An Investigation into the Use of Trace Metals for the Determination of Geographical Origin of Heroin

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Abstract

A review of the literature revealed that investigations into the use of trace metals to determine the geographical origin of heroin have been performed in the past. However, the findings of these studies could not be substantiated due to a lack of seized heroin samples of known provenance with which comparisons could be made. This study involves a novel approach in which opium poppy plants (*Papaver somniferum* L.) were grown in pots of soil each containing different concentrations of copper, lead and zinc. Leaf and resin samples were collected from each of the plants along with a sample of the soil in which they were grown and, after appropriate sample pre-treatment, the concentrations of copper, lead and zinc were determined using differential pulse anodic stripping voltammetry and atomic absorption spectroscopy .

Hierarchical clustering and discriminant function analysis were used to investigate two hypotheses. Firstly, whether resin samples that were known to have originated from plants that were grown in the same soil type could be clustered together and secondly, whether resin samples could be linked back to the soil from which the corresponding poppy plants were grown. The findings showed that when soil types with a greater difference in concentrations of copper, lead and zinc were considered, metal concentration ratios in resin could be used to determine the soil in which the corresponding poppy plants had been grown. However, the classification techniques proved to be more successful when attempting to cluster together resin samples that were known to have originated from poppy plants that were grown in the same soil type.

In comparison to the findings of this study, previous studies demonstrated a greater ability to distinguish between seized heroin samples of different origins using the same classification techniques. This suggests that the addition of metals from sources other than the soil contributed towards making the samples of heroin unique.

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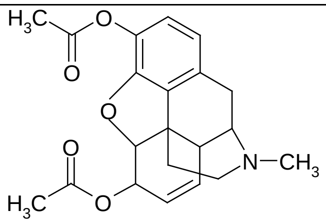
Chapter 1 : Introduction

1.1 Heroin

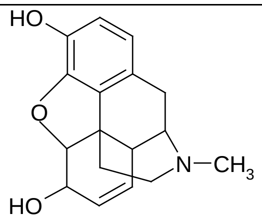
1.1.1 What is Heroin?

Heroin is derived from the opium poppy, *Papaver somniferum* L.; a hardy annual flowering plant that sheds its petals to reveal a seed capsule [1]. The coagulated juice (known as “raw opium”) collected from the seed capsule is dried and added to boiling water to produce opium resin. This resin is then processed so that morphine (Figure 1-1) can be extracted [2]. Diacetylmorphine (Figure 1-1) is then prepared by the acetylation of morphine [3]. Acetylation describes the process by which an acetyl group ($-COCH_3$) replaces another functional group on an organic compound; in this case it is the hydrogen atoms from the two hydroxyl groups of morphine that are replaced.

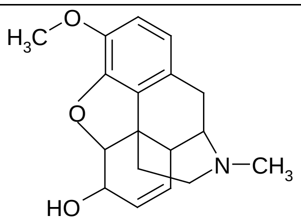
In the United States of America (USA), the name Heroin refers only to diacetylmorphine whereas in the United Kingdom (UK), the name Heroin is a collective term for diacetylamorphine and other opiate compounds that are either naturally occurring or have been produced during the preparation or hydrolysis of diacetylmorphine. These other compounds include O^6-monoacetylmorphine, morphine, codeine and acetylcodeine (Figure 1-1). Throughout this report, the use of the word “heroin” refers to the UK definition.



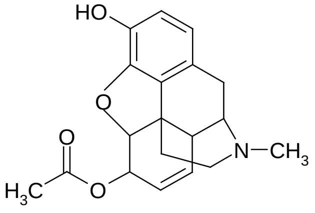
Diacetylmorphine
(CAS#561-27-3)



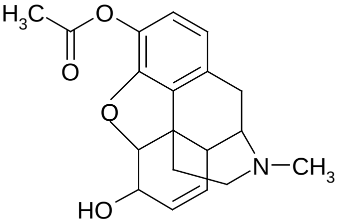
Morphine
(CAS#57-27-2)



Codeine
(CAS#76-57-3)



O^6-monoacetylmorphine
(CAS#2784-73-8)



O^3-monoacetylmorphine
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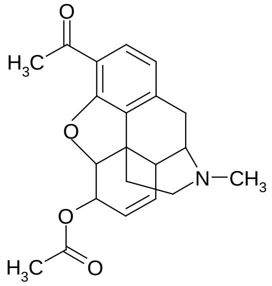
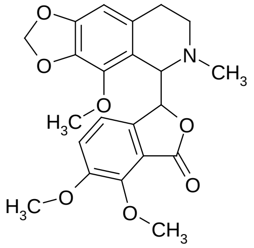
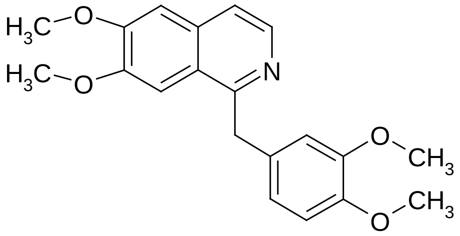


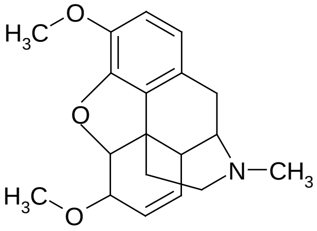
Acetylcodeine
(CAS#6703-27-1)



Noscapine
(CAS#128-62-1)



Papaverine
(CAS#58-74-2)



Thebaine
(CAS#115-37-7)

Figure 1-1: Structure of opiates

Diacetylmorphine is an analgesic (painkiller) that results in both psychological and physical dependence. Diacetylmorphine can pass into the brain more quickly than morphine and therefore produces a more dramatic and intense euphoria. However, most of the effects of diacetylmorphine are as a result of it being changed into morphine in the brain. The three major classes of opiate receptors are mu (μ), delta (δ) and kappa (κ). These receptors are found in the spinal cord and in the periaqueductal grey matter (PAG) of the brain [4]; μ receptors produce analgesia, respiratory depression, euphoria and some sedation, δ receptors produce some analgesia and respiratory depression and κ receptors produce sedation and some analgesia. Morphine is selective for μ and κ receptors only. In the spine the major receptors are present on the dorsal horn pain transmission neuron and opiates directly inhibit this neuron producing the analgesic effect [5].

1.1.2 The Opium Trade

The use of opium (raw or resin) has been mentioned in ancient Assyrian, Egyptian, Greek and Roman pharmacopoeia [6]. In the eighth century A.D. the opium poppy spread along trade routes from the eastern Mediterranean through to the mountains of India and China. In the fifteenth century A.D., the work of Wang Hi included the first reference to the method of extracting the raw opium from the seed capsule [7]. By the sixteenth century A.D. the inhabitants of both Persia (now Iran) and India were eating and drinking mixtures of opium for recreational purposes and the Portuguese were shipping increasing quantities of opium from India to China [1]. In the seventeenth century A.D. the Dutch succeeded the Portuguese traders and as well as increasing the scale of shipments between India and China, they also introduced the practice of smoking opium using a tobacco pipe. These European shipments of smoking opium created one of the world's most lucrative trade triangles. In 1770 the emperor of China banned opium but this failed to stop the British trading India's opium for China's tea and by the end of the eighteenth century A.D., the opium trade had accelerated [1]. In 1839 and 1858, Britain successfully fought two wars with the Chinese to force the empire to rescind the opium ban [8]. By the late nineteenth century A.D., there were tens of millions of opium addicts in China [9] but opium use had also spread to southeast Asia and Europe [1].

1.1.3 Legislation and Control

In 1909, representatives from fourteen different countries including the UK, USA and China, met in Shanghai to form the International Opium Commission. It was at this meeting that the idea to restrict the manufacture, sale and distribution of opium and morphine was first introduced [10]. It was the meeting in Shanghai that led to the signing of the International Opium Convention in The Hague in 1912 [9]. The treaty formalized the ideas from the International Opium Commission but as well as opium and morphine, cocaine and heroin were also included [11].

When the UK's Defence of the Realm Act was updated in 1916, rigorous controls were imposed governing the possession, distribution and sale of opium (and cocaine) in the UK [12]. In 1919, more countries signed the International Opium Convention when it became part of World War I peace treaties such as the Treaty of Versailles [11].

In the UK, the Dangerous Drugs Act of 1920 made it illegal to be in possession of opium for personal consumption [12]. In 1961, all existing international drug control agreements were merged into the Single Convention on Narcotic Drugs [13]. The Misuse of Drugs Act was introduced by the UK government in 1971 to control the use, possession and supply of any substance or product that may cause harm to society [14]. Under the Misuse of Drugs Act 1971, heroin and other major opiates are currently described as being Class A drugs [15].

1.1.4 Medicinal Uses

The healing properties of opium were first described by Hippocrates and later by the Roman physician, Claudius Galen [1]. As early as the second century B.C., Egyptian and Persian doctors treated patients with opium [16]. In the early sixteenth century A.D., Paracelsus created Laudanum by dissolving opium in alcohol; this was then widely used in the Middle Ages [4]. In the nineteenth century A.D., other opium-containing medicines were popular such as Dover's Powder (created by Dr Thomas Dover) and Godfrey's Cordial, a form of Laudanum for children [16].

In the early 1800s, a German pharmacist called Friedrich Sertürner was working to isolate the active ingredient present in opium [17]. In 1805 Sertürner extracted a white powder from opium that was named Morphium (later changed to morphine) after the Greek god of dreams [18]. However, it was not until 1827 that Germany's E. Merck & Co. began to commercially manufacture morphine [1]. After the development of the hypodermic syringe and hollow needle in the late 1850s, morphine became popular as an anesthetic [19].

In 1874, an English researcher named C.R. Wright isolated diacetylmorphine by boiling morphine with acetic anhydride [1]. Twenty four years later the German company Bayer began the mass production of diacetylmorphine using the brand name "Heroin"[4]. By now the dependency-inducing properties of morphine were understood and in 1906, the American Medical Association approved the replacement of morphine with heroin as it was believed to be an non-addictive substitute [1]. In 1911, it was stated in the British Pharmaceutical Codex that heroin was as addictive as morphine [20].

1.1.5 Current Trends

Opium and its related products are used by an estimated twelve to twenty-one million people world-wide, with heroin accounting for seventy-five percent of cases [21]. In 2009, an estimated 375 tonnes of heroin were consumed globally and the market was valued at $68 billion. Although the consumption of heroin in Europe has stabilized, it is believed that African consumption is increasing [21]. Between 2009 and 2010, heroin was the second highest seized drug in England and Wales with a total of 1.5 tonnes and since 2008, the purity of the seized heroin has increased [15]. In 2009 there were 2092 drug-related deaths recorded in the UK, with opiates being the main class of drug responsible. The UK has an estimated 260,000 opiate users and statistics suggest that the UK's illicit heroin market results in social and economic costs of £1.3 billion per year [22].

In the early 1990s, Afghanistan overtook Myanmar as the leading supplier of the world's opium [23] and the 2011 World Drug Report [21] indicated that the main four countries responsible for the cultivation of opium poppies were Afghanistan, Myanmar, Mexico and Lao PDR. Although the 2011 World Drug Report only named other global regions (rather than individual countries) that were also believed to produce opium, the 2009 World Drug Report [24] named the following countries: Balkan countries, Bangladesh, Bolivarian Republic of

Venezuela, Colombia, Ecuador, Egypt, Guatemala, India, Iraq, Lebanon, Nepal, Pakistan, Peru, Russian Federation (and other CIS countries), Thailand, Ukraine and Vietnam.

Between 2009 and 2010, the global cultivation of opium poppies had increased from below 186,000 ha to just below 196,000 ha, with Afghanistan alone contributing 123,000 ha. Although opium poppy cultivation is now concentrated in only a few main countries, the global production of heroin rose by 80 % between 1998 and 2009. In 2010 a fungus, suspected of being *pleospora papaveracea* [25], affected opium poppies in Afghanistan which resulted in a reduction in global opium production (although an increase in cultivation in both Myanmar and Mexico helped to minimize this). However it was anticipated that levels would once again increase in 2011 [21].

In 2000, the Taliban government began to enforce the ban on opium poppy growing and this resulted in a significant reduction in opium produced in 2001 compared with the yield of 2000 [26]. The Taliban government was removed from power at the end of 2001 and from then until 2007, the opium yields of Afghanistan have continued to increase [24]. Between 2007 and 2008, opium poppy cultivation in Afghanistan decreased by 22 %. However, although there was a decrease in yield, this was not as great as expected due to the farmers extracting more opium per bulb [27]. In 2010, the price of opium rose sharply and as a result, provinces of Afghanistan that had previously been poppy-free were shown in 2011 to be cultivating opium poppies with Hilmand province providing the largest amount of opium [28].

It is thought that as much as 90 % of the UK’s heroin originated in Afghanistan [29]. The majority of the heroin arriving in the UK is thought to come in through sea and air ports located in the south east of England [29]. The main route from Afghanistan to the UK is across land via countries such as Iran, Pakistan, Turkey and the Balkans to British organized crime groups based in Spain and the Netherlands. Much of the heroin trade in the UK is controlled by Kurdish and Turkish criminals operating out of London but criminals from Pakistan exploit the strong family and business ties that that country has with both Afghanistan and the UK to supply and distribute to the Midlands and the north of England [30].

1.2 Heroin Analysis

Due to the different classes of drugs, and their corresponding penalties for possession and supply, it is essential that seized drugs are correctly identified and quantified [31, 32]. Traditionally, Gas Chromatography (GC) coupled with a Flame Ionisation Detector (FID) has been used to identify a number of drugs including heroin. This technique was chosen because of its ability to separate and quantify the major components of opium as well as any additional compounds present in seized heroin [33]. GC also provides good reproducibility and sensitivity [34]. Although the concentration of diacetylmorphine is important, more information is required to be able to compare different seized samples and determine whether or not they originated from the same batch [33]. This information can be used locally to provide intelligence on dealers but also nationally and internationally to provide intelligence on trafficking routes [35]. Table 1-1 details the gas chromatography methods that have been used to compare batches of seized heroin by examining the organic components present.

Table 1-1: Use of gas chromatography to analyse organic components of seized heroin

Author(s):	Analytical Technique(s):	Classification Technique(s):	Summary of Findings:
Narayanaswami et al (1979) [36]	Gas Chromatography-Flame Ionisation Detector (GC-FID)	n/a	28 seized opium samples were analysed and % morphine as well as ratios of codeine/thebaine and narctotine/papaverine were determined. The results were triangulated and it was found that heroin that originated from inner Mongolia, Manchuria, China and Korea was grouped together as was heroin that originated from Turkey and Greece. Heroin that originated from Yugoslavia, Japan and Ecuador was all found to be completely different to anything else. Heroin that originated from different regions of India could also be differentiated in this way.
Narayanaswami (1985) [37]	Gas Chromatography-Flame Ionisation Detector (GC-FID)	n/a	Opium samples of known origin and 14 seized heroin samples were analysed and the percentages of morphine, codeine, diacetylmorphine and acetylcodeine were determined. Ratios of morphine/codeine and diacetylmorphine/acetylcodeine were then calculated. Comparison of the two sets of data enabled the origin of the seized heroin samples to be determined.
Barnfield et al (1988) [35]	Gas Chromatography-Flame Ionisation Detection (GC-FID)	n/a	This paper provides information on the development of a method for the profiling of seized heroin samples. The final method enables the elution of the major components of heroin in less than 12 minutes. By producing ratios of the peak area of the major components to the peak area of diacetylmorphine, a method for comparing batches was proposed.

Table 1-1: Continued

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Neumann (1994) [33]	Gas Chromatography-Flame Ionisation Detection (GC-FID)	Hierarchical Cluster Analysis (HCA)	This paper provides a summary of the methods used to compare seizures of heroin in Germany in the early 1990s. The techniques stated were used to examine the ratios of whole morphine (diacetylmorphine and O^6-monoacetylmorphine)/papaverine as well as trace organic impurities. Heroin impurity profiles were separated into four classes.
Besacier et al (1997) [38]	Gas Chromatography-Flame Ionisation Detection (GC-FID) and Gas Chromatography – Isotope Ratio Mass Spectrometry (GC-IRMS)	Principal Component Analysis (PCA) & *K*-means Clustering	GC-FID was used to calculate the ratios of whole morphine (diacetylmorphine & O^6-monoacetylmorphine)/acetylcodeine, whole morphine/papaverine and narcotine/papaverine and GC-IRMS was used to measure the $^{13}C/^{12}C$ isotope ratio of diacetylmorphine and acetylcodeine. 8 samples of seized heroin and 1 control sample were analysed and the same four groups were distinguished using both techniques.
Janhunen and Cole (1999) [39]	Gas Chromatography-Flame Ionisation Detection (GC-FID)	Fisher's Linear Discriminant Analysis	31 street samples of heroin were analysed and the use of Fisher's Linear Discriminant Analysis was explored. It was found that the methods used may be used in the future to provide information regarding batch membership.
Myors et al (2001) [40]	Gas Chromatography – Mass Spectrometry (GC-MS)	K-means Clustering and Hierarchical Cluster Analysis	The acidic and neutral impurities in 54 seized heroin samples were identified and quantified. Out of 649 impurities identified, clustering was performed to produce 18 parameters that enabled the discrimination of samples of purified Southeast Asia heroin (PSEA) and non-PSEA.

Table 1-1: Continued

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Zhang et al (2004) [41]	Gas Chromatography-Flame Ionisation Detection (GC-FID) and Gas Chromatography – Mass Spectrometry (GC-MS)	n/a	500 seized heroin samples were analysed and ratios of acetylcodeine/diacetylmorphine and acetylcodeine/O^6-monoacetylmorphine were calculated. The samples were divided into 9 groups representing the 9 regions of China from the samples were trafficked.
Esseiva et al (2005) [34]	Gas Chromatography-Flame Ionisation Detection (GC-FID)	Principal Component Analysis (PCA) & Correlation Analysis	3371 samples of seized heroin analysed and the peak areas of 6 target alkaloids (meconine, acetylcodeine, acetylthebaol, O^6-monoacetylmorphine, papaverine and noscapine) were each normalised to the peak area of diacetylmorphine. A database was created and PCA and Correlation Analysis were used to group together samples with a similar chemical profile; 20 "classes" were identified.
Sharma et al (2005) [42]	Gas Chromatography – Mass Spectrometry (GC-MS)	n/a	21 seized heroin samples were analysed and the ratio of diacetylmorphine/acetylcodeine was calculated for each. The information was used to identify the possible heroin source as China, Hong Kong or Pakistan.
Dufey et al (2007) [43]	Gas Chromatography-Flame Ionisation Detection (GC-FID)	Correlation Scores	The peak areas of papaverine, noscapine, acetylcodeine, O^6-monoacetylmorphine and acetylthebaol were normalised against the peak area of diacetylmorphine and the resulting information was added to a database. A correlation score between two chromatograms could be calculated to determine whether or not the samples originate from the same batch.

Table 1-1: Continued

Author(s):	Analytical Technique(s):	Classification Technique(s):	Summary of Findings:
Morello and Meyers (1995) [44]	Static Headspace Gas Chromatography –Mass Spectrometry (SHS-GC-MS)	n/a	826 seized heroin samples were analysed and the concentrations of residual solvents were determined. The information can help to determine which chemicals should be controlled but could also prove useful when comparing batches of seized drugs.
Klemenc (2000) [45]	Gas Chromatography – Mass Spectrometry (GC-MS)	n/a	From the analysis of seized heroin in Slovenia it was found that the presence of high levels of noscapine was as a result of it being added as an adulterant. This information could prove useful when comparing batches.
El-Haj et al (2004) [46]	Gas Chromatography – Mass Spectrometry (GC-MS)	n/a	This report investigates the presence of mannitol hexaacetate in brown heroin. Through testing it was found that the presence of mannitol hexaacetate results from the addition of mannitol before the acetylating step rather than after it. As this is uncustomary it could prove to be useful when attempting to connect batches.

As well as the opiate compounds present in heroin samples, cutting agents and adulterants added to heroin by the traffickers and dealers need to be investigated. The addition of certain compounds (as detailed in Table 1-1) or the use of particular solvents during the production of diacetylmorphine, can provide markers that enable batches of drug to be compared. In addition, Klemenc [45] identified that noscapine may be being added as an adulterant and therefore the ratio of noscapine to whole morphine (morphine O^6-monoacetylmorphine and diacetylmorphine) cannot be reliably used to compare samples of seized heroin.

Although GC provides excellent peak capacity, it is unsuitable for the analysis of non-volatile, thermally unstable and highly polar compounds [47] and problems associated with the transacetylation and thermal degradation of diacetylmorphine were observed by Dybowski and Gough [48]. If the amounts or actual presence of the major components of heroin are altered during analysis, the ratios that are calculated and used to compare samples could be inaccurate. These problems can be overcome by the addition of a derivatising agent [2] prior to analysis but this increases sample preparation time and alternative methods have been investigated.

As alternatives to GC-FID and gas chromatography – mass spectrometry (GC-MS), techniques such as High Performance Liquid Chromatography (HPLC) and Capillary Electrophoresis (CE) have been used; Table 1-2 details methods other than GC that have been used to compare batches of seized heroin by examining the organic components present.

Table 1-2: Use of techniques other than gas chromatography to analyse organic components of seized heroin

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Huizer (1983) [49]	High Performance Liquid Chromatography (HPLC) with UV & fluorescence Detection	n/a	25 seized heroin samples were analysed and with a combination of UV and fluorescence detection, grouping was possible. When two case studies were investigated it was found that batches could be linked using this procedure.
Law et al (1984) [50]	High Performance Liquid Chromatography (HPLC) with UV & Fluorescence Detection	n/a	Over 100 seized heroin samples were analysed and phenanthrene-based impurities were identified. Although the same impurities were identified in all samples, the ratios were different and the information could be used to identify common batches.
Johnston and King (1998) [51]	High Performance Liquid Chromatography (HPLC) with UV Detection	Discriminant Analysis	Data analysis was performed on results of 505 previously analysed seized heroin samples from Turkey, Pakistan, India and southeast Asia. Using the information on 8 components (acetylcodeine, O6-monoacetylmorphine, diacetylmorphine, noscapine, papaverine, methaqualone, paracetamol and phenobarbitone) the source of the heroin could be identified. Overall the misclassification rate was 17%.
Lurie and Toske (2008) [47]	Ultra-Performance Liquid Chromatography – Tandem Mass Spectrometry (UPLC-MS-MS)	n/a	This was a preliminary study to investigate the potential for this technique to be used for the profiling of heroin. 20 components were identified including morphine, codeine, O3-monoacetylmorphine, O6-monoacetylmorphine, thebaine, acetylcodeine, diacetylmorphine, papaverine and noscapine as well as previously unseen impurities. Further work is required.

Table 1-2: Continued

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Walker et al (1995) [52]	Micellar Electrokinetic Capillary Chromatography (MECC)	n/a	Heroin separation was achieved in 5 minutes. The method was tested using 14 prepared samples and 6 seized samples of heroin. The results using MECC were comparable to gas chromatography (GC) but provided better resolution.
Lurie et al (2004) [53]	Micellar Electrokinetic Capillary Chromatography (MECC) with Photodiode Array-UV (PDA-UV) Detection	n/a	This paper describes the development of a method for the analysis of seized heroin; hundreds of samples of seized heroin have been routinely analysed using the method described.
Collins et al (2006) [54]	High Performance Liquid Chromatography (HPLC), Capillary Electrophoresis – Diode Array Detection (CE-DAD) and Gas Chromatography –Mass Spectrometry (GC-MS)	Principal Component Analysis (PCA)	100 blocks of seized heroin were analysed and the ratios of whole codeine/whole morphine, whole noscapine/whole morphine and whole papaverine/whole morphine were determined. The data was compared to that of heroin of known origin and all 100 blocks was found to be southeast Asia heroin. Solvent analysis corroborated these findings but the presence of acidic/neutral impurities showed the seized heroin to be more consistent with heroin from southwest Asia. The origin was therefore unknown.
Rendle (2003) [55]	X-Ray Diffraction (XRD)	n/a	When analysing seized heroin samples, XRD was used to determine the precise chemical form as well as to identify diluents/adulterants present. The information obtained can be used to compare one seizure with another.

During the last few years, seized drugs have generally become purer and the organic contaminants tend to be present in almost constant proportions [56]. This has meant that chromatograms produced from different batches of drug are very similar, making profiling by chromatography and CE difficult if not impossible. Degradation of the major and minor constituents of heroin over time has also been observed [57], and this could affect the ability to successfully use ratios of opiates to differentiate between batches.

Because of these factors, new techniques have been employed, mostly to look at the metal ion content of drugs. Table 1-3 details the methods that have been used to compare batches of seized heroin by examining the inorganic components present. A number of authors have suggested that the information could be used to predict the country of origin but without heroin samples of known provenance, the success of the proposed methods cannot be tested [58, 59]. A couple of similar studies have been also been conducted using cocaine instead of heroin [60, 61].

Although not related to drug analysis, metal ions have been successfully used to determine the geographic origin of various foodstuffs, details of which can be found in Table 1-4. These results could be due to the regional variation in trace metal concentration in near-surface soil [62] and the success of these studies can be attributed to the availability of samples of known provenance.

To date, the author has not found any studies that attempt to link the metal ions found in heroin to the metal ions found in the soil where *Papaver somniferum L.* plants were grown.

Table 1-3: Summary of methods used to analyse inorganic components of seized heroin

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Infante et al (1999) [63]	Atomic Absorption Spectrophotometry (AAS)	Correlation Scores and Discriminant Analysis	198 seized heroin samples were analysed. 6 metals were measured. Although the concentrations of the metals were determined, no attempt was made to use the information to compare batches.
Bora et al (2002) [59]	Electrothermal Atomic Absorption Spectrometry (ETAAS) and Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)	Principal Component Analysis (PCA)	44 seized heroin samples were analysed. 10 elements were measured. Findings suggest that the information obtained could be useful for comparative work.
Violante et al (1992) [61]	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)	Correlation scores	35 seized heroin samples were analysed. 11 elements were measured. As well as concentrations of the individual elements, ratios of the elements were also calculated. Unfortunately, all samples of heroin were analysed as one data set so no comparisons could be made.
Wells et al (1995) [64]	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	Hierarchical Clustering Analysis	126 seized heroin samples from 96 different seizures were analysed. 35 different elements were measured. Samples known to have the same origin were clustered together. A number of different blocks from one seizure were analysed and were clustered separately suggesting that the seizure was not homogeneous and consisted of heroin with different origins.

Table 1-3: Continued

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Ekangaki et al (1998) [65]	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	Principal Component Analysis (PCA)	139 seized heroin samples were analysed. 69 elements were measured. A model based on just 8 of those elements was used to distinguish between heroin that had been smuggled into Australia from China and from countries other than China.
Myors et al (1998) [66]	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	Hierarchical Cluster Analysis (HCA), *K*-means Clustering, Correlation Scores and Principal Component Analysis (PCA)	188 seized heroin samples were analysed. In total, 73 elements were measured and using a combination of classification techniques, southeast Asia (SEA) and non-SEA heroin could be distinguished.
Budic and Klemenc (2000) [67]	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)	n/a	This article reports the development of a more sensitive technique for the analysis of trace elements in heroin. 14 elements were measured; the method is expected to prove useful for profiling purposes.

Table 1-3: Continued

Author(s):	Analytical Technique(s):	Classification Technique(s):	Summary of Findings:
Besacier et al (1997) [68]	Gas Chromatography – Isotope Ratio Mass Spectrometry (GC-IRMS)	n/a	31 samples of seized heroin were analysed and the mean ^{13}C enrichment of diacetylmorphine was measured. 17 of the seized samples were then deacetylated and the mean ^{13}C enrichment of morphine was measured. Differences were seen between the values obtained from the diacetylmorphine and the deacetylated morphine; this demonstrates that the information from the morphine provides a better indication of origin whereas the information from the diacetylmorphine can provide information on the source of the acetic anhydride used in the production of diacetylmorphine.
Ehleringer et al (1999) [69]	Isotope Ratio – Mass Spectrometry (IR-MS)	n/a	76 seized heroin samples were analysed and ratios of ^{13}C/^{12}C and ^{15}N/^{14}N were determined. Samples from southwest Asia, southeast Asia, Mexico and south America could be differentiated.
Zhang et al (2004) [70]	Neutron Activation Analysis (NAA)	Hierarchical Cluster Analysis (HCA)	62 seized heroin samples were analysed and 15 elements were measured. The samples could be separated into two distinct groups.
Muratsu et al (2002) [71]	Synchrotron Radiation Total Reflection X-Ray Fluorescence (SR-TXRF)	n/a	3 seized samples of heroin were analysed with differences observed; sample one showed the presence of Ca Kα and Zn Kα, sample two showed the presence of Ca Kα, Ca Kβ and Zn Kα whilst sample three showed the presence of I Lα, I Lβ1, I Lβ2, I Lγ1 and Zn Kα.

Table 1-4: Examples of studies where metal concentrations have been successfully used to determine geographical origin

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Marcos et al (1998) [72]	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	Principal Component Analysis (PCA)	15 types of tea from 10 different countries were analysed and 28 elements were measured. African and Asian tea could be differentiated and Chinese tea could be differentiated from other Asian teas.
Anderson et al (1999) [73]	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)	Principal Component Analysis (PCA), Canonical Discriminant Analysis (CDA), discriminant function analysis and *k*-nearest neighbour	608 potato samples were analysed and could be categorised as either Idaho-grown or non-Idaho-grown.

Table 1-4: Continued

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Martin et al (1999) [74]	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), Flame Atomic Absorption Spectroscopy (FAAS), Site-specific Natural Isotope Fractionation Nuclear Magnetic Resonance (SNIF-NMR), Isotope Ratio – Mass Spectrometry (IR-MS)	Principal Component Analysis (PCA)	162 wine samples (collected over four years) from 10 sub-regions of Bordeaux were analysed. It was found that the techniques used enabled the characterisation of both vineyard and vintage.
Latorre et al (2000) [75]	Atomic Absorption Spectrometry (AAS) and Atomic Emission Spectrometry (AES)	Cluster Analysis, Principal Component Analysis (PCA), Bayesian Method, Partial Least-Squares Regression (PLS) and Neural Networks.	42 honey samples were analysed and 9 metals were measured. Galician and non-Galician honey could be differentiated with PLS being the most accurate classification technique.

Table 1-4: Continued

Author(s):	Analytical Technique(s):	Classification Technique(s):	Summary of Findings:
Anderson and Smith (2002) [76]	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)	Principal Component Analysis (PCA), Discriminant Function Analysis and Neural Networks.	160 coffee samples from Indonesia, East Africa and central/south America were analysed and 18 elements measured. The study showed that the determination of geographical origin of coffee may be possible and further work is being conducted to examine differences in sub-regions.
Branch et al (2003) [77]	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)and Isotope Ratio – Mass Spectrometry (IR-MS)	n/a	Wheat samples from USA, Canada and Europe were analysed. It was found that only the value of $\delta^{13}C$ (determined using IR-MS) could be used to distinguish between the three geographical sources.
Moreda-Pineiro et al (2003) [78]	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) and Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	Cluster Analysis, Principal Component Analysis (PCA), Linear Discriminant Analysis (LDA) and Soft Independent Modelling of Class Analogy (SIMCA).	85 tea samples were analysed and 17 elements measured. All classification techniques were able to differentiate between African and Asian tea whereas only LDA was able to differentiate between teas from China, India and Sri Lanka.

Table 1-4: Continued

Author(s):	**Analytical Technique(s):**	**Classification Technique(s):**	**Summary of Findings:**
Nikkarinen et al (2004) [79]	Inductively Coupled Plasma – Mass Spectrometry (ICP-MS)	n/a	Edible mushrooms from two different regions of Finland were analysed. 33 elements were measured and it was found that the metal ion content varied according to the species of mushroom and the region from where it was sampled.
Anderson and Smith (2005) [80]	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES)	Principal Component Analysis (PCA), Canonical Discriminant Analysis (CDA), Linear Discriminant Analysis and Neural Networks.	Almost 400 pistachios samples from Turkey, Iran and California were analysed and 19 elements measured. The study showed that the determination of geographical origin of pistachios was possible.
Kelly et al (2005) [81]	Various	Various	This article summarises the use of various analytical techniques to determine the geographical origin of various foodstuffs such as meat, dairy products, beverages, cereals and wine.
Zeiner et al (2005) [82]	Inductively Coupled Plasma – Atomic Emission Spectrometry (ICP-AES) and Electrothermal Atomic Absorption Spectrometry (ETAAS)	n/a	Olive oil from different regions of Croatia were analysed and 7 elements were measured. The information was used to determine the geographical origin.

1.3 Voltammetry

Voltammetry is an electrochemical technique involving the electrolysis of a sample solution; voltage is applied to an electrode, a redox reaction occurs and the resulting current flow is measured [83]. The current is proportional to the concentration of the electroactive species present in the sample solution. The equipment used is called a polarograph and the data are shown on a polarogram, with voltage plotted against current.

1.3.1 Instrumentation

Typical instrumentation of a polarograph consists of a working electrode, reference electrode, auxiliary electrode, supporting electrolyte, potentiostat, voltmeter and ammeter as shown in Figure 1-2.

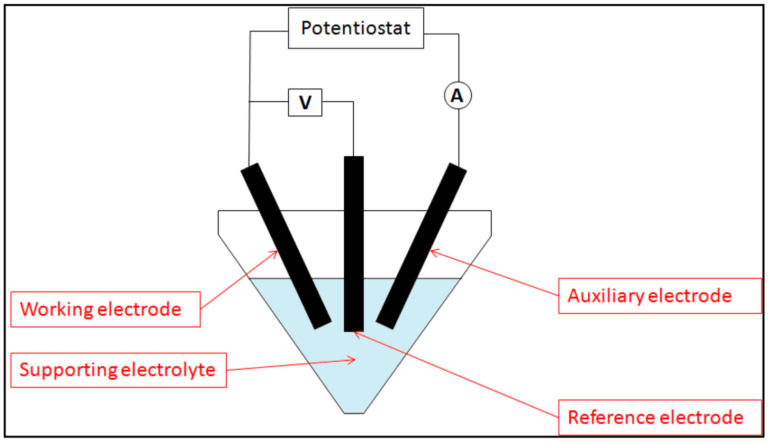


Figure 1-2: Polarograph instrumentation

1.3.1.1 Working Electrode

The working electrode is the place where the redox reaction occurs [84]. Examples of working electrodes include dropping mercury electrode (DME) and hanging mercury drop electrode (HMDE), as well as various types of solid electrode [84].

Both the DME and HMDE make use of a reservoir of mercury attached to a glass capillary; mercury travels down the centre of the capillary and forms a small drop on the end. The HDME makes use of a single mercury drop whereas in the case of the DME, each small drop of mercury only lasts for four to five seconds before falling off to be replaced by a new drop. This occurs at a constant rate. Whilst still attached to the mercury reservoir via a column of mercury in the capillary, each drop of mercury acts as the working electrode. This is true for both DME and HDME however, when a DME is used, the technique is referred to as polarography rather than voltammetry [83].

1.3.1.2 Reference Electrode

The role of the reference electrode is to measure and control the potential at the working electrode [85]. The most common reference electrodes are the saturated calomel electrode (SCE) and the silver-silver chloride electrode [86].

1.3.1.3 Auxiliary Electrode

An auxiliary electrode (also known as a counter electrode) is made from an inert metal or carbon and is used to complete the circuit with the working electrode so that no current flows through the reference electrode [85]. If current were to flow through the reference electrode then its potential might be altered.

1.3.1.4 Supporting Electrolyte

A supporting electrolyte is added to the solution being analysed to decrease the electrical resistance of the cell so that the species of interest moves by diffusion, not by migration [83].

The supporting electrolyte must be a strong electrolyte that either completely or almost completely dissociates in water. An example of a strong electrolyte is potassium chloride: $KCl_{[aq]} \rightarrow K^{+}_{[aq]} + Cl^{-}_{[aq]}$ [86].

1.3.1.5 Potentiostat

This controls the potential of the working electrode with respect to the reference electrode [86].

1.3.2 Process

In conventional voltammetry, the electrodes are immersed in the sample solution and a difference in potential across the electrodes is applied. The potential difference is then gradually increased. Reduction of ions at the cathode removes the ions from the solution, creating a concentration gradient that results in the diffusion of ions. This diffusion process creates a current. To begin with, this current is quite small and known as a residual current. As the applied potential exceeds the decomposition potential (the minimum potential required for electrolysis to occur), small increases in applied potential result in large increases in the current [87]. Once the voltage is sufficiently negative that all of the analytes reaching the electrode are reduced, the current ceases to increase and is known as the limiting or diffusion current [83]. The diffusion current is proportional to the concentration of the analyte in the solution.

1.3.3 Anodic Stripping

Stripping voltammetry introduces a pre-concentration step to increase the sensitivity of the technique [86]. Initially, a fixed potential is applied to the hanging drop mercury electrode which is sufficiently negative to reduce a metal ion to its metallic form. This results in a metal-mercury amalgam forming on the mercury drop [88]. Once sufficient time has passed to allow all of the metal ions to be reduced, a linear sweep will begin. This linear sweep involves the increase in potential to a more positive value and as this occurs, the metal is re-oxidised back to its ionic form and is stripped from the electrode [89]. As the metal ions dissolve back into solution, there will be an exponential rise in the current that is proportional to the amount of metal ions present [86]. The results are produced in the form of a peak, see Figure 1-3.

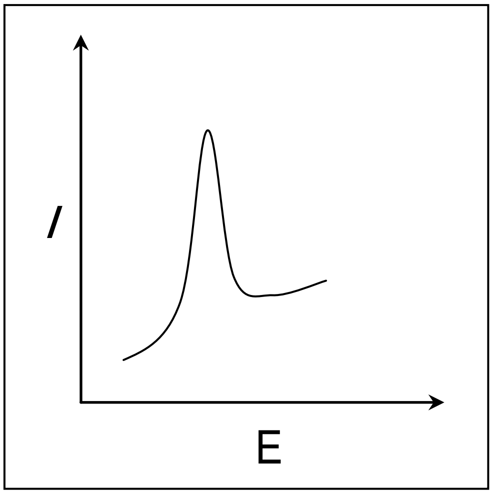


Figure 1-3: Current signal measured in linear sweep stripping voltammetry

As can be seen from Figure 1-3, the problem with this technique is the sloping baseline that is produced as a result of the current not returning to its original value.

1.3.4 Differential Pulse

By increasing the ratio of faradaic: residual current, the detection limit can be lowered as in the case of differential pulse (DP) [83]. In conventional voltammetry, a gradually increasing potential difference is applied (as mentioned in section 1.3.2) but with DP, a pulsed voltage is experienced [90]. The current is measured twice; firstly just before the pulse is applied and secondly, towards the end of the pulse. The difference in the two current readings is then plotted as a function of the voltage [90]. When used in conjunction with anodic stripping, even greater sensitivity is achieved compared with when anodic stripping alone is used [86] and it is during the voltage sweep that these pulses occur (approximately every second). The resultant peak can be seen in Figure 1-4.

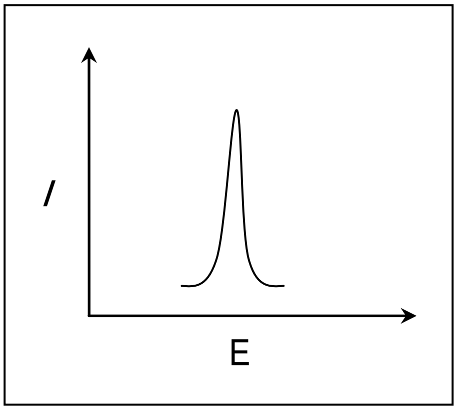


Figure 1-4: Current signal measured in differential pulse stripping

Compared with anodic stripping along (Figure 1-3), differential pulse anodic stripping produces peaks with a much improved baseline.

1.4 Aims and Objectives

The aim of this project was to determine whether or not it was possible to identify the geographical source of seized heroin based on its metal ion content.

Objectives:

- Identify which metal ions would be used in the study
- Design an experiment to simulate the growing of opium poppies in different environments
- Design a method for extracting and analysing metal ions in soil
- Design a method for extracting and analysing metal ions in opium poppy leaves
- Design a method for extracting and analysing metal ions in opium resin
- Explore links between metal concentration data from soil and poppy plant material

Chapter 2 : Materials

All chemicals used were analytical grade or better unless stated otherwise.

2.1 Supporting Electrolytes

Lithium Chloride

A 0.1 M solution was made by dissolving 4.24 g of lithium chloride (obtained from Fisons Scientific Apparatus) in 1 L of deionised water.

Potassium Chloride

A 1.0 M potassium chloride solution was made by dissolving 74.55 g potassium chloride (obtained from Sigma Aldrich) in 1 L of deionised water. A 0.1 M potassium chloride solution was made by diluting a 1.0 M potassium chloride solution with deionised water.

Tetramethyl Ammonium Chloride

A 0.1 M solution was made by dissolving 10.96 g tetramethyl ammonium chloride (obtained from Aldrich Chemical Company) in 1 L of deionised water.

2.2 Metal Solutions

All solutions were made by diluting aliquots of 1000 mgL^{-1} stock solutions with deionised water. Details of where solutions were obtained can be found in Table 2-1.

Table 2-1: Source of metal solutions used

Metal	Molecular Form	Source	
		Company	Location
Aluminium	aluminium nitrate 6-hydrate	BDH Laboratory Supplies	Poole, UK
Barium	n/a	Fisher Scientific	Loughborough, UK
Calcium	calcium nitrate 4-hydrate	BDH Laboratory Supplies	Poole, UK
Cobalt	cobalt nitrate 6-hydrate	BDH Laboratory Supplies	Poole, UK
Copper	copper nitrate 3-hydrate	BDH Laboratory Supplies	Poole, UK
Lead	lead nitrate	BDH Laboratory Supplies	Poole, UK
Magnesium	magnesium nitrate 6-hydrate	BDH Laboratory Supplies	Poole, UK
Manganese	manganese nitrate 4-hydrate	BDH Laboratory Supplies	Poole, UK
Nickel	nickel nitrate 6-hydrate	BDH Laboratory Supplies	Poole, UK
Sodium	sodium nitrate 4-hydrate	BDH Laboratory Supplies	Poole, UK
Zinc	zinc nitrate 4-hydrate	BDH Laboratory Supplies	Poole, UK

2.3 Mixed Standard of Copper, Lead and Zinc

A 20 mgL^{-1} mixed standard was made using 0.2 mL of 1000 mgL^{-1} copper nitrate (obtained from BDH Laboratory Supplies, Poole, UK), 0.2 mL of 1000 mgL^{-1} lead (obtained from Fisher Scientific, Loughborough, UK) and 0.2 mL of 1000 mgL^{-1} zinc nitrate (obtained from BDH Laboratory Supplies, Poole, UK) made up to 10 mL using deionised water.

2.4 Solutions Added to Soil

Copper

A 10 mgL^{-1} copper solution was made by dissolving 0.950 g copper (II) nitrate 3-hydrate (obtained from BDH Laboratory Supplies, Poole, UK) in 25 L deionised water.

Lead

A 33 mgL^{-1} lead solution was made by dissolving 1.320 g lead nitrate (obtained from BDH Laboratory Supplies, Poole, UK) in 25 L deionised water.

Potassium

A 13 mgL^{-1} potassium solution was made by dissolving 0.505 g potassium nitrate (obtained from BDH Laboratory Supplies, Poole, UK) in 15 L deionised water.

Zinc

An 11 mgL^{-1} zinc solution was made by dissolving 1.251 g zinc (II) nitrate 6-hydrate (obtained from BDH Laboratory Supplies, Poole, UK) in 25 L deionised water.

2.5 Acid Digestion

Nitric Acid

A 2 M nitric acid solution was made by diluting 17 M nitric acid (obtained from BDH Laboratory Supplies, Poole, UK) with deionised water.

2.6 Determination of Cation Exchange Capacity (CEC)

Ammonium Chloride

A 50 mM solution was made by dissolving 2.6745 g ammonium chloride (obtained from Acros Organics, New Jersey, USA) in 1 L of deionised water. A 2 mM solution was made by dissolving 0.1070 g ammonium chloride in 1 L of deionised water. Both ammonium chloride solutions were then adjusted to the pH of the soil using a 35 % solution of ammonia (obtained from BDH Laboratory Supplies, Poole, UK).

Boric Acid

A solution was made by dissolving 10 g boric acid (obtained from Janssen Chimica, Belgium) in 500 mL of deionised water.

Hydrochloric Acid

A 0.05 M solution was made by diluting 2 M hydrochloric acid with deionised water. A 0.01 M solution was made by diluting 0.05 M hydrochloric acid with deionised water.

Indicator

The indicator was prepared using 0.1 g laboratory grade Methyl Red (obtained from Gallenkamp and Co, Widnes, UK) and 0.2 g Bromocresol Green (obtained from Sigma-Aldrich, Germany) made up to 250 mL using ethanol.

Potassium Nitrate

A 20 mM solution was made by dissolving 2.022 g potassium nitrate (obtained from BDH Laboratory Supplies, Poole, UK) in 1 L of deionised water.

Sodium Hydroxide

A solution was made by dissolving 100 g sodium hydroxide (obtained from Fisher Scientific, Loughborough, UK) in 200 mL of deionised water.

2.7 Determination of Available Metals in Soil

Ammonia Solution

A 35 % solution of ammonia was obtained from BDH Laboratory Supplies, Poole, UK.

Nitric Acid

A 1 M nitric acid solution was made by diluting 17 M nitric acid (obtained from BDH Laboratory Supplies, Poole, UK) with deionised water.

Ammonium-EDTA

A 0.05 M solution was made by dissolving 14.6 g ethylenediaminetetraaceticacid disodium salt dihydrate (obtained from Janssen Chimica, Geel, Belgium) in 8 mL of ammonia solution (see above) and approximately 950 mL of deionised water. The pH of the solution was adjusted to 7.0 by addition of 1 M nitric acid (see above). The solution was then made up to 1 L using deionised water.

Mixed Metal Solutions

Mixed solutions of concentration 10, 20, 40, 80 and 160 mgL^{-1} were made using dilutions of 1000 mgL^{-1} copper nitrate (obtained from BDH Laboratory Supplies, Poole, UK), 1000 mgL^{-1} lead (obtained from Fisher Scientific, Loughborough, UK) and 1000 mgL^{-1} zinc nitrate (obtained from BDH Laboratory Supplies, Poole, UK) made up to volume using deionised water.

Zinc Solutions

Zinc solutions were made using dilutions of 1000 mgL^{-1} zinc nitrate (obtained from BDH Laboratory Supplies, Poole, UK) made up to volume using deionised water.

2.8 *Papaver somniferum L.*

Seeds from *Papaver somniferum L.* were obtained from Macfarlan Smith , Edinburgh, UK.

2.9 Compost

All of the compost used was John Innes Potting Compost N$^{\underline{o}}$ 2. Throughout the rest of the report, this compost was referred to as soil.

2.10 Equipment

2.10.1 Polarograph

All voltammetric analyses were carried out on a 797 VA Computrace polarograph from Metrohm UK Ltd (Buckingham, UK). The polarograph was attached to a computer equipped with 797 VA Computrace software (also from Metrohm UK Ltd). The working electrode was a hanging mercury drop electrode (HMDE), with a drop area of 0.25 mm^2, the reference electrode was a silver-silver chloride (Ag/AgCl) electrode in a 3 $molL^{-1}$ solution of potassium chloride (KCl) and the auxiliary electrode was a platinum electrode. All three electrodes were obtained from Metrohm UK Ltd. Polarography-grade Fluka mercury (Hg) obtained from Sigma-Aldrich (Germany) was used.

2.10.2 Flame AAS

An S Series atomic absorption spectrometer from Thermo Fisher Scientific Inc (Waltham, USA) was used for analyzing copper, lead and zinc in an air-acetylene flame. A Deuterium lamp (as supplied by Thermo Fisher Scientific Inc) was used for background correction and Cathodeon hollow cathode lamps (obtained from Spectronic Camspec Ltd, Garforth, UK) were used for each of the elements. Table 2-2 shows the instrumental parameters used for each metal.

Table 2-2: Instrument parameters for AAS

Element:	**Wavelength (nm):**	**Lamp Current (nA):**	**Slit (nm):**	**Gas:**
Copper	324.8	4	0.5	Air/C_2H_2
Lead	217.0	5	1.0	Air/C_2H_2
Zinc	213.9	5	1.0	Air/C_2H_2

All determinations were made using dissolved acetylene (obtained from BOC Tradequip, Stoke-on-Trent, UK) and compressed air as fuel and oxidant gas, respectively.

2.10.3 Other

pH Meter

pH measurements were made with a Denver Instrument (Downham Market, UK) Model 50 pH/ion/conductivity meter. The apparatus was calibrated before use using 3 buffers solutions at pH 4.00, 7.00 and 9.00.

Muffle Furnace

A model TC100 muffle furnace from Stanton Pottery Supplies Ltd (Stoke-on-Trent, UK) was used.

Flask Shaker

A flask shaker from Stuart Scientific (Stone, UK) was used at a speed of 300 oscillations per minute.

Chapter 3 : Methods

3.1 Pilot studies

3.1.1 Investigation into Which Metals to Analyse

Methods for analyzing each of the metals were obtained from Kolthoff and Lingane [91] and Schröder and Kahlert [92]. All of the metals were analysed with the same basic voltammetric method (detailed in Table 3-2) with differing values of A and B as indicated in Table 3-3. For each analysis, 10 mL of electrolyte along with 10 mL of metal solution was used. For each metal, solutions of concentration 50 mgL^{-1}, 5 mgL^{-1} and 50 ugL^{-1} were analysed.

3.1.2 Development of the Voltammetric Method

Limit of Detection for Potassium Chloride

To test the limit of detection for copper, 20 ml of 1.0 M KCl was analysed. Standard additions (x3) were then performed using 5 µl of 1000 mgL^{-1} copper solution. This process was then repeated 10 times. The operating conditions used are shown in

Table 3-1. To test the limit of detection for lead and zinc, this entire process was then repeated using 5 µl of 1000 mgL^{-1} lead solution and 5 µl of 1000 mgL^{-1} zinc solution, respectively.

Table 3-1: Voltammetric conditions for determination of limit of detection

Highest current range:	10 mA
Lowest current range:	100 nA
Electrode:	HDME
Drop Size (1-9):	3
Stirrer speed (rpm):	2000
Initial purge time (s):	60
Conditioning cycles	
Start potential (V):	0
End potential (V):	0
No. of cycles:	0
Hydrodynamic (measurement):	No
Cleaning potential (V):	-1.600
Cleaning time (s):	5.000
Deposition potential (V):	-1.600
Deposition time (s):	60
Sweep	
Equilibration time (s):	5.000
Start potential (V):	-1.600
End potential (V):	0.000
Voltage step (V):	0.006
Voltage step time (s):	0.500
Sweep rate (V/s):	0.012
Pulse amplitude (V):	0.050
Pulse time (s):	0.040
Cell off after measurement:	Yes

Effect of Deposition Potential

A 5 mgL^{-1} solution of zinc was used to establish the effect that changing the deposition potential had on the peak height. In each case, 10 mL of the zinc solution and 10 mL of 1.0 M KCl was analysed. The operating conditions were described by Schröder and Kahlert [92] and are shown in Table 3-4.

Table 3-2: Initial voltammetric conditions

Highest current range:	10 mA
Lowest current range:	100 nA
Electrode:	HDME
Drop Size (1-9):	3
Stirrer speed (rpm):	2000
Initial purge time (s):	60
Conditioning cycles	
Start potential (V):	0
End potential (V):	0
No. of cycles:	0
Hydrodynamic (measurement):	No
Cleaning potential (V):	0
Cleaning time (s):	0
Deposition potential (V):	A
Deposition time (s):	60.000
Sweep	
Equilibration time (s):	5.000
Start potential (V):	A
End potential (V):	B
Voltage step (V):	0.005
Voltage step time (s):	0.400
Sweep rate (V/s):	0.013
Pulse amplitude (V):	0.050
Pulse time (s):	0.040
Cell off after measurement:	Yes

Table 3-3: Experimental conditions specific to each metal

Metal:	Electrolyte:	E ½ (V):	A (V):	B (V):
Aluminium	1.0 M Potassium chloride	-1.59	-1.80	-1.50
Barium	0.1 M Lithium chloride	-1.90	-2.00	-1.50
Calcium	0.1 M Tetramethyl ammonium chloride	-2.20	-2.75	-1.80
Cobalt	1.0 M Potassium chloride	-1.38	-1.85	-1.30
Copper	1.0 M Potassium chloride	-0.18	-0.30	0.00
Lead	1.0 M Potassium chloride	-0.41	-0.50	-0.30
Magnesium	0.1 M Tetramethyl ammonium chloride	-2.20	-2.75	-1.80
Manganese	1.0 M Potassium chloride	-1.55	-1.60	-1.30
Nickel	1.0 M Potassium chloride	-1.03	-1.10	-0.80
Sodium	0.1 M Tetramethyl ammonium chloride	-2.15	-2.30	-1.90
Zinc	1.0 M Potassium chloride	-1.02	-1.50	-0.90

Table 3-4: Voltammetric conditions used to investigate effect of deposition potential

Highest current range:	10 mA
Lowest current range:	100 nA
Electrode:	HDME
Drop Size (1-9):	3
Stirrer speed (rpm):	2000
Initial purge time (s):	300
Conditioning cycles	
Start potential (V):	0
End potential (V):	0
No. of cycles:	0
Hydrodynamic (measurement):	No
Cleaning potential (V):	0
Cleaning time (s):	0
Deposition potential (V):	
Deposition time (s):	180.000
Sweep	
Equilibration time (s):	3.000
Start potential (V):	-1.5
End potential (V):	-0.9
Voltage step (V):	0.005
Voltage step time (s):	0.500
Sweep rate (V/s):	0.010
Pulse amplitude (V):	0.050
Pulse time (s):	0.040
Cell off after measurement:	Yes

As the half-wave potential of zinc in 1.0 M KCl is -1.022 V, the following deposition potentials were investigated: -1.00 V, -1.10 V, -1.20 V, -1.30 V, -1.40 V, -1.50 v, -1.60 V, -1.70 V, -1.80 V and -1.90 V.

Effect of Deposition Time

A 5 mgL^{-1} solution of zinc was used to establish the effect that changing the deposition time has on the peak height. In each case, 10 mL of the zinc solution and 10 mL of 1.0 M KCl was analysed. The operating conditions were described by Schröder and Kahlert [92] and are shown in Table 3-5.

Table 3-5: Voltammetric conditions used to investigate effect of deposition time

Highest current range:	10 mA
Lowest current range:	100 nA
Electrode:	HDME
Drop Size (1-9):	3
Stirrer speed (rpm):	2000
Initial purge time (s):	300
Conditioning cycles	
Start potential (V):	0
End potential (V):	0
No. of cycles:	0
Hydrodynamic (measurement):	No
Cleaning potential (V):	0
Cleaning time (s):	0
Deposition potential (V):	-1.6
Deposition time (s):	
Sweep	
Equilibration time (s):	3.000
Start potential (V):	-1.5
End potential (V):	-0.9
Voltage step (V):	0.005
Voltage step time (s):	0.500
Sweep rate (V/s):	0.010
Pulse amplitude (V):	0.050
Pulse time (s):	0.040
Cell off after measurement:	Yes

The following deposition times were investigated: 30 s, 60 s, 120 s, 240 s, 480 s, 600 s, 900 s and 1200 s.

Effect of Initial Purge Time

A 5 mgL^{-1} solution of zinc was used to establish the effect that changing the initial purge time has on the peak height. In each case, 10 mL of the zinc solution and 10 mL of 1.0 M KCl was analysed. The operating conditions were described by Schröder and Kahlert [92] and are shown in Table 3-5.

Table 3-6: Voltammetric conditions used to investigate effect of initial purge time

Highest current range:	10 mA
Lowest current range:	100 nA
Electrode:	HDME
Drop Size (1-9):	3
Stirrer speed (rpm):	2000
Initial purge time (s):	
Conditioning cycles	
Start potential (V):	0
End potential (V):	0
No. of cycles:	0
Hydrodynamic (measurement):	No
Cleaning potential (V):	0
Cleaning time (s):	0
Deposition potential (V):	-1.6
Deposition time (s):	600
Sweep	
Equilibration time (s):	3.000
Start potential (V):	-1.5
End potential (V):	-0.9
Voltage step (V):	0.005
Voltage step time (s):	0.500
Sweep rate (V/s):	0.010
Pulse amplitude (V):	0.050
Pulse time (s):	0.040
Cell off after measurement:	Yes

The following initial purge times were investigated: 30 s, 60 s, 90 s, 100 s, 120 s, 180 s, 240 s.

Repeatability

To test the repeatability when using KCl, 10 mL of 1.0 M KCl plus 20 µL of 200 mgL^{-1} copper, lead and zinc mixed standard was analysed 10 times. This process was then repeated 5 times. The operating conditions used are shown in Table 3-6.

Table 3-7: Voltammetric conditions used to investigate repeatability

Highest current range:	10 mA
Lowest current range:	100 nA
Electrode:	HDME
Drop Size (1-9):	3
Stirrer speed (rpm):	2000
Initial purge time (s):	60
Conditioning cycles	
Start potential (V):	0
End potential (V):	0
No. of cycles:	0
Hydrodynamic (measurement):	No
Cleaning potential (V):	-1.200
Cleaning time (s):	5.000
Deposition potential (V):	-1.200
Deposition time (s):	60.000
Sweep	
Equilibration time (s):	5.000
Start potential (V):	-1.200
End potential (V):	0.000
Voltage step (V):	0.006
Voltage step time (s):	0.500
Sweep rate (V/s):	0.012
Pulse amplitude (V):	0.050
Pulse time (s):	0.040
Cell off after measurement:	Yes

3.1.3 Sample Preparation

Dry Ashing

Leaf

This work was conducted by Sarah Davies under the author’s direction. Initially leaf samples were ashed in a muffle furnace at a temperature of 450 °C [93] but subsequently a temperature of 550 °C was used.

Acid Digestion

Leaf

This work was conducted by Sarah Davies under the author's direction. The ashed sample was put into a 25 mL beaker and placed on a steam bath, then 2 mL of 2 M nitric acid was added [94]. After 5 minutes, a further 2 mL of 2 M nitric acid was added and after a total of 10 minutes, the solution was then filtered and made up to 25 mL using deionised water.

Soil

It was decided to investigate for how long the soil samples should be on the steam bath. After ashing at 550 °C for 90 minutes, the soil was ground using a pestle and mortar and ~2 g was accurately weighed into a 100 mL conical flask and 10 mL 2 M nitric acid was added. The sample was placed on a steam bath for 5 minutes. The process was repeated (using the same soil sample) and the following times were investigated: 10, 15, 20, 30, 40, 50 and 60 minutes.

3.1.4 Calculation of Cation Exchange Capacity of Soil

Measurement of pH

It was necessary to determine the pH of the soil so that the ammonium chloride solution, necessary for the leaching of the soil, could be adjusted to the same pH. 10 g of air-dry soil was placed in a 50 mL conical flask with 25 mL of deionised water and the conical flask was placed on a flask shaker for 15 minutes at 300 oscillations per minute. Once the soil had finished being shaken, the pH electrode was placed into the solution and the pH recorded after 30 seconds [95].

Leaching of Soil

A dry syringe, capillary tubing and two nylon discs were weighed. 4 g of air-dried soil was accurately weighed out, added to the syringe and compressed as shown in Figure 3-1.

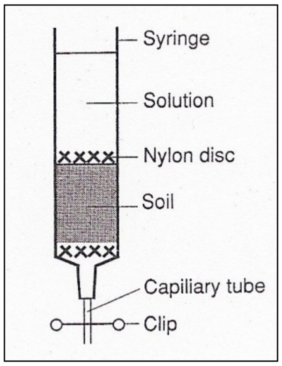


Figure 3-1: Experimental set-up for leaching of soil [95]

To ensure that the effective cation exchange capacity (ECEC) sites were saturated with NH_4^+, 25 mL of 50 mM NH_4Cl was poured into the tube and allowed to drain. To thoroughly wet the soil, 5 mL of 2 mM NH_4Cl was poured into the tube and as it began to run from the capillary tubing, a clip was applied and it was allowed to sit overnight. The following day, successive 5 mL volumes of 2 mM NH_4Cl were added over a period of 3 hours to leach the soil. This solution was allowed to run to waste.

After drainage had ceased, the syringe and capillary tubing was re-weighed to determine the volume of solution held in the soil. The soil was then leached with successive 5 mL volumes of 20 mM KNO_3 over a period of 3 hours. In this instance, all leachate was collected in a 100 mL volumetric flask then made up to volume using deionised water [95].

Distillation

The equipment was set up as shown in Figure 3-2.

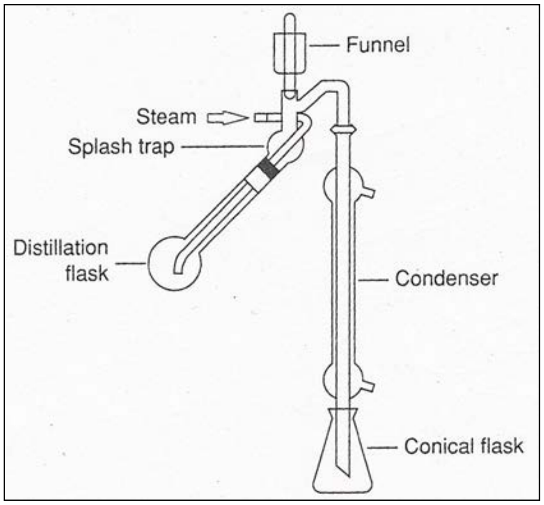


Figure 3-2: Equipment set-up for steam distillation [95]

Prior to analysis, the equipment was tested using a standard solution of known ammonia concentration.

In a 250 mL conical flask, 10 mL boric acid solution, 100 mL deionised water and a few drops of mixed indicator were added and the conical flask placed under the condenser. Into the funnel, 25 mL of the leachate was added and released into the distillation flask. The funnel was rinsed with deionised water and this was also released into the distillation flask. In the same way, 10 mL of NaOH solution was added to the distillation flask. Steam was passed and after the boric acid solution had changed colour from pink to green, 10 mL of distillate was collected. The end of the condenser was rinsed with deionised water into the conical flask containing the distillate [95].

The process was repeated using 25 mL of the 2 mM NH_4Cl solution in place of the leachate.

Titration

Both the distillate from the leachate and the distillate from the NH_4Cl were titrated against 0.01 M HCl until an end point was reached (when the indicator changed from a green colour through colourless to a pale pink [95]).

3.2 Main study

3.2.1 Soil preparation

The soil was air-dried to constant mass before being weighed into plastic buckets (5 kg of soil for each soil type). The appropriate volume of each metal solution was then added according to Table 3-8.

Table 3-8: Concentration and volume of each metal solution added to soil

	Volume of metal solution (L)			
	Copper (10 mgL^{-1})	Lead (33 mgL^{-1})	Potassium (13 mgL^{-1})	Zinc (11 mgL^{-1})
AA	10	0	0	0
AB	5	5	0	0
AC	5	0	0	5
AD	5	0	5	0
BB	0	10	0	0
BC	0	5	0	5
BD	0	5	5	0
CC	0	0	0	10
CD	0	0	5	5
EE	0	0	0	0

Once all of the metal solution had been absorbed, the soil for each soil type was divided between 4 plant pots.

3.2.2 Growing of *Papaver somniferum* L.

The pots of soil were all placed in a greenhouse. Initially, a large number of seeds were sown in each plant pot before being covered with a thin layer of soil from that pot. The plant pots were all placed on saucers so that none of the metals could be lost through watering. The plants were always watered on the same day and using triple distilled water from the same container. After 1 month of growing, excess seedlings were removed from each pot to leave 5 seedlings in each plant pot (a total of 20 in each soil type).

3.2.3 Collection of Samples

Soil

There were four plants pots for each of the soil types detailed in Table 3-8; from each of these pots, two soil samples were collected providing a total of eight soil samples for each soil type. The soil samples were named according to their soil type, plant pot number and repeat e.g. AA1_a was the name given to the first soil sample taken from soil type AA, plant pot 1.

Leaf

Two leaves were collected from each plant that successfully grew to maturity, i.e. flowered and produced a seed pod. The leaves collected were youngest fully expanded leaves (YFEL) as their trace metal concentrations are viewed as being independent of age [96]. The leaves were named according to their soil type, plant pot number, plant number and leaf number e.g. AA3_12 was the name given to leaf 2 taken from plant 1 from plant pot 3 for soil type AA.

Resin

Two weeks after the petals fell from the seed pod, the seed pods were examined; if the seed pods were dark green in colour and the points of the crown (see Figure 3-3) were either curving upwards or pointing straight out then the pods were ready to be scored. The blade (N^{o}11) of a Retractaway knife (obtained from Swann Morton, Sheffield, UK) was set to 1 mm and this was used to make a number of vertical scores in each seed pod. The scoring was done in the late afternoon so that the resin could seep out and dry on the surface of the seeds pod during the night; if this was done during the day the sun would cause the resin to coagulate and the flow would cease. The following morning, the resin was scraped from the seed pod. The process was repeated until the seed pods stopped producing resin [97]. All resin from the same plant was collected in the same container.

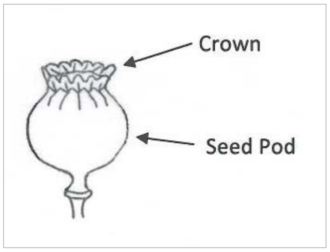


Figure 3-3: Diagram of a mature poppy seed pod

The resin samples were named according to their soil type, plant pot number and plant number e.g. AA3_1 was the name given to the resin sample taken from plant 1 from plant pot 3 for soil type AA.

3.2.4 Sample Preparation

Dry Ashing

Soil

The soil sample was placed into a porcelain crucible and placed in a furnace at 550 °C for 90 minutes. The sample was then ground using a pestle and mortar.

Leaf

The leaf sample was placed into a porcelain crucible and placed in a furnace at 550 °C for 90 minutes.

Acid Digestion

Soil

Approximately 1 g of oven-dried and sieved soil was accurately weighed into a 100 mL conical flask. 5 mL of 2 M nitric acid was added and the conical flask was placed on a steam bath for 30 minutes. The sample was filtered into a 25 mL volumetric flask using Whatman Nº 541 filter paper and then made up to volume using deionised water.

Leaf

Each individual leaf was air-dried to constant mass and then accurately weighed into a 25 mL beaker. 2 mL of 2 M nitric acid was added and the beaker was placed on a steam bath for 5 minutes after which another 2 mL of 2 M nitric acid was added to the beaker and it was kept on the steam bath for another 5 minutes. The sample was filtered into a 25 mL volumetric flask using Whatman Nº 541 filter paper and then made up to volume using deionised water.

Resin

Each individual resin sample was air-dried to constant mass and then accurately weighed into a 25 mL beaker. 2 mL of 2 M nitric acid was added and the beaker was placed on a steam bath for 5 minutes after which another 2 mL of 2 M nitric acid was added to the beaker and it was kept on the steam bath for another 5 minutes. The sample was filtered into a 10 mL volumetric flask using Whatman Nº 541 filter paper and then made up to volume using deionised water.

Blanks

In order to prepare blank solutions for analysis, the methods described above were repeated in all details except that the matrix being analysed (i.e. soil, leaf or resin) was omitted.

3.2.5 Voltammetric Conditions

The conditions shown in Table 3-9 were used for all analyses of soil, leaf, resin and blank solutions.

Table 3-9: Voltammetric conditions used for main study

Highest current range:	10 mA
Lowest current range:	100 nA
Electrode:	HDME
Drop Size (1-9):	3
Stirrer speed (rpm):	2000
Initial purge time (s):	60
Conditioning cycles	
Start potential (V):	0
End potential (V):	0
No. of cycles:	0
Hydrodynamic (measurement):	No
Cleaning potential (V):	-1.200
Cleaning time (s):	5.000
Deposition potential (V):	-1.200
Deposition time (s):	60.000
Sweep	
Equilibration time (s):	5.000
Start potential (V):	-1.200
End potential (V):	0.000
Voltage step (V):	0.006
Voltage step time (s):	0.500
Sweep rate (V/s):	0.012
Pulse amplitude (V):	0.050
Pulse time (s):	0.040
Cell off after measurement:	Yes

3.2.6 Standard Addition Method

For all analyses, 1 mL of sample was mixed with 9 mL 1.0 M KCl and analysed. Three standard additions were then performed using 20 µl of a mixed standard containing 20 mgL^{-1} each of copper, lead and zinc.

