, with several vegetation variables grouped around the heavy metal variables. It may be assumed that these types of vegetation are more tolerant to the concentrations of heavy metals found in the soil samples. The plant families grouped with the heavy metal variables include the species white clover (*Trifolium repens)*, red clover (*Trifolium pratense)* and birds-foot trefoil (*Lotus* *corniculatus)* (all legumes in the family Fabaceae); meadow buttercup (*Ranunculus acris)* (family Ranunculaceae), milkwort (*Polygala vulgaris)* (family, Polygalaceae), common mouse-ear (*Cerastium fontanum)* (family Caryophyllaceae) and mountain pansy (*Viola lutea)* (family, Violaceae), a known metallophyte.

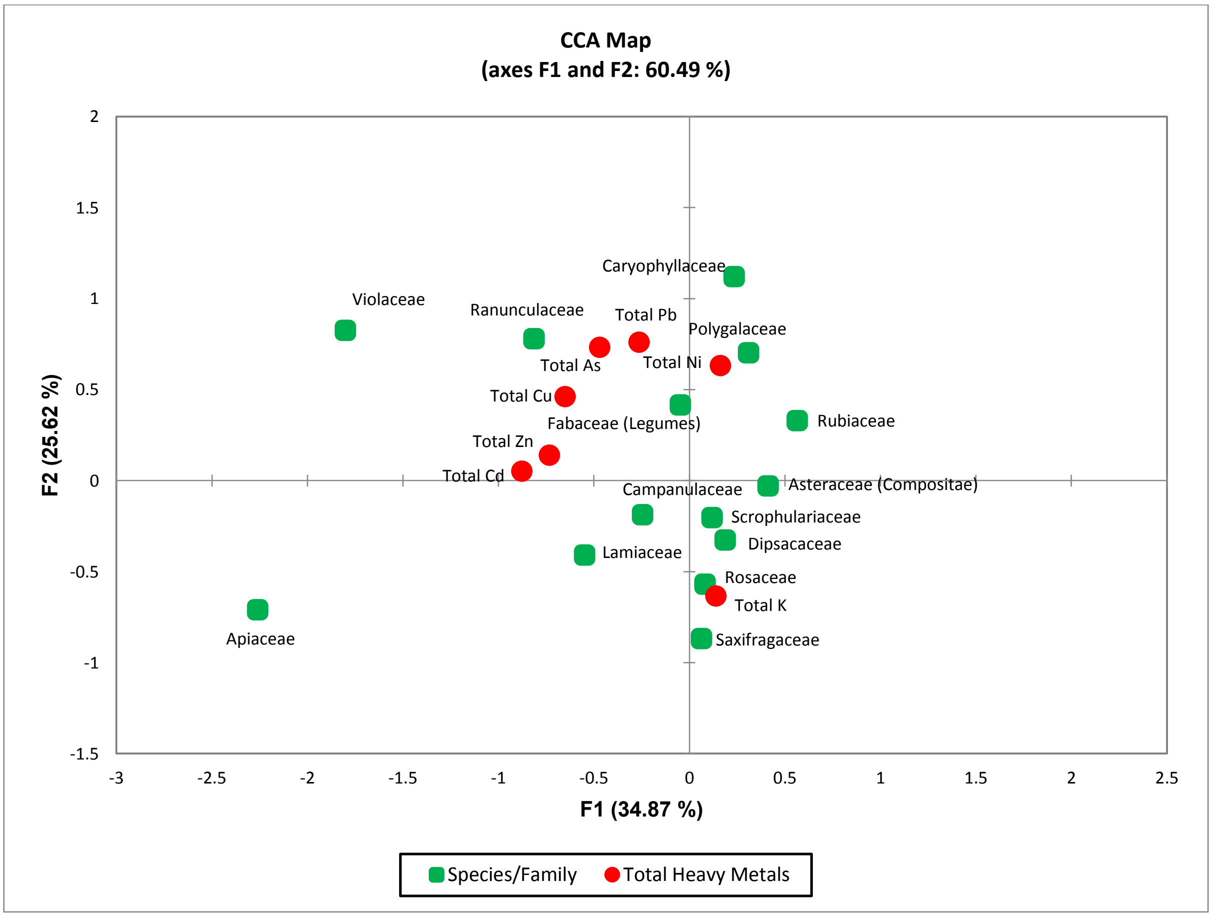


Figure 5:2. CCA of plant families and total heavy metal concentrations from all sites with the inclusion of total K concentrations

The inclusion of total K concentration in the dataset in *Figure* 5:2 seems to compound the assumption that metal tolerant species are located close to the heavy metal variables and other species/families are pulled further from the heavy metals towards a naturally occurring and important element for plant growth (Dubbin, 2001). The biplot in *Figure* 5:2 represents 60.49% of the variance for this dataset. The same vegetation variables are grouped with the heavy metal variables as with *Figure* 5:1.

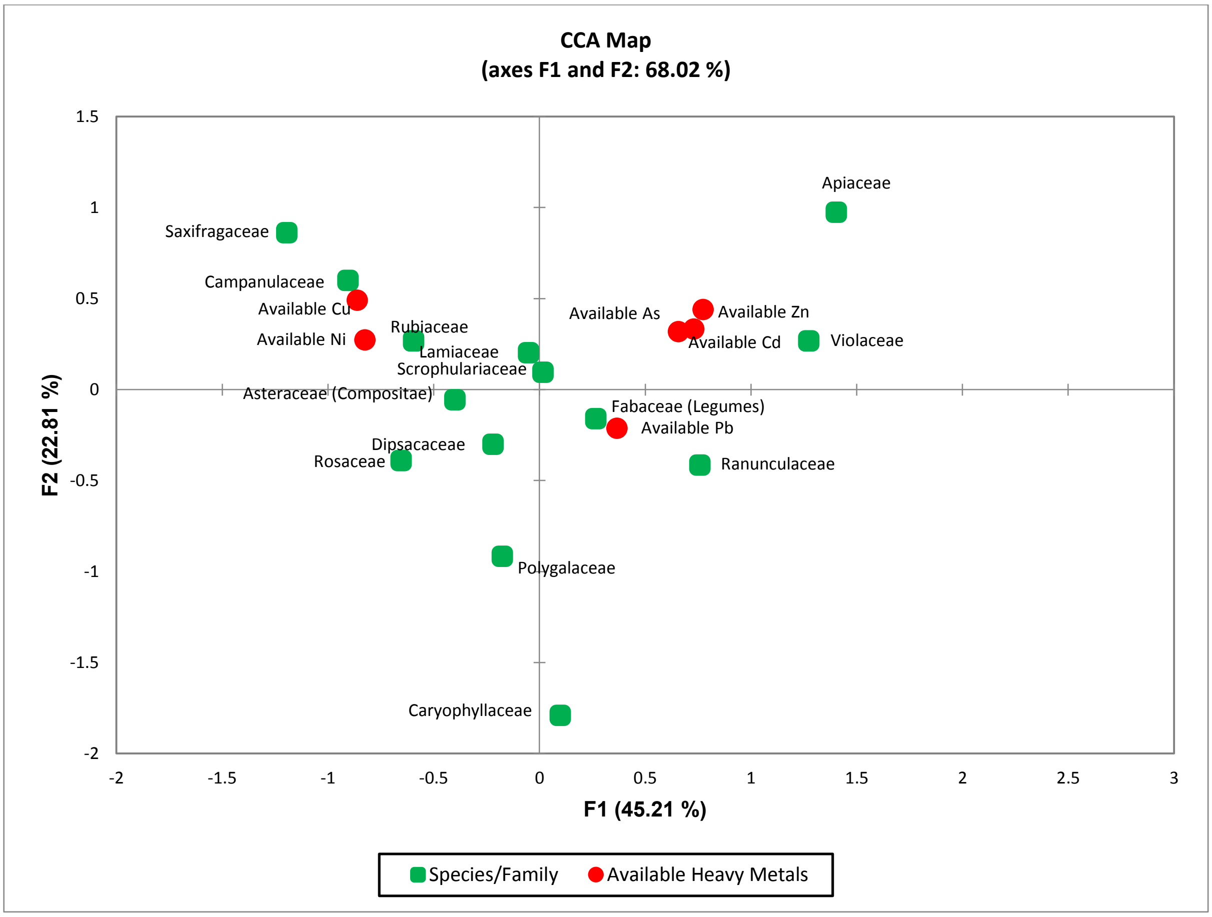


Figure 5:3. CCA of plant families and available heavy metal concentrations from all sites

Figure 5:3 displays a CCA biplot using the same plant family variables, whilst the environmental variables are the extracted available fractions of heavy metals measured in the soil samples. The two axes on the biplot represent 68.02 of the variance in the dataset. Plant families, Fabaceae, Ranunculaceae and Violaceae are grouped with the available Pb, As, Zn and Cd. Cu and Ni are separated from this group of heavy metals and this may be attributed to the group containing Pb, As, Zn and Cd of an elevated concentration caused by anthropogenic activity at the survey sites, whilst Cu and Ni are closer to naturally occurring background concentrations of these elements in this type of habitat.

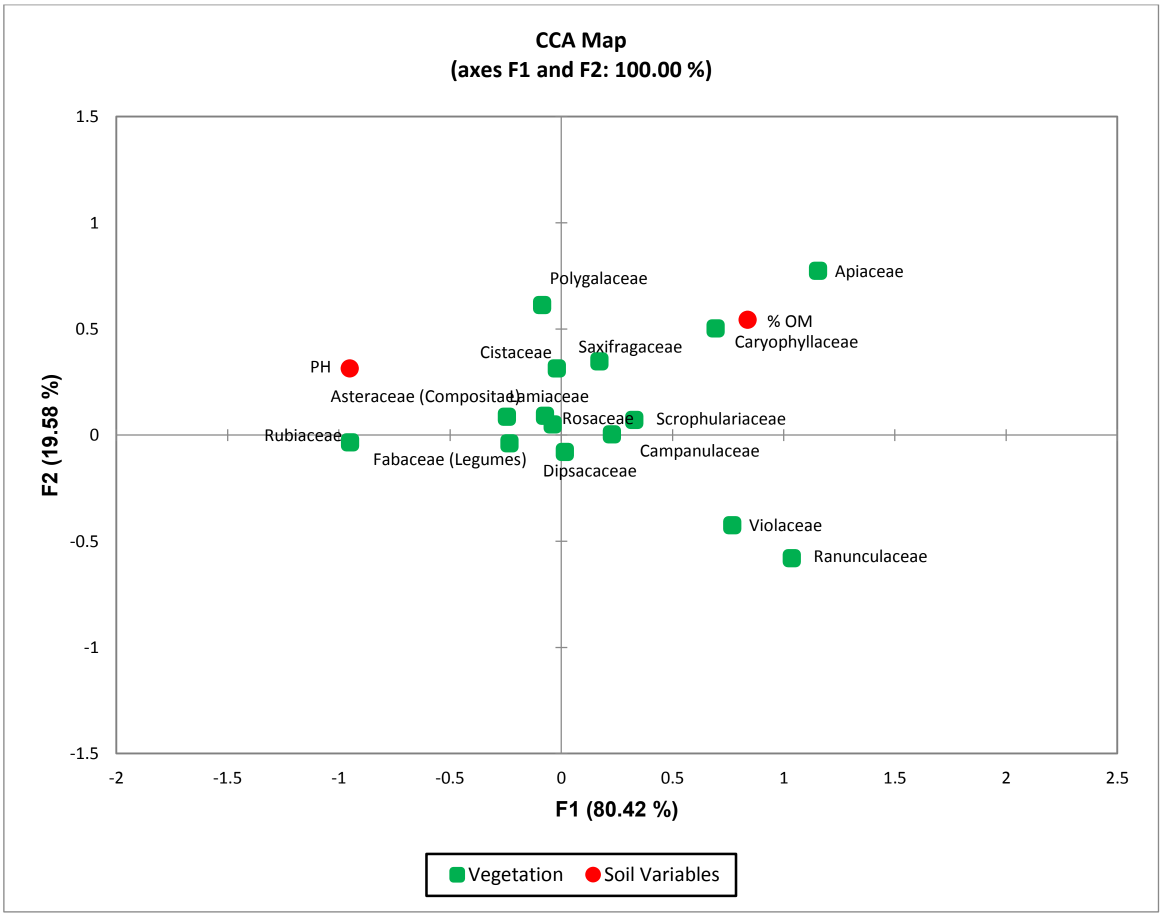


Figure 5:4. CCA plot displaying pH, SOM and plant families

The CCA biplot in *Figure* 5:4 represents 100% of the variance in this dataset and plots the vegetation variables with pH and SOM% measured in the soil samples. The majority of plant families are grouped centrally between the two environmental variables. Rubiaceae (containing the species lady's bedstraw, *Galium verum*) is influenced by pH to the left of the biplot, whilst Caryophyllaceae (species common mouse ear, *Cerastium fontanum*) and Apaiceae (species, burnet saxifrage, *Pimpinella saxifraga*) are influenced by SOM. Violaceae and Ranunculaceae are distanced from both environmental variables.

5.1.2 Cluster analysis

Cluster analysis was carried out using the plant family variables. Firstly, the variables were standardized using z-scores, after which Euclidean distances were calculated to find similarities in the variables (Li & Feng, 2012). The Ward linkage method was utilised to carry out hierarchical clustering on the standardised data set. The cluster analysis results for the vegetation variables are displayed in *Figure* 5:5 as a dendrogram, which displays a very similar pattern to the CCA analysis. Three distinct clusters can be observed in the dendrogram. Cluster I includes Rosaceae, Saxifragaceae, Campanulaceae, Dipsacaceae and Cistaceae. Cluster II consists of Caryophyllaceae, Polygalaceae, Asteraceae and Rubiaceae. Clusters I and II tend to be distanced from Pb, As, Zn, Cd heavy metal concentrations in CCA. Cluster III in the dendrogram contains plant families that showed greater association with Pb, As, Zn and Cd and those more dissociated from families in cluster I and II. Cluster III correspondingly contains two sub-clusters, Fabaceae, Violaceae and Ranunculaceae, tended to be more closely grouped with Pb, As, Zn and Cd, and Lamiaceae, Scrophulariaceae and Apiaceae, further detached from flora in clusters I and II.

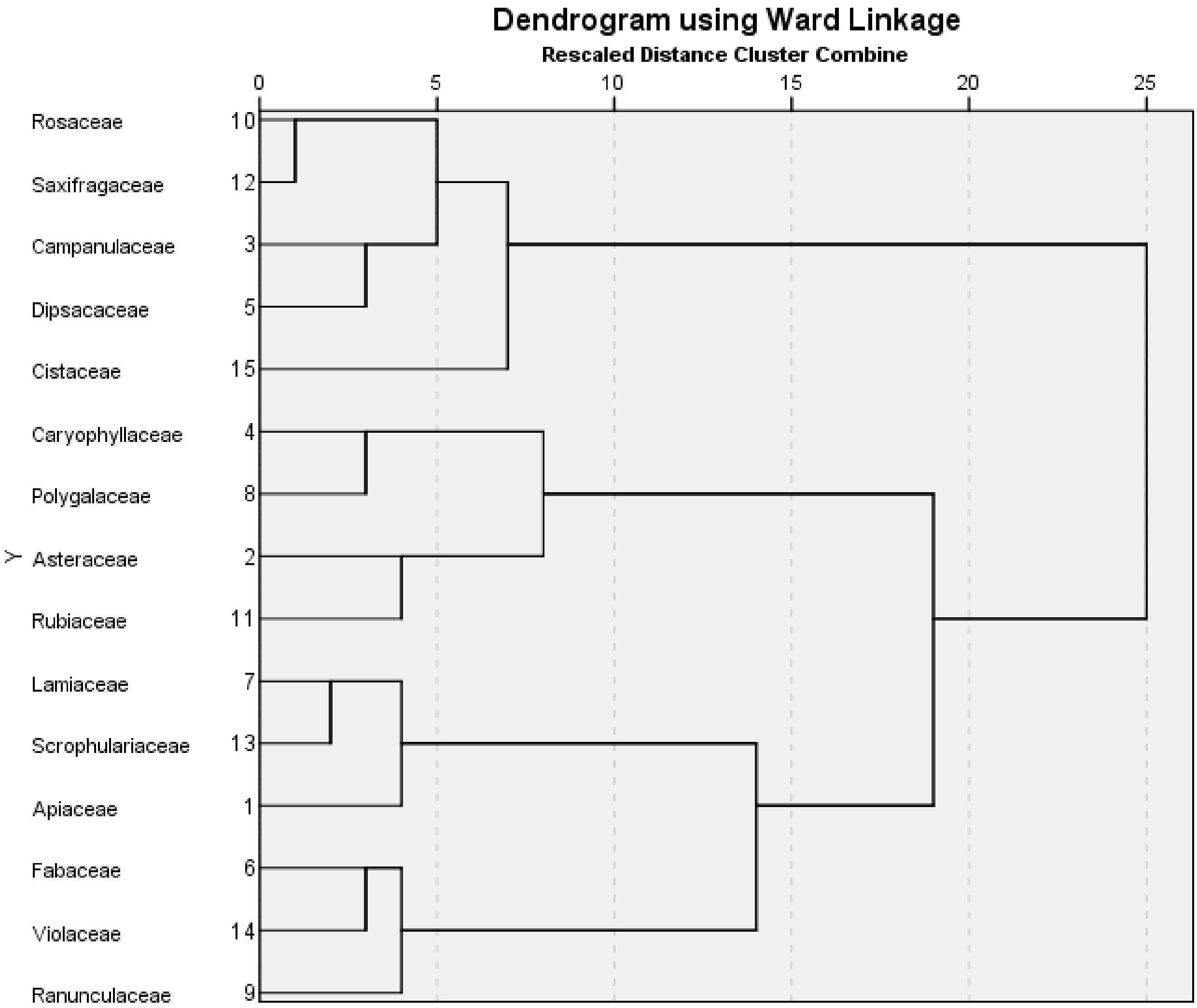


Figure 5:5. Dendrogram displaying clustering of those plant families closely related

5.2 Relationship between total and available heavy metal fractions

5.2.1 Relationships between soil variable measurements

A main objective of this study was to determine the bioavailable fraction of the heavy metals and metalloids in soil samples taken from the study sites. This section presents scatterplots to explore any correlation between total and available heavy metals measured in all 155 soil samples taken from all eight sites.

5.2.2 Total and available heavy metal relationship

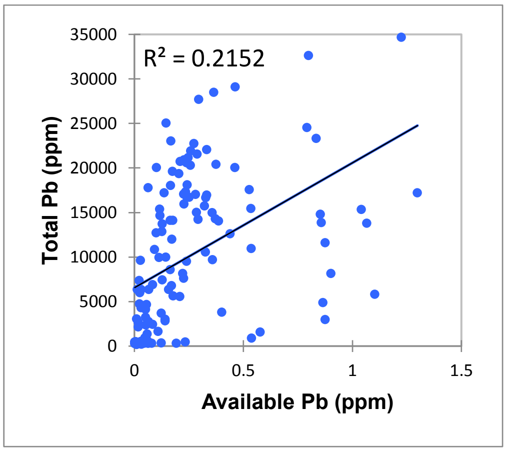


Figure 5:6. Scatterplot displaying relationship between total and available Pb (y = 14021x + 6554.5)

*Fig.*5:6 displays a slight positive correlation between total and available Pb concentrations.

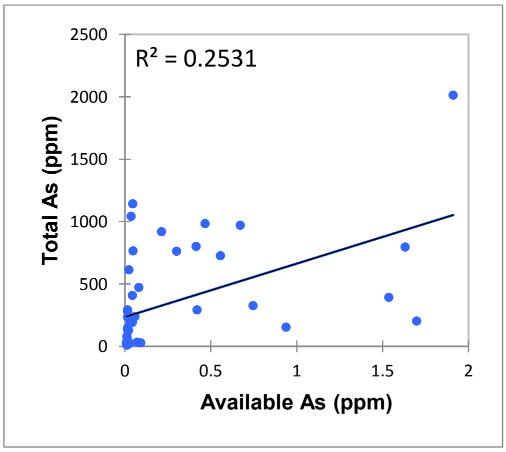


Figure 5:7. Scatterplot displaying relationship between total and available As (y = 427.58x + 235.38)

Fig. 5:7 presents a slight positive relationship between total and available As.

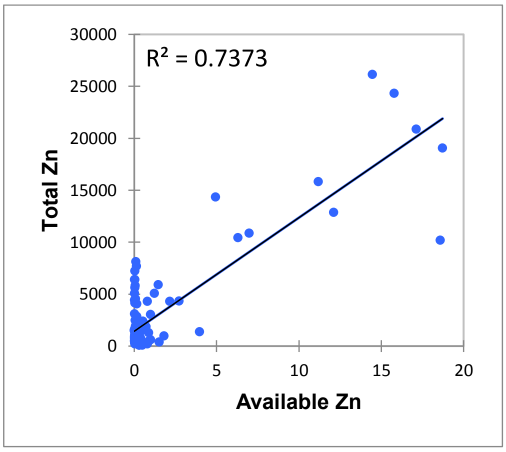


Figure 5:8. Scatterplot displaying relationship between total and available Zn (y = 1,092.97x + 1,431.03)

Fig. 5:8 shows a very strong association between concentrations of total and available Zn.

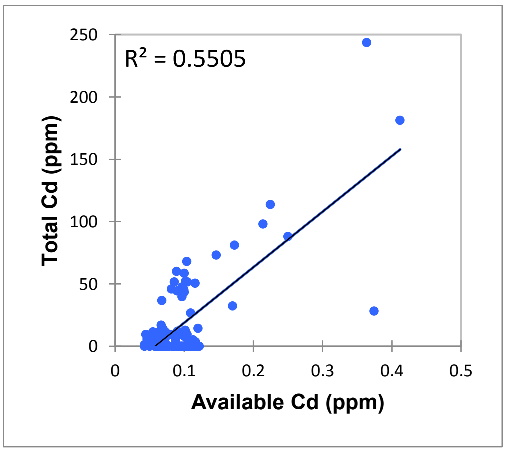


Figure 5:9. Scatterplot displaying relationship between total and available Cd (y = 445.36x - 25.651)

Total and available Cd display a good positive correlation in *Fig*. 5:9.

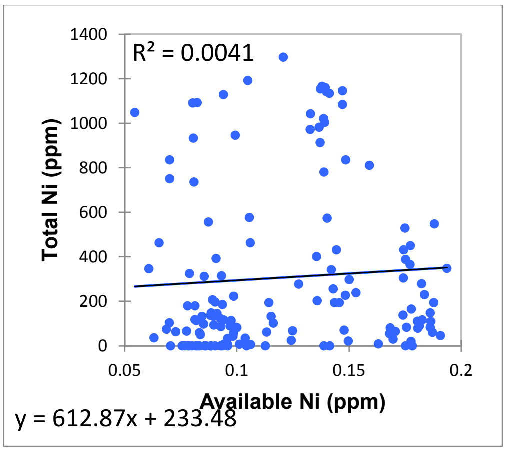


Figure 5:10. Scatterplot displaying relationship between total and available Ni (y = 612.87x + 233.48)

Fig. 5:10 shows total and available Ni to have no correlation.

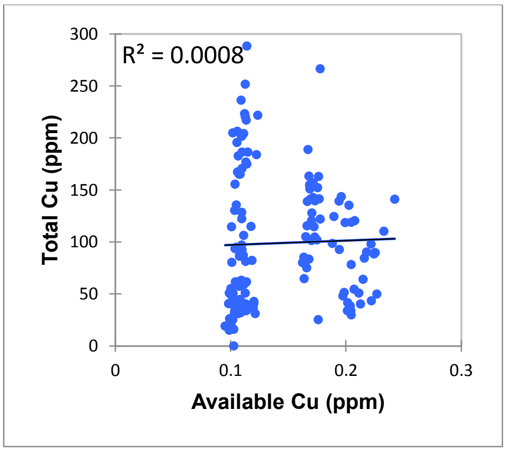


Figure 5:11. Scatterplot displaying relationship between total and available Cu (y = 40.08x + 93.209)

Fig. 5:11 displays no correlation between total and available Cu. It also illustrates a distinct split into two groups of points in the scatterplot.

5.2.3 Spearman's rank order correlation: Heavy metal variables

The following matrix displays two-tailed Spearman's rank order correlation and *P*-values for the associations of all total and available heavy metal concentrations. These results illustrate significant positive and negative relationships between many of the combinations of total and available metals. The matrix has been colour coded for ease of interpretation. Pink** squares on the matrix represent correlations that are significant at the *P*-value = 0.01 level and the blue* squares illustrate the correlations at the *P*-value = 0.05 level. Pb, Ni and Cu exhibit the least association with other metals [*Table.*5:1]. Ni and Cu display significant negative correlations with most other metals/metalloids.

****. Correlation is significant at the 0.01 level (2-tailed).**

***. Correlation is significant at the 0.05 level (2-tailed).**

Table 5:1. Spearman's rank order correlation matrix for total and available metals

Spearman's rho		Total Lead (Pb)	Total Zinc (Zn)	Total Arsenic (As)	Total Cadmium (Cd)	Total Copper (Cu)	Total Nickel (Ni)	Available Lead (Pb)	Available Zinc (Zn)	Available Arsenic (As)	Available Cadmium (Cd)	Available Copper (Cu)	Available Nickel (Ni)
Total Lead (Pb)	**Corr Coef**		.604**	.471**	.661**	.689**	.602**	.702**	.418**	.178*	.216**	-.162*	-.234**
	Sig. (2-tailed)		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.027	0.007	0.043	0.003
Total Zinc (Zn)	**Corr Coef**	.604**		.390**	.791**	.722**	.305**	.277**	.291**	.397**	.243**	0.125	0.023
	Sig. (2-tailed)	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.121	0.776
Total Arsenic (As)	**Corr Coef**	.471**	.390**		.391**	.347**	0.064	.462**	.497**	.293**	.635**	-.424**	-.416**
	Sig. (2-tailed)	0.000	0.000		0.000	0.000	0.426	0.000	0.000	0.000	0.000	0.000	0.000
Total Cadmium (Cd)	**Corr Coef**	.661**	.791**	.391**		.534**	.209**	.399**	.433**	.327**	.316**	-0.120	-.180*
	Sig. (2-tailed)	0.000	0.000	0.000		0.000	0.009	0.000	0.000	0.000	0.000	0.139	0.025
Total Copper (Cu)	**Corr Coef**	.689**	.722**	.347**	.534**		.580**	.417**	.266**	.410**	.214**	.184*	0.022
	Sig. (2-tailed)	0.000	0.000	0.000	0.000		0.000	0.000	0.001	0.000	0.008	0.022	0.790
Total Nickel (Ni)	**Corr Coef**	.602**	.305**	0.064	.209**	.580**		.358**	-0.135	-0.060	-.209**	.188*	0.150
	Sig. (2-tailed)	0.000	0.000	0.426	0.009	0.000		0.000	0.095	0.461	0.009	0.019	0.062
Available Lead (Pb)	**Corr Coef**	.702**	.277**	.462**	.399**	.417**	.358**		.660**	0.116	.293**	-.424**	-.393**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.149	0.000	0.000	0.000
Available Zinc (Zn)	**Corr Coef**	.418**	.291**	.497**	.433**	.266**	-0.135	.660**		.296**	.628**	-.603**	-.570**
	Sig. (2-tailed)	0.000	0.000	0.000	0.000	0.001	0.095	0.000		0.000	0.000	0.000	0.000
Available Arsenic (As)	**Corr Coef**	.178*	.397**	.293**	.327**	.410**	-0.060	0.116	.296**		.380**	0.070	0.005
	Sig. (2-tailed)	0.027	0.000	0.000	0.000	0.000	0.461	0.149	0.000		0.000	0.388	0.953
Available Cadmium (Cd)	**Corr Coef**	.216**	.243**	.635**	.316**	.214**	-.209**	.293**	.628**	.380**		-.529**	-.563**
	Sig. (2-tailed)	0.007	0.002	0.000	0.000	0.008	0.009	0.000	0.000	0.000		0.000	0.000
Available Copper (Cu)	**Corr Coef**	-.162*	0.125	-.424**	-0.120	.184*	.188*	-.424**	-.603**	0.070	-.529**		.689**
	Sig. (2-tailed)	0.043	0.121	0.000	0.139	0.022	0.019	0.000	0.000	0.388	0.000		0.000
Available Nickel (Ni)	**Corr Coef**	-.234**	0.023	-.416**	-.180*	0.022	0.150	-.393**	-.570**	0.005	-.563**	.689**	
	Sig. (2-tailed)	0.003	0.776	0.000	0.025	0.790	0.062	0.000	0.000	0.953	0.000	0.000	

5.2.4 Principal component analysis of total and available heavy metals

PCA was undertaken using total and available heavy metals to further validate the relationship between them. PCA was an effective technique to verify these correlations. This PCA, using varimax rotation, identified two components using data from all sites, and the percentage of variance explained by each component is shown in *Table.*5:3. The resulting component plot [*Fig.* 5:12] as well as the eigenvalues [*Table.*5:2 & *Fig*.5:13], revealed two eigenvalues greater than one and two components explaining 79.061% of the total variance of the dataset [*Table.*5:3]. The first component with the highest eigenvalue explains 60.262% of the total variance with high loadings on available Cd, Zn, As, Pb and total Zn, Cu, As, Pb [*Table.*5:2]. Principal component two is dominated by total Ni and Pb and accounts for 18.799% variance [*Table.*5:3]. The relationships between the heavy metals using these two principal components are displayed in *Figure* 4:39 on a two-dimensional plot. Total Ni and available Ni and Cu are distanced from the other variables and two distinct groupings are formed from the rest of the heavy metal variables [*Fig.* 5:12]. The eigenvalues are also illustrated on a scree plot [*Fig.* 5:13].

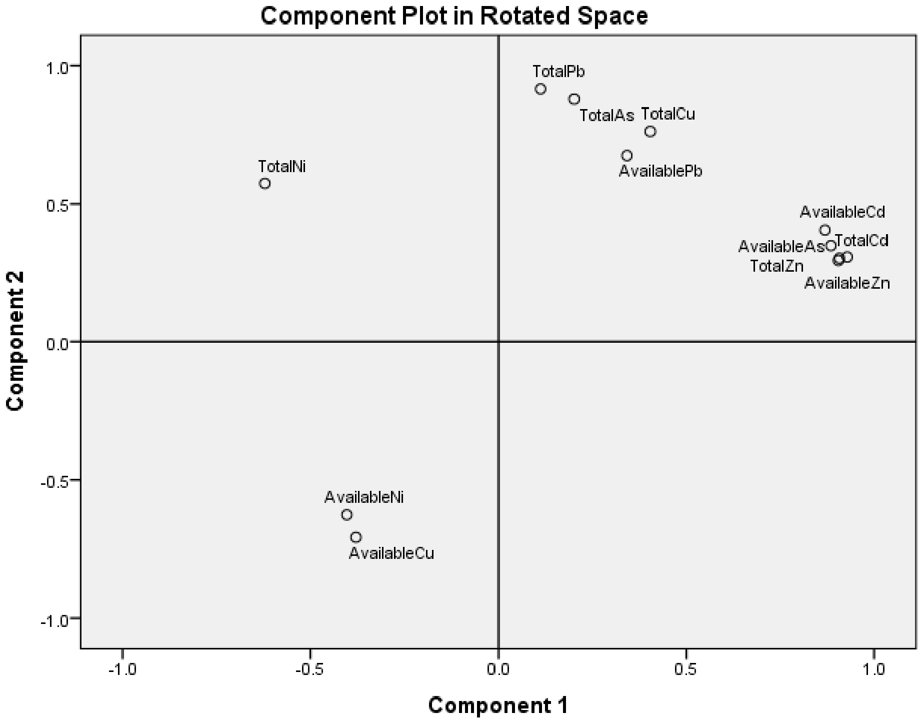


Figure 5:12. PCA component plot for available and total heavy metals

Table 5:2. Component loadings of individual factors for total and available metals

	Component	
	1	2
Available Cd	.919	
Available Zn	.900	-.382
Available As	.894	-.322
Total Cd	.881	-.372
Total Zn	.874	-.376
Total Cu	.806	.306
Available Cu	-.752	
Total As	.731	.527
Available Ni	-.717	
Available Pb	.702	
Total Pb	.688	.614
Total Ni		.841

Table 5:3. Loadings and variance of principal components for total and available metals

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	7.231	60.262	60.262	7.231	60.262	60.262	5.065	42.212	42.212
2	2.256	18.799	79.061	2.256	18.799	79.061	4.422	36.849	79.061
3	.939	7.827	86.888						
4	.735	6.121	93.009						
5	.267	2.227	95.237						
6	.192	1.598	96.834						
7	.143	1.190	98.024						
8	.125	1.043	99.068						
9	.061	.508	99.576						
10	.032	.264	99.840						
11	.016	.131	99.971						
12	.003	.029	100.000						

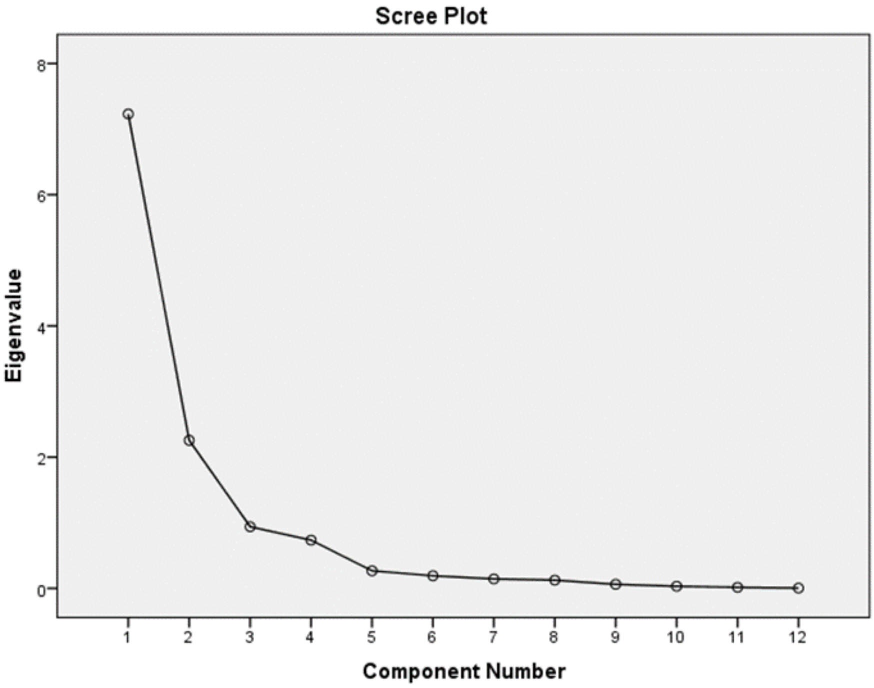


Figure 5:13. Scree plot illustrating the eigenvalues of extracted components

5.3 Principal component analysis of particle size and heavy metals

5.3.1 Particle size analysis and total heavy metal concentrations

To investigate whether values generated during particle size analysis can be used as indicators of heavy metal contamination, PCA was carried out. The PCA shown in *Fig.* 5:14 plots total heavy metal concentrations alongside the calculated PSA span. This analysis extracted two components for all sites and these explain 73.180% of the variance in the dataset [*Table.* 5:5]. The resulting loadings with a varimax rotation, in addition to the eigenvalues, reveal three eigenvalues greater than one, explaining 88.384% of the total variance [*Table.* 5:5]. However, to avoid plotting these onto a harder to interpret three-dimensional component plot, just the two highest loaded components were plotted. The first component with the greatest eigenvalue explains 48.323% of the total variance, and all variables except Ni have high loadings [*Table.* 5:4]. The second component was loaded primarily by Ni (0.847) [*Table.* 5:4] and accounts for 24.856% of the variance [*Table.* 5:5]. The relationships among the heavy metals and PSA span are illustrated in *Fig.* 5:14 on a two-dimensional plot and show the PSA span variable grouped with the heavy metal variables. The eigenvalues are illustrated on a scree plot in *Fig.* 5:15.

It must be noted that any statements made in regards to particle size distribution in relation to heavy metal contents must consider the proportion of clay-sized particle contained in soil samples. A high proportion of clay-sized particles within a soil sample will generally indicate a greater heavy metal concentration adhered to the clay particles (Alloway, 1995). Hence, any relationships between heavy metal concentrations and particle size shown here (section 5.3 and throughout this thesis) must be take into account the clay-sized materials within the soil sample. Access to all PSA raw data is available by clicking the following link: https://www.dropbox.com/sh/bdbxz67kjgw6vay/AADY6rj5JxDpe_oSiP0wXs93a?dl=0

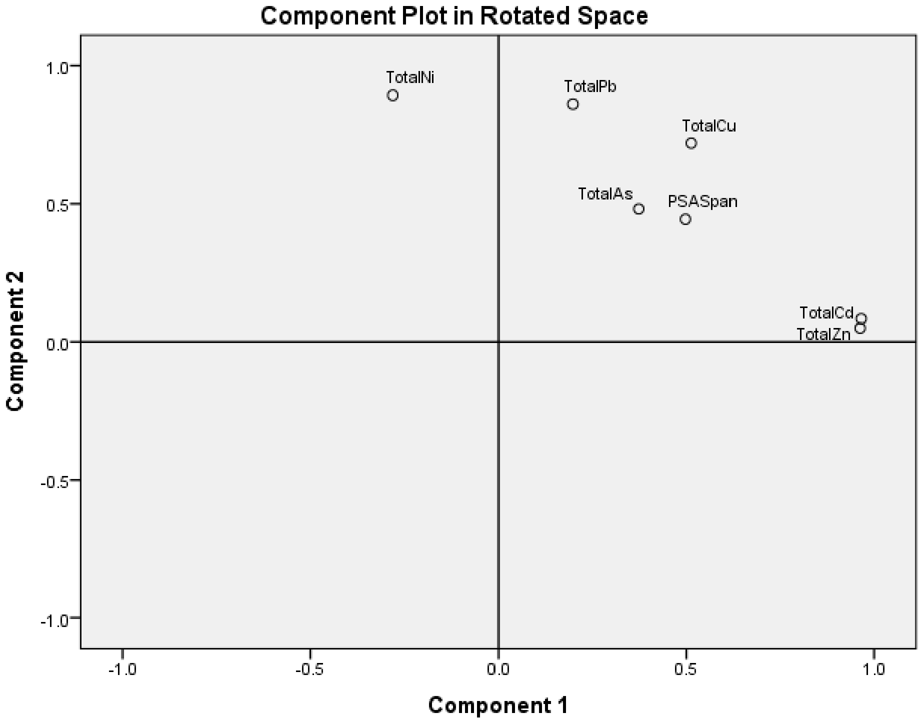


Figure 5:14. PCA component plot for total heavy metals and particle size span

Table 5:4. Component loadings of individual factors for total metals and PSA span

Component Matrix^a

	Component	
	1	2
Total Cu	.865	
Total Zn	.766	-.593
Total Cd	.741	-.616
Total Pb	.730	.499
PSA Span	.667	
Total As	.601	
Total Ni	.399	.847

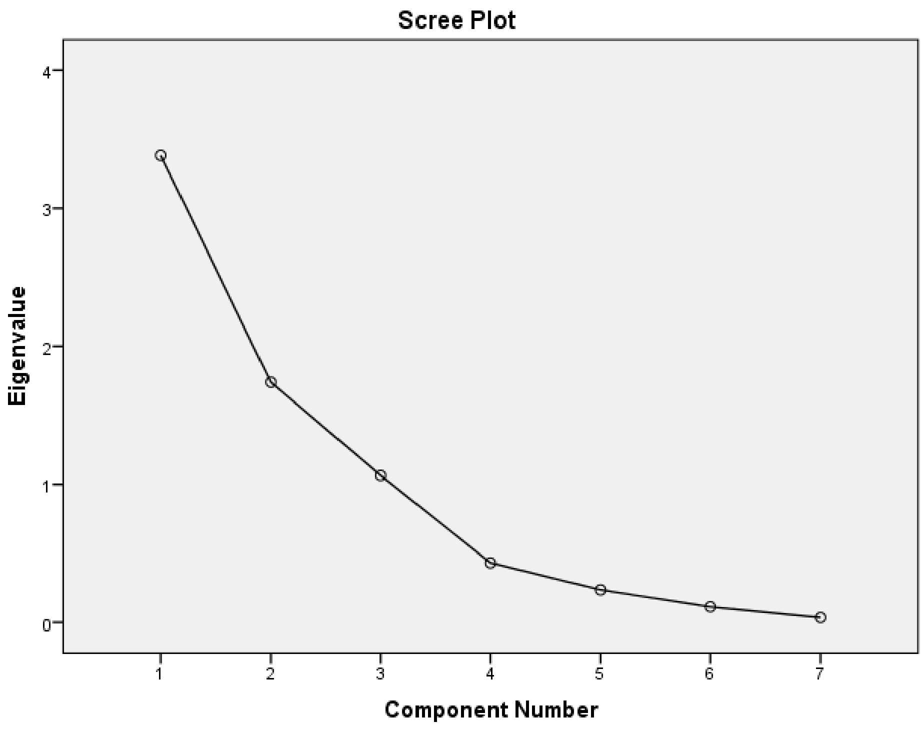


Figure 5:15. Scree plot illustrating the eigenvalues of extracted components

Table 5:5. Loadings and variance of principal components for total metals and PSA span

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.383	48.323	48.323	3.383	48.323	48.323	2.627	37.532	37.532
2	1.740	24.856	73.180	1.740	24.856	73.180	2.495	35.647	73.180
3	1.064	15.204	88.384						
4	.431	6.154	94.538						
5	.235	3.360	97.898						
6	.112	1.605	99.503						
7	.035	.497	100.000						

5.3.2 Particle size analysis and available heavy metal content

PCA was also carried out using PSA span and available heavy metal variables. This indicates a close association of PSA span and available Pb on the component plot. Available Zn, Cd, and As form a close grouping, as do available Cu and Ni [*Fig.* 5:16]. This analysis extracted two components for all sites and these explain 73.180% of the variance in the dataset [*Table.* 5:7]. The results of the eigenvalues indicate that there were three eigenvalues greater than one [*Table.* 5:7 & *Fig*. 5:17]. The first component with the highest eigenvalue describes 61.980% of the total variance [*Table.* 5:7] and available Zn, As and Cd have high loadings [*Table.* 5:6]. The second component has relatively low loadings and accounts for only 15.302% of the variance [*Table.* 5:7]. The relationships among the heavy metals and PSA span are illustrated in *Fig*. 5:16 on a two-dimensional plot and the eigenvalues are illustrated on a scree plot [*Fig.* 5:17].

