Characterising the nature and extent of contamination at a restored coal mining area in Staffordshire and assessment of remediation options

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'In the Name of Allah, Most Gracious, Most Merciful'

"Read! and thy Lord is Most Bountiful,
He Who taught the use of the pen,
Taught man that which he knew not"

Al Quran, Sura XCVI 3-5

ABSTRACT

Mine site management, monitoring and restoration are important, long term environmental activities that are needed in many regions of the UK and around the world. This project characterised the nature and level of contamination at a former coal mining site in Staffordshire, UK, that was partially restored and is currently used for agriculture and recreation but which was understood to still have problems of mine-affected soils, sediment and water. The characterisation included analysing elemental contents, the mobility and fractionation of meta(loid)s in soils using single ($CaCl_2$) and sequential (BCR) extraction methods, metal content in soil solution, the total and dissolved fraction of meta(loid)s (0.45µm filtered) in water, and testing ecotoxicology of mining impacted water using the crustacean *Daphnia magna*. The characterisation results revealed probable Al toxicity and Ca, K and Mg deficiency in the mine-affected soils as well as potential Co, Fe, Mn, Ni and Zn toxicity risks in soils and water. The aquatic sediment was found to have elevated concentrations of elements associated with mining pollution, such as Fe. The aquatic toxicity tests revealed the stream waters to be hostile to crustaceans (100% mortality was observed in many samples), while measurements of turbidity, elemental concentrations and other water quality parameters indicated that the stream was now uninhabitable by fish. The characterisation work identified likely points of mine drainage input to the stream and thus established a basis to build further hydrological investigations upon. The soils of the most severely impacted positions in the area, which were apparent mining drainage upwelling or seep points, were shown to be in need of remediation if they were to be rendered useful for agricultural or other purposes. A soil remediation trial was thus embarked upon.

Iron and Aluminium water treatment residuals (Fe-WTR and Al-WTR), by-products of drinking water clarification, are generated in vast quantities around the world and have potential beneficial uses in environmental applications (e.g. soil remediation). This study first successfully demonstrated metal (Pb and Zn) sorption and retention capacity of WTRs in batch experiments (single metal and in combination), from the point of view of verifying their capacity for immobilising potentially toxic elements in treated soils, and then investigated directly WTR-based remediation potential for the soils at the site through conducting a series of laboratory trials on field collected soils. Remediation trial treatments comprised non-amended controls, Al-WTR, Fe-WTR and lime amendments (10% w/w), with subsequent outcomes for soil properties (e.g. pH), plant yield, earthworm survival, tissue element concentrations and soil solution chemistry all assessed. Effects of a wetting-drying cycle on subsequent plant yield, earthworm survival, and element uptake were also examined to investigate longer term aspects. The remediation trials revealed that WTRs generated significant improvements in pH, plant yield and element content, earthworm survival, and soil solution properties that were comparable to, and in some cases better than, those achieved by liming. These positive effects generally persisted after a wetting-drying cycle. Importantly, the WTRs applied to less impacted reference soils also showed quality indicator improvements or maintenance of good conditions. The remediation trials were therefore highly successful and provide strong evidence that field trials, which are the next logical step, should be conducted to examine the remediation benefits of adding WTRs to mining impacted soils at the field scale.

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Abbreviations

Al – Aluminium

ALAD- aminolaevulinic acid dehydratase

Al-WTR - Aluminium Water Treatment Residual

AMD – Acid Mine Drainage

ANOVA -One-way analysis of variance

AOD - Above the Ordnance Datum

Aqua regia aqua regia (3:1 concentrated $HCl:HNO_3$)

ARCHE - Assessing Risks of Chemicals consulting

ARCS - Assessment & Remediation of Contaminated Sediments

As - Arsenic

As(III) - Arsenite

As(V) - Arsenate

AT - Temkin isotherm equilibrium constant

ATR-FTIR - Attenuated total reflection - Fourier-transform infrared

B - Langmuir isotherm constant

B.D - Below detection,

BAF - Bioaccumulation Factor

BCR - Community Bureau of Reference

C0 - Initial concentration of adsorbate

Ca – Calcium

Calgon - Sodium hexa-metaphosphate

CCME - Canadian Council of Ministers of the Environment

Cd - Cadmium

Ce - Equilibrium concentration of adsorbate in solution

CEC - Cation Exchange Capacity

COPC - Contaminant of potential concern

Cr - Chromium

Cr(III) - Trivalent Chromium

Cr(VI) – Hexavalent Chromium

Cu - Copper

DO - Dissolved oxygen

DOM - Dissolved organic matters

D-R - Dubinin-Radushkevich

DTPA - Diethylene triamine pentaacetic acid

Ɛ - Change in chemical potentials

E°- Standard electrode potential

EC - Electrical conductivity

EC10 - 10% effect concentration

eCEC - Effective cation exchange capacity

EDTA - Ethylene diamine tetra acetic acid

EDX - Energy Dispersive X-Ray Spectroscopy

Eh - Electron activity

EM - Electron microprobe

EQS - Environmental quality standards

ESSL - Ecological soil screening level

Fe - Iron

Fe-WTR - Iron Water Treatment Residual

FWQG - Canadian Federal Water Quality Guideline

GCL - Geosynthetic clay liner

GSC - Geological Survey of Canada

ICP-MS - Inductively Coupled Plasma Mass Spectroscopy

ICP-OES - Inductively Coupled Plasma Optical Emission Spectroscopy

Igeo - Geo-accumulation index

ISO - International Organization for Standardization

Kad - Dubinin–Radushkevich constant

Labile- Easy Broken Down or Displaced

LOI - Loss on ignition

MABC - Median ambient background concentration

MAC - Maximum allowable concentrations

M-BAT - Metal Bioavailability Assessment tool

Metal(loid)s- Metals and Metalloids

Mn - Manganese

N.A. - Not available

NOEC - No Observed Effect Concentration

NR - Not reported

NTU - Nephelometric Turbidity Units

OM - Organic Matter

P - Phosphate

PAF - potentially affected fraction

Pb - Lead

PEL - Predicted effect level

PLI - Pollution load index

PMF - Potential mobility fraction

PNEC - Predicted No Effect Concentration

PTEs - Potential toxic elements

Qe - Amount adsorbed at equilibrium

Qm - Maximum monolayer coverage capacity

Qs - Theoretical isotherm saturation capacity

R2(adj) - Adjusted r-squared

RAC - Risk assessment code index

RCR - Risk Characterisation Ratio

Redox- Oxidation-Reduction potential

Revers Aqua regia - 1:3 concentrated $HCl:HNO_3$

RM - Red mud

RSD - Relative standard deviation

S - Sulphur

SD - Standard deviation

SEM - Scanning electron microscopy

SEPA - Scottish Environment Protection Agency

SOC - Soil organic carbon

SOM - Soil organic matter

SSVs - Soil screening values

TDS - Total dissolved solids

TEA - Triethanolamine

TELs - Threshold effect levels

TF - Translocation factor

UKTAG - UK Technical Advisory Group

USEPA - United States Environmental Protection Agency

WFD - Water Framework Directive

WHC – Water Holding Capacity

WTR - Water Treatment Residual

WTR - Water treatment residuals

XAFS - X-ray absorption fine structure

XRD - X-ray diffraction

Zn - Zinc

ΔH - Change in enthalpy

ΔG° - Change in standard Gibbs free energy

1 Introduction and literature review:

1.1 Mining as a current and historic source of significant environmental pollution

Lives rely directly or indirectly on a healthy, clean environment. The quality of water, air and soil are of essential importance, but there are many pressures that threaten it. One of those pressures is the pollution legacy of mines.

Mining activities, especially those concerning base coal and metal ores, are an important economic source for people all over the world. For example, it has been estimated that coal still supplies 41% of global electricity generation and directly employs 7 million people, according to the Society for Mining and Metal Exploration (2012).

On the other hand, mining can have many negative environmental impacts. Some of them are immediately obvious such as the initial radical land use change and associated future land use restrictions post mine closure, the total removal of vegetation, and waste management requirements. Air pollution impacts can include dust generation and dispersal as well as toxic components that contribute to smog and haze impairing visibility and causing human health issues linked to respiratory tract damage (Aneja et al., 2012, Marcus, 1997).

Hydrological impacts of mines are less often appreciated including that river abstractions or diversions can occur during mining operations (which affect flow regimes) and expansion, or the collapse of mining shafts in the subsurface can create alternative flow paths and therefore affect aquifer recharge and connectivity (Larson and Powell, 1986). The topography of the land surface in mined areas can be affected even by underground operations through subsidence that can occur following underground mining (Jung et al., 2007)

Environmental damage can arise from mining accidents, such as the disastrous collapse of a mine spoil tip in Aberfan in Wales (Taylor, 1971), leaking of cyanide from gold mining near Baia Mare (Michnea and Gherhes, 2001), and tailing dam failure, 32 tone m^3 of tailing, was recorded in Sanmarco, Brazil, in December 2015. That slurry resulted in polluting North Gualaxo River, Carmel River, and Rio Doce over 663 Km, destroying 15 Km^2 of land along the rivers and cutting residents off from potable water supply (Espindola et al., 2019).

Significant environmental and health concerns linked with coal mining (and mining more generally) can occur that cannot be ignored, including the release of elements that can build up to potentially toxic levels in soil and water and biota because elements may spread from mining operations to the pedosphere, biosphere, atmosphere, and hydrosphere and consequently cause environmental effects (Lottermoser, 2010).

A common impact that will be examined closely in this PhD study is the discoloration and pollution of streams and/or river beds, such as what happened in the Fal Estuary in England, in which orange layers of metal-bearing iron hydroxides were established (Banks et al., 1997, Hudson-Edwards et al., 2008a). This mine drainage (see next section) is a major issue with coal and ore mines. These all have clear environmental and socio-economic consequences.

1.1.1 Mine drainage and ochre deposits in water and sediment

Water is actively managed during mine operations, through pumping out, drains or other mechanisms to prevent water build up and submergence of ore and coal seams. However, upon cessation of mining such water management steps are typically discontinued, allowing water to build up which may eventually seep or burst out and enter surface or ground water. Abandoned mines, of which there are many in the UK and elsewhere around the world, therefore are a common source of water pollution. The water draining from mines is often very acidic (~pH 2 or lower) (Johnson and Hallberg, 2003), because of the chemical processes occurring when exposed sulphide bearing minerals are oxidised and dissolved. This process includes the following reactions:

1. Oxidation reactions involving the sulphide of soluble and insoluble pyrite minerals by catalysing of acidophilic microbial microbes (Nordstrom and Southam, 1997, Schippers, 2004) in combination with the gas oxygen in air or in the water. The oxidation process generates acidity and water-soluble ions (Chen et al., 2014, Jacobs et al., 2014) as represented in chemical Equation 1-1.

$$FeS_2 + \frac{7}{2}\, O_{2_{(g)}} + H_2O_{(l)} \rightarrow \mathrm{Fe}^{2+}_{(aq)} + 2\mathrm{SO}_4^{2-}{}_{(aq)} + 2\mathrm{H}^{+}_{(aq)} \qquad \text{Equation 1-1}$$

At mine abandonment, water pumping ceases so water levels gradually increase inside shafts and this can dissolve solubilised ions. Consequently, the water becomes acidic. Therefore many metals are capable of undergoing hydrolysis, and the level of oxygen can also be low (Price, 2005).

2. Further oxidation of ferrous iron into ferric iron can easily happen (see Equation 1-2) when adequate oxygen is dissolved in water or once the water is exposed to enough atmospheric oxygen such as when groundwater discharges at the surface (Mayes et al., 2008).

$$4\mathrm{Fe}^{2+}_{(aq)} + \mathrm{O}_{2_{(g)}} + 4\mathrm{H}^{+}_{(aq)} \rightarrow 4\mathrm{Fe}^{3+}_{(aq)} + 2\mathrm{H}_2O_{(l)} \qquad \text{Equation 1-2}$$

Iron-oxidizing bacteria can increase the rate of ferrous oxidation by a factor of a million times (Singer and Stumm, 1970, Nordstrom and Southam, 1997, Schippers, 2004). This oxidation can lead to “ochre” formation, where the oxidised iron now in a ferric state precipitates out as an orange coloured solid. According to Younger and Wolkersdorfer (2004), this precipitation of ferric ions means that the ferrous ions then predominate as the remaining dissolved iron species in the minewater discharges and receiving waters, i.e. because the pH of surface water in catchments is usually about neutral and in this condition the solubility of ferric ion is very low.

3. The ochre deposits start appearing when ferric iron hydrolyses to introduce the ferric hydroxide precipitates, as shown Equation 1-3.

$$2\mathrm{Fe}^{3+}_{(aq)} + 6\mathrm{H}_2\mathrm{O}_{(l)} \leftrightarrow 2\mathrm{Fe(OH)}_{3_{(s)}} + 6\ \mathrm{H}^{+}_{(aq)} \qquad \text{Equation 1-3}$$

This is a reversible precipitation-dissolution reaction that acts as a source or sink of ferric ions. This reaction also raises the chemical oxygen demand of the water and possibly decreases the pH of watercourses receiving mine water (Mayes et al., 2008).

Simultaneous processes can occur with the first step when pH of mine waters is less than 4.5. The sulphide of pyrite can be oxidized by the ferric iron (Equation 1-4) much faster than the oxygen and more rapidly than oxygen oxidizes the ferrous iron (Nordstrom, 1982; Jacobs et al., 2014) so that the reaction shown in Equation 1-2 is known to be the rate-limiting step in pyrite oxidation.

$$FeS_{2(s)} + 14Fe^{3+}_{(aq)} + 8H_2O_{(l)} \rightarrow 15Fe^{2+}_{(aq)} + 2SO_4^{2-}{}_{(aq)} + 16\,H^{+}_{(aq)} \qquad \text{Equation 1-4}$$

Ochre deposits in rivers are now common problems in coal and metal mining areas in the UK and around the world, e.g. the River Don in South Yorkshire (Amisah and Cowx, 2000) , the River Gaunless in the northeast of England (Mayes et al., 2008) and in the River Akagawa and the River Kitakami in northern Japan(Sasaki et al., 2005).

As stated above, while mine water drainage is often acidic, this is not always the case. Neutral mine drainage (NMD), with pH typically 6–8, also occurs and can arise in two ways; i) alkaline components of host rock or surrounding soil (e.g., carbonates or hydroxides) neutralize the acidity of acid mine drainage emerging from sulphide ores (Shahhosseini et al., 2017), or ii) the drainage originates from non-sulphide ores or minerals (e.g., oxides, halides, carbonates, borates, etc.) that do not generate acidity in leach water (Bright and Sandys, 2016, Younger, 2000). Both forms of neutral drainage occur in the UK and can have detrimental impacts on the quality of receiving water and on ecology (Younger, 2000).

Dissolution of some minerals such as carbonate and aluminosilicate in the host rocks neutralizes the acidity produced by pyrite or marcasite (Nordstrom, 2011). Examples for neutral drainage where ores contain insufficient amounts of pyrite for the drainage to be acidic are Cligga Head Mine, Perranporth, Perranzabuloe, Cornwall, England, UK, in which the mine waters have near-neutral pH (6.5), (Palumbo-Roe and Colman, 2010), and the Frongoch mine site in mid Wales in which the pH values are closer to neutrality (Bearcock et al., 2010). In Northern Pennines mines, low amounts of pyrite, coupled with large quantities of acid-buffering minerals, e.g., carbonate rock, make mine waters with a neutral pH (Palumbo-Roe and Colman, 2010).

1.1.2 Impacts on biota from mining related metals and metalloids in water

The environmental impacts of the ochre formations described above are many and include accumulation of ochre on fish gills, which leads to damage to the gills. A study carried out by Larsen and Olsen (1950 as cited in Jones, 2013) to investigate the impact of iron-rich mining wastes on some fish showed that accumulations of ochre occurred on the gills of the dead fish (suggesting that ochre deposition had played a role in the death of the fish). They thought that the ochre precipitate on the fish gill prevents oxygen exchange between blood vessels on lamellae with water, resulting in respiratory distress and then fish death.

Ochre suspended particles are also known to cause a pronounced turbidity (Johnston et al., 2008). According to Utne-Palm (2002), elevated turbidity in water scatters the light, decreases clarity and so interferes with detection of prey. The mechanism is thought to include that scattering of light leads to a degrading of the target’s brightness and, as a result, it impacts on the contrast between prey and background. This phenomenon impairs the ability of both predators and prey to detect each other. Although a turbid environment may offer fish larvae some protection from predators, it can still cause them respiratory problems and is not optimum for adult predatory fish and thus, as a consequence, increased turbidity can cause structural changes in a fish community. This may play an important role in the distribution of species. This explains observed changes in diversity of species and consequently changes in community structure as a function of turbidity (see below).

According to Younger and Wolkersdorfer (2004), in receiving watercourses heavy loadings of suspended sediments can be caused by ochre particle formations. These particles decrease light penetration which leads to inhibition of photosynthesis, which negatively impacts the primary producers such as submerged higher plants, algae and various organisms in the periphyton in aquatic ecosystems. Consequently, it decreases the availability of food for the macroinvertebrate community and for the fish population that feeds on them. Furthermore, ochre particles contribute to the disruption of mating and territorial behaviour patterns, which rely highly on visual cues and subsequently affect strongly the reproduction, abundance, and population size (Younger and Wolkersdorfer, 2004).

Ferruginous minewaters, which typically cause major ochre precipitation upon mixing with surface water and/or oxidation, can cause smothering of fish and invertebrate eggs in the sediment. Ochre particles smother the interstitial pore space of permeable sediment fines or the gravel beds where the eggs are laid, and as a consequence the passage of oxygenated water to the eggs is inhibited (Mayes et al., 2008). For example, there was a large decrease in salmon in the water column in the Pelenna catchment in south Wales which happened due to smothering of salmonid spawning areas because of excess iron (ochre) particulates formation and sedimentation (Edwards and Maidens, 1995).

In addition to the problems associated with iron and ochre formation, other metals and metalloids including lead, zinc, copper, chromium, cadmium and arsenic are known to reach levels in mining affected rivers that are harmful to the environment.

In surficial water samples of mine-affected sites (pH 1.5-3.5) from Aljustrel mining region, Portugal, high metal concentrations of Pb (136 µg/L), Cu (68,795 µg/L), Cd (455 µg/L), Zn (264,377 µg/L), and As 6,837 µg/L were found which have potential direct and indirect impacts on the diversity in diatom communities (Luís et al., 2009).

In Korea, a study done by Lee et al. (2005) showed that the mean concentrations of As, Cd, and Zn in stream waters of the Songcheon abandoned mine areas were 246 μg/l, 161 μg/l and 3899 μg/l, respectively. The levels of As and Cd in drinking water were 10 and 4 times, respectively, greater than those considered safe. Additionally, the cancer risk posed by As for exposed individuals through the drinking water pathway was 5 times greater than that stipulated for regulatory purposes.

The Iron Mountain Mine activity in California, USA, led to increases in the concentration of zinc in the surface water. Many of the samples exceed the toxicity limits of US-EPA for zinc (Seal II et al., 1998).

According to the UK Environment Agency (2012), metal pollution (mainly Fe, Cd, Pb and Zn) from abandoned mines is a widespread issue in England and Wales and is responsible for 8% of the water bodies that fail to achieve good ecological and chemical status under the Water Framework Directive (WFD). For example, the concentrations of cadmium and zinc in Carnon river, England, exceeded the environmental quality standards (EQS) by several hundred times. The river was muted and aquatic life was not generally abundant (Morris et al., 2003).

In an Australian study of the ecological impacts of metals mine effluents on the benthic macroinvertebrates in the South Esk River, Tasmania, even at 80 km downstream from the source of the metals negative impact was observed. Relative composition of the fauna drifting reveals that the numbers of the helminthid beetles and baetid mayflies were lower in the contaminated section of the river. The relative number (number collected per 24 h at a flow rate of 1 m^3/s) of Baetidae: Baetis baddamsae decreased from 500 (above the source of the metals) to about 150 (below source of the metals); and Helminthidae:

Austrolimnius sp. (adults) reduced from 300 (above the source of the metals) to less than 100 (below source of the metals) (Norris et al., 1982).

Historic mining within the Leadhills area, Scotland, has heavily affected the Glengonnar Water. The levels of Cd, Pb and Zn in the water body exceed the environmental quality standards and have caused abnormalities and related effects on fish, such as the appearance of blackened tails of trout (Salmo trutta) which is a sign of chronic lead toxicity (SEPA, 2011).

Waste rock and untreated water from the chromite mines in Sukinda, India, pollute local water supplies with hexavalent chromium as well as the air and soils. Not only it is highly toxic to plants and animals but also causes human health problems such as infertility and birth defects are common in the workers and inhabitants; in addition locals suffer from increased prevalence of gastrointestinal bleeding, tuberculosis, and asthma (Das and Mishra, 2008, Iyer and Mastorakis, 2010, Das and Singh, 2011).

High concentrations of heavy metals, especially dissolved Cu (e.g. 3.4 µM) were found in the aquatic environment in the Copperbelt area in Zambia. This is attributed to the mining and smelting operations in the region. The high Cu concentrations measured could affect humans, livestock and wildlife living in the area (Ikenaka et al., 2010, Pettersson and Ingri, 2001).

In the case of acid mine drainage, low pH itself can have negative impacts on biota. Most species have a defined pH tolerance range, which, if exceeded, can cause damage to tissues and/or biological functions. A high concentration of H^+ ion in mineral soils can be toxic to plants; in addition to that, it can adversely impact ion transport mechanisms across cell membranes like membrane-bound ATPases (Shabala, 2017). Low pH affects the salmonid fishes in particular; the main effects are on mucous secretion ability and gill structure and ion and acid–base regulatory mechanisms at the gills, all of which have potential severe negative effects on organism fitness (McDonald, 1983, Wood, 2017). In the case of plants growing in mine impacted soils or sediments where the pH is decreased, the excess of hydrogen ions causes the general effect sometimes referred to as Proton rhizotoxicity (i.e. proton induced toxicity of substrate in the immediate vicinity of roots). This adversely affects root growth in many plants and applies its toxic consequences through disturbing the antioxidant defense system, damaging the plasma membrane H^+-ATPase activity, damaging the metabolic process, decreasing the nutrient availability by changing solubity and mobility of nutrients, and yielding damaging reactive oxygen species (Borhannuddin Bhuyan et al., 2019). There are many examples of those effects; e.g. *Erica andevalensis* and *Erica australis* were adversely affected by low pH (Oliva et al., 2018).

1.1.3 Accumulation of metals and metalloids in soils linked to mining

Soils in mining areas can become contaminated through aerial deposition of dusts, use of contaminated water for irrigation, surface water flow across exposed ores and mine tailings that subsequently enters soils, or subsurface percolation bringing contaminated water to soils. These have environmental and human health impacts and many cases have been described in the literature.

According to a reconnaissance study by Reuer et al. (2012), metal mining and smelting over 80 years had led to a significant lead and arsenic contamination of soils in the area. They found that the depositional history of metal aerosols from the La Oroya smelter in Peru has made a significant contribution to the level of high contaminants 1,658 ppm As, 127 ppm Cd and 3,177 ppm Pb. The high soil Pb and As levels are potential causes of serious health problems, according to the exposure and dose-response modeling performed and therefore are in need of remediation.

Irrigation water polluted by mine drainage of the Wanshan mining area in Guizhou Province, China, has contaminated important rice paddy soils. Total mercury THg and Cd ranged up to 130 (mg/kg) and 1.3 (mg/Kg) respectively from non-rhizosphere paddy soils near the mine. In brown rice, THg and Cd (mg/kg) were 0.21 and 0.47, respectively. Assessment of the health risks of consuming the rice from the area has concluded that there is a potential threat to human health (Li et al., 2014).

In Mae Tao, northwest Thailand, a study into mining contaminated paddy soils by Kosolsaksakul et al. (2014) found that there is a strong relationship between soil cadmium concentrations [up to 200 mg/kg] and rice grain Cd (0.05– 8.00 mg/kg). They thought that fine clay particles bearing Cd are being transferred especially during the rainy season onto the paddy fields, which are irrigated from creek and canal waters impacted by a large zinc mine. Consequently, locals suffer from kidney as well as other problems because of the Cd levels in rice.

High leachability of metals in the acidic conditions of mine water discharges or run-off has been shown to facilitate the spread of metals into rivers and onto soils. One study showed Cu at 10371 µg/l, Zn 44900 µg/l, Cr 489 µg/. and Pb 1587 µg/l in the extremely low pH (~2) tailings water samples from north-west of a gold-silver enrichment plant at Mitsero, Cyprus (Lortzie et al., 2015). The dominant contaminant transport mechanism in the area was shown to be fluvial in nature, in addition to a degree of chemical weathering/precipitation, but not aeolian. It has been argued that the high leachability combined with pH~2 water may generate toxic runoff during storm events, which could lead to the destruction of aquatic life, crops, and livestock in the surrounding environment (Kostarelos et al., 2015).

Mine wastes mainly include wastes rocks, tailings, and mine water. Flooding can mobilise and redistribute mine wastes both laterally and vertically within a soil profile and into and along a river or floodplain. As an example, such flooding events transferred and dispersed a large amount of material from the mine heaps close to the Xiropotamos stream, which passes through an region of Mn mines located adjacent to the Drama district in Macedonia, northern Greece. The heaps material was highly contaminated by As, Pb, Cu, Zn, Cd, and Mn, all of which were dispersed to the western edge of the Drama plain and around the Xiropotamos river course, with soils covering an area of about 50 to 200m laterally found to have a black colour as well as contamination because of the input of mine wastes (Sofianska et al., 2008, Sofianska and Michailidis, 2013, Sofianska and Michailidis, 2016).

1.2 Forms of metals and metalloids in water

1.2.1 Total, dissolved and particulate fractions

Mobility and toxicity of metal(loid)s in surface water are affected by the physical forms and chemical species in which the element is found (Namieśnik and Rabajczyk, 2010), so it is essential to understand what is meant by terms like dissolved and particulate.

Metals and metalloids can exist in surface water in various forms or fractions. Forms in water and are often described in terms of being in dissolved or particulate (Namieśnik and Rabajczyk, 2010). Combining the dissolved and particulate contents gives the total amount for any given element across all forms (Abbott and Price, 2014).

Dissolved forms include free cations such as Ca^{2+}, Fe^{2+}, Al^{3+}, Ag^{+}, or organometallics, for instance, $Hg(CH_3)_2$, $B(C_2H_5)_3$, $Al(C_2H_5)_3$, or complexes with dissolved organic matter, or inorganic complexes for example $Zn(OH)_4^{2+}$, $Au(CN)_2^{-}$, $Ca(P_2O_7)^{2-}$. Typically, dissolved concentrations are defined as the concentration in solution after filtering through 0.45 µm pore size membranes (Stumm and Bilinski, 1973).

Particulate forms can be solid metal compounds formed by weathering of minerals. Metals in particulate form can also be precipitated sulphide oxides, silicates, hydroxides or carbonates. Particulate metal forms also include cations sorbed to organic matter and mineral sediments (Namieśnik and Rabajczyk, 2010).

Colloids are finer particles with diameters that are less than 0.45 µm and so they exist in samples that have been filtered through 0.45µm filters, i.e. in the fraction considered by convention to be the dissolved component or phase. Colloidal metals refer to metals dispersed on/within colloids. They are highly mobile and remain suspended in water and are mobilized by water movement (Caspers, 1981). Colloidal metal forms are important because metals bound to colloids might not be readily available to organisms, or on the other hand colloids might mobilise metals in solution bring them into contact with organisms (Bradl, 2004).

1.2.2 Influence of water pH, Eh, hardness and organic matter concentration on element toxicity

The role of water chemistry in influencing element mobility and toxicity has already been alluded to in relation to ochre formation (see above), but the importance of these water quality parameters need to be fully appreciated.

Water pH in particular is a key variable for metal behaviour in the environment. Changing the pH affects heavy metal solubility (as shown in Equation 1-5, Equation 1-6 and Equation 1-7), and so the metal speciation which impacts the bioavailability and thus ecotoxicity (van Leeuwen and Vermeire, 2007).

$$\mathrm{M}^{n+} + \mathrm{H_2O} \leftrightarrow [\mathrm{M}(H_2\mathrm{O})_6]^{n+} \qquad \text{Equation 1-5}$$

$$[\mathrm{M}(H_2\mathrm{O})_6]^{n+}_{\text{Hydrated Metals}} + \mathrm{H_2O} \leftrightarrow [\mathrm{M}(H_2O)_5OH]^{n-1}_{\text{low-solubility polyhydroxides}} + H_3O^+ \qquad \text{Equation 1-6}$$

$$[\mathrm{M}(H_2O)_5OH]^{n-1} + \mathrm{H_2O} \leftrightarrow [\mathrm{M}(H_2O)_4OH_2]^{n-2}_{\text{low-solubility polyhydroxides}} + H_3O^+ \qquad \text{Equation 1-7}$$

where M is a mono or polyvalent cation (e.g., Fe^{3+}, Zn^{2+}, Mn^{2+} and Cr^{3+}); n is the number of positive charges on the cation; and the number of water molecules in the innermost hydration shell is with a maximum of 6 for most cations. Note that Equation 1-7 will continue up to n times, not all of the metal ions will necessarily be precipitated out as solid and some will remain in solution (van Leeuwen and Vermeire, 2007).

Water molecules form a hydration shell around dissolved metal cations (Foy, 2000) and this also influences their behaviour. The availability of cation forming heavy metals (which are the great majority) is therefore increased at low pH levels because of the greater solubility of the metal. The pH can also have indirect impacts on toxic metal solubility, mobility and availability through its effects on other parameters such as organic matter and nutrients (Ripley and Redmann, 1995). The pH also has an effect on the functionality of some ligands and thus on their ability to form complexes with the metals; for instance COO- becomes non-functional as a metal binding site by coordination with a hydrogen ion (COOH). This means that the H^+ is a competitor with the metals for such coordination sites and affects inorganic as well as organometallic interaction. This gives further explanation as to why metal cations become more soluble and more available at low pH; the additional H^+ in solution occupy binding sites on solids that might otherwise bind metals, keeping more of the metals in solution. Examples for how pH affects metal binding and solubility have been provided for Cu (Stumm and Morgan, 1970), Cd (Morel et al., 1973) and Fe (Hem, 1985).

According to Benjamin and Leckie (1981), there is a rapid change of adsorption capacity as a function of pH and concentrations of adsorbent and adsorbate. The order of sorption of metals on humic acids is affected by the pH; a study done by Kerndorff and Schnitzer (1980) showed sharp rises in sorption efficiencies with a pH increase from 2 to 6.

The pH and redox potential significantly control the relative stabilities of different metal-containing minerals and the order of metal solubility (Krumbein and Garrels, 1952). For example; under high Eh (i.e. very oxidising conditions), the ferric hydroxide precipitate is highly insoluble while under low Eh (or reducing) conditions the iron is reduced to ferrous iron, and its affinity to precipitate as the sulphide is low compared to many other elements (Elder, 1988). The redox potential also affects concentrations of dissolved components such as sulphide that can in turn control or influence precipitate formation, and so at reducing conditions the sulphides are of prime importance whereas in oxidising waters the oxides are one of the major precipitates (Berner, 1981).

The ionization state of metals can change based on the proton activity (pH) and the redox state of the metals can vary based on the electron activity (Eh) simultaneously, so the speciation is a sensitivity to both redox potential and pH in a net effect. A metal speciation distribution across different forms for iron is presented Figure 1-1, called a pourbaix or Eh-pH diagram. It is based on the fact that metal speciation is simultaneously sensitive to (i.e. influenced by) both Eh and pH. This double variable distribution diagram is used to describe the speciation of iron among various oxidized and reduced as well as protonated and deprotonated conditions (Hem and Cropper, 1960).

The low Eh means reducing condition while high Eh means oxidizing condition. Each region is characterised by a particular range of Eh-pH and indicates the species that has highest concentration as a function of the Eh-pH variables of an aqueous electrochemical system. The lines, which are calculated according to the Nernst equation or and equilibrium constants, refer to boundaries of a region of the dominant ion (Hem and Cropper, 1960).

As displayed in Figure 1-1, iron solubility is very low under a condition of pH above 4 with moderate oxidation where FeOH may precipitate, as well as a condition of very low Eh at a broad range of pH in the presence of S, resulting in precipitation of S^- . Under low pH (i.e. pH<2) the iron is comparatively soluble as free Fe $^{2+}$ under very low Eh conditions or complexes of F^{3+} under high Eh condition (Hem and Cropper, 1960).

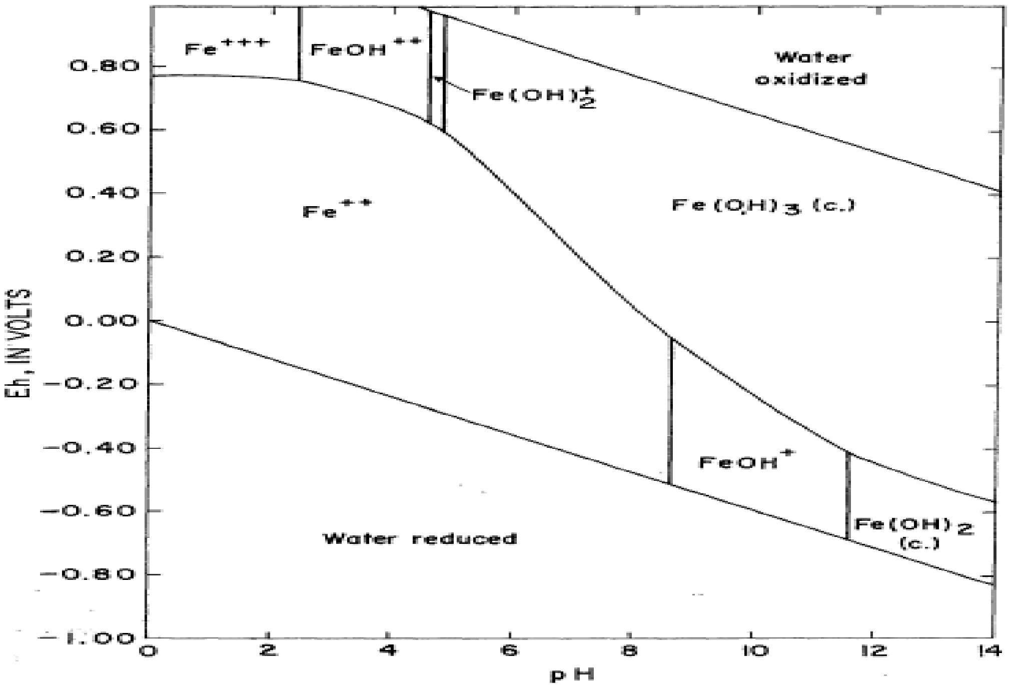


Figure 1-1: Pourbaix diagram for 1 M iron solutions (Hem and Cropper, 1960)

According to O'Connor and Kester (1975), high water hardness exerts significant control on metal speciation. It is commonly thought that the degree of water hardness impacts the toxicity by forming insoluble carbonates or by adsorption on calcium carbonate, especially for the mixing zone (Prosi, 1981). The complexation and adsorption presumably are affected by some major ions, especially magnesium and calcium, by competing with trace metals for surface binding sites, including those on fish gills and other biotic surfaces (Millward and Moore, 1982). Furthermore, a study carried out by Oduleye (1976) showed that the permeability of cell membranes to solutes seem to be controlled by some alkaline-earth ions, particularly calcium. Consequently, it has been repeatedly reported that there are decreases in metal toxicity which are attributable to increases in water hardness (Howarth and Sprague, 1978, Dunlop and Chapman, 1981, Stendahl and Sprague, 1982).

Charged dissolved organic matter present in water can act as ligands that can form some complexes and compounds with some of the metals. The organic matters have effects on the solubility of metals by controlling or influencing the solubility product, which can be represented by the equilibrium reactions (see Equation 1-8-Equation 1-11) (Elder, 1988).

$$\mathrm{MA}_{n_{(s)}} \leftrightarrow \mathrm{M}^{2+} + \mathrm{nA}^{-2/n} \qquad \text{Equation 1-8}$$

$$K_{sp} = [\mathrm{M}^{2+}][\mathrm{A}]^{n} \qquad \text{Equation 1-9}$$

Where M is a divalent metal; A is an anion e.g. (OH^-, CO_3^{2-}, or S^-), K_{sp} is solubility product.

In presence of charged dissolved organic ligands (L), side complexation reaction can occur between the ligands (competing ligand) and the free metals ions (Equation 1-10), however, this side complexation reaction forces more still dissolution. Theoretically, this would end with equilibrium not only comply with solubility product constant of the solid phase of metals but also comply with stability constant of organic matters-metals complexes.

$$\mathrm{M}^{2+}_{produced\ from\ equation\ 1-8} + \mathrm{L} \leftrightarrow \mathrm{ML}_{(aq)} \qquad \text{Equation 1-10}$$

$$K_s = \frac{\left[ML_{(aq)}\right]}{[\mathrm{M}^{2+}][L]} \qquad \text{Equation 1-11}$$

where Ks is stability constant.

Similarly, presence of charged dissolved organic ligands (L) can compete with metals sorbed on sorbing surface, metals complexed with ligands and also with sorbing surface to ligands or even with other system constituents. The results of this competition would be based on the concentration of these reactants and based on relative association constants of the complexes (Elder, 1988). Davis and Leckie (1978 as cited in Elder, 1988) observed that some ligands such as glutamic acid and ethylene diamine can adsorb in a particular configuration in which binding sites directed outward toward the solution are strongly able to bind the metal. Contrary to this, some ligands such as picolinic acid or salicylic acid form complexes in such a way that functional groups involved in the surface bonding of metals are not available, leaving no binding sites available for metal ions. So the extent and even the trend of the influence of organic ligands on metal speciation is variable and depends on factors such as pH, Eh, metal concentration, organic matter concentration and type of organic matter present.

1.2.3 Environmental quality standards for mining related metal(oid)s in water

In recognition of the potential harmful effects of mining related pollutants in waters, environmental quality standards (EQS) have been developed to identify threshold concentrations below which no adverse effects are expected. Different regions have different EQS and associated regulations, but in the European Union (including the UK) the EQS are established under the Water Framework Directive (WFD; European Commission, 2000). The EQS for the various elements that are relevant to mining pollution in water are briefly outlined in Table 1-1 below, with all EQS values stated being those set down in the Water Framework Directive (Standards and Classification) Directions (England and Wales) 2015.

Table 1-1: Environmental Quality Standards (EQS) for selected mining pollution related elements (WFD standards England and Wales (2015).

Element	Concentration (µg/l)	Application detail
Arsenic (As)	50	A mean (long term average of monitoring) applying to freshwater considering only the dissolved fraction obtained by filtration through a 0.45 µm filter.

Cadmium (Cd)	≤0.08; ≤0.45 (Class 1) 0.08; 0.45 (Class 2) 0.09; 0.6 (Class 3) 0.15; 0.9 (Class 4) 0.25; 1.5 (Class 5)	The EQS for Cd are determined according to water hardness class, in $CaCO_3$ equivalents. The first (left) values are set as means (long term average of monitoring) applying to freshwater. The second (right) values are separate EQS set as the maximum allowable concentration in freshwater. The water hardness classes are: Class 1: < 40 mg/l, Class 2: 40 to < 50 mg/l, Class 3: 50 to < 100 mg/l, Class 4: 100 to < 200 mg/l and Class 5: ≥ 200 mg/l).
Chromium (Cr) III	4.7; 32	The value 4.7 is the EQS set as a mean (long term average of monitoring) applying to freshwater. The value 32 is a separate EQS set as the maximum value that the 95th percentile of monitoring data can reach and still comply. These values are EQS specific for the III (+3) valence state of Cr.
Chromium (Cr) VI	3.4	A mean (long term average of monitoring) applying to freshwater. This is an EQS specific for the VI (+6) valence state of Cr.
Iron (Fe)	1000	Applies to all surface waters, considering total concentration.
Lead (Pb)	1.2; 14	The value 1.2 is the EQS set as a mean (long term average of monitoring) applying to freshwater. The value 14 is a separate EQS set as the maximum allowable concentration in freshwater. Both values apply to dissolved, bioavailable concentrations of Pb as calculated using the bioavailability calculator tool developed for the UKTAG*.
Zinc (Zn)	10.9	A mean (long term average of monitoring) applying to freshwater that is set for dissolved ‘bioavailable’ metal and to which the ambient background concentration should be added. The bioavailable fraction is calculated using the bioavailability calculator tool developed for the UKTAG*. The ambient background concentration for the Stoke-on-Trent area (Humber basin/catchment area) is 2.9 µg/l.

* UKTAG = UK Technical Advisory Group; see

https://www.wfduk.org/tagged/bioavailability-assessment-tool.

1.3 Forms of metals in soils and sediments

1.3.1 Inner and outer sphere complexes and ‘Total’ vs 'available' metals

Metals and metalloids are present in soils and sediments from natural sources as well as from pollution inputs and they occur in multiple forms. They can be associated with the mineral solids, the organic solids (or both), and be present in soil porewater. Metals can be directly bound to surfaces or be bound via intervening molecules or ions.

Water molecules can readily act as ligands to bond or complex with most types of multivalent cations in the soil solution to form aquo-complexes, hence water can play an important role in interactions between ions and soil surfaces. When there is one or more water molecules inter-posed between the functional group binding site of soil and the metal cation this is described as an outer-sphere surface complex (Tabatabai et al., 2005). When there is no intervening water molecule but rather a direct bond between ion and soil surface this is referred to as inner-sphere surface complexes, which typically involve a covalent or ionic bond strongly connecting metal(loid) ions with the electron-donating group in the soil surface such as oxygen or hydroxide groups (Stumm, 1995). Electrostatic bonding mechanism is proposed as the main driver in outer-sphere surface complexes, so they are less stable than inner-sphere complexes that necessarily involve either ionic or covalent bonding or some combination of the two (Johnston et al., 2002). This has significance for how mobile or available a metal(loid) is in the soil system, with those bound by outer-sphere complexation being held less strongly than those held by inner-sphere binding.

The total metal(liod) content refers to the sum of free and bound metal form in the soil. Total metal contents of soils therefore indicate the degree of contamination, but not necessarily the risk (as not all metals will be available to organisms, see later). Typically, digestion processes of soil sample are used to release the metals in acid solution and after that the samples are analysed by one of many analytical techniques to determine the total metal content. Several procedures are described by the United States Environmental Protection Agency (USEPA) such as 3050, 3050B, 3051B and 3052 methods, which all have subtle variations in specifications and operation conditions and the recommended analytical technique but all follow the same overall process of acid digestion and analysis. Spectrometry, chromatography, and colorimetry are analytical techniques that are used to determine the total metal(liod) concentration in digest solutions (Sparks, 2003).

The ability to speciate metal-contaminated soils is critical in predicting mobility and bioavailability of the metals and enhancing the remediation of contaminated soil. Although total metal contents of soils indicate the degree of contamination, it reveals no information on speciation or availability. Chemical extraction techniques, such as sequential extractions, provide useful information on quantities of metals species and/or their associations with certain soil components (Sparks, 2003).

1.3.2 Associations in soil fractions: selective & sequential extractions; soil porewater

The fraction of soil with which metals and metalloids are associated can strongly influence how readily they dissolve and come into soil solution and/or are accessible by plants and other organisms. Understanding these associations can help to reveal how available the elements are. For this reason, there have been many procedures and schemes designed to estimate the available metal concentrations and to determine the associations of metals within soils.

Soil porewater is often used as a measure of the available metal and metalloids (e.g. Lock and Janssen, 2001), while single solution extracts have also been widely used to quantify the elements in a soil sample that can be easily shifted into solution and are therefore readily available. Common single solution extractants include neutral salts such as 0.001M or 0.01M $CaCl_2$ (e.g. (Degryse et al., 2007, Houben et al., 2013, Wendling et al., 2009) or organic chelators such as ethylene diamine tetra acetic acid (EDTA) (Kim et al., 2003). Multiple or sequential extraction schemes have been developed to determine associations of elements across multiple soil fractions. Common schemes include the following:

The Tessier procedure includes five steps (Tessier et al., 1979). The steps in this scheme include determining the fractions of exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter and the final step is residual. The problem is the comparability of the metals contents results of the exchangeable and carbonates bound forms that are considered to be the most important bioavailability and that means there is a problem with the precision (Ure et al., 1993). Frentiu et al. (2008) found poorer reproducibility of Pb extraction due to a great variance in the mineralogical structure of soil. The steps in this scheme are shown in Table 1-2.

The Community Bureau of Reference BCR procedure has achieved a harmonization to the extractants and procedures for chemical speciation (or fractionation) of elements in soils and sediments. Certified standard soils have been prepared which are used for making certification of their EDTA and acetic acid extractable contents of some heavy metals; consequently, they can help to make agreed sequential extraction procedure (Ure et al., 1993). However, in the second step of the BCR scheme, the concentration of the extractant, the pH of the extracting solution and the speed of the centrifugation is not optimum for all elements and this can have a negative effect on the reproducibility and extractability especially for Cr, Cu and Pb extraction (Gwendy, 1996, Rauret et al., 1999, Quevauviller, 2002). The steps in this scheme are shown in Table 1-3.

The Maiz extraction procedure includes three steps, targeting the so-called mobile, mobilisable and residual fractions. A significant time saving was accomplished by using this method compared with e.g. the BCR approach (Maiz et al., 1997). However, there was a difficulty to perform a direct comparison between the result of this method with Tessier and BCR procedures because each method uses particular extractants with specific concentrations (Zimmerman and Weindorf, 2010), and this may be the reason why the Maiz method has not been widely used in soil studies. The steps in this scheme are shown in Table 1-4. The Geological Survey of Canada (GSC) procedure includes specifically targeting the Fe and Mn oxide fractions within the amorphous oxyhydroxides and crystalline oxides (Gwendy, 1996). The chemical reactivity of amorphous iron oxyhydroxides [$Fe\ (OH)_3\ .nH_2O$] is more than crystalline forms. The procedure uses the similarity of the chemical properties and ionic radius of Fe and Mn to target both metals in their oxides. This results in the extraction of amorphous iron oxyhydroxides, together with that of manganese oxides (Gwendy, 1996). This sequential extraction scheme achieves a good precision for a given test soil, with the range 2-10% RSD typically reported. However, metals bound to the organic fraction are potentially underestimated because they are extracted after the extraction of the amorphous compounds and Mn oxides. This is because the reagent involved, hydroxylamine, is also able to extract some metals bound to the organic matter (Benitez and Dubois, 1999). The steps in this scheme are shown in Table 1-5.

The modified GCS Benitez-Dubois procedure includes six steps for exchangeable, carbonate, organic matter, Mn-oxides and amorphous compounds, Fe-oxides and residual fractions. It also uses a different order in the extraction reagents, with the reagent targeting the organic matter being moved in the sequence to before the step that targets Mn-oxide bound metals so to avoid the problem of underestimating the organic bound fraction (Benitez and Dubois, 1999). However, the selectivity of extractant is still not high. This is because part of the metals bound to organic matter can again be dissolved by the hydroxylamine reagent. Neither $Na_4P_2O_7$, nor H_2O_2/HNO_3, have been shown to be optimum extracting reagents in the procedure for organic bound metals. The reasons are that $Na_4P_2O_7$ is unable to dissolve some organic matter forms, while H_2O_2/HNO_3 can dissolve other mineral phases, for instance, some sulphide compounds (Benitez and Dubois, 1999). The steps in this scheme are shown in Table 1-6.

Therefore, no single or sequential extraction procedure can be considered as ideal for every investigation, and thus a decision needs to be made in each investigation about the most appropriate and useful approach. All of the schemes produce operationally defined fractions rather than truly reflecting the target fraction, which must always be kept in mind. The decision about which extraction procedure to employ in a particular investigation therefore must be based on considerations such as the principal soil fraction(s) of interest, the speed (and thus time and expense) of the procedure, the compatibility of extractant reagents with available analysis equipment, and the comparability of the results with those from other studies. The last point, comparability, is one of the main reasons why the BCR and, to a lesser extent, the Tessier schemes remain very widely used and reported.

Table 1-2: Tessier sequential extraction scheme, OAc symbols to CH_3COO.

