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Phytoremediation strategies and nutrient availability of the arsenic contaminated tailings in Devon Great Consols

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Abstract

Improper waste disposal and management of mining tailings in the past has led to heavy and extensive contamination of land making it prone to erosion, limited biodiversity and general environmental damage that poses serious risks for the environment and consequently, directly or indirectly, to human health.

This study focuses on two heavily contaminated (As, Cu, Fe) and toxic waste heaps (tailings) at the Devon Great Consols (DGC) located in the region of the Tamar Valley in the Southwest of England. One of these heaps has been reprocessed at the beginning of the 20th century and has been slowly recolonised by some species such as *Calluna vulgaris* and *Agrostis capillaris*. The other heap has not been re-worked and, after 100 years, it remains largely unvegetated. This provides an excellent opportunity to investigate the environmental factors and soil conditions that have led to the recolonisation of some areas as well as an opportunity to assess the feasibility of phytoremediation in the stabilisation of the heaps; as other methods of remediation such as removal, solidification and chemical oxidation amongst others, have the disadvantage of being very costly and the risk of contamination of other environments increases when treatment is done ex-situ.

For this study nineteen (19) soil samples and fourteen (14) plant samples were collected and analysed for arsenic (As) by inductively coupled plasma optical emission spectrometry (ICP-OES). Additionally, the soil samples were analysed for organic matter, pH and nutrient content (P, K, Ca, Mg and Na) by ICP-OES and for NO_3-N by segmented flow analysis ("Skalar").

Results suggest that the main growth limiting factors are severe nutrient deficiency due to low pH values, low organic matter content and a coarse soil texture that limits water retention. The vegetation community at the DGC appears to have successfully developed As tolerance mechanisms and therefore As toxicity is unlikely to be the growth limiting factor in itself. Additionally, soil amendment strategies are proposed to encourage vegetation growth and thus increase stability.

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Tiivistelmä

Kaivosjätteen puutteellinen käsittely ja hävittäminen menneinä vuosina ovat johtaneet maaperän vakavaan ja laajaan saastumiseen, joka on jättänyt maaperän alttiiksi eroosiolle, rajoittuneelle monimuotoisuudelle ja yleisille ympäristöhaitoille, jotka uhkaavat vakavasti ympäristöä ja tämän seurauksena suorasti tai epäsuorasti ihmisten terveyttä.

Tässä opinnäytetyössä keskitytään kahteen vakavasti saastuneeseen (As, Cu, Fe) ja myrkylliseen kaivosjätekasaan, jotka sijaitsevat Devon Great Consols –kaivosalueella (DGC) Tamar-laaksossa Etelä-Englannissa. Toinen jätekasoista on uudelleenkäsitelty 1900-luvun alkupuolella ja jotkin kasvilajit, kuten *Calluna vulgaris* ja *Agrostis capillaris*, ovat hitaasti alkaneet rekolonisoida aluetta. Toista jätekasaa ei ole uudelleenkäsitelty ja vielä 100 vuoden jälkeenkin se on pysynyt pääasiassa kasvittomana. Nämä olosuhteet tarjoavat erinomaisen tilaisuuden tutkia niitä ympäristötekijöitä ja maaperän ominaisuuksia, jotka ovat johtaneet joidenkin alueiden rekolonisaatioon, ja arvioida fytoremediaation toteutettavuutta kaivosjätekasojen stabilisoinnissa. Muilla puhdistusmenetelmillä, kuten poistolla, kiinteytyksellä ja kemiallisella hapettamisella, on haittapuolina kalleus sekä muun ympäristön kontaminaatioriski, kun maaperää käsitellään ex-situ.

Tätä tutkimusta varten kerättiin yhdeksäntoista (19) maaperänäytettä ja neljätoista (14) kasvinäytettä, joiden arsenikkipitoisuudet analysoitiin plasmaemissiospektrometrilla (ICP-OES). Lisäksi maaperänäytteistä määriteltiin orgaanisen aineen määrä sekä pH ja analysoitiin ravinnesisältö (P, K, Ca, Mg ja Na) ICP-OES-spektrometrilla sekä NO3-N Skalar- autoanalysaattorilla.

Tulokset viittaavat siihen, että matalasta pH:sta johtuva vakava ravinnepuutos, matala orgaanisen aineen määrä sekä maan vedenpidätyskykyä rajoittava karkearakenteinen maaperä ovat pääasiallisia kasvua rajoittavia tekijöitä. Devon Great Consols –kaivosalueella esiintyvä kasvillisuus näyttäisi kehittäneen onnistuneesti arsenikkitoleranssimekanismeja ja siten on epätodennäköistä, että arsenikki itsessään olisi kasvua rajoittava tekijä. Lisäksi tutkimuksessa tuodaan esille maaperän korjausehdotuksia, joilla kasvillisuuden kasvua voidaan edistää ja sitä kautta parantaa kaivosjätekasojen stabiiliutta.

Asiasanat (avainsanat)

Phytoremediation, tailings, arsenic, nutrients, availability

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1 INTRODUCTION

Intensive metal mining during the last centuries has left a legacy of not only heavily contaminated sites around the world but also of sites prone to erosion, limited biodiversity and environmental damage that ultimately can affect human health.

The southwest of England is well-known for its mining heritage and geology. Mineral deposits in the area led to exhaustive mining of lead (Pb), tin (Sn), copper (Cu) and particularly arsenic (As) during the 18th and 19th centuries (Fig. 1) (Mining Heritage Project, 2010). The area became the biggest supplier of As in the world leaving behind large amounts of waste high in As and metal concentrations, which have been eroded, atmospherically dispersed to neighbouring areas, and hydrogeologically carried into water systems (Fig. 2) (Mighanetara, 2008; Green, 2012). In the Tamar Valley at the Devon Great Consols (DGC) mine site, waste heaps have been left untouched for almost 100 years and little obvious biological recolonisation has occurred. However, some indigenous species of e.g., bryophytes, grasses and heathers have adapted to these harsh environments providing an outstanding opportunity to study their feasibility in the stabilisation or remediation of As contaminated waste heaps by understanding the soil conditions in which these plants are able to grow.

Figure 1. Devon Great Consols (DGC) waste heaps. ***Source: Author, 2014***

The purpose of this research is to investigate the degree of arsenic contamination, its bioavailability and bioaccumulation (if any) in the indigenous vegetation of the tailings at the DGC site. Although the site is known to be highly contaminated with metals and metalloids, a variety of plant species and other biota appear to be developing successfully on the area, particularly in one of the heaps.

For this reason, the main aim of this research is a) to understand what is the growth limiting factor (if any) that determines the slow rate of vegetation recolonisation at the site, b) to investigate the use of local vegetation in phytoremediation strategies and their feasibility at the site, and finally, c) use gathered information in order to propose a strategy to improve the containment of contaminants and the stability of the tailings at the DGC. Thus, decrease future risks of contaminated runoff, percolation and erosion and as a result minimise pollutant migration to the Tamar River and reduce contamination pathways to visitors.

In order to meet the objectives of this research, it is vital to measure the nutrient availability in addition to arsenic bioavailability, as it has been assumed that the high concentration levels of arsenic is the growth limiting factor for plants at the site, yet this might not be the case.

Figure 2. Erosion and leachate at the DGC. ***Source: Author, 2014***

2 LITERATURE REVIEW

2.1 Geological setting

The Tamar valley area is located in South West of England. In this region approximately 300 million years ago granitic batholithic intrusions metamorphosed rocks from Upper Devonian and Lower Carboniferous periods, filling fractures in the country rock producing thus lodes and metalliferous mineral veins rich in lead, tin, copper and arsenic ores. Devon Great Consols (DGC) is located north of a significant mineral vein in Gunnislake, where valuable products such as copper (Cu) and arsenic (As) were located. (Klinck et al., 2005 & Rieuwerts et al., 2014)

2.2 Site description

2.2.1 History

The DGC is the result of the merging of five neighbouring mines (Fig. 3) on the east bank of the River Tamar in Devon. Copper ore ($CuFeS_2$) was the main product at the beginning stages of the mine; however arsenic production took over with an output of over 1150 tons per year between 1848 and 1909. (Dines, 1956)

Arsenic (As) was extracted as arsenopyrite (FeAsS) as it was the only economically viable form of As (Dines, 1956). The process of As extraction was performed in the Calciner complex, whither the ore was brought, mechanically crushed, washed and sieved. This was followed by a two-stage roasting process (calcining) in pre-heated furnaces at 538-593 °C, where arsenic and sulphur sublimated. These arsenic-rich fumes from the furnace were diverted and precipitated on a labyrinth condenser (Fig. 4) as arsenic oxides (As_2O_5 & As_2O_3) and were scraped from the walls of the labyrinth by workmen with limited protection. (Haswell, 1983 & Klinck et al., 2005)

The residues of the mined materials (a.k.a tailings, waste heaps, waste tips) after extraction processes were dumped on a slope on site facing the Tamar River.

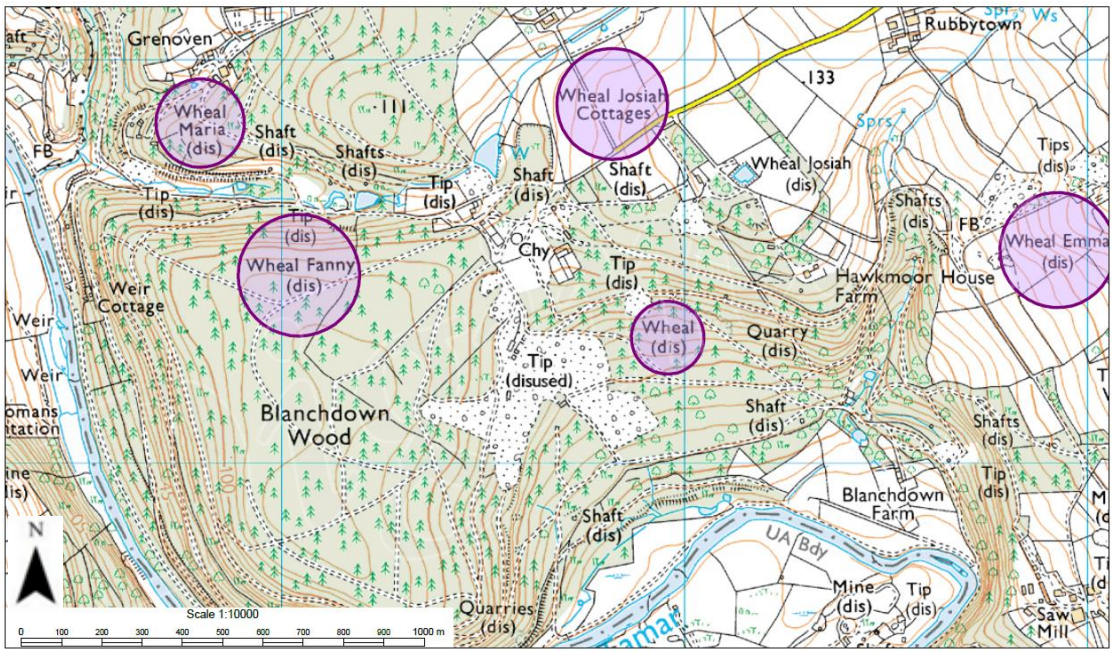

Figure 3. Map of Devon Great Consols showing the five mines in purple. ***Source: created by author based on Digimap database, 2015.***

Figure 4. The remains of the labyrinth condenser at DGC. ***Source: Author, 2014***

2.2.2 Current state

Presently, the land covers ca. 67.6 ha (Kavanagh et al., 1997) and it is mainly covered with mixed plantation forest, aside from the main works and tailings which are at some areas lightly vegetated with mainly bryophytes, grass (*Agrostis capillaris*) and

heather (*Calluna vulgaris*). The Tamar Valley Mining District receives approximately 1000 – 1400 mm of rainfall annually (Met Office, 2010); the runoff and drainage of the area are discharged into the Tamar River, which surrounds the Devon Great Consols flowing east and south of the complex (Fig. 3) towards the English Channel.

The site is currently owned by the Duke of Bedford and has been a World Heritage Site since 2006 (UNESCO, 2014). Consequently, it has undergone some redevelopment for tourism and recreation activities (Mining Heritage Project, 2010), which has resulted in the alteration of surface drainage pattern throughout the site, leading to dispersal and transportation of contaminants such as Al, As, Cd, Cu, Ni, Pb and Zn into the River Tamar (Green, 2012).

This study focuses on the two waste heaps (Fig. 5) with the approximate coordinates 50°32'16"N, 4°13'14"W and 50°32'13"N, 4°13'09"W, near the Calciner Complex. The Upper waste heap (Northern, referred as Top Heap) is composed of coarse grained material and is larger than the bottom waste heap (South heap, referred as Bottom Heap). The Bottom Heap is finer grained material as it was reworked in 1902-1925 to re-extract arsenic using more sophisticated processes (Mighanetara, 2008).

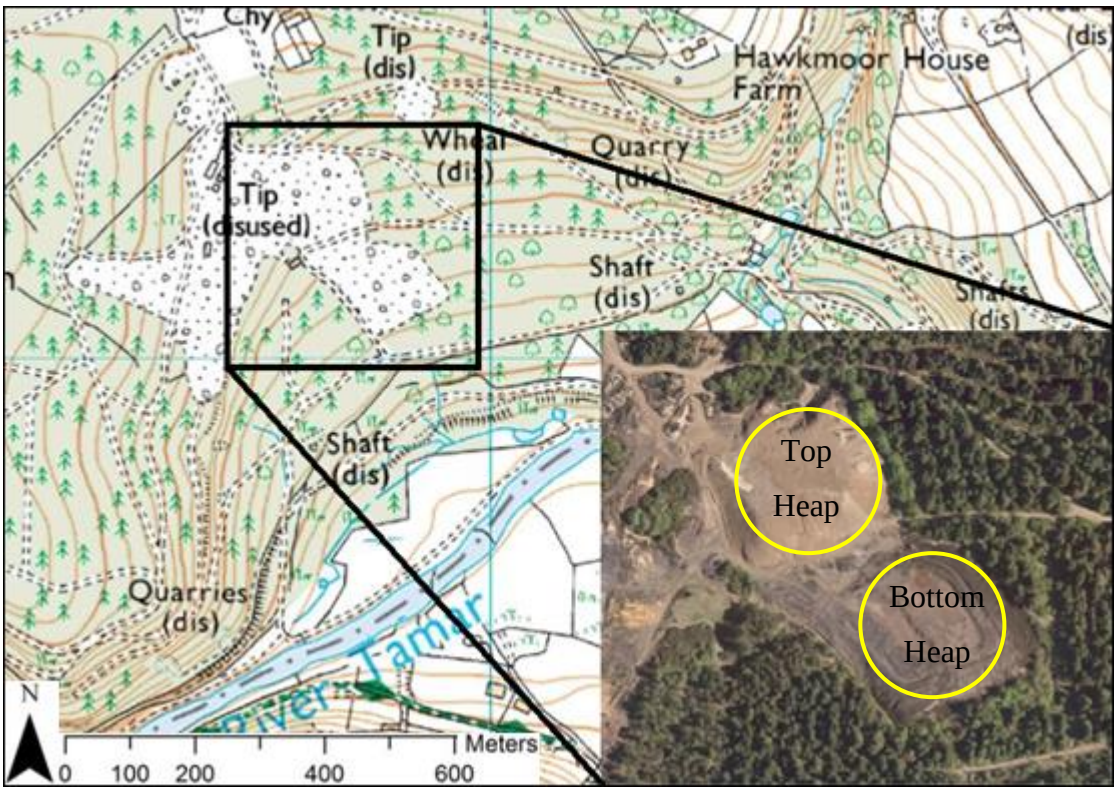

Figure 5. Aerial view of the Devon Great Consols waste heaps (circled in yellow).
Source: Created by author from Google maps & digimap database, 2015 .

The stability of the waste heaps has been comprehensively studied in 2008 by Mighanetara and the relevant findings are shown summarised in Table 1. In this study it was found that the Top Heap, unlike the Bottom Heap, was fairly permeable, permitting the infiltration of water and enhancing oxidation.

Table 1. Properties of the waste heaps. ***Data from: Mighanetara, 2008***

	Top Heap	Bottom Heap
Volume (m^3)	160 000 - 192 000	86 500 - 110 000
surface pH	4.7	5.4
Sand %	~39	No Data
Gravel %	~34	17
Silt %	~25	72
Clay %	< 2	11
Texture	Coarse sandy material	Homogeneous coarse silt, fine sand
Density g/cm^3	1.5	1.3
Erosion rate (cm/annum)	4.0 ± 0.5	Non observed
Export of material (m^3/annum)	280 - 300	Non observed

Additionally, it was suggested that both heaps are geotechnically stable. (Mighanetara, 2008) However, recently some areas in the Bottom Heap have shown signs of instability i.e., a deep gully on the side facing the River Tamar has formed (Fig. 6).

Figure 6. Deep gully in the Bottom heap. ***Source: Author, 2014***

The spatial distribution of arsenic in the DGC environment has been studied by the British Geological Survey (Klinck et al., 2005). The study reports values for As and Fe concentrations at the DGC area shown in Table 2. Additionally, mean pH values of

3.88 for the soils within the mine area were reported, whereas Mighanetara (2008) reported values of 4.7 - 5.4 (Table 1).

Table 2. Summary of As concentrations. ***Data source: Klinck et al., 2005***

		Tailings	DGC soils	Outside DGC soils (mineralisation)	Background soils (No mineralisation)
As [mg/kg]	Min	1279	249	252	17
	Mean	30842	8081	2019	82
	Median	19170	2105	1695	71
	Max	204478	68924	4482	172
	Std. Dev.	44685	12753	1481	57

The background soil values are from areas unaffected by neither mineralisation nor mining near the village of Bere Alston. The outside DGC soil values come from an area located 2.5 km southwest of the DGC, where mineralisation occurred but without mining. (Klink et al., 2005)

2.3 Arsenic biogeochemistry

Arsenic is a naturally occurring metalloid which belongs to the nitrogen family (Group Va) and was traditionally used in paints, dyes and due to its toxicity as pesticide. However, recently it has been rather used in photoelectric devices and as an alloy to improve corrosion and tensile strength (Kabata-Pendias, 2011). According to Environment Agency (2007) the As concentration in English rural soils ranges from 1.37 to 143 mg/kg with a mean value of 13.9 mg/kg. Its biogeochemistry is fairly complex as it undergoes adsorption and desorption processes, as well as reduction-oxidation and acid-alkaline reactions and biomethylation.

Arsenic bonds easily with oxygen and metals such as Fe, Pb and Cu and its most stable inorganic forms are as arsenites As^{III} (AsO_3^{3-}) and arsenates As^{V} (AsO_4^{3-}). Moreover, the most abundant As mineral is arsenopyrite (FeAsS) and once exposed to the environment, As minerals are easily soluble. Furthermore, the toxicity of As is associated with its solubility; Arsenite (As^{3+}) is more soluble than arsenate (As^{5+}) and thus more toxic and mobile. (Kabata-Pendias, 2011) However, studies agree that arsenate solubility also increases as pH increases. (Fitz and Wenzel, 2002)

Arsenic migration in soil is hence essentially limited by sorption on clays, organic matter and hydroxides. (Violante et al., 2008 & Ghosh and Bhattacharya, 2004) Additionally, the redox potential and the pH of soil appear to be the most significant parameters controlling As sorption, as they facilitate the bonding of As ions with iron

(Fe) and aluminium (Al) producing Fe and Al-oxyhydroxides (Bissen and Frimmel, 2003). Moreover, aerobic conditions promote the oxidation of As to less toxic compounds (Bissen and Frimmel, 2003), which might be favourable for biotic recolonisation.

Zandsalimi et al., (2011) ponder how improbable it is that plants play a major role in geochemical cycling of arsenic as only a limited fraction of As is available to them due to its strong bonds with Fe and Al oxides. On the other hand, evidence shows that anaerobic methanobacterium could methylate arsenate to dimethylarsine, suggesting that microorganisms present in soil may play a role in the toxicity and availability of arsenic. (Porter and Peterson, 1977)

2.4 Bioavailability and plant uptake of As

Bioavailability describes the portion of the total concentration of a contaminant that is available for adsorption by biological systems (Paustenbach, 2000). Likewise, the term bioaccessibility is used to describe the fraction that is soluble solely by the digestive system. (Klink et al., 2005)

Notwithstanding that arsenic (As) concentrations in soils at the DGC are very high, it is their chemical and mineral state what determines their bioavailability or biological exposure potential. Uptake of arsenic is therefore influenced by factors such as arsenic concentration and its oxidation state (solubility), species-specific uptake mechanisms, the characteristics of the environment (e.g. organic matter) and the abundance of competing ions e.g., phosphates and sulphates. (Wang and Mulligan, 2006 & Hartley et al., 2009) Additionally, iron (Fe) oxyhydroxides crystallinity is believed to represent a significant influence of As bioaccessibility and bioavailability (Kavanagh et al., 1997; Palumbo-Roe and Klinck 2007 & Hartley et al., 2009). The median fraction of As available to organisms at the DGC is 408 mg/kg (Klinck et al., 2005). Moreover, recent studies (Rieuwerts et al., 2006; Palumbo-Roe and Klinck, 2007 & Rieuwerts et al., 2014) discuss in-depth the soil and mineralogical factors that influence As bioaccessibility at the site. Klinck et al., (2005) estimate the bioaccessible fraction to be ~19%, which is close to the 15% found by Palumbo-Roe and Klinck (2007). Furthermore, Kavanagh et al., (1997) found that the proportion of As soluble in water of topsoils was 0.02 – 1.2% and that ≈ 93% of total As was found as Fe-organic and residual fractions. On the other hand, Palumbo-Roe et al., (2007) report that only

0.0004% of the total As at the DGC from the tailings is water soluble and that higher arsenic mobility ($As_{water\ soluble}$ 3.7% of total arsenic) was found in the alkaline waste material near the calciner. These findings suggest that As migration is most likely due to surface erosion and atmospheric dispersion.