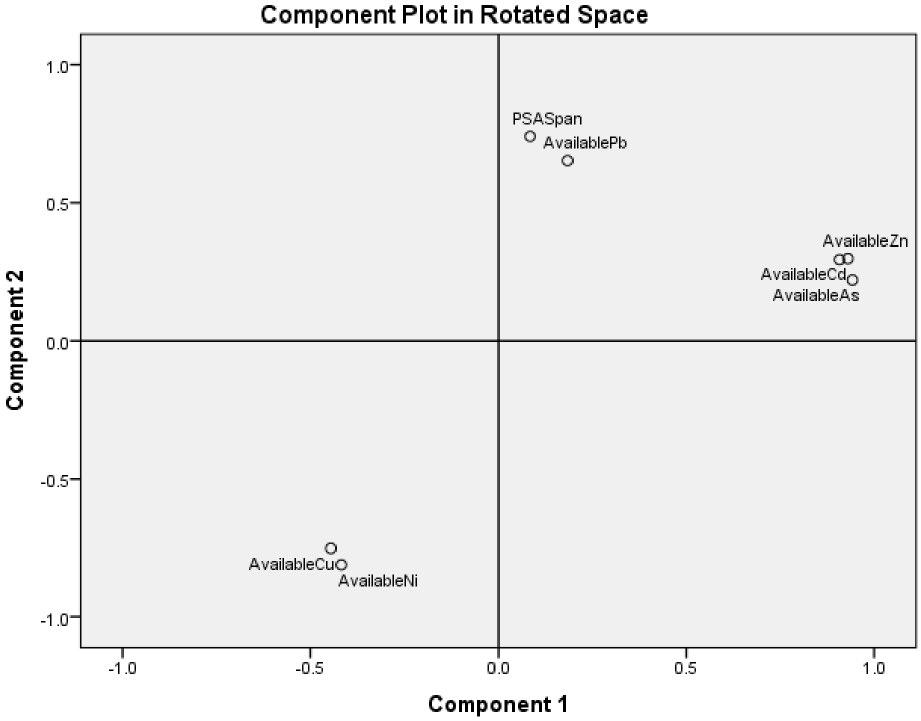


Figure 5:16. PCA component plot for available heavy metals and particle size span

Table 5:6. Component loadings of individual factors for available metals and PSA span

	Component	
	1	2
Available Zn	.904	.369
Available Cd	.884	.357
Available As	.863	.437
Available Ni	-.842	.353
Available Cu	-.824	
Available Pb	.560	-.381
PSA Span	.540	-.513

Table 5:7. Loadings and variance of principal components for available metals and PSA span

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.339	61.980	61.980	4.339	61.980	61.980	2.989	42.703	42.703
2	1.071	15.302	77.282	1.071	15.302	77.282	2.421	34.579	77.282
3	.729	10.414	87.696						
4	.604	8.626	96.322						
5	.170	2.428	98.750						
6	.077	1.099	99.849						
7	.011	.151	100.000						

Extraction Method: Principal Component Analysis.

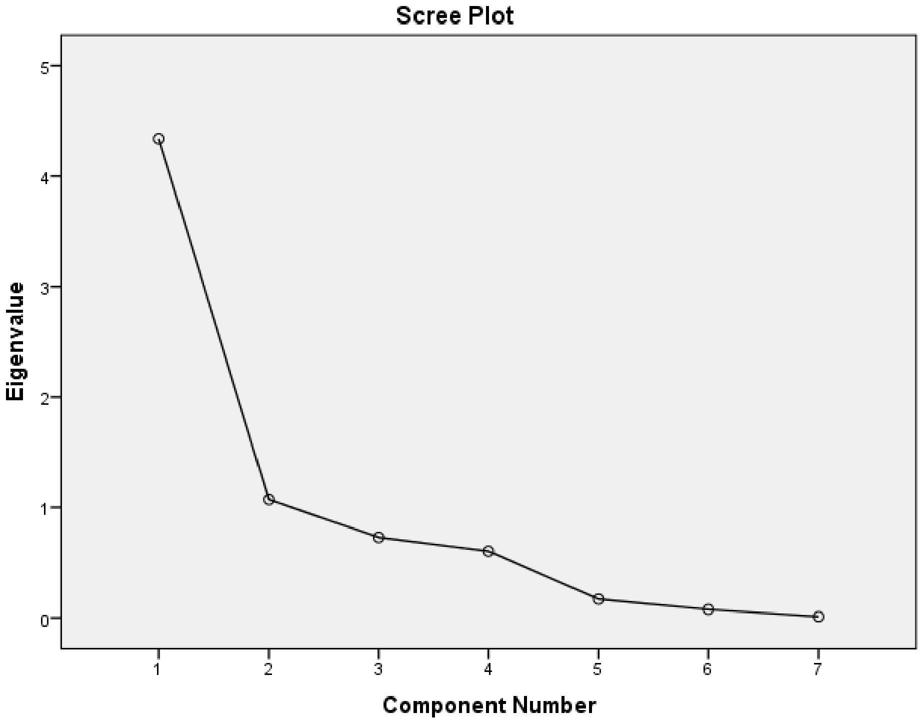


Figure 5:17. Scree plot illustrating the eigenvalues of extracted components

5.3.3 Comparing particle size span and median (total heavy metals)

To compare the practicality of both PSA median and span, both PSA variables were plotted alongside total heavy metal variables. *Fig.* 5:18 illustrates that PSA span has a closer association with the total heavy metal variables than PSA median. The first two components in *Table.* 5:9 explain 66.260% of the variance. Component one has the highest eigenvalue of 3.528 and explains 44.095% of the variance [*Table.* 5:9]. All variables have high loadings on the first extracted component, except PSA median, which does not *Figure* in either of the extracted components [*Table.* 5:8]. The second component extracted has an eigenvalue of 1.773 and explains 22.165% of the variance. Eigenvalues are illustrated on the scree plot in *Fig.* 5:19.

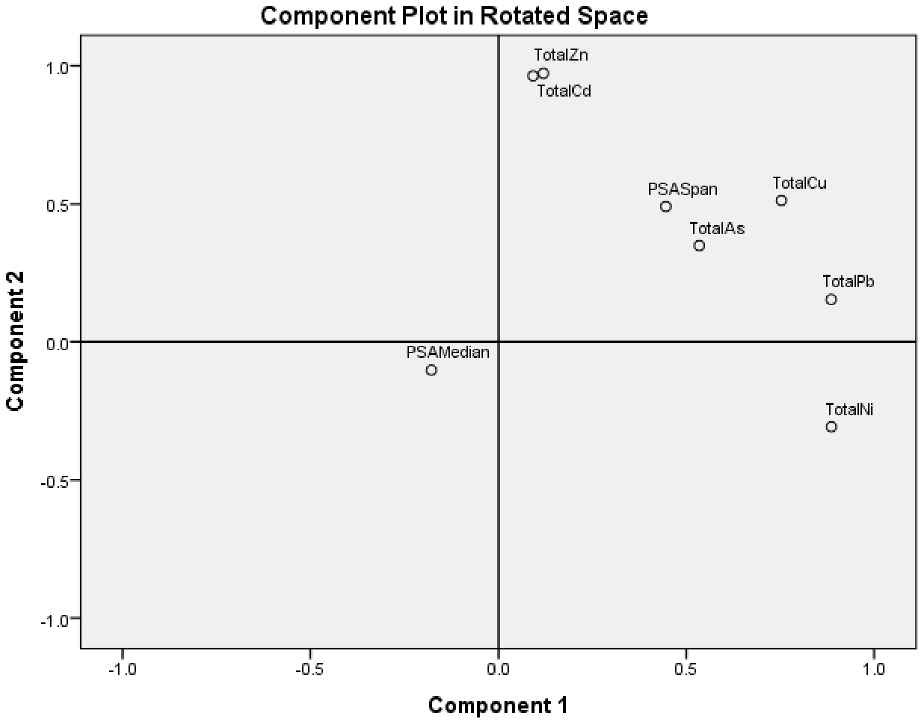


Figure 5:18. PCA component plot for total heavy metals and particle size mean and span

Table 5:8. Component loadings of individual factors for total metals, PSA span and median

Component Matrix^a

	Component	
	1	2
Total Cu	.897	
Total Zn	.764	-.613
Total Pb	.741	.508
Total Cd	.738	-.626
PSA Span	.661	
Total As	.626	
PSA Median		
Total Ni	.419	.839

Table 5:9. Loadings and variance of principal components for total metals, PSA span and median

Total Variance Explained

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.528	44.095	44.095	3.528	44.095	44.095	2.673	33.417	33.417
2	1.773	22.165	66.260	1.773	22.165	66.260	2.627	32.843	66.260
3	1.113	13.918	80.179						
4	.861	10.761	90.940						
5	.359	4.493	95.433						
6	.226	2.829	98.262						
7	.111	1.391	99.652						
8	.028	.348	100.000						

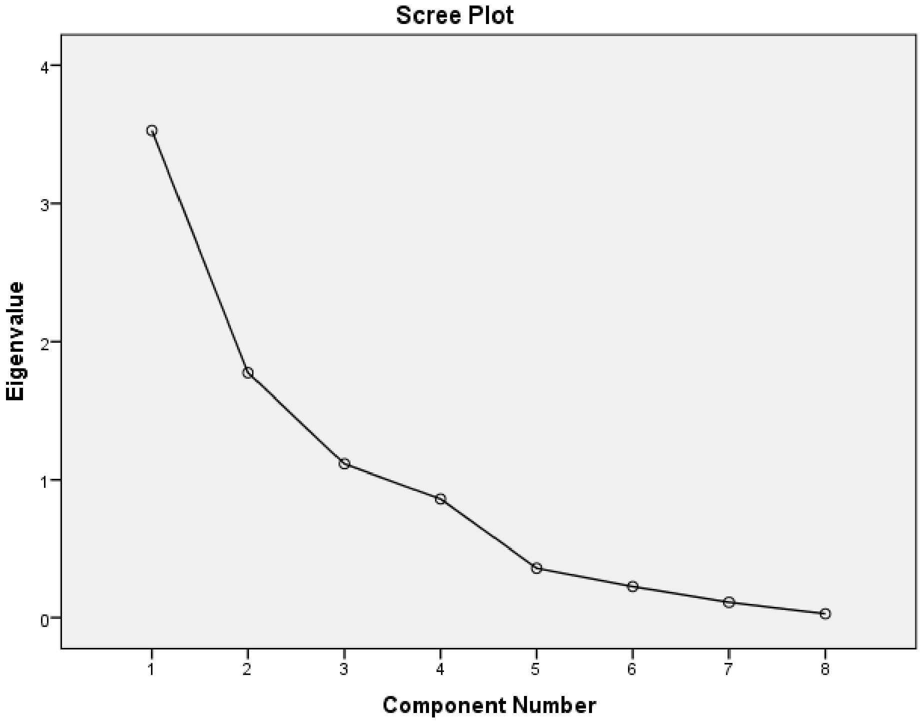


Figure 5:19. Scree plot illustrating the eigenvalues of extracted components

5.3.4 Comparing particle size span and median (available heavy metals)

Both PSA variables were plotted with available heavy metal variables on a two-dimensional component plot and the generated results are displayed in *Fig*. 5:20. PSA span is shown to be more closely associated with available Pb, Zn, As and Cd, whereas PSA median is grouped with Available Ni and Cu [*Fig.* 5:20]. The two extracted components were responsible for 71.752% of the variance in the dataset [*Table.* 5:11]. Component one shows high weightings for available Zn, Cd and As which is contrasted against negative loadings of available Ni, Cu and PSA median [*Table.* 5:10]. It has an eigenvalue of 4.643 which explains 58.035% of the variance [*Table.* 5:11]. Component two shows moderate weightings for available Zn, Cd, As and Ni and contrasting negative weightings for available Pb and PSA span [*Table.* 5:10], explaining 13.716% of the variance [*Table.* 5:11]. Eigenvalues are displayed on a scree plot in *Fig.* 5:21.

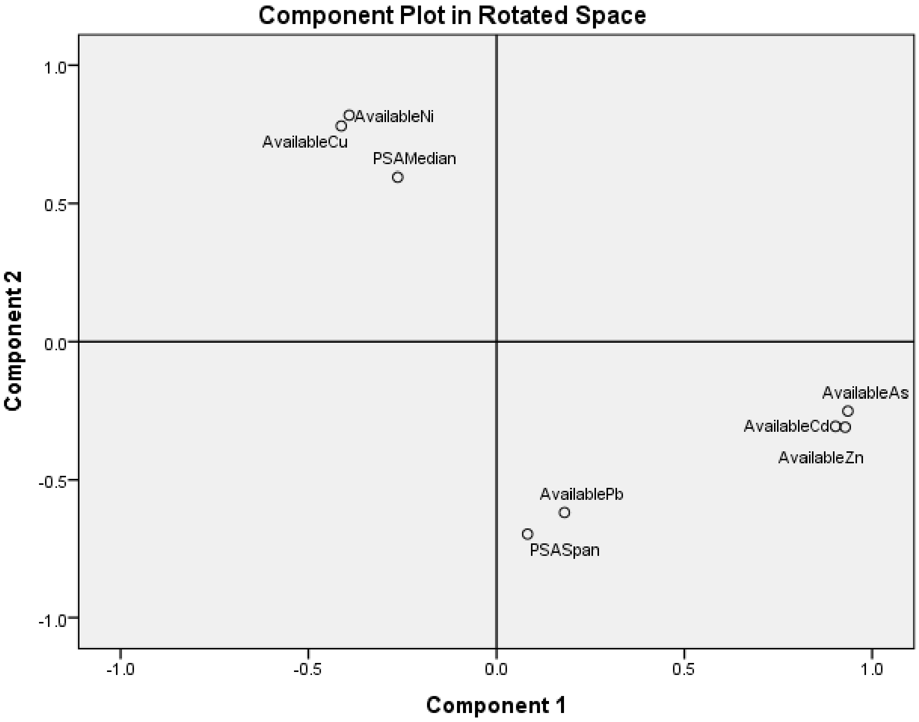


Figure 5:20. PCA component plot for available heavy metals and particle size span

Table 5:10. Component loadings of individual factors for available metals, PSA span and median

	Component	
	1	2
Available Zn	.889	.409
Available Cd	.868	.394
Available As	.854	.457
Available Ni	-.847	.329
Available Cu	-.835	
PSA Median	-.599	
Available Pb	.556	-.327
PSA Span	.538	-.452

Table 5:11. Loadings and variance of principal components for available metals, PSA span and median

Total Variance Explained

	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
Component	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	4.643	58.035	58.035	4.643	58.035	58.035	2.982	37.275	37.275
2	1.097	13.716	71.752	1.097	13.716	71.752	2.758	34.477	71.752
3	.745	9.311	81.063						
4	.728	9.100	90.163						
5	.538	6.722	96.885						
6	.162	2.024	98.909						
7	.077	.959	99.868						
8	.011	.132	100.000						

Extraction Method: Principal Component Analysis.

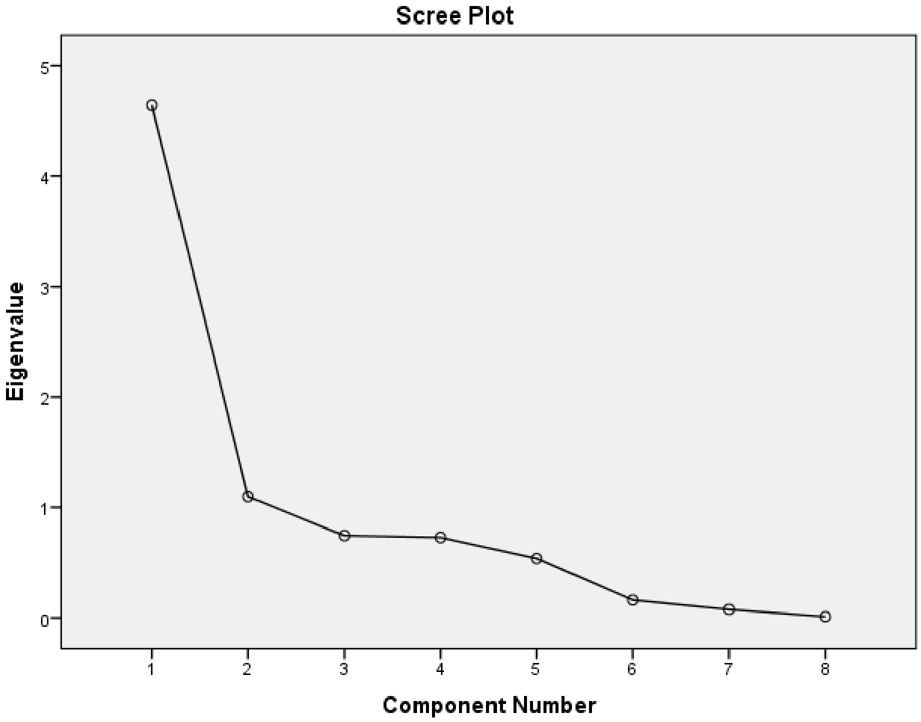


Figure 5:21. Scree plot illustrating the eigenvalues of extracted components

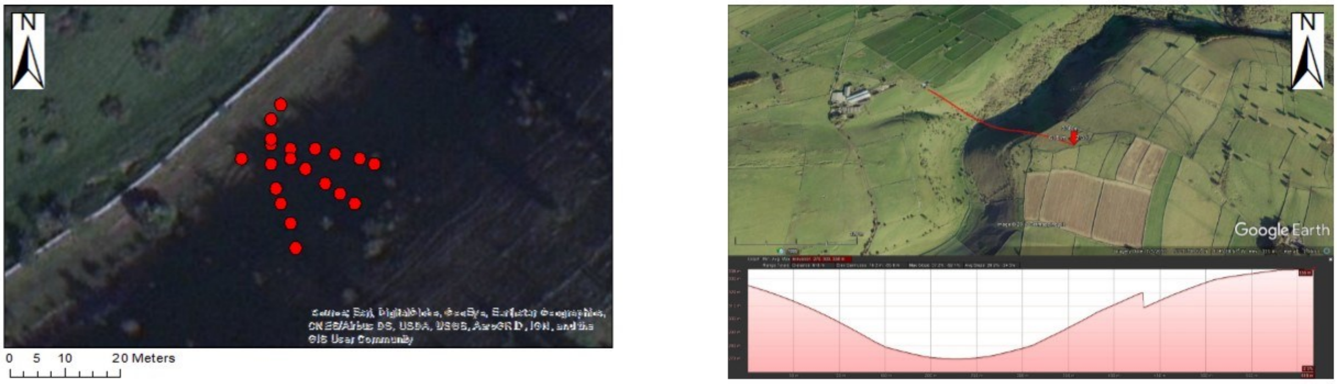
5.4 Spatial distribution of heavy metals

5.4.1 Introduction

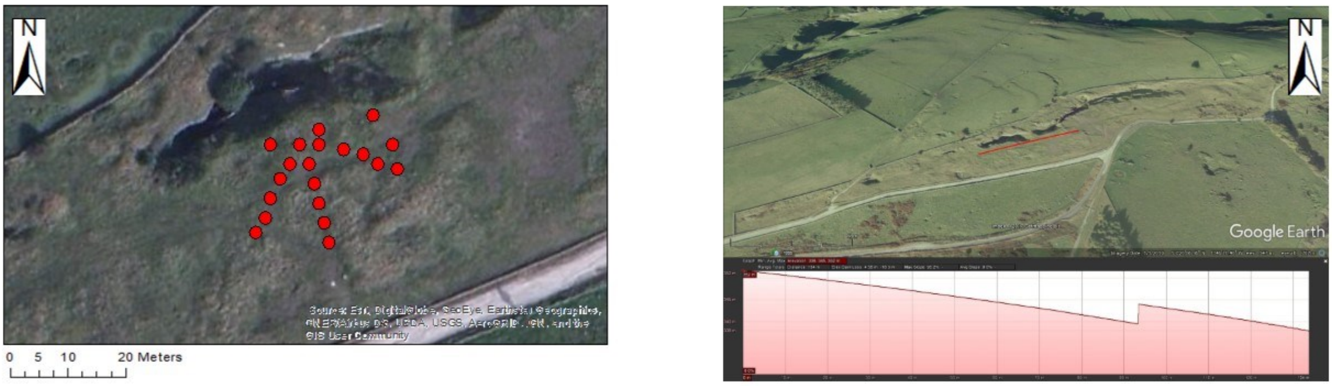
An objective of this research was to evaluate the distribution of any heavy metals and other elements found in topsoil samples and to assess their influence on vegetation structure. To achieve this objective universal kriging was undertaken, utilising ArcGIS 10.5 geostatistical analyst software. Kriging is a statistical method that generates an approximation of a surface by calculating a weighted average of the data. These weights decrease as the distance between the estimated points and the known point data increase (Hengl, Heuvelink, & Stein, 2004). In this case, kriging produces an isopleth map of the modeled prediction of heavy metal concentration between the known concentrations of sample points at the study sites.

Kriging distribution isopleth maps were produced for all soil variables, species richness and cover/abundance. Kriging distribution isopleth maps revealed a very similar distribution pattern for heavy metals Pb, Zn, As and Cd at all sites across their sampled areas. Equally, Ni and Cu displayed similar distribution patterns. To prevent repetitiveness only total Pb and vegetation cover/abundance variables will be presented in this chapter. Significant findings illustrating similar distribution patterns between variables are presented and considered in Chapter 6: Discussion. Kriging prediction isopleth maps for vegetation species richness, Total Zn, Cd, Ni, Cu and available Pb, Zn and Cd at each study area are contained within Appendix C-J. Kriging prediction isopleth maps for soil variables are plotted using all transect sample points, plus the extra samples collected (19-20 samples per site). Vegetation variables are analysed using data from quadrats plotted along transect points 1-16 (16 samples per site).

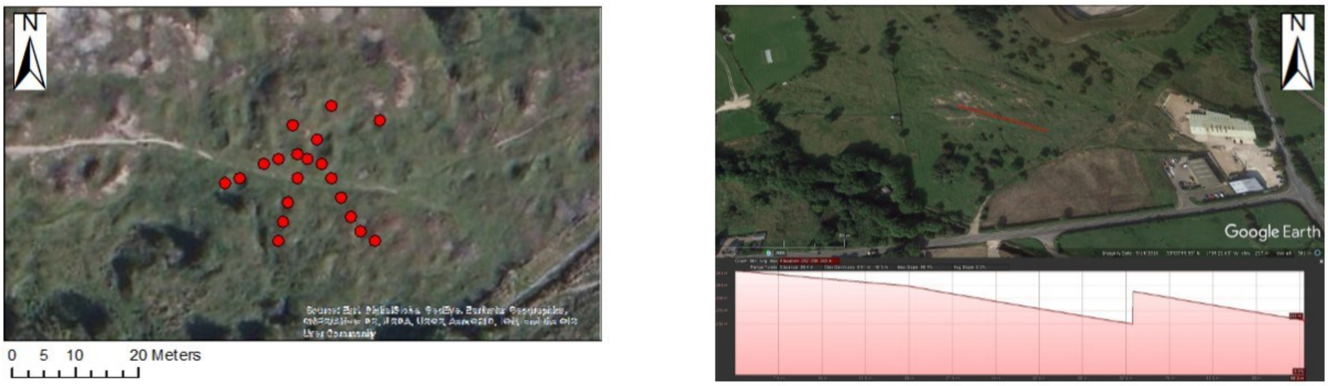
To assist the visualisation of the isopleth maps, an initial overview of each sampling site will be presented including a topographical profile is displayed in *Figures* 5:22 & 5:23 below.



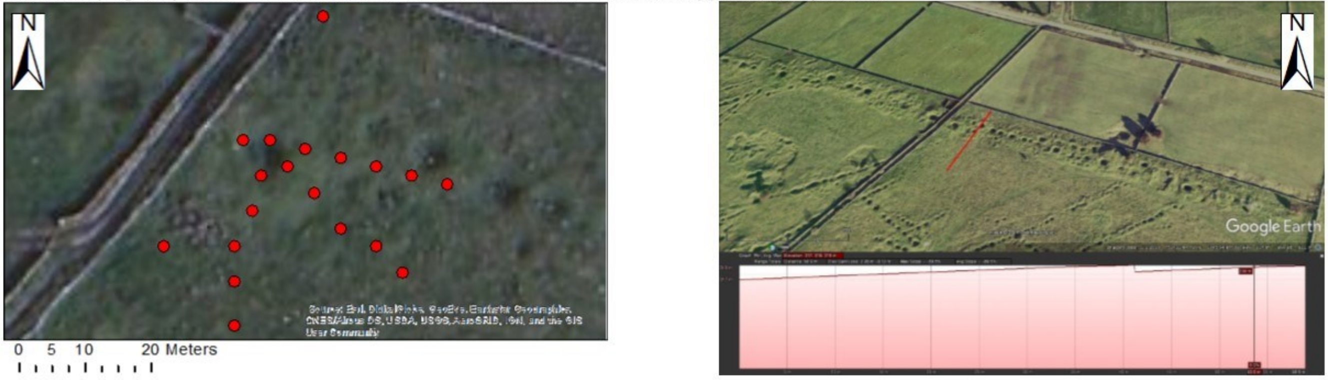
Deep Dale. Deep Dale is steep sided dale with average slope of 20.2% and a maximum slope of 37.2%. Samples were taken up the dale side and extra samples from the dale floor. The average elevation of sample points was 268.56m.



Dirtlow Rake. Samples at Dirtlow Rake were taken directly adjacent to a capped mine shaft at the edge of a ravine caused by past mining activities. The site slopes gently North-east with an average slope of 0.3%. The average elevation of samples was 348.75m.

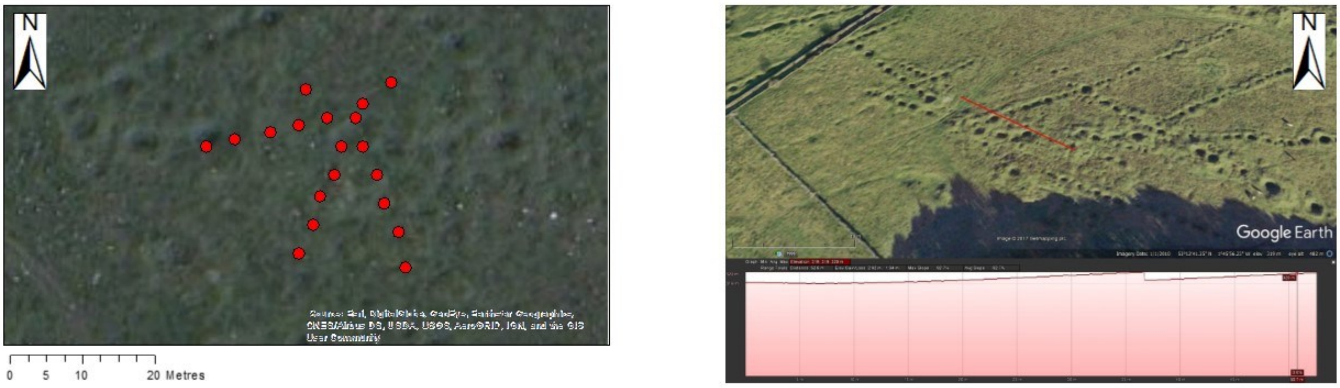


Gang Mine. Gang Mine samples were taken from the edge of a bell pit in a roughly Southerly direction across a footpath that crosses the site. The site dips gently to the East and has the lowest elevation of all sites at approximately 238m.

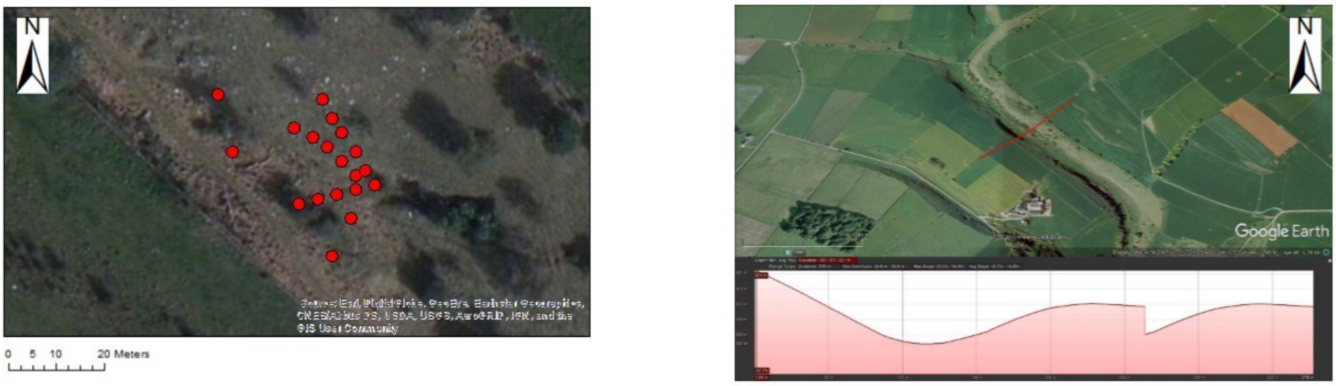


Hard Rake 1. Hard Rake consists of a heavily pitted surface due to historic mining activity. Samples were taken from the edge of a large bell pit and extend approximately South-east. The topography is relatively flat with the exception of the cratered landscape. The elevation of samples was 318m.

Figure 5:22. Site sampling overview and elevation profile 1



Hard Rake 2. Samples at Hard Rake 2 were taken from a rim of a bell pit and extend across several other bell pits. As with Hard Rake 1 the area is relatively flat with no noticeable slope. Extra samples were taken around the pit where sampling originated. Elevation is 319m.



Hay Dale. Hay Dale is a fairly steep sided dale with an average slope of 12.5% and an average elevation of 298m. Transects originated at the entrance to a mine and extra samples were taken from the dale floor.

High Rake. Sample transects originated close to a historic, excavated ore crushing circle and extend up 1m onto a plateau of earth towards the West. The sampling area is fairly flat except for a slope down onto agricultural fields to the North. Elevation at High Rake is an average of 332.5m.

Tansley Dale. Tansley Dale is a steep sided dale and the central pathway declines in elevation to the East. Average elevation at the sampling area was 284m. The central transect and extra soil samples were taken along the dale bottom and close to the trackway.

Figure 5:23. Site sampling overview and elevation profile 2

5.4.2 Deep Dale

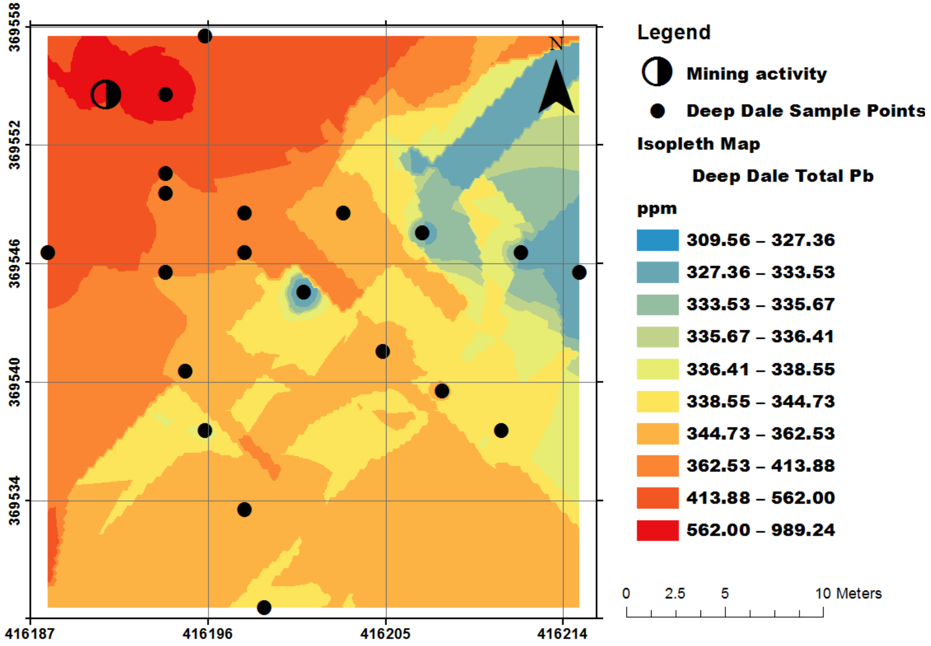


Figure 5:24. Isopleth map of total Pb at Deep Dale

Total Pb recordings at Deep Dale display greater concentrations at the lower elevation sample points on the dale bottom (NW). The dale side shows lower concentrations of Pb.

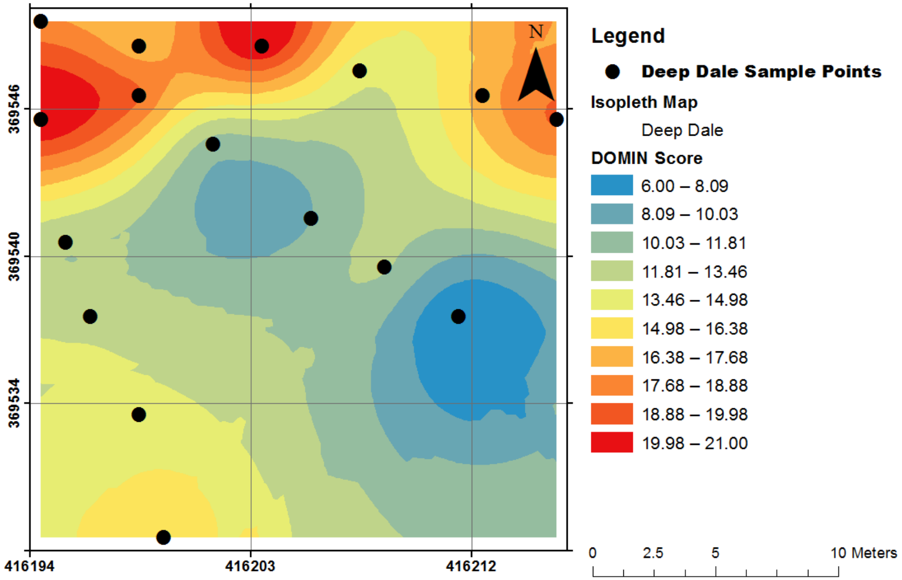


Figure 5:25. Isopleth map of species cover/abundance at Deep Dale

Deep Dale species abundance is higher to the north of this isopleth map, towards the dale bottom. This is a similar distribution to that of the heavy metal concentrations at Deep Dale.

5.4.3 Dirtlow Rake

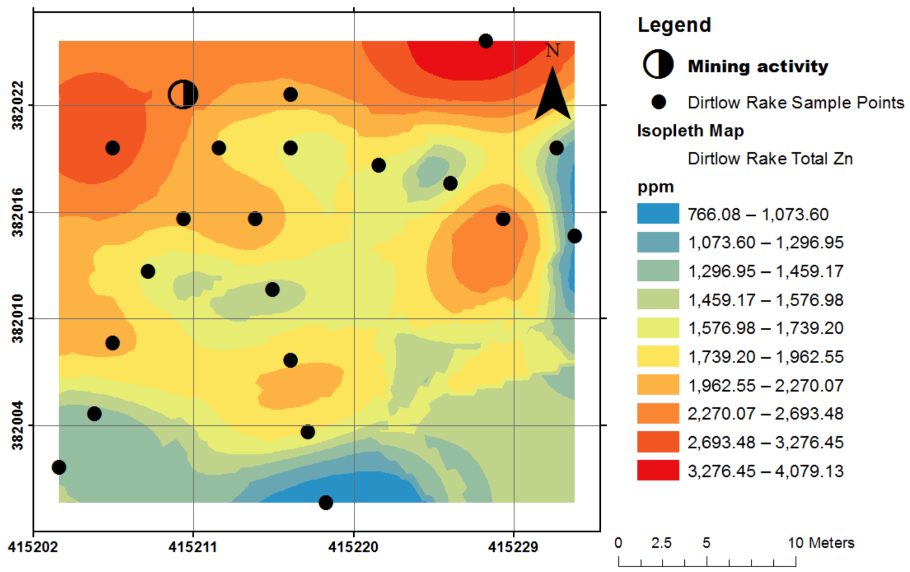


Figure 5:26. Isopleth map of total Pb at Dirtlow Rake

Dirtlow Rake exhibits higher Pb concentrations around the transect origin point, next to the capped mine shaft (N). Lower concentrations are evident with distance from the transects' source point.