Steps	Fraction label	Reagent
1	Exchangeable	1M $MgCl_2$ at pH 7
2	Bound to Carbonates	1M NaOAc adjusted to pH 5.0 with HOAc
3	Bound to Fe and Mn Oxides	0.04 M $NH_2OH.HCl$ in 25% HOAc
4	Bound to organic matter	$HNO_3+H_2O_2$ then 3.2 M NH_4OAc in 20% HNO_3
5	Residual Metals	HF-$HClO_4$

Table 1-3: BCR sequential extraction scheme, OAc symbols to CH_3COO.

Steps	Fraction label	Reagent
1	Exchangeable and Bound to Carbonates	0.11 M HOAc
2	Bound to Fe and Mn Oxides	0.5 M $NH_2OH.HCl$ at pH 1.5 with HNO_3
3	Bound to organic matter	H_2O_2 then 1.0 M NH_4OAc
4	Residual Metals.	*Aqua regia*

Table 1-4: Maiz extraction scheme, TEA symbols triethanolamine and DTPA symbols diethylene triamine pentaacetic acid.

Steps	Fraction label	Reagent
1	mobile	0.01 M $CaCl_2$
2	mobilisable	0.005 M DTPA+0.01M $CaCl_2$+0.1 M TEA at pH 7.3
3	Residual Metals.	*Aqua regia*

Table 1-5: GSC sequential extraction scheme, OAc symbols to CH_3COO.

Steps	Fraction label	Reagent
1	Adsorbed, Exchangeable, and carbonate	1.0 M NaOAc adjusted to pH 5, conduct a second leach with 1.0 M NaOAc
2	Amorphous Fe, Mn oxyhydroxide	0.25 M $NH_2OH.HCl$ in 0.05 M HCl

3	Crystalline Fe, Mn oxyhydroxide	1 M $NH_2OH.HCl$ in in 25% HOAc
4	Oxidisable (organic matter and sulphide fractions)	0.75 g of $KClO_3$, 12 M HCl, 4 M HNO_3
5	Residual Metals.	12 M HCl, 16 M HNO_3

Table 1-6: Modified GCS Benitez-Dubois sequential extraction scheme, OAc symbols to CH_3COO.

Steps	Fraction label	Reagent
1	Exchangeable	0.1 M $NaNO_3$
2	Adsorbed	1M NaOAc in pH 5, with HOAc
3	Bound to organic matter	0.1 M $Na_4P_2O_7$
4	Amorphous Fe, Mn Oxyhydroxides	0.25M $NH_2OH.HCl$ in 0.5 M HCl
5	Crystalline Oxyhydroxides	1M $NH_2OH.HCl$ in 25% HOAc
6	Residual Metals.	HF, HNO_3, $HClO_4$

In addition to determining associations and fractionations by extractions, direct observation methods can also be employed such as molecular fluorescence spectroscopy, Infrared spectroscopy IR, X-ray diffraction and, synchrotron X-ray methods. The X-ray Absorption Near-Edge Structure spectroscopy (XANES) is a technique used to obtain information about the local structure around a specific a metal. Minkina et al. (2019) investigated the local structure of Haplic Chernozem saturated with Cu^{2+} and Pb^{2+} ions using X-ray absorption spectroscopy (XANES). They determined the mechanism of Cu^{2+} and Pb^{2+} ions interaction with soil phases and the nature of bonds formed by metal atoms. However, as with other methods of assessing element or mineral associations, the mentioned direct techniques may introduce artifacts due to sample alterations; additionally, their detection limits are often far above the background concentrations of the metals contained in the soils (Sparks, 2017). Molecular fluorescence spectroscopy and Infrared spectroscopy IR methods are limited to those compounds that fluoresce or have IR active bonds, while the XRD is limited to crystalline forms (D'amore et al., 2005).

1.3.3 Importance of texture, pH, Eh, and organic matter for metal forms & availability

The texture, pH, oxidation-reduction potential (Eh) and organic matter content of soils are very important in determining how readily soil elements come into soil solution.

The effect of soil texture on metal solubility in soils is best expressed in terms of the partition of soils into clay, silt and sand portions which are in turn defined according to particle size fractions of the soil (Qian et al., 1996). These components are significant adsorption surfaces for heavy metals in the soils. According to Andersson (1979), clay soil holds a high amount of metals when compared to sandy soil because of the increased surface area (i.e. potential binding area) of the much smaller clay sized particles and because of the greater charge density on clay particles; for example, the adsorption of lead and other metals to the soil components separately can be ranked as clay > silt > sand.

The high surface area given by clay for adsorption results in the accumulation of metal in the clay fraction and metals are naturally part of clay minerals, being within the clay lattice, but adsorbed metals on clay minerals can also become occluded in the clay lattice over time. This can complicate distinction of whether metals are adsorbed to or bound within clay. A high degree of extractability is found in sand fractions of the soil and this can be because of the low surface binding strength of these fractions for the metals (Qian et al., 1996).

Soil pH is considered the master variable concerning metal behaviour in soil systems (McBride, 1994) and is the most important factor affecting metal speciation in soils (Sposito et al., 1982). Generally, increasing pH results in increasing cation binding over a pH range of 1 to 2 units called adsorption edge (Davis and Hayes, 1986) while anion adsorption tends to decrease with increasing pH. According to Yin et al. (1996), the solubility of metals may increase at higher pH despite this, due to increasing binding with dissolved organic matters (DOM) which can mobilise metals in soil; the solubility of solid organic matter can increase as the pH increases (You et al., 1999). The mechanism behind the increased binding of cations at higher pH is that increasing pH leads to creating more deprotonated sites on soil solids and then metals in solution can react at these sites. In addition, the proton competition for fixed charge sites is less at higher pH values (Selim and Sparks, 2001). At high pH, metals may simply precipitate out of solution onto soil solids (Barrow, 1986).

Redox reactions in soils are controlled by the aqueous free electron activity (Sposito, 1983). Redox potentials in dry, well-aerated soils are typically high (e.g. an Eh>300 mV), while Eh of soils prone to waterlogging and rich-organic matter soils are low (e.g. an Eh<300 mV) (Evans, 1989). Several impacts have been recorded at different Eh. Sims and Patrick (1978), found decreasing in the concentration of soluble Zn in the soil pore water at low Eh (0-150) mV. Reducing of metal sulphates to insoluble sulphide occurs at low Eh<170 mV by acting of bacteria (Bauld, 1986, Sposito and Page, 1984) as cited by (Clark et al., 1997); contrary, sulphide minerals are normally oxidised to sulphates at higher Eh levels(Evans, 1989). The exchangeable forms of some metals in particular cases increase at low Eh (Bjerre and Schierup, 1985). Chuan et al. (1996) thought that under reducing conditions, the solubility of Pb, Cd and Zn in soils increase because of dissolution of Fe–Mn oxyhydroxides which lead to release of adsorbed metals as a mechanism. Therefore several effects occur for Eh simultaneously with pH on the metal form in the soil (John and Leventhal, 1995), which is logical considering that pH and Eh are linked as shown in the full version of the Nernst equation (Stumm and Morgan, 2012) (see Equation 1-12 and Equation 1-13).

$$a\mathrm{M}_{oxidised} + mH^{+} + ze^{-} \leftrightarrow bM_{reduced} + H_2O \qquad \text{Equation 1-12}$$

$$\mathrm{E} = \mathrm{E}^{0} + \frac{0.0591}{z}\, log \frac{[\mathrm{M}_{oxidised}]^{a}}{[\mathrm{M}_{reduced}]^{b}} - \frac{m}{z} 0.0591pH \qquad \text{Equation 1-13}$$

Organic matter has an important influence on metal binding although the amount of binding by organic matter is small compared to that of clay in mineral soils typically used for agriculture (Zimdahl and Skogerboe, 1977). This is not the case in organic matter rich soils or peat soils (i.e. soils with organic matter >30%), as in these soils organic matter can bind more metals than the clay components. The tendency of metals to bind with organic components in both the solid and solution phases of soil are very significant in metal binding by organic matter (Rieuwerts et al., 1998). Metals may form complexes with ligands which have varying affinities for adsorption onto the soil surface, in addition complexation of metals with solid phase organic matter, as well as complexes of metals with low molecular weight organic components for example fulvic acids (Rieuwerts et al., 1998). As a consequence, organic matters are an important factor in the mobilisation of metals in soil. The retention of metals by organic matter seems to involve both adsorption and complexation; specifically inner sphere reactions may happen in addition to ion exchange (Evans, 1989).

1.3.4 Soil guideline levels, thresholds or PNECs for appropriate metal(oid)s

Guidelines and regulations for metal/metalloids in soils are limited and are disputed (and this is even more so for sediment), but some thresholds have been determined.

The UK Environment Agency recognised the need to develop assessment criteria to protect soil and organisms live in it, therefore, soil screening values (SSVs) were proposed to assess the ecological risk for soil contamination. The UK Environment Agency defined SSVs as "concentrations of chemical substances found in soils below which there [were] not expected to be any adverse effects on wildlife such as birds, mammals, plants and soil invertebrates, or on the microbial functioning of soils.' (Ian Martin, 2017).

Soil predicted no effect concentration (PNEC) is a concentration in the soil below which a chemical or element is not anticipated to cause harm to the terrestrial ecosystem. PNEC or soil screening values can be used as a trigger value to prompt evaluation of the potential for environmental risk; i.e. concentrations above these values may indicate that an ecological risk is posed by the element concerned. Site-specific soil PNECs can be generated for Co, Pb, Ni, Cu and Zn using a spreadsheet that has recently been developed by the consulting company Assessing Risks of Chemicals consulting (ARCHE) and measured site-specific soil properties (ARCHE, 2014). The spreadsheet calculator can then be used to calculate the potentially affected fraction (PAF) of soil organisms as well as the Risk Characterisation Ratio (RCR, i.e. measured concentration/PNEC) for those metals in relation to the test soils.Non-site-specific screening levels for Cd, As, Fe and Mn for different protection goals are shown in Table 1-7.

Table 1-7: Non-site-specific screening levels for Cd, As, Fe and Mn for different protection goals

Element	ESSL* (mg/kg)	Goal of protection	Reference
Cd	0.77	Wildlife-Avian	(EPA, 2005b)
Cd	0.36	Wildlife-Mammalian	(EPA, 2005b)
As	60	Earthworm	(Efroymson et al., 1997)
As	50	Plant on grassland areas	(BBodSch, 1999).
Fe	200	Soil microorganisms and microbial processes	(Efroymson et al., 1997)
Mn	100	Soil microorganisms and microbial processes	(Efroymson et al., 1997)
Mn	220	Plants	(EPA, 2007)
Mn	450	Soil invertebrates	(EPA, 2007)

* ESSL = Ecological soil screening level.

1.4 Remediation of water impacted by mines

A number of approaches have been used to remediate mining impacted waters. A popular and successful approach is to channel mine drainage into lagoons and reed beds to promote removal of the suspended sediment and metals prior to entry into a river. Table 1-8 lists some examples of successful reed bed treatments (Brown et al., 2002).

The reed bed lagoon system works by performing some process. The land is planted with reeds which slow the flow and allow iron to oxidise, hydrolyse and precipitate in a 'natural' environment. Filtration of minewater within the soil and subsoil as well as through the reed stem bases and roots occur. Ochre becomes confined and stays within the reed filter, while the resulting low-particle water flows onwards. A Second process is settlement of the particles formed during first step by moving the particles downward to the ground of the reed bed (Wiseman, 2002). Reed bed systems can be highly successful, with another example being Mouse Water in South Lanarkshire, Scotland, where inflowing iron concentrations above 25 mg/l were reduced to below 1 mg/l (Johnston et al., 2008). One limitation, however, is that reed beds require considerable land area to be available for them to be constructed and implemented (e.g. 8400 m^2 for the Mouse Water example).

Table 1-8: Successful reed bed treatments schemes for mine waters

treatment system location	minewater type	type of system	year of completion
Acomb, Northumberland	net alkaline drift minewater*	settlement lagoons and reed beds	2001
Blaenavon, Gwent	neutral pH deep coal minewater	surface flow wetland	2001
Polkemmet, West Lothian	deep minewater, pumped	hydrogen peroxide addition, settlement and reed beds	1999
Wheal Jane, Cornwall	acidic metal mine drainage	Aerobic reed beds, anaerobic cells and rock filters.	1994
Old Meadows, Bacup, Lancashire	acidic drift mine discharge	pH adjustment with NAOH , settlement lagoons and reed bed	1999

*Net Alkaline minewater is characterised by fact that the total alkalinity>total acidity (Hedin et al., 1994).

Although iron has often been the focus of mine water treatment processes such as the cases described above, the other contaminants outlined in Table 1-1 (e.g. As, Cd, Cr, Pb, Zn, and additional contaminants) are of concern and remediation efforts also target these. The treatments for Fe do remove these elements also to a greater or lesser degree, and there are some additional treatments in use or testing. For example, As removal from acid mine drainage by encouraging activity of sulphate reducing bacteria has been demonstrated

(Serrano and Leiva, 2017), while nano-metal oxides have proven effective for removal of Cd and other metals from waste water via adsorption (Kumar and Chawla, 2014). Contamination of water and soil by Cd relesed from Zn mines has also been a major problem in some areas, such as north west Thailand, and this has caused harmful impacts to human health and the environment and has triggered remediation trials using biochar (Kosolsaksakul et al., 2018, Kosolsaksakul et al., 2014).

1.5 Remediation of soils impacted by mines

Soils impacted by mining related pollution (e.g. those described in section 1.3) need to be remediated to render them less of a risk to human health and the environment. The success of any remediation will depend on both the extent of the pollution and on the desired end use of the site. That is, a site to be used for agriculture or human dwellings requires a much higher level of remediation and restoration than a site to be used as a parkland or as an industrial site. The intended end use therefore needs to be considered in any remediation strategy (Wagner et al., 2016). However, regardless of the end use, there are some common issues that need to be addressed in the remediation process of any mining impacted site, including the issue of making the surface soil suitable for some use. Some of the more widely used remediation strategies are outlined in the following sections.

1.5.1 Soil removal or capping

For small areas, contaminated soils can be removed and placed in special landfills, but more typically contaminated soils or mining wastes are capped with a lining material such as clay and then ‘clean soils’ are placed over top (Guide, 2009).

What materials are available at or near the site as well as the toxicity of the material to be capped are very important in choosing the type of cap. The kinds of cover or cap can be divided broadly into categories of simple, composite and complex. Simple cover comprises optimally 30 cm of soil obtained at/near the site. The cap should be graded to a gentle slope to encourage runoff, and should be sufficiently compacted. The roughness of the surface of the slope should enhance vegetation, and not promote pooling of water and it should be vegetated by seed sowing or direct planting once it is in place (Lottermoser, 2010). Composite covers include at least two layers of various soil types. The lower layer lying next to the waste rock or tailings is characterized by fine-grained, high density and low permeability (e.g. clay material). It prevents water from the surface from seeping into the contaminated pile and forming acid drainage, while the upper layer is formed of a coarser material and its density is lower (e.g. soil/rock layer which holds moisture and encourages plant growth, as well as avoids erosion). The upper layer should be vegetated once it is in place (Hutchison and Ellison, 1992). Complex cover contains interlayered synthetic filter fabrics and fine and coarse material. Examples for synthetic geomembrane liner are polyester geotextile, polypropylene geotextile, polyolefin geomembranes and Sodium bentonite geosynthetic clay liner (GCL). The idea behind this cap is also to block water infiltration into the reactive material below as well as to encourage plant growth on the top (Bill Owens, 2002).

Ideally, at least for mines operated in recent times and in the future, the clean soils would come from the site originally; i.e. after having been removed and stored prior to the main mining operations starting. However, this can be problematic depending on budget and local conditions, as it has been noted that restored mining sites often have poor soil fertility because of the inability to preserve local, removed topsoil securely during the mining process (Ghose, 2004). It may also be that significant variations happen in the chemical and physical nature of the wider land system due to the disturbance of the overburden that is caused by surface mining (Rai et al., 2011). Soils may therefore alternatively be imported from elsewhere. Soil fertility issues still often need to be addressed in such remediation measures, with pH and organic matter being important factors. Organic matter is an important source of nutrients and influences soil structure, while an optimum pH of ~ 6.5 maximises nutrients availability levels and minimises toxic metal availability to plants. As discussed earlier, the pH controls the plant growth by its influence on the solubility of the chemical parameters such as nutrients (Harris et al., 1996).

1.5.2 Soil phytoremediation

In cases where capping or other remediation approaches are not viable or available, an approach of phytoremediation may be considered. Much research has been undertaken into phytoremediation of mining contaminated soils.

Phytoremediation is a plant-based remediation approach in which the toxic wastes are stabilized and/or concentrated and extracted from the soil by certain plants. Plants that are able to extract and concentrate high levels of pollutants are called hyperaccumulators (i.e. plants that can assimilate unusually high levels of metals) (McIntyre, 2003). Phytoremediation can be considered to have five strategies; phytoextraction, phytodegradation, phytostabilisation, phytovolatilization, and rhizofiltration. However, the commercially important strategies to date are only phytoextraction, rhizofiltration, and phytostabilization (Thakur et al., 2016, Salt et al., 1995).

In the phytoextraction process, particular plants called hyperaccumulators uptake some toxic ions from soil; toxic ions are directly translocated into the plant biomass through the roots (Cornish et al., 1995). By relatively inexpensive ways, the harvested plant parts can be combusted and disposed of as small amounts of ash and some elements can be recovered through extraction procedures if they are commercial valuable. The best candidates for phytoextraction are plants which have a sizable ground biomass and that can be harvested several times a season to eliminate toxic elements from the site (Brown, 1995).

Degradation of some contaminants can occur certain plants with the help of enzymes produced by the plants and plant-associated microorganisms (Singh and Singh, 2019). Phytostabilisation is the process by which the growth of plants, particularly their roots, stabilises waste mounds or contaminated soil. This prevents or reduces wind and water erosion of the contaminated materials and thus inhibits their further distribution within the environment. Plants that are tolerant to high element concentrations are suitable for this, regardless of whether they are hyperaccumulators. Plants vary in the internal mechanisms that allow them to cope with elevated levels of contaminants. Typical processes involve enzyme activity followed by storing of contaminants in plant vacuoles or exclusion from uptake (Pilon-Smits and Freeman, 2006, Champagne, 2007).

Phytovolatilization is an elimination pathway in which some contaminants can be removed by some plants. For example, Cattail was found to be a suitable species to completely removed Se from the aqueous system (Nattrass et al., 2019).

Some contaminants can be absorbed and accumulated in the rhizosphere using plant roots. This process is called rhizofiltration. For example, *Zea mays* was found to have a high potential for concentrated of the Mercury metal (Benavides et al., 2018).

Phytoremediation has been successfully employed at sites with contaminated soils or static water. For example, lead concentration in surface soil in Bayonne, New Jersey, USA decreased by grown three crops (harvested after six weeks) of *Brassica juncea* from 2,300 to 420 mg/kg (Henry, 2000), another example, in Dorchester site, Boston, USA Pb concentration was reduced from 984 to 644 mg/kg in surface soil, after three crops of (harvested after six weeks) *Brassica juncea* (Blaylock, 2000). Many plants are inserted in Table 1-9 the table below have proven to be successful at toxic waste sites.

Table 1-9: List of some hyperaccumulators plants.

Plant species	Metal	Metal accumulation mg /kg	Reference
Alyssum corsicum	Ni	18100	(Li et al., 2003)
Schima superba	Mn	62412.3	(Yang et al., 2008)
Azolla pinnata	Cd	310 - 740	(Rai, 2008)
Euphorbia cheiradenia	Pb	1138	(Chehregani and Malayeri, 2007)
Pteris vittata.	As Cr	8331 20675	(Ma et al., 2001) (Kalve et al., 2011)
P. cretica	As	2200–303	(Zhao et al., 2002)

Eleocharis acicularis	Zn Cu As Cd	14200 20200 1740 239	(Sakakibara et al., 2011)

The efficiency of a plant for phytoextraction, i.e. the capacity of the plant to accumulate metal, and its viability/limitations for phytoremediation depend on some factors corresponding to each specific plant such as Extraction Coefficient; Translocation Factor TF, Bioaccumulation Factor BAF, the level of metal uptake of hyperaccumulating plant to a certain metal compared with non-hyperaccumulating plant grown in the contaminated soil, (Ali et al., 2013, Rascio and Navari-Izzo, 2011).

Translocation factor (TF) of metals from root to different parts of plant was defined as ratio of metals in plant shoot to that in plant root The values of TF >1 for a metal indicate sufficient metal translocation of that metal from root to shoot of a certain plant. Extraction Coefficient refers to as ratio of total metals in plant to that in soil, hyperaccumulating plant uptake should be also sufficient, it should be higher than the total metal concentration in the soil i.e. the value of Extraction Coefficient greater than one. Or, as an alternative, the used hyperaccumulating plant at least should hyperaccumulate an equivalent to the phytoavailable mount of the contaminant. Therefore, the bioaccumulation Factor BAF, which was described as ratio of total metals in plant to the bioavailable in soil, should be greater than one (Khaokaew and Landrot, 2015). The threshold concentration for defining hyperaccumulation by a plant is generally 2 or 3 orders of magnitude greater than that of most species growing on “normal” soils and at least one order of magnitude greater than the usual range found in other plants from metal-enriched soils (Neilson and Rajakaruna, 2015).

Furthermore, the resistance of the plant to different types of environmental stress, that is the survival rate of plant species up to the final harvest day, is an important factor. The hyperaccumulating plants that have high survival rates up until the final harvest day are likely to be the most efficient for treating the impacted fields. The numbers of harvest repeats required to remove a target metal from most impacted fields are affected by the planting densities and biomass of the plants (Khaokaew and Landrot, 2015).

1.5.3 Immobilisation of metal(oid)s

An alternative approach to removing metals by phytoremediation or by soil removal is to immobilise metals in place within soils. In this approach the aim is not to remove them but to restrict their mobility and availability to the point that they are no longer environmentally active and therefore no longer a risk to ecosystem health (Bolan et al., 2014). A number of materials have been/ are being trialled for this approach, including red mud (a bauxite processing residue), hematite, biochar, drinking water treatment residuals (WTR) and zeolites (e.g. Gibbons and Gagnon (2011); Garau et al. (2007); Garau et al. (2014); Makris et al. (2009); Silvetti et al. (2014)). The successes of the immobilisation treatments, however, have been inconsistent. For example, the long-term efficiency of gravel sludge, a waste product of the gravel industry, with red mud, that have been trialled for immobilising Cd, Pb, and Zn in contaminated arable soils close to a former Pb/Zn smelter, appeared to be a realistic and practical measure for the mentioned land and it is best in combination with metal-excluding cultivars (Friesl-Hanl et al., 2009). A study carried out by Garau et al. (2014) aimed to evaluate effectiveness of amendments at stabilising As, Cd, Cu, Pb and Zn in a sub-alkaline contaminated soil, with the amendments including red mud (RM), hematite (Fe_2O_3), an iron-rich water treatment residual (Fe-WTR) and amorphous Al hydroxide (Al-OH). The authors found that all the amendments except RM immobilised As whilst only Al-OH immobilised the heavy metals examined. In another study was carried out by Kumpiene et al. (2013), As-contaminated soil was treated with Fe without combination and in combination with peat, biosolids from sewage sludge, coal fly ash, or gypsum. Soil amendment with only iron showed 99 % efficiency for As immobilization, but only down to a 50 cm depth. The risk of As dissolution was substantially increased as a result of the slightly reduced soil conditions in deeper layers. The soil amendments Fe combined with peat was best that reduced dissolved As in soil solution to what was considered as the safe levels (Kumpiene et al., 2013).

The effects of these amendments on soil properties and processes (including microbial processes) have not been fully explored. However, the addition of organic amendments can increase enzyme activity such as urease, phenylboronic acid-protease, β-glucosidase, alkaline phosphatase and arylsulphatase (Park et al., 2011). Kumpiene et al. (2009) found that the amendment combination also helped soil recovery from contamination through increased sustained plant growth, soil enzyme activity, and microbial biomass and respiration (all of which are indicative of increased soil fertility and microbial function). Even after ten years of the treatment by the fly ash, which has a long-term neutralizing capacity, the pH of the treated soil stayed neutral rather than decreasing to pre-treatment low pH levels (Bolan et al., 2014). This indicates that in-situ immobilisation with amendments such as these can be a potential long term resolution to a soil contamination problem.

1.5.4 Using ecotoxicology to assess impacts and remediation success of contaminants in water and soil

Ecotoxicology is the study of the harmful influence of contaminants upon ecosystems and includes influences on the individuals and consequential influences at the levels of population and above (Walker et al., 2016). A dose-response relationship, that is, the relationship between the adverse effect on living organisms as a result of exposure to a chemical and its concentration, is of central importance in both toxicology and ecotoxicology. It is based on the concept that a chemical (or substance) is poisonous only when its concentration rises high enough to cause harm (Walker et al., 2016). This approach can also be used to assess toxicity (harm) of mining impacted water or soil, i.e. the mine contamination can be evaluated through its ecotoxicity. Ecotoxicity testing can be carried out with terrestrial organisms or aquatic organisms, depending on the environmental media that is thought or known to be contaminated. For terrestrial assays with a focus on soil environments, commonly employed test organisms include plants such as ryegrass and soil dwelling invertebrates including earthworms, springtails, and other beneficial arthropods. Toxicity testing with aquatic organisms, i.e. to test toxicity of impacted aquatic environments, can be conducted to assess impacts arising from direct absorption of contaminants from water or tests concerned with sediment toxicity (Valavanidis and Vlachogianna, 2015). Common aqatic test organisms are crustaceans such as Daphnia (water fleas) and fish such as rainbow trout (*Oncorhynchus mykiss*).

Several ways are used as an endpoint in testing the toxicity. The most commonly recognised measure of ecotoxicity tests (test endpoint) is lethality (i.e. death), which can be quantified by e.g. the median lethal concentration or ‘LC50’. However, more sensitive and subtle endpoints are also important and are routinely used. Behavioural effects of a chemical on reproductive, biochemical, and physiological processes are also used as measures of toxicity, with the endpoint quantified as the concentration causing the median the median adverse response or decreasing the biological response by 50% (known as the 'EC50'). It is thought that population drops may be the result of sublethal, such as growth, instead of the lethal effects of chemicals (Council, 2014). The methods mainly depend on exposing a certain living organism to different concentrations of contaminants and observed the impact on the organism. The collected data from this method is used to extrapolate the concentration of the contaminant that lead to an adverse impact, such as mortal, sublethal effects, such as those on growth and reproduction of the organism. The method can also be used in an approach of exposing a selected test organism to effluent or waste at different dilution levels and observe its toxicity or the hostility of the resulting environment to the test organism (Walker et al., 2016). This has been done successfully to assess the toxicity of mining waste effluents to rainbow trout (Gerhardt, 1998).

The success of the soil remediation process can also be assessed through ecotoxicological testing, for example, Yoon et al. (2019) examined weight increase, mortality, and bioaccumulation of heavy metal in the earthworm (*Eisenia fetida*) to assess remediation of heavy metal polluted soil by applying limestone, acid mine drainage sludge, and steel slag with varied application ratio (1, 3, 5%).

Similarly, ecotoxicological approaches can have an essential role as an indicator in quantifying the risks of metals to biota before and after remediation of metals contaminated water. Chalkley et al. (2019) applied Macroalgae as spatial and temporal bioindicators of coastal metal pollution following remediation and diversion of acid mine drainage.

The ecotoxicological approaches based on the use of biomonitors and biomarkers (particularly biomarkers that are highly specific for a metal) are valuable for knowing the exposure and possible effects of that metal. This is a significant reason for utilizing biomarkers in environmental risk assessment; for example, Araujo et al. (2019) studied bioaccumulation and morphological of lead on inhibition of aminolaevulinic acid dehydratase (ALAD).

1.6 Conclusions and knowledge gaps

This literature review has indicated the current state of understanding on this topic. It has aimed to identify the key processes leading to contamination from mining areas and the factors that control contaminant metals and metalloids in the soil and water environments. The literature review has identified the following research gaps:

1.6.1 Previously under-researched location of the study

Few studies have examined the metal contamination in mining-affected sites at Staffordshire. Bradley and Cox (1986), (1987) studied the distribution and partitioning of base metals contamination at historic mining area in North Staffordshire. However, the researchers sampled the floodplain soils, which have a different scenario from that in this study. Mehra et al. (1999) examined the distribution and bioavailability of metals in soils in the vicinity of a copper works in Staffordshire, UK. The researchers investigated soil contamination of only Cd, Cu, and Zn. The contamination source was through aerial deposition, not through acid (or other) mine drainage as the case in this study. The literature review indicates that no previous research has investigated the metal contamination in the study site (Parrot’s Drumble, in Talke Pits in Kidsgrove, Staffordshire).

Therefore, what the extent and the nature of the contamination in Parrot's Drumble, Talke Pits area is unknown and considered as a contextual research gap, which is crucial for monitoring and restoration of this area.

1.6.2 Unknown extent of environmental impact

Although the study site has not been the subject of scientific investigation, it is clear to agricultural and recreational users of the area that an ongoing environmental impact exists and that this is likely linked to the former mining activities conducted there. The extent and environmental implications of that impact therefore need to be determined and understood in order for best management practises to be identified and implemented.

As previously mentioned in the literature review, when oxidation of sulphide-minerals occurs, acid mine derange is generated. The acid mine derange is characterised by low pH and high concentrations of metal(loid)s, therefore the study site may have high levels of metal contaminants in its soils and waters and they may also have adversely low pH. However, several factors, which vary from site-to-site, can have a significant impact on the acidity of any mine drainage or seepage generated and on the processes that govern metals contamination in the mining-affected area. Of those factors, the available amount of oxygen and water and the mineralogy of local rock material at the site are important to determine when developing an understanding of mining legacy impacts and the metals contamination (Akcil and Koldas, 2006). In a former Pb and Zn mining in North Yorkshire, UK, carbonate-rich rocks reacted with and neutralized the acidity generated by sulphide oxidation (Jones et al., 2013). While issues of acid and other polluting forms of mine drainage were a major problem at the Wheal Jane mine, Cornwall, UK (Whitehead and Neal, 2005). As Mulligan and Yong (2004) stated, metals can be fixed onto different minerals through different mechanisms, for example, Co and Zn can embed in hexagonal cavities of Montmorillonite while Zn, Cd, Cu, and Pb adsorb onto illite through chemisorption at edges mechanism. Consequently, every mining impacted site is unique to some degree in terms of the factors of underlying geology, ore type, resource extracted, closure activities (if any), over bearing soil type and hydrology, and therefore too the extent and nature of the associated risk. Therefore, to get precise knowledge about the nature and extent of mining-impact at the study site, there is a need for characterising the location across all of these aspects and particularly in terms of contaminant metals(loid)s and their toxicity.

1.6.3 Pooling characterisation tools for evaluating the contamination in mine-affected sites

It is crucially important to understand what the problems are or what makes the water and soils in the mining-affected sites ecologically vulnerable. There is a need for assessing the contamination to understand the scenario

Ecotoxicology deals with toxicological impacts on ecology caused by chemicals or conditions. Assessment of contaminated sites, long term monitoring programs, remedial action plans, and assessment of the success of remediation processes all require or can be enhanced by, the use of ecotoxicity and bioaccumulation tests. Furthermore, limit values designed to protect ecology and ecosystems (e.g., ecological screening levels values) for some chemicals have been derived by some environmental agencies based on ecotoxicology tests. A potential risk is posed by a metal (or other substance) when the water contain higher levels than their ecological limit value. Ecotoxicity tests can include assays for microorganisms, plants, invertebrates and vertebrates (Plants et al., 2009). However, the mining-affected sites can pose a risk to a nearby aquatic ecosystem. The risk can be assessed by the evaluation of the physical and chemicals parameters of water (Jørgensen et al., 2015). However, when aquatic animals are exposed to a toxic mixture, one toxin may increase (antagonism) or decrease (synergism) the effect of another, or the combined effects may be merely additive (Parker, 1979, Damasceno et al., 2017). Therefore, evaluation of the risks posed by of contamination, based only on physical-chemical indicators without examining aquatic biota cannot provide a precise understanding of the extent of the problems and thus gives an incomplete picture of the needs regarding remediation options. The risks must also be assessed by evaluation of the biological indicators (Beane et al., 2016).

A highly controllable and precise method to assess biological indicators is through ecotoxicity tests,e.g., using *Daphnia Magna* to assess toxiticy of assess toxiticy of mining-affected water samples. This directly indicates toxicity to individuals and also can provide useful information related to the population and to the health of the entire aquatic ecosystem (Norberg-King et al., 2018). However, such ecotoxicity tests evaluate the toxicity of mining impacted environments considering effect as total from of all pollutants contained. That is, identification of the primary toxicant(s) in effluent or drainage (or soil) cannot be achieved using such ecotoxicity tests alone but rather a combination of ecotoxicity tests and environmental contaminant chemical characterisation is needed.

The literature review has identified the research questions that need to be addressed when investigating a site that is potentially affected by a mining history and when evaluating how best to remediate such a site if required. This includes determining: the current levels of the metal(loid)s in the soil, soil pore water and surface water at the site; the bioavailability of the metal(loid)s and their mobility (i.e., whether they are easily shifted into soil solution and are therefore readily mobile and available); the fraction within the soil solids that the contaminants are bound to (i.e., speciation/fractionation); toxicity of soils and waters at the affected sites to organisms that are important for ecosystem functions; and identifiying the appropriate remediation options according to the information generated and the specific site conditions.

Quantification of the intensity of enrichment or accumulation of metals on surface soils is one of the approaches for assessing metals contamination in soil. The approach compares the measured pseudo-total content of the examined metal at the contaminated site with that of the background levels of that metal at the nearby uncontaminated area. Environmental indexes such as Enrichment Factor, and geo-accumulation index detailed in Manta et al. (2002) and Okedeyi et al. (2014), are used in this approach. However, this approach provides no information about the mobility or bioavailability of metals, which is essential in assessing the contamination risk at the study site. Therefore, it should be accompanied by other tools.

Description of the mobility of metals (i.e., whether they are easily shifted into soil solution and are therefore readily mobile and available) is crucial in the evaluation of the environmental risks of metal in soils in addition to assess the success of metal-contaminated soils remediation. The metals mobility varies with differing metals distribution in the soil constituents, as described in section 1.3.2. Researchers use sequential extractions procedures to understand metals fractionation. The metal speciation (or fractionation) determined via the BCR sequential extraction procedure has been and is still being used widely (Ure et al., 1993, Asmoay et al., 2019).

As shown in Figure 1-2, the bioaccumulation of metals, metals content in soil solution and metals toxicity test are linked together (Harmsen, 2008). Methods to measure or predict the bioavailable fraction of metals are based on either chemical extraction or mechanistic modelling, see Figure 1-3, (Kim et al., 2015).

Metals phytoavailability can be examined using single extractants such as 0.01 M $CaCl_2$ that simulates the natural soil solution (Barber, 1984, Marković et al., 2018, Houba et al., 2000). In terms of the potential for pollutant transition from soil to plant on agricultural land, trigger values were developed and used under Germany's Federal Soil Protection Act for extracted metal(loid)s. These values have been set concerning the growth weakening of cultivated plants (BBodSch, 1999). Although single extraction is widely employed in studies of soil contamination assessment, there is still a need to use another method to investigate the risk of contamination for protection each of soil organisms.

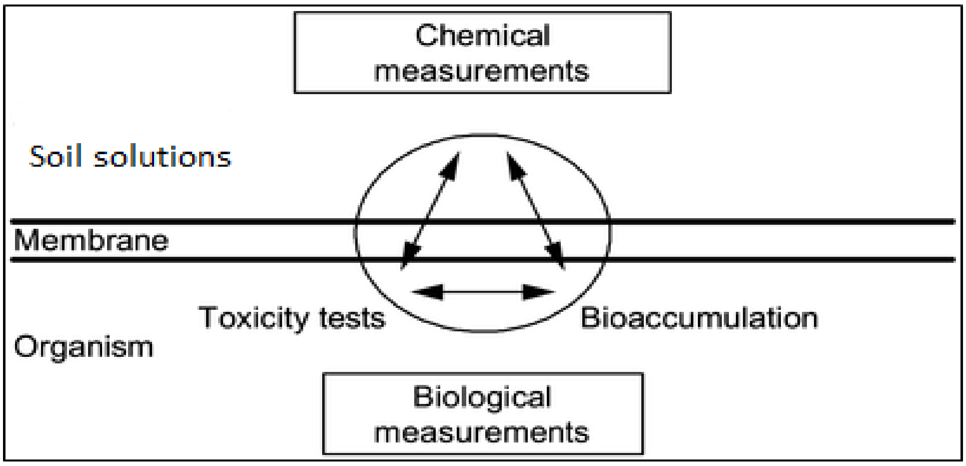


Figure 1-2: Relation between chemical and biological assays and bioaccumulation (Harmsen, 2008).

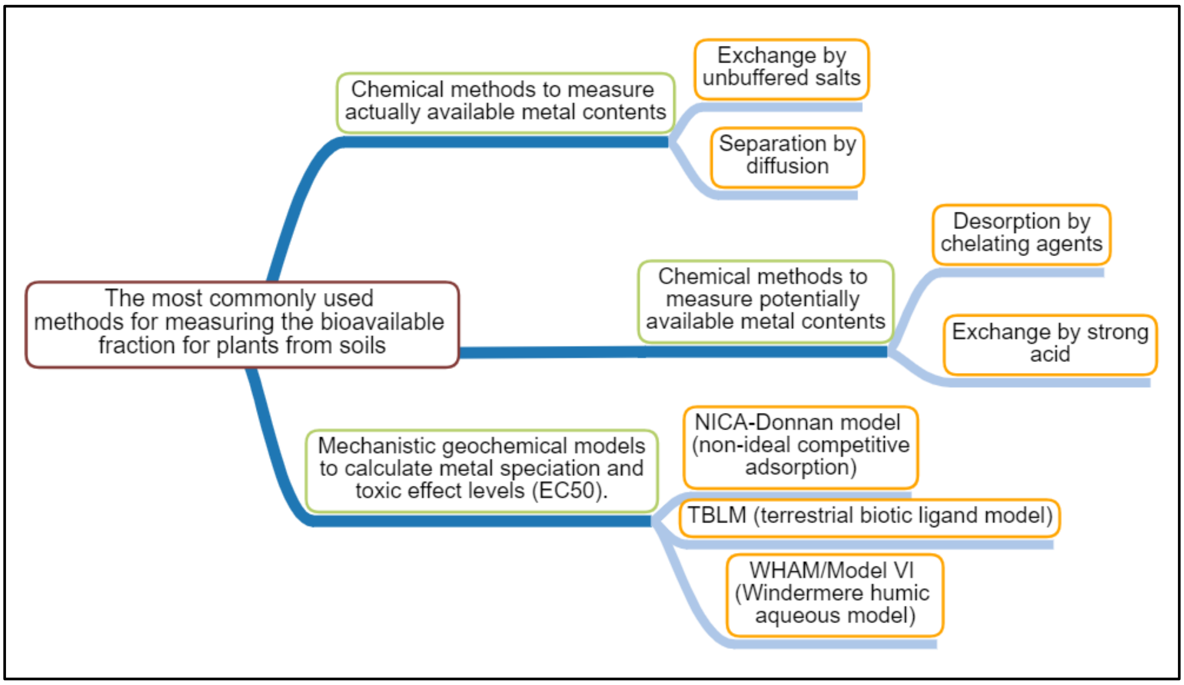


Figure 1-3: Summary of the most commonly used methods for measuring the bioavailable fraction for plants from soils (Kim et al., 2015).

Site-specific soil PNEC calculator has developed by the consulting company Assessing Risks of Chemicals consulting, ARCHE (ARCHE, 2014) As described in 1.3.4, pseudo-total metal concentrations in the contaminated soil, uncontaminated background levels of that metal, soil pH, organic carbon%, clay % and effective cation exchange capacity (eCEC, cmole/kg) can be used to predict soil-specific ecotoxicological threshold concentrations (expressed as pseudo-total metal concentrations in soil mg/kg dry weight). The calculator generates a PNEC, estimates the potentially affected fraction (PAF) of soil organisms, and calculates the Risk Characterisation Ratio (RCR, i.e., measured concentration/site-specific PNEC). However, this approach benefits for few metals, which are Cd, Co, Cu, Pb, Mo, Ni, and Zn, also, this approach reveals no information about the mobility and the distribution of metals in soil constituents and thus needs combination with biotic measurements and soil porewater measurements that do indicate mobility.

To conclude, the site contamination knowledge gap needs to be examined by assessing the metals enrichment index based on the pseudo-total metal content, examining the metals fractionation, studying the phytoavailability using $CaCl_2$ extractant, examining the metals in soil solution, and assessing the Risk Assessment Ratio.

The literature review has also highlighted that there is no universally applicable single approach to investigating and remediating a site that is impacted by previous mining activities but that, rather, there are a variety of options available that must be considered and carefully decided upon. It is also clear that any final approach adopted will likely only be arrived at through trial and error in order to determine the most appropriate measurements, tests, and remediation strategies.

1.6.4 Evaluation of remediation sites options

The literature review has also identified the research questions that need to be addressed when evaluating how best to remediate such a site if required.

Soil washing methods including both chemical methods, e.g., using chelating agents or physical methods, e.g., attrition scrubbing and wet-screening (Liu et al., 2018). They are not preferable in the study site because they generate secondary waste products that require additional hazardous waste treatments.

Bolan et al. (2014) reviewed the remediation of heavy metal(loid)s contaminated soils through manipulating the bioavailability utilizing a range of soil amendments. The amendments are either mobilising the metal(loid)s, e.g., chelating and desorbing agents or immobilising the metal(loid)s, e.g., precipitating agents and sorbent materials. A mobilising amendment increases the mobility and the bioavailability for a contaminant, while an immobilising amendment decreases the mobility and the bioavailability for a certain contaminant. Remediation of the mining-affected site in this study by applying mobilising amendments with the aid of plant uptake and soil wash is not a preferable option for the study site because cattle are grazing in part of the land and there is a risk of metal accumulation in the cattle. Therefore, in this study, remediation of the study site will be through using immobilising agents.

The need for plentiful, active, low-cost materials for use in mine site remediation has, therefore, stimulated interest in finding new applications for readily available by-products that might otherwise simply be discarded; in this study, the utility of using water treatment residuals (WTRs) in the remediation of mining-impacted soils will be tested. The most common soil application of WTRs reported in the literature to date has been as a general amendment and as a means to limit P mobility in over-fertilised or otherwise over-enriched soils (Oliver et al., 2011). While several studies have investigated their effects on some of soil microbes following soil amendment with WTRs (e.g., Garau et al. (2014), very few, if any, have examined the influence of WTR application on plants, earthworms, and soil solution chemistry in mining-affected soils after the application. This is a gap in current understanding of the benefits of using these materials in mining-affected soils, especially considering that plants and earthworms are widely recognized as essential ecosystem factors.

2 Study Locations, aims and objectives

2.1 Site history, geography, current use and concerns

Coal mining has a long history in Staffordshire, England, dating back to the 13th century but with most of the large scale extraction occurring in the 19th and 20th century (Coal Mining in North Staffordshire, nd, Talke Info, 2008). The location of the present study is a nature reserve (called Parrot's Drumble) owned by Staffordshire County Council that is in a restored coal mining area. The region is in the heart of the coal mining area of the English Midlands (Figure 2-1). The location is in the borough of Newcastle-under-Lyme, very near to Talke Pits (Figure 2-2). This area previously had multiple collieries and related activities (Table 2-1, and Figure 2-2).

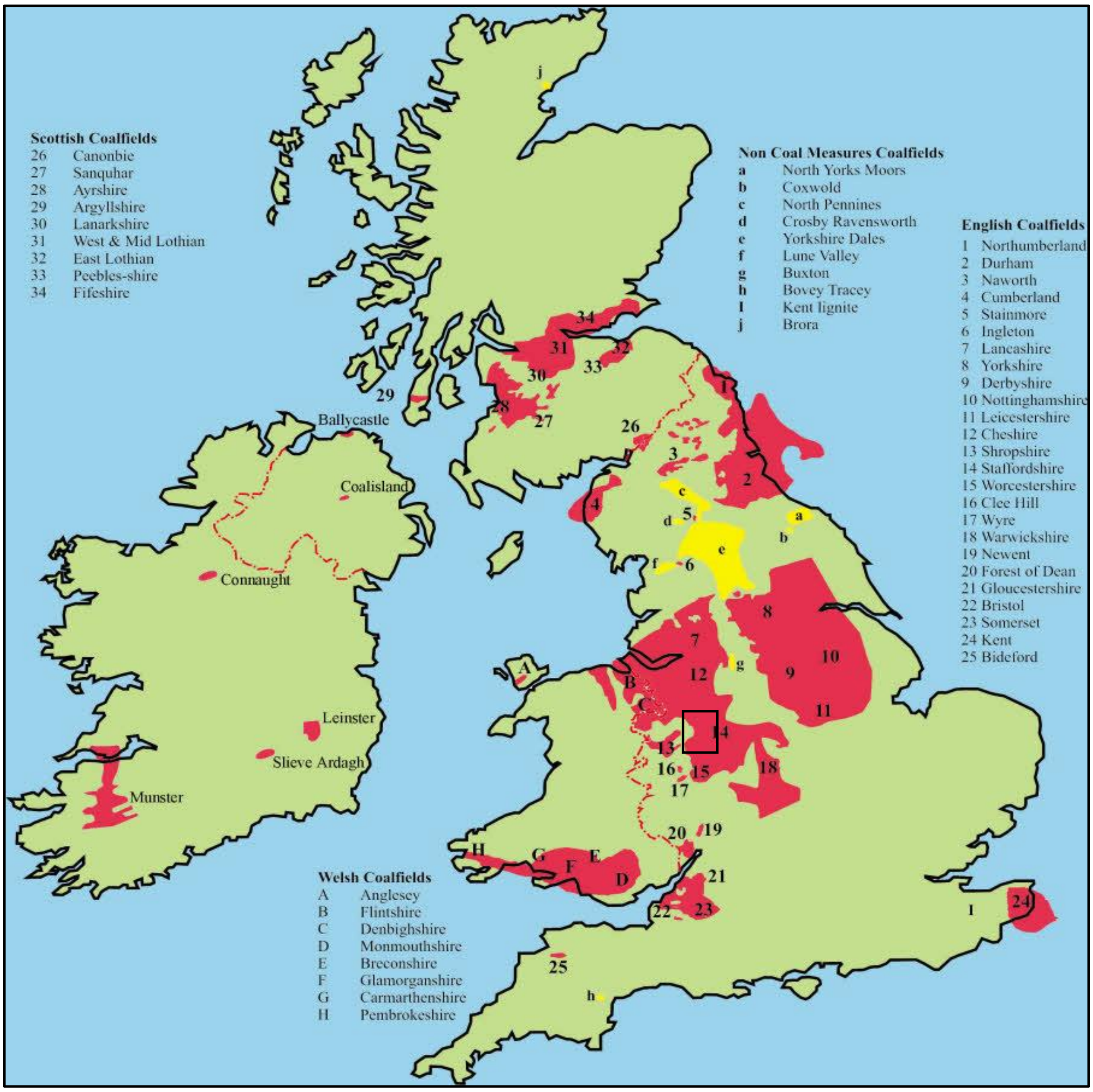


Figure 2-1: Coal map of the British Isles, showing the Staffordshire fields at point 14 (Northern Mine Research Society, n.d. available from http://www.nmrs.org.uk).