3.2.7 Determination of Available Metals in Soil

Soil Preparation

The soil was air-dried to constant mass before being weighed into 11 plastic beakers (~5 g of soil per beaker). Metal solutions were then added to each beaker as detailed in Table 3-10. Once all of the metal solutions had been absorbed, and left for 24 hours, the soils were left to air-dry before being extracted.

Table 3-10: Details of solutions added to soil for soil metal availability study

Beaker	Mass of Soil (g)	Solution Added	Volume Added (mL)	Concentration of Each Metal (mgL^{-1})
1	4.9664	Deionised water	10	0
2	4.9431	Mixed metal solution containing copper, lead and zinc in deionised water	10	10
3	4.9919		10	20
4	5.0139		10	40
5	5.1510		10	80
6	5.3486		10	160
7	4.9486	Zinc solution in deionised water	10	10
8	4.9249		10	20
9	4.9608		10	40
10	5.0622		10	80
11	5.0934		10	160

Extraction

Each air-dried soil sample (from Table 3-10) was placed in a bottle along with 25 mL of ammonia-EDTA solution. The solution was shaken for 1 hour at 20 °C then filtered through Whatman No. 40 filter paper. The filtrate was made back up to 25 mL using deionised water and retained for analysis [95]. The process was followed for the contents of all 11 beakers mentioned in “soil preparation” above. A “blank” was made following the same method as above but with the absence of soil.

Measurement by Flame Atomic Absorption Spectrometry

The blank, calibration standards and samples were all aspirated into the flame AAS. Three absorption readings were obtained for each solution and a mean reading calculated. Details of the flame AAS can be found in section 2.9.2.

3.2.8 Statistical Analysis

Unless stated otherwise, all statistical tests were carried out using Predictive Analytics SoftWare (PASW) Statistics 18 or IBM Statistical Package for the Social Sciences (SPSS) Statistics 19.

Removal of Outliers

A datum point was identified as being an outlier if it lay further than 1.5 interquartile ranges (IQR) beyond the 75th or 25th percentile [98]. If an outlier was identified, Grubbs’s Test was manually used to determine whether or not the value should be rejected. If the value of G was greater than $G_{critical}$, the value was rejected [99].

Assessing Normality

To test for normal distribution, the Kolmogorov-Smirnov One-Sample Test was used with a critical value, $\alpha = 0.05$. If the calculated *p*-value was greater than the critical value, the distribution of data was not found to be significantly different from a set of normally distributed data and was subsequently treated as if it were normally distributed.

Homogeneity of Variance

To test for homogeneity of variance, the Levene's test was used with a critical value, $\alpha = 0.05$. If the calculated *p*-value was greater than the critical value, the assumption of homogeneity of variance could not be rejected at a 95 % confidence level and was accepted as correct [100].

Multivariate Analysis of variance (MANOVA)

This test was performed with a critical value, $\alpha = 0.05$ but if the assumption of homogeneity of variance could be rejected at a confidence level of 95 % or more, a more conservative alpha level of 0.01 [101] was set. If the calculated *p*-value was less than or equal to the critical value, there was a significant difference somewhere amongst the mean scores. Due to the small sample sizes, Pillai's trace was used as it was more robust under these circumstances [100]. If a significant difference was found, the tests of between-subjects effects were consulted and if a significant difference was found, a *post-hoc* test was required to identify between which pair of results the difference had occurred.

Post-Hoc Tests

If a significant difference was found in a MANOVA, a *post-hoc* test was performed to identify between which pair of results the difference had occurred. The Tukey HSD *post-hoc* test was performed if the sample sizes were equal and the variance was homogeneous and if the sample sizes were unequal but the variance was homogeneous the Tukey-Kramer *post-hoc* test was performed [102]. The Games-Howell *post-hoc* test was performed if the sample sizes were unequal and the variance was heterogeneous at a confidence level of 95 % or greater [103]. As the Games-Howell *post-hoc* test makes allowances for number of comparisons, an alpha level of 0.05 was used.

Chapter 4 : Results and Discussion of Pilot Studies

4.1 Investigation into Which Metals to Analyse

According to Budic and Klemenc [67], the most abundant metals found in heroin were barium, calcium, magnesium and zinc whereas Zhang et al [70] found calcium, zinc and sodium to be the most abundant. Based on this information, it was decided to begin by devising approaches for analysing barium, calcium, magnesium, sodium and zinc using voltammetry as the analytical technique.

With both barium and sodium a peak was produced initially consistent with the published half-wave potential (E½) but when repeat analyses were performed the peak response was found to be variable especially when lower concentrations were considered. Zinc was found to be very repeatable; regardless of the concentration analysed, a peak was seen that was consistent with the published E½. No peak was observed for calcium or magnesium and it was decided to pursue the investigation of other metals that might be present in soil and be taken up by poppy plants during growth.

After further consideration of the findings of Budic and Klemenc [67] and Bora et al [59], it was decided to consider the metals aluminium, copper, manganese, nickel, cobalt and lead (in addition to zinc).

With both aluminium and cobalt a voltammetric peak was produced which was consistent with the published half-wave potential (E½) but the height of the peak was inconsistent, tending to increase in size as repeat analyses were performed at the same metal concentration. Copper, lead and nickel were found to be repeatable. A peak was seen that was consistent with the published E½, but this was not the case for manganese for which there were problems with obtaining a peak. In a personal communication it was suggested that the pulse amplitude should be a negative value rather than a positive value when analysing manganese [104]. However, even when this was changed, the results were still not repeatable.

Based on the above results, it was found that the most repeatable results were produced in the analysis of zinc, copper, nickel and lead. Of these metals, it was decided that zinc, lead and copper would be used for the study. The reason for not including nickel was that its half-wave potential was too similar to that of zinc to be able to differentiate between them; from the work of Budic and Klemenc [67] it was found that zinc was more abundant in heroin samples than nickel therefore it would be more appropriate to use zinc rather than nickel. The decision to use zinc, lead and copper was further justified after consulting the Afghanistan Mineral Occurrence Table [105] as these were found to be the most abundant metals found across the various provinces of Afghanistan, which at the time of writing was the world’s largest producer of heroin [21].

It was decided that *Papaver somniferum L*. plants would be grown in the soil types detailed in Table 4-1.

Table 4-1: Different soil types to be used

	Metal added			
	Copper	Lead	Potassium	Zinc
AA	●			
AB	●	●		
AC	●			●
AD	●		●	
BB		●		
BC		●		●
BD		●	●	
CC				●
CD			●	●
EE				

Potassium was added to some of the pots of soil to see if its presence had any effect on the uptake of the other metals being added.

4.2 Development of the Voltammetric Method

4.2.1 Determination of Limit of Detection for Potassium Chloride

An equation taken from Skoog et al [106], was used to calculate the copper, lead and zinc detection limit s (at a 95 % confidence level) of the method for a single analysis. The number of degrees of freedom was 9 therefore t = 2.26.

Table 4-2: Results for limit of detection of copper

Concentration values (μgL^{-1}):	12.6, 27.1, 27.3, 41.8, 40.3, 51.7, 39.4, 46.3, 33.1, 37.8
Mean (μgL^{-1}):	35.74
Standard deviation:	11.20
Δx_{min} (μgL^{-1}):	26.55

Table 4-3: Results for limit of detection of lead

Concentration values (μgL^{-1}):	17.4, 8.3, 18.3, 18.2, 20.4, 24.7, 0.3, 29.0, 16.3, 20.1
Mean (μgL^{-1}):	17.3
Standard deviation:	8.04
Δx_{min} (μgL^{-1}):	19.1

Table 4-4: Results for limit of detection of zinc

Concentration values (μgL^{-1}):	2.7, 18.0, 6.2, 5.2, 6.5, 20.1, 9.5, 8.4, 9.7, 10.8
Mean (μgL^{-1}):	9.71
Standard deviation:	5.49
Δx_{min} (μgL^{-1}):	13.01

This means that 95 times out of 100, a result greater than 26.55μgL^{-1} would indicate the presence of copper, a result greater than 19.1μgL^{-1} would indicate the presence of lead and a result greater than 13.01μgL^{-1} would indicate the presence of zinc.

4.2.2 Effect of Deposition Potential

Figure 4-1 shows the results of this study. Initially, as the deposition potential became more negative, the peak height increased but after a deposition potential of -1.60 V, the peak height began to decrease as the deposition potential became more negative. The decrease in peak height may have been due to the mercury drop getting saturated at such a high deposition potential [107]

The chosen deposition potential should be sufficiently negative so as to reduce the metal ions to the metal [86] at the surface of the mercury drop and, since it is really only necessary to select a deposition potential that would provide a significant peak height without saturating the mercury drop at higher concentrations, a value of -1.2 V was chosen as the deposition voltage. It should be noted that the data points on Figure 4-1 are based on single measurements rather than mean values which may account for the unexpectedly lower peak height obtained for -1.5 V.

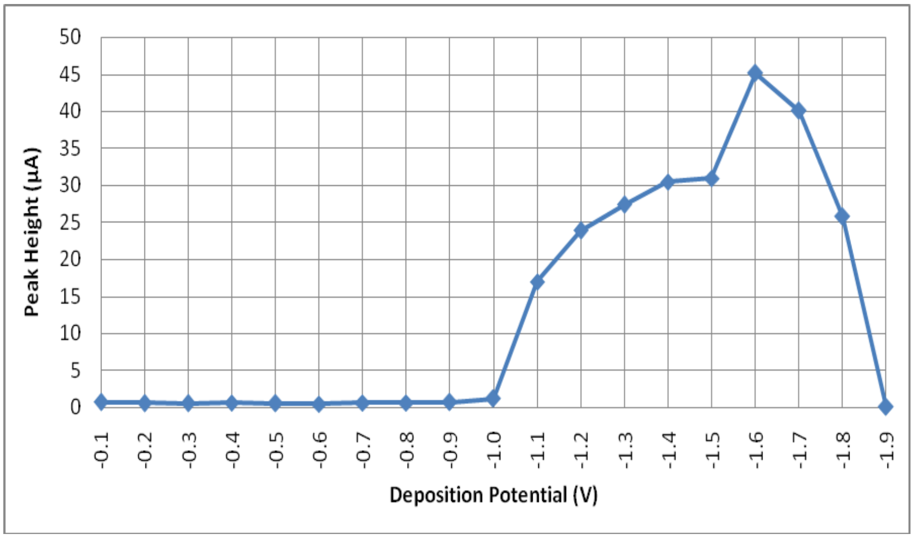


Figure 4-1: Effect of deposition potential on peak height of zinc

4.2.3 Effect of Deposition Time

The results of this study are displayed in Figure 4-2. Initially, as the deposition time increased, the peak height increased but after a deposition time of 900s, the peak height began to decrease with increased deposition time. The decrease in peak height was probably as a result of the mercury drop becoming saturated at a longer deposition time.

As with any analytical method, it is important that the total run time is as efficient as possible but to avoid additional problems associated with the deposition step, long deposition times should be avoided [86] . It was important to select a deposition time that, in combination with deposition potential, would provide a sufficient peak height without resulting in a long run time, therefore a deposition time of 60 seconds was chosen. It should be noted that the data points on Figure 4-2 are based on single measurements rather than mean values.

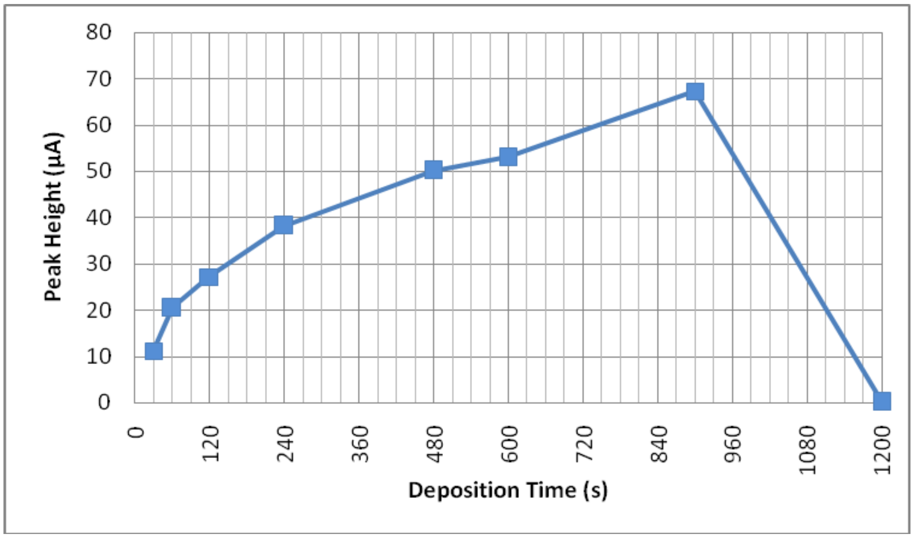


Figure 4-2: Effect of deposition time on peak height of zinc

4.2.4 Effect of Initial Purge Time

The results are depicted in Figure 4-3. There was a significant difference in peak height between 30 s and 60 s, but thereafter between 60 s and 20 s the values become relatively constant. Based on analytical efficiency, the shortest time of 60s when results became constant was adopted. It should be noted that the data points on Figure 4-3 are based on single measurements rather than mean values.

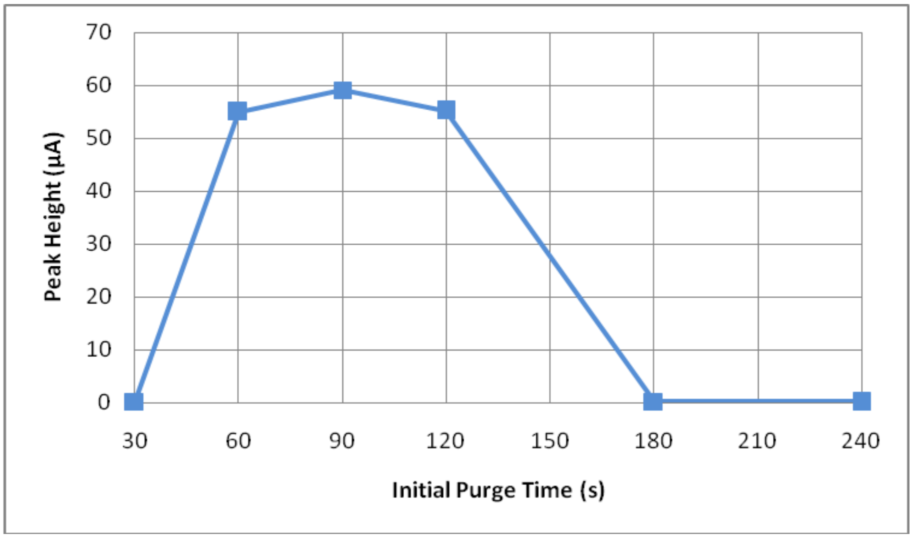


Figure 4-3: Effect of initial purge time on peak height of zinc

4.3 Repeatability

Tables 4-5 – 4-9 show the individual results with Table 4-10 showing the overall results.

Table 4-5: Repeatability results 1

Metal	E ½ (v)			I (µA)		
	Mean	SD	% RSD	Mean	SD	% RSD
Zinc	-1.010	0.0052	0.5	1.17	0.0343	2.9
Lead	-0.438	0.0000	0.0	0.65	0.0231	3.6
Copper	-0.200	0.0000	0.0	0.45	0.0127	2.8

Table 4-6: Repeatability results 2

Metal	E ½ (v)			I (µA)		
	Mean	SD	% RSD	Mean	SD	% RSD
Zinc	-1.010	0.0000	0.0	1.31	0.0300	2.3
Lead	-0.438	0.0000	0.0	0.63	0.0103	1.6
Copper	-0.200	0.0000	0.0	0.43	0.0074	1.7

Table 4-7: Repeatability results 3

Metal	E ½ (v)			I (µA)		
	Mean	SD	% RSD	Mean	SD	% RSD
Zinc	-1.010	0.0032	0.3	1.37	0.1036	7.6
Lead	-0.438	0.0000	0.0	0.69	0.0151	2.2
Copper	-0.200	0.0000	0.0	0.46	0.0105	2.3

Table 4-8: Repeatability results 4

Metal	E ½ (v)			I (µA)		
	Mean	SD	% RSD	Mean	SD	% RSD
Zinc	-1.010	0.0000	0.0	1.29	0.0576	4.5
Lead	-0.438	0.0000	0.0	0.65	0.0099	1.5
Copper	-0.200	0.0000	0.0	0.44	0.0095	2.2

Table 4-9: Repeatability results 5

Metal	E ½ (v)			I (µA)		
	Mean	SD	% RSD	Mean	SD	% RSD
Zinc	-1.010	0.0000	0.0	1.34	0.0183	1.4
Lead	-0.438	0.0000	0.0	0.66	0.0143	2.2
Copper	-0.200	0.0000	0.0	0.45	0.0084	1.9

Table 4-10: Overall repeatability results

Metal	E ½ (v)			I (µA)		
	Mean	SD	% RSD	Mean	SD	% RSD
Zinc	-1.010	0.0030	0.3	1.29	0.0865	6.7
Lead	-0.438	0.0000	0.0	0.65	0.0228	3.5
Copper	-0.200	0.0000	0.0	0.44	0.0141	3.2

According to the modified Horwitz equation [108], the acceptable % RSD for this method is 12.3 %. The results in Table 4-10 show that when using the method described in Section 3.1.2

and potassium chloride as an electrolyte, the results for zinc, lead and copper were less than the acceptable % RSD and are therefore considered to be repeatable. When compared to the % RSD values for copper, lead and zinc obtained using ICP-MS [66] (Table 4-11), the values obtained using voltammetry were lower for copper and lead but slightly higher for zinc.

Table 4-11: Comparison of % RSD values for voltammetry and ICP-MS

Metal	**% RSD**	
	Voltammetry	**ICP-MS**
Copper	3	5
Lead	4	94
Zinc	7	3

4.4 Sample Preparation

4.4.1 Dry Ashing

Leaf

According to Karam [109], dry ashing can be used to oxidize organic materials using a muffle furnace then ions can be extracted using a solution of acid. When a muffle furnace temperature of 450°C was used (and acid digest was performed), the resulting solution had a green-yellow appearance which was attributed to the presence of chlorophyll [110].

When the sample was analysed using the polarograph, it was found that the peaks for copper and zinc did not increase, even with the addition of a mixed standard. Also, the zinc peak was present at a more negative half-wave potential than was expected, suggesting that a zinc complex had been formed [106]; according to Petrovic et al [111], zinc and copper both form complexes with chlorophyll. It was therefore decided to try ashing the samples at a higher temperature to ensure that the chlorophyll had been destroyed and would consequently not interfere with analysis.

Following the same procedure as above but at the higher muffle furnace temperature of 550°C, a colourless solution was obtained. When the sample was analysed (using the polarograph), the metal peaks were as expected and they increased accordingly when the mixed standard was added. It was therefore decided that for “main study” analyses, a muffle furnace temperature of 550°C (for 90 minutes) would be used.

Soil

It was decided to apply the method that had been developed for the leaf samples to the soil samples. It was found that ashing the soil samples at 550°C for 90 minutes was sufficient to ash all of the sample if the crucible was initially half-filled with soil.

Resin

Due to the small masses of resin collected, it was decided not to ash the resin samples prior to digesting them with the acid.

4.4.2 Acid Digestion

Leaf

It was found that the method described in Section 3.2.4 was suitable for the preparation of the leaf samples.

Soil

It was decided to investigate for how long the soil samples should be on the steam bath. The method used is described in Section 3.1.3 and the results are shown graphically in Figure 4-4.

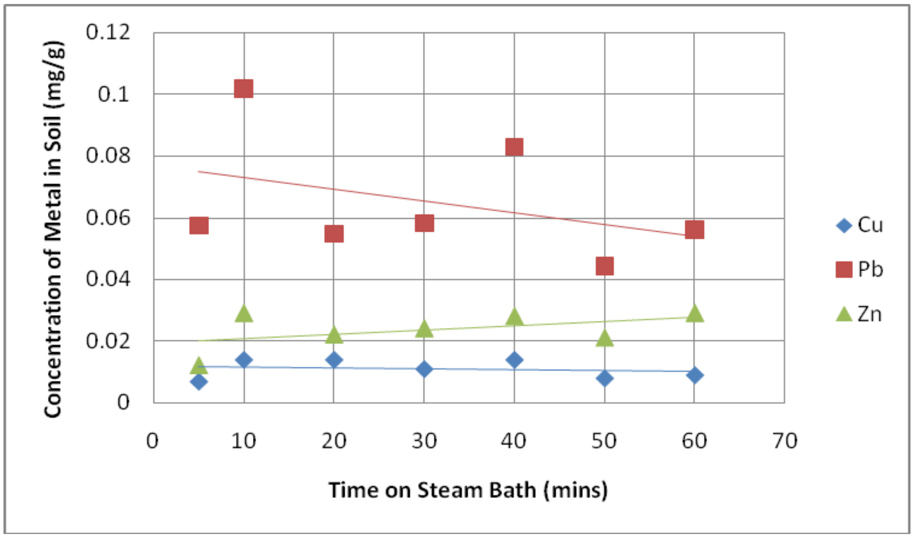


Figure 4-4: Effect of acid digest time on amount of metal present

For zinc and copper it can be seen that between 5 and 10 minutes, there is a gradual increase in the amount of metal present but after 10 minutes, the amount of metal present does not appear to change. For lead, the same trend was mainly observed. From the graph it can be seen that the values for 10 minutes and 40 minutes were higher than expected and it was thought that pockets of lead were forming in the soil.

Based on the results above, an acid digest time of 30 minutes was used for all other soil sample analyses. Due to the lead results showing greater variation than the other two metals, all soil samples were analysed in duplicate and the potential for pockets of lead noted.

Resin

Due to the small quantities of resin available, it was decided to try the same method of digestion that had been used for the leaf samples. After 10 minutes, most of the resin had dissolved and only a small amount of orange-coloured precipitate remained. As this precipitate remained even when longer acid digestion times were tested, it was decided that the method used for the preparation of the leaf samples was adequate to use for the preparation of the resin samples.

4.5 Calculation of Cation Exchange Capacity of Soil

It was necessary to determine the pH of the soil so that the ammonium chloride solution, necessary for the leaching of the soil, could be adjusted to the same pH. The pH of the soil was found to be 5.21.

The weights of soil as a result of leaching can be seen in Table 4-12 and the titre values from the titration can be seen in Table 4-13.

Table 4-12: Resulting weights from soil leaching

Weight soil:	4.0555 g
Weight dry syringe:	24.7561 g
Weight wet syringe:	28.2798 g
Difference:	3.5237 g

Table 4-13: Titration results

Sample:	Titre (mL)		
	1	2	Mean
Leachate	6.60	6.75	6.68
NH_4Cl	5.10	5.00	5.05

The cation exchange capacity of the soil was found to be 6.41 $cmolkg^{-1}$ for monovalent ions (the calculations for determining cation exchange capacity can be found in Appendix 1).

4.6 Amount of soil required

To grow 20 *Papaver somniferum* L. plants in each soil type, a total of 40 pots of soil were required (4 for each soil type). For each soil type, a total of 5kg of air-dried soil would be required.

4.7 Metal solutions added to soil

It was decided that the volume of metal solution added to each 5 kg of soil (10 L) and the resulting percentage of cation exchange sites occupied (1 %) were kept constant. The concentration and volume of each of the metal solutions used are shown in Table 3-8 (calculations are shown in Appendix 2).

Chapter 5 : Results and Discussion of Main Study

5.1 Use of Voltammetry

Voltammetry is not widely used in forensic science but its main application is in the analysis of gunshot residue as demonstrated by Liu et al [112], Woolever et al [113], Brihaye [114] and Woolever and Dewald [115]. There have also been studies related to the use of voltammetry in the determination of cadmium poisons in blood [116] as well as the identification and quantification of drugs; de Carvalho [117] made use of a hanging mercury dropping electrode (HMDE) to detect the use of 1,4-benzodiazepines as adulterants in slimming formulations, Oiye et al [118] detected cocaine using a cobalt hexacyanoferrate film-modified electrode and El-Maali [84] made use of adsorptive stripping voltammetry to analyse various pharmaceuticals . Voltammetry has also been used by Sharma and Rajpal [119]to analyse trace metals in soil and by Locatelli [120] to determine trace metals in food, plant tissue and soil.

There are many advantages of using voltammetry compared to other techniques. The technique is non-destructive [121] and field-portable [122] which are important attributes for a technique used in forensic analyses. The ability to be able to analyse a sample in under three minutes helps to improve sample through-put and hence reduce cost but it is also useful when analysing samples that can deteriorate with time [123]. Voltammetric methods also offer high sensitivity as anodic stripping voltammetry allows determinations down to 10^{-10} M [119].

Voltammetry is able to simultaneously determine a number of heavy metals [122] and whereas techniques such as AA can only provide information relating to the total concentration of an element, voltammetry can also provide information relating to the oxidation state of the analyte [123].

The instrumentation used in voltammetry costs a fraction of that required for analysis by GC, HPLC or AA [123] and modern voltammetric instrumentation has enabled analysis to become automated.

5.2 Removal of Outliers

It was decided that any datum points identified and confirmed as being an outlier would be removed. A number of the statistical tests used are sensitive to outliers [100] and it was also felt that the multiple stages of preparation involved made it more likely that a human error had occurred. Tables showing descriptive statistics for soil, leaf and resin data before and after outlier removal can be found in Appendix 3.

5.3 Metal Availability

Flame AAS data and calibration graphs can be found in Appendix 4.

Figure 5-1 and Figure 5-2 both show that there was a positive, linear relationship between the concentration of metal added to the soil and the concentration available to plants. This demonstrated that the more metal that was present in the soil, the more metal would be available to the plants. However the graphs showed that the concentrations of available metal were less than the concentration of metals added to the soil indicating that some of the metals were becoming bound in the soil and were therefore not all available to the plants.

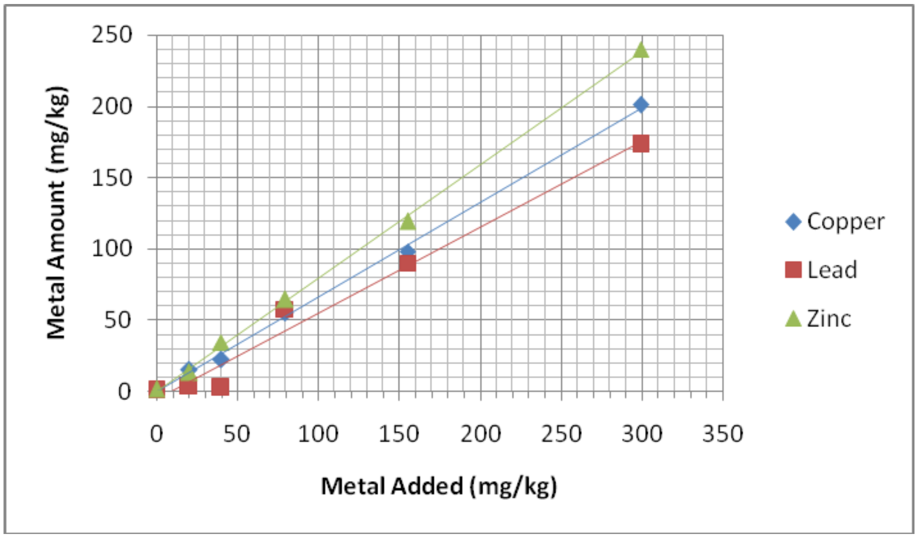


Figure 5-1: The relationship between the concentration of metal added to the soil and the concentration of available metal in the soil when copper, lead and zinc were added

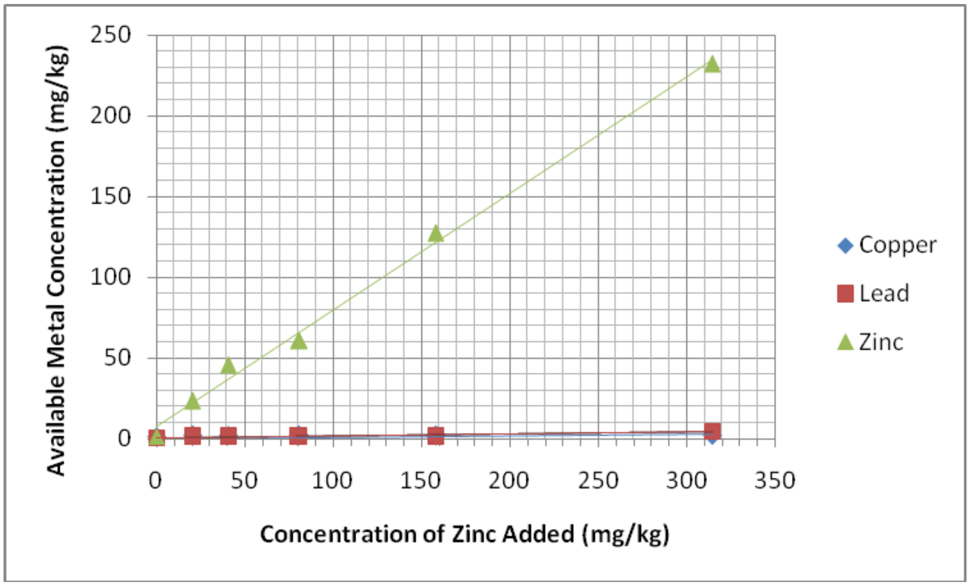


Figure 5-2: The relationship between the concentration of metal added to the soil and the concentration of available metal in the soil when zinc was added

5.4 Metal Concentrations in Soil

All raw soil data can be found in Appendix 5. In this section, all concentrations (in µmolg^{-1}) are of oven-dried soil.

As shown in Figure 5-3, in all oven-dried soil samples (except AA) the mean total zinc concentrations were significantly higher (at a confidence level ≥95 %) than the mean total copper concentrations and in all soil types (except BB) there were significantly higher zinc concentrations than lead. However, as the results of EE (no metals added) showed, this was to be expected as there was a greater concentration of zinc present in the soil to begin with compared with copper and lead.

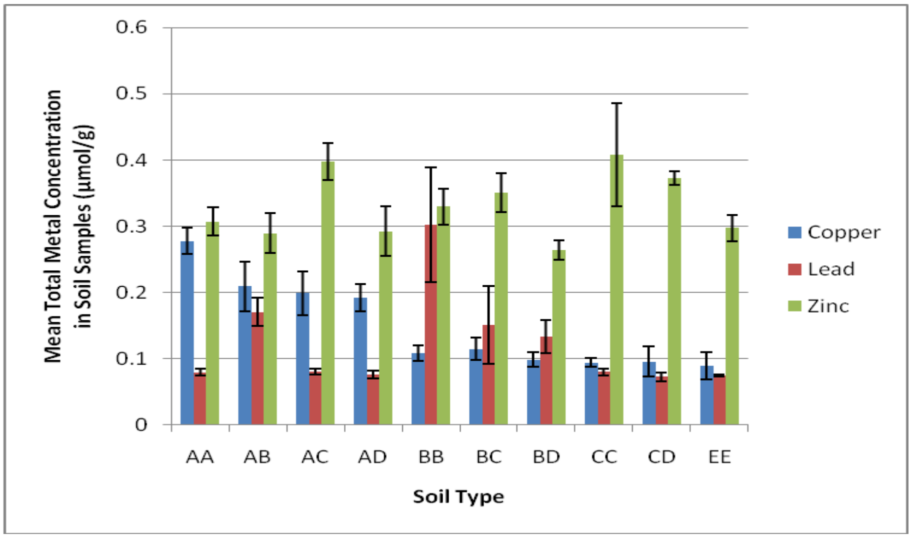


Figure 5-3: Mean total concentrations of copper, lead and zinc in oven-dried soil samples

A one-way between-groups multivariate analysis of variance (MANOVA) was performed to investigate differences in copper, lead and zinc concentration of different soil types. Three dependent variables were used; copper concentration, lead concentration and zinc concentration (all micromoles per gram). The independent variable was soil type. There was a statistically significant difference between soil types on the combined dependent variables (Table 5-1) and when the results for the dependent variables were considered separately, all three showed a statistically significant difference using a Bonferroni adjusted alpha level of 0.001 (Table 5-2). Partial eta squared represents the proportion of the variance of the dependent variable that can be explained by the independent variable, in this case soil type. According to Cohen [124], a partial eta squared value of 0.138 or more demonstrates a large effect size therefore, as all effect sizes shown are large, it was assumed that the differences in copper, lead and zinc concentration were responsible for the significant differences seen between soil types.

Table 5-1: Results of the multivariate test performed on soil data

Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type – Pillai's Trace	2.18	20.00	27	204	0.000	0.73

Table 5-2: Results of the tests of between-subjects effects performed on soil data

Source	Dependant Variable	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type	Copper	50.36	9	68	0.000	0.87
Soil Type	Lead	23.63	9	68	0.000	0.76
Soil Type	Zinc	10.97	9	68	0.000	0.59

Post-hoc comparisons using Games-Howell test were performed and the results are shown in Table 5-3, Table 5-4 and Table 5-5.

Table 5-3: Results of Games-Howell *post-hoc* test showing significance values for copper concentration of soil samples

	AA									
AB	0.061	AB								
AC	0.011	1.000	AC							
AD	0.000	0.989	1.000	AD						
BB	0.000	0.006	0.004	0.000	BB					
BC	0.000	0.008	0.006	0.000	0.999	BC				
BD	0.000	0.003	0.002	0.000	0.905	0.688	BD			
CC	0.000	0.003	0.002	0.000	0.366	0.303	0.996	CC		
CD	0.000	0.002	0.002	0.000	0.969	0.854	1.000	1.000	CD	
EE	0.000	0.001	0.001	0.000	0.622	0.430	0.984	1.000	1.000	EE

🟨 Significant difference, expected

🟩 Significant difference, not expected

🟦 No significant difference, expected

🟥 No significant difference, not expected

Figure 5-4: Key for understanding colours used in results of *post-hoc* tests

Table 5-4: Results of Games-Howell *post-hoc* test showing significance values for lead concentration of soil samples

	AA									
AB	0.000	AB								
AC	1.000	0.000	AC							
AD	0.971	0.000	0.808	AD						
BB	0.009	0.123	0.009	0.008	BB					
BC	0.263	0.998	0.272	0.222	0.093	BC				
BD	0.011	0.228	0.013	0.007	0.037	0.999	BD			
CC	1.000	0.000	1.000	0.919	0.009	0.269	0.012	CC		
CD	0.752	0.000	0.510	0.998	0.007	0.194	0.005	0.637	CD	
EE	0.586	0.000	0.108	1.000	0.008	0.208	0.007	0.361	1.000	EE

Table 5-5: Results of Games-Howell *post-hoc* test showing significance values for zinc concentration of soil samples

	AA									
AB	0.974	AB								
AC	0.001	0.001	AC							
AD	0.996	1.000	0.004	AD						
BB	0.851	0.431	0.023	0.663	BB					
BC	0.208	0.080	0.225	0.193	0.949	BC				
BD	0.043	0.749	0.000	0.812	0.009	0.002	BD			
CC	0.232	0.132	1.000	0.157	0.500	0.811	0.050	CC		
CD	0.002	0.005	0.586	0.022	0.107	0.777	0.000	0.975	CD	
EE	0.997	1.000	0.000	1.000	0.443	0.065	0.123	0.163	0.000	EE

5.4.1 Copper

The total concentration of copper in the soil samples corresponded to the concentration of copper added to the soil. As shown by Figure 5-5, AA had the highest concentration of copper, AB, AC and AD had similar concentrations of copper and the remaining samples also had similar concentrations of copper (but less than AB, AC and AD). However, the results of the *post-hoc* test (Table 5-3) showed that there was no significant difference between the concentration of copper in AA and the concentration of copper in AB; this finding was unexpected as a higher concentration of copper was added to AA than was added to AB. All of the other results were in line with expectations in terms of the concentration of copper added to the soil.

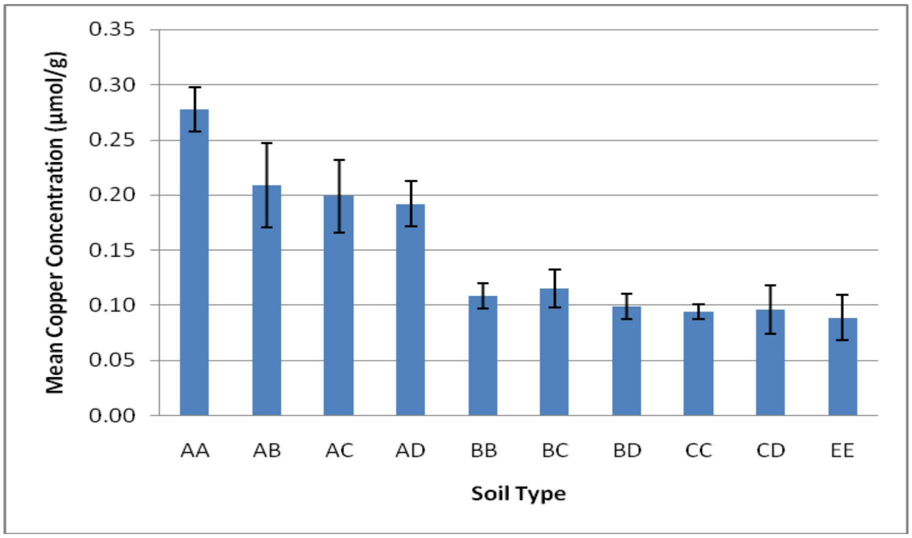


Figure 5-5: Mean total concentration of copper in soil samples (with 95 % confidence intervals)

5.4.2 Lead

The total concentration of lead in the soil samples also corresponded to the concentration of lead added to the soil. As shown by Figure 5-6. BB had the highest concentration of lead, AB, BC and BD had similar concentrations of lead and the remaining samples also had similar concentrations of lead (but less than AB, BC and BD). However, the results of the *post-hoc* test (Table 5-4) showed that there was no significant difference between the concentration of lead in BB and AB and due to it’s large variance, the concentration of lead, BC was not significantly different from the lead concentration in samples AA, AC, AD, BB, CC, CD or EE. All of the other results were as expected in terms of the concentration of lead added to the soil.

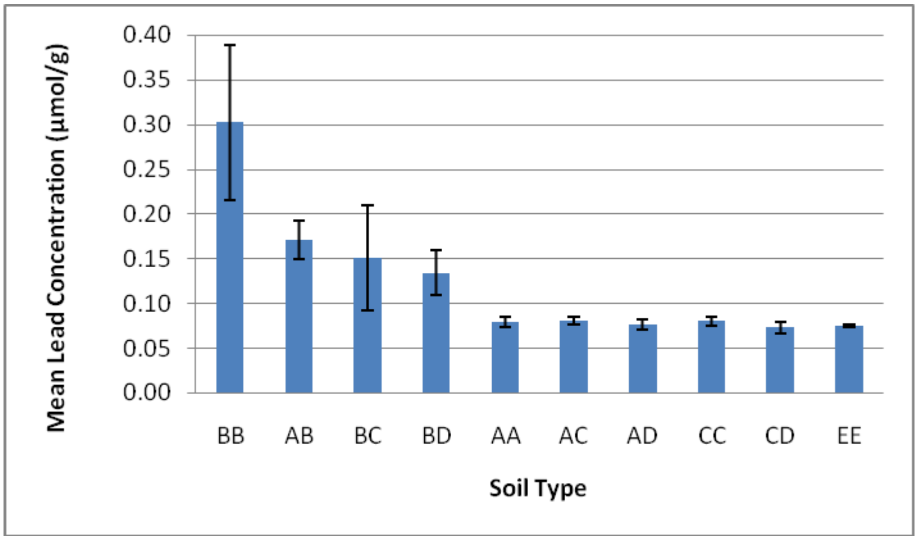


Figure 5-6: Mean total lead concentration in soil samples (with 95 % confidence intervals)

5.4.3 Zinc

The trends in results for zinc were not as clear as those for copper and lead. Although CC should have had the highest concentration of zinc, Figure 5-7 showed that the value was not significantly greater than the concentration of zinc in AC, BC, CD or BB and the results of the *post-hoc* test (Table 5-5) showed that the concentration of zinc in CC was not significantly greater than the concentration of zinc in any of the other samples. As expected, the zinc concentrations for AC and CD were significantly greater than the zinc concentrations for the other samples (except CC) but the concentration of zinc for BC was not significantly greater than the zinc concentrations of AA, AB, AD, BB and EE. This was probably due to the higher concentration of zinc present in the soil to begin with (as shown in Figure 5-3), lessening the impact of the zinc that was added.

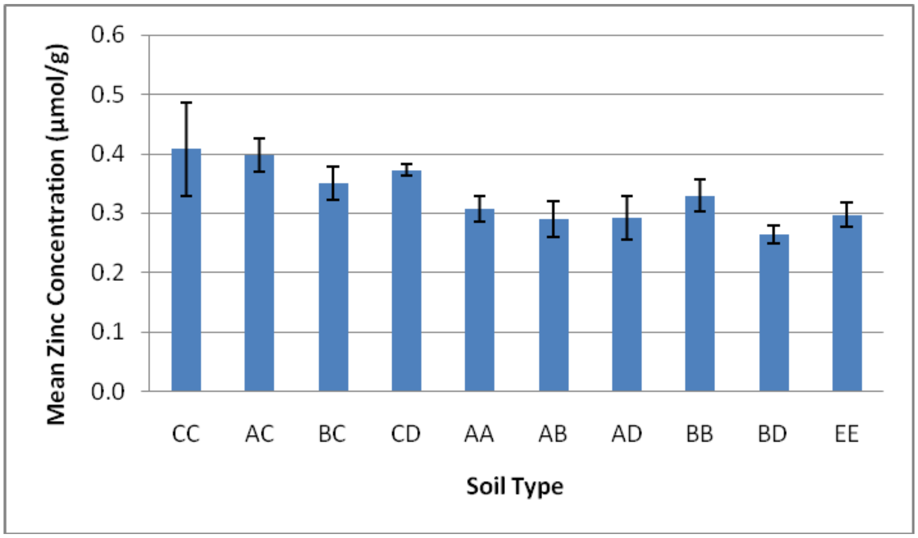


Figure 5-7: Mean total zinc concentration in soil samples (with 95 % confidence intervals)

5.4.4 Assessment of Soil Types

Most soil types can be distinguished between using the concentration of one or more metal. However, according to these results, BB & BC, BC & CC, BC & EE, CC & CD and CC & EE could not be distinguished by their copper, lead or zinc concentrations.

Due to the inability to differentiate between the different soil samples mentioned above, the MANOVA was re-calculated with soil types BC and CC excluded. The results are shown in Table 5-6 and Table 5-7.

Table 5-6: The results of the multivariate test performed on soil data (excluding BC & CC)

Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type – Pillai's Trace	2.34	27.14	21	162	0.000	0.78

Table 5-7: Results of the tests of between-subjects effects performed on soil data (excluding BC & CC)

Source	Dependant Variable	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type	Copper	47.90	7	54	0.000	0.86
Soil Type	Lead	31.86	7	54	0.000	0.81
Soil Type	Zinc	17.64	7	54	0.000	0.70

There was a statistically significant difference between soil types on the combined dependent variables (Table 5-6) and when the results for the dependent variables were considered separately, all three showed a statistically significant difference using a Bonferroni adjusted alpha level of 0.001 (Table 5-7). As before, large effect sizes indicated that the concentrations of copper, lead and zinc were responsible for the significant differences observed between soil types.

Post-hoc comparisons using Games-Howell test were performed and the results are shown in Table 5-8, Table 5-9 and Table 5-10.Reference should be made to Figure 5-4 for an explanation of the colours used.

Table 5-8: Results of Games-Howell *post-hoc* test showing significance values for copper concentration of soil samples (excluding BC & CC)

	AA							
AB	0.045	AB						
AC	0.008	1.000	AC					
AD	0.000	0.972	1.000	AD				
BB	0.000	0.004	0.003	0.000	BB			
BD	0.000	0.002	0.002	0.000	0.838	BD		
CD	0.000	0.001	0.001	0.000	0.936	1.000	CD	
EE	0.000	0.001	0.001	0.000	0.530	0.962	0.999	EE

Table 5-9: Results of Games-Howell *post-hoc* test showing significance values for lead concentration of soil samples (excluding BC & CC)

	AA							
AB	0.000	AB						
AC	1.000	0.000	AC					
AD	0.937	0.000	0.722	AD				
BB	0.006	0.095	0.007	0.006	BB			
BD	0.008	0.174	0.009	0.005	0.028	BD		
CD	0.661	0.000	0.425	0.991	0.005	0.003	CD	
EE	0.499	0.000	0.082	0.999	0.006	0.005	0.999	EE

Table 5-10: Results of Games-Howell *post-hoc* test showing significance values for zinc concentration of soil samples (excluding BC & CC)

	AA							
AB	0.942	AB						
AC	0.001	0.000	AC					
AD	0.988	1.000	0.002	AD				
BB	0.772	0.346	0.016	0.566	BB			
BD	0.031	0.660	0.000	0.731	0.007	BD		
CD	0.001	0.003	0.498	0.017	0.081	0.000	CD	
EE	0.990	0.999	0.000	1.000	0.359	0.092	0.000	EE

Although AB & BB could not be distinguished by their lead values (Table 5-9), they could be distinguished by their copper values (Table 5-8). Moreover, although BB & CD could not be distinguished by their zinc values (Table 5-10), they could be distinguished by their lead values (Table 5-9).

As a result of these findings, only the data from soil types AA, AB, AC, AD, BB, BD, CD and EE were considered when classification techniques were explored.

5.5 Metal Concentrations in Leaf

All raw leaf data can be found in Appendix 6. All concentrations (in μmolg^{-1}) are of air-dried leaf.

Figure 5-8 shows that only leaf sample AD had a significantly higher (at a confidence level ≥95 %) mean concentration of total copper compared with lead and zinc. Only samples BB, CC and CD contained a significantly higher mean concentration of total zinc compared with lead.

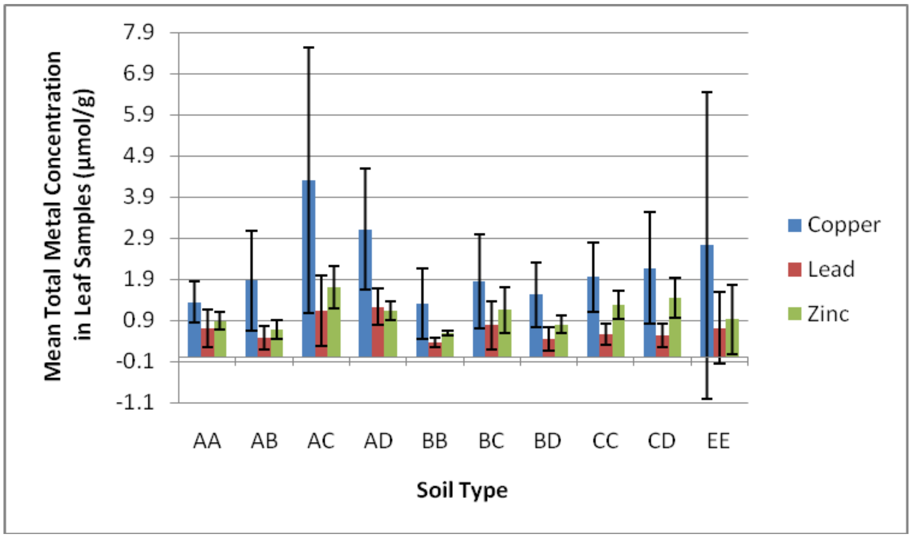


Figure 5-8: Mean total concentrations of copper, lead and zinc in air-dried leaf samples

As copper is an essential micronutrient that is used by plants in various functions such as oxidation and photosynthesis [125], it was expected that the mean concentration of total copper in the leaf samples would have been significantly higher than the mean concentration of total lead (which does not serve a purpose in plants [126]) for all soil types. Zinc is also an essential micronutrient and is used by plants to metabolise carbohydrates and proteins [125] so it was also expected that the mean concentration of total zinc present in the leaves would have been significantly greater than the mean concentration of total lead for all soil types.

One explanation for the greater concentration of copper compared with zinc was that the two metals use the same carrier sites for adsorption and therefore would be in direct competition with one another [127]. Also, as copper plays a significant role in photosynthesis [125], (the main site of which is in the leaves), it was not unexpected to find more copper than zinc in the leaf samples.

A one-way between-groups multivariate analysis of variance was performed to investigate differences in copper, lead and zinc concentration of leaves from plants grown in different soil types. Three dependent variables were used; copper concentration, lead concentration and zinc concentration (all micromoles per gram). The independent variable was soil type. There was a statistically significant difference between the leaves from the plants grown in the different soil types on the combined dependent variables (Table 5-11) but when the results for the dependent variables were considered separately, all three showed statistically no significant difference using a Bonferroni adjusted alpha level of 0.001 (Table 5-12) although it should be noted that for zinc concentration, the significance was exactly 0.001.

Table 5-11: The results of the multivariate test performed on leaf data

Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type – Pillai's Trace	0.79	2.20	27	165	0.001	0.26

Table 5-12: Results of the tests of between-subjects effects performed on leaf data

Source	Dependant Variable	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type	Copper	1.53	9	55	0.161	0.20
Soil Type	Lead	2.06	9	55	0.049	0.25
Soil Type	Zinc	3.65	9	55	0.001	0.37

5.5.1 Copper

Figure 5-9 shows that with the exception of EE, all leaf samples contained a significantly higher (at a confidence level ≥95 %) mean total copper concentration than the corresponding soil samples. This demonstrated that bioaccumulation of copper took place.

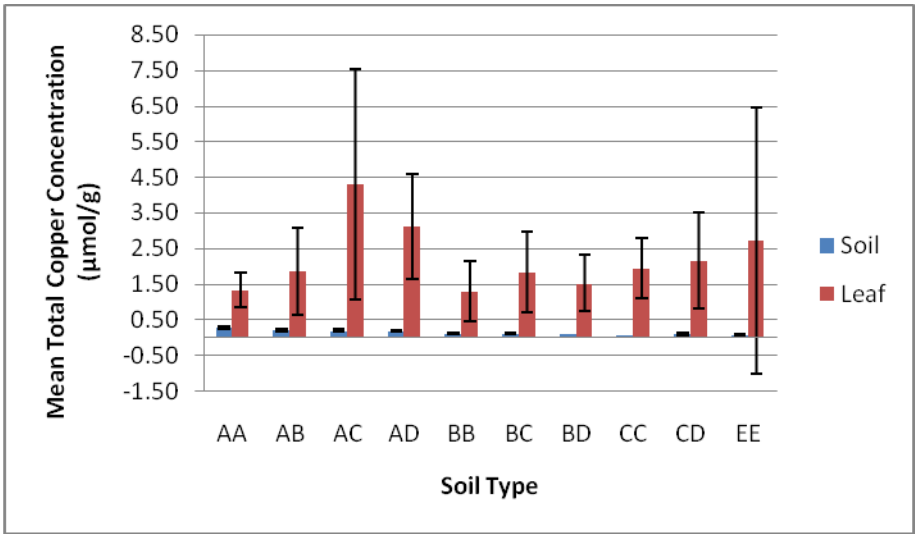


Figure 5-9: Mean total concentration of copper in oven-dried soil and air-dried leaf samples (with 95 % confidence intervals)

The concentration of copper in the leaf samples was not what was expected; leaves from soil type AA should have had the highest copper concentration, leaves from soil types AB, AC and AD should have had equal concentrations and the leaves from the remaining soil types should also have contained equal concentrations (but less than AB, AC and AD).

As can be seen from Figure 5-9, there was no significant difference in the mean concentration of total copper present in the leaf samples from the different soil types. This could have been due to all soil types containing a greater concentration of copper than was required by the plants and the plants only taking up as much as was necessary rather than taking up as much as was available.

5.5.2 Lead

Figure 5-10 shows that with the exception of BB, BC and EE, all leaf samples contained a significantly higher (at a confidence level of ≥95%) mean total lead concentration than the corresponding soil samples. This demonstrated that in most cases, bioaccumulation of lead took place.

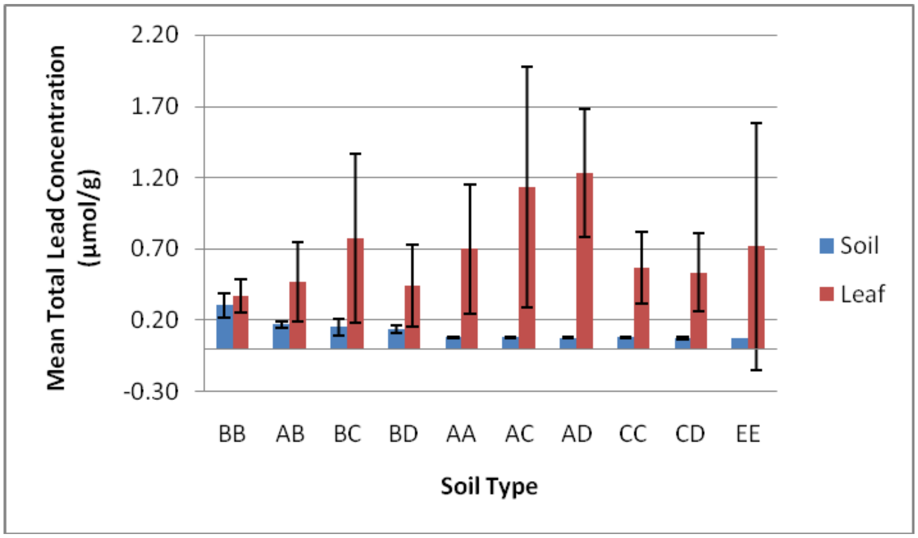


Figure 5-10: Mean total concentration of lead in oven-dried soil and air-dried leaf samples (with 95 % confidence intervals)

The concentration of lead in the leaf samples was not as might have been expected; leaves from soil type BB should have had the highest lead concentration, leaves from soil types AB, BC and BD should have had equal concentrations and the leaves from the remaining soil types should also have contained equal concentrations (but less than AB, BC and BD). However, as shown in Figure 5-10, there was a significant difference between the leaves from soil types BB and AD.

5.5.3 Zinc

Figure 5-11 shows that with the exception of EE, all leaf samples contained a significantly higher (at a confidence level of ≥95 %) mean total zinc concentration than the corresponding soil samples. This demonstrated that bioaccumulation of zinc took place.

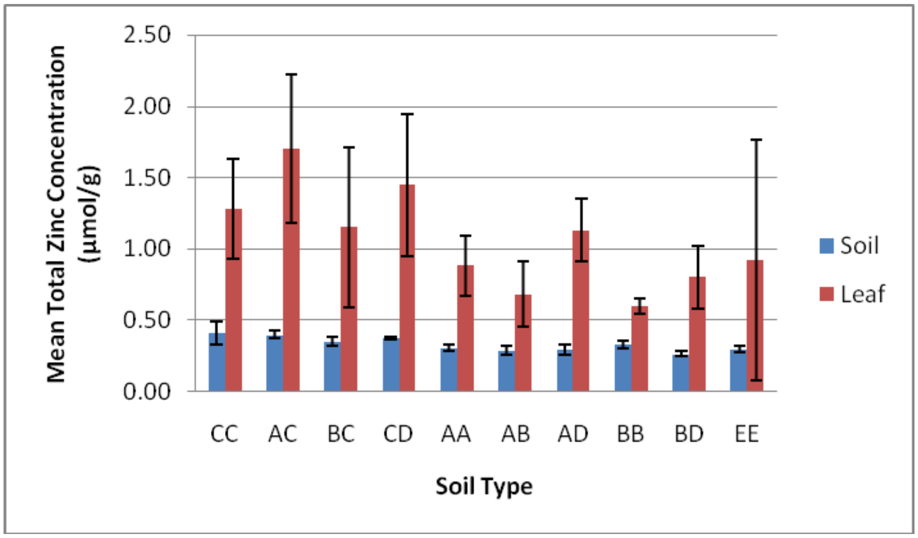


Figure 5-11: Mean total concentration of zinc in oven-dried soil and air-dried leaf samples (with 95 % confidence intervals)

The concentration of zinc in the leaf samples was unexpected; leaves from soil type CC should have had the highest zinc concentration, leaves from soil types AC, BC and CD should have had equal concentrations and the leaves from the remaining soil types should also have contained equal concentrations (but less than AC, BC and CD). As can be seen from Figure 5-11, there was only a significant difference between the concentration of zinc present in AB and AC, AC and BB and BB and CC. There was also a significant difference between the concentration of zinc present in AD and BB even though the concentration of zinc in these two samples was expected to have been the same.

5.5.4 Overall

There were no differences detected between plants based on their mean total concentration of copper, lead or zinc in the leaf samples as shown by the results of the MANOVA. This may have been due to the intra-sample variation being large in the context of the inter-sample variation. This intra-sample variation may have been due to the small masses of leaf available (average leaf weighed 0.08 g) compared with soil (average soil sample weighed 1.02 g). As can be seen from Table 5-13, the mean % RSD values for soil are lower than for leaf. Paired t-tests revealed that these differences were significant at a confidence level ≥95 %.

Table 5-13: Summary of mean % RSD values for copper, lead & zinc concentrations of soil and leaf samples

Sample	**Mean % RSD**		
	Copper	**Lead**	**Zinc**
Soil	17	16	10
Leaf	64	62	35

The masses of leaves from soil type EE were some of the largest and the smallest analysed; when the data from the heavier samples were isolated, the % RSD values were found to be 5.8 %, 0.68 % and 2.4 % for copper, lead and zinc respectively, and when the data from the lighter samples were isolated, the % RSD values were found to be 54 %, 24 % and 33 % for copper, lead and zinc respectively. However, a paired t-test showed that these differences were not significant (at a confidence level ≥95 %) but as the data set was so small, the likelihood of achieving a significant result was small [128]. This demonstrated that the mass of sample could have affected the % RSD and therefore may have been responsible for the intra-sample variation observed. Any variation due to small masses used would almost certainly have been caused by random errors associated with the balance used to weigh the samples; weighing errors increase as mass decreases [129].

5.6 Metal Concentrations in Resin

All raw resin data can be found in Appendix 7. All concentrations (in $\mu molg^{-1}$) are of air-dried resin.

Figure 5-12 shows that only sample AA contained a significantly higher (at a confidence level ≥95 %) mean concentration of total copper than lead and only samples AD and CC contained significantly higher mean concentrations of total copper than zinc. None of the samples showed a significant difference between mean total lead concentrations and mean total zinc concentrations. These metal concentrations in the resins were not consistent with the metal concentrations contained within the leaf samples suggesting that bioaccumulation of the metals was not consistent throughout the plant.

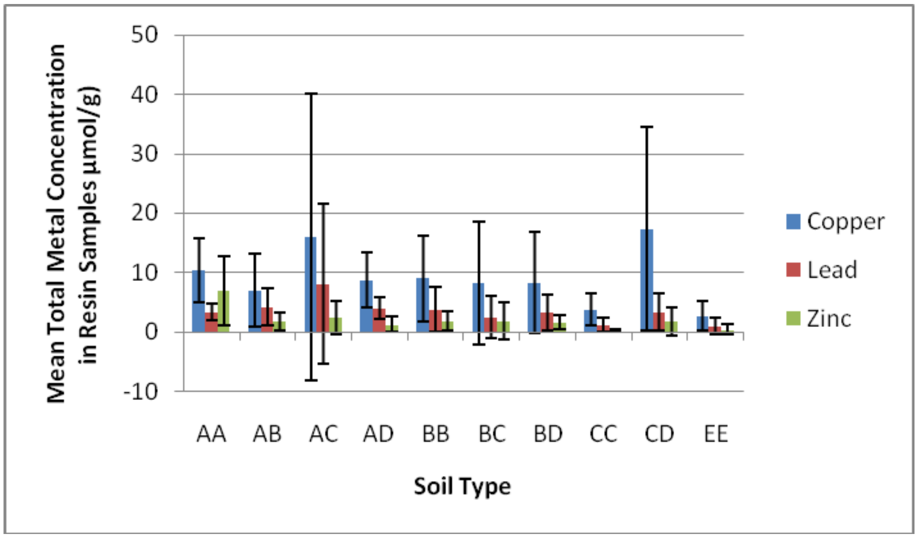


Figure 5-12: Mean total concentration of copper, lead and zinc in air-dried resin samples

A one-way between-groups multivariate analysis of variance was performed to investigate differences in copper, lead and zinc concentration of resin from plants grown in different soil types. Three dependent variables were used; copper concentration, lead concentration and zinc concentration (all micromoles per gram). The independent variable was soil type. There was a statistically significant difference between the resin from the plants grown in the different soil types on the combined dependent variables (Table 5-14) but when the results for the dependent variables were considered separately, all three showed statistically no significant difference using a Bonferroni adjusted alpha level of 0.001 (Table 5-15). However, it should be noted that for zinc concentration, the significance was exactly 0.001.

Table 5-14: The results of the multivariate test performed on resin data

Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type – Pillai's Trace	1.14	2.45	27	108	0.001	0.38

Table 5-15: Results of the tests of between-subjects effects performed on resin data

Source	Dependant Variable	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type	Copper	1.43	9	36	0.214	0.26
Soil Type	Lead	1.43	9	36	0.213	0.26
Soil Type	Zinc	4.15	9	36	0.001	0.51

5.6.1 Copper

Figure 5-13 shows that with the exception of AC, BC and BD, all resin samples contained significantly higher (at a confidence level ≥95 %) mean total copper concentrations than the corresponding soil samples. Only resin sample AA contained a significantly higher mean total copper concentration than the corresponding leaf sample. These findings were consistent with those of Cataldo and Wilding [127] who found that leaves were the major site of deposition for essential nutrients such as copper and zinc.

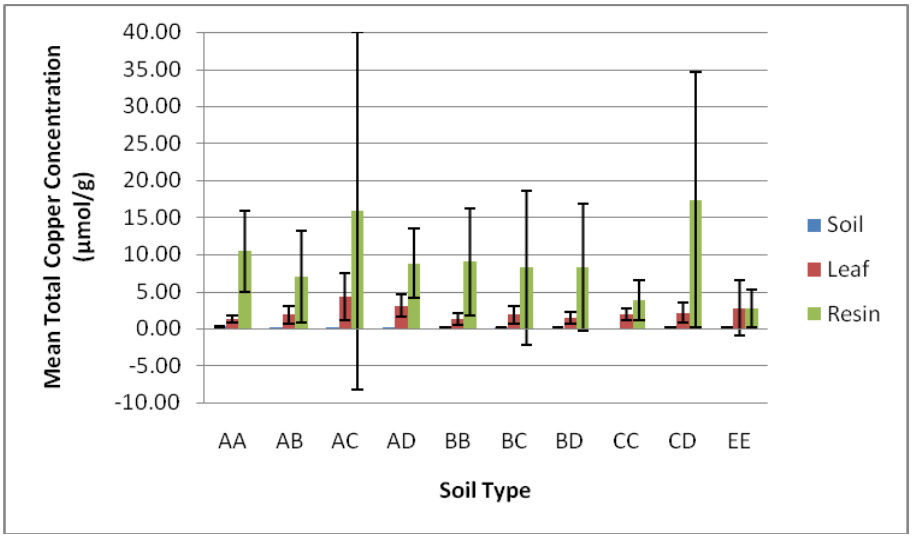


Figure 5-13: Mean total concentration of copper in oven-dried soil, air-dried leaf and air-dried resin samples

The concentration of copper in the resin samples was unexpected; resin from soil type AA should have had the highest copper concentration, resin from soil types AB, AC and AD should have had equal concentrations and the resin from the remaining soil types should also have had equal concentrations (but less than AB, AC and AD). As can be seen from Figure 5-13, there was no significant difference in the concentration of copper between any of the resin samples.

5.6.2 Lead

Figure 5-14 shows that only resin samples AA, AB and AD contained significantly higher (at a confidence level ≥95 %) mean total lead concentrations than the corresponding leaf samples and only resin samples AA, AB, AD, BD, CC and CD contained significantly higher mean total lead concentrations than the corresponding soil samples.

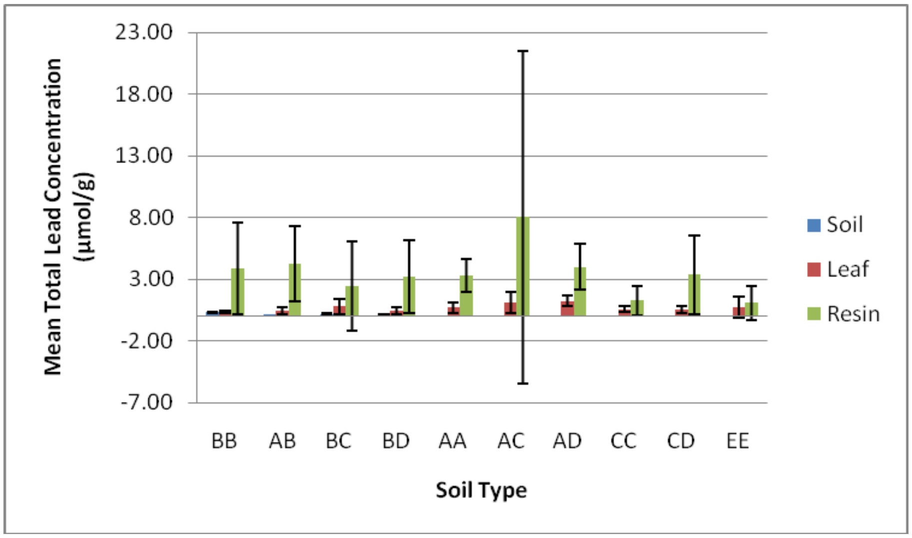


Figure 5-14: Mean total concentration of lead in oven-dried soil, air-dried leaf and air-dried resin samples

The concentration of lead in the resin samples was not as might have been expected; resin from soil type BB should have contained the highest lead concentration, resin from soil types AB, BC and BD should have contained equal concentrations and the resin from the remaining soil types should also have had equal concentrations (but less than AB, BC and BD). As can be seen from

Figure 5-14, there was no significant difference in the concentration of lead between any of the resin samples.

5.6.3 Zinc

Figure 5-15 shows that only resin sample AA contained a significantly higher (at a confidence level ≥95 %) mean total zinc concentration than the corresponding leaf sample and only resin samples AA and BD contained significantly higher mean total zinc concentrations than the corresponding soil samples. As with copper, these findings were consistent with those of Cataldo and Wilding [127].

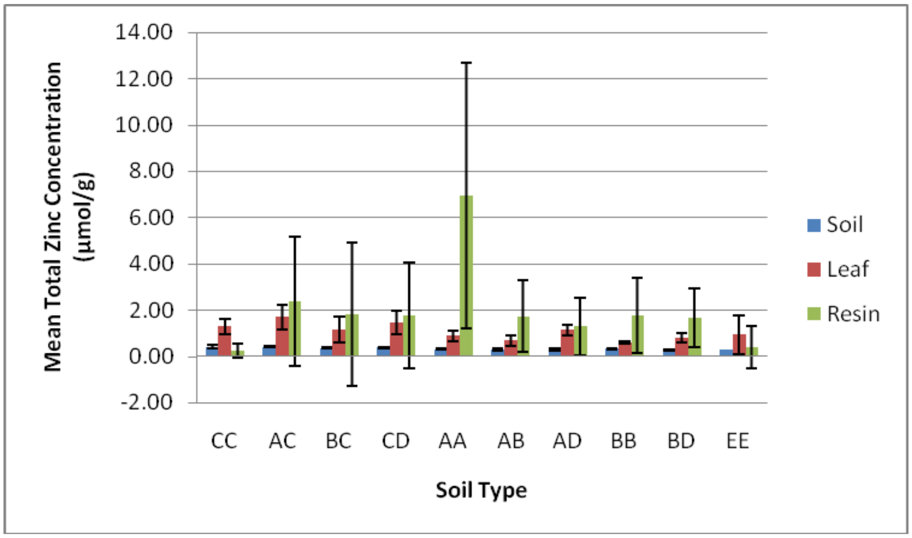


Figure 5-15: Mean total concentration of zinc in oven-dried soil, air-dried leaf and air-dried resin samples

Overall it was expected that resin from soil type CC should have had the highest zinc concentration, resin from soil types AC, BC and CD should have had equal concentrations and the resin from the remaining soil types should also have had equal concentrations (but less than AC, BC and CD). Therefore the results did not observe the expected trend.

5.6.4 Overall

There were no differences detected between plants based on the mean total concentration of copper, lead or zinc in the resin samples as shown by the results of the MANOVA. As with the leaf data, this may have been due to a large intra-sample variation as a result of the small amount of resin available (average resin sample weighed 0.01 g). As can be seen from Table 5-16, the mean % RSD values for resin were higher than for soil. Results of a paired t-test showed that these differences were significant at a confidence level ≥95 %.