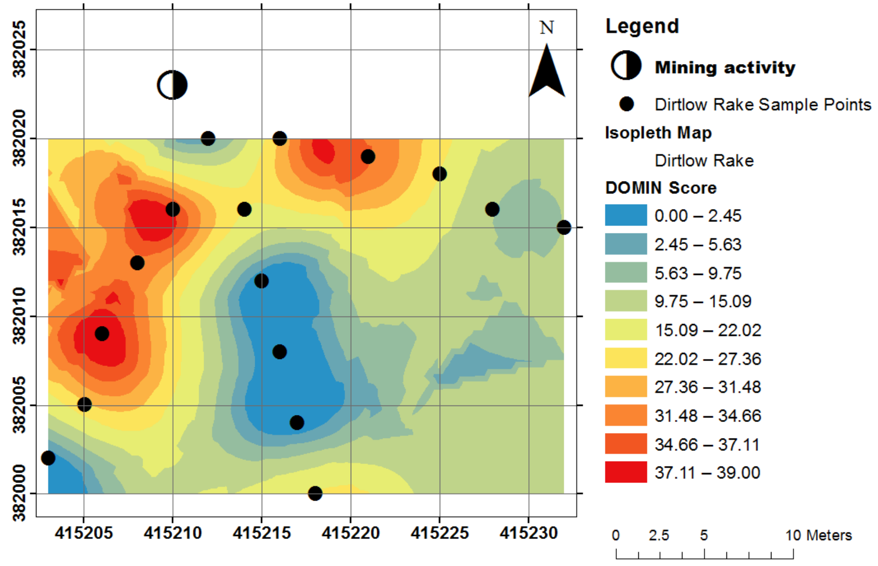


Figure 5:27. Isopleth map of species cover/abundance at Dirtlow Rake

Higher DOMIN scores at Dirtlow Rake are present to the north and west. This also shows a similar pattern to that of the higher metal concentrations at Dirtlow Rake.

5.4.4 Gang Mine

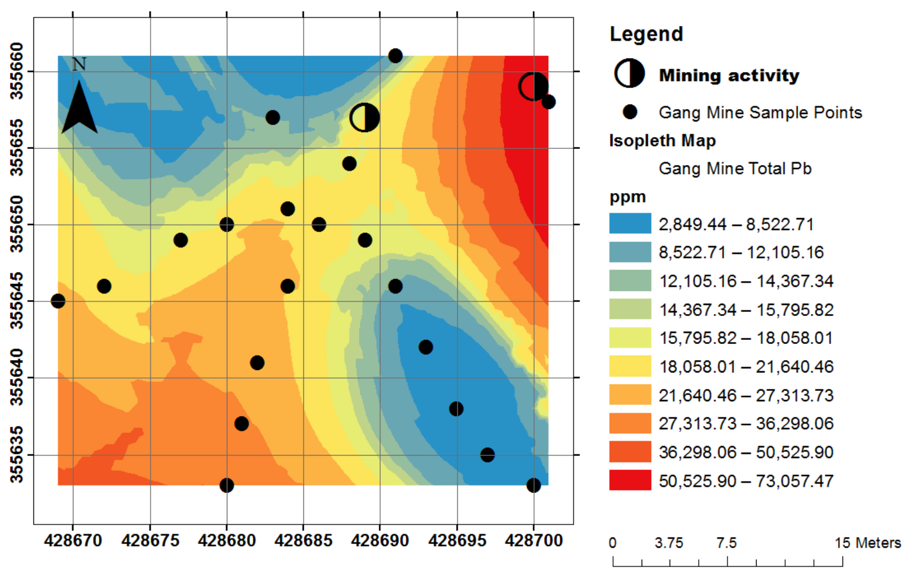


Figure 5:28. Isopleth map of total Pb at Gang Mine

Distribution of Pb at Gang Mine is elevated either side of the informal pathway that cuts diagonally across the sample transects (central). The higher Pb concentrations occur on the more heavily worked areas.

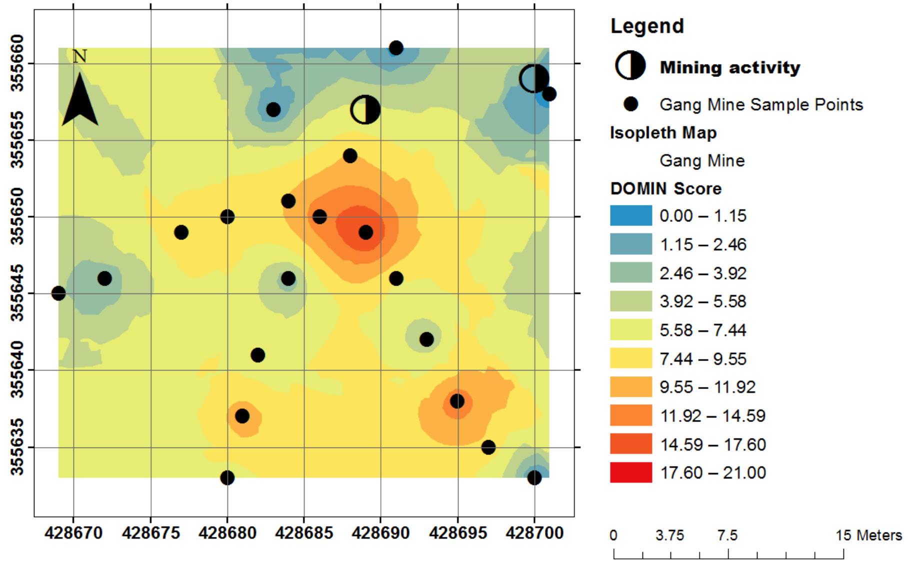


Figure 5:29. Isopleth map of species cover/abundance at Gang Mine

Gang Mine displays slightly higher species abundance to the north-east, closer to the zone of higher Pb and Zn concentrations.

5.4.5 Hard Rake 1

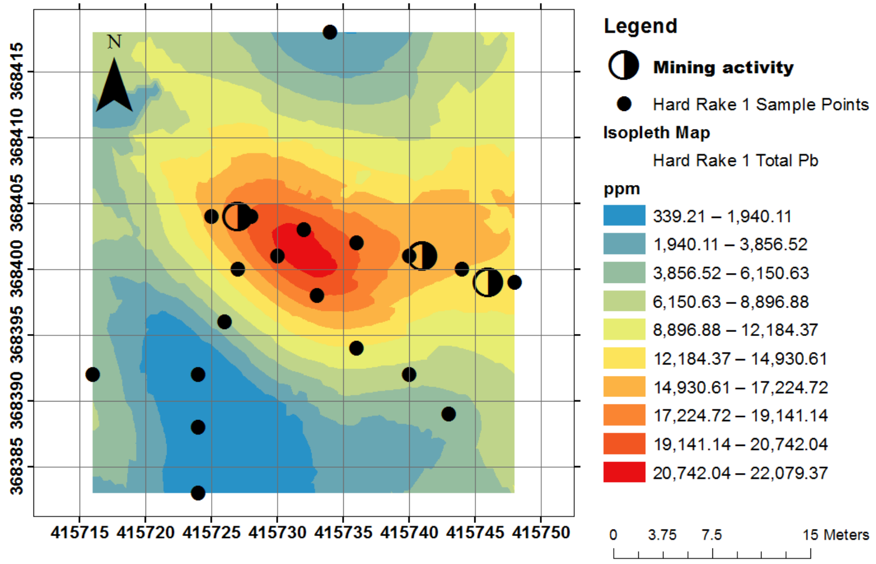


Figure 5:30. Isopleth map of total Pb at Hard Rake 1

The Hard Rake 1 map reveals peak Pb concentrations around the origin of the transects at the central bell pit (central) and lower concentrations in the surrounding sampling zone.

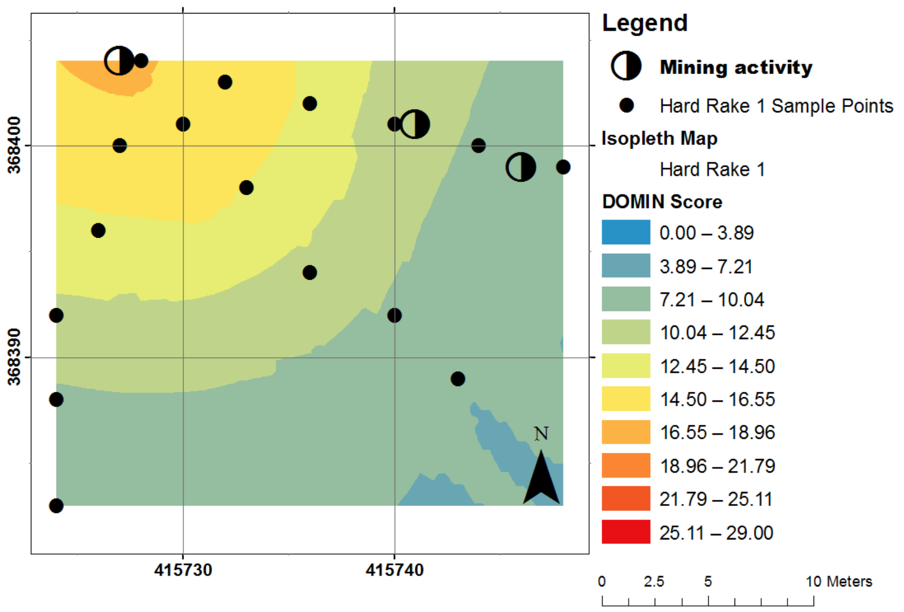


Figure 5:31. Isopleth map of species cover/abundance at Hard Rake 1

Hard Rake 1 exhibits its highest species cover close to the edge of the bell pit (NW) where metal concentrations were greatest; and species abundance decreases with distance from it.

5.4.6 Hard Rake 2

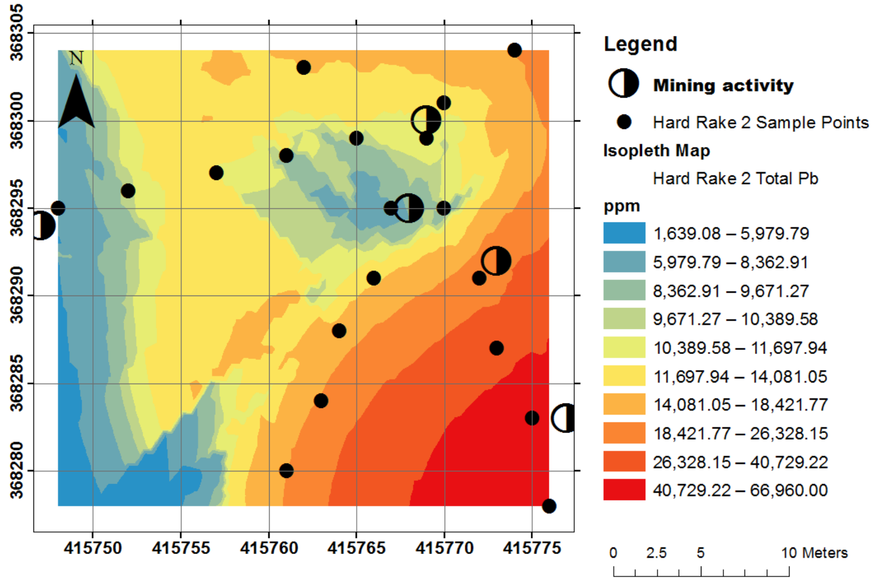


Figure 5:32. Isopleth map of total Pb at Hard Rake 2

Hard Rake 2 shows Pb concentrations to be higher to the east of the sample area, where evidence of abundant of mining activity is present (SE).

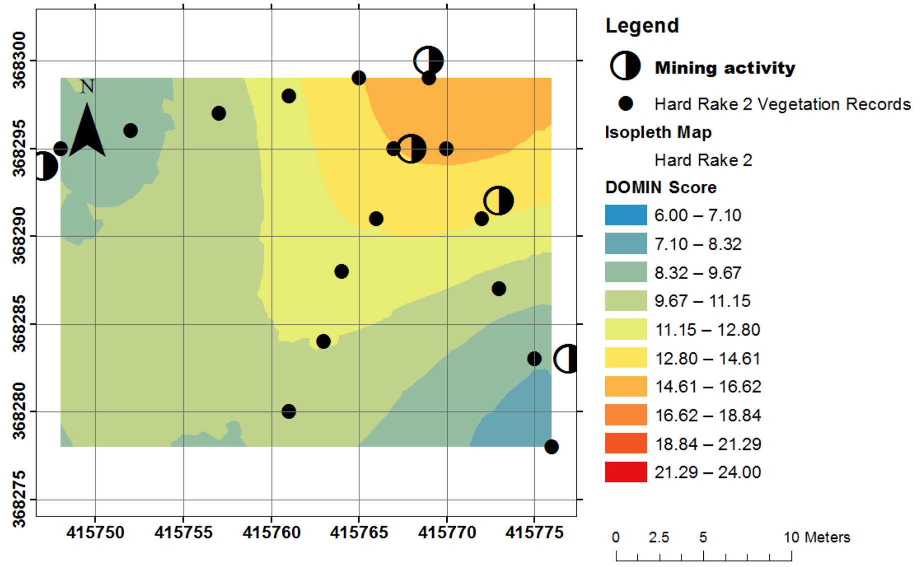


Figure 5:33. Isopleth map of species cover/abundance at Hard Rake 2

Hard Rake 2 displays lower species abundance in the areas with the greatest heavy metal contamination. This increases towards the transect origin point, close to the bell pit.

5.4.7 Hay Dale

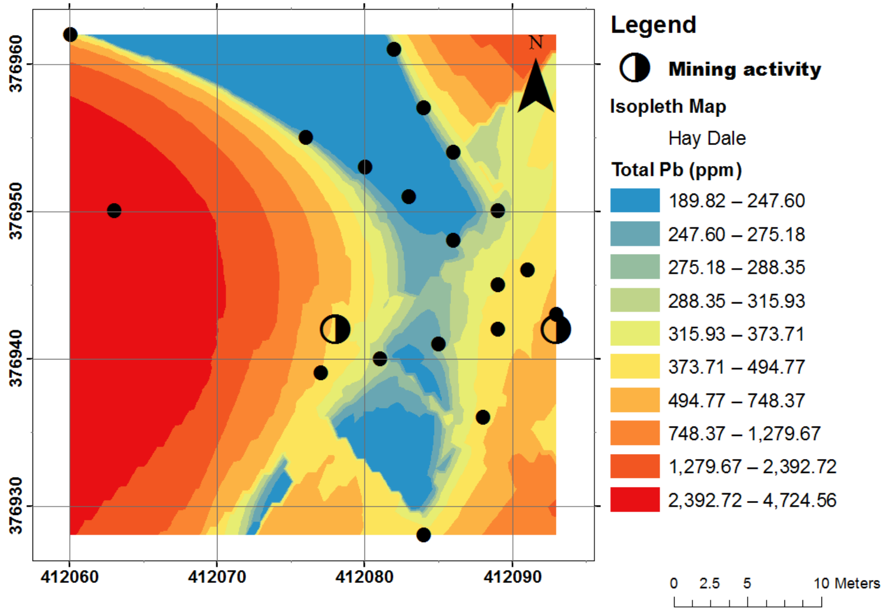


Figure 5:34. Isopleth map of total Pb at Hay Dale

The Hay Dale isopleth map presents higher Pb contamination levels close to the mine entrance (NE) and the dale bottom (E).

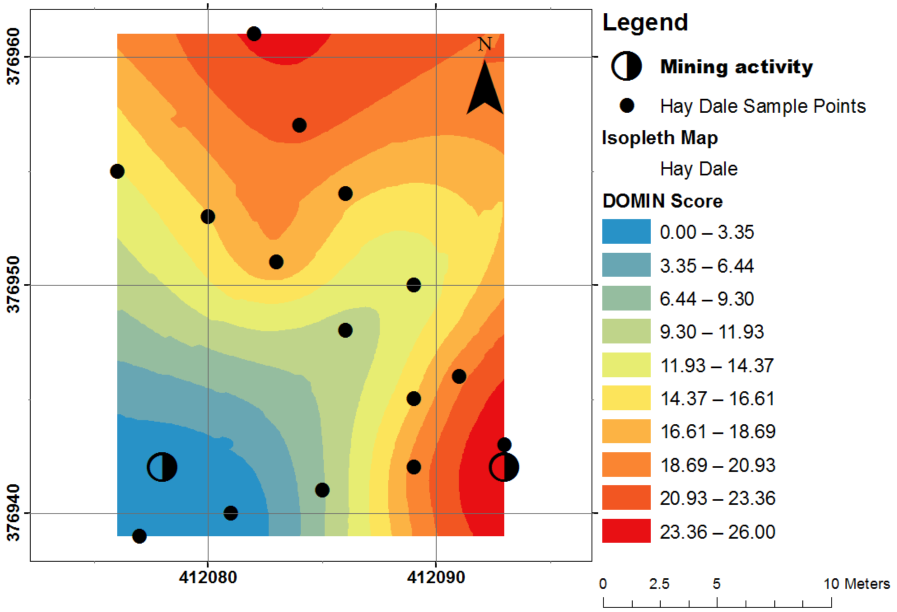


Figure 5:35. Isopleth map of species cover/abundance at Hay Dale

Hay Dale species abundance was lowest towards the dale floor, with its greatest floristic cover/abundance higher up the dale-side.

5.4.8 High Rake

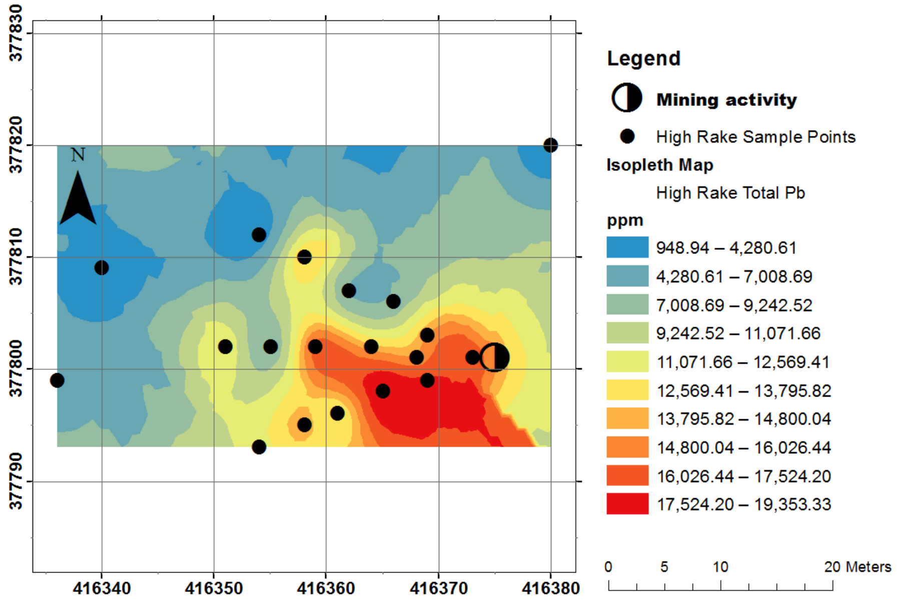


Figure 5:36. Isopleth map of total Pb at High Rake

The distribution of Pb at High Rake shows a higher concentration of Pb at the transect source point, next to the disused chimney base (SE).

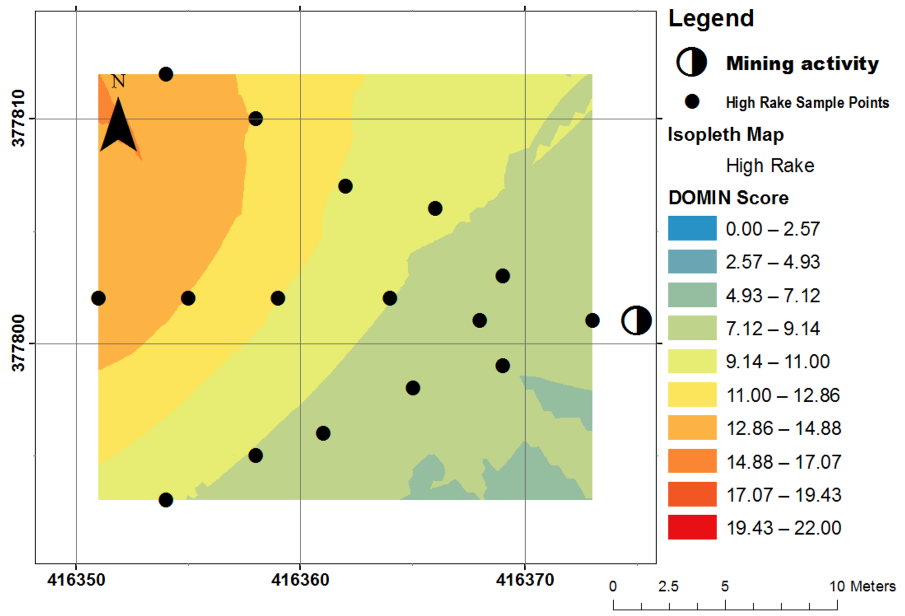


Figure 5:37. Isopleth map of species cover/abundance at High Rake

High Rake displays its lowest species cover close to the chimney base where heavy metal concentrations are highest. The species cover/abundance here seems to increase with distance from it.

5.4.9 Tansley Dale

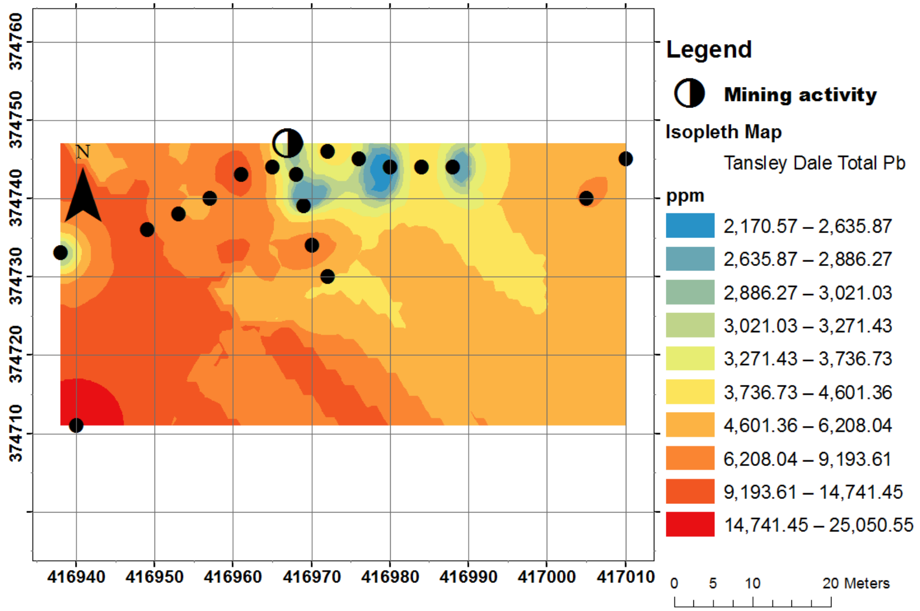


Figure 5:38. Isopleth map of total Pb at Tansley Dale

Tansley Dale displays high Pb concentrations across the sampling area, with the dale bottom exhibiting the greater levels of Pb.

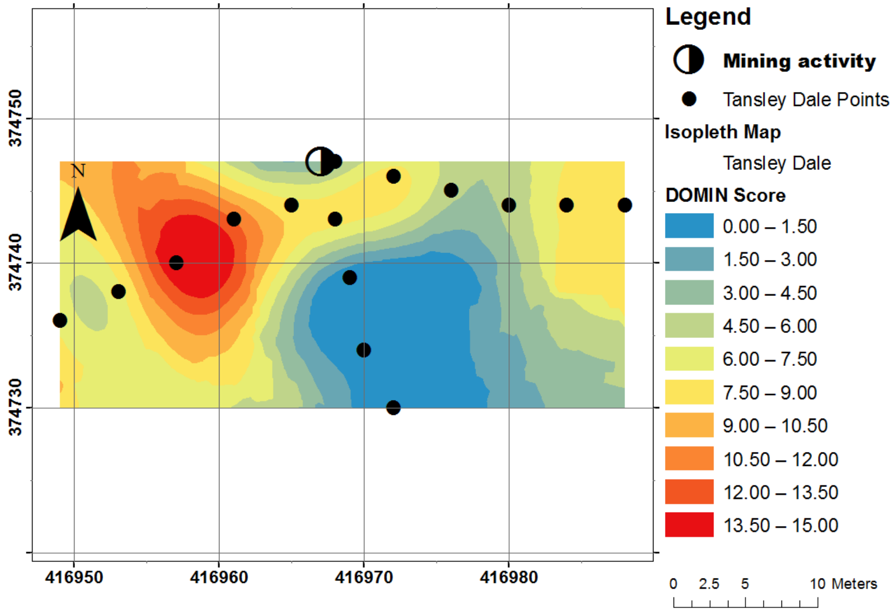


Figure 5:39. Isopleth map of species cover/abundance at Tansley Dale

Tansley Dale displays just one concentrated area of high species abundance, to the east, further up the dale’s track.

5.5 Summary of results

Chapters 4 and 5 have presented several sets of results in order to achieve the aims and objectives of this research. The descriptive statistics indicated that the more extensively worked sites (Dirtlow Rake, Gang Mine, Hard Rake and High Rake) consistently exhibited the greater concentrations of both total and available heavy metal contaminants. Gang Mine and Hard Rake sample areas produced extremely elevated levels of contamination, whilst High Rake was the least contaminated of the rakes. Of the three sites that occurred in naturally formed steep-sided dales, Tansley Dale demonstrated significantly greater heavy metal concentrations than Deep Dale and Hay Dale, with some samples surpassing the contamination levels present at some of the rakes. Deep Dale and Hay Dale consistently displayed the lowest heavy metal concentrations of all study sites. Many outliers from the box and whisker plots presented increased concentrations of heavy metals at lesser elevations or the dale floors contained within samples 17-20.

Particle size analysis descriptive statistics indicated no noteworthy results, with no uniformity between particle size span or particle size median and no sites noticeably distinctive. However, when investigating particle size and heavy metal variables further with PCA, the particle size span displayed a close association with several heavy metals on the biplots produced. The median particle size results were shown to be distanced from the heavy metal variables on these same plots.

pH results were generally uniform across sites exhibiting moderately basic soils of pH 7 to 8. SOM measurements displayed fairly similar results at all sites, although High Rake and Dirtlow Rake frequently presented samples with lower SOM.

Results from Xrf analysis and the heavy metal extraction procedure, analysed with ICP, displayed many significant relationships between total and available heavy metal variables. Total and available Zn, Cd and As in particular were revealed to have strong relationships with each other, as did available Cu and Ni. Total Zn and Cd displayed strong positive correlations with their available fraction and total Pb and As had a slight positive correlation with their available fraction. PCA of heavy metal variables also illustrated these strong associations and revealed total and available Ni and available Cu to be disassociated from the other grouped heavy metals on the biplots.

Exploration of associations between vegetation and heavy metal variables using CCA indicated associations between several plant families and heavy metal variables, particularly the plant families Violaceae, Fabaceae and Ranunculaceae, which contain the plant species;

- Red clover
- White clover
- Birds-foot trefoil
- Meadow buttercup
- Mountain pansy

These associations were evident with both total and available metal concentrations. The cluster analysis carried out on the plant family data also emphasised these relationships between the plant families, grouping the heavy metal associated vegetation together on the dendrogram.

Spatial distribution of both total Pb and vegetation cover/abundance was presented on kriging prediction isopleth maps. These maps demonstrated the mobility and distribution of Pb in relation to the topography and other features present at the study sites. The isopleth maps also presented some similar patterns of distribution between Pb and vegetation cover/abundance indicative of a relationship between them. Significant relationships between the distribution of soil and vegetation variables are discussed in detail in Chapter 6 Discussion, along with further analysis of other findings in greater detail.

Chapter 6: Discussion

6.1 Introduction

This chapter discusses the findings from the collection and analysis of soil samples and vegetation data from seven sites with a history of mining activity in the White Peak area of the Southern Peak District. The results from the data analysis are presented in Chapter 4: Results. The discussion focuses on several main themes:

- The assessment of soil and vegetation characteristics and the analysis of any relationship between them
- The evaluation of the distribution of these soil characteristics and whether any patterns have an influence on the structure of vegetation at the sample sites
- Any relationship between the bioavailable and total fractions of heavy metal concentrations and other soil properties
- The use of particle size analysis as a predictor of heavy metal concentrations with the use of principal component analysis
- Any relationships between results and links to onsite observations and literature review findings will also be discussed

Many results saw distinct differences between Hay Dale, Deep Dale and Tansley Dale and Gang Mine, Dirtlow, Hard and High Rakes. These distinctly different areas will be described as the dales and the rakes [*Table.* 6:1].

Table 6:1. Study sites descriptive terms

Study site	**Descriptive term**
Deep Dale	The dales
Hay Dale	
Tansley Dale	
Dirtlow Rake	The rakes
Gang Mine	
Hard Rake	
High Rake	

6.2 Soil analysis results

6.2.1 Heavy metals and other soil properties

Results from the analysis of soil samples demonstrated significant differences across all eight sites. In particular, the samples taken from areas located in naturally formed steep-sided dales tended to have lower concentrations of heavy metals than the lead rakes, with the exception of Tansley Dale. This may be for several reasons, such as evidence showing that Hay Dale was a calcite mine (and not mined for lead) that was operated for a relatively short period of time (Ford, 2002), and that the Deep Dale site may have been an abandoned test pit for the extensive lead workings directly above on the dale tops (Heathcote, 2004), and hence no great amounts of galena were discovered lower down the dale-side. Tansley Dale, whilst also a steep-sided dale, has extensive lead workings located along its tops and the head of the dale and several ore processing areas located within it (Barnatt & Penny, 2004). Consequently, Tansley Dale samples displayed some high heavy metal concentrations. Of all sites, the samples taken from both Gang Mine and Hard Rake 1 and 2 consistently displayed extremely high heavy metal concentrations, and this could be due to the type of historic practices used for mineral extraction at these sites. Both sites had extensive areas of spoil heaps and hollows [*Fig.* 6:1 & 6:2] which is likely to be due to the extraction processes used or more recent reworking of the gangue materials left at the sites (Ford & Rieuwerts, 2000; Gilbert, 2007).

Figure. 6:1. Image of the hollow at Hard Rake 1 sample area. Source (Author, 2016)

Figure. 6:2. The expanse of spoil heaps and hollows at Hard Rake 2 sample area. Source (Author, 2016)

Gang Mine and Hard Rake displayed maximum Pb concentrations of 73,057ppm and 66,960ppm respectively, arsenic levels above 2,000ppm; Zn concentrations for Gang Mine reached an extremely high maximum reading of 140,226ppm and at Hard Rake more than 10,000ppm, and Cd at Gang Mine presented a reading of over 1,000ppm [*Fig.* 6:3 & 6:4].

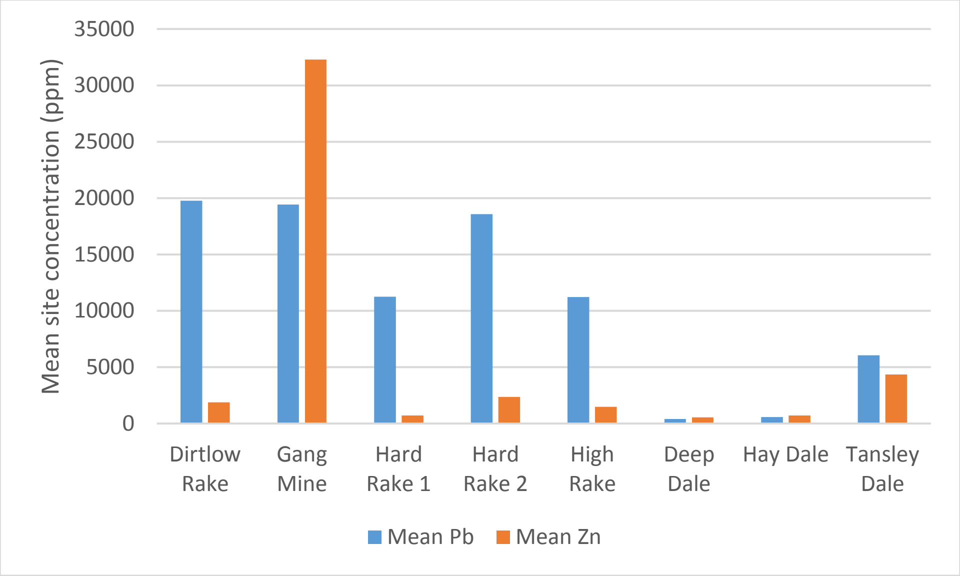


Figure 6:3. Site mean Pb and Zn concentrations

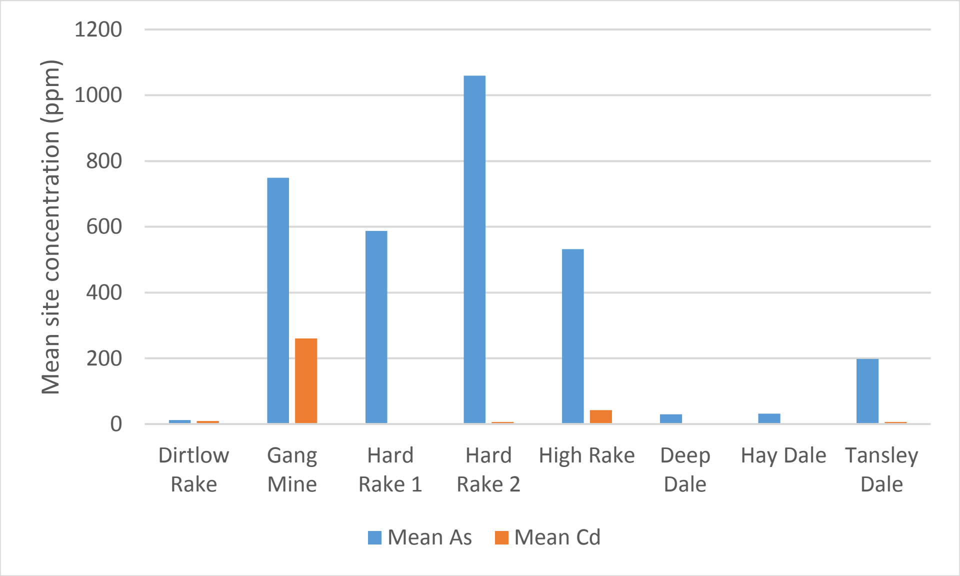


Figure 6:4. Site mean As and Cd concentrations

No significant correlations between total heavy metal concentrations and pH or SOM% were evident utilising all 155 sample results from all sites; however, some interesting heavy metal hot-spots in the distribution patterns at several of the sites seemed to be caused by individual mining features or other geochemical processes (see 2.5.1).

6.2.2 Soil variable relationships and distribution

In addition to the spoil heaps and hollows that feature at Hard Rake and Gang Mine, other objects of historic mining infrastructure and equipment were present at the research areas, and these seem to have influenced the concentrations of several heavy metals in soil samples near to them. This would seem to correspond with what Kossoff *et al.* (2016) theorised - that the archaeological remnants found at historic metal mining sites are linked to higher concentrations of heavy metals, in particular Pb. This could be due to environmental changes such as pH, and the decay of buildings and structures which can lead to mobilisation of metals into the surrounding area (Kossoff *et al.*, 2016). Other research is also consistent with these findings. Mills, Simpson, and Adderley (2014) found correlations between hot-spots of contamination and the post mining landscape features in the UK, as did Rieuwerts, Austin, and Harris (2009), who found higher concentrations around the hillocks present at mining areas; similar to those present at Hard Rake and Gang Mine. Alibrahim *et al.* (2017) used multivariate geospatial analysis technique, similar to this research, to discover peak concentrations of heavy metals around anthropogenic features at a mining area in Ecton, Derbyshire.

The four samples within 5 metres of the grilled shaft and opencast chasm present at Dirtlow Rake [*Fig.* 6:5] exhibited higher mean Pb, Zn and Cd concentrations than the site total mean concentrations. The isopleth map in *Figure*. 6:6 illustrates the higher concentrations of Pb directly adjacent to the mining structures present at Dirtlow rake, which is likely to suggest higher contamination is directly associated with the intensity of mining activity or the residual remains and the geochemical processes that interact with them (see 2.5.1).

Figure. 6:5. Grilled shaft at Dirtlow Rake, with opencast chasm to the right. Source (Author, 2016)

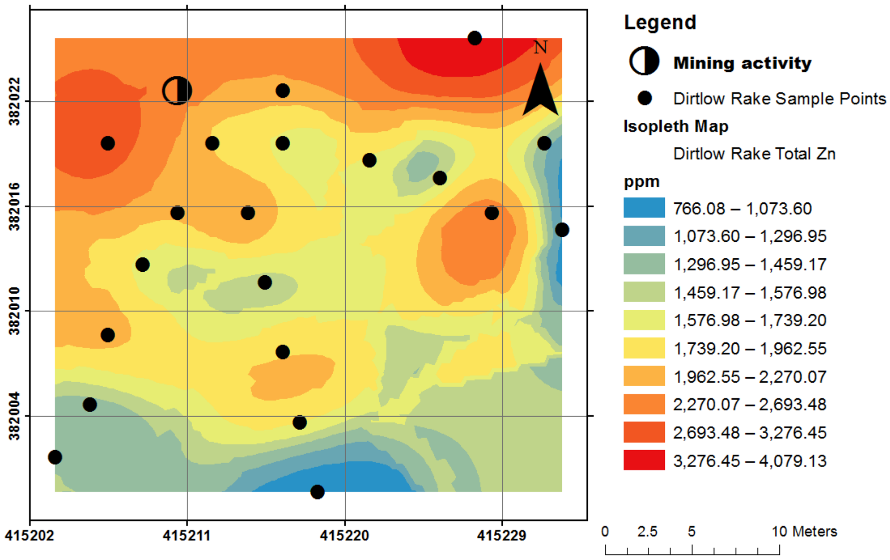


Figure. 6:6. Isopleth map of Pb distribution at Dirtlow Rake

Hay Dale calcite mine had several disused mining structures: the remains of an old shed, a wagon and an ore chute dropping onto the dale floor [*Fig.* 6:7] (AditNow Mining History Society (ANMHS), 2017). Samples taken from beneath the ore chute on the dale floor, and those taken from close to the mine entrance, displayed the highest concentrations of heavy metals at the Hay Dale sample area [*Fig.* 6:8]. The blue area on the isopleth map in *Figure* 5:8 represents the lower concentrations of Pb on the steep dale side, with higher concentrations around the mine, access track and dale floor.

Figure.6:7. Ore chute dropping onto dale floor at Hay Dale. Source (AditNow Mining History Society (ANMHS), 2017)

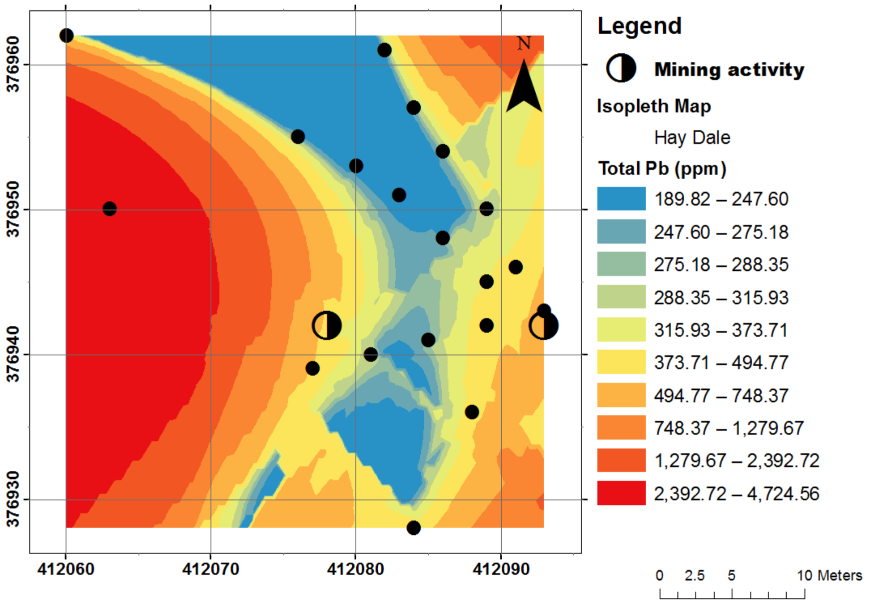


Figure.6:8. Isopleth map displaying concentrations of total Pb

High Rake had an abundance of historic mining structures. Sampling at High Rake originated close to the remains of an old chimney [*Fig.* 6:9] and this area contained the highest concentrations of Pb, Zn, As and Cd at the site. The four samples within five metres of the chimney displayed higher mean concentrations than the total site mean. As an example, Pb close to the structure was 45% higher than the site total mean, and Zn was 50% higher close to the chimney than across the rest of the site [*Fig.* 6:10].