Table 2-1: Location and years of operation of some important coal mines near the study site

Mine	Coordinates (Easting/Northing)	Years of operation
Talk-o'-th'-Hill	E 382305 N 352730	1855-1940
Jamage	E 382218 N 351670	1910-1925
Jamage Main	E 382575 N 351670	1910-1925
Jamage Footrail	E 382635 N 351495	1915-1922
Rookery	E 381678 N 351685	1855-1900
Talke Green No.5	E 381875 N 353065	1930-1953
New Peacock Hay	E 383355 N 351965	1955-1970
Bignall Hill	E 382234 N 351673	1855-1900
Wedgewood	E 382550 N 351176	1947-1980
Mitchell’s Wood	E 383455 N 351355	1945-1960
Apedale	E 382250 N 348440	1855-1969
Silverdale	E 381348 N 346742	1855-1998

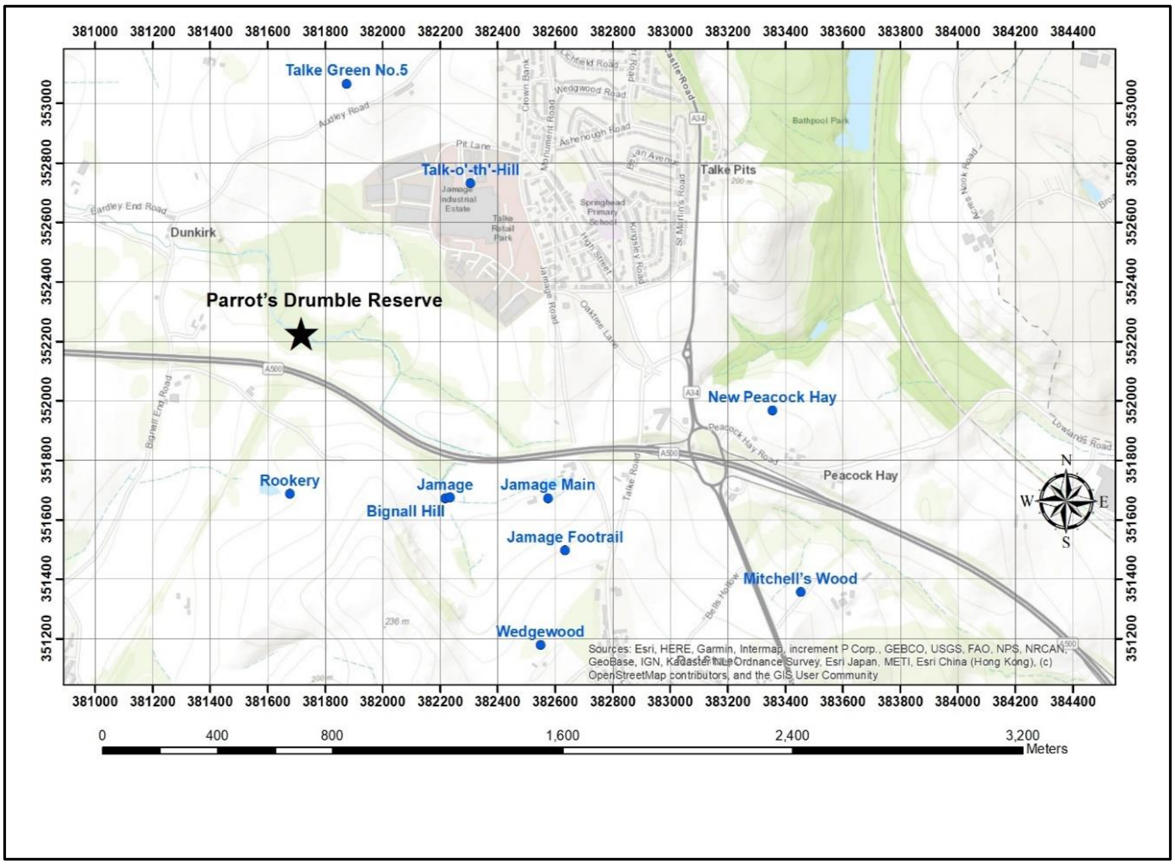


Figure 2-2: Map showing locations of former mine sites near the study location. It is from 1951-1953, Using: EDINA Digimap Ordnance Survey Service, <http://digimap.edina.ac.uk/>, Created: Jan 2017. It shows location of study area (Parrot's Drumble Reserve) in relation to Talke o' th' Hill Colliery and Carbon Works. Rookery and Jamage Collieries are also visible.

Other industries, in addition to coal mining and processing, were also in operation in this region. Talke oil works included two retorts that opened around 1865. Moreover, it was reported that two blast furnaces, which were owned by North Staffordshire Coal and Iron Company, were fully operational to produce pig iron from the coal, coke, and ironstone (Oliver, 2005). Furthermore, it is also assumed that the two retorts operated to produce oil which was used in coke ovens, blast furnaces and a brick works in Talke. These activities may also contribute to a pollution legacy for the area. Figure 2-2 showing the location of the study area and its proximity to Talke o' th' Hill Colliery and Carbon Works.

Topographically, the study area lies in Kidsgrove east to Dunkirk and near to Talke Pits (Figure 2-3). Most of this land is over 150 m AOD with the highest ground reaching over 165 m AOD. There is a considerable range of elevation (185 m AOD) in the north and the east of it.

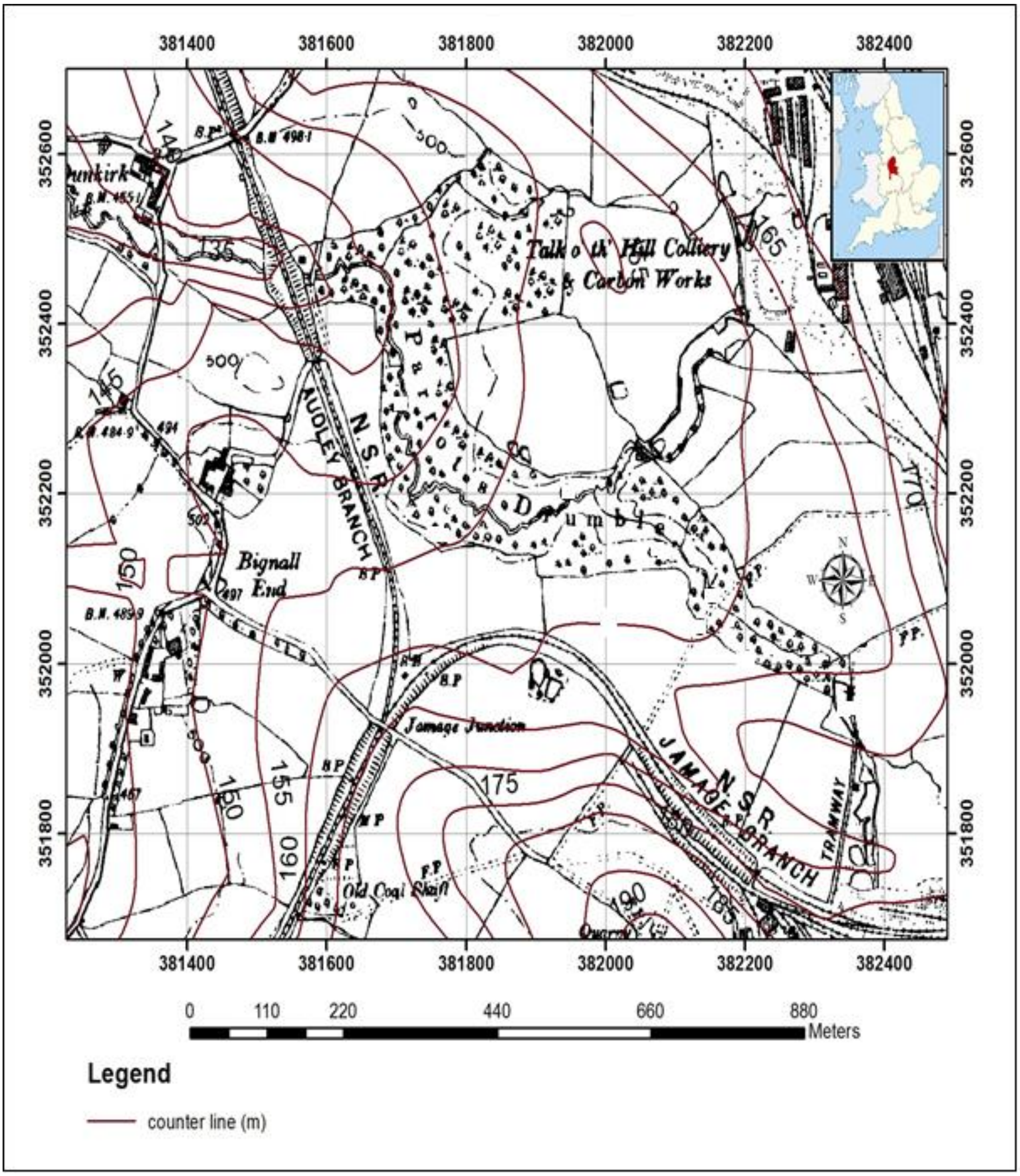


Figure 2-3: Topographic map shows the contour lines of the study area. Updated June 2016. Using: EDINA Digimap Ordnance Survey Service, Master Map® Topography Layer, <http://digimap.edina.ac.uk/>, downloaded on Jan 2017.

The superficial deposits of the study area and surrounding region are described as Till derived from erosion of material by the moving ice of a glacier, Alluvium containing sand with clay and gravel, and Peat which is an accumulation of partly decayed plants or organic matter (Figure 2-4).

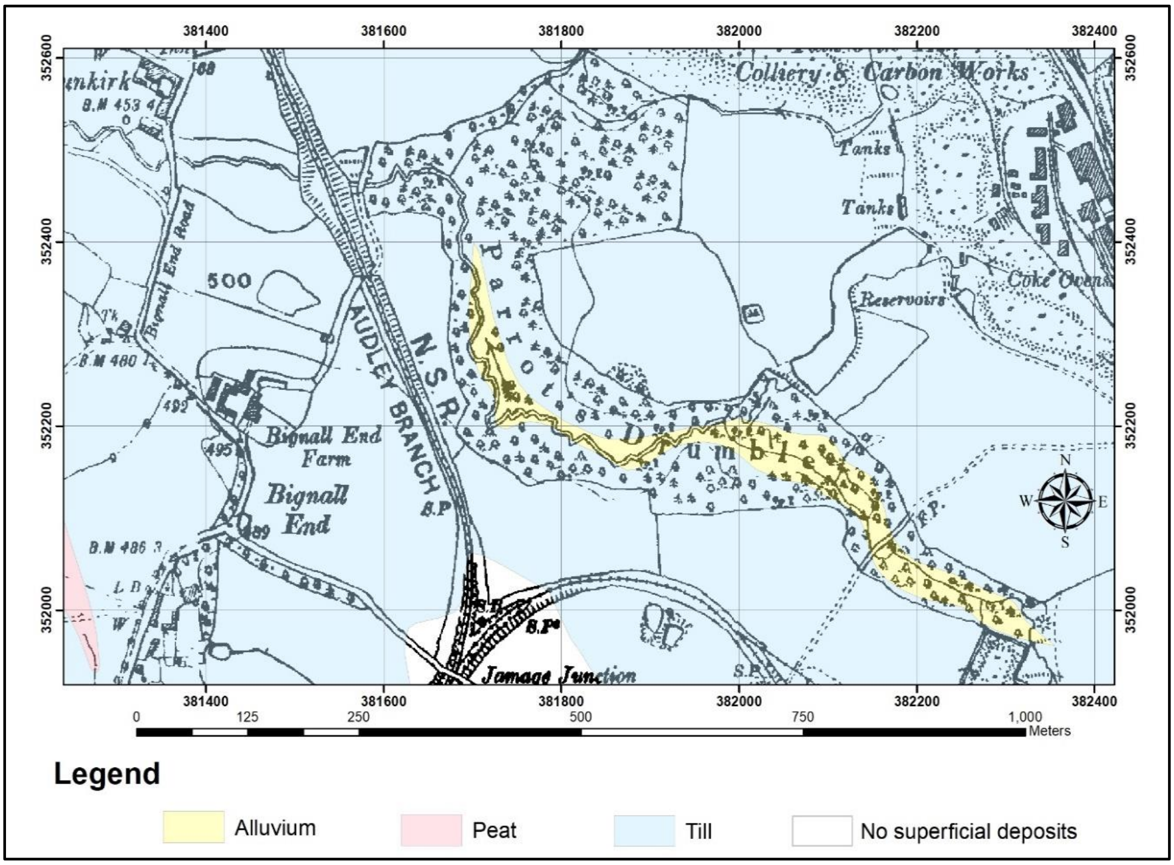


Figure 2-4: Superficial deposits map of the study area and surrounds. Updated: June 2011, BGS, Using: EDINA Geology Digimap Service, <http://digimap.edina.ac.uk>, downloaded: Jan.2017.

According to (Rees and Wilson, 1998) the bedrock geology description of study area is Pennine Middle Coal Measures Formation.

The Coal Measures comprise

- claystones,
- mudstones,
- siltstones,
- sandstones,
- ironstones,
- coals and
- rare limestones.

They were deposited in delta or alluvial plain settings in the Pennine Basin. The formation thickness is up to 600m thick across the North Staffordshire Coalfield. Many faults seem clear from the bedrock map of the study area (Figure 2-5). Blackband and clayband ironstones a sedimentary rock occur in the coal measures formation of the area. The ore minerals in the area that are commonly known are:

- Lead sulphide known as galena PbS.
- Zinc sulphide known as sphalerite.

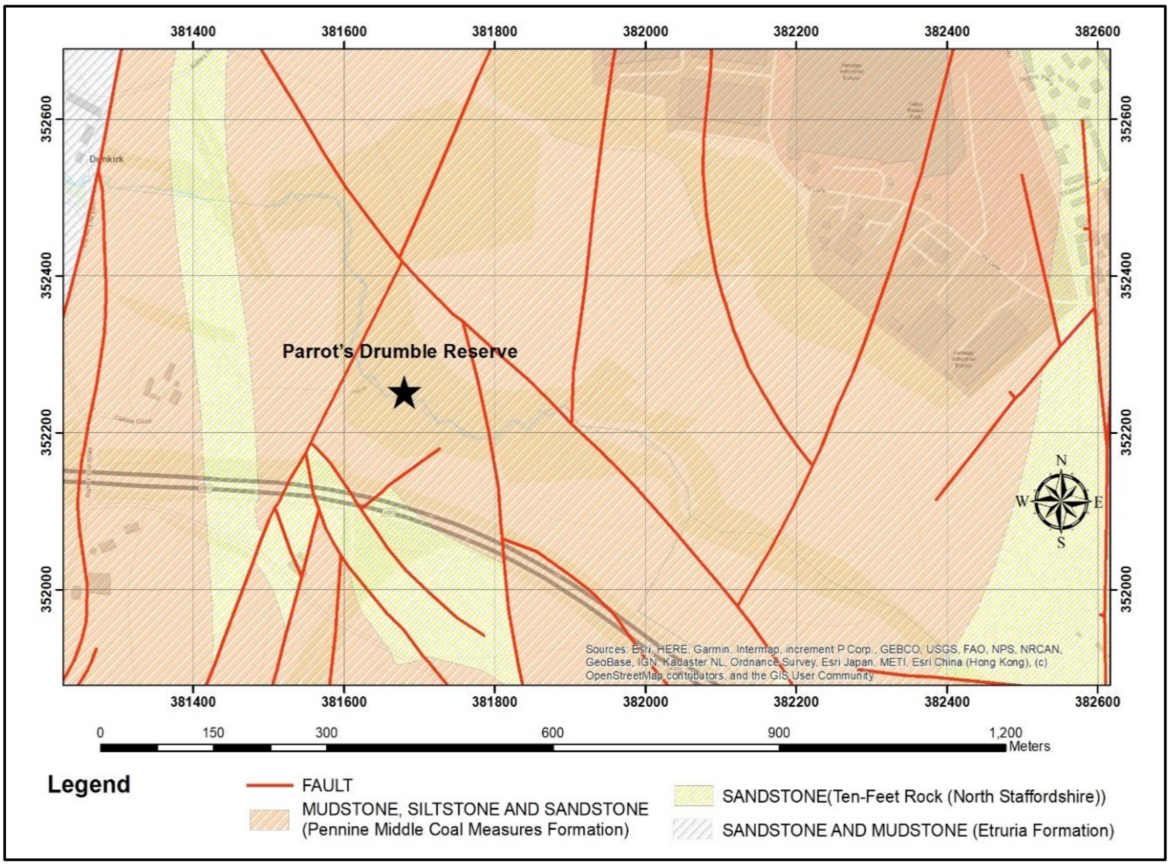


Figure 2-5: Bedrock map of the study area. Updated: June 2011, BGS, Using: EDINA Geology Digimap Service, <http://digimap.edina.ac.uk>, downloaded: Jan.2017.

According to the Climate-data website, the area experiences significant rainfall throughout the year even during the driest months. Precipitation averages 858 mm and the temperature averages 8.8 °C (Climate-data, 2014).

After the closure of the mines in the area, site restoration was by back-filling, capping and covering with topsoil. Across the region, restored sites became used for agriculture, grassland, and commercial operations including industry. The specific location of this study, Parrot’s Drumble Reserve, is used for recreation (e.g. dog walking and hiking) and is immediately bounded by pasture land grazed by cattle. A water course runs through the reserve, entering at the eastern end via a poorly defined culvert beneath the A500 road and then flowing east to west. At points along the water course the water often appears orange (Figure 2.7), suggesting the possibility that ochre (precipitated iron) is accumulating that may originate from seepage from mine spoil material (i.e. acid mine drainage). This has potentially major consequences for the ecology of the stream. The watercourse is not large enough to warrant monitoring under the Water Framework Directive by the Environment Agency and therefore the quality of this water has not been previously monitored or evaluated.

Although the stream is not subject to regular water quality monitoring and classification by the Environment Agency under the Water Framework Directive (WFD), on account of its modest size, the site is within the North West River Basin District of England which alone has over a dozen waterbody locations that do not meet the criteria for good status because of pollution from abandoned mines (Environment Agency Catchment Data Explorer). Similarly, the adjacent River Basin District of Humber, which encompasses Stoke on Trent, has more than 30 waterbodies monitored under WFD that have poor classifications linked to abandoned mines (Environment Agency Catchment Data Explorer).

Figure 2-6: Water in the Parrot's Drumble with indications of iron precipitation and possible water quality problems (photo taken in July 2014 by I.W. Oliver).

In addition to potential water quality issues, the soils surrounding the Drumble show indications in places that they may be adversely affected by contamination linked to the previous mining activities of the area (i.e. bare patches with poor or no growth of plants; Figure 2-7). The direct cause is unknown, but the problem may be linked to possible mine slag heap deposits that were poorly disposed of or to seepage or upwelling of water that percolates through mine spoil or other materials beneath. The existence of some spoil materials (i.e. slag heaps) are known in the area, as shown in maps dating from the 1960s (Figure 2-8). The impacted soil means less grass production than would otherwise be possible and therefore this limits the agricultural productivity of the area (i.e. reduced grazing capacity).

Figure 2-7: Areas of soil apparently impacted by previous mining related use of the area. The upper panel shows the area designated as Seepage Point 2 and the lower panel the area designated Seepage Point 3 (see descriptions later).

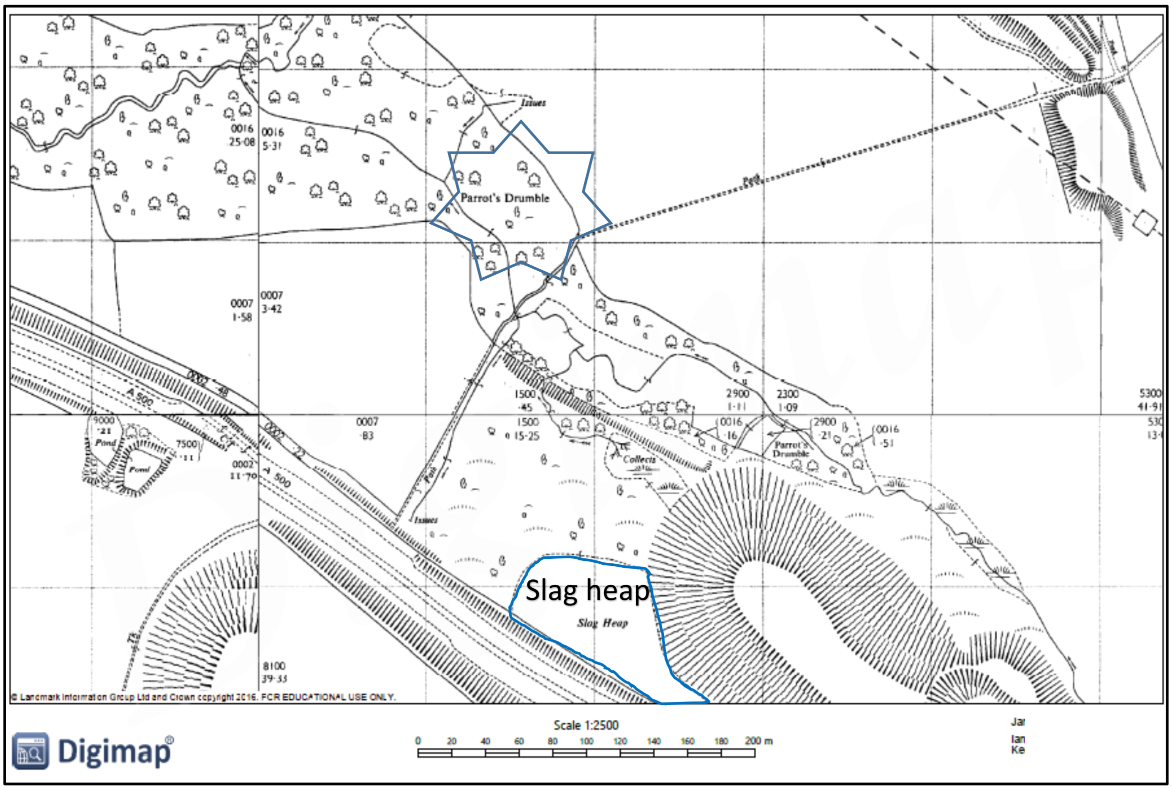


Figure 2-8: Map from the 1960s showing location of a known mine slag heap in the field immediately south of the study site. The slag heap is in the lower part of the image, above (and in line with) the middle of the scale. Note that in the electronic version of the map is created using: EDINA Geology Digimap Service, <http://digimap.edina.ac.uk>, downloaded: Jan.2016.

The extent of the contamination of soil, sediment and water at the site, and the ecological and agronomic implications of it, are not currently known and therefore investigations of these environmental media need to be made. To achieve that, sites along the Parrot's Drumble Reserve have been selected for an initial sampling and analysis campaign Figure 2-9.

The sampling was designed to investigate and evaluate the range of sites observed in the location, i.e. water upstream and downstream of suspected points of contaminant entry, seemingly unafected soils, and bare/ damaged areas of soil potentially affected by mining spoil hotspots and/or seepage and upwelling of water moving through mining spoil at depth. The sampling points were therefore selected to correspond with the conceptual model of the site that was conceived to visualise the likely factors that led to the current situation (Figure 2-10).

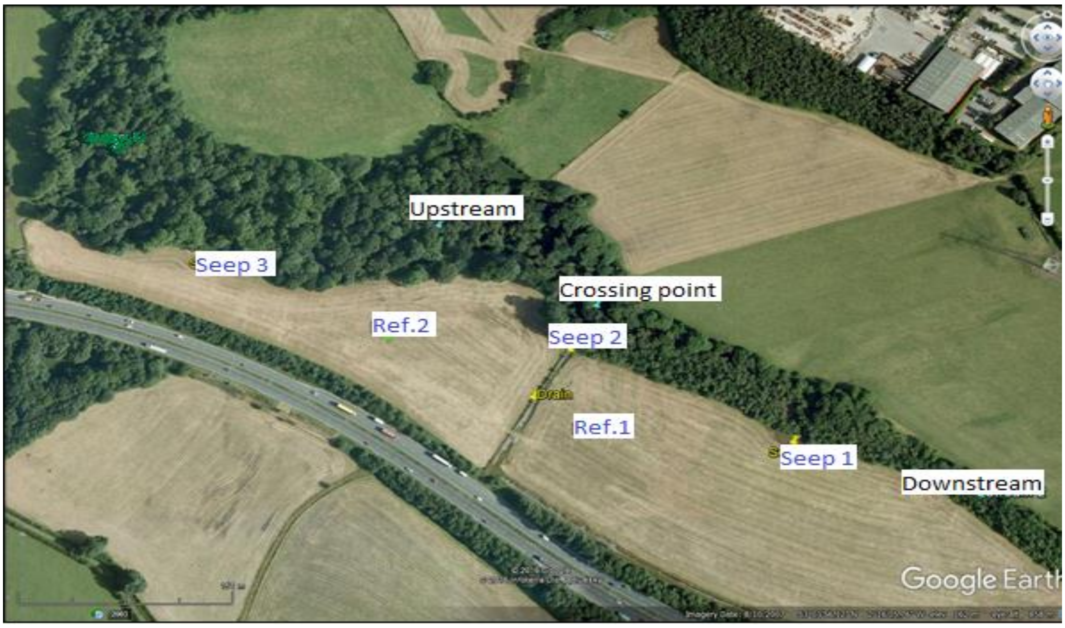


Figure 2-9: Map (aerial photograph) shows selected sites for the sampling of water, sediment and soil, including a soil reference site (Ref 1) where grass grows well and soil appears normal. Note Stream site 1 is just out of map coverage. The road visible is the A500.

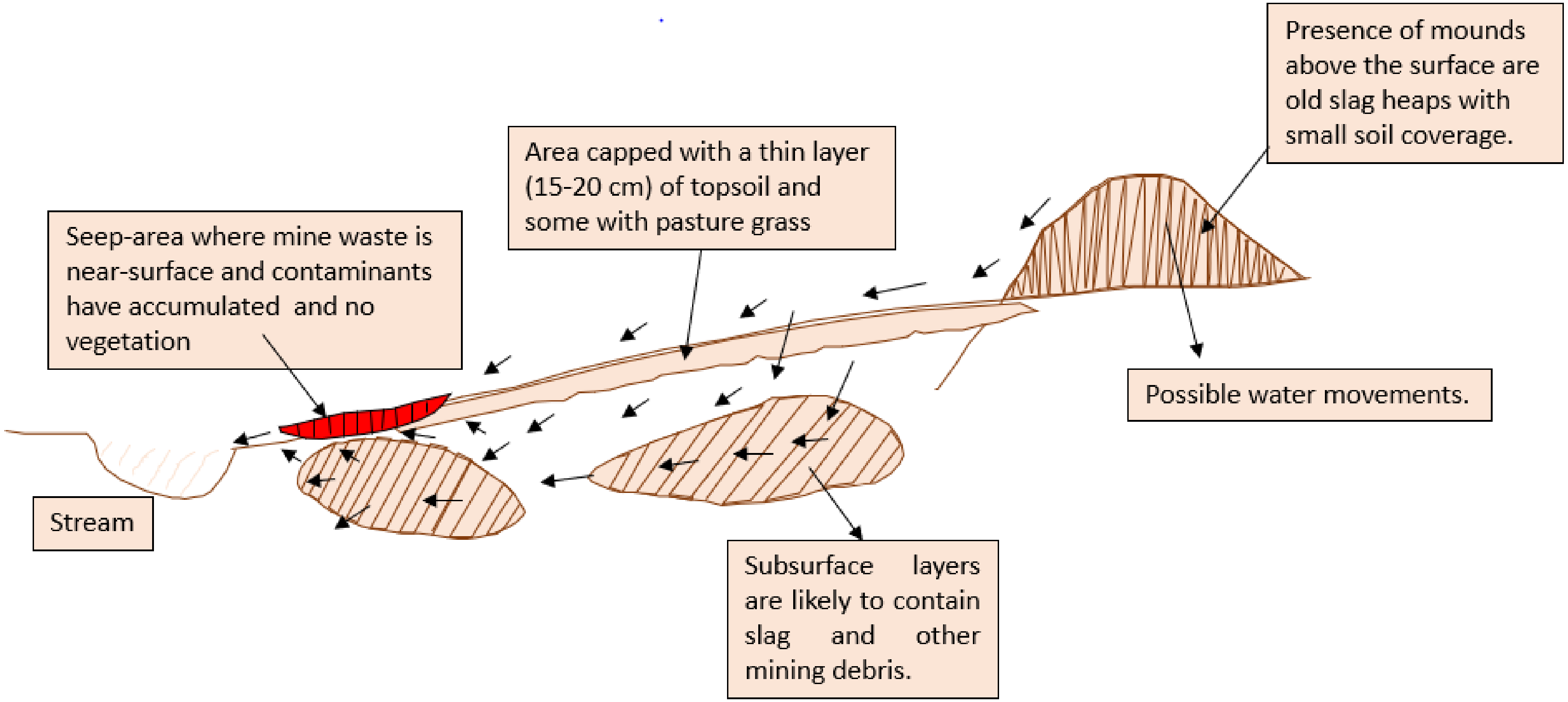


Figure 2-10: Conceptual model of the site. Cross-section (idealised) to indicate likely water movement and possible pathways of contaminants dispersion from old mining waste and debris to soil and stream.

2.2 Aims and objectives

2.2.1 Aims

Generally, acid or neutral mine drainage, fluvial deposition, and spoil material movement can all result in contamination of restored or partially restored coal mine areas. Added to this, the area experiences typical UK weathering cycles and so serves as a good model site for mining-affected soils across the UK and more widely across temperate Europe. Therefore, this site is highly representative of restored or partially restored coal mines in England, wider Europe and other temperate zones and thus development of an approach for its characterisation and assessment for remediation has wide implications in terms of serving as a model to apply elsewhere. The aims of the project are to:

I. Characterize the study sites in terms of metal and metalloid contaminants in the stream water, stream sediment and soil.
II. Quantify and assess the significance of the level of any contamination.
III. Attempt to identify the positions where the water becomes more polluted.
IV. Determine the forms and associations of contaminants in soil and water.
V. Examine the potential for site soil remediation using novel amendments.

2.2.2 Objectives

I. To determine and assess water quality along the stream by examining turbidity, pH, dissolved (0.45 µm) and total element concentrations. And to assess sediment pseudo total element concentrations along the stream
II. To determine total (aqua regia extractable) element contents in soil and determine element associations using the BCR sequential extraction scheme. This will include soil from the damaged areas (seep points) and reference points away from damaged areas.

III. To examine the toxicity of the soil and water samples using ecotoxicology methods including earthworm survival tests (e.g. OECD 207 Earthworm Acute Toxicity Test for soils) and Daphnia magna assays (e.g. OECD 202 Daphnia Acute Immobilisation Test for waters).

IV. To assess the capacity for using water treatment residuals to ameliorate the contaminated soil to make it more fit for plant growth and other biological activity.

3 Evaluation of soil contamination

3.1 Introduction

Metal pollution of soils is a common issue in many mining-affected areas. Bioavailability, mobility, and toxicity of metals in the soil are controlled or influenced by soil biological (i.e., bacterial properties), physical (i.e., soil texture) and chemical (i.e., soil pH) properties and chemical properties of the various metals present. Key processes such as ion exchange, adsorption and desorption, complexation, precipitation and dissolution, oxidation-reduction, diffusion and migration, metal competition, biological immobilization and mobilization, and plant uptake can all have influence. Soil properties such as soil pH, moisture content, organic matter content, and particle size distribution (texture) can all directly or indirectly affect these processes (Alamgir, 2016). For example, organic matters contain ligands or functional groups which have an affinity to form complexes with metals. The metals bind strongly to the ligand and consequently that can decrease the concentration of the free metal ion in soil solution (Bolan et al., 2014). However, increasing the solubility of OM might lead to mobilisation of the associated metals (Gangloff et al., 2014). Therefore, it is important to assess the physicochemical properties of soil to understand the extent and the context of the contamination of metals.

The degree of metals contamination, pollution or enrichment in terrestrial environment has been evaluated based on the level of the examined metal in the soil compared with the uncontaminated background levels of that metal at that area. Enrichment factor, detailed in Manta et al. (2002), and geo-accumulation index, described in Okedeyi et al. (2014), are used for quantifying the presence and intensity of enrichment or accumulation of a metal on surface soils, or to assess the overall site-specific pollution as the pollution load index

(PLI), explained in Onwuka et al. (2018). The calculated numerical results can be interpreted based on their fit within categorises from unpolluted to very strongly polluted intensity, as detailed in Manta et al. (2002), Okedeyi et al. (2014), and Onwuka et al. (2018).

Recently, the available ecotoxicity data and bioavailability models for metals were used in a flexible framework for the derivation of environmental quality standards or guidelines in soil. Soil-specific ecotoxicological threshold concentrations for the metals Cd, Co, Cu, Pb, Mo, Ni and Zn, expressed as pseudo-total metal concentrations in soil (mg/kg dry weight), can be derived using measured soil properties and the site specific soil PNEC calculator developed by the consulting company Assessing Risks of Chemicals consulting, ARCHE (ARCHE, 2014) described in the literature review (chapter 1). The calculator generates a PNEC, estimates the potentially affected fraction (PAF) of soil organisms and calculates the Risk Characterisation Ratio (RCR, i.e. measured concentration/site-specific PNEC) for those metals in relation to the test soils. The required input data are the measured pseudo-total metal concentrations in the soil, uncontaminated background levels of that metal, soil pH, organic carbon%, clay % and effective cation exchange capacity (eCEC, cmole/kg).

Understanding the mobility and bioavailability of the metals is important for the evaluation of metal-contaminated soils. Metals in contaminated soils can be distributed in exchangeable fraction, reducible fraction, and oxidisable fraction and residual fraction. Sequential extractions can be utilised to provide useful information on quantities of metals distributed with those phases. The metal speciation (or fractionation) determined via the BCR sequential extraction procedure has been, and is still being, used widely to evaluate the environmental risks due to mobility and availability of the different forms of the metals in the soils (Ure et al., 1993, Asmoay et al., 2019). As introduced in the literature review (section 1.3.2), such sequential extraction schemes have their limitations, e.g DeVivo et al. (2017) found that during extraction procedures, readsorption and redistribution of metals can occur among soil constituents. Nevertheless, as concluded in their review titled *Is there a future for sequential chemical extraction?*, Bacon and Davidson (2008) concluded that the sequential extraction approach is still an excellent option to get a better understanding of metals distribution amongst soil constituents.

Single extractants are also widely used to assess mobility and availability; Barber (1984), Houba et al.(2000), and Marković et al. (2018) found that the phytoavailability of metals was related to the amounts extractable by a reagent that simulates the natural soil solution such as 0.01 M $CaCl_2$. Indeed, it has been argued that metal(loid)s content within the extractable fraction determined by a dilute neutral salt solution can be more useful than the BCR scheme fractions (or equivalent) in terms of prediction of bioavailability because the latter does not directly correspond to the available content as seen by organisms exposed to elements via the soil solution (Ahnstrom and Parker, 2001). Nevertheless, both single and sequential extractions are widely employed in studies of bioavailability and soil assessment.

Paquin et al. (2002) described the inter-relationships of chemistry, physiology and toxicology. Toxicity of a metal to an organism occurs when an excessive amount of metal ions bind to specific receptor sites, so-called biotic ligands. The chemistry of soil solution plays an important role in the toxicity. Ligands in the soil solution such as carbonate and natural dissolved organic matter compete with biotic ligands to take up the metal ions; for example, fewer metal ions would bind to biotic ligands in contact with soil solution that has high organic matter content compared with one that has low organic matter content and, consequently, there would be less metal toxicity in that solution for a given metal concentration. Another aspect of importance of the chemistry of soil solution lies in the competition that occurs between positive ions such as H^+, Ca^{2+}, Mg^{2+} and potentially toxic cationic metals for binding sites on the biotic ligands. For example, less metal ions would bind to biotic ligands in contact with soil solution that has high Ca^{2+} content compared with one that has low Ca^{2+} content and, consequently, there would be less metal toxicity in that solution. Furthermore, comparing the measured element content in soil solution with what has been reported in the literature for metals contaminated soil can also provide a clue as to whether metals contamination is evident at a site.

3.1.1 Aims and objectives

The management, monitoring and restoration of former coal mines are important, long term environmental activities that are a pressing concern in many regions and countries around the world. Contamination at coal mines, whether they are not yet restored or partially restored or fully restored, generally occurs as a result of acid or neutral mine drainage, fluvial deposition, and spoil material movement. The aim of the study was to characterise the nature and extent of contamination at a restored coal mining area in Staffordshire which, because the area experiences typical UK weathering cycles, would serve as a good model to understand what may occur in mining-affected soils across the UK and more widely across temperate Europe.

Therefore, the objectives were:

1. To characterise the study site in terms of physicochemical properties of the soil by determining the soil pH, soil organic matter and soil particle size distribution.

2. To quantify and assess the significance of the metal(loid)s contaminants in the soil by determining total (aqua regia extractable) element contents in the soil which are going to be used to assess the level of accumulation of metals in the soils as well as going to be used in combination with some soil properties to predicted soil-specific ecotoxicological threshold concentrations for the some of the metals.
3. To assess the forms and associations of contaminants in soils using the BCR sequential extraction scheme as well as to evaluate the potential bioavailabilty of contaminants in soil based on $CaCl_2$-extractable metals contents and, in addition, soil solution metals contents. This will include soil from the damaged areas (seep points) and reference points away from damaged areas.

3.2 Methods

3.2.1 Soil sampling

The characterisation of mine-effected areas starts by designing a sampling programme which focuses on locating points and determining sample quantity, proper methods of collecting, containing, storing and transporting samples. The sample collection strategy in this study was designed according to ISO 10381-1 (ISO, 2002).

Soil samples were collected from five sites that were thought to have been exposed to varying levels of acid mine drainage (AMD) or other mining impacts arising from the site's coal mining history. They include three areas seemingly vulnerable to seepages (as evidenced by lack of vegetation cover and discoloured soil, see figure 2-8 in the previous chapter), and two sites that seem in a good ecological condition that were sampled as reference positions. Within each of the seepage impacted areas, samples were collected from different zones to assess and capture variability. The relative positions of the sampling points are shown in Figure 3-1. Approximately 1 kg of each soil sample was collected with a trowel from the ~10cm surface depth and placed in a plastic zip-lock bag for transportation to the laboratory. After soil samples were delivered they were air dried and ground to <2 mm and put in polyethylene containers pending further experimentation and analyses.

Figure 3-1: Map of the sampling points; Seep 1, 2 and 3 appeared to be zones impacted by some type of mine water seepage while Ref 1 and 2 were selected as reference points that appeared less (or not) impacted.

3.2.2 Basic soil physicochemical properties

3.2.2.1 Soil moisture content

Soil water or moisture content refers to the water which might be evaporated by heating the soil at 105±5 °C. The thermogravimetric method was used to analyse the moisture content percent. It is based on removing soil moisture by oven-drying of soil samples that were placed in crucibles at a constant temperature of 105 °C for 24 hours; moisture content (%) is computed from the sample weight before and after drying using a five-decimal place balance (Smith, 2000).

3.2.2.2 Soil pH

Soil pH is a measurement of the acidity and alkalinity in soils. Aqueous solutions were prepared according to ISO-10390 (ISO, 2005), where a portion from each soil sample (as a volume ~5 ml) was placed in a 50 mL centrifuge tube, 25 ml of deionized water added and then shaken for ~1h on a rotating shaker after which the sample was allowed to stand for one hour. The pH value was measured in the supernatant solution using a Jenway 3510 pH probe and meter that was calibrated at the begging and every five measurements using pH 4 and 7 buffer solutions at about 20 °C.

3.2.2.3 Soil organic matter content and organic carbon

Carbon can exist in soils as carbonate or bicarbonate or other mineral forms, as elemental organic carbon such as in charcoal, graphite, or coal, or as humus which is organic residues of plants, animals, and microorganisms as well as the little-changed (fresh) organic residues of soil biota. Soil organic carbon content can be stated as the percentage of total C or can be figured from the soil organic matter content based on the widely used assumption that organic carbon forms 58 % of soil organic matter. Soil organic matter percentage (SOM%) was determined by mass loss on ignition (LOI), where oven dry samples were placed in a muffle furnace at 400 °C for at least 4h and the reduction in mass (attributed to ignition of organic matter) determined by weighing before and after.

The procedure included the following steps (Nelson and Sommers, 1996).

1. Recording the weight of furnace -dried crucibles,
2. Adding soil samples (that had been already air-dried, ground, sieved to 2 mm then 105 °C oven-dried) to the crucible,
3. Recording accurately in sensitive balance the weight of crucible with sample ‘’weight 105’’,
4. Igniting samples in a muffle furnace at 400 °C for >4 (max. 16 hours),
5. Obtaining the weight of furnace-dried sample ‘’weight 400’’
6. Calculating the LOI content of the samples using Equation 3-1.

$$Loss\ of\ ignition\% = \frac{Weight\ 105 - Weight\ 400}{Weight\ 105 - crucible\ mass} * 100 \quad \text{Equation 3-1}$$

3.2.2.4 Soil particle size distribution

Splitting of the soil mineral parts into different size fractions and determination of the proportion of these fractions is the principle of soil particle-size distribution studies. Sand, silt, and clay content of a soil determine it textural type according to the textural triangle Figure 3-2.

The laser diffraction method was used to determine the soil particle-size distribution. Following combustion in the muffle furnace to remove organic matter, the soil samples were further prepared by adding sodium hexa-metaphosphate (Calgon) liquid (50g/l) as dispersant solution to help disaggregation and dispersion. Samples were then allowed to soak in the calgon solution for at least 24h. Analyses of particle size distributions were done with Beckman Coulter LS 230 Particle Size Analyser, which measures particles from 0.04-2000 µm.

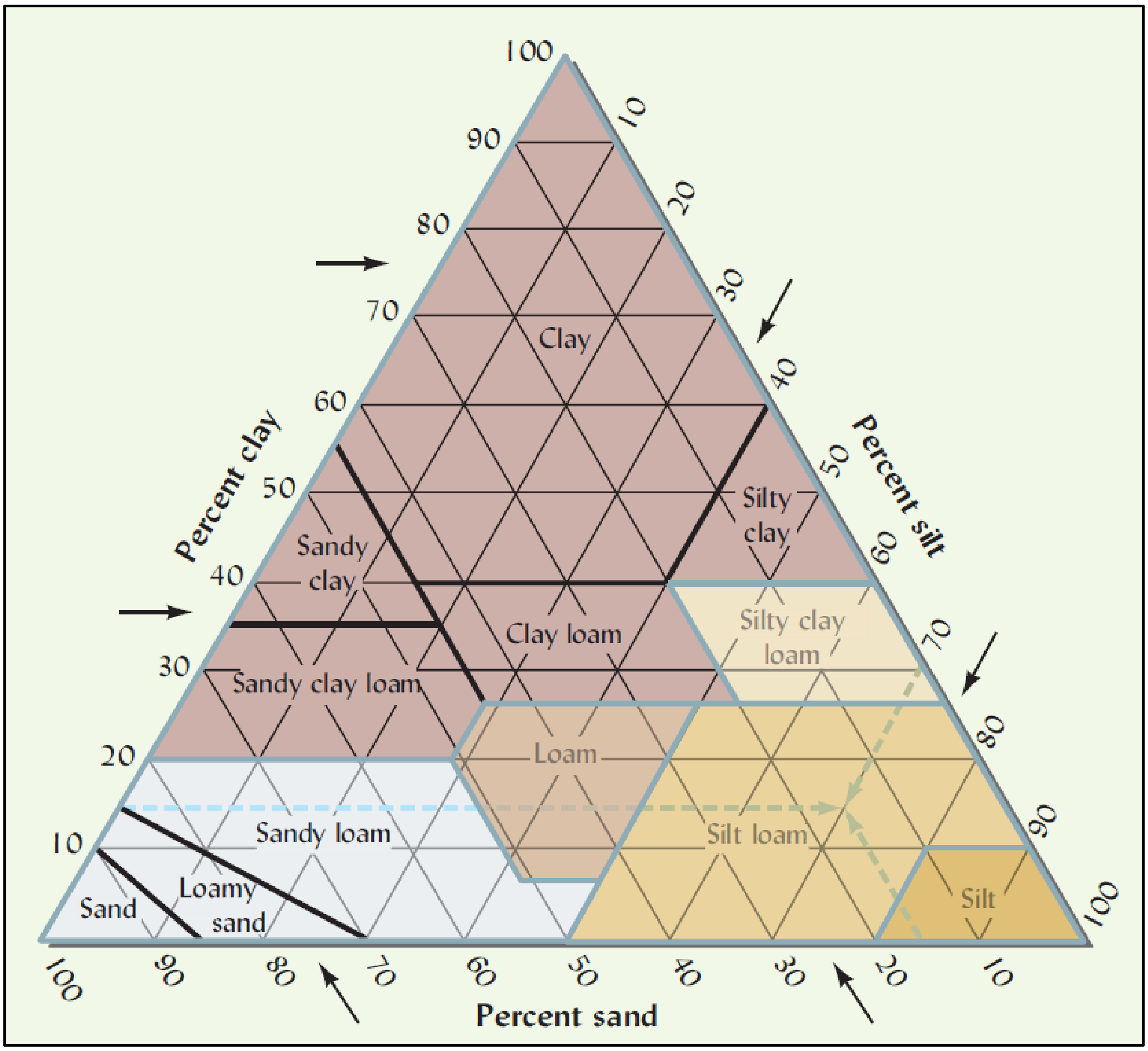


Figure 3-2: The textural triangle (Brady and Weil, 2013)

3.2.2.5 Calculation of Effective Cation Exchange Capacity

Cation exchange capacity (CEC) is the total capacity of a soil to hold positively exchangeable cations by electrostatic force. It measures negatively charged sites on the surface of clay minerals and organic matter. It is important because it influences the soil's ability to maintain essential nutrients, other metals, and provides a buffer against soil acidification (Sharma et al., 2015). Cation exchange capacity CEC of soil is often measured at a buffered pH, usually pH 7 or pH 8.2 (e.g. US EPA method 9081), but which may not adequately reflect the natural pH for all soil samples. However, cation exchange capacity can be estimated at the measured soil pH; that is the so-called effective cation exchange capacity (eCEC) of soils. According to the study by Helling et al. (1964), as cited by McGrath and Zhao (2006), eCEC can be calculated based on organic matter, clay content percentages and pH of the soil as shown in Equation 3-2.

$$eCEC_{(\frac{cmole}{Kg})} = (30 + 4.4pH) * \frac{Clay\,\%}{100} + (-59 + 51pH) * \frac{OC\,\%}{100} \quad \text{Equation 3-2}$$

3.2.3 Soil pseudo-total element concentrations

3.2.3.1 Method refinement procedures

Initial measurements for soil element concentrations via concentrated HNO_3 digestion and ICP-OES analysis were inconsistent and therefore a method evaluation and refinement process were conducted to optimise the procedure for the available equipment. This involved a range of digestion method variations performed on a certified standard reference soil (CRM033 Loamy Sand Soil; Trace Metals - Loamy Sand 10, Sigma-Aldrich) and selected site samples.

The first approach (termed approach 1) trialled involved digestions carried out according to EPA 3051 by adding 10 ml of trace metal grade nitric acid to about 0.4 g (accurate mass recorded) of soil samples in Teflon microwave vessels that were then sealed and placed in the microwave system (Mars 6). After cooling, the vessel contents were decanted to beakers (acid washed), dried down to near dryness on a hot plate, then made up to 20 ml and filtered via 0.45 micron syringe filters. Filtered solutions were analysed by ICP-OES (Vista MPS ICP-OES) using settings shown in Table 3.1.

The second digestion approach (approach 2) used reverse aqua regia (6 ml nitric acid and 2 ml hydrochloric acid, both trace metal grade, with 2 ml de-ionised water also added as per guidance from MARS 6 manufacturers) rather than using only HNO_3. The operating conditions of the microwave digestion system were also changed by setting the power at 1200 W. However, adjusting the power setting of the microwave made little difference to the temperature, as under both settings the desired temperature of 180°C was achieved and maintained. After microwave digestion, the samples were processed and analysed as per the first approach (i.e. dried down on a hotplate then taken up to 20 mL, filtered at 0.45 µm and analysed by ICP-OES).

The third digestion approach (approach 3) also used reverse aqua regia and the same microwave conditions, however the post digestion treatment was altered such that the soil digest solution of each sample was divided into two parts. One part (in what was termed approach 3b) was diluted by ten times using 2% HNO_3, filtered at 0.45 µm and then analysed by ICP-MS (Perkin Elmer NexION 300D), while the other part of the digestion solution (termed approach 3a) was transferred to a beaker, dried down on a hot plate to near dryness, diluted to 20 ml by 2% HNO_3 and then filtered (0.45 µm) and analysed by ICP-OES.