Table 5-16: Summary of mean % RSD values for copper, lead & zinc concentrations of soil and resin samples

Sample	Mean % RSD		
	Copper	Lead	Zinc
Soil	17	16	10
Resin	65	68	79

As for leaf samples (Section 5.5.4), the large % RSD values obtained for resin were probably caused by random errors associated with the weighing of samples with a small mass [129]. Myors et al [66] only used 0.02-0.04 g heroin for analysis and achieved % RSD values of 5 %, 94 % and 3 % for copper, lead and zinc respectively, when using ICP-MS. However, the values achieved using ICP-MS were based on repeatedly analysing the same heroin sample, rather than a number of samples that had supposedly originated from the same batch therefore it cannot be ruled out that the high % RSD values obtained in this study were due to plant variation and not due to random error. Unfortunately, there was no published data referring to the intra-batch variation in metal concentration of heroin samples.

5.7 Effect of Potassium

5.7.1 Leaf

As shown in Figure 5-9, there was no significant difference in the concentration of copper in samples AB, AC and AD (with each soil type having 10 $mgKg^{-1}$ copper added) or in samples BB, BC, BD, CC, CD and EE (each having 0 $mgKg^{-1}$ copper added). This indicated that the presence of potassium did not affect the concentration of copper taken up by the plant and present in the leaves.

As shown in Figure 5-10, there was no significant difference in the concentration of lead in samples AB, BC and BD (with each soil type having 33 $mgKg^{-1}$ lead added) or in samples AA, AC, AD, CC, CD and EE (each having 0 $mgKg^{-1}$ lead added) leading to the conclusion that the presence of potassium had no affect on the concentration of lead taken up by the plant and present in the leaves.

As shown in Figure 5-11, there was no significant difference in the concentration of zinc in samples AC, BC and CD (with each soil type having 11 $mgKg^{-1}$ zinc added). In the remaining samples (each having 0 $mgKg^{-1}$ zinc added), there were no significant differences except that sample AD was found to have significantly more zinc than sample BB but this was considered an exception and did not alter the overall conclusion that the presence of potassium did not influence the concentration of zinc taken up by the plant and present in the leaves.

5.7.2 Resin

As shown in Figure 5-13, Figure 5-14 and Figure 5-15, the trends for resin mirrored those found for leaf so that again it can be concluded that the presence of potassium did not affect the concentration of copper, lead or zinc taken up by the plant and present in the resin.

5.7.3 Overall

As commercial compost was used, the absence of any significant differences due to the addition of potassium was probably due to a large amount of potassium present in the soil to begin with.

5.8 Relationship between Concentration of Metals in Soil, Leaf and Resin

5.8.1 Copper

Figure 5-16 shows that overall there was no trend evident in the relationship between the mean concentration of available copper in soil and the mean concentration of total copper in the corresponding leaf samples.

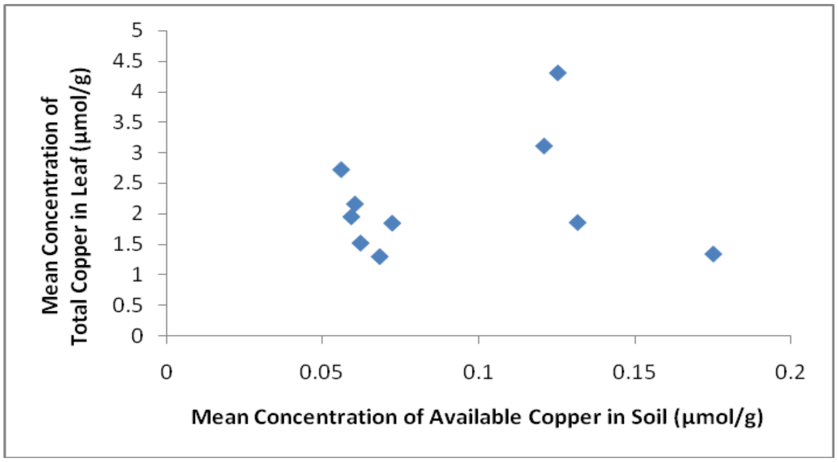


Figure 5-16: Relationship between mean concentration of available copper in oven-dried soil and mean concentration of total copper in air-dried leaf samples

Figure 5-17 shows that there was no trend evident in the relationship between the mean concentration of available copper in the soil and the mean concentration of total copper in the corresponding resin samples.

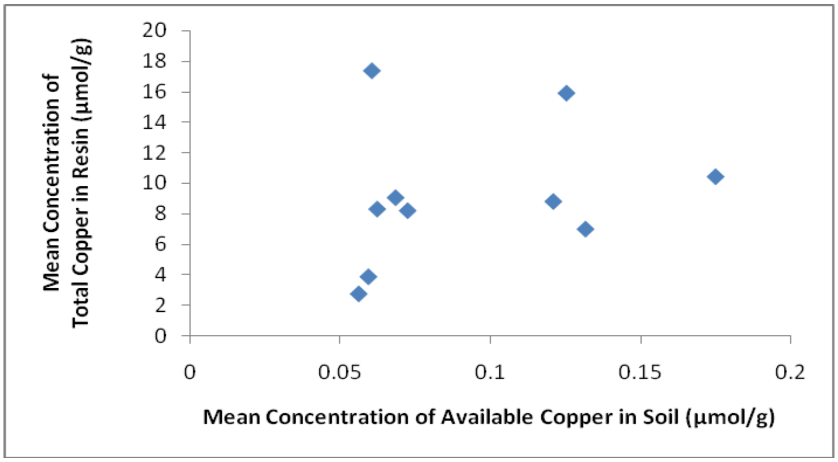


Figure 5-17: Relationship between mean concentration of available copper in oven-dried soil and mean concentration of total copper in air-dried resin samples

The data in Table 5-17also showed that there was a large variation in the leaf:soil and the resin:soil ratios for copper suggesting that it would be difficult to define an accurate formula for determining the concentration of available copper in the soil from the concentration of total copper in seized heroin.

Table 5-17: Ratio data for copper concentrations in soil, leaf and resin

Soil Type	Ratio	
	Leaf:Soil	Resin:Soil
AA	10	34
AB	36	34
AC	20	87
AD	16	33
BB	24	109
BC	20	58
BD	15	86
CC	19	48
CD	22	166
EE	54	24
Mean	24	68
SD	13	45
% RSD	53	66

5.8.2 Lead

Figure 5-18 shows that there was no trend evident in the relationship between the mean concentration of available lead in soil and the mean concentration of total lead in the corresponding leaf samples.

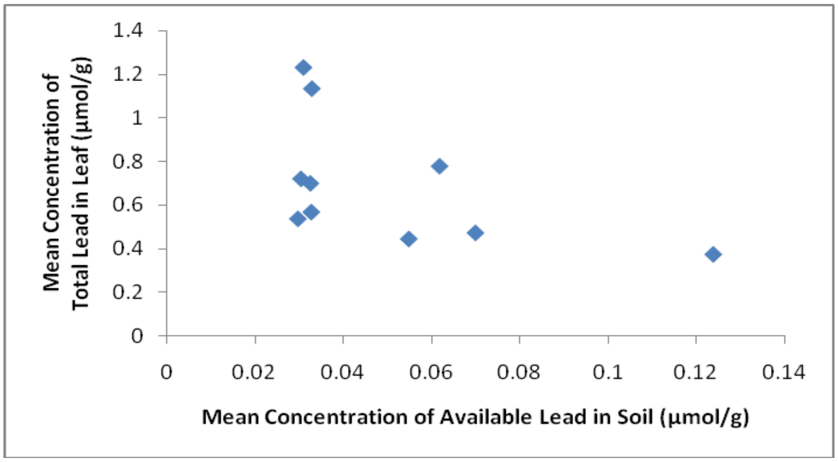


Figure 5-18: Relationship between mean concentration of available lead in oven-dried soil and mean concentration of total lead in air-dried leaf samples

Figure 5-19 shows that there was no trend evident in the relationship between the mean concentration of available lead in the soil and the mean concentration of total lead in the corresponding resin samples.

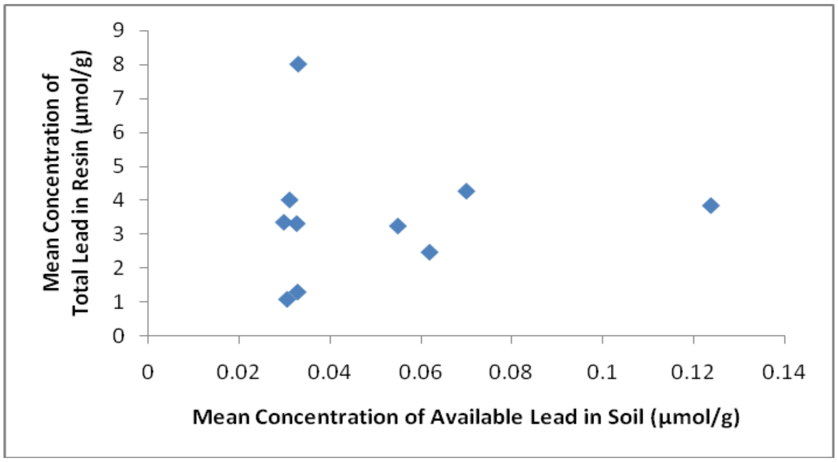


Figure 5-19: Relationship between mean concentration of available lead in oven-dried soil and mean concentration of total lead in air-dried resin samples

The data in Table 5-18 also showed that there was a large variation in the leaf:soil and resin:soil ratios for lead suggesting that it would be difficult to define an accurate formula for determining the concentration of available lead in the soil from the concentration of total lead in seized heroin.

Table 5-18: Ratio data for lead concentrations in soil, leaf and resin

Soil Type	Ratio	
	Leaf:Soil	Resin:Soil
AA	12	40
AB	9	18
AC	13	94
AD	15	42
BB	4	17
BC	10	19
BD	3	26
CC	7	16
CD	7	47
EE	15	11
Mean	9	33
SD	4	25
% RSD	48	76

5.8.3 Zinc

Figure 5-20 shows that there was a positive trend evident in the relationship between the mean concentration of available zinc in soil and the mean concentration of total zinc in the corresponding leaf samples.

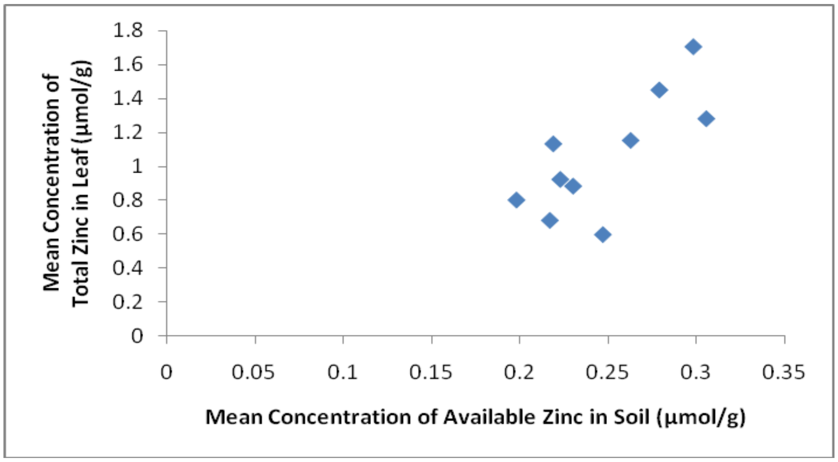


Figure 5-20: Relationship between mean concentration of available zinc in oven-dried soil and mean concentration of total zinc in air-dried leaf samples

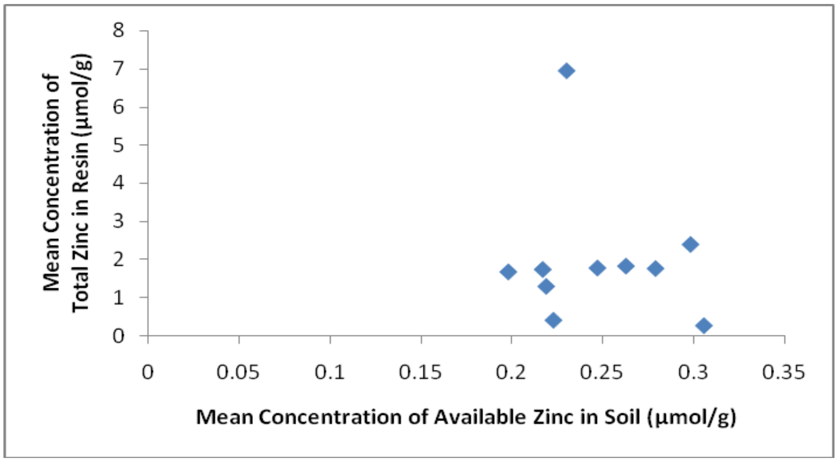


Figure 5-21 shows that there was no trend evident in the relationship between the mean concentration of available zinc in the soil and the mean concentration of total zinc in the corresponding resin samples.

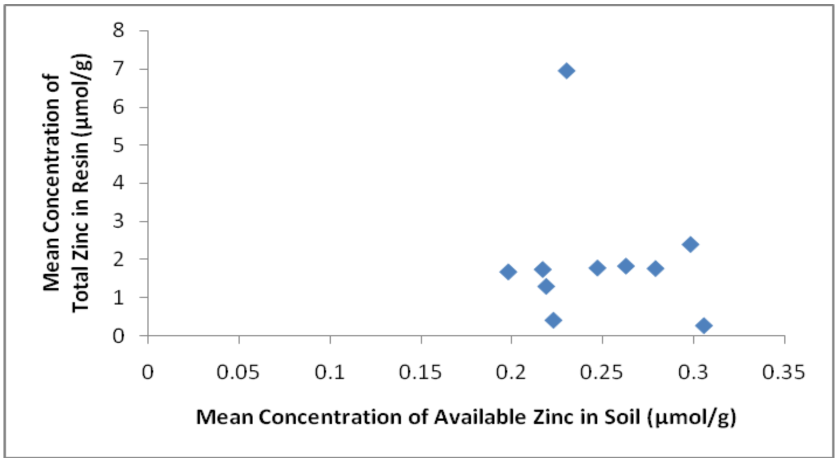


Figure 5-21: Relationship between mean concentration of available zinc in oven-dried soil and mean concentration of total zinc in air-dried resin samples

The data in Table 5-19 also showed that there was a large variation in the resin:soil ratio for zinc suggesting that it would be difficult to define an accurate formula for determining the concentration of available zinc in the soil from the concentration of total zinc in seized heroin.

Table 5-19: Ratio data for zinc concentrations of soil, leaf and resin

Soil Type	Ratio	
	Leaf:Soil	Resin:Soil
AA	4	20
AB	7	8
AC	4	5
AD	4	3
BB	3	10
BC	4	5
BD	3	7
CC	3	1
CD	4	5
EE	5	1
Mean	4	7
SD	1	6
% RSD	27	86

5.8.4 Overall

There were no reliable relationships evident between the soil and resin samples for any of the metals looked at. Therefore, graphical methods could not be used to determine the concentration a metal in the soil where the opium poppies were grown based on the concentration of said metal in a sample of seized heroin.

5.9 Metal Ion Ratios

As the different samples of leaf and resin could not be discriminated using the concentrations of copper, lead and zinc, it was decided to investigate the use of metal concentration ratios as used by Violante et al [61]. The metal ion data were created using the concentrations of copper, lead and zinc (in µmolg^{-1}) in soil, leaf and resin samples as referred to in Section 5.4, Section 5.5 and Section 5.6 respectively. The raw concentration ratio data for soil, leaf and zinc can be found in Appendix 8.

5.9.1 Soil

After plotting a scatterplot matrix (Figure 5-22) of all the possible metal concentration ratio combinations, the ratios of Cu:Zn, Cu:Pb and Zn:Pb were chosen as these concentration ratios produced data that were more spread out and therefore were more likely to produce clusters that could be distinguished from one another.

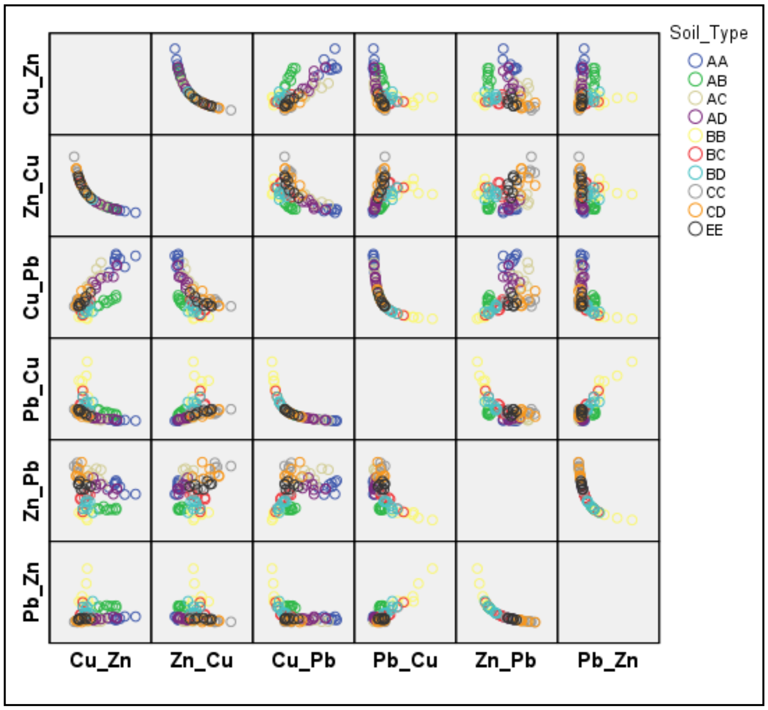


Figure 5-22: Scatterplot matrix for soil metal concentration ratios

A one-way between-groups multivariate analysis of variance was performed to investigate differences in three different metal concentration ratios. Three dependent variables were used: Cu:Zn, Cu:Pb and Zn:Pb. The independent variable was soil type.

Table 5-20: The results of the multivariate test performed on soil ratio data

Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type – Pillai's Trace	2.429	32.162	27	204	0.000	0.81

Table 5-21: Results of the tests of between-subjects effects performed on soil ratio data

Source	Dependant Variable	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type	Cu:Zn	41.990	9	68	0.000	0.848
Soil Type	Zn:Pb	52.565	9	68	0.000	0.874
Soil Type	Cu:Pb	69.439	9	68	0.000	0.902

There was a statistically significant difference between soil types on the combined dependent variables (Table 5-20) and when the results for the dependent variables were considered separately, all three showed a statistically significant difference using a Bonferroni adjusted alpha level of 0.001 (Table 5-21). The large effect sizes showed that the significant differences observed were due to the ratios of Cu:Zn, Zn:Pb and Cu:Pb. *Post-hoc* comparisons using Games-Howell test were performed and the results are shown in Table 5-22, Table 5-23 and table 5-24.

Table 5-22: Results of Games-Howell *post-hoc* test showing significance values for Cu:Zn concentration ratios of soil samples

	AA									
AB	0.177	AB								
AC	0.000	0.038	AC							
AD	0.150	1.000	0.339	AD						
BB	0.000	0.000	0.038	0.008	BB					
BC	0.000	0.000	0.032	0.008	1.000	BC				
BD	0.000	0.001	0.156	0.019	0.882	0.802	BD			
CC	0.000	0.000	0.002	0.002	0.272	0.301	0.026	CC		
CD	0.000	0.000	0.003	0.002	0.469	0.512	0.065	1.000	CD	
EE	0.000	0.000	0.021	0.004	0.994	0.998	0.562	0.889	0.974	EE

Table 5-23: Results of Games-Howell *post-hoc* test showing significance values for Zn:Pb concentration ratios of soil samples

	AA	AB	AC	AD	BB	BC	BD	CC	CD	EE
AB	0.000									
AC	0.042	0.000								
AD	1.000	0.000	0.028							
BB	0.000	0.041	0.000	0.000						
BC	0.019	0.102	0.000	0.021	0.007					
BD	0.000	0.556	0.000	0.000	0.016	0.662				
CC	0.234	0.001	1.000	0.200	0.000	0.003	0.001			
CD	0.017	0.000	0.999	0.012	0.000	0.000	0.000	1.000		
EE	1.000	0.000	0.043	0.999	0.000	0.008	0.000	0.280	0.020	

Table 5-24: Results of Games-Howell *post-hoc* test showing significance values for Cu:Pb concentration ratios of soil samples

	AA	AB	AC	AD	BB	BC	BD	CC	CD	EE
AB	0.000									
AC	0.020	0.004								
AD	0.014	0.001	1.000							
BB	0.000	0.000	0.000	0.000						
BC	0.000	0.305	0.000	0.000	0.067					
BD	0.000	0.007	0.000	0.000	0.010	0.997				
CC	0.000	1.000	0.004	0.001	0.000	0.376	0.004			
CD	0.000	0.999	0.010	0.002	0.006	0.342	0.091	0.983		
EE	0.000	1.000	0.003	0.001	0.003	0.594	0.115	1.000	0.998	

🟨 Significant difference, expected

🟩 Significant difference, not expected

🟦 No significant difference, expected

🟥 No significant difference, not expected

Figure 5-23: Key for understanding colours used in results of *post-hoc* tests

Based on the concentrations of copper, lead and zinc already present in the soil (according to the results from soil type EE) and the concentrations of copper, lead and zinc that were added to the soil, predicted ratios that should have been produced for each soil type were produced. The observed and predicted concentration ratios for Cu:Zn, Zn:Pb and Cu:Pb can be seen in Figure 5-24, Figure 5-25 and
Figure 5-26 respectively.

With a few exceptions, the predicted and the observed concentration ratios for Cu:Zn, Zn:Pb and Cu:Pb were found not to be significantly different (at a confidence level ≥95 %) for the soil types considered. As the predicted values were calculated using the concentration data obtained for soil type EE, any errors in these values would have caused the predicted values to be incorrect accounting for the instances where significant differences were observed.

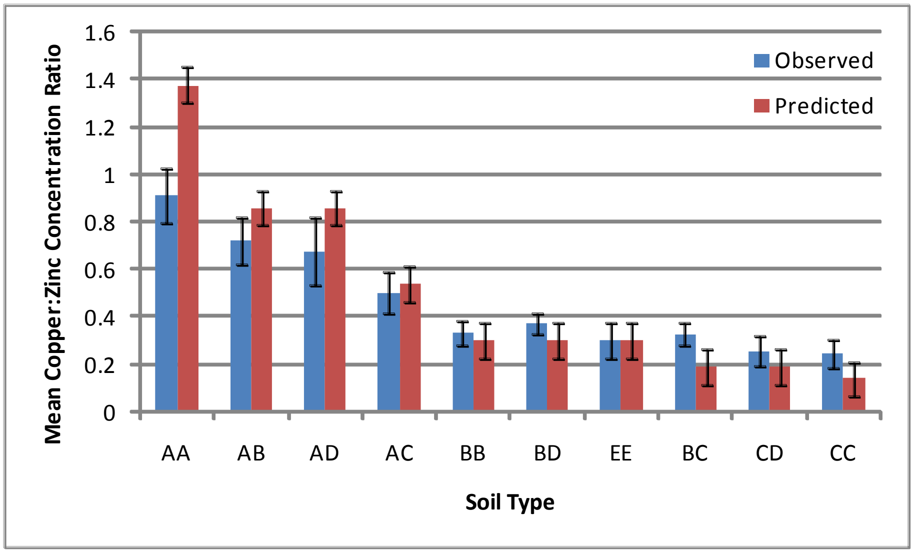


Figure 5-24: Mean Cu:Zn concentration ratios (observed & predicted) for soil samples (with 95 % confidence intervals)

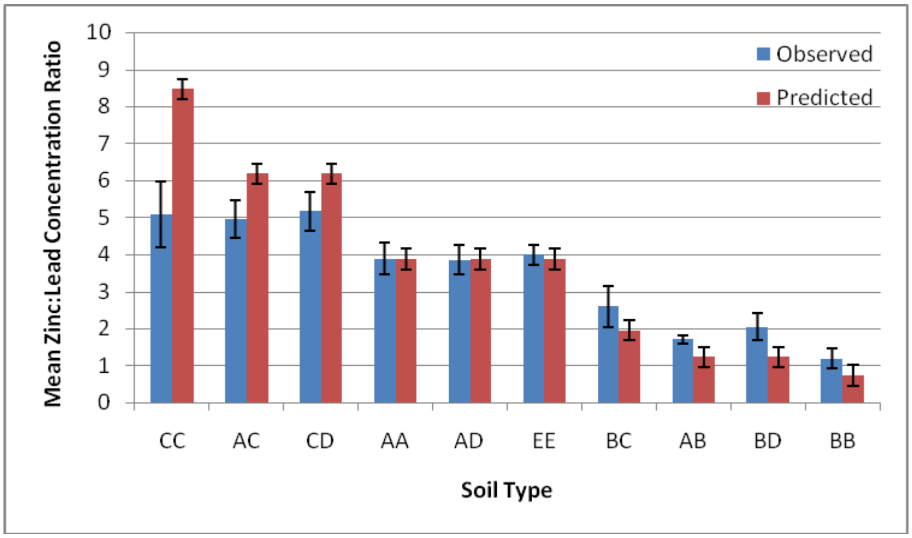


Figure 5-25: Mean Zn:Pb concentration ratios (observed & predicted) for soil samples (with 95 % confidence intervals)

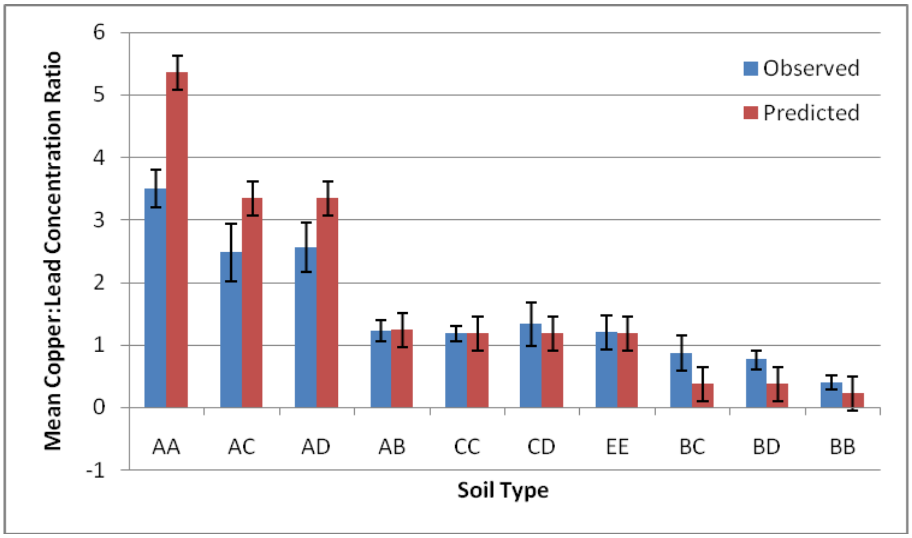


Figure 5-26: Mean Cu:Pb concentration ratios for soil samples (with 95 % confidence intervals)

5.9.2 Leaf

A one-way between-groups multivariate analysis of variance (MANOVA) was performed to investigate differences in three different metal concentration ratios. Three dependent variables were used: Cu:Zn, Cu:Pb and Zn:Pb. The independent variable was soil type.

Table 5-25: Results of the multivariate test performed on leaf ratio data

Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type – Pillai's Trace	0.741	1.896	27	156	0.008	0.247

Table 5-26: Results of the tests of between-subjects effects performed on leaf ratio data

Source	Dependant Variable	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type	Cu:Zn	1.915	9	52	0.070	0.249
Soil Type	Zn:Pb	2.764	9	52	0.010	0.324
Soil Type	Cu:Pb	1.616	9	52	0.135	0.219

There was a statistically significant difference between soil types on the combined dependent variables (Table 5-25) but when the results for the dependent variables were considered separately, all three showed that statistically there was no significant difference using a Bonferroni adjusted alpha level of 0.001 (
Table 5-26).

Cu:Zn

The results of the MANOVA indicated that there was no significant difference (at a confidence level ≥ 95 %) between the leaf samples from poppy plants grown in the different soil types based on the ratio of total copper concentration to total zinc concentration. The results can be seen visually in Figure 5-27.

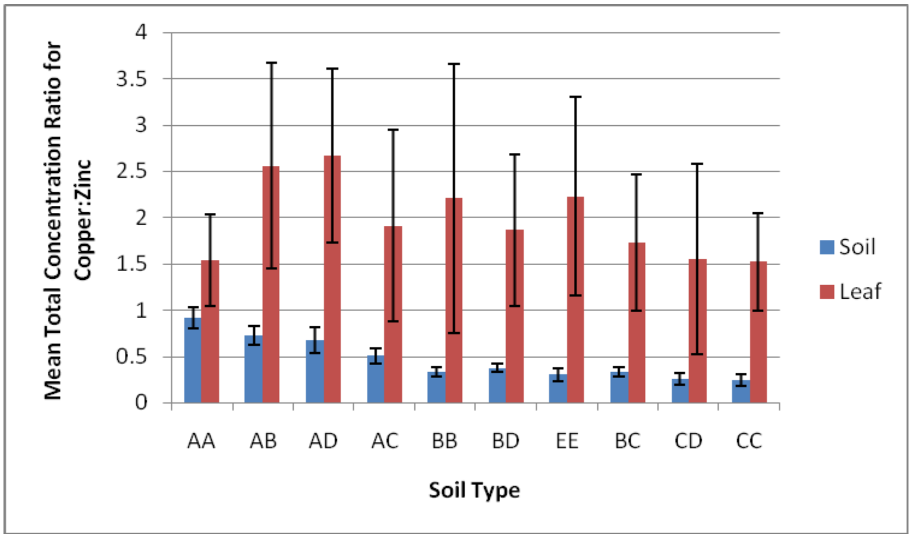


Figure 5-27: Mean concentration ratios of total Cu:Zn for air-dried leaf samples and oven-dried soil samples (with 95 % confidence intervals)

As shown in Figure 5-27, all mean total concentration ratios of Cu:Zn for air-dried leaf samples were significantly higher than the mean total concentration ratios of Cu:Zn for oven-dried soil samples. This indicated that more copper was accumulated in the leaves of the poppy plants than zinc. As mentioned previously, copper plays a significant role in photosynthesis [125] therefore it would be expected that there would be more copper in the leaves than zinc.

Zn:Pb

The results of the MANOVA indicated that there was no significant difference (at a confidence level ≥ 95 %) between the leaf samples from poppy plants grown in the different soil types based on the ratio of total zinc concentration to total lead concentration. However, as shown in Figure 5-28, there were significant differences (at a confidence level ≥ 95 %) between leaf sample AD and leaf samples CC, CD, EE and BB.

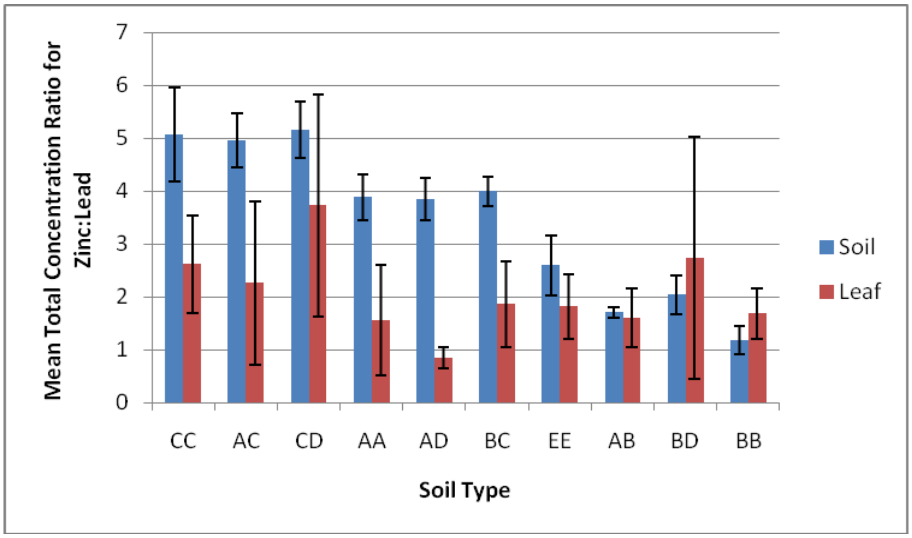


Figure 5-28: Mean concentration ratios of total Zn:Pb for air-dried leaf samples and oven-dried soil samples (with 95 % confidence intervals)

Unlike for Cu:Zn, none of the mean total concentration ratios for Zn:Pb in the air-dried leaf samples were significantly higher (at a confidence level ≥ 95 %) than the corresponding ratios in the oven-dried soil. In fact, as shown in Figure 5-28, the ratio of Zn:Pb in the oven-dried soil samples for soil types CC, AC, AA, AD and BC were significantly higher (at a confidence level ≥ 95 %) than the corresponding ratios in the air-dried leaf samples. This indicated that for those samples, more lead was accumulated in the leaves than zinc.

Cu:Pb

The results of the MANOVA indicated that there was no significant difference (at a confidence level ≥ 95 %) between the leaf samples from poppy plants grown in the different soil types based on the ratio of total copper concentration to total lead concentration. The results can be seen visually in Figure 5-29.

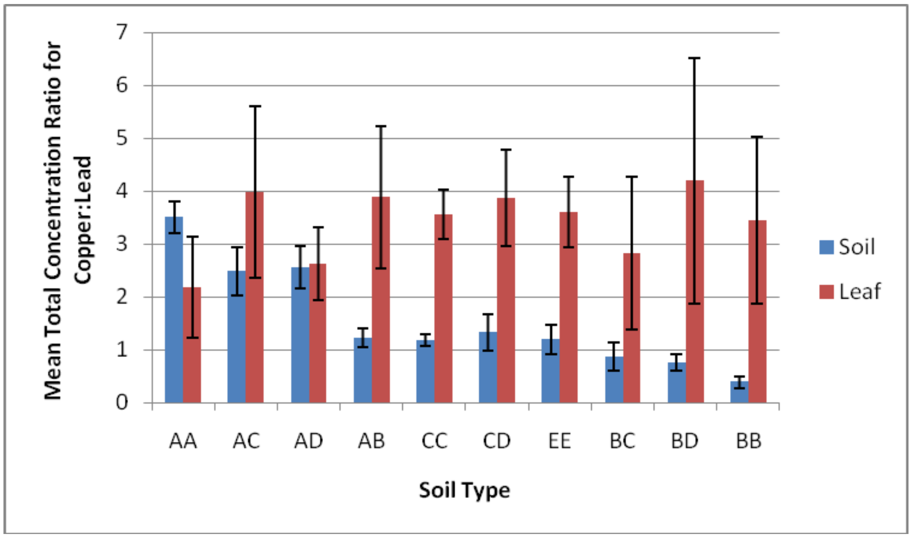


Figure 5-29: Mean concentration ratios of total Cu:Pb for air-dried leaf samples (with 95 % confidence intervals)

As shown in Figure 5-29, with the exception of soil types AA, AC and AD, all mean total concentration ratios of Cu:Pb for air-dried leaf samples were significantly higher than the mean total concentration ratios of Cu:Pb for oven-dried soil samples. This indicated that in those samples, more copper was accumulated in the leaves of the poppy plants than lead. As for Cu:Zn, this was probably due to role that copper plays in photosynthesis [125].

5.9.3 Resin

A one-way between-groups multivariate analysis of variance (MANOVA) was performed to investigate differences in three different metal concentration ratios. Three dependent variables were used: Cu:Zn, Cu:Pb and Zn:Pb. The independent variable was soil type.

Table 5-27: Results of the multivariate test performed on resin ratio data

Effect	Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type – Pillai's Trace	0.890	1.547	27	99	0.063	0.297

Table 5-28: Results of the tests of between-subjects effects performed on resin ratio data

Source	Dependant Variable	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Soil Type	Cu:Zn	1.324	9	33	0.263	0.265
Soil Type	Zn:Pb	1.571	9	33	0.165	0.300
Soil Type	Cu:Pb	1.892	9	33	0.088	0.340

There was not a statistically significant difference between soil types on the combined dependent variables (Table 5-27) and when the results for the dependent variables were considered separately, all three showed that statistically there was no significant difference using a Bonferroni adjusted alpha level of 0.001 (Table 5-28).

Cu:Zn

The results of the MANOVA indicated that there was no significant difference (at a confidence level ≥ 95 %) between the resin samples from poppy plants grown in the different soil types based on the ratio of total copper concentration to total zinc concentration. The results can be seen visually in Figure 5-30 and Figure 5-31.

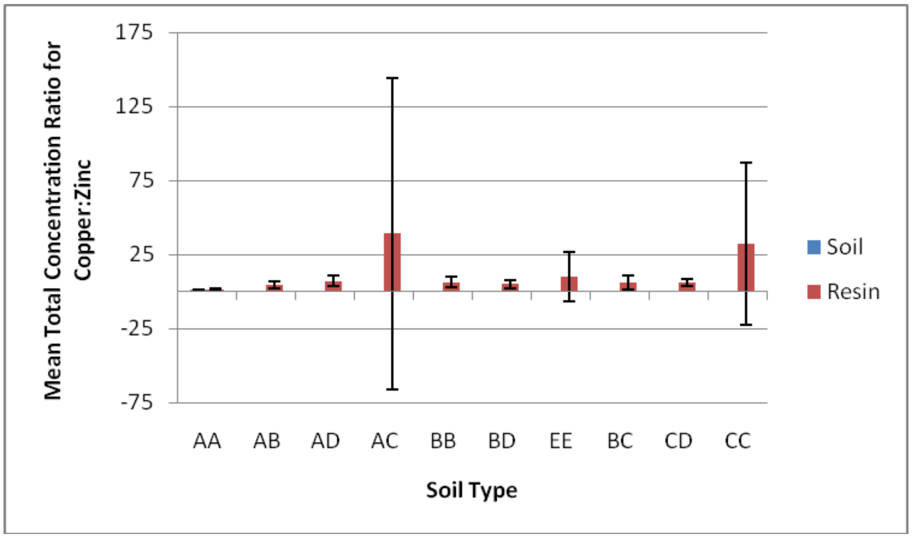


Figure 5-30: Mean concentration ratios of total Cu:Zn for air-dried resin samples and oven-dried soil samples (with 95 % confidence intervals)

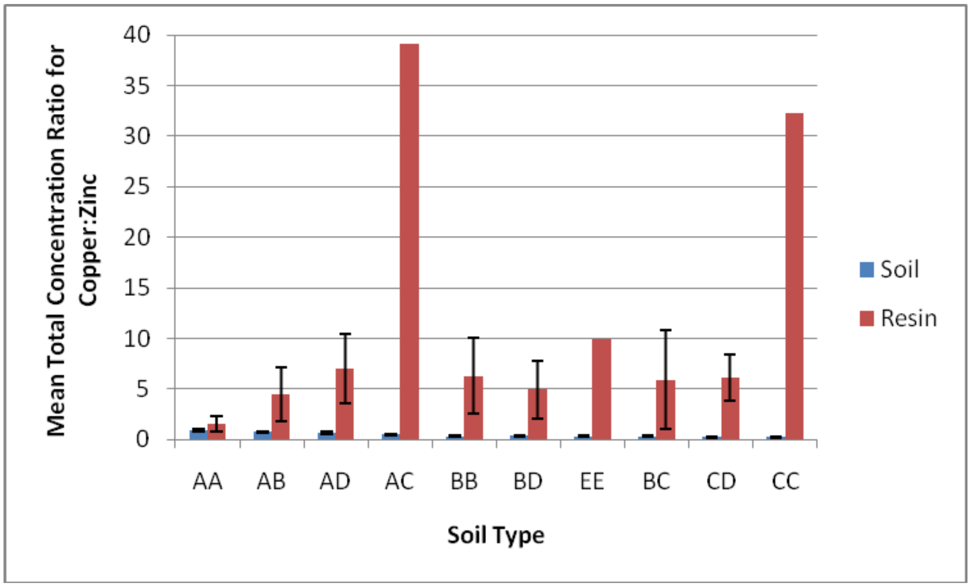


Figure 5-31: Mean concentration ratios of total Cu:Zn for air-dried resin samples and oven-dried soil samples (rescaled to show selected 95 % confidence intervals)

As shown in Figure 5-30 and Figure 5-31, with the exception of soil types AA, AC, EE and CC, all mean total concentration ratios of Cu:Zn for air-dried resin samples were significantly higher (at a confidence level ≥ 95 %) than the mean total concentration ratios of Cu:Zn for oven-dried soil samples. This indicated that in those samples, more copper was accumulated in the resin of the poppy plants than zinc. This may have been due to a tendency for copper to accumulate in the reproductive organs of some plants [125].

Zn:Pb

The results of the MANOVA indicated that there was no significant difference (at a confidence level ≥ 95 %) between the resin samples from poppy plants grown in the different soil types based on the ratio of total zinc concentration to total lead concentration. The results can be seen visually in Figure 5-32.

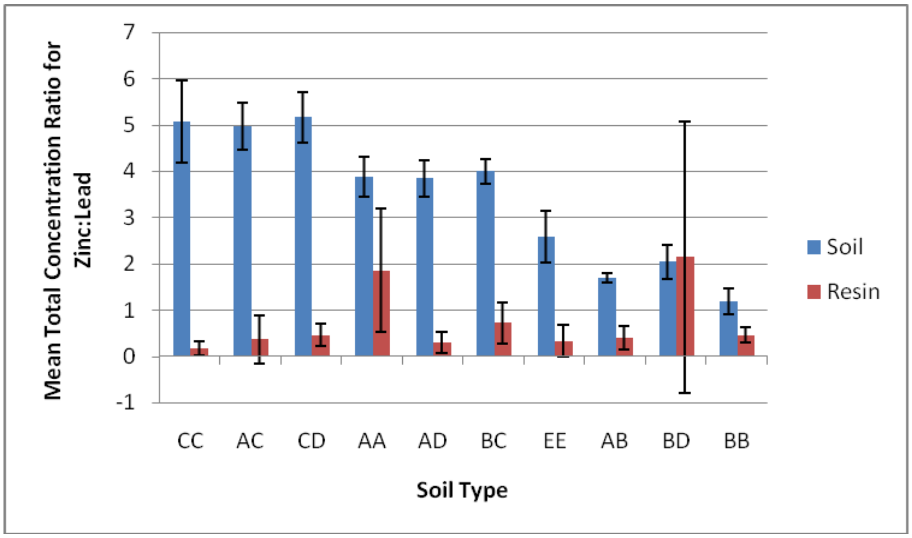


Figure 5-32: Mean total concentration ratios of Zn:Pb for air-dried resin samples and oven-dried soil samples (with 95 % confidence intervals)

As shown in Figure 5-32, with the exception of soil type BD, all mean total concentration ratios of Zn:Pb for oven-dried soil samples were significantly higher (at a confidence level ≥ 95 %) than the mean total concentration ratios of Zn:Pb for air-dried resin samples. This indicated that in those samples, more lead was accumulated in the resin of the poppy plants than zinc. These findings were consistent with other studies that found cadmium (which, like lead, is an element not essential to plants) accumulated more in poppy seeds than in other parts of the plant [130].

Cu:Pb

The results of the MANOVA indicated that there was no significant difference (at a confidence level ≥ 95 %) between the resin samples from poppy plants grown in the different soil types based on the ratio of total copper concentration to total lead concentration. The results can be seen visually in Figure 5-33.

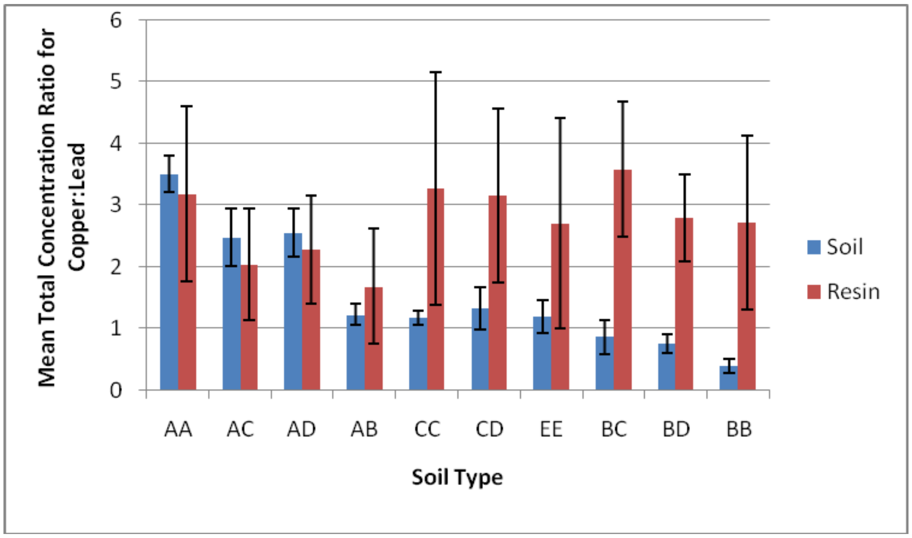


Figure 5-33: Mean total concentration ratios of Cu:Pb for air-dried resin samples and oven-dried soil samples (with 95 % confidence intervals)

As shown in Figure 5-33, soil types CC, CD, BC, BD and BB all had mean total concentration ratios of Cu:Pb for air-dried resin samples that were significantly higher (at a confidence level ≥ 95 %) than the mean total concentration ratios of Cu:Pb for the corresponding oven-dried soil samples. This indicated that in those samples, more copper was accumulated in the resin of the poppy plants than lead. As mentioned previously, this may have been due to a tendency for copper to accumulate in the reproductive organs of some plants [125].

5.9.4 Overall

As the mean total concentrations of copper, lead and zinc could not be used to differentiate between the different leaf samples (Section 5.5) and resin samples (Section 5.6), it was not unexpected that the mean total concentration ratios could not differentiate between the different leaf and resin samples.

5.10 Relationship between Metal Concentration Ratios in Soil, Leaf and Resin

5.10.1 Cu:Zn

Figure 5-34 shows that with the exception of one point, there was a positive trend evident in the relationship between the mean concentration ratio of available copper/zinc in soil and the mean concentration ratio of total copper/zinc in the corresponding leaf samples.

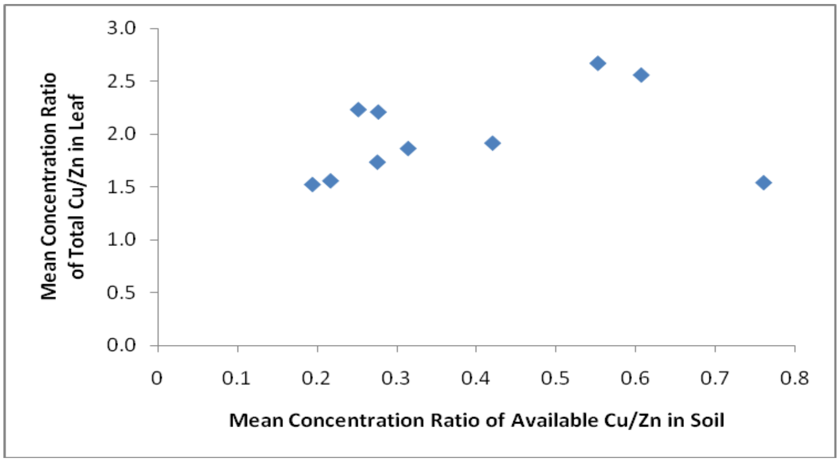


Figure 5-34: Relationship between mean concentration ratio of available Cu/Zn in oven-dried soil and mean concentration ratio of total Cu/Zn in air-dried leaf samples

Figure 5-35 shows that there was no trend evident in the relationship between the mean concentration ratio of available copper/zinc in the soil and the mean concentration ratio of total copper/zinc in the corresponding resin samples. These results suggested that the concentration ratio of copper/zinc in seized heroin could not be used to determine the concentration ratio of copper/zinc in the soil where the opium poppies were grown.

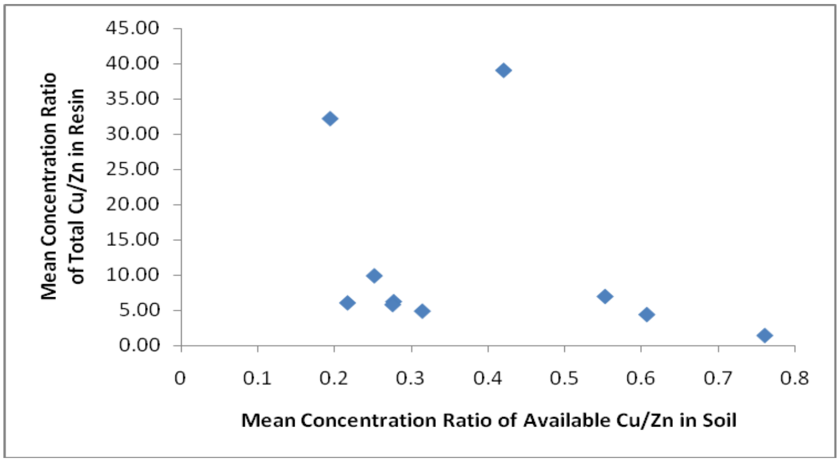


Figure 5-35: Relationship between mean concentration ratio of available Cu/Zn in oven-dried soil and mean concentration ratio of total Cu/Zn in air-dried resin samples

5.10.2 Zn:Pb

Figure 5-36 shows that there was no trend evident in the relationship between the mean concentration ratio of available zinc/lead in soil and the mean concentration ratio of total zinc/lead in the corresponding leaf samples.

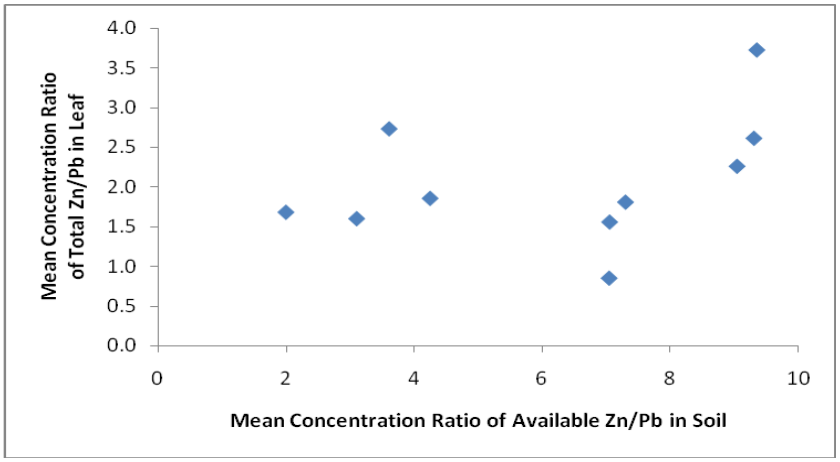


Figure 5-36: Relationship between mean concentration ratio of available Zn/Pb in oven-dried soil and mean concentration ratio of total Zn/Pb in air-dried leaf samples

Figure 5-37 shows that there was no trend evident in the relationship between the mean concentration ratio of available zinc/lead in the soil and the mean concentration ratio of total zinc/lead in the corresponding resin samples.

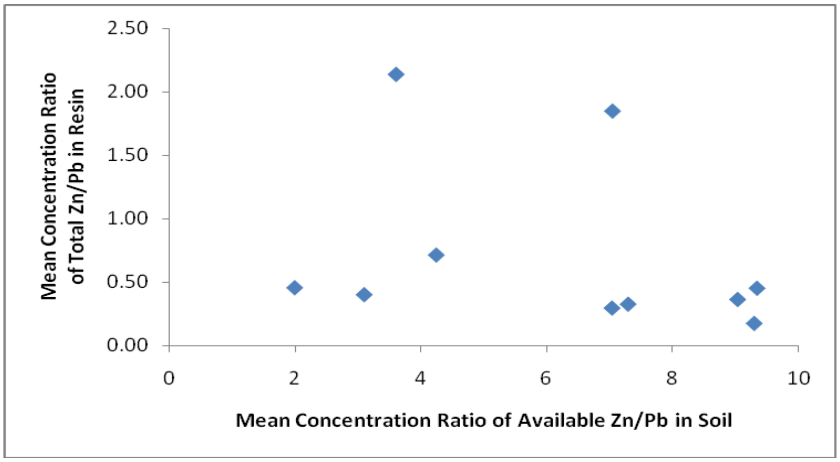


Figure 5-37: Relationship between mean concentration ratio of available Zn/Pb in oven-dried soil and mean concentration ratio of total Zn/Pb in air-dried resin samples

These results suggested that the concentration ratio of zinc/lead in seized heroin could not be used to determine the concentration ratio of zinc/lead in the soil where the opium poppies were grown.

5.10.3 Cu:Pb

Figure 5-38 shows that there was no trend evident in the relationship between the mean concentration ratio of available copper/lead in soil and the mean concentration ratio of total copper/lead in the corresponding leaf samples.

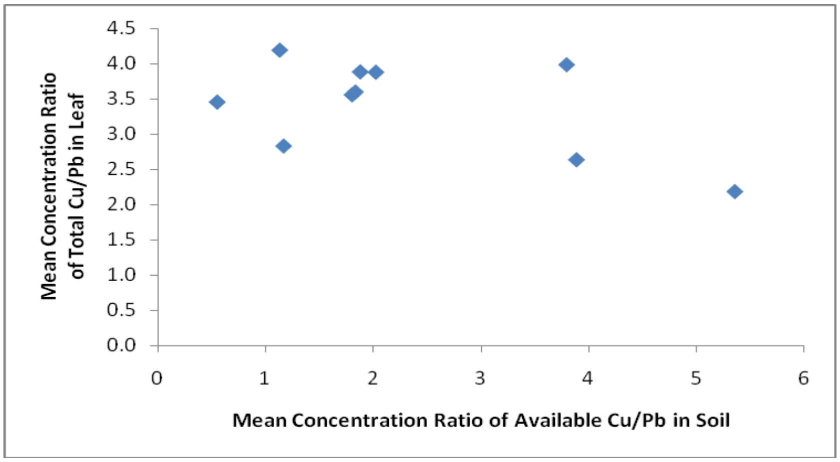


Figure 5-38: Relationship between mean concentration ratio of available Cu/Pb in oven-dried soil and mean concentration ratio of total Cu/Pb in air-dried leaf samples

Figure 5-39 shows that there was no trend evident in the relationship between the mean concentration ratio of available copper/lead in the soil and the mean concentration ratio of total copper/lead in the corresponding resin samples. These results suggested that the concentration ratio of zinc/lead in seized heroin could not be used to determine the concentration ratio of available zinc/lead in the soil where the opium poppies were grown.

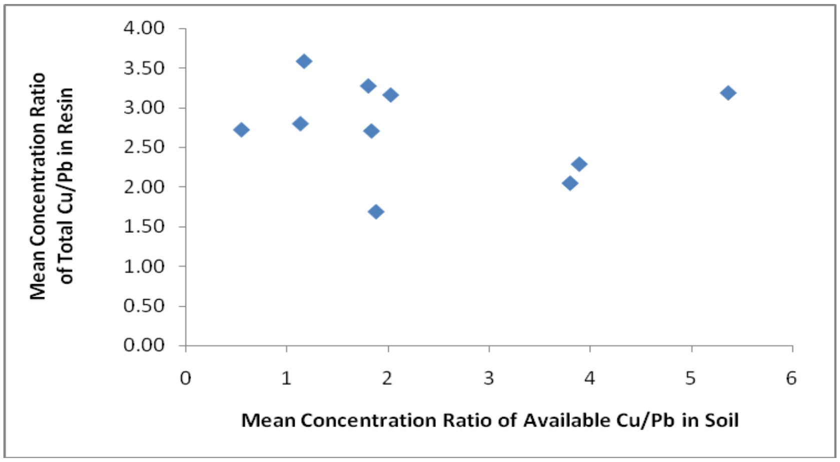


Figure 5-39: Relationship between mean concentration ratio of available Cu/Pb in oven-dried soil and mean concentration ratio of total Cu/Pb in air-dried resin samples

5.10.4 Overall

There were no reliable relationships evident between the soil and resin samples for any of the metal concentration ratios looked at. Therefore, graphical methods could not be used to determine the concentration ratio a particular metal combination in the soil where the opium poppies were grown based on the concentration ratio of said metals in a sample of seized heroin.

5.11 Classification of Data

A number of the studies that investigated the use of metal concentrations to determine geographical origin (of heroin and foodstuffs) made use of classification techniques. The applications of these techniques are referred to in Table 1-3 and Table 1-4 and the main techniques considered for use with this study are shown in Table 5-29.

Table 5-29: Summary of classification techniques considered for use with this study

Classification Technique	**References**	**Used in Study?**
Principal Component Analysis	[59], [60], [65], [66], [70], [72], [73], [74], [75], [76], [78], [80]	No
Hierarchical Cluster Analysis	[60], [64], [66], [70]	Yes
Discriminant Analysis	[60], [63], [73], [75], [76], 69], [80]	Yes

Principal components analysis (PCA) is a technique used to reduce the amount of data present from multivariate analysis by grouping together data that are correlated to form principal components with principal component 1 accounting for the greatest variation in the data [99]. As a result of this, PCA typically only presents a percentage of the variation in contrast with other classification techniques [131]. The previous studies that made use of PCA all involved a large number of variables as a result of multi-elemental analysis using ICP techniques therefore a form of data reduction was required. As this study only involved three variables in the form of concentrations of copper, lead and zinc, data reduction was not necessary and therefore, PCA was not employed.

Cluster analysis can be performed even when the number of groups is not known [132] and therefore potentially provides a useful tool for the classification of heroin samples when the origin is not known. The main method of cluster analysis is hierarchical cluster analysis (HCA) which measures the distances between individual data points to form clusters, the results of which may be depicted in a dendrogram. There are various methods for performing hierarchical clustering that differ in the way in which the distances are measured [133].

Unlike hierarchical clustering, discriminant function analysis (DA) requires that the number of groups is determined prior to analysis [128]. However, a function of DA provides a probability of group membership for individual data points that were not initially assigned to a particular group [134]. This function could be useful in heroin analysis; if seized samples had previously been clustered, any new seizures could be tested to see the likelihood that they have come from the same batch.

5.11.1 Concentration Data

Based on previous studies as shown in Table 5-29, hierarchical clustering and discriminant function analysis were selected as the most appropriate classification techniques to be applied to the soil, leaf and resin concentration data. The results of MANOVAs in Section 5.4 had already shown an inability to distinguish soil types BC and CC from the other soil types therefore data from soil types BC and CC were not included when applying classification techniques to the concentration data.

For hierarchical cluster analysis, there were a number of methods that could have been used for the analysis of multivariate data but for unequal samples sizes, complete linkage and average linkage have proven successful [134] whereas Ward's method has also proven to be more successful than other methods [135, 136] therefore these methods were compared.

It was necessary to develop a tool to compare the relative abilities of different HCA methods to place the data into meaningful clusters. A meaningful cluster was one in which the data points it contained had originated from the same soil type. The tool used produced a single percentage for each method referred to as the HCA success rating. The tool used is described in Table 5-30, with reference to Figure 5-40.

Table 5-30: Method for calculating HCA success rating

Step	Method	Example using Figure 5-40
1	The rescaled distance (shown on the Y-axis of dendrograms) that produced sensible clusters was identified (usually a cluster would contain a minimum of four samples [132] but as some resin groups only contained three samples this was accepted as the minimum number per cluster)	See red vertical line on Figure 5-40 (A). Clusters viewed to the left of this line.
2	Clusters assigned according to the majority of points from same soil type within a cluster	First red-circled cluster from top of Figure 5-40 (B): This was assigned to soil type AD even though it contained 2 data points from soil type AC and 1 from AA.
3	The total number of (correct) samples that were present in the clusters identified in step 2 was calculated.	From the top of Figure 5-40 (B): The first red-circled cluster contained 7 samples from soil type AD (soil types AC and AA were not counted as these samples were seen as incorrect as soil type AD had the majority of samples in this cluster), the second red-circled cluster contained 7 samples from soil type AA, the third red-circled cluster contained 4 samples from soil type AB etc... 45 in total.
4	The value from step 3 was divided by the total number of samples and then multiplied by 100 to give a %.	The value from step 3 (45) was divided by the total number of samples (62) and multiplied by 100 to give 73 %.

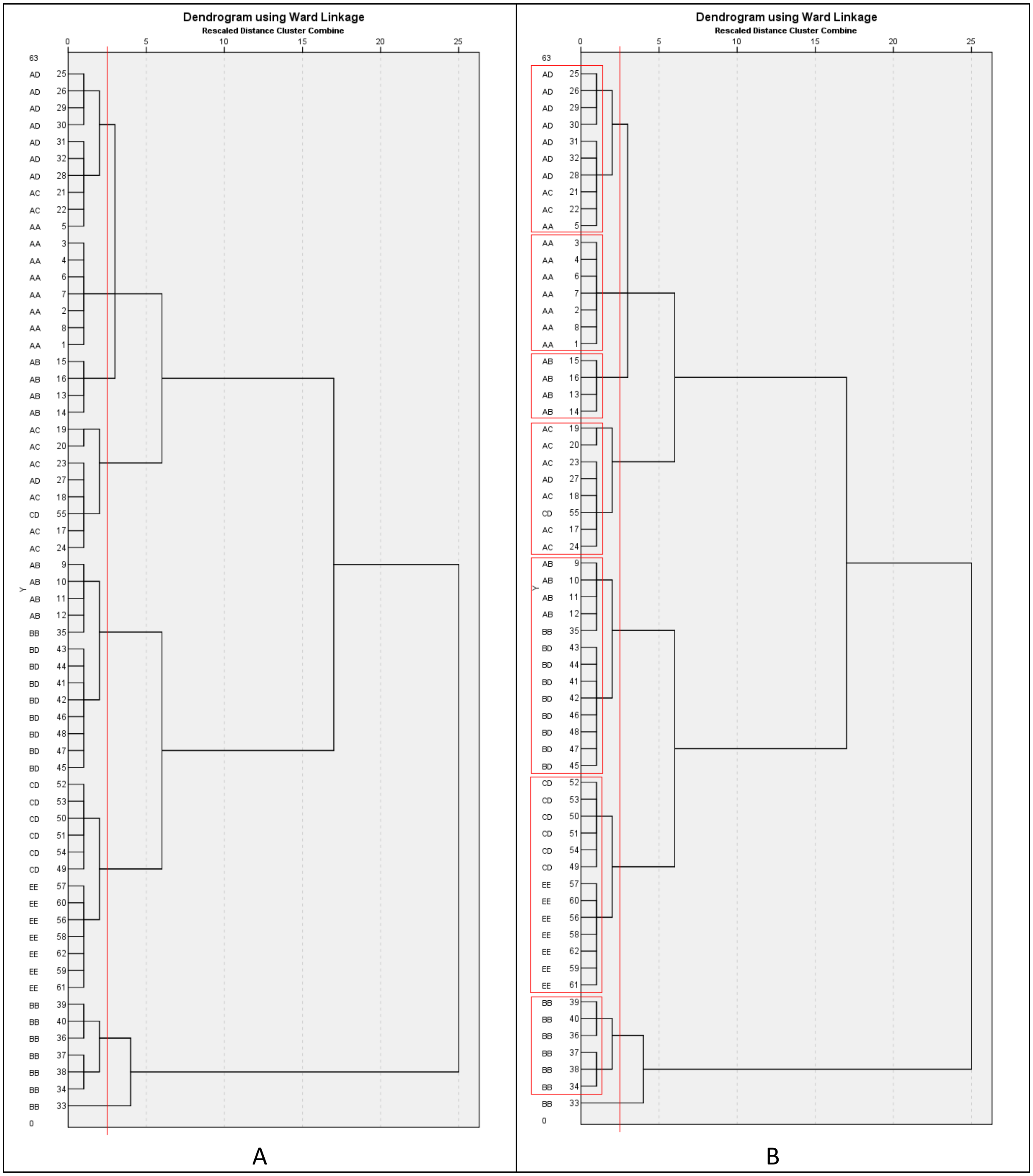


Figure 5-40: Dendrogram using Ward linkage produced from copper, lead & zinc concentrations of soil

The dendrograms of the hierarchical cluster analyses performed on the soil, leaf and resin data (Appendix 9) were examined and for each method, the HCA success rating was calculated. The HCA success ratings for soil, leaf and resin data can be seen in Table 5-31.

Table 5-31: Results of hierarchical clustering of soil, leaf and resin concentration data

Hierarchical Clustering Method	Distance Used	HCA Success Rating		
		Soil (n=62)	Leaf (n=51)	Resin (n=38)
Complete Linkage	Squared Euclidean	66 %	8 %	24 %
Complete Linkage	Manhattan*	68 %	27 %	8 %
Average Linkage	Squared Euclidean	58 %	4 %	11 %
Ward Linkage	Squared Euclidean	73 %	22 %	8 %

*as used by Myors et al [66]

As shown in Table 5-31, there was variation between the different hierarchical clustering methods therefore it was difficult to determine which would be the most appropriate method to use. For the soil data, Ward linkage proved to be slightly more successful than complete linkage whereas complete linkage (with Manhattan distances) and complete linkage (with squared Euclidean distances) were best for leaf and resin data respectively.

The results of discriminant function analysis for soil, leaf and resin data can be seen in Table 5-32 (and Appendix 10). Classification was considered acceptable if the percentage obtained was 25 % larger than that due to chance [132]. The results showed that the soil data were classified the most accurately, with 92 % of the cases correctly assigned to their group. As expected based on the results of Section 5.5 and Section 5.6, the leaf and resin data were not as successfully classified as the soil data.

Table 5-32: Results of discriminant function analysis for soil, leaf and resin data

Sample	Percentage of original grouped cases correctly classified	Percentage value 25 % larger than that due to chance	Acceptable classification?
Soil (n=62)	92 %	16 %	✓
Leaf (n=51)	26 %	16 %	✓
Resin (n=38)	42 %	16 %	✓

5.11.2 Optimisation of Concentration Data

As the initial results produced using the concentration data did not provide a 100 % correct classification for all soil types, it was decided to try to optimise the analysis by selecting particular soil types and/or variables. A scatterplot matrix (Figure 5-41) was created to investigate which combination of soil types would provide the largest spread of data.

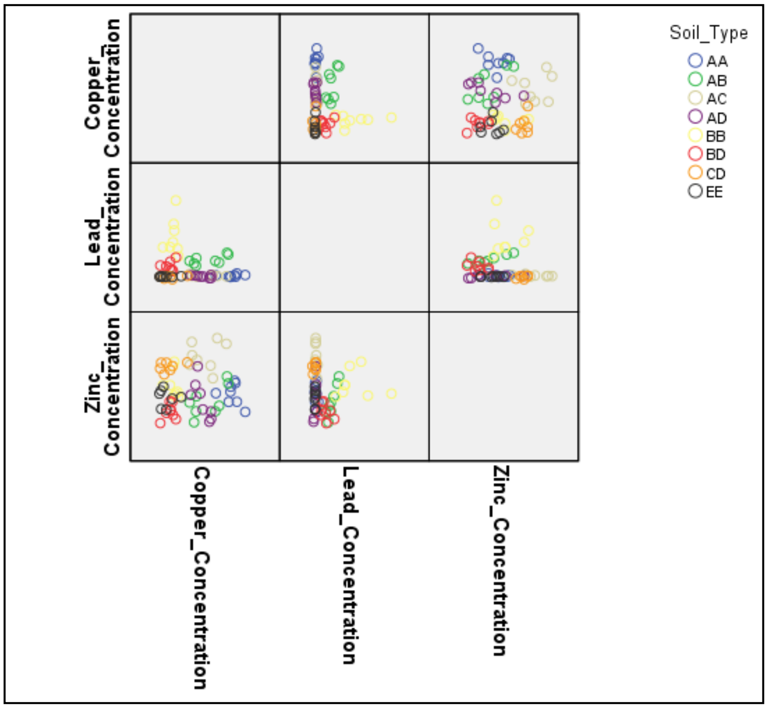


Figure 5-41: Scatterplot matrix of all soil data for concentrations of copper, lead & zinc

On the basis of the data shown in Figure 5-41, it can be seen that the most distinguishable soil types are AA, BB and EE. Cross-referencing this information with the concentrations of each metal that were added to the soil (Table 3-8), shows that soil types AA, BB and EE were those that were most different from each other i.e. AA had 20mg/kg copper added, BB had 66mg/kg lead added and EE had no metals added. As a result of Figure 5-41, it was decided to apply classification techniques to data consisting soil types AA, BB and EE only.

A second scatterplot matrix (Figure 5-42) was created to investigate whether the concentrations from all three metals should be used.