Experiments conducted on the As tolerance of grass suggest that the major form of As available to plants is arsenate, where 60 – 94% of water soluble As is in the form of arsenate. (Porter and Peterson, 1977)

2.4.1 Uptake mechanisms and effects

The uptake, translocation and accumulation, if any, of As into plants depend on the species and habitat. Arsenic is however considered readily bioavailable (Prasad, 2003) and although plant roots can additionally secrete H^+ ions which can solubilise cationic metals in soil into solution, anionic As mobility is unaffected. (Ali et al., 2013)

Studies suggest that As is taken up by plants through the apoplast (Chen et al., 2005). As a result of arsenate showing similar chemical properties with phosphates, arsenate might be carried by the phosphate transport system and compete with phosphate during the production of adenosine triphosphate (ATP) causing adenosine diphosphate arsenate (ADP-arsenate) complexes, which in turn alter the balance of biochemical equilibria (Park et al., 2012). In the cytoplasm peptides, such as glutathione, reduce arsenate to arsenite, allowing it to bind with enzymes and proteins hindering cellular functions which lead to oxidative stress and thus DNA and cell damage (Slejkovec et al., 2010) and ultimately apoptosis (cell death). As a result of this, physical manifestations of cell damage can be observed as e.g., growth reduction, leaf wilting (loss of rigidity due to loss of tugor pressure), cell plasmolysis, violet coloration and root discoloration. (Kabata-Pendias 2011)

There appears to be some disagreement in whether phosphorus concentration in soils affects the plant uptake of arsenic (Otte et al., 1990; Wang et al., 2002; Cao and Ma, 2004; Poynton et al., 2004; Huang et al., 2007; Hartley et al., 2009; Vetterlein et al., 2009 & Karczewska et al., 2013).

A study (Karczewska et al., 2013) carried a pot experiment to evaluate the modification of soil with phosphate and sewage sludge. They suggested that application of

phosphates and sewage sludge increased solubility of As in soil but without any significant uptake change of As. However, sewage sludge appeared to have a favourable impact on the growth of grass (*Holcus lanatus L.*). Other studies (Vetterlein et al., 2009) suggest that there is no difference or that differences are only significant if P concentrations vary largely. On the other hand, there are studies that suggest a clear inverse correlation (P increase leads to As uptake decrease), suggesting that the amount of phosphorus bioavailable in the soil will determine the effects of arsenic on plants. (Otte et al. 1990; Poynton et al., 2004; Wang et al., 2002) Hartley et al., (2009) suggested that arsenic mobility was significantly related to phosphate.

A recent study in 2013 by Bolan et al. reviews this disagreement and suggests that P addition competes for As absorption by plant roots decreasing its arsenic uptake. However, in soils P addition facilitates desorption and bioavailability of As (Fig. 7).

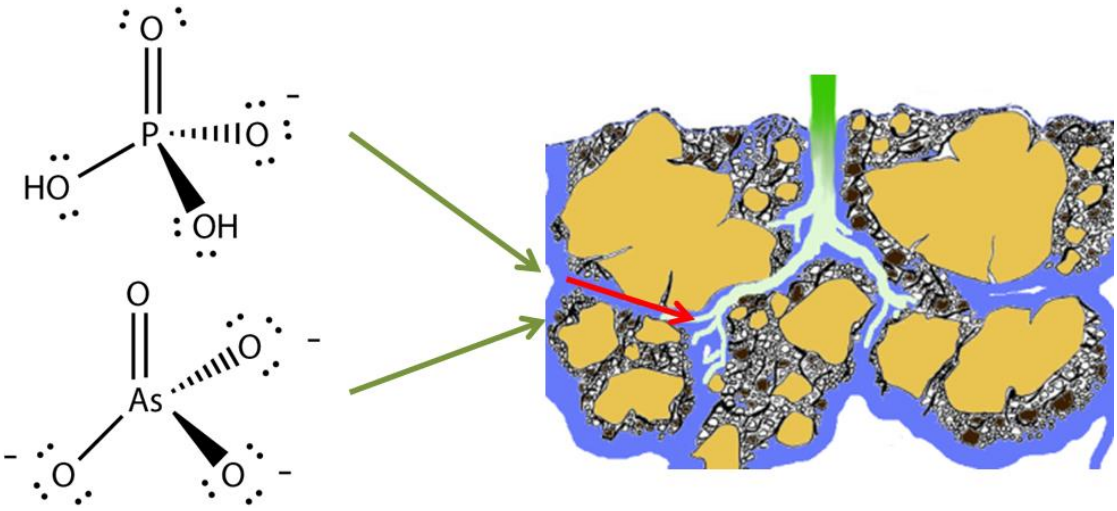

Figure 7. Arsenate ions competing with phosphate ions for adsorption by soil particles and absorption by roots. ***Created by Author. Adapted from: Bolan et al, 2013***

Several plant species have been shown to accumulate As to toxic degrees or show resistance to various metals and metalloids. Species known to accumulate As are, amongst many others:

- *Agrostis castellana* and *A. delicatula* (De Koe, 1994)
- *Agrostis capillaris* and *Deschampsia cespitosa* (Meharg et al., 1991)
- *Calluna vulgaris* (Sharples et al., 2000)
- Conifers (more specifically *Pseudotsuga menziesii*) have been shown to uptake As in high quantities (Porter and Peterson, 1975).
- *Holcus lanatus* (MacNair et al., 1987)

- *Leymus cinereus* (Knudson et al., 2003)
- *Pteris vittata* (hyperaccumulator) (Vetterlein et al., 2009)

2.4.2 Exclusion and tolerance mechanisms

Due to the high concentrations of arsenic found at Devon Great Consols, many plants have devised strategies to cope with these toxic levels of arsenic. The primary two strategies used are exclusion and accumulation.

Exclusion occurs by actively reducing the uptake of arsenic and by moving toxic compounds out of the cell (efflux). Uptake is minimised by suppressing the function of proteins (aquaporins) in the root membranes, thereby reducing the intake of phosphate and arsenate and at the same time, efflux is actively excreting arsenite from the cell back into the soil through ion channels (Zhao et al, 2009). These strategies allow keeping arsenic concentrations relatively low.

Other studies (Sharples et al., 2000; Fomina et al., 2005 & Zhang et al., 2015) suggest that the symbiotic relationships of plants (e.g., heathers and > 80% of land plants) with arbuscular mycorrhizas fungi (AMF) e.g., *Hymenoscyphus ericae* increase the tolerance of plants to As. Furthermore, it was suggested that P concentrations in plants increased whereas As concentrations decreased thanks to AMF. Moreover, AMF is very likely involved in methylating inorganic As into less toxic organic dimethylarsenic acid, suggesting the potential use of this mechanism in bioremediation (Zhang et al., 2015). Fomina et al., (2005) have shown the possibility to solubilise metals and create a tolerance to them with AMF. Additionally, it has been demonstrated that the microfungus (*Microascus brevicaulis*) is able to produce trimethylarsine, which was proven to be exceptionally far less toxic than inorganic arsenic (Porter and Peterson, 1977 & Bentley and Chasteen, 2002). Thus, mycorrhizal fungi growth enhancement might pose an advantage in terms of stabilisation and perhaps the production of less toxic arsenic compounds by e.g., methylation. A study investigating the phytoremediation consequences of applying a consortium of rhizobacteria (2% v/v) in addition to nitrogen-phosphate-potassium (NPK) fertilizer (0.02% w/w), suggested that it could ease the toxic effects of As and thus increase the effectiveness of phytoremediation (Titah et al., 2013).

Accumulators, on the other hand, appear to translocate arsenic into their shoots and leaves and store it in cell vacuoles away from proteins leading to limited damage. Some accumulators depend on the bonding of peptides (phytochelatins) to arsenite ions to prevent them from bonding to more metabolically important proteins (Zhao et al, 2009). Porter and Peterson (1977) argue that grass species have evolved tolerance to arsenate ions rather than to arsenite ions, since the former does not affect important proteins. This tolerance to arsenate is believed to derive from the fact that its behaviour resembles that of phosphates (PO_4^{3-}) and therefore it is able to take part in phosphate biochemical mechanisms of the plant (Porter and Peterson, 1977).

2.5 Remediation

Contaminated land is an environmental risk issue (Petts et al., 1997); hence remediation is carried out generally for the following reasons (Nathanail and Bardos, 2004):

- Regulatory requirements
- Protection of the environment and ultimately human health
- Land availability for reuse
- In order to avoid potential liabilities
- Good environmental practice

Especially since the redevelopment of the DGC, leachates (e.g., acid drainage) and the dispersal of toxic contaminants represent a significant source of contaminants that can impose harmful effects not only for the local area but also for the Tamar River and ultimately to human health (Green, 2012).

Commonly, remediation methods concentrate in either source control or migration control. Source control is the prevention of contaminant migration by either containment or neutralisation, whereas migration control concentrates in minimising the impact of the pollution to the environment (Johnson and Hallberg, 2005). Source control techniques such as removal, solidification and chemical oxidation amongst others have been extensively used due their efficacy and wide operational range. However, they have the disadvantage of being costly, usually can be hazardous and may produce secondary waste; especially when treatment is performed ex-situ. (Favas et al., 2014)
Remediation techniques such as liming have been used in the past in an effort to increase the soil pH and minimise acidic drainage; however high metal concentrations can coat limestone surfaces and increase neutralization time (Sun et al., 2000). Addi-

tionally, higher pH levels appear to increase the water solubility of As (Palumbo-Roe and Klinck, 2007).

2.6 Phytoremediation strategies

Phytoremediation is the usage of plants and their related microorganisms for the treatment or containment of environmental contaminants. (Favas et al., 2014) Therefore, it has the ability of minimising environmental and human exposure to contaminants by decreasing soil erosion, runoff, dust generation and migration as well as minimising skin contact (Nathanail and Bardos, 2004).

In order to design an effective phytoremediation approach, the nature of the contaminants (toxicity, salinity, metals), the characteristics of suitable and available plants as well as the nature of the soil, such as its physical and chemical properties, should be considered. Physical properties include soil water content, temperature and texture, whereas chemical characteristics include pH and the availability of macronutrients and micronutrients.

Table 3. Common advantages and limitations of phytoremediation. ***Source: Raskin and Ensley, 2000, pp. 16***

Advantages	Disadvantages
• Low operational and capital costs • Permanent treatment solution • In-situ remediation: lower risks of further contamination, less invasive • Improve hydraulic and soil stability • Public acceptance; aesthetically pleasing	• Slower than other alternatives • Seasonally dependent • Soil phytoremediation applicable only to surface soils • Complex • Lack of recognized economic performance data

The low costs associated with phytoremediation have been an important driving factor of this technology, as it can provide an economically viable solution to the remediation of some sites. However, phytoremediation carries some limitations (Table 3) such as being slower than other treatment techniques and usually applicable only to topsoils. (Raskin and Ensley, 2000, pp. 16) Nevertheless, several different strategies of phytoremediation have been developed and are described briefly in sections 2.6.1-2.6.5. In situ phytoremediation strategies in the past have been focused in both the unaided and aided stabilisation of tailings. (Antonovics and Bradshaw, 1970; Hart and Luckai, 2010 & Perez-Sanz et al., 2013) Additionally, revegetation trials can be conducted either in or ex situ with different kind of amendments to the soil to reduce metal and metalloids mobility and bioavailability. Hart and Luckai (2010) conducted amendments for alkaline, coarse textured former copper-mine tailings with fertilizer,

wood chips, wood mulch, wood ash, charcoal and natural forest soil. Additionally their vegetation mix contained grasses and nitrogen fixing species. It was found that fertilizer and natural forest soil amendments improved plant growth significantly while other treatments did not have any beneficial effects. Moreover, forest soil had the additional benefit of providing dormant seeds and propagules.

2.6.1 Phytoextraction

Also known as phytoaccumulation is the uptake and fixation of contaminants from the roots to the aerial parts of the plants. Although this technique is frequently used for metals, other elements (e.g., As, Se) and compounds (e.g., organic compounds) can also be accumulated (Favas et al., 2014). If the arsenic concentration in a plant surpasses 1000 mg/kg (dry-weight) it is considered a hyperaccumulator (Branquinho et al., 2007).

Some mushrooms (e.g., amongst others *Laccaria spp., Thelephora terrestris, Boletus cavipes*) seem to be high As accumulators (Slekovec and Irgolic, 1996). Some As hyperaccumulating plants such as ferns (*Pteris vittata, Pityrogramma calomelanos*) have been found to accumulate high concentrations of arsenic and therefore have been recommended for phytoextraction and even phytomining (Slejkovec et al, 2010).

2.6.2 Phytostabilisation and vegetation cover

Phytostabilisation is an analogue of the more traditional technique of contaminant containment (Raskin and Ensley, 2000, pp. 71). Phytostabilisation is therefore the incorporation of contaminants into the structure of the plants or humus. Additionally, phytostabilisation provides structural stability to slopes and loose wastes by plant roots and vegetation cover resulting in the containment of contaminants and therefore limiting their mobility and diffusion in the soil (Favas et al., 2014). Vegetation cover is therefore one of the most effective techniques to stabilise disturbed land and prevent soil erosion. It additionally, reduces contaminated dust-blown particulates and therefore exposure pathways (Hartley et al., 2009). However, Bradshaw and Chadwick (1980) established that vegetation cover on mine heaps encounters several difficulties such as low nutrient content and low water holding capacity.

2.6.3 Degradation

Phytodegradation is the transformation of organic contaminants through metabolism or mineralisation in the plant by enzymes into other, usually less toxic or mobile, compounds. (Favas et al., 2014)

Rhizodegradation is the enhancement of root growth to stimulate microorganism growth which can utilise plant metabolites as a source of energy and breakdown pollutants in soil. (Kuiper et al., 2004)

2.6.4 Phytofiltration

Phytofiltration is the absorption or concentration of contaminants by roots in hydroponic systems with a continuous effluent flow. Plants with high root surface area and tolerance to contaminants are optimal for this method. (Favas et al., 2014)

2.6.5 Phytovolatilization

Phytovolatilization is the usage of a plant's ability to absorb an element through its roots, convert it into a less toxic and volatile form and release it to the atmosphere. This technique can be applied for some metals and metalloids as well as for organic compounds. (Favas et al., 2014)

2.7 Genus specific implementation in phytoremediation

For this investigation some species of the following genera were investigated and the sections 2.7.1 to 2.7.5 provide a basic context on their tolerance and accumulation (if any) to arsenic and their previous implementation in phytoremediation (if any).

2.7.1 Calluna vulgaris

The common heather (*Calluna vulagaris*) appears to be a pseudo-pioneer plant found in a wide range of soil types and on lead, copper and china clay mine spoil heaps (Young, 1973; Bradshaw et al., 1975) According to studies made in arsenic-contaminated soils by Slejkovec (2010) arsenic concentrations found in common heathers range from 11.9 – 38.6 mg/kg. Porter and Peterson (1975) report highest concentrations of As found in C. vulgaris of 4130 mg/kg with mean values of 1260 mg/kg (n=25-50) in highly contaminated sites. Additionally, soil-to-plant transfer factors of

0.01 – 1.3% were reported in common heather indicating that arsenic is efficiently excluded from uptake (Slejkovec et al., 2010).

2.7.2 Grasses

Several studies have been performed in order to assess the feasibility of grass usage as a phytoremediation strategy (Porter and Peterson, 1977; Meharg et al., 1991; De Koe, 1994 & Hartley et al., 2009). Studies (Porter and Peterson, 1977) on grassland include the evolution of As tolerance (to arsenate ions) of some grass species, and their As accumulation, where it was found that some species accumulate As in high quantities (460 - 3470 mg/kg) in highly As contaminated sites and that total As is correlated with total Fe (Porter and Peterson, 1977). The grass species *Agrostis capillaris* is known to grow in heavily contaminated sites and to accumulate As in high quantities. (Porter and Peterson, 1977 & De Koe, 1994)

2.7.3 Lichens

Nieboer et al., (1984a) studied the uptake of arsenate by some lichen species to investigate anion uptake. In their paper it was suggested that the uptake might be linked to the non-photosynthetic fungal symbiont and that active uptake of arsenate is a likely mechanism at pH 4 - 6. In a following paper (Nieboer et al., 1984b) they investigated the competition between arsenate and phosphates in lichens and arsenate toxicity. It was reported that equimolar amounts of phosphate and arsenate had no effect on arsenate uptake; a 100-fold excess of phosphate however ceased the uptake of arsenate, therefore reducing its toxicity. Additionally, it was suggested that As accumulation by lichens might reach 1000 mg/kg near smelters.

2.7.4 Mosses

Wells and Richardson (1985) investigated the uptake and competition of anions in moss species. It was found that arsenate uptake was optimal at pH 3-5. It was also found that the presence of phosphate decreased the uptake of arsenate. However, mosses are used as bioindicators for monitoring atmospheric metal deposition (Niemela et al., 2003), suggesting that there might be little uptake of metals from the substrate and elements are obtained through precipitation or deposition. (Harmens et al., 2007).

2.7.5 Picea spp.

Only a few studies have assessed the performance of As accumulation in conifers. Nevertheless, pine and spruce species analysed in Poland and Norway for arsenic content show concentrations in the needles ranging from 0.3 - 1.01 mg/kg (Pine) and 0.3 - 0.68 mg/kg (Spruce) (Pohl et al., 2003). They also reported literature values of As in conifer needles of 0.3 - 4.1 mg/kg and 0.003 - 0.68 mg/kg in pine and spruce species respectively. Soil concentrations were not reported. (Pohl et al., 2003) Another study suggests that pine species e.g., *Pseudotsuga menziesii* are able to accumulate high quantities of As. (King et al., 1985, pp. 18)

2.8 Growth limiting factors

2.8.1 Arsenic as a growth limiting factor

Plant growth might be limited by the total concentration of As in soil. (Kabata-Pendias, 2011) However, according to Woolson et al. (1973) the soil properties have an important influence too, where “heavy soils” rich in organic matter and high water retention show growth limitation at much higher concentrations of As (90% growth reduction at 1000 mg/kg), whereas “light sandy soils” low in organic matter show similar growth reduction at 100 mg/kg.

Studies (Sharples et al., 2000; Fomina et al., 2005; Slejkovec et al., 2010 & Zhang et al., 2015) suggest that due to the mycorrhizal fungus acting as a filter and due to the ability of some plants to metabolise a portion of the inorganic arsenic taken from soil, it seems unlikely that arsenic is a growth-limiting factor in itself.

2.8.2 Nutrients

Nutrients are essential minerals for plants found in the soils as inorganic ions or molecules. These nutrients (Table 4) are directly involved in plant metabolism and are divided into macronutrients and micronutrients. (Ridge, 2002, pp. 168)

Table 4. Summary of essential nutrients, their function and symptoms when limited availability. *Source: Ridge, 2002, pp.168-170.*

Element	Form absorbed	Function	Deficit
Nitrogen (N)	NO_3^- (Nitrate) NH_4^+ Ammonium	Component of proteins and nucleotides	Plant light green to yellow
Potassium (K)	K^+	Osmoregulation Electrochemical equilibria Regulation of enzyme activity (protein synthesis)	Mottled or chlorotic (yellowing) leaves with smalls spots of dead tissue at tips.
Calcium (Ca)	Ca^{2+}	Stabilizes cell walls and membranes	Young leaves of terminal bud hooked then dying
Magnesium (Mg)	Mg^{2+}	Constituent of enzymes (chlorophyll)	Mottled or chlorotic leaves, may redden
Phosphorus (P)	PO_4^{3-} (Phosphate) Also as HPO_4^{2-} , $H_2PO_4^-$	Constituent of nucleic acids, phospholipids, ATP and ADP	Plant dark green, developing red and purple colours
Sodium (Na)	Na+	Essential in a few plants	Excess = salty soils

The availability of nitrogen and phosphorus is frequently the growth limiting factor in terrestrial ecosystems. Liebig's law (1840) of the minimum suggest that the relative nutrient requirement of a plant is limited by the nutrient in least supply. However, a more recent hypothesis (Bloom et al., 1985) suggests that a plant's growth is adjusted by the plant simultaneously by several resources (co-limitation). Thus, co-limitation occurs at strict N:P ratios and not necessarily at concentration values ranges; increasing the availability of one nutrient might induce an increased access to the other nutrient as the plant does not need to allocate many resources into attaining the former nutrient. (Agren et al., 2012)

Nitrogen is available to plants as ammonium (NH_4^+) or nitrate (NO_3^--N) and their concentration ranges in soil significantly; Reisenaur (1964) summarised the concentration of almost 900 soil samples and found that the majority of the values fall within the 50 and 150 mg/l range. Inorganic nitrogen is nevertheless usually present as NO_3^- in the soils. The minimum concentration (C_{min}) at which nitrogen influx stops, differs with species (Barber, 1995, pp. 189-190). However, Warncke and Barber (1974) investigated grass species (Sorghum, grain sorghum and bromegrass) and found C_{min} values of 1.7, 2.7 and 1.4 μmol/l respectively (≈ 0.10, 0.17 and 0.08 mg/l as NO_3^- ≈ 62 g/mol).

Phosphorus concentrations in soils vary from 0.02 to 0.5 % with a mean value of 0.05% (Kovar and Barber, 1988). However, only a small fraction of P is readily available for plants, usually as inorganic orthophosphate PO_4 (Barber, 1995, pp. 202-203). Reisenauer (1964) analysed around 150 soil samples from the US and found that the majority of the values fell within the 0.0 and 0.15 mg/l range. A study by Janssens et al., (1998) investigates the relationship between nutrients and grassland diversity.

They suggest an optimum value range for P for plant nutrition of 50-80 mg/kg. They additionally found that if P concentration goes above this range, floral biodiversity decreases. According to Janssens et al., (1998) potassium is a primary nutrient for plants together with nitrogen and phosphorus and it was found that the optimum K concentration is 200 mg/kg and in contrast with P, higher values are found to be compatible with biodiversity.

2.8.3 Nutrient availability

An available nutrient is the amount of ions present in the soil that are able to move to the plant root through diffusion and be absorbed (Barber, 1995, pp. 4). The availability of nutrients to plants is correlated with the soil pH and therefore soil pH is an important indicator of nutrient deficiency (Kumar and Kumar, 2013, pp. 18). In Table 5, pH values at which nutrients are most and least available are summarised, indicating that nutrients are generally very limited in acidic soils (pH < 5 – 6). On the other hand, amendments such as infection with arbuscular mycorrhizal fungi (AMF) have shown to improve the access of plants to nutrients (Smith and Gianazzi-Pearson, 1988). In contrast, Ietswaar et al., (1992) found only a small impact of AMF infection on nutrient concentration of *Agrostis capillaris.*

Table 5. Soil pH and nutrient availability. ***Adapted from: Kumar& Kumar., 2013, pp. 18***

Nutrient	Sufficiently available	Moderately low availability	Severely low availability
Nitrogen	6.0-8.0	5.5-6.0	<5.5
Phosphorus	6.0-7.5	5.0-6.0	<5.0
Potassium	>6.0	5.5-6.0	<5.5
Calcium	6.5-8.5	6.0-6.5	<6.0
Magnesium	6.5-8.5	6.0-6.5	<6.0

2.8.4 Organic Matter (OM)

Organic Matter content and composition can vary significantly from 0.1% in desert soils to 50% (w/w) in histosols, and is usually composed of 50% C, 39% O, 5% H, 5% N, 0.5% P and 0.5% S (Barber, 1995, pp. 20). OM present in soil together with the clay fraction has an important influence on soil properties and therefore nutrient retention and availability due to its cation exchange capacity. Furthermore, organic matter may release nitrogen, phosphorus and trace elements by microbial mineralisation. (McBride, 1994, pp. 56)

3 METHODS

A combination of both fieldwork and literature research was essential to assure a holistic coverage and understanding of the research subject. The methods employed were carefully chosen prior to the collection of samples and good practice techniques were utilised and are detailed where relevant.