The fourth digestion approach (approach 4) was designed to evaluate any errors introduced in the first protocol during the drying down step, so involved digestion with nitric acid alone but with no dry down step. This fourth approach therefore involved adding 10 ml nitric acid (trace metal grade), microwaving as per previous, transfer of the digest solutions to beakers and dilution with 2% HNO_3 to 20 ml, filtering (0.45 µm) via syringe filters and placing in the refrigerator pending analysis via ICP-MS.

For all ICP-OE analysis runs, mixed calibration standard solutions (100, 50, 25, 10, 5, 2, 1, 0.5 mg/l) were prepared by combining appropriate volumes of the stock solutions in 2% HNO_3 matrix that were used immediately or stored briefly in the fridge before analysis. These standards were checked in term of possible impurities present or spectral interferences. Calibrations blanks were used to establish the analytical calibration curve and instrument detection limits for each element of interest, which were determined by multiples of the standard deviation of the concentration determined for three replicates of method reagent blanks. The operation conditions were set for the ICP-OES as describe in Table 3-1.

Table 3-1: The operation conditions were set for the ICP-OES

Parameter	Setting	Parameter	Setting
Read time	20 S	Replicates	3
Sample uptake delay	35 S	Rinse time	3 S
Stabilization time	10 S	RF power	1.4 KW
Fast pump	80 rpm	Nebulizer flow	0.70 L/min

Plasma flow	12.0 L/min	Aux flow	1.0 L/min
Viewing height	8 mm		

The results of analysis following the various digestion procedures on the certified reference soil are displayed in Table 3-2 to Table 3-6. All tests were done in 3-5 replicates, hence the table shows mean ± standard deviation, recovery percent and relative standard deviation (RSD). NR refers to not reported.

The recovery percent (i.e. measured concentration as a percentage of certified concentration) was used as an indicator for the accuracy while the relative standard deviation percent was used as an indicator for the precision of analysis of metal(loid)s in soil samples, and together the data were used to identify the best digestion and analysis approach for the elements of interest.

Comparison of approach 1 and approach 4 is made in order to assess the benefit of not doing the dry-down step following a nitric acid only digestion. The recovery percentages of Ca, Cd, Fe, Mg, Pb and Zn using approach 1 were better than that using approach 4. While the recovery percentages of Cr and Cu were better in approach 4 than that by approach 1. There was not a significant improvement in recovery of As when digested in approach 4 from that in approach 1. High relative standard deviation percentage RSD% was noted in the analysis of Co using approach 1, indicating unacceptable precision, therefore accurate comparison cannot be done between approach 1 and 4 for that element (see Table 3-2, to Table 3-6).

In order to assess benefit from doing a dry-down step or instead direct dilution, when using reverse aqua-regia as digestion solutions, a comparison between the data from approach

3a and that from 3b was carried out. The recovery percentages of As, Cd, Co, Cr, Cu and Zn using approach 3b (direct dilution) were better than that using approach 3a, while the recovery % of Pb in approach 3a was better than that by approach 3b. There was not a big difference in recovery of Ca, Fe, Mn and Mg when digested in approach 3b from that in approach 3a, see Table 3-2, to Table 3-6.

To assess the performances of HNO_3 or reverse aqua-regia as digestion solutions, the following comparison will be useful. The value of recovery % of As, Cd, Cr, Cu, Mn and Zn using approach 3b (reverse aqua regia) were greater than that using approach 1 (nitric acid only). Comparison of RSD% and recovery % values of Co when using approach 1 and 3b, approach 3b seems more successful (accurate) than approach 1. The recovery % of Ca and Mn using approach 1 were better than that using approach 3b, however, the differences were not substantial; the recovery % using approach 1 and 3b were for Mg 102% and 93% respectively and for Ca were 93% and 75%, respectively. The data indicates that the recovery percentages of Fe using approach 1, 3a, 3b were all relatively similar. The recovery percentages of Cr and Mn using approach 3a were better than that using approach 1, while the recovery % of Cd and digested in approach 1 better than that by approach 3a. There was not a big difference in recovery of Al, As, Ca, Cu, Fe, Mg, Pb and Zn when digested in approach 1 from that in approach 3a, see Table 3-2, to Table 3-6.

For each element, there are several line emission spectra which provide different sensitivity, linear ranges and detection limits; they are produced by different wavelengths. Selection of an appropriate wavelength depends on factors such as sensitivity and the concentration of the element in solution, and the presence or absence of any interference. For some elements in this study, e.g. Cd, detection capability is a major performance attribute that must be considered and therefore the wavelength which has highest sensitivity is required. By contrast, elements at higher concentrations such as Al, Fe and Ca, the lines with lower sensitivity are needed to be chosen to maximize the concentration range over which they can be calibrated and measured. However, the problem is that these lines can have interferences. To avoid the overwhelming correction process, the procedure that has been used in this study includes choosing 2 or 3 wavelengths for each element (e.g. 238.204, 239.562 and 259.940 nm for iron), inspecting visually the shape of emission peaks (symmetric, Gaussian) for the data collected and examining for interferences (e.g. the spectra of cobalt 238.892 nm suffered from Fe interference); lines deemed unsuitable were eliminated from further consideration (e.g. line 228.803nm for cadmium, which appeared to have a significant interference from arsenic) (EPA, 2014).

The energy and stability of plasma are affected by plasma operating conditions such as radio-frequency power, plasma gas flow, auxiliary gas flow, pump speed, sample delay, stabilization time, rinse time, replicate time and number of replicates. Each of these parameters were set to improve results and to achieve maximum intensities (Charles and Fredeen, 1997).

Certified reference solutions of all elements of interest have been analysed to check the validity of the analytical method. To reduce memory interferences a 5% nitric acid rinse blank has been utilised for flushing the nebulizer and instrument uptake system between samples, standards, and check solutions (EPA, 2014).

While many analytical quality control steps were implemented (see below), an internal standard calibration method was not used for metal determination in digested soil samples. This is not unusual for soil element determinations using ICP-OES/MS due to the often unavailability of suitable internal standard elements, complicated metal chemistry in aqueous solution, and undefined sample matrix. Here, an internal standard calibration Iridium solution was initially tried for metal determination in soil samples but it did not succeed because of Iridium being present within the samples. Instead, quality assurance was achieved via use of certified standard solutions used to make the calibration curves, in addition to other different external certified standard solutions that were analysed as samples within sample runs. The calibration curves were repeated every teen samples and were compared by plotting them together every single run. When calibration curves did not correspond closely, which indicated a degree of instrument drift, the data were not used but rather samples were reanalysed with new standards.

The conclusion drawn from the method refinement procedures was that the best digestion and analysis approach for the key elements of interest at the study site was 3b. The primary factors deciding that was the accuracy for elements As, Ca, Cd, Co, Cr, Cu , Fe, Mg, Mn and Zn relative to the certified standard soil, repeatability of the results and minimisation of the number of steps (i.e. to avoid introduction of contaminants).

Table 3-2: Total element concentrations of As, Cd and Co for certified reference soil CRM033 Loamy Sand determined via different digestion method variations (mean mg/kg ± standard deviation, n = 3-5), Note: the choice of emission line for each element is fully explained in the pages of text that above the tables. Approach 1) HNO_3, 800 W, Dry down; measured by ICP-OES. Approach 2) reverse aqua-regia, 1600 W, Dry down; measured by ICP-OES. Approach 3a) reverse aqua-regia, 800 W, Dry down; measured by ICP-OES. Approach 3b) reverse aqua-regia, 800 W, Direct dilute; measured by ICP-MS. Approach 4) HNO_3, 800 W, without dry down, Direct dilute; measured by ICP-MS.

Approach 1			Approach 2		Approach 3a		Approach 3b			Approach 4		
Element	Recovery%	RSD%	Recovery %	RSD%	Recovery%	RSD%	Element	Recovery%	RSD%	Element	Recovery%	RSD%
As-188	78	11	64	2	88	9	As-75	90	8	As -75	83	9
As-197	87	18	86	11	82	11						
Cd-214	82	10	54	10	56	9	Cd-111	83	6	Cd -111	64	9
Cd-226	82	11	55	10	59	8						
Co-238	126	67	40	18	22	15	Co-59	93	7	Co-59	85	10

Table 3-3: Total element concentrations of Cr and Cu for certified reference soil CRM033 Loamy Sand determined via different digestion method variations (mean mg/kg ± SD, n = 3-5). Note: the choice of emission line for each element is fully explained in the pages of text that above the tables. . Approach 1) HNO_3, 800 W, Dry down; measured by ICP-OES. Approach 2) reverse aqua-regia, 1600 W, Dry down; measured by ICP-OES. Approach 3a) reverse aqua-regia, 800 W, Dry down; measured by ICP-OES. Approach 3b) reverse aqua-regia, 800 W, Direct dilute; measured by ICP-MS. Approach 4) HNO_3, 800 W, without dry down, Direct dilute; measured by ICP-MS.

Approach 1			Approach 2		Approach 3a		Approach 3b			Approach 4		
Element	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%	Element	Recovery%	RSD%	Element	Recovery%	RSD%
Cr-205	53	9	45	11	38	7	Cr-52	91	7	Cr-52	67	10
Cr-267	27	24	42	11	36	9						
Cu-324	77	2	64	7	71	9	Cu-63	99	7	Cu-63	95	19
Cu-327	78	2	64	7	71	9						

Table 3-4: Total element concentrations of Fe and Mn for certified reference soil CRM033 Loamy Sand determined via different digestion method variations (mean mg/kg ± standard deviation, n = 3-5), NR means not reporting. Note: the choice of emission line for each element is fully explained in the pages of text that above the tables. . Approach 1) HNO_3, 800 W, Dry down; measured by ICP-OES. Approach 2) reverse aqua-regia, 1600 W, Dry down; measured by ICP-OES. Approach 3a) reverse aqua-regia, 800 W, Dry down; measured by ICP-OES. Approach 3b) reverse aqua-regia, 800 W, Direct dilute; measured by ICP-MS. Approach 4) HNO_3, 800 W, without dry down, Direct dilute; measured by ICP-MS.

Approach 1			Approach 2		Approach 3a		Approach 3b			Approach 4		
Element	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%	Element	Recovery%	RSD%	Element	Recovery%	RSD%
Fe-238	77	5	66	7	79	4	Fe-238	69	4	Fe-238	27	12
Fe-239	78	5	67	7	79	4	Fe-239	69	3	Fe-239	27	10
Fe-259	78	6	67	7	78	4	Fe-259	70	3	Fe-259	28	10
Mn-257	13	12	52	6	74	23	Mn-257	70	11	Mn-257	NR	NR
Mn-259	16	9	54	7	77	21	Mn-259	76	11	Mn-259	NR	NR

Table 3-5: Total element concentrations of Pb and Zn for certified reference soil CRM033 Loamy Sand determined via different digestion method variations (mean mg/kg ± standard deviation, n = 3-5). Note: the choice of emission line for each element is fully explained in the pages of text that above the tables. Approach 1) HNO_3, 800 W, Dry down; measured by ICP-OES. Approach 2) reverse aqua-regia, 1600 W, Dry down; measured by ICP-OES. Approach 3a) reverse aqua-regia, 800 W, Dry down; measured by ICP-OES. Approach 3b) reverse aqua-regia, 800 W, Direct dilute; measured by ICP-MS. Approach 4) HNO_3, 800 W, without dry down, Direct dilute; measured by ICP-MS.

Approach 1			Approach 2		Approach 3a		Approach 3b			Approach 4		
Element	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%	Element	Recovery%	RSD%	Element	Recovery%	RSD%
Pb-182	61	9	79	11	82	11	Pb-208	66	7	Pb-208	56	7
Pb-220	77	9	66	12	66	9						
Zn-330	85	2	49	10	69	7	Zn-66	87	5	Zn-66	60	7
Zn-334	87	15	66	13	72	2						

Table 3-6: Total element concentrations of Al, Ca and Mg for certified reference soil CRM033 Loamy Sand determined via different digestion method variations (mean mg/kg ± standard deviation, n = 3-5), NR = not reported. Note: the choice of emission line for each element is fully explained in the pages of text that above the tables. Approach 1) HNO_3, 800 W, Dry down; measured by ICP-OES. Approach 2) reverse aqua-regia, 1600 W, Dry down; measured by ICP-OES. Approach 3a) reverse aqua-regia, 800 W, Dry down; measured by ICP-OES. Approach 3b) reverse aqua-regia, 800 W, Direct dilute; measured by ICP-MS. Approach 4) HNO_3, 800 W, without dry down, Direct dilute; measured by ICP-MS.

Approach 1			Approach 2		Approach 3a		Approach 3b			Approach 4		
Element	Recovery%	RSD%	Recovery%	RSD%	Recovery%	RSD%	Element	Recovery%	RSD%	Element	Recovery%	RSD%
Al 237	73	47	53	13	72	9		NR	NR		NR	NR
Al 396	73	48	59	13	71	9		NR	NR		NR	NR
Ca 396	93	8	71	8	83	5	Ca 396	75	5	Ca 396.	77	1
Ca 422	94	11	68	11	78	8	Ca 422	66	2	Ca 422	64	2
Mg 279	102	4	63	9	94	13	Mg 279	93	9	Mg 279	92	7
Mg 383	100	4	63	11	87	9	Mg 383	73	8	Mg 383	77	14

3.2.3.2 Evaluation of the Soil Contamination using Geo-accumulation index (Igeo)

Geo-accumulation index (Igeo) has been used to estimate the metal(loid)s contamination in sediments and soils by Barbieri (2016) and Dung et al.(2013). This can be calculated by comparing the measured pseudo-total metal(loid)s contents in the present study with median ambient background concentration (MABC) of them for soil in England. The Equation 3-3 was used to calculate the load of the metal(loid)s in the soils Müller (1981).

$$Igeo = \log_2 \frac{\text{Element concentration in test soil}}{1.5 * \text{background concentration}} \qquad \text{Equation 3-3}$$

The MABC of some heavy metal(loid)s in England for a sandy-loam soil comparable to that of the sites of the present study have been published in the National soil inventory by McGrath and Zhao (2006) as shown in Table 3-7. The background concentration of arsenic, which was not recorded in that inventory, is 14.1 mg/kg according to statistical summary of As in topsoil at Stoke-on-Trent by British Geological Survey and Department for Environment Food and Rural Affairs report (Ander et al., 2011).

Table 3-7: Median ambient background concentration MABC (mg/Kg) for comparable soils in England according to McGrath and Zhao (2006).

Element	Cd	Co	Cr	Cu	Ni	Pb	Zn
concentration (mg/Kg)	0.5	7.1	27.4	14.7	15.9	36	65

The Igeo index values here have been calculated using the mean of each measured element concentration for the sample sites and MABC. The resulting I geo values have been interpreted using the seven-category scale as shown in Table 3-8 which is published by Müller (1981) as cited in Barbieri (2016).

Table 3-8: I geo-accumulation scales Müller (1981).

Class	Value	Soil quality
0	Igeo ≤ 0	not accumulated
1	0< Igeo <1	not accumulated to moderately accumulated
2	1< Igeo <2	Moderately accumulated
3	2< Igeo <3	Moderately to heavily accumulated
4	3< Igeo <4	Heavily accumulated
5	4< Igeo <5	Heavily to extremely accumulated
6	Igeo ≥ 5	Extremely accumulated

3.2.3.3 Prediction of ecological risks of metals based on site-specific properties

Soil predicted no effect concentration (PNEC) values can be used as a trigger value to evaluate the potential for environmental risk. PNEC is the concentration of a certain element below which no ecological risk is anticipated. Site-specific soil PNECs were generated for Co, Pb, Ni, Cu and Zn using the calculator from the consulting company Assessing Risks of Chemicals consulting (ARCHE) and measured site-specific soil properties (ARCHE, 2014), as outlined in the introduction.

3.2.4 Metal(loid)s fractionation using BCR scheme and $CaCl_2$- extractable contents of metal(loid)s in soil

3.2.4.1 BCR three-step sequential extraction procedure

The BCR three-step sequential extraction procedure which is described by Quevauviller et al. (1997) is followed to determine bound forms of trace metals in soil samples. As with all sequential extraction schemes, the fractions identified by the BCR scheme are operationally defined by the reagent and the order of reagents used. However, even so, identifying which fractions metals are associated with is useful because it does provide a relative measure of availability as well as facilitating comparisons with the many other studies that have employed the BCR scheme. The steps in the scheme are described below (Quevauviller et al., 1997).

Step 1 (exchangeable fraction):

Air-dried soil samples (1 g) were treated with 40 ml 0.11 M acetic acid (pH 2.8) in 50 ml centrifuge tubes and the samples shaken on a mechanical shaker for 16 h at room temperature, centrifuged (3,000 rpm, 15 min), the supernatant decanted and filtered (0.45 µm). The filtrate aliquots were stored in the fridge until analysing them by ICP-OES or ICP-MS for measuring the elements of interest. The solid residues were washed with 10 mL of de-ionised water, shaken for 15 min, centrifuged (3,000 rpm, 15 min) and the supernatant discarded.

Step 2 (reducible fraction):

40 ml 0.1 M hydroxylamine hydrochloride, acidified to pH 2 with nitric acid, was added to the residues from the exchangeable fraction step and then at room temperature were shaken for 16 h followed by centrifuging and filtering them as in the first step. The filtrate aliquots were stored in the fridge until analysing them by ICP-OES or ICP-MS. The residues also were washed with water as in step 1.

Step 3 (oxidisable fraction):

The residues from step 2 were cautiously treated with 10 ml 35% hydrogen peroxide, acid-stabilised with nitric acid to pH 2-3. The contents were digested at room temperature with occasional shaking. Then, in a water bath, at about 85 °C, the digestion was continued and the volume was lowered to approximately 2 ml by additional heating of the uncapped tube. Then, an extra 10 ml of hydrogen peroxide was added, the tubes were again heated to about 85 °C, the volume reduced to approximately 2 ml and allowed to cool. Then 50 ml 1M ammonium acetate solution was added to the cool residue, shaken for 16 h at room temperature and then they were centrifuged, transferred and filtered as in steps 1 and 2. The filtrate aliquots were stored in the fridge until analysing them by ICP-OES or ICP-MS. The residues were washed with water as in steps 1 and 2.

Step 4 (residual fraction):

The residues from step 3 were subjected to acid digestion for an internal check on the procedure, as recommended in the literature (Rauret et al., 2000) and reverse aqua regia reagent was used for the digestion. This additional step allows a mass balance to be assessed (i.e. sum of all BCR fractions can be compared to the total element concentration previously determined in a separate digested sample). Blank reagent samples were also analysed in each extraction step.

The BCR scheme was carried out on samples from Ref. 1 and Ref. 2 (i.e. sites in apparent good ecological condition and used as reference positions) and two of the sites vulnerable to seepages, namely seep 1 and seep 3. It was originally intended to do BCR on seeps 1, 2 and 3 and Ref 1 sites, but a handling error (a mistake) resulted in the Ref 1 and Ref 2 and seeps 1 and 3 being subjected to the BCR scheme. By the time this error was identified it was no longer practical to complete the BCR on seep 2 (ICP instrument access being the main limitation at the time).

3.2.4.2 $CaCl_2$- extractable contents of metal(loid)s in soil

According to Menzies et al. (2007) neutral salt extractants (such as 0.01 M $CaCl_2$ and 0.1 M $NaNO_3$) provide the most useful prediction of bioavailability of metals for plants. Extraction of the chemical elements from the soil using calcium chloride, at a 1:10 soil: extracting reagent ratio, can to some extent mimic the availability of metals to plants via the soil solution. This is because a 0.01M $CaCl_2$ solution has the same approximate ionic strength as soil solution and because Ca is often a major cation present in soils and soil solutions (Houba et al. 2000). In the extraction step conducted here, 2 g of air-dried soil samples were treated with 20 ml of 0.01 M $CaCl_2$ solution in 50 ml polyethylene tubes and then shaken; using an end over end shaker at room temperature, for at least 2 h (Houba et al. 2000). Samples were then centrifuged for at least 10 minutes at about 1,800 rpm. The supernatants were filtered via 0.45 µm syringe filter and after the clear supernatants were transferred into test tubes and 0.1 ml 1 M HCl (high purity) was added to them to prevent growth of bacteria and also to prevent elemental adsorption to the containers as well. The acidified supernatant aliquots were stored in a refrigerator until determination of the element concentrations using ICP-OES and ICP-MS.

The samples extracted by 0.01M $CaCl_2$ were Ref 1 and Ref 2 (in good ecological condition as reference positions) and seeps 1, 2 and 3. A minimum of two replicates were conducted for each.

3.2.5 Soil solution element concentrations

Immediately after the soil samples were transferred to zip-lock plastic bags and transported to the laboratory, two subsamples for each site were placed into 50 mL centrifuge tubes and centrifuged at ~ 4000 rpm for 20 minutes, after which the supernatant soil solution was removed and filtered through a syringe filter with aperture 0.45 µm (Di Bonito et al., 2008). The soil solution samples for each site were acidified to pH < 2 with high purity HCl (Primar plus, Fisher) and then were analysed by ICP-MS (Perkin Elmer NexION 300D x). This procedure successfully yielded soil solution for only a small number of samples, because of the dryness of the samples at time of collection; hence an additional process was conducted to generate simulated soil solution for the samples. Following a commonly applied approach (Ardestani and van Gestel, 2013, Ma et al., 2006), simulated soil solution was extracted from the soil samples by adding deionized water at 100% of water holding capacity for 3 days and then extracting via centrifugation after which samples were filtered and acidified as above.

3.3 Results and discussion

3.3.1 Basic soil physicochemical properties

3.3.1.1 Soil moisture, soil organic matter (SOM %) and organic carbon (SOC %)

As shown in Table 3-9, the soil moisture content of air dried samples ranged from 2.17 to 9.62 %, while organic matter content ranged between 7.26 and 39.01 %. Soil organic carbon (SOC) was calculated from soil organic matter values and the assumption that 58 % of soil organic matter is organic carbon (Perie and Ouimet, 2008).

It is apparent that the soil moisture at seep 1 and Ref. 2 is significantly higher than that at Ref. 1, seep 2 and seep 3. The moisture content in the soil depends on some soil properties, they are the particle-size distribution, the dry bulk density, and the carbon content (Vereecken et al., 1989). Therefore, the variances in the soil moisture content might be related to those properties. There was a significant positive correlation between moisture % and SOC% (Pearson correlation of Moisture % and SOC% = 0.793; P-Value = 0.000), this might be a reason for moisture findings. In various extents, the soil moisture content affects the adsorption of elements on the soil surface, which in turn can modify the bioavailability of toxic and nutrient elements. The amount of soil moisture affects the bringing of dissolved nutrient elements (e.g., Ca, K, N, and P) to plant roots (Brady and Weil, 2013). Soil fauna can also be impacted by soil moisture, (Verhoef and Nagelkerke, 1977, Kaczmarek, 1975) found that behaviour and survival of Collembola as well as population size were influenced by soil moisture.

Figure 3-3 and Table 3-9 show that SOM% and SOC% at Ref. 2 (i.e. the site with vegetation immediately next to the bare area) was significantly higher than that at the rest of the sites ($p<0.0001$, Tukey's multiple comparisons test) while there was not any significant difference between SOM% and SOC% at Seep 3 and at Ref. 1 ($p=0.99$, Tukey's multiple comparisons test).

Factors such as topography, soil moisture and water saturation, salinity and acidity, vegetation and biomass production and drainage affect the soil organic matter (Bot and Benites, 2005). According to Emmett et al.(2010), SOM% in the general area of the study site typically ranged between 10% and 20%. The current study found low SOC% at seep 2 and seep 3 (un-vegetated sites) comparison with that at Ref. 2 site. One unanticipated finding was that SOC% at Ref 1 site (vegetated site) is lower than that at Ref. 2, seep 1 and seep 2, the reason is not clear. The possible explanation for these high findings might be related to the presence of coal residuals in the seep samples (which would burn off during the ignition process and therefore give an elevated SOM% result because of lost mass) or, in the case of Ref 2, might be related to soil moisture content (i.e. better moisture retention can encourage more growth in this vegetated area). The latter can be drawn from the positive correlation between SOC% and soil moisture content. Several reports on comparable sites have shown that the metals toxicity was decreased due to some soil organic matter (Beesley et al., 2014, Ondrasek et al., 2018).

Table 3-9: Mean of soil moisture, SOM% and SOC% ±SEM.

sample label	Moisture%	SOM%	SOC%
Ref.1	3.95±1.10	7.65±0.38	4.44±0.22
Seep 1	9.62±1.54	27.63±0.57	16.02±0.33
Ref. 2	8.23±0.11	39±0.2	22.62±0.12
Seep 2	5.11±0.98	21.51±0.54	12.47±0.31
Seep 3	2.17±0.25	8.19±0.44	4.75±0.25

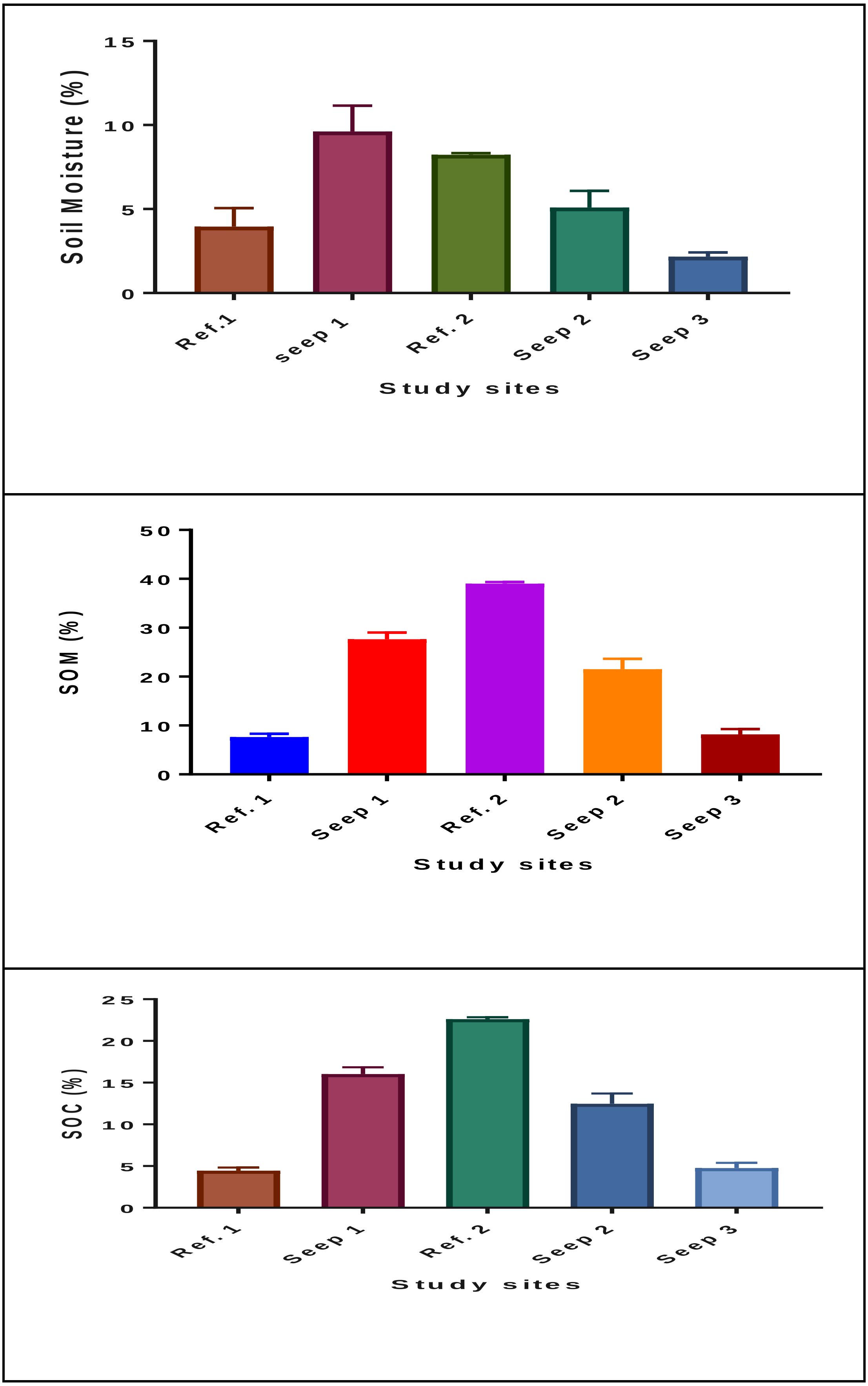


Figure 3-3: Percentages for soil moisture, SOM and SOC at study sites, error bars show standard deviation (SD).

3.3.1.2 Soil pH

Low pH was dominant in the study sites except at the two fully vegetated points of Ref. 1 and Ref. 2, which had pH values ~5.9 and 6.3 respectively compared with all of the seep sites whose pH was <3 (Table 3-10). From Table 3-10 and Figure 3-4, it is evident that the soil pH at Ref. 1 and Ref. 2 are significantly higher than that at seep 1, seep 2 and seep 3 ($p<0.05$, Dunn's multiple comparisons test). It is clear that soil pH at seep 1, seep 2 and seep 3 is not significantly different amongst these sites as confirmed by statistical assessment ($p >0.9999$ Dunn's multiple comparisons test). According to Emmett et al. (2010) soil pH in this general area of England typical ranged between 6.0 and 6.5, hence the seep sites have been impacted by something that has led to their lower pH values.

These results seem to be consistent with those of Dybowska et al. (2005) who studied soils from a mining site in Devon, UK, and found the soil pH ranged from 2.9 to 6.8 and also in agreement with those obtained by Bradley and Cox (1986) who found that soil pH values from a mining site elsewhere in Staffordshire, UK, were between 2.6 and 7.3.

According to U.S. Environmental Protection Agency (2003), if the soil pH of a site is less than 5.5 aluminium is known as a contaminant of potential concern (COPC) therefore it seems clear that only sites Ref. 1 and Ref. 2 can be considered safe regarding aluminium ecotoxicity (see Figure 3-4). Very high H^+ activity can be toxic to plants, another effect is that it can affect membrane-bound ATPases which in turn can affect transport mechanisms of ions across membranes (Shabala, 2017).

Negative and positive charges such as those associated with allophane, 1:1-type clays, iron and aluminum oxides, and humus are soil pH-dependent, therefore, soil cation exchange capacity changes with the soil pH changes, and at low pH the soil cation exchange capacity decreases since exchangeable nutrient cations are displaced by H^+ ions. Iron and aluminium ions and some other toxic metals are more bioavailable at low pH in the acidic soils (Brady and Weil, 2013).

Table 3-10: Mean ± SD (n≥3) of soil pH for the study sites.

Ref.1	Seep 1	Ref. 2	Seep 2	Seep 3
5.91±0.23	2.91±0.08	6.28±0.05	2.87±0.41	2.73±0.08

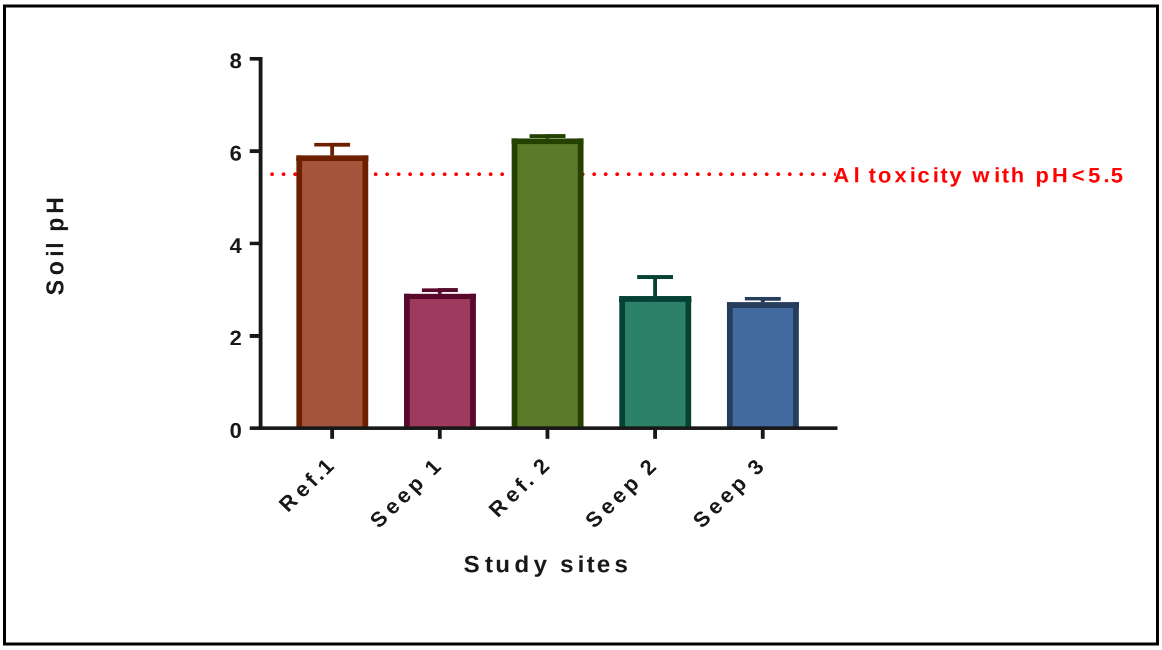


Figure 3-4: Soil pH for the study sites, illustrating the potential issue in the study soils of aluminium toxicity that can occur in soils with a soil pH less than 5.5. The US-EPA identifies Al as a contaminant of potential concern (COPC) in such situations (EPA, 2003).

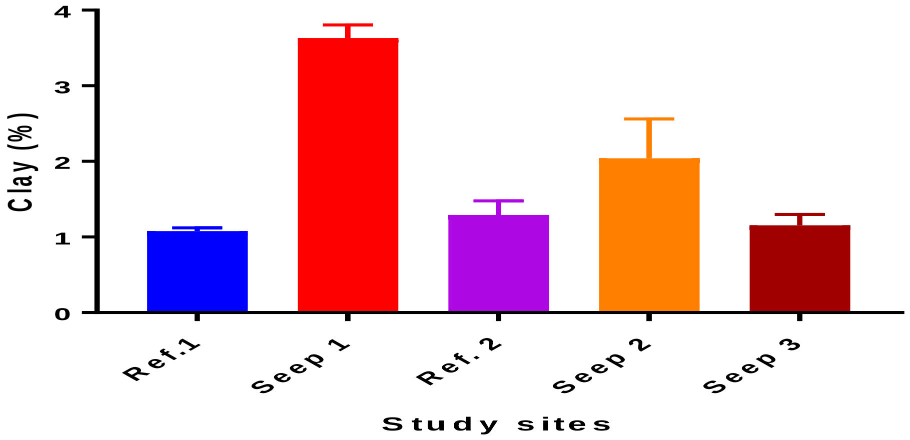
3.3.1.3 Soil particle size distribution

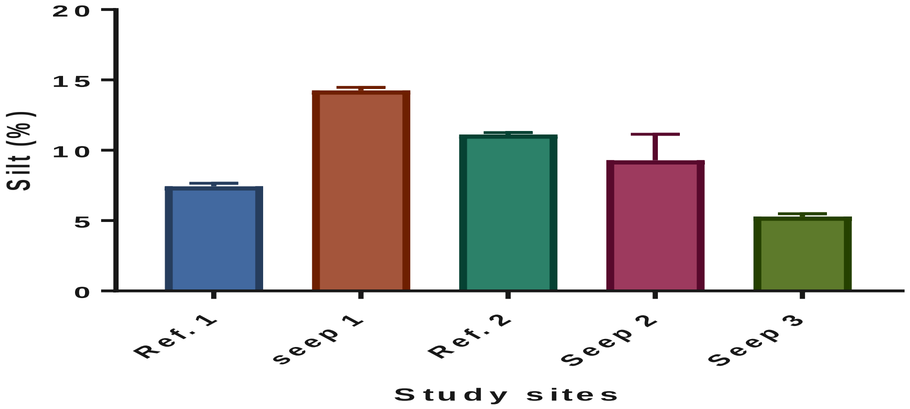
The percentages of clay, silt and sand at the study sites ranged from 1.04 to 3.62, 5.19 to 14.26 and 82.10 to 93.67 respectively, as shown in Table 3-11. As show in Figure 3-5, although the clay percentages were low across the soils, the values at seep 1 and seep 2 were greater than that at Ref. 1, Ref. 2 and seep 3. There were not identifiable differences in the clay content at Ref. 1, Ref. 2 and seep 3. The clay content at seep 1 is significantly greater than that at seep 2 ($p<0.05$, Tukey's multiple comparisons test). It is clear from Figure 3-5 that silt content at Ref. 1 and seep 3 is significantly lower than that the rest of sites ($p<0.05$, Dunn's multiple comparisons test). It can be seen that the percentages of sand content at study sites were high across all locations. Even so, there were identifiable differences; the sand content at Ref. 1 and at seep 3 were greater than that at the rest of the sites whilst the lowest sand content were at Ref. 2 and at seep 1 sites ($p<0.05$, Brown-Forsythe test followed by Benjamini, Krieger and Yekutieli test). Soil texture is majorly impacted by the types of the weathering which take place within the mineral content of that soil. For example, soils are likely to be richer in clay, when chemical weathering dominates (Earle, 2018), this might be a reason for that differences.

Table 3-11: Mean of fractions persentage ($n \geq 3$) ± SD at the study sites.

Site / Fraction%	Ref.1	Seep 1	Ref. 2	Seep 2	Seep 3
Clay%	1.08±0.05	3.63±0.18	1.29±0.19	2.04±0.52	1.16±0.14
Silt%	7.43±0.22	11.13±0.12	14.26±0.22	9.30±1.84	5.29±0.20
Sand%	91.52±0.25	85.26±3.45	87.60±0.22	88.71±2.18	93.57±0.22

The results of this study indicate that soil texture is classified as sandy-loam and that is in line with what has been published by Emmett et al. (2010) for the soil texture in the same geographic area. The retention of metals in soil by both adsorption reactions and precipitation reactions can strongly be affected by the charged surfaces of soil particles particularly clay particles (Evans, 1989). According to the findings found by Smolders and co-workers (2003), it is expected, regarding the clay content, that the metal toxicity threshold in soil seep 1 to be more than that at seep 2 and at seep 3. In other words, seep 1 can be considered as a less sensitive soil and the amount of the metal to reach the toxic level in seep 1 soil is thus expected to be greater than that at seep 2 and at seep 3. Regarding the influence of texture on the nutrients available for the plant, the nutrient-holding capacity is known to increase by a small increase of clay particles because of a large surface area provided by clay particles (Moore and Bradley, 2018). Therefore, even small differences can be important.


Clay (%)
0
1
2
3
4
Ref.1
Seep 1
Ref. 2
Seep 2
Seep 3
Study sites


Silt (%)
0
5
10
15
20
Ref. 1
seep 1
Ref. 2
Seep 2
Seep 3
Study sites

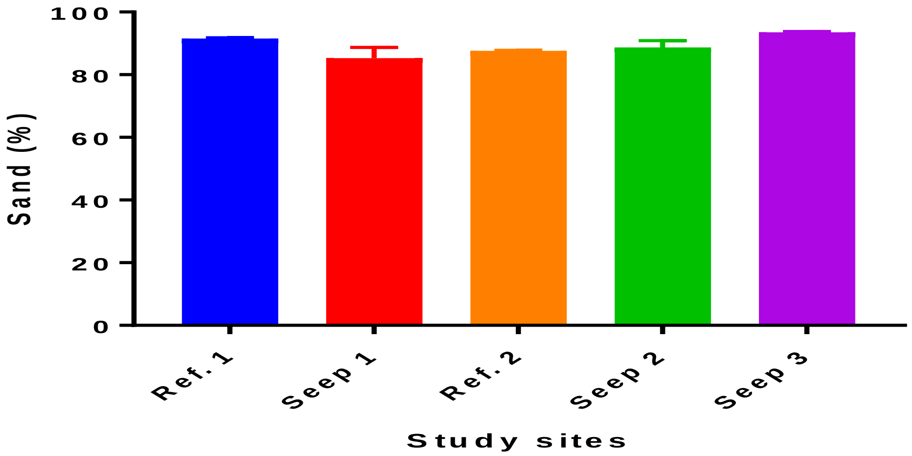

Sand (%)
0
20
40
60
80
100
Ref. 1
Seep 1
Ref. 2
Seep 2
Seep 3
Study sites

Figure 3-5: Comparison of mean of clay content, silt content and sand content at sites, error bars represent SD, (n≥3).

3.3.1.4 The calculated effective Cation Exchange Capacity

The effective cation exchange capacity (eCEC) values of soils were calculated based on the study by Helling et al. (1964), using Equation 3-2 as described in section 3.2.2.5. The eCEC is related to the variation in soil pH, organic matter and clay content within the samples. Table 3-12 shows calculated soil eCECs. Figure 3-6 shows a comparison of eCEC at the study sites. It is clear that eCEC at Ref. 2 is greater than that at the rest of sites. It can be seen that eCEC at Seep 3 soils is significantly lower than that at the rest of sites ($p<0.05$, Tukey's multiple comparisons test). These data should be interpreted with some caution, however, because of a possible uncertainty of the soil organic carbon data linked to coal fragment residues (as stated in section 3.3.1). It seems clear that the eCEC values at the un-vegetated soils decrease in order Seep 1> Seep 2> Seep 3, and this is related to the differences in soil pH, organic matter and clay content within those samples.

Table 3-12: Mean (cmol/Kg) of eCEC for study sites ± SD, (n≥3).

Ref. 1	Seep 1	Ref. 2	Seep 2	Seep 3
11.06±0.70	16.14±0.69	59.55±0.19	11.47±3.01	4.20±0.43

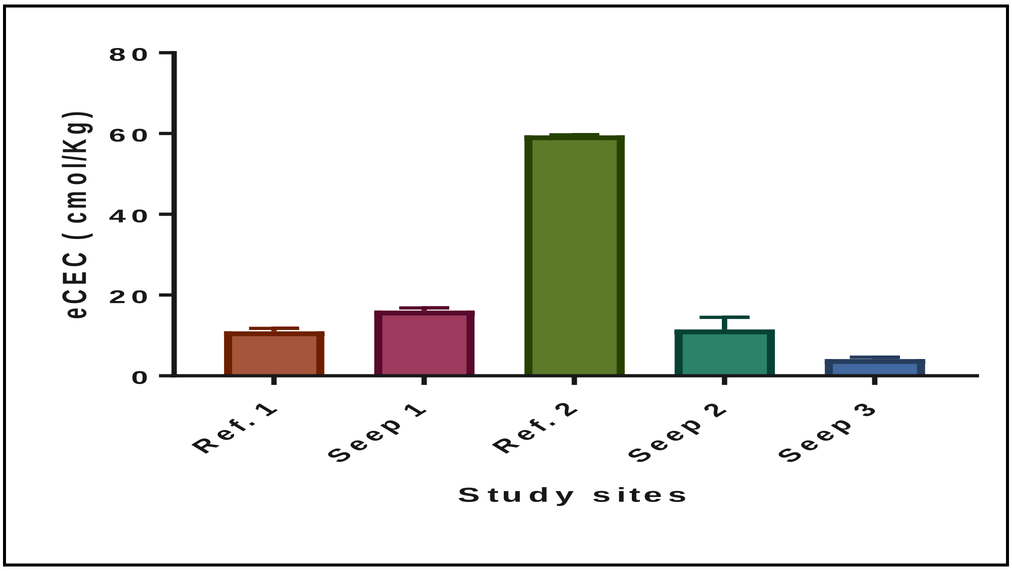


Figure 3-6: Mean of eCEC at study sites, error bars show SD from the mean, (n≥3).

The number of cation adsorption sites in the soil depends on eCEC value (Robertson, 1999). Strong correlations between toxicity thresholds for a number of metals and effective cation exchange capacity as function of clay%, soil pH and organic matter, have been revealed by a many researchers such as Smolders et al. (2003), Smolders et al. (2004a), Oorts et al. (2006) and Rooney et al. (2006).

According to work done by Smolders et al. (2009), the soil can be classified as a high, median and weak sensitive soils depending on the effective cation exchange capacity eCEC, pH, organic carbon (OC) percentage and clay percentage of soil as shown in Table 3-13. Comparison of the soil properties at the study site with the mentioned classification criteria indicates that all the seep soils investigated in the present study are considered as highly sensitive to metals toxicity based primarily on their pH and organic carbon content. The Ref.1 and Ref. 2 soil sites would fall within the weakly and median sensitive soil groups, respectively. Therefore, it is expected that the metal toxicity threshold in soil at seep 3, seep 2 and seep 1 would be less than that at Ref. 2; in other words, the amount of the metal to reach the toxic level in Ref. 2 soil is expected to be greater than that at seep 3, seep 2 and at seep 1.

Table 3-13: High, median and weak sensitive soil groups based on soil properties as mention in (Smolders et al., 2009).

Soil class	Soil eCEC (cmole/ kg)	Soil pH	Soil OC%	Soil clay%
Highly sensitive	4	4.5	1	5
Median sensitive	15	5.5	2.9	15
Weakly sensitive	35	7	12	30

3.3.2 Soil pseudo-total element concentrations

The pseudo-total (aqua regia digested) element concentrations varied widely amongst the soil samples, as shown in Table 3-14. Variances in factors such as parent material of the soil, soil texture, chemistry and regime of water in the soil, soil microbiology activities, growing crop in the soil, rate of element leaching in the soil can all cause significant variances in metal(loid)s concentrations in soil because those factors impact immobilisation/ mobilisation processes (Bolan et al., 2014). Generally, the concentration of Al, Ca, Cd, Cr, Co, Cu, Fe, Mn, Ni, and Zn in the soils at seep 1, 2 and 3 are significantly less than that at Ref. 1 and Ref. 2 soils. This might be related to leaching of those elements with acid mine drainage at the seep points. As Ceto and Mahmud (2000) comment, the dissolved metal(loid)s can migrate from the soils to the local water bodies, so those soils might be eroded and washed to stream water. RoyChowdhury et al. (2015) state that the plants' root systems protect the soils against erosion and leaching, this might be a reason why the reference samples from vegetated soils Ref. 1 and Ref. 2 contain grater metals than seep 1, 2 and 3 (i.e. the seep soils lack vegetation and thus have no protection from plant roots).

The enrichment of metal(loid)s in soil can be estimated based on comparison of their measured concentrations with the known background levels, using enrichment indexes such as geo-accumulation.

Table 3-14: Pseudo total element concentrations for metal(loid)s in soils examined. Values are means (n=3) ± SD, B.D refers to below limit of detection.

Site Element	Ref. 1	Seep 1	Ref. 2	Seep 2	Seep 3
Al%	0.71±0.07	0.35±0.20	0.54±0.05	0.28±0.13	0.39±0.06
As mg/kg	7.14±0.78	18.95±3.47	42.21±0.71	34.21±10.90	10.99±1.28
Ca%	0.27±0.03	0.07±0.01	0.62±0.02	0.28±0.27	0.08±0.03
Cd mg/kg	0.99±0.45	0.28±0.01	1.60±0.13	0.22±0.10	B.D
Co mg/kg	4.80±0.20	2.17±0.92	25.54±0.40	3.54±0.52	2.93±0.43
Cr mg/kg	11.93±0.64	3.51±0.95	9.59±0.10	4.91±1.68	10.86±0.86
Cu mg/kg	14.57±0.86	33.48±5.03	106.60±10.19	23.55±9.95	8.00±0.72
Fe%	1.22±0.06	7.10±1.55	2.20±0.12	6.06±2.11	4.28±0.78
K%	0.31±0.36	0.50±0.20	0.21±0.12	0.30±0.12	0.12±0.04
Mg%	0.23±0.02	0.01±0.01	0.09±0.01	0.06±0.03	0.17±0.03
Mn mg/kg	183±16.98	168.70±20.61	892.4±59.90	143.3±25.48	152.2±37.78
Ni mg/kg	12.35±0.45	4.98±3.51	58.23±1.60	7.87±2.70	10.12±6.48
Pb mg/kg	24.04±1.25	56.18±5.98	97.19±2.05	36.16±17.29	20.58±2.40
Zn mg/kg	54.06±3.80	15.25±4.63	81.36±2.66	24.33±15.9	27.79±5.32

3.3.2.1 Evaluation of the Soil Contamination using geo-accumulation index (Igeo)

Geo-accumulation index (Igeo) was calculated by comparing the measured pseudo-total metal(loid)s contents in the present study with median ambient background concentration (MABC) of them for soil in England. The Igeo index values here have been calculated using the mean of each measured element concentration for the sample sites and the MABC. The resulting Igeo values have been interpreted using the seven-category scale as shown in Table 3-7, detailed in section 3.2.3.2, and the results are shown in Table 3-15.