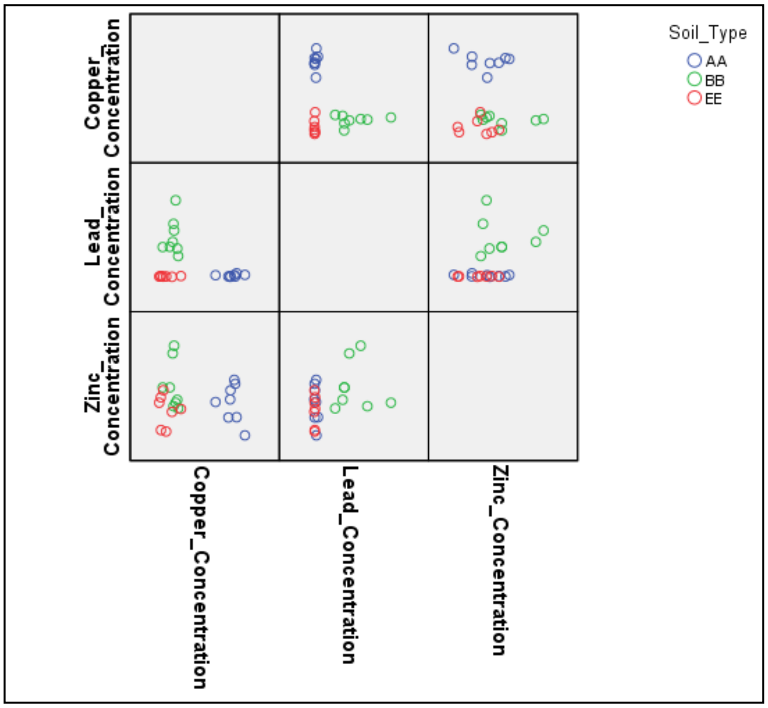


Figure 5-42: Scatterplot matrix of selected soil data for concentrations of copper, lead & zinc

As a result of Figure 5-42, only soil, leaf and resin data that originated from soil types AA, BB and EE would be tested using the clustering methods, using copper and lead concentrations only. As before, the different hierarchical clustering methods were investigated as shown in Table 5-33 (the dendrograms can be found in Appendix 9).

Table 5-33: Results of hierarchical clustering for soil, leaf & resin concentration data from soil types AA, BB & EE

Hierarchical Clustering Method	Distance Used	HCA Success Rating		
		Soil (n=23)	Leaf (n=16)	Resin (n=13)
Complete Linkage	Squared Euclidean	87 %	0 %	46 %
Complete Linkage	Manhattan	87 %	44 %	46 %
Average Linkage	Squared Euclidean	96 %	0 %	46 %
Ward Linkage	Squared Euclidean	100 %	13 %	46 %

Table 5-33 shows that for the soil data, Ward linkage was the most successful with 100 % of the data assigned to a unique cluster. The resin data gave consistent results with all four tests and for the leaf data, complete linkage with Manhattan distances proved to be the most successful.

The results of discriminant function analysis for soil, leaf and resin data can be seen in Table 5-34 (and Appendix 10). The results showed that the soil data were classified more accurately than the resin data, with 100 % of the cases correctly assigned to their group. The leaf data was not considered to have been classified successfully. From the point of view of heroin profiling, it is important that the resin data were able to be classified even if they were not to the same extent as the soil data.

Table 5-34: Results of discriminant function analysis for soil, leaf & resin concentration data from soil types AA, BB & EE

Sample	Percentage of original grouped cases correctly classified	Percentage value 25 % larger than that due to chance	Acceptable classification?
Soil (n=23)	100 %	41 %	✓
Leaf (n=16)	38 %	41 %	✗
Resin (n=13)	62 %	41 %	✓

Overall, the percentages obtained using soil types AA, BB and EE and the concentrations of copper and lead were higher than when all of the soil types and the concentrations of copper, lead and zinc were considered (Table 5-32).

5.11.3 Concentration Ratio Data

When classification techniques were applied to the soil, leaf and resin ratio data for the ratios selected in Section 5.9.1, the data from all ten soil types were considered. As with the concentration data (Section 5.11.1), the four different hierarchical clustering methods were investigated and the results shown in Table 5-35 (the dendrograms can be found in Appendix 9). The results of discriminant function analysis for soil, leaf and resin ratio data can be seen in Table 5-36 (and Appendix 10).

Table 5-35: Results of hierarchical clustering for all soil, leaf & resin ratio data

Hierarchical Clustering Method	Distance Used	HCA Success Rating		
		Soil (n=78)	**Leaf (n=65)**	**Resin (n=46)**
Complete Linkage	Squared Euclidean	58 %	18 %	16 %
Complete Linkage	Manhattan	55 %	18 %	19 %
Average Linkage	Squared Euclidean	53 %	15 %	16 %
Ward Linkage	Squared Euclidean	44 %	18 %	16 %

Table 5-36: Results of discriminant function analysis for all soil, leaf & resin ratio data

Sample	Percentage of original grouped cases correctly classified	Percentage value 25 % larger than that due to chance	Acceptable classification?
Soil (n=78)	72 %	13 %	✓
Leaf (n=65)	31 %	13 %	✓
Resin (n=46)	21 %	13 %	✓

As can be seen from Table 5-35, the most successful hierarchical clustering method for all sample types was complete linkage. Using discriminant function analysis, the soil data were classified the most accurately, with 72 % of the cases correctly assigned to their group. As expected based on the results of Section 5.9.2 and Section 5.9.3, the leaf and resin data were not as successfully classified. The results obtained differed from those obtained when the corresponding concentration data were clustered (Table 5-32); for soil and resin the results were lower whereas the result for leaf was slightly higher. As soil and resin were the more significant two sample types for the purpose of this study, it appeared that at this stage, the concentration data were the most useful.

5.11.4 Optimisation of Concentration Ratio Data

As with the concentration data, the initial classification using the ratio data did not provide a 100 % successful classification for all soil types. It was therefore decided to re-classify the data using the soil types that had the greatest differences in soil metal concentration, i.e. soil types AA, BB and EE. A scatterplot matrix (Figure 5-43) was created to investigate which combination of ratios would provide the greatest spread of data.

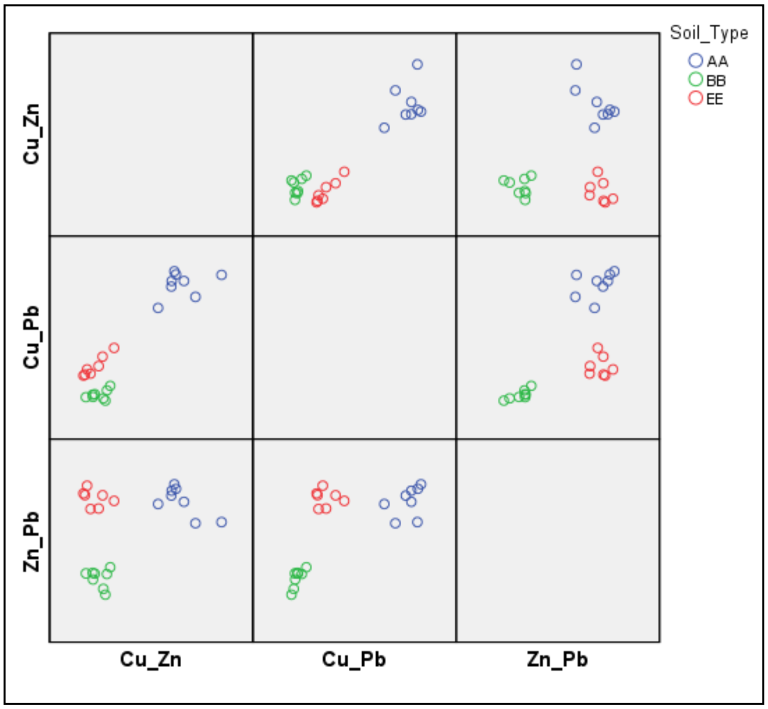


Figure 5-43: Scatterplot matrix for selected soil ratio data for Cu:Zn, Cu:Pb & Zn:Pb

Figure 5-43 showed that the ratio combinations of Cu:Pb with Zn:Pb and Cu:Zn with Zn:Pb both produced clusters that were clearly distinguishable from one another but the individual clusters were more compact with the combination of Cu:Pb with Zn:Pb; these were therefore the variables chosen for the remainder of the analyses.

As previously, the four different methods of hierarchical clustering were performed (the dendrograms can be found in Appendix 9), this time on the ratio data for soil, leaves and resin (Table 5-37); discriminant function analysis (Table 5-38 and Appendix 10) was also carried out.

Table 5-37: Results of hierarchical clustering for soil, leaf & resin ratio data from soil types AA, BB & EE

Hierarchical Clustering Method	Distance Used	HCA Success Rating		
		Soil (n=23)	Leaf (n=16)	Resin (n=13)
Complete Linkage	Squared Euclidean	100 %	56 %	62 %
Complete Linkage	Manhattan	100 %	56 %	62 %
Average Linkage	Squared Euclidean	100 %	56 %	62 %
Ward Linkage	Squared Euclidean	100 %	63 %	46 %

Table 5-38: Results of discriminate function analysis for soil, leaf & resin ratio data from soil types AA, BB & EE

Sample	Percentage of original grouped cases correctly classified	Percentage value 25 % larger than that due to chance	Acceptable classification?
Soil (n=23)	100 %	41 %	✓
Leaf (n=16)	56 %	41 %	✓
Resin (n=13)	69 %	41 %	✓

From Table 5-37, it can be seen that, with the exception of the resin data, there was not much variation in the results obtained using the different hierarchical clustering methods. For both hierarchical clustering and discriminant function analysis, the soil ratio data were all correctly classified. As seen previously, the ratio data for leaf and resin were not as successfully classified as soil but the results obtained were higher than when all of the soil types and all three ratios were considered (Table 5-36). Also, the results between hierarchical clustering and discriminant function analysis were more consistent than they were for the previous comparisons.

5.11.5 Summary of Classification Results

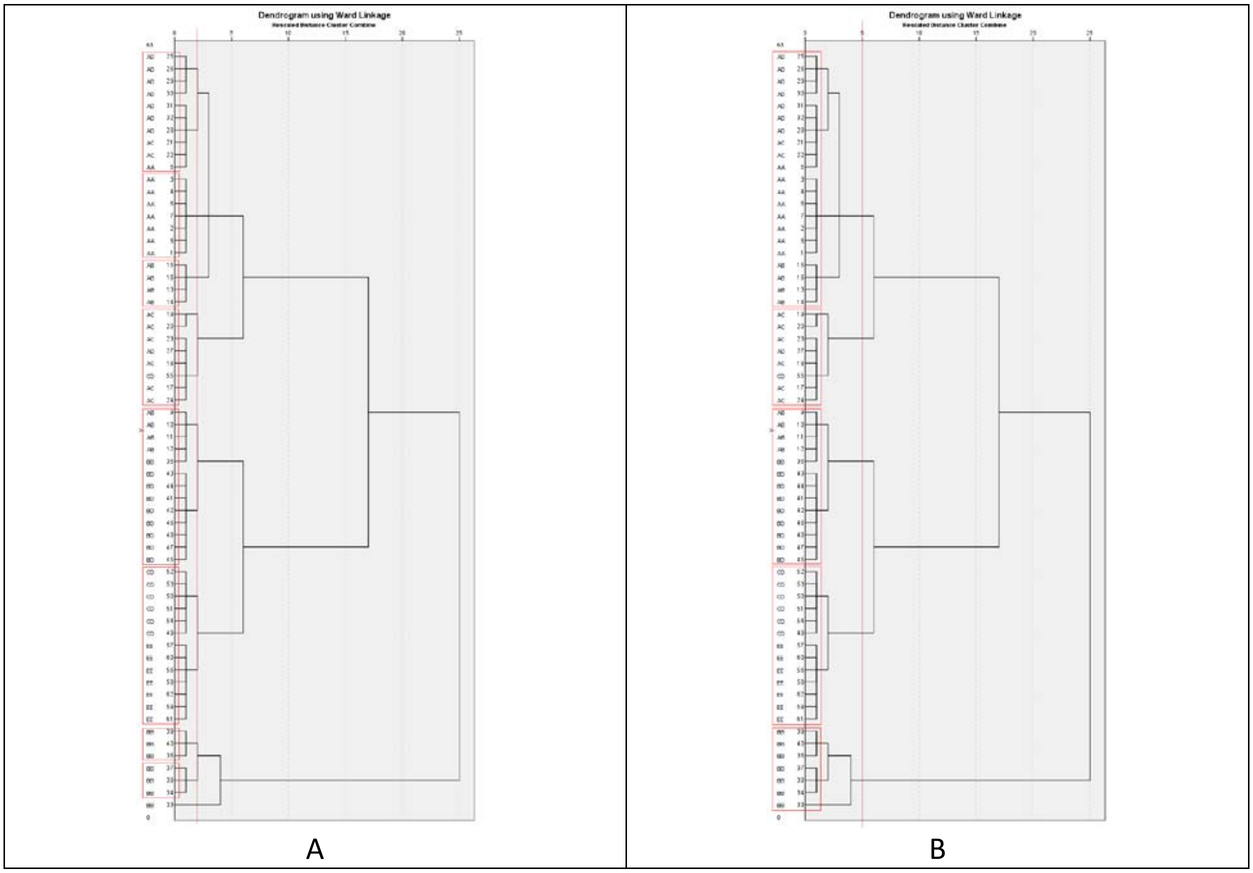
The results produced using soil types AA, BB and EE were better than when all ten soil types were considered. This showed that the success of these methods relied on there being a larger difference in the concentration of metals in the soils than may have been present between some of the soil types used in this study. This suggested that the use of metal concentrations in heroin would not be useful for differentiating between regions that only had a small variation in soil metal concentration.

After looking at the results of the clustering analysis for both the concentration data (Table 5-34) and the ratio data (Table 5-38) it was seen that the ratio data provided the most successful results. Although the classification methods correctly classified 100 % of the data points for both the soil concentration data and the soil ratio data, the leaf and resin ratio data produced greater classification percentages than the concentration data. Overall the resin data were more successfully classified than the leaf data.

There are a number of problems associated with the use of hierarchical clustering. As shown above, there are several different methods available that in some cases can produce quite different results to one another and it is the decision of the analyst which one to choose. This can present a problem when different results need to be compared as the best method for one data set may not be the best method for another. Another problem that can occur when the analyst has prior knowledge of group membership is that of post-rationalisation. The interpretation of hierarchical clustering methods relies on the analyst determining at which distance to observe the clusters and depending upon where this line is drawn, the results can vary (see Table 5-39 and Figure 5-44). In this study, any post-rationalisation did not affect the results; hierarchical clustering was used as a test to see if the soil, leaf and resin samples could be clustered therefore the optimum clustering was looked for in order for comparisons to be made between methods and between different sample types. However, in the real world it would be important that any post-rationalisation did not occur and therefore samples should be anonymised before clustering occurs to enable only true clusters to be seen.

Table 5-39: Effect of line position on classification % for hierarchical clustering

HCA Success Rating	
Line drawn at a rescaled distance 2 (Figure 5-40 A)	**Line drawn at a rescaled distance of 5 (Figure 5-40 B)**
73 %	45 %



A

B

Figure 5-44: Dendrograms using Ward linkage produced from copper, lead & zinc concentrations of soil

Another problem that can occur due to the nature of hierarchical clustering is the effect of sample order when clustering is performed [137]. However, after repeating analyses with the data randomly ordered, no differences were seen so the sample order was found not to affect the results of this study.

As previously mentioned, the use of discriminant function analysis requires a prior knowledge of group membership. As group membership was known, this technique was able to be used to assess the successful classification of samples without the influence of post-rationalisation and therefore, in this study, the results of this technique were more reliable than those obtained using hierarchical clustering. However, the use of this technique in the differentiation of heroin samples would rely either on the results of clustering techniques (such as HCA) or intelligence data from law enforcement agencies, to inform the analyst of the number of clusters present.

Comparison of resin data with soil data

To be able to use data pertaining to the concentration of metals in heroin to determine geographical origin, the resin data would need to be successfully classified into the same group as the corresponding soil data. As the use of metal concentration ratios was found to produce the most successful classification of both soil and resin samples, hierarchical clustering (the dendrograms can be found in Appendix 9) and discriminant function analysis were performed on this data. For discriminant function analysis, the percentage of successful classification was calculated from the resin data alone, with a correct classification viewed as one in which the resin data was grouped in with the corresponding soil data.

The results in Table 5-40 and Table 5-41 showed that overall, hierarchical clustering was more successful than discriminant function analysis in classifying the resin ratio data into the group for the corresponding soil ratio data. However, as the hierarchical clustering results were influenced by post-rationalisation, the results from the discriminant function analysis were viewed as being more reliable. The value of 54 % for discriminant function analysis demonstrated that a link could to be made between some of the resin samples and their corresponding soil samples when using the ratios of Cu:Pb and Zn:Pb.

Table 5-40: Results of hierarchical clustering for soil & resin ratio data from soil types AA, BB & EE

Sample	**Percentage of resin data points assigned to the correct group based on classification of soil**		
	Complete Linkage (Squared Euclidean)	**Complete Linkage (Manhattan)**	**Average Linkage (Squared Euclidean)**
Soil (n=23) & Resin (n=13)	62 %	23 %	62 %

Table 5-41: Results of discriminant function analysis for soil & resin ratio data from soil types AA, BB & EE

Sample	**Percentage of original grouped cases correctly classified**	**Percentage value 25 % larger than that due to chance**	**Acceptable classification?**
Soil (n=23) & Resin (n=13)	54 %	41 %	✓

The main reason why the percentage of correct classification was not higher was due to a lack of correlation between the concentrations of copper, lead and zinc in the resin of *Papaver somniferum* L. and the concentrations of copper, lead and zinc present in the soil in which they were grown, as shown in Figure 5-17, Figure 5-19 and Figure 5-21. When comparing concentration data, the plant/soil ratios were > 1, suggesting that bioaccumulation had taken place [138] but when the metal concentration ratio data were considered [Section 5.10], there was poor relationship between plant and soil suggesting a variation in the metal uptake between individual plants. Studies that investigated the variation in metal uptake of plants reported similar intra-species variation [139-142] and intra-species variation was also observed in the metal content of poppy seeds [143]. There was also the possibility of contamination of the plants from aerial sources [144] but as the plants were all grown together in a greenhouse, exposure to aerial contamination was minimised and it was assumed that any contamination would have been consistent between plants.

These values suggested that trace metal concentration ratio data from seized heroin samples could be classified with soil data (from different regions of the world where opium poppies are known to be grown) to produce clusters indicating the likely geographical origin.

Comparison of seized heroin samples

Although the resin samples from this study were classified with an accuracy of 69 % (Table 5-38), this value was not as high as those stated in previous studies; both Zhang et al [70] and Myors et al [66] were able to classify heroin into two groups with 100 % accuracy using hierarchical clustering. There were a number of possible explanations for why the classification of data in this study was not as successful as those studies mentioned above in terms of classification using trace metal data.

As previously acknowledged (Section 5.11.2), the success of the classification of the data in this study was dependant on the soil types used; only those soil types that demonstrated a greater difference in trace metal concentration were suitable. Therefore, in the previous studies, the heroin samples from the two regions may have differed more significantly in the concentrations of metals analysed. Unfortunately, it was not possible to investigate this further as the relevant literature did not report the necessary raw data.

Coupled with this is the effect that a difference in concentration may have on the effect of classification. In this study, results from MANOVAs demonstrated that there was no significant difference between the different resin samples yet they were still able to be classified. This would indicate that potentially even a slightly greater difference in metal concentrations could produce a more successful grouping of data using the classification techniques explored in this study. A difference in near-surface parent material and soil-forming processes between regions results in considerable variability in the trace metal concentration of soil [62]. Other factors, such as pollution from mining, could also result in more localized variation due to an increase in the concentration of specific metals [145, 146]. However, if more samples were analysed (to reduce the effect of random errors caused by weighing small masses (Section 5.5.4)), data with a smaller difference in metal concentrations may be able to be classified more successfully.

Another reason that the previous studies were able to classify more successfully may have been due to the use of more metals, rather than just the three as in this study. Moreover, other metals may have been more discriminating; for example, Ekangaki et al [65] found that lithium, beryllium, yttrium, zirconium, samarium, gadolinium, dysprosium, thorium, germanium, lead and arsenic were found to be the most discriminating when distinguishing between samples of Chinese and non-Chinese heroin and Myors et al [66] found palladium, platinum and ruthenium to be useful when distinguishing between heroin samples.

The final explanation for why better classification was achieved in other studies involves the influence of metals from sources other than the soil in which *Papaver somniferum* L. were grown. As previously mentioned, opium poppies may have been exposed to aerial contamination but there may also be genetic variations in the species [147] as a result of selective breeding to improve opium yield [148]. Any genetic variations that have arisen may have affected the uptake of metals by the plants therefore contributing to the differences in metal concentrations in heroin from plants that were grown in different regions of the world. Depending upon the region where grown, fertilisers may be added to the soil to improve growth and increase productivity; the use of such fertilisers may have impacted upon the rate at which metals are taken up by the plants. Finally, there are a number of post-harvest factors that may have affected the concentration of trace metals in the resulting heroin. The process of synthesising the heroin from morphine involves the use of metal containers which, during acetylation, will have been exposed to high temperatures and extremes of pH, resulting in metals from the containers having leached out into the heroin [59, 63]. The process of acetylation also involved the addition of water and solvents which may also have contributed to the metals present in the final product [70]. After processing, the heroin is packed into blocks using metal presses [97] and dealers will have added diluents and cutting agents [63] to the drug which may also have resulted in the presence of metals additional to those that originated from the soil.

Chapter 6 : Conclusion

Previous studies have been conducted that have investigated the potential for the use of trace metals in heroin for determining geographical origin [64-66, 70] yet without samples of heroin of known provenance, the success of such studies could not be substantiated [59]. This study provided a novel approach by growing opium poppies (*Papaver somniferum* L.) in different soil conditions to produce samples of resin that could be compared to the soil from which the corresponding plants were grown.

When statistical tests (MANOVAs) were applied to the metal concentration data and the metal concentration ratio data (for Cu:Zn, Cu:Pb and Zn:Pb), eight out of the ten soil types were found to be significantly different from one another (at a confidence level ≥95 %) but neither the leaf samples nor the resin samples from the corresponding soil types were found to be significantly different from one another. This may have been due to random errors associated with the weighing of small masses of leaves and resin that resulted in intra-sample variations that were large in comparison with the inter-sample variations. However, there may have also been intra-plant variation in the uptake of metals, as reported for other plant species by Wang et al [139], Zhu et al [140], Deng et al [141] and Landberg and Greger [142].

Hierarchical clustering (HCA) and discriminant function analysis were performed to test the ability to cluster the soil, leaf and resin data according to their originating soil type. When such classification techniques were used, optimum variables were selected; for metal concentration data these were data from soil types AA, BB and EE with the concentrations of copper and lead only. For metal concentration ratio data these were data from soil types AA, BB and EE only with the ratios of Cu:Pb and Zn:Pb.

A tool was developed to measure the success of clustering using hierarchical clustering (known as HCA success rating). When this tool was used, along with the results of the discriminant function analysis, the metal concentration ratio data were found to cluster more successfully than the metal concentration data. The metal concentration ratio data for soil, leaf and resin samples were found to cluster with an HCA success rating of 100 %, 63 % and 62 % respectively. These results were mostly consistent with the results of discriminant function analysis which returned results of 100 %, 56 % and 69 % for soil, leaf and resin samples, respectively.

It was found that using hierarchical clustering and discriminant function analysis, data from resin that originated from poppy plants grown in the different soil types could be placed into the same clusters as the data that corresponded to the soil in which the poppy plants were grown. However, because of the poor relationships that existed between the individual ratios of metals in the soil and the resin samples, the ability to determine geographical origin would still rely on the presence of heroin samples or soil samples of known origin to compare to.

Using hierarchical clustering and discriminant function analysis, the clustering of resin samples was successful which supports the findings of Ekangaki et al [65], Myors et al [66] and Zhang et al [70] that trace metals in heroin can be useful for the comparison of batches. However, the findings of Zhang et al [70] and Myors et al [66] produced 100 % success when classifying batches of heroin using hierarchical clustering analysis compared with only 69 %

for the resin samples in this study. Although random errors and/or intra-plant variation may have been responsible for these differences, another factor that may have been responsible was a difference between the metals present in heroin compared with the metals present in resin. This suggested that a number of post-harvest factors such as the use of metal containers [59, 63], contamination from solvents and water [70] and the addition of cutting agents [63] were responsible for contributing to the trace metals present in the heroin and for creating differences in the batches of heroin analysed. Also, in this study, the poppy seeds used came from the same source whereas around the world, genetic differences in *Papaver somniferum* L. [147] could affect the uptake and accumulation of trace metals.

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Appendices

Appendix 1: Calculation of Cation Exchange Capacity

Concentration of NH_4^+ in NH_4Cl

= mean titre x molarity HCl

= ($5.05cm^3$ /1000) x 0.01M

= $5.05x10^{-3}$ L x 0.01 $molL^{-1}$

= $5.05x10^{-5}$ moles in 25ml therefore in 1L = ($5.05x10^{-5}$) x 40 = 0.00202

= 0.00202 $molL^{-1}$

Amount of NH_4^+ in solution in soil

= weight of wet syringe – weight dry syringe

= 28.2798g – 24.7561g

= 3.5237g

Assuming mass = volume, 3.5237g = 3.5237ml of NH_4Cl

Concentration = (3.5237ml / 1000) x 0.00202 $molL^{-1}$

= $7.12x10^{-6}$ moles of NH_4^+

Amount of NH_4^+ extracted in KNO_3

= mean titre x molarity HCl

= (6.68cm3 / 1000) x 0.01M

= $6.68x10^{-3}$ L x 0.01 $molL^{-1}$

$6.68x10^{-5}$ moles in 25ml of leachate

$6.68x10^{-5}$ x 4 = $2.67x10^{-4}$ moles in 100ml of leachate (total volume)

NH_4^+ bound in soil

= NH_4^+ in leachate – NH_4^+ in NH_4Cl added

= ($2.67x10^{-4}$) – ($7.12x10^{-6}$)

= $2.60x10^{-4}$ moles NH_4^+ in 4.0555g soil

= 0.0641 moles kg^{-1} soil

= 6.41 $cmolkg^{-1}$

As NH_4^+ is monovalent, 1 cation exchange site would be occupied by 1 mole. The same is true for potassium.

Copper, lead and zinc are all divalent therefore 2 cation exchange sites would be occupied by 1 mole. Therefore ECEC of soil for divalent metals:

= 6.41 $cmolkg^{-1}$ / 2

= 3.2 $cmolkg^{-1}$

Appendix 2: Calculation of Concentration of Each Metal Added to Each Soil Type

For monovalent metals: if the ECEC of the soil is 6.4 cmolKg^{-1}, in 5kg there would be 32 cmol (0.32 moles). For divalent metals: if the ECEC of the soil is 3.2 cmolKg^{-1}, in 5kg there would be 16 cmol (0.16 moles).

The concentration of each metal solution was then calculated as follows:

Copper

In 5kg soil, 1% of the ECEC would be $1.6\text{x}10^{-3}$ moles.
Mass of copper required = RMM copper x $1.6\text{x}10^{-3}$ moles
= 63.5 gmol^{-1} x $1.6\text{x}10^{-3}$ moles
= 0.1016g copper
If 10L of solution is required, 0.1016g / 10L = 0.0102 gL^{-1}
= 10mgL^{-1}

Lead

In 5kg soil, 1% of the ECEC would be $1.6\text{x}10^{-3}$ moles.
Mass of lead required = RMM lead x $1.6\text{x}10^{-3}$ moles
= 207.2 gmol^{-1} x $1.6\text{x}10^{-3}$ moles
= 0.3315g lead
If 10L of solution is required, 0.3315g / 10L = 0.033 gL^{-1}
= 33 mgL^{-1}

Potassium

In 5kg soil, 1% of the ECEC would be $3.2\text{x}10^{-3}$ moles.
Mass of potassium required = RMM potassium x $3.2\text{x}10^{-3}$ moles
= 39.1 gmol^{-1} x $3.2\text{x}10^{-3}$ moles
= 0.1250g potassium

If 10L of solution is required, 0.1250g / 10L = 0.0125 gL^{-1}

= 13 mgL^{-1}

Zinc

In 5kg soil, 1% of the ECEC would be $1.6x10^{-3}$ moles.

Mass of zinc required = RMM zinc x $1.6x10^{-3}$ moles

= 65.4 $gmol^{-1}$ x $1.6x10^{-3}$ moles

= 0.1046 g zinc

If 10L of solution is required, 0.1046g / 10L = 0.0105 gL^{-1}

= 11 mgL^{-1}

Appendix 3: Descriptive Statistics for Soil, Leaf and Resin Data Before and After Removal of Outliers

Concentration Data

Soil

Descriptive data for soil copper concentrations (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	8	0.2779	0.0241	8.67	0.595	0.804	0.2309	-1.9502	2.1266
AB	8	0.2091	0.0456	21.81	0.771	0.508			
AC	8	0.1990	0.0396	19.90	0.604	0.790			
AD	8	0.1920	0.0219	11.41	0.559	0.858			
BB	8	0.1086	0.0142	13.08	0.638	0.733	0.0790	-2.0845	2.1266
BC	8	0.1150	0.0204	17.74	0.693	0.638			
BD	8	0.0988	0.0135	13.66	0.690	0.642	0.0716	-2.0148	2.1266
CC	8	0.0942	0.0083	8.81	0.466	0.958			
CD	8	0.1123	0.0521	46.39	0.843	0.399	0.2261	2.1843	2.1266
EE	8	0.0865	0.0219	25.32	0.646	0.718			

Descriptive data for soil copper concentrations (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	8	0.2779	0.0241	8.67	0.595	0.804	0.2309	-1.954	2.127
AB	8	0.2091	0.0456	21.81	0.771	0.508			
AC	8	0.1990	0.0396	19.90	0.604	0.790			
AD	8	0.1920	0.0219	11.41	0.559	0.858			
BB	8	0.1086	0.0142	13.08	0.638	0.733	0.0790	-2.086	2.127
BC	8	0.1150	0.0204	17.74	0.693	0.638			
BD	8	0.0988	0.0135	13.66	0.690	0.642	0.0716	-2.012	2.127
CC	8	0.0942	0.0083	8.81	0.466	0.958			
CD	7	0.0960	0.0266	27.71	0.557	0.858	0.1489	1.992	2.020
EE	7	0.0890	0.0224	25.17	0.577	0.829			

Descriptive data for soil lead concentrations (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p*-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	8	0.0797	0.0068	8.53	0.575	0.835			
AB	8	0.1709	0.0258	15.10	0.604	0.790			
AC	8	0.0806	0.0045	5.58	0.474	0.952			
AD	8	0.0759	0.0066	8.70	0.504	0.924			
BB	8	0.3022	0.1039	34.38	0.665	0.686			
BC	8	0.1511	0.0700	46.33	1.146	0.107			
BD	8	0.1341	0.0270	20.13	0.514	0.914			
CC	8	0.0802	0.0060	7.48	0.625	0.754			
CD	8	0.0738	0.0080	10.84	0.906	0.313			
EE	8	0.0736	0.0032	4.35	0.568	0.844	0.0669	-2.1288	2.1266

Descriptive data for soil lead concentrations (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p*-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	8	0.0797	0.0068	8.53	0.575	0.835			
AB	8	0.1709	0.0258	15.10	0.604	0.790			
AC	8	0.0806	0.0045	5.58	0.474	0.952			
AD	8	0.0759	0.0066	8.70	0.504	0.924			
BB	8	0.3022	0.1039	34.38	0.665	0.686			
BC	8	0.1511	0.0700	46.33	1.146	0.107			
BD	8	0.1341	0.0270	20.13	0.514	0.914			
CC	8	0.0802	0.0060	7.48	0.625	0.754			
CD	7	0.0729	0.0082	11.25	0.799	0.459			
EE	7	0.0746	0.0017	2.28	0.487	0.938			

Descriptive data for soil zinc concentrations (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	8	0.3071	0.0256	8.34	0.423	0.982			
AB	8	0.2896	0.0365	12.60	0.452	0.967			
AC	8	0.3980	0.0333	8.37	0.526	0.901			
AD	8	0.2922	0.0451	15.43	0.542	0.881			
BB	8	0.3297	0.0324	9.83	0.759	0.528			
BC	8	0.3506	0.0344	9.81	0.564	0.851			
BD	8	0.2643	0.0181	6.85	0.450	0.968			
CC	8	0.4078	0.0936	22.95	0.641	0.728			
CD	8	0.3709	0.0108	2.91	0.625	0.755			
EE	8	0.2880	0.0330	11.46	0.604	0.790			

Descriptive data for soil zinc concentrations (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	8	0.3071	0.0256	8.34	0.423	0.982			
AB	8	0.2896	0.0365	12.60	0.452	0.967			
AC	8	0.3980	0.0333	8.37	0.526	0.901			
AD	8	0.2922	0.0451	15.43	0.542	0.881			
BB	8	0.3297	0.0324	9.83	0.759	0.528			
BC	8	0.3506	0.0344	9.81	0.564	0.851			
BD	8	0.2643	0.0181	6.85	0.450	0.968			
CC	8	0.4078	0.0936	22.95	0.641	0.728			
CD	7	0.3723	0.0108	2.90	0.637	0.732			
EE	7	0.2974	0.0210	7.06	0.480	0.945			

Leaf

Descriptive data for leaf copper concentrations (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	6	3.4052	5.0592	148.57	1.100	0.127	13.7073	2.036	1.887
AB	7	4.5304	6.7112	148.14	1.217	0.071	19.6200	2.248	2.020
AC	7	4.3143	3.4885	80.86	0.582	0.822			
AD	11	3.8376	3.0832	80.34	0.714	0.614	11.0432	2.337	2.355
BB	7	2.5449	2.8988	113.90	1.010	0.200	8.9329	2.204	2.020
BC	8	2.3216	1.7517	75.45	0.798	0.465			
BD	6	1.5245	0.7543	49.48	0.447	0.965			
CC	7	1.9553	0.9060	46.33	0.680	0.656			
CD	7	2.1654	1.4648	67.65	0.504	0.922			
EE	7	5.1390	7.1527	139.18	0.801	0.455	19.5970	2.021	2.020

Table 0-1: Descriptive data for leaf copper concentrations (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	5	1.3448	0.3928	29.21	0.707	0.600			
AB	5	1.8617	0.9859	52.95	0.737	0.547			
AC	7	4.3143	3.4885	80.86	0.582	0.822			
AD	10	3.1170	2.0534	65.88	0.829	0.425			
BB	5	1.3028	0.6828	52.41	0.381	0.993			
BC	7	1.8508	1.2292	66.42	0.770	0.503	4.1385	1.861	2.020
BD	6	1.5245	0.7543	49.48	0.447	0.965			
CC	7	1.9553	0.9060	46.33	0.680	0.656			
CD	7	2.1654	1.4648	67.65	0.504	0.922			
EE	6	2.7294	3.5524	130.16	0.932	0.275	9.4030	1.879	1.887

Descriptive data for leaf lead concentrations (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	p-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	6	1.1052	1.0503	95.03	0.752	0.525	3.1448	1.942	1.887
AB	7	1.1957	1.3625	113.95	0.933	0.277	3.9380	2.013	2.020
AC	7	1.1322	0.9119	80.54	1.049	0.167			
AD	11	1.4207	0.8726	61.42	0.554	0.871			
BB	7	1.0240	1.1685	114.11	1.006	0.203	3.2479	1.903	2.020
BC	8	1.1807	1.2877	109.07	0.715	0.600	4.0121	2.199	2.127
BD	6	0.4422	0.2758	62.38	0.473	0.947			
CC	7	0.5653	0.2745	48.56	0.558	0.857			
CD	7	0.5343	0.2983	55.83	0.661	0.689			
EE	7	1.2422	1.5779	127.02	0.818	0.429	4.3871	1.993	2.020

Descriptive data for leaf lead concentrations (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	p-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	5	0.6973	0.3618	51.88	0.596	0.790	1.2866	1.629	1.715
AB	5	0.4700	0.2237	47.60	0.514	0.901			
AC	7	1.1322	0.9119	80.54	1.049	0.167			
AD	10	1.2292	0.6308	51.32	0.457	0.966			
BB	5	0.3713	0.0942	25.38	0.500	0.916			
BC	7	0.7762	0.6384	82.25	0.741	0.550	2.0267	1.959	2.020
BD	6	0.4422	0.2758	62.38	0.473	0.947			
CC	7	0.5653	0.2745	48.56	0.558	0.857			
CD	7	0.5343	0.2983	55.83	0.661	0.689			
EE	6	0.7181	0.8246	114.84	0.947	0.258			

Descriptive data for leaf zinc concentrations (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p*-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	6	1.1576	0.6886	59.49	0.909	0.301	2.5282	1.990	1.887
AB	7	1.3886	1.8505	133.26	1.246	0.061	5.5715	2.260	2.020
AC	7	1.7031	0.5656	33.21	0.582	0.822			
AD	11	1.2930	0.6067	46.92	0.841	0.411	2.8995	2.648	2.355
							0.5528	-1.220	2.355
BB	7	0.9445	0.8100	85.76	1.066	0.154	2.7672	2.250	2.020
BC	8	1.3410	0.7757	57.84	0.420	0.983			
BD	6	0.8020	0.2095	26.12	0.453	0.961			
CC	7	1.2800	0.3778	29.51	0.663	0.686	2.0102	1.933	2.020
CD	7	1.4487	0.5366	37.04	0.666	0.681			
EE	7	1.3999	1.4594	104.25	0.794	0.467	4.2598	1.960	2.020

Descriptive data for leaf zinc concentrations (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	p-value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	5	0.8834	0.1707	19.32	0.681	0.645			
AB	5	0.6822	0.1813	26.58	0.681	0.647			
AC	7	1.7031	0.5656	33.21	0.582	0.822			
AD	10	1.1323	0.3058	27.01	0.563	0.856	1.7134	1.900	2.290
							0.5528	-1.895	2.290
BB	5	0.5987	0.0430	7.18	0.602	0.780	0.5263	-1.683	1.715
BC	7	1.1523	0.6080	52.76	0.449	0.967			
BD	6	0.8020	0.2095	26.12	0.453	0.961			
CC	7	1.2800	0.3778	29.51	0.663	0.686	2.0102	1.933	2.020
CD	7	1.4487	0.5366	37.04	0.666	0.681			
EE	6	0.9233	0.8046	87.15	0.887	0.329			

Resin

Descriptive data for resin copper concentrations (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	5	10.4298	4.3522	41.73	0.460	0.952			
AB	5	9.6116	6.7467	70.19	0.592	0.796			
AC	4	15.9178	15.1754	95.34	0.512	0.893			
AD	5	8.8036	3.7730	42.86	0.485	0.931			
BB	6	15.0226	15.5165	103.29	0.781	0.478	44.8702	1.924	1.887
BC	4	8.1988	6.4955	79.23	0.746	0.526			
BD	6	8.3010	8.1179	97.79	0.586	0.732			
CC	5	5.2738	3.4915	66.20	0.812	0.556	10.9354	1.622	1.715
							1.3483	-1.124	1.715
CD	5	17.3868	13.8432	79.62	0.405	0.987			
EE	3	2.7347	1.0322	37.74	0.458	0.955			

Descriptive data for resin copper concentrations (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	5	10.4298	4.3522	41.73	0.460	0.952			
AB	4	6.9900	3.8562	55.17	0.552	0.841			
AC	4	15.9178	15.1754	95.34	0.512	0.893			
AD	5	8.8036	3.7730	42.86	0.485	0.931			
BB	5	9.0531	5.8041	64.11	0.483	0.933			
BC	4	8.1988	6.4955	79.23	0.746	0.526			
BD	6	8.3010	8.1179	97.79	0.586	0.812			
CC	4	3.8585	1.7025	44.12	0.695	0.614			
CD	5	17.3868	13.8432	79.62	0.405	0.987			
EE	3	2.7347	1.0322	37.74	0.458	0.955			

Descriptive data for resin lead concentrations (before Grubb's Test)

Soil Type	**n**	**Mean**	**Standard Deviation**	**% RSD**	**Kolmogorov-Smirnov**		**Grubb's Test**		
					Z-value	***p*-value**	**Outlier Value (μmolg^{-1})**	**G**	**$G_{critical}$**
AA	5	3.3118	1.0985	33.17	0.429	0.975			
AB	5	4.0990	1.7186	41.93	0.569	0.831			
AC	4	8.0249	8.4669	105.51	0.666	0.663			
AD	5	4.0114	1.5052	37.52	0.277	1.000			
BB	6	7.9710	10.4605	131.23	0.816	0.424	28.6081	1.973	1.887
BC	4	2.4647	2.2602	91.70	0.772	0.482			
BD	6	3.2391	2.8124	86.82	0.485	0.937			
CC	5	1.4187	0.7152	50.42	0.673	0.661			
CD	5	3.3511	2.5833	77.09	0.508	0.908	7.5111	1.610	1.715
EE	3	1.0711	0.5621	52.48	0.626	0.701			

Descriptive data for resin lead concentrations (after Grubb's Test)

Soil Type	**n**	**Mean**	**Standard Deviation**	**% RSD**	**Kolmogorov-Smirnov**		**Grubb's Test**		
					Z-value	***p*-value**	**Outlier Value (μmolg^{-1})**	**G**	**$G_{critical}$**
AA	5	3.3118	1.0985	33.17	0.429	0.975			
AB	4	4.2695	1.9350	45.32	0.406	0.986			
AC	4	8.0249	8.4669	105.51	0.666	0.663			
AD	5	4.0114	1.5052	37.52	0.277	1.000			
BB	5	3.8436	3.0020	78.10	0.650	0.701			
BC	4	2.4647	2.2602	91.70	0.772	0.482			
BD	6	3.2391	2.8124	86.82	0.485	0.937			
CC	4	1.2870	0.7526	58.48	0.501	0.906			
CD	5	3.3511	2.5833	77.09	0.508	0.908	7.5111	1.610	1.715
EE	3	1.0711	0.5621	52.48	0.626	0.701			

Descriptive data for resin zinc concentrations (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	5	6.9425	4.6270	66.65	0.342	0.998			
AB	5	3.2006	3.3920	105.98	0.736	0.549	9.0775	1.733	1.715
AC	4	2.3882	1.7563	73.54	0.415	0.982			
AD	5	1.2882	0.9954	77.27	0.474	0.941			
BB	6	4.9569	7.8921	159.21	1.014	0.192	20.8931	2.019	1.887
BC	4	1.8197	1.9406	106.65	0.728	0.557			
BD	6	1.6689	1.2021	72.03	0.390	0.991			
CC	5	0.5213	0.6129	117.56	0.743	0.538	1.5766	1.722	1.715
CD	5	1.7565	1.8397	104.74	0.629	0.736			
EE	3	0.3992	0.3607	90.35	0.576	0.781			

Descriptive data for resin zinc concentrations (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p*-value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	5	6.9425	4.6270	66.65	0.342	0.998			
AB	4	1.7314	0.9748	56.30	0.466	0.948			
AC	4	2.3882	1.7563	73.54	0.415	0.982			
AD	5	1.2882	0.9954	77.27	0.474	0.941			
BB	5	1.7697	1.2914	72.97	0.453	0.957			
BC	4	1.8197	1.9406	106.65	0.728	0.557			
BD	6	1.6689	1.2021	72.03	0.390	0.991			
CC	4	0.2575	0.1920	74.55	0.331	0.999			
CD	5	1.7565	1.8397	104.74	0.629	0.736			
EE	3	0.3992	0.3607	90.35	0.576	0.781			

Concentration Ratio Data

Soil

Descriptive data for concentration ratio of Cu:Zn (before & after Grubb's Test)

Soil Type	**n**	**Mean**	**Standard Deviation**	**% RSD**	**Kolmogorov-Smirnov**		**Grubb's Test**		
					Z-value	***p* -value**	**Outlier Value (µmolg^{-1})**	**G**	**$G_{critical}$**
AA	8	0.9129	0.1363	14.93	0.675	0.668	1.1939	2.062	2.127
AB	8	0.7213	0.1221	16.93	0.362	0.997			
AC	8	0.5022	0.1026	20.42	0.506	0.923			
AD	8	0.6783	0.1675	24.69	0.554	0.865			
BB	8	0.3328	0.0602	18.08	0.547	0.874			
BC	8	0.3289	0.0574	17.46	0.504	0.925			
BD	8	0.3743	0.0523	13.97	0.593	0.807			
CC	8	0.2445	0.0713	29.18	0.873	0.356			
CD	7	0.2575	0.0678	26.35	0.548	0.870	0.3894	1.944	2.020
EE	7	0.3010	0.0785	26.08	0.556	0.860			

Descriptive data for concentration ratio of Zn:Cu (before & after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	p-value	Outlier Value (µmolg-1)	G	Gcritical
AA	8	1.1148	0.1497	13.42	0.579	0.829			
AB	8	1.4239	0.2550	17.91	0.423	0.982			
AC	8	2.0659	0.4208	20.37	0.491	0.937			
AD	8	1.5567	0.3852	24.74	0.547	0.874			
BB	8	3.0975	0.5922	19.12	0.621	0.762			
BC	8	3.1208	0.5284	16.93	0.408	0.987			
BD	8	2.7156	0.3641	13.41	0.458	0.963			
CC	8	4.3804	1.1759	26.84	0.706	0.615			
CD	7	4.0885	0.9304	22.76	0.426	0.979			
EE	7	3.5031	0.8202	23.41	0.429	0.977			

Descriptive data for concentration ratio of Pb:Zn (before & after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	p-value	Outlier Value (µmolg-1)	G	Gcritical
AA	8	0.2618	0.0379	14.48	0.645	0.720			
AB	8	0.5894	0.0382	6.48	0.729	0.577			
AC	8	0.2041	0.0259	12.68	0.500	0.929			
AD	8	0.2632	0.0314	11.94	0.520	0.907			
BB	8	0.9256	0.3519	38.02	0.883	0.344	1.6657	2.103	2.127
BC	8	0.4196	0.1492	35.56	1.013	0.200			
BD	8	0.5120	0.1245	24.32	0.712	0.605			
CC	8	0.2060	0.0497	24.11	0.691	0.641			
CD	7	0.1956	0.0197	10.05	0.855	0.377	0.1588	-1.871	2.020
EE	7	0.2518	0.0189	7.52	0.600	0.793			

Descriptive data for concentration ratio of Zn:Pb (before & after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	p-value	Outlier Value (µmolg-1)	G	Gcritical
AA	8	3.8848	0.5174	13.32	0.501	0.928			
AB	8	1.7032	0.1181	6.93	0.757	0.532			
AC	8	4.9654	0.6062	12.21	0.512	0.917			
AD	8	3.8487	0.4763	12.37	0.618	0.767			
BB	8	1.1855	0.3247	27.39	0.827	0.421			
BC	8	2.5898	0.6739	26.02	0.792	0.474			
BD	8	2.0452	0.4395	21.49	0.705	0.618			
CC	8	5.0756	1.0610	20.90	0.686	0.650			
CD	7	5.1635	0.5817	11.27	0.904	0.312	6.2992	1.9524	2.0200
EE	7	3.9909	0.2972	7.45	0.549	0.869			

Descriptive data for concentration ratio of Cu:Pb (before & after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	p-value	Outlier Value (µmolg-1)	G	Gcritical
AA	8	3.5020	0.3528	10.07	0.644	0.723			
AB	8	1.2256	0.2057	16.78	0.453	0.966			
AC	8	2.4845	0.5504	22.15	0.439	0.974			
AD	8	2.5608	0.4699	18.35	0.652	0.709			
BB	8	0.3913	0.1334	34.08	0.587	0.816			
BC	8	0.8682	0.3271	37.67	0.527	0.899			
BD	8	0.7610	0.1781	23.41	0.575	0.835			
CC	8	1.1803	0.1387	11.76	0.387	0.993			
CD	7	1.3304	0.3756	28.23	0.630	0.743			
EE	7	1.1938	0.2930	24.54	0.550	0.868			

Descriptive data for concentration ratio of Pb:Cu (before & after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	p-value	Outlier Value (µmolg-1)	G	Gcritical
AA	8	0.2884	0.0321	11.12	0.702	0.621			
AB	8	0.8373	0.1469	17.54	0.607	0.785			
AC	8	0.4201	0.0927	22.08	0.462	0.960			
AD	8	0.4018	0.0715	17.80	0.475	0.951			
BB	8	2.8071	0.8945	31.87	0.365	0.996			
BC	8	1.3327	0.5754	43.17	0.498	0.931			
BD	8	1.3804	0.3300	23.90	0.499	0.930			
CC	8	0.8583	0.1084	12.63	0.489	0.940			
CD	7	0.8011	0.2083	26.00	0.430	0.977			
EE	7	0.8765	0.1873	21.37	0.518	0.907			

Leaf

Descriptive data for concentration ratio of Cu:Zn (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p* -value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	5	1.54	0.405	26.35	0.437	0.969			
AB	5	2.56	0.895	35.00	0.547	0.861			
AC	7	2.63	2.105	80.03	0.648	0.712	6.94	2.048	2.020
AD	10	2.67	1.312	49.21	0.620	0.769			
BB	5	2.21	1.164	52.75	0.424	0.978			
BC	7	1.73	0.792	45.72	0.419	0.982			
BD	6	1.86	0.783	42.08	0.564	0.845			
CC	7	1.52	0.569	37.46	0.751	0.533			
CD	7	1.56	1.114	71.58	0.644	0.720			
EE	6	2.23	1.022	45.85	0.771	0.494			

Descriptive data for concentration ratio of Cu:Zn (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p* -value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	5	1.54	0.405	26.35	0.437	0.969			
AB	5	2.56	0.895	35.00	0.547	0.861			
AC	6	1.91	0.991	51.82	0.491	0.932			
AD	10	2.67	1.312	49.21	0.620	0.769			
BB	5	2.21	1.164	52.75	0.424	0.978			
BC	7	1.73	0.792	45.72	0.419	0.982			
BD	6	1.86	0.783	42.08	0.564	0.845			
CC	7	1.52	0.569	37.46	0.751	0.533			
CD	7	1.56	1.114	71.58	0.644	0.720			
EE	6	2.23	1.022	45.85	0.771	0.494			

Descriptive data for concentration ratio of Zn:Pb (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p* -value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	5	1.56	0.844	54.02	0.599	0.785	2.89	1.57	1.72
AB	5	1.60	0.436	27.25	0.495	0.921			
AC	7	2.26	1.662	73.41	0.569	0.841	5.50	1.95	2.02
AD	10	1.24	0.894	72.23	1.212	0.079	0.42 2.10 3.45	-0.91 0.96 2.47	2.29 2.29 2.29
BB	5	1.68	0.391	23.21	0.474	0.941			
BC	7	1.86	0.879	47.31	0.545	0.874			
BD	6	2.74	2.178	79.60	0.720	0.581	6.80	1.87	1.89
CC	7	2.62	1.003	38.32	0.382	0.993			
CD	7	3.73	2.273	60.91	0.633	0.738			
EE	6	1.81	0.584	32.21	0.824	0.412			

Descriptive data for concentration ratio of Zn:Pb (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p* -value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	5	1.56	0.844	54.02	0.599	0.785	2.89	1.57	1.72
AB	5	1.60	0.436	27.25	0.495	0.921			
AC	7	2.26	1.662	73.41	0.569	0.841	5.50	1.95	2.02
AD	8	0.85	0.233	27.26	0.689	0.644			
BB	5	1.68	0.391	23.21	0.474	0.941			
BC	7	1.86	0.879	47.31	0.545	0.874			
BD	6	2.74	2.178	79.60	0.720	0.581	6.80	1.87	1.89
CC	7	2.62	1.003	38.32	0.382	0.993			
CD	7	3.73	2.273	60.91	0.633	0.738			
EE	6	1.81	0.584	32.21	0.824	0.412			

Descriptive data for concentration ratios (before & after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p* -value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	5	2.18	0.765	35.10	0.420	0.980			
AB	5	3.88	1.090	28.05	0.527	0.886	2.52 5.52	-1.252 1.502	1.715 1.715
AC	7	3.98	1.747	43.89	0.566	0.845			
AD	10	2.63	0.962	36.60	0.714	0.612			
BB	5	3.45	1.273	36.89	0.703	0.608			
BC	7	2.83	1.569	55.49	1.124	0.116	1.87	-0.610	2.020
BD	6	4.19	2.212	52.76	0.632	0.736	8.35	1.880	1.887
CC	7	3.55	0.502	14.13	0.527	0.897	2.67	-1.761	2.020
CD	7	3.88	0.984	25.36	0.504	0.922			
EE	6	3.60	0.639	17.77	0.465	0.953			

Resin

Descriptive data for concentration ratio of Cu:Zn (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb’s Test		
					Z-value	*p* -value	Outlier Value (μmolg^{-1})	G	$G_{critical}$
AA	5	4.65	7.08	152.40	0.985	0.213	17.29	1.786	1.715
AB	4	4.46	1.66	37.29	0.608	0.759			
AC	4	39.11	66.21	169.28	0.824	0.399			
AD	5	11.59	10.39	89.66	0.861	0.351	29.87	1.759	1.715
BB	5	6.29	3.04	48.24	0.442	0.966			
BC	4	5.87	3.08	52.52	0.746	0.526			
BD	7	4.94	3.08	62.41	0.670	0.674			
CC	4	32.26	34.66	107.46	0.782	0.467			
CD	5	30.57	54.70	178.90	1.037	0.168	128.39	1.788	1.715
EE	3	9.96	6.70	67.28	0.529	0.872			

Descriptive data for concentration ratio of Cu:Zn (after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p* -value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	4	1.49	0.48	32.45	0.442	0.967			
AB	4	4.46	1.66	37.29	0.608	0.759			
AC	4	39.11	66.21	169.28	0.824	0.399			
AD	4	7.02	2.18	31.09	0.505	0.900			
BB	5	6.29	3.04	48.24	0.442	0.966			
BC	4	5.87	3.08	52.52	0.746	0.526			
BD	7	4.94	3.08	62.41	0.670	0.674			
CC	4	32.26	34.66	107.46	0.782	0.467			
CD	4	6.12	1.46	23.91	0.592	0.783			
EE	3	9.96	6.70	67.28	0.529	0.872			

Descriptive data for concentration ratio of Zn:Pb (before & after Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p* -value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	5	1.85	1.07	57.90	0.675	0.657			
AB	4	0.40	0.16	39.63	0.588	0.789			
AC	4	0.37	0.33	90.22	0.579	0.803			
AD	5	0.30	0.18	61.30	0.526	0.888			
BB	5	0.46	0.13	29.35	0.442	0.966			
BC	4	0.72	0.28	39.48	0.756	0.509			
BD	7	2.14	3.16	147.55	1.100	0.131	8.10	1.886	2.020
CC	4	0.17	0.10	57.66	0.560	0.830			
CD	5	0.45	0.20	43.34	0.527	0.886			
EE	3	0.33	0.14	41.56	0.373	0.994			

Descriptive data for concentration ratio of Cu:Pb (before Grubb's Test)

Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p* -value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	5	3.18	1.14	35.81	0.627	0.740	5.08	1.663	1.715
AB	4	1.68	0.59	34.84	0.368	0.995			
AC	4	2.05	0.57	27.68	0.577	0.805			
AD	5	2.29	0.70	30.64	0.638	0.722	1.11	-1.679	1.715
BB	5	2.72	1.13	41.56	0.596	0.790			
BC	4	3.58	0.69	19.24	0.496	0.912			
BD	7	2.80	0.76	27.31	0.772	0.500			
CC	4	3.27	1.18	36.14	0.726	0.560			
CD	5	6.69	7.95	118.77	0.921	0.278	20.85	1.781	1.715
EE	3	2.70	0.69	25.42	0.617	0.715			

Descriptive data for concentration ratio of Cu:Pb (after Grubb's Test)

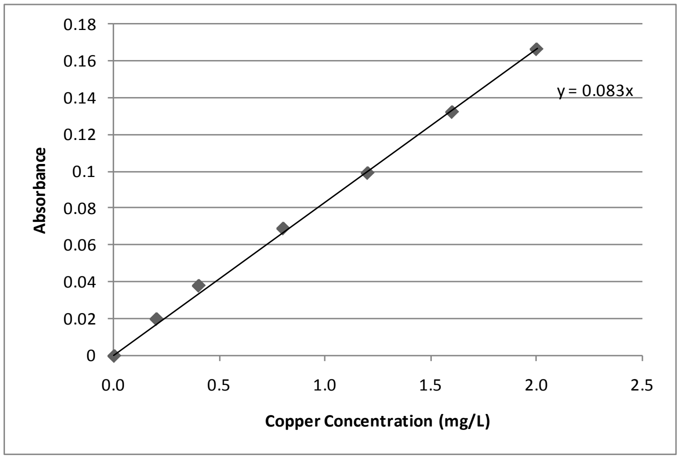
Soil Type	n	Mean	Standard Deviation	% RSD	Kolmogorov-Smirnov		Grubb's Test		
					Z-value	*p* -value	Outlier Value (µmolg^{-1})	G	$G_{critical}$
AA	5	3.18	1.14	35.81	0.627	0.740	5.08	1.663	1.715
AB	4	1.68	0.59	34.84	0.368	0.995			
AC	4	2.05	0.57	27.68	0.577	0.805			
AD	5	2.29	0.70	30.64	0.638	0.722	1.11	-1.679	1.715
BB	5	2.72	1.13	41.56	0.596	0.790			
BC	4	3.58	0.69	19.24	0.496	0.912			
BD	7	2.80	0.76	27.31	0.772	0.500			
CC	4	3.27	1.18	36.14	0.726	0.560			
CD	4	3.16	0.88	28.01	0.586	0.793			
EE	3	2.70	0.69	25.42	0.617	0.715			

Appendix 4: Determination of Available Metals in Soil Using Flame AAS

Copper

Flame AAS readings for copper calibration standards

Copper Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.004	0.004	0.004	0.004
0.2	0.023	0.024	0.024	0.024
0.4	0.042	0.042	0.041	0.042
0.8	0.073	0.073	0.073	0.073
1.2	0.103	0.102	0.103	0.103
1.6	0.136	0.135	0.137	0.136
2.0	0.170	0.170	0.171	0.170



Calibration graph for copper using flame AAS

Flame AAS readings for copper when copper, lead & zinc were added to soil

Added Copper Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.026	0.026	0.026	0.026
10	0.118	0.117	0.118	0.118
20	0.175	0.176	0.176	0.176
40	0.035	0.035	0.035	0.035
80	0.032	0.032	0.032	0.032
160	0.034	0.034	0.034	0.034

Determination of concentration of available copper when copper, lead & zinc were added to soil

Added Copper Concentration (mg/L)	Concentration from Graph (mg/L)	Dilution Factor	Volume (L)	Copper Amount (mg)	Mass of Soil Used (Kg)	Copper Concentration (mg/Kg)
0	0.313	1.05	0.025	0.008	0.0049664	1.656
10	1.422	2.1	0.025	0.075	0.0049431	15.100
20	2.120	2.1	0.025	0.111	0.0049919	22.301
40	0.422	26.25	0.025	0.277	0.0050139	55.193
80	0.386	52.5	0.025	0.506	0.0051510	98.238
160	0.410	105	0.025	1.075	0.0053486	201.043

Flame AAS readings for copper when zinc was added to soil

Added Zinc Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.026	0.026	0.026	0.026
10	0.031	0.030	0.030	0.030
20	0.034	0.034	0.034	0.034
40	0.033	0.033	0.033	0.033
80	0.031	0.031	0.030	0.031
160	0.036	0.036	0.037	0.036

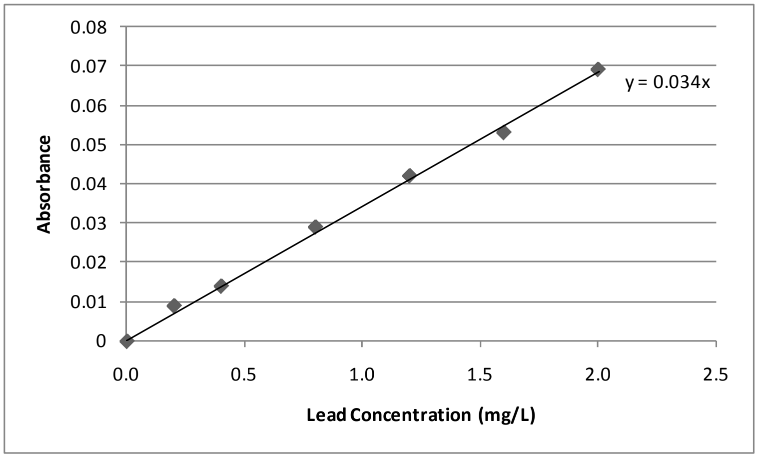
Determination of concentration of available copper when zinc was added to soil

Added Zinc Concentration (mg/L)	Concentration from Graph (mg/L)	Dilution Factor	Volume (L)	Copper Amount (mg)	Mass of Soil Used (Kg)	Copper Concentration (mg/Kg)
0	0.313	1.05	0.025	0.008	0.0049664	1.656
10	0.361	1.05	0.025	0.009	0.0049431	1.919
20	0.410	1.05	0.025	0.011	0.0049919	2.154
40	0.398	1.05	0.025	0.010	0.0050139	2.082
80	0.373	1.05	0.025	0.010	0.0051510	1.903
160	0.434	1.05	0.025	0.011	0.0053486	2.129

Lead

Flame AAS readings for lead calibration standards

Lead Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.000	0.000	0.002	0.001
0.2	0.010	0.009	0.010	0.010
0.4	0.014	0.015	0.014	0.014
0.8	0.029	0.030	0.030	0.030
1.2	0.044	0.042	0.042	0.043
1.6	0.054	0.054	0.054	0.054
2.0	0.069	0.070	0.070	0.070



Calibration graph for lead using flame AAS

Flame AAS readings for lead when copper, lead & zinc were added to soil

Added Lead Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.005	0.005	0.005	0.005
10	0.010	0.011	0.011	0.011
20	0.008	0.007	0.008	0.008
40	0.015	0.015	0.015	0.015
80	0.013	0.012	0.012	0.012
160	0.012	0.011	0.012	0.012

Determination of concentration of available lead when copper, lead & zinc were added to soil

Added Lead Concentration (mg/L)	Concentration from Graph (mg/L)	Dilution Factor	Volume (L)	Lead Amount (mg)	Mass of Soil Used (Kg)	Lead Concentration (mg/Kg)
0	0.147	1.05	0.025	0.004	0.0049664	0.777
10	0.324	2.1	0.025	0.017	0.0049431	3.436
20	0.235	2.1	0.025	0.012	0.0049919	2.475
40	0.441	26.25	0.025	0.290	0.0050139	57.744
80	0.353	52.5	0.025	0.463	0.0051510	89.931
160	0.353	105	0.025	0.926	0.0053486	173.217

Flame AAS readings for lead when zinc was added to soil

Added Zinc Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.005	0.005	0.005	0.005
10	0.007	0.009	0.009	0.008
20	0.006	0.006	0.008	0.007
40	0.006	0.007	0.007	0.007
80	0.008	0.008	0.007	0.008
160	0.032	0.031	0.032	0.032

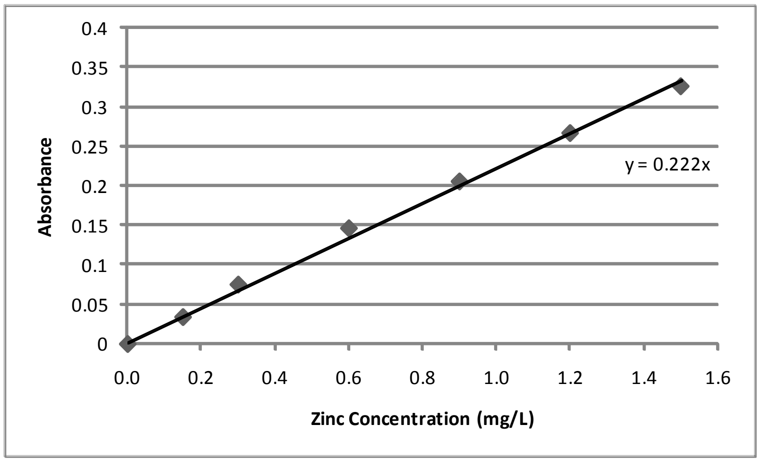
Determination of concentration of available lead when zinc was added to soil

Added Zinc Concentration (mg/L)	Concentration from Graph (mg/L)	Dilution Factor	Volume (L)	Lead Amount (mg)	Mass of Soil Used (Kg)	Lead Concentration (mg/Kg)
0	0.147	1.05	0.025	0.004	0.0049664	0.777
10	0.235	1.05	0.025	0.006	0.0049431	1.250
20	0.206	1.05	0.025	0.005	0.0049919	1.083
40	0.206	1.05	0.025	0.005	0.0050139	1.078
80	0.235	1.05	0.025	0.006	0.0051510	1.199
160	0.941	1.05	0.025	0.025	0.0053486	4.619

Zinc

Flame AAS readings for zinc calibration standards

Zinc Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.013	0.013	0.013	0.013
0.2	0.047	0.047	0.046	0.047
0.4	0.089	0.088	0.088	0.088
0.8	0.159	0.160	0.158	0.159
1.2	0.219	0.218	0.218	0.218
1.6	0.280	0.279	0.279	0.279
2.0	0.340	0.338	0.337	0.338



Calibration graph for zinc using flame AAS

Flame AAS readings for zinc when copper, lead & zinc were added to soil

Added Zinc Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.069	0.069	0.069	0.069
10	0.279	0.279	0.279	0.279
20	0.058	0.059	0.058	0.058
40	0.110	0.109	0.109	0.109
80	0.105	0.104	0.103	0.104
160	0.217	0.217	0.218	0.217

Determination of concentration of available zinc when copper, lead & zinc were added to soil

Added Zinc Concentration (mg/L)	Concentration from Graph (mg/L)	Dilution Factor	Volume (L)	Zinc Amount (mg)	Mass of Soil Used (Kg)	Zinc Concentration (mg/Kg)
0	0.311	1.05	0.025	0.008	0.0049664	1.643
10	1.257	2.1	0.025	0.066	0.0049431	13.348
20	0.261	1.05	0.025	0.007	0.0049919	1.374
40	0.491	26.25	0.025	0.322	0.0050139	64.264
80	0.468	52.5	0.025	0.615	0.0051510	119.368
160	0.977	52.5	0.025	1.283	0.0053486	239.864

Flame AAS readings for zinc when zinc was added to soil

Added Zinc Concentration (mg/L)	Absorbance			
	Reading 1	Reading 2	Reading 3	Mean
0	0.069	0.069	0.069	0.069
10	0.098	0.098	0.095	0.097
20	0.076	0.076	0.075	0.076
40	0.102	0.101	0.102	0.102
80	0.109	0.109	0.108	0.109
160	0.100	0.101	0.100	0.100

Determination of concentration of available zinc when zinc was added to soil

Added Zinc Concentration (mg/L)	Concentration from Graph (mg/L)	Dilution Factor	Volume (L)	Zinc Amount (mg)	Mass of Soil Used (Kg)	Zinc Concentration (mg/Kg)
0	0.311	1.05	0.025	0.008	0.0049664	1.643
10	0.437	10.5	0.025	0.115	0.0049431	23.203
20	0.342	26.25	0.025	0.225	0.0049919	45.005
40	0.459	26.25	0.025	0.302	0.0050139	60.137
80	0.491	52.5	0.025	0.644	0.0051510	125.107
160	0.450	105	0.025	1.182	0.0053486	221.073