A summary of the analytical tests and their methods utilised for plants and soils is shown in Table 6. Additionally, a table for the analytical instruments used (Table 7). A more detailed table on the operational conditions of the instruments can be found in Appendix 1(1-3).

Table 6. Summary of analytical tests and methods

PLANTS	Test	Methods
General	Species	Observation
Physical	Condition	Observation
Chemical	Arsenic (As) concentration	Digestion with HNO_3, ICP-OES Analysis.
SOILS	**Test**	**Methods**
General	Type	Observation
	Organic Matter (OM)	Loss on Ignition
Physical	Structure	Observation / Literature research
	Texture	Observation / Literature research
	Conductivity	Electrical conductivity meter
Chemical	Arsenic total (As)	Aqua-Regia Digestion, ICP-OES Analysis
	Available phosphorus (PO_4)	Mehlich 1 extraction, ICP-OES Analysis
	Available nitrogen (NO_3)	KCl extraction Analysis with SKALAR (Segmented flow analysis)
	Potassium (K)	Mehlich 1 extraction, ICP-OES Analysis
	Calcium (Ca)	Mehlich 1 extraction, ICP-OES Analysis
	Magnesium (Mg)	Mehlich 1 extraction, ICP-OES Analysis
	Sodium (Na)	Mehlich 1 extraction, ICP-OES Analysis
	pH	pH Meter

Table 7. Analytical instruments employed

Device	Name	Used in	Readability / Detection limits
pH meter	Oakton pH 6 meter	pH determination	0.01 pH
Balance	Precisa 2200c	Loss on Ignition	0.01 g
Precision balance	Oxford A2204	Soil and plants samples	0.0001 g
Inductively coupled plasma optical emission spectrometer	Thermo Scientific iCAP™ 7400 series, ICP-OES Analyser	As, P, K, Ca, Mg, Na determination	~0.1 mg/l
Automatic segmented flow analyser	SKALAR	N	0.02 – 5 mg/kg

3.1 Literature research

The methodology for the literature review was based principally on the critical selection of secondary data both qualitative as well as quantitative. This was made with the intention to compliment and create a strong foundation upon which it would be possible to base this study as well as to develop analytical and critical skills.

3.2 Field work

Previous research (Klinck et al., 2005) indicated the presence of high concentrations of arsenic throughout the site and very high concentrations of arsenic in the tailings. Additionally, there has been limited vegetation recolonisation on these tailings. Therefore, the sampling strategy was targeted on the two waste heaps on site and on their autochthonous flora.

3.2.1 Sampling strategy

A sampling strategy was developed from which it could be possible to investigate the differences in the soil quality of bare soil against recolonized soil, calculate the uptake of arsenic by endemic plants and thus assess the feasibility and application of each species on the stabilisation of the site.

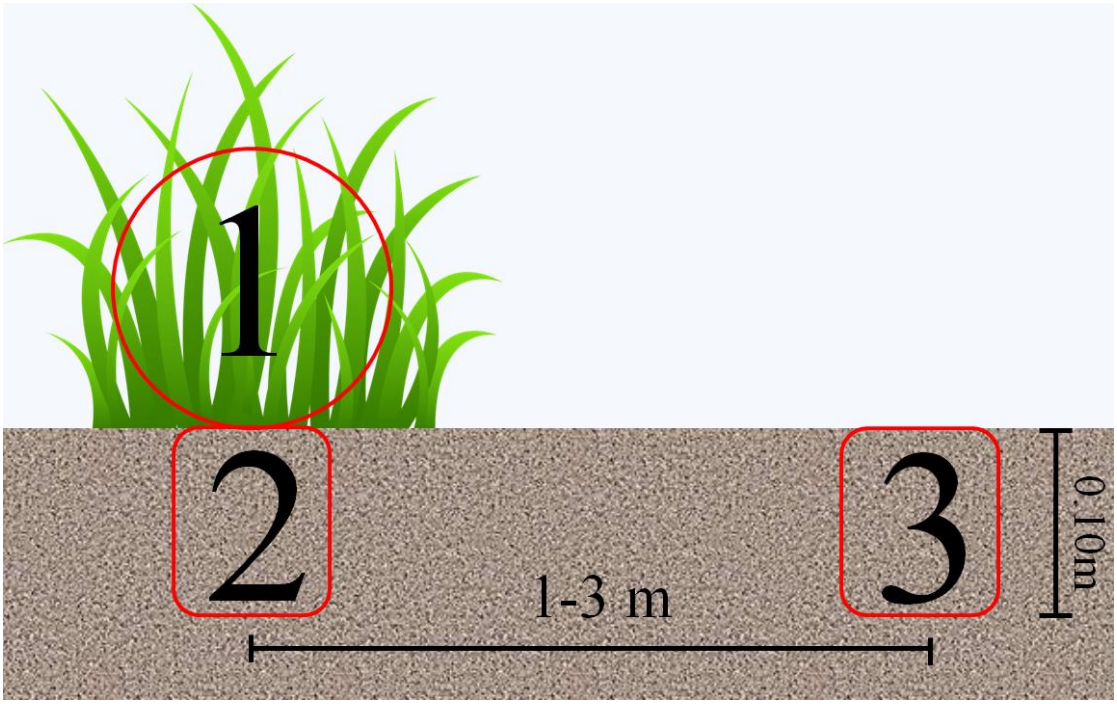

Figure 8. Sampling strategy, where 1 is vegetation sample, 2 is soil underneath and 3 is bare soil. ***Created by: Author, 2015***

The sampling strategy was devised as presented in Fig. 8, where a plant sample was taken (1), followed by the soil where the plant was growing (2) and from bare soil 1 to 3 meters from the vegetated area (3). In order to extract the soils a plastic shovel was used to collect and transfer the soil into waxed paper "Kraft" samples bags. The shovel was cleaned between samples with a wet towel to minimise cross-contamination.

The samples constituted of approximately the upper 10 cm of the soil strata, where roughly 200 grams of dried soil were collected per sample and around 20 grams of dried plant samples.

The vegetation varieties sampled at the DGC consisted of grasses (*Agrostis capillaris*, Fig. 9-1), lichen (*Cladonia spp*. Fig. 9-2), spruce (*Picea spp*. Fig. 9-3) heather (*Calluna vulgaris*, Fig. 9-4), and other unidentified grass and moss (possibly (*Barbula spp.*) species.

Figure 9. Vegetation sampled at the DGC *Source: Author, 2014*

The samples were numbered as they were taken (Table 8).

Table 8. Sample location, number and sample type. (Bottom Heap)

Sample	Location	Sample type	Sample	Location	Sample type
1	Bottom Heap	Heather 1	11	Bottom Heap	Grass 2
2	Bottom Heap	Soil vegetated	12	Bottom Heap	Soil vegetated
3	Bottom Heap	Soil bare	13	Bottom Heap	Soil vegetated
4	Bottom Heap	Grass 1	14	Bottom Heap	Lichen
5	Bottom Heap	Soil vegetated	15	Bottom Heap	Heather 3
6	Bottom Heap	Soil bare	16	Bottom Heap	Soil bare
7	Bottom Heap	Heather 2	17	Bottom Heap	Soil vegetated
8	Bottom Heap	Moss	18	Bottom Heap	Grass 3
9	Bottom Heap	Soil vegetated	19	Bottom Heap	Grass 4
10	Bottom Heap	Soil bare			

Table 8. (Continuation) Sample location, number and sample type. (Top Heap)

Sample	Location	Sample type	Sample	Location	Sample type
20	Top Heap	Heather 4	27	Top Heap	Soil vegetated
21	Top Heap	Soil vegetated	28	Top Heap	Soil bare
22	Top Heap	Soil bare	29	Top Heap	Grass 5
23	Top Heap	Heather 5	30	Top Heap	Soil vegetated
24	Top Heap	Soil vegetated	31	Top Heap	Soil bare
25	Top Heap	Soil bare	32	Top Heap	Conifer
26	Top Heap	Heather 6	33	Top Heap	Soil vegetated

In Fig. 10 it is possible to visualise the approximate geographical location of the samples taken as well as to understand the relation between them.

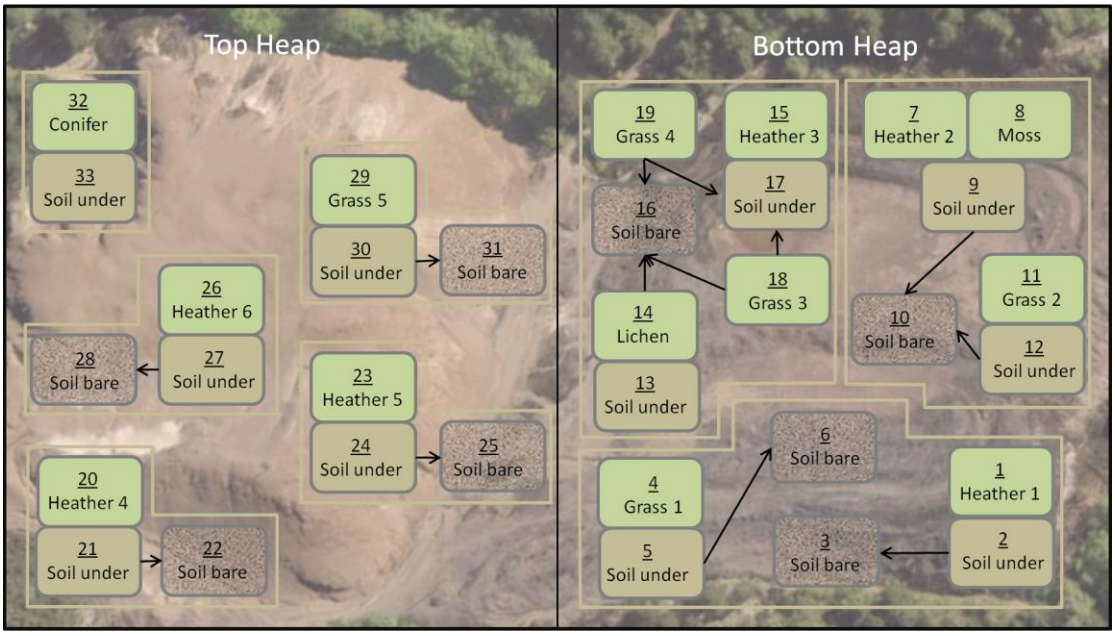

Figure 10. Aerial representation of samples. Green squares represent plant samples, khaki represent soil under corresponding plant and sandy represent bare soil samples as described in Fig. 8. *Created by Author, 2015. Source of background image: Google, 2015*

3.2.2 Sample handling

In total 33 samples were taken, 19 of which were soil samples and 14 plant samples (Table 8). The samples were acquired on 09.12.2014 and brought the same day to air-dry for approximately 4 days at ~50 - 60°C. After the samples had dried the plant samples were thoroughly rinsed under running water to minimise soil contamination and air-dried again followed by grinding using a coffee grinder and stored in re-sealable plastic bags. The soil samples were grounded with a mortar and a pestle and sieved into two fractions: < 2 mm and < 180 µm fractions and subsequently stored in re-sealable plastic bags. The grinding and sieving instruments were cleaned between samples with moist towels.

3.3 Laboratory work

For this study plants were only analysed for their arsenic content in order to calculate their arsenic uptake, whereas the soil was analysed, in addition to physical observations, for a wider range of chemical properties and nutrient availability.

3.3.1 pH

The soil pH has a significant effect on many chemical properties of the soil. It additionally limits the range of plants, if any, which are able to grow at those particular pH values. Therefore, a simple pH test was devised, where approximately 10.0±0.2 ml of milli-Q water was added to ca. 4.00±0.05 grams of soil of the < 2 mm sample fraction and rigorously mixed. While letting the suspensions settle for ~1 hour, the instrument was calibrated and subsequently the pH was measured and corrected automatically for temperature.

3.3.2 Electrical conductivity (EC)

Electrical conductivity is a quick and important indicator of soil health, as it is a measure of the soil properties such as soluble salts e.g. Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- (micronutrients), clay content and organic matter (Corwin and Lesch, 2005). Therefore, an EC test was performed on the same samples used in pH analysis after having added further 10.0±0.2 ml of milli-Q water (4.00±0.05 grams in 20.00±0.4 ml in total, 1:5 soil:water ratio). The results given by the instrument were corrected automatically for temperature.

3.3.3 Organic matter (OM)

A critical factor in soil function and soil quality is soil organic matter as its presence or absence influences chemical reactions in the soil. For this study a loss on ignition method was used due to the less hazardous nature of the method and its simplicity.

For the loss on ignition test approximately 15.00±0.01 g, < 2 mm were weighed with a Precisa 2200c balance into dry porcelain crucibles and placed into a furnace for 6 hours at 450 °C. The samples were left to cool down overnight inside the furnace and weighed in the morning with the same balance. The results were calculated with the following formula (1):

$$OM[\%] = \left(\frac{\Delta Weight\ [g]}{Measured\ weight\ [g]}\right) \cdot 100 \qquad (1)$$

where, ΔWeight is the weight difference and Measured weight is the original weight.

3.3.4 Arsenic analyses

For the determination of arsenic content in soil, each soil sample (0.1000 g, < 180 µm) was digested for ca. 2 hours in approximately 4 ml of aqua regia (HNO_3 + 3 HCl) in watch glass covered glass beakers (25 ml) to prevent evaporation, and heated

(~120°C) on a hot plate until most of the material was broken down (~3 hours). Aqua regia was added when necessary to prevent drying and the loss of sample material. After cooling, the digest was transferred into Fisher volumetric flasks (25±0.04 ml) and adjusted with milli-Q water and left to settle overnight. Subsequently the samples were analysed by inductively coupled plasma - optical emission spectrometer (ICP-OES) (Fig. 11), where the samples are sprayed with a nebuliser into argon (Ar) gas plasma, where the sample elements are excited and as their energy decreases, they emit photons at particular wavelengths, whose intensity and spectra can be accurately measured by the ICP's sensor. The results of these intensities were plotted on a calibration curve (R^2=1.000) from standard solution to acquire a concentration value, corrected for the dilution factor and verified against certified reference material and an additional reference material for quality assurance.

Figure 11. ICP-OES used for As, P, K, Ca, Mg and Na determination. ***Source: Author***

For the determination of arsenic content in plants approximately 2.0000 g of each grounded plant sample were digested for ca. 2 hours in ~20 ml of HNO_3 in watch glass covered glass beakers (50 ml) to prevent evaporation and subsequently heated (~120°C) on a hot plate (Fig. 12) until the material was broken down (~3 hours). The digests were left for cooling and filtered with Whatman No. 54 into Fisher volumetric flasks (50±0.08 ml) and adjusted with milli-Q water. The samples were analysed next morning by ICP-OES. The results were plotted on a calibration curve from standard solution (R^2=1.000) and corrected for the dilution factor.

Figure 12. Beakers on hot plate with watch glass to prevent evaporation *Source: Author*

3.3.5 Phosphorus, potassium, calcium, magnesium and sodium

In order to analyse the above mentioned nutrients present in the soil, all glassware was acid washed in 10% v/v HCl prior to sample preparation for at least 24 hours in order to minimize contamination. One litre of Mehlich 1 (0.05 N HCl and 0.025 N H_2SO_4) solution was prepared in a volumetric flask by adding ~4 ml of HCl and ~0.7 ml of H_2SO_4 and adjusted to 1 litre with milli-Q water. Approximately 5.0000 grams of the < 2 mm soil fraction was measured and 25.00±0.05 ml of Mehlich solution added, followed by 5 minutes on the reciprocating mechanical shaker. Afterwards, the suspension was filtered using Whatman No. 42 filter papers. The extractions were left in the fridge overnight and analysed the following day by ICP-OES. Results were plotted on a calibration curve (R^2=1.000) and corrected for the dilution factor.

Additionally the formula (2) was used to determine the exchangeable sodium percentage (ESP). (van de Graaff and Patterson, 2001)

$$ESP[\%] = \left(\frac{C_{Na}\,[mg/kg]}{(C_{Ca} + C_{Mg})[mg/kg]}\right) \cdot 100 \qquad (2)$$

Where C_{Na} is the concentration of Na, C_{Ca} the concentration of Ca and C_{Mg} of Mg.

3.3.6 Nitrogen

Nitrate concentrations were determined by automated segmented flow analysis (SKALAR instrument (Fig. 13)). To minimize contamination, all glassware was acid washed prior to sample preparation in 10% v/v HCl for at least 24 hours. A 2M KCl solution was prepared by adding 298.2±0.1 grams of KCl salt into a 2 litre volumetric flask and adjusted with milli-Q water. 25 ml of the extraction solution were added to 5.0000 grams of the < 2 mm soil fraction. Subsequently it was shaken for 5 minutes on a reciprocating mechanical shaker and filtered with Whatman No. 42 filter papers. Due to the low operational range (0.02 – 5 mg/L) of the analytical instrument (SKALAR), a preliminary analysis was required to identify the approximate nitrogen concentration of the samples. For this analysis a multiparameter handheld colorimeter HACH DR900 was used. For the HACH analysis only 6 samples were analysed (3 from each waste heap) and 1 blank to obtain rough results. This method however, produced elevated and likely unreliable results (See results and discussion 4.2.4).

Afterwards, the extractions were stored frozen for approximately 1 week before analysis. The analysis was carried out by SKALAR automated segmented flow analyser (Fig. 13), which essentially reduces NO_3 into NO_2 using a copperised-Cadmium (Cd) column, followed by the addition of a colour reagent, and measures its intensity spectrophotometrically. The intensity values are then plotted on a calibration curve (R^2 = 0.9996) to obtain the concentration of $NO_2 + NO_3$ (as NO_2 might be originally present in the solution).

Figure 13. SKALAR instrument used for nitrogen analysis. ***Source: Author***

3.4 Uncertainties and quality assurance

Measurements and their interpretations are meaningless without the knowledge of their uncertainty. Therefore it is important to acknowledge the uncertainties associated with the techniques used during the preparation of samples and during the analytical measurements, due to the own limitations of the instruments. Efforts were made in order to maximize precision and accuracy in every way possible.

3.4.1 Procedural quality assurance

Plants on site are very likely to be contaminated, especially when soil As concentrations are as high as in the current site. Therefore, an effort was made to carefully pick only the aerial parts of the plant and obtain samples with no obvious soil contamination. Additionally, after drying in the laboratory, the plant samples were subjected to rigorous rinsing to minimise contamination from soil. If a sample seemed nevertheless contaminated, the less contaminated parts were stored and used for analysis and the rest stored separately as backups.During the preparation of samples, the same precision instruments were used if available; same type of volumetric flasks with similar accuracy tolerances and same precision balance. Additionally, a systematic way of work for each analysis was prepared before sample preparation.

3.4.2 Blanks and detection limits

For all performed chemical analyses, at least 2 or 3 procedural blanks were prepared in exactly the same way as the rest of the samples. It is possible to see from the appendix 2(4) (Table 27) that most blanks are under the detection limits of the instruments and their values are most probably only analytical noise. When the blank signals were relevant, a simple correction was made by subtracting the blank value from the raw value given by the instrument in order to adjust the rest of the samples. The limits of detection (LOD) are calculated according to the values given by the instrument (Appendix 1(1-3)) and, if needed, accommodated for the dilution factor.

3.4.3 Replicates

One or two replicates were prepared for each sample in parallel, i.e., sample 1a, 1b and 1c. Additionally, each sample was analytically analysed 3 times. The sample values shown in the results section (4.2.2 – 4.2.5) are the average of the procedural and analytical replicates.

3.4.4 Arsenic recovery (accuracy) and precision

Certified reference material (CRM) and reference material (RM) (Appendix 3) were prepared and analysed in parallel together with the other samples. The results of this analysis allow the calculation of As recovery of the method employed and hence its accuracy. The calculation was performed with formula (3) and presented in Table 9:

$$Recovery_{As}\,[\%] = \left(\frac{As_{result}[\frac{mg}{kg}]}{As_{CRM}[\frac{mg}{kg}]}\right) \cdot 100 \qquad (3)$$

where As_{result} is the value obtained from the analysis and As_{CRM} is the actual certified value of the material.

Table 9. Recovery of As from certified reference material (CRM) and an additional reference material (RM).

	Concentration		Concentration
Analysis of CRM	24.9±3.8	Analysis of RM	11800±550
CRM certified value	20.7±1.1	RM value	12250
Recovery mean (%)	120.3	Recovery mean (%)	96.4
Max recovery (%)	138.6	Max recovery (%)	100.8
Min recovery (%)	101.9	Min recovery (%)	91.9
Max. difference (Max recovery-Δrecovery)	18.4	Max. difference (Max recovery-Δrecovery)	4.4
Recovery value	(120±19)%	Recovery value	(96±5)%
Combined value (CRM+RM)			(108±12)%

It is important to note that the recovery value of CRM ≈ (120±19) % might indicate that the As concentrations of the samples is lower than those reported in the results section. However, the recovery value of the additional RM ≈ (96±5) % provides additional confidence in the reported results; particularly since the concentration of the RM is likely to be more representative of the ranges of As concentration of the soils on site.

Additionally, from the replicates (section 3.4.3) it is possible to calculate the overall precision of the methodology employed with the following formula (4):

$$Precision\,[\%] = \left(\frac{Sample_{Std.Dev}[\frac{mg}{kg}]}{Sample_{Mean}[\frac{mg}{kg}]}\right) \cdot 100 \qquad (4)$$

Where $Sample_{Std.Dev}$ indicates the standard deviation of a set of replicates and it is divided by the mean of those samples ($Sample_{mean}$).