It can be seen that As, Co, Cr, Cu, Ni, Pb and Zn fall in class 0 in Ref.1 which means there has been no substantial accumulation beyond background in Ref.1 soil, while Cd in Ref.1 was within the not accumulated to moderately accumulated range. In seep 1 soil, the results of Igeo of As, Cd, Co, Cr, Ni and Zn were within not accumulated range. The data indicate that Cu and Pb were within not accumulated to moderately accumulated range. Moderately accumulated were indicated for Cd, Cu, and Ni in Ref.2 soil. In this site, the data also showed moderate to heavy Cu accumulation occurred, while there were no Cr and Zn accumulation. As and Pb were within not accumulated to moderately accumulated range. The Igeo value of As and Cu in seep 2 fall in the not accumulated to moderately accumulated class, while Cd, Co, Cr, Ni, Pb and Zn in seep 2 were within the not accumulated range. In seep 3, As, Cd, Co, Cr, Cu, Ni, Pb and Zn fall in the not accumulated range.

The vegetated soils (Ref.1 and Ref.2), for some elements, had more accumulation than unvegetated soils (seep 1, 2 and 3). As noted in the previous section, a possible explanation for this might be that leaching of those elements with acid mine drainage occurred in seep 1, 2 and 3 (as had been suggested elsewhere, e.g. (Ceto and Mahmud, 2000) compared with Ref.1 and Ref.2 soils. One issue emerging from these findings relate to leaching of metals from seeps soils to local stream water affecting the aquatic biota, while another issue might be a rise in metals accumulation in plant tissue in nearby soils if mobile metals migrate there, which might lead to metals accumulation in livestock living there.

The risk of metal to the soil biota, however, cannot be determined depending only on the comparison with the background concentrations; therefore, prediction of ecological risks of some metals based on the predicted site-specific soil PNEC at the study sites will be the next step.

Table 3-15: Igeo-accumulation index classes for As, Cd, Co, Cr, Cu, Ni, Pb and Zn at study sites,

Site Element	Ref. 1	Seep 1	Ref. 2	Seep 2	Seep 3
As	0	0	1	1	0
Cd	1	0	2	0	0
Co	0	0	2	0	0
Cr	0	0	0	0	0
Cu	0	1	3	1	0
Ni	0	0	2	0	0
Pb	0	1	1	0	0
Zn	0	0	0	0	0

3.3.2.2 Prediction of ecological risks from metals at the study sites

Site-specific soil PNECs and Risk Characterisation Ratio (RCR) for Co, Pb, Ni, Cu and Zn were calculated using a spreadsheet (ARCHE, 2014) for all sites. Table 3-16 shows that there are potential risks of Zn and Ni at seep 3. Although no such site specific PNEC calculator has been developed for other elements, initial screening for Cd, As, Fe and Mn can be done by comparing pseudo-total concentrations in this study with non-site-specific ecological soil screening levels (ESSL) which have been published in the literature as shown in Table 3-17. RCR for these elements have been calculated and presented in Table 3-18.

Values of RCR for Cd at Ref. 1 and Ref. 2 soils indicate that there are potential risks of Cd for avian and mammalian organisms. Chronic oral exposure of avian and mammals to Cd can be a problem (Beyer, 2000). Mammals can accumulate Cd concentrations in some of their organs such as kidneys. Świergosz et al. (1998) found that 54% mortality of bank voles at accumulated about 40 mg/kg of renal Cd from dietary exposure. Furthermore, bioaccumulation of Cd between trophic levels of terrestrial food chains has been found in many studies (Baudrot et al., 2018).

Based on values of predicted RCR, high-risk by Fe and Mn at all studied soils for soil microbiome and microbial function are expected. The influence might be on the processes of nodulation and nitrogen fixation, or the process of developing of the root-nodule bacteria in the root zone (Alexander, 1980). This means that size or activity of some of the microbial communities might be affected by the Mn and Fe. For example, Mn might affect soil symbiotic nitrogen fixing bacteria, aerobic- heterotrophic bacterial and actinomycetes as it was found in some studies (Ahmad et al., 2005).

The data also suggest that Mn likely poses a toxic risk for soil invertebrates and plants at Ref. 2 soil. Although the concentrations of Mn and Fe were not as high in the seep soils, their lower pH may result in toxicity issues. The solubility of Mn and Fe increase at pH less than 5.5, consequently Mn and Fe can become easily phytoavailable (Kabata-Pendias, 2010). This can lead to a decrease in plant growth which is attributed to the resulting chlorosis from excessive metal exposure (Foy et al., 1998).

Assessments of total or pseudo-total element concentrations relative to generic or site-specific PNECs can, however, only indicate potential risks. To examine risks more closely, the mobility and bioavailability of the elements need to be investigated (and this is investigated in later parts of the study).

Table 3-16: Risk Characterisation Ratio RCR (mean ±SD) and potentially affected fraction PAF % of soil organisms for Co, Cu, Ni, Pb and Zn at study sites.

Site Element		Ref. 1	Seep1	Ref. 2	Seep2	Seep3
Co	RCR	0.23±0.01	0.05±0.01	0.17±0.01	0.18±0.04	0.55±0.18
	PAF	0	0	0	0.2	2.1
Cu	RCR	0.26±0.01	0.36±0.18	0.64±0.01	0.39±0.23	0.32±0.04
	PAF	0	0.8	1.7	2.7	0.4
Ni	RCR	0.47±0.01	0.08±0.04	0.34±0.07	0.32±0.08	1.36±0.83
	PAF	0.6	0	0.2	1.6	10.3
Pb	RCR	0.10±0.01	0.17±0.01	0.18±0.03	0.21±0.03	0.26±0.02
	PAF	0	0.1	0.1	0.6	0.5
Zn	RCR	0.39±0.04	0.12±0.02	0.24±0.05	0.23±0.06	1.05±0.08
	PAF	0.4	0	0.1	1.2	4.9

Table 3-17: Non-site-specific screening levels ESSL (mg/kg for Cd, As, Fe and Mn for different protection goals

Element	ESSL	Goal of protection	Reference
As	60	Earthworm	(Efroymson et al., 1997)
As	50	Plant on grassland areas	(BBodSch, 1999).
Cd	0.77	Wildlife-Avian*	(EPA, 2005a)
Cd	0.36	Wildlife-Mammalian*	(EPA, 2005a)
Fe	200	Soil microorganisms and microbial processes	(Efroymson et al., 1997)
Mn	100	Soil microorganisms and microbial processes	(Efroymson et al., 1997)
Mn	220	Plants	(EPA, 2007)
Mn	450	Soil invertebrates	(EPA, 2007)

* For clarity, it is stated that these values are for soil and not for concentrations in food sources of the birds and mammals (see cited reference for details)

Table 3-18: RCR for Cd, Fe, As and Mn for several protection goals at study sites

Site / RCR-Element	Ref. 1	Seep 1	Ref. 2	Seep2	Seep3
RCR-Cd Wildlife-Avian	1.29 ±0.58	0.37 ±0.02	2.08 ±0.21	0.28 ±0.15	N.R
RCR-Cd-Wildlife-Mammalian	2.75 ±1.25	0.78 ±0.04	4.45 ±0.44	0.60 ±0.31	N.R
RCR-As earthworm	0.12 ±0.02	0.34 ±0.05	0.70 ±0.01	0.62 ±0.20	0.19 ±0.01
RCR-As Plant	0.14 ±0.03	0.40 ±0.06	0.84 ±0.02	0.74 ±0.24	0.22 ±0.00
RCR-Fe Soil microorganisms and microbial processes	61.00 ±3.00	355.00 ±77.01	110.00 ±6.02	303.00 ±105.05	214.00 ±39.06
RCR-Mn-soil Microbiome & microbial processes	1.83 ±0.18	1.67 ±0.26	8.92 ±1.00	1.49 ±0.34	1.38 ±0.22
RCR-Mn Plant	0.83 ±0.08	0.76 ±0.12	4.05 ±0.51	0.68 ±0.16	0.63 ±0.10
RCR-Mn soil invertebrates	0.41 ±0.04	0.37 ±0.06	1.98 ±0.17	0.33 ±0.08	0.31 ±0.05

3.3.3 Metals fractionation of soils, extractable contents of metals in soils and metal(loid)s contents in soil solution.

3.3.3.1 Metals fractionation in soil using BCR scheme

Fractionation of Ni, As, Cd, Co, Cr, Cu, Al, Fe, Mn, Pb, Zn, Ca, and Mg in soils was studied using Community Bureau of Reference (BCR) sequential extraction scheme (Ure et al., 1993). It provides information about the potential bioavailability and mobility of elements in the studied soils by attempting to determine the amounts of each element that are associated with various target soil fractions. Each successive step in the BCR scheme uses a more aggressive chemical reagent so that overall the scheme can identify elements that are in easily mobile through to resistant (or residual) fractions. The first fraction has been defined as targeting the most readily bioavailable and the most mobile forms of elements in soil and it is intended to represent the weakly bound (at cation-exchange sites) and that bound to carbonates. Step two of the BCR includes metal(loid)s associated to iron and manganese oxides that at low soil pH can be mobilised. Organic-bound metals are targeted by the third BCR step which are stable unless under strong oxidising environment. The final step includes silicates and residual oxides phase which are considered immobile. For simplicity of representation, elements found at lower than the limit of detection (using ICP-OES) were treated as zero.

Findings of fractionation of some metal(loid)s in soils collected from Ref.1 site, which is vegetated soil, are shown in Table 3-19 as individual mean concentrations of fractions and in Figure 3-7 as the percentages of individual fractions. It can be seen that Ni, Al, Cr, Cu, Fe, Pb and Zn are found to be dominantly within the non-labile phase. At this site, Ca and Mg labile fractions are at dramatically high concentrations (1953.58 mg/kg and 203.00 mg/kg, respectively). There is a significant labile fraction of As and Mn (40%, 6.8 mg/kg and 30%, 69.2 mg/kg respectively) at this is site. Although there is a high percentage of Co in the labile fraction, in reality this represents a small total amount at less than 1 mg/kg.

Table 3-19: Individual BCR fractions concentrations of metal(loid)s extracted from Ref.1 site; based on the mean ± SD; (mg/kg dried basis).

Fraction Element	Exchangeable	Reducible	Oxidisable	Residual
Al	57.82±5.37	513.07±10.93	359.26±4.10	6799.07±542.73
As	6.80±0.56	6.60±0.78	7.47±4.97	3.87±0.61
Ca	1953.58±30.43	537.13±1.24	36.07±1.32	50.57±2.85
Cd	0.00±0.00	0.67±0.71	0.00±0.00	0.40±0.01
Co	0.98±0.07	0.00±0.00	0.00±0.00	0.00±0.00
Cr	1.46±0.21	3.87±1.77	1.11±0.09	11.87±1.29
Cu	0.66±0.02	0.00±0.00	0.00±0.00	9.21±1.70
Fe	251.80±1.41	2372.10±246	1821.33±98.55	22462.33±2362.33
Mg	203.72±3.29	69.07±20.58	109.41±16.67	1017.15±44.17
Mn	69.21±3.68	44.09±1.75	25.29±10.20	93.87±4.89
Ni	0.00±0.00	5.04 ±1.94	4.17 ±NR	19.60±2.40
Pb	3.87±0.61	7.25±4.83	2.85±0.31	61.40±24.04
Zn	1.40±0.28	5.72±3.47	0.00±0.00	87.47±7.86

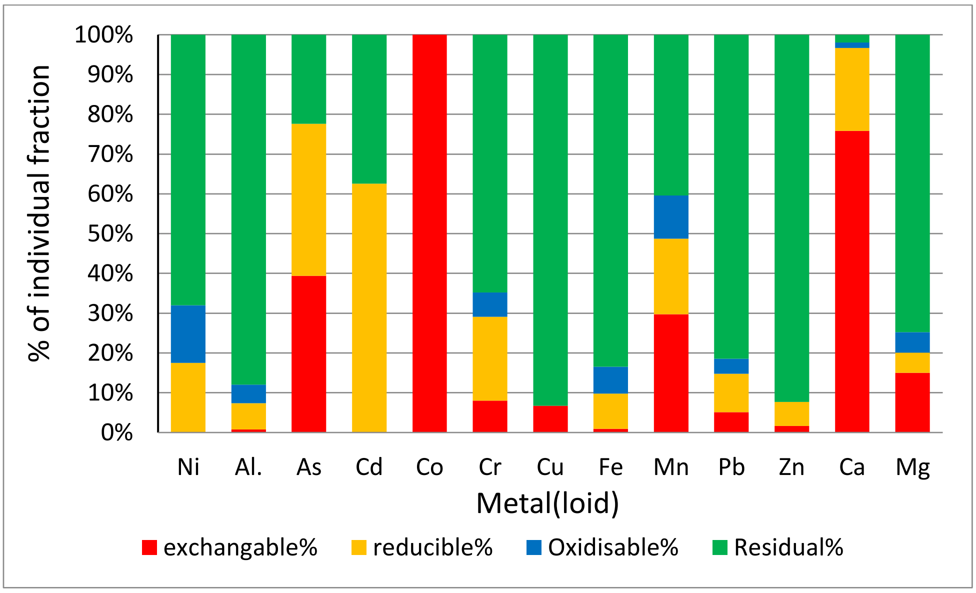


Figure 3-7: Percentages of individual fractions of some metal(loid)s extracted from Ref.1 site.

The results of fractionation of some metal(loid)s in soils at seep 1 site, which is unvegetated soil, are presented in Table 3-20 as individual mean concentrations of fractions and in Figure 3-8 as percentages of individual fractions. It is clear that Al, As Cd, Co, Cr, Cu, Fe, Pb and Zn distribute within non-labile (residual) fractions. Interestingly, the labile fraction of Mn and As are significant, representing 43% and 26% of totals and with concentrations of 59.00 mg/kg and 8.80 mg/kg, respectively. There is a small percentage of the respective total amounts of Al and Fe in the labile fraction (16% and 0.1% respectively), however, it should be noted that those small percentages of the total represent great amounts (200.00 mg/kg and 257.00 mg/kg respectively).

Table 3-20: Individual BCR fractions concentrations of some metal(loid)s extracted from seep 1 site; based on the mean ± SD; (mg/kg dried basis).

Fraction Element	Exchangeable	Reducible	Oxidisable	Residual
Al	200.37±1.84	226.39±12.74	163.11±0.00	3458.20±728.32
As	8.80±2.26	2.28±0.00	10.22±1.61	15.60±0.40
Ca	736.80±24.46	55.92±3.71	9.36±0.69	18.36±3.87
Cd	0.13±0.02	0.00±0.00	0.00±0.00	3.50±0.21
Co	0.27±0.004	0.00±0.00	0.00±0.00	9.20±0.40
Cr	0.32±0.05	1.09±NR	0.16±NR	5.01±0.81
Cu	6.71±0.14	1.20±NR	0.00±0.00	60.53±1.22
Fe	257.00±2.55	14878.07±32.60	38760.00±2870.76	209600.80±1455.43
Mg	37.49±0.25	0.00±0.00	1.46±0.21	35.40±2.81
Mn	59.00± NR	5.31±3.16	42.13±5.24	59.01±3.11
Ni	0.00±0.00	0.00±0.00	20.16±4.17	8.93±1.85
Pb	3.40±0.28	0.00±0.00	5.93±0.71	77.20±2.83
Zn	2.0±1.13	1.63±NR	0.00±0.00	90.27±13.79

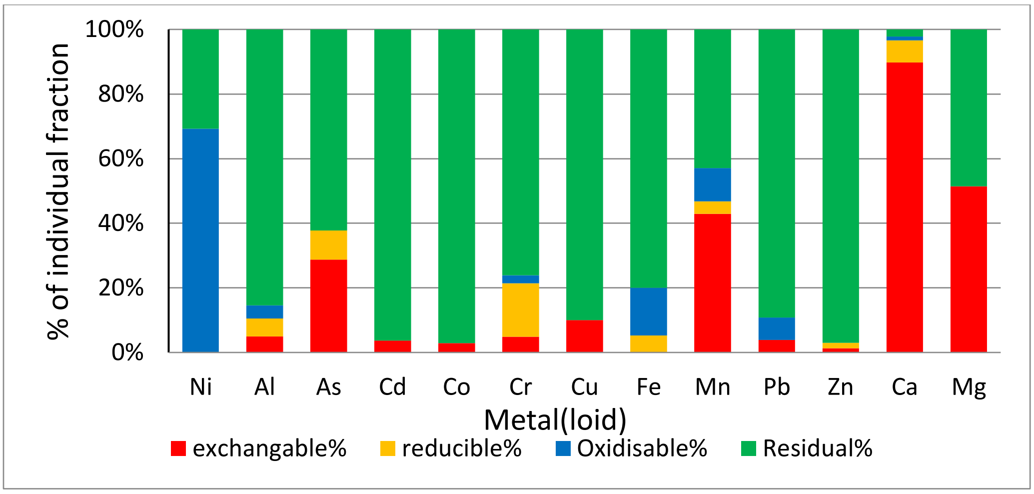


Figure 3-8: Percentages of individual fractions of the interested metal(loid)s extracted from seep 1 site

Data of fractionation of some metal(loid)s in Ref. 2 soils, which is vegetated soil, are shown in Table 3-21 as individual mean concentrations of fractions and in Figure 3-9 as percentages of individual fractions. Ni, Al, Cd, Cr, Fe, Pb and Zn are within the non-labile phase. There are high concentrations of exchangeable Co and As (5.1 mg/kg and 6.80 mg/kg, respectively). On the other hand, the concentrations of labile fractions of Ca and Mg are considerably high at 4964.17 mg/kg and 340.05 mg/kg respectively.

Table 3-21: Individual BCR fractions concentrations of metal(loid)s extracted from Ref. 2 site; based on the mean ± SD; (mg/kg dried basis).

Fraction Element	Exchangeable	Reducible	Oxidisable	Residual
Al	88.22±0.93	1429.77±164.74	687.47±120.19	5068±774.99
As	6.80±0.56	27.86±3.49	19.34±2.27	34.80±3.20
Ca	4964.17±60.32	2597.61±25.90	105.10±6.33	41.88±0.21
Cd	0.00±0.00	0.00±0.00	0.00±0.00	1.00±0.28
Co	5.10±0.24	11.31±1.86	0.00±0.00	0.00±0.00
Cr	1.58±0.04	0.00±0.00	2.38±0.18	10.03±1.83

Cu	2.70±0.05	51.56±7.94	50.67±5.46	54.93±7.89
Fe	248.40±NR	6976.33±1097.50	12706.67±1314.74	36607.33±2815.79
Mg	340.05±4.94	93.41±2.41	75.50±10.55	182.04±15.87
Mn	396.79±7.75	782.58±12.31	65.70±26.59	118.81±36.20
Ni	3.90±0.70	24.39±3.62	3.99±1.82	38.66±3.49
Pb	4.53±0.46	31.85±1.03	8.01±.92	76.80±9.05
Zn	4.20±0.28	25.09±1.16	7.25±1.20	124.00±21.51

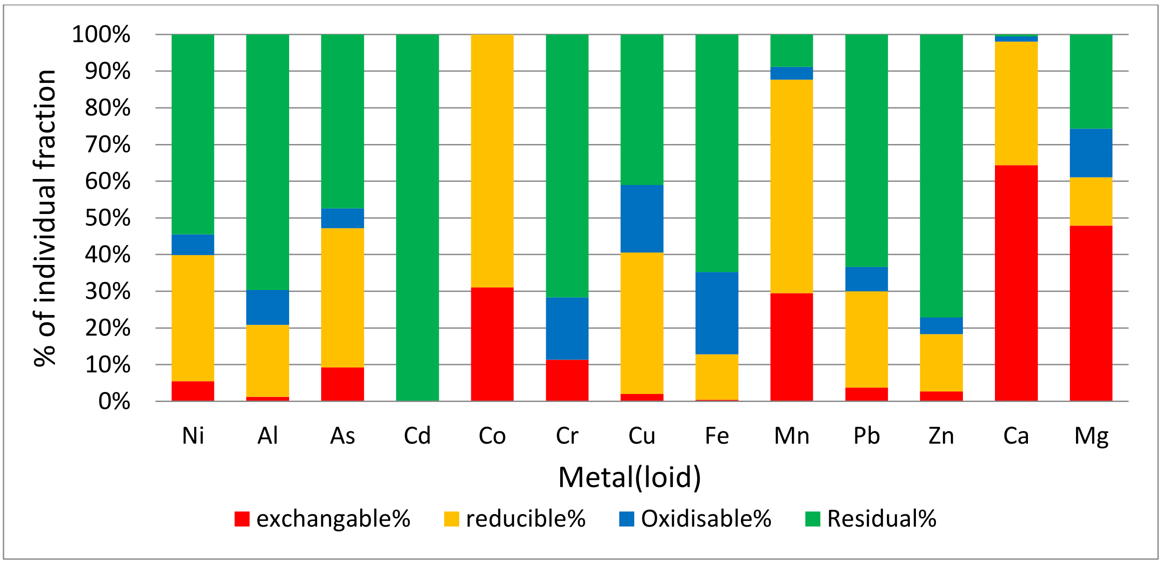


Figure 3-9: Percentages of individual fractions of metal(loid)s extracted from Ref. 2 site.

Findings of fractionation of some metal(loid)s in seep 3 soils, which is unvegetated soil, are presented in Table 3-22 as individual mean concentrations of fractions and Figure 3-10 as percentages of individual fractions. It is clear that, Al, Ni, Cd, Cr, Co, Fe, Mn, Pb and Zn are mainly associated with the residual phase. Similar to that found in seep 1, the exchangeable fraction concentrations of Al, Mn and Fe in are high at 57.44 mg/kg, 22.51 mg/kg and

447.60 mg/kg respectively. Interestingly, the majority of Cu is found to be associated with exchangeable and reducible fractions (7% and 63%, respectively).

Table 3-22: Individual BCR fractions concentrations of some metal(loid)s extracted from seep 3 site soil; based on the mean ± SD; (mg/kg dried basis).

Fraction / Element	Exchangeable	Reducible	Oxidisable	Residual
Al	57.44±1.87	223.46±31.62	211.76±9.20	6763.07±664.80
As	0.40±0.06	6.70±0.85	0.00±0.00	8.27±1.22
Ca	502.27±6.03	95.42±6.47	5.55±1.21	24.09±2.13
Cd	0.00±0.00	0.00±0.00	0.00±0.00	2.80±0.01
Co	0.74±0.09	0.00±0.00	0.00±0.00	3.20±0.01
Cr	0.90±0.12	0.00±0.00	1.18±0.16	12.40±0.81
Cu	0.68±0.06	6.34±1.08	0.00±0.00	7.01±0.04
Fe	447.60±67.88	5878.32±148.19	19346.67±563.01	98627.60±1740.11
Mg	162.18±3.67	26.64±4.34	39.05±0.26	909.04±42.29
Mn	22.51±0.67	5.06±NR	9.25±NR	102.80±1.06
Ni	0.00±0.00	1.48±0.21	0.00±0	16.93±2.03
Pb	3.20±2.12	0.00±0.00	4.38±0.52	36.40±0.57
Zn	2.40±2.55	6.54±NR	0.00±0.00	84.53±12.37

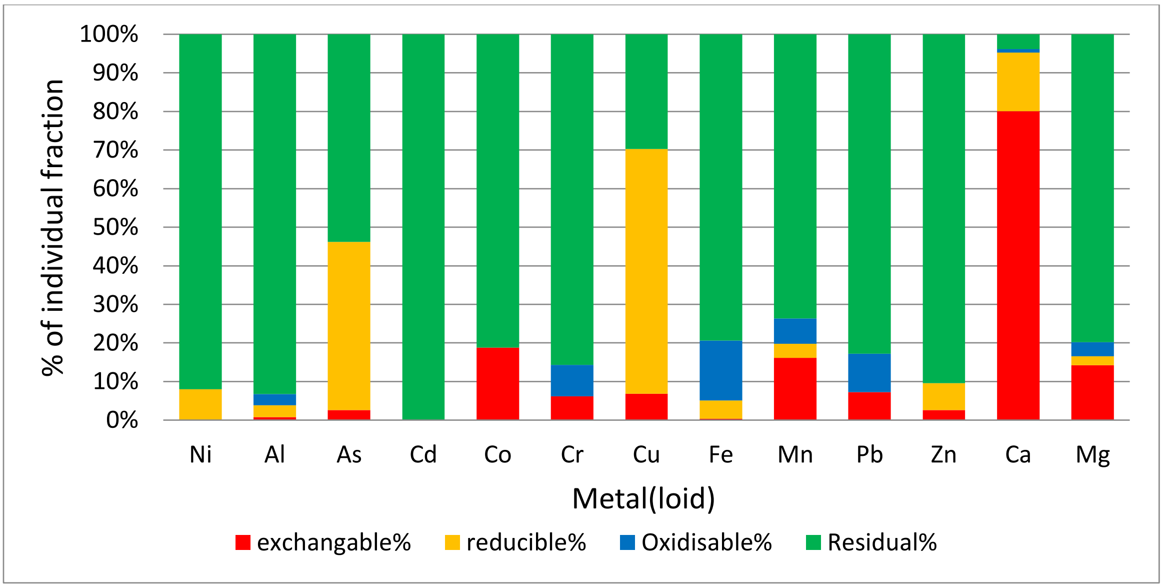


Figure 3-10: Percentages of individual fractions of some metal(loid)s with extracted from seep 3 site.

Assessment of potential environmental risk of the elements of interest can also be conducted based on the percentage of metal associated with the soil in the exchangeable and carbonate fractions. This approach has been referred to as a risk assessment code (RAC) index (Yang et al., 2018). The criteria of the assessment is that RAC less than 1% means no (or little) risk; low risk is referred to RAC between 1% and 10%; medium risk indicated when RAC is 11%-30% and 31%-50% represent high risk. Of course, a common sense evaluation based on what the percentage actually represents in terms of the total amount of mobile metal must also be factored in when using this RAC approach, but nevertheless it can be useful and the BCR results of the present study can therefore be evaluated in these terms. In other words, based on risk assessment code (RAC) the risk can be considered to be high if the BCR1 fraction is above 30% of total. Closer inspections of Table 3-23 show that in these terms there is As, Co and Mn risk at Ref.1 site, risks of As and Mn at seep 1 site, and risks of Co and Mn at Ref. 2 site.

Table 3-23: Risk assessment code (RAC) index of some metal(loid)s based on BCR fraction 1 as a percentage of total

Site / Element	Ref. 1	Seep 1	Ref. 2	Seep 3
Al	1%	5%	1%	1%
As	39%	29%	9%	3%
Ca	76%	90%	64%	80%
Cd	0%	4%	0%	0%
Co	100%	3%	31%	19%
Cr	8%	5%	11%	6%
Cu	7%	10%	2%	7%
Fe	1%	0%	0%	0%
Mg	15%	51%	48%	14%
Mn	30%	43%	29%	16%
Ni	0%	0%	5%	0%
Pb	5%	4%	4%	7%
Zn	2%	1%	3%	3%

The BCR extraction results can also be probed by examining what has been referred to as the potential mobility fraction (PMF) in soil; PMF is the summation of BCR1+BCR2+BCR3 (Rinklebe and Shaheen, 2014). What is interesting in the data shown in Table 3-24, is that PMF for Cu at seep 1 and seep 3 sites might mean that the sources of these metals are of acid mine weathering origin since the natural originated metal(liod)s distribute mostly within silicates and residual phase.

Table 3-24: The potential mobility fraction (PMF) of some metal(loid)s based on sum of BCR fractions 1-3 as percentage of total

Site Element	Ref. 1	Seep 1	Ref. 2	Seep 3
Al	12%	15%	30%	7%
As	78%	38%	53%	46%
Ca	98%	98%	99%	96%
Cd	63%	4%	0%	0%
Co	100%	3%	100%	19%
Cr	35%	24%	28%	14%
Cu	7%	10%	59%	70%
Fe	17%	20%	35%	21%
Mg	25%	51%	74%	20%
Mn	60%	57%	91%	26%
Ni	32%	69%	46%	8%
Pb	19%	11%	37%	17%
Zn	8%	3%	23%	10%

The order of metal(loid)s mobility in sites in term of the percentage of total in labile BCR1 fraction were:

In Ref. 1 site Ni=Cd<Al=Fe<Zn<Pb<Cu<Cr<Mn<As<Co.

In seep 1site Ni=Fe< Zn< Co< Cd=Pb< Cr=Al< Cu< As< Mn.

In Ref. 2 site Cd=Fe< Al< Cu< Zn< Pb< Ni< As< Cr< Mn< Co.

In seep 3 site Ni=Cd=Fe< Al< Zn< As< Cr< Cu=Pb< Mn< Co.

The mobility of metal(loid)s are governed by their interactions with microorganisms, organic matter, phyllosilicates and variable charge minerals. Those factors are different in the studied heterogeneous soils and these lead to variances in the order of mobility of metal(loid)s (Caporale and Violante, 2016).

In general, Al, Cr, Cd, Co, Fe, Ni, Pb and Zn are within the non-labile phase, this possibly related to the nature of the adsorption of those elements to edge sites of phyllosilicates, in which those elements chemically bind to SiOH or AlOH groups in phyllosilicates (Sparks, 2013, Sparks and Ginder-Vogel, 2013).

An exception of that is Co in Ref.2 site in which part of Co was weakly bound. Gál et al. (2008) stated that during weathering processes, Co is mobilised relatively readily. Co is susceptible to oxidation-reduction reactions and involved in dissolution–precipitation processes in the soil (Barker and Pilbeam, 2015). Below pH 7 outer-sphere surface Co complexes (weakly bound) onto some soil component were found (Woodward et al., 2018, O'Day et al., 1994). Palit et al. (1994),Alloway (2012),Gál et al. (2008) stated that adsorption and coprecipitation of Co occur with MnO_2. Weathering of the Mn-bearing minerals consequently impact Co mobility.

Exchangeable arsenic concentration in Ref.1, seep 1 and Ref. 2 sites were in agreement with work by Catalano et al. (2008). They found that arsenic can be attached to some soil component in a way which is easy to desorb. Again, those are not only important for potential stream water and groundwater contamination (Ceto and Mahmud, 2000) but also pose risk for soil biota (USEPA, 2003).

A high Mn proportion was found in the labile fraction in Ref. 1, seep 1 and Ref. 2 sites, and this might be related to the fact that when oxidation of sulphide minerals occur, Mn-bearing minerals dissolve and then Mn ions replaced the Mg and Ca ion on the exchange sites in soils (Goulding, 2016). As Khattak and Page (2017) comment, in many studies, the electron spin resonance spectroscopy data have suggested that Mn^{2+} is adsorbed to clay minerals through outer-sphere complexation mechanisms. Those are important for potential groundwater and stream water contamination (Tiankao and Chotpantarat, 2018, McMahon et al., 2018) as well as a potential risk for soil biota (Wu et al., 2015).

Comparison among the labile fractions of the elements at the four sites could be useful to assess the potential bioavailable and mobile elements that could be at a level of concern, Table 3-25.

Table 3-25: Comparison among the labile fractions (0.11M acetic acid extractable) of the elements at four sites, the data is presented as mean (mg/kg) ± SD.

Fraction / Element	Ref.1	seep 1	Ref. 2	seep 3
Ni	0.00±0.00	0.00±0.00	3.90±0.70	0.00±0.00
Al	57.82±5.37	200.37±1.84	88.22±0.93	57.44±1.87
As	6.80±0.56	8.80±2.26	6.80±0.56	6.40±1.41
Cd	0.00±0.00	0.13±0.02	0.00±0.00	0.00±0.00
Co	0.98±0.07	0.27±0.004	5.10±0.24	0.74±0.09
Cr	1.46±0.21	0.32±0.05	1.58±0.04	0.90±0.12
Cu	0.66±0.02	6.71±0.14	2.70±0.05	0.68±0.06
Fe	251.80±1.41	257.00±2.55	248.40±28.00	447.60±67.88
Mn	69.21±3.68	59.00±7.21	396.79±7.75	22.51±0.67
Pb	3.87±0.61	3.40±0.28	4.53±0.46	3.20±2.12
Zn	1.40±0.28	2.0±1.13	4.20±0.28	2.40±2.55
Ca	1953.58±30.43	736.80±24.46	4964.17±60.32	502.27±6.03
Mg	203.72±3.29	37.49±0.25	340.05±4.94	162.18±3.67

As a consequence of the oxidation of sulphide minerals, the Al-bearing minerals then Fe-bearing minerals in the soil are dissolved after that Al^{3+} and Fe^{2+} ions replace the exchangeable base cations such as Ca, Mg, K through the cation exchange process (Goulding, 2016). This would be an explanation for increase of exchangeable Al concentration at seep 1 which is about 4 and 2 times greater than that at Ref. 1 and Ref. 2, respectively, similarly, the increase of exchangeable Fe concentration at seep 3 which is about 2 times greater than that at Ref. 1 and Ref. 2. This can be therefore, supporting for the findings of potential Al toxicity in seep 1 that was mentioned in section 3.3.1.2 and findings of the potential risk of Fe in seep 3 site in section 3.3.2.2.

Ca and Mg at Ref. 2 and Ref. 1 sites are considerably higher than those at seep 1 and seep 3 sites. Cover plant act as a phytostabilization in Ref. 2 and Ref. 1 sites, which leads to a reduction of Ca and Mg losing via erosion and leaching (Chibuike and Obiora, 2014), whereas, in seep 1 and seep 3 Ca and Mg might be washed to the nearby stream by rainwater or upwelling seepage.

The exchangeable concentrations of Ni, Co, Mn, Pb and Zn at Ref. 2 site are greater than those at the rest of the sites; however, higher soil pH in this site as well as the amount of the exchangeable Ca and Mg might be a reason for enhancing the soil condition and reducing the toxicity of metals present. Furthermore, metal(loid)s may have both antagonistic and synergistic interactions in terms of toxic effects, for example, the antagonistic effects of Ca on trace elements such as Cd, Pb, and Ni as well as the antagonistic effects of Fe on Zn, Mn, and Co (Kabata-Pendias, 2010). Similarly, Al toxicity is decreased by the presence of Mg (Rengel et al., 2016). However, such possible antagonistic or synergistic effects on toxicity cannot be evaluated just by extraction methods, but rather by the use of direct measurements of toxicity such as by using plants or other test organisms (as is addressed later in this study).

The recovery percentages, or element mass balances, were computed by comparing the sum of all BCR fractions with the pseudo-total element concentrations previously determined using reverse aqua regia (for Ref.1, seep 1, Ref. 2 and seep 3 soils) as shown in Table 3-26. There are good recoveries for Al, As, Cd (for Ref.1 and Ref. 2), Cr, Cu (except for soil Ref.1), Mn, Ca and Mg. Recoveries for Pb and Fe were high but still mostly within a factor of 2 of the pseudo-totals. The inconsistent recovery for Ni, Co, Zn, Pb and Fe, together with the relatively tight precision for the BCR fractions and the previous pseudo-total concentration measurements, show how heterogeneous the soils at the sites can be for those elements. This heterogeneity therefore should be taken into account when evaluating the sites and considering any remediation or management recommendations.

The chemical sequential extraction methods, e.g., BCR scheme, can bring the individual geochemical phases into the solution at the experimentally defined stages. Based on the findings of this study, an evaluation of the general chemical extraction methods and particularly of the BCR procedure will be presented hereafter.

Certified reference materials (CRMs) with certified extractable contents are commercially available and their use can provide additional confidence in the extraction procedure and analytical measurements, however they are problematic and often prohibitively expensive because they cover only a limited range of elements and are often in a matrix that is not a precise match for the environmental samples under study (e.g. CRM BCR701 is a lake bed sediment matrix). For these reasons they were not used in the present study. Even with the use of such CRMs there is often still the issue of suboptimal recovery of elements across the sequential extraction steps relative to the previously determined total element concentration (Horvath et al., 2010), and the reasons can vary. Depending on the soil types, readsorption, and redistribution of metals bound at exchangeable, carbonate, Fe, Mn oxide, and organic fractions can occur onto the other solid geochemical phases during sequential extraction (Shan and Chen, 1993).

The reagents can even create and expose new surfaces within residual solid phase materials that can bind elements more strongly and prevent their solubiisation, potentially even resisting strong mineral acids. Natural heterogeneity of environmental samples is also a factor. Nevertheless, use of sequential extraction techniques such as BCR does provide highly useful insight into the relative distribution of elements across increasingly recalcitrant soil components and therefore provides information about likely mobility and potential bioavailability. That is, they can be a reasonable screening tool for estimating the mobilizable pool of elements in soils. Although these extractions do have limitations and cannot directly be 100% related to the bioavailability of elements as experienced by plant roots or soil dwelling invertebrates and microbes, the findings can be utilized for assessing the risk, e.g. using risk assessment code RAC, and as an indication and point of relative comparison.

Concerning the difficulties of achieving quality control of the sequential extraction method, it can not be considered as a completely precise, specific, and efficient tool to quantify metals partitioning for all the complex soil systems without accompanying another assessment tool.

Table 3-26: Recoveries percentage (mass balances of BCR fractions sum relative to pseudo-total element concentrations previously determined) of elements at the four sites

Site / Element	Ref.1	Seep1	Ref.2	Seep3
Al	69%	69%	73%	81%
As	79%	141%	143%	137%
Ca	103%	140%	124%	110%
Cd	90%	>150%	75%	NR
Co	20%	>150%	64%	28%
Cr	96%	61%	90%	74%
Cu	35%	81%	92%	126%
Fe	>150%	>150%	>150%	>150%
Mg	66%	93%	106%	90%
Mn	95%	104%	138%	66%
Ni	41%	>150%	63%	8%
Pb	>150%	>150%	>150%	>150%
Zn	>150%	>150%	>150%	>150%

3.3.3.2 $CaCl_2$-extractable contents of metal(loid)s in soil

The extractable metal(loid)s fraction determined by a dilute neutral salt solution have been said to be more correlated than the BCR scheme fractions (or equivalent) with phytoavailability (Ahnstrom and Parker, 2001). The idea of Barber (1984) of using an extractant which simulates the natural soil solution for predicting the metals' phytoavailability was further developed by Houba et al. (2000). Common neutral salts extractants are $CaCl_2$, $MgCl_2$, $Sr(NO_3)_2$, and NH_4NO_3. They offer a good indication of metal(loid)s availability to the plant (Kabata-Pendias, 2004, Menzies et al., 2007).

In terms of the potential for pollutant transition from soil to plant on agricultural land, trigger values were developed and used under Germany's Federal Soil Protection Act for extracted metal(loid)s (using neutral salt reagents and thus similar to some extent to what have been used in this study), as shown in Table 3-27. These values have been set with regard to growth weakening of cultivated plants (BBodSch, 1999).

Table 3-27: Trigger values, mg/kg, for As, Cu, Ni and Zn using ammonium nitrate extract as employed under the German Federal Soil Protection Act.

Element	Trigger value mg/kg
Arsenic	0.4
Copper	1
Nickel	1.5
Zinc	2

The concentration of metal(loid)s extractable using 0.01M $CaCl_2$-extractant are presented in Table 3-28. It is apparent from the table that concentrations of Al-extractable and Fe-extractable at seep 1, seep 2 and seep 3 were considerably higher than those at Ref 1 and Ref. 2 which were less than the detection limit using ICP-OES for Al and less than 1 mg/kg for Fe. Similarly, Mn content is considerably high at seep 2 and seep 3, those results might be related to replacing of Ca ions from the extractant with Al, Fe and Mn which are weakly bound to the ions exchange site in the soil as described in Goulding (2016). Hume et al. (1988) found that 0.02M $CaCl_2$-extractable Al contents of less than 3.3 mg/kg were unlikely to affect white clover. $CaCl_2$-extractable Al content in seep 1, seep 2 and seep 3 are quite greater than 3.3 mg/kg, based on that, Al phytotoxicity is likely in those sites. Duncan (2012) stated that $CaCl_2$-extractable Mn levels that are associated with Mn toxicity occurred as low as 10 mg/kg when Al-extractable amounts were greater than 1.80 mg/kg.

From the table (3-28), it can be seen that the extractable Co content at seep 2 and seep 3 were significantly higher than those at the rest of the sites, which might be attributed to low soil pH mobilising the Co and this might cause concern to soil biota (Barker and Pilbeam, 2015).

Closer inspection of the table shows an increase in Pb content at seep 2 relative to other sites but statistically, that was not significant. Those values were higher than what has been found in a study by Pueyo et al. (2004) which were below the detection limit in three sandy loam soils that contained 96.00, 245.00 and 403.00 mg Pb/kg aqua regia extractable contents that are greater than European range of maximum allowable concentrations MAC[1].

Statistical tests reveal that Zn concentration in sites seep 1, seep 2 and seep 3 were significantly higher than those at the rest of sites. The values of extractable Zn content in the present study were within the range of those found in a study done by Pueyo et al. (2004). They examined the concentration of Zn-extractable in three sandy loam soils (contain 776.00, 911.00 and 1248.00 mg Zn/kg (extracted using aqua regia which are greater than the Netherlands intervention limits[2]) and the extractable Zn contents were 2.5, 0.31 and 0.29 mg/kg. However, compared with the trigger value which developed and

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[1] As describe in KABATA-PENDIAS, A. 2010. *Trace elements in soils and plants*, CRC press.

[2] PRONK, J. 2000. Circular on target values and intervention values for soil remediation. *Ministry of Housing, Spatial Planning and Environment Rep. No. DBO,* 1999226863.

used under Germany’s Federal Soil Protection Act for extracted Zn, there is not risk for Zn transition from soil to plant, Table 3-27 and Table 3-28.

Cu $CaCl_2$-extractable contents were greatest at seep 1 and seep 2 sites. Extractable Cu contents were also examined by Pueyo et al. (2004). Three sandy loam soils contained 278.00, 214.00, 308.00 mg Cu/kg (extracted using aqua regia which are greater than the Netherlands intervention limits) have Cu $CaCl_2$-extractable 0.13, 0.11, and 0.14 mg/kg respectively, those were less than that measured on seep 1, seep 2 and seep 3 in this study. Compared with the trigger value which based on Germany’s Federal Soil Protection Act for extracted Cu, there is potential risk for Cu transition from soil to plant at seep 1 and seep 2, Table 3-27 and Table 3-28.

Extractable Ni contents were greatest at seep 1 and seep 2 sites, and if compared with the trigger value for neutral salt extractable Ni used under Germany’s Federal Soil Protection Act there is potential risk for Ni transition from soil to plant at seep 2 (Table 3-27 and Table 3-28). Interestingly, Mg content is considerably low at seep 1 and seep 3. It might be possible that, if plants could be made to grow here through a remediation step, Mg deficiency may occur because of this. The long-term Mg depletion of soils as a consequence of Mg leaching due to the chemical weathering processes in which the soil cation exchange sites were saturated with H^+ ions and resulting Mg leaching has been noted elsewhere (Gransee and Führs, 2013).

Table 3-28: The concentration (mg/Kg) of some metal(loid)s using $CaCl_2$-extractant, the data is presented as mean ± SD, B.D refers to bellow limit of the detection, N.R refers to not.

Sites / Element	Ref. 1	Seep 1	Ref. 2	Seep 2	Seep 3
Al	B.D	91.42±0.68	B.D	71.94±0.39	40.19±0.46
Co	0.03±0.03	0.12±0.02	B.D	0.98±0.07	0.13±0.08
Cd	0.010±0.002	B.D	B.D	B.D	B.D
Cr	0.003±0.001	B.D	B.D	B.D	B.D
Cu	0.07±0.01	1.40±0.04	0.33±0.01	1.64±0.05	0.31±0.07
Fe	0.48±0.06	11.58±0.36	0.50±0.11	124.13±3.38	82.34±1.38
Ni	0.09±0.01	0.36±0.00	0.25±0	1.91±0.05	0.24±0.00
Mn	3.28±0.07	3.25±0.12	5.93±0.08	34.65±0.52	29.34±0.51
Zn	0.71±0.03	1±0.01	0.67±0	1.42±0.07	1.20±0.04
Pb	1.10±0.10	0.87±0.65	1.03±0.34	1.56±0.40	1.11±0.25
As	0.12±0.01	0.09±0.00	0.09±0.00	0.11±0.00	0.07±0.01
Mg	173.98±3.84	35.33±0.79	208.34±2.59	200.1±4.43	160.5±16.42

3.3.3.3 Soil solution metal(loid)s contents

Dissolved metals in the soil solution are the most easily available form of metals to soil biota (Hamon et al., 1995, Lorenz et al., 1997, Sauve et al., 2000). It is considered as an indication of the actual mobility of metal(loid)s (Cappuyns and Swennen, 2008). Concentrations in the directly obtained soil solutions, to the extent that they could be determined, are shown in Table 3-29, while concentrations measured in the simulated soil solutions are shown in Table 3-30 and Table 3-31. Average natural abundance of some metal(loid)s in soil solution have been cited by Wolt (1994) in µmol/l which have been converted to be µg/l as presented in Table 3-32 for comparison. Corresponding values for other elements have been estimated by Kabata-Pendias (2010), Table 3-33.

Table 3-29: Mean concentration (mg/l or µg/l) ± SD of Al, Fe, Mn, Ca and Mg content in directly obtained soil solution samples, B.D refers to bellow limit of the detection, N.R refers to not reported (i.e. soil solution was not obtained).

Site / Element	Ref. 1	Seep1	Ref. 2	Seep 2	Seep 3
Al mg/l	N.R	N.R	N.R	4.91±0.38	54.56±0
Fe mg/l	N.R	N.R	N.R	396.03±46.27	363.35±56.21
Mn mg/l	N.R	N.R	N.R	24.37±0.26	37.46±0.10
Ca mg/l	N.R	N.R	N.R	517.23± 8.53	290±28.6
Mg mg/l	N.R	N.R	N.R	N.R	309.03±3.54
Cd µg/l	N.R	N.R	N.R	18.00±0.92	50±6.16
Cr µg/l	N.R	N.R	0.12±0.05	21.73±6.03	5.37±0.02
Cu µg/l	N.R	N.R	N.R	B.D	42.50±3.54
Pb µg/l	N.R	N.R	1.28±0.36	4.88±1.56	3.89±0.93
Zn µg/l	N.R	N.R	N.R	44.50±3.93	207.80±15.30
As µg/l	N.R	N.R	2.12±0.14	3.04±0.29	N.R

Table 3-30: Mean concentration (mg/l) ± standard error of K, Fe, Mn, Ca and Mg content in simulated soil solution samples.

Site / Element	Seep 1	Seep 2	Seep 3
Ca	201.21±17.68	247.4±0.62	251.37±1.8
Fe	913.32±240.74	327.42±76.24	395.29±109.75
K	8.32±2.38	254.96±18.65	51.57±3.42
Mg	93.96±7.18	261.17±1.73	231.68±1.31
Mn	9.99±0.76	69.24±3.66	45.49±1.75

Table 3-31: Mean (µg/l) ± standard error of Cr, Cu, As, Pb, Cd, Co, Ni and Zn content in simulated soil solution samples.

Site Element	Seep 1	Seep2	Seep 3
Cr	3.54±0.67	1.25±0.1	17.19±4.08
Cu	48.52±16.21	8.48±2.49	24.58±11.12
As	2.86±0.29	19.68±5.82	12.24±1.56
Pb	0.54±0.2	4.86±1.43	5.69±1.45
Cd	5.89±2.61	1.57±0.98	11.21±5.64
Co	99.76±10.87	550.27±94.01	538.89±63.46
Ni	962.35±73.95	2425.22±350.76	2289.93±261.57
Zn	1182.15±188.66	1453.84±482.68	2420.86±666.62

Table 3-32: Average natural abundance of some metal(loid)s in soil solution in µmol/l after Wolt (1994), which have been converted to be in µg/l.

Element	µmol/l	µg/l
As	0.01	0.75
Cd	0.04	4.50
Cr	0.01	0.52
Cu	1.00	63.55
Pb	0.005	1.04
Zn	0.08	7.30

Table 3-33: Arithmetic means of trace elements concentration (µg/L) in soil solution obtained by centrifugation for acid sandy and sandy contaminated soils after Kabata-Pendias (2010).