Appendix 5: Soil Data

Concentration of copper in soil samples

Soil Sample	Soil Dry Weight (g)	Soil Ashed Weight (g)	Copper Concentration (μg/L) in 25mL			Ashed Soil		Dry Soil	
			Gross	Blank	Net	Weight Used (g)	Copper Conc (μg/g)	Copper Conc (μg/g)	Copper Conc (μmol/g)
AA1_a	11.3540	9.5120	977.123	12.753	964.370	1.0073	23.935	20.023	0.315
AA1_b	11.3540	9.5120	949.842	27.848	921.994	1.0412	22.138	18.515	0.291
AA2_a	9.1342	7.6851	880.733	12.753	867.980	1.0076	21.536	18.089	0.285
AA2_b	9.1342	7.6851	882.077	12.753	869.324	1.0011	21.709	18.257	0.287
AA3_a	11.1515	9.4595	710.386	12.753	697.633	1.0109	17.253	14.675	0.231
AA3_b	11.1515	9.4595	854.880	12.753	842.127	1.0293	20.454	17.390	0.274
AA4_a	9.8072	7.7611	894.659	12.753	881.906	1.0087	21.857	17.331	0.273
AA4_b	9.8072	7.7611	882.042	12.753	869.289	1.0107	21.502	17.014	0.268
AB1_a	11.0358	9.3780	539.414	12.753	526.661	1.0091	13.048	11.047	0.174
AB1_b	11.0358	9.3780	531.989	25.252	506.737	1.0073	12.577	10.707	0.168
AB2_a	8.5189	6.9660	500.037	12.753	487.284	1.0088	12.076	9.894	0.156
AB2_b	8.5189	6.9660	559.764	12.753	547.011	1.0032	13.632	11.121	0.175
AB3_a	6.8664	5.4367	783.401	8.068	775.333	1.0053	19.281	15.281	0.240
AB3_b	6.8664	5.4367	747.168	8.068	739.100	1.0021	18.439	14.569	0.229
AB4_a	9.5126	7.4304	910.693	25.252	885.441	1.0344	21.400	16.716	0.263
AB4_b	9.5126	7.4304	913.929	25.252	888.677	1.0249	21.677	16.950	0.267
AC1_a	7.9867	6.5602	517.687	8.068	509.619	1.0102	12.612	10.350	0.163
AC1_b	7.9867	6.5602	496.743	8.068	488.675	1.0082	12.118	9.939	0.156
AC2_a	8.5346	6.7749	934.968	25.252	909.716	1.0875	20.913	16.591	0.261
AC2_b	8.5346	6.7749	762.820	8.068	754.752	1.0030	18.812	14.924	0.235
AC3_a	11.9620	9.9423	705.682	8.068	697.614	1.0268	16.985	14.130	0.222
AC3_b	11.9620	9.9423	720.710	25.252	695.458	1.0566	16.455	13.714	0.216
AC4_a	9.7671	7.6139	587.599	8.068	579.531	1.0034	14.439	11.225	0.177
AC4_b	9.7671	7.6139	533.493	27.848	505.645	0.9588	13.184	10.290	0.162
AD1_a	11.6982	9.0183	744.313	8.068	736.245	1.0528	17.483	13.491	0.212
AD1_b	11.6982	9.0183	739.339	8.068	731.271	1.0230	17.871	13.799	0.217
AD2_a	12.2526	10.3079	570.329	8.068	562.261	1.0397	13.520	11.357	0.179
AD2_b	12.2526	10.3079	491.533	8.068	483.465	1.0041	12.037	10.095	0.159
AD3_a	10.7976	8.8550	686.764	8.068	678.696	1.0108	16.786	13.778	0.217
AD3_b	10.7976	8.8550	608.433	8.068	600.365	1.0030	14.964	12.301	0.194
AD4_a	8.8177	7.2570	586.065	8.068	577.997	1.0024	14.415	11.851	0.186
AD4_b	8.8177	7.2570	553.569	27.848	525.721	0.9887	13.293	10.946	0.172
BB1_a	8.5368	6.6337	369.890	8.068	361.822	0.9489	9.533	7.382	0.116
BB1_b	8.5368	6.6337	420.282	25.252	395.030	1.0966	9.006	6.994	0.110
BB2_a	8.1678	6.8224	409.691	25.252	384.439	1.0216	9.408	7.852	0.124
BB2_b	8.1678	6.8224	384.765	8.068	376.697	1.0209	9.225	7.685	0.121
BB3_a	8.9550	7.4753	326.004	27.848	298.156	0.9074	8.215	6.845	0.108
BB3_b	8.9550	7.4753	358.707	8.524	350.183	1.0348	8.460	7.095	0.112
BB4_a	11.2659	9.7489	322.634	25.252	297.382	1.0155	7.321	6.317	0.099
BB4_b	11.2659	9.7489	243.422	8.524	234.898	1.0095	5.817	5.019	0.079

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Soil Sample	Soil Dry Weight (g)	Soil Ashed Weight (g)	Copper Concentration (µg/L) in 25mL			Ashed Soil		Dry Soil	
			Gross	Blank	Net	Weight Used (g)	Copper Conc (µg/g)	Copper Conc (µg/g)	Copper Conc (µmol/g)
BC1_a	11.3688	9.6326	392.312	25.252	367.060	1.0637	8.627	7.287	0.115
BC1_b	11.3688	9.6326	462.677	8.524	454.153	1.0092	11.250	9.574	0.151
BC2_a	9.9539	7.7996	302.620	8.524	294.096	1.0602	6.935	5.407	0.085
BC2_b	9.9539	7.7996	301.256	8.524	292.732	1.0009	7.312	5.720	0.090
BC3_a	10.3163	8.3660	389.334	8.524	380.810	1.0149	9.380	7.623	0.120
BC3_b	10.3163	8.3660	397.328	8.524	388.804	1.0134	9.592	7.785	0.123
BC4_a	11.1186	9.2925	388.933	8.524	380.409	1.0356	9.183	7.689	0.121
BC4_b	11.1186	9.2925	374.869	8.524	366.345	1.0430	8.781	7.355	0.116
BD1_a	9.9056	7.7282	379.736	8.524	371.212	1.0582	8.770	6.866	0.108
BD1_b	9.9056	7.7282	374.613	8.524	366.089	1.1093	8.250	6.476	0.102
BD2_a	11.4847	9.5990	335.786	8.524	327.262	1.0200	8.021	6.686	0.105
BD2_b	11.4847	9.5990	309.250	8.524	300.726	1.0036	7.491	6.269	0.099
BD3_a	7.7877	5.3260	453.214	8.524	444.690	1.0260	10.836	7.386	0.116
BD3_b	7.7877	5.3260	382.231	25.252	356.979	0.9620	9.277	6.360	0.100
BD4_a	11.6795	9.8408	245.417	25.252	220.165	1.0113	5.443	4.550	0.072
BD4_b	11.6795	9.8408	279.157	8.524	270.633	1.0039	6.740	5.645	0.089
CC1_a	9.9272	8.3602	313.671	8.524	305.147	1.0198	7.481	6.316	0.099
CC1_b	9.9272	8.3602	306.399	8.524	297.875	1.0239	7.273	6.148	0.097
CC2_a	9.0166	7.3521	282.329	25.252	257.077	0.8725	7.366	6.034	0.095
CC2_b	9.0166	7.3521	349.636	17.876	331.760	1.0074	8.233	6.686	0.105
CC3_a	13.1404	11.4152	282.250	17.876	264.374	1.0141	6.517	5.647	0.089
CC3_b	13.1404	11.4152	330.883	17.876	313.007	1.0583	7.394	6.428	0.101
CC4_a	9.8694	7.8742	334.308	25.252	309.056	1.1273	6.854	5.505	0.087
CC4_b	9.8694	7.8742	279.799	17.876	261.923	1.0247	6.390	5.106	0.080
CD1_a	9.0383	6.6884	302.348	17.876	284.472	1.0013	7.103	5.254	0.083
CD1_b	9.0383	6.6884	444.467	25.252	419.215	1.1529	9.090	6.734	0.106
CD2_a	10.0791	8.3078	247.621	17.876	229.745	1.0479	5.481	4.533	0.071
CD2_b	10.0791	8.3078	332.825	17.876	314.949	1.0285	7.656	6.347	0.100
CD3_a	10.4648	8.4842	312.165	17.876	294.289	1.0447	7.042	5.675	0.089
CD3_b	10.4648	8.4842	252.555	17.876	234.679	1.0062	5.831	4.702	0.074
CD4_a	14.8853	12.5787	570.099	25.252	544.847	1.2143	11.217	9.464	0.149
CD4_b	14.8853	12.5787	731.496	25.252	706.244	1.0370	17.026	14.366	0.226
EE1_a	13.2026	11.1173	268.643	17.876	250.767	1.0195	6.149	5.137	0.081
EE1_b	13.2026	11.1173	254.305	25.252	229.053	1.0967	5.221	4.379	0.069
EE2_a	13.0760	10.8525	231.364	17.876	213.488	1.0130	5.269	4.399	0.069
EE2_b	13.0760	10.8525	301.084	17.876	283.208	1.0347	6.843	5.644	0.089
EE3_a	9.8584	7.6889	454.171	25.252	428.919	1.0043	10.677	8.345	0.131
EE3_b	9.8584	7.6889	265.796	17.876	247.920	1.0347	5.990	4.680	0.074
EE4_a	12.4278	10.4383	341.017	17.876	323.141	1.0064	8.027	6.719	0.106
EE4_b	12.4278	10.4383	249.015	17.876	231.139	1.0299	5.611	4.704	0.074

Concentration of lead in soil samples

Soil Sample	Soil Dry Weight (g)	Soil Ashed Weight (g)	Lead Concentration (µg/L) in 25mL			Ashed Soil		Dry Soil	
			Gross	Blank	Net	Weight Used (g)	Lead Conc (µg/g)	Lead Conc (µg/g)	Lead Conc (µmol/g)
AA1_a	11.3540	9.5120	874.336	38.892	835.444	1.0073	20.735	17.342	0.084
AA1_b	11.3540	9.5120	979.728	27.174	952.554	1.0412	22.872	19.185	0.093
AA2_a	9.1342	7.6851	862.486	38.892	823.594	1.0076	20.435	17.164	0.083
AA2_b	9.1342	7.6851	770.161	38.892	731.269	1.0011	18.262	15.397	0.074
AA3_a	11.1515	9.4595	844.289	38.892	805.397	1.0109	19.918	16.881	0.081
AA3_b	11.1515	9.4595	805.987	38.892	767.095	1.0293	18.631	15.778	0.076
AA4_a	9.8072	7.7611	803.25	38.892	764.358	1.0087	18.944	14.957	0.072
AA4_b	9.8072	7.7611	828.796	38.892	789.904	1.0107	19.539	15.432	0.074
AB1_a	11.0358	9.3780	1377.82	38.892	1338.93	1.0091	33.171	28.213	0.136
AB1_b	11.0358	9.3780	1502.1	27.218	1474.88	1.0073	36.605	31.102	0.150
AB2_a	8.5189	6.9660	1722.15	38.892	1683.25	1.0088	41.714	34.099	0.165
AB2_b	8.5189	6.9660	1884.99	38.892	1846.1	1.0032	46.005	37.615	0.182
AB3_a	6.8664	5.4367	1670.07	30.847	1639.22	1.0053	40.764	32.305	0.156
AB3_b	6.8664	5.4367	1763.21	30.847	1732.36	1.0021	43.218	34.205	0.165
AB4_a	9.5126	7.4304	2347.04	27.218	2319.82	1.0344	56.067	43.820	0.211
AB4_b	9.5126	7.4304	2226.44	27.218	2199.22	1.0249	53.645	41.868	0.202
AC1_a	7.9867	6.5602	857.28	30.847	826.433	1.0102	20.452	16.839	0.081
AC1_b	7.9867	6.5602	936.499	30.847	905.652	1.0082	22.457	18.481	0.089
AC2_a	8.5346	6.7749	903.279	27.218	876.061	1.0875	20.139	15.956	0.077
AC2_b	8.5346	6.7749	843.313	30.847	812.466	1.0030	20.251	16.114	0.078
AC3_a	11.9620	9.9423	883.803	30.847	852.956	1.0268	20.767	17.288	0.083
AC3_b	11.9620	9.9423	893.826	27.218	866.608	1.0566	20.505	17.039	0.082
AC4_a	9.7671	7.6139	829.798	30.847	798.951	1.0034	19.906	15.513	0.075
AC4_b	9.7671	7.6139	827.762	27.174	800.588	0.9588	20.875	16.293	0.079
AD1_a	11.6982	9.0183	772.536	30.847	741.689	1.0528	17.612	13.568	0.065
AD1_b	11.6982	9.0183	771.612	30.847	740.765	1.0230	18.103	13.954	0.067
AD2_a	12.2526	10.3079	832.47	30.847	801.623	1.0397	19.275	16.237	0.078
AD2_b	12.2526	10.3079	838.113	30.847	807.266	1.0041	20.099	16.910	0.082
AD3_a	10.7976	8.8550	887.799	30.847	856.952	1.0108	21.195	17.386	0.084
AD3_b	10.7976	8.8550	791.257	30.847	760.41	1.0030	18.953	15.582	0.075
AD4_a	8.8177	7.2570	785.931	30.847	755.084	1.0024	18.832	15.472	0.075
AD4_b	8.8177	7.2570	829.813	27.174	802.639	0.9887	20.295	16.707	0.081
BB1_a	8.5368	6.6337	5220.4	30.847	5189.55	0.9489	136.725	106.226	0.513
BB1_b	8.5368	6.6337	4438.6	27.218	4411.38	1.0966	100.569	78.173	0.377
BB2_a	8.1678	6.8224	1971.93	27.218	1944.71	1.0216	47.590	39.759	0.192
BB2_b	8.1678	6.8224	2410	30.847	2379.15	1.0209	58.261	48.697	0.235
BB3_a	8.9550	7.4753	2486.45	27.174	2459.27	0.9074	67.756	56.597	0.273
BB3_b	8.9550	7.4753	3512.58	31.86	3480.72	1.0348	84.091	70.204	0.339
BB4_a	11.2659	9.7489	2411.42	27.218	2384.2	1.0155	58.695	50.796	0.245
BB4_b	11.2659	9.7489	2387.63	31.86	2355.77	1.0095	58.340	50.450	0.243

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Soil Sample	Soil Dry Weight (g)	Soil Ashed Weight (g)	Lead Concentration (µg/L) in 25mL			Ashed Soil		Dry Soil	
			Gross	Blank	Net	Weight Used (g)	Lead Conc (µg/g)	Lead Conc (µg/g)	Lead Conc (µmol/g)
BC1_a	11.3688	9.6326	1308.07	27.218	1280.85	1.0637	30.104	25.503	0.123
BC1_b	11.3688	9.6326	1176.02	31.86	1144.16	1.0092	28.343	23.978	0.116
BC2_a	9.9539	7.7996	1406.52	31.86	1374.66	1.0602	32.415	25.388	0.123
BC2_b	9.9539	7.7996	1315.8	31.86	1283.94	1.0009	32.070	25.153	0.121
BC3_a	10.3163	8.3660	2977.28	31.86	2945.42	1.0149	72.554	58.875	0.284
BC3_b	10.3163	8.3660	2511.49	31.86	2479.63	1.0134	61.171	49.630	0.240
BC4_a	11.1186	9.2925	1080.88	31.86	1049.02	1.0356	25.324	21.145	0.102
BC4_b	11.1186	9.2925	1065.61	31.86	1033.75	1.0430	24.778	20.727	0.100
BD1_a	9.9056	7.7282	1223.81	31.86	1191.95	1.0582	28.160	22.001	0.106
BD1_b	9.9056	7.7282	1352.43	31.86	1320.57	1.1093	29.761	23.250	0.112
BD2_a	11.4847	9.5990	1177.9	31.86	1146.04	1.0200	28.089	23.486	0.113
BD2_b	11.4847	9.5990	1261.97	31.86	1230.11	1.0036	30.642	25.576	0.123
BD3_a	7.7877	5.3260	2330.24	31.86	2298.38	1.0260	56.003	38.298	0.185
BD3_b	7.7877	5.3260	1896.35	27.218	1869.13	0.9620	48.574	33.237	0.160
BD4_a	11.6795	9.8408	1405.43	27.218	1378.21	1.0113	34.070	28.732	0.139
BD4_b	11.6795	9.8408	1350.47	31.86	1318.61	1.0039	32.837	27.636	0.133
CC1_a	9.9272	8.3602	815.196	31.86	783.336	1.0198	19.203	16.169	0.078
CC1_b	9.9272	8.3602	776.757	31.86	744.897	1.0239	18.188	15.327	0.074
CC2_a	9.0166	7.3521	799.384	27.218	772.166	0.8725	22.125	18.020	0.087
CC2_b	9.0166	7.3521	929.209	33.232	895.977	1.0074	22.235	18.102	0.087
CC3_a	13.1404	11.4152	810.647	33.232	777.415	1.0141	19.165	16.679	0.080
CC3_b	13.1404	11.4152	784.43	33.232	751.198	1.0583	17.745	15.376	0.074
CC4_a	9.8694	7.8742	899.397	27.218	872.179	1.1273	19.342	15.398	0.074
CC4_b	9.8694	7.8742	953.354	33.232	920.122	1.0247	22.449	17.872	0.086
CD1_a	9.0383	6.6884	741.748	33.232	708.516	1.0013	17.690	13.098	0.063
CD1_b	9.0383	6.6884	793.062	27.218	765.844	1.1529	16.607	12.284	0.059
CD2_a	10.0791	8.3078	813.044	33.232	779.812	1.0479	18.604	15.331	0.074
CD2_b	10.0791	8.3078	837.797	33.232	804.565	1.0285	19.557	16.155	0.078
CD3_a	10.4648	8.4842	877.981	33.232	844.749	1.0447	20.215	16.377	0.079
CD3_b	10.4648	8.4842	835.695	33.232	802.463	1.0062	19.938	16.134	0.078
CD4_a	14.8853	12.5787	965.16	27.218	937.942	1.2143	19.310	16.309	0.079
CD4_b	14.8853	12.5787	845.426	27.218	818.208	1.0370	19.725	16.647	0.080
EE1_a	13.2026	11.1173	766.274	33.232	733.042	1.0195	17.976	15.157	0.073
EE1_b	13.2026	11.1173	823.55	27.218	796.332	1.0967	18.153	15.325	0.074
EE2_a	13.0760	10.8525	708.12	33.232	674.888	1.0130	16.656	13.860	0.067
EE2_b	13.0760	10.8525	800.523	33.232	767.291	1.0347	18.539	15.354	0.074
EE3_a	9.8584	7.6889	844.94	27.218	817.722	1.0043	20.356	15.911	0.077
EE3_b	9.8584	7.6889	877.309	33.232	844.077	1.0347	20.394	15.911	0.077
EE4_a	12.4278	10.4383	749.57	33.232	716.338	1.0064	17.795	14.950	0.072
EE4_b	12.4278	10.4383	795.38	33.232	762.148	1.0299	18.501	15.538	0.075

Concentration of zinc in soil samples

Soil Sample	Soil Dry Weight (g)	Soil Ashed Weight (g)	Zinc Concentration (µg/L) in 25mL			Ashed Soil		Dry Soil	
			Gross	Blank	Net	Weight Used (g)	Zinc Conc (µg/g)	Zinc Conc (µg/g)	Zinc Conc (µmol/g)
AA1_a	11.3540	9.5120	839.305	9.368	829.937	1.0073	20.598	17.258	0.264
AA1_b	11.3540	9.5120	944.066	6.980	937.086	1.0412	22.500	18.850	0.288
AA2_a	9.1342	7.6851	1069.063	9.368	1059.695	1.0076	26.293	22.128	0.338
AA2_b	9.1342	7.6851	1046.895	9.368	1037.527	1.0011	25.910	21.791	0.333
AA3_a	11.1515	9.4595	972.109	9.368	962.741	1.0109	23.809	20.189	0.309
AA3_b	11.1515	9.4595	1040.582	9.368	1031.214	1.0293	25.046	21.207	0.324
AA4_a	9.8072	7.7611	1051.767	9.368	1042.399	1.0087	25.835	20.417	0.312
AA4_b	9.8072	7.7611	972.039	9.368	962.671	1.0107	23.812	18.835	0.288
AB1_a	11.0358	9.3780	831.521	9.368	822.153	1.0091	20.368	17.336	0.265
AB1_b	11.0358	9.3780	746.504	5.854	740.650	1.0073	18.382	15.636	0.239
AB2_a	8.5189	6.9660	932.305	9.368	922.937	1.0088	22.872	18.726	0.286
AB2_b	8.5189	6.9660	976.153	9.368	966.785	1.0032	24.093	19.707	0.301
AB3_a	6.8664	5.4367	929.460	6.361	923.099	1.0053	22.956	18.211	0.278
AB3_b	6.8664	5.4367	884.043	6.361	877.682	1.0021	21.896	17.340	0.265
AB4_a	9.5126	7.4304	1211.974	5.854	1206.120	1.0344	29.150	22.808	0.349
AB4_b	9.5126	7.4304	1144.982	5.854	1139.128	1.0249	27.786	21.715	0.332
AC1_a	7.9867	6.5602	1289.953	6.361	1283.592	1.0102	31.766	26.120	0.399
AC1_b	7.9867	6.5602	1217.293	6.361	1210.932	1.0082	30.027	24.642	0.377
AC2_a	8.5346	6.7749	1536.395	5.854	1530.541	1.0875	35.185	27.942	0.427
AC2_b	8.5346	6.7749	1463.833	6.361	1457.472	1.003	36.328	28.816	0.441
AC3_a	11.9620	9.9423	1120.356	6.361	1113.995	1.0268	27.123	22.524	0.344
AC3_b	11.9620	9.9423	1254.393	5.854	1248.539	1.0566	29.541	24.519	0.375
AC4_a	9.7671	7.6139	1314.541	6.361	1308.180	1.0034	32.594	25.413	0.389
AC4_b	9.7671	7.6139	1394.638	6.980	1387.658	0.9588	36.182	28.220	0.432
AD1_a	11.6982	9.0183	865.552	6.361	859.191	1.0528	20.403	15.727	0.241
AD1_b	11.6982	9.0183	863.937	6.361	857.576	1.023	20.957	16.189	0.248
AD2_a	12.2526	10.3079	1213.167	6.361	1206.806	1.0397	29.018	24.397	0.373
AD2_b	12.2526	10.3079	959.884	6.361	953.523	1.0041	23.741	19.938	0.305
AD3_a	10.7976	8.8550	859.988	6.361	853.627	1.0108	21.113	17.304	0.265
AD3_b	10.7976	8.8550	867.204	6.361	860.843	1.003	21.457	17.632	0.270
AD4_a	8.8177	7.2570	989.663	6.361	983.302	1.0024	24.524	20.164	0.308
AD4_b	8.8177	7.2570	1041.122	6.980	1034.142	0.9887	26.149	21.480	0.328
BB1_a	8.5368	6.6337	988.251	6.361	981.890	0.9489	25.869	20.126	0.308
BB1_b	8.5368	6.6337	1125.596	5.854	1119.742	1.0966	25.528	19.815	0.303
BB2_a	8.1678	6.8224	966.840	5.854	960.986	1.0216	23.517	19.629	0.300
BB2_b	8.1678	6.8224	1003.413	6.361	997.052	1.0209	24.416	20.381	0.312
BB3_a	8.9550	7.4753	1069.790	6.980	1062.810	0.9074	29.282	24.459	0.374
BB3_b	8.9550	7.4753	1253.760	6.703	1247.057	1.0348	30.128	25.126	0.384
BB4_a	11.2659	9.7489	1013.207	5.854	1007.353	1.0155	24.799	21.461	0.328
BB4_b	11.2659	9.7489	1008.873	6.703	1002.170	1.0095	24.818	21.461	0.328

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Soil Sample	Soil Dry Weight (g)	Soil Ashed Weight (g)	Zinc Concentration (µg/L) in 25mL			Ashed Soil		Dry Soil	
			Gross	Blank	Net	Weight Used (g)	Zinc Conc (µg/g)	Zinc Conc (µg/g)	Zinc Conc (µmol/g)
BC1_a	11.3688	9.6326	1142.244	5.854	1136.390	1.0637	26.708	22.622	0.346
BC1_b	11.3688	9.6326	1113.435	6.703	1106.732	1.0092	27.416	23.216	0.355
BC2_a	9.9539	7.7996	1151.896	6.703	1145.193	1.0602	27.004	21.156	0.324
BC2_b	9.9539	7.7996	1143.947	6.703	1137.244	1.0009	28.406	22.253	0.340
BC3_a	10.3163	8.3660	1305.127	6.703	1298.424	1.0149	31.984	25.950	0.397
BC3_b	10.3163	8.3660	1334.279	6.703	1327.576	1.0134	32.751	26.599	0.407
BC4_a	11.1186	9.2925	1030.256	6.703	1023.553	1.0356	24.709	20.643	0.316
BC4_b	11.1186	9.2925	1054.996	6.703	1048.293	1.043	25.127	20.978	0.321
BD1_a	9.9056	7.7282	917.995	6.703	911.292	1.0582	21.529	16.774	0.257
BD1_b	9.9056	7.7282	1004.901	6.703	998.198	1.1093	22.496	17.554	0.268
BD2_a	11.4847	9.5990	925.204	6.703	918.501	1.02	22.512	18.806	0.288
BD2_b	11.4847	9.5990	915.062	6.703	908.359	1.0036	22.628	18.889	0.289
BD3_a	7.7877	5.3260	975.557	6.703	968.854	1.026	23.608	16.140	0.247
BD3_b	7.7877	5.3260	989.137	5.854	983.283	0.962	25.553	17.508	0.268
BD4_a	11.6795	9.8408	751.227	5.854	745.373	1.0113	18.426	15.503	0.237
BD4_b	11.6795	9.8408	823.561	6.703	816.858	1.0039	20.342	17.104	0.262
CC1_a	9.9272	8.3602	845.125	6.703	838.422	1.0198	20.554	17.348	0.265
CC1_b	9.9272	8.3602	1472.125	6.703	1465.422	1.0239	35.780	30.149	0.461
CC2_a	9.0166	7.3521	1267.811	5.854	1261.957	0.8725	36.159	29.517	0.451
CC2_b	9.0166	7.3521	1661.884	33.532	1628.352	1.0074	40.410	32.942	0.504
CC3_a	13.1404	11.4152	960.647	33.532	927.115	1.0141	22.856	19.893	0.304
CC3_b	13.1404	11.4152	1101.689	33.532	1068.157	1.0583	25.233	21.891	0.335
CC4_a	9.8694	7.8742	1600.649	5.854	1594.795	1.1273	35.368	28.244	0.432
CC4_b	9.8694	7.8742	1748.649	33.532	1715.117	1.0247	41.844	33.350	0.510
CD1_a	9.0383	6.6884	1288.196	33.532	1254.664	1.0013	31.326	23.162	0.354
CD1_b	9.0383	6.6884	1528.218	5.854	1522.364	1.1529	33.012	24.420	0.373
CD2_a	10.0791	8.3078	1255.826	33.532	1222.294	1.0479	29.161	24.068	0.368
CD2_b	10.0791	8.3078	1222.432	33.532	1188.900	1.0285	28.899	23.821	0.364
CD3_a	10.4648	8.4842	1319.307	33.532	1285.775	1.0447	30.769	24.971	0.382
CD3_b	10.4648	8.4842	1272.947	33.532	1239.415	1.0062	30.794	24.971	0.382
CD4_a	14.8853	12.5787	1444.277	5.854	1438.423	1.2143	29.614	25.013	0.383
CD4_b	14.8853	12.5787	1162.784	5.854	1156.930	1.037	27.891	23.577	0.361
EE1_a	13.2026	11.1173	1059.236	33.532	1025.704	1.0195	25.152	21.220	0.325
EE1_b	13.2026	11.1173	1053.268	5.854	1047.414	1.0967	23.876	20.125	0.308
EE2_a	13.0760	10.8525	743.719	33.532	710.187	1.013	17.527	14.524	0.222
EE2_b	13.0760	10.8525	912.770	33.532	879.238	1.0347	21.244	17.595	0.269
EE3_a	9.8584	7.6889	1012.994	5.854	1007.140	1.0043	25.071	19.576	0.299
EE3_b	9.8584	7.6889	1125.592	33.532	1092.060	1.0347	26.386	20.590	0.315
EE4_a	12.4278	10.4383	961.165	33.532	927.633	1.0064	23.043	19.318	0.295
EE4_b	12.4278	10.4383	903.663	33.532	870.131	1.0299	21.122	17.722	0.271

Concentration of copper, lead and zinc in soil samples (outliers removed)

Soil Sample	Metal Concentration of Dry Soil (µmol/g)			Soil Sample	Metal Concentration of Dry Soil (µmol/g)		
	Copper	Lead	Zinc		Copper	Lead	Zinc
AA1_a	0.315	0.084	0.264	**BB4_b**	0.079	0.243	0.328
AA1_b	0.291	0.093	0.288	**BC1_a**	0.115	0.123	0.346
AA2_a	0.285	0.083	0.338	**BC1_b**	0.151	0.116	0.355
AA2_b	0.287	0.074	0.333	**BC2_a**	0.085	0.123	0.324
AA3_a	0.231	0.081	0.309	**BC2_b**	0.090	0.121	0.340
AA3_b	0.274	0.076	0.324	**BC3_a**	0.120	0.284	0.397
AA4_a	0.273	0.072	0.312	**BC3_b**	0.123	0.240	0.407
AA4_b	0.268	0.074	0.288	**BC4_a**	0.121	0.102	0.316
AB1_a	0.174	0.136	0.265	**BC4_b**	0.116	0.100	0.321
AB1_b	0.168	0.150	0.239	**BD1_a**	0.108	0.106	0.257
AB2_a	0.156	0.165	0.286	**BD1_b**	0.102	0.112	0.268
AB2_b	0.175	0.182	0.301	**BD2_a**	0.105	0.113	0.288
AB3_a	0.240	0.156	0.278	**BD2_b**	0.099	0.123	0.289
AB3_b	0.229	0.165	0.265	**BD3_a**	0.116	0.185	0.247
AB4_a	0.263	0.211	0.349	**BD3_b**	0.100	0.160	0.268
AB4_b	0.267	0.202	0.332	**BD4_a**	0.072	0.139	0.237
AC1_a	0.163	0.081	0.399	**BD4_b**	0.089	0.133	0.262
AC1_b	0.156	0.089	0.377	**CC1_a**	0.099	0.078	0.265
AC2_a	0.261	0.077	0.427	**CC1_b**	0.097	0.074	0.461
AC2_b	0.235	0.078	0.441	**CC2_a**	0.095	0.087	0.451
AC3_a	0.222	0.083	0.344	**CC2_b**	0.105	0.087	0.504
AC3_b	0.216	0.082	0.375	**CC3_a**	0.089	0.080	0.304
AC4_a	0.177	0.075	0.389	**CC3_b**	0.101	0.074	0.335
AC4_b	0.162	0.079	0.432	**CC4_a**	0.087	0.074	0.432
AD1_a	0.212	0.065	0.241	**CC4_b**	0.080	0.086	0.510
AD1_b	0.217	0.067	0.248	**CD1_a**	0.083	0.063	0.354
AD2_a	0.179	0.078	0.373	**CD1_b**	0.106	0.059	0.373
AD2_b	0.159	0.082	0.305	**CD2_a**	0.071	0.074	0.368
AD3_a	0.217	0.084	0.265	**CD2_b**	0.100	0.078	0.364
AD3_b	0.194	0.075	0.270	**CD3_a**	0.089	0.079	0.382
AD4_a	0.186	0.075	0.308	**CD3_b**	0.074	0.078	0.382
AD4_b	0.172	0.081	0.328	**CD4_a**	0.149	0.079	0.383
BB1_a	0.116	0.513	0.308	**EE1_a**	0.081	0.073	0.325
BB1_b	0.110	0.377	0.303	**EE1_b**	0.069	0.074	0.308
BB2_a	0.124	0.192	0.300	**EE2_b**	0.089	0.074	0.269
BB2_b	0.121	0.235	0.312	**EE3_a**	0.131	0.077	0.299
BB3_a	0.108	0.273	0.374	**EE3_b**	0.074	0.077	0.315
BB3_b	0.112	0.339	0.384	**EE4_a**	0.106	0.072	0.295
BB4_a	0.099	0.245	0.328	**EE4_b**	0.074	0.075	0.271

Appendix 6: Leaf Data

Concentration of copper in leaf samples

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Copper Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Copper Conc (µg/g)	Copper Conc (µg/g)	Copper Conc (µmol/g)
AA1_11	0.0537	0.0170	154.291	20.462	133.829	301.417	95.421	1.502
AA1_12	0.0562	0.0090	132.768	44.900	87.868	228.823	36.644	0.577
AA1_21	0.0365	0.0045	245.871	44.900	200.971	728.156	89.773	1.413
AA1_22	0.0409	0.0087	209.905	44.900	165.005	528.862	112.496	1.770
AA2_11	0.0733	0.0250	241.292	20.462	220.830	303.338	103.458	1.628
AA2_12	0.0763	0.0191	142.845	44.900	97.945	122.431	30.648	0.482
AA2_21	0.0103	0.0013	267.104	44.900	222.204	2923.737	369.015	5.807
AA2_22	0.0200	0.0063	839.949	20.462	819.487	4358.973	1373.077	21.608
AA3_11	0.0381	0.0081	176.527	20.462	156.065	650.271	138.247	2.176
AA3_12	0.0549	0.0104	281.079	44.900	236.179	557.026	105.520	1.661
AA4_11	0.0643	0.0181	183.789	20.462	163.327	171.562	48.293	0.760
AA4_12	0.0669	0.0168	302.654	44.900	257.754	374.642	94.081	1.481
AB1_11	0.1060	0.0234	241.811	20.462	221.349	245.943	54.293	0.854
AB1_12	0.1041	0.0236	173.285	44.900	128.385	134.858	30.573	0.481
AB1_21	0.0430	0.0093	315.800	44.900	270.900	752.500	162.750	2.561
AB1_22	0.0295	0.0045	313.280	44.900	268.380	1242.500	189.534	2.983
AB2_11	0.0237	0.0074	898.359	44.900	853.459	3386.742	1057.464	16.641
AB2_12	0.0063	0.0030	286.162	44.900	241.262	3015.775	1436.083	22.599
AB3_11	0.1353	0.0307	337.700	44.900	292.800	240.789	54.636	0.860
AB3_12	0.1137	0.0304	317.729	44.900	272.829	233.586	62.454	0.983
AB3_21	0.0405	0.0053	262.842	44.900	217.942	756.743	99.031	1.558
AB3_22	0.0455	0.0072	373.525	32.359	341.166	1353.833	214.233	3.371
AB4_11	0.0679	0.0165	493.575	32.359	461.216	900.813	218.901	3.445
AB4_12	0.1113	0.0237	451.498	44.900	406.598	453.792	96.630	1.521
AB4_21	0.0559	0.0102	128.085	20.462	107.623	266.394	48.608	0.765
AB4_22	0.0355	0.0062	493.714	32.359	461.355	1747.557	305.207	4.803
AC1_11	0.1084	0.0286	585.482	44.900	540.582	544.942	143.776	2.263
AC1_12	0.0785	0.0193	1331.153	32.359	1298.794	1699.992	417.960	6.577
AC1_21	0.0463	0.0093	392.093	20.462	371.631	1106.045	222.164	3.496
AC1_22	0.0225	0.0046	492.933	32.359	460.574	3289.814	672.584	10.584
AC2_11	0.0563	0.0136	317.911	32.359	285.552	575.710	139.070	2.188
AC2_12	0.0455	0.0074	144.030	20.462	123.568	417.459	67.895	1.068
AC2_21	0.0704	0.0174	411.248	32.359	378.889	627.300	155.043	2.440
AC2_22	0.0790	0.0183	729.357	20.462	708.895	968.436	224.334	3.530
AC3_11	0.1053	0.0325	438.293	32.359	405.934	356.082	109.902	1.729
AC3_12	0.0685	0.0195	423.870	44.900	378.970	560.607	159.589	2.511
AC3_21	0.0170	0.0037	673.928	20.462	653.466	5105.203	1111.132	17.485
AC3_22	0.0119	0.0019	156.987	32.359	124.628	1639.842	261.824	4.120
AC4_11	0.0558	0.0132	118.310	20.462	97.848	209.077	49.459	0.778
AC4_12	0.0524	0.0108	249.205	32.359	216.846	501.958	103.457	1.628

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Copper Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Copper Conc (µg/g)	Copper Conc (µg/g)	Copper Conc (µmol/g)
AD1_11	0.0809	0.0219	100.747	20.462	80.285	92.494	25.039	0.394
AD1_12	0.0704	0.0247	472.630	32.359	440.271	516.750	181.303	2.853
AD1_21	0.0393	0.0081	113.219	20.462	92.757	322.073	66.381	1.045
AD1_22	0.0288	0.0097	323.771	32.359	291.412	1175.048	395.763	6.228
AD2_11	0.0539	0.0108	219.777	44.900	174.877	437.193	87.601	1.379
AD2_12	0.0485	0.0146	446.294	32.359	413.935	892.101	268.550	4.226
AD2_21	0.0424	0.0089	225.275	20.462	204.813	602.391	126.445	1.990
AD2_22	0.0278	0.0112	419.774	32.359	387.415	1181.143	475.856	7.488
AD2_31	0.0229	0.0051	298.637	32.359	266.278	1479.322	329.456	5.185
AD2_32	0.0233	0.0048	415.000	32.359	382.641	2733.150	563.052	8.861
AD2_41	0.0122	0.0032	352.088	32.359	319.729	3633.284	952.993	14.997
AD2_42	0.0200	0.0046	322.251	32.359	289.892	1958.730	450.508	7.089
AD2_51	0.0310	0.0061	413.161	32.359	380.802	1983.344	390.271	6.142
AD2_52	0.0254	0.0052	317.468	32.359	285.109	1549.505	317.222	4.992
AD3_11	0.0670	0.0167	347.689	32.359	315.330	498.940	124.363	1.957
AD3_12	0.0700	0.0178	215.897	20.462	195.435	284.063	72.233	1.137
AD3_21	0.0901	0.0155	169.565	20.462	149.103	260.670	44.843	0.706
AD3_22	0.0548	0.0126	354.177	32.359	321.818	718.344	165.167	2.599
AD4_11	0.1432	0.0575	189.721	20.462	169.259	74.761	30.019	0.472
AD4_12	0.1226	0.0543	486.275	32.359	453.916	216.977	96.100	1.512
AD4_21	0.0675	0.0124	104.655	20.462	84.193	183.028	33.623	0.529
AD4_22	0.0561	0.0094	353.527	32.359	321.168	1003.650	168.170	2.646
BB1_11	0.1072	0.0300	170.617	20.462	150.155	131.715	36.861	0.580
BB1_12	0.1205	0.0291	326.201	32.359	293.842	267.129	64.510	1.015
BB1_21	0.3279	0.0063	336.510	20.462	316.048	1234.563	23.720	0.373
BB1_22	0.0366	0.0065	407.733	32.359	375.374	1538.418	273.216	4.300
BB2_11	0.2069	0.0520	326.182	20.462	305.720	151.347	38.038	0.599
BB2_12	0.1012	0.0239	494.606	32.359	462.247	495.973	117.132	1.843
BB3_11	0.1015	0.0218	571.765	32.359	539.406	670.903	144.095	2.268
BB3_12	0.1126	0.0298	234.308	20.462	213.846	198.006	52.403	0.825
BB3_21	0.0834	0.0172	85.778	20.462	65.316	100.178	20.660	0.325
BB3_22	0.1236	0.0241	293.167	32.359	260.808	293.703	57.267	0.901
BB4_11	0.0764	0.0187	327.277	44.900	282.377	476.988	116.750	1.837
BB4_12	0.0493	0.0140	353.958	32.359	321.599	648.385	184.126	2.898
BB4_21	0.0135	0.0042	199.854	20.462	179.392	1661.037	516.767	8.132
BB4_22	0.0155	0.0047	334.253	32.359	301.894	2039.824	618.527	9.734
BC1_11	0.1032	0.0362	139.104	20.462	118.642	85.231	29.897	0.470
BC1_12	0.0857	0.0234	310.738	32.359	278.379	316.340	86.375	1.359
BC1_21	0.0185	0.0049	101.153	20.462	80.691	611.295	161.911	2.548
BC1_22	0.0144	0.0048	237.703	32.359	205.344	1656.000	552.000	8.687
BC2_11	0.0362	0.0127	83.612	20.462	63.150	138.487	48.585	0.765
BC2_12	0.0573	0.0162	308.922	32.359	276.563	553.126	156.381	2.461
BC2_21	0.0445	0.0107	139.275	20.462	118.813	315.992	75.980	1.196
BC2_22	0.0322	0.0076	296.471	32.359	264.112	1222.741	288.597	4.542

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Copper Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Copper Conc (µg/g)	Copper Conc (µg/g)	Copper Conc (µmol/g)
BC3_11	0.0780	0.0193	153.715	20.462	133.253	189.280	46.835	0.737
BC3_12	0.0961	0.0193	199.768	32.359	167.409	241.921	48.585	0.765
BC3_21	0.0824	0.0172	204.233	20.462	183.771	308.341	64.362	1.013
BC3_22	0.0863	0.0198	273.335	32.359	240.976	322.160	73.914	1.163
BC4_11	0.1143	0.0294	804.917	44.900	760.017	685.936	176.435	2.776
BC4_12	0.1124	0.0213	143.151	32.359	110.792	130.038	24.642	0.388
BC4_21	0.0220	0.0057	204.908	20.462	184.446	1072.360	277.839	4.372
BC4_22	0.0130	0.0028	161.386	32.359	129.027	1152.027	248.129	3.905
BD1_11	0.1217	0.0308	229.389	20.462	208.927	178.876	45.270	0.712
BD1_12	0.1097	0.0192	196.234	32.359	163.875	213.379	37.346	0.588
BD1_21	0.0416	0.0070	266.064	44.900	221.164	801.319	134.837	2.122
BD1_22	0.0720	0.0120	191.018	32.359	158.659	330.540	55.090	0.867
BD2_11	0.6167	0.1165	635.753	32.359	603.394	134.207	25.353	0.399
BD2_12	0.2766	0.0593	823.046	44.900	778.146	343.098	73.556	1.158
BD3_11	0.0832	0.0211	372.481	44.900	327.581	391.843	99.374	1.564
BD3_12	0.1420	0.0372	507.854	32.359	475.495	339.639	88.976	1.400
BD4_11	0.0677	0.0186	342.824	32.359	310.465	482.089	132.450	2.084
BD4_12	0.0541	0.0129	357.828	44.900	312.928	597.191	142.399	2.241
BD4_21	0.0444	0.0083	343.201	44.900	298.301	877.356	164.010	2.581
BD4_22	0.0403	0.0097	242.006	32.359	209.647	680.672	163.834	2.578
CC1_11	0.0654	0.0177	487.510	32.359	455.151	734.115	198.682	3.127
CC1_12	0.0868	0.0215	407.021	44.900	362.121	517.316	128.137	2.016
CC1_13	0.0420	0.0070	266.898	44.900	221.998	685.179	114.197	1.797
CC1_14	0.0645	0.0113	333.652	44.900	288.752	577.504	101.175	1.592
CC1_15	0.0706	0.0139	311.189	44.900	266.289	465.540	91.657	1.442
CC1_16	0.0767	0.0219	389.976	44.900	345.076	403.126	115.104	1.811
CC1_17	0.0872	0.0286	162.262	44.900	117.362	111.987	36.730	0.578
CC1_18	0.0354	0.0119	429.749	44.900	384.849	907.663	305.118	4.802
CC1_21	0.0309	0.0067	327.383	44.900	282.483	1103.449	239.259	3.765
CC1_22	0.0435	0.0101	304.652	32.359	272.293	840.410	195.130	3.071
CC2_11	0.0690	0.0167	236.216	44.900	191.316	289.873	70.158	1.104
CC2_12	0.0511	0.0152	248.514	32.359	216.155	412.510	122.703	1.931
CC2_21	0.0926	0.0168	265.203	44.900	220.303	348.581	63.241	0.995
CC2_22	0.0908	0.0279	466.002	32.359	433.643	416.964	128.120	2.016
CC3_11	0.0880	0.0169	160.472	44.900	115.572	182.867	35.119	0.553
CC3_12	0.0865	0.0147	576.747	32.359	544.388	829.860	141.028	2.219
CC3_21	0.0525	0.0120	281.298	44.900	236.398	523.004	119.544	1.881
CC3_22	0.0299	0.0095	305.966	44.900	261.066	767.841	243.963	3.839
CC4_11	0.2080	0.0458	350.413	44.900	305.513	176.393	38.840	0.611
CC4_12	0.1352	0.0301	418.126	32.359	385.767	313.123	69.711	1.097

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Copper Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Copper Conc (µg/g)	Copper Conc (µg/g)	Copper Conc (µmol/g)
CD1_11	0.1934	0.0419	334.964	44.900	290.064	180.838	39.178	0.617
CD1_12	0.2163	0.0419	463.700	32.359	431.341	246.763	47.801	0.752
CD1_21	0.0480	0.0107	498.392	44.900	453.492	1318.291	293.869	4.625
CD1_22	0.0407	0.0056	501.776	32.359	469.417	1862.766	256.302	4.033
CD2_11	0.0857	0.0188	183.270	44.900	138.370	186.986	41.019	0.646
CD2_12	0.0729	0.0221	506.434	44.900	461.534	544.262	164.996	2.596
CD2_21	0.1005	0.0203	333.998	44.900	289.098	374.479	75.641	1.190
CD2_22	0.0514	0.0132	325.148	44.900	280.248	714.918	183.598	2.889
CD3_11	0.0291	0.0067	430.725	44.900	385.825	1663.039	382.899	6.026
CD3_12	0.0780	0.0208	335.502	32.359	303.143	375.177	100.047	1.574
CD3_21	0.0412	0.0103	280.162	44.900	235.262	582.332	145.583	2.291
CD3_22	0.0413	0.0116	246.813	32.359	214.454	501.061	140.734	2.215
CD4_11	0.2118	0.0394	272.557	44.900	227.657	147.065	27.358	0.431
CD4_12	0.2019	0.0415	243.939	31.034	212.905	133.399	27.420	0.431
EE1_11	0.1676	0.0342	264.007	44.900	219.107	162.061	33.070	0.520
EE1_12	0.1641	0.0287	400.962	31.034	369.928	337.526	59.031	0.929
EE1_21	0.0235	0.0046	288.788	44.900	243.888	1354.933	265.221	4.174
EE1_22	0.0228	0.0053	240.914	31.034	209.880	1140.652	265.152	4.173
EE2_11	0.1750	0.0348	337.857	44.900	292.957	216.045	42.962	0.676
EE2_12	0.1319	0.0524	265.358	31.034	234.324	114.640	45.543	0.717
EE2_21	0.0159	0.0037	420.467	44.900	375.567	3237.647	753.415	11.856
EE2_22	0.0188	0.0043	339.967	31.034	308.933	1930.831	441.626	6.950
EE3_11	0.2184	0.0397	285.483	44.900	240.583	162.118	29.469	0.464
EE3_12	0.1642	0.0345	379.131	31.034	348.097	259.774	54.581	0.859
EE4_11	0.1963	0.0489	366.690	31.034	335.656	171.603	42.748	0.673
EE4_12	0.1751	0.0518	362.498	31.034	331.464	163.767	48.447	0.762
EE4_21	0.0100	0.0019	501.337	44.900	456.437	6339.403	1204.487	18.955
EE4_22	0.0082	0.0028	332.358	31.034	301.324	3766.550	1286.139	20.239

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Concentration of lead in leaf samples

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Lead Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Lead Conc (µg/g)	Lead Conc (µg/g)	Lead Conc (µmol/g)
AA1_11	0.0537	0.0170	269.007	27.238	241.769	544.525	172.382	0.832
AA1_12	0.0562	0.0090	276.635	33.033	243.602	634.380	101.591	0.490
AA1_21	0.0365	0.0045	248.931	33.033	215.898	782.239	96.440	0.465
AA1_22	0.0409	0.0087	673.617	33.033	640.584	2053.154	436.734	2.108
AA2_11	0.0733	0.0250	275.933	27.238	248.695	341.614	116.512	0.562
AA2_12	0.0763	0.0191	256.511	33.033	223.478	279.348	69.928	0.337
AA2_21	0.0103	0.0013	253.249	33.033	220.216	2897.579	365.714	1.765
AA2_22	0.0200	0.0063	586.768	27.238	559.530	2976.223	937.510	4.525
AA3_11	0.0381	0.0081	251.586	27.238	224.348	934.783	198.733	0.959
AA3_12	0.0549	0.0104	263.126	33.033	230.093	542.672	102.801	0.496
AA4_11	0.0643	0.0181	271.013	27.238	243.775	256.066	72.081	0.348
AA4_12	0.0669	0.0168	245.909	33.033	212.876	309.413	77.700	0.375
AB1_11	0.1060	0.0234	278.559	27.238	251.321	279.246	61.645	0.298
AB1_12	0.1041	0.0236	236.063	33.033	203.030	213.267	48.349	0.233
AB1_21	0.0430	0.0093	234.064	33.033	201.031	558.419	120.774	0.583
AB1_22	0.0295	0.0045	263.052	33.033	230.019	1064.903	162.443	0.784
AB2_11	0.0237	0.0074	250.264	33.033	217.231	862.028	269.156	1.299
AB2_12	0.0063	0.0030	261.976	33.033	228.943	2861.788	1362.756	6.577
AB3_11	0.1353	0.0307	291.323	33.033	258.290	212.410	48.196	0.233
AB3_12	0.1137	0.0304	254.400	33.033	221.367	189.527	50.674	0.245
AB3_21	0.0405	0.0053	266.124	33.033	233.091	809.344	105.914	0.511
AB3_22	0.0455	0.0072	331.963	30.335	301.628	1196.937	189.405	0.914
AB4_11	0.0679	0.0165	264.427	30.335	234.092	457.211	111.104	0.536
AB4_12	0.1113	0.0237	350.264	33.033	317.231	354.052	75.391	0.364
AB4_21	0.0559	0.0102	281.385	27.238	254.147	629.077	114.787	0.554
AB4_22	0.0355	0.0062	1161.013	30.335	1130.678	4282.871	747.994	3.610
AC1_11	0.1084	0.0286	308.376	33.033	275.343	277.564	73.232	0.353
AC1_12	0.0785	0.0193	611.146	30.335	580.811	760.224	186.909	0.902
AC1_21	0.0463	0.0093	267.294	27.238	240.056	714.452	143.508	0.693
AC1_22	0.0225	0.0046	699.343	30.335	669.008	4778.629	976.964	4.715
AC2_11	0.0563	0.0136	306.216	30.335	275.881	556.212	134.360	0.648
AC2_12	0.0455	0.0074	277.376	27.238	250.138	845.061	137.438	0.663
AC2_21	0.0704	0.0174	544.398	30.335	514.063	851.098	210.357	1.015
AC2_22	0.0790	0.0183	279.386	27.238	252.148	344.464	79.794	0.385
AC3_11	0.1053	0.0325	327.796	30.335	297.461	260.931	80.534	0.389
AC3_12	0.0685	0.0195	309.316	33.033	276.283	408.703	116.346	0.562
AC3_21	0.0170	0.0037	254.342	27.238	227.104	1774.250	386.160	1.864
AC3_22	0.0119	0.0019	277.731	30.335	247.396	3255.211	519.739	2.508
AC4_11	0.0558	0.0132	256.301	27.238	229.063	489.451	115.784	0.559
AC4_12	0.0524	0.0108	288.215	30.335	257.880	596.944	123.034	0.594

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Lead Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Lead Conc (µg/g)	Lead Conc (µg/g)	Lead Conc (µmol/g)
AD1_11	0.0809	0.0219	285.205	27.238	257.967	297.197	80.453	0.388
AD1_12	0.0704	0.0247	332.452	30.335	302.117	354.597	124.411	0.600
AD1_21	0.0393	0.0081	269.476	27.238	242.238	841.104	173.357	0.837
AD1_22	0.0288	0.0097	308.529	30.335	278.194	1121.750	377.812	1.823
AD2_11	0.0539	0.0108	235.331	33.033	202.298	505.745	101.337	0.489
AD2_12	0.0485	0.0146	1225.275	30.335	1194.940	2575.302	775.245	3.742
AD2_21	0.0424	0.0089	277.135	27.238	249.897	734.991	154.279	0.745
AD2_22	0.0278	0.0112	323.459	30.335	293.124	893.671	360.040	1.738
AD2_31	0.0229	0.0051	307.321	30.335	276.986	1538.811	342.705	1.654
AD2_32	0.0233	0.0048	321.536	30.335	291.201	2080.007	428.499	2.068
AD2_41	0.0122	0.0032	342.881	30.335	312.546	3551.659	931.583	4.496
AD2_42	0.0200	0.0046	320.281	30.335	289.946	1959.095	450.592	2.175
AD2_51	0.0310	0.0061	295.902	30.335	265.567	1383.161	272.170	1.314
AD2_52	0.0254	0.0052	520.253	30.335	489.918	2662.598	545.099	2.631
AD3_11	0.0670	0.0167	1236.954	30.335	1206.619	1909.207	475.877	2.297
AD3_12	0.0700	0.0178	257.575	27.238	230.337	334.792	85.133	0.411
AD3_21	0.0901	0.0155	260.268	27.238	233.030	407.395	70.085	0.338
AD3_22	0.0548	0.0126	710.924	30.335	680.589	1519.172	349.299	1.686
AD4_11	0.1432	0.0575	265.711	27.238	238.473	105.333	42.295	0.204
AD4_12	0.1226	0.0543	460.311	30.335	429.976	205.533	91.032	0.439
AD4_21	0.0675	0.0124	272.581	27.238	245.343	533.354	97.979	0.473
AD4_22	0.0561	0.0094	310.574	30.335	280.239	875.747	146.738	0.708
BB1_11	0.1072	0.0300	275.875	27.238	248.637	218.103	61.036	0.295
BB1_12	0.1205	0.0291	311.981	30.335	281.646	256.042	61.833	0.298
BB1_21	0.3279	0.0063	293.617	27.238	266.379	1040.543	19.992	0.096
BB1_22	0.0366	0.0065	300.824	30.335	270.489	1108.561	196.876	0.950
BB2_11	0.2069	0.0520	250.206	27.238	222.968	110.380	27.742	0.134
BB2_12	0.1012	0.0239	399.122	30.335	368.787	395.694	93.450	0.451
BB3_11	0.1015	0.0218	358.191	30.335	327.856	407.781	87.583	0.423
BB3_12	0.1126	0.0298	269.804	27.238	242.566	224.598	59.441	0.287
BB3_21	0.0834	0.0172	268.301	27.238	241.063	369.729	76.251	0.368
BB3_22	0.1236	0.0241	417.979	30.335	387.644	436.536	85.117	0.411
BB4_11	0.0764	0.0187	303.328	33.033	270.295	456.579	111.754	0.539
BB4_12	0.0493	0.0140	1329.000	30.335	1298.665	2618.276	743.527	3.588
BB4_21	0.0135	0.0042	259.030	27.238	231.792	2146.222	667.714	3.223
BB4_22	0.0155	0.0047	361.357	30.335	331.022	2236.635	678.205	3.273
BC1_11	0.1032	0.0362	281.153	27.238	253.915	182.410	63.985	0.309
BC1_12	0.0857	0.0234	320.802	30.335	290.467	330.076	90.126	0.435
BC1_21	0.0185	0.0049	265.502	27.238	238.264	1805.030	478.089	2.307
BC1_22	0.0144	0.0048	470.974	30.335	440.639	3553.540	1184.513	5.717
BC2_11	0.0362	0.0127	228.932	27.238	201.694	442.311	155.176	0.749
BC2_12	0.0573	0.0162	313.229	30.335	282.894	565.788	159.961	0.772
BC2_21	0.0445	0.0107	255.284	27.238	228.046	606.505	145.834	0.704
BC2_22	0.0322	0.0076	350.911	30.335	320.576	1484.148	350.296	1.691

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Lead Concentration (μg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Lead Conc (μg/g)	Lead Conc (μg/g)	Lead Conc (μmol/g)
BC3_11	0.0780	0.0193	248.466	27.238	221.228	314.244	77.755	0.375
BC3_12	0.0961	0.0193	336.126	30.335	305.791	441.895	88.747	0.428
BC3_21	0.0824	0.0172	253.821	27.238	226.583	380.173	79.356	0.383
BC3_22	0.0863	0.0198	346.421	30.335	316.086	422.575	96.952	0.468
BC4_11	0.1143	0.0294	260.268	33.033	227.235	205.086	52.752	0.255
BC4_12	0.1124	0.0213	258.122	30.335	227.787	267.356	50.664	0.245
BC4_21	0.0220	0.0057	259.288	27.238	232.050	1349.128	349.547	1.687
BC4_22	0.0130	0.0028	285.301	30.335	254.966	2276.482	490.319	2.366
BD1_11	0.1217	0.0308	250.143	27.238	222.905	190.843	48.299	0.233
BD1_12	0.1097	0.0192	287.645	30.335	257.310	335.039	58.639	0.283
BD1_21	0.0416	0.0070	254.045	33.033	221.012	800.768	134.745	0.650
BD1_22	0.0720	0.0120	303.475	30.335	273.140	569.042	94.840	0.458
BD2_11	0.6167	0.1165	362.430	30.335	332.095	73.865	13.954	0.067
BD2_12	0.2766	0.0593	294.227	33.033	261.194	115.165	24.690	0.119
BD3_11	0.0832	0.0211	255.647	33.033	222.614	266.285	67.531	0.326
BD3_12	0.1420	0.0372	340.632	30.335	310.297	221.641	58.064	0.280
BD4_11	0.0677	0.0186	350.984	30.335	320.649	497.902	136.794	0.660
BD4_12	0.0541	0.0129	267.828	33.033	234.795	448.082	106.844	0.516
BD4_21	0.0444	0.0083	261.104	33.033	228.071	670.797	125.397	0.605
BD4_22	0.0403	0.0097	324.034	30.335	293.699	953.568	229.519	1.108
CC1_11	0.0654	0.0177	346.527	30.335	316.192	509.987	138.024	0.666
CC1_12	0.0868	0.0215	283.314	33.033	250.281	357.544	88.562	0.427
CC1_13	0.0420	0.0070	301.017	33.033	267.984	827.111	137.852	0.665
CC1_14	0.0645	0.0113	292.497	33.033	259.464	518.928	90.913	0.439
CC1_15	0.0706	0.0139	330.868	33.033	297.835	520.691	102.516	0.495
CC1_16	0.0767	0.0219	250.815	33.033	217.782	254.418	72.644	0.351
CC1_17	0.0872	0.0286	270.750	33.033	237.717	226.829	74.396	0.359
CC1_18	0.0354	0.0119	285.528	33.033	252.495	595.507	200.185	0.966
CC1_21	0.0309	0.0067	304.976	33.033	271.943	1062.277	230.332	1.112
CC1_22	0.0435	0.0101	299.245	30.335	268.910	829.969	192.705	0.930
CC2_11	0.0690	0.0167	240.870	33.033	207.837	314.905	76.216	0.368
CC2_12	0.0511	0.0152	310.489	30.335	280.154	534.645	159.033	0.768
CC2_21	0.0926	0.0168	225.354	33.033	192.321	304.305	55.209	0.266
CC2_22	0.0908	0.0279	448.434	30.335	418.099	402.018	123.528	0.596
CC3_11	0.0880	0.0169	262.742	33.033	229.709	363.464	69.802	0.337
CC3_12	0.0865	0.0147	374.764	30.335	344.429	525.044	89.227	0.431
CC3_21	0.0525	0.0120	235.514	33.033	202.481	447.967	102.392	0.494
CC3_22	0.0299	0.0095	282.020	33.033	248.987	732.315	232.675	1.123
CC4_11	0.2080	0.0458	259.793	33.033	226.760	130.924	28.828	0.139
CC4_12	0.1352	0.0301	326.886	30.335	296.551	240.707	53.589	0.259

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Lead Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Lead Conc (µg/g)	Lead Conc (µg/g)	Lead Conc (µmol/g)
CD1_11	0.1934	0.0419	243.237	33.033	210.204	131.050	28.392	0.137
CD1_12	0.2163	0.0419	417.712	30.335	387.377	221.612	42.929	0.207
CD1_21	0.0480	0.0107	254.584	33.033	221.551	644.044	143.568	0.693
CD1_22	0.0407	0.0056	382.583	30.335	352.248	1397.810	192.328	0.928
CD2_11	0.0857	0.0188	257.361	33.033	224.328	303.146	66.501	0.321
CD2_12	0.0729	0.0221	318.491	33.033	285.458	336.625	102.050	0.493
CD2_21	0.1005	0.0203	269.646	33.033	236.613	306.494	61.909	0.299
CD2_22	0.0514	0.0132	276.060	33.033	243.027	619.967	159.213	0.768
CD3_11	0.0291	0.0067	270.784	33.033	237.751	1024.789	235.948	1.139
CD3_12	0.0780	0.0208	330.885	30.335	300.550	371.968	99.191	0.479
CD3_21	0.0412	0.0103	307.030	33.033	273.997	678.210	169.553	0.818
CD3_22	0.0413	0.0116	305.757	30.335	275.422	643.509	180.744	0.872
CD4_11	0.2118	0.0394	288.967	33.033	255.934	165.332	30.756	0.148
CD4_12	0.2019	0.0415	315.481	30.148	285.333	178.780	36.748	0.177
EE1_11	0.1676	0.0342	247.184	33.033	214.151	158.396	32.322	0.156
EE1_12	0.1641	0.0287	319.161	30.148	289.013	263.698	46.119	0.223
EE1_21	0.0235	0.0046	256.262	33.033	223.229	1240.161	242.755	1.172
EE1_22	0.0228	0.0053	314.404	30.148	284.256	1544.870	359.114	1.733
EE2_11	0.1750	0.0348	279.057	33.033	246.024	181.434	36.079	0.174
EE2_12	0.1319	0.0524	350.719	30.148	320.571	156.835	62.306	0.301
EE2_21	0.0159	0.0037	271.271	33.033	238.238	2053.776	477.923	2.307
EE2_22	0.0188	0.0043	291.494	30.148	261.346	1633.413	373.600	1.803
EE3_11	0.2184	0.0397	241.380	33.033	208.347	140.396	26.000	0.125
EE3_12	0.1642	0.0345	357.003	30.148	326.855	243.922	51.250	0.247
EE4_11	0.1963	0.0489	311.002	30.148	280.854	143.586	35.769	0.173
EE4_12	0.1751	0.0518	319.043	30.148	288.895	142.735	42.225	0.204
EE4_21	0.0100	0.0019	231.693	33.033	198.660	2759.167	524.242	2.530
EE4_22	0.0082	0.0028	333.260	30.148	303.112	3788.900	1293.771	6.244

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Concentration of zinc in leaf samples

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Zinc Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Zinc Conc (µg/g)	Zinc Conc (µg/g)	Zinc Conc (µmol/g)
AA1_11	0.0537	0.0170	73.795	4.255	69.540	156.622	49.582	0.758
AA1_12	0.0562	0.0090	137.492	8.134	129.358	336.870	53.947	0.825
AA1_21	0.0365	0.0045	80.557	8.134	72.423	262.402	32.351	0.495
AA1_22	0.0409	0.0087	105.600	8.134	97.466	312.391	66.450	1.016
AA2_11	0.0733	0.0250	119.722	4.255	115.467	158.609	54.096	0.827
AA2_12	0.0763	0.0191	141.996	8.134	133.862	167.328	41.887	0.641
AA2_21	0.0103	0.0013	78.793	8.134	70.659	929.724	117.344	1.795
AA2_22	0.0200	0.0063	131.553	4.255	127.298	677.117	213.292	3.262
AA3_11	0.0381	0.0081	82.743	4.255	78.488	327.033	69.527	1.063
AA3_12	0.0549	0.0104	172.266	8.134	164.132	387.104	73.331	1.121
AA4_11	0.0643	0.0181	198.374	4.255	194.119	203.907	57.398	0.878
AA4_12	0.0669	0.0168	224.859	8.134	216.725	315.007	79.105	1.210
AB1_11	0.1060	0.0234	141.497	4.255	137.242	152.491	33.663	0.515
AB1_12	0.1041	0.0236	115.118	8.134	106.984	112.378	25.477	0.390
AB1_21	0.0430	0.0093	62.995	8.134	54.861	152.392	32.959	0.504
AB1_22	0.0295	0.0045	105.814	8.134	97.680	452.222	68.983	1.055
AB2_11	0.0237	0.0074	257.416	8.134	249.282	989.214	308.869	4.723
AB2_12	0.0063	0.0030	78.655	8.134	70.521	881.513	419.768	6.419
AB3_11	0.1353	0.0307	211.907	8.134	203.773	167.576	38.024	0.581
AB3_12	0.1137	0.0304	140.937	8.134	132.803	113.701	30.400	0.465
AB3_21	0.0405	0.0053	117.086	8.134	108.952	378.306	49.507	0.757
AB3_22	0.0455	0.0072	107.294	8.325	98.969	392.734	62.147	0.950
AB4_11	0.0679	0.0165	144.148	8.325	135.823	265.279	64.464	0.986
AB4_12	0.1113	0.0237	178.271	8.134	170.137	189.885	40.434	0.618
AB4_21	0.0559	0.0102	75.886	4.255	71.631	177.304	32.353	0.495
AB4_22	0.0355	0.0062	105.354	8.325	97.029	367.534	64.189	0.982
AC1_11	0.1084	0.0286	480.673	8.134	472.539	476.350	125.679	1.922
AC1_12	0.0785	0.0193	306.659	8.325	298.334	390.490	96.006	1.468
AC1_21	0.0463	0.0093	198.935	4.255	194.680	579.405	116.382	1.780
AC1_22	0.0225	0.0046	123.896	8.325	115.571	825.507	168.770	2.581
AC2_11	0.0563	0.0136	136.615	8.325	128.290	258.649	62.480	0.955
AC2_12	0.0455	0.0074	92.974	4.255	88.719	299.726	48.747	0.745
AC2_21	0.0704	0.0174	259.845	8.325	251.520	416.424	102.923	1.574
AC2_22	0.0790	0.0183	245.647	4.255	241.392	329.770	76.390	1.168
AC3_11	0.1053	0.0325	628.577	8.325	620.252	544.081	167.926	2.568
AC3_12	0.0685	0.0195	421.301	8.134	413.167	611.194	173.989	2.661
AC3_21	0.0170	0.0037	57.023	4.255	52.768	412.250	89.725	1.372
AC3_22	0.0119	0.0019	62.460	8.325	54.135	712.303	113.729	1.739
AC4_11	0.0558	0.0132	245.111	4.255	240.856	514.650	121.745	1.862
AC4_12	0.0524	0.0108	206.727	8.325	198.402	459.264	94.657	1.448

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Zinc Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Zinc Conc (µg/g)	Zinc Conc (µg/g)	Zinc Conc (µmol/g)
AD1_11	0.0809	0.0219	94.210	4.255	89.955	103.635	28.054	0.429
AD1_12	0.0704	0.0247	269.629	8.325	261.304	306.695	107.605	1.646
AD1_21	0.0393	0.0081	74.472	4.255	70.217	243.809	50.251	0.768
AD1_22	0.0288	0.0097	105.352	8.325	97.027	391.238	131.771	2.015
AD2_11	0.0539	0.0108	113.496	8.134	105.362	263.405	52.779	0.807
AD2_12	0.0485	0.0146	106.745	8.325	98.420	212.112	63.852	0.976
AD2_21	0.0424	0.0089	62.789	4.255	58.534	172.159	36.137	0.553
AD2_22	0.0278	0.0112	116.420	8.325	108.095	329.558	132.772	2.030
AD2_31	0.0229	0.0051	82.669	8.325	74.344	413.022	91.983	1.407
AD2_32	0.0233	0.0048	98.096	8.325	89.771	641.221	132.097	2.020
AD2_41	0.0122	0.0032	91.129	8.325	82.804	940.955	246.808	3.774
AD2_42	0.0200	0.0046	93.514	8.325	85.189	575.601	132.388	2.025
AD2_51	0.0310	0.0061	65.625	8.325	57.300	298.438	58.725	0.898
AD2_52	0.0254	0.0052	89.674	8.325	81.349	442.114	90.512	1.384
AD3_11	0.0670	0.0167	201.692	8.325	193.367	305.960	76.262	1.166
AD3_12	0.0700	0.0178	205.100	4.255	200.845	291.926	74.233	1.135
AD3_21	0.0901	0.0155	196.950	4.255	192.695	336.879	57.954	0.886
AD3_22	0.0548	0.0126	161.441	8.325	153.116	341.777	78.584	1.202
AD4_11	0.1432	0.0575	394.490	4.255	390.235	172.365	69.211	1.058
AD4_12	0.1226	0.0543	366.307	8.325	357.982	171.120	75.789	1.159
AD4_21	0.0675	0.0124	53.242	4.255	48.987	106.493	19.563	0.299
AD4_22	0.0561	0.0094	109.035	8.325	100.710	314.719	52.734	0.806
BB1_11	0.1072	0.0300	152.435	4.255	148.180	129.982	36.376	0.556
BB1_12	0.1205	0.0291	217.331	8.325	209.006	190.005	45.885	0.702
BB1_21	0.3279	0.0063	87.446	4.255	83.191	324.965	6.244	0.095
BB1_22	0.0366	0.0065	110.695	8.325	102.370	419.549	74.510	1.139
BB2_11	0.2069	0.0520	233.727	4.255	229.472	113.600	28.551	0.437
BB2_12	0.1012	0.0239	201.533	8.325	193.208	207.305	48.958	0.749
BB3_11	0.1015	0.0218	189.437	8.325	181.112	225.264	48.382	0.740
BB3_12	0.1126	0.0298	87.699	4.255	83.444	77.263	20.448	0.313
BB3_21	0.0834	0.0172	101.210	4.255	96.955	148.704	30.668	0.469
BB3_22	0.1236	0.0241	242.584	8.325	234.259	263.805	51.438	0.787
BB4_11	0.0764	0.0187	129.867	8.134	121.733	205.630	50.331	0.770
BB4_12	0.0493	0.0140	114.837	8.325	106.512	214.742	60.981	0.933
BB4_21	0.0135	0.0042	47.203	4.255	42.948	397.667	123.719	1.892
BB4_22	0.0155	0.0047	124.574	8.325	116.249	785.466	238.174	3.642
BC1_11	0.1032	0.0362	343.003	4.255	338.748	243.353	85.362	1.305
BC1_12	0.0857	0.0234	199.532	8.325	191.207	217.281	59.328	0.907
BC1_21	0.0185	0.0049	59.836	4.255	55.581	421.068	111.526	1.706
BC1_22	0.0144	0.0048	96.337	8.325	88.012	709.774	236.591	3.618
BC2_11	0.0362	0.0127	108.040	4.255	103.785	227.599	79.848	1.221
BC2_12	0.0573	0.0162	330.061	8.325	321.736	643.472	181.924	2.782
BC2_21	0.0445	0.0107	111.274	4.255	107.019	284.625	68.438	1.047
BC2_22	0.0322	0.0076	156.366	8.325	148.041	685.375	161.766	2.474