Table 10. Relative standard deviation (RSD) or precision % for ICP-OES and Skalar analyses

ICP-OES		Skalar	
Average %RSD	6.1	Average %RSD	39.7
SD	2.0	SE	6.6
SE	0.8	Final %RSD	39.7±7.0
Final %RSD	6.1±0.8		

The precision for all ICP-OES analyses fell under 8.3% with an average value of Precision ≈ (6.1±0.8) %, whereas for Skalar analysis was Precision ≈ (39±7.0) % due to the very low results obtained and their high variance.

3.5 Mathematical and statistical analysis

For this study Microsoft office Excel 2010 and Minitab 16 were utilised for data management as well as for graphical and statistical analyses. Each data set was checked for normality (Table 11), where the highest p-value indicated the most probable distribution. A low p-value, e.g., < 0.05, indicates that data do not follow that particular distribution. In table 11 the distribution with the best fit is noted together with its corresponding p-value. Additionally, the p-value of the data as normal distribution is shown.

Table 11. Best fit and P-values

Data	Best Fit	Best fit p-value	Normal distribution p-value
OM	Normal	0.571	0.571
pH	Box-Cox Transformation	0.220	0.133
EC	Box-Cox Transformation	0.835	<0.005
As	3-parameter weibull	0.487	0.254
P	3-parameter weibull	>0.500	0.399
Ca	Johnson transformation	0.420	<0.005
Mg	Log Normal / Box cox	0.945	0.193
K	Normal	0.506	0.506
N	Johnson transformation	0.402	<0.005

One of the main questions of this study was to understand why some soil has been recolonized and some has not. Therefore, paired t-tests were used for vegetated soil samples and their corresponding bare soil. Additionally, 2-sample t-tests (unpaired) were utilised to compare differences between Bottom Heap and Top Heap as one is far more vegetated than the other. Also, Pearson correlation tests were performed in order to understand plausible relationships between analytes and parameters. Finally, the

arsenic uptake of each plant was calculated to assess the feasibility of bioaccumulation by indigenous plants with the following formula:

$$Uptake\ [\%] = \left(\frac{As_{plant}[\frac{mg}{kg}]}{As_{soil}[\frac{mg}{kg}]}\right) \cdot 100 \quad (5)$$

where As_{plant} is the concentration of arsenic in the plant and As_{soil} is the arsenic concentration in the soil under that plant.

4 RESULTS AND DISCUSSION

4.1 Physical observations

The texture, composition and layout of both heaps differ considerably. However, both heaps are on a slope and have a south-easterly aspect. Additionally, the vegetation in both heaps showed signs of stress such as limited growth and foliosity, chlorotic needles and leaves as well as the browning of some grass leaves (Fig. 14).

Figure 14. Browning and chlorotic vegetation

4.1.1 Top Heap

The Top Heap is a hilly area characterised by sandy coarse grained material (≈ 73% of material = sand/gravel (Mighanetara, 2008)) with areas of seemingly finer material in

some of the lower level surfaces (Fig. 15), likely due to the transport of sediments by water into these hollows. Patches of grass, moss, heather and thin microbial mats grow on some of these lower level surfaces as well as on the west edges of the heap (West edge on right side of Fig. 15). This might suggest that plants are better able to grow in areas where even a smidgen of sedimentation has occurred; smaller material size offers more surface area which could increase nutrient availability and water retention capabilities. Additionally, the microbial mats present might be assisting sedimentation as well as fixing nitrogen and adding organic material (Michel and Henein, 2007).

Figure 15. Hilly surface of the Top Heap with patches of grass predominantly in lower levels and in the west edge. Microbial mat pic no. 1. *Source: Author, 2014.*

4.1.2 Bottom Heap

The Bottom Heap is composed by finer grained re-processed waste material (≈ 72% silt (Mighanetara, 2008)) with seemingly higher macrobiotic diversity e.g., taller, more foliose and abundant heathers, more grass species, lichen, moss and small conifers. However, the conifers were chlorotic and the grass showed in general poor health. In addition, the presence of puddles on the Bottom Heap (Fig. 16) suggests better water retention than the Top Heap. Moreover, the Bottom Heap has banks on its edges (Fig. 16), which may provide additional hydrological and growing advantages such as wind protection and less surface erosion.

Figure 16. View of Bottom Heap from Top Heap. Presence of puddles and banks on edges and centre. *Source: Author, 2014*.

4.1.3 Organic Matter

The amount of organic matter (OM) found in the tailings area ranges from 1.6 *to* 6.3 %, with an average value of $\boldsymbol{OM \approx 3.3 \pm 0.3}$ % and a median value of 3.4%. Values in Fig. 18 are presented to contrast the two heaps as well as the vegetated and bare soil.

Figure 17. Manure in coarse Top Heap with grass patches. *Source: Author, 2014*

In both heaps animal droppings were found near most grass and heather patches (Fig. 17) contributing to the OM in vegetated areas. The results likewise show that the soil in vegetated areas had significantly ($p < 0.05$) more OM than the non-vegetated (bare soil) areas (Fig. 18). Additionally, the results indicate that soil samples (vegetated and non-vegetated) in the Bottom Heap have significantly higher OM content than the Top Heap samples (Fig. 18). Moreover, in the Bottom Heap OM is significantly ($p < 0,05$) and highly correlated with Mg, K and negatively correlated with PO_4, which likely indicate that plants are incorporating PO_4. On the other hand, the absence of correlation with NO_3-N was unexpected (Tables 12-13).

Finally, it is possible to observe graphically (Fig. 18) that the majority of the values fall between the literature value ranges (McGrath and Loveland, 1992) in the vegetated area, especially in the Bottom heap. However, the majority of bare soil values, especially in the Top heap, fall under average soil OM values in the Southwest.

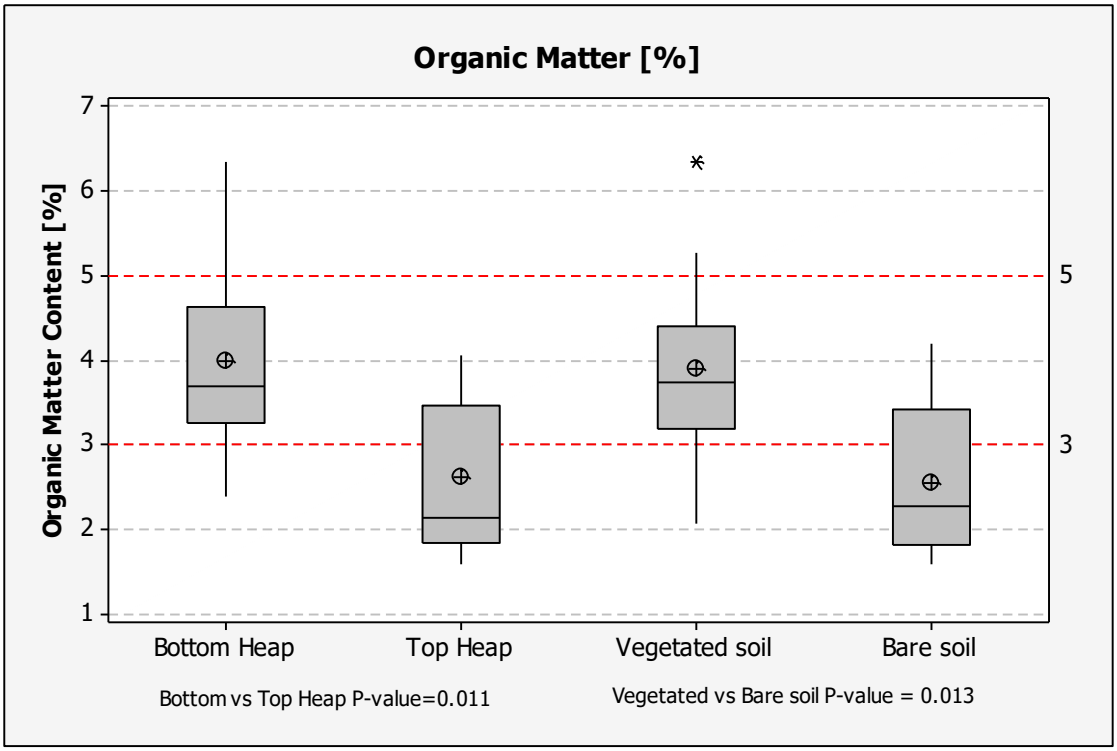

Figure 18. Comparisons of organic matter in bottom vs top heap and vegetated vs bare soils. The circles inside boxes represent the average values and the central horizontal lines represent median values. The red dotted lines represent the literature value range.

4.2 Chemical observations

The individual results for each sample and analyte can be found in in Appendix 2(1-4) (Table 22) and summary figures with averages and median values can be found in each section (4.2.1 - 4.2.5). Additionally, 3 correlation matrices were devised (One

matrix for the overall correlation of all samples (Appendix 2(4), Table 26), a second Table (12) for the samples from the Bottom Heap, and a third Table (13) for the samples from the Top Heap. In these correlation matrices, the correlation coefficients (r) are set in bold, with their corresponding p-values underneath them. The correlation coefficients were arbitrarily highlighted if R-values < -0.5 or > 0.5 and p-values < 0.1 were also highlighted. Correlation values are discussed more in detail where relevant.

Table 12. Correlation matrix for Bottom Heap soils

BH	OM	pH	EC	As	PO_4	Ca	Mg	Na	K
pH	**-0.553**								
	0.097								
EC	**0.944**	**-0.716**							
	0.000	0.020							
As	**0.600**	**-0.341**	**0.546**						
	0.067	0.335	0.102						
PO_4	**-0.645**	**0.743**	**-0.626**	**-0.569**					
	0.044	0.014	0.053	0.086					
Ca	**-0.146**	**0.578**	**-0.143**	**-0.149**	**0.756**				
	0.687	0.080	0.694	0.681	0.011				
Mg	**0.933**	**-0.478**	**0.923**	**0.496**	**-0.464**	**0.118**			
	0.000	0.162	0.000	0.145	0.177	0.746			
Na	**0.436**	**-0.049**	**0.404**	**-0.061**	**-0.003**	**0.412**	**0.678**		
	0.208	0.894	0.247	0.867	0.993	0.237	0.031		
K	**0.751**	**-0.261**	**0.705**	**0.629**	**-0.224**	**0.121**	**0.707**	**0.188**	
	0.012	0.467	0.023	0.051	0.534	0.738	0.022	0.603	
NO_3-N	**-0.033**	**0.210**	**-0.076**	**-0.157**	**0.503**	**0.404**	**0.062**	**0.333**	**0.320**
	0.928	0.560	0.835	0.665	0.138	0.247	0.865	0.348	0.367

Table 13. Correlation matrix for Top Heap soils

Top Heap	OM	pH	EC	As	PO_4	Ca	Mg	Na	K
pH	**-0.229**								
	0.553								
EC	**0.055**	**-0.473**							
	0.889	0.199							
As	**0.022**	**-0.446**	**-0.144**						
	0.954	0.229	0.711						
PO_4	**-0.366**	**0.354**	**-0.510**	**-0.281**					
	0.332	0.351	0.161	0.464					
Ca	**0.272**	**0.259**	**-0.118**	**0.155**	**0.275**				
	0.480	0.502	0.763	0.690	0.474				
Mg	**0.433**	**0.298**	**0.484**	**-0.363**	**-0.239**	**0.502**			
	0.244	0.436	0.187	0.337	0.536	0.169			
Na	**0.511**	**0.307**	**0.142**	**-0.748**	**0.091**	**0.170**	**0.657**		
	0.159	0.422	0.716	0.020	0.816	0.662	0.055		
K	**0.850**	**-0.158**	**-0.086**	**0.095**	**-0.131**	**0.231**	**0.342**	**0.357**	
	0.004	0.684	0.826	0.808	0.737	0.550	0.367	0.346	
NO_3-N	**0.154**	**-0.228**	**-0.207**	**0.107**	**0.431**	**0.511**	**-0.212**	**-0.103**	**0.027**
	0.693	0.556	0.594	0.785	0.246	0.160	0.584	0.791	0.946

4.2.1 pH

The pH values found (Fig. 19) agreed with literature values of 3.1 - 5.6 (Rieuwerts et al., 2014) and ranged from 3.4 to 5.2 in agreement with Mighanetara's (2008) reported values (4.7-5.4). A mean value of $\boldsymbol{pH \approx 4.6 \pm 0.2}$ and a median value of 4.8 was found, suggesting that the soils range from very acidic (3.0 - 5.0) to acidic (5.1-6.0) (RHS, 2015). Additionally, these findings suggest that nutrient bioavailability may be severely limited (see Table 5 in section 2.8.3) and that the solubility of metals might reach biologically toxic levels e.g., phytotoxicity $Fe > 1000\, mg/kg$, $Cu > 20 - 100\, mg/kg$. (McBride, 1994, pp. 169, 326).

Additionally, it is possible to see from (Fig. 19) that the pH levels between heaps differ significantly ($p<0.001$) supporting that, during the re-processing of the Bottom Heap for arsenic extraction, limestone was added during the process to reduce acidity. (Palumbo-Roe and Klinck, 2007)

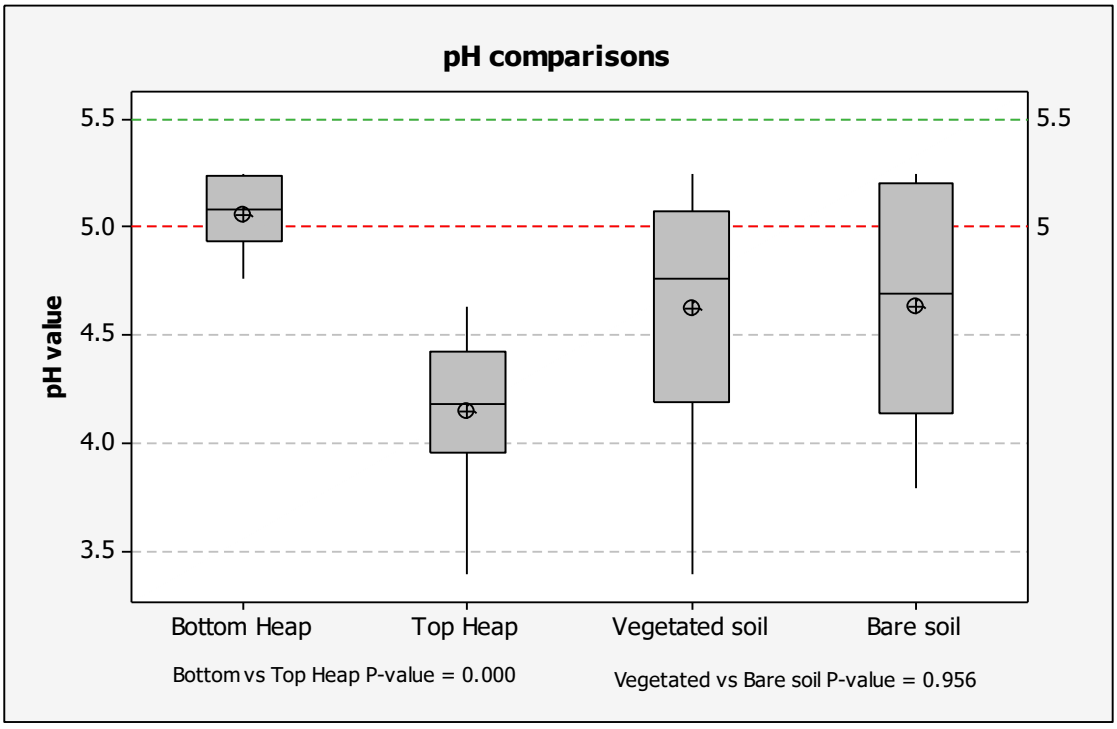

Figure 19. pH values where the red dotted line is the threshold for very acidic soils and the green line the average threshold for severely low nutrient availability. Nutrients generally very limited in soils pH < 5 – 6. (Kumar et al., 2013, 18)

In the Top Heap there appears to be no correlation between analytes and pH; however in the Bottom Heap, pH values appear to be directly correlated with PO_4. If all data from both heaps is taken into account, pH is significantly ($p=0.004$) and inversely correlated ($r = -0.625$) with As and directly correlated with PO_4, Ca, Na and K. The

pH - As inverse correlation probably arises due to the formation of As and Fe oxyhydroxides minerals under acidic conditions (Rieuwerts et al., 2014). Therefore, the significant pH difference in the Heaps and its correlation with nutrients indicates that low pH affects significantly nutrient availability, particularly in the Top Heap.

4.2.2 Arsenic content

The arsenic content values (pseudo-total) represent the amount of extractable As that is likely to be in the inert phase as well as the potentially mobile fraction (Rieuwerts et al., 2014). The As content in the waste heaps (tailings) ranged from $10\,620\ to\ 27\,010\ mg/kg$ with an average value of $\boldsymbol{As \approx 18700 \pm 1200\ mg/kg}$ and a median value of $17\,100\ mg/kg$. The heaps appear to be ca. 1340 times more contaminated relative to rural areas in England (Rural background $As_{concentration}$ = 13.9 mg/kg, Env. Agency, 2007) and around 9 times richer in As relative to areas with mineralisation but no mining (Mineralisation areas $As_{concentration}$ = 2019 mg/kg, Klinck et al., 2005). A summary of the arsenic content is presented (Fig. 20) to contrast these values.

Overall, As is significantly inversely correlated with PO_4 (r=-0.579, p=0.009), perhaps suggesting ion competition in soils (Bolan et al, 2013). In the Top Heap arsenic shows a significant (P=0.020) inverse correlation with Na (Table 13), unlike the Bottom Heap which shows a significant (P=0.051) correlation with K.

The results (Fig. 20) show that the Bottom Heap is significantly (p=0.037) less contaminated by As than the Top Heap as expected due to the reprocessing and re-extraction of As of the Bottom Heap. The concentrations of As in vegetated soils are noticeably (p=0.396) lower than in their paired bare soils. Additionally, the median value obtained in this study is compared with the median value reported by Klinck et al. (2005) in Fig. 20 with a yellow and a red line respectively. The ~2000 mg/kg difference might lie in the sampling strategy used; this study was targeted to vegetated areas and their surroundings (19 soil samples), whereas the Klinck et al. (2005) sampling strategy appears to be untargeted and randomised (10 soil samples). Moreover, this difference could indicate the following: vegetation “prefers” generally areas with less As contamination or that vegetation has removed a portion of the As from the soil underneath. However, it is also possible to be a combination of both.

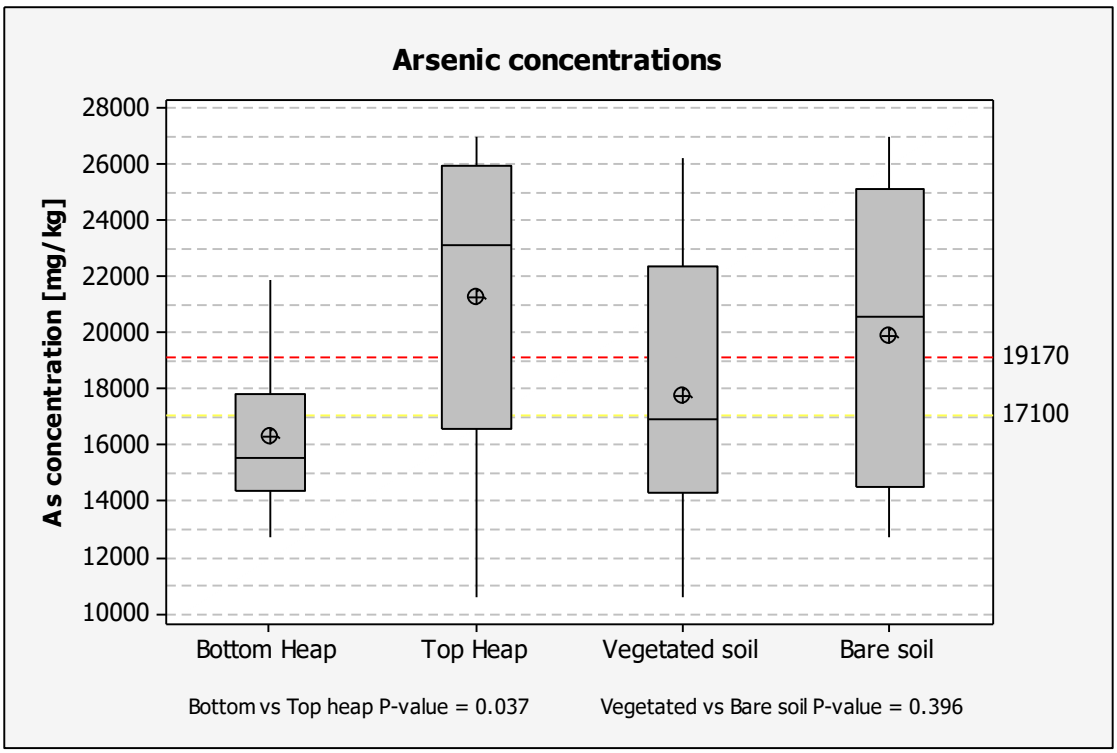

Figure 20. Arsenic comparisons. Red line = Klinck et al., 2005 median value and yellow = median value in this study.

4.2.3 Uptake of arsenic

All vegetation samples appear to intake arsenic into their aerial parts to some extent and these values are summarised by genus in Fig. 22 as arsenic content in dry biomass as well as a percentage of uptake (Formula 5).