Figure 6:9. The remnants of the chimney base at High Rake. Source (Author, 2016)

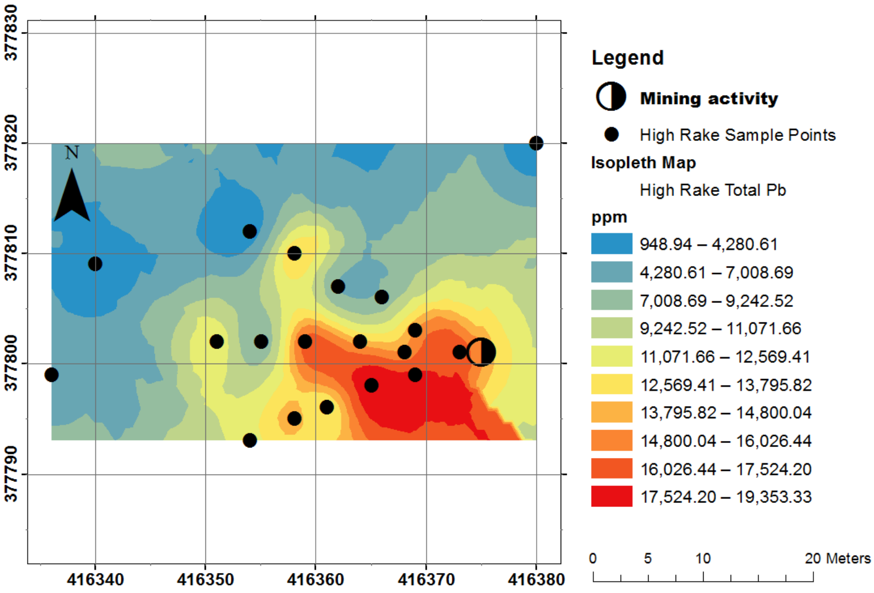


Figure 6:10. Isopleth map shows higher concentrations of Pb around the base of the chimney base at High Rake (SE)

Sampling points at Tansley Dale originated close to a backfilled sough or adit [*Fig.* 6:11], and these displayed higher concentrations of Zn close to the feature than the mean Zn for the whole sampling area (35% higher). The isopleth map in *Figure* 6:12 displays hot-spots of Zn concentrations in the north central area of the map.

Figure 6:11. An ancient looking adit or sough at Tansley Dale. Source (Author, 2016)

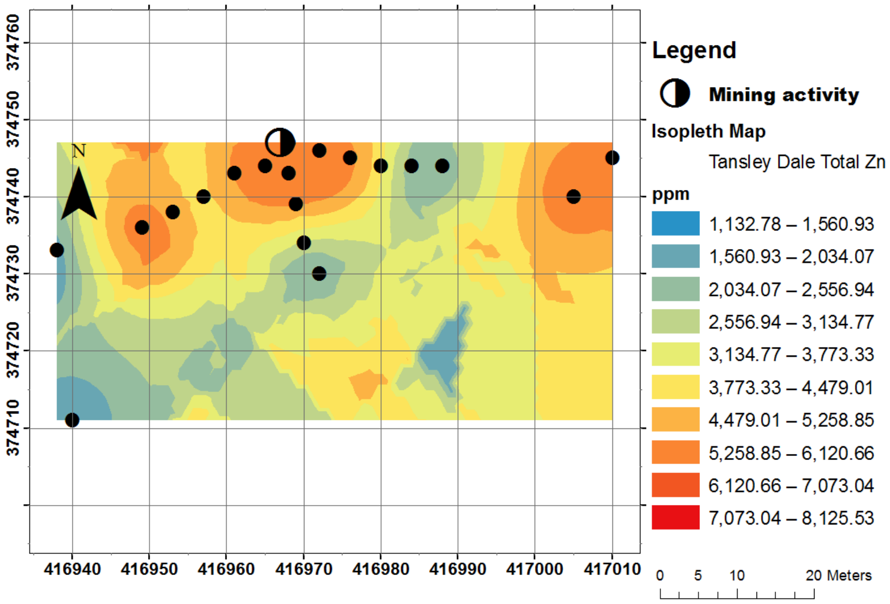


Figure 6:12. Isopleth map for total Zn concentrations at Tansley Dale

6.2.3 Potassium (K) and heavy metals

K distribution patterns at the sites tended to be almost opposite to Pb, Zn, Cd and As, in some cases almost a negative image. Heavy metal contamination in soils often indicates low organic matter, and low levels of the essential nutrients nitrogen, potassium and phosphorus (Cooke & Johnson, 2002; Elouear, Bouhamed, Boujelben, & Bouzid, 2016). According to Tu, Zheng, and Chen (2000), heavy metal contamination can displace K from cation exchange sites, causing desorption of K into the soil solution. They state the heavy metal contamination can degrade soil K and change its behaviour in soils. Yang and Skogley (1990) also report that contamination of soils with heavy metals and the geochemical processes associated with them, could impact soil K by altering buffering capacity, adsorption and distribution. This and the fact that oven dried samples were analysed for elements and thus little soil solution, may explain these distribution patterns, although literature for the effects of heavy metals directly on K in soils was limited. Some examples of K distribution at sample sites compared to heavy metal concentrations are displayed in *Figure*s 6:13 to 6:16.

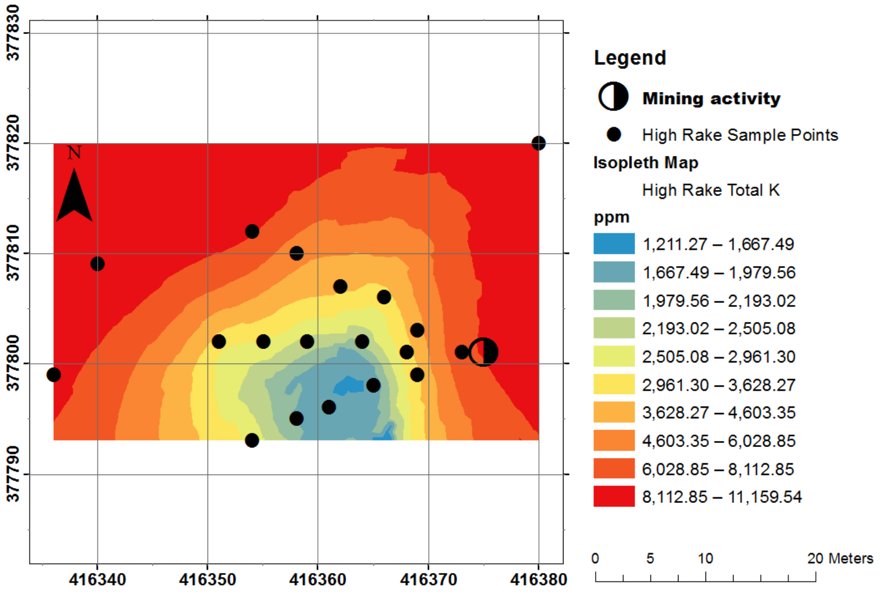


Figure 6:13. The distribution of K at High Rake is lowest next to the area nearest the chimney base

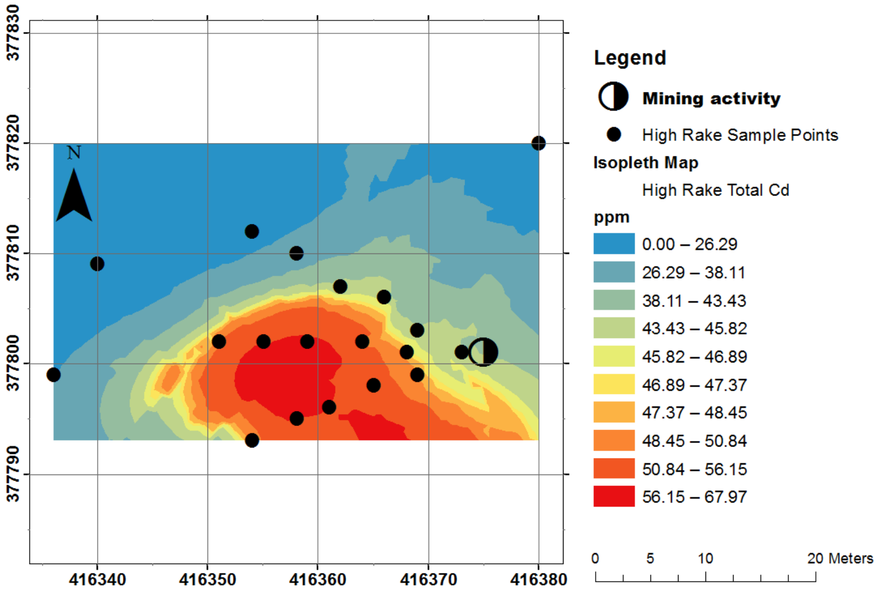


Figure 6:14. The distribution of Cd at High Rake is almost opposite to that of K

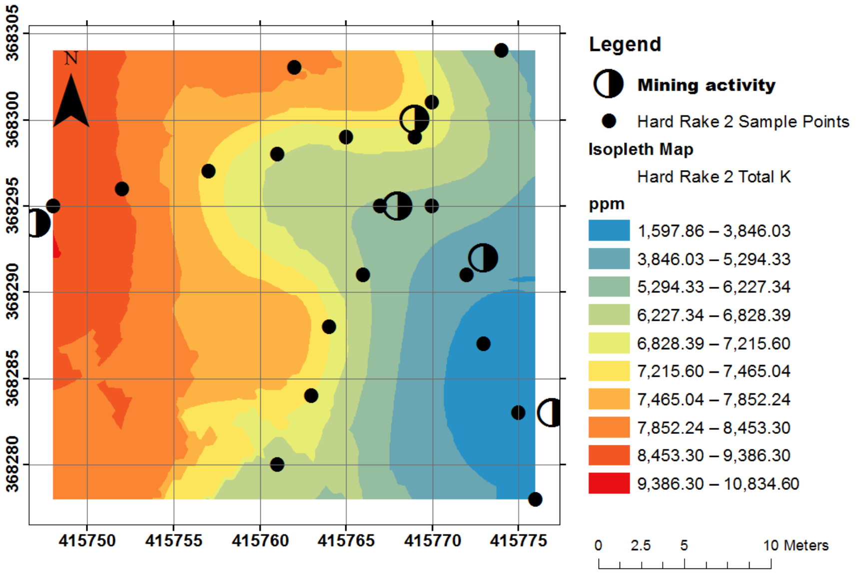


Figure 6:15. K distribution at Hard Rake 2 is lowest to the East of the isopleth map

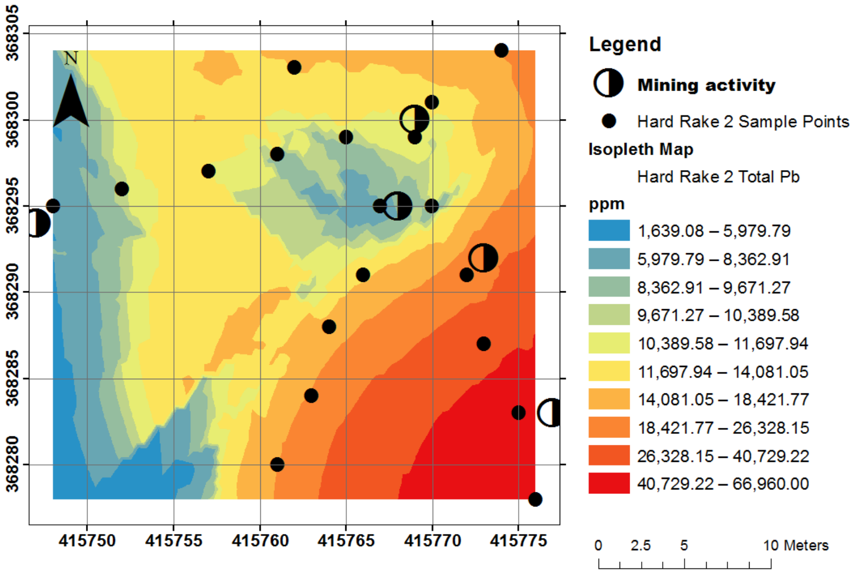


Figure 6:16. Pb concentrations are highest to the east of the isopleth map, opposite to K distribution

6.2.4 pH and bioavailable Pb

Whilst no direct correlations of pH with total metal content were revealed, some patterns of distribution at some sites suggested that lower pH affected the bioavailability of Pb (however, this could also be due to greater presence of sulphides within the soil matrix and the associated geochemical processes). This was especially true of bioavailable Pb at the Gang Mine and Hard Rake 2 sample areas, where extremely high levels of heavy metals were present, and mean pH was lower at 6.5, compared to all other sites where pH was 7 or greater. This implies that a lower pH influences the bioavailability of Pb and other heavy metals, however, it must be noted that decomposition of sulphides (lead and zinc sulphides here, amongst others), are a key factor in the mobilisation of Pb and other metals and pH within the soil phases at mining sites (Alloway, 1995; Dubbin, 2001; Ellis & Mellor, 1995; Kim *et al.*, 2015). These findings also suggest that the single extraction procedure used during this research is a valid method of analysing the freely available fraction of heavy metals in soils. This extraction method is also advocated by several other sources including Kim *et al.* (2015), Luo, Verweij, and van Gestel (2014), Houba *et al.* (1986), Pueyo *et al.* (2004). Some examples of the greater availability of Pb in slightly acidic soils are displayed in *Figures* 6:17 to 6:20.

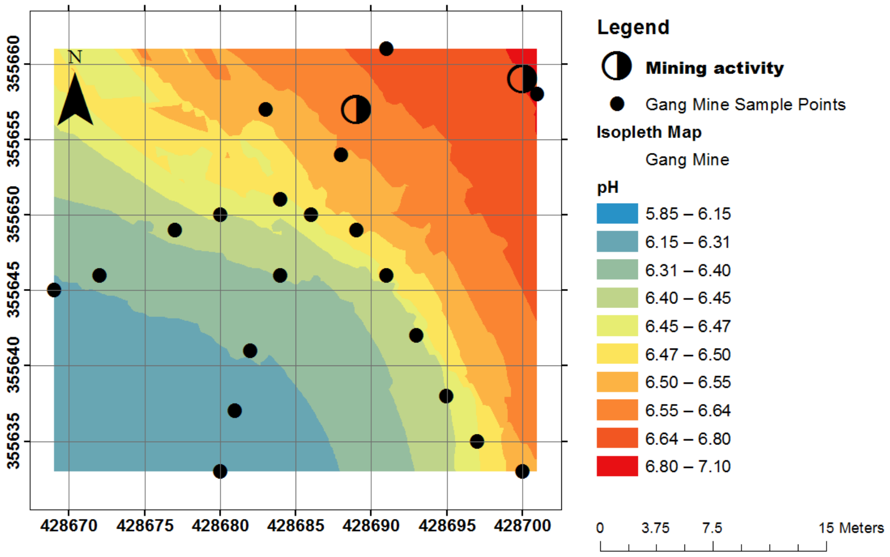


Figure 6:17. pH distribution at Gang Mine sample area

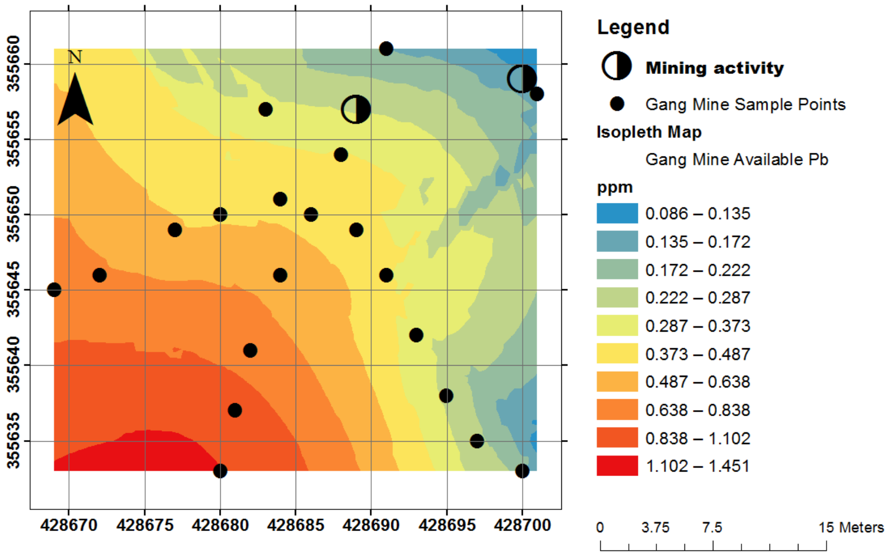


Figure 6:18. Bioavailable Pb concentrations at Gang Mine sample area

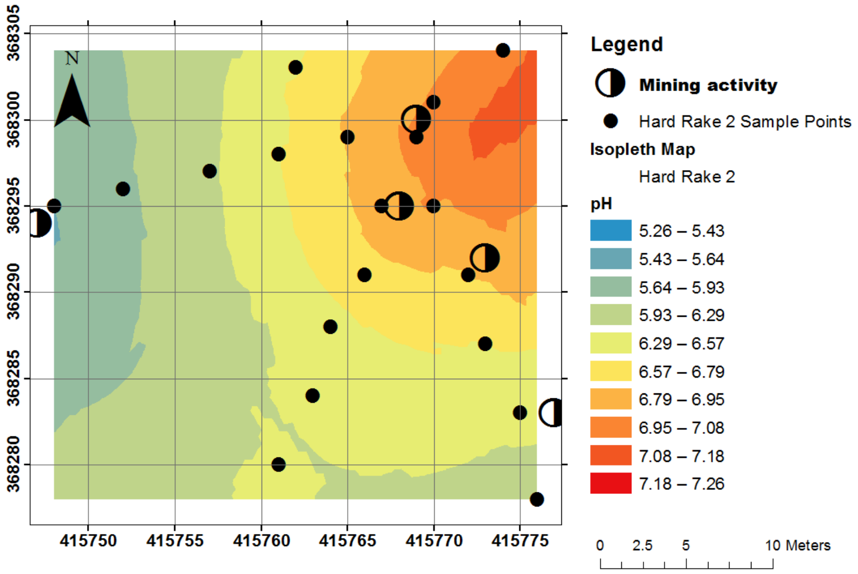


Figure 6:19. pH distribution at Hard Rake 2 sample area

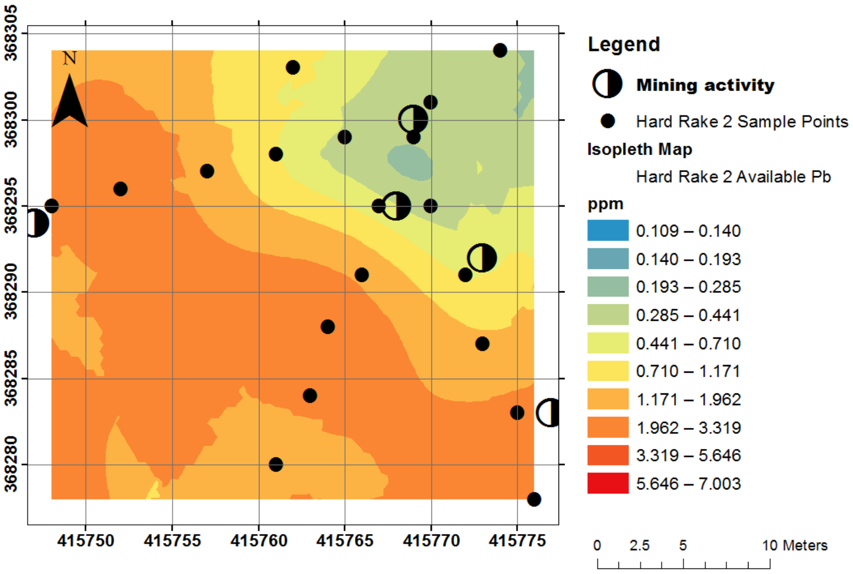


Figure 6:20. Bioavailable Pb concentrations at Hard Rake 2 sample area

6.2.5 Notable relationships between total and bioavailable heavy metals

As illustrated in the Spearman's correlation matrix in Chapter 5: Descriptive Results, *Table* 5:1, many of the heavy metal concentrations had significant correlations. This was accentuated further by separating the sample sites into two groups: the sites situated in the steep sided dales and those located along heavily worked rakes. Of these two groups, the rake sites displayed some very strong positive correlations between several metals, both available and total. The strongest relationships were between Zn and Cd of both total and available fractions [Figs. 6:21 to 6:24].

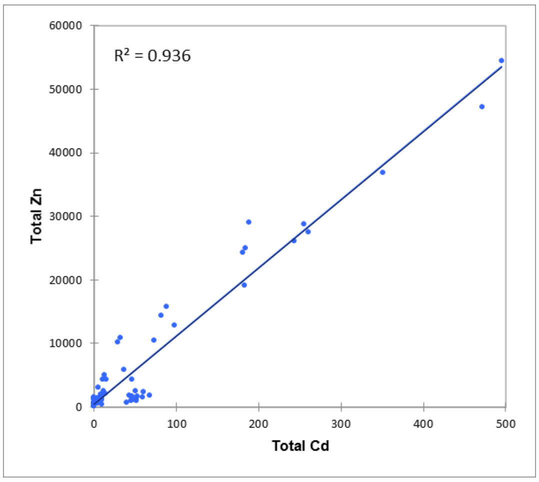


Figure 6:21. Strong positive correlation between total Zn and Cd (y = 107.15x + 488.61)

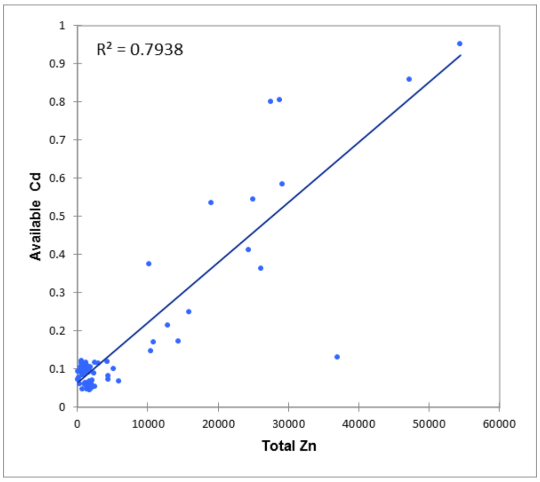


Figure 6:22. Strong positive correlation between total Zn and available Cd (y = 2E-05x + 0.0639)

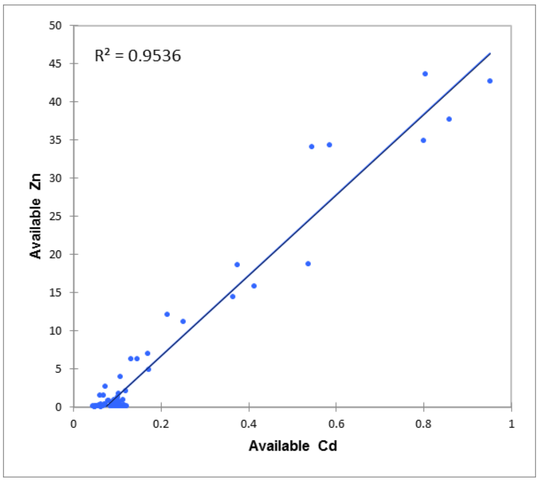


Figure 6:23. Strong positive correlation between available Zn and Cd (y = 52.735x - 3.822)

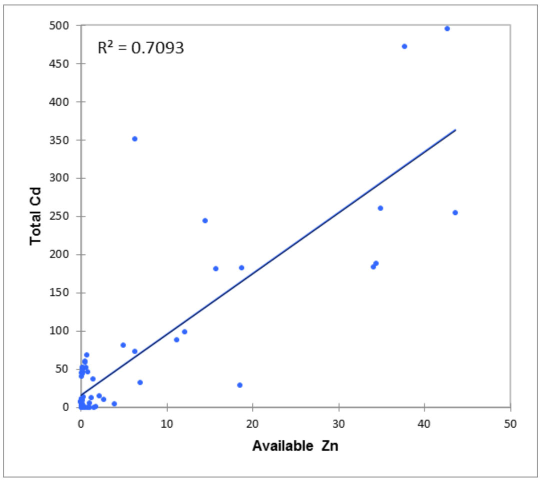


Figure 6:24. Strong positive correlation between available Zn and total Cd (y = 7.9577x + 15.733)

Alloway (1995) explains this relationship, writing that Cd and Zn are closely linked due to their geochemical and ionic structures and electronegativities, which are influential in the ionisation potential of these elements. They are also easily bonded with sulphur to form sulphide minerals. It is these sulphide ore minerals in the form of ZnS (sphalerite) that have been extracted at the research areas along with other minerals (Ford & Rieuwerts, 2000), and consequently large amounts of Zn and Cd are present in soil samples. Alloway (1995) also states that these elements usually have a ratio of approximately 500:1, although this can vary. Available arsenic also showed strong positive correlations with both available and total Cd and Zn results (Av. Cd R^2=0.9361, Av. Zn R^2=0.8717, Tot. Cd R^2=0.6801 and Tot. Zn R^2=0.6871) and this too can be attributed to arsenic being notably associated with sulphide mineralisation (Alloway, 1995).

6.2.6 Particle size analysis

Several studies have explored the relationship between particle size distribution and heavy metal contamination (Abouelnasr 2010). Hao, Jiang, Zhang, and Tang (2010) found higher concentrations of heavy metals and increased leachability of metal linked with smaller particle sizes in fly-ash; Ikegami, Yoneda, Tsuji, Bannai, and Morisawa (2014) discovered greater heavy metal quantities associated with smaller particle sizes in their study on metal ingestion in children; Qian, Shan, Wang, and Tu (1996) examined the particle size and texture class of soil and how it effects plant uptake, and found higher metal uptake correlated with smaller particle size. Other studies observed a link between particle size and heavy metal concentrations in road dust (Li, Shi, & Zhang, 2015) and house dust (Wang, Willis, Buckley, Rhoads, & Lioy, 1996). The results of these studies show higher amounts of heavy metals associated with the larger surface areas existing on smaller particles. They also found associations between grain size and heavy metals, whilst this research only found a relationship between particle size span and heavy metal concentrations.

The results of this research observed some relationship between particle size and heavy metal concentrations in soil samples. Utilising all 155 soil samples, data analysis explored the relationships of mean, median, span, < D10 and <D90 (those particles in the lower 10% and 90% of the spread). Results only showed some positive correlations between PSA span and total Pb, Zn, Cd, and Cu [Figs. 6:25 to 6:28], although none are strong enough to draw conclusions.

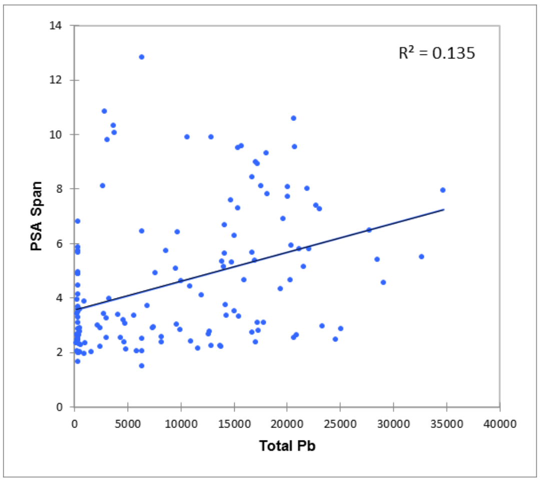


Figure 6:25. Slight positive relationship between PSA span and total Pb (y = 0.0001x + 3.5519)

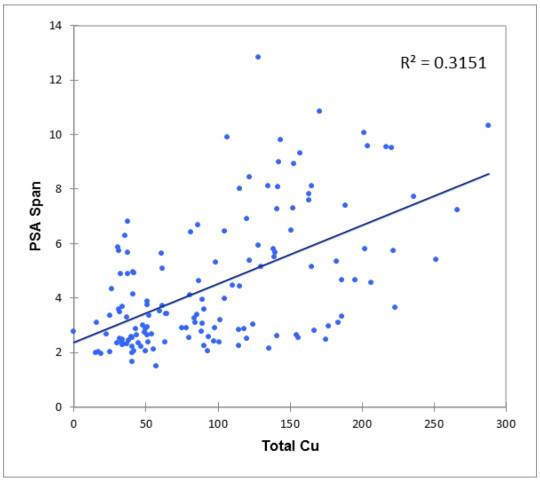


Figure 6:27. Slight positive relationship between PSA span and total Cu (y = 0.0215x + 2.3668)

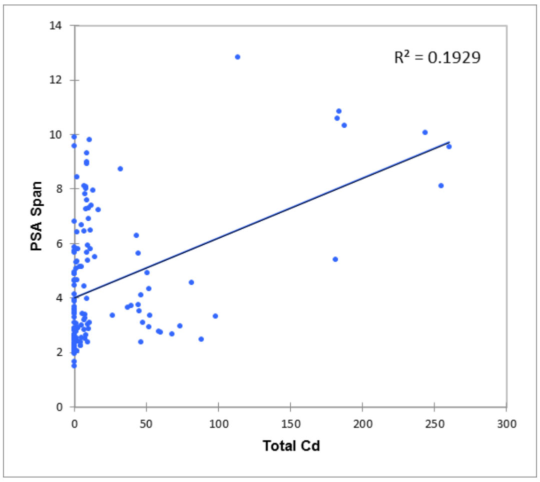


Figure 6:26. Slight positive relationship between PSA span and total Cd (y = 0.0219x + 4.0197)

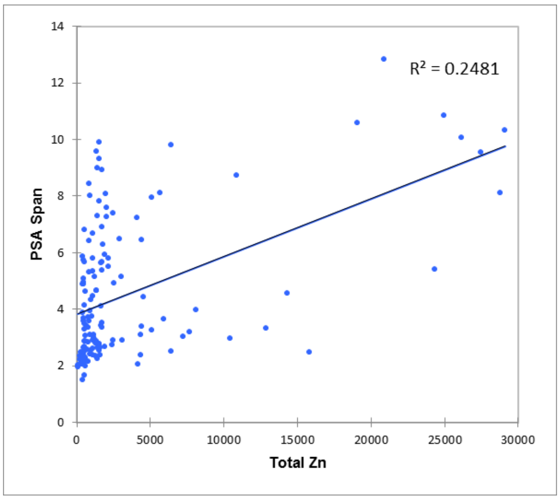


Figure 6:28. Slight positive relationship between PSA span and total Zn (y = 0.0002x + 3.8276)

Further analysis of particle size span using PCA revealed a close association with total heavy metals, although correlations were very weak in most cases. Bioavailable metals, whilst not displaying significant correlations on scatter plots, showed a close association with available Pb and particle size span using PCA. Romero-Freire, Martin Peinado, and van Gestel (2015) also found correlations between available Pb and particle size, although not PSA span, and that this relates to cation exchange capacity and surface area. In a study of the bioavailability of Pb at lead mining sites, Hemphill, Ruby, Beck, Davis, and Bergstrom (1991) conclude that studies of Pb availability must also include the distribution of particle sizes. The results here are inconclusive and further investigation is needed.

6.3 Vegetation data

Vegetation records at the research areas highlighted differences between the species composition and distribution at both the rake and dale sites. Five species were only present at the dales and eight species were exclusive to the rakes at the time of survey. Some species were more universal and were present across 7-8 of the sites, and these represented four of the five most abundant species. These included common birds-foot trefoil (*Lotus corniculatus*), eyebright (*Euphrasia officinalis)*, harebell (*Campanula rotundifolia)* and red clover (*Trifolium pratense*). These abundant species, along with several of those recorded only at the rake sites, showed close associations with several heavy metals when analysed using canonical correspondence analysis (CCA). CCA of plant families and both total and available heavy metals concentrations indicated that there was a close relationship between them. The CCA plot in *Figure 6*:29 illustrates a close association with total heavy metals for plant families Fabaceae, Ranunculaceae, Polygalaceae, Caryophyllaceae and Violaceae. When plotted against available heavy metals [*Fig.* 6:30], three plant families (Fabaceae, Ranunculaceae and Violaceae) clearly have a closer association than other vegetation families. The species contained within these plant families are presented in *Table.* 6:2.

Table 6:2. Plant species contained within plant families found associated to heavy metals

Plant Family	Common Name	Latin Name
Fabaceae (Legumes)	COMMON BIRDSFOOT TREFOIL	*Lotus corniculatus*
	WHITE CLOVER	*Trifolium repens*
	RED CLOVER	*Trifolium pratense*
Violaceae	MOUNTAIN PANSY	*Viola lutea*
Ranunculaceae	MEADOW BUTTERCUP	*Ranunculus acris*
Caryophyllaceae	COMMON MOUSE EAR	*Cerastium fontanum*
Polygalaceae	MILKWORT	*Polygala vulgaris*

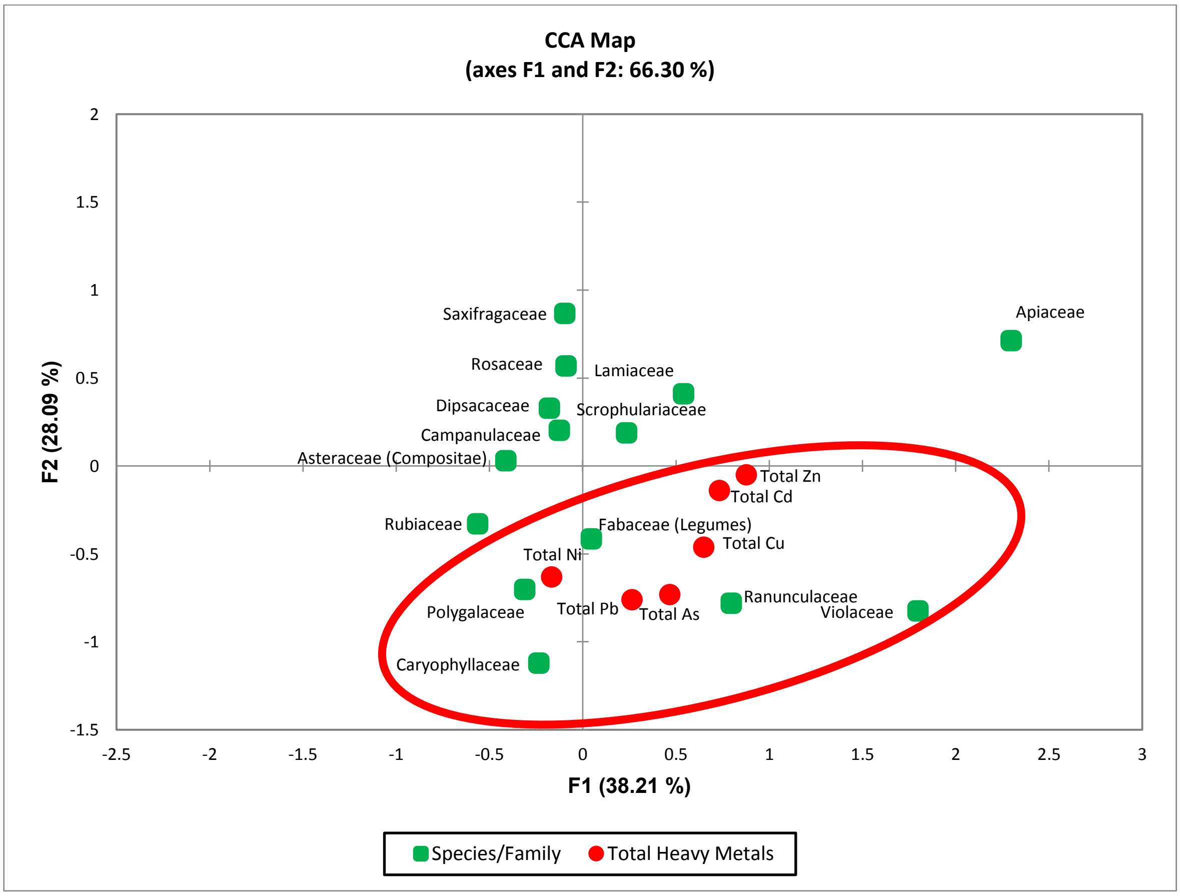


Figure 6:29. CCA plot showing plant family and total heavy metal associations

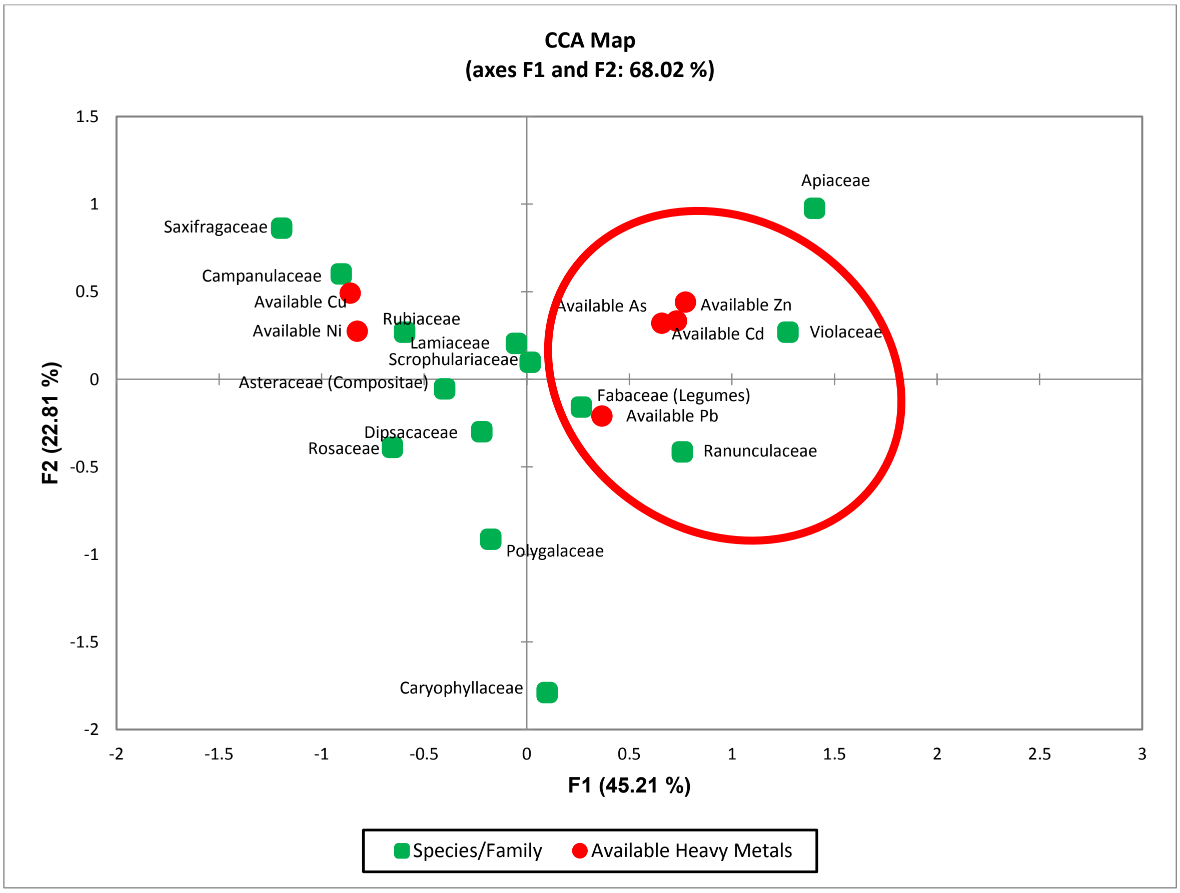


Figure 6:30. CCA plot showing plant family and available heavy metal associations

Further investigation of these plant species revealed several studies associating them with heavy metal tolerance and accumulation at historical mining sites and other contaminated areas.