Element	Acid Sandy, pH (2.5-4)	Sandy, pH (4-4.5)
Fe	2223	1000
Mn	5965	8000

A comparison of studied metal(loid)s content in simulated soil solution from the soils of the present study with the typical values described in the literature and shown in Table 3-32 and Table 3-33 indicate that the levels of most elements in soil solutions of the study site are elevated, even though the total concentrations in the soil solid may not be considered high. This provides one measure of the relative risk of most elements present. However, the toxicity of elements can be soil and organism specific. For example, based on Al^{3+} thresholds in soil solution, (Poschenrieder et al., 2008) plant species can be classified in terms of their Al resistance: very sensitive < 0.03 mg/l e.g. *Bromus wildenowii*; sensitive 0.03–0.05 mg/l e.g. *Poa pratensis*; moderately sensitive 0.05–0.14 mg/l e.g. *Dactylis glomerata*; tolerant> 0.14 mg/l e.g. *Phaseolus vulgaris* and highly tolerant> 0.81 mg/l e.g. *Agrostis tenuis.* Thus specific tests are needed to fully assess the environmental toxicity of the site in terms of habitability for soil organisms.

The concentrations of metals released in solution are quite significant for a number of the elements investigated. Fluxes of those metals from soil to soil solution and to other soil compartments can pose a risk to soil organisms as well as affecting soil biochemical processes. For example, the concentrations of Ni in all seep location soil solutions were well above the level reported as the 10% effect concentration (EC10) for inhibiting root elongation in barley seedlings (i.e. 200 µg/L; Zhang et al., 2013b), while the concentrations of Zn were an order of magnitude above EC20 soil porewater concentrations determined for the microbial functions of soil nitrification and maize residue respiration (Smolders et al. 2004). Similarly, the concentrations of Cd were above the EC5 value for reproduction (number of juveniles produced) of the springtail *Folsomia candida* in one particularly sensitive soil (~1 µg/L; Bur et al., 2010) and the Pb concentrations were approaching the

EC50 values determined for reproduction of the earthworm *Eisenia fetida* in 7 European soils (the most sensitive soil EC50 value was ~15 µg/L; Lanno et al., 2019), meaning that the concentrations of these elements would impair plant, microbial and invertebrate development in the soils of the study site. There are also wider ecosystem and food chain implications, as any organisms that are able to become established in the seep soils of the site would pass on any assimilated contaminants to consumers higher up the food chain. Furthermore, surface water and groundwater can be affected by those contaminants if they move through the soils, which has potential environmental and human health (i.e. groundwater as a drinking water source) implications.

3.4 Chapter conclusions

The aims of the chapter:

In many regions and countries around the world, long term environmental legacies of former coal mines are a pressing concern. In this investigation, the aim was to characterise the study site in terms of soil physicochemical properties, the significance of the metal(loid)s contaminants in the soil, and the forms and associations of contaminants in soils sampled from the damaged areas (seep points) compared with those from reference points away from damaged areas.

The main findings:

Soil property assessments have identified aluminium toxicity related to low soil pH (less than 5.5) as a likely major problem in seep 1, seep 2, and seep 3 sites; moreover, the findings indicate that these soils are highly sensitive to metals toxicity because of their low pH and eCEC, while Ref.1 and Ref. 2 soil sites were placed in the weakly and median sensitive soil groups respectively.

The pseudo-total element contents, when assessed using the geo-accumulation index approach indicate that As, Cd, Co, Cr, Ni, and Zn were within the not accumulated range in seep 1, seep 2, and seep 3, while higher accumulations were found in Ref. 1 and Ref. 2, which is concerning because leaching of metals with rainwater or mine seepage from seeps soils to local stream water is likely which in turn could be affecting the aquatic biota. Furthermore, comparison of the measured contents with the predicted soil-specific ecotoxicological threshold concentrations or with non-soil-specific ecotoxicological threshold concentrations for some of the metal(loid)s indicate that potential risk of Zn and Ni in seep 3, risk of Cd for avian and mammals in Ref. 1 and Ref. 2, and potential risk of Mn and Fe in all sites.

The BCR fractionation data has shown that, in general, most metals in the studied sites were dominantly found within the non-labile phase, except Mn, As, and Co, which might pose risks either to surface/ground waters or to soil biota. High concentrations of exchangeable Al, Mn, Fe as well as low Ca and Mg are likely to pose risks in some seep sites. The results of the assessment of $CaCl_2$-extractable metals contents revealed that seep 1, seep 2, and seep 3 soils have high extractable Al and Fe contents compared with Ref.1 and Ref.2. Similarly, soil in seep 2 and seep 3 sites also have considerably high extractable Mn contents; this might be attributed to chemical weathering of minerals contained Al, Fe, and Mn. Furthermore, a comparison of extractable Cu, Co, Pb, and Zn contents in this study with extractable Cu, Co, Pb, and Zn contents of some contaminated soils, there is a possible posed a risk to some soil organisms. The Concentration of Mg in seep 1 soil would suggest a possible risk of Mg deficiency. Those findings might cause concern for the soil biota. The soil solution data has shown that the levels of most elements in soil solutions of the study site were, in general, greater than the typical values described in the literature which might indicate the relative risk of As, Cr, Cu, Cd, Co, Fe, Mn, Ni, Pb, and Zn, but it is recognised that the toxicity of metal(loid)s in many cases are soil-specific and organism-specific and so further assessment is warranted.

Therefore, to summarise, the main potential problems with the soils of the seep sites (i.e., soils in which plants cannot seem to grow in the field) might be the low soil pH and the associated Al, Fe, and Mn toxicity in addition to Ca and Mg deficiency as well as possibly Co, Cu, Pb, and Zn toxicity.

The implications of the study:

The results of the refinement study of pseudo-total metal content analysis methods indicate that dilution of the digested solution of soil samples followed by direct analysis provides a more precise and consistent result that is an environmentally friendly and time-saving alternative to drying down the digested solution of soil samples option.

In relation to assessment of soils at the site, in general, it seems that oxidation of sulphide minerals leading to a decrease in soil pH is the main contributor to the ecological vulnerability in the area. That highlights the importance of monitoring and controlling the sulphide oxidation and resulting low pH of the exposed soils and also the need for a remediation strategy that particularly targets the most severely impacted seep sites. Also, these findings have significant implications for the understanding of how the pattern of the mining contamination may occur in the restored or partially restored coal mining area.

Furthermore, the results of this research support the idea of focusing on bioavailable-based assessment tools for evaluating soil contamination instead of the bulk metals content-based assessment tools that are commonly relied upon. Finally, these data suggest that comprehensive, precise assessment of mine contamination and protection of an ecosystem in the mining-affected area can be achieved through combining data from various assessment tools.

The significance of the findings or contribution:

The findings of the refinement study of metal analysis in soils will be of interest to all researchers who are analysing metals in soil samples and who want to achieve more precise data with fewer interefences and fewer procedural steps. In terms of the site study, the findings highlight the importance of longer term monitoring and assessment of abandoned mine sites and the continued environmental implications of sulphide oxidation and related processes. The findings also show the need for effective remediation of mining sites. This can have importance for mining related laws and policies in terms of contributing to developing best practices required for good environmental stewardship within the mining sector. Understanding the pattern of the mining contamination provided in this study is also useful for environmental protection agencies as a possible scenario of mining contamination in similar situations (i.e. a case study with relevance to other sites). Furthermore, focusing on bioavailable-based assessment tool instead of the bulk metals content-based assessment tool, and combining data from various assessment tools may also be important for environmental protection agencies to ensure successful protection of the ecosystem at mining areas.

Limitations:

Examining the chemistry alone cannot capture important factors potentially in effect such as synergistic, antagonistic and additive effects of multiple metals stressors; assessments that include living organisms are needed for that. Thus, there is a need to test the toxicity of the soils using living organisms, which is addressed in subsequent chapters. It is also important to recognise that this is the first investigation of the site and thus a conceptual model of likely contaminat dispersion patterns was developed based on theories in order to design the sampling approach. A more comprehensive investigation of the hydrology of the site (both surface and subsurface flows) would greatly inform further development of conceptual moels of contaminant movement pathways. Nevertheless, despite its limitations, the study certainly adds to the understanding of the nature and the extent of the contamination in the study site.

Recommendation:

A key policy priority in the mining sector should, therefore, be to plan for the long-term care of mining-affected areas.

4 Evaluation of water quality, aquatic ecotoxicology and sediment contamination

4.1 Introduction

4.1.1 Important water quality parameters

A healthy catchment or riverine environment has good water quality that is fit for multiple uses and which provides suitable habitat for a range of wildlife. According to the Water Framework Directive 60/EC (2000) of the European Union, the quality of the surface water can be studied and assessed through determining both the chemical status and ecological status. The biological quality, hydromorphological quality, and physico-chemical quality are all considered and assessed against standards when determining the ecological status (Munné, 2006).

Hydromorphological elements such as the assessment of depth fluctuations, channel patterns, and dynamics of flow are a part of the ecological status of the aquatic system because they influence such things as the habitats and conditions (niches) available to organisms (Munné, 2006). Chemical quality aspects such as Dissolved Oxygen (DO) are also important as most aquatic organisms need to access some oxygen via the water and so to be of good status the DO should be at an adequate level in order to support the aquatic life. Several factors such as biochemical activity, chemical and physical factors (i.e. turbulence of flow and the temperature of the water) affect the level of the dissolved oxygen in surface water. The fish absorb DO via their gills and many fish require the concentration to be >3 mg/l (Vaquer-Sunyer and Duarte, 2008). The DO is also necessary for the decomposition process of the organic materials (such as the residues of dead organisms) in water. These materials are broken down by other microorganisms (called the decomposers). The decomposers consume the DO during the decomposition process (Dugan, 2012), hence suitable DO is an essential component of a healthy river environment.

Physical water quality elements, as alluded to above, comprise parameters such as temperature, turbidity, suspended solid, colour, odour and taste. Temperature affects some important parameters such as the dissolved oxygen through its influence of the oxygen solubility as well as its impact on the rate of growth of aquatic microorganism and as a consequence affects the level of DO. Temperature also affects the nutrient content through its effect on the growth rate of cyanobacteria (Philippe Quevauviller, 2008).

The chemical characteristics include parameters such as inorganic minerals, organic materials, radionuclides, dissolved gases, state of acidification which is indicated by pH and alkalinity, and the state of nutrients like total phosphorus, soluble reactive phosphorus, total nitrogen, nitrogen-NO_3, nitrogen-NO_2, nitrogen-NH_4 (Carr and Neary, 2008).

Chemical status along with ecological status needs to be assessed to characterise the status of the aquatic systems. The chemical quality can be assessed by comparing with environmental quality standards set down in the Water Framework Directive; the standards can vary in format for different parameters, but typically are set either as maximum allowable concentrations or as maximum annual average concentrations permitted for particular water contaminants.

4.1.2 Importance of sediment quality

The sediment can be defined as relatively heterogeneous materials which include organic, inorganic and detrital particles which in the end will settle on the bed of a water body (Burton, 2018).

It is essential to study the sediment quality if one is to form an overall understanding of the status of the aquatic environment because in many cases, most contaminants bind in different nature to the particles of the sediments. The sediments can be considered either as acute or chronic sources of contaminants to the surrounding water or to other sediment components and thus can directly impact the sedimentary environment and organisms within it. The dangers posed to sedimentary organisms can occur by exposure to the contaminated sediment as well as the contaminated sediment pore water. Furthermore, dangers can occur to wildlife and humans by exposure via these sedimentary organisms (Burton, 2018), e.g. via food chain propagation of contaminants.

Determination of element concentrations in sediments via acid digestion and analysis (i.e. pseudo-total content) and comparison with the ambient element content can indicate the gross contamination level. However, it is widely recognised that the hazardousness of metal contamination to sedimentary biota is not necessarily reflected accurately based on the content of the pseudo-total contaminants alone. The biologically available content of the metals is a more reliable indication of the immediate threat than the pseudo-total content for assessing the sediment toxicity to biota. Therefore, combining the measurements of total element content (i.e. the total potential toxic load of an element) with the bioavailable content or with the outcome of toxicity tests on organisms is considered a more accurate and robust way to assess the sediment quality (Greenstein et al., 2013).

Several methods can be used to provide information about the sediment quality, one way is by assessment of the freshwater community structure. Toxicity tests of freshwater sediments can be a method to assess the toxicity of sediment for fish, plankton, amphibian and macrophyte. Hazards of contaminated sediments can be also assessed using biomarkers (i.e. protein, enzyme or genetic markers) (Martın-Dıaz et al., 2004). Field assessment of contaminants and their impacts is also routinely accomplished using benthic or epibenthic invertebrates surveys (i.e. presence/absence and or abundance/diversity of certain groups with varying sensitivities to contaminants, e.g. (Byrne et al., 2013), and these are considered a successful technique for evaluation of contaminated sediment (Diepens et al., 2014).

4.1.3 Use of ecotoxicology to evaluate water quality

Understanding the toxic effect of unsafe substances on the aquatic environment can be gained from ecological toxicology (ecotoxicology) studies. These studies can be performed in a laboratory and typically include studying the toxic effects that occur with set ranges of concentrations of the substance of interest. Alternatively, rather than creating different concentrations of a test substance and measuring toxic effects that arise, samples of water from a suspected contaminated site can be brought into the laboratory and be tested for their toxicity to organisms. Ecotoxicology studies can also be performed under a variety of ecological conditions in complex field ecosystems which can be distinguished from the more common laboratory studies by being referred to as field-base ecotoxicology studies. Ecotoxicology laboratory tests are useful for several aspects, these are shown in Figure 4-1, (Chapman, 1995).

The tests that measure lethality to aquatic organisms usually within 24 hours are called acute toxicity tests while the chronic aquatic toxicity tests are used to measure sub-lethal and lethal impacts over a partial life cycle or life cycle of organisms so they provide information about the possible long-term impacts of contamination (Posthuma et al., 2001).

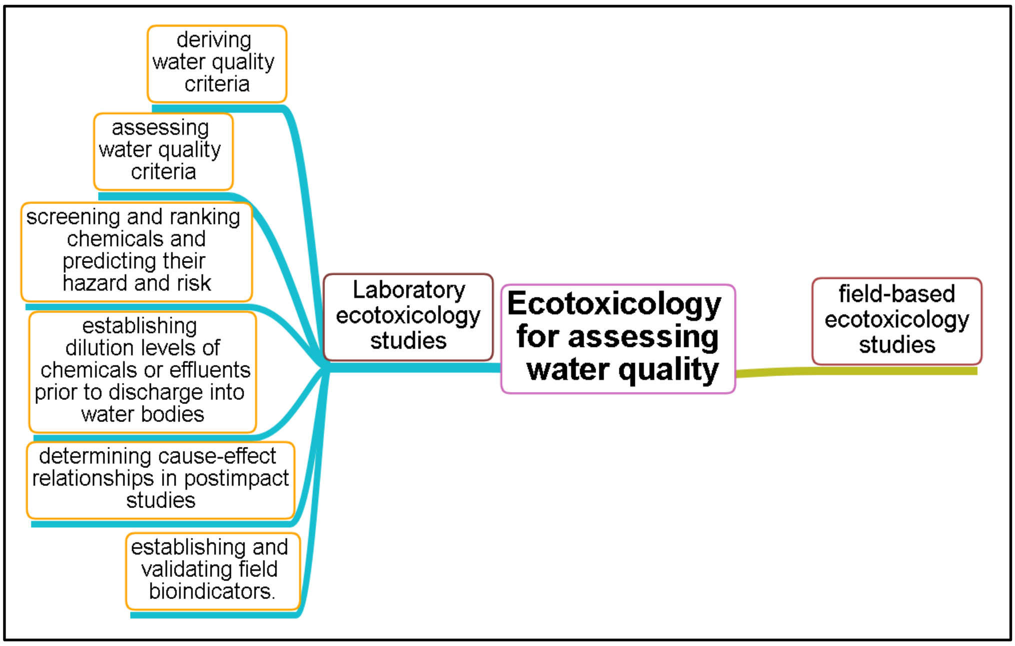

Figure 4-1: Applications of ecotoxicology laboratory tests, (Chapman, 1995).

4.1.4 Aims and objectives

The aims of this study, therefore, were to:

1. Characterise the study site in terms of metal and metalloid contaminants in the water and sediment.
2. Quantify and assess the significance of the level of any contamination.
3. Attempt to identify the positions where the water and sediment become more polluted.
4. Assess aquatic ecotoxicology with *Daphnia* in water.

Therefore, the objectives were set as:

1. Examining water quality along the stream by measuring turbidity, pH, dissolved (0.45 µm filtered) and total element concentrations of the collected water samples.

2. Determining the total (aqua regia extractable) element contents of sediment samples collected from the affected areas (crossing point and downstream) and from a reference point before affected area (upstream).
3. Testing the toxicity of the stream water samples using ecotoxicology method including *Daphnia magna* assays (OECD 202 Daphnia Acute Immobilisation Test for waters).

4.2 Methods

4.2.1 Water sampling and analysis for pH, turbidity and total element concentrations

4.2.1.1 Sampling

Stream water samples were collected to study the water quality of a mine-affected stream in a restored coal mining area in Staffordshire, UK. As shown in the map in Figure 4-2, three sites along the stream were sampled. The first point is from the stream before it comes into proximity or contact with the area of suspected seepage from the coal mining area which was detailed in chapter three (i.e. site 1 is the upstream location); the second point is where the stream appears to meet or be influenced by the seepage point (it is also the crossing point for the stream and so is referred to as crossing point, see Figure 4-3; the third point was approximately 200 m (downstream) from the second point and was selected to assess continued influence of the seepages downstream.

The design of sampling programmes and sampling techniques of water samples were done based on the guidance detailed in ISO 5667-1 (ISO, 2006). The water sampling process was performed in selected locations from specific sites while factors such as the depth and time of sampling were as constant as possible according to ISO 5667-6 (ISO, 2014). The sampling protocol involved multiple samples collected in polypropylene bottles that were pre-rinsed three times with stream water prior to sample collection. The bottles were completely filled to minimise head space and any gas interactions. The samples were stored and transported within 2 hours to the laboratory according to ISO 5667-3 (ISO, 2018). Water samples were collected, as described, on multiple occasions: samples collected in November 2016 were analysed for dissolved content of Cr, Ca, Fe, K, Mg, Mn, P, Zn and for total content of Al, Ca, Cd, Cr, Fe, Mg, Mn, P, Pb. Samples collected in May 2017 were analysed for pH, turbidity, dissolved content of As, Cr, Co, Cu, Ca, Fe, Mg, Mn, Ni, P, Zn and for total content of As, Co, Cr, Cu, Fe, Mg, Mn, Ni, P as well as aquatic ecotoxicology test with *Daphnia*. Samples collected in August 2018 were analysed for pH, DO, EC and total dissolved solids, dissolved content of As, Co, Cu, Fe, K, Mg, Mn, Ni, Zn and total content of Al, Ca, Co, Cu, Fe, K, Mg, Mn, Ni as well as aquatic ecotoxicology test with *Daphnia*.

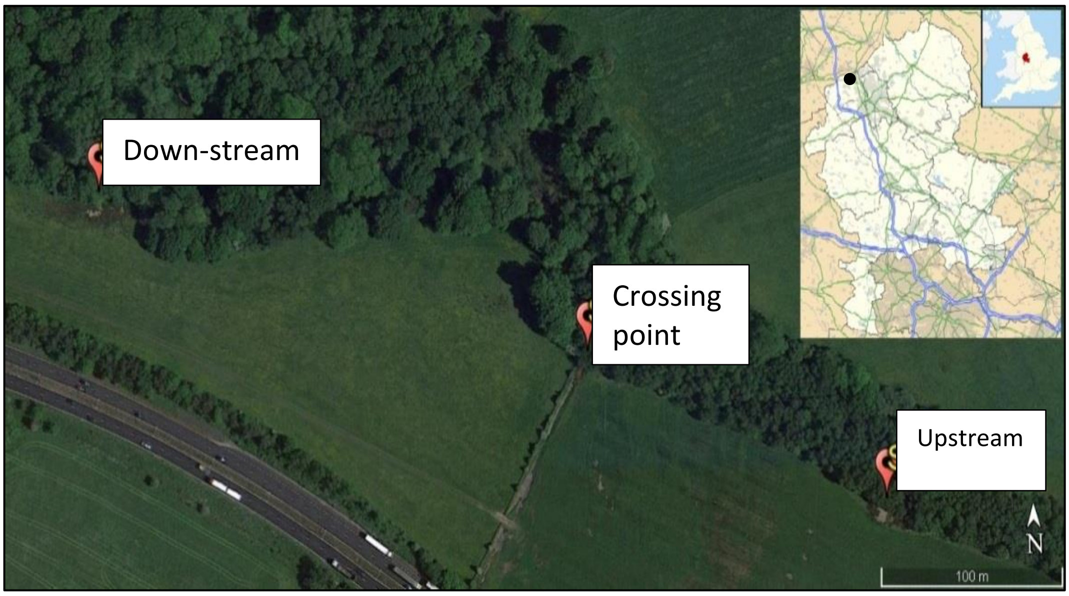

Figure 4-2: Location map shows the position of the tested stream water samples and sediments samples. In the top right corner, the study area have been shown as a black dot within Staffordshire county.

Figure 4-3: View of the stream at the Crossing Point (site 2), near the first seepage point, where the water is visibly more coloured than upstream.

4.2.1.2 Stream water pH

The water pH value is always important to determine because high and low pH values can cause toxicity, directly or indirectly, for aquatic organisms. Water pH values can indicate the corrosive properties of an aquatic environment.

The method is based on measuring the potential variance of an electrochemical cell which involves two half-cells, one of them is a selective electrode for measuring the hydrogen ion activity and a reference electrode is the other (Buck et al., 1985).

Measuring the activity of hydrogen ions in a solution is done according to ISO 10523:2008 (DIN, 2012). Approximately 5 ml of stream water, from the collected samples, were placed in a sample vial and then the pH values were read after stabilization of the values at approximately 20 °C. Precision to two decimal places were recorded for each measurement. The Jenway 3510 pH (probe and meter) used for the analysis was calibrated at the beginning and after five measurements using the pH 4 and 7 buffer solutions at approximately 20 °C.

4.2.1.3 Turbidity

The turbidity of the samples were analysed either in the field or as soon as possible back in the laboratory using a portable Hannah Turbidimeter which had been fully calibrated previously in accordance with ISO 7027-1 (ISO, 2016). The measurements were recorded as nephelometric turbidity units (NTU).

4.2.1.4 Dissolved Oxygen, Electrical Conductivity and Total Dissolved Solids

The dissolved oxygen (DO), electrical conductivity (EC) and total dissolved solids (TDS) were measured on site using a Hanna multi-parameter probe which had been calibrated previously. The DO measurements were recorded as a percentage of total saturation, the EC measurements were recorded in units of µS/cm, and the TDS was recorded in mg/l. Water temperature was also measured using the Hannah multi-probe.

4.2.1.5 Dissolved element and total element concentrations

Collected stream water samples were filtered using 0.45 micron filter syringes then acidified using high purity grade nitric acid (i.e. Primar Plus trace analysis). Samples were then kept in a refrigerator for analysis of dissolved element concentration using ICP-MS Perkin Elmer NexION 300D and Vista MPS ICP-OES (EPA, 1994).

For total elements, a 20 ml aliquot of homogenised (but not filtered) water sample was quantitatively transferred to an acid-cleaned microwave digestion vessel to which 2 ml of concentrated nitric acid and 0.5 ml concentrated hydrochloric acid (both high purity grade) were added in the fume hood. Samples were digested, transferred to plastic tubes and then analysed for element content via ICP-MS (EPA, 1994).

4.2.1.6 Aquatic ecotoxicology assays with *Daphnia*

The scientific classification of *Daphnia magna* is that it belongs to Animalia kingdom, *Arthropod* phylum, *Daphniidae* family, *Daphnia* genus (Gheju et al., 2006) . *Daphnia magna* has a wide distribution covering fresh and brackish waters across much of the Northern Hemisphere. It forms an important part of many food-webs and is also a popular fish food. They have been used extensively in ecotoxicology because they are easy to breed and maintain and are very sensitive to toxic chemicals and other water impurities. Therefore, in this study the water quality at the sites was assessed biologically using *Daphnia magna* as the test species.

The upstream, crossing point, and downstream sites as well as water from a clean Reference point were tested. Five replicates (n=5) were tested from each site, with 10 *Daphnia* placed in a 150 ml container for each replicate according to protocol OECD 222 (OECD, 2016). To determine mortality at the end of the test period, according to the protocol, when the test container is gently agitated the *Daphnia* should be able to swim within 15 seconds otherwise they are considered as having died. Mortality percentage of *Daphnia magna* at crossing point and downstream samples were compared with that at upstream. The validity of the tests was considered confirmed by the 90% survival rate in the control (a clean reference site) samples.

4.2.2 Sampling and analysis of the sediment samples

Stream sediment samples were collected to study the extent of the metal(loid)s dispersion in the study area. The design of sampling programmes and sampling techniques of water sediment samples were done in accordance with ISO 5667-12 (ISO, 2017). Sampling preservation and handling of sediment samples were carried out in accordance with the method ISO 5667-15 (ISO, 2009). The sampling involved scooping out sediment using stainless steel scoops and transferring them to separate zip-lock sealable plastic bags. Samples were taken to the laboratory and were dried, ground finely and homogenised (within each separate sample) by hand. Total element contents of sediment samples were determined via microwave assisted acid digestion (reverse aqua regia, high purity acids) and analysis by ICP-OES and, where necessary, ICP-MS. Certified reference materials were used to test the recovery of the acid digestion process.

4.3 Results and Discussion

4.3.1 Results of the Water samples (pH, turbidity, DO, EC and Total Dissolved Solids, temperature, dissolved elements, total element and aquatic ecotoxicology assays)

4.3.1.1 Water pH

The stream water pH was measured in May 2017 and August 2018. Within each site, the pH was quite consistent across the two time periods, but the crossing Point and downstream locations had lower pH than the upstream location (Figure 4-4). The differences between upstream and the other sites were significant according to an ANOVA, Brown-Forsythe Test of Variances ($p = <0.0001$) for both sampling periods. Although there are differences, at all sites and at all times the pH was within the range of 6 to 9, which is the target range for ‘Good status’ under the WFD (Water Framework Directive (Standards and Classification) Directions (England and Wales) 2015). However, the standards are intended to be applied to year-long (i.e. monthly measurement across a whole year) data sets and so it might still be possible that the pH dips below this good status range at certain times of year (e.g. possibly during low rainfall periods when a larger proportion of the flow comes from below ground upsurges). The pH of the stream is far healthier than the values reported for untreated streams impacted by acid mine drainage; e.g. the Ely Creek watershed (Lee County, VA, USA) had pH < 3 prior to remediation work, after which it had risen to 7.1 (Simon et al., 2006). Taken together, such comparisons indicate that the pH of the stream in the present study, if the measurements reflect average conditions, is not likely to present an environmental problem.

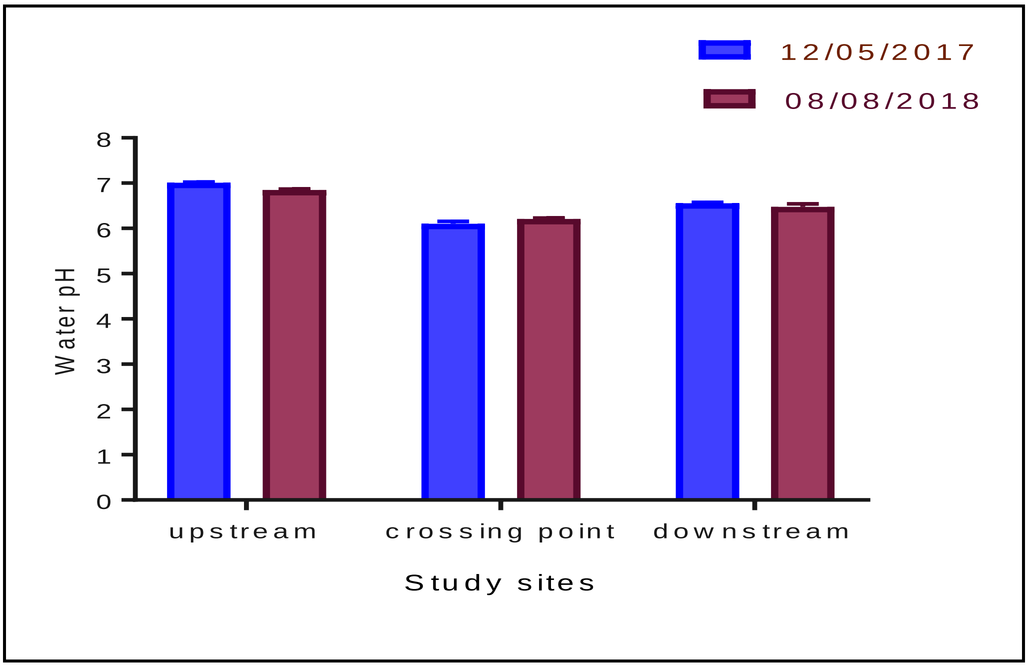

Figure 4-4: Mean of water pH and the error bars represent the standard deviation of the mean.

4.3.1.2 Turbidity

It is clear that the turbidity increased significantly at the crossing point and downstream sites compared with the turbidity value at upstream (Figure 4-5). There is no current turbidity quality standard for UK rivers under the WFD, but there has been a substantial amount of work done elsewhere toward determining indicative thresholds. According to benchmarks based on works by Newcombe and Macdonald (1991) and Newcombe and Jensen (1996), the upstream turbidity value of ~400 NTU may already make the stream water unsuitable for prolonged residence of freshwater fish. For example, when fish are exposed to that level of NTU value for a period of days, it is likely that effects including increased respiration, reduced feeding, and avoidance behaviour will be observed, while if the period of exposure extended to several weeks then more severe effects such as delayed hatching and reduced growth would occur. The turbidity was even higher at the crossing point and downstream (~1500 NTU and ~800 NTU, respectively, Figure 5), and was at levels that could induce death in fish if exposure extended to a period of months (Newcombe and MacDonald, 1991). Therefore, the turbidity at the crossing point and downstream location would make the sites unable to sustain a population of fish.

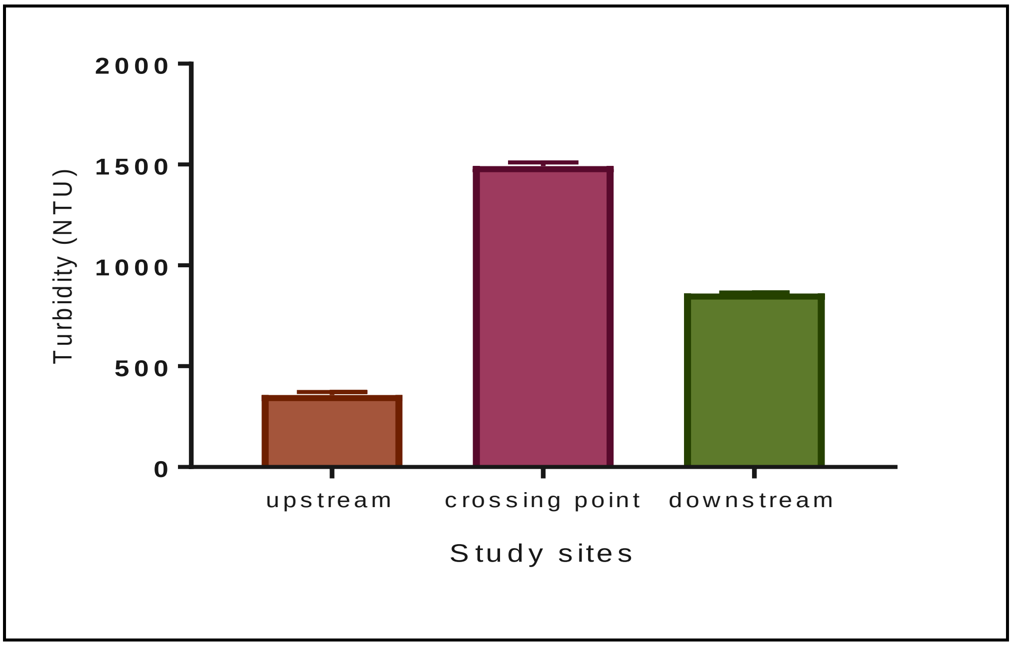

Figure 4-5: Mean of the water turbidity measured on 15-5-2017, the error bars represent the standard deviation of the mean.

4.3.1.3 Dissolved Oxygen

There were significant increases in the mean of the dissolved oxygen percentages at crossing point and downstream sites comparison with that at upstream site (ANOVA, $p< 0.05$) (Figure 4-6). This may be linked to the higher turbulence the water experiences as it flows over a small cascade near the crossing point. The EQS for dissolved oxygen used in England under the WFD is a 10th percentile of monitored values being above 60%

saturation, meaning that the upstream point would not meet the EQS if the values observed here were typical throughout the year. However, the DO of the crossing point and the downstream location would meet the EQS.

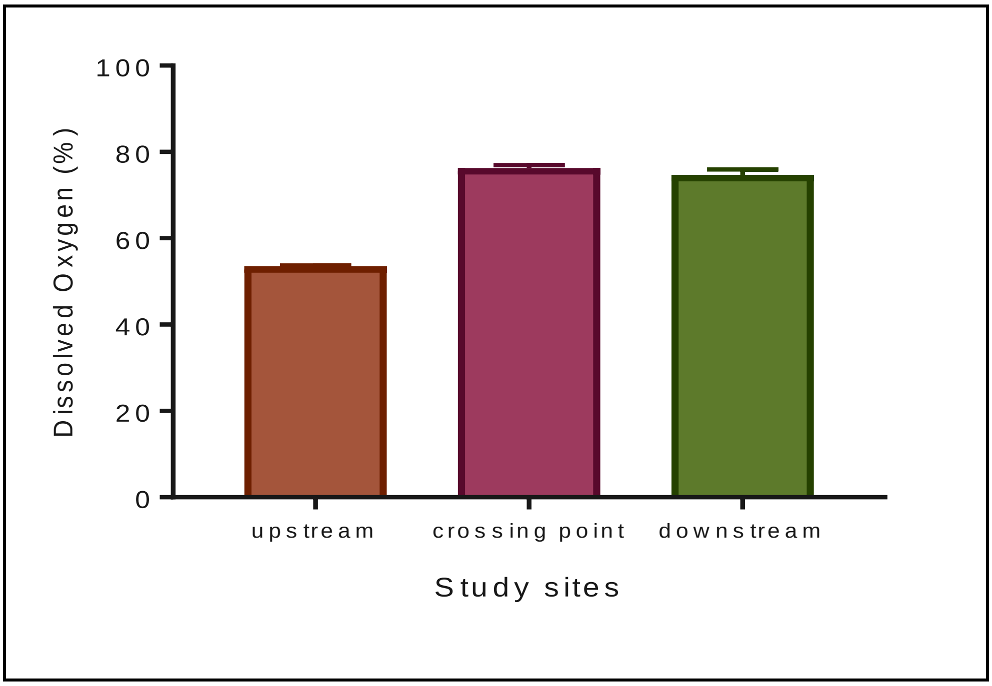

Figure 4-6: Mean of the dissolved oxygen percentage, error bars represent the SD of the mean.

4.3.1.4 Electrical Conductivity, Total Dissolved Solids and Temperature

The electrical conductivity (EC) and total dissolved solid values in the crossing point site were more than double those observed upstream, while the downstream site also had substantially higher values than upstream, see Figure 4-7 and Figure 4-8. This suggests some level of input arising from the seepage points that may have negative impacts on ecology. For example, the US EPA advises that EC values above 500 µs/cm may render the water unsuitable for fish and certain important invertebrates; all three sites of this study exceed this value with the crossing point exceeding it by more than three-fold and the downstream site by almost three-fold. The temperature (Figure 4-9) was fairly uniform and unremarkable across sites at ~13°C and so was considered not to pose any problem.

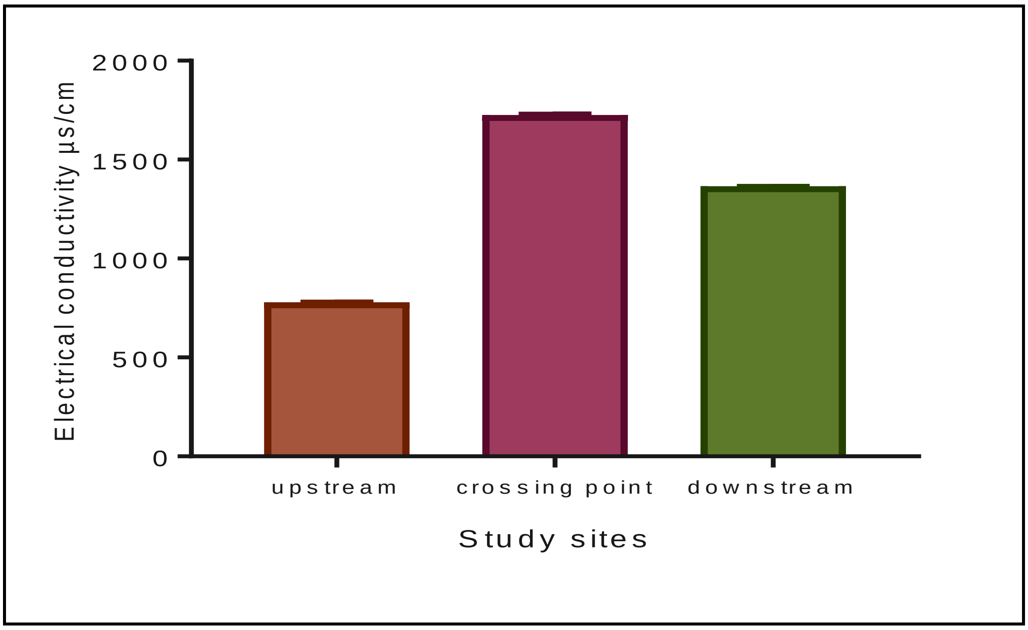

Figure 4-7: Mean of electrical conductivity and error bars represent the standard deviation of the mean.

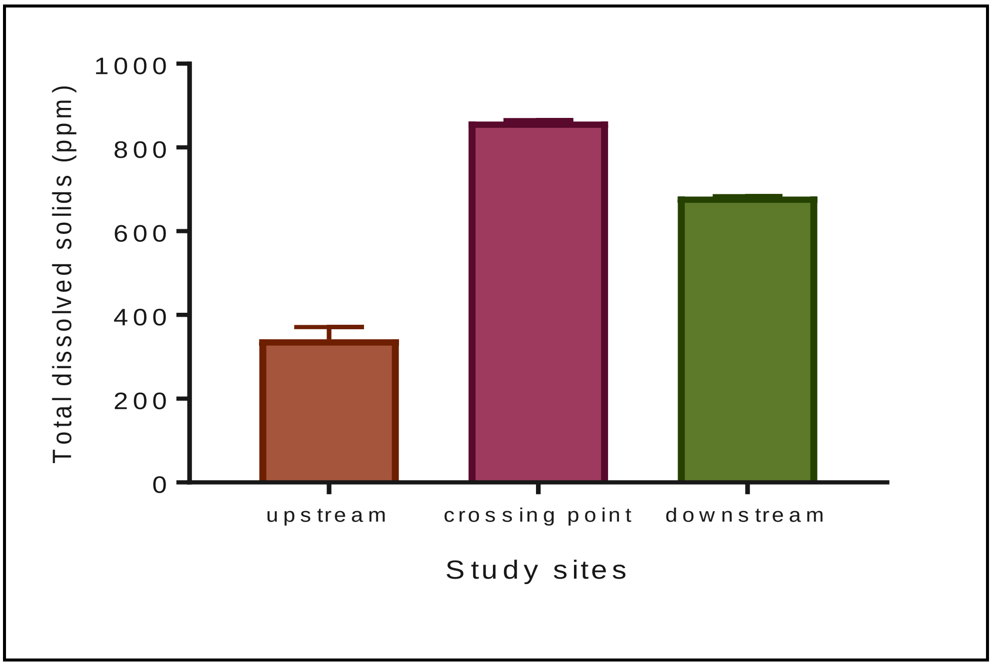

Figure 4-8: Mean of total dissolved solid and error bars represent the standard deviation of the mean.

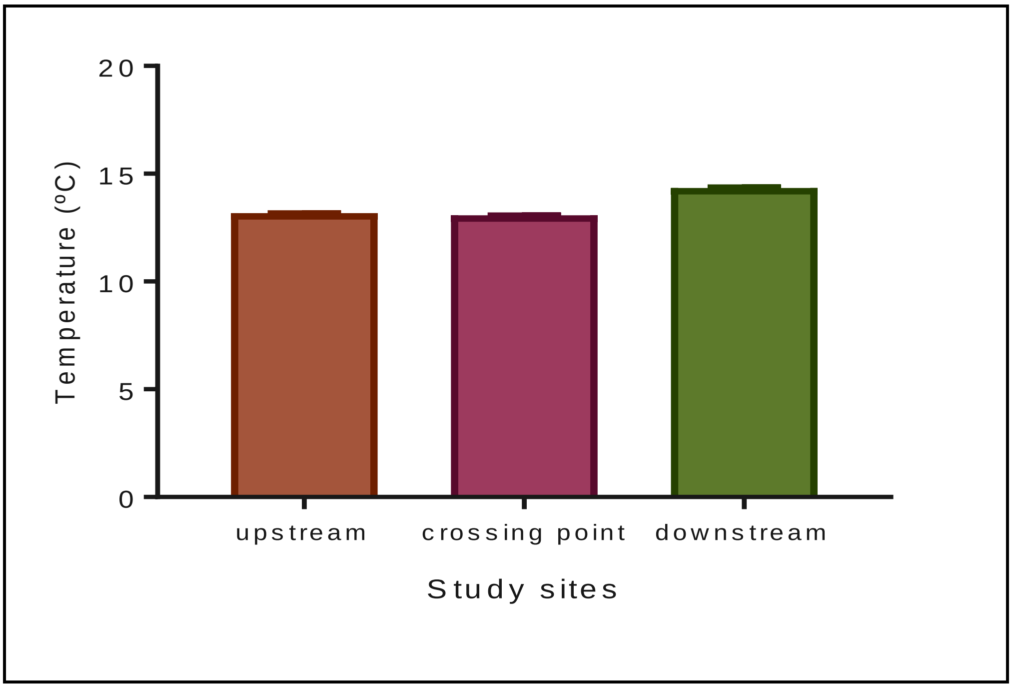

Figure 4-9: Mean temperature of the stream water in the study sites (°C) and error bars represent standard deviation of the mean.

4.3.1.5 Dissolved elements concentrations

Water samples were collected three times during the study for element analysis. Important elements are presented and discussed individually in turn below, but the overall general finding was that the concentrations of dissolved Zn, Fe, Mn, Mg and Ca at the crossing point and downstream sites were significantly higher than that at the upstream site, indicating some level of input along the stream and likely coming from the seepage points. Contrastingly, the concentrations of dissolved Cr and P at the crossing point and downstream sites were significantly less than that in the upstream site, possibly indicating sorption to or precipitation into sediments.

For As, at all locations and on all occasions it was measured, the concentration values were below 1.5 µg/l, and were mostly < 1.0 µg/l (Figure 4-10). This is in line with typical background concentrations reported for England (10th percentile values for various rivers ranged 0.50 – 2.00 µg/l (Peters et al., 2012). Environmental quality standard (EQS) for As under the WFD, moreover, is set as a dissolved (filtration through a 0.45 µm filter) concentration of 50 µg/l (WFD-UK, 2015). This EQS is set as the mean of long term average of monitoring and so, if the values recorded in this study are representative, then the EQS would be comfortably met at the study site. Therefore, because all the As concentration values observed are substantially below the As EQS value, adverse effects attributable to dissolved As are not expected to arise at the study site. The variance of As content with different time of sampling (Figure 4-10) might be related to something simple such as dilution by rainfall.

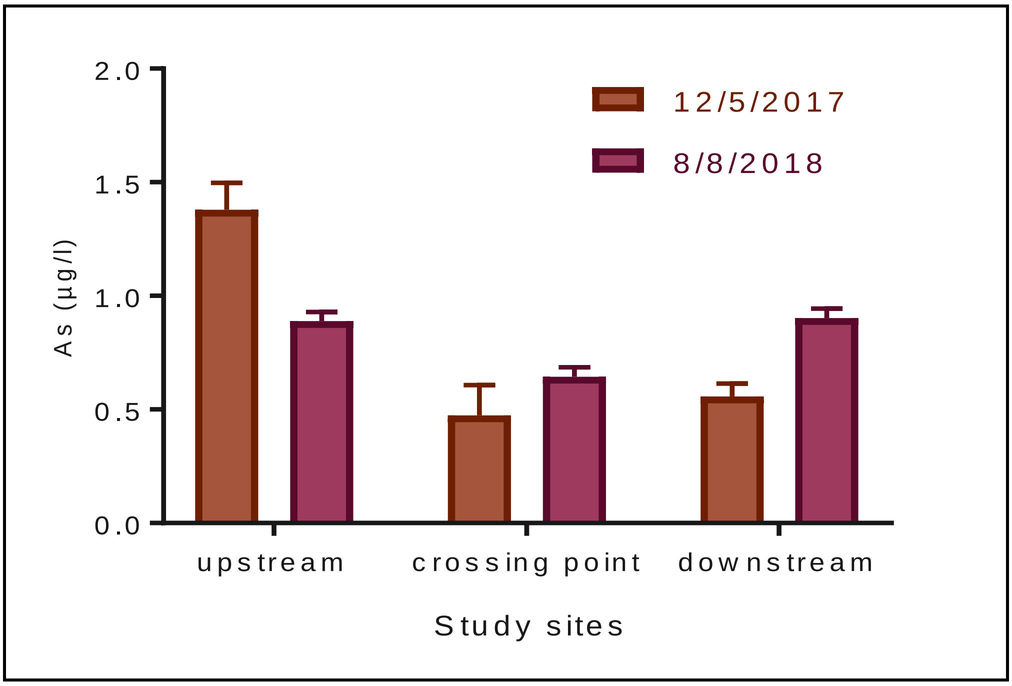

Figure 4-10: Mean of the As dissolved concentration in stream water collected from study sites, error bars represent SD of the mean. Samples were collected in 12/5/2017 and 8/8/2018.

For Cr (Figure 4-11), all concentrations measured were below 0.5 µg/l with a number of samples having been below the limit of detection. Water Framework Directive standards in England and Wales (2015) establish specific EQS values that are set as a mean (long term average of monitoring) for each common valence state of Cr. The EQS 4.7 µg/l value is specific for the trivalent state of Cr and the EQS 3.4 µg/l is specific for the hexavalent state of Cr. The species (oxidation state) specific values recognise the increased toxicity of hexavalent Cr compared with trivalent Cr. Here, the Cr concentrations could not be speciated and so represent total dissolved Cr. However, because both EQS values are far greater than the total dissolved Cr concentrations determined for the collected samples, consequently there is no need for measuring the trivalent and hexavalent Cr content individually because the measured total dissolved concentrations easily meet both EQS values. Although having little ecological importance, it was noted that, for samples collected on 2016, the dissolved Cr concentrations at crossing point and downstream sites were less than that at upstream site while for samples collected on 2017 there was detected amounts of Cr only for the downstream site Figure 4-11.

On both occasions when water was analysed for cobalt (Co), the concentrations of the dissolved Co at the crossing point and downstream sites were greater than that at upstream site (AOVA, Brown-Forsythe test, P value<0.0001; Figure 4-12). The crossing point had concentrations above 15 µg/l, whereas the upstream site had values of ~7 µg/l and ~2 µg/l on the two sampling times respectively. There is no EQS established for Co under current WFD monitoring and classification, however, guideline values are established in Canada and can be used for comparison. The Canadian Federal Water Quality Guideline (FWQG) for Co is set to a value dependent on water hardness, ranging from 0.78 µg/l (for hardness at 52 mg/l) to 1.8 µg/l (for hardness at 396 mg/l), and was established following a review of ecotoxicology data (Canada Department of the Environment, 2017). Using this Canadian FWQG as a point of reference, the data for the stream studied here indicates that there is a risk of Co toxicity to aquatic biota, particularly at the crossing point where the concentration was ~10 times above the guideline value.