Figure 21. Grass 3 and grass 5 respectively

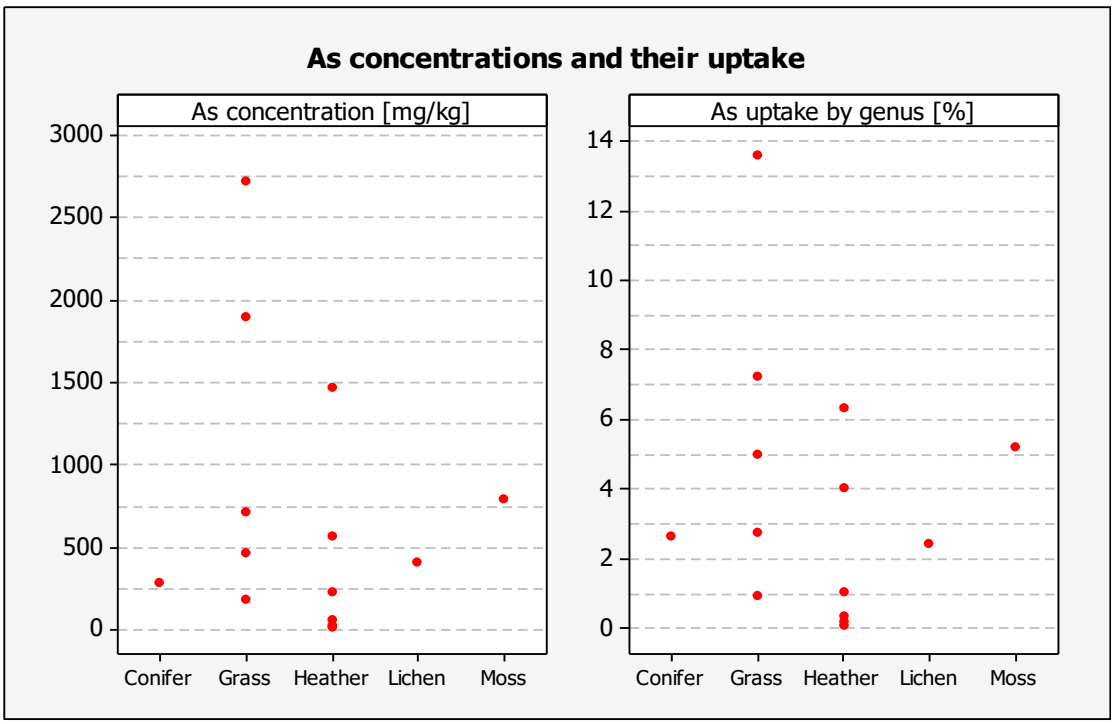

Figure 22. As concentration in different genera and their uptake

Grasses sampled appear to be the highest accumulators ranging from 185 – 2720 mg/kg of As (0.93 - 13.60 % As uptake) with an average value of $\boldsymbol{Uptake \approx 6.0 \pm 3.0}$ % and a median value of 4.99 % (Table 14). These results agree with literature values (Porter and Peterson, 1977 & De Koe, 1994). The big differences in uptake may lie in the fact that not all species belonged to the *Agrostis* genera (e.g., grass 4, Fig. 23) and remain unidentified. Grass 3 and 5 (Fig. 21) however, were both identified as *Agrostis capillaris* and both appear to be hyperaccumulating. Additionally, although grass samples were carefully rinsed, cross contamination cannot be ruled out completely.

Figure 23. Grass 4

The second highest accumulator seems to be moss with an uptake of $\approx \mathbf{5.4}\%$. According to the literature "incorporation" is a more appropriate term, since mosses do not interact sufficiently with the underlying substrate and accumulation via uptake appears to be unlikely (Harmens et al., 2007). Other mechanisms might however play a role in the incorporation of arsenic from dust and although the moss sample was also thoroughly rinsed; cross-contamination may have influenced the results.

The conifer sampled was growing in the least contaminated area sampled and was found to accumulate $\boldsymbol{As} \approx \mathbf{283.8} \pm \mathbf{1.4}\, \boldsymbol{mg/kg}$ in the needles, which equals to an $\boldsymbol{Uptake} \approx \mathbf{2.7}\,\%$. The conifer sample was also rinsed and cross-contamination is very unlikely.

Lichen was found to uptake ca. $\approx \mathbf{414} \pm \mathbf{13}\, \boldsymbol{mg/kg\ or}\ \mathbf{2.4}\,\%$ and it is likely to be accumulated by the fungal symbiont (Nieboer et al., 1984a). The lichen sample was very carefully picked and rinsed; cross-contamination should not have affected the results significantly.

Finally, As concentrations in heathers ranged from 19 – 1470 mg/kg (0.09 – 6.32 % uptake) with a mean value of $\approx 396 \pm 240\, mg/kg$ ($2.0 \pm 1.1\,\%$) and a median value of 147 mg/kg (0.71%). Although literature suggests that heathers in highly contaminated areas can accumulate up to 4130 mg/kg (Porter and Peterson, 1975), the values found in heather 4 differ almost 2.6 std. dev., suggesting sample contamination. Therefore, the uptake should be considered as ranging from 19 – 570 mg/kg (0.09 – 4.10%) with a mean value of $\boldsymbol{As} \approx \mathbf{180} \pm \mathbf{100}\, \boldsymbol{mg/kg}$ ($\mathbf{1.2} \pm \mathbf{0.8}\,\%\, \boldsymbol{uptake}$) and a median value of 60 mg/kg (0.38%), in agreement with literature range values of 0.01 – 1.3 % (Slejkovec et al., 2010).

Table 14. Arsenic concentration per genus

		N samples	Average	Median	Min	Max	Std. Dev
As concentration in plants [mg/kg]	Heather	6*	396	147	19	1470	566
	Grass	5	1198	713	185	2720	1072
	Moss	1	793.6	-	-	-	-
	Lichen	1	413.7	-	-	-	-
	Conifer	1	283.8	-	-	-	-
Uptake [%]	Heather	6*	2.02 %	0.71 %	0.09 %	6.32 %	2.59 %
	Grass	5	5.92 %	4.99 %	0.93 %	13.60 %	4.91 %
	Moss	1	5.24 %	-	-	-	-
	Lichen	1	2.42 %	-	-	-	-
	Conifer	1	2.67 %	-	-	-	-

*Including likely contaminated sample

Additionally, heathers and grasses uptake of As concentration was analysed for correlation against analytes in their corresponding vegetated soil samples (Table 15); no evident correlation was found in the uptake of As by grasses.

Table 15. Correlation matrix for uptake of As and corresponding soil analytes

Uptake	OM %	pH	EC	As	P	Ca	Mg	Na	K	N
Heather	-0.537	-0.958	0.844	0.285	-0.476	-0.828	-0.834	-0.541	-0.775	0.227
	0.272	0.003	0.035	0.584	0.340	0.042	0.039	0.268	0.070	0.666
Grass	0.343	-0.255	0.375	0.258	-0.379	-0.154	0.335	0.278	0.071	-0.376
	0.657	0.745	0.625	0.742	0.621	0.846	0.665	0.722	0.929	0.624

On the other hand, heathers showed significant (p=0.003) inverse correlation (r=-0.958) between As uptake and soil pH, suggesting an increase in uptake of arsenic as soil pH decreases. However, the solubility of Fe-Arsenate minerals decreases with lower pH, whereas the solubility of Fe-Arsenite increases with lower pH (Tu and Ma, 2003), suggesting that As uptake is likely in the form of arsenite, which could explain the visible stress. Additionally, significant inverse correlations were found between uptake of As in heathers and soil nutrients, where higher nutrient availability corresponded to a decrease in As uptake in heathers. This might suggest that "healthier" soil allows more efficient uptake exclusion either due to the mycorrhizal fungi or heathers' own exclusion mechanism.

Table 16. Correlation matrix of As in plants [mg/kg] versus arsenic and PO_4 in vegetated soil.

	As in Plants	As in vegetated soil	PO_4 in vegetated soil
As in vegetated soil	0.468 0.091		
PO_4 in vegetated soil	-0.348 0.222	-0.503 0.067	
Uptake of As	0.970 0.000	0.267 0.355	-0.254 0.381

Additionally, no significant influence was found between phosphates in soil and As uptake. However, the results moderately suggest that in general higher concentration of As in soil increases the amount of As in plants. (Table 16)

4.2.4 Nutrients

All nutrients, with the exception of NO_3-N, were found in significantly ($p< 0.05$) higher concentrations in the Bottom Heap than in the Top Heap. On the other hand, the differences in nutrient availability between vegetated and non-vegetated soils were not highly significant but noticeable; all nutrients with the exception of PO_4 and Ca were found in slightly higher quantities in vegetated soils (Fig. 24 - 30).

Nitrogen

The nitrogen concentrations in both heaps ranged from 0.12 - 0.63 mg/kg with a mean value of $\boldsymbol{NO3 - N \approx 0.31 \pm 0.04\, mg/kg}$ and a median value of 0.27 mg/kg.

The preliminary analysis that was required to identify the approximate nitrogen concentration of the samples (HACH analysis) produced highly elevated results (Blank ≈ 4.5 mg/l ≈ 22.5 mg/kg). These high results may have resulted from the interference of the inorganic salt (KCl) anions (Cl^-) having a much higher concentration relative to the soil organic anions (NO_3^-). However, the 6 samples analysed (3 from each waste heap) produced data that correlates with the corresponding samples of the Skalar data (r=0.835, p=0.038), suggesting that the absolute values of the HACH analysis, when 2M KCl is used for extraction, are not reliable but the relative values might be good approximations if properly corrected. On the other hand no other correlation was found between NO_3-N and any other analyte in this study. The lack of correlation with OM could indicate the absence of microbial mineralisation in the tailings.

According to Barber (1995, pp. 189-190) the minimum concentration at which nitrogen influx stops differs with plant species. However, grass species investigated by Warncke and Barber (1974) suggest a minimum average value represented as a red dotted line in Fig. 24. It is clear that the high majority of NO_3-N results were below this threshold, which could indicate that nitrogen availability is seriously deficient at the DGC. Additionally, according to the U.S.A. Environmental Protection Agency (2013) an acceptable range of total nitrogen is 2 to 6 mg/l, but for this thesis neither total nitrogen nor ammonia were measured.

Notwithstanding, there is the possibility that the air-dried storage method utilised might have rendered the results untrustworthy; studies have shown this method to be unreliable for soil sample storage as the NO_3-N content might change. (Esala, 1996).

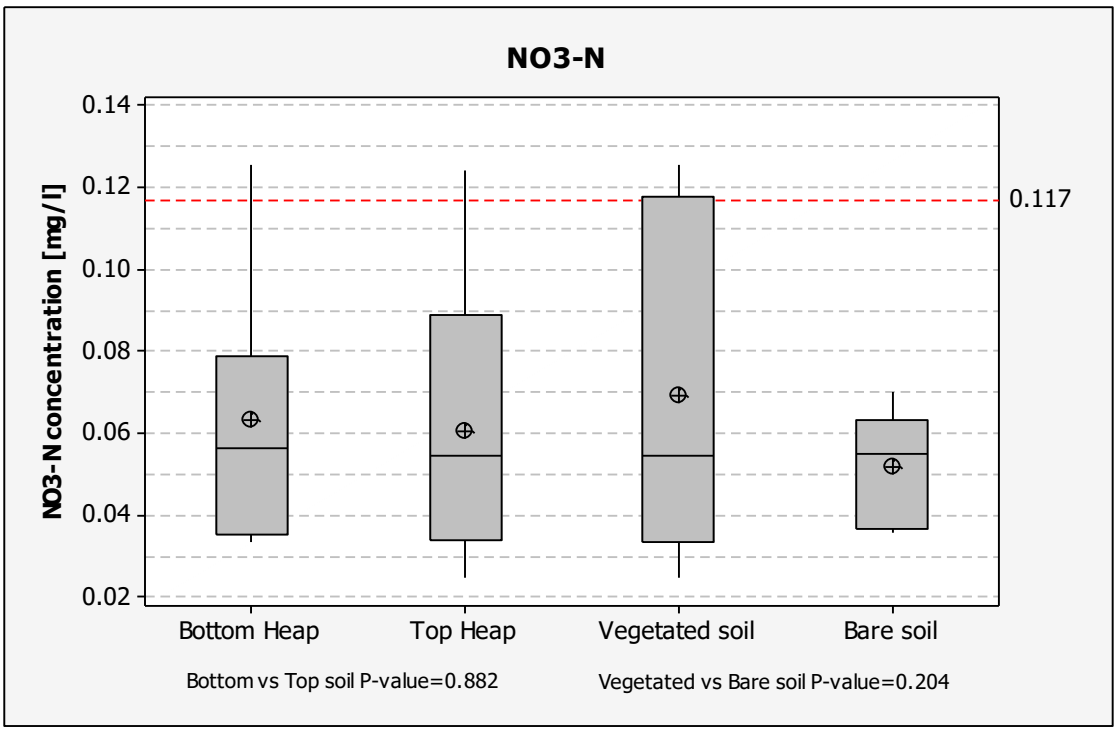

Figure 24. NO_3 – N in mg/L boxplot comparison. Red dotted line represent average minimum influx concentration

Phosphorus

The orthophosphate concentrations in both heaps ranged from 8.2 – 52.1 mg/kg with an average of $\boldsymbol{PO_4 \approx 28.3 \pm 4.0\, mg/kg}$ and a median value of 22.9 mg/kg. A summary of all results can be found in Fig. 25.

Overall phosphates (PO_4) show a highly significant direct correlation with Ca (r=0.827, p<0.001) and a significant but moderate correlation with K (r=0.535, p=0.018). Furthermore the Bottom Heap alone shows a correlation (r=0.756, p=0.011) between PO_4 and Ca, whereas the Top Heap does not (r=0.275, p=0.474). This could indicate a common source of PO_4 and Ca in the Bottom Heap perhaps as a result of the reprocessing and adding of limestone. Additionally, the PO_4 and Ca concentrations were found to be significantly more abundant in the Bottom Heap (Fig. 25), which supports the assumption of a common source. Phosphate concentrations were found to be slightly lower in vegetated areas suggesting that vegetation is taking up phosphates from these soils. However, most values fall under the lower range of optimum P content (Fig. 25) for plant nutrition of 50-80 mg/kg (Janssens et al., 1998), suggesting that PO_4 is somewhat deficient, especially in the Top Heap (Fig. 25). Notwithstanding, phosphate does not appear to be the growth limiting factor at the DGC.

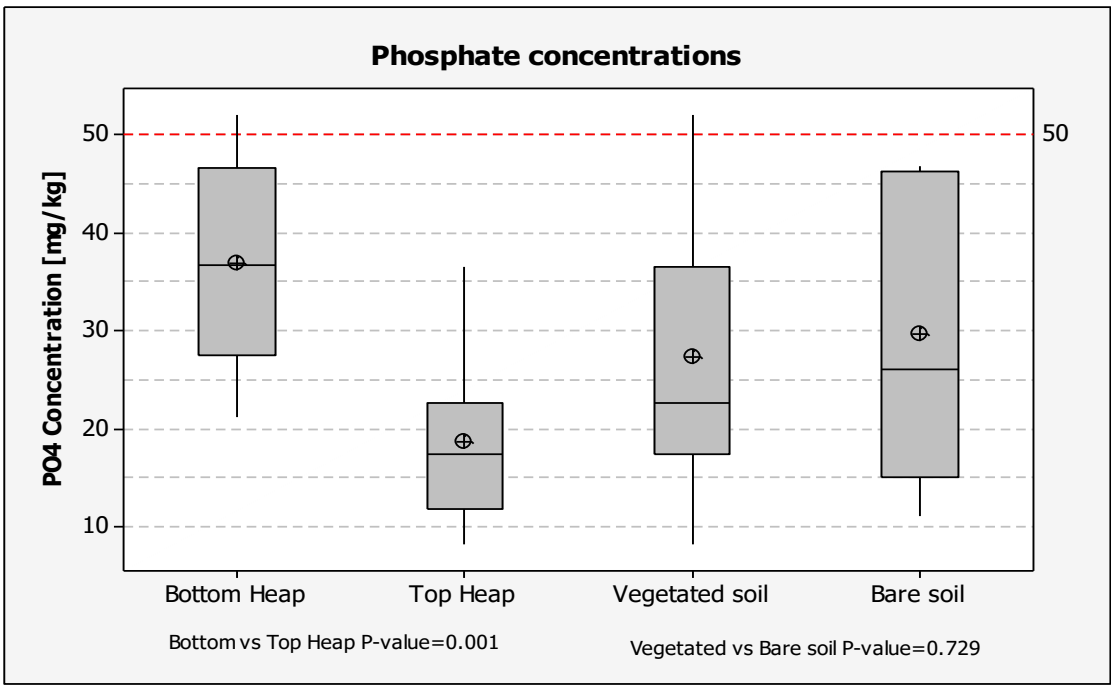

Figure 25. Phosphate Boxplots. Red dotted line represent the lower range of P concentration optimum value

NO3:PO4 Ratio

At the DGC the highest ratio occurs at the Top Heap not because of its NO_3-N abundance but because of its PO_4 deficiency. According to the literature (Janssens et al., 1998 & Agren et al., 2012), optimum N:P ratios should be skewed towards nitrogen as it is required by plants in the largest quantities. The ratios are on the other hand heavily skewed towards PO_4 (Fig. 26), supporting the assumption that NO_3 is deficient at the DGC.

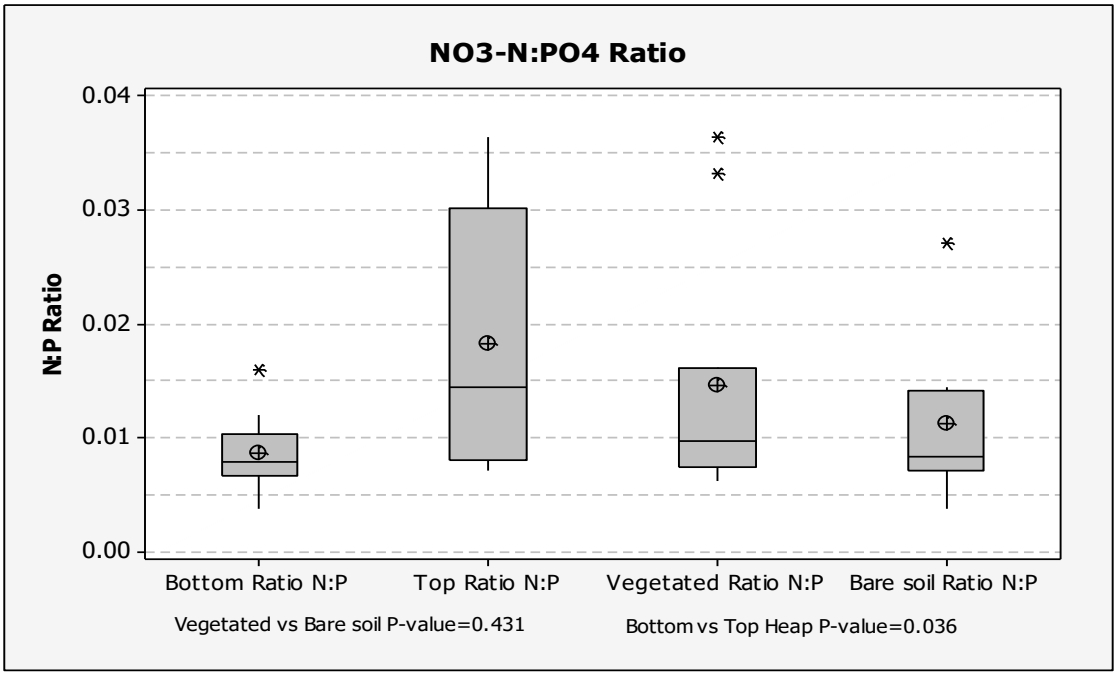

Figure 26. N:P Ratio

Potassium

The potassium concentrations in both heaps ranged from 1.6 – 59.1 mg/kg with an average of $\boldsymbol{K \approx 27.0 \pm 5.0\, mg/kg}$ and a median value of 23.6 mg/kg. A summary of all K results can be found in Fig. 27.

Potassium (K) is the third most important macronutrient for vegetation growth (Yang et al., 2014). The concentration values found at the DGC fall short of Janssens et al. (1998) optimum K concentration of 200 mg/kg, where higher values are more compatible with higher biodiversity. Nevertheless, the concentrations in the Bottom Heap appear to be only 4 - 5 times deficient, whereas the Top Heap is ~20 times more deficient (Fig. 27). When taking into account both heaps, potassium is highly correlated with most analytes with the exception of NO_3-N and As, the latter being only weakly correlated in the Bottom Heap (r=0.629, p=0.051) with K. This absence of correlation between As and K in the Top Heap but borderline significant correlation in the Bottom Heap could indicate that K levels are also related somehow to the reprocessing. Nevertheless, in the Bottom Heap no evident correlation between K and Ca or PO_4 was found, ruling out the obvious common source that is limestone. The source of K on the Bottom Heap thus remains unclear, yet a significant difference in concentration with Top Heap suggests that the difference cannot be explained by chance. In the Top Heap however, K is correlated with OM (r=0.850, p=0.004).

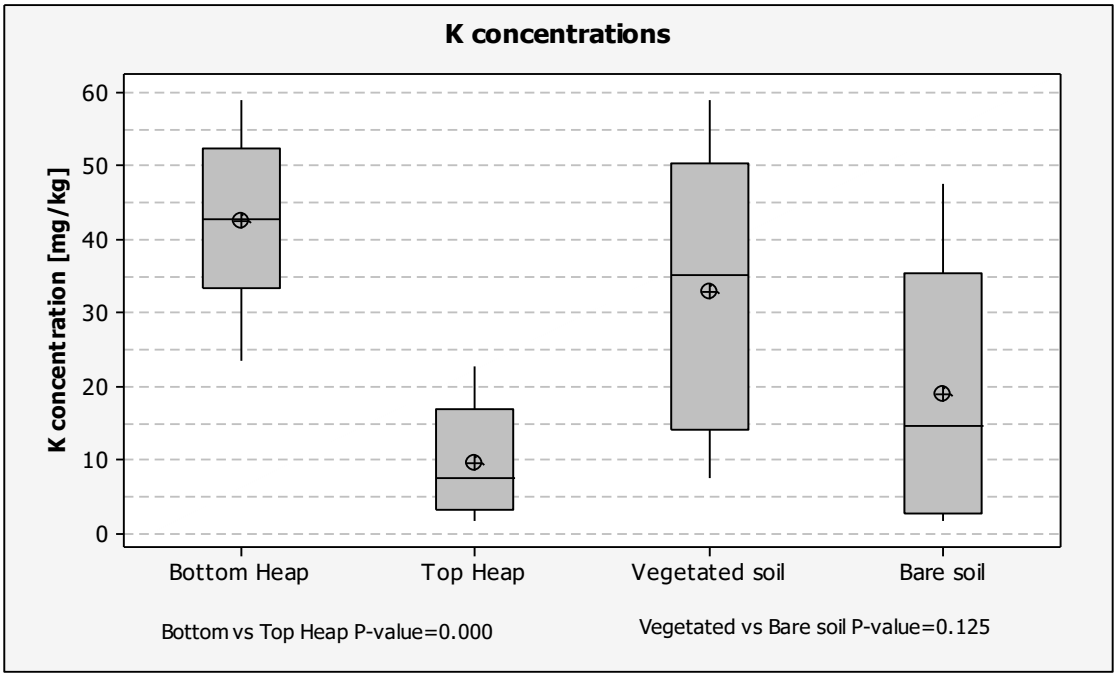

Figure 27. K boxplot comparison

Calcium

The calcium concentrations in both heaps ranged from 117 – 707 mg/kg with an average of $\boldsymbol{Ca \approx 400 \pm 50\ mg/kg}$ and a median value of 317 mg/kg. A summary of Ca results can be found in Fig. 28.