6.3.1 Meadow buttercup. *Ranunculus acris*

Meadow buttercup, *Ranunculus acris,* is a widely distributed plant throughout Europe and is commonly found in pastures, meadows, grassland and roadside verges but is especially common on agricultural land (Marchand, Lamy, Bert, Quintela-Sabaris, & Mench, 2016). However, it is also frequently found growing on metalliferous soils throughout Europe (Pawlowska, Błaszkowski, & Rühling, 1996). According to Baker *et al.* (2010) it is a pseudo-metallophyte which is moderately tolerant of heavy metal contaminated soils, although not restricted to them like some species of true metallophytes. Cooke *et al.* (1976) also state that is an established species on areas of gangue waste materials, especially those containing quantities of fluorspar. Similar to this research, Schipper *et al.* (2011) discovered a clear positive relationship between *Ranunculus acris* and contaminated soils, whilst analysing the relationships between vegetation and heavy metals using CCA. Odat, Jetschke, and Hellwig (2004) also found a close positive association using CCA whilst studying the genetic diversity of *Ranunculus acris.* They also conclude that the type of habitat this species grows in is correlated with its genetic diversity and that this genetically diverse species is highly adaptable to the metal enriched soil and is certainly influenced by it. Wojcik, Sugier, and Siebielec (2014) found *Ranunculus acris* to have accumulated up to 446ppm Zn, 42ppm Pb and 10ppm Cd within its shoots whilst growing on heavy metal contaminated land, although this may not be representative of the accumulative capabilities as Marchand *et al.* (2016) state that *Ranunculus acris* is an excluder of heavy metals and stores the majority of accumulated metals in its roots.

6.3.2 Common birds-foot trefoil, *Lotus corniculatus*

Birds-foot trefoil, *Lotus corniculatu*s, a legume, has a worldwide distribution and is found growing in a diverse range of environmental settings (Escaray *et al.*, 2012). In the UK it is a species that is commonly found on calcareous (Streeter *et al.*, 2009) and metal enriched grasslands (Simkin, 2011). *Lotus corniculatus* has repeatedly been recorded growing on mine spoils in studies of heavy metal contamination and vegetation (Cooke *et al.*, 1976; Johnson *et al.*, 1977; Pawlowska *et al.*, 1996; Prasad, Pratas, & Freitas, 2006; Shimwell & Laurie, 1972; Tordoff *et al.*, 2000) and Shimwell and Laurie (1972) describe it as widespread on the waste heaps and extensively worked lead mining sites where reworking for gangue materials has taken place. They noted the species as preferring a neutral to alkaline substrate of between pH 6.3-7.4 and recorded an accumulation of 320ppm of Zn and 68ppm Pb, as well as Cooke *et al.* (1976) recording 281ppm Fluorspar in *Lotus corniculatus* tissues; Prasad *et al.* (2006) recorded 63ppm of Pb in its above ground tissues. Although not a hyperaccumulator, the species is so adept at acclimatising to soils with a low nutrient status and high contamination (Tordoff *et al.*, 2000), along with its capacity to establish symbiotic relationship with nitrogen (N) fixing bacteria, that it is frequently used to help restore or reclaim metalliferous mining areas (Bradshaw, 1997; Escaray *et al.*, 2012). Once established in a metal-enriched area, the genetically diverse species (Escaray *et al.*, 2012) can supply the vegetation community with N and allow other species to thrive (Tordoff *et al.*, 2000), especially in conjunction with other legumes such as white clover, *Trifolium repens* (Bradshaw, 1997).

6.3.3 White Clover, *Trifolium repens*

White clover, *Trifolium repens*, is a widely distributed species and is commonly found in meadows, pastures and calcareous grasslands (Streeter *et al.*, 2009). It is also known to spontaneously inhabit heavily metal contaminated soil (Corrales, Barceló, Bech, & Poschenrieder, 2014), and many studies on vegetation communities on mine tailings have recorded this species (Andrews *et al.*, 1989; Bidar *et al.*, 2007; Cooke *et al.*, 1976) or utilised it for revegetation or remediation of contaminated mining sites (Bradshaw, 1997; Chen, Zhu, Duan, Xiao, & Smith, 2007; Ye, Wong, Wong, Lan, & Baker, 1999). Bidar *et al.* (2007) reported concentrations of Zn 1563ppm, Pb 167ppm and Cd 127ppm present in the roots of *Trifolium repens,* whilst lower concentrations were present in the shoots (Zn 97ppm, Pb 36ppm and Cd 9ppm). He states that this species has limited metal translocation into shoot tissues. Lopareva-Pohu *et al.* (2011) found similar concentrations in the above and below ground tissues and reports that heavy metals are better accumulated in the presence of several contaminants rather than pollution with a single element. Lanier *et al.* (2016) also found that most Pb accumulates within the fluids of the plant, whilst Cd is stored in the cell walls and that the ability of *Trifolium repens* to accumulate Cd increases with time. *Trifolium repens* has also been found to inhabit mining spoil heaps containing gangue materials, on which it has been recorded to have accumulated substantial amounts of fluoride (6429ppm in the roots and 4308ppm in the leaves) (Cooke *et al.*, 1976). Whilst the above studies have focused on the metal uptake of *Trifolium repens*, most literature concerns the symbiotic nitrogen fixation abilities of this legume (Bidar *et al.*, 2007) and it has often been used as a pioneer plant to revegetate mined areas (Bradshaw, 1997; Hao *et al.*, 2014).

6.3.4 Red clover, *Trifolium pratense*

Red clover, *Trifolium pratense*, is found throughout Europe, commonly on pastures, meadows and rough grasslands (Streeter *et al.*, 2009). It is also a legume and has adapted to many environmental conditions due to a large number of locally adapted genotypes instead of a single universal genotype (Dias, Julier, Sampoux, Barre, & Dall'Agnol, 2008). Like *Trifolium repens* and *Lotus corniculatus,* this species is commonly found on mine spoils (Szarek-Lukaszewska & Grodzinska, 2007; Turnau, Gawroński, Ryszka, & Zook, 2012), and is often used for remediation of contaminated sites due to its N fixing capabilities (Bradshaw, 1997). Hao *et al.* (2014) write that although having an advantage when establishing on metalliferous soils through the legume-rhizobia symbiosis and N fixation, most legumes are sensitive to very high Zn concentrations. However, Tlustoš *et al.* (2006) found *Trifolium pratense* to be successful in the uptake of Pb when compared to other high biomass producing plants, especially in early stages of growth, and Kos, Grčman, and Leštan (2003) recorded concentrations of 398ppm Pb, 179ppm Zn and 3ppm Cd in its plant tissues whilst examining the phytoextraction of heavy metals in soils.

6.3.5 Milkwort, Polygala vulgaris

Milkwort, *Polygala vulgaris*, is found throughout most of Europe and its main habitats are calcareous grasslands, heaths and sand dunes (Streeter *et al.*, 2009). The species is also frequently observed growing on metalliferous soils and spoil heaps throughout Europe (Pawlowska *et al.*, 1996; Reeves, Schwartz, Morel, & Edmondson, 2001), particularly Pb and Zn wastes (Barnatt *et al.*, 2013; Richards & Swan, 1976). *Polygala vulgaris* is also described by Antonovics *et al.* (1971) and Baker and Proctor

(1990) as a pseudo-metallophyte species. Baker *et al.* (2010) and Brown (1994) state that species such as *Polygala vulgaris* are mildly tolerant of elevated heavy metal concentrations and are an accompanying species for true metallophytes, capable of existing in both contaminated soils and uncontaminated soils.

6.3.6 Common mouse-ear, *Cerastium fontanum*

Common mouse-ear, *Cerastium fontanum*, is widespread throughout Europe, a very diverse species with many sub-species. It typically exists on meadows, pastures, grassland, agricultural land and road-side verges (Streeter *et al.*, 2009). It is also commonly found on mine spoils and disturbed sites with elevated heavy metal concentrations (Barnatt *et al.*, 2013; Pawlowska *et al.*, 1996; Tierney, 1998). Whilst no direct evidence of *Cerastium fontanum* being a pseudo-metallophyte could be found in literature, the family *Caryophyllaceae* to which it belongs contains many true and pseudo-metallophytes (Baker *et al.*, 2010; Baker & Proctor, 1990; Mengoni *et al.*, 2001). An updated inventory of metal and gangue mining sites in the Peak District by Barnatt *et al.* (2013) describes *Cerastium fontanum* as being found at disturbed sites where re-working of gangue materials has previously taken place, and on lead rakes, where it typically occurs on well drained and south facing areas. One article by Thompson and Proctor (1983) analysed the species for its metal accumulation, and found maximum concentrations of 470ppm Pb, 310ppm Zn and 180ppm Cu contained within its plant tissues. These findings suggest that *Cerastium fontanum* is a mildly tolerant pseudo-metallophyte.

6.3.7 Mountain pansy, *Viola lutea*

The mountain pansy species recorded at Gang Mine and Hard Rake 2 [*Fig.* 5:30] sample areas were *Viola lutea* ssp. Calaminaria (yellow zinc violet), and was the only true metallophyte recorded during this research. This species is restricted to heavy metal spoil heaps (Hildebrandt, Regvar, & Bothe, 2007; Kuta *et al.*, 2012), particularly those with high concentrations of Zn, and consequently it is also known as the zinc violet (Bizoux *et al.*, 2004; Hermann *et al.*, 2013; Kuta *et al.*, 2012). The association of this species with Zn and Cd is clear on the CCA plot for bioavailable metals and plant variables [*Fig.* 6:30]. Also, the close association with Cd on the CCA plot may be explained by a) the close relationship between Zn and Cd from a geochemical standpoint (Alloway, 1995) and b) the fact that plant species are known to uptake Cd alongside Zn (Benavides *et al.*, 2005; Smolders, 2001). Kuta *et al.* (2012) speculates that this restriction to metalliferous sites may be the cause of genetic mutations which have allowed its adaptation to extremely metal enriched sites, and that the species may have been dispersed to other mining areas on miners' tools and clothing, or by livestock on large areas of agricultural land with evidence of metal extraction. This specialised species in typically recorded alongside other metallophytes and accompanying pseudo-metallophytes (Bizoux *et al.*, 2004) and frequently displays a Zn concentration of greater than 1% within its plant tissues (dry weight) (Ufimtseva, 2015). *Viola lutea* ssp. Calaminaria have also been known to accumulate more metals in plant tissues than is present in the soil surrounding them in areas of low contamination (Hermann *et al.*, 2013). However, Hermann *et al.* (2013) report that there has been some controversy about whether this species is a heavy metal excluder or a heavy metal accumulator. They state that some literature has reported heavy metal concentrations lower than non-metallophyte species (Ernst, 1982, cited in Hermann et al., 2013), and other studies had found very high concentrations (Bizoux *et al.*, 2004). Their research discovered mean concentrations of 9433ppm Zn and 1737ppm Pb in root tissues, and 5827ppm Zn and 547ppm Pb in shoot tissues. Hermann *et al.* (2013) concluded that although very high concentrations of both Zn and Pb were found in roots and shoots, that *Viola lutea* ssp. Calaminaria was a heavy metal excluder. This is because typical heavy metal accumulators contain a higher shoot to root ratio and store the mass of metals in their above-ground parts.

Figure 6:31. Mountain pansy at Hard Rake. Source (Author)

The relationship between the plant families and total heavy metals in the CCA plot in *Figure 5*:29 is supported by the many sources discussed above which have described how the plant families and the species they contain are all commonly associated with heavy metal polluted sites alongside other metal tolerant species, and are either pseudo-metallophytes, accompanying species such as the legumes, or in the case of mountain pansy, *Viola lutea*, a true metallophyte (Baker *et al.*, 2010). The CCA plot containing plant variables and the extracted available heavy metal values [*Fig.* 6:30] highlights further the relationship between mountain pansy, *Viola lutea*, and metals Zn and Cd (Hermann *et al.*, 2013), as well as the tolerance of Meadow buttercup, *Ranunculus acris* (Odat *et al.*, 2004; Schipper *et al.*, 2011) and the legume species (Bradshaw, 1997; Hao *et al.*, 2014) to the available concentrations of heavy metals at the study sites. Conversely, it is also worth noting than other plant family variables *Asteraceae, Rosaceae,* and *Apiaceae* which were less associated with the heavy metals in the CCA plots [*Fig.* 6:29 & 6:30] were also found to be poorly represented in areas of elevated heavy metal contamination by Baker and

Proctor (1990). They conclude that these large plant families may not have the genetic variability to evolve metal tolerance to the same level as others.

6.4 Soil and vegetation variable distribution similarities

Some interesting patterns of species cover seem to display similar distributions to either total and available Pb and Zn, and more interestingly Cd. Six sites showed some similar pattern of distribution between Cd and vegetation cover and five sites displayed associations between either total or available Pb, and vegetation cover.

Isopleth maps of the study sites associating vegetation and metal distribution are displayed below. These isopleth maps correspond to the same sixteen sample points where both vegetation recording and soil sampling was undertaken, and hence the isopleth maps for metals exclude the extra sample points 17-20 where no vegetation recording took place. This gives a clearer view of how vegetation variables relate to the distribution of elements in soil samples within the same 1m quadrat.

6.4.1 Deep Dale

Deep Dale displays greater species cover to the north-west of the isopleth map, where available Pb and Zn are also of a higher concentration [Figs. 6:32 to 6:34]. This area is situated around the perceived lead mining test pit used as an origin point for sample transects.

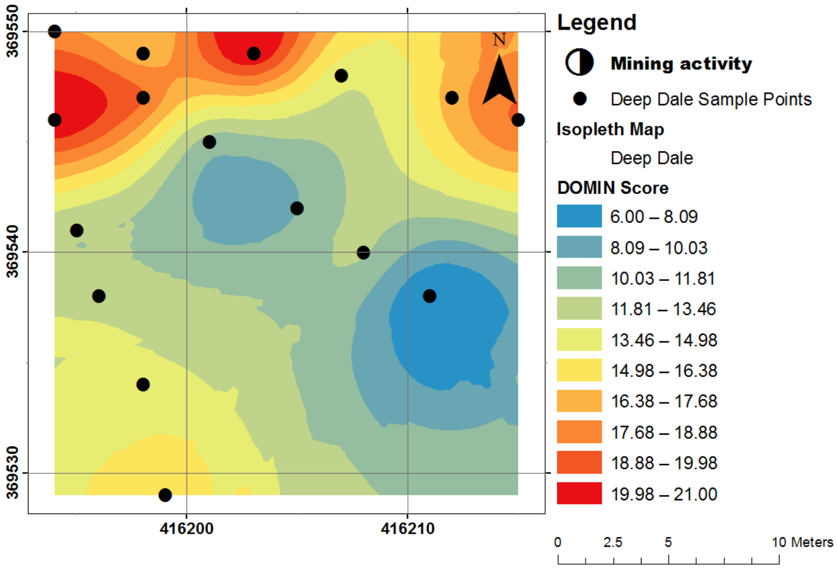


Figure 6:32. Distribution of species cover at Deep Dale

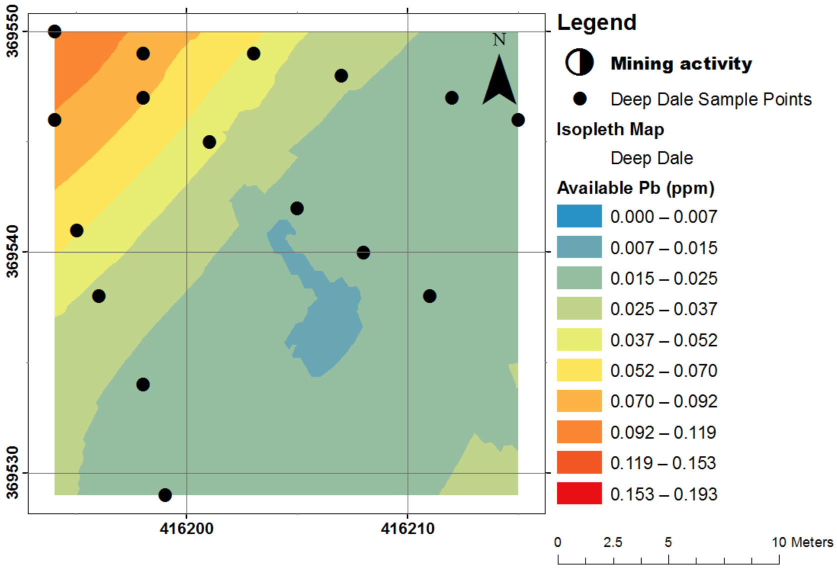


Figure 6:33. Spatial distribution of available Pb at Deep Dale

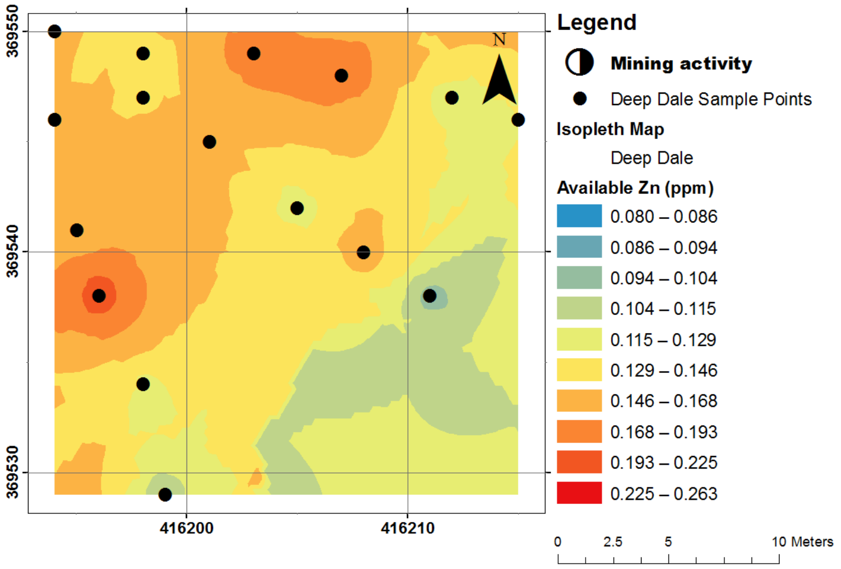


Figure 6:34. Spatial distribution of available Zn at Deep Dale

6.4.2 Dirtlow Rake

Dirtlow Rake maps display a greater vegetation cover to the north-west of the isopleth map where available Cd and total Pb are more elevated [Figs. 6:35 to 6:37]. This area is situated next to a grilled mine shaft and opencast mine which were used as the origin of the sample point transects.

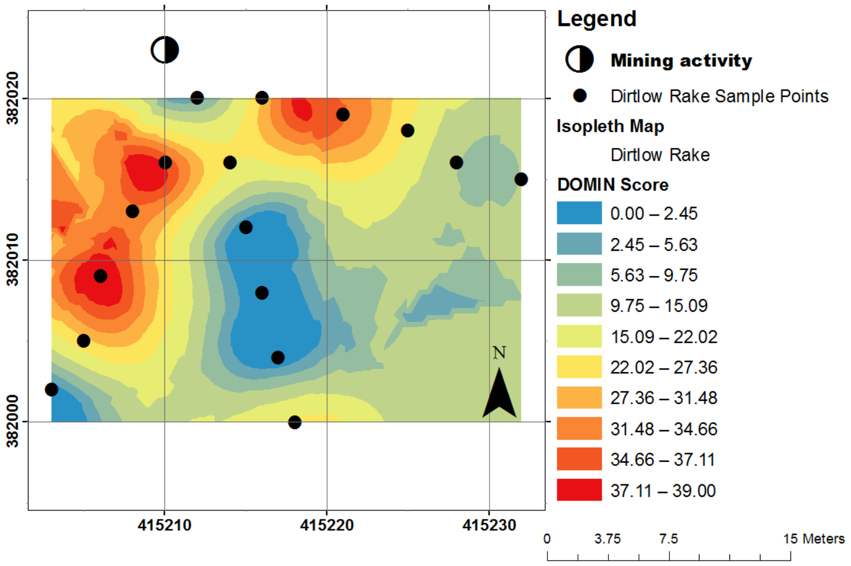


Figure 6:35. Distribution of species cover at Dirtlow Rake

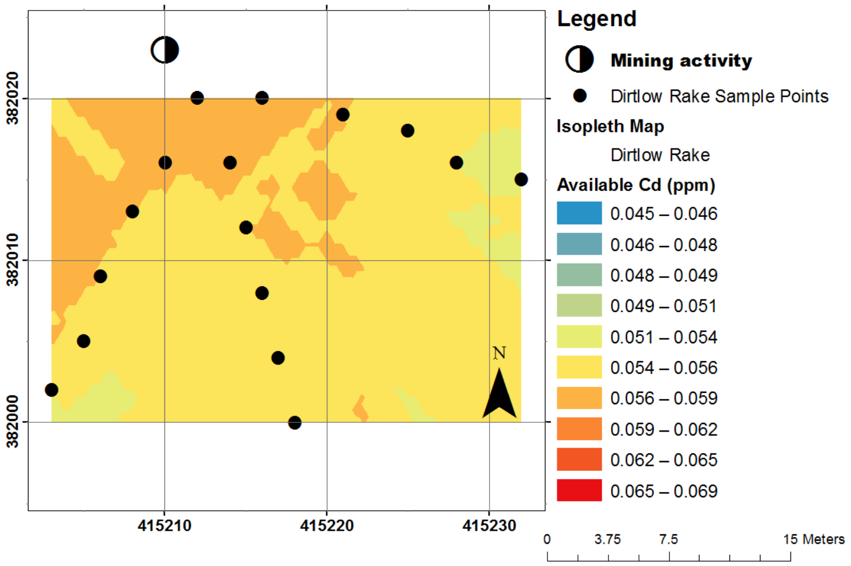


Figure 6:36. Spatial distribution of available Cd at Dirtlow Rake

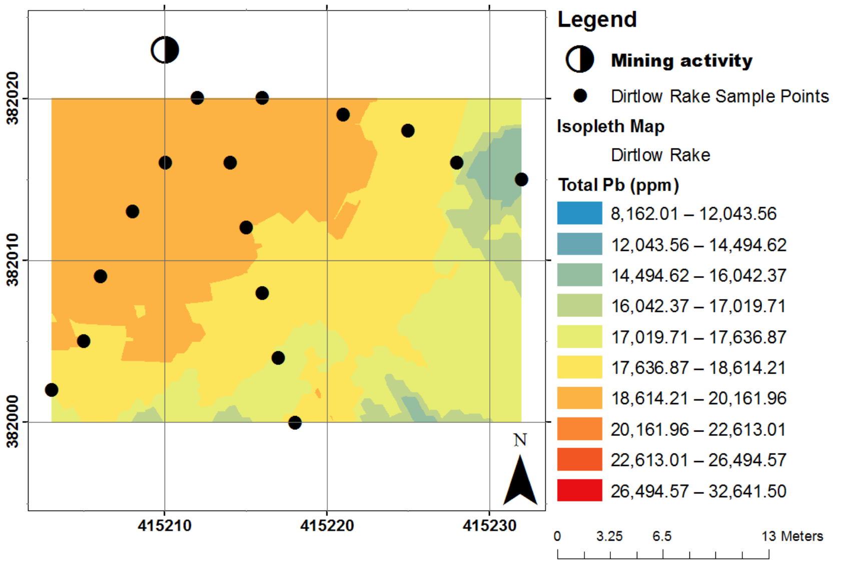


Figure 6:37. Spatial distribution of total Pb at Dirtlow Rake

6.4.3 Gang Mine

At Gang Mine, vegetation species cover is greatest to the east of the isopleth maps, which is also where the peak concentrations of available Cd and Zn are located [Figs. 6:38 to 6:40]. This area is located along the edge of the hollow leftover from mineral extraction processes, which is close to the transect origin point.

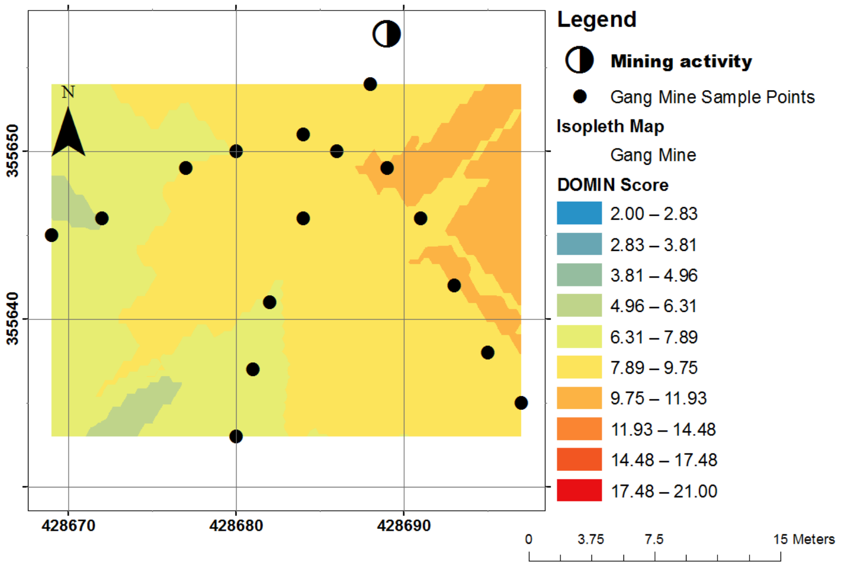


Figure 6:38. Distribution of species cover at Gang Mine

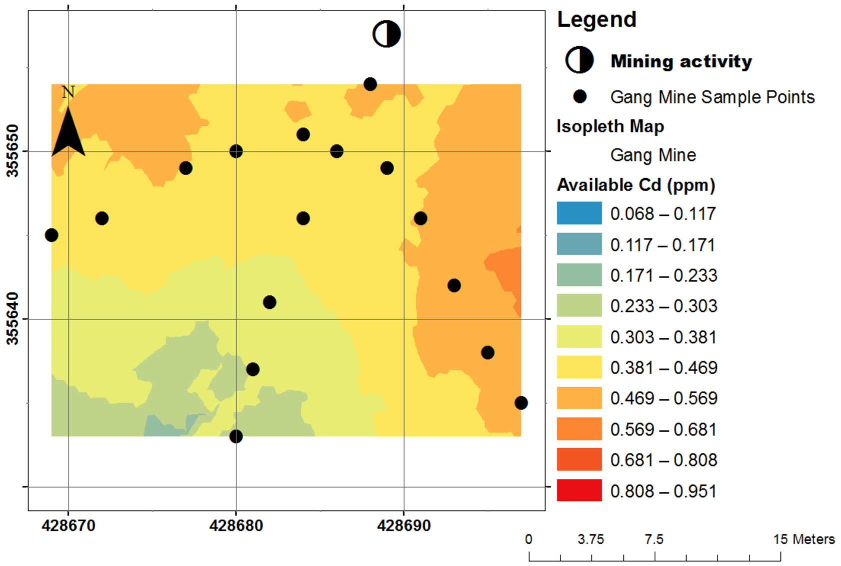


Figure 6:39. Spatial distribution of available Cd at Gang Mine

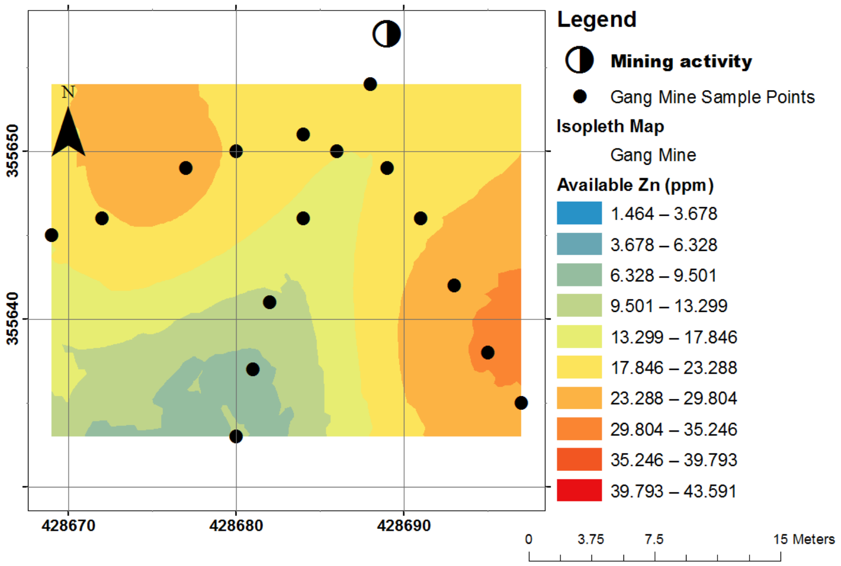


Figure 6:40. Spatial distribution of available Zn at Gang Mine

6.4.4 Hard Rake 1

Hard Rake 1 species cover is greater to the north-west of the isopleth maps, in the same zone as peak elevations of available Cd and Pb [Figs. 6:41 to 6:43]. This area is located at the edge of a large hollow, which was the origin point of sample transects.

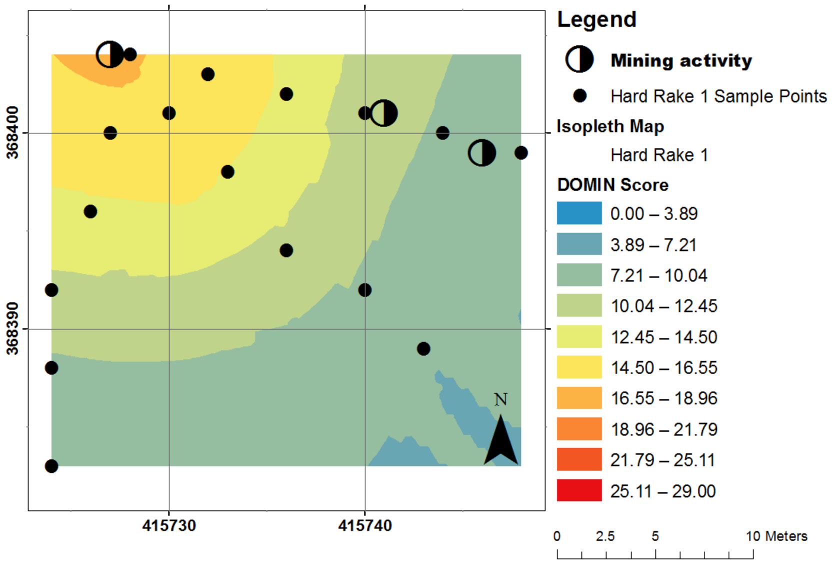


Figure 6:41. Distribution of species cover at Hard Rake 1

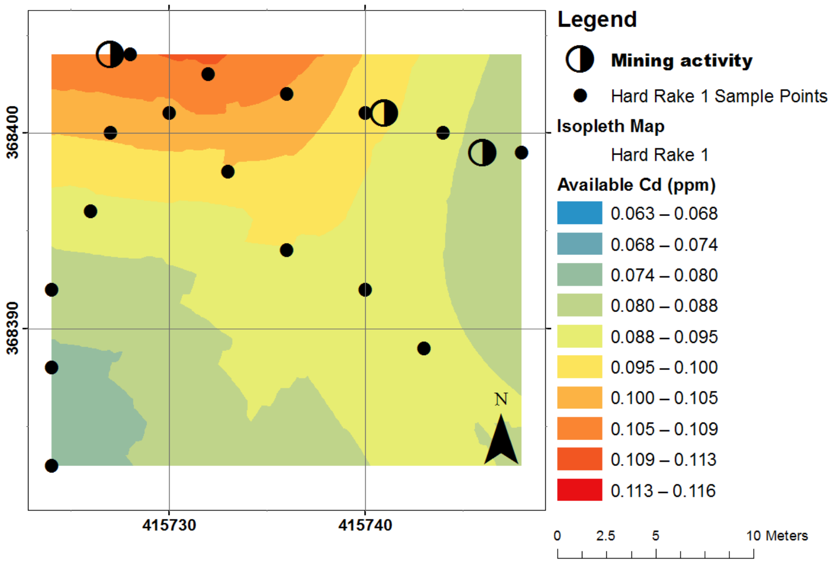


Figure 6:42. Spatial distribution of available Cd Hard Rake 1

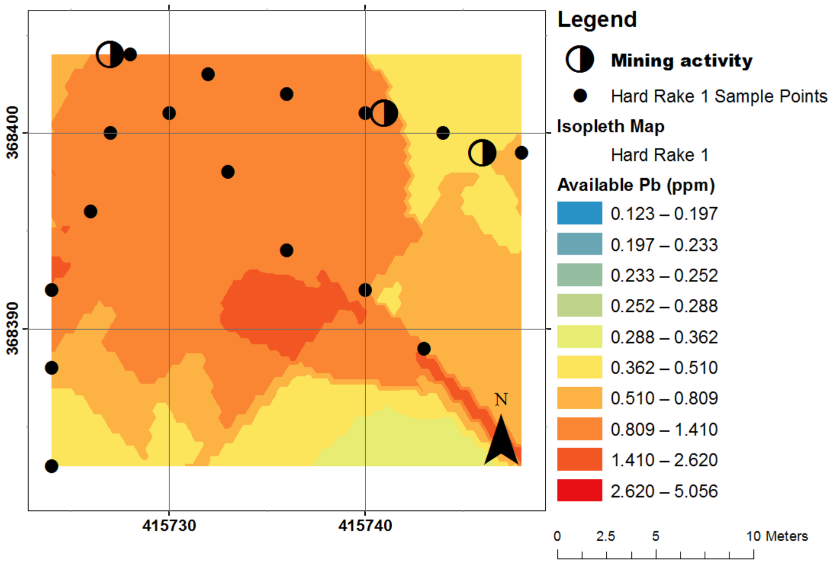


Figure 6:42. Spatial distribution of available Pb at Hard Rake 1

6.4.5 Hard Rake 2

Hard Rake 2 sample area is the only site that shows no significant patterns of distribution between vegetation and metal concentrations. The vegetation cover is greater to the north-east of the isopleth map in *Figure 6*:43, whereas available Pb and Cd are of lower concentrations in that same area [Figs. 6:44 & 6:45]. The origin point for soil and vegetation samples was at the edge of a bell pit, although the landscape around this origin point was extensively disturbed in relation to Hard Rake 1, and this may explain the negative patterns in vegetation and soil relationships here. The concentrations of heavy metals were also found to be extremely high here.

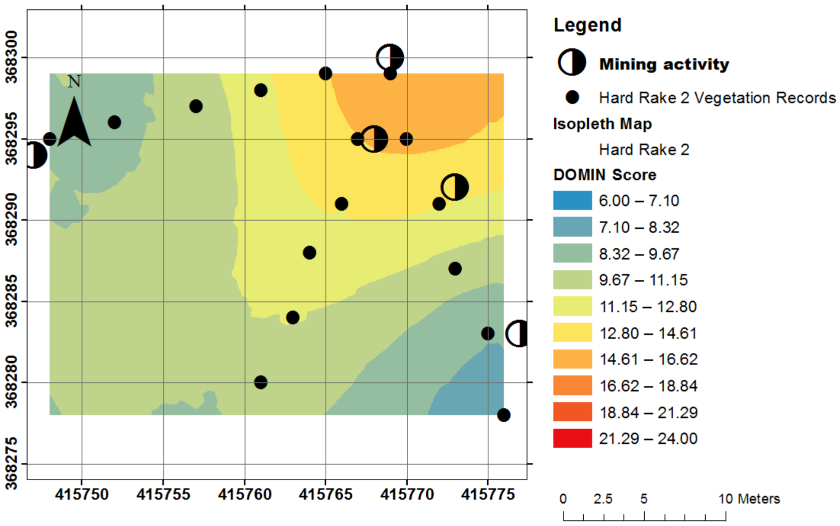


Figure 6:43. Distribution of species cover at Hard Rake 2

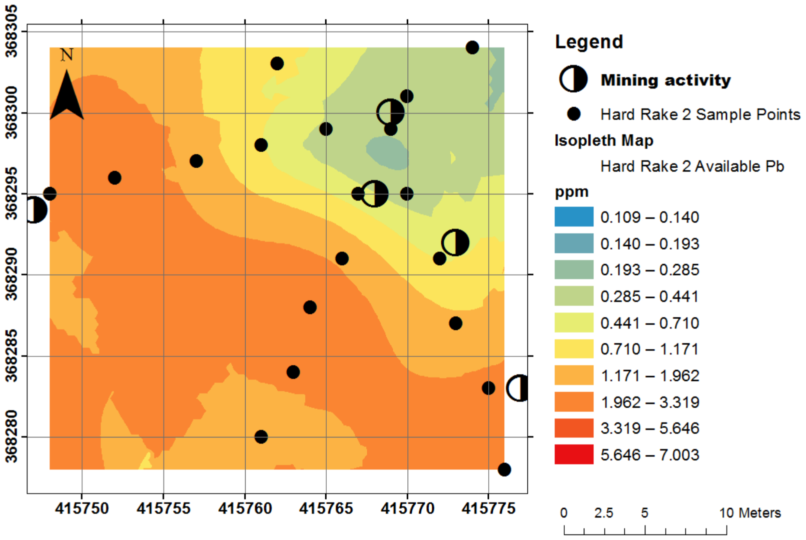


Figure 6:44. Spatial distribution of available Pb at Hard Rake 2

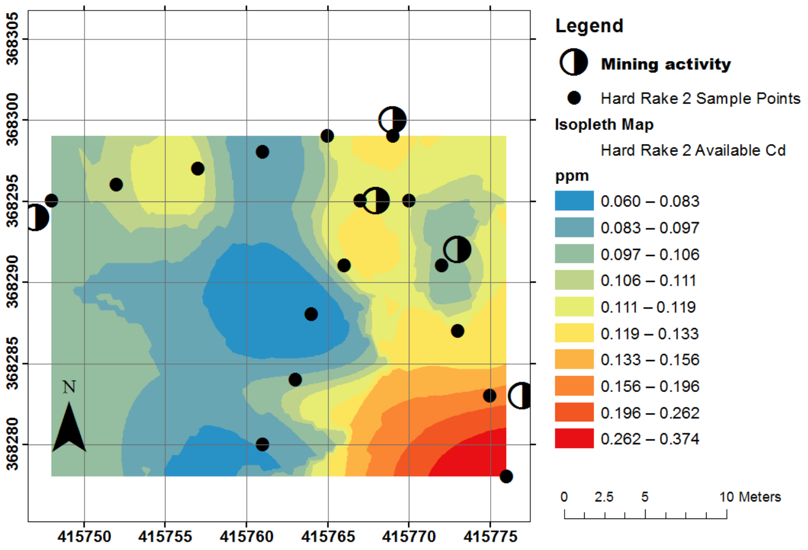


Figure 6:45. Spatial distribution of available Cd at Hard Rake 2

6.4.6 Hay Dale

Hay Dale contained some of the lowest metal concentrations of all sites; however, some relationship between the distribution of available Cd and total Pb can still be seen. The north and eastern areas of the isopleth maps [Figs. 6:46 to 6:48] illustrate higher species cover and available Cd and a slightly similar pattern for total Pb in the south-eastern areas of the map. The north and eastern area of the map was the area of the transect origin point, just a short distance from the calcite mine entrance.