Site-specific water predicted no effect concentration (PNECs) can be generated for Cu, Zn, Ni and Mn using a spreadsheet tool sometimes referred to as the Metal Bioavailability Assessment Tool (M-BAT) that has recently been developed by United Kingdom Technical Advisory Group (UKTAG) and key measured or estimated site-specific water properties of pH, Ca and dissolved organic matter (DOC) (WFD-UKTAG, 2014). The spreadsheet calculator can then be used to calculate the site-specific PNEC Dissolved (µg/l) of Zn, Cu, Ni and Mn (µg/l) and bioavailable concentration (µg/l) of those elements as well as the Risk Characterisation Ratio (RCR, i.e. measured concentration/PNEC) for those metals in relation to the test water samples. It was not possible to measure DOC in this study so the default assumed DOC value for the English Midlands (Trent) of 4.2 mg/l, as recommended by the Water Framework Directive-United Kingdom Technical Advisory Group (2012) was used. The water pH was not measured for samples collected in 2016, alternatively, water pH values in 2017 were used (this is more appropriate to avoid overestimated of toxicity, since pH values in 2017 were higher than pH in 2018) for the prediction of site-specific PNEC Dissolved Cu, Mn, Zn and Ni in 2016. Ca content was not measured for samples collected in 2017, so the values for Ca content in 2018 were used for prediction of site-specific PNEC Dissolved Cu, Mn, Zn and Ni 2017.

The Cu concentration was always < 3 µg/l (Figure 4-13). The EQS for Cu under the WFD is set for 1 µg/l ‘bioavailable Cu’ and so the site specific PNEC value derived by the tool is calculated with consideration of bioavailability (i.e. the M-BAT calculates a dissolved Cu concentration that would result in a ‘bioavailable Cu’ concentration at that site that is equivalent to the 1 µg/l 'bioavailable Cu' EQS). The site specific PNECs for Cu, serving as EQS for samples collected in 2016, 2017 and 2018, were calculated and are shown in Table 4-1. The results indicate that the levels of Cu observed at the sites are unlikely to pose any ecological threat to water species.

Table 4-1: Site-specific Cu PNEC (µg/l), as dissolved Cu, mean ± SD for each of sites samples collected in 2016, 2017 and 2018

Year / Site	2016	2017	2018
Upstream	17.28±0.12	17.38±0.1	15.87±0.04
Crossing point	5.17±0.31	5.17±0.31	6.17±0.17
Downstream	11.32±0.24	11.32±0.24	9.58±0.39

As show in Figure 4-14, Figure 4-16 and Figure 4-17, there were significant increases of Ca, Mg and K dissolved concentrations in crossing point and downstream sites compared with Ca, Mg and K dissolved concentrations in upstream.

On the three occasions that water samples were measured for dissolved Fe, the samples from the upstream location had very low or undetectable Fe (Figure 4-15). At the crossing point, however, this rose to between 70 and 150 mg/l indicating inputs of Fe at or near this location, most likely from around the seep point following mine drainage processes described in earlier chapters. The downstream site had values below 50 mg/l, suggesting that precipitation had removed some of the Fe from the dissolved phase by the time the water reached that point. Environmental quality standards (EQS) for Fe are somewhat confused, with no EQS for Fe appearing in the current WFD Directions for England and Wales (WFD-UK, 2015) but with a value of 1.0 mg/l dissolved Fe listed in the Water Framework Directive (Standards and Classification) Directions (England and Wales) (2015).

An EQS of 1.0 mg/l dissolved Fe is also listed in the current Scottish equivalent directions (SEPA, 2019). On top of this, the WFD UK TAG also proposed an EQS of 0.016 mg/l dissolved Fe (Maycock et al., 2007). Clearly, the dissolved Fe at the crossing point was far higher (more than 100 times) than the old EQS and was many orders of magnitude above the UK TAG's proposed lower EQS. This would indicate that there is a potential risk to aquatic organisms posed by the concentrations of Fe at the crossing point and, to a lesser degree, at the downstream site, see Figure 4-15.

There were increasing Mn dissolved concentrations at crossing point and downstream sites compared with that at upstream site (i.e. ≥4 mg/l compared with ~2 mg/l; Figure 4-18). Site specific PNEC values calculated with the M-BAT for Mn, for use as EQS for samples collected in 2016, 2017 and 2018, were predicted, see Table 4-2. The RCR values mostly were greater than 1 for samples collected in 2016, 2017 and 2018, and this would suggest that the Mn concentration in the water of the upstream, crossing point and downstream locations could potentially pose a threat of Mn toxicity to aquatic species, Table 4-3.

Table 4-2: Site-specific Mn PNEC (µg/l), as dissolved Mn, mean ± SD for each of sites samples collected in 2016, 2017 and 2018

Year Site	2016	2017	2018
Upstream	1499.09±32.41	1499.09±32.4	1976.04±13.52
Crossing point	3459.26±301.95	4663.3±23.96	4665.29±23.97
Downstream	3539.82±47.35	3603±106.79	4173.22±27.56

Table 4-3: Mean ±SD of Risk Characterisation Ratio RCR for Mn samples collected in 2016, 2017 and 2018.

Year / Site	2016	2017	2018
Upstream	1.69±0.05	1.85±0.10	1.10±0.01
Crossing point	1.39±0.29	1.05±0.03	1.83±0.01
Downstream	1.08±0.23	0.93±0.03	1.40±0.01

Ni dissolved concentrations at crossing point and downstream sites were increased compared with that at upstream site (Figure 4-19). Site specific PNEC values for Ni calculated with the M-BAT for use as EQS for samples collected in 2016, 2017 and 2018 were predicted, see Table 4-4. The RCR values at crossing point were greater than 1 for samples collected in 2017 and 2018, and this would suggest that the Ni concentration in the water of the crossing point location is a potential threat of causing Ni toxicity to aquatic species, see Table 4-5.

Table 4-4: Site-specific Ni PNEC (µg/l), as dissolved Ni, mean ± SD for each of sites samples collected in 2016, 2017 and 2018

Year / Site	2016	2017	2018
Upstream	20.09±0.31	19.81±0.12	21.38±0.04
crossing point	32.53±0	32.53±0	32.53±0
Downstream	25.01±0.19	25.01±0.19	32.53±0

Table 4-5: Mean ±SD of Risk Characterisation Ratio RCR for Ni samples collected in 2016, 2017 and 2018. N.R refers to not reported because the dissolved Ni in samples collected in 2016 could not be measured.

Year Site	2016	2017	2018
Upstream	N.R	0.39±0.00	0.18±0.09
Crossing point	N.R	1.27±0.03	1.49±0.02
Downstream	N.R	0.90±0.03	0.83±0.02

Environmental quality standard (EQS) of Zn in waters has been reported to be 10.9 µg/l ‘bioavailable’ Zn plus the ambient background concentration (WFD Standards Directions for England and Wales 2015). What this means in reality, as explained in the UKTAG guidance for WFD (UKTAG 2014) is that the mean (long term average of monitoring) dissolved Zn value is determined and the ambient background concentration is subtracted from that mean before it is used in the RCR assessment using the site specific PNEC. The EQS for Zn includes consideration of the background concentration while the EQS for other metals do not because Zn is considered a special case because it is ubiquitous and is commonly naturally at much higher concentrations than that observed for other metals (UKTAG 2014). According to WFD standards England and Wales (2015), the ambient background Zn concentration for the Stoke-on-Trent area (Humber basin/catchment area) is 2.9 µg/l. Site specific PNEC values for Zn calculated with the M-BAT, to serve as EQS for samples collected in 2016, 2017 and 2018, were predicted, see Table 4-6. Based on the outcomes from the RCR determined by the M-BAT calculations, a potential risk is posed by Zn in the aquatic environment at crossing point and downstream since the RCR values were greater than 1 for samples collected in 2017 and 2018, sees Table 4-7.

Table 4-6: Site-specific Zn PNEC (µg/l), as dissolved Zn, mean ± SD for each of sites samples collected in 2016, 2017 and 2018

Year Site	2016	2017	2018
Upstream	23.34±0.07	23.5±0.04	23.07±0.01
Crossing point	21.31±0.44	21.63±0.11	21.92±0.04
Downstream	22.75±0.08	22.92±0.04	22.61±0.07

Table 4-7: Mean ± SD of Risk Characterisation Ratio RCR for Zn samples collected in 2016, 2017 and 2018. N.R refers to not reported because the dissolved of Zn in Upstream location in 2018 were undetectable.

Year Site	2016	2017	2018
Upstream	0.06±0.07	0.83±0.36	N.R
Crossing point	0.6±0.49	1.4±0.04	2.26±0.03
Downstream	0.34±0.22	1.15±0.2	3.12±0.08

For phosphorus (P), the concentrations were low (<0.1 mg/l) during the December sampling period but were higher (0.20-0.25 mg/l; Figure 4-20) when sampled in May, possibly reflecting a build up towards the summer P peaks commonly observed in UK rivers (e.g. (Bowes et al., 2003). The way that EQS for P in rivers is set under the WFD has been changed in recent years, with a system now in place that determines a river-specific P EQS based on a calculation that factors in river typology and on-site ecology (diatom and macrophyte). This makes it difficult to establish the correct EQS and would require information not available here. However, the previous set of EQS set under WFD and implemented in England had a more straightforward approach for P EQS in rivers, and under that previous regulation the appropriate EQS would be an annual average P concentration of 0.12 mg/l for this type of stream (Ryder and Bennett, 2010). Although only two sampling periods were undertaken, it would appear likely that the P in the river would meet or be close to meeting this annual average EQS and thus P would not be considered as a major problem in the stream at the study site.

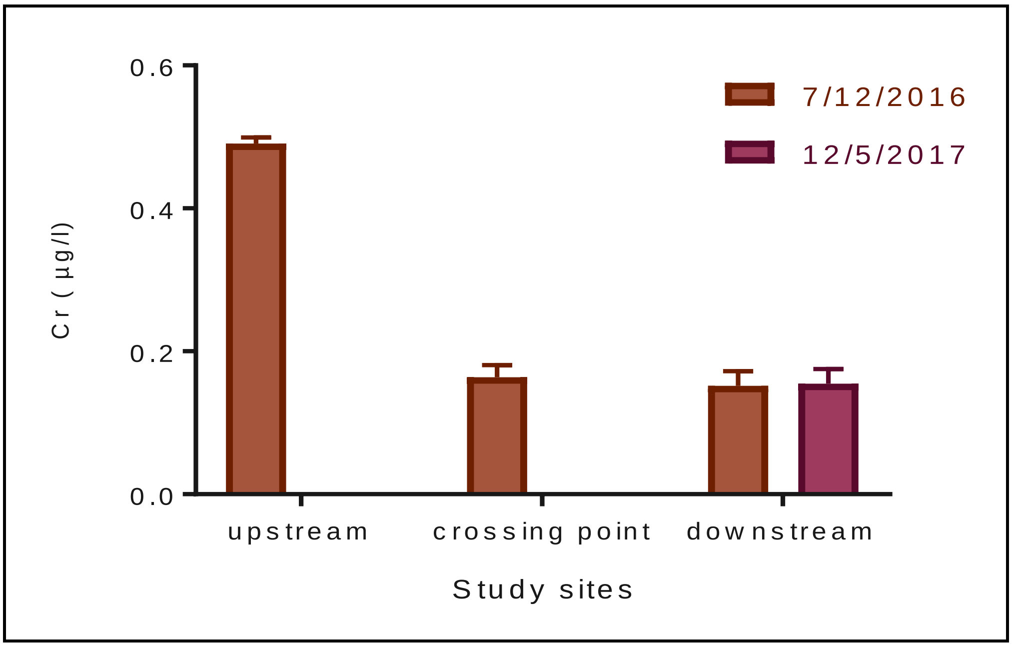

Figure 4-11: Mean of Cr dissolved concentration in stream water collected from study sites, error bars represent SD of mean. Samples were collected in 7/12/2016 and 12/5/2017

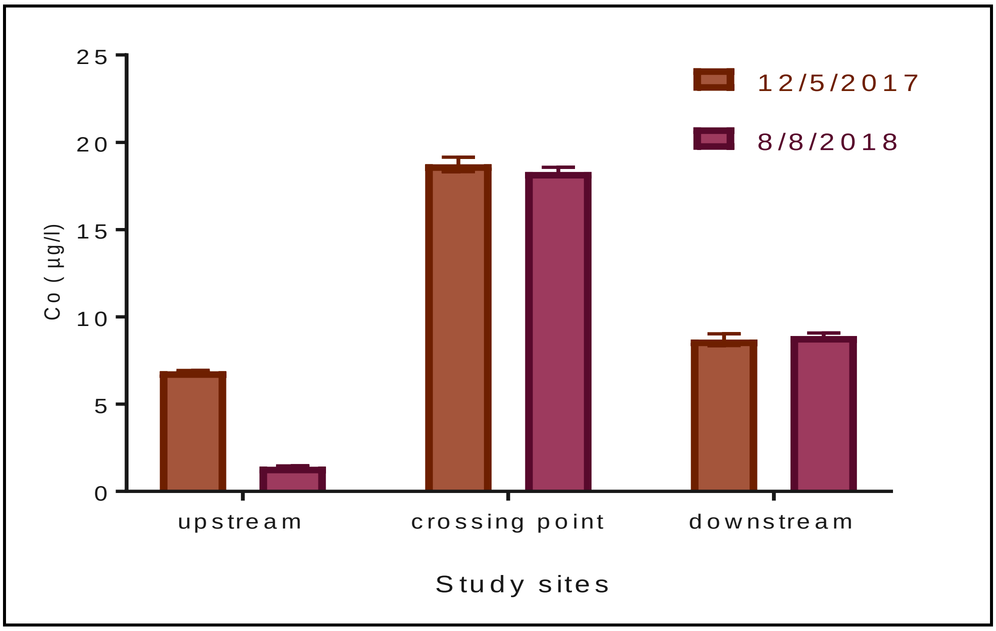

Figure 4-12: Mean of Co dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 12/5/2017 and 8/8/2018

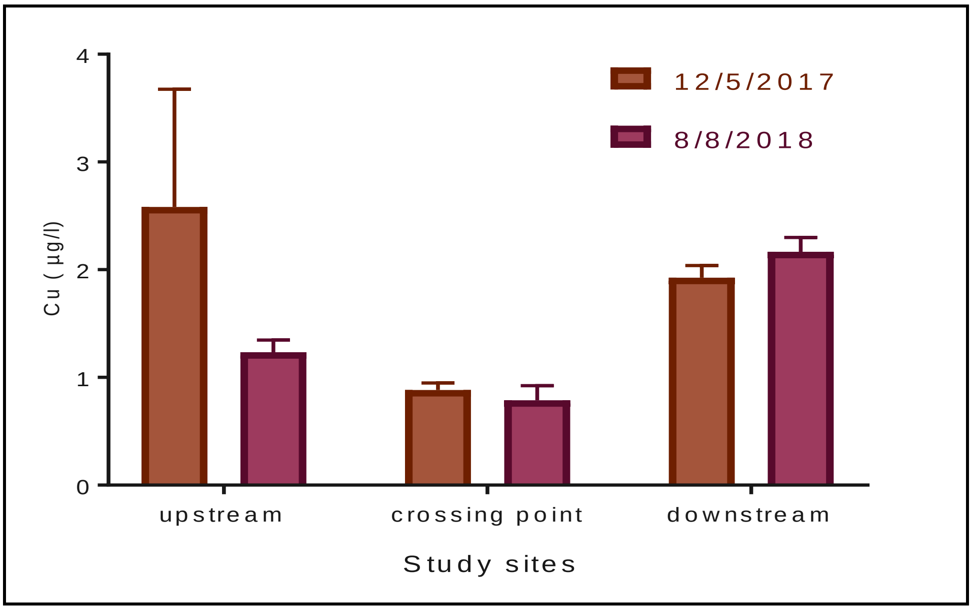

Figure 4-13: Mean of Cu dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 12/5/2017 and 8/8/2018

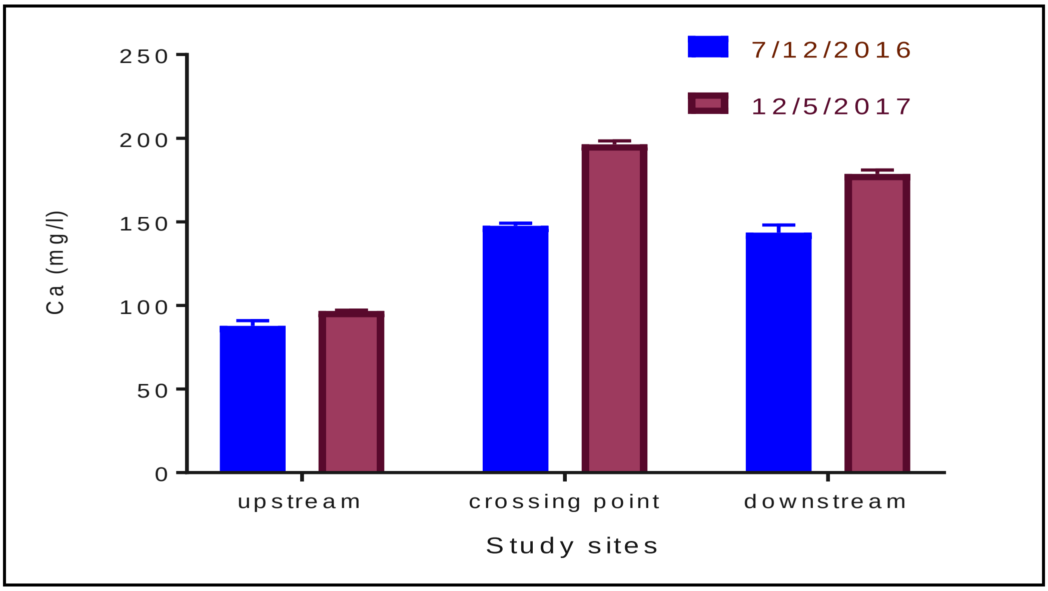

Figure 4-14: Mean of Ca dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 7/12/2016 and 12/5/2017.

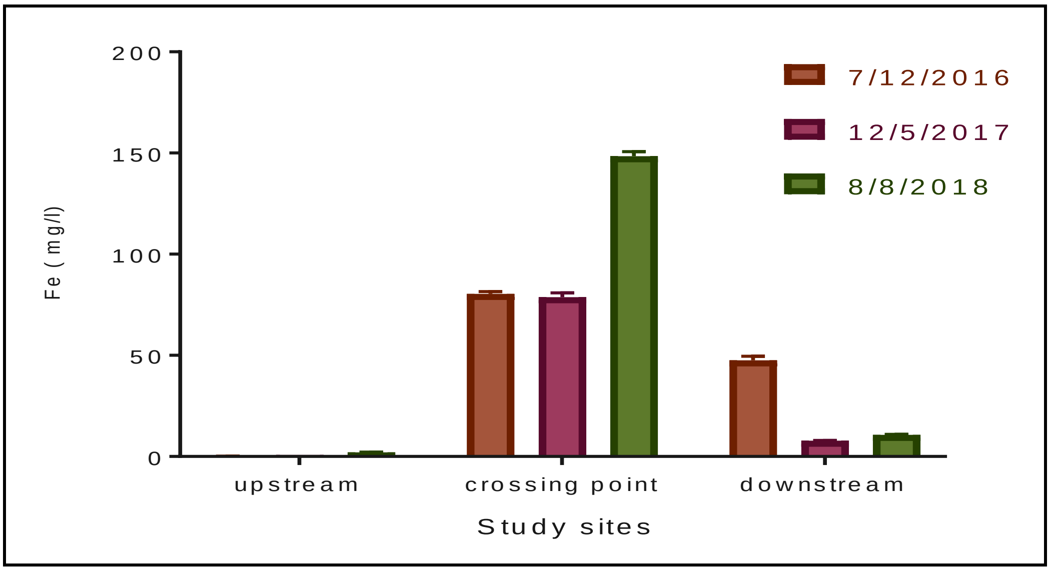

Figure 4-15: Mean of Fe dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 7/12/2016, 12/5/2017 and 8/8/2018.

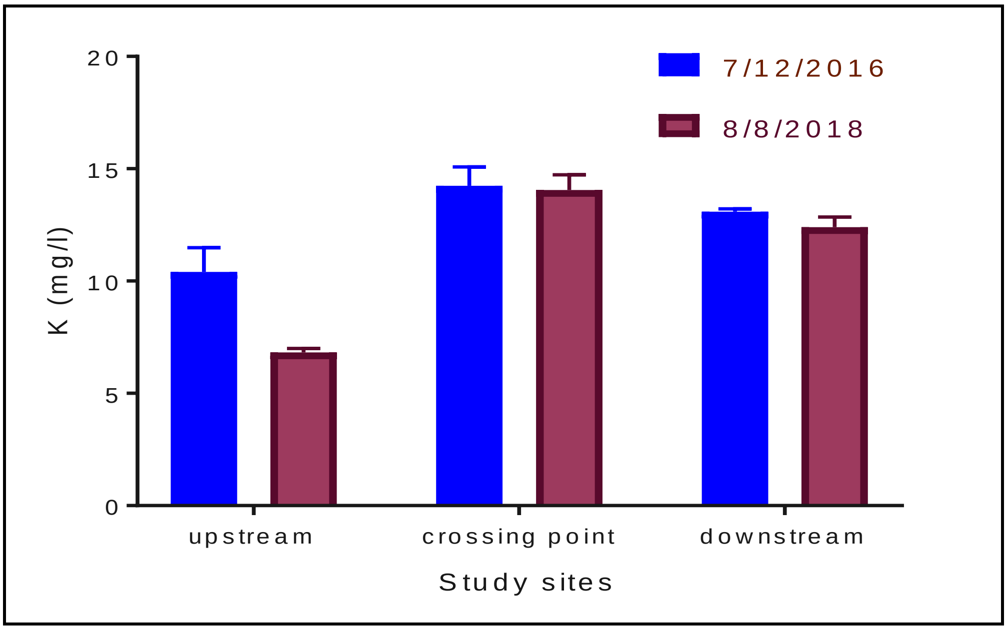

Figure 4-16: Mean of K dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 7/12/2016 and 8/8/2018.

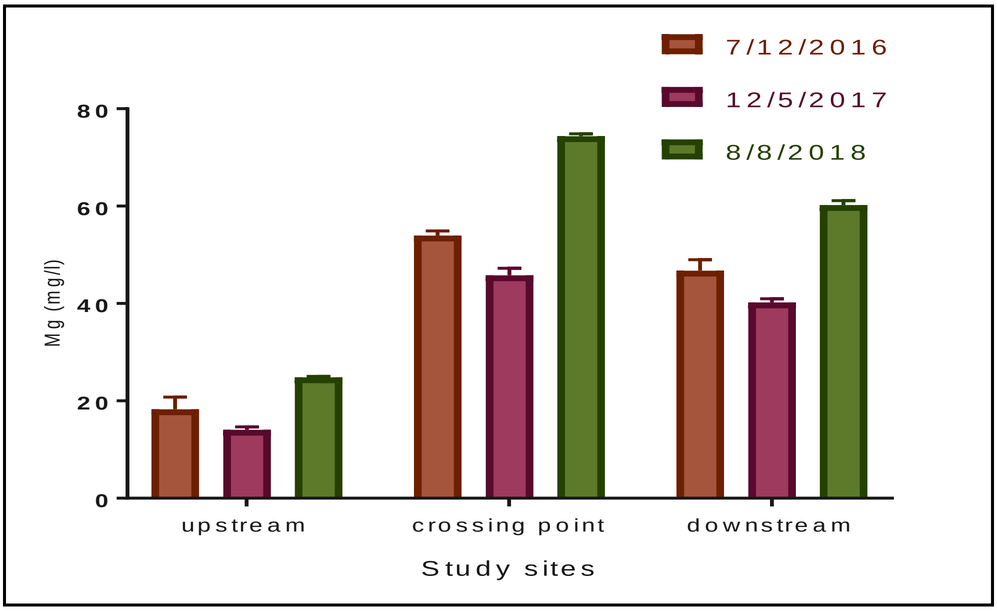

Figure 4-17: Mean of Mg dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 7/12/2016, 12/5/2017 and 8/8/2018.

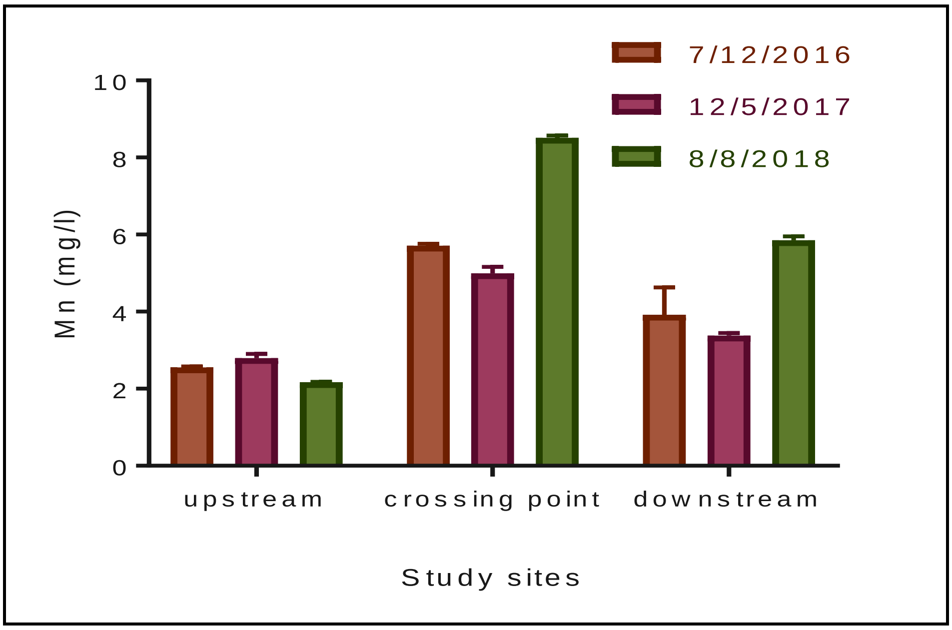

Figure 4-18: Mean of Mn dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 7/12/2016, 12/5/2017 and 8/8/2018.

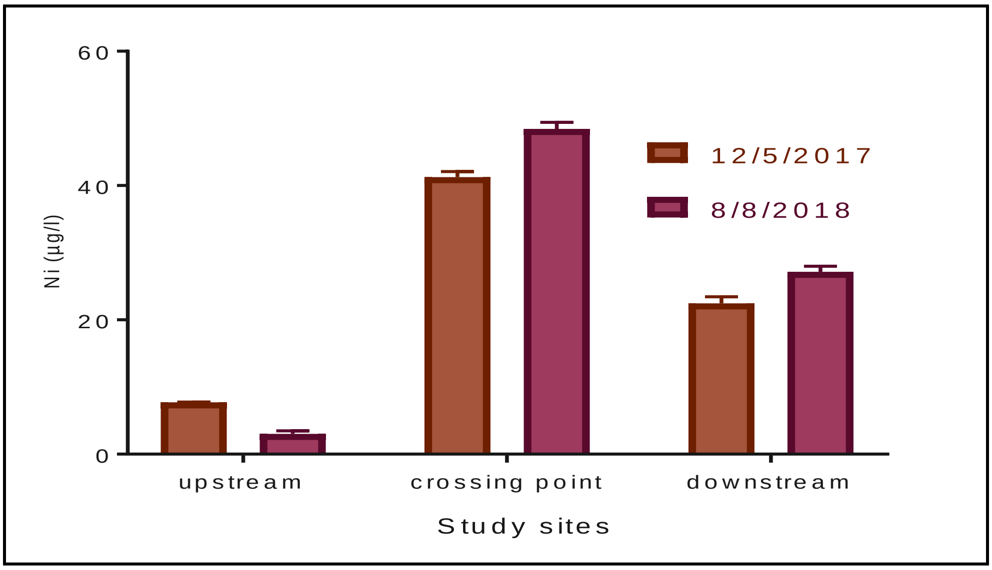

Figure 4-19: Mean of Ni dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 12/5/2017 and 8/8/2018.

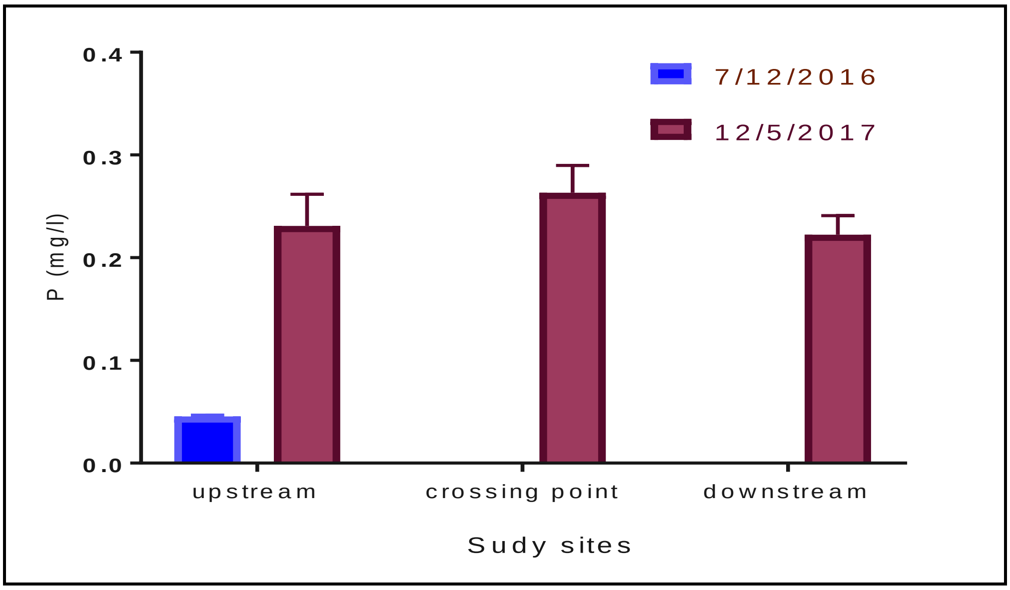

Figure 4-20: Mean of P dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 7/12/2016 and 12/5/2017.

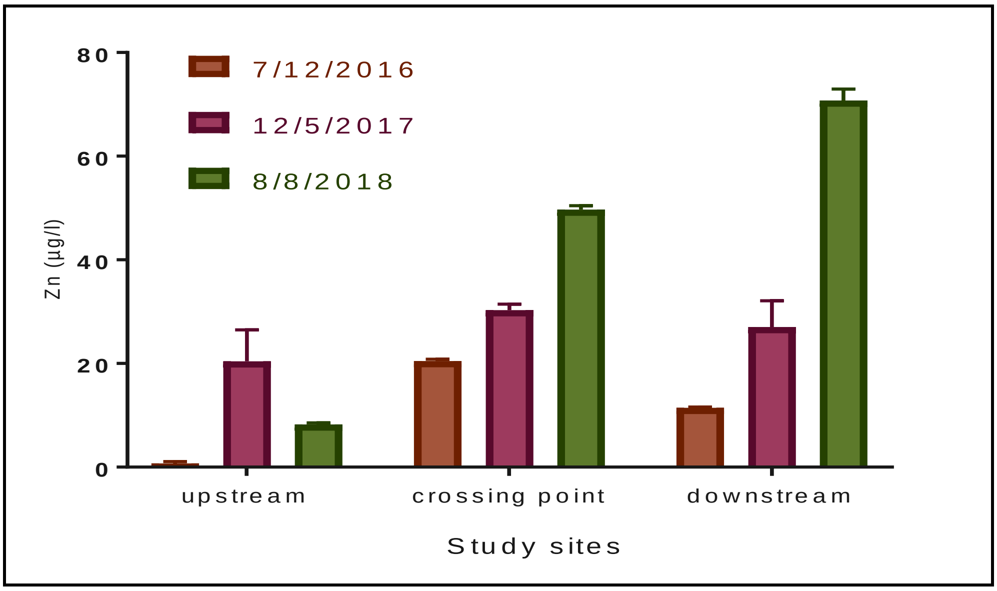

Figure 4-21: Mean of Zn dissolved concentration in stream water collected from study sites; error bars represent SD of mean. Samples were collected in 7/12/2016, 12/5/2017 and 8/8/2018.

4.3.1.6 Total elements in stream water

The total recoverable concentrations of the Al, As, Zn, Ni, Co, Cd, Fe, Mn, K, Mg and Ca at the crossing point and some of them also in downstream sites were significantly higher than that in the upstream site, whereas the total recoverable concentrations of Cr, Cu and P at crossing point and downstream sites were significantly less than that in the upstream site (Figure 4-22 to Figure 4-34). Although EQS for these elements are set for the dissolved (< 0.45 µm) fraction, examining the total amount allows an understanding of the total load and allows an assessment of the distribution between dissolved and particulate phases. This is important for understanding how the elements are distributed through the system. Table 4-8 summarises this by displaying the total concentration and the proportions in the dissolved and particulate phases for each element.

Al was mostly in the particulate phase at all sites and Al proportions were consistent across the sites. This is consistent with the known solubility of Al, which is very limited at the pH observed in the stream water compared with waters of lower pH. Similar findings have been reported in former mining areas elsewhere; e.g. in the Animas River watershed in Colorado, USA, which was an area of intensive mining activity, the streams with pH <4 had the majority of their Al and associated metals in the dissolved phase while in other streams with higher pH (e.g. 6.35 in Mineral Creek) the Al was mostly or entirely in the colloidal and particulate phase (Church et al., 1997).

Proportions of Ni were mostly in the dissolved phase for the samples collected in 2017. Similarly, the proportions of Ca were mostly in the dissolved phase for the samples collected in 2016 but, contrastingly, they were mostly in the particulate phase for the samples collected in 2018. This might be simply related to very low input of Ca from the seep area on that occasion. Proportions of Cr were mostly in the dissolved phase for the samples collected in 2016. Likewise, the proportions of Co were mostly in the dissolved phase for the samples collected in 2017. Contrastingly, the particulate phase was the dominant form in the samples collected in 2017 for Cr and 2018 for Co, see Table 4-8. For Cr, this variability in dominant phase may reflect different speciation and associations with differing levels of turbidity and DOC, as has been shown for Cr in surface and groundwaters of ore processing affected areas such as parts of Glasgow (Farmer et al., 2002). However, it may also just be a quirk of the rather low concentrations observed here at the study site (e.g. typically <2 mg/l and often less than 1 mg/l).

Fe was more strongly associated with the dissolved forms at the crossing point for the samples collected in 2016 and 2017, while the proportions of Fe were more strongly associated with particulate forms at all sites for the samples collected in 2018. The proportions of Fe were more evenly spread between the two phases in downstream site for the samples collected in 2017. Considering the elevated concentrations of Fe observed here, as is typical for mining impacted areas, it was anticipated that the majority of the Fe would be in the particulate phase. This is more common because Fe tends to precipitate out as Fe^{3+} particulates such as oxides and hydroxides when the water has sufficient dissolved oxygen and the pH is above ~4 or 5 (Pulford and Flowers, 2006), which gives a characteristic 'ochre' formation in mining impacted rivers. Nevertheless, at the present study site there was still a substantial amount of particulate Fe that would contribute to the high turbidity at the crossing point site and would have implications for fish in terms of gill blockage, prey obscuration and stomach problems following inadvertent ingestion.

Proportions of Mg and Mn were mostly in the dissolved phase for the samples collected in 2016 and 2017 at all sites but, in contrast, they were mostly in the particulate phase for the samples collected in 2018 at all sites Table 4-8. The change in dominance for Mn might be associated with the shifts in the proportions of particulate Fe observed, as Mn can often become associated with Fe. Other studies in mining impacted catchments have reported a dominance of Mn in the dissolved phase (e.g. Church et al. (1997), Kimball et al. (1995), thus the results of the present study are partially in line with such results.

The particulate forms of Zn in water from upstream were the dominant form in 2016, 2017 and 2018. Proportions of Zn were mostly in the dissolved phase for the samples collected in 2016 at crossing point and downstream sites; contrastingly Zn was mostly in the particulate phase for the samples collected in 2017 at crossing point and downstream sites. Zn was more strongly associated with the particulate forms at the crossing point for the samples collected in 2018; while, Zn was more evenly spread between the two phases in downstream site for the samples collected in 2018, see Table 4-8. A shifting distribution of Zn between the dissolved and particulate phase is a common observation in mining impacted streams, with the shifts often being linked to inputs and within-stream processes. For example, an investigation of the Glengonnar Water river in Scotland (SEPA, 2011) found that fresh inputs from mining sources had high proportions of dissolved Zn but that there was in increase in particulate Zn downstream, possibly because of precipitation or complex formation with river water constituents. The variation observed amongst the sites and sampling periods in the present study may also reflect different proportions of input sources and changing levels of binding agents in the stream at different times.

Arsenic (As) was mostly in the particulate phase (i.e. always > 70%) for the samples collected in 2017 at all sites, but with a clear increase in the particulates proportion beyond the upstream site, i.e. upstream had 74% while crossing point and downstream sites had >94% of As in the particulate phase. The increase might be due to the increased Fe concentrations that are evident at the crossing point site, perhaps with an oxidation of As form being involved. Pb was mainly associated with particulate forms at all sites for the samples collected in 2016. This is commonly the case for Pb because of its strong affiliation with organic matter and tendency to precipitate. Domination of particulate Pb is routinely observed in pristine river environments (e.g. in peat catchments; Graham et al. (2006) as well as in mining contaminated river environments (e.g. SEPA (2011).

P was more evenly spread between the two phases in upstream site for the samples collected in 2016 however; P became more strongly associated with dissolved forms at the crossing point and downstream site for the samples collected in 2017. It is possible that inputs from the agricultural fields adjacent to the crossing point and downstream site contributed to the dissolved P observed. Cu was mostly in the particulate phase for the samples collected in 2017 at upstream and crossing point sites. However, the dissolved concentration of P and Cu in downstream site for the samples collected in 2017 were greater than the respective total concentrations which, logically, cannot be the true situation. This odd result is likely to be related to the majority of the Cu and P at this location having been in the dissolved phase but that the level of error from analytical techniques was not able to discern consistent amounts, especially when the amount of these elements was quite low (e.g. <0.21 mg/l for P; see Table 4-8).

Table 4-8: Total element concentrations and particulate / dissolved phase distribution of water samples collected from different locations (upstream, crossing point and downstream) and sampled on different times (2016, 2017 and 2018).

2016									
Element	Total			% Particulate			% Dissolved		
	upstream	crossing point	Downstream	Upstream	crossing point	downstream	upstream	crossing point	downstream
Al mg/l	0.18	1.09	0.11	100.00	99.46	100.00	0.00	0.54	0.00
Ca mg/l	96.30	158.42	156.07	7.00	6.25	6.26	93.00	93.75	93.74
Cr µg/l	0.56	0.24	0.22	14.03	30.64	23.97	85.97	69.36	76.03
Fe mg/l	4.37	117.42	13.01	99.92	31.00	71.43	0.08	69.00	28.57
Mg mg/l	16.04	39.62	35.08	0.00	0.00	0.00	100.00	100.00	100.00
Mn mg/l	2.11	4.09	2.58	0.00	0.00	0.00	100.00	100.00	100.00
P mg/l	0.08	0.00	0.00	53.81	0.00	0.00	46.19	0.00	0.00
Pb µg/l	0.23	0.07	0.08	100.00	100.00	100.00	0.00	0.00	0.00
Zn µg/l	4.00	3.98	3.38	86.74	0.00	0.00	13.26	100.00	100.00

2017									
Element	Total			% Particulate			% Dissolved		
	upstream	crossing point	downstream	Upstream	crossing point	downstream	upstream	crossing point	downstream
Al mg/l	0.29	2.32	0.11	100.00	100.00	100.00	0.00	0.00	0.00
As µg/l	5.31	8.50	16.65	74.13	94.47	96.61	25.87	5.53	3.39
Co µg/l	10.50	25.66	12.12	34.24	26.98	28.53	65.76	73.02	71.47
Cr µg/l	2.59	1.36	1.45	100.00	100.00	100.00	0.00	0.00	0.00
Cu µg/l	7.39	3.59	2.10	64.55	75.50	0.00	35.45	24.50	120.93
Fe mg/l	8.40	85.61	16.03	90.12	8.04	51.25	9.88	91.96	48.75
Mg mg/l	10.96	34.67	31.22	0.00	0.00	0.00	100.00	100.00	100.00
Mn mg/l	2.31	3.76	2.64	0.00	0.00	0.00	100.00	100.00	100.00
Ni µg/l	12.82	54.66	31.08	39.67	24.52	27.84	60.33	75.48	72.16
P mg/l	1.94	0.25	0.21	87.76	0.00	0.00	12.24	101.92	107.94
Zn µg/l	176.01	179.09	145.88	88.98	82.86	81.36	11.02	17.14	18.64

2018									
Element	Total			% Particulate			% Dissolved		
	upstream 2018	crossing point	downstream	Upstream	crossing point	downstream	upstream	crossing point	downstream
Al mg/l	0.37	3.76	0.24	85.40	98.50	80.23	14.60	1.50	19.77
Ca mg/l	393.36	670.14	657.48	75.47	70.48	72.41	24.53	29.52	27.59
Co µg/l	5.49	63.21	27.17	73.98	71.05	67.30	26.02	28.95	32.70
Fe mg/l	15.69	588.47	42.82	86.60	74.75	74.91	13.40	25.25	25.09
K mg/ l	18.19	43.66	40.96	62.57	68.13	69.50	37.43	31.87	30.50
Mg mg/l	96.09	291.77	249.17	74.10	74.49	75.79	25.90	25.51	24.21
Mn mg/l	8.28	29.56	21.77	73.82	71.12	73.06	26.18	28.88	26.94
Ni µg/l	37.15	148.82	87.11	89.13	67.42	68.90	10.87	32.58	31.10
Zn µg/l	61.19	145.62	149.64	100.00	65.52	52.18	0.00	34.48	47.82

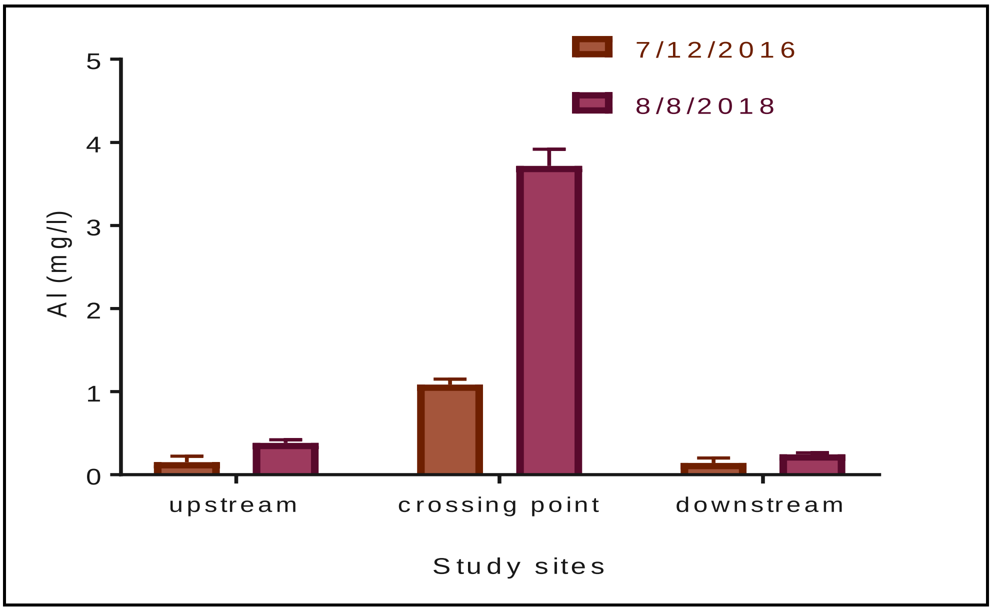

Figure 4-22: Total recoverable metals of Al in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016 and 8/8/2018.

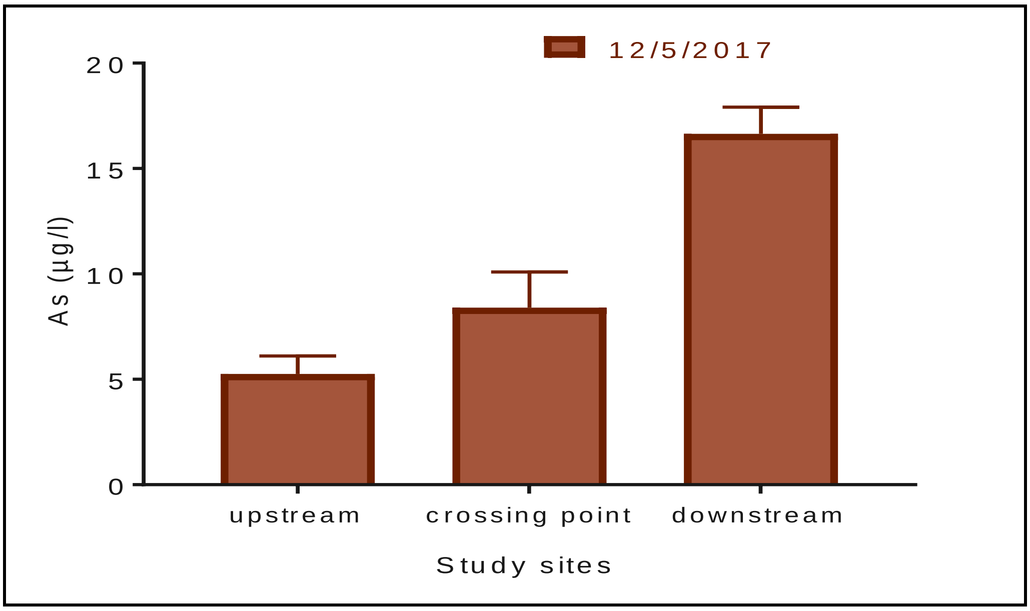

Figure 4-23: Total recoverable metals of As in stream water collected from study sites, mean,error bars represent SD of mean. Samples were collected in 12/5/2017.

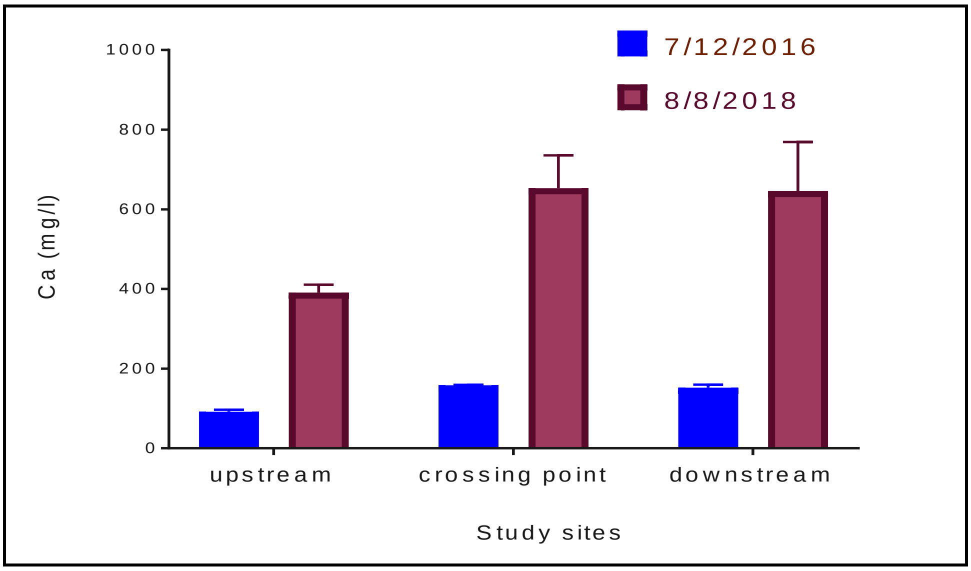

Figure 4-24: Total recoverable metals of Ca in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016 and 8/8/2018.