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Zinc Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Zinc Conc (µg/g)	Zinc Conc (µg/g)	Zinc Conc (µmol/g)
BC3_11	0.0780	0.0193	72.522	4.255	68.267	96.970	23.994	0.367
BC3_12	0.0961	0.0193	91.515	8.325	83.190	120.217	24.143	0.369
BC3_21	0.0824	0.0172	121.498	4.255	117.243	196.716	41.062	0.628
BC3_22	0.0863	0.0198	198.183	8.325	189.858	253.821	58.235	0.891
BC4_11	0.1143	0.0294	253.274	8.134	245.140	221.245	56.908	0.870
BC4_12	0.1124	0.0213	123.099	8.325	114.774	134.711	25.528	0.390
BC4_21	0.0220	0.0057	52.363	4.255	48.108	279.698	72.467	1.108
BC4_22	0.0130	0.0028	68.586	8.325	60.261	538.045	115.887	1.772
BD1_11	0.1217	0.0308	146.384	4.255	142.129	121.686	30.796	0.471
BD1_12	0.1097	0.0192	173.231	8.325	164.906	214.721	37.581	0.575
BD1_21	0.0416	0.0070	98.424	8.134	90.290	327.138	55.047	0.842
BD1_22	0.0720	0.0120	147.024	8.325	138.699	288.956	48.159	0.736
BD2_11	0.6167	0.1165	898.972	8.325	890.647	198.098	37.422	0.572
BD2_12	0.2766	0.0593	489.392	8.134	481.258	212.195	45.492	0.696
BD3_11	0.0832	0.0211	266.857	8.134	258.723	309.477	78.485	1.200
BD3_12	0.1420	0.0372	338.862	8.325	330.537	236.098	61.851	0.946
BD4_11	0.0677	0.0186	153.850	8.325	145.525	225.970	62.083	0.949
BD4_12	0.0541	0.0129	159.271	8.134	151.137	288.429	68.775	1.052
BD4_21	0.0444	0.0083	103.540	8.134	95.406	280.606	52.456	0.802
BD4_22	0.0403	0.0097	73.801	8.325	65.476	212.584	51.168	0.783
CC1_11	0.0654	0.0177	341.770	8.325	333.445	537.815	145.555	2.226
CC1_12	0.0868	0.0215	369.731	8.134	361.597	516.567	127.952	1.957
CC1_13	0.0420	0.0070	240.796	8.134	232.662	718.093	119.682	1.830
CC1_14	0.0645	0.0113	286.438	8.134	278.304	556.608	97.514	1.491
CC1_15	0.0706	0.0139	464.156	8.134	456.022	797.241	156.964	2.400
CC1_16	0.0767	0.0219	259.216	8.134	251.082	293.320	83.751	1.281
CC1_17	0.0872	0.0286	479.032	8.134	470.898	449.330	147.372	2.254
CC1_18	0.0354	0.0119	226.071	8.134	217.937	514.002	172.786	2.642
CC1_21	0.0309	0.0067	116.057	8.134	107.923	421.574	91.409	1.398
CC1_22	0.0435	0.0101	143.652	8.325	135.327	417.676	96.978	1.483
CC2_11	0.0690	0.0167	192.841	8.134	184.707	279.859	67.734	1.036
CC2_12	0.0511	0.0152	175.296	8.325	166.971	318.647	94.783	1.450
CC2_21	0.0926	0.0168	147.244	8.134	139.110	220.111	39.934	0.611
CC2_22	0.0908	0.0279	343.921	8.325	335.596	322.688	99.152	1.516
CC3_11	0.0880	0.0169	210.045	8.134	201.911	319.479	61.355	0.938
CC3_12	0.0865	0.0147	357.743	8.325	349.418	532.649	90.520	1.384
CC3_21	0.0525	0.0120	188.427	8.134	180.293	398.878	91.172	1.394
CC3_22	0.0299	0.0095	84.891	8.134	76.757	225.756	71.728	1.097
CC4_11	0.2080	0.0458	419.079	8.134	410.945	237.266	52.244	0.799
CC4_12	0.1352	0.0301	295.563	8.325	287.238	233.148	51.906	0.794

Leaf Sample	Leaf Dry Weight (g)	Leaf Ashed Weight (g)	Zinc Concentration (µg/L) in 25mL			Ashed Leaf	Dry Leaf	
			Gross	Blank	Net	Zinc Conc (µg/g)	Zinc Conc (µg/g)	Zinc Conc (µmol/g)
CD1_11	0.1934	0.0419	694.243	8.134	686.109	427.749	92.672	1.417
CD1_12	0.2163	0.0419	582.225	8.325	573.900	328.318	63.599	0.973
CD1_21	0.0480	0.0107	187.123	8.134	178.989	520.317	115.987	1.774
CD1_22	0.0407	0.0056	130.831	8.325	122.506	486.135	66.888	1.023
CD2_11	0.0857	0.0188	595.380	8.134	587.246	793.576	174.087	2.662
CD2_12	0.0729	0.0221	395.590	8.134	387.456	456.906	138.513	2.118
CD2_21	0.1005	0.0203	272.078	8.134	263.944	341.896	69.060	1.056
CD2_22	0.0514	0.0132	288.025	8.134	279.891	714.008	183.364	2.804
CD3_11	0.0291	0.0067	139.133	8.134	130.999	564.651	130.006	1.988
CD3_12	0.0780	0.0208	136.726	8.325	128.401	158.912	42.377	0.648
CD3_21	0.0412	0.0103	144.220	8.134	136.086	336.847	84.212	1.288
CD3_22	0.0413	0.0116	97.301	8.325	88.976	207.888	58.390	0.893
CD4_11	0.2118	0.0394	439.517	8.134	431.383	278.671	51.840	0.793
CD4_12	0.2019	0.0415	438.133	9.242	428.891	268.729	55.236	0.845
EE1_11	0.1676	0.0342	191.173	8.134	183.039	135.384	27.626	0.422
EE1_12	0.1641	0.0287	198.689	9.242	189.447	172.853	30.231	0.462
EE1_21	0.0235	0.0046	79.287	8.134	71.153	395.294	77.377	1.183
EE1_22	0.0228	0.0053	98.444	9.242	89.202	484.793	112.693	1.723
EE2_11	0.1750	0.0348	175.256	8.134	167.122	123.246	24.508	0.375
EE2_12	0.1319	0.0524	227.777	9.242	218.535	106.915	42.474	0.650
EE2_21	0.0159	0.0037	88.146	8.134	80.012	689.759	160.510	2.455
EE2_22	0.0188	0.0043	110.841	9.242	101.599	634.994	145.238	2.221
EE3_11	0.2184	0.0397	184.728	8.134	176.594	118.999	21.631	0.331
EE3_12	0.1642	0.0345	208.014	9.242	198.772	148.337	31.167	0.477
EE4_11	0.1963	0.0489	220.572	9.242	211.330	108.042	26.914	0.412
EE4_12	0.1751	0.0518	174.104	9.242	164.862	81.454	24.096	0.369
EE4_21	0.0100	0.0019	76.907	8.134	68.773	955.181	181.484	2.775
EE4_22	0.0082	0.0028	97.243	9.242	88.001	1100.013	375.614	5.744

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Mean concentration of copper, lead and zinc in leaf samples

Leaf Sample	Metal Concentration of Dry Leaf (µmol/g)		
	Copper	Lead	Zinc
AA1_1	1.039	0.661	0.792
AA1_2	1.592	1.287	0.755
AA2_1	1.055	0.450	0.734
AA2_2	13.707	3.145	2.528
AA3_1	1.918	0.728	1.092
AA4_1	1.120	0.361	1.044
AB1_1	0.668	0.265	0.452
AB1_2	2.772	0.683	0.779
AB2_1	19.620	3.938	5.571
AB3_1	0.921	0.239	0.523
AB3_2	2.465	0.713	0.854
AB4_1	2.483	0.450	0.802
AB4_2	2.784	2.082	0.738
AC1_1	4.420	0.628	1.695
AC1_2	7.040	2.704	2.180
AC2_1	1.628	0.656	0.850
AC2_2	2.985	0.700	1.371
AC3_1	2.120	0.475	2.614
AC3_2	10.803	2.186	1.556
AC4_1	1.203	0.576	1.655
AD1_1	1.624	0.494	1.037
AD1_2	3.636	1.330	1.392
AD2_1	2.802	2.115	0.892
AD2_2	4.739	1.241	1.292
AD2_3	7.023	1.861	1.713
AD2_4	11.043	3.335	2.899
AD2_5	5.567	1.972	1.141
AD3_1	1.547	1.354	1.151
AD3_2	1.652	1.012	1.044
AD4_1	0.992	0.322	1.109
AD4_2	1.588	0.591	0.553
BB1_1	0.798	0.296	0.629
BB1_2	2.336	0.523	0.617
BB2_1	1.221	0.292	0.593
BB3_1	1.546	0.355	0.526
BB3_2	0.613	0.389	0.628
BB4_1	2.367	2.064	0.851
BB4_2	8.933	3.248	2.767
BC1_1	0.915	0.372	1.106
BC1_2	5.617	4.012	2.662
BC2_1	1.613	0.760	2.002
BC2_2	2.869	1.197	1.760
BC3_1	0.751	0.402	0.368
BC3_2	1.088	0.425	0.759
BC4_1	1.582	0.250	0.630
BC4_2	4.138	2.027	1.440
BD1_1	0.650	0.258	0.523
BD1_2	1.494	0.554	0.789
BD2_1	0.778	0.093	0.634
BD3_1	1.482	0.303	1.073
BD4_1	2.163	0.588	1.001
BD4_2	2.580	0.856	0.792
CC1_1	2.146	0.546	2.010
CC1_2	3.418	1.021	1.440
CC2_1	1.517	0.568	1.243
CC2_2	1.506	0.431	1.064
CC3_1	1.386	0.384	1.161
CC3_2	2.860	0.809	1.246
CC4_1	0.854	0.199	0.796
CD1_1	0.684	0.172	1.195
CD1_2	4.329	0.811	1.398
CD2_1	1.621	0.407	2.390
CD2_2	2.040	0.534	1.930
CD3_1	3.800	0.809	1.318
CD3_2	2.253	0.845	1.090
CD4_1	0.431	0.163	0.819
EE1_1	0.725	0.189	0.442
EE1_2	4.173	1.452	1.453
EE2_1	0.696	0.237	0.512
EE2_2	9.403	2.055	2.338
EE3_1	0.661	0.186	0.404
EE4_1	0.718	0.188	0.390
EE4_2	19.597	4.387	4.260

Mean concentration of copper, lead and zinc in leaf samples (outliers removed)

Leaf Sample	Metal Concentration of Dry Leaf (µmol/g)		
	Copper	Lead	Zinc
AA1_1	1.039	0.661	0.792
AA1_2	1.592	1.287	0.755
AA2_1	1.055	0.450	0.734
AA3_1	1.918	0.728	1.092
AA4_1	1.120	0.361	1.044
AB1_1	0.668	0.265	0.452
AB1_2	2.772	0.683	0.779
AB3_1	0.921	0.239	0.523
AB3_2	2.465	0.713	0.854
AB4_1	2.483	0.450	0.802
AC1_1	4.420	0.628	1.695
AC1_2	7.040	2.704	2.180
AC2_1	1.628	0.656	0.850
AC2_2	2.985	0.700	1.371
AC3_1	2.120	0.475	2.614
AC3_2	10.803	2.186	1.556
AC4_1	1.203	0.576	1.655
AD1_1	1.624	0.494	1.037
AD1_2	3.636	1.330	1.392
AD2_1	2.802	2.115	0.892
AD2_2	4.739	1.241	1.292
AD2_3	7.023	1.861	1.713
AD2_5	5.567	1.972	1.141
AD3_1	1.547	1.354	1.151
AD3_2	1.652	1.012	1.044
AD4_1	0.992	0.322	1.109
AD4_2	1.588	0.591	0.553
BB1_1	0.798	0.296	0.629
BB1_2	2.336	0.523	0.617
BB2_1	1.221	0.292	0.593
BB3_1	1.546	0.355	0.526
BB3_2	0.613	0.389	0.628
BC1_1	0.915	0.372	1.106
BC2_1	1.613	0.760	2.002
BC2_2	2.869	1.197	1.760
BC3_1	0.751	0.402	0.368
BC3_2	1.088	0.425	0.759
BC4_1	1.582	0.250	0.630
BC4_2	4.138	2.027	1.440
BD1_1	0.650	0.258	0.523
BD1_2	1.494	0.554	0.789
BD2_1	0.778	0.093	0.634
BD3_1	1.482	0.303	1.073
BD4_1	2.163	0.588	1.001
BD4_2	2.580	0.856	0.792
CC1_1	2.146	0.546	2.010
CC1_2	3.418	1.021	1.440
CC2_1	1.517	0.568	1.243
CC2_2	1.506	0.431	1.064
CC3_1	1.386	0.384	1.161
CC3_2	2.860	0.809	1.246
CC4_1	0.854	0.199	0.796
CD1_1	0.684	0.172	1.195
CD1_2	4.329	0.811	1.398
CD2_1	1.621	0.407	2.390
CD2_2	2.040	0.534	1.930
CD3_1	3.800	0.809	1.318
CD3_2	2.253	0.845	1.090
CD4_1	0.431	0.163	0.819
EE1_1	0.725	0.189	0.442
EE1_2	4.173	1.452	1.453
EE2_1	0.696	0.237	0.512
EE2_2	9.403	2.055	2.338
EE3_1	0.661	0.186	0.404
EE4_1	0.718	0.188	0.390

Appendix 7: Resin Data

Concentration of copper in resin samples

Resin Sample	Resin Dry Weight (g)	Copper Concentration (µg/L)			Volume (L)	Dry Resin	
		Gross	Blank	Net		Copper Conc (µg/g)	Copper Conc (µmol/g)
AA1_2	0.0029	263.362	19.263	244.099	0.010	841.721	13.246
AA2_1	0.0106	438.893	31.302	407.591	0.025	961.300	15.128
AA2_3	0.0024	196.148	19.263	176.885	0.010	737.021	11.598
AA3_1	0.0038	210.523	19.263	191.260	0.010	503.316	7.920
AA4_1	0.0149	422.321	19.263	403.058	0.010	270.509	4.257
AB1_1	0.0040	336.849	19.263	317.586	0.010	793.965	12.494
AB1_2	0.0032	427.955	19.263	408.692	0.010	1277.163	20.098
AB3_1	0.0077	255.918	19.263	236.655	0.010	307.344	4.837
AB4_1	0.0124	243.725	31.302	212.423	0.025	428.272	6.740
AB4_2	0.0079	214.521	19.263	195.258	0.010	247.162	3.889
AC1_2	0.0044	516.691	19.263	497.428	0.010	1130.518	17.791
AC2_1	0.0060	225.342	19.263	206.079	0.010	343.465	5.405
AC2_2	0.0058	159.809	19.263	140.546	0.010	242.321	3.813
AC4_1	0.0105	1009.795	31.302	978.493	0.025	2329.745	36.662
AD1_2	0.0041	159.772	19.263	140.509	0.010	342.705	5.393
AD2_3	0.0020	187.644	19.263	168.381	0.010	841.905	13.249
AD2_5	0.0028	233.863	19.263	214.600	0.010	766.429	12.061
AD3_1	0.0115	275.387	31.302	244.085	0.025	530.620	8.350
AD3_2	0.0065	224.339	19.263	205.076	0.010	315.502	4.965
BB1_2	0.0091	240.782	19.263	221.519	0.010	243.427	3.831
BB1_3	0.0030	271.089	19.263	251.826	0.010	839.420	13.210
BB1_4	0.0009	275.882	19.263	256.619	0.010	2851.322	44.870
BB2_1	0.0161	454.841	31.302	423.539	0.025	657.669	10.349
BB3_1	0.0032	336.274	19.263	317.011	0.010	990.659	15.590
BB4_1	0.0148	234.263	19.263	215.000	0.010	145.270	2.286
BC1_1	0.0134	560.584	35.853	524.731	0.010	391.590	6.162
BC3_1	0.0116	283.756	19.263	264.493	0.010	228.011	3.588
BC3_2	0.0086	305.320	19.263	286.057	0.010	332.624	5.234
BC4_2	0.0022	268.254	19.263	248.991	0.010	1131.777	17.810

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Resin Sample	Resin Dry Weight (g)	Copper Concentration (µg/L)			Volume (L)	Dry Resin	
		Gross	Blank	Net		Copper Conc (µg/g)	Copper Conc (µmol/g)
BD1_2	0.0027	424.537	35.853	388.684	0.010	1439.570	22.654
BD2_1	0.0607	463.927	35.853	428.074	0.010	70.523	1.110
BD2_1	0.0607	463.927	35.853	428.074	0.010	70.523	1.110
BD2_1	0.0607	581.000	35.853	545.147	0.010	89.810	1.413
BD3_1	0.0354	354.453	35.853	318.600	0.010	90.000	1.416
BD4_1	0.0158	455.429	35.853	419.576	0.010	265.554	4.179
BD4_1	0.0158	455.429	35.853	419.576	0.010	265.554	4.179
BD4_2	0.0064	452.151	35.853	416.298	0.010	650.466	10.236
BD4_2	0.0064	452.151	35.853	416.298	0.010	650.466	10.236
BD4_3	0.0066	464.103	35.853	428.250	0.010	648.864	10.211
BD4_3	0.0066	464.103	35.853	428.250	0.010	648.864	10.211
CC1_1	0.0130	372.714	19.263	353.451	0.010	271.885	4.279
CC2_2	0.0065	216.419	19.263	197.156	0.010	303.317	4.773
CC3_1	0.0083	284.754	19.263	265.491	0.010	319.869	5.034
CC3_2	0.0062	450.101	19.263	430.838	0.010	694.900	10.935
CC4_1	0.0406	383.708	35.853	347.855	0.010	85.679	1.348
CD1_1	0.0074	1769.492	19.263	1750.229	0.010	2365.174	37.220
CD1_2	0.0028	334.941	19.263	315.678	0.010	1127.421	17.742
CD2_1	0.0044	205.051	19.263	185.788	0.010	422.245	6.645
CD3_1	0.0018	282.173	19.263	262.910	0.010	1460.611	22.985
CD4_1	0.0231	379.744	35.853	343.891	0.010	148.871	2.343
EE1_1	0.0169	285.481	19.263	266.218	0.010	157.525	2.479
EE2_1	0.0288	375.240	35.853	339.387	0.010	117.843	1.854
EE3_1	0.0068	186.521	19.263	167.258	0.010	245.968	3.871

Concentration of lead in resin samples

Resin Sample	Resin Dry Weight (g)	Lead Concentration (µg/L)			Volume (L)	Dry Resin	
		Gross	Blank	Net		Lead Conc (µg/g)	Lead Conc (µmol/g)
AA1_2	0.0029	259.485	23.918	235.567	0.010	812.300	3.920
AA2_1	0.0106	332.128	70.397	261.731	0.025	617.290	2.979
AA2_3	0.0024	246.926	23.918	223.008	0.010	929.200	4.485
AA3_1	0.0038	304.815	23.918	280.897	0.010	739.203	3.568
AA4_1	0.0149	520.122	23.918	496.204	0.010	333.023	1.607
AB1_1	0.0040	450.094	23.918	426.176	0.010	1065.440	5.142
AB1_2	0.0032	250.484	23.918	226.566	0.010	708.019	3.417
AB3_1	0.0077	540.741	23.918	516.823	0.010	671.199	3.239
AB4_1	0.0124	740.058	70.397	669.661	0.025	1350.123	6.516
AB4_2	0.0079	380.849	23.918	356.931	0.010	451.811	2.181
AC1_2	0.0044	593.939	23.918	570.021	0.010	1295.502	6.252
AC2_1	0.0060	458.401	23.918	434.483	0.010	724.138	3.495
AC2_2	0.0058	254.040	23.918	230.122	0.010	396.762	1.915
AC4_1	0.0105	1848.947	70.397	1778.550	0.025	4234.643	20.437
AD1_2	0.0041	438.032	23.918	414.114	0.010	1010.034	4.875
AD2_3	0.0020	266.968	23.918	243.050	0.010	1215.250	5.865
AD2_5	0.0028	261.911	23.918	237.993	0.010	849.975	4.102
AD3_1	0.0115	383.946	70.397	313.549	0.025	681.628	3.290
AD3_2	0.0065	283.234	23.918	259.316	0.010	398.948	1.925
BB1_2	0.0091	453.447	23.918	429.529	0.010	472.010	2.278
BB1_3	0.0030	392.139	23.918	368.221	0.010	1227.403	5.924
BB1_4	0.0009	557.402	23.918	533.484	0.010	5927.600	28.608
BB2_1	0.0161	383.946	70.397	313.549	0.025	486.877	2.350
BB3_1	0.0032	552.847	23.918	528.929	0.010	1652.903	7.977
BB4_1	0.0148	235.269	23.918	211.351	0.010	142.805	0.689
BC1_1	0.0134	441.120	65.410	375.710	0.010	280.381	1.353
BC3_1	0.0116	264.000	23.918	240.082	0.010	206.967	0.999
BC3_2	0.0086	322.819	23.918	298.901	0.010	347.559	1.677
BC4_2	0.0022	289.646	23.918	265.728	0.010	1207.855	5.829

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Resin Sample	Resin Dry Weight (g)	Lead Concentration (µg/L)			Volume (L)	Dry Resin	
		Gross	Blank	Net		Lead Conc (µg/g)	Lead Conc (µmol/g)
BD1_2	0.0027	484.354	65.410	418.944	0.010	1551.644	7.489
BD2_1	0.0607	410.833	65.410	345.423	0.010	56.907	0.275
BD2_1	0.0607	410.833	65.410	345.423	0.010	56.907	0.275
BD2_1	0.0607	564.514	65.410	499.104	0.010	82.225	0.397
BD3_1	0.0354	550.594	65.410	485.184	0.010	137.058	0.661
BD4_1	0.0158	648.260	65.410	582.850	0.010	368.892	1.780
BD4_1	0.0158	648.260	65.410	582.850	0.010	368.892	1.780
BD4_2	0.0064	712.865	65.410	647.455	0.010	1011.648	4.882
BD4_2	0.0064	712.865	65.410	647.455	0.010	1011.648	4.882
BD4_3	0.0066	659.887	65.410	594.477	0.010	900.723	4.347
BD4_3	0.0066	659.887	65.410	594.477	0.010	900.723	4.347
CC1_1	0.0130	253.372	23.918	229.454	0.010	176.503	0.852
CC2_2	0.0065	265.714	23.918	241.796	0.010	371.994	1.795
CC3_1	0.0083	374.847	23.918	350.929	0.010	422.806	2.041
CC3_2	0.0062	273.839	23.918	249.921	0.010	403.098	1.945
CC4_1	0.0406	452.553	65.410	387.143	0.010	95.355	0.460
CD1_1	0.0074	297.671	23.918	273.753	0.010	369.936	1.785
CD1_2	0.0028	256.226	23.918	232.308	0.010	829.671	4.004
CD2_1	0.0044	253.142	23.918	229.224	0.010	520.964	2.514
CD3_1	0.0018	304.051	23.918	280.133	0.010	1556.294	7.511
CD4_1	0.0231	515.638	65.410	450.228	0.010	194.904	0.941
EE1_1	0.0169	272.490	23.918	248.572	0.010	147.084	0.710
EE2_1	0.0288	533.667	65.410	468.257	0.010	162.589	0.785
EE3_1	0.0068	266.083	23.918	242.165	0.010	356.125	1.719

Concentration of zinc in resin samples

Resin Sample	Resin Dry Weight (g)	Zinc Concentration (µg/L)			Volume (L)	Dry Resin	
		Gross	Blank	Net		Zinc Conc (µg/g)	Zinc Conc (µmol/g)
AA1_2	0.0029	198.784	17.672	181.112	0.010	624.524	9.551
AA2_1	0.0106	217.715	17.565	200.150	0.025	472.052	7.219
AA2_3	0.0024	213.825	17.672	196.153	0.010	817.304	12.499
AA3_1	0.0038	146.828	17.672	129.156	0.010	339.884	5.198
AA4_1	0.0149	41.657	17.672	23.985	0.010	16.097	0.246
AB1_1	0.0040	94.896	17.672	77.224	0.010	193.060	2.952
AB1_2	0.0032	207.617	17.672	189.945	0.010	593.578	9.078
AB3_1	0.0077	99.743	17.672	82.071	0.010	106.586	1.630
AB4_1	0.0124	75.061	17.565	57.496	0.025	115.919	1.773
AB4_2	0.0079	47.137	17.672	29.465	0.010	37.297	0.570
AC1_2	0.0044	135.907	17.672	118.235	0.010	268.716	4.109
AC2_1	0.0060	104.012	17.672	86.340	0.010	143.900	2.201
AC2_2	0.0058	18.718	17.672	1.046	0.010	1.803	0.028
AC4_1	0.0105	105.863	17.565	88.298	0.025	210.233	3.215
AD1_2	0.0041	33.113	17.672	15.441	0.010	37.661	0.576
AD2_3	0.0020	53.577	17.672	35.905	0.010	179.525	2.745
AD2_5	0.0028	44.099	17.672	26.427	0.010	94.382	1.443
AD3_1	0.0115	62.983	17.565	45.418	0.025	98.735	1.510
AD3_2	0.0065	24.738	17.672	7.066	0.010	10.871	0.166
BB1_2	0.0091	79.860	17.672	62.188	0.010	68.338	1.045
BB1_3	0.0030	83.407	17.672	65.735	0.010	219.117	3.351
BB1_4	0.0009	140.630	17.672	122.958	0.010	1366.200	20.893
BB2_1	0.0161	77.935	17.565	60.370	0.025	93.742	1.434
BB3_1	0.0032	76.526	17.672	58.854	0.010	183.919	2.813
BB4_1	0.0148	37.630	17.672	19.958	0.010	13.485	0.206
BC1_1	0.0134	151.568	41.414	110.154	0.010	82.204	1.257
BC3_1	0.0116	80.374	17.672	62.702	0.010	54.053	0.827
BC3_2	0.0086	45.880	17.672	28.208	0.010	32.800	0.502
BC4_2	0.0022	85.191	17.672	67.519	0.010	306.905	4.693

Gross = Total metal concentration in sample
Blank = Total metal concentration in blank
Net = Total metal concentration in sample - total metal concentration in blank

Resin Sample	Resin Dry Weight (g)	Zinc Concentration (µg/L)			Volume (L)	Dry Resin	
		Gross	Blank	Net		Zinc Conc (µg/g)	Zinc Conc (µmol/g)
BD1_2	0.0027	103.196	41.414	61.782	0.010	228.822	3.499
BD2_1	0.0607	924.229	41.414	882.815	0.010	145.439	2.224
BD2_1	0.0607	924.229	41.414	882.815	0.010	145.439	2.224
BD2_1	0.0607	839.616	41.414	798.202	0.010	131.500	2.011
BD3_1	0.0354	84.564	41.414	43.150	0.010	12.189	0.186
BD4_1	0.0158	97.233	41.414	55.819	0.010	35.328	0.540
BD4_1	0.0158	97.233	41.414	55.819	0.010	35.328	0.540
BD4_2	0.0064	123.798	41.414	82.384	0.010	128.725	1.969
BD4_2	0.0064	123.798	41.414	82.384	0.010	128.725	1.969
BD4_3	0.0066	110.219	41.414	68.805	0.010	104.250	1.594
BD4_3	0.0066	110.219	41.414	68.805	0.010	104.250	1.594
CC1_1	0.0130	36.078	17.672	18.406	0.010	14.158	0.217
CC2_2	0.0065	31.509	17.672	13.837	0.010	21.288	0.326
CC3_1	0.0083	43.289	17.672	25.617	0.010	30.864	0.472
CC3_2	0.0062	81.592	17.672	63.920	0.010	103.097	1.577
CC4_1	0.0406	45.678	41.414	4.264	0.010	1.050	0.016
CD1_1	0.0074	31.700	17.672	14.028	0.010	18.957	0.290
CD1_2	0.0028	62.168	17.672	44.496	0.010	158.914	2.430
CD2_1	0.0044	43.270	17.672	25.598	0.010	58.177	0.890
CD3_1	0.0018	72.810	17.672	55.138	0.010	306.322	4.685
CD4_1	0.0231	115.162	41.414	73.748	0.010	31.926	0.488
EE1_1	0.0169	33.312	17.672	15.640	0.010	9.254	0.142
EE2_1	0.0288	87.491	41.414	46.077	0.010	15.999	0.245
EE3_1	0.0068	53.750	17.672	36.078	0.010	53.056	0.811

Concentration of copper, lead and zinc in resin samples (outliers removed)

Resin Sample	Metal Concentration of Resin (µmol/g)		
	Copper	Lead	Zinc
AA1_2	13.246	3.920	9.551
AA2_1	15.128	2.979	7.219
AA2_3	11.598	4.485	12.499
AA3_1	7.920	3.568	5.198
AA4_1	4.257	1.607	0.246
AB1_1	12.494	5.142	2.952
AB3_1	4.837	3.239	1.630
AB4_1	6.740	6.516	1.773
AB4_2	3.889	2.181	0.570
AC1_2	17.791	6.252	4.109
AC2_1	5.405	3.495	2.201
AC2_2	3.813	1.915	0.028
AC4_1	36.662	20.437	3.215
AD1_2	5.393	4.875	0.576
AD2_3	13.249	5.865	2.745
AD2_5	12.061	4.102	1.443
AD3_1	8.350	3.290	1.510
AD3_2	4.965	1.925	0.166
BB1_2	3.831	2.278	1.045
BB1_3	13.210	5.924	3.351
BB2_1	10.349	2.350	1.434
BB3_1	15.590	7.977	2.813
BB4_1	2.286	0.689	0.206
BC1_1	6.162	1.353	1.257
BC3_1	3.588	0.999	0.827
BC3_2	5.234	1.677	0.502
BC4_2	17.810	5.829	4.693
BD1_2	22.654	7.489	3.499
BD2_1	1.110	0.275	2.224
BD2_2	1.413	0.397	2.011
BD3_1	1.416	0.661	0.186
BD4_1	4.179	1.780	0.540
BD4_2	10.236	4.882	1.969
BD4_3	10.211	4.347	1.594
CC1_1	4.279	0.852	0.217
CC2_2	4.773	1.795	0.326
CC3_1	5.034	2.041	0.472
CC4_1	1.348	0.460	0.016
CD1_1	37.220	1.785	0.290
CD1_2	17.742	4.004	2.430
CD2_1	6.645	2.514	0.890
CD3_1	22.985	7.511	4.685
CD4_1	2.343	0.941	0.488
EE1_1	2.479	0.710	0.142
EE2_1	1.854	0.785	0.245
EE3_1	3.871	1.719	0.811

Appendix 8: Metal Concentration Ratio Data

Soil (Pre & Post Grubb's Test)

Soil Sample	**Ratios**					
	Zn:Cu	**Zn:Pb**	**Pb:Cu**	**Pb:Zn**	**Cu:Zn**	**Cu:Pb**
AA1_a	0.84	3.15	0.27	0.32	1.19	3.76
AA1_b	0.99	3.11	0.32	0.32	1.01	3.15
AA2_a	1.19	4.09	0.29	0.24	0.84	3.44
AA2_b	1.16	4.48	0.26	0.22	0.86	3.87
AA3_a	1.34	3.79	0.35	0.26	0.75	2.83
AA3_b	1.19	4.26	0.28	0.23	0.84	3.59
AA4_a	1.14	4.33	0.26	0.23	0.87	3.78
AA4_b	1.08	3.87	0.28	0.26	0.93	3.60
AB1_a	1.52	1.95	0.78	0.51	0.66	1.28
AB1_b	1.42	1.59	0.89	0.63	0.70	1.12
AB2_a	1.84	1.74	1.06	0.57	0.54	0.95
AB2_b	1.72	1.66	1.04	0.60	0.58	0.96
AB3_a	1.16	1.79	0.65	0.56	0.86	1.54
AB3_b	1.16	1.61	0.72	0.62	0.86	1.39
AB4_a	1.33	1.65	0.80	0.61	0.75	1.24
AB4_b	1.24	1.64	0.76	0.61	0.80	1.32
AC1_a	2.45	4.92	0.50	0.20	0.41	2.00
AC1_b	2.41	4.22	0.57	0.24	0.42	1.75
AC2_a	1.64	5.55	0.29	0.18	0.61	3.39
AC2_b	1.88	5.67	0.33	0.18	0.53	3.02
AC3_a	1.55	4.13	0.38	0.24	0.65	2.66
AC3_b	1.74	4.56	0.38	0.22	0.58	2.62
AC4_a	2.20	5.19	0.42	0.19	0.45	2.36
AC4_b	2.67	5.49	0.49	0.18	0.38	2.06
AD1_a	1.13	3.67	0.31	0.27	0.88	3.24
AD1_b	1.14	3.68	0.31	0.27	0.88	3.22
AD2_a	2.09	4.76	0.44	0.21	0.48	2.28
AD2_b	1.92	3.74	0.51	0.27	0.52	1.95
AD3_a	1.22	3.15	0.39	0.32	0.82	2.58
AD3_b	1.39	3.59	0.39	0.28	0.72	2.57
AD4_a	1.65	4.13	0.40	0.24	0.60	2.50
AD4_b	1.91	4.07	0.47	0.25	0.52	2.14
BB1_a	2.65	0.60	4.41	1.67	0.38	0.23
BB1_b	2.75	0.80	3.43	1.25	0.36	0.29
BB2_a	2.43	1.56	1.55	0.64	0.41	0.64
BB2_b	2.58	1.33	1.94	0.75	0.39	0.51
BB3_a	3.47	1.37	2.54	0.73	0.29	0.39
BB3_b	3.44	1.13	3.03	0.88	0.29	0.33
BB4_a	3.30	1.34	2.47	0.75	0.30	0.41
BB4_b	4.16	1.35	3.08	0.74	0.24	0.32

Soil Sample	**Ratios**					
	Zn:Cu	**Zn:Pb**	**Pb:Cu**	**Pb:Zn**	**Cu:Zn**	**Cu:Pb**
BC1_a	3.02	2.81	1.07	0.36	0.33	0.93
BC1_b	2.36	3.07	0.77	0.33	0.42	1.30
BC2_a	3.80	2.64	1.44	0.38	0.26	0.69
BC2_b	3.78	2.80	1.35	0.36	0.26	0.74
BC3_a	3.31	1.40	2.37	0.72	0.30	0.42
BC3_b	3.32	1.70	1.96	0.59	0.30	0.51
BC4_a	2.61	3.09	0.84	0.32	0.38	1.19
BC4_b	2.77	3.21	0.86	0.31	0.36	1.16
BD1_a	2.37	2.42	0.98	0.41	0.42	1.02
BD1_b	2.63	2.39	1.10	0.42	0.38	0.91
BD2_a	2.73	2.54	1.08	0.39	0.37	0.93
BD2_b	2.93	2.34	1.25	0.43	0.34	0.80
BD3_a	2.12	1.34	1.59	0.75	0.47	0.63
BD3_b	2.68	1.67	1.60	0.60	0.37	0.62
BD4_a	3.31	1.71	1.94	0.58	0.30	0.52
BD4_b	2.94	1.96	1.50	0.51	0.34	0.67
CC1_a	2.67	3.40	0.79	0.29	0.37	1.27
CC1_b	4.77	6.23	0.76	0.16	0.21	1.31
CC2_a	4.75	5.19	0.92	0.19	0.21	1.09
CC2_b	4.79	5.77	0.83	0.17	0.21	1.20
CC3_a	3.42	3.78	0.91	0.26	0.29	1.10
CC3_b	3.31	4.51	0.73	0.22	0.30	1.36
CC4_a	4.99	5.81	0.86	0.17	0.20	1.17
CC4_b	6.35	5.91	1.07	0.17	0.16	0.93
CD1_a	4.28	5.60	0.76	0.18	0.23	1.31
CD1_b	3.52	6.30	0.56	0.16	0.28	1.79
CD2_a	5.16	4.97	1.04	0.20	0.19	0.96
CD2_b	3.65	4.67	0.78	0.21	0.27	1.28
CD3_a	4.28	4.83	0.89	0.21	0.23	1.13
CD3_b	5.16	4.90	1.05	0.20	0.19	0.95
CD4_a	2.57	4.86	0.53	0.21	0.39	1.89
EE1_a	4.01	4.44	0.90	0.23	0.25	1.10
EE1_b	4.47	4.16	1.07	0.24	0.22	0.93
EE2_b	3.03	3.63	0.83	0.28	0.33	1.20
EE3_a	2.28	3.90	0.58	0.26	0.44	1.71
EE3_b	4.28	4.10	1.04	0.24	0.23	0.96
EE4_a	2.79	4.09	0.68	0.24	0.36	1.47
EE4_b	3.66	3.61	1.01	0.28	0.27	0.99

Leaf (Pre Grubb’s Test)

Leaf Sample	**Ratios**					
	Cu:Zn	**Zn:Cu**	**Pb:Zn**	**Zn:Pb**	**Cu:Pb**	**Pb:Cu**
AA1_1	1.31	0.76	0.84	1.20	1.57	0.64
AA1_2	2.11	0.47	1.70	0.59	1.24	0.81
AA2_1	1.44	0.70	0.61	1.63	2.35	0.43
AA3_1	1.76	0.57	0.67	1.50	2.64	0.38
AA4_1	1.07	0.93	0.35	2.89	3.10	0.32
AB1_1	1.48	0.68	0.59	1.70	2.52	0.40
AB1_2	3.56	0.28	0.88	1.14	4.06	0.25
AB3_1	1.76	0.57	0.46	2.19	3.86	0.26
AB3_2	2.89	0.35	0.83	1.20	3.46	0.29
AB4_1	3.10	0.32	0.56	1.78	5.52	0.18
AC1_1	2.61	0.38	0.37	2.70	7.04	0.14
AC1_2	3.23	0.31	1.24	0.81	2.60	0.38
AC2_1	1.91	0.52	0.77	1.30	2.48	0.40
AC2_2	2.18	0.46	0.51	1.96	4.26	0.23
AC3_1	0.81	1.23	0.18	5.50	4.46	0.22
AC3_2	6.94	0.14	1.41	0.71	4.94	0.20
AC4_1	0.73	1.38	0.35	2.87	2.09	0.48
AD1_1	1.57	0.64	0.48	2.10	3.28	0.30
AD1_2	2.61	0.38	0.96	1.05	2.73	0.37
AD2_1	3.14	0.32	2.37	0.42	1.32	0.75
AD2_2	3.67	0.27	0.96	1.04	3.82	0.26
AD2_3	4.10	0.24	1.09	0.92	3.77	0.27
AD2_5	4.88	0.20	1.73	0.58	2.82	0.35
AD3_1	1.34	0.74	1.18	0.85	1.14	0.88
AD3_2	1.58	0.63	0.97	1.03	1.63	0.61
AD4_1	0.90	1.12	0.29	3.45	3.08	0.32
AD4_2	2.87	0.35	1.07	0.94	2.69	0.37
BB1_1	1.27	0.79	0.47	2.12	2.69	0.37
BB1_2	3.78	0.26	0.85	1.18	4.46	0.22
BB2_1	2.06	0.49	0.49	2.03	4.17	0.24
BB3_1	2.94	0.34	0.67	1.48	4.36	0.23
BB3_2	0.98	1.02	0.62	1.61	1.57	0.64
BC1_1	0.83	1.21	0.34	2.97	2.46	0.41
BC2_1	0.81	1.24	0.38	2.63	2.12	0.47
BC2_2	1.63	0.61	0.68	1.47	2.40	0.42
BC3_1	2.04	0.49	1.09	0.92	1.87	0.54
BC3_2	1.43	0.70	0.56	1.78	2.56	0.39
BC4_1	2.51	0.40	0.40	2.53	6.34	0.16
BC4_2	2.87	0.35	1.41	0.71	2.04	0.49

Leaf Sample	**Ratios**					
	Cu:Zn	**Zn:Cu**	**Pb:Zn**	**Zn:Pb**	**Cu:Pb**	**Pb:Cu**
BD1_1	1.24	0.80	0.49	2.03	2.52	0.40
BD1_2	1.89	0.53	0.70	1.42	2.70	0.37
BD2_1	1.23	0.81	0.15	6.80	8.35	0.12
BD3_1	1.38	0.72	0.28	3.54	4.89	0.20
BD4_1	2.16	0.46	0.59	1.70	3.68	0.27
BD4_2	3.26	0.31	1.08	0.93	3.01	0.33
CC1_1	1.07	0.94	0.27	3.68	3.93	0.25
CC1_2	2.37	0.42	0.71	1.41	3.35	0.30
CC2_1	1.22	0.82	0.46	2.19	2.67	0.37
CC2_2	1.42	0.71	0.41	2.47	3.49	0.29
CC3_1	1.19	0.84	0.33	3.03	3.61	0.28
CC3_2	2.30	0.44	0.65	1.54	3.54	0.28
CC4_1	1.07	0.93	0.25	4.00	4.29	0.23
CD1_1	0.57	1.75	0.14	6.94	3.98	0.25
CD1_2	3.10	0.32	0.58	1.73	5.34	0.19
CD2_1	0.68	1.47	0.17	5.88	3.99	0.25
CD2_2	1.06	0.95	0.28	3.62	3.82	0.26
CD3_1	2.88	0.35	0.61	1.63	4.70	0.21
CD3_2	2.07	0.48	0.78	1.29	2.67	0.38
CD4_1	0.53	1.90	0.20	5.03	2.65	0.38
EE1_1	1.64	0.61	0.43	2.34	3.83	0.26
EE1_2	2.87	0.35	1.00	1.00	2.87	0.35
EE2_1	1.36	0.74	0.46	2.16	2.93	0.34
EE2_2	4.02	0.25	0.88	1.14	4.58	0.22
EE3_1	1.64	0.61	0.46	2.17	3.55	0.28
EE4_1	1.84	0.54	0.48	2.07	3.81	0.26

Leaf (Post Grubb’s Test*)

Leaf Sample	Ratios					
	Cu:Zn	**Zn:Cu**	**Pb:Zn**	**Zn:Pb**	**Cu:Pb**	**Pb:Cu**
AA1_1	1.31	0.76	0.84	1.20	1.57	0.64
AA1_2	2.11	0.47	1.70	0.59	1.24	0.81
AA2_1	1.44	0.70	0.61	1.63	2.35	0.43
AA3_1	1.76	0.57	0.67	1.50	2.64	0.38
AA4_1	1.07	0.93	0.35	2.89	3.10	0.32
AB1_1	1.48	0.68	0.59	1.70	2.52	0.40
AB1_2	3.56	0.28	0.88	1.14	4.06	0.25
AB3_1	1.76	0.57	0.46	2.19	3.86	0.26
AB3_2	2.89	0.35	0.83	1.20	3.46	0.29
AB4_1	3.10	0.32	0.56	1.78	5.52	0.18
AC1_1	2.61	0.38	0.37	2.70	7.04	0.14
AC1_2	3.23	0.31	1.24	0.81	2.60	0.38
AC2_1	1.91	0.52	0.77	1.30	2.48	0.40
AC2_2	2.18	0.46	0.51	1.96	4.26	0.23
AC3_1	0.81	1.23	0.18	5.50	4.46	0.22
AC3_2		0.14	1.41	0.71	4.94	0.20
AC4_1	0.73	1.38	0.35	2.87	2.09	0.48
AD1_1	1.57	0.64	0.48		3.28	0.30
AD1_2	2.61	0.38	0.96	1.05	2.73	0.37
AD2_1	3.14	0.32	2.37	0.42	1.32	0.75
AD2_2	3.67	0.27	0.96	1.04	3.82	0.26
AD2_3	4.10	0.24	1.09	0.92	3.77	0.27
AD2_5	4.88	0.20	1.73	0.58	2.82	0.35
AD3_1	1.34	0.74	1.18	0.85	1.14	0.88
AD3_2	1.58	0.63	0.97	1.03	1.63	0.61
AD4_1	0.90	1.12	0.29		3.08	0.32
AD4_2	2.87	0.35	1.07	0.94	2.69	0.37
BB1_1	1.27	0.79	0.47	2.12	2.69	0.37
BB1_2	3.78	0.26	0.85	1.18	4.46	0.22
BB2_1	2.06	0.49	0.49	2.03	4.17	0.24
BB3_1	2.94	0.34	0.67	1.48	4.36	0.23
BB3_2	0.98	1.02	0.62	1.61	1.57	0.64
BC1_1	0.83	1.21	0.34	2.97	2.46	0.41
BC2_1	0.81	1.24	0.38	2.63	2.12	0.47
BC2_2	1.63	0.61	0.68	1.47	2.40	0.42
BC3_1	2.04	0.49	1.09	0.92	1.87	0.54
BC3_2	1.43	0.70	0.56	1.78	2.56	0.39
BC4_1	2.51	0.40	0.40	2.53	6.34	0.16
BC4_2	2.87	0.35	1.41	0.71	2.04	0.49

* Only the data for Cu:Zn, Zn:Pb and Cu:Pb were examined for outliers.

Leaf Sample	Ratios					
	Cu:Zn	Zn:Cu	Pb:Zn	Zn:Pb	Cu:Pb	Pb:Cu
BD1_1	1.24	0.80	0.49	2.03	2.52	0.40
BD1_2	1.89	0.53	0.70	1.42	2.70	0.37
BD2_1	1.23	0.81	0.15	6.80	8.35	0.12
BD3_1	1.38	0.72	0.28	3.54	4.89	0.20
BD4_1	2.16	0.46	0.59	1.70	3.68	0.27
BD4_2	3.26	0.31	1.08	0.93	3.01	0.33
CC1_1	1.07	0.94	0.27	3.68	3.93	0.25
CC1_2	2.37	0.42	0.71	1.41	3.35	0.30
CC2_1	1.22	0.82	0.46	2.19	2.67	0.37
CC2_2	1.42	0.71	0.41	2.47	3.49	0.29
CC3_1	1.19	0.84	0.33	3.03	3.61	0.28
CC3_2	2.30	0.44	0.65	1.54	3.54	0.28
CC4_1	1.07	0.93	0.25	4.00	4.29	0.23
CD1_1	0.57	1.75	0.14	6.94	3.98	0.25
CD1_2	3.10	0.32	0.58	1.73	5.34	0.19
CD2_1	0.68	1.47	0.17	5.88	3.99	0.25
CD2_2	1.06	0.95	0.28	3.62	3.82	0.26
CD3_1	2.88	0.35	0.61	1.63	4.70	0.21
CD3_2	2.07	0.48	0.78	1.29	2.67	0.38
CD4_1	0.53	1.90	0.20	5.03	2.65	0.38
EE1_1	1.64	0.61	0.43	2.34	3.83	0.26
EE1_2	2.87	0.35	1.00	1.00	2.87	0.35
EE2_1	1.36	0.74	0.46	2.16	2.93	0.34
EE2_2	4.02	0.25	0.88	1.14	4.58	0.22
EE3_1	1.64	0.61	0.46	2.17	3.55	0.28
EE4_1	1.84	0.54	0.48	2.07	3.81	0.26

Resin (Pre Grubb's Test)

Resin Sample	Ratios					
	Cu:Zn	Zn:Cu	Pb:Zn	Zn:Pb	Cu:Pb	Pb:Cu
AA1_2	1.39	0.72	0.41	2.44	3.38	0.30
AA2_1	2.10	0.48	0.41	2.42	5.08	0.20
AA2_3	0.93	1.08	0.36	2.79	2.59	0.39
AA3_1	1.52	0.66	0.69	1.46	2.22	0.45
AA4_1	17.29	0.06	6.53	0.15	2.65	0.38
AB1_1	4.23	0.24	1.74	0.57	2.43	0.41
AB3_1	2.97	0.34	1.99	0.50	1.49	0.67
AB4_1	3.80	0.26	3.68	0.27	1.03	0.97
AB4_2	6.82	0.15	3.82	0.26	1.78	0.56
AC1_2	4.33	0.23	1.52	0.66	2.85	0.35
AC2_1	2.46	0.41	1.59	0.63	1.55	0.65
AC2_2	138.26	0.01	69.43	0.01	1.99	0.50
AC4_1	11.40	0.09	6.36	0.16	1.79	0.56
AD1_2	9.36	0.11	8.46	0.12	1.11	0.90
AD2_3	4.83	0.21	2.14	0.47	2.26	0.44
AD2_5	8.36	0.12	2.84	0.35	2.94	0.34
AD3_1	5.53	0.18	2.18	0.46	2.54	0.39
AD3_2	29.87	0.03	11.58	0.09	2.58	0.39
BB1_2	3.67	0.27	2.18	0.46	1.68	0.59
BB1_3	3.94	0.25	1.77	0.57	2.23	0.45
BB2_1	7.22	0.14	1.64	0.61	4.40	0.23
BB3_1	5.54	0.18	2.84	0.35	1.95	0.51
BB4_1	11.09	0.09	3.34	0.30	3.32	0.30
BC1_1	4.90	0.20	1.08	0.93	4.55	0.22
BC3_1	4.34	0.23	1.21	0.83	3.59	0.28
BC3_2	10.44	0.10	3.34	0.30	3.12	0.32
BC4_2	3.79	0.26	1.24	0.81	3.06	0.33
BD1_2	6.47	0.15	2.14	0.47	3.03	0.33
BD2_1	0.50	2.00	0.12	8.10	4.04	0.25
BD2_2	0.70	1.42	0.20	5.07	3.56	0.28
BD3_1	7.60	0.13	3.55	0.28	2.14	0.47
BD4_1	7.73	0.13	3.30	0.30	2.35	0.43
BD4_2	5.20	0.19	2.48	0.40	2.10	0.48
BD4_3	6.40	0.16	2.73	0.37	2.35	0.43
CC1_1	19.76	0.05	3.93	0.25	5.02	0.20
CC2_2	14.66	0.07	5.51	0.18	2.66	0.38
CC3_1	10.66	0.09	4.32	0.23	2.47	0.41
CC4_1	83.95	0.01	28.65	0.03	2.93	0.34

Resin Sample	Ratios					
	Cu:Zn	Zn:Cu	Pb:Zn	Zn:Pb	Cu:Pb	Pb:Cu
CD1_1	128.39	0.01	6.16	0.16	20.85	0.05
CD1_2	7.30	0.14	1.65	0.61	4.43	0.23
CD2_1	7.47	0.13	2.83	0.35	2.64	0.38
CD3_1	4.91	0.20	1.60	0.62	3.06	0.33
CD4_1	4.80	0.21	1.93	0.52	2.49	0.40
EE1_1	17.52	0.06	5.02	0.20	3.49	0.29
EE2_1	7.58	0.13	3.21	0.31	2.36	0.42
EE3_1	4.77	0.21	2.12	0.47	2.25	0.44

Resin (Post Grubb's Test*)

Resin Sample	Ratios					
	Cu:Zn	Zn:Cu	Pb:Zn	Zn:Pb	Cu:Pb	Pb:Cu
AA1_2	1.39	0.72	0.41	2.44	3.38	0.30
AA2_1	2.10	0.48	0.41	2.42	5.08	0.20
AA2_3	0.93	1.08	0.36	2.79	2.59	0.39
AA3_1	1.52	0.66	0.69	1.46	2.22	0.45
AA4_1		0.06	6.53	0.15	2.65	0.38
AB1_1	4.23	0.24	1.74	0.57	2.43	0.41
AB3_1	2.97	0.34	1.99	0.50	1.49	0.67
AB4_1	3.80	0.26	3.68	0.27	1.03	0.97
AB4_2	6.82	0.15	3.82	0.26	1.78	0.56
AC1_2	4.33	0.23	1.52	0.66	2.85	0.35
AC2_1	2.46	0.41	1.59	0.63	1.55	0.65
AC2_2	138.26	0.01	69.43	0.01	1.99	0.50
AC4_1	11.40	0.09	6.36	0.16	1.79	0.56
AD1_2	9.36	0.11	8.46	0.12	1.11	0.90
AD2_3	4.83	0.21	2.14	0.47	2.26	0.44
AD2_5	8.36	0.12	2.84	0.35	2.94	0.34
AD3_1	5.53	0.18	2.18	0.46	2.54	0.39
AD3_2		0.03	11.58	0.09	2.58	0.39
BB1_2	3.67	0.27	2.18	0.46	1.68	0.59
BB1_3	3.94	0.25	1.77	0.57	2.23	0.45
BB2_1	7.22	0.14	1.64	0.61	4.40	0.23
BB3_1	5.54	0.18	2.84	0.35	1.95	0.51
BB4_1	11.09	0.09	3.34	0.30	3.32	0.30

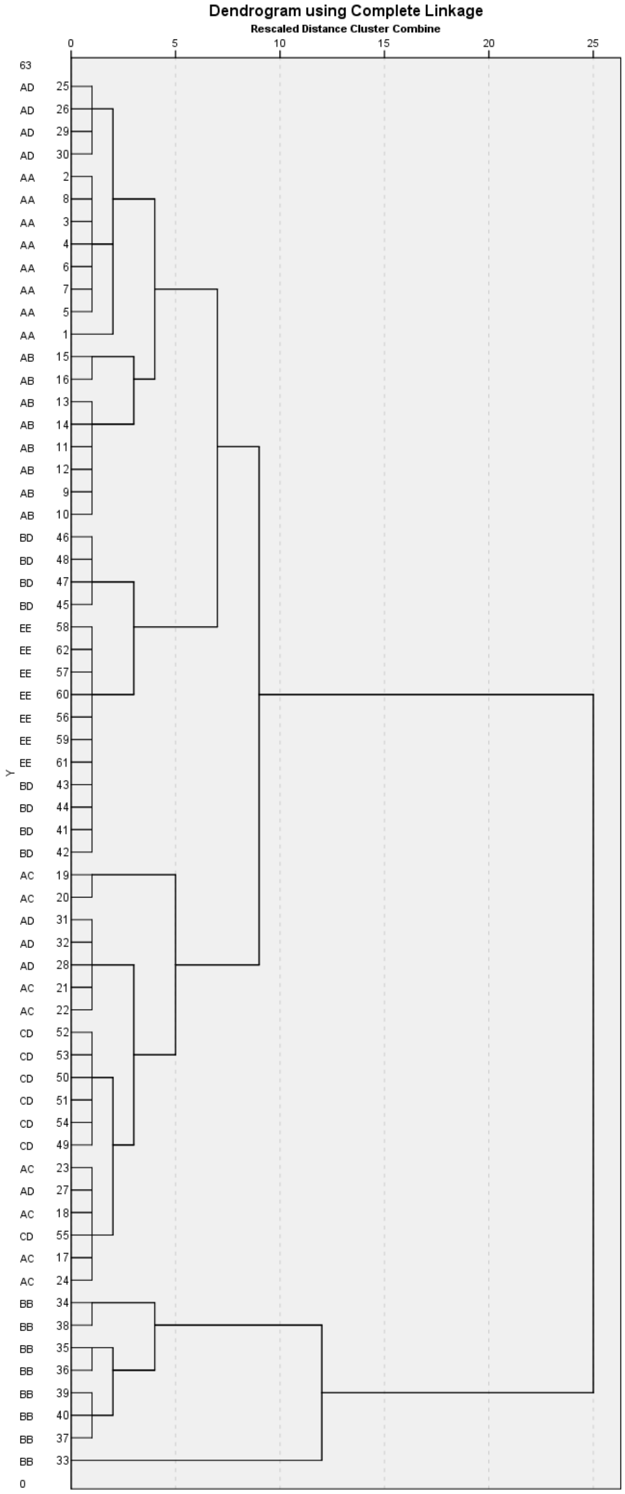
* Only the data for Cu:Zn, Zn:Pb and Cu:Pb were examined for outliers.

Resin Sample	**Ratios**					
	Cu:Zn	**Zn:Cu**	**Pb:Zn**	**Zn:Pb**	**Cu:Pb**	**Pb:Cu**
BC1_1	4.90	0.20	1.08	0.93	4.55	0.22
BC3_1	4.34	0.23	1.21	0.83	3.59	0.28
BC3_2	10.44	0.10	3.34	0.30	3.12	0.32
BC4_2	3.79	0.26	1.24	0.81	3.06	0.33
BD1_2	6.47	0.15	2.14	0.47	3.03	0.33
BD2_1	0.50	2.00	0.12	8.10	4.04	0.25
BD2_2	0.70	1.42	0.20	5.07	3.56	0.28
BD3_1	7.60	0.13	3.55	0.28	2.14	0.47
BD4_1	7.73	0.13	3.30	0.30	2.35	0.43
BD4_2	5.20	0.19	2.48	0.40	2.10	0.48
BD4_3	6.40	0.16	2.73	0.37	2.35	0.43
CC1_1	19.76	0.05	3.93	0.25	5.02	0.20
CC2_2	14.66	0.07	5.51	0.18	2.66	0.38
CC3_1	10.66	0.09	4.32	0.23	2.47	0.41
CC4_1	83.95	0.01	28.65	0.03	2.93	0.34
CD1_1		0.01	6.16	0.16		0.05
CD1_2	7.30	0.14	1.65	0.61	4.43	0.23
CD2_1	7.47	0.13	2.83	0.35	2.64	0.38
CD3_1	4.91	0.20	1.60	0.62	3.06	0.33
CD4_1	4.80	0.21	1.93	0.52	2.49	0.40
EE1_1	17.52	0.06	5.02	0.20	3.49	0.29
EE2_1	7.58	0.13	3.21	0.31	2.36	0.42
EE3_1	4.77	0.21	2.12	0.47	2.25	0.44

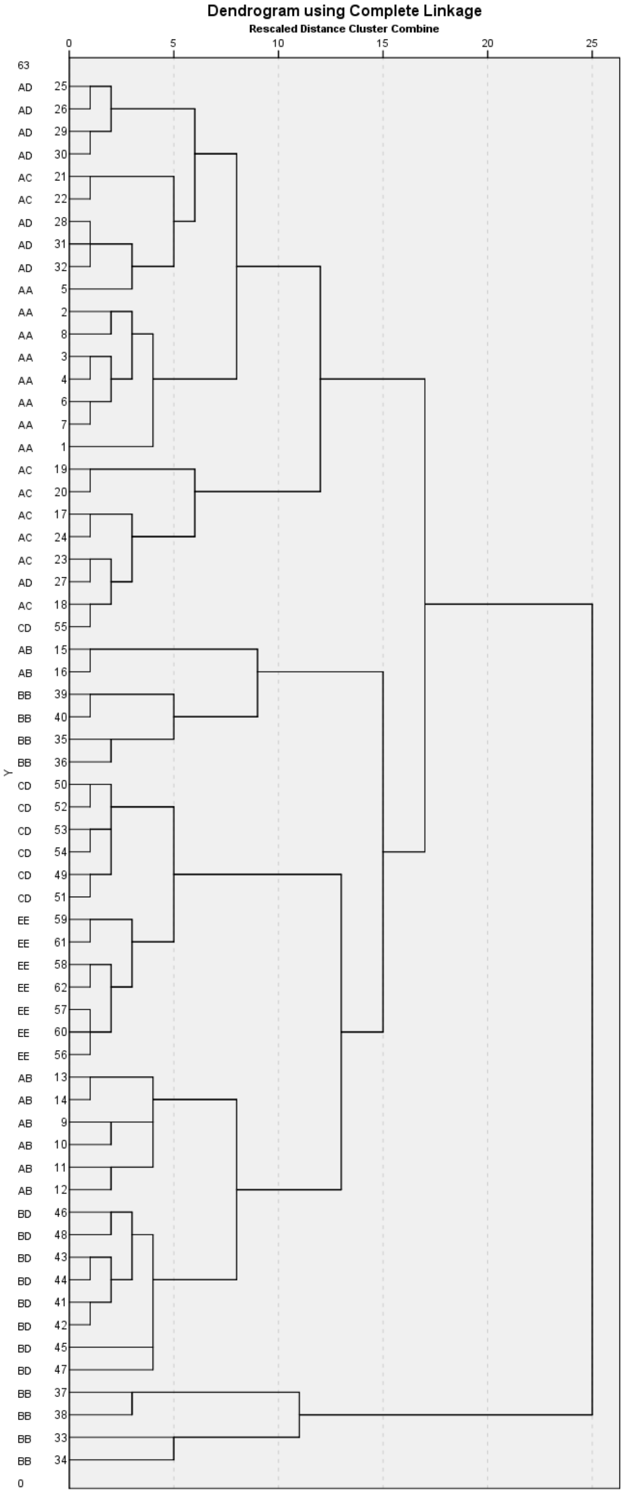
Appendix 9: Hierarchical Clustering Dendrograms

Soil - Concentration Data

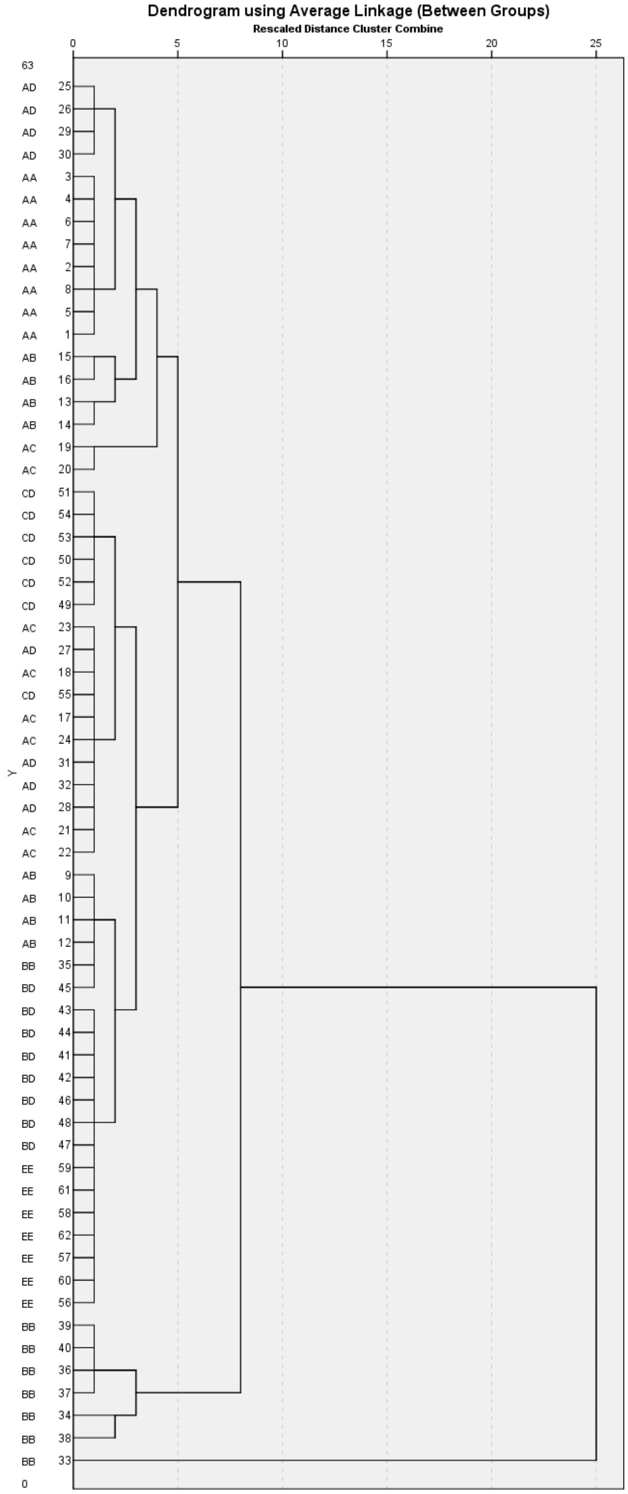
Complete Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



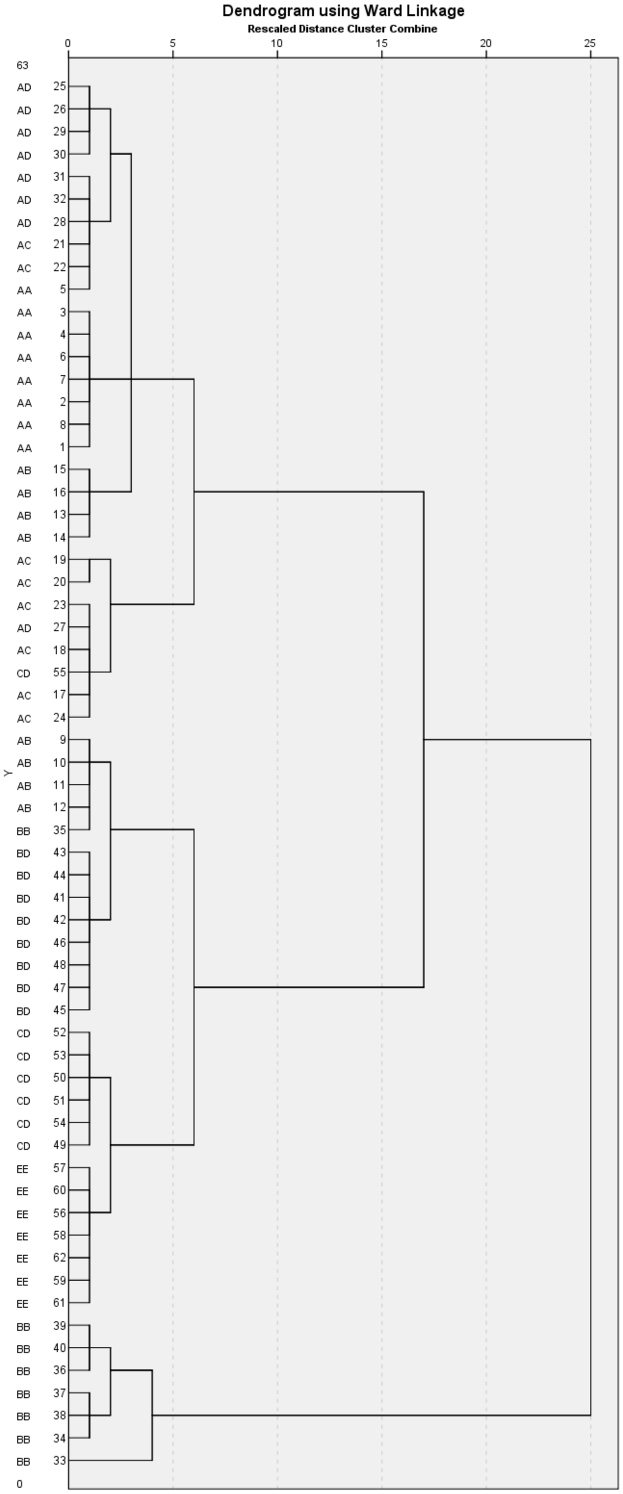
Complete Linkage (Manhattan distance) for copper, lead & zinc of all soil types (except BC & CC)