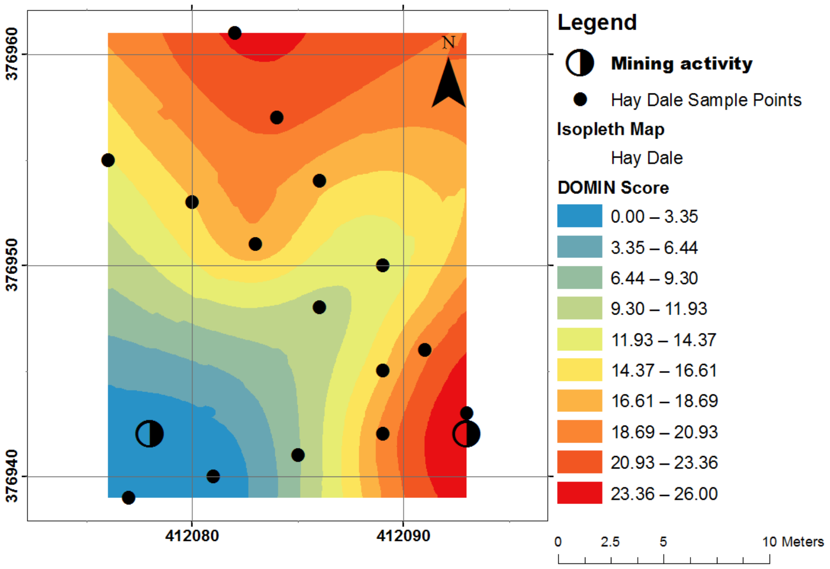


Figure 6:46. Distribution of species cover/abundance at Hay Dale

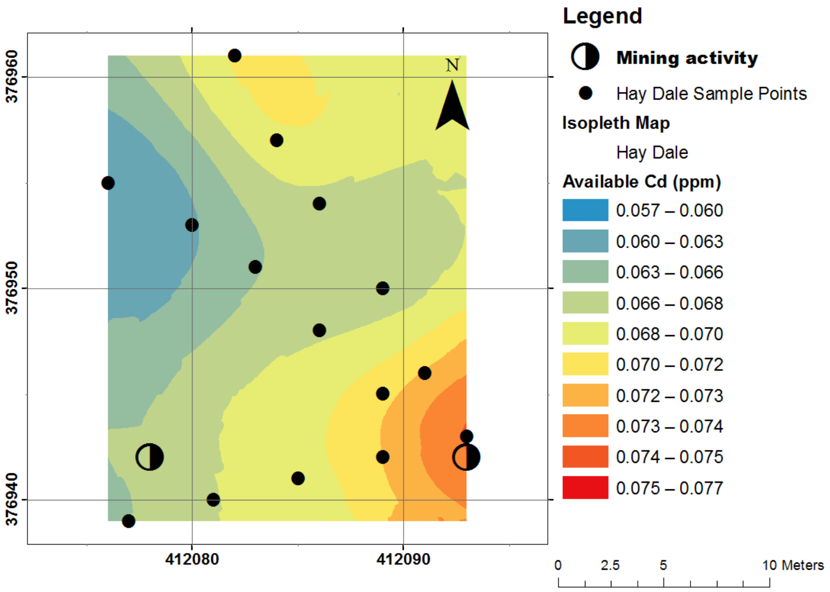


Figure 6:47. Spatial distribution of available Cd at Hay Dale

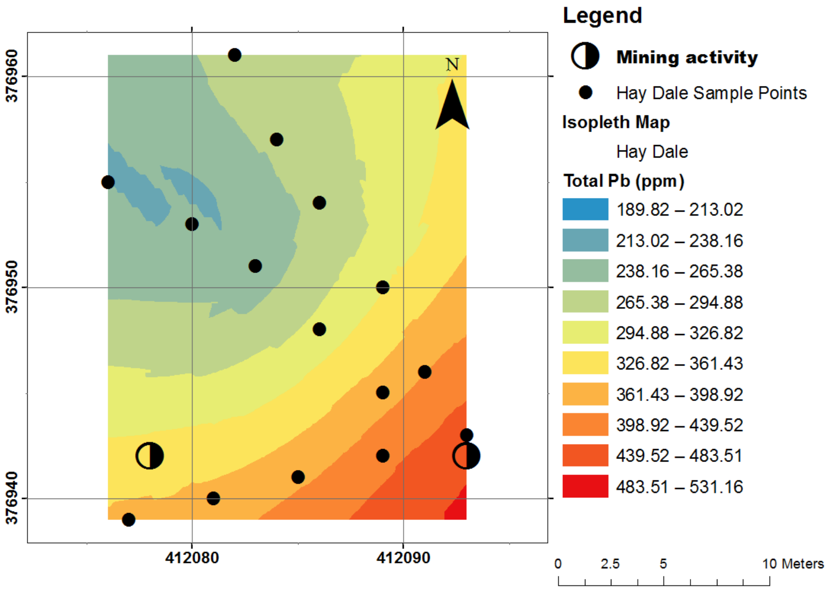


Figure 6:48. Spatial distribution of total Pb at Hay Dale

6.4.7 High Rake

Recent comprehensive excavations at High Rake (Barnatt, 2011; Barnatt & Penny, 2004) suggest that the vegetation community here is still being established. However, there is still a slight similarity between vegetation cover and available Cd at the site [Figs. 6:49 & 6:50]. The isopleth map for available Pb shows an opposite distribution pattern to vegetation, and available Pb is elevated close to the old chimney present on site (transect origin point) [*Fig.* 6:51]. At the time of survey, the soil substrate here was very granular, containing limestone gravel, and consequently displayed a very large particle size, very low organic matter content (mean 5.79%) and a high pH (mean 8.16) [see Chapter 3: Results]. However, SOM% increases with distance from the transect origin point [*Fig.* 6:52]. This could explain the lack of relationship between vegetation and metal distribution. Firstly SOM contains all of the nutrients considered essential for plant growth, suggesting increased vegetation cover (Bot & Benites, 2005) in comparison to the areas containing lower SOM. Additionally, when organic matter in soils decrease, contamination is likely to increase (Bot & Benites, 2005) as Pb tends to be strongly adsorbed to SOM especially at elevated pH levels and this would increase with additional SOM (Kerndorff & Schnitzer, 1980). Consequently, the isopleth maps display contrasting distribution between SOM% and species cover and available Pb concentrations.

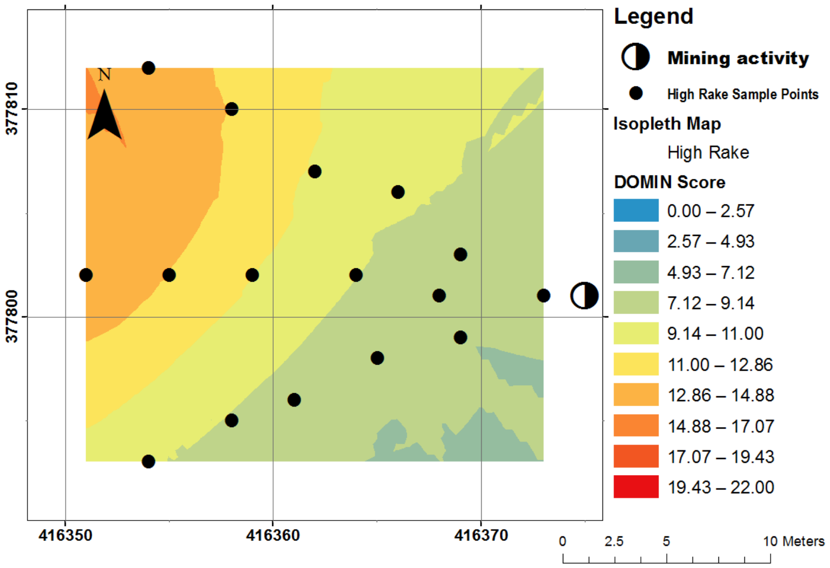


Figure 6:49. Distribution of species cover/abundance at High Rake

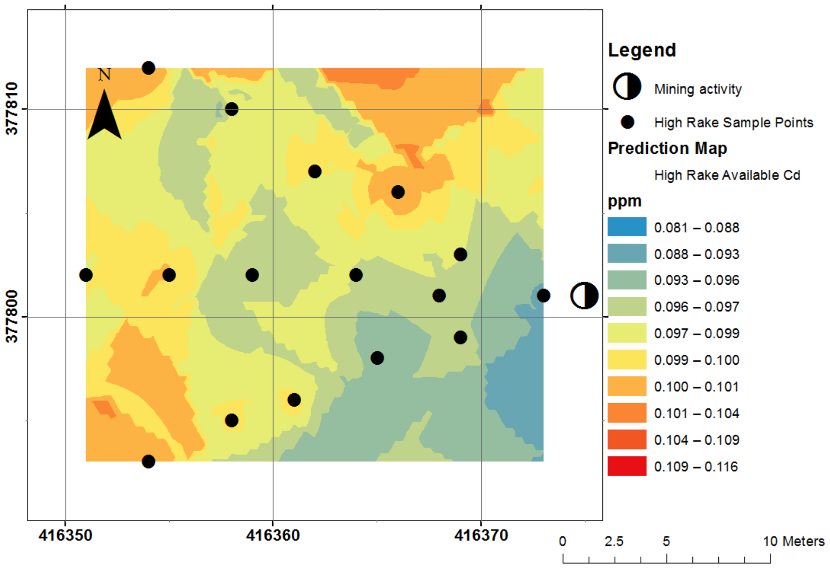


Figure 6:50. Spatial distribution of available Cd at High Rake

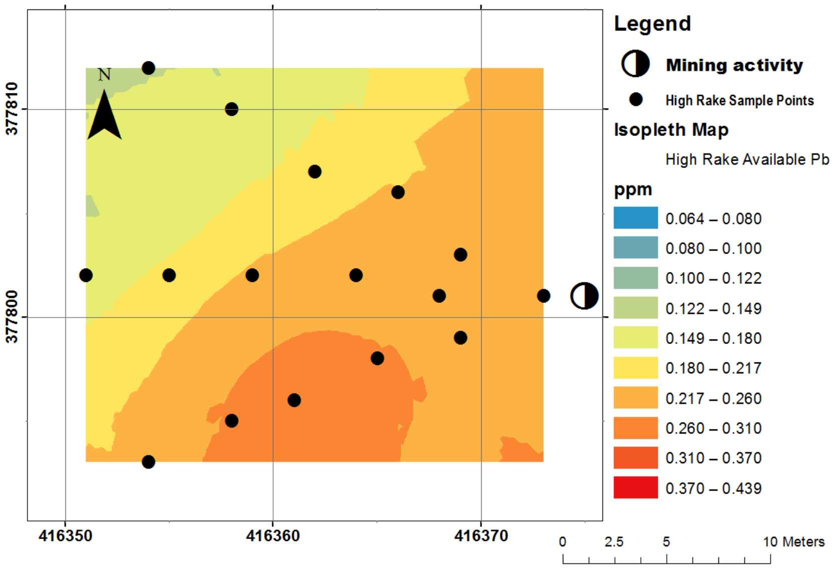


Figure 6:51. Spatial distribution of available Pb at High Rake

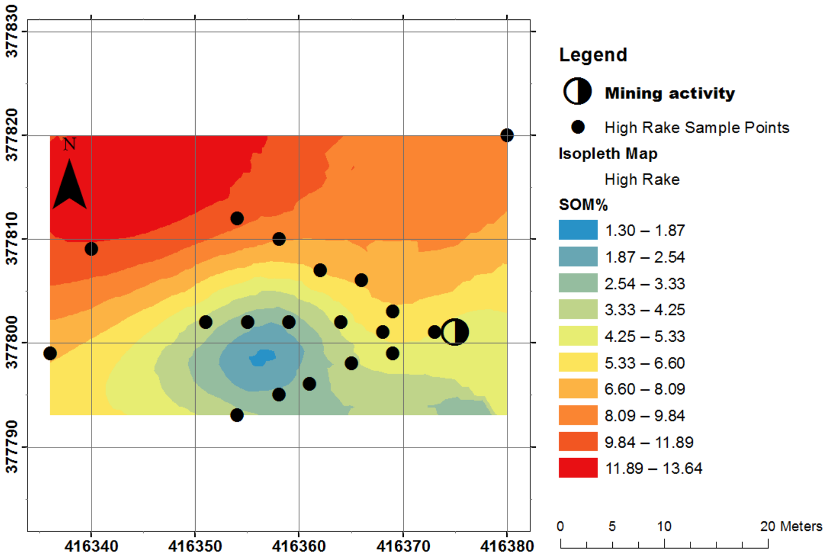


Figure 6:52. Spatial distribution of SOM% at High Rake (includes extra samples 17-19)

6.4.8 Tansley Dale

Tansley Dale showed similar patterns of distribution for species cover and total Pb concentration, and a slight similarity with total Cd concentrations too [Figs. 6:53 to 6:55]. The sample transect origin point next to a historic mining structure was to the north central area of the isopleth maps, and the area of maximum total Cd concentration is situated at the same location.

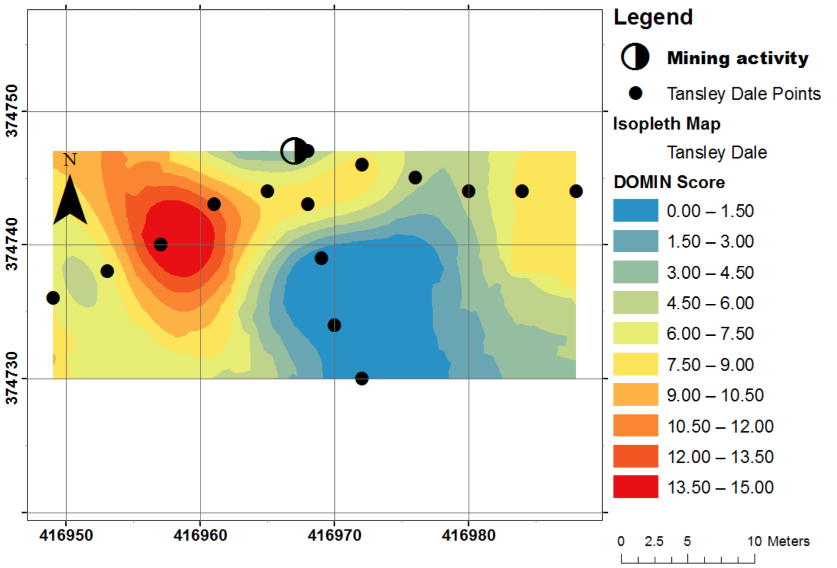


Figure 6:53. Tansley Dale species cover/abundance distribution

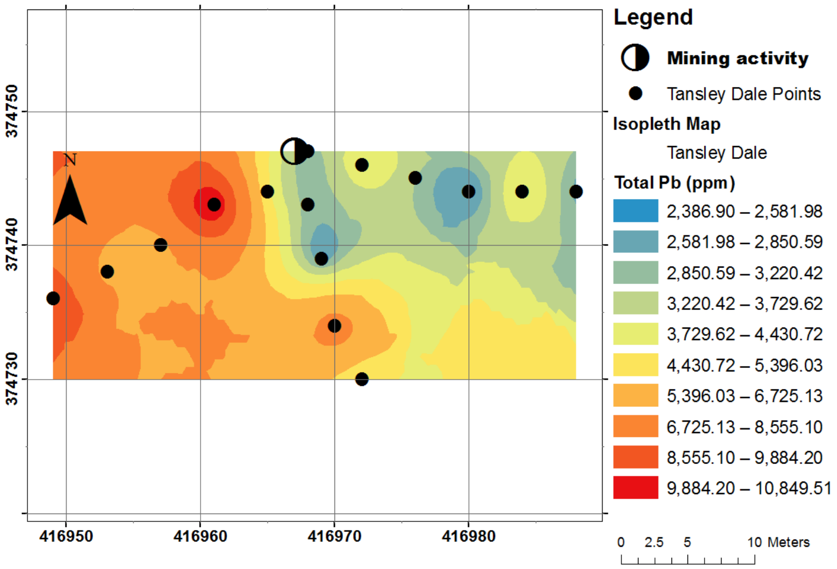


Figure 6:54. Spatial distribution of total Pb at Tansley Dale

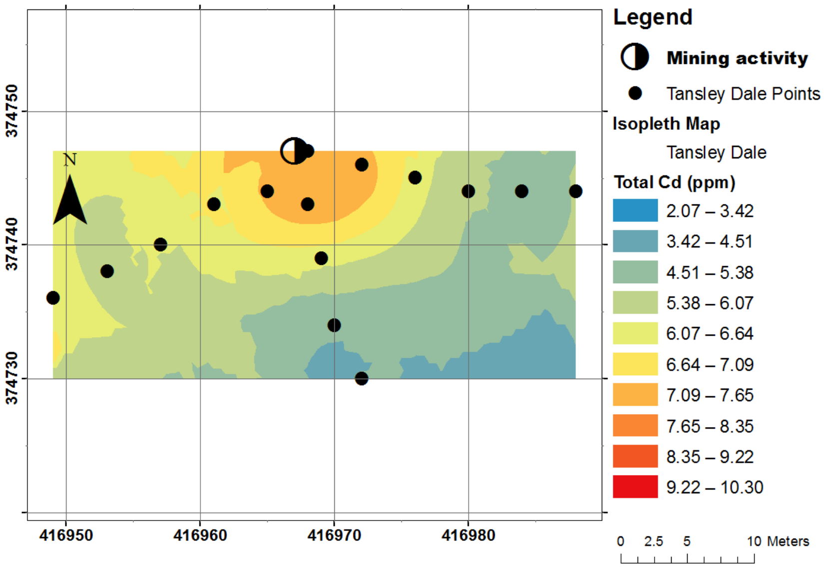


Figure 6:55. Spatial distribution of total Cd at Tansley Dale

The similarities in the distribution of vegetation and Pb, Zn and Cd may be explained by the presence of certain plant species with the ability to tolerate or accumulate certain heavy metals (Simon, 1978; Woch *et al.*, 2016). These plants will have undergone natural selection processes which have created an adapted genotype (Baker *et al.*, 2010), allowing them to exist in areas of high metal concentrations, with little competition from other plant families which do not have the genetic variability (Baker & Proctor, 1990) of the pseudo-metallophytes, accompanying species such as legumes or true metallophytes found at the study sites.

The interesting patterns of vegetation and Cd distribution, which were similar at six of the eight study sites, may also be explained by the high concentrations of Pb and other metals present. Alloway (1995) explains how if high levels of Pb and Cd are present in soils, Pb is preferentially absorbed by plants and that this reaction can leave more Cd in the soil solution. If this is the case, then it would explain the higher levels of Cd, especially available Cd, found at the areas of greater plant cover, especially considering the adaptations of the plant species here. He also states that excess levels of other heavy metals can also inhibit the uptake of Cd, and that areas situated on calcareous substrates that have undergone extensive mining activities have shown lower accumulated heavy metals in vegetation than other areas.

6.5 Summary

The results discussed here have indicated that the type and intensity of historic metalliferous ore extraction and processing have left varying degrees of contamination at the study areas, with sites located on extensively mined lead rakes having much higher concentrations of heavy metals. Also, the spatial distribution of several heavy metals at the study sites may be influenced by the modifications to the landscape or the presence of structures that are left behind from these historic mining activities, which show higher concentrations in close proximity to them (Alibrahim *et al.*, 2017; Kossoff *et al.*, 2016; Mills *et al.*, 2014). Younger (1997) states that these patterns of contamination distribution will change over time and that remediation may be necessary. Kossoff *et al.* (2016), however, argues that although hotspots of contamination may need remediation in some instances, the structures associated with them are important heritage assets. He goes on to state that the areas are also often populated by rich and diverse vegetation communities that contain rare metallophyte plant species, which is another reason for the conservation of these mining areas. This is also reflected in work by Barnatt and Penny (2004), Barnatt *et al.* (2013), Batty (2005) and others.

Close associations between total and available heavy metal concentrations were also identified using single extraction procedure and multivariate analysis. The heavy metal spatial distribution has also been shown here to affect other soil properties such as pH and K or vice versa, and that there is some relationship between the particle size and metal concentrations.

This research also found that the landscape and structures and the associated elevated metal content have influenced the type of vegetation communities present, with greater species cover in areas of elevated contamination (Penny, 2009), especially Pb, Zn and Cd contamination. CCA also showed that several individual species, shown to be metal-tolerant in the literature, were closely associated with these elevated heavy metals at the study areas (Baker *et al.*, 2010).

The assessment, characterisation, and monitoring of ecological and mineralogical aspects of historical mining areas is not only important for the measurement of the distribution and mobilisation of heavy metals (Kossoff *et al.*, 2016; Stefanowicz *et al.*, 2014; Younger, 1997), but also to improve knowledge of the important ecological aspects of these areas (Barnatt & Penny, 2004; Batty, 2005).

The results from this research have demonstrated that the use of geostatistical analysis utilising isopleth maps and multivariate analysis allows easy visualisation of the spatial distribution of abiotic and biotic variables within the same area.

Chapter 7: Conclusion

7.1 Introduction

This research concentrated on two main areas: the assessment of the distribution of metal/metalloids and their bioavailable fractions and the observation of vegetation distribution at the study areas, along with determining if these were associated in any way. This chapter discusses the main conclusions drawn from the analysis and synthesis of the research findings and includes limitations to this research and recommendations.

7.2 Research objective 1: Findings, conclusions and recommendations

Research objective one was to assess the soil characteristics and vegetation at sites of historical mining activity. The assessment of soil characteristics at the study sites presented neutral to basic soil pH levels, typical of the limestone geology on which these areas exist (Rodwell *et al.*, 2007), and soil organic matter (SOM%) content that was homogenous at five of the seven areas. High Rake and Dirtlow Rake frequently presented samples with lower SOM%, and this may be attributed to more recent ground disturbances at both sites (Barnatt, 2011; Waltham, 2006).

Particle size analysis was undertaken to explore if any association between heavy metal concentration and particle size existed. Results exhibited a slight positive correlation between particle size span and heavy metal concentration and a close association in PCA plots, although no direct conclusions can be drawn. Other research, however, found strong links between particle size and heavy metals (Abouelnasr 2010; Hao *et al.*, 2010; Li, Shi, *et al.*, 2015; Wang *et al.*, 1996), although none using the calculated span measurement.

Heavy metal content in soil samples from the study areas separated the sites into two groups; greatly elevated concentrations were present where documented signs of extensive mineral extraction were evident, at Dirtlow Rake (Waltham, 2006), Gang Mine (Baker *et al.*, 2010), Hard Rake (Ford, 2002) and High Rake (Barnatt, 2011). Maximum metal concentrations detected were at Dirtlow Rake (Zn 4,079ppm, Pb 34,504ppm), Gang Mine (Zn 140,226ppm, Pb 73,057ppm), Hard rake (Zn 10,859ppm, Pb 66,960ppm) and High Rake (Zn 4,323ppm, Pb 19,353ppm), whilst lower metal concentrations were evident at Deep Dale, Hay Dale and Tansley Dale, although sporadic hot-spots of Zn and Pb were present at Tansley Dale (Zn 8,125ppm, Pb 25,050ppm), particularly around the mining feature on site and the dale bottom. These differences in concentrations may be explained by the level of mining activity observed at the sites. Although some heavy metal concentrations at Deep Dale (Pb 989ppm) and Hay Dale (Pb 4724ppm) were slightly elevated, Deep Dale contained only what was perceived to be a test pit in the sample area, and Hay Dale was occupied by a little used calcite mine (Ford, 2002). Tansley Dale is an area that is rich in mining history along its tops, at the head of the dale and further down its length (Barnatt & Penny, 2004), and the sampling was conducted around a back-filled sough or adit. These elevated concentrations of heavy metals at the study areas, left behind from anthropogenic activities, were found not to have significantly negatively impacted vegetation communities, and vegetation surveys revealed many species which are typically found in calcareous grassland habitats (Streeter *et al.*, 2009). Several of these species were infrequent and confined to a single study area; however, others were widespread, appearing at most sites, including the legumes, common birds-foot trefoil (*Lotus corniculatus*) and red clover (*Trifolium pratense)*. One species was restricted to the most contaminated areas at Gang Mine and Hard Rake and this was discovered to be the metallophyte species mountain pansy, *viola lutea*, also known as zinc violet. Incidentally, this species was found at the areas with the greatest heavy metal contamination.

These findings demonstrate the elevated heavy metal content at the study areas and may inform land owners of contamination and particle size, especially for those areas used as grazing pastures; this would be useful in light of the research indicating the toxicity problems in livestock associated with Pb and fluorine ingestion at areas of historic mining activity (Abrahams & Blackwell, 2013; Geeson *et al.*, 1998; Smith *et al.*, 2010). Further research to determine fluorine levels would be needed, particularly in those areas reworked for gangue materials.

7.3 Research objective 2 and 4: Findings, conclusions and recommendations

Objective two and four aimed to analyse the relationship between the measured soil properties and the vegetation species recorded at the study sites, and investigate how the spatial distribution of heavy metals influenced vegetative structure. Whilst the geographical location, the physical and chemical soil properties and other environmental factors undoubtedly play an important role in influencing the vegetation communities that exist in these areas (Jefferson *et al.*, 2014), this research discovered close associations between several species and the heavy metals Pb, Zn, Cd and arsenic. The use of canonical correspondence analysis (CCA) indicated that the plant families Violaceae, Fabaceae and Ranunculaceae, which contain the plant species red clover, white clover, birds-foot trefoil, meadow buttercup and mountain pansy, have positive associations with heavy metal concentrations. Both CCA and cluster analysis emphasised the relationships between the plant families. Cluster analysis grouped the heavy metal associated vegetation together on a dendrogram, suggesting taxonomic similarities (Dytham, 2011), whilst CCA indicated a close relationship with the heavy metals Pb, Zn, Cd and As. These associations were evident with both total and available metal concentrations. Additionally, some similarities in the spatial distribution of both heavy metals and plant families were illustrated with the use of geospatial analysis. Kriging prediction isopleth maps not only demonstrated the mobility and distribution of heavy metals in relation to the topography and other features present at the study sites, but also revealed similar patterns of distribution of some heavy metals (Pb, Zn and Cd) and plant species, indicative of a relationship between them. These plant families and species were revealed in the literature to be metallophytes and pseudo-metallophytes which have evolved to be metal tolerant species, able to populate metalliferous soils (Baker & Proctor, 1990; Batty, 2005; Penny, 2009). Other species which exhibited less association with the heavy metals present were also shown to be less able to genetically adapt to some of the conditions present (Baker & Proctor, 1990).

Furthermore, the heavy metal concentrations were shown to be substantially increased close to areas containing landscape modifications and other mining structures, suggesting that the species demonstrating more metal tolerance were more abundant in close proximity to them. Further research would be needed to verify this deduction. The methods employed proved to be effective techniques for allowing relatively quick and easy visualisation of the relationships and spatial distribution of abiotic and biotic variables within the same area and the assessment of areas of heavy metal pollution.

7.4 Research objective 3: Findings, conclusions and recommendations

Objective three sought to determine the bioavailable fraction of heavy metals found in soils at the study areas. It is the bioavailable fraction of heavy metals that can influence vegetation both negatively by toxicity and positively as essential nutrients (Chojnacka *et al.*, 2005). A simple single extraction procedure was employed to extract the freely available fraction of heavy metals. The available fraction of heavy metals corresponded to the concentration of total metals, with the sites containing higher total metal concentrations also exhibiting higher available fractions. The bioavailable heavy metal fractions also displayed mostly similar distribution patterns to those of total heavy metals and were shown to be influenced by pH, SOM and the presence of other metals, especially evident with available Pb, Cd and Zn. These results were easily visualised with the use of isopleth maps.

These findings may inform decision makers of the potential mobility of available metals in these areas through leaching and transport of dust particles, particularly in relation to Pb and Cd (Gutierrez *et al.*, 2016). This point also highlights the importance of the metallophyte and pseudo-metallophyte species recorded at the study sites, as these species can play a role in the natural phytoremediation of the mining areas (Bradshaw, 1997).

7.5 Research objective 5: Findings, conclusions and recommendations

Objective five intended to generate an understanding of any issues concerning the distribution of any soil contamination that may cause harm to or decline in flora and fauna at the study sites and to suggest any remediation procedures. Several of the study areas displayed heavy metal concentrations far exceeding those from uncontaminated soils (Ross *et al.*, 2007). The availability of these metals is greatly influenced by many environmental factors and geochemical processes, including the increased buffering capacity, elevated pH and the cation exchange capacity and adsorption capability of the organic and mineral content in the soils (Dubbin, 2001). These abiotic factors and the identification of several plant species at the study sites that have evolved ecotypes able to tolerate the metalliferous conditions, demonstrate that the heavy metal contamination observed caused no significant harm or decline in species present at the study areas. On the contrary, the metalliferous conditions found at the study sites provide a niche habitat capable of supporting well-established, species rich vegetation communities, including some rare and threatened plant species (Batty, 2005; Penny, 2009). Furthermore, the landscape modifications and structures left behind after the cessation of mining activities have been shown to influence not only the distribution of contaminants at the study areas, but also the distribution of vegetation cover/abundance. These patterns of vegetation distribution may partly be due to the abundance of legume species across the sites that were shown to be metal tolerant by their close associations with heavy metals when analysed with CCA. The nitrogen fixing capabilities of these metal tolerant species may also have improved nutrient deficient soil conditions, increasing the opportunities for other species to adapt and become established in the metal-rich areas (Bradshaw, 1997; Hernandez & Pastor, 2008). With respect to the suggestion of remediation processes mentioned in objective five, the associations between the vegetation, heavy metals, topography and structures revealed whilst conducting this research, would suggest that these areas of historic mining activity should be the focus of conservation rather than remediation. This is also the view of several others including (Baker *et al.*, 2010), Barnatt and Penny (2004), Barnatt *et al.* (2013), Penny (2009) and Batty (2005). Furthermore, the results of this research have demonstrated that geostatistical analytic tools such as kriging and the use of multivariate analysis can support the characterisation, assessment and monitoring of the ecological and mineralogical features present in areas of historical mining (Ersoy, Yunsel, & Cetin, 2004). These tools also allow the visualisation of the spatial distribution of abiotic and biotic variables within the same area. Further recommendations for conservation or remediation are discussed in the following section (7.6), along with recommendations for future research (7.7).

7.6 Recommendations for conservation and remediation

The Peak District naturally contains areas of high background metal soil concentrations, in particular Pb and Zn (Kossoff *et al.*, 2016). In terms of the overall contaminant load, however, historical metal mining wastes can significantly increase background soil concentrations to levels which can often exceed regulatory limits (e.g. Hard Rake and Gang Mine) (Kossoff *et al.*, 2016). However, mining in the Peak District has a lengthy history, maybe as far back as late prehistory on the basis of evidence in other areas in the Peak District (Barnatt *et al.*, 2013; Barnatt & Penny, 2004). Moreover, these remnants of the rich history of lead mining in the Peak District not only provide important heritage sites, but also support for interesting and rare assemblages of plants and animals. The Lead Rakes Project has revealed further sites where metallophytes exist, and this is increasing the understanding of these rare plants and the metal rich grassland on which their communities thrive (Penny, 2009). The Lead Rakes Project has enabled the prioritisation of some sites for conservation efforts, though many remain without (Barnatt & Penny, 2004). Whilst it is recommended here that these important sites be the target of further conservation efforts, such as through designation of scheduled monument (i.e. High Rake), SSSI (Cressbrook Dale, Gang Mine, Deep Dale), or agri-environment schemes, which provide funding to farmers and other land managers in return for managing the environmental aspects of their land (Hard Rake), it is also recommended that regular monitoring of these sites be undertaken.

The extremely high concentrations of both total and available heavy metals discovered during this research should be noted (Gang Mine and Hard Rake in particular), as there could be future risk of mobilisation of these metals, especially with the on-set of climate change and predicted extreme weather events (IPCC, 2007; Kossoff *et al.*, 2016). Extreme flood events and drought could mobilise these contaminants, either through leaching or as airborne particles. Consequently, as there will undoubtedly be remobilisation of contaminants in the future, regular sampling and analysis should be undertaken to accurately assess the risk of heavy metal contamination and mobilisation, into surrounding areas and up the food chain via livestock. In addition, the likelihood of future mobilisation of heavy metal contaminants should be established. Should an unacceptable risk of contamination be found, the various costs and benefits of taking action should be stringently taken into account with a particular focus on the local circumstances, especially with regard to the many values these places have to offer. This is in line with the

Environmental Protection Act 1990: Part 2A Contaminated Land Statutory Guidance (Defra, 2012).

It is further recommended that grazing on these sites be limited to smaller livestock, as larger cattle can not only cause damage to the remnants of historic mining, but they also have a destructive method of consuming vegetation which can expose ore and gangue materials, cause surface erosion and expose them to poisoning through the ingestion of mining spoil.

7.7 Future research

The results presented in this thesis, from studies performed in the field and in the laboratory, have added to the understanding of the distribution of total and available heavy metals at contaminated historical mining sites in the Peak District. However, more research is needed to provide a more comprehensive knowledge and understanding of these and their interactions with biotic organisms. Therefore, a number of possible ideas for further research are recommended.

Future research should provide more thorough vegetation surveys and identification of all species within the study area, rather than in the immediate vicinity of mining activity. Furthermore, research could also benefit from plant tissue (roots, shoots and leaves) analysis to examine uptake and accumulation of heavy metals in these areas, to give a more accurate representation of the relationships between the distribution of heavy metals and vegetation species richness and diversity.

Concerning soil sampling and analysis, further research investigating heavy metal distribution across the whole of a site would provide invaluable data. This could perhaps be achieved with a case study of one of the sites researched here; Hard Rake and Gang Mine would provide a fascinating study. Samples taken from a 5m grid of sample points, for instance, would provide a much more comprehensive overview of the distribution of contaminants in a mining area, especially analysed with a more rigorous spatial correlation method than the kriging method used here.

Analytically, another interesting area of research would see a sequential extraction procedure undertaken to extract specific metals bound to certain exchange sites or associated with the organic or mineral phases of soil samples. This would not only provide invaluable information for stakeholders, but also go some way towards interpreting the geochemical processes underway at sites such as Hard Rake, especially if a more comprehensive particle size analysis accompanied it.

7.8 Final thought and critical reflections

The contamination resulting from historic mining activities does not inevitably mean that areas containing elevated heavy metal concentrations will be ecologically poor; quite the reverse, as many historical mining sites are a valuable habitat for some rare and important plant species, some of which depend on the metal-rich conditions that would be considered toxic to other more widespread species (Batty, 2005). These areas of historic mining in the Peak District, and the specialised vegetation communities that occur on them, would likely not exist if not for the mineral extraction processes and land disturbances that were carried out there in the past (Baker *et al.*, 2010). Additionally, the introduction of stringent environmental legislation, together with the implementation of more efficient and cleaner mining technologies, means that the future creation of heavily disturbed and contaminated landscapes like those present in the study areas has become an unlikely prospect (Hilson, 2000). This, coupled with the losses and further threats to historic mining areas by way of improvement for agricultural and recreational purposes, for instance (Penny, 2009), means that these metalliferous habitats containing species rich vegetation communities (including nationally rare metallophyte species) should have protective measures and management regimes put in place to ensure their future survival (Barnatt *et al.*, 2013).

7.8.1 Critical reflections

This research project was undertaken to investigate the relationships between spatial distribution of total and available heavy metals and other abiotic variables in relation to the distribution of vegetation species at seven areas of historical mining activity in the Southern Peak District. To accomplish these aims, several processes were involved. The following text provides a critical reflection on these processes, their design, implementation and hindsight on completion.

Firstly, the sampling design; the aim here was to carry out both soil sampling and vegetation surveys at each site. The sample design chosen was initially selected to provide a range of samples emanating away from sources of mining activity, to provide an indication of contamination concentrations both close to, and further away from, the perceived mining activity. This choice of sampling design was chosen without prior knowledge of the site topography and historical structures present at the selected study sites (other than aerial images). In hindsight, a preliminary site survey should have been undertaken in order to better aid in this sampling design. Several of the sites produced unforeseen obstacles and challenges when sample collection was underway. These included unsafe or restricted structures preventing sample collection from the preferred areas close to activity, and steep topography and geological features that prevented sampling within the strict parameters of the sampling design, especially in the steep sided dales. Furthermore, a more comprehensive coverage of sample points, in all directions, rather than emanating in one direction from source, would have provided a better overview of the distribution of soil variables measured at each site. Vegetation surveying presented no barriers, except for some difficulty with the identification of species.

Secondly, laboratory analysis of soil samples proved somewhat difficult at times; lack of available equipment and breakdown of apparatus caused some setbacks, and certain procedures required more than one individual to undertake i.e. loss on ignition (to assess soil organic matter content), which required further planning. Further reading and training on some of the procedures undertaken in the laboratory would also have provided an improved overview of other techniques available (and more suitable in some cases) and an understanding of any shortcomings and limitations of chosen methods, as well as a better basis for understanding any uncertainties and biases in the resulting data. It is also important to make notes on the technical specifications and operating guidelines of apparatus used. This would have saved time and provided a much more comprehensive methodology, enabling further research to be undertaken to the same specifications. This research would also have benefitted from the use of a sequential extraction procedure and a more thorough particle size analysis, had time been permitting.

The same applies to the analysis of the data collected; further reading around alternative data analysis methods could have provided an overview of more suitable and rigorous methods which would provide a more comprehensive understanding of the data collected and allow a superior presentation of results. Further training on some of the software utilised here, and an understanding of the processes involved (rather than relying on the software to do all the work), would have proved invaluable for the improved presentation and interpretation of results. Some further research involving the correct presentation of maps, tables, graphs and images would also have proved helpful. It is also important not just to rely on advice and comments from peers, but to ensure a thorough personal understanding of methods, concepts and all other aspects of the research at hand, before interpreting and incorporating their input and thoughts into your own thoroughly researched ideas and final outputs.

The literature review included in this study provides an overview of the study area, basic soil principles, mineral extraction processes and their interaction with the environment, the common heavy metals and elements found in the study area and the habitats that exist alongside them. On reflection, further research into surrounding areas of expertise, such as the geology and the geochemical processes at play in the study areas, would have proved invaluable, especially in the reporting of results and the discussion of perceived interactions between biotic and abiotic variables. It should not be considered that the variables measured here have direct two-way interactions with each other, rather that processes that are far more complicated are involved in these complex relationships. More consideration of the parent materials, ores, gangue materials and mine tailings present throughout the study areas would have permitted a superior understanding of the behaviour of heavy metals at the study areas.

In conclusion, this study has proved to be an enjoyable challenge, further enhancing my knowledge and leading to an understanding of research that will enable me to progress in my academic career with more vigour and allow me to analyse, synthesise and interpret subjects and themes far more comprehensively in the future.