The calcium concentrations in the Bottom Heap differ significantly with the concentrations of the Top Heap. As previously suggested, higher Ca values may arise from the reprocessing of the Bottom Heap for additional As extraction, in which reportedly limestone was added to decrease acidity. There is no significant difference between vegetated and bare soil concentrations but like PO_4, Ca was found in slightly lower concentrations in vegetated soils (Fig. 28).

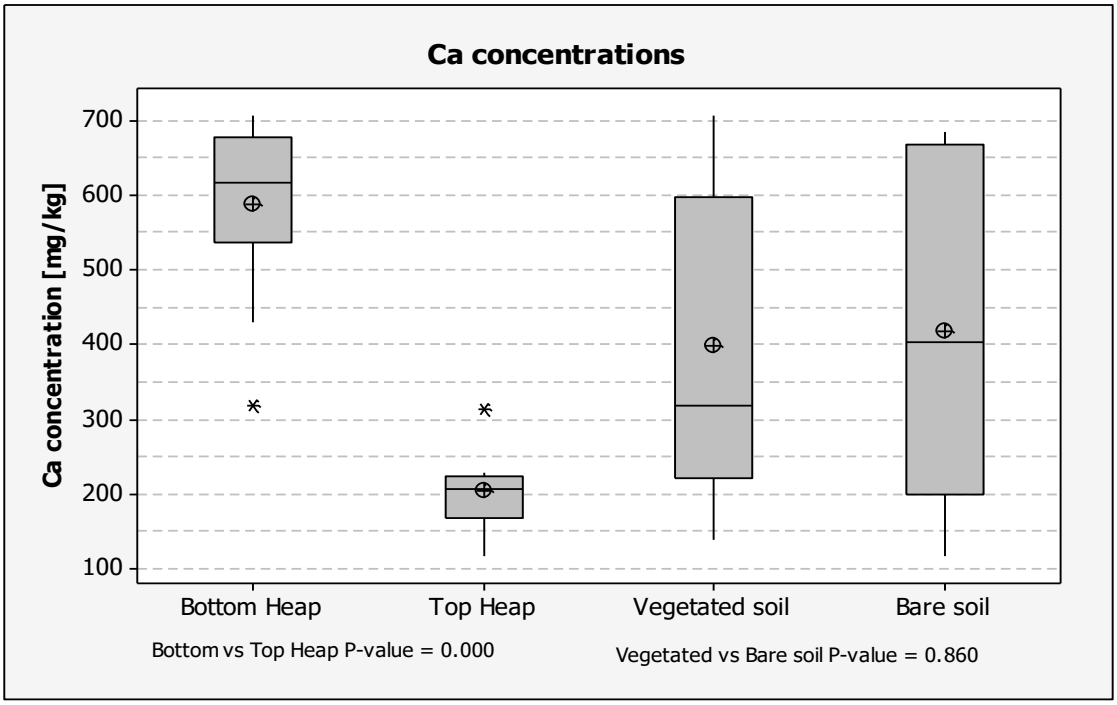

Figure 28. Ca boxplot comparison

Magnesium

The magnesium, concentrations in both heaps ranged from 6.8 – 50.7 mg/kg with an average of $\boldsymbol{Mg \approx 22.8 \pm 3.0\ mg/kg}$ and a median value of 20.4 mg/kg. A summary of all Mg results can be found in Fig. 29.

Mg concentrations are significantly (p=0.041) higher in the Bottom Heap and higher in vegetated soils, similar to other nutrients (Fig. 29). Taking into account all soil

samples magnesium showed a significant correlation with organic matter (r=0.821, p<0.001). In the Bottom Heap, in addition to Mg being very highly correlated with OM (r=0.933, p<0.001), Mg also appears to be correlated with K (r=0.707, p=0.022), however no correlation between Mg and K was found in the Top Heap. Mg, K and OM show significant correlations (P≤0.022) in the Bottom Heap indicating a common source.

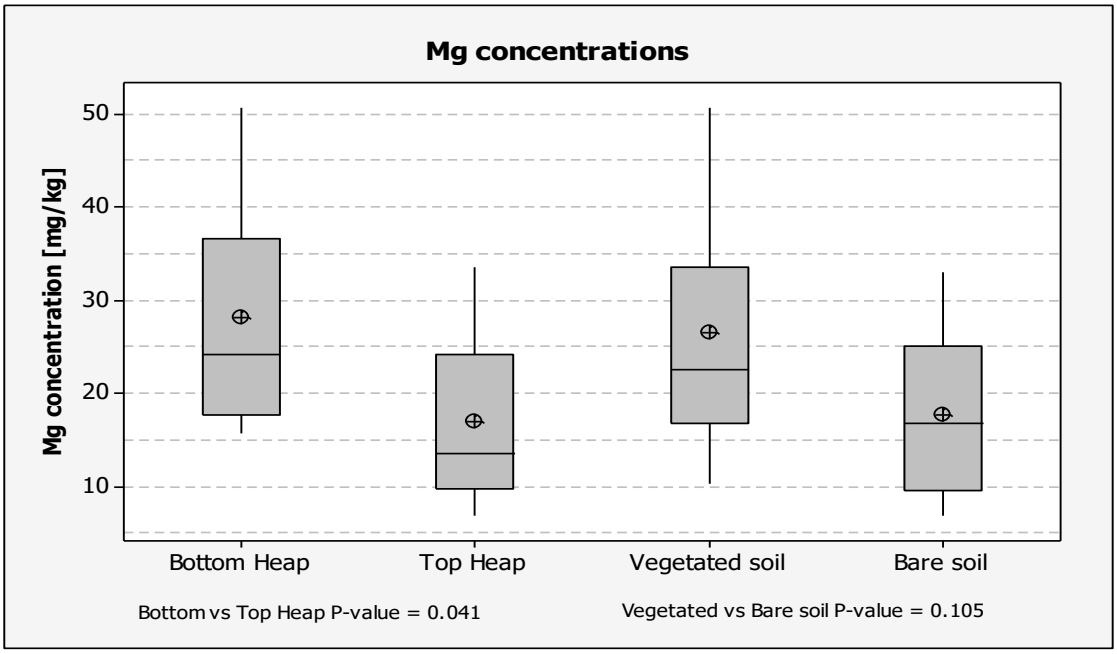

Figure 29. Mg boxplot comparison

Sodium

The sodium concentrations in both heaps ranged from 1.3 – 28.8 mg/kg with an average of $\boldsymbol{Na \approx 12.8 \pm 2.0\, mg/kg}$ and a median value of 11.9 mg/kg. A summary of all Na results can be found in Fig. 30.

Sodium appears to be moderately correlated with Mg in both Heaps (R=0.715, P=0.001) and inversely correlated with As in the Top Heap (r=-0.748, p=0.020). Similarly to other nutrients, the Bottom Heap showed significantly higher concentrations of Na and slightly higher concentrations in the vegetated soil (Fig. 30).

Although sodium (Na) is not a vital nutrient for many plants, exchangeable sodium percentage (ESP) is associated with poor structural stability properties. Soils high in ESP are susceptible to swelling, surface crusting, sealing and erosion. (McBride, 1994, pp. 282) On average, the soils at the DGC heaps result in a value of $ESP \approx 3.0 \pm 1.0$ % remaining well under 6% which is when soils are considered to have

structural problems (van de Graaff and Patterson, 2001) and thus it indicates that the soil salinity is unlikely a growth limiting factor. This is additionally corroborated by the low average EC value of $EC \approx 46.0 \pm 9.0\ \mu S/cm$, which is clearly under the value of "barely saline soil" of $EC \approx 4000\ \mu S/cm$. (McBride, 1994, pp. 302)

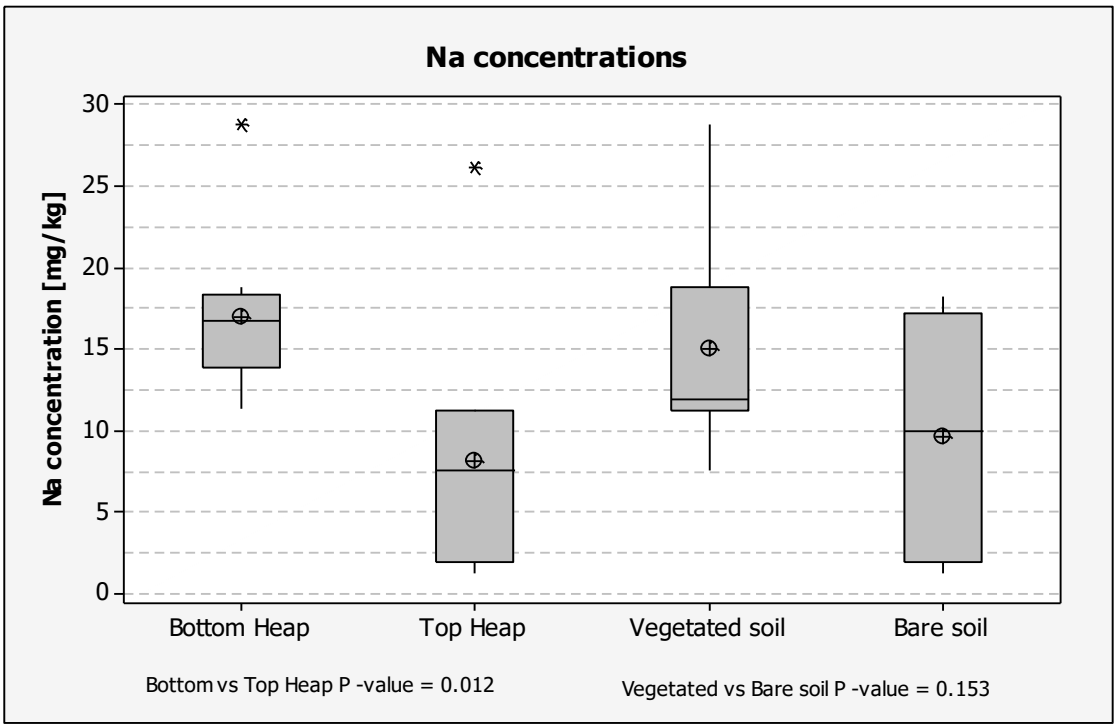

Figure 30. Na boxplot comparison

4.2.5 Species/genera specific results

Two tables (17 and 18) were devised, where the average values of the vegetated soil on which each particular plant was growing are reported in order to give a broad view of what environments appear to be suitable for their growth. Heather and grass results should be more statistically significant and representative results than the moss, conifer and lichen values due to the fact that 6 heather samples and 5 grass samples were taken, in comparison to 1 moss, 1 conifer and 1 lichen sample.

Heathers in general appear to better withstand lower pH than other vegetation types sampled. However, overall grass appears to be able to grow where higher As concentrations were present, as well as to accumulate higher levels of As. Moss appears to need or bring to the system higher OM content than other vegetation. Moss additionally appears to be growing where higher concentrations of all nutrients are to be found.

Table 17. Summary of uptake, OM, pH and As content of soil under each genus.

Plant		Uptake %	Organic Matter %	pH	As [mg/kg]
Heather	Average	**2.0 ± 1.2**	**4.2 ± 0.7**	**4.5 ± 0.3**	**18000 ±2 000**
	Median	0.7	4.2	4.7	17971
	Range	0.1 - 6.3	2.1 - 6.3	3.4 - 5.1	13930 - 23240
	Std. Dev	2.6	1.5	0.6	3958
Grass	Average	**5.9 ± 3.0**	**4.7 ± 0.9**	**4.8 ± 0.2**	**19 000 ± 3 000**
	Median	5.0	3.7	4.9	19993
	Range	0.9 -13.6	3.4 - 6.3	4.2 - 5.2	14300 - 26230
	Std. Dev	4.9	1.5	0.4	4450
Moss		**5.2**	**5.3**	**4.9**	**15148**
Lichen		**2.4**	**3.8**	**5.1**	**17085**
Conifer		**2.7**	**3.1**	**4.6**	**10616**

Conifers on the other hand appear to grow where lower concentrations of nutrients are found relative to other vegetation, but this could also indicate that it has removed from the soil a fraction of the available nutrients.

Table 18. Summary of soil nutrient content of vegetated soil

Plant		P [mg/kg]	N [mg/kg]	K [mg/kg]	Ca [mg/kg]	Mg [mg/kg]	Na [mg/kg]
Heather	Average	**26.5 ± 6.0**	**0.33 ± 0.08**	**34.0 ± 9.0**	**400 ± 100**	**29.8 ± 7.0**	**15.7 ± 3.0**
	Median	27.0	0.26	35.7	401.0	28.4	13.5
	Min	8.2 - 40.7	0.16 - 0.60	7.7 - 59.1	140 - 630	10.3 - 50.7	8.7 - 28.8
	Std. Dev	12.3	0.18	20.7	227.5	16.8	7.3
Grass	Average	**28.8 ± 8.0**	**0.38 ± 0.12**	**45.3 ± 11.0**	**520 ± 80**	**32.7 ± 9.0**	**14.4 ± 3.0**
	Median	21.3	0.31	59.0	572.7	22.5	14.6
	Min	17.1 - 52.1	0.17 - 0.60	14.0 - 59.1	310 - 710	19.2 - 50.7	7.6 - 18.8
	Std. Dev	14.2	0.23	20.3	150.9	16.5	4.8
Moss		**32.8**	**0.52**	**48.7**	**597.9**	**47.4**	**28.8**
Lichen		**22.6**	**0.17**	**36.5**	**317.3**	**16.9**	**11.4**
Conifer		**17.4**	**0.12**	**9.0**	**220.7**	**33.6**	**26.2**

The values obtained by the species *Calluna vulgaris* (Heathers) can be compared with the values from Marrs and Bannister (1978), where they analysed the soils in which heather is known to grow (Table 19).

Table 19. Soil properties of places where Calluna vulgaris is known to grow. ***Data source: Marrs and Bannister, 1978***

	pH	OM (%)	N [mg/kg]	P [mg/kg]	K [mg/kg]	Na [mg/kg]	Mg[mg/kg]	Ca[mg/kg]
Range	3.6 - 6.9	1.2 - 12.8	1260 - 4100	1.3 - 5.3	101 - 170	97 - 227	20 - 1653	2.1 - 2776

It can be seen that the values obtained for pH, OM, Ca and Mg, fall well within the ranges in which heathers usually grow. However, nitrogen values and potassium appear to be considerably lower in the Devon Great Consols than at the usual range in which heathers are known to grow suggesting at least severe nitrogen deficiency.

5 CONCLUSIONS

5.1 Conclusions

The assessment of the tailings at the Devon Great Consols mining area indicates serious As pollution in agreement with previous investigations. However, the largest fraction of As appears to be strongly bonded with iron oxyhydroxides reducing its mobility. Notwithstanding, the mobile fraction $As_{mobile} \approx$ ~29% of pseudo-total As (Rieuwerts et al., 2014) is a substantial amount of As under these circumstances.

The arsenic concentrations vary on site, yet the Top Heap appears to be significantly more contaminated than the Bottom Heap: $As \approx 21\,000 \pm 2000\ and\ 16\,300 \pm 900\ mg \cdot kg^{-1}$ respectively. Additionally, the vegetated soils appear to contain in general less As than the bare soils: $As \approx 17\,800 \pm 1\,500$ and $20\,000 \pm 2\,000\ mg \cdot kg^{-1}$ respectively, which could indicate that vegetation generally succeeds in areas with less As contamination or that vegetation has removed a portion of the As in the soil underneath. It may however be a combination of both.

In regards to the soil structure, the re-processing of the Bottom Heap for As extraction appears to have brought different kind of advantages such as different soil texture, pH and composition due to the addition of limestone. It appears that the advantage in the Bottom Heap is due to the less coarse texture of the soil and higher pH value which increase significantly both water retention capacity and nutrient availability, respectively. Thus, a trend was found where nutrient availability is higher in the Bottom Heap and in vegetated soils, with the exception of Ca and PO_4, whose content in vegetated soils are slightly lower than in bare soils. The lower content of PO_4 under vegetated soil could be explained by plant usage and uptake. Additionally, the significant negative correlation between PO_4 and As could indicate that their ions are competing in soil for absorption as suggested in the literature (Bolan et al, 2013).

The heather community growing in the tailings appear to have developed tolerance to As mainly through exclusion strategies as most plants showed low As content in their aerial tissues: $As\ uptake \approx 1.2 \pm 0.8\ \%$. While this makes them inadequate for As extraction, they are very valuable in the phytostabilisation of the heaps due to both their As resistance and their ability to grow in very acidic and relatively lower nutrient

availability conditions. Notwithstanding, nitrogen and potassium concentrations were clearly under the normal range in which heathers grow and could explain the limited growth at the DGC. Nonetheless, heathers provide a relatively safe browsing opportunity to animals due to low aerial As accumulation.

Grasses also appear to have developed a tolerance to arsenic as it appears that they are able to grow at relatively higher As concentrations than heathers and to accumulate As to a higher extent, making them highly suitable for the stabilisation of the heaps through phytoaccumulation and phytostabilisation. Unlike grasses and heathers, it is not very clear how conifers, mosses and lichen have adapted to these environments. However it appears that they were able to grow where soil conditions were the least harsh. In particular, conifers and their corresponding soil environment should be investigated more in detail as they appear to accumulate As to some extent and provide valuable stability to the heaps and therefore a decrease As leaching.

There appears to be two likely scenarios that would explain the nitrogen results. Either the tailings really are very NO_3-N deficient making it the obvious growth limiting factor for the vegetation or that the methods for extraction were inadequate i.e., air-dried storage and long thawing (overnight) of samples after extraction. However, even the results provided by the HACH analysis, which showed more elevated concentrations (see results and discussion 4.2.4), showed relatively low nitrate concentrations, suggesting that mostly long thawing time and time before analysis could have interfered with the results and/or that anyway available nitrogen is heavily deficient at the DGC.

In conclusion, the agreement of the results with available literature provide confidence to suggest that the major limiting factors for vegetation growth on the site include moisture retention on the Top Heap due to the coarse texture of the soil and low organic content as well as limited nutrient content and availability in both heaps, more specifically nitrogen, possibly due to low pH values. The vegetation community at the DGC appears to have successfully developed tolerance mechanisms to As and therefore is unlikely to be the main growth limiting factor in itself. However, it may contribute to some extent to the stress on the vegetation. Perhaps also, due to the low pH, the toxicity of other metals (Al, Cu, Fe) in the tailings might play a role to some extent and should be looked into more carefully.

5.2 Recommendations

By analysing the available data, it appears that the Bottom Heap only needs minor soil amendments to continue developing successfully; however, the restoration of the recently developed gully will be necessary in order to avoid further soil erosion. On the other hand, the revegetation of the Top Heap appears to need more extensive soil amendments if it is to support a full vegetation cover. Feasible and possibly inexpensive solutions would comprise the addition of Calcium carbonate ($CaCO_3$). Trials should be carried out on site, where e.g., ~100kg of $CaCO_3/m^2$ are added and mixed with the top 10 cm of the soil. This amount of $CaCO_3$ should increase the Ca content to the same amount of Ca in the Bottom Heap and increase its pH to a more comparable level with the Bottom Heap without affecting too much As solubility. A small increase in pH values would not stop plant uptake of As (grasses and heathers), but it would be suited for better nutrient release and therefore more successful revegetation of conifers and grasses. Consequently, mosses, lichen and other bryophytes will have a better chance to develop due to increases in OM and water retention. Additionally, low cost solutions such as the addition of sewage sludge and forest soils have shown to have favourable impacts on vegetation growth in As contaminated sites (Karczewska et al., 2013 & Hart and Luckai, 2012) and could be carried out in a trial experiment to provide the soils with additional sources of OM content and hence higher water content and nutrient availability.

According to the chemical results obtained and physical observations NO_3-N is severely deficient; therefore nitrogen fixing vegetation or microbial mats should be investigated in order to assess their feasibility to bring nitrogen into the system or to determine whether the addition of N fertilizer is necessary. The incorporation of fungi (AMF) and rhizobacteria into trials should be implemented and not be overlooked; as they have shown to provide many advantages in many different levels, from arsenic filtration to detoxification by methylation, and the increase of the nutrient available fraction (Porter and Peterson, 1977; Sharples et al., 2000; Bentley and Chasteen, 2002; Fomina et al., 2005; Titah et al., 2013 & Zhang et al., 2015).

Future research could be concentrated in NO_3-N availability to verify these results and to improve soil conditions and fertility. Additionally N:P ratios in biomass could be investigated to conclude specific growth limiting factors. Moreover, the biological investigation of the soils could provide some valuable insight into the niches that

could be amended to improve soil quality. Finally, it could be investigated whether other metals (Al, Cu, Fe) are also contributing to phytotoxicity under present environmental conditions at the Devon Great Consols.

REFERENCES

Agren, G., Wetterstedt, M. and Billberger, M. (2012). Nutrient limitation on terrestrial plant growth – modeling theinteraction between nitrogen and phosphorus. *New Phytologist*. Vol. 194, 953–960.

Ali, H., Khan, E. And Sajad, M. (2013). Phytoremediation of heavy metals—Concepts and applications. *Chemosphere*. Vol. 91, 869-881.

Antonovics, J., and Bradshaw, A. (1970). Evolution in closely adjacent plant populations, VIII. Clinal patterns at a mine boundary. *Heredity*. Vol., 2, 349–362.

Barber, S. (1995). Soil Nutrient Bioavailability. A Mechanistic Approach. Second Edition. John Wiley & Sons, Inc. ISBN: 0-471-58747-8

Bentley, R. and Chasteen, T. (2002). Microbial Methylation of Metalloids: Arsenic, Antimony, and Bismuth. Microbiology and Molecular Biology Reviews. Vol. 66, pp. 250-271

Bissen, M. and Frimmel, F. (2003). Arsenic – A review: Part I. Occurences, toxicity, speciation and mobility. *Acta Hydrochimica et Hydrobiologica.* Vol. 31:1, 9-18

Bloom, A., Chapin, F. and Mooney, H. (1985). Resource limitation in plants – an economic analogy. *Annual Review of Ecology and Systematics.* Vol. 16, 363–392.