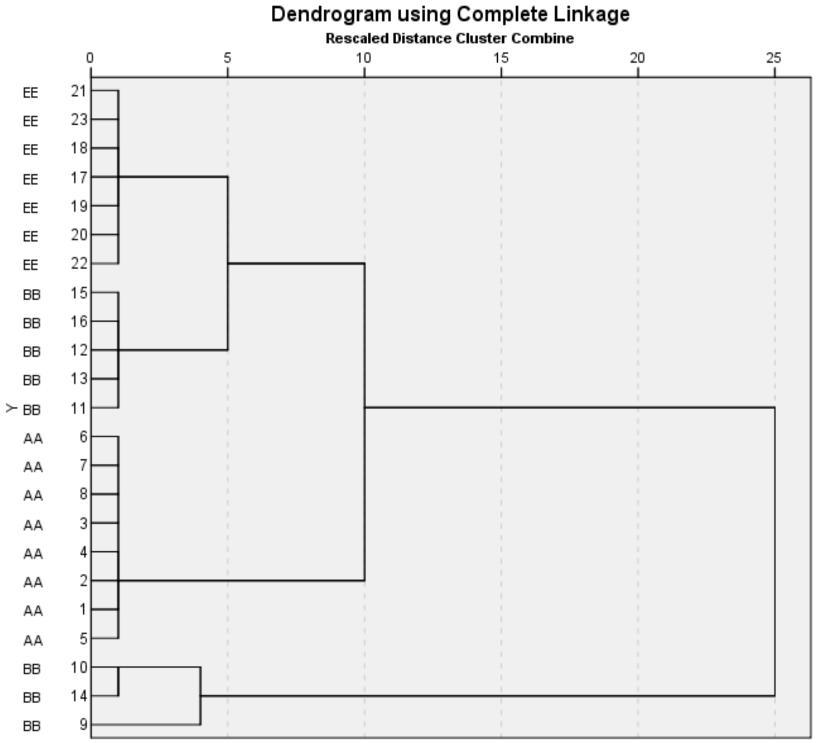
Average Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



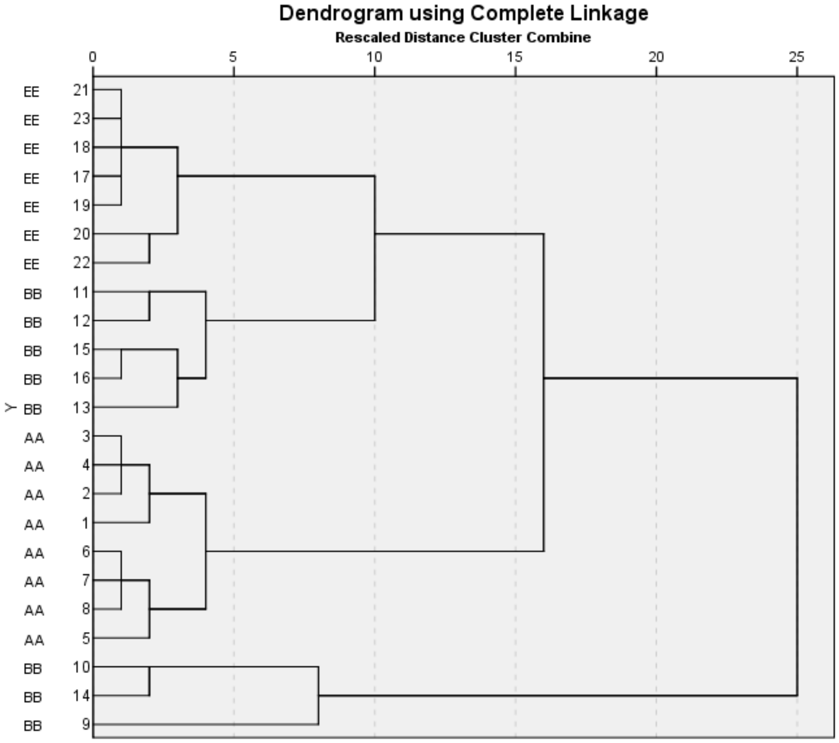
Ward Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



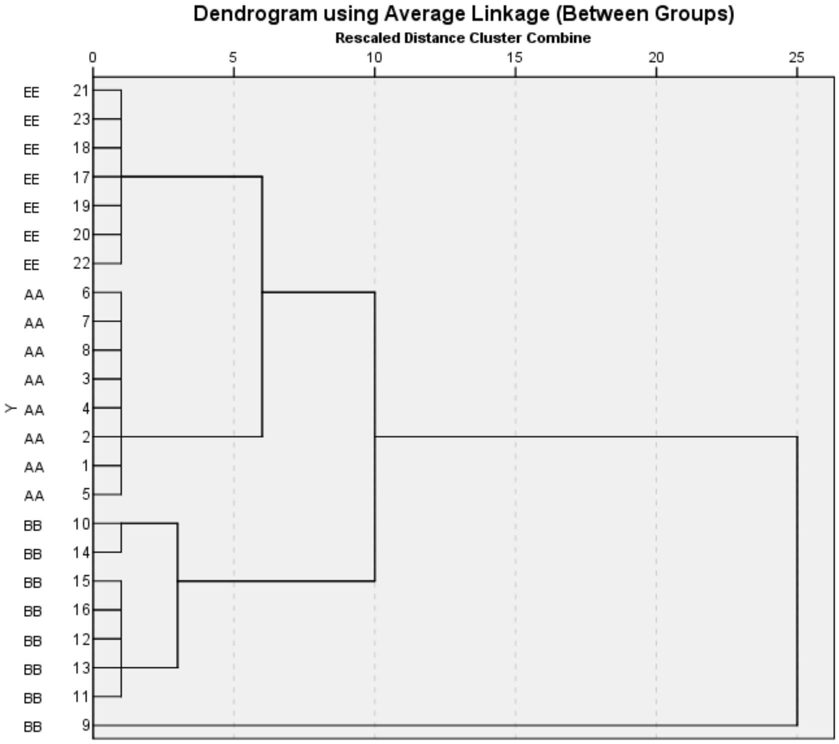
Complete Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE



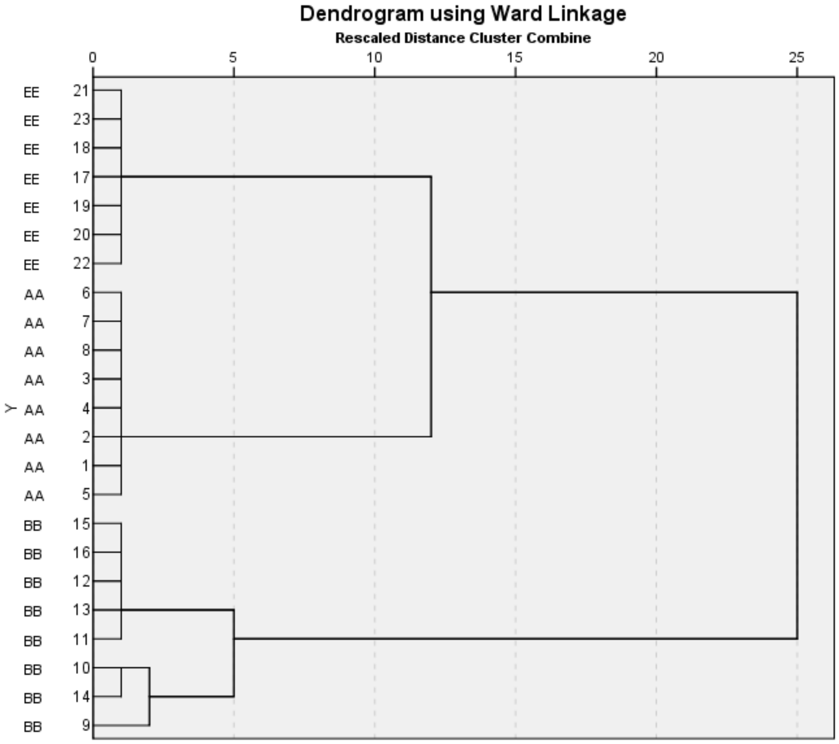
Complete Linkage (Manhattan distance) for copper & lead of soil types AA, BB & EE



Average Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE

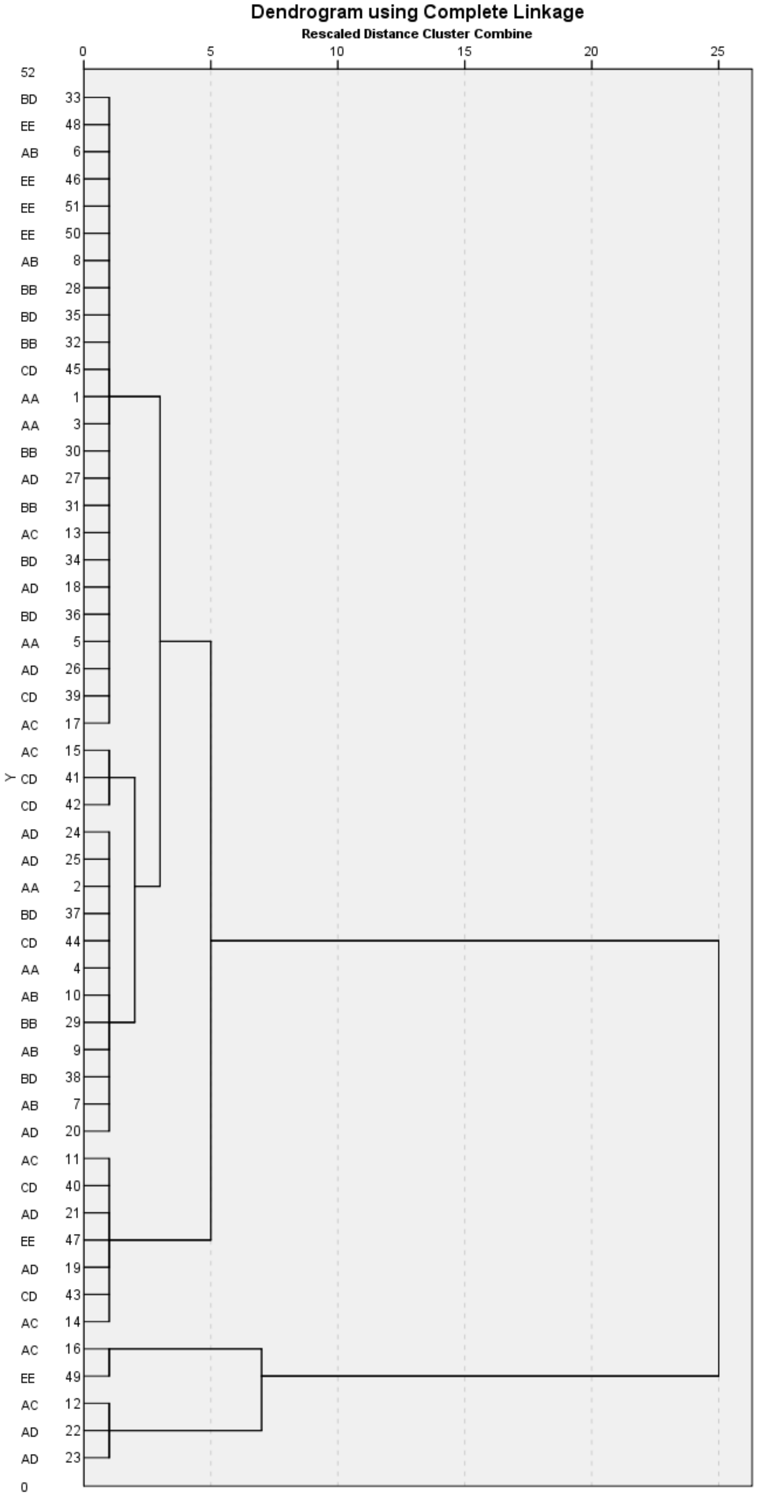


Ward Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE

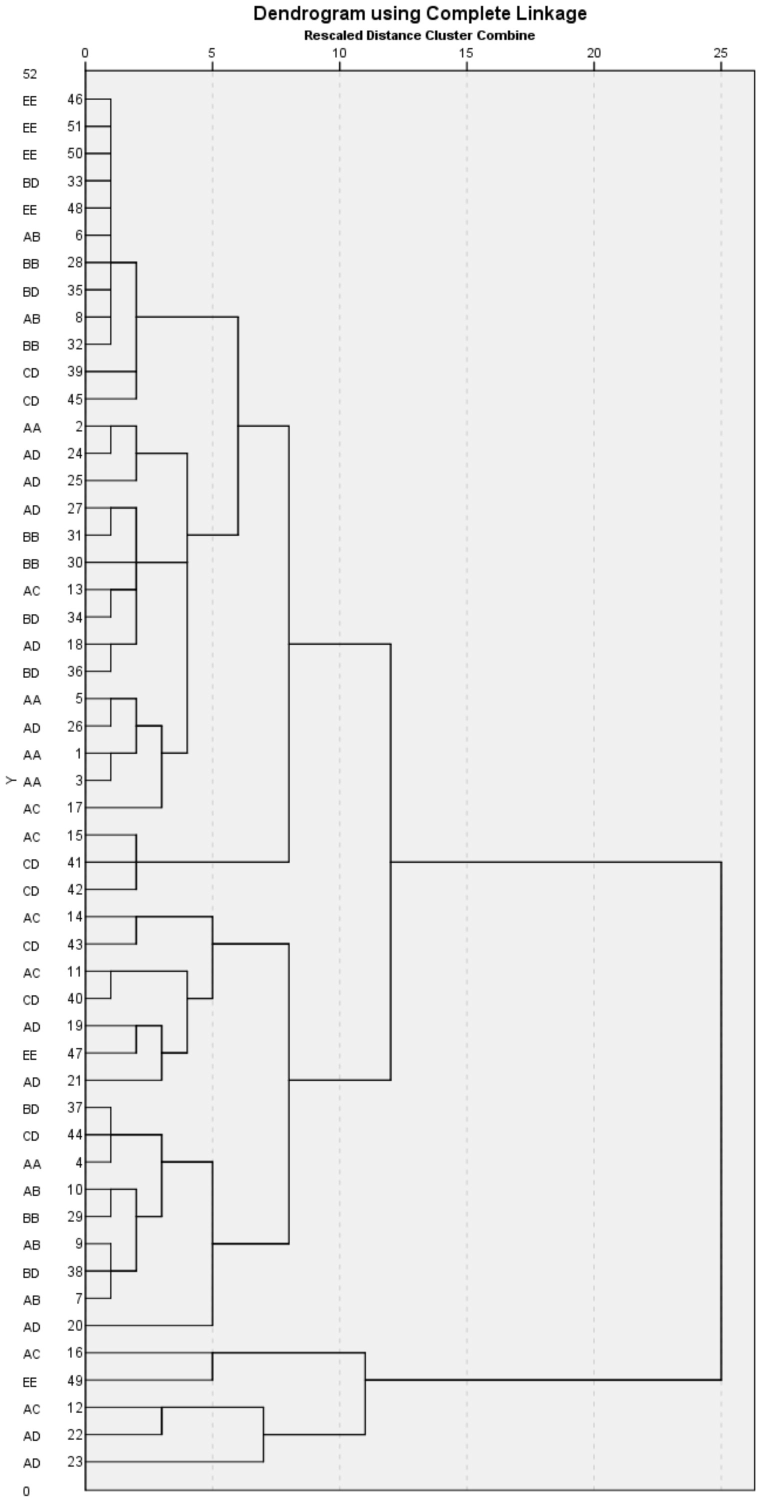


Leaf – Concentration Data

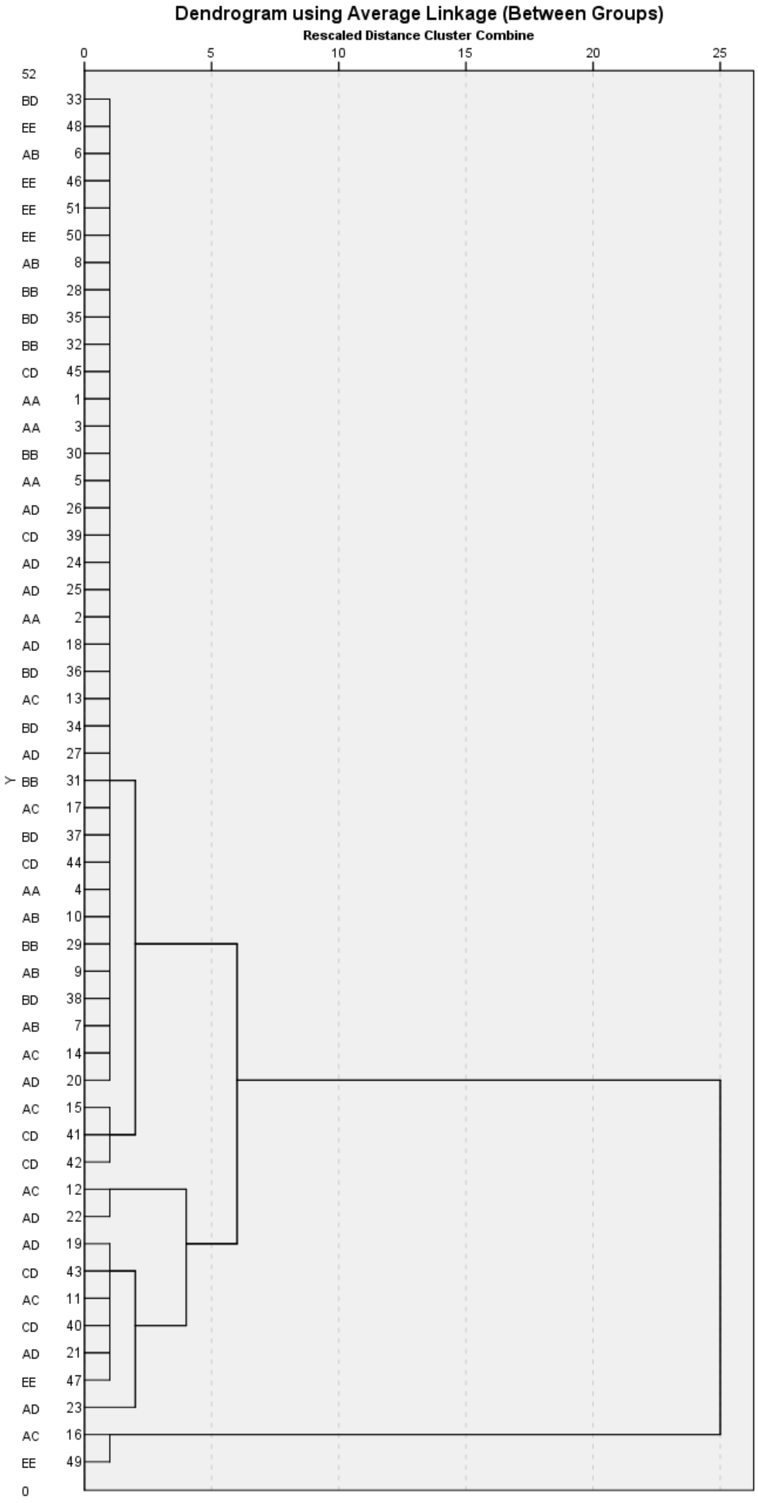
Complete Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



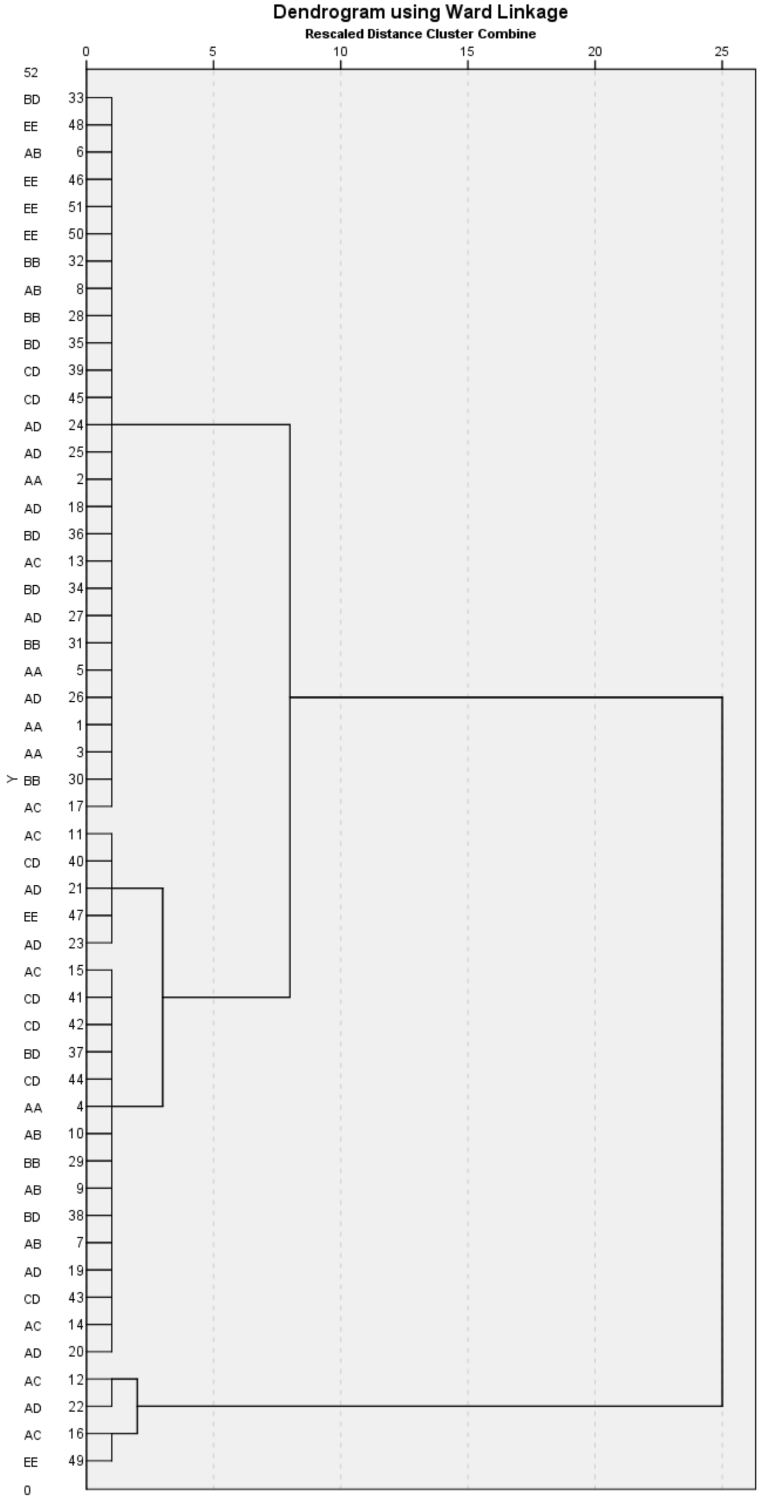
Complete Linkage (Manhattan distance) for copper, lead & zinc of all soil types (except BC & CC)



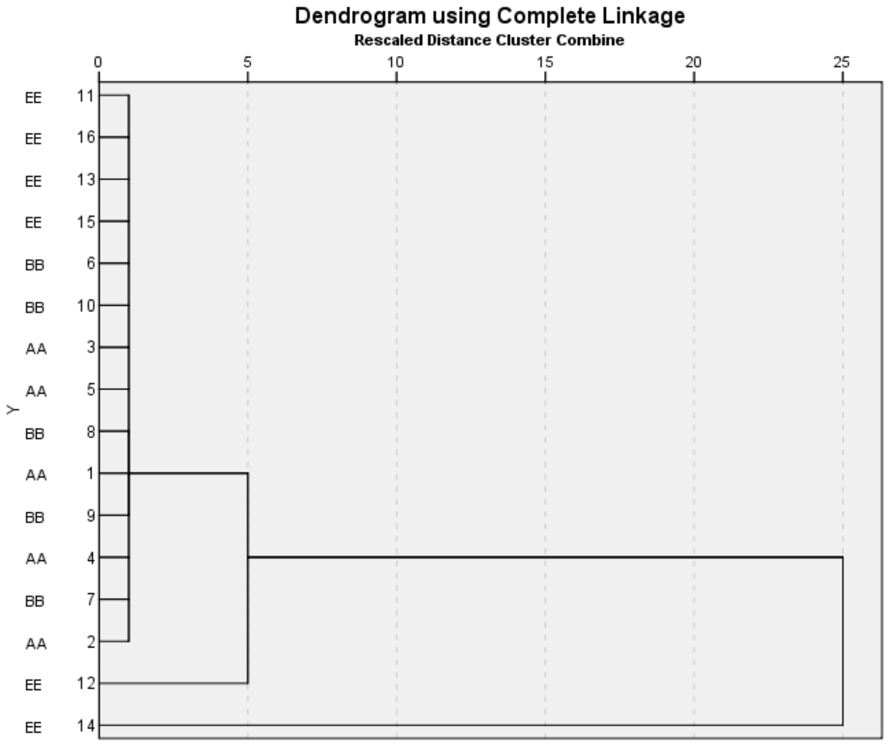
Average Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



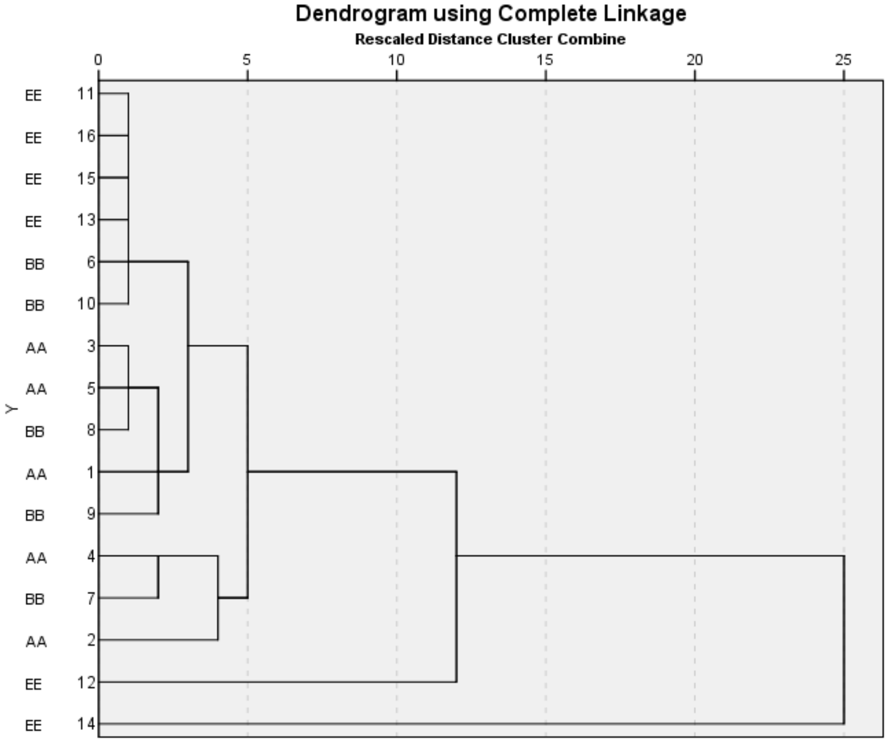
Ward Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



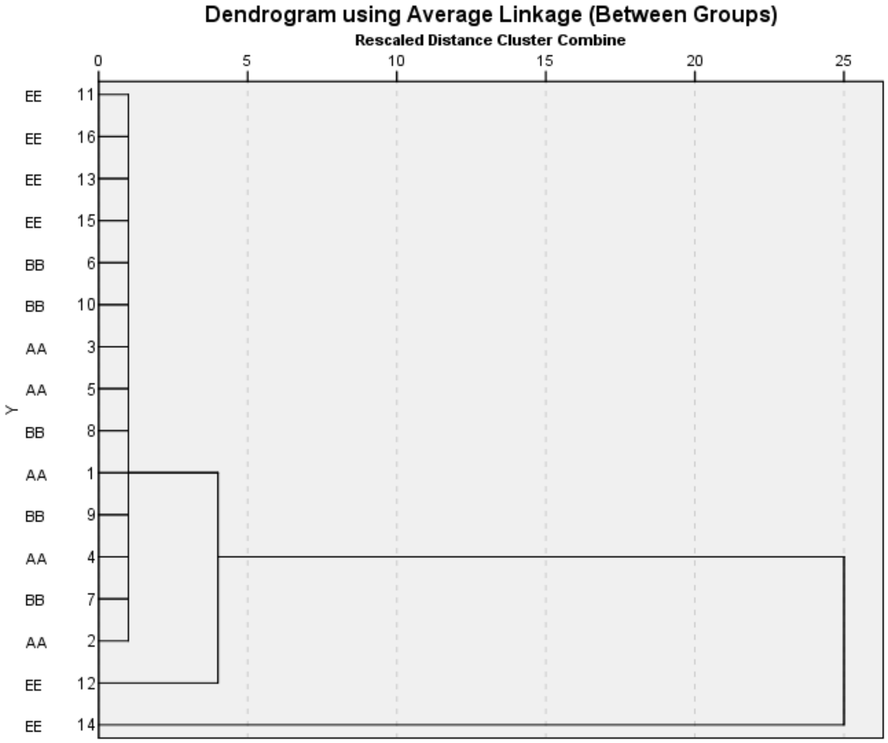
Complete Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE



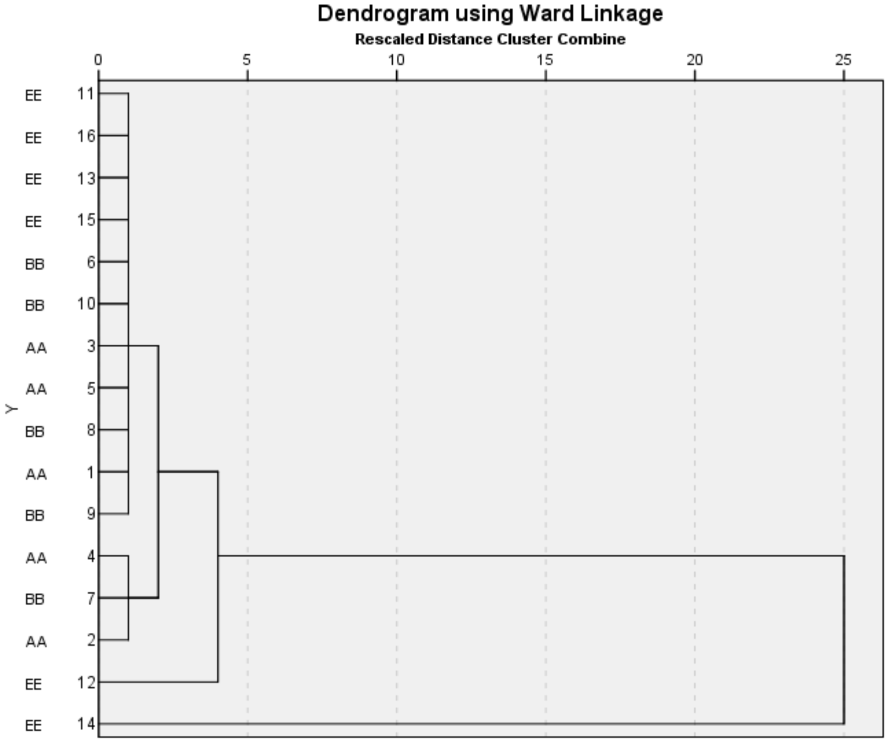
Complete Linkage (Manhattan distance) for copper & lead of soil types AA, BB & EE



Average Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE

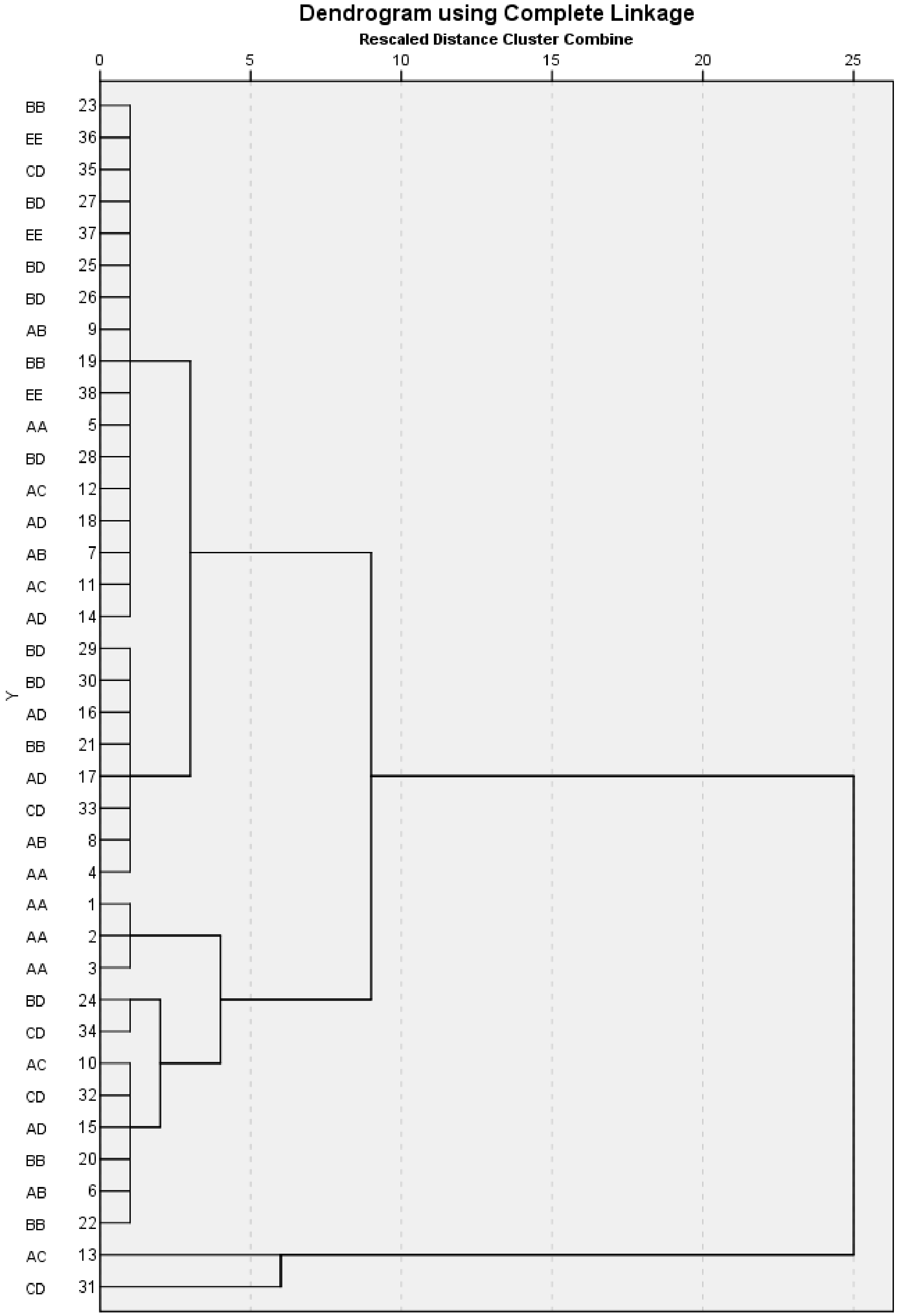


Ward Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE

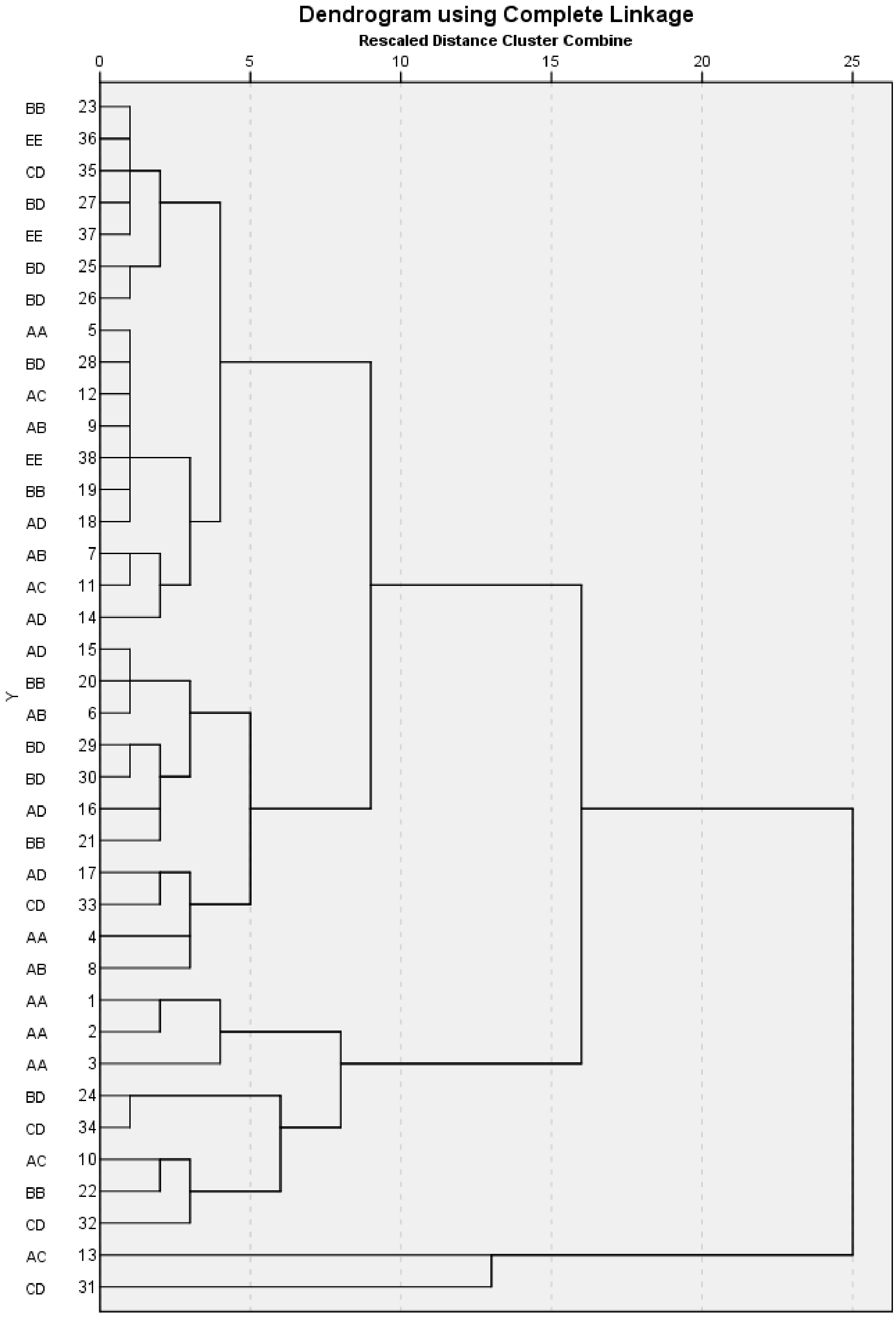


Resin – Concentration Data

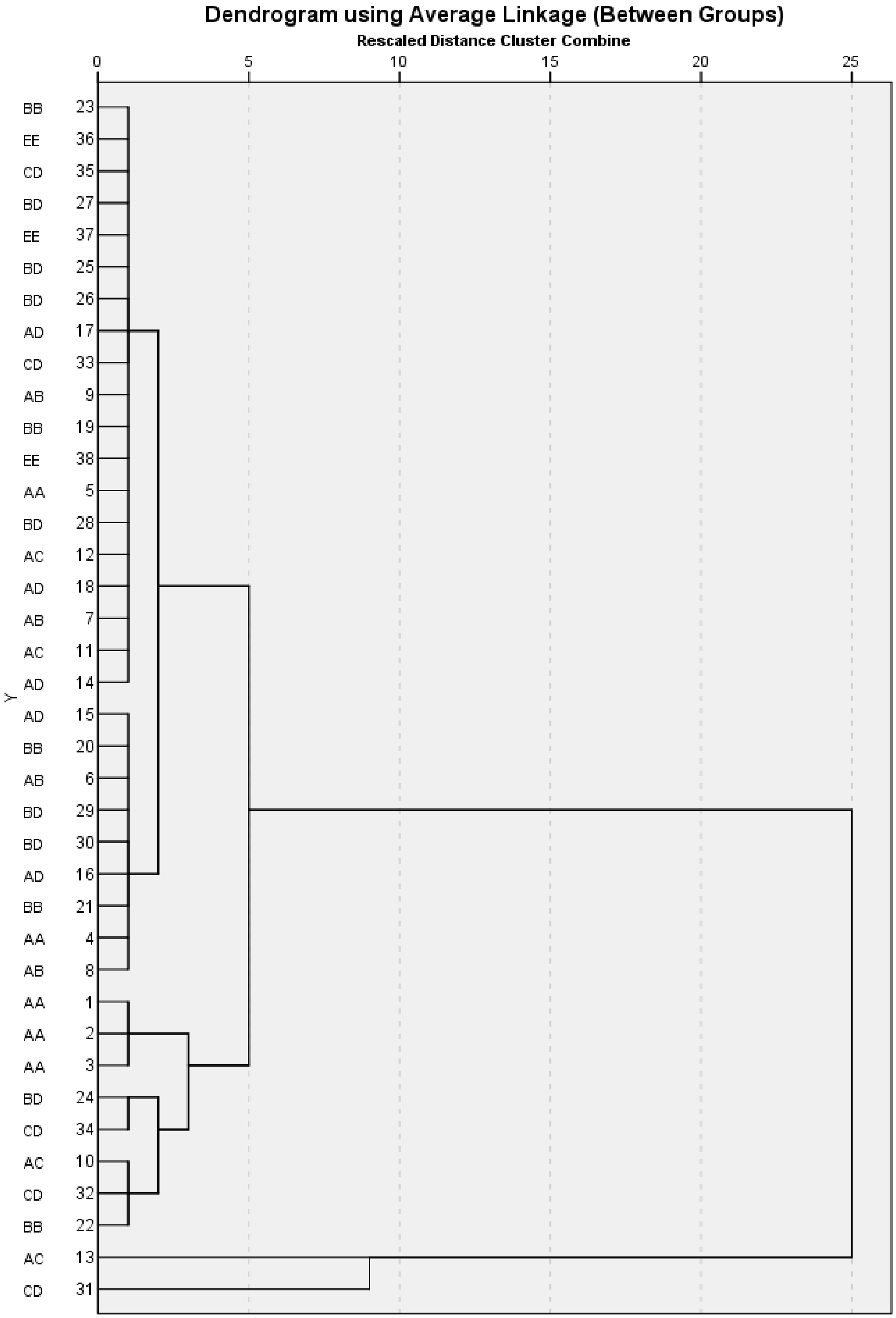
Complete Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



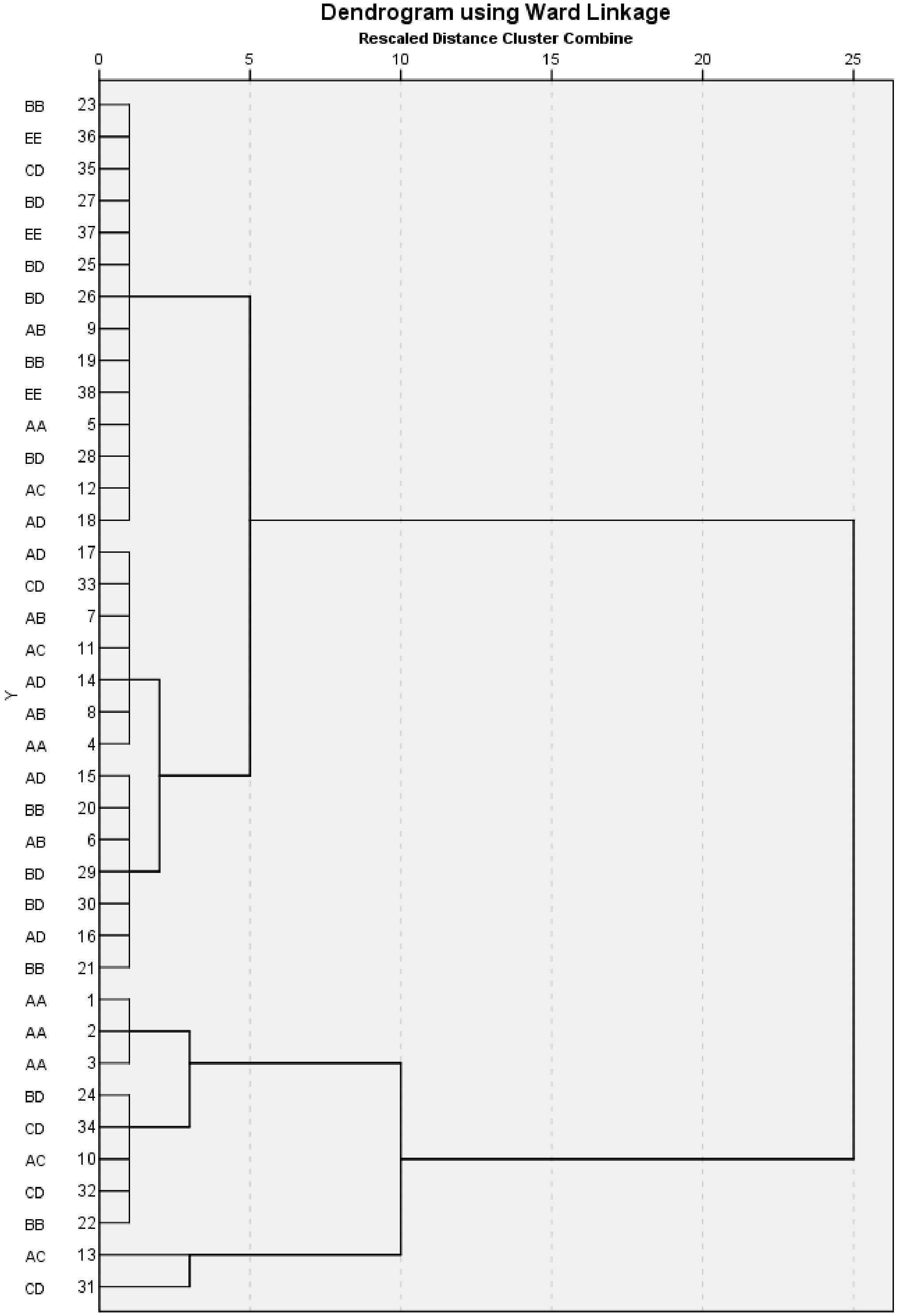
Complete Linkage (Manhattan distance) for copper, lead & zinc of all soil types (except BC & CC)



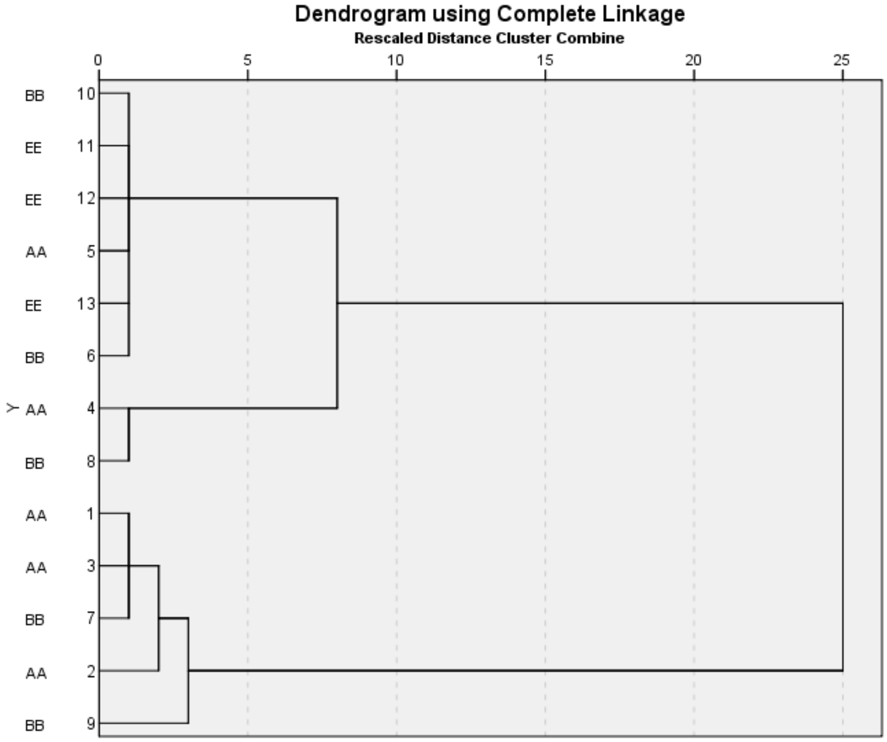
Average Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



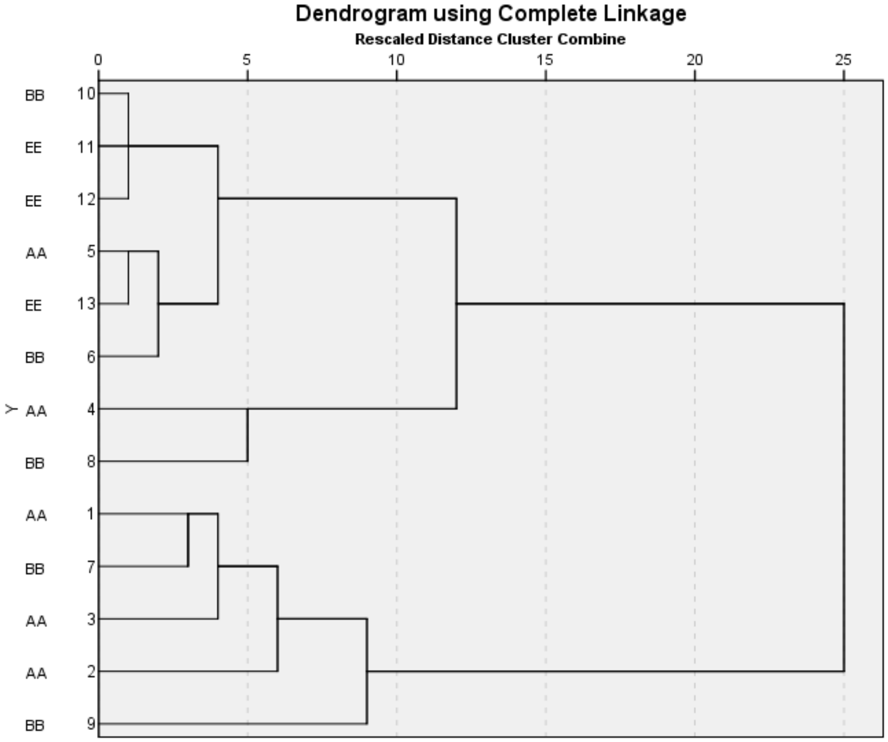
Ward Linkage (squared Euclidean distance) for copper, lead & zinc of all soil types (except BC & CC)



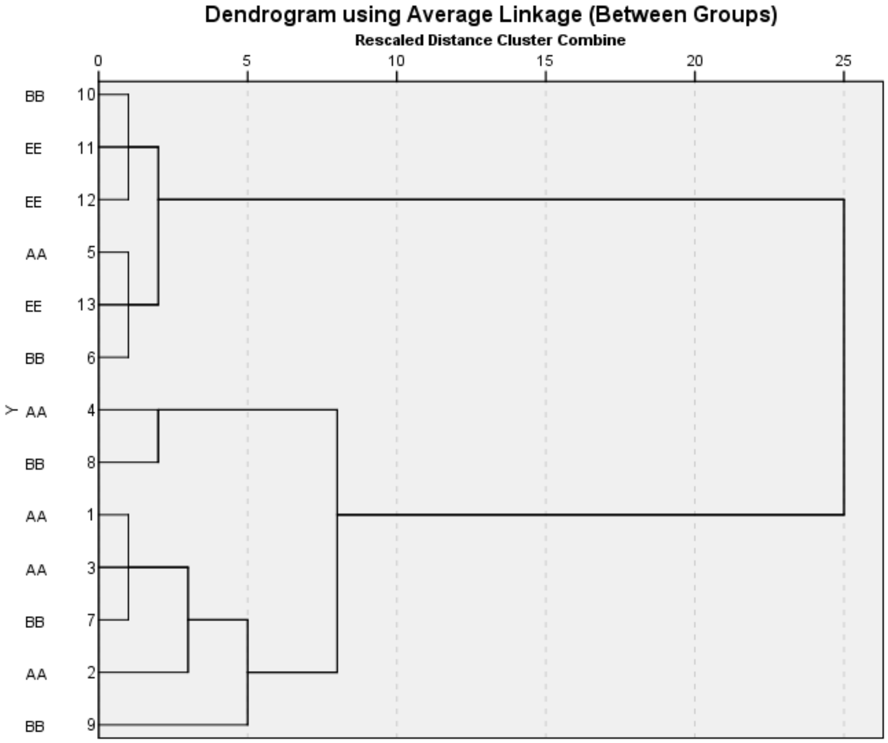
Complete Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE



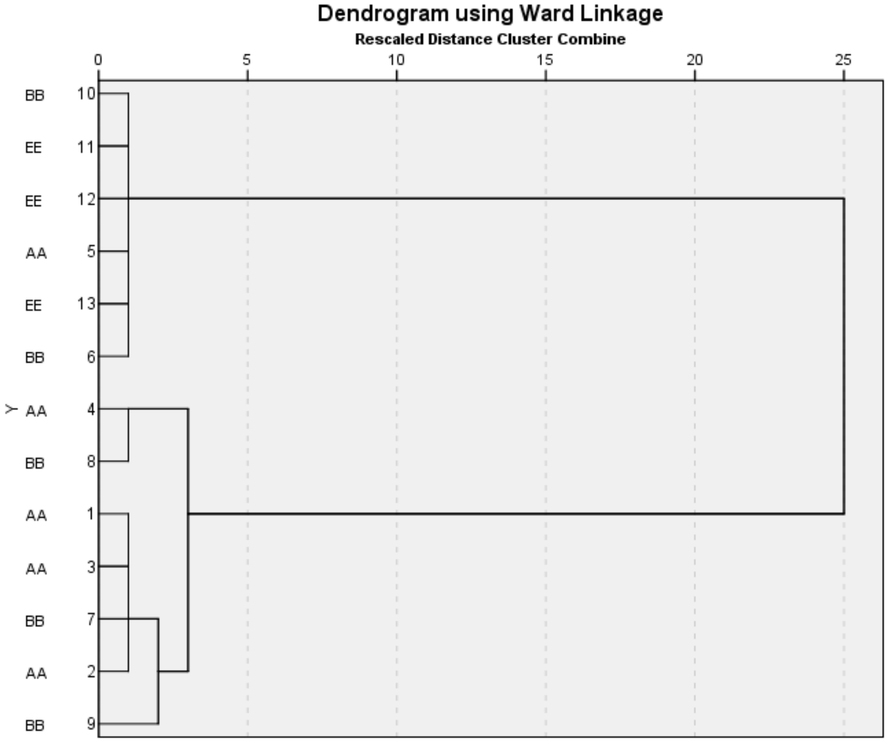
Complete Linkage (Manhattan distance) for copper & lead of soil types AA, BB & EE



Average Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE

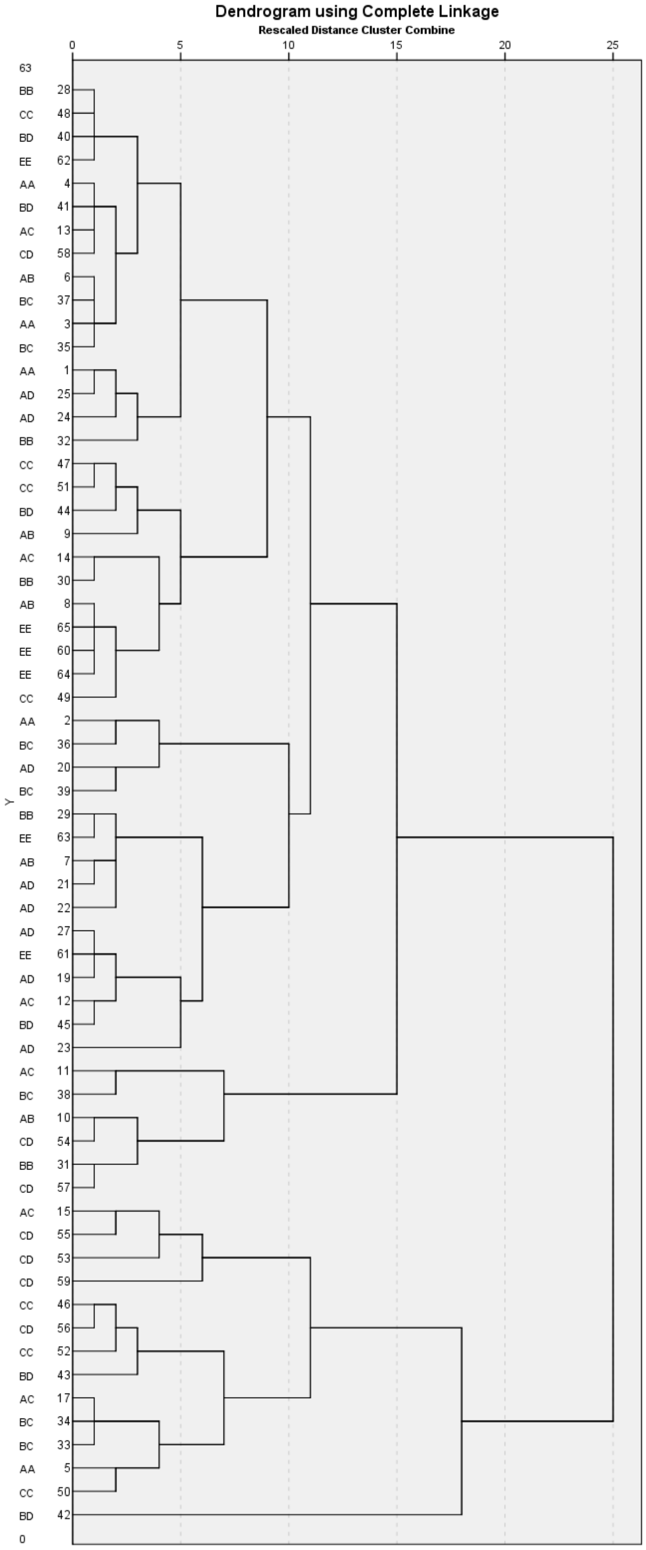


Ward Linkage (squared Euclidean distance) for copper & lead of soil types AA, BB & EE

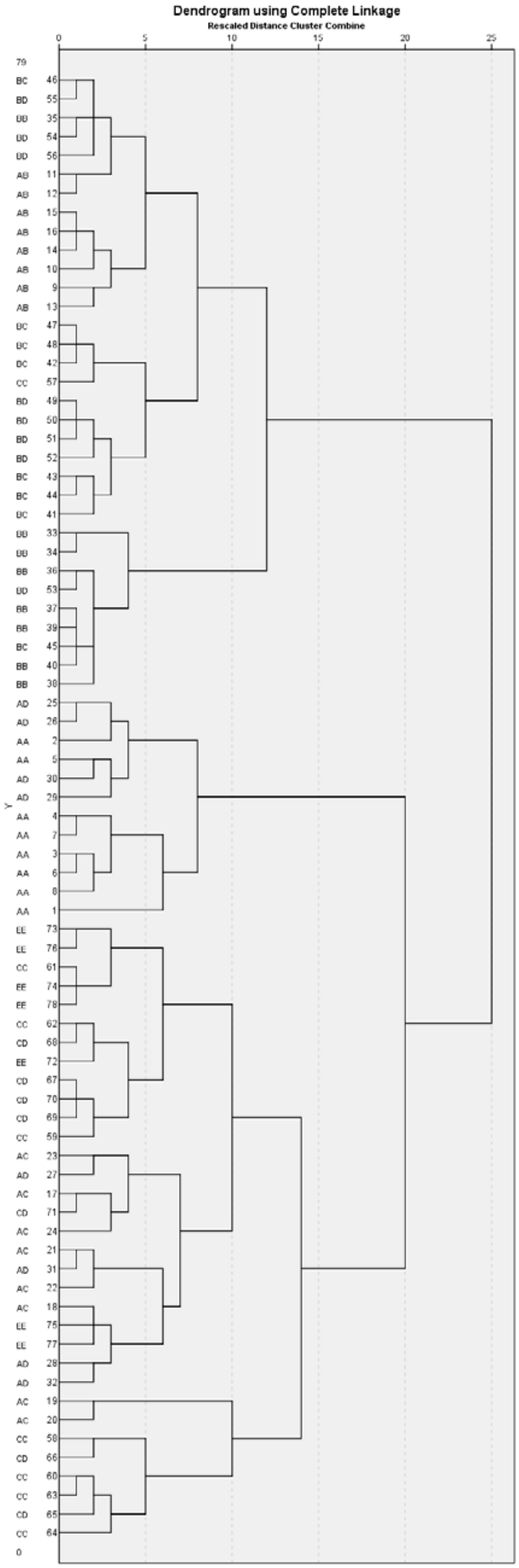


Soil – Concentration Ratio Data

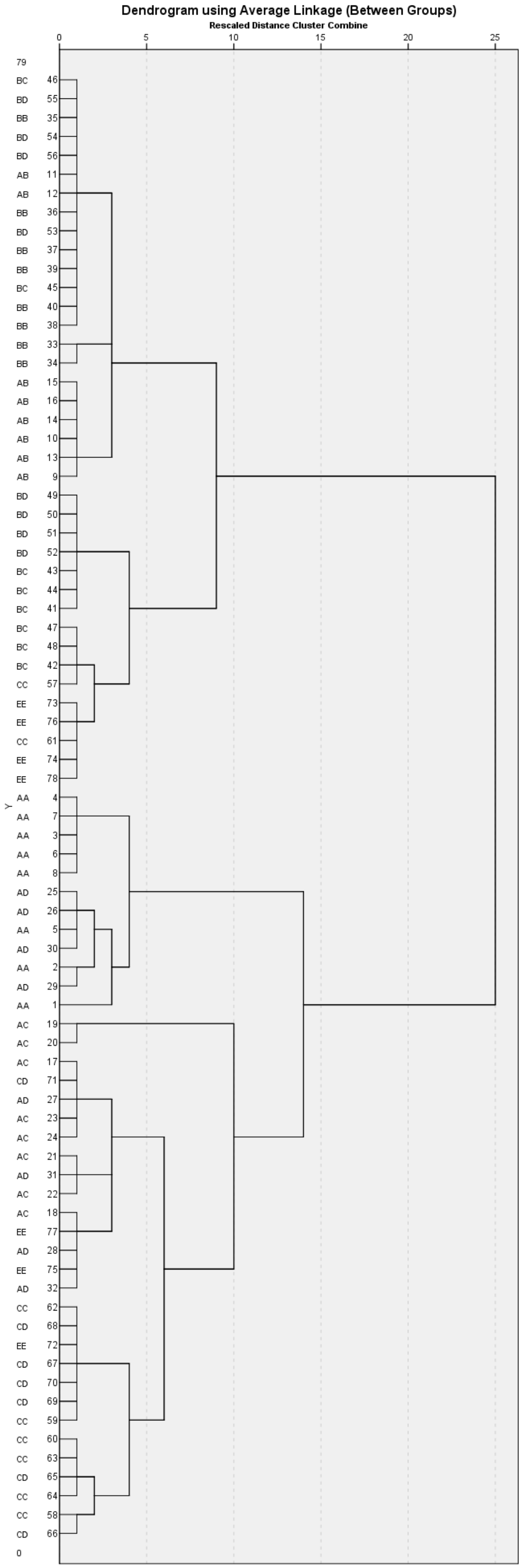
Complete Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



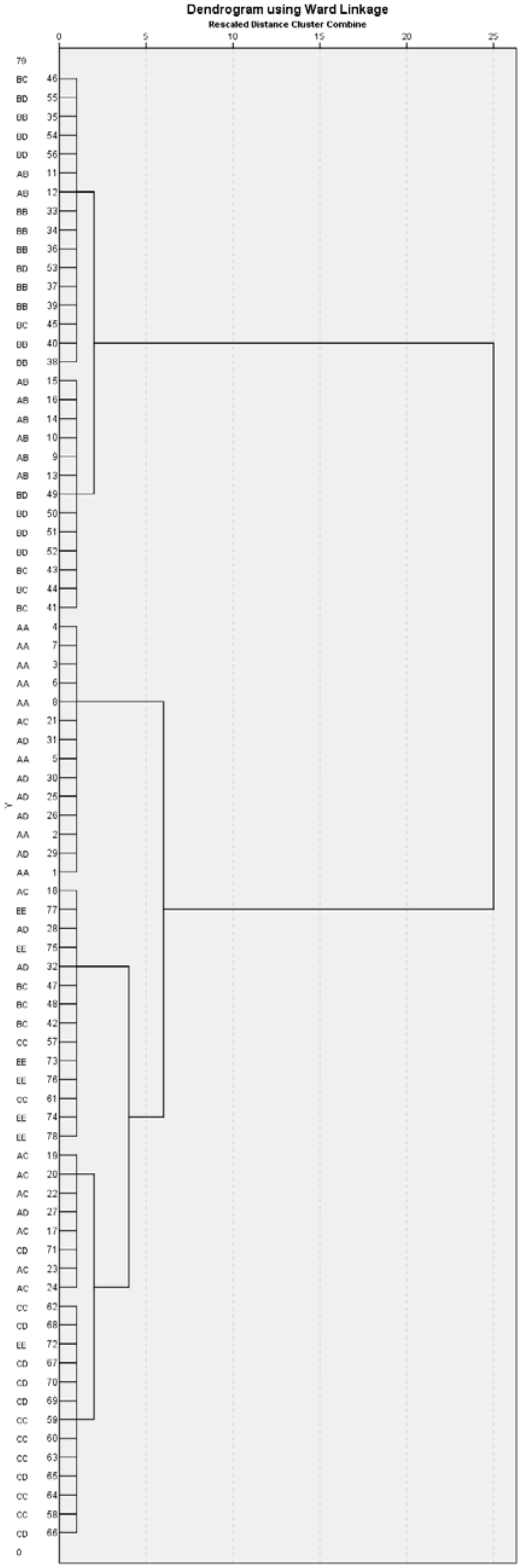
Complete Linkage (Manhattan distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



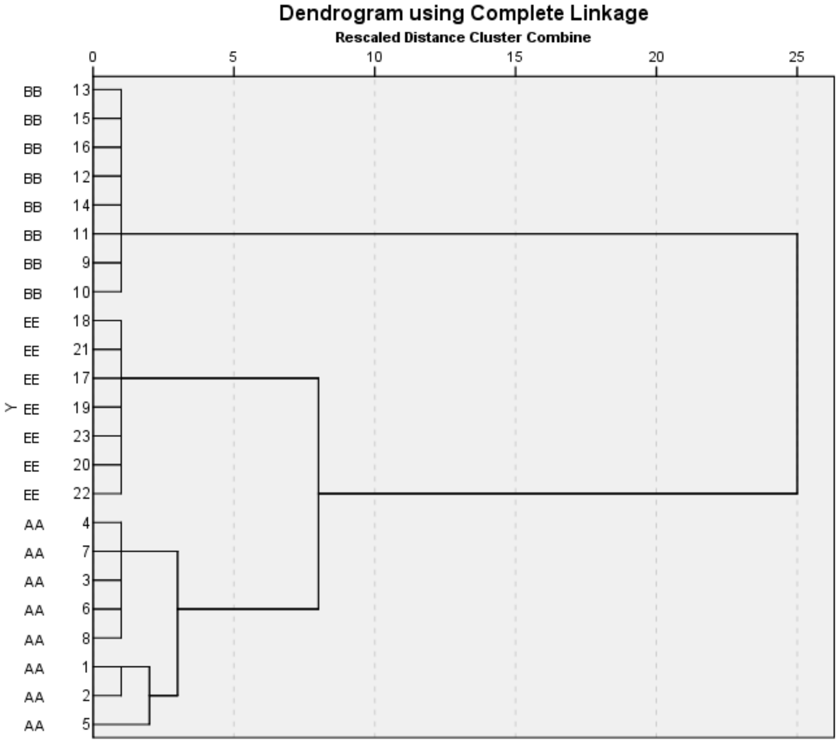
Average Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



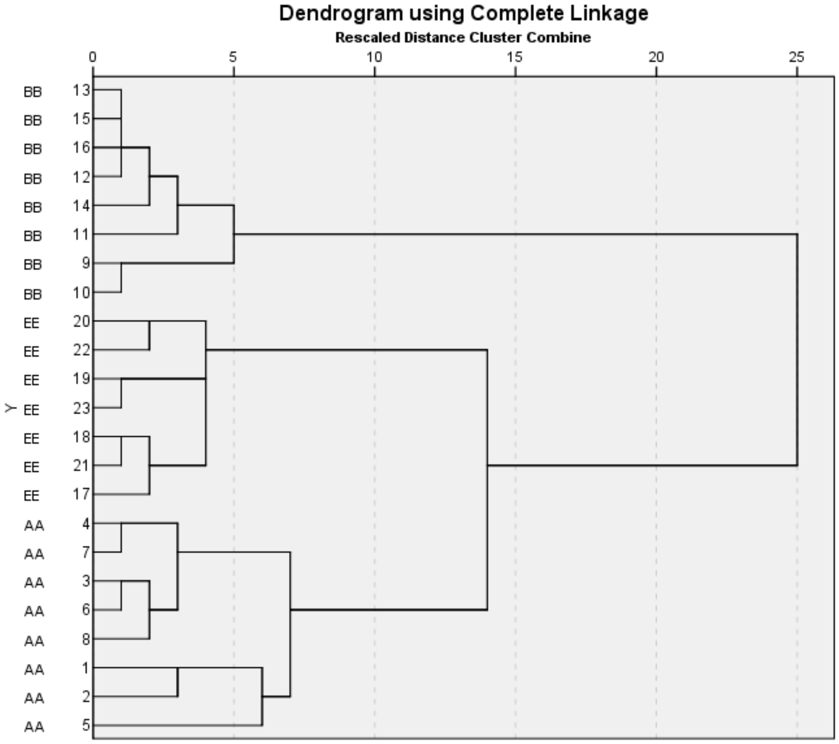
Ward Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