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Appendices

Appendix A-Isopleth prediction maps pH

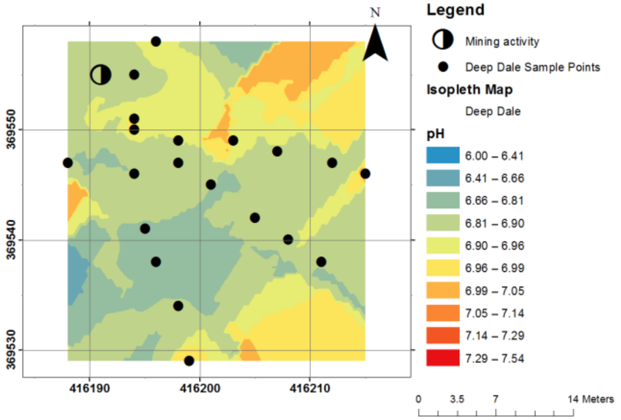

Figure A:1. Deep Dale pH spatial distribution

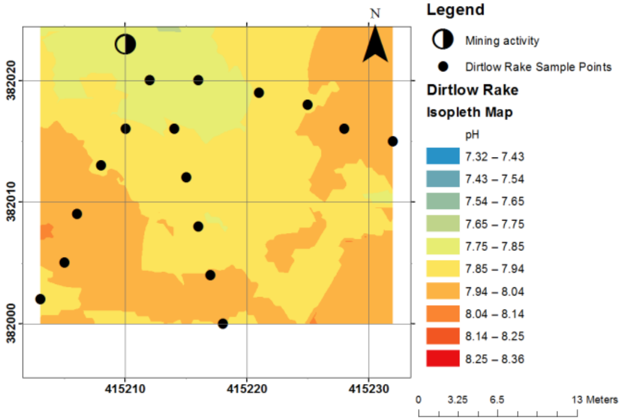

Figure A:2. Dirtlow Rake pH spatial distribution

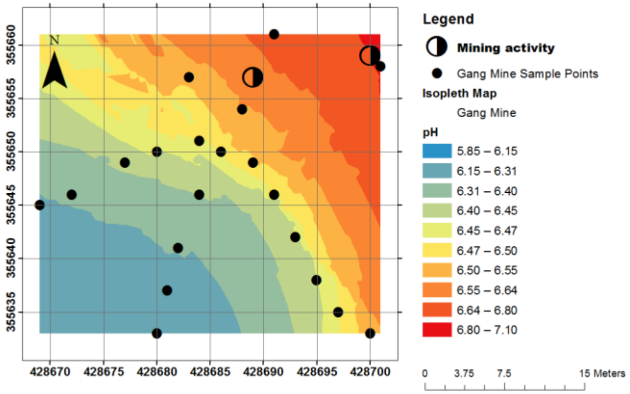

Figure A:3. Gang Mine pH spatial distribution

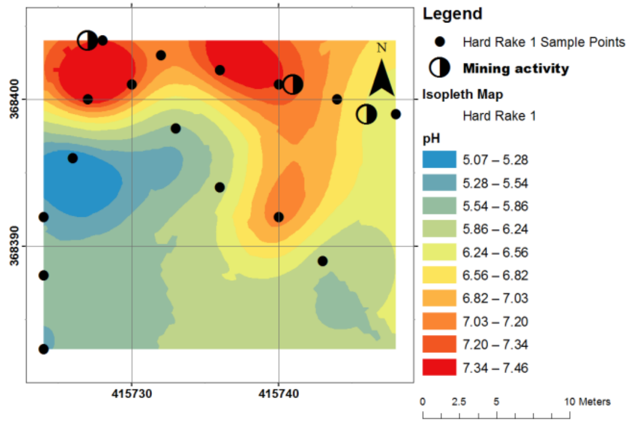

Figure A:4. Hard Rake 1 pH spatial distribution

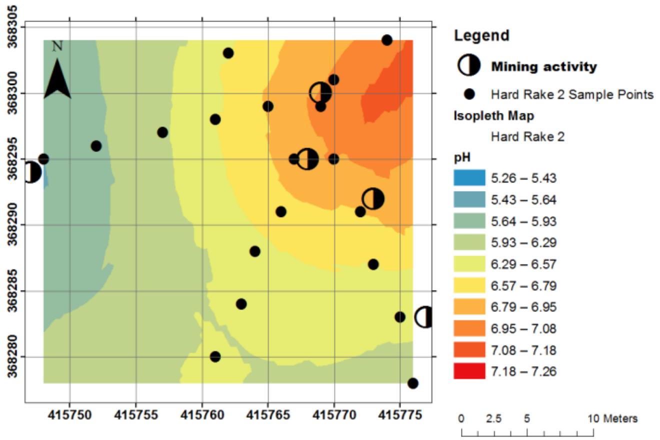

Figure A:5. Hard Rake 2 pH spatial distribution

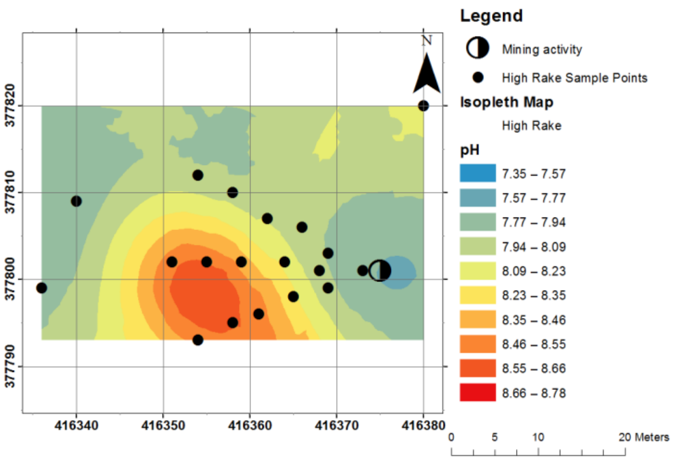

Figure A:7. High Rake pH spatial distribution

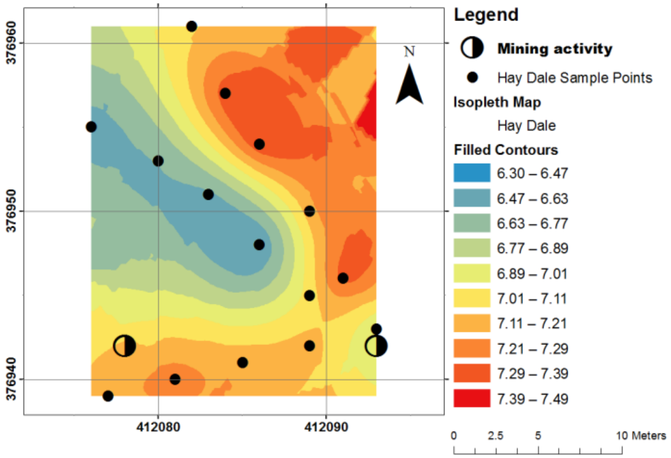

Figure A:6. Hay Dale pH spatial distribution

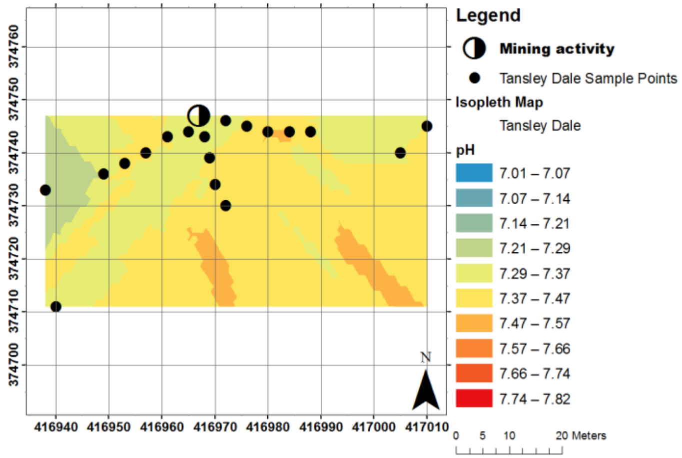

Figure A:8. Tansley Dale pH spatial distribution

Appendix B-Isopleth prediction maps SOM %

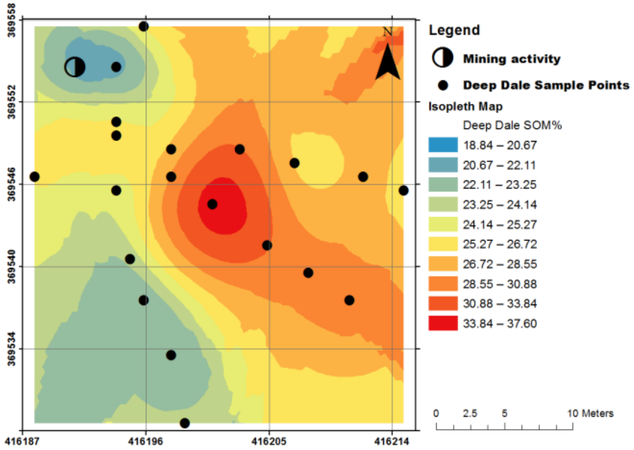

Figure B:1. SOM% spatial distribution at Deep Dale

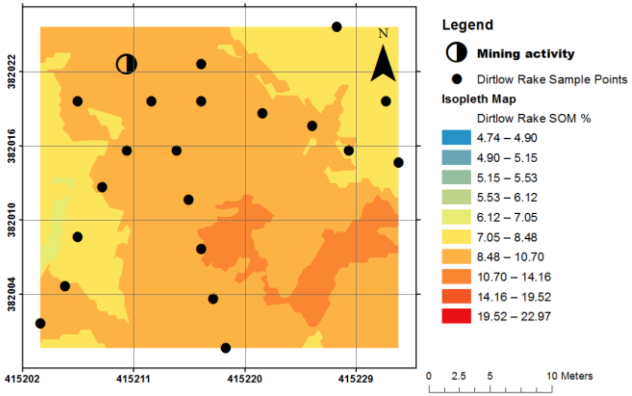

Figure B:2. SOM% spatial distribution at Dirtlow Rake

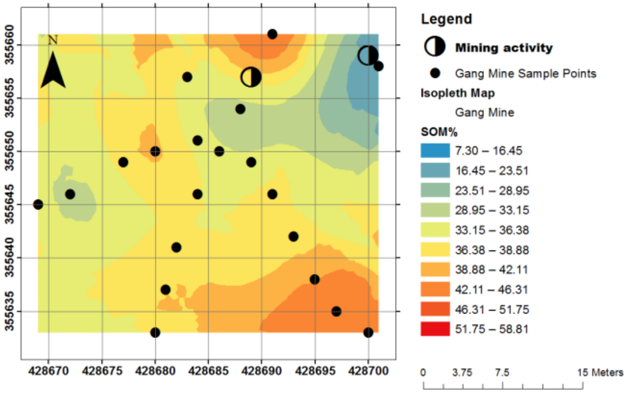

Figure B:3. SOM% spatial distribution at Gang Mine

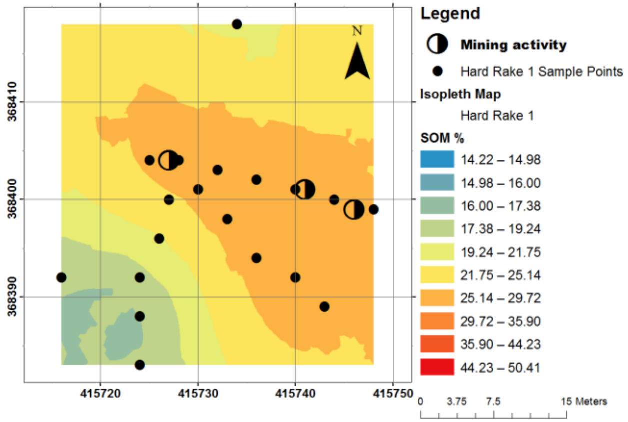

Figure B:4. SOM% spatial distribution at Hard Rake 1

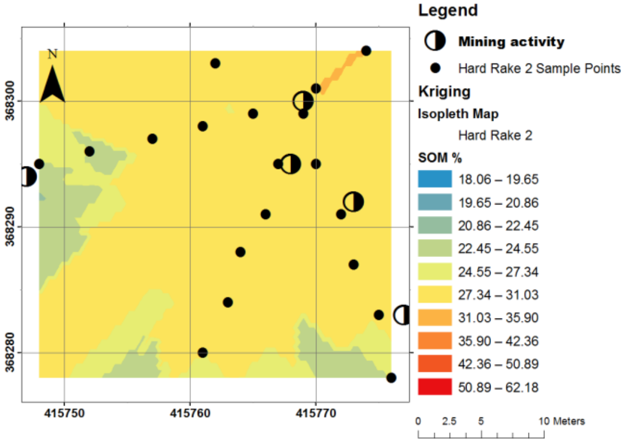

Figure B:5. SOM% spatial distribution at Hard Rake 2

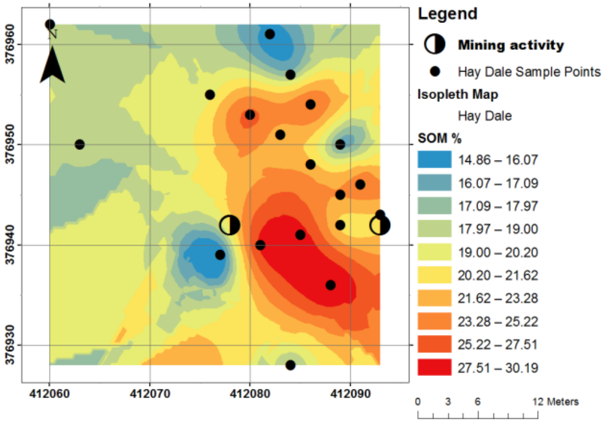

Figure B:6. SOM% spatial distribution at Hay Dale

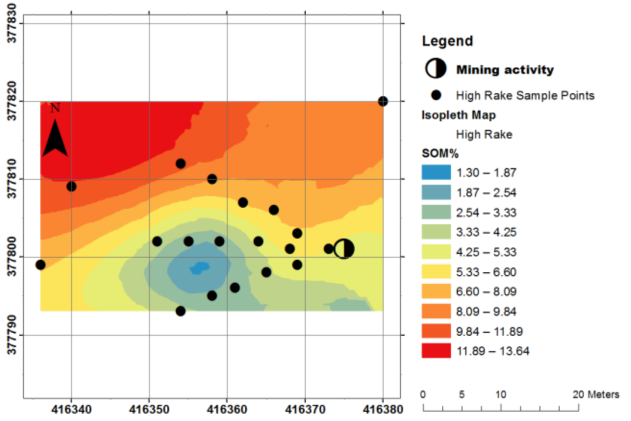

Figure B:7. SOM% spatial distribution at High Rake

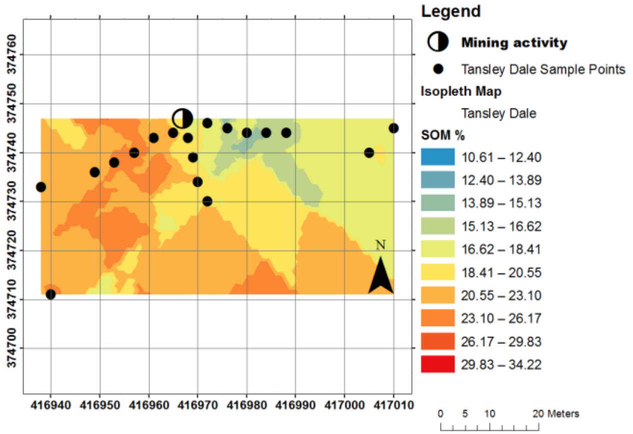

Figure B:8. SOM% spatial distribution at Tansley Dale

Appendix C-Isopleth prediction maps: Deep Dale

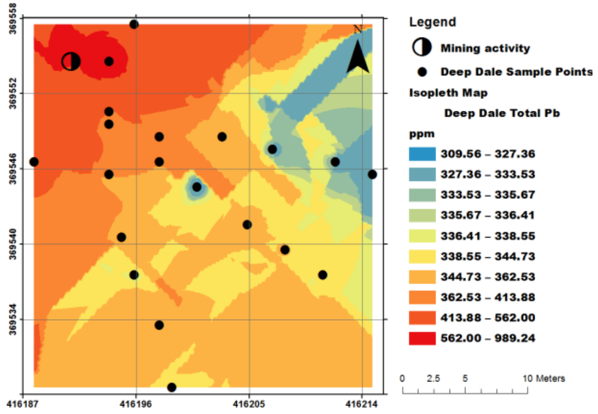

Figure C:1. Isopleth map of total Pb at Deep Dale

Total Pb recordings at Deep Dale display greater concentrations at the lower elevation sample points on the dale bottom (NW). The dale side shows lower concentrations of Pb.

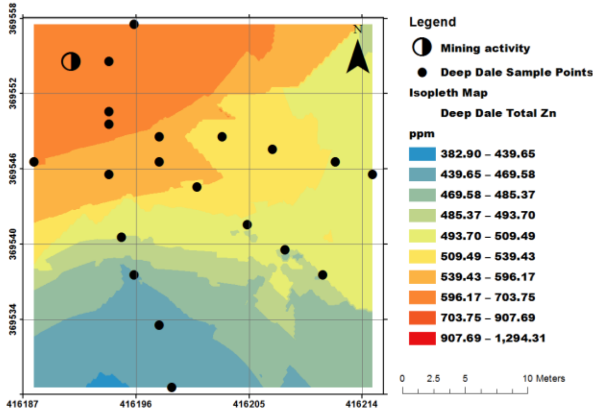

Figure C:2. Isopleth map of total Zn at Deep Dale

Like Pb distribution, Zn is also most elevated along the dale bottom (NE), and concentrations diminish up the steeper dale side.

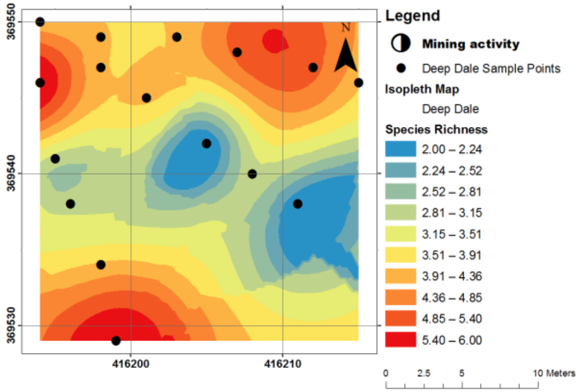

Figure C:3. Isopleth map of species richness at Deep Dale

Species richness at Deep Dale shows similar distribution to species abundance, except for a cluster of species richness further up the dale-side (S).

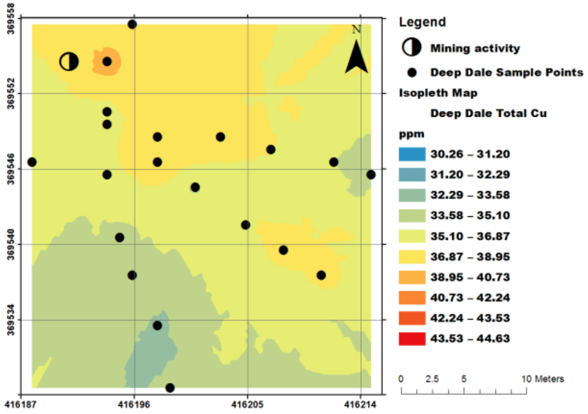

Figure C:4. Isopleth map of total Cu at Deep Dale

Cu concentrations are comparatively low compared to other heavy metals at Deep Dale, there is only a slight increase in concentration on the dale floor.

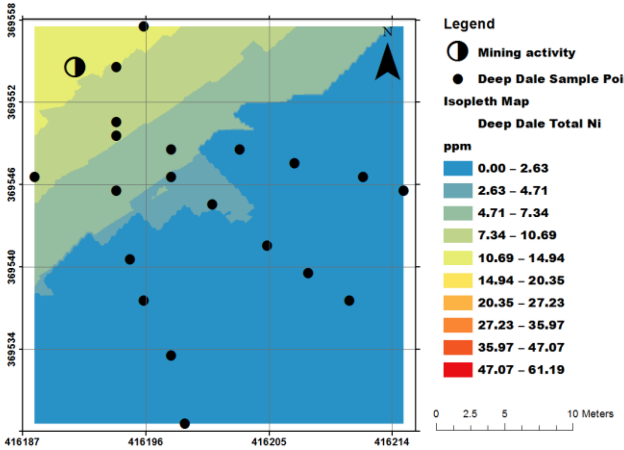

Figure C:5. Isopleth map of total Ni at Deep Dale

Ni at Deep Dale displays a distinct graduation from very low concentrations higher up the dale-side to higher levels on the dale floor (NW).

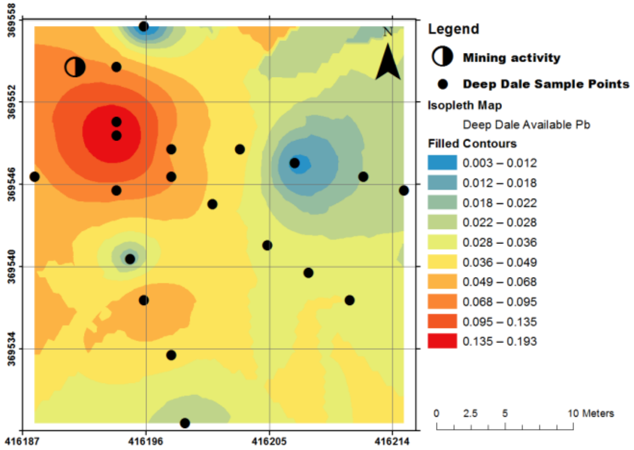

Figure C:6. Isopleth map of available Pb at Deep Dale

Available Pb concentrations display a similar pattern to that of total Pb, with higher levels on the dale floor and lower amounts up the dale-side.

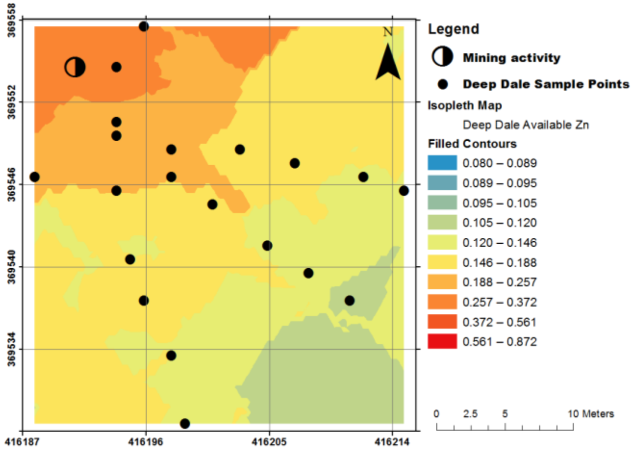

Figure C:7. Isopleth map of available Zn at Deep Dale

Available Zn concentration at Deep Dale is evidently higher at the dale bottom (NW) and significantly lower higher up the dale-side.

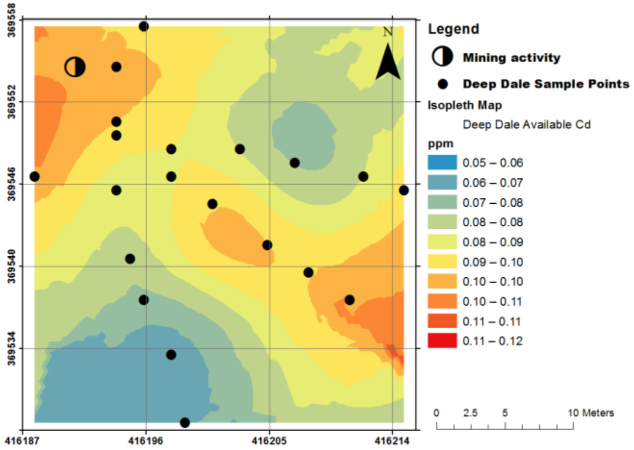

Figure C:8. Isopleth map of available Cd at Deep Dale

Available Cd concentrations are elevated towards the dale floor and to further up the dale-side.

Appendix D-Isopleth prediction maps: Dirtlow Rake

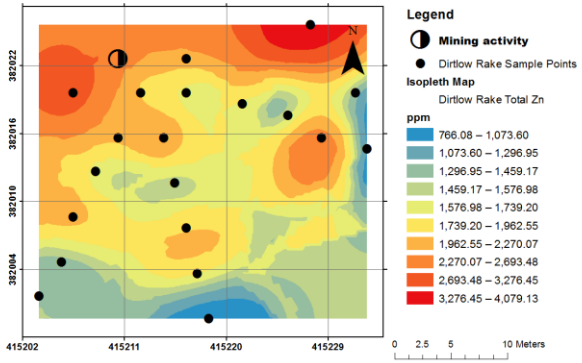

Figure D:1. Isopleth map of total Zn at Dirtlow Rake

Greater concentrations of Zn are evident along the north edge of the sampling area, close to the capped mine shaft and excavated gully present at Dirtlow Rake.

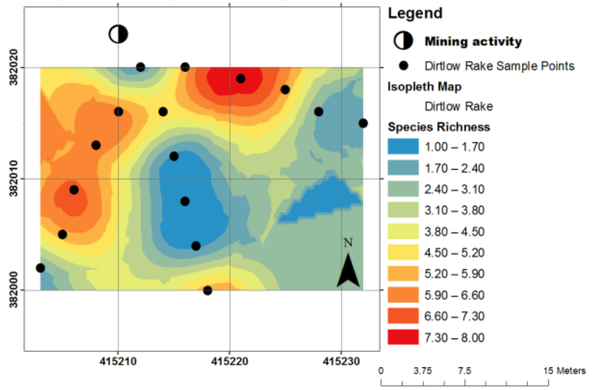

Figure D:2. Isopleth map of species richness at Dirtlow Rake

Species richness at Dirtlow Rake reveals a very similar distribution to both species abundance and heavy metals Pb and Zn.

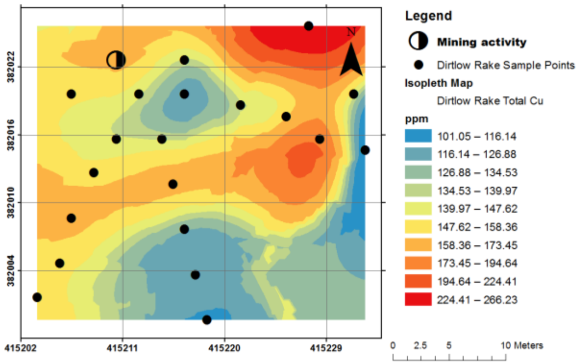

Figure D:3. Isopleth map of total Cu at Dirtlow Rake

Dirtlow Rake displays higher concentrations of Cu at the north-eastern extremities of the sampling area. This is along the edge of the previously mined open pit and slightly downhill from the mine shaft.

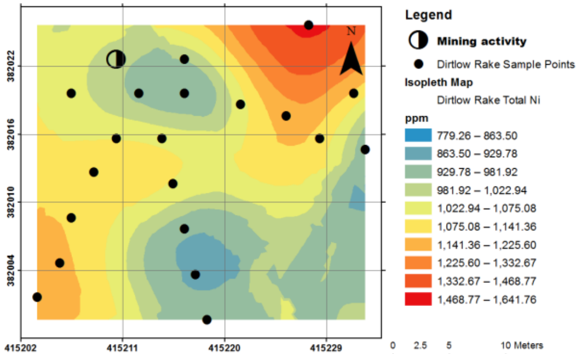

Figure D:4. Isopleth map of total Ni at Dirtlow Rake

The distribution of Ni at Dirtlow Rake shows a very similar pattern to that of Cu, with the greater concentrations to the North-east of the isopleth map.

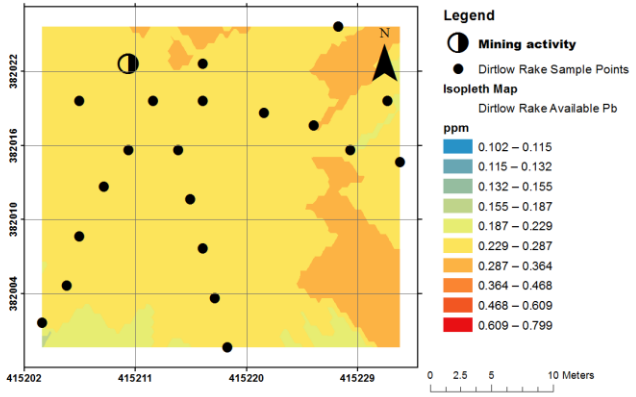

Figure D:5. Isopleth map of available Pb at Dirtlow Rake

Available Pb concentrations at Dirtlow Rake are quite homogenous across the sampling area.

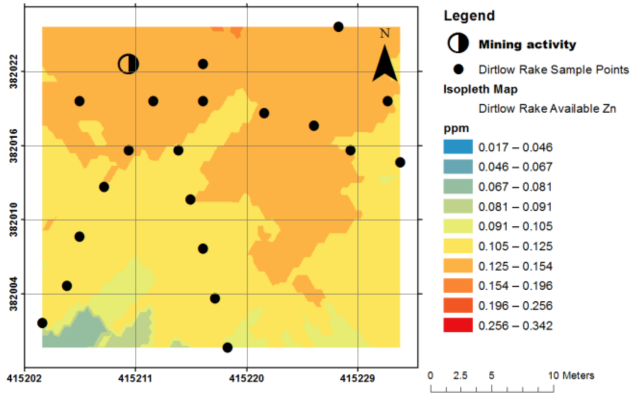

Figure D:6. Isopleth map of available Zn at Dirtlow Rake

Relatively low concentrations of available Zn are found across Dirtlow Rake's sample area, although higher concentrations are found closer to the mine shaft and open pit (N).

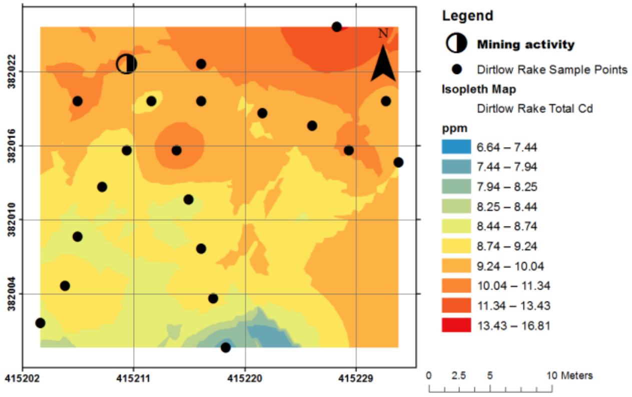

Figure D:7. Isopleth map of total Cd at Dirtlow Rake

Total Cd displaying higher concentrations to the north and east of the survey area.

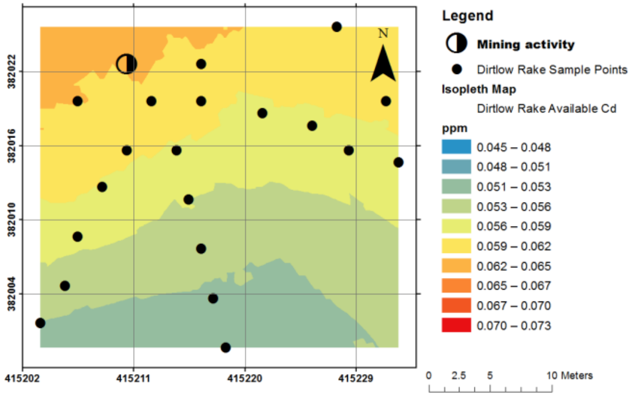

Figure D:8. Isopleth map of available Cd at Dirtlow Rake

Available Cd id elevated to the north of the sample area close to mining structures.

Appendix E-Isopleth prediction maps: Gang Mine

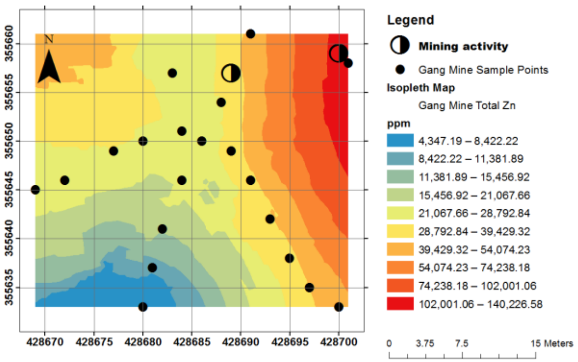

Figure E:1. Isopleth map of total Zn at Gang Mine

The more heavily worked area of Gang Mine (NE) to the east of the sampling area shows much higher concentrations of Zn.

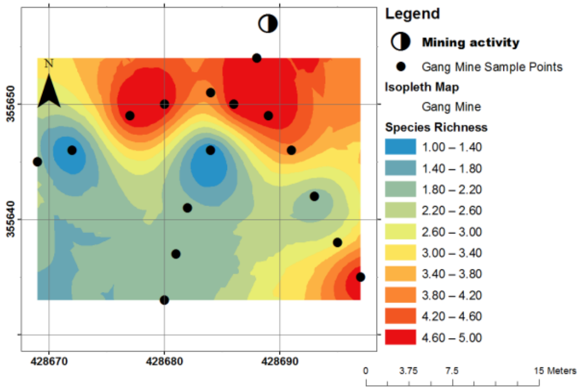

Figure E:2. Isopleth map of species richness at Gang Mine

More species are present per quadrat to the north of the sample area, around the edge of the bell pit (N).

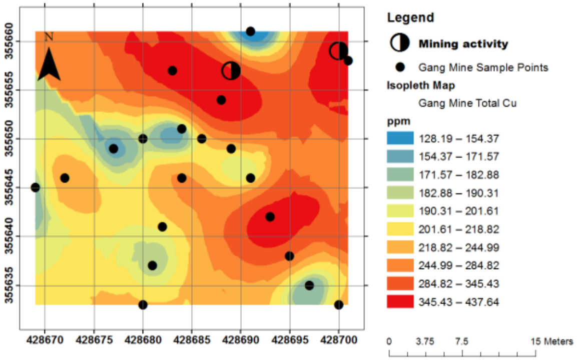

Figure E:3. Isopleth map of total Cu at Gang Mine

Gang Mine exhibits high concentrations of Cu around the more extensively worked areas on the site. There are lower levels of Cu to the south-west across its informal pathway.

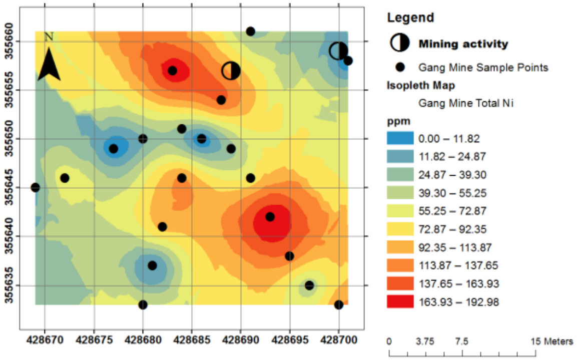

Figure E:4. Isopleth map of total Ni at Gang Mine

The distribution of Ni concentration at Gang Mine is almost a reverse pattern to Pb, displaying low concentrations of Ni where Pb is high and vice versa.

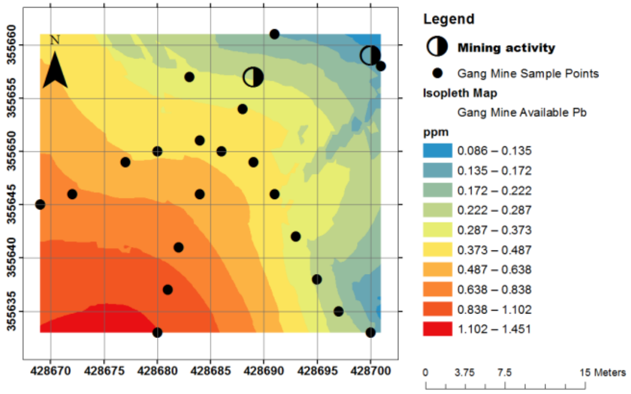

Figure E:5. Isopleth map of available Pb at Gang Mine

The available Pb concentrations at Gang Mine show some contrasting spatial distribution to total Pb, with lowest available Pb levels across the heavily worked area on site.

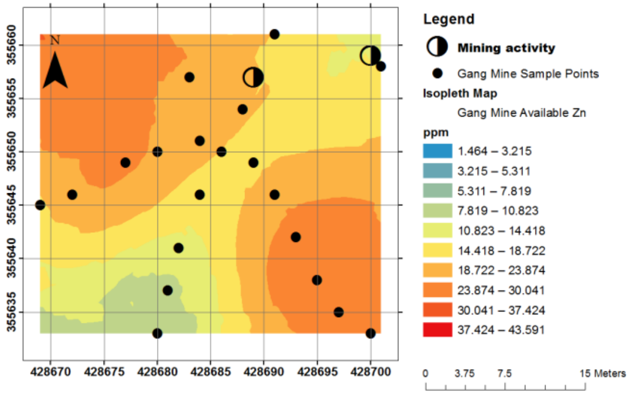

Figure E:6. Isopleth map of available Zn at Gang Mine

Available Zn concentrations at Gang Mine are elevated in two areas of the sampling area (NW and SE).

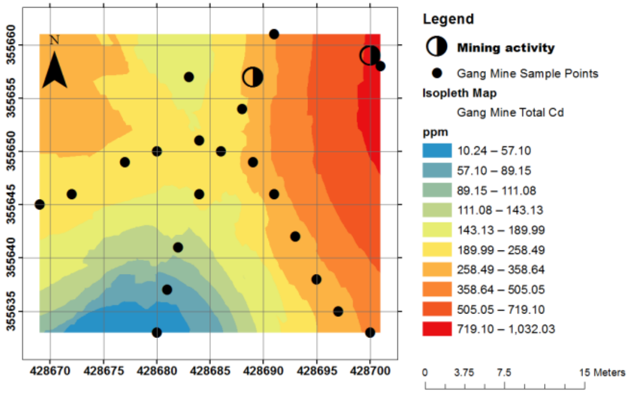

Figure E:7. Isopleth map of total Cd at Gang Mine

Total Cd at Gang Mine is elevated to the eastern area where land is most disturbed.

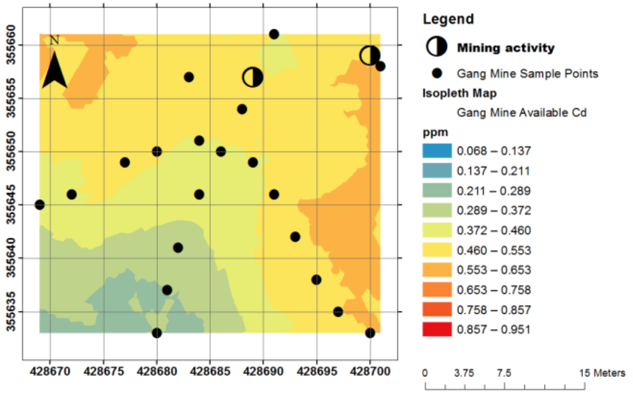

Figure E:8. Isopleth map of available Cd at Gang Mine

Available Cd distribution shows similar patterns to those of total Cd.

Appendix F- Isopleth prediction maps: Hard Rake 1

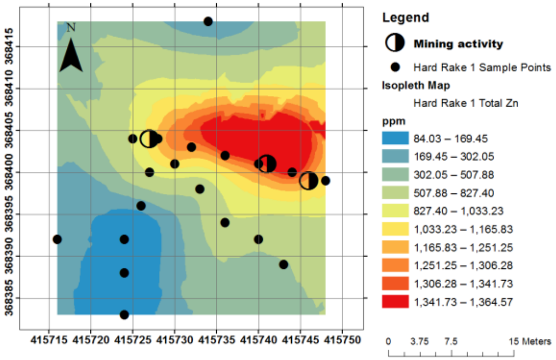

Figure F:1. Isopleth map of total Zn at Hard Rake 1

Zn concentration is higher originating around the central bell pit present at Hard Rake 1 sample area, and appears to extend towards other bell pits along this mineral vein.

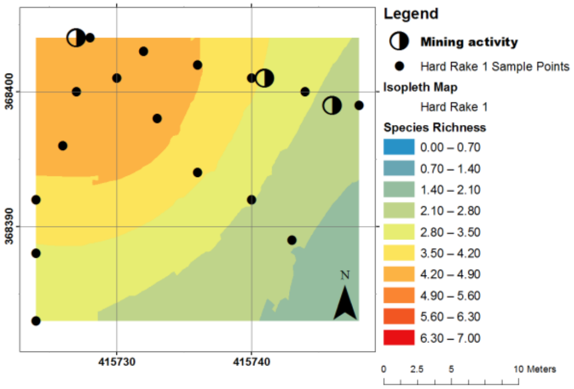

Figure F:2. Isopleth map of species richness at Hard Rake 1

Species richness across Hard Rake 1 sample area advances toward the north-west extent. This is comparable to species abundance.

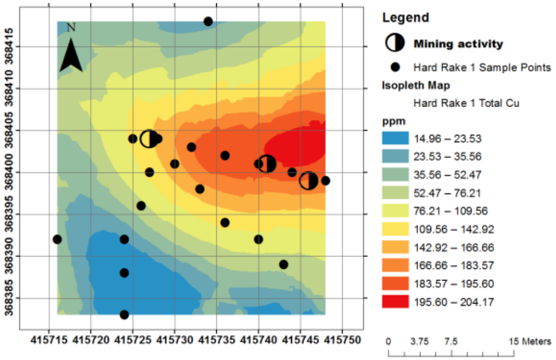

Figure F:3. Isopleth map of total Cu at Hard Rake 1

Hard Rake site 1 displays a similar spatial distribution of Cu to that of Pb. Lower levels of Cu are seen around edges of the bell pit.