Bolan, N., Mahimairaja, S., Kunhikrishnan, A. and Choppala, G. (2013). Phosphorus–arsenic interactions in variable-charge soils in relation to arsenic mobility and bioavailability. *Science of the Total Environment*. Vol. 463–464, 1154–1162.

Bradshaw, A. and Chadwick, M. (1980). The restoration of land. The ecology and reclamation of derelict and degraded land. Blackwell, Oxford.

Bradshaw, A., Dancer, W., Handley, J. and Sheldon, J. (1975). Biology of land revetation and reclamation of china clay wastes. *The Ecology of Resource Degradation and Renewal*. Blackwell Scientific Publications, Oxford, p. 363.

Branquinho, C., Serrano, H., Pinto, M. and Martins-Loucao, M. (2007). Revisiting the plant hyperaccumulation criteria to rare plants and earth abundant elements. *Environmental pollution*. Vol. 146, 437–443.

Cao, X., and Ma, L. (2004). Effects of compost and phosphate on plant arsenic accumulation from soils near pressure-treated wood. *Environmental Pollution*. Vol. 132, pp. 435-442.

Cave, M., Wragg, J., Palumbo-Roe, B. and Klinck, B. (2003). Measurement of the Bioaccessibility of Arsenic in UK Soils. *British Geological Survey R&D Technical Report.*

Chen, T., Yan, X., Liao, X., Xiao, X., Huang, Z., Xie, H. and Zhai, L. (2005). Subcellular distribution and compartmentalization of arsenic in Pteris vittata L. *Chinese science bulletin*. Vol. 50, 2843-2849.

Corwin, D., Lesch, S. (2005). Apparent soil electrical conductivity measurement in agriculture. Computers and Electronics in agriculture 46, 11-43.

De Koe. T. (1994). Agrostis castellana and Agrostis delicatula on heavy metal and arsenic enriched sites in NE Portugal. *The Science of Total Environment*. Vol. 145, pp. 103-109.

Dines, H., Phemister, J. and Beer, K. (1956). The metalliferous mining region of south-west England, Her Majesty's Stationary Office. British Geological Survey. London. Vol. 11 (1996 reprint).

Environment Agency. (2007). UK Soil and Herbage Pollutant Survey. UKSHS Report No. 7. Pdf-document: http://www.doeni.gov.uk/niea/uk_soil_herbage_ pollutant_survey_report7.pdf Viewed: 02.02.2015

Esala, M. (1996). Is deep-freezing a safe method for storing soil samples for inorganic nitrogen determination? *O. Van Cleemput et al. (eds.). Progress in Nitrogen Cycling Studies. Developments in Plant and Soil Sciences.* Vol. 68, 705-708. PDF-document: http://eurekamag.com/pdf/003/003184334.pdf Viewed: 11.04.2015

Favas,P., Pratas, J. Varun, M., et al. (2014). Phytoremediation of Soils Contaminated with Metals and Mettalloids at Mining Areas: Potential of Native Flora. www-document: http://www.intechopen.com/books/environmental-risk-assessment-of-soil-contamination/phytoremediation-of-soils-contaminated-with-metals-and-metalloids-at-mining-areas-potential-of-nativ Viewed: 10.12.2014

Fitz, W. and Wenzel, W. (2002). Arsenic transformations in the soil–rhizosphere–plant system: Fundamentals and potential application to phytoremediation. *Journal of Biotechnolology.* Vol. 99, 259–278.

Fomina, M., Alexander, J., Colpaert, J. and Gadd, G. (2005). Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. *Soil Viology & Biochemistry*. Vol. 37, pp. 851-866.

Ghosh, A. and Bhattacharya, P. (2004). Arsenate sorption by reduced and reoxidised rice soils under the influence of organic matter amelioration. *Environmental Geology*. Vol 45, 1010-1016.

Green, T. (2012). The impact of surface drainage alteration on sediment dispersal and contaminant release at disused copper – arsenic mine in the Tamar Valley, Devon. Available at: http://hdl.handle.net/10026.2/1777 Viewed: 17.11.2014

Harmens, H., Norris, D., Koerber, G., Buse, A., Steinnes, E. and Ruhling, A. (2007). Temporal trends in the concentration of arsenic, chromium, copper, iron, nickel, vanadium and zinc in mosses across Europe between 1990 and 2000. *Atmospheric Environment*. Vol. 41, 6673–6687.

Hart, S. and Luckai, N. (2010). Mine tailing revegetation. Pdf-document: https://www.ceaa-acee.gc.ca/050/documents_staticpost/54755/80473/Supporting_Document_18_-_Appendix_J.pdf. Forest resources and soil testing laboratory, Lakehead University. Viewed: 3.04.2015

Hartley, W., Dickinson, N., Clemente, R., French, C., Piearce, T., Sparke, S. and Lepp, N. (2009). Arsenic stability and mobilization in soil at an amenity grassland overlying chemical waste (St. Helens, UK). *Environmental Pollution*. Vol. 157, pp. 847-856

Haswell, S. (1983). Studies of Arsenic, Copper and Lead in the soils of the Tamar Valley. Pdf-document: http://hdl.handle.net/10026.1/498 Viewed:15.01.2015

Huang, Z., An, Z, Chen, T., Lei, M., Xiao, X. and Liao, X. (2007). Arsenic uptake and transport of Pteris vittata L. as influenced by phosphate and inorganic arsenic species under sand culture. *Journal of Environmental Sciences*. Vol. 19, pp. 717-718.

Ietswaart, J., Griffioen, W. and Ernst, W. (1992). Seasonality of VAM infection in three populations of Agrostis capillaris (Gramineae) on soil with or without heavy metal enrichment. *Plant and Soil*. Vol. 139, 67-73.

Jansses, G., Peeters, A., Tallowing, J., Bakker, J., Bekker, R., Fillat, F. and Oomes, M. (1988). *Plant and Soil*. Vol. 202, 69-78.

Johnson, D. and Hallberg, K. (2005). Acid mine drainage remediation options: a review. *Science of the Total Environment*. Vol. 338, 3 – 14.

Kabata-Pendias, A. (2011). Trace elements in Soils and Plants. 4th Edition. Taylor and Francis Group, LLC. Available at: http://www.petronet.ir/documents/10180/2323242/Trace_Elements_in_Soils_and_Plants Viewed: 20.04.2015

Karczewska, A., Lewinska, K. and Galka, B. (2013). Arsenic extractability and uptake by velvetgrass Holcus lanatus and ryegrass Lolium perenne in variously treated soils polluted by tailing spills. *Journal of Hazardous Materials.* Vol. 262, 1014-1021.

Kavanah, P., Farago, M., Thornton, I. and Braman, R. (1997). Bioavailability of arsenic in soil and mine wastes of the Tamar valley, SW England. *Chemical Speciation & Bioavailability*. Vol. 9:3, 77-81

King, H., Curtin, G. and Shacklette, H. (1985). Metal uptake by young conifer trees. *Dept. of the Interior, (U.S.) Geological Survery.* pp. 18.

Klinck, B., Palumbo, B., Cave, M. and Wragg, J. (2005).. Arsenic dispersal and bioaccessibility in mine contaminated soils: a case study from an abandoned arsenic mine in Devon, UK. *British Geological Survey Research Report.* Viewed: 18.11.2014

Kovar, J. and Barber, S. (1988). Phosphorus supply characteristics of 33 soils as influenced by seven rates of phosphorus addition. *Soil Science Society of America Journal*. Vol. 52, 160-165.

Knudson, J., Meikle, T. and De Luca, T. (2003). Role of mychorrhizal fungi and phosphorus in the arsenic tolerance of basin wild rye. *Journal of Environmental Quality*. Vol. 32, 2001-2006.

Kuiper, I., Lagendijk, E., Bloemberg, G. and Lugtenberg, B., (2004). Rhizoremediation: a beneficial plant-microbe interaction. *Molecular Plant-Microbe Interactions*. Vol. 17, 6–15.

Kumar, P. and Kumar, M. (2013). Nutrient Deficiencies of Field Crops: Guide to diagnosis and Management. CAB International. pp 18. ISBN: 978-1-78064-278-9

Liebig, J. (1840). Die organische Chemie in ihrer Anwendung auf Agrikultur und Physiologie. Braunschweig, Germany: Friedrich *Vieweg und Sohn Publ. Co*.

MacNair, M. and Cumbes, Q. (1987). Evidence that arsenic tolerance in Holcus lanatus L. is caused by an altered phosphate uptake system. *New Phytologist*. Vol. 107, 387–394.

Marrs, R. and Bannister, P. (1978). The Adaptation of Calluna Vulgaris (L.) Hull to contrasting soil types. *New Phytologist*. Vol. 81, 753-761.

McBride, M. (1994). Environmental Chemistry of Soils. Oxford University Press, Oxford. ISBN 0-19-507011-9

McGrath, S. and Loveland, P. (1992). The Soil Geochemical Atlas of England and Wales. Glasgow: Blackie. ISBN 0751400882

Meharg, A. and MacNair, M. (1991). Uptake, accumulation and translocation of arsenate in arsenate tolerant and non-tolerant Holcus lanatus L. *New Phytologist*. Vol 117, 225–231.

Met Office. (2010). Tamar Valley Mining District Valley climate. www-document: http://www.metoffice.gov.uk/public/weather/climate/gbvp2neyw Viewed: 10.03.2015

Michel, F. and Henein, K. (2007). Natural revegetation of arsenic-bearing alkaline tailings at cobalt, Ontario. PDF-document: http://www.researchgate.net/ publication/228851805_NATURAL_RE-VEGETATION_OF_ARSENIC-BEARING_ ALKALINE_TAILINGS_AT_COBALT_ONTARIO. Viewed: 06.04.2015

Mighanetara, K. (2008). Impact of metal mining on the water quality in the Tamar Catchment. University of Plymouth. Available at: http://hdl.handle.net/10026.1/824

Mining Heritage Project. (2010). Tamar Valley. www-document: http://www.tamarvalley.org.uk/projects/project-archive/miningheritage/ Viewed: 12.11.2014

Nathanail, C. and Bardos, R. (2004). Reclamation of Contaminated Land. John Wiley & Sons, Ltd. ISBN: 9780471985600

Nieboer, E., Lavoie, P. and Padovan, D. (1984a). Anion accumulation by Lichens. I. The characteristics and kinetics of arsenate uptake by Umbilicaria muhlenbergii. *New Phytologist.* Vol. 96, 71-82.

Nieboer, E., Padovan, D. and Lavoie, P. (1984b). Anion accumulation by Lichens. II. Competition and toxicity studies involving arsenate, phosphate, sulphate and sulphite. *New Phytologist*. Vol. 96, 83-93.

Niemela, M., Peramaki, P., Kola, H. and Piispanen, J. (2003). Determination of arsenic, iron and selenium in moss samples using hexapole collision cell, inductively coupled plasma–mass spectrometry. *Analytica Chimica Acta*. Vol. 493, 3–12.

Otte, M., Rozema, J., Beek, M, Kater, B. and Broekman, R. (1990). Uptake of arsenic by estuarine plants and interactions with phosphate, in the field (Rhine estuary) and under outdoor experimental conditions. *The Science of the Total Environment*. Vol. 97-98, 83-854.

Palumbo-Roe, B. and Klinck. B. (2007). Bioaccessibility of arsenic in mine waste-contaminated soils: A case study from an abandoned arsenic mine in SW England. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering.* Vol. 42:9, 1251-1261.

Palumbo-Roe, B., Klinck, B. and Cave, M. (2007). Arsenic speciation and mobility in mine wastes from a copper–arsenic mine in Devon, UK: a SEM, XAS, sequential chemical extraction study. *Trace Metals and other Contaminants in the Environment Volume.* Vol. 9, 441–471

Park, J., Rhee, Y., Kim, M. (2012). Can Adenosine Triarsenate Role as an Energy Carrier? *Bull. Korean Chem. Soc.*Vol. 34, No. 2, 361.

Paustenbach, D. (2000). The practice of exposure assessment: A state-of-the-art review. *Journal of Toxicology and Environmental Health, Part B: Critical Reviews*, Vol. 3 (3), 179-291

Perez-Sanz, A., Vazquez, S., Lobo, M, Moreno-Jimenez, E., Garcia, P. and Carpena, R. (2013). Soil Factors Controlling Arsenic Availability for Silene vulgaris. *Communications in Soil Science and Plant Analysis*. Vol. 44:14, 2152-2167

Petts, J., Cairney, T. and Smith, M. (1997). Risk-Based Contaminated Land Investigation and Assessment. John Wiley & Sons, Chichester, England.

Pohl, P., Lesniewicz, A. and Zyrnicki, W. (2003). Determination of As, Bi, Sb and Sn in conifer needles from various locations in Poland and Norway by hydride generation inductively coupled plasma atomic emission spectrometry. *International Journal of Environmental Analytical Chemistry.* Vol. 83:11, 963-970.

Porter, E. and Peterson, P. (1975). Arsenic accumulation by plants on mine waste (UK). *The Science of Total Environment*. Vol. 4, 365-371.

Porter, E. and Peterson, P. (1977). Arsenic tolerance in grasses growing on mine waste. *Environ. Pollut*. Vol. 14. Viewed: 20.11.2014

Poyton, C., Huang, J., Blaylock, M., Kochian, L., Elles, M. (2004). Mechanism of arsenic hyperaccumulation in Pteris species: root As influx and translocation. *Planta*. Vol. 219, pp. 1080-1088

Prasad, M. (2003). Phytoremediation of Metal-Polluted Ecosystems: Hype for Commercialization. *Russian Journal of Plant Physiology*. Vol. 50- 5, 686–700.

Raskin, I. and Ensley, B. (2000). Phytoremediation of Toxic metals: Using Plants to Clean up the Environment. John Wiley & Sons Ltd.

Reisenauer, H. (1964). Mineral nutrients in soil solution. Federation of American Societies for Experimental Biology. *Environmental Biology*. Pp. 507-508.

RHS. Royal Horticultural Society. Soil: understanding pH and testing soil. www-document: https://www.rhs.org.uk/advice/profile?pid=239. Viewed: 30.03.2015

Ridge, I. (2002). Plants. Oxford University Press. The Open University. ISBN: 0-19-925548-2

Rieuwerts, J., Searle, P. and Buck, R. (2006). Bioaccessible arsenic in the home environment in southwest England. *Science of the Total Environment*. Vol. 371, 89-98.

Rieuwerts, J., Mighanetara, K., Braungardt, C., Rollinson, G., Pirrie, D. and Azizi, F. (2014). Geochemistry and mineralogy of arsenic in mine wastes and stream sediments in a historic metal mining area in the UK. *Science of Total Environment*. Vol. 472, 226-234.

Sharples, J., Meharg, A., Chambers, S. and Cairney, J. (2000). Evolution: Symbiotic solution to arsenic contamination. *Nature.* Vol. 404, 951-952

Slejkovec, Z., van Elteren, J., Glass, H., Jeran, Z., & Jacimovic, R. (2010). Speciation analysis to unravel the soil-to-plant transfer in highly arsenic-contaminated areas in cornwall (UK). *International Journal of Environmental Analytical Chemistry*. Vol. 90:10, 784-796.

Slekovec, M. and Irgolic, K. (1996) Uptake of arsenic by mushrooms from soil. *Chemical Speciation & Bioavailability*. Vol. 8:3-4, 67-73.

Smith, S. and Gianazzi-Pearson, V. (1988). Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. *Annual Review of Plant Physiology and Plant Molecular Biology.* Vol. 39, 221-244.

Sun, Q., McDonald, L. and Skousen, J. (2000). Effects of Armoring on Limestone Neutralization of AMD. *Proceedings of the 2000 West Virginia Mine Drainage Task Force Symposium*. Morgantown, West Virginia. p. 10

Titah, H., Abdullah, S., Mushrifah, I., Anuar. N., Basri, H. and Mukhlisin, M. (2013). Effect of applying rhizobacteria and fertilizer on the growth of Ludwigia octovalvis for arsenic uptake and accumulation in phytoremediation. *Ecological Engineering.* Vol. 58. 303-313.

Tu, S. and Ma, L. (2003). Interactive effects of pH, arsenic and phosphorus on uptake of As and P and growth of the arsenic hyperaccumulator Pteris vittata L. under hydroponic conditions. *Environmental and Experimental Botany.* Vol. 50, 243-251

U.S.A. Environmental Protection Agency. (2013). Total Nitrogen. PDF-document: http://www.epa.gov/region9/water/tribal/training/pdf/TotalNitrogen.pdf Viewed: 07.04.2015

UNESCO. (2014). Cornwall and West Devon Mining Landscape. www-document: http://whc.unesco.org/en/list/1215 Viewed: 12.11.2014

van de Graaff, R. and Patterson, R. (2001). Explaining the Mysteries of Salinity, Sodicity, SAR and ESP in On-site Practice. Lanfax Laboratories, Armidale. PDF-document: http://www.lanfaxlabs.com.au/papers/P47-mysteries.PDF. Viewed : 08.04.2015

Vetterlein, D., Wesenberg, D., Nathan P., Brautigam, A., Schierhorn, A., Mattusch, J. and Jahn, R. (2009). *Environmental Pollution*. Vol. 157, 3016-3024.

Violante, A., Del Gaudio, S., Pigna, M., Pucci, M. and Amalfitano, C. (2008). Soprtion and desoprtion of arsenate by soil minerals and soils in the presence of nutrients and organics. *Soil Mineral-microbe-organic Interactions, Springer-Verlang*. pp. 39-69

Wang, J., Zhao, F., Meharg, A., Raab, A., Feldman, J. and McGrath, S. (2002). Mechanism of arsenic hyperaccumulation in Pteris vittata: Uptake kinetics, interactions with phosphate, and arsenic speciation. *Plant Physiology*. Vol. 130, pp. 1552–1561.

Wang, S. and Mulligan, C. (2006). Occurrence of arsenic contamination in Canada: Sources, behavior and distribution. *Science of the Total Environment*. Vol. 366, 701–721.

Warncke, D and Barber, S. (1974). Nitrate uptake effectiveness of four plant species. *Journal of Environmental Quality*. Vol. 3, 28-30.

Wells, J. and Richardson, D. (1985). Anion accumulation by the moss Hylocomium splendens: Uptake and competition studies involving arsenate, selenite, selenite, phosphate, sulphate and sulphite. *New Phytologist*. Vol. 101, 571-583.

Woolson, E., Axley, J. and Kearney, P. (1973). The Chemistry and Phytotoxicity of Arsenic in Soils: II. Effects of Time and Phosphorus. *Soil Science Society of America, Proceedings*. Vol.37, 254-259.

Yang, T., Zhang, S., Hu, Y., Wu, F., Hu, Q., Chen, G., Cai, J., Wu, T., Moran, N, Yu, L. and Xu, G. (2014). The Role of a Potassium Transporter OsHAK5 in Potassium Acquisition and Transport from Roots to Shoots in Rice at Low Potassium Supply Levels. *Plant Physiology*. Vol. 166, 945-959.

Young, D. (1973). Edaphic ecotypes of Calluna vulgaris. B.A. (Hons.) Thesis, Univ. of Stirling.

Zandsalimi, S., Karimi, N. and Kohandel, A. (2011). Arsenic in soil, vegetation and water of a contaminated region. *International Journal of Environmental Science and Technology*. Vol. 8 (2), pp. 331-338,

Zhang, X., Ren, B., Wu, S., Sun, Y-Q., Lin, Ge and Chen, B. (2015) Arbuscular mycorrhizal symbiosis influences arsenic accumulation and speciation in Medicago truncatula L. in arsenic-contaminated soil. *Chemosphere*. Vol. 119, 224-230.

Zhao, F., Ma, J., Meharg, A. and McGrath, S. (2009). Arsenic uptake and metabolism in plants. Tansley review. *New Phytologist*. Vol. 181, 777-794.

APPENDIX 1(1)

Operating conditions

Table 20. Operating conditions for continuous flow inductively coupled plasma atomic emission spectrometry (ICP-OES) measurements.