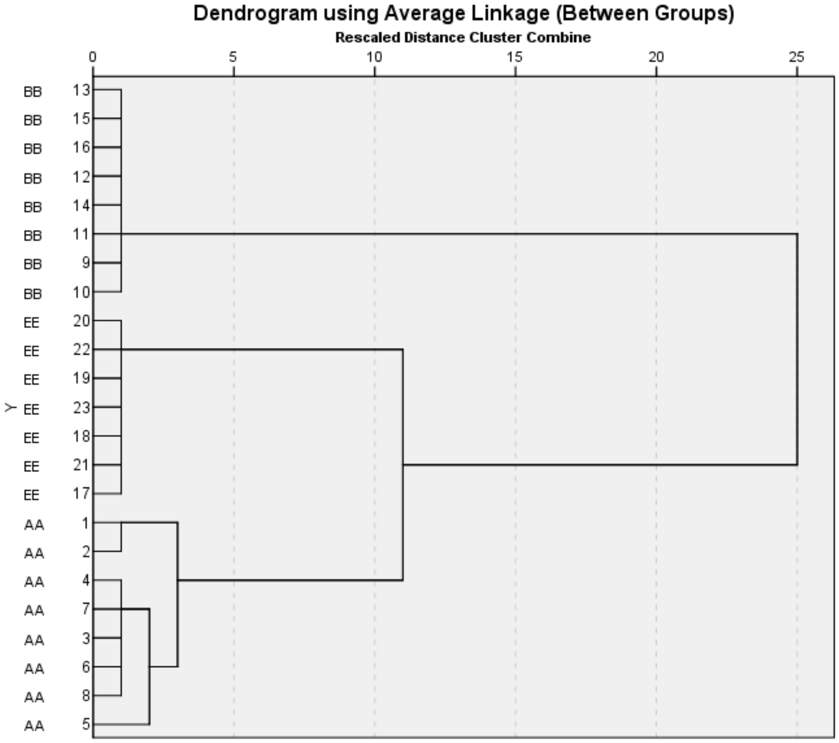
Complete Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



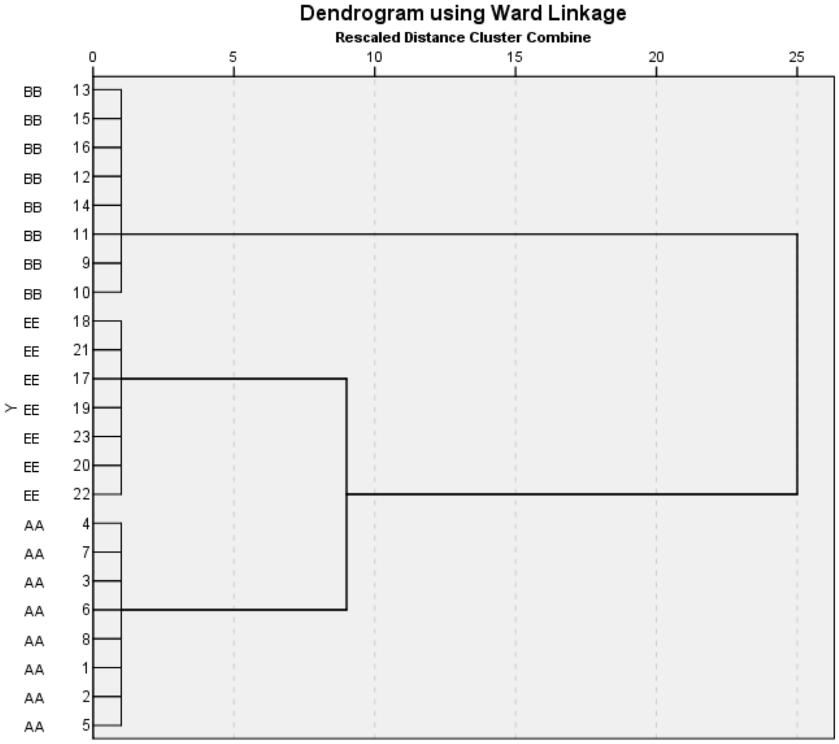
Complete Linkage (Manhattan distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



Average Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE

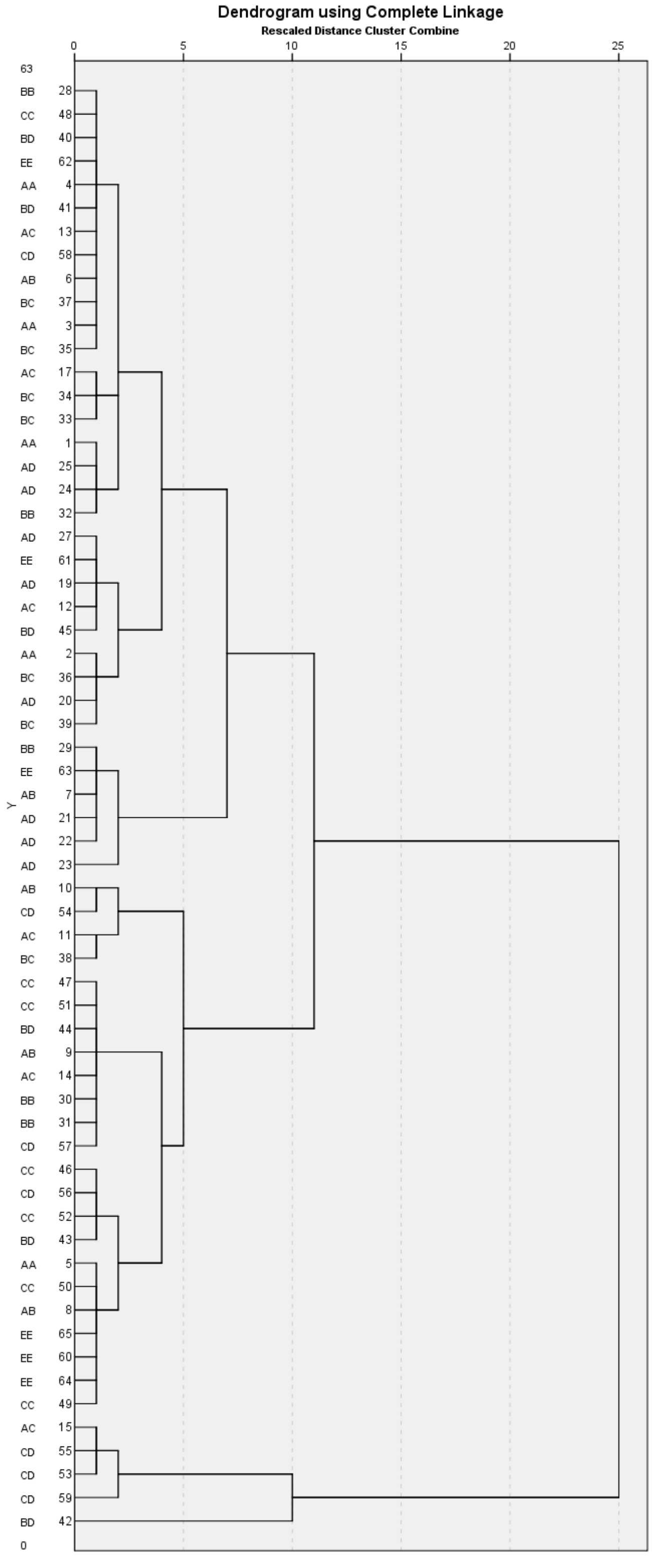


Ward Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE

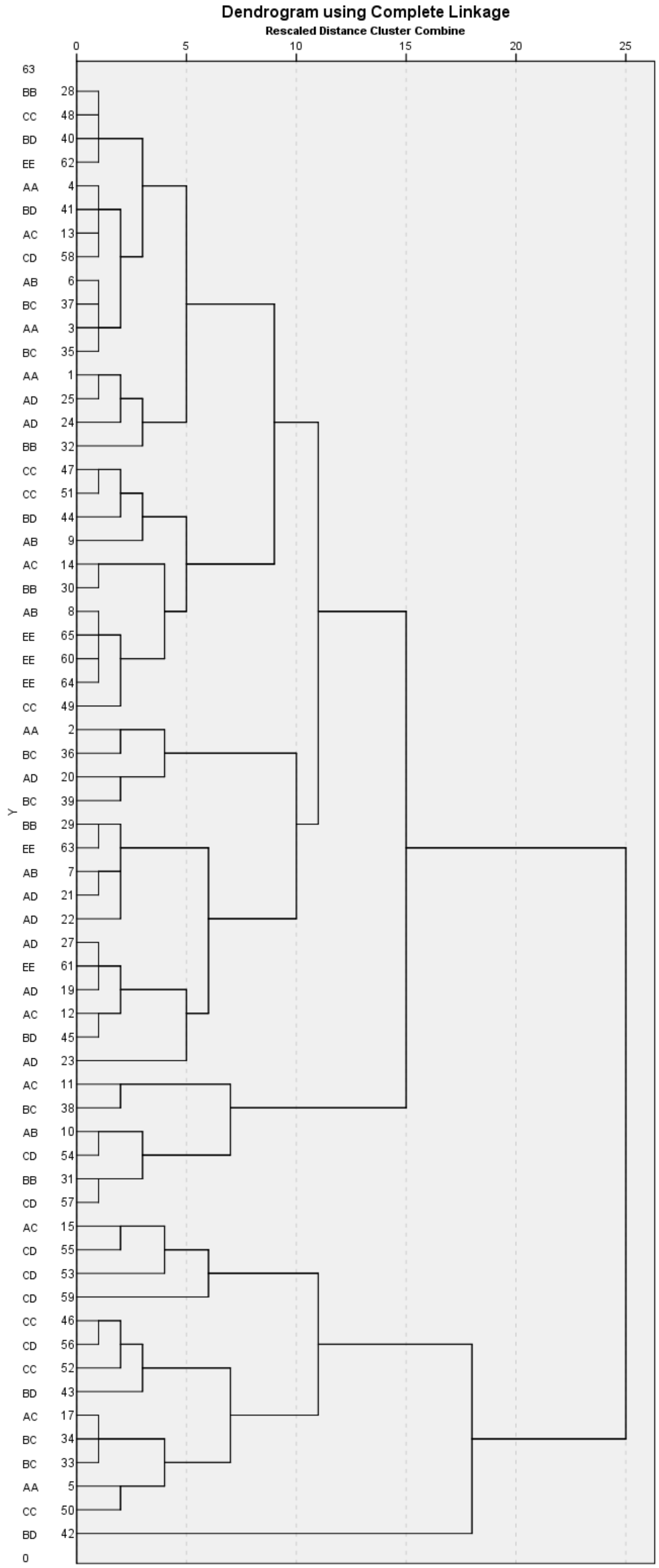


Leaf - Concentration Ratio Data

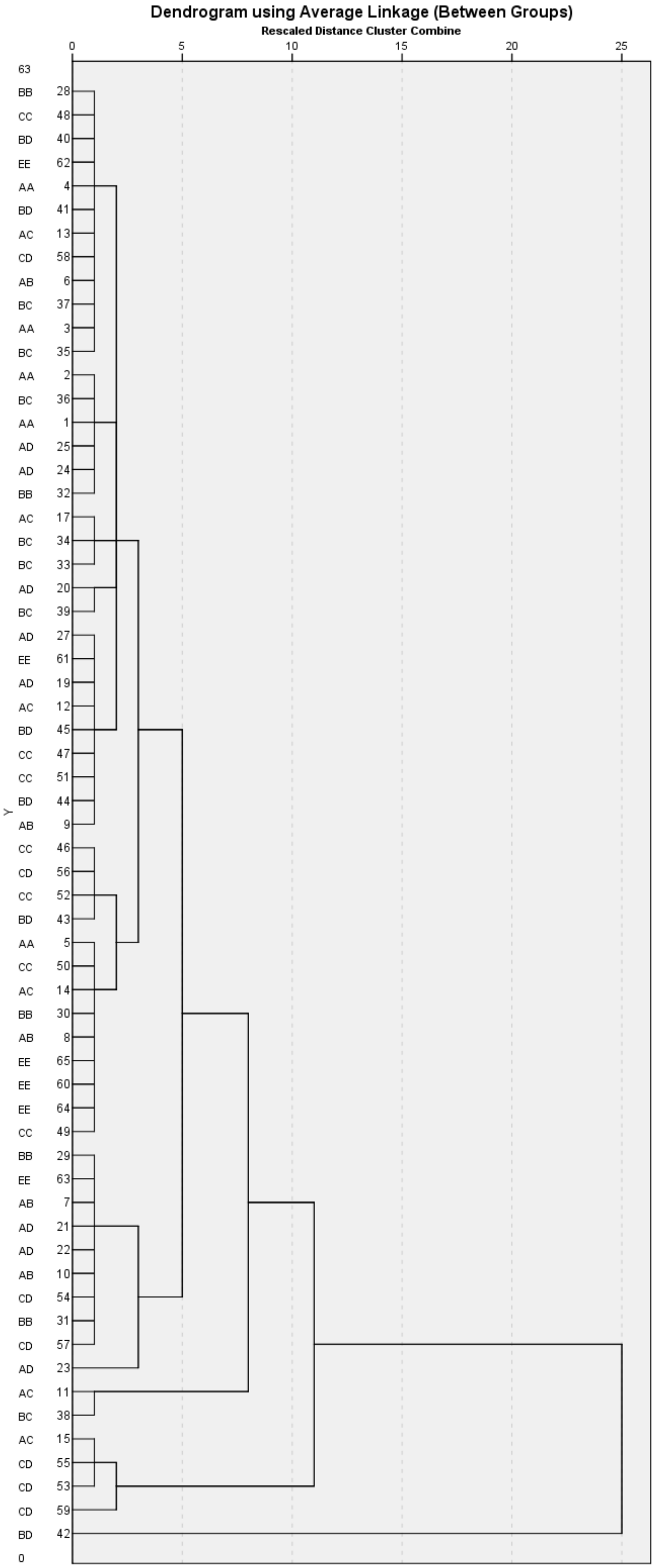
Complete Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



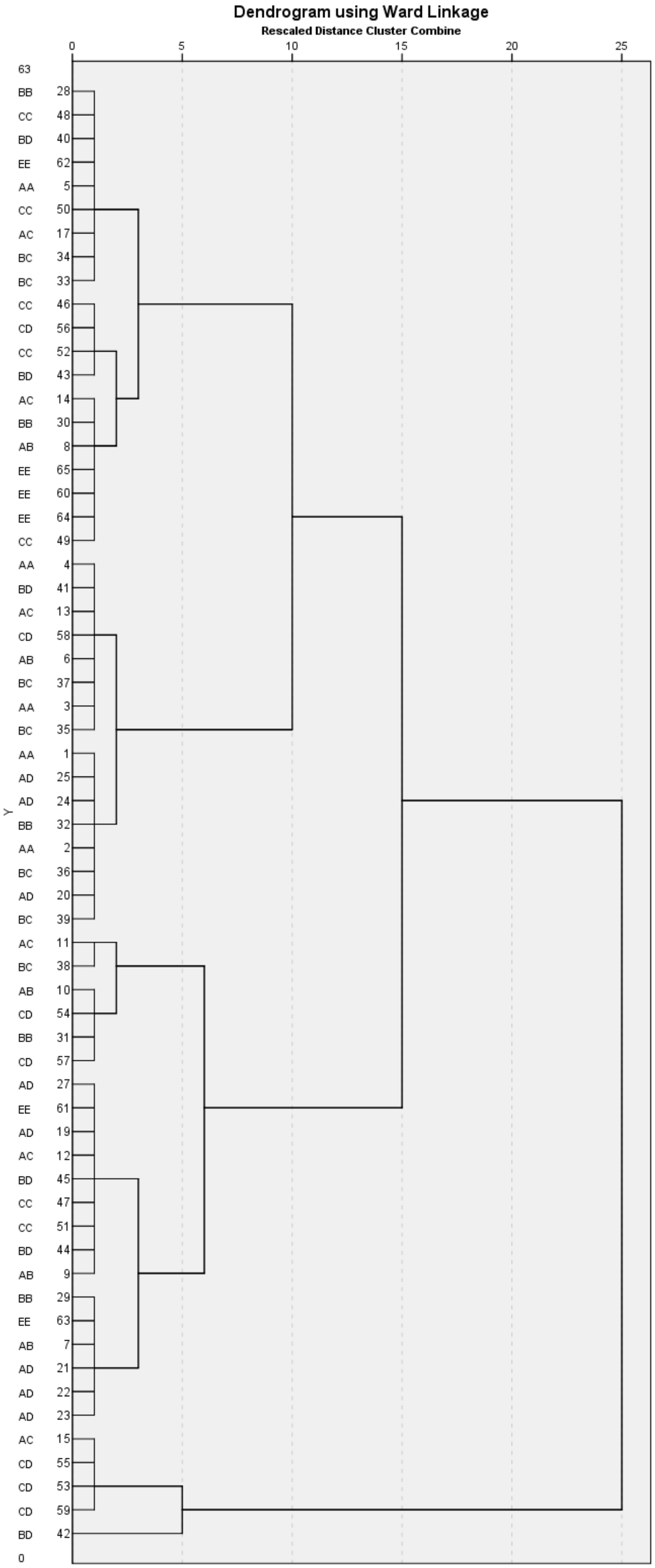
Complete Linkage (Manhattan distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



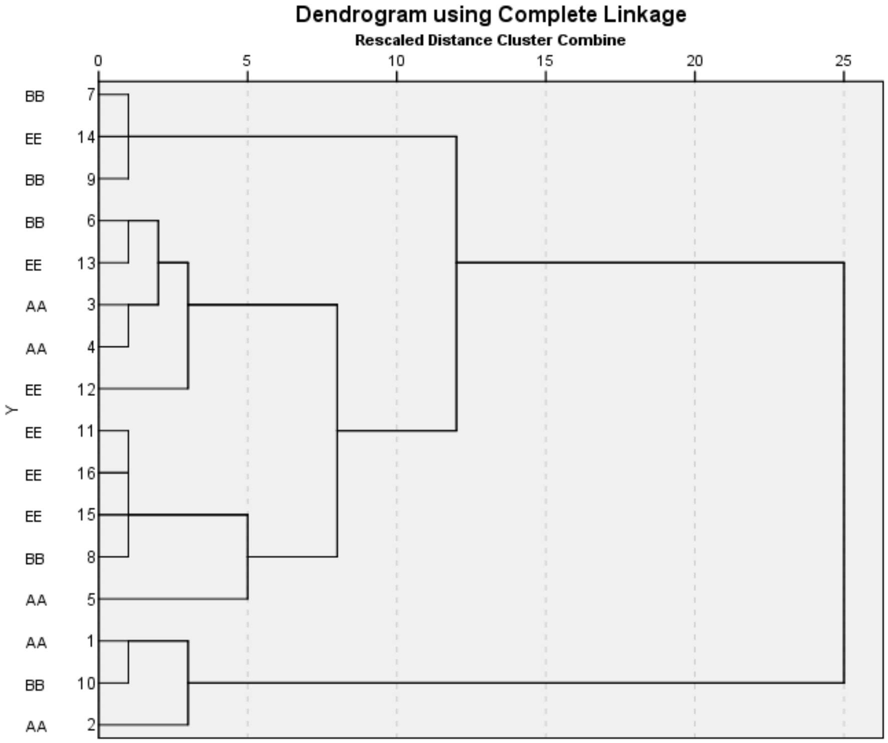
Average Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



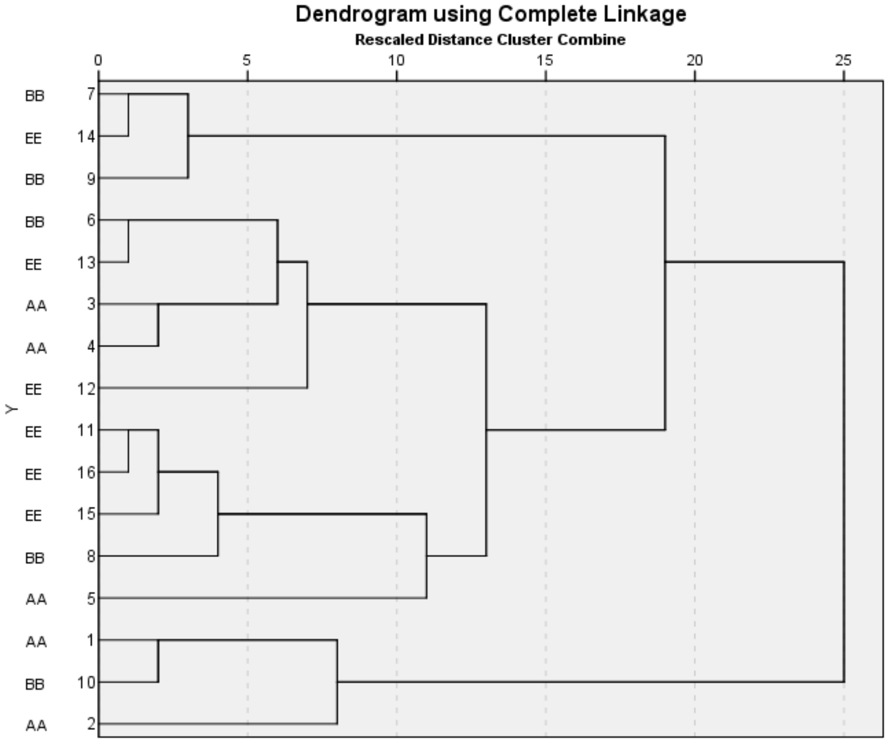
Ward Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



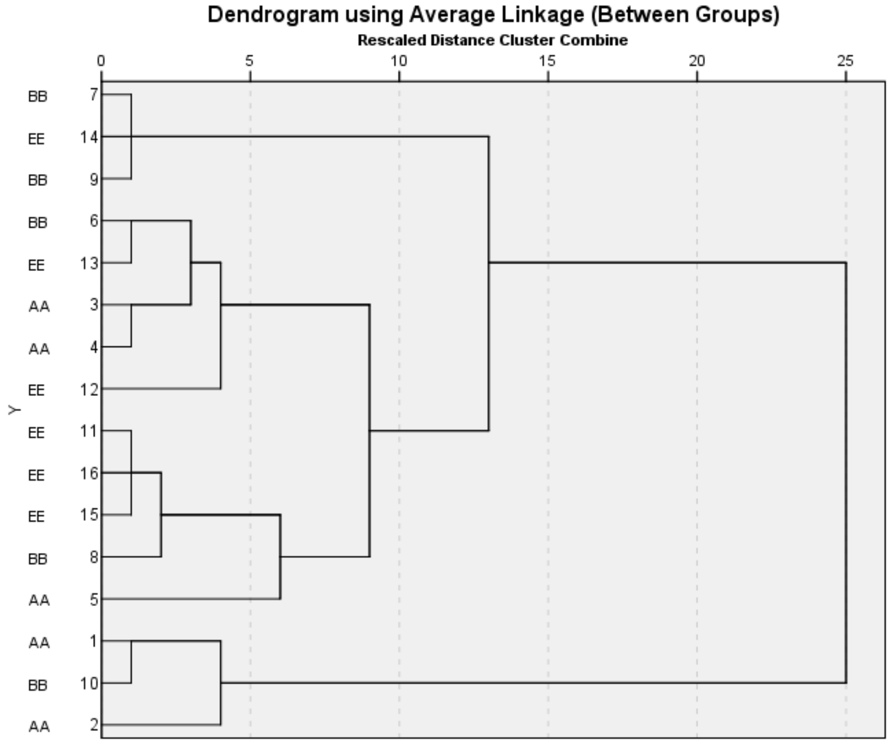
Complete Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



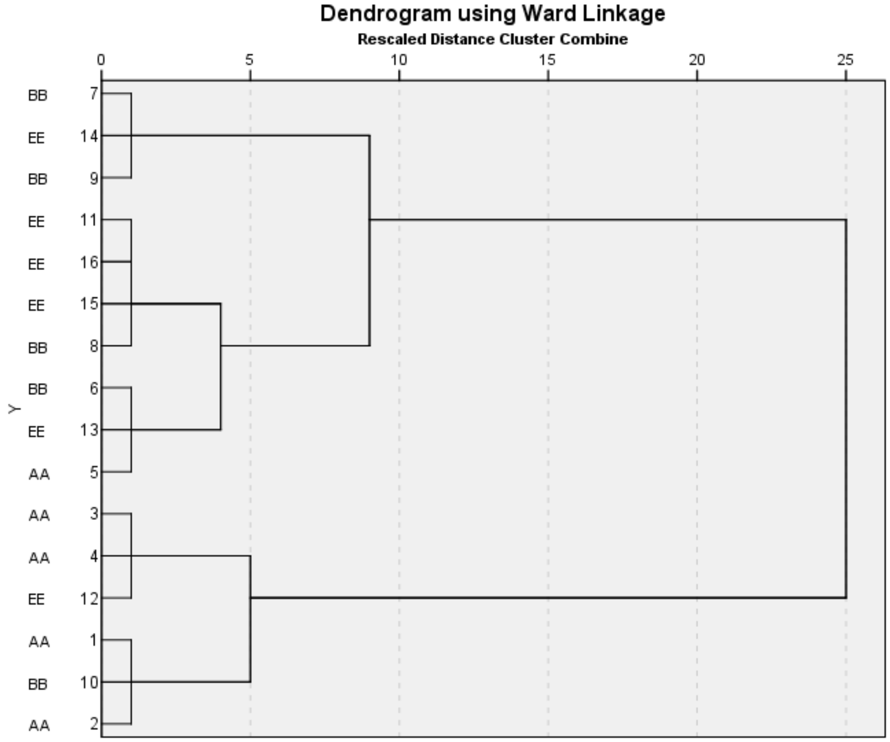
Complete Linkage (Manhattan distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



Average Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE

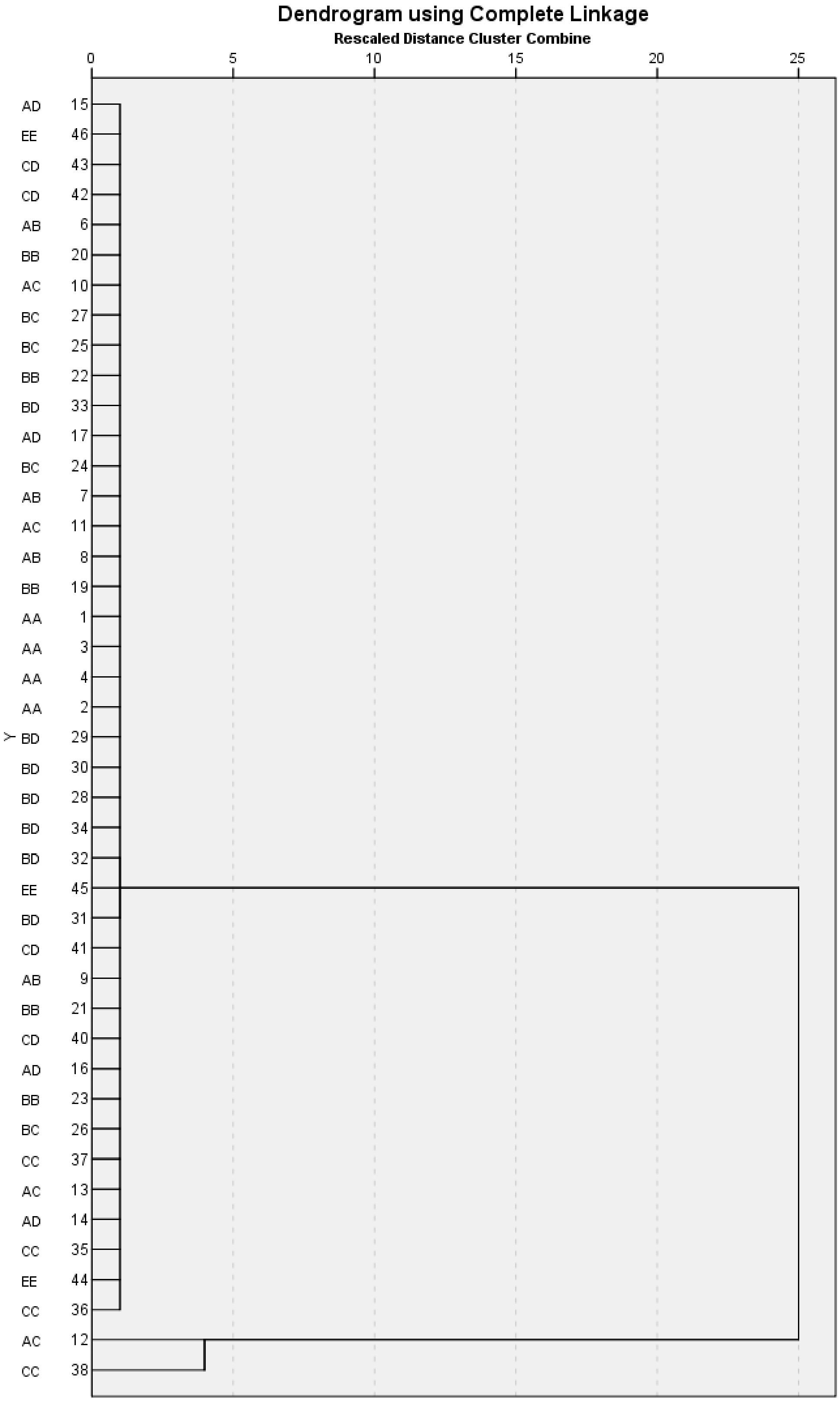


Ward Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE

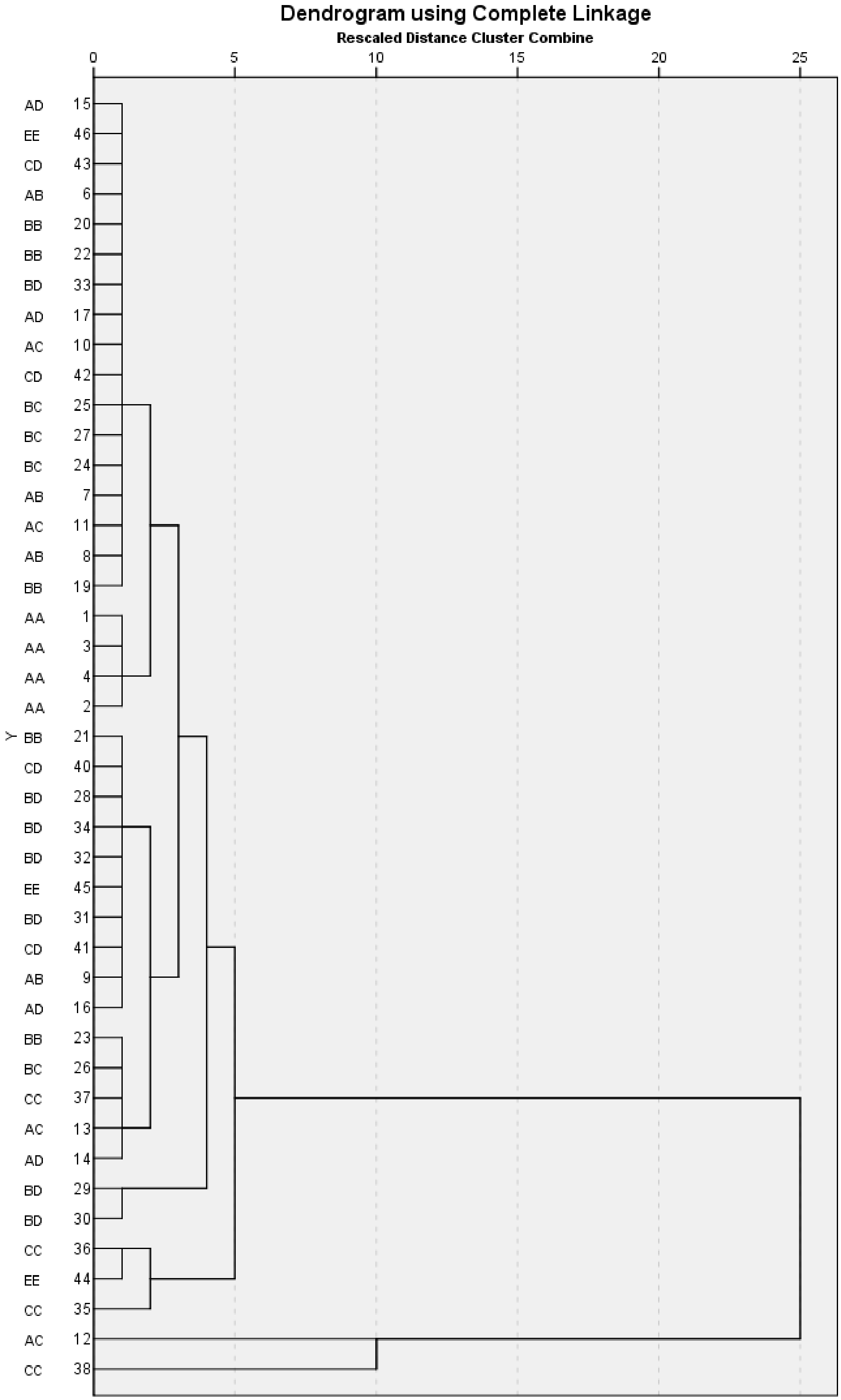


Resin - Concentration Ratio Data

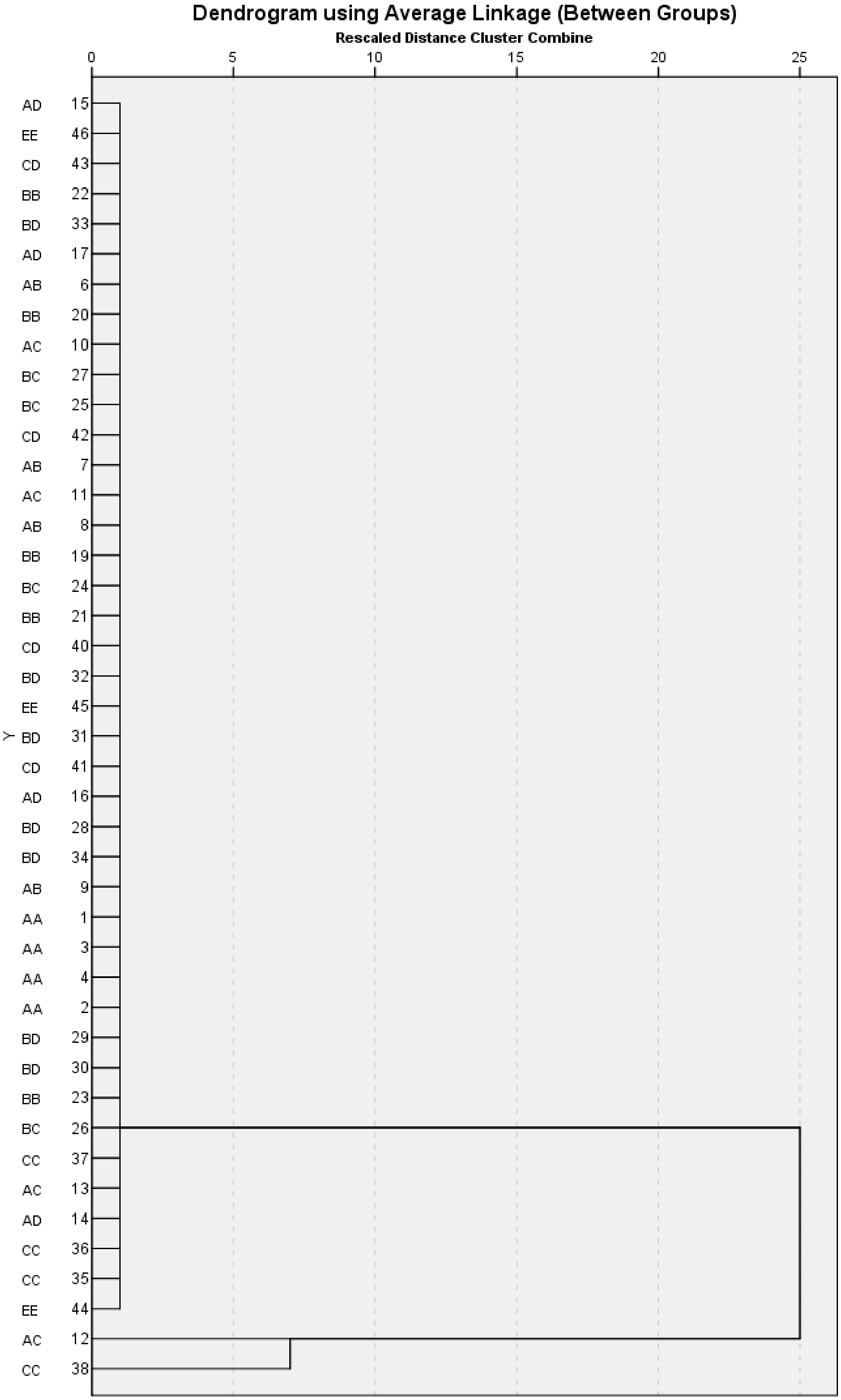
Complete Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



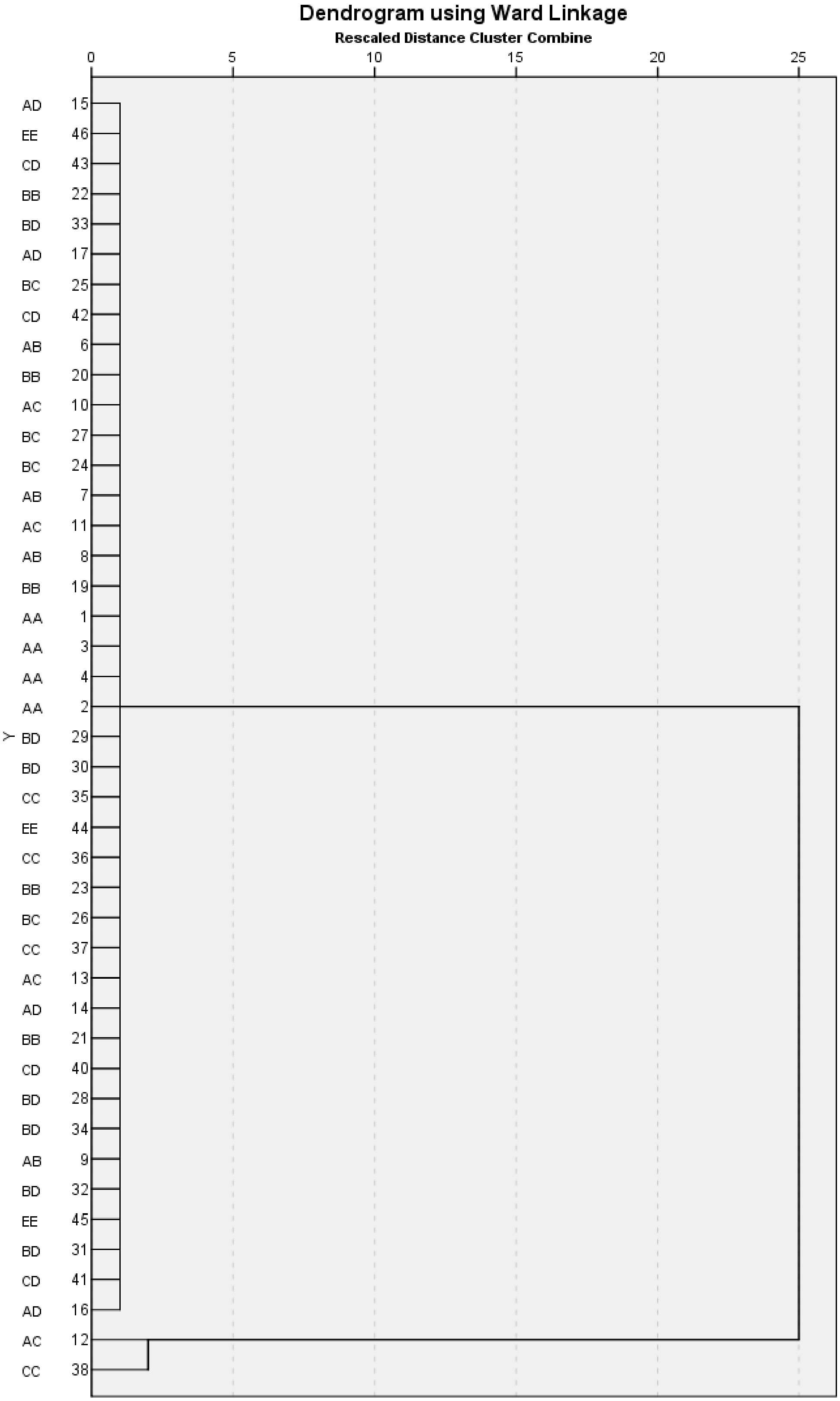
Complete Linkage (Manhattan distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



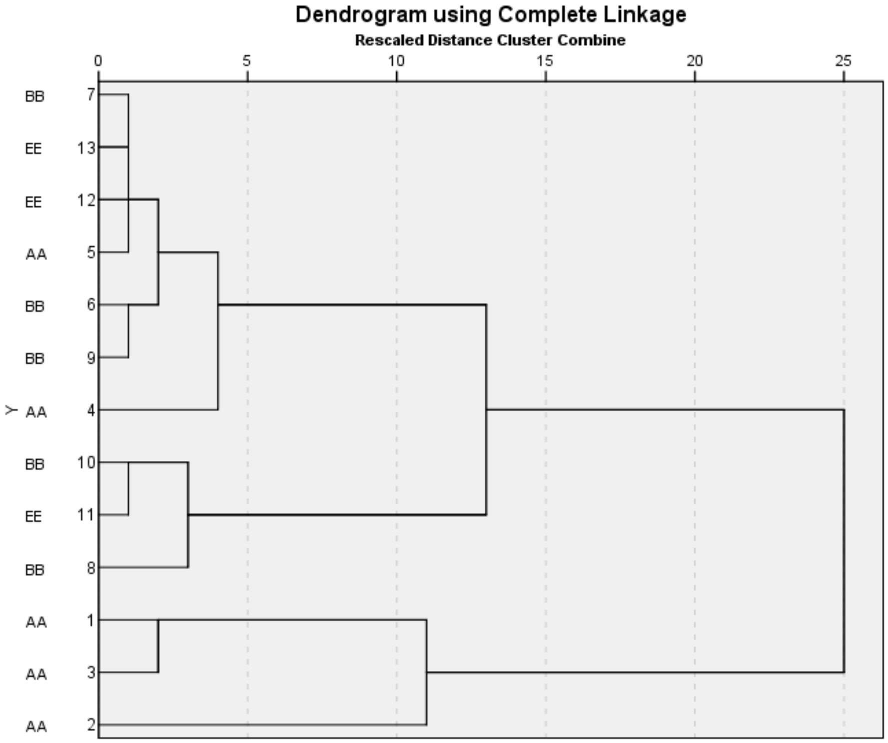
Average Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



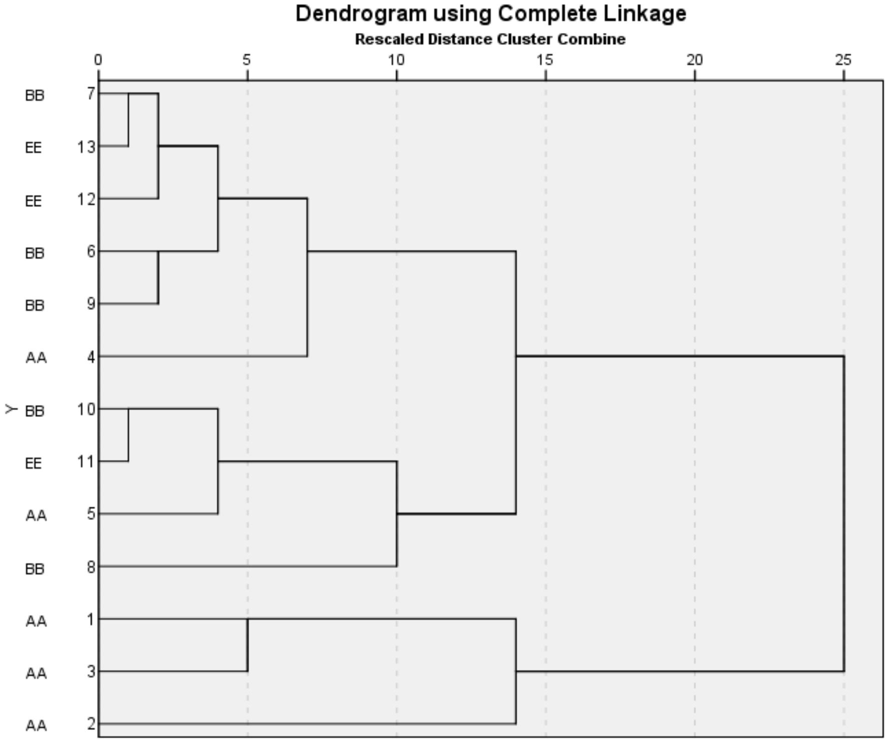
Ward Linkage (squared Euclidean distance) for Cu:Zn, Cu:Pb & Zn:Pb of all soil types



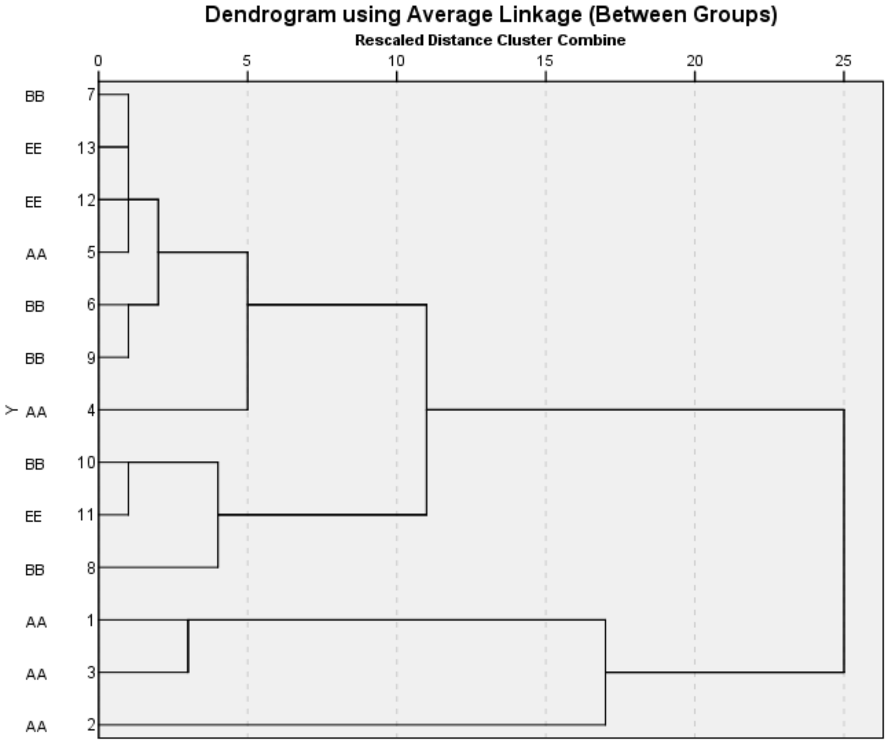
Complete Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



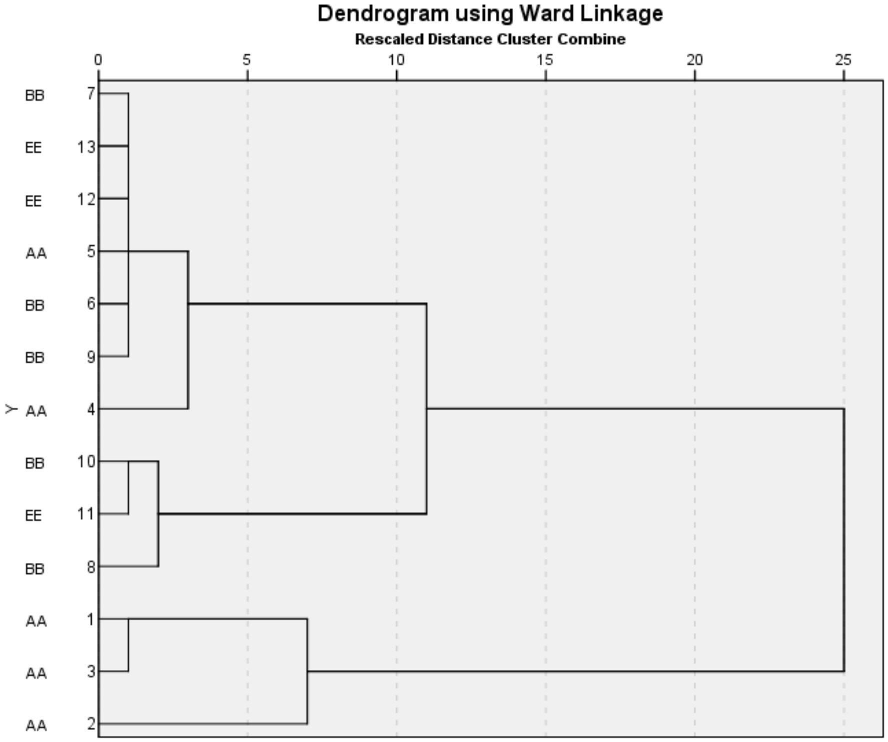
Complete Linkage (Manhattan distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



Average Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE

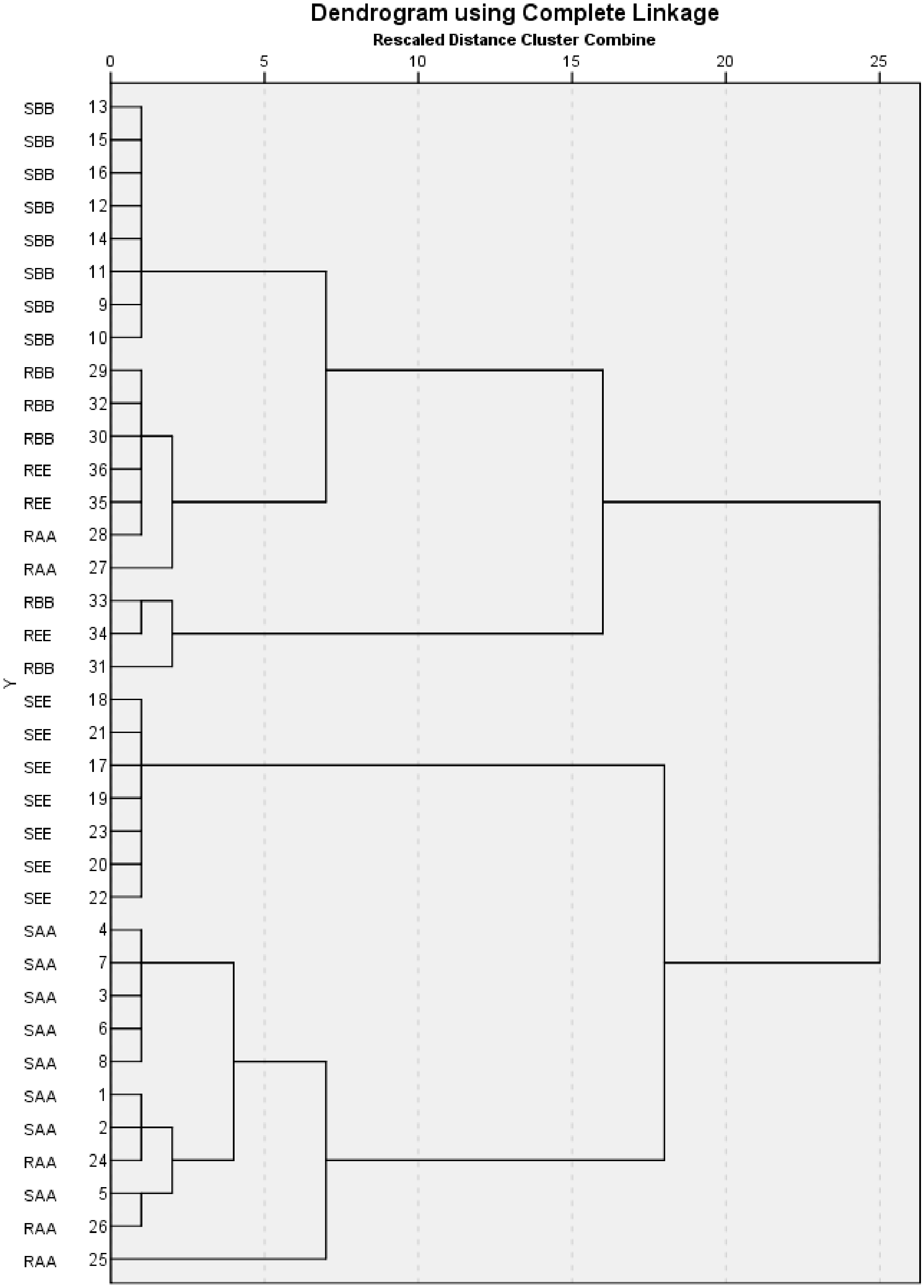


Ward Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE

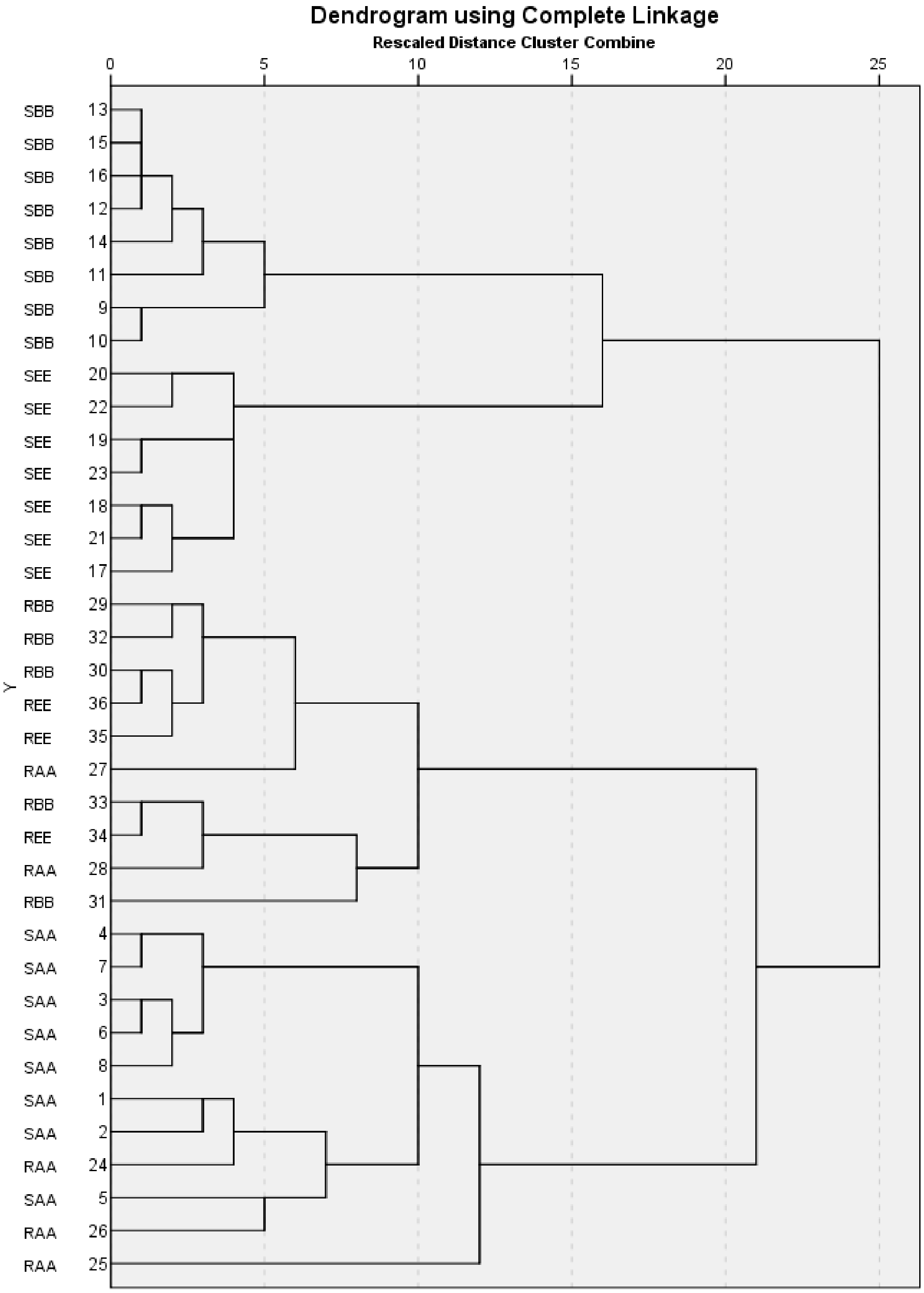


Soil & Resin – Concentration Ratio Data

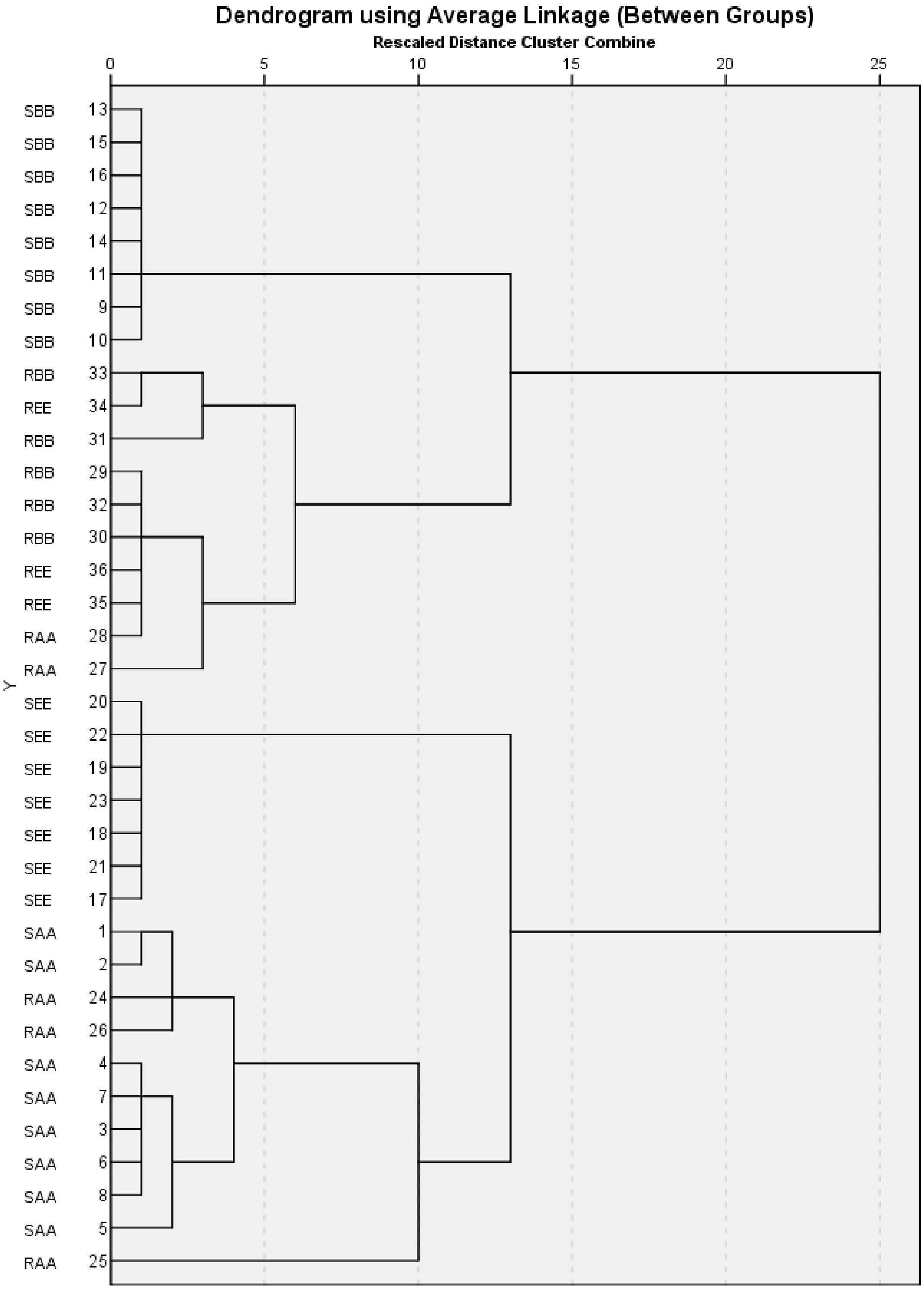
Complete Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



Complete Linkage (Manhattan distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



Average Linkage (squared Euclidean distance) for Cu:Pb & Zn:Pb of soil types AA, BB & EE



Appendix 10: Discriminant Function Analysis

Soil – Concentration Data (all soil types except BC & CC)

Soil Type	Predicted Group Membership								Total
	AA	AB	AC	AD	BB	BD	CD	EE	
AA	7	0	0	1	0	0	0	0	8
AB	0	8	0	0	0	0	0	0	8
AC	0	0	7	1	0	0	0	0	8
AD	0	0	1	7	0	0	0	0	8
BB	0	0	0	0	7	1	0	0	8
BD	0	0	0	0	0	8	0	0	8
CD	0	0	1	0	0	0	6	0	7
EE	0	0	0	0	0	0	0	7	7

91.9% of original grouped cases correctly classified.

Soil – Concentration Data (AA, BB & EE)

Soil Type	Predicted Group Membership			Total
	AA	BB	EE	
AA	8	0	0	8
BB	0	8	0	8
EE	0	0	7	7

100.0% of original grouped cases correctly classified.

Leaf – Concentration Data (all soil types except BC & CC)

Soil Type	Predicted Group Membership								Total
	AA	AB	AC	AD	BB	BD	CD	EE	
AA	0	0	0	3	0	1	1	0	5
AB	0	0	0	0	2	0	0	3	5
AC	0	0	1	2	0	0	4	0	7
AD	0	0	1	6	0	2	1	0	10
BB	0	1	0	0	1	3	0	0	5
BD	0	0	0	1	0	3	1	1	6
CD	0	0	2	1	0	1	3	0	7
EE	0	0	1	1	3	1	0	0	6

27.5% of original grouped cases correctly classified.

Leaf – Concentration Data (AA, BB & EE)

Soil Type	Predicted Group Membership			Total
	AA	BB	EE	
AA	3	2	0	5
BB	1	2	2	5
EE	1	4	1	6

37.5% of original grouped cases correctly classified.

Resin – Concentration Data (all soil types except BC & CC)

Soil Type	Predicted Group Membership								Total
	AA	AB	AC	AD	BB	BD	CD	EE	
AA	4	0	0	0	0	1	0	0	5
AB	0	1	0	0	0	3	0	0	4
AC	0	0	1	0	0	3	0	0	4
AD	0	0	0	1	0	4	0	0	5
BB	0	0	1	0	0	4	0	0	5
BD	0	0	0	0	0	6	1	0	7
CD	0	0	0	0	0	2	3	0	5
EE	0	0	0	0	0	3	0	0	3

42.1% of original grouped cases correctly classified.

Resin – Concentration Data (AA, BB & EE)

Soil Type	Predicted Group Membership			Total
	AA	BB	EE	
AA	2	2	1	5
BB	1	3	1	5
EE	0	0	3	3

61.5% of original grouped cases correctly classified.

Soil – Concentration Ratio Data (all soil types)

Soil Type	Predicted Group Membership										Total
	AA	AB	AC	AD	BB	BC	BD	CC	CD	EE	
AA	7	0	0	1	0	0	0	0	0	0	8
AB	0	7	0	0	0	0	1	0	0	0	8
AC	0	0	6	1	0	0	0	0	0	1	8
AD	2	0	1	5	0	0	0	0	0	0	8
BB	0	0	0	0	7	0	1	0	0	0	8
BC	0	0	0	0	2	6	0	0	0	0	8
BD	0	0	0	0	1	3	4	0	0	0	8
CC	0	0	0	0	0	0	0	5	0	3	8
CD	0	0	1	0	0	0	0	4	2	0	7
EE	0	0	0	0	0	0	0	0	0	7	7

71.8% of original grouped cases correctly classified.

Soil – Concentration Ratio Data (AA, BB & EE)

Soil Type	Predicted Group Membership			Total
	AA	BB	EE	
AA	8	0	0	8
BB	0	8	0	8
EE	0	0	7	7

100.0% of original grouped cases correctly classified.

Leaf – Concentration Ratio Data (all soil types)

Soil Type	Predicted Group Membership										Total
	AA	AB	AC	AD	BB	BC	BD	CC	CD	EE	
AA	1	0	0	1	0	2	0	1	0	0	5
AB	0	1	0	2	0	1	0	1	0	0	5
AC	0	0	0	1	0	2	1	0	1	1	6
AD	2	0	0	6	0	0	0	0	0	0	8
BB	1	1	0	1	0	1	0	1	0	0	5
BC	0	0	0	1	0	4	1	1	0	0	7
BD	0	0	0	1	0	2	1	0	1	1	6
CC	0	0	0	0	0	1	0	3	1	2	7
CD	0	2	0	0	0	1	0	1	3	0	7
EE	0	0	0	2	0	1	0	3	0	0	6

30.6% of original grouped cases correctly classified.

Leaf – Concentration Ratio Data (AA, BB & EE)

Soil Type	Predicted Group Membership			Total
	AA	BB	EE	
AA	4	0	1	5
BB	2	0	3	5
EE	0	1	5	6

56.3% of original grouped cases correctly classified.

Resin – Concentration Ratio Data (all soil types)

Soil Type	Predicted Group Membership										Total
	AA	AB	AC	AD	BB	BC	BD	CC	CD	EE	
AA	0	0	0	0	0	1	3	0	0	0	4
AB	0	3	0	0	1	0	0	0	0	0	4
AC	0	2	1	0	1	0	0	0	0	0	4
AD	0	1	0	0	3	0	0	0	0	0	4
BB	0	1	0	1	1	1	0	0	1	0	5
BC	0	0	0	0	2	2	0	0	0	0	4
BD	0	0	0	1	4	0	2	0	0	0	7
CC	0	0	1	0	2	1	0	0	0	0	4
CD	0	0	0	0	3	1	0	0	0	0	4
EE	0	0	0	0	2	1	0	0	0	0	3

20.9% of original grouped cases correctly classified.

Resin – Concentration Ratio Data (AA, BB & EE)

Soil Type	Predicted Group Membership			Total
	AA	BB	EE	
AA	4	1	0	5
BB	0	5	0	5
EE	0	3	0	3

69.2% of original grouped cases correctly classified.

Soil & Resin Concentration Ratio Data (AA, BB & EE)

Soil Type	Predicted Group Membership			Total
	AA	BB	EE	
AA	11	2	0	13
BB	1	12	0	13
EE	0	3	7	10

83.3% of original grouped cases correctly classified.

Soil & Resin Concentration Ratio Data (AA, BB & EE)

(Breakdown of results from table above)

Soil Type:	Matrix:	Soil Type Classification	Correct?
AA	Soil	AA	✓
AA	Soil	AA	✓
AA	Soil	AA	✓
AA	Soil	AA	✓
AA	Soil	AA	✓
AA	Soil	AA	✓
AA	Soil	AA	✓
AA	Soil	AA	✓
BB	Soil	BB	✓
BB	Soil	BB	✓
BB	Soil	BB	✓
BB	Soil	BB	✓
BB	Soil	BB	✓
BB	Soil	BB	✓
BB	Soil	BB	✓
BB	Soil	BB	✓
EE	Soil	EE	✓
EE	Soil	EE	✓
EE	Soil	EE	✓
EE	Soil	EE	✓
EE	Soil	EE	✓
EE	Soil	EE	✓
EE	Soil	EE	✓
		% Correct (soil)	100%
AA	Resin	AA	✓
AA	Resin	AA	✓
AA	Resin	AA	✓
AA	Resin	BB	✗
AA	Resin	BB	✗
BB	Resin	BB	✓
BB	Resin	BB	✓
BB	Resin	AA	✗
BB	Resin	BB	✓
BB	Resin	BB	✓
EE	Resin	BB	✗
EE	Resin	BB	✗
EE	Resin	BB	✗
		% Correct (resin)	54%