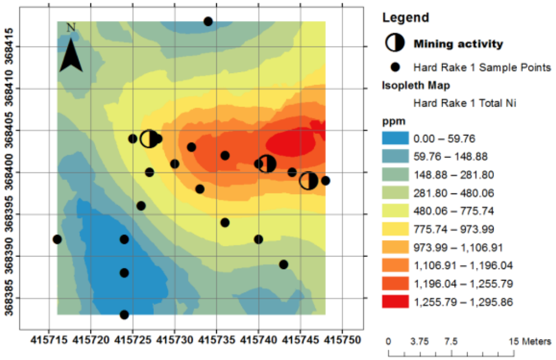

Figure F:4. Isopleth map of total Ni at Hard Rake 1

Ni distribution at Hard Rake 1 sampling area corresponds to the spatial patterns of the other total heavy metal concentrations.

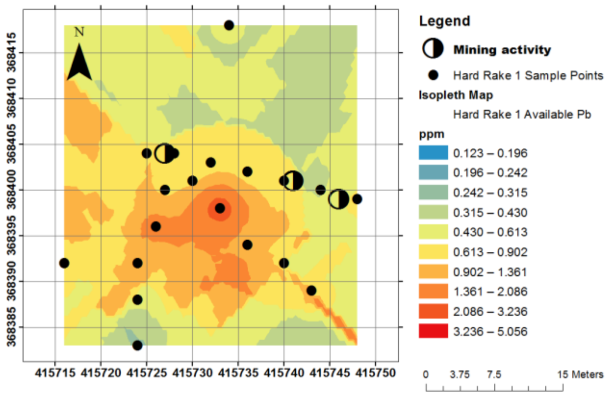

Figure F:5. Isopleth map of available Pb at Hard Rake 1

The greatest amounts of available Pb are present in the central area of this isopleth map, close to the bell pit at Hard Rake 1.

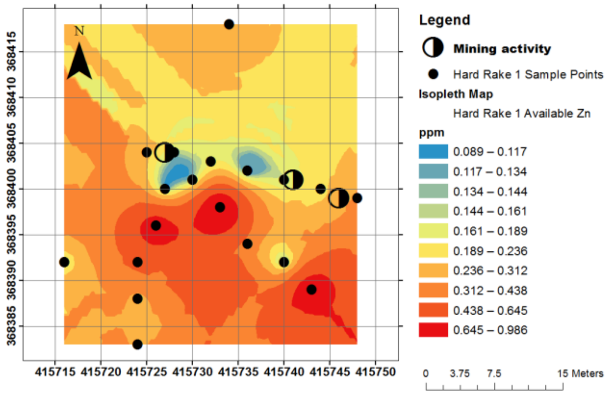

Figure F:6. Isopleth map of available Zn at Hard Rake 1

Available Zn distribution at Hard Rake 1 is not comparable to total heavy metal dispersal and is more concentrated to the southern edge of the bell pit.

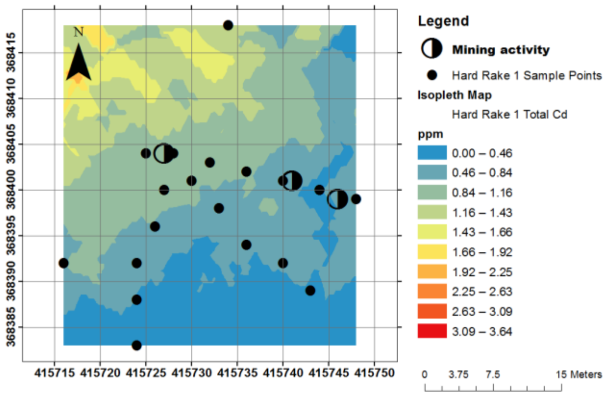

Figure F:7. Isopleth map of total Cd at Hard Rake 1

Total Cd displaying relatively low concentrations that are evenly distributed.

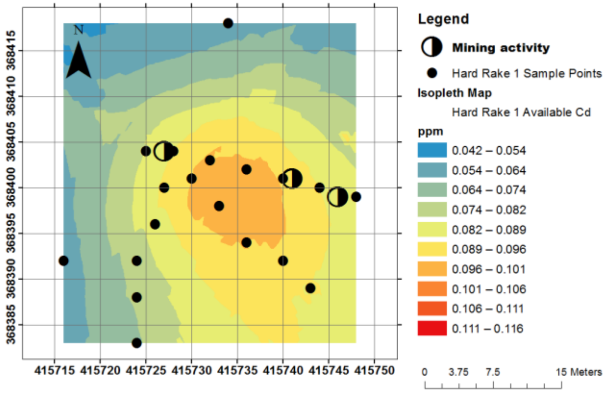

Figure F:8. Isopleth map of available Cd at Hard Rake 1

Available Cd is elevated around the hollow caused by mining activities.

Appendix G- Isopleth prediction maps: Hard Rake 2

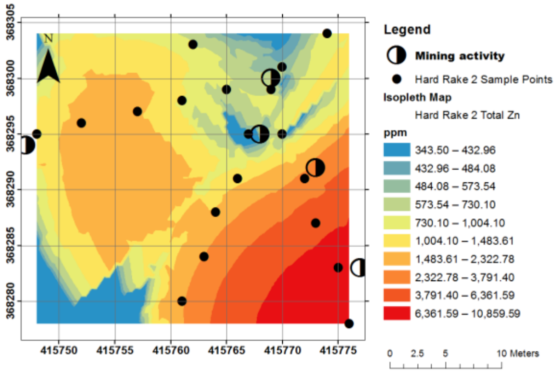

Figure G:1. Isopleth map of total Zn at Hard Rake 2

Hard Rake 2 sample area displays greater concentrations of Zn towards the south-east limit. Lower concentrations are present to the north and east of the sampling zone. This is similar to Pb distribution.

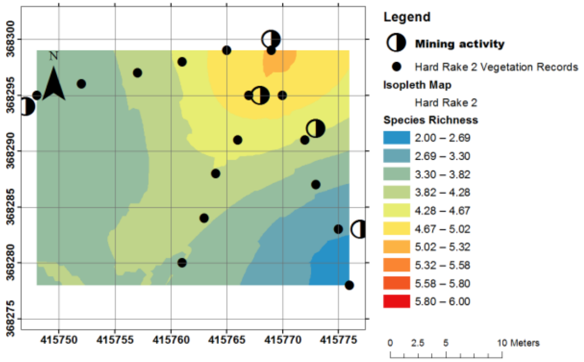

Figure G:2. Isopleth map of species richness at Hard Rake 2

Species richness is lowest in the areas of greater metal concentrations. The pattern of distribution is similar to that of species abundance.

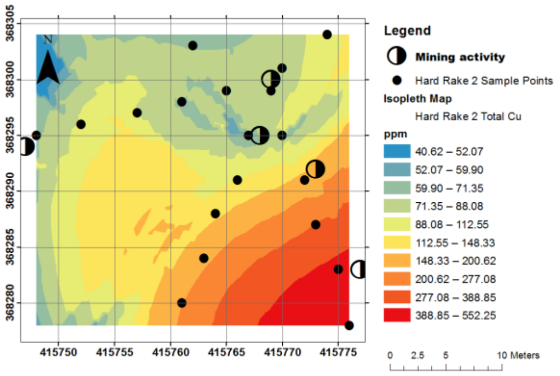

Figure G:3. Isopleth map of total Cu at Hard Rake 2

Again, Hard Rake 2 demonstrates higher concentrations of its metals to the south-east of the sampling area, around the heavily mined areas.

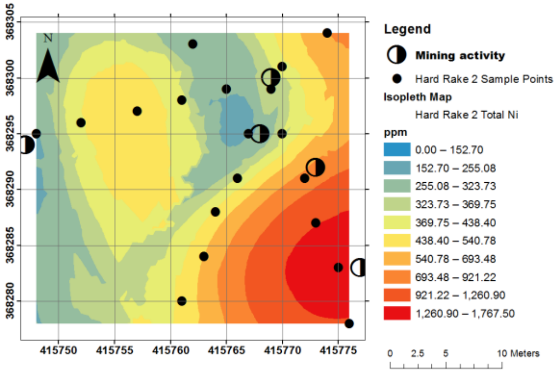

Figure G:4. Isopleth map of total Ni at Hard Rake 2

Ni concentrations display a very similar distribution to Pb. Zn and Cu at Hard Rake 2.

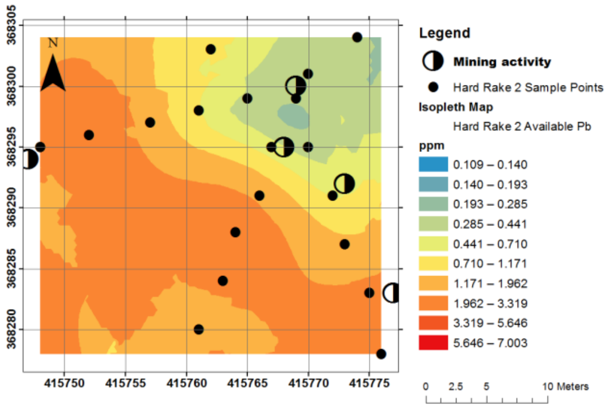

Figure G:5. Isopleth map of available Pb at Hard Rake 2

The higher concentrations of available Pb are distributed across the south-western area of the map with lower concentrations close to the transects' origin point (NE).

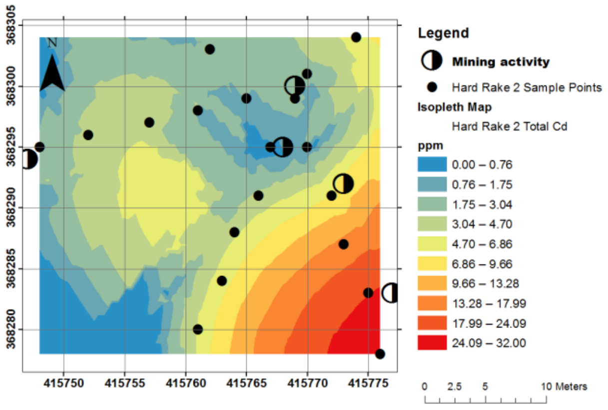

Figure G:7. Isopleth map of total Cd at Hard Rake 2

Elevated total Cd concentrations to the south-east of the isopleth map.

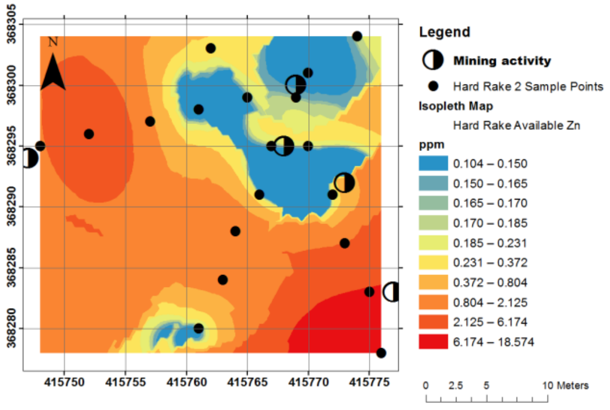

Figure G:6. Isopleth map of available Zn at Hard Rake 2

Available Zn has a comparable spatial distribution to that of available Pb, with elevated concentrations across the south-west of the map.

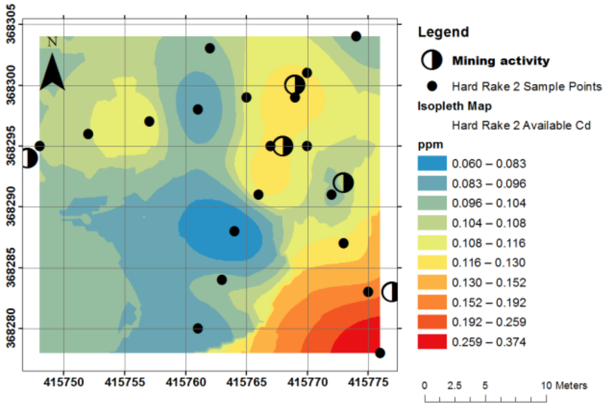

Figure G:8. Isopleth map of available Cd at Hard Rake 2

Available Cd at Hard Rake 2 showing similar distribution patterns to those of total Cd.

Appendix H-Isopleth prediction maps: Hay Dale

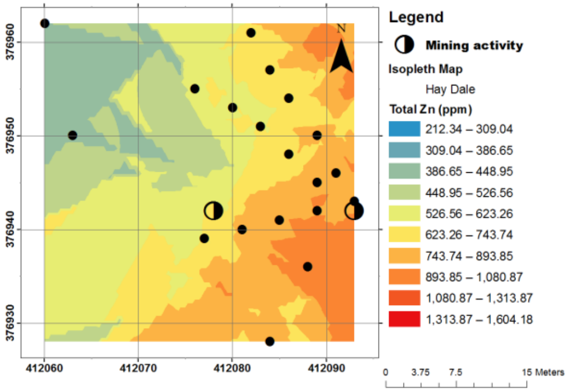

Figure H:1. Isopleth map of total Zn at Hay Dale

Unlike Pb concentrations at Hay Dale, Zn levels are lower on the dale bottom and display higher concentrations at the eastern sampling area.

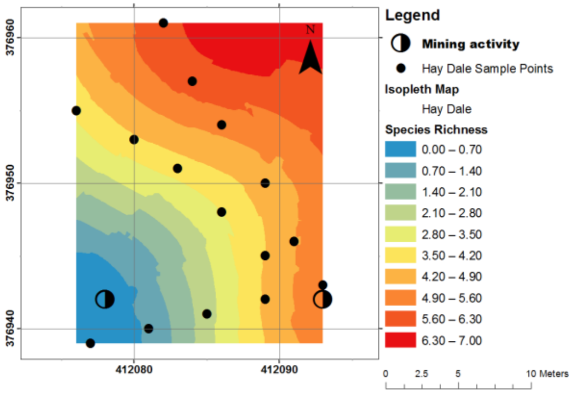

Figure H:2. Isopleth map of species richness at Hay Dale

Comparable to species abundance, species richness is lower on the dale bottom and much higher close to the mine entrance (NE) and on the dale-side.

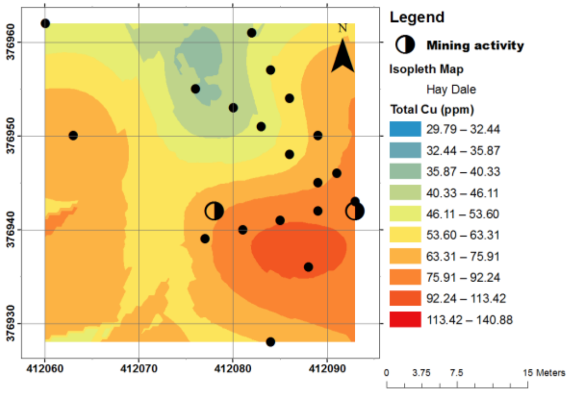

Figure H:3. Isopleth map of total Cu at Hay Dale

This Hay Dale isopleth map shows higher Cu levels around the entrance to the mine and down at the dale bottom. Less Cu is present up the dale-side to the north.

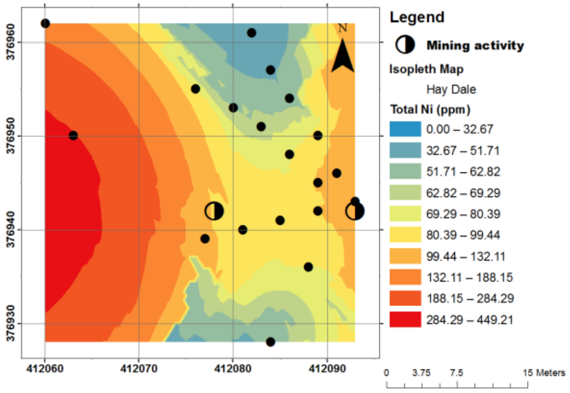

Figure H:4. Isopleth map of total Ni at Hay Dale

The highest Ni concentrations are present to the east, towards the extremities of the sample area.

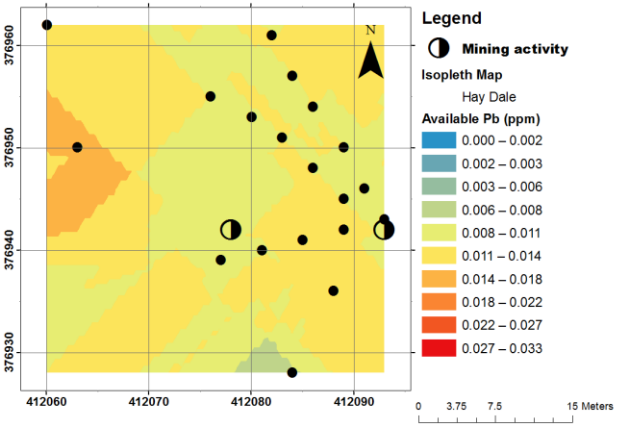

Figure H:5. Isopleth map of available Pb at Hay Dale

Low concentrations of available Pb are present across the whole of the sampling area at Hay dale.

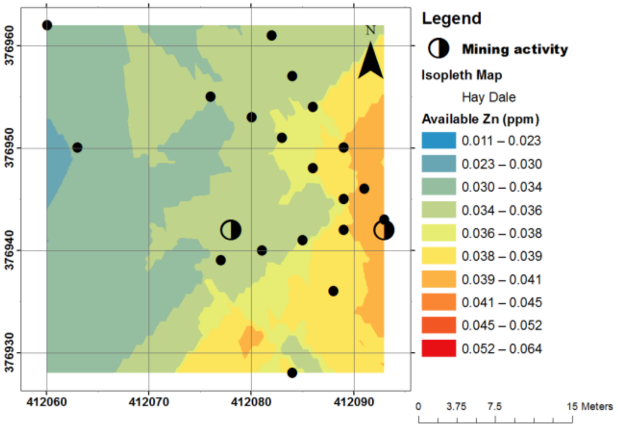

Figure H:6. Isopleth map of available Zn at Hay Dale

Available Zn exhibits comparable patterns of distribution to total Zn, with greater quantities to the west of the map.

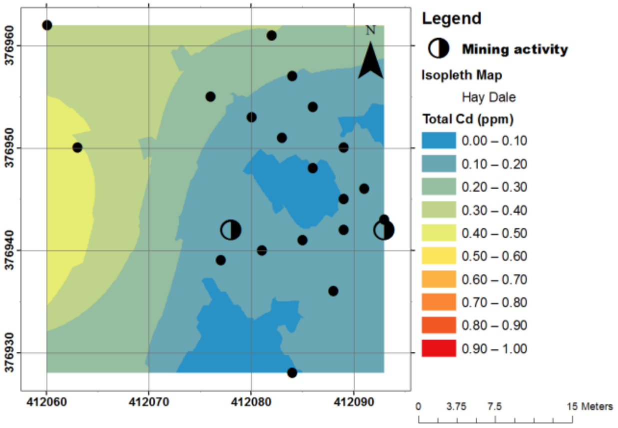

Figure H:7. Isopleth map of total Cd at Hay Dale

Total Cd at Hay Dale has a regular distribution across Hay Dale study area.

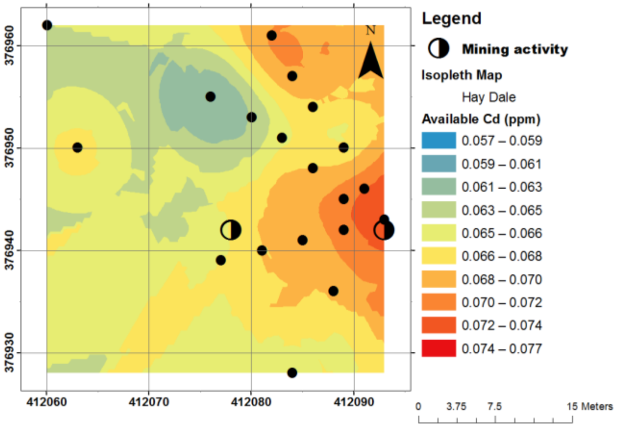

Figure H:8. Isopleth map of available Cd at Hay Dale

Available Cd is elevated close to the mine entrance at Hay Dale.

Appendix I- Isopleth prediction maps: High Rake

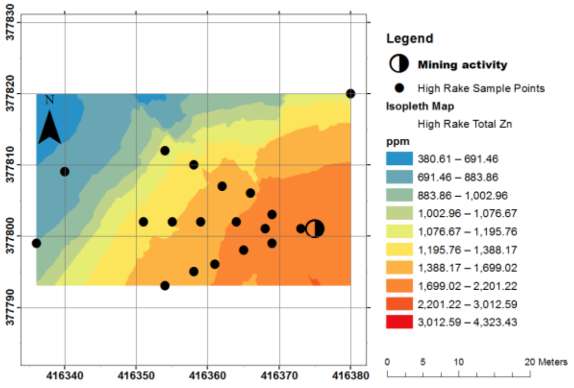

Figure I:1. Isopleth map of total Zn at High Rake

The High Rake isopleth map illustrates much greater concentrations of Zn in the close to the chimney base, and this diminishes with distance from it.

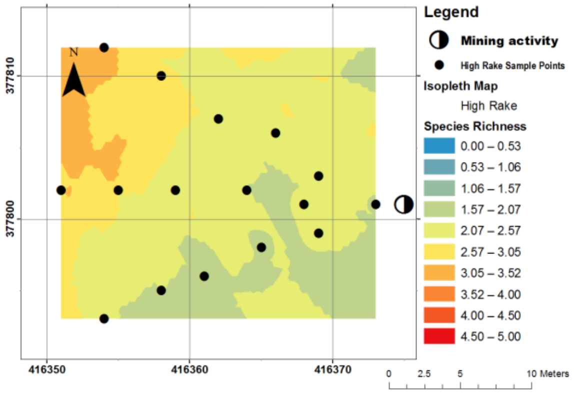

Figure I:2. Isopleth map of species richness at High Rake

Species richness shows a similar pattern of distribution to abundance and is lower in the areas of greater Pb and Zn contamination.

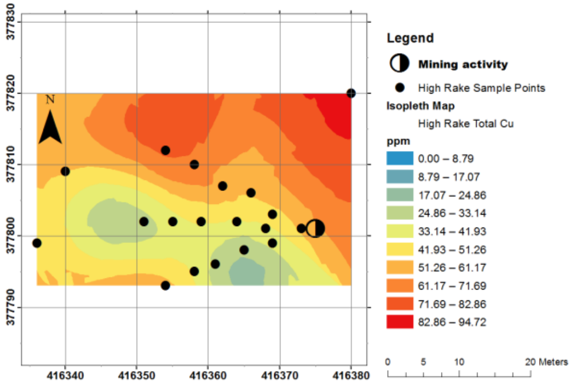

Figure I:3. Isopleth map of total Cu at High Rake

High Rake displays lower Cu concentrations around the chimney base and greater levels in the direction of the more fertile agricultural land to the north.

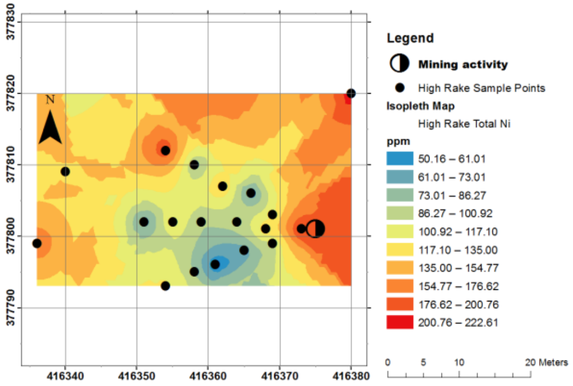

Figure I:4. Isopleth map of total Ni at High Rake

Like Cu distribution, Ni presents lower concentrations close to the chimney base, where greater Pb and Zn concentrations are present.

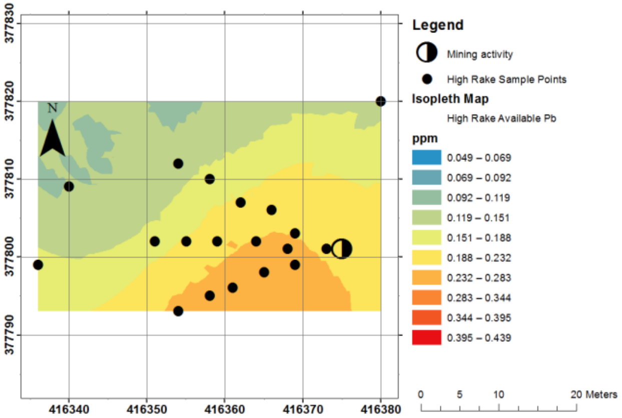

Figure I:5. Isopleth map of available Pb at High Rake

High Rake displays a similar spatial distribution of available Pb to that of its total Pb levels, with elevated levels close to the chimney base.

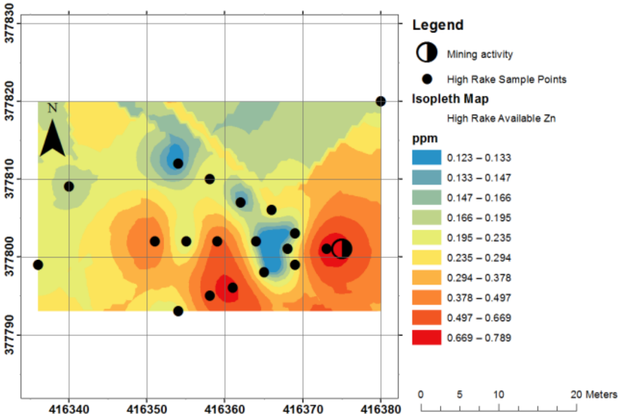

Figure I:6. Isopleth map of available Zn at High Rake

Available Zn displays increased concentrations close to the area of the transects' origin point.

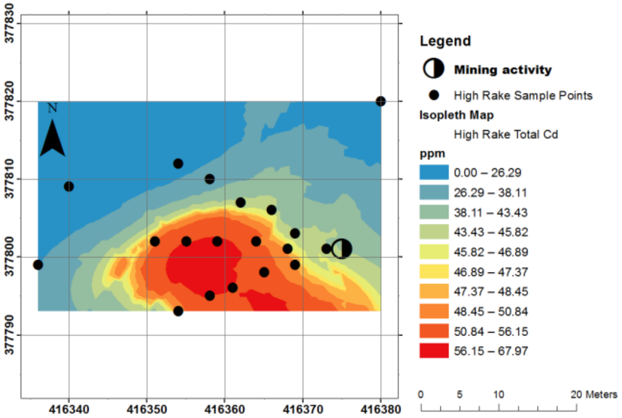

Figure I:7. Isopleth map of total Cd at High Rake

Greatest total Cd concentrations are close to the mining structure on site at High Rake.

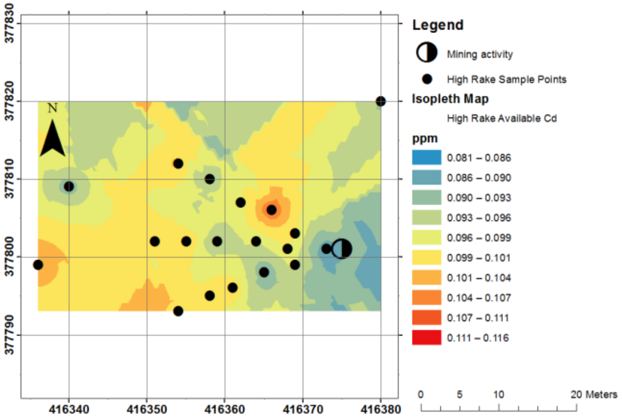

Figure I:8. Isopleth map of available Cd at High Rake

Available Cd at High Rake displays sporadic distribution patterns.

Appendix J- Isopleth prediction maps: Tansley Dale

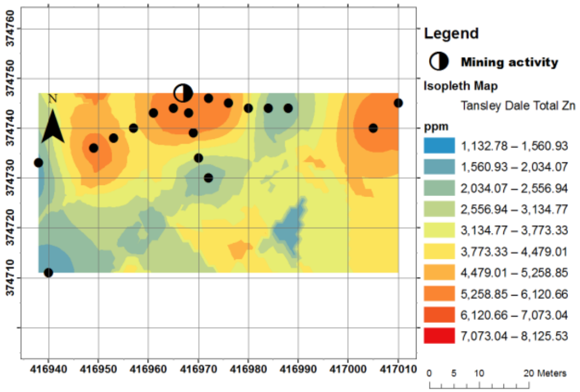

Figure J:1. Isopleth map of total Zn at Tansley Dale

Similar to Hay Dale, Tansley Dale seems to show lower concentrations of Zn on the dale floor, which is the opposite to Pb concentrations at both Hay Dale and Tansley Dale.

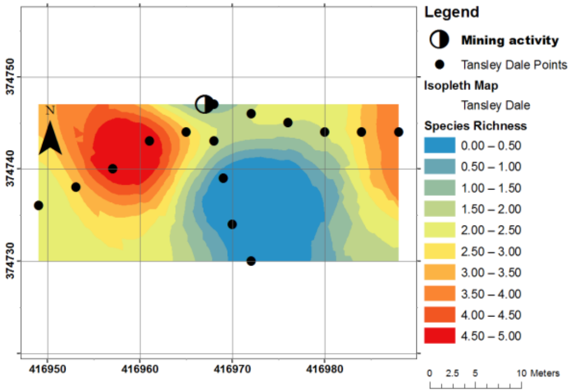

Figure J:2. Isopleth map of species richness at Tansley Dale

Species richness displays a very similar distribution to species abundance and total Zn concentrations.

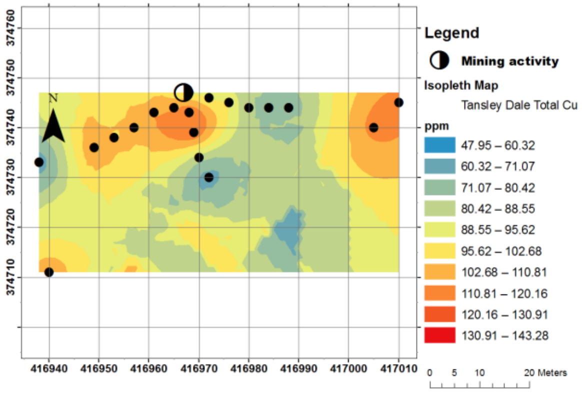

Figure J:3. Isopleth map of total Cu at Tansley Dale

Tansley Dale Cu levels are lower towards the dale floor (S) and elevated pockets further up the dale-side.

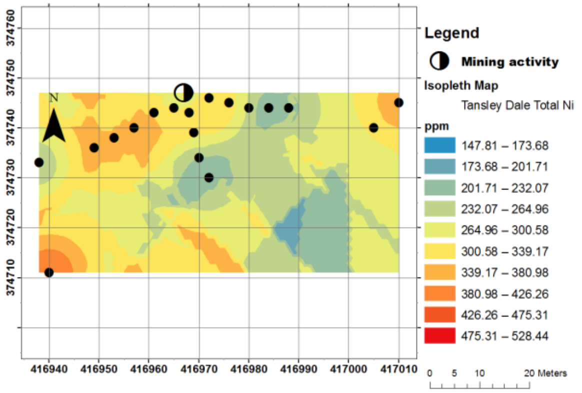

Figure J:4. Isopleth map of total Ni at Tansley Dale

Ni concentration at Tansley Dale shows a very similar spatial distribution to Cu and displays three principal areas of elevated levels.

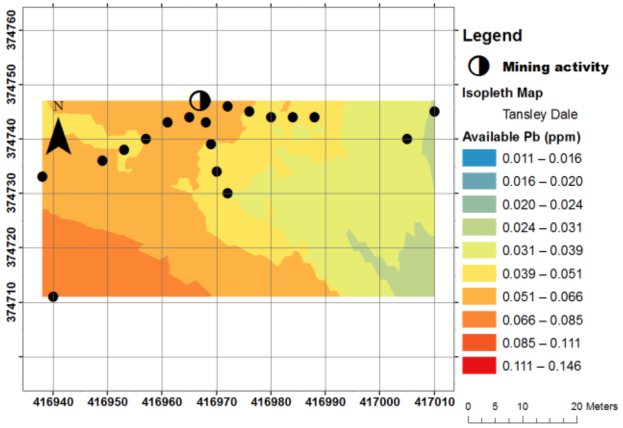

Figure J:5. Isopleth map of available Pb at Tansley Dale

Tansley Dale also presents a similar pattern of available Pb distribution to that of its total Pb with higher concentrations on the dale bottom and further up the dale (E), and lower readings up the dale-side.

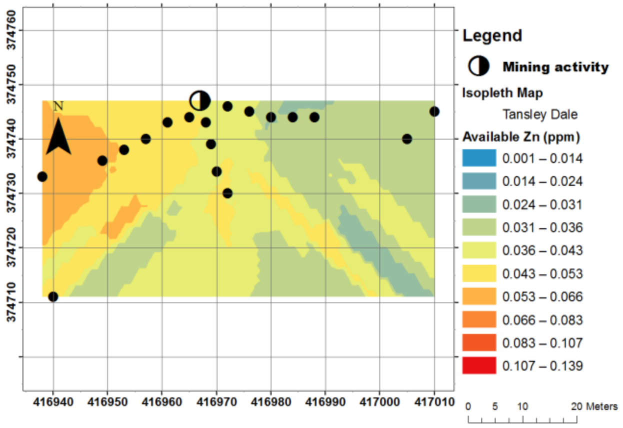

Figure J:6. Isopleth map of available Zn at Tansley Dale

Low concentrations of available Zn are present across the study area with a slight increase further up the dale.

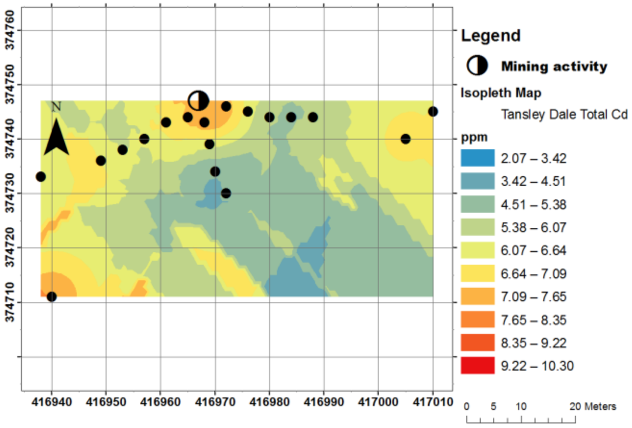

Figure J:7. Isopleth map of total Cd at Tansley Dale

Total Cd at Tansley Dale is elevated to the north, east and west and lower on the dale floor.

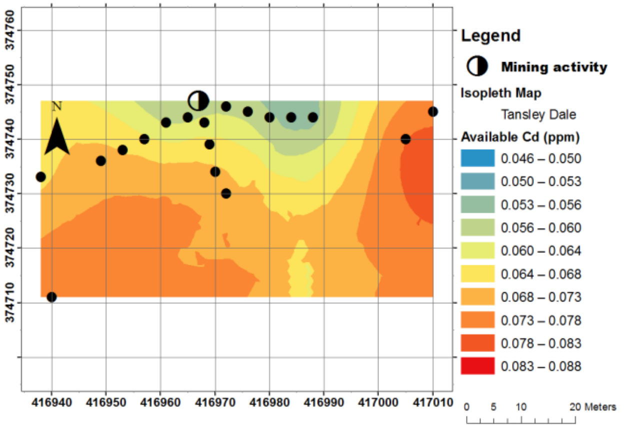

Figure J:8. Isopleth map of available Cd at Tansley Dale

Available Cd displays higher concentrations on the dale floor area of the study area at Tansley Dale.

Appendix K-Braun-Blanquet vegetation data

Table K:1. Braun-Blanquet score by quadrat

Braun-Blanquet Score	Deep Dale	Dirtlow Rake	Gang Mine	Hard Rake1	Hard Rake 2	Hay Dale	High Rake	Tansley Dale
Quadrat 1	9	2	5	8	12	11	5	4
Quadrat 2	11	11	7	7	5	9	5	2
Quadrat 3	6	2	1	5	8	5	6	2
Quadrat 4	7	2	4	6	4	11	6	4
Quadrat 5	9	2	6	9	7	8	11	4
Quadrat 6	8	14	3	0	4	7	2	4
Quadrat 7	9	15	5	9	10	10	7	7
Quadrat 8	11	17	5	9	7	7	4	7
Quadrat 9	8	11	9	5	3	9	6	3
Quadrat 10	8	5	1	1	3	13	7	4
Quadrat 11	10	6	2	4	3	13	8	3
Quadrat 12	10	18	12	14	5	12	0	0
Quadrat 13	5	17	4	4	6	6	1	0
Quadrat 14	5	19	2	7	4	0	10	0
Quadrat 15	6	16	5	2	4	0	0	0
Quadrat 16	3	2	8	4	6	3	2	0
Total	**125**	**159**	**79**	**94**	**91**	**124**	**80**	**44**
Mean	**7.81**	**9.94**	**4.94**	**5.88**	**5.69**	**7.75**	**5.00**	**2.75**
Max	**11.00**	**19.00**	**12.00**	**14.00**	**12.00**	**13.00**	**11.00**	**7.00**
Min	**3.00**	**2.00**	**1.00**	**0.00**	**3.00**	**0.00**	**0.00**	**0.00**
Std Dev	**2.32**	**6.75**	**3.00**	**3.54**	**2.60**	**4.14**	**3.33**	**2.35**

Table K:2. Braun-Blanquet score by species

Common Name	**Latin Name**	**Deep Dale**	**Dirtlow Rake**	**Gang Mine**	**Hard Rake 1**	**Hard Rake 2**	**Hay Dale**	**High Rake**	**Tansley Dale**
BURNET-SAXIFRAGE	*Pimpinella saxifraga*	0	0	12	0	0	0	1	0
CARLINE THISTLE	*Carlina vulgaris*	0	0	0	0	0	2	3	0
COMMON BIRDSFOOT TREFOIL	*Lotus corniculatus*	3	3	17	8	7	0	2	3
COMMON KNAPWEED	*Centaurea nigra*	20	0	0	0	0	0	0	0
COMMON MOUSE EAR	*Cerastium fontanum*	0	0	0	6	0	0	0	0
DEVILS BIT SCABIOUS	*Succisa pratensis*	40	0	0	0	0	0	0	0
DROPWORT	*Filipendula vulgaris*	2	0	0	0	0	7	0	1
DWARF THISTLE	*Cirsium acaule*	0	0	0	1	0	0	0	0
EYEBRIGHT	*Euphrasia officinalis*	1	23	17	10	22	32	0	12
HAREBELL	*Campanula rotundifolia*	2	2	2	4	1	14	11	10
LADYS BEDSTRAW	*Galium verum*	0	21	0	2	2	6	1	0
LESSER HAWKBIT	*Leontodon saxatilis*	28	0	0	0	0	0	0	0
MEADOW BUTTERCUP	*Ranunculus acris*	0	0	6	11	4	0	0	0
MILKWORT	*Polygala vulgaris*	0	2	0	4	3	0	0	0
MOUNTAIN PANSY	*Viola lutea*	0	0	5	0	6	0	0	0
MOUSE EAR HAWKWEED	*Pilosella officinarum*	0	0	0	2	6	7	0	0
RED CLOVER	*Trifolium pratense*	2	22	1	15	9	20	30	0
ROCK ROSE	*Helianthemum nummularium*	0	0	0	0	0	0	0	2
ROUGH HAWKBIT	*Leontodon hispidus*	0	22	0	0	0	0	2	0
SALAD BURNET	*Sanguisorba minor*	22	0	0	1	0	5	0	3
SMALL SCABIOUS	*Scabiosa columbaria*	0	0	0	0	0	2	0	2
TORMENTIL	*Potentilla erecta*	0	13	7	0	0	8	13	2
WHITE CLOVER	*Trifolium repens*	0	0	0	0	7	6	0	0
WILD THYME	*Thymus polytrichus*	0	0	6	13	12	0	4	0
YARROW	*Achillea millefolium*	0	10	7	0	3	9	0	11
Total Species Recorded at Site		0	31	0	7	10	5	16	0
	Total Species Recorded at Site	**10**	**10**	**10**	**15**	**12**	**13**	**11**	**9**

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