	UV	Visible
Exposure time (s)	2.0	2.0
Radiofrequency power (W)	1150.0	1150.0
Nebuliser gas flow (L/min)	0.5	0.5
Viewing Height (mm)	12.0	12.0
Capture full frame	No	No
Coolant gas flow (L/min)	12	
Aux. gas flow (L/min)	0.5	
Add. Gas flow (L/min)	0	

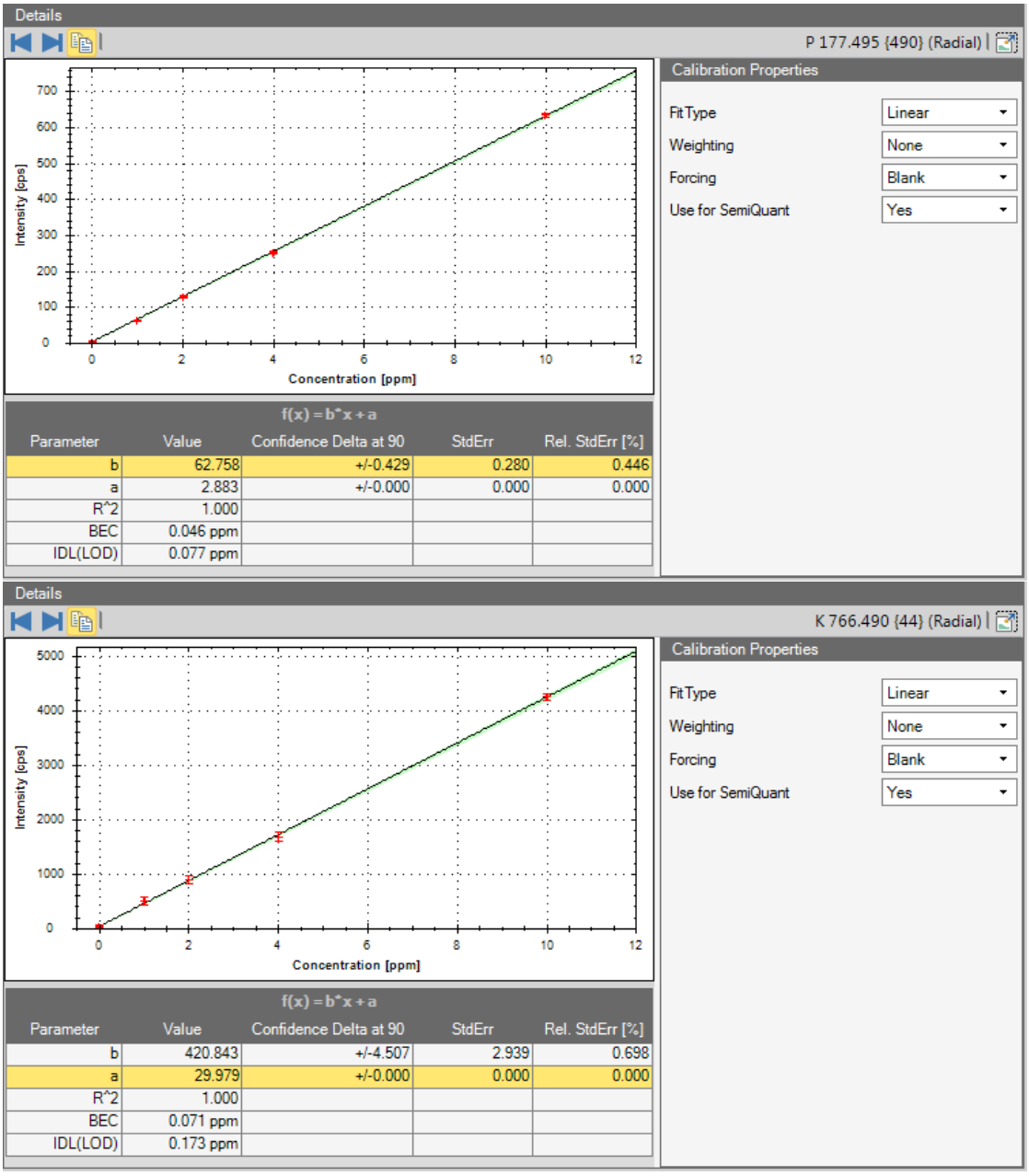

Figure 31. Calibration curves of nutrients and wavelengths

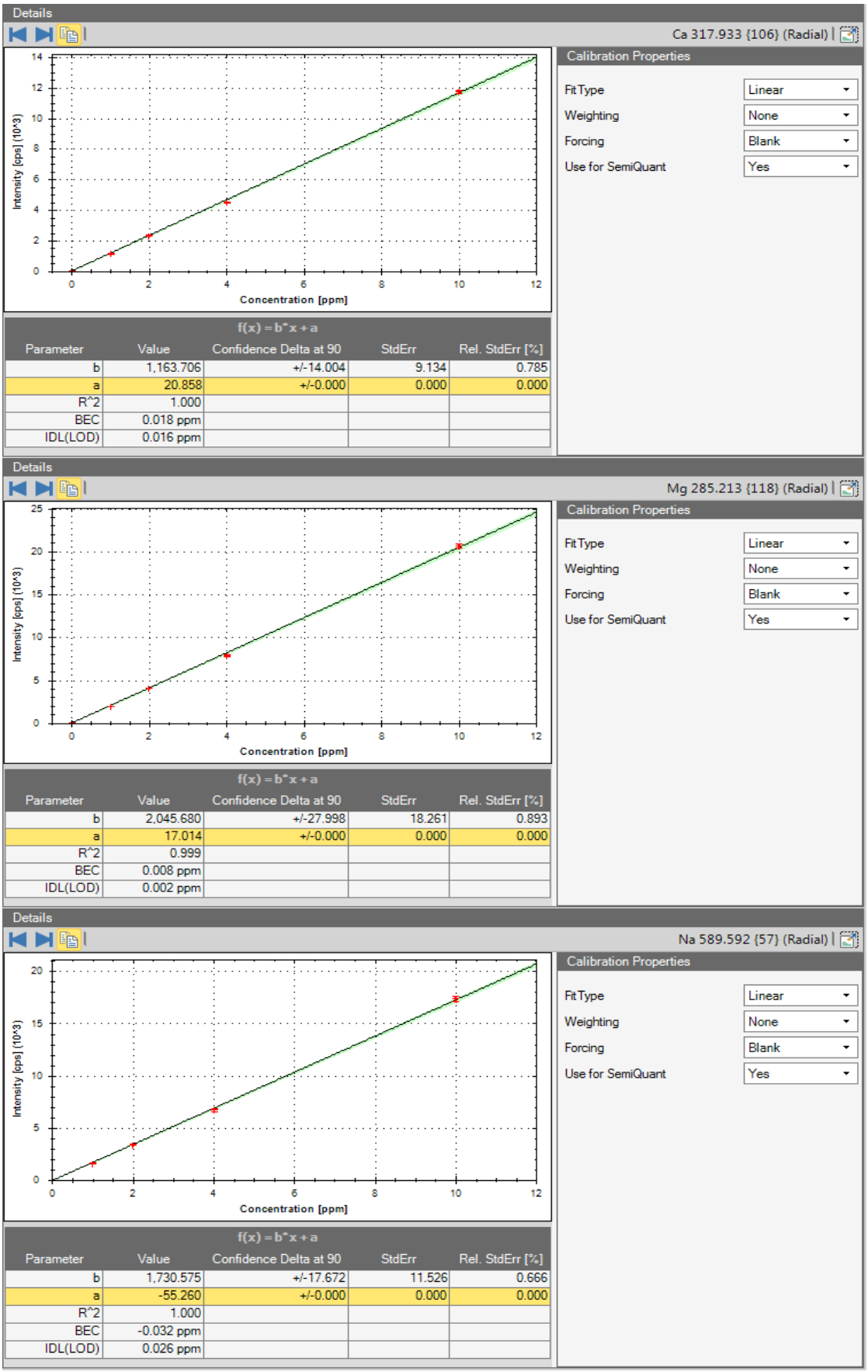

Figure 32. Calibration curves (continuation)

APPENDIX 1(3)

Operating conditions

Table 21. Operating conditions for segmented flow analyser measurements (SKALAR).

Sensitivity (mg/L)	0.2 - 5
Sample time (s)	60.0
Wash time (s)	60.0
Air time (s)	0

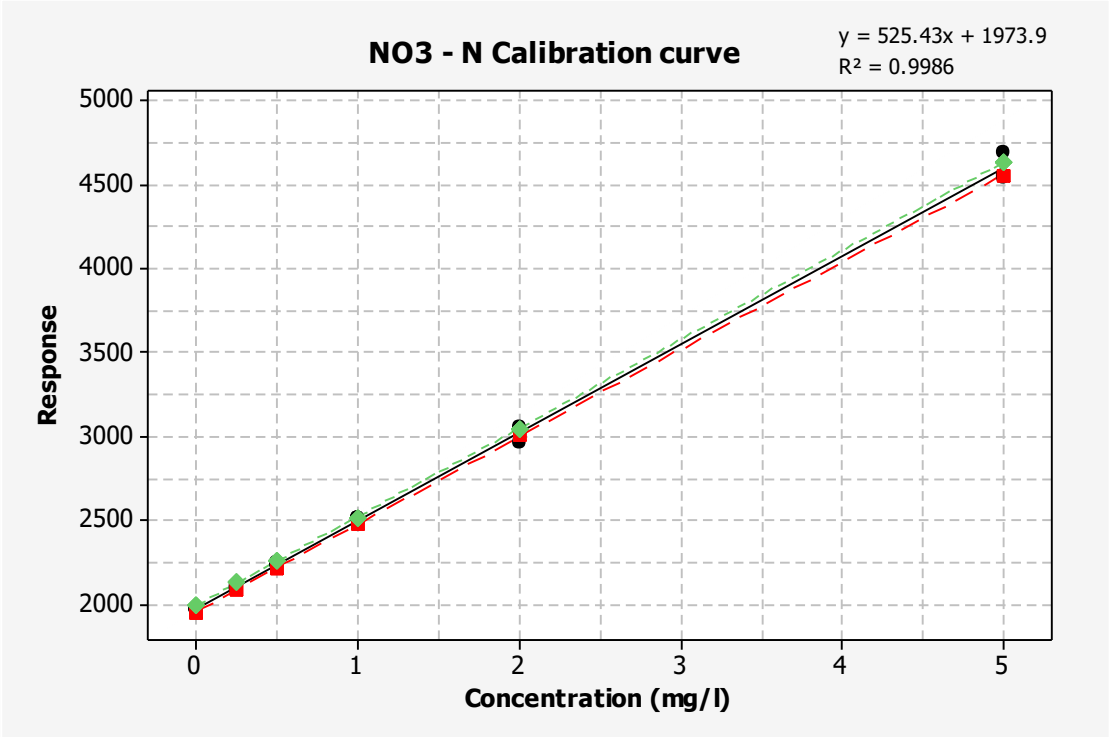

Figure 33. Calibration curve for Skalar analysis

$$y = 525.43x + 1973.9$$

$$x = \frac{y - 1973.9}{525.43}$$

APPENDIX 2(1).

Result tables (Individual values)

Table 22. Results

Sample	Location	Sample type	OM %	pH	EC (µS/cm)	As (mg/kg)		P (mg/kg)		Ca (mg/kg)		Mg (mg/kg)		Na (mg/kg)		K (mg/kg)		N (mg/kg)	
						Concentration	Error	Concentration	Error	Concentration	Error	Concentration	Error	Concentration	Error	Concentration	Error	Concentration	Error
1	Bottom Heap	Heather 1				60	8												
2	Bottom Heap	Soil vegetated	4.4	5.1	29.2	15949	1	40.7	0.3	635.2	3.1	31.5	1.1	15.6	0.3	50.4	1.7	0.25	0.03
3	Bottom Heap	Soil bare	2.4	5.2	20.4	12705	898	46.8	1.5	684.1	3.3	17.9	0.2	18.3	0.2	28.0	0.2	0.18	0.03
4	Bottom Heap	Grass 1				467	17												
5	Bottom Heap	Soil vegetated	3.5	5.3	24.2	16935	131	52.1	0.8	706.7	38.9	22.5	1.3	14.6	0.6	59.0	1.9	0.63	0.20
6	Bottom Heap	Soil bare	2.9	5.3	18.9	14435	178	45.2	1.1	673.3	10.2	15.7	0.1	17.3	0.1	23.6	0.8	0.35	0.10
7	Bottom Heap	Heather 2				26	2												
8	Bottom Heap	Moss				794	55												
9	Bottom Heap	Soil vegetated	5.3	5.0	35.1	15148	382	32.8	0.5	597.9	3.2	47.4	0.4	28.8	1.2	48.7	1.2	0.52	0.07
10	Bottom Heap	Soil bare	3.6	5.1	28.1	14900	56	46.5	1.0	652.5	19.9	25.8	0.7	16.6	0.4	38.0	1.4	0.32	0.02
11	Bottom Heap	Grass 2				713	13												
12	Bottom Heap	Soil vegetated	3.4	4.8	29.0	14305	98	32.1	0.1	430.7	11.6	19.2	0.5	11.9	0.9	35.3	0.3	0.31	0.01
13	Bottom Heap	Soil vegetated	3.8	5.1	24.0	17085	8	22.6	1.5	317.3	15.0	16.9	0.8	11.4	0.2	36.5	1.7	0.17	0.06
14	Bottom Heap	Lichen (White)				414	13												
15	Bottom Heap	Heather 3				19	1												
16	Bottom Heap	Soil bare	4.2	5.0	30.6	21933	223	29.2	0.5	595.5	8.1	33.1	1.0	16.9	0.1	47.5	0.7	0.25	0.04
17	Bottom Heap	Soil vegetated	6.3	4.9	42.3	19993	119	21.3	0.5	572.7	13.7	50.7	1.2	18.8	0.6	59.1	1.4	0.17	0.06
18	Bottom Heap	Grass 3				2721	86												
19	Bottom Heap	Grass 4				185	6												

Table 23. Results (continuation)

Sam-ple	Location	Sample type	OM %	pH	EC (µS/cm)	As (mg/kg)		P (mg/kg)		Ca (mg/kg)		Mg (mg/kg)		Na (mg/kg)		K (mg/kg)		N (mg/kg)	
						Concen-tration	Error	Concen-tration	Error	Concen-tration	Error	Concen-tration	Error	Concen-tration	Error	Concen-tration	Error	Concen-tration	Er-ror
20	Top Heap	Heather 4				1472	10												
21	Top Heap	Soil vege-tated	4.1	3.4	100.1	23245	137	8.2	1.3	138.6	9.3	13.5	0.4	11.3	1.5	19.7	0.2	0.27	0.06
22	Top Heap	Soil bare	2.1	4.1	39.7	27012	144	22.4	1.2	197.4	12.0	10.4	0.7	3.3	0.4	5.5	0.3	0.30	0.12
23	Top Heap	Heather 5				566	26												
24	Top Heap	Soil vege-tated	2.1	4.2	45.0	13933	163	36.5	6.4	207.5	26.1	10.3	1.3	11.3	0.5	7.7	1.7	0.59	0.27
25	Top Heap	Soil bare	1.9	4.4	35.0	19214	498	12.7	2.5	116.5	17.9	6.8	0.8	1.9	0.4	2.0	0.4	0.18	0.17
26	Top Heap	Heather 6				234	3												
27	Top Heap	Soil vege-tated	3.2	4.4	52.7	22372	363	19.4	1.2	229.2	4.9	25.4	1.1	8.7	0.3	22.7	2.7	0.16	0.06
28	Top Heap	Soil bare	1.8	3.8	162.5	23154	338	11.1	0.7	209.8	10.0	22.8	2.3	1.3	0.4	1.6	0.2	0.30	0.05
29	Top Heap	Grass 5				1896	84												
30	Top Heap	Soil vege-tated	3.7	4.2	33.6	26230	572	17.1	1.4	313.3	17.4	20.4	0.2	7.6	0.1	14.0	0.1	0.62	0.16
31	Top Heap	Soil bare	1.6	4.2	35.4	25786	346	22.9	0.5	205.1	14.5	9.4	0.5	2.1	0.2	4.7	0.3	0.18	0.07
32	Top Heap	Conifer				284	2												
33	Top Heap	Soil vege-tated	3.1	4.6	93.3	10616	67	17.4	2.1	220.7	11.7	33.6	0.4	26.2	0.8	9.0	0.1	0.12	0.07

APPENDIX 2(3).

Result tables (Individual values)

Table 24. Concentration of Arsenic in plants, soil and uptake.

	As in Plants [mg/kg]		As soil under [mg/kg]		As bare soil [mg/kg]		Plants As uptake [%]	
Plant	Concen-tration	Error	Concentra-tion	Error	Concen-tration	Error	Uptake	Error
Heather 1	60	8	15950	10	12700	900	0.38	0.05
Heather 2	26.3	1.5	15100	400	14900	60	0.17	0.02
Heather 3	18.7	0.2	19990	120	21900	300	0.09	0.01
Heather 4	1470	10	23250	140	27000	150	6.32	0.08
Heather 5	570	30	13900	200	19200	500	4.1	0.3
Heather 6	234	3	22400	400	23200	400	1.04	0.03
Grass 1	470	20	16940	140	14400	200	2.77	0.13
Grass 2	713	13	14300	100	14900	60	4.99	0.13
Grass 3	2720	90	19990	120	21900	300	13.6	0.6
Grass 4	185	6	19990	120	21900	300	0.93	0.04
Grass 5	1900	90	26200	600	25800	400	7.3	0.5
Moss	790	60	15100	400	14900	60	5.20	0.6
Lichen	414	13	17084	8	21900	300	2.42	0.08
Conifer	283.8	1.4	10620	70	Not sampled	Not sampled	2.67	0.04

Table 25. Uptake of Arsenic per genus. EC [uS/cm], As, P, Ca, Mg, Na, K and N [mg/kg]

Sam-ple	Plant	Up-take [%]	Organic Matter [%]	pH	EC	As	P	Ca	Mg	Na	K	N
2	Heather	0.4	4.4	5.1	29.2	15949	40.7	635.2	31.5	15.6	50.4	0.3
9	Heather	0.2	5.3	5.0	35.1	15148	32.8	597.9	47.4	28.8	48.7	0.5
17	Heather	0.1	6.3	4.9	42.3	19993	21.3	572.7	50.7	18.8	59.1	0.2
21	Heather	6.3	4.1	3.4	100.1	23245	8.2	138.6	13.5	11.3	19.7	0.3
24	Heather	4.1	2.1	4.2	45.0	13933	36.5	207.5	10.3	11.3	7.7	0.6
27	Heather	1.0	3.2	4.4	52.7	22372	19.4	229.2	25.4	8.7	22.7	0.2
5	Grass	2.8	3.5	5.3	24.2	16935	52.1	706.7	22.5	14.6	59.0	0.6
12	Grass	5.0	3.4	4.8	29.0	14305	32.1	430.7	19.2	11.9	35.3	0.3
17	Grass	13.6	6.3	4.9	42.3	19993	21.3	572.7	50.7	18.8	59.1	0.2
17	Grass	0.9	6.3	4.9	42.3	19993	21.3	572.7	50.7	18.8	59.1	0.2
30	Grass	7.3	3.7	4.2	33.6	26230	17.1	313.3	20.4	7.6	14.0	0.6
9	Moss	5.2	5.3	5.0	35.1	15148	32.8	597.9	47.4	28.8	48.7	0.5
13	Lichen	2.4	3.8	5.1	24.0	17085	22.6	317.3	16.9	11.4	36.5	0.2
33	Conifer	2.7	3.1	4.6	93.3	10616	17.4	220.7	33.6	26.2	9.0	0.1

APPENDIX 2(4).

Result tables (Individual values)

Table 26. Correlation matrix for all analyses (samples)

	OM	pH	EC	As	PO_4	Ca	Mg	Na	K
pH	**0.360**								
	0.130								
EC	**-0.204**	**-0.674**							
	0.401	0.002							
As	**-0.124**	**-0.625**	**0.220**						
	0.612	0.004	0.365						
PO_4	**0.081**	**0.768**	**-0.613**	**-0.579**					
	0.743	0.000	0.005	0.009					
Ca	**0.496**	**0.848**	**-0.520**	**-0.464**	**0.827**				
	0.031	0.000	0.022	0.046	0.000				
Mg	**0.821**	**0.397**	**0.033**	**-0.233**	**0.087**	**0.499**			
	0.000	0.092	0.894	0.338	0.725	0.029			
Na	**0.632**	**0.598**	**-0.220**	**-0.697**	**0.439**	**0.622**	**0.715**		
	0.004	0.007	0.365	0.001	0.060	0.004	0.001		
K	**0.814**	**0.699**	**-0.448**	**-0.310**	**0.535**	**0.806**	**0.675**	**0.605**	
	0.000	0.001	0.054	0.196	0.018	0.000	0.002	0.006	
NO_3-N	**0.063**	**-0.015**	**-0.154**	**0.000**	**0.353**	**0.205**	**-0.031**	**0.067**	**0.131**
	0.798	0.951	0.529	0.999	0.139	0.399	0.900	0.784	0.594

Table 27. Blank results vs analytical detection limits

	N. of Blanks	Average [mg/kg]	Blanks SD	Detection limit [mg/kg]	Corrected
As soil	4	0.04	0.02	~25	No
As plants	2	-0.04	0.00	~2.5	No
P	4	0.00	0.00	0.04	No
Ca	4	0.20	0.06	0.01	Yes
Mg	4	0.01	0.00	0.001	No
Na	4	0.35	0.04	0.13	Yes
K	4	0.20	0.11	0.85	Yes
N	3	0.05	0.02	0.02 mg/l	Yes

Table 28. NO_3 comparison SKALAR and HACH analysis [mg/kg]

	Skalar					HACH (Blank corrected)	
	Bottom Heap	Top Heap	Vegetated soil	Bare soil	Overall	Bottom Heap	Top Heap
N Samples	10	9	11	8	19	3	3
Average	0.32±0.05	0.30±0.07	0.35±0.07	0.26±0.03	0.31±0.04	10.7±4.0	12.3±5.0
Median	0.28	0.27	0.27	0.28	0.30	9.0	13.5
Range	0.17 - 0.63	0.12 - 0.62	0.12 - 0.63	0.18 - 0.35	0.12 - 0.63	5.5 - 17.5	3.5 - 20.0
Std. Dev.	0.15	0.18	0.20	0.07	0.16	6.2	8.3

Certificate of Analysis

Catalogue Number: 140-025-001
Description: Certified Reference Standard
*Enviro*MAT Contaminated Soil (SS-1)
Lot Number: SC0063618
Date of Initial Certification: May 3rd, 2010
Date of Last Verification: N/A

Consensus Values :

Elements	Reference Value (mg/kg)	Confidence Interval (mg/kg)	Tolerance Interval (mg/kg)
Ag	0.88	0.85 – 0.91	0.72 – 1.04
Al	12 163	11 753 – 12 572	9 579 – 14 746
As	20.7	19.7 – 21.8	14.0 – 27.5
B	26.9	18.5 – 35.2	0.0 – 77.8
Ba	464	448 – 480	359 – 569
Be	0.48	0.43 – 0.53	0.22 – 0.74
Ca	50 265	49 052 – 51 478	42 222 – 53 308
Cd	3.2	3.0 – 3.5	1.8 – 4.7
Ce	(40.1)	----	----
Co	12.9	12.5 – 13.4	10.2 – 15.7
Cr	103	97.9 – 109	66.6 – 140
Cu	403	393 – 413	334 – 472
Fe	72 000	69 728 – 74 273	57 212 – 86 789
Hg	0.41	0.39 – 0.43	0.29 – 0.53
K	2232	2082 – 2382	1257 – 3208
Li	14.3	12.9 – 15.8	6.4 – 22.3
Mg	9690	9459 – 9920	8141 – 11 239
Mn	737	718 – 756	605 – 869
Mo	6.8	6.5 – 7.2	4.7 – 9.0
Na	650	587 – 714	235 – 1066
Ni	59.2	57.9 – 60.5	50.4 – 68.0
P	1552	1518 – 1586	1329 – 1775
Pb	764	749 – 779	665 – 863
S	1916	1776 – 2057	1045 – 2787
Sb	5.5	4.4 – 6.6	0.0 – 12.0
Se	0.78	0.64 – 0.92	0.02 – 1.54
Sn	340	324 – 357	245 – 436
Sr	114	113 - 116	106 – 122
Ti	530	473 – 587	195 – 865
Tl	(0.19)	----	----
U	(0.76)	----	----
V	27.2	25.9 – 28.6	18.8 – 35.7
Zn	1114	1078 – 1151	860 – 1369

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REFERENCE MATERIAL DATA

Materials all ground to pass a 180µm sieve.

Data based on hot aqua regia extraction and analysis by ICP.

	Material Code		
Element	A	B	C
As (mg/Kg)	12,250	620	1840
Ca (mg/Kg)	25,650	5,750	2,270