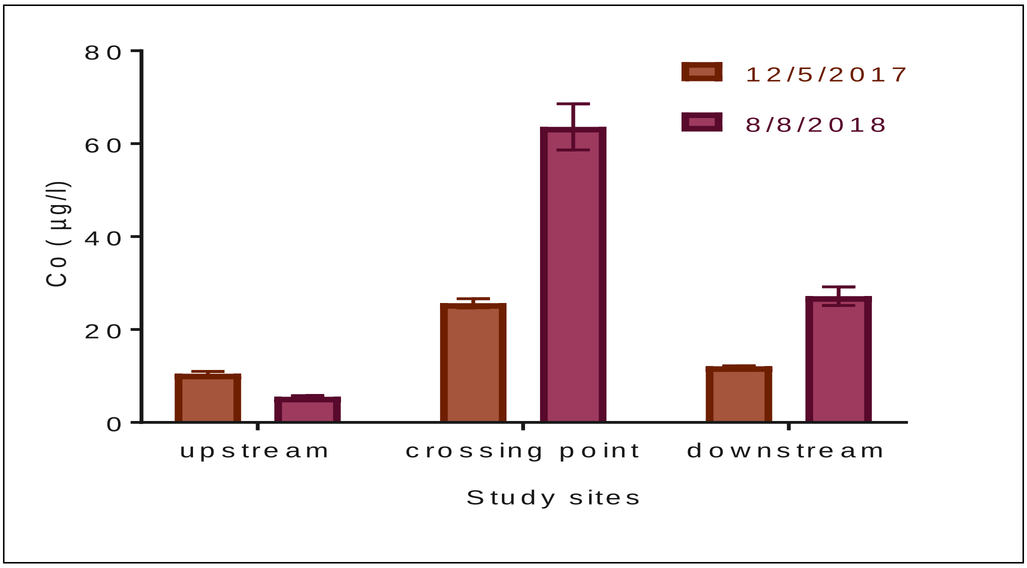

Figure 4-25: Total recoverable metals of Co in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 12/5/2017 and 8/8/2018.

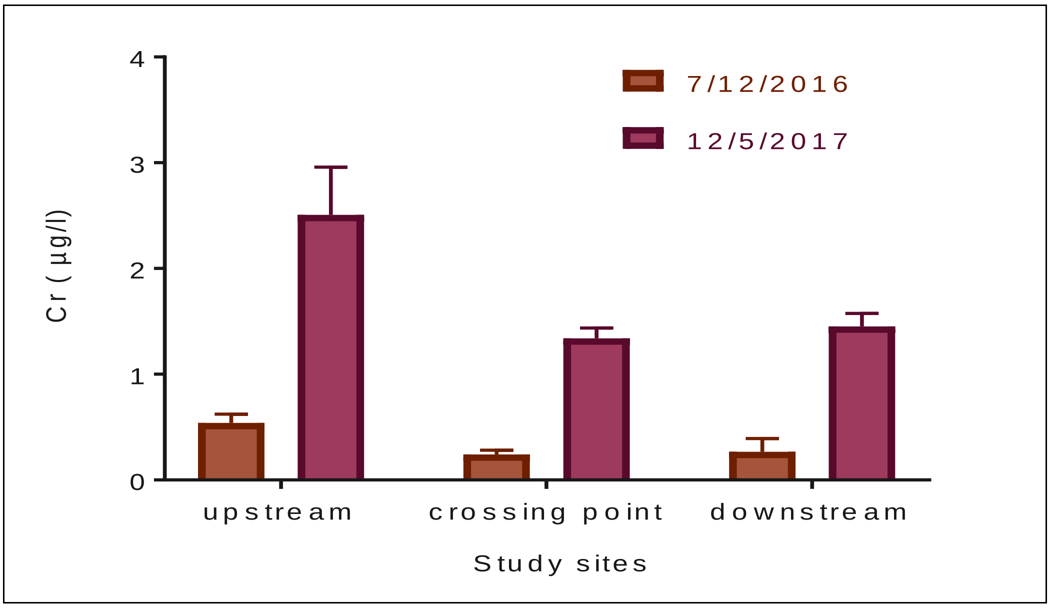

Figure 4-26: Total recoverable metals of Cr in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016 and 12/5/2017.

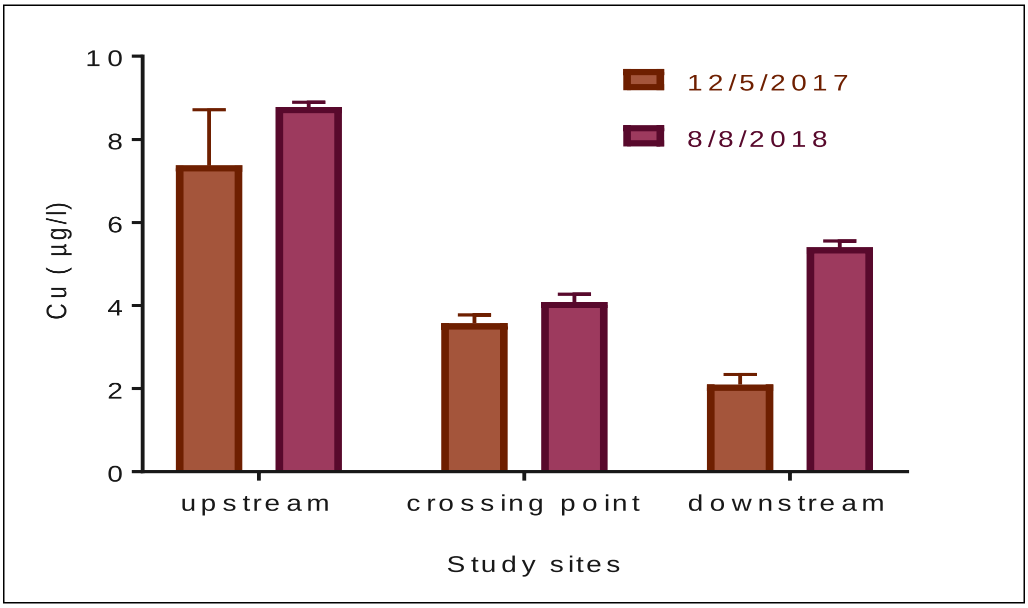

Figure 4-27: Total recoverable metals of Cu in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 12/5/2017 and 8/8/2018.

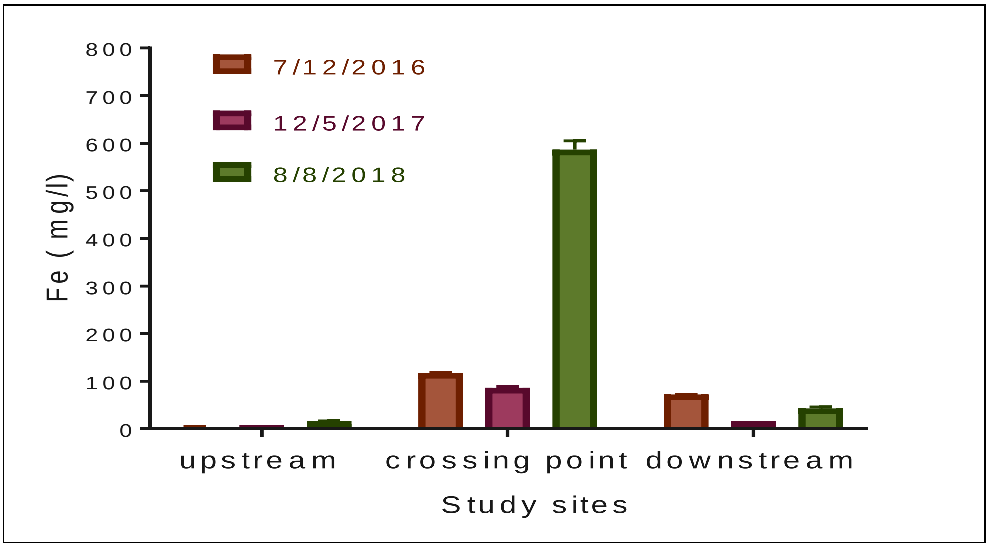

Figure 4-28: Total recoverable metals of Fe in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016, 12/5/2017 and 8/8/2018.

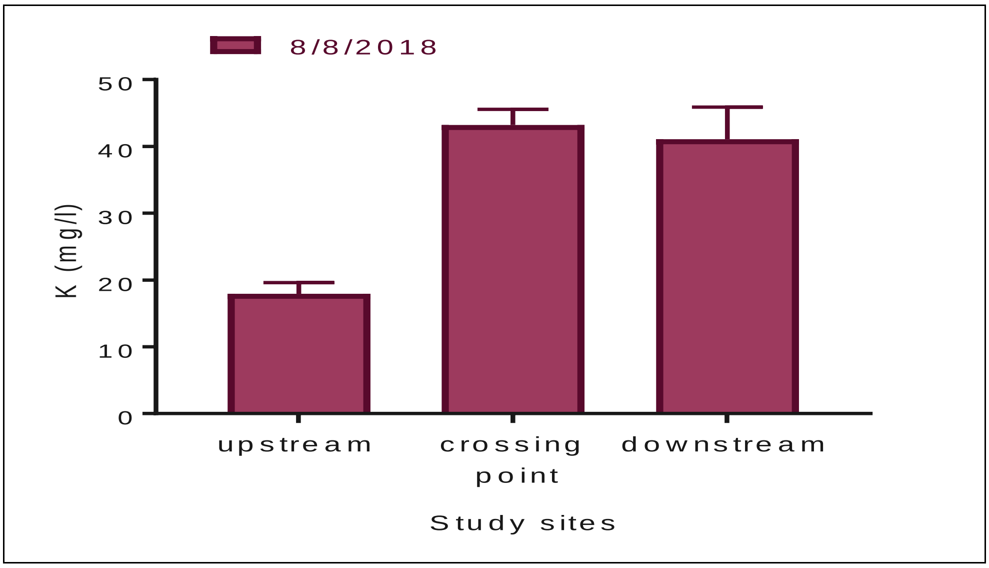

Figure 4-29: Total recoverable metals of K in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 8/8/2018.

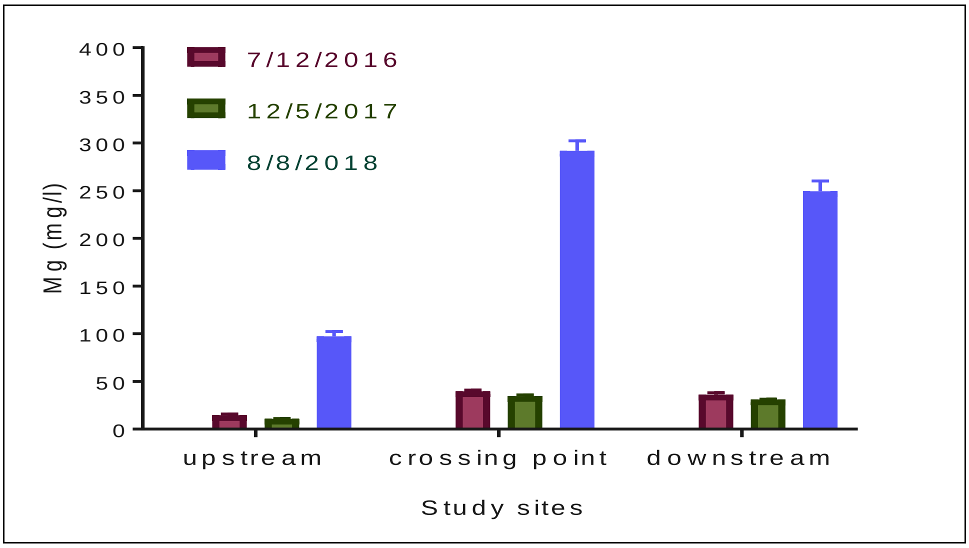

Figure 4-30: Total recoverable metals of Mg in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016, 12/5/2017 and 8/8/2018.

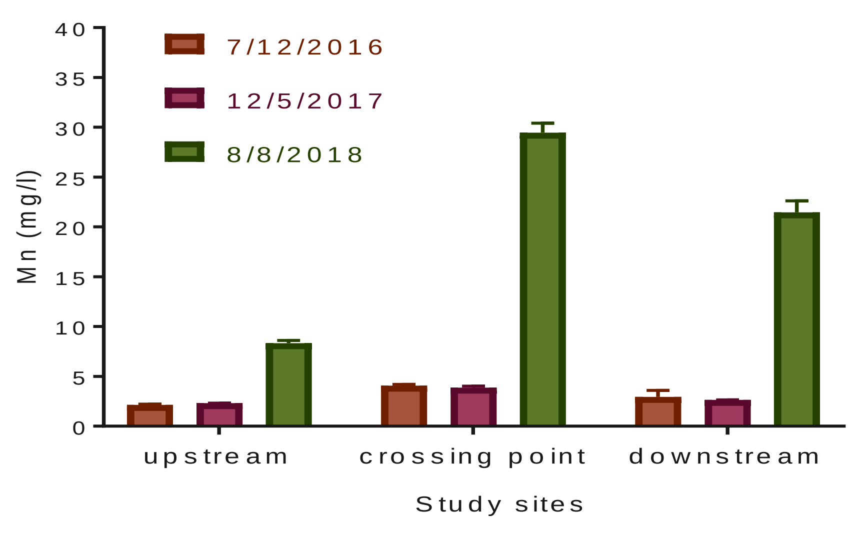

Figure 4-31: Total recoverable metals of Mn in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016, 12/5/2017 and 8/8/2018.

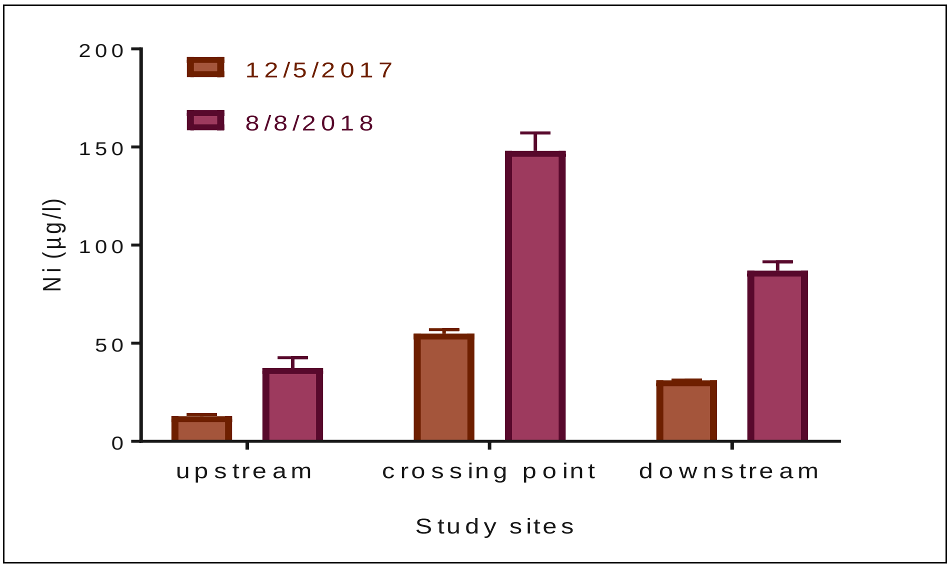

Figure 4-32: Total recoverable metals of Ni in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 12/5/2017 and 8/8/2018.

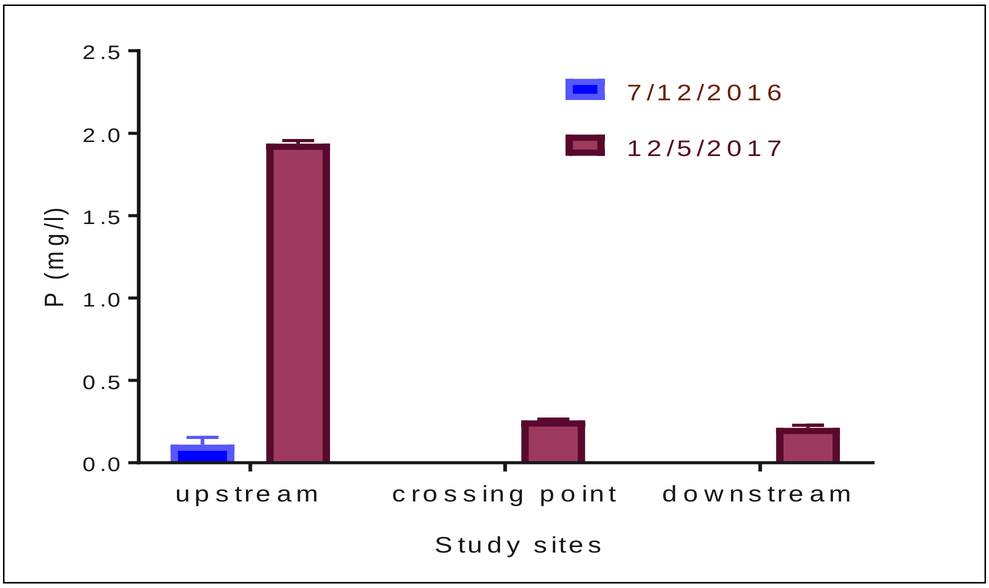

Figure 4-33: Total recoverable metals of P in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016 and 12/5/2017.

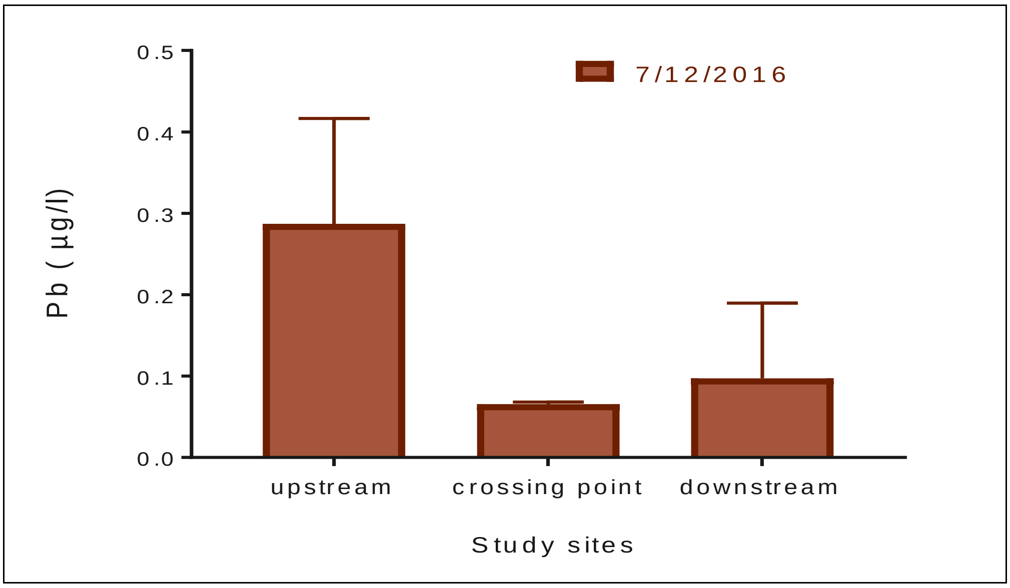

Figure 4-34: Total recoverable metals of Pb in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016.

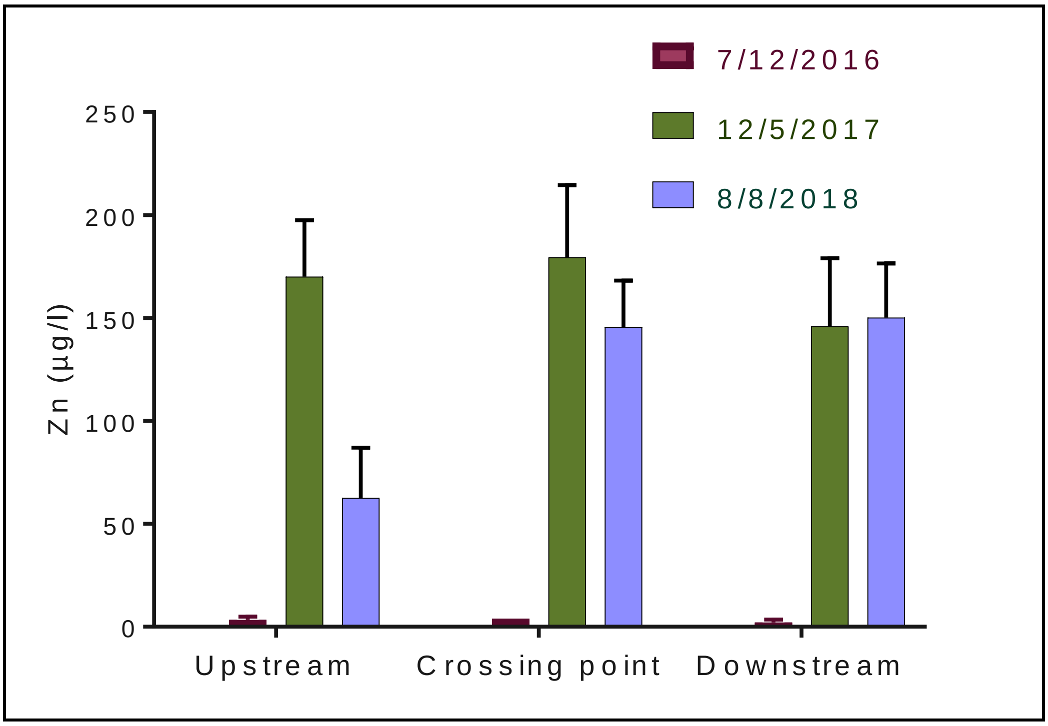

Figure 4-35: Total recoverable metals of Zn in stream water collected from study sites, mean, error bars represent SD of mean. Samples were collected in 7/12/2016, 12/5/2017, and 8/8/2018.

4.3.1.7 Aquatic ecotoxicology assays with *Daphnia*

The *Daphnia* was used to directly study the toxicity of the whole stream water without filtration. Not filtering was important because *Daphnia* are exposed to the dissolved form of the contaminants and to the particulate forms, e.g. from contact and dietary assimilation, in the field. *Daphnia* are also useful for examining the overall toxicity of the aquatic system because the concentrations in the water are likely to be in equilibrium with those in the sediment (at least during low flow periods).

The mortality percentage of *Daphnia magna* exposed to stream water for 24h and 48h, respectively, for the 2016 sampling and testing are shown in Figure 4-36 and Figure 4-37. Consistently, the mortality percentages of *Daphnia magna* were 100% at crossing point site, showing that the water here is entirely hostile to small crustaceans. The mortality was lower (at ca. 40-55%) for the other two sites and the differences were statistically significant ($p<0.05$). This indicates that the water was less toxic at these locations but still induced substantial mortality.

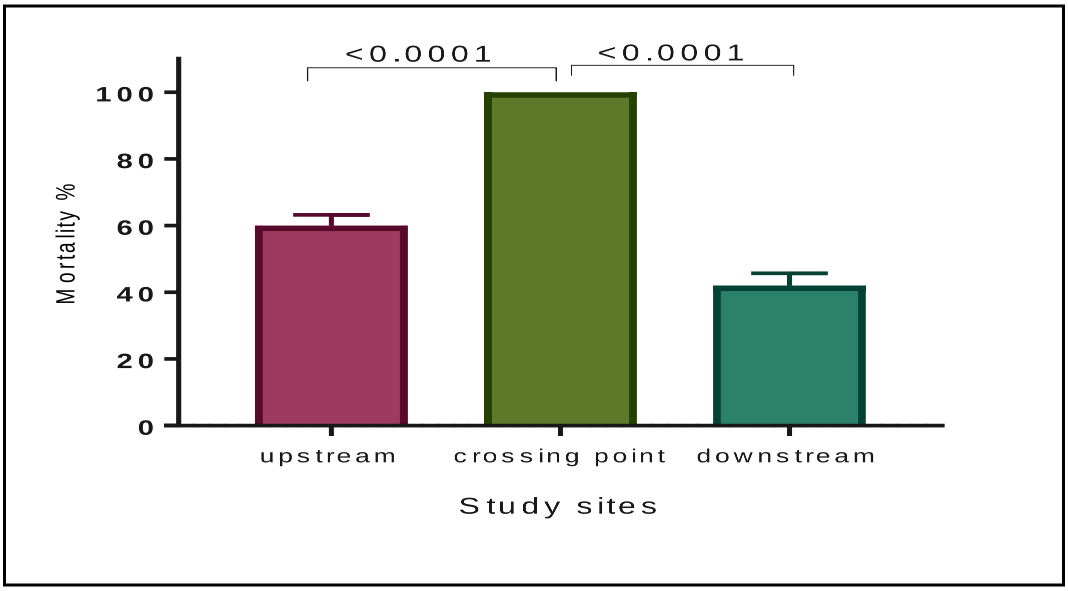

Figure 4-36: Mortality percentage of Daphnia magna exposed to streamwater sites within 24 hours in 2016.

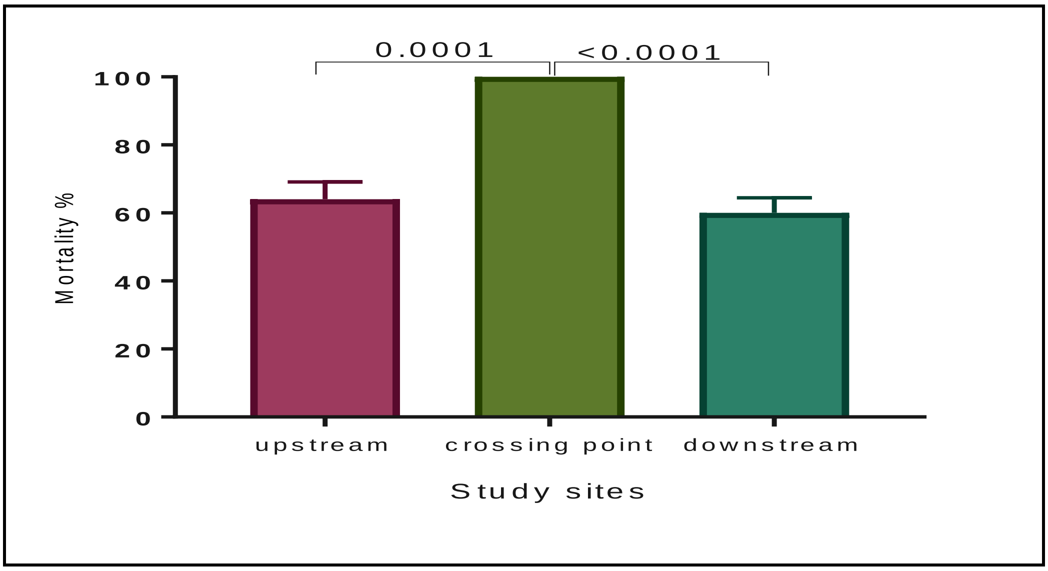

Figure 4-37: Mortality percentage of Daphnia magna exposed to streamwater sites within 48 hours in 2016.

The mortality percentage of *Daphnia magna* exposed to streamwater sites for 24h and 48h, respectively, for the 2018 sampling and testing are shown in Figure 4-38 and Figure 4-39. Again, the highest mortality by far (100%) was observed in water from the crossing point, confirming that the water here is extremely hostile to *Daphnia* and, presumably, other similar crustaceans and invertebrates. The toxicity (mortality) observed for the upstream water was similar to that observed in the 2016 testing, but that in the downstream water was much higher in 2018 than it was in 2016 (i.e. ~70-90%). This suggests that at some times of year or in some periods of time the upwelling discharges from the seepage area adjacent to the crossing point or other related inputs in the area exert an increased toxicity that persists downstream, whereas at other times the increased negative impacts do not persist that far downstream. This variation in persistence of increased toxicity to *Daphnia* is likely caused by the changing level of dominance of inputs from the seepage points that could be linked to relative flow level of the stream, or to amount of rainfall dilution, or to the level of groundwater seepage input or some other factor not identified. It is important to note that the control samples run in parallel showed <10% mortality (data not shown) and so the assay met the validity criteria and can be considered to show toxicity at the study sites. Other studies have similarly used assays with *Daphnia* or similar zooplankton to evaluate toxicity of rivers influenced by mining history (Balistrieri et al., 2007), some of which have concluded that particular metal or non-metal elements might have been the primary toxicant responsible for the mortality observed while others concluded that it was likely a mixtures effect. Here, either case is possible because a number of elements were determined to have concentrations above guideline thresholds (as discussed in previous sections). It is also possible that the turbidity, EC and TDS of the study sites may have had a major negative impact on *Daphnia* because zooplankton have been shown to be sensitive to these parameters at levels approximating those observed here (van Dam et al., 2014, Chapman and McPherson, 2016).

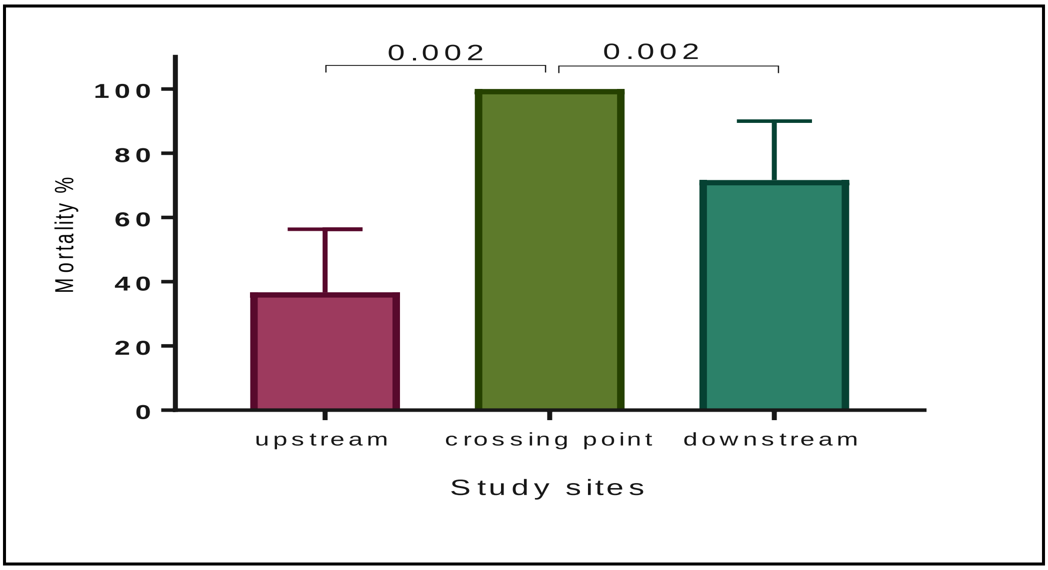

Figure 4-38: Mortality percentage of Daphnia magna exposed to streamwater sites within 24 hours in 2018.

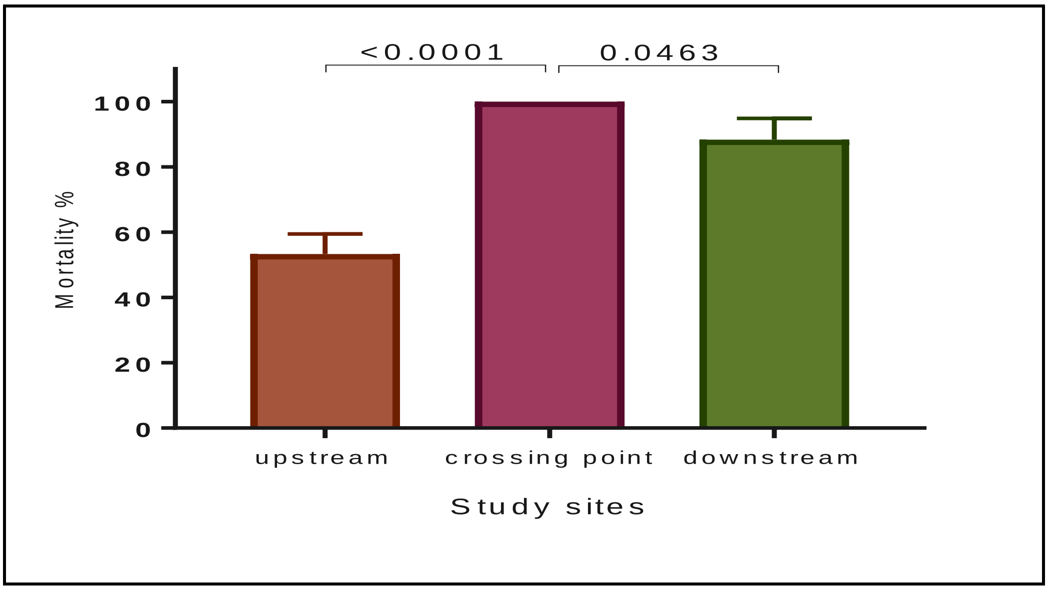

Figure 4-39: Mortality percentage of Daphnia magna exposed to streamwater sites within 48 hours in 2018.

4.3.2 The pseudo-total element concentrations of sediment

Table 4-9 shows the pseudo-total element concentration; it is clear that there is an increase in the Al and Fe content (ANOVA p <0.05) in the crossing points and downstream sites compared with upstream site, while the concentrations of As, Co and Mn were decreased in the crossing points and downstream sites compared with upstream site.

The concentrations of Cr, Cu, Ni, Pb and Zn in downstream site were greater than that at upstream and crossing point sites.

The increase in Al and Fe concentration, in particular, would be consistent with the suspected inputs of mine drainage/ upwell water at or near the seep area in close proximity to the crossing point. The numbers would suggest that while Al (and also Cu and Mg) takes some time and distance downstream to precipitate out of water and into the sediment (i.e. the concentration is even higher downstream), Fe precipitates out more evenly resulting in a steady concentration range from the crossing point to the downstream point. The precipitation of Fe, in what is sometimes called ‘ochre’ deposits, can have negative impacts on aquatic life such as changing the surface and the texture of the substrate (or even fully coating it) which can reduce suitability for egg laying and incubation (for fish and invertebrates) and for plant growth (Mayes et al., 2008).

Table 4-9: Mean of pseudo-total element concentration ± MES

Site / Element	Upstream	Crossing point	Downstream
Al%	1.64±0.26	3.74±0.34*	15.33±0.18*
As mg/kg	42.19±1.05	31.73±2.69*	38.76±0.66
Ca mg/kg	166.22±4.37	216.46±4.64*	786.33±10.82*
Co mg/kg	11.87±0.83	2.34±0.06*	6.38±0.08*
Cr mg/kg	16.26±0.91	12.83±0.81*	19.97±0.38*
Cu mg/kg	15.72±0.57	14.04±0.33*	51.16±0.41*
Fe%	0.32±0.00	5.37±0.15*	5.41±0.03*
Mg mg/kg	100.65±5.45	103.82±9.3	240.49±3.58*
Mn mg/kg	2473.28±202.81	207.53±8.44*	697.66±13.06*
Ni mg/kg	16.31±1.1	6.24±0.16	17.18±0.21
Pb mg/kg	13.38±0.58	13.34±0.59	24.81±0.03
Zn mg/kg	68.47±3.54	42.26±1.63*	113.04±1.21*

* Significantly different from upstream location

The geo-accumulation index (I_{geo}) has been used to estimate the relative metal(loid)s contamination in sediments by Barbieri (2016) and Dung et al.(2013). This can be calculated for the sites investigated here by comparing the measured pseudo-total metal(loid)s contents in the crossing point ($C_{crossing\ point.}$) or downstream site ($C_{downstream.}$) with concentration of them for sediment from upstream site ($C_{upstream}$).

The calculation of geo-accumulation index were based on Equation 4-1 and the resulting I geo values have been interpreted using the seven-category scale as shown in Table 4-10 which is published by Müller (1981). The results indicate that Cu in sediments of the downstream site would be considered to fall in the moderately burdened class. The Al outcomes in downstream site were within the moderately to heavily loaded class, furthermore, geo-accumulation index findings revealed that Fe in both crossing point and downstream sites would fall in the heavily loaded class, whilst the results for the rest of elements fall within practically unloaded class at the concerned sites.

$$\mathrm{I_{geo}} = \log 2\left(\frac{(\mathrm{C_{crossing\ point.}})\ \mathrm{or}\ (\mathrm{C_{downstream.}})}{(1.5 * \mathrm{C_{upstream}})}\right) \qquad \text{Equation 4-1}$$

Table 4-10: $\mathrm{I_{geo}}$-accumulation scales (obtained from Müller (1981).

Class	Value	Sediment quality
0	I geo ≤ 0	practically unloaded
1	0< I geo -1	unloaded to moderately burdened
2	1< I geo- 2	moderately burdened
3	2< I geo -3	moderately - heavily loaded
4	3< I geo -4	heavily loaded
5	4< I geo -5	strong – overburdened
6	I geo ≥ 5	Overburdened

Table 4-11: Geo-accumulation index (I_{geo}) of elements in sediments.

Site Element	Crossing point	Downstream
I geo of Cr	-0.93±0.05	-0.29±0.09
I geo of Mn	-4.15±0.15	-2.4±0.15
I geo of Co	-2.92±0.12	-1.47±0.15
I geo of Ni	-1.97±0.1	-0.5±0.14
I geo of Cu	-0.75±0.09	1.12±0.07
I geo of Zn	-1.28±0.05	0.14±0.1
I geo of As	-1.01±0.16	-0.71±0.02
I geo of Pb	-0.59±0.04	0.31±0.1
I geo of Al	0.62±0.59	2.67±0.36
I geo of Fe	3.47±0.08	3.49±0.03

However, whilst informative and useful, I_{geo} values reveal only relative contaminant loads based on total element concentrations and so are linked to (and limited by) the point of reference. Moreover, those values do not directly inform about the level of the bioavailable and toxic components of the element in the sediment (as it is well established that not the entire amount of an element in sediment is ecologically relevant).

To address the risks that might be posed by the elements for the sedimentary biota, sediment screening level thresholds that are used by environmental agencies in various parts of the world can be used because these have been based on ecotoxicity evidence (i.e. biological assessments of bioavailability and toxicity).

The threshold effect levels (TELs), the concentration at or below which adverse effects are expected only rarely (MacDonald et al., 2000), for Aluminium in sediment for *Hyalella* *azteca* (a shrimp-like amphipod crustacean that is widespread and abundant) has been reported by the Assessment & Remediation of Contaminated Sediments (ARCS) Program of the US EPA (1993) to be 2.55% w/w. This indicates that there is a potential for negative effects on sediment organisms from Al at the crossing point and downstream site.

Arsenic contents of sediment in all of the studied sites were >30 mg/kg and so were greater than the Sediment Screening Benchmark for arsenic of 9.8 mg/kg which has been published by the US EPA (2006). The As concentrations in the sediment at all the sites were also above the Predicted effect level (PEL) of 17 mg/kg, which is the lower limit of the range of concentrations associated with adverse biological effects, that is used in the guidelines of Canadian Council of Ministers of the Environment (CCME, 2001). This same PEL value has also been considered in draft freshwater sediment quality guidelines in England and Wales (Hudson-Edwards et al., 2008b). This would indicate a potential toxicity problem for aquatic sediment dwelling biota from As in the sediment of this former mining area.

The Cu concentrations ranged from ~15 mg/kg at the upstream and crossing point sites to ~51 mg/kg at the downstream site. The TEL for Cu, once considered by the Environment Agency as a draft sediment quality guideline but not finally implemented, has been stated as 37 mg/kg whereas the PEL has been stated as 197 mg/kg (Hudson-Edwards et al., 2008b). This would suggest that the Cu concentration in the sediment of the upstream and crossing point locations poses no direct threat of toxicity, whereas that in the sediment of the downstream location falls between the value that would not be expected to cause adverse effects and the value that would be expected to cause harm. Therefore, while not a definite danger, the Cu concentration in the downstream location could potentially cause a risk to sediment dwelling biota.

The Mn concentration was notable because it was highest, by far, at the upstream site (2473 mg/kg), with lower values at the crossing point (207 mg/kg) and downstream site (698 mg/kg). This suggests that any input of Mn from the seepage points are not the only source of Mn entering the stream and that the sediment load is high upstream. The natural background level of Mn can be variable in different rivers and can be quite high (e.g. 400 mg/kg, (Buchman, 2008), however the concentrations observed at the upstream and downstream sites exceed the TEL threshold for *H. azteca* of 630 mg/kg reported by the ARCS program (Ingersoll et al., 1996). This indicates a potential risk of Mn toxicity to sediment dwelling organisms at these sites.

By contrast, the concentrations of Ni at the three sites were all below 18 mg/kg Table 4-9, which happens to be the TEL for Ni and is well below the PEL of 36 mg/kg (Buchman, 2008). This means that Ni is unlikely to pose any ecological threat to sediment species. For Zn, upstream and crossing point concentrations were below 70 mg/kg and so are below any relevant TEL or PEL values, however the downstream concentration of 113 mg/kg is above the 98 mg/kg TEL determined for *H. azteca* (Ingersoll et al., 1996). The Zn value is, however, still below the general TEL (123 mg/kg) and the PEL (315 mg/kg) for sediment dwellers reported by Buchman (2008), so any negative effects from Zn to sediment dwellers is likely to be minimal.

4.4 Chapter conclusions

The aims of the chapter:

Long term environmental legacies of former coal mines are a pressing concern in many regions and countries around the world. The stream adjacent to the former mining area , which was thought to have been exposed to varying levels of acid mine drainage (AMD) or other mining impacts arising from the site's coal mining history, was seemingly vulnerable to seepage as evidenced by discoloured water. This chapter aimed to characterise and evaluate the water and sediment contamination at three sites.

The main findings:

The findings of this investigation have led to the following understandings: analysis of the physical parameters of stream water showed that a high level of the turbidity, electrical conductivity, and total dissolved solids at the crossing point and downstream location would make the sites unable to sustain a population of fish.

The dissolved concentrations of Fe, Mn, Ni, and Co were above EQS or other guidelines, thereby posing a serious potential threat to ecological integrity, and the increases were apparently mainly linked to inputs at or near the seeps at the Crossing point. Zn was also above quality standards to a small degree.

Al was mostly in particulate form, and this was consistent over time and location, and the particulate phase was also the main phase for As, Pb, and Cu. This has implications for any filter-feeding organisms and will contribute to the deterioration of sediment quality when deposited. Geo-accumulation index findings suggest that Fe was heavily loaded in both crossing point and downstream, while other important outcomes from the geo-accumulation index are that Al and Cu were moderately-heavily loaded and moderately loaded, respectively. Further continued deterioration of sediment quality from seepage inputs is therefore an ongoing concerning.

Comparing the measured pseudo-total elements concentrations in sediment samples with the threshold effect levels (TELs), there is a potential for negative effects on sediment organisms from Al, As and Mn at the crossing point and downstream site.

The results from the *Daphnia* ecotoxicology test confirm that the sites are hostile to aquatic life, particularly the crossing point (100% mortality). This makes it difficult for any type of aquatic ecosystem to develop here because crustaceans are a vital link in the food chain of aquatic systems.The implications of the study:

These findings have significant implications for the understanding of how mining-affected areas can pose an ongoing risk of metals contamination to the adjacent aquatic environment. Also, the data suggest that the sources of contamination seem to be diffuse pollution from the seepage area and this provides additional justification for conducting future hydrological studies at the site and at similar sites in the wider region where only limited mine-site restoration attempts were made. The conclusions regarding the stream being uninhabitable by fish also has wider implications in terms of restricting species dispersion options across the region and indicating that this entire part of the stream is not able to function as a fish refuge or as a source for repopulating fish that have become absent elsewhere. Furthermore, the results of this chapter suggest that a more comprehensive and robust assessment of the contaminant impacts on the aquatic environment can be achieved through accompanying chemical characterisation of water and sediment with a biological assessment via e.g. an ecotoxicological assay with important crustaceans such as Daphnia. Together, such a combined approach would give a much better understanding of the implications of the measured parameters and their consequences for the environment.

The significance of the findings:

The findings will be of interest to environmental agencies for effective river basin management and the remediation and restoration of mining-impacted river systems. The findings of this study will also be of interest to researchers who wanted to do future research into examining aquatic communities in examining the impacts of mine water contamination on the study site. That is, the multi-component approach developed here that covers chemical and physical characterisation of the site as well as biological assessment will be of interest to researchers as a method for understanding the actual parameter or process that might be driving the ecotoxicity.

Limitations:

Examining subterranean sources and flow pathways (i.e., shallow and deeper groundwater upwellings and movement, and hyporheic zone exchanges, etc., via wells and lysimeters) was beyond the capacity of the project, which was the first examination of the site and thus was restricted to a certain scale. Also, it is unfortunate that the study did not include measurement or estimation of flow rates or periodic flow volumes to make calculations of contaminant loads possible. It is unfortunate that in this study, no speciation analysis was possible. That is a limitation because speciation is essential for assessing the relative toxicities of Cr and As in water, and of these and other elements in sediment. Notwithstanding these limitations, the study has clearly demonstrated that the stream is impacted and that mining-affected land immediately adjacent to it is overwhelmingly likely to form a large proportion of the source of that impact.

Further work:

Further work needs to be done on the metals partitioning in the sediments to establish whether the metals are bound to exchangeable and reducible sediment fractions (i.e. the components that would more readily release elements to water and therefore have more profound environmental implications). Examining metals partitioning in the sediments is also important to predict whether the sediment could potentially be additional sources of contamination to stream water when changing hydrochemistry occurs, e.g., due to changes in ionic strength or the oxidation events in water because of flood/drought events or inputs from storm flows or even through changes in activity of microbes brought about by climate change. Also, further work needs to be done to determine the speciation of chromium forms and Arsenic forms in the stream water, as this has important implications for toxicity. Additionally, as indicated above, greater characterisation of hydrological pathways and flows within and around the site are needed to more fully understand contaminant dispersion mechanisms. Further work should also focus on remediation strategies for the stream.

Recommendations:

The combined approach of chemical and physical characterisation as well as biological assessment of sites should be adopted more broadly for investigation of stream and river quality. Some environmental protection agencies do a combined approach in certain areas but it needs to be adopted more widely by those types of organisations and other researchers to ensure a comprehensive investigation or assessment is made. For this site and others like it with similar histories, the future work described above should be enacted.

5 Soil ecotoxicity assessments and remediation trials with Water Treatment Residuals

5.1 Introduction

Resource extraction (mining) and use of the obtained materials has been a fundamental part of human history and a crucial process in the development of nations and technologies. The extraction of coal, for example, largely powered what is thought of as the Industrial Revolution (Reed, 2002) and provided economic stimulus and employment for vast numbers of people. For example, at the peak of coal mining activity in the UK (ca. 1913), a million people were directly employed across 1600 active mines producing ~300 million tonnes of coal per year (Johnston et al., 2008).

However, despite its importance and contributions, coal mining has left a legacy of pollution in many parts of the world that continues to cause environmental concerns. This can particularly be the case at old or abandoned mines that pre-date modern effective environmental controls. Mine spoil, waste rock and other solid and liquid wastes are generated during coal extraction and processing and all can become future sources of soil and water pollution in the surrounding environment. Thus acid or neutral mine drainage, aerial or fluvial movement and deposition of spoil materials and other mechanisms can all result in wider contamination of former mine areas. Often this contamination arises because sulphide minerals in the solid phases react with oxygen and water to produce sulphuric acid and in turn, dissolve potential toxic elements (PTEs) from the solid phase of minerals and soil. The pH of any receiving soil and water can also be impacted, affecting the mobility and toxicity of PTEs. Indeed, PTEs at elevated concentrations arising from mining activities can adversely affect soil and aquatic biota communities, habitat and ecosystems (e.g. (Anawar, 2015, Kostarelos et al., 2015).

The adsorption technique, which is accumulation of molecules or ions called adsorbate onto surface call adsorbents, has environment uses. The uses include treatment of deterioration of water quality of lakes, dams and rivers using biological activated carbon for reduction of soluble organic matter and ammonium nitrogen in water sources, treatment of ground water contaminated with volatile organic carbon compounds such as tetrachloroethylene and/or trichloroethylene using a combination of stripping and adsorption processes, or removal and recovery of nutrients such as ammonium and phosphate ions from wastewater by activated carbon in aqueous phase (Suzuki, 1996). Adsorbates placed into soil could similarly bind and retain contaminants, reducing their mobility and bioavailability to organisms and therefore reducing the negative impacts they have on the environment.

Remediation and restoration of mining impacted areas has become a major undertaking, with many different approaches and options available. The cost, long-term effectiveness/ permanence, and commercial availability of remediating materials must be considered when planning a remediation effort, as must the public general acceptance of them and their capacity to deal with potentially high metal concentrations that may have considerable toxicity and mobility. The remediation approach adopted must therefore consider all such points and strike a balance (Abdullahi, 2015). The need for plentiful, effective, low cost materials for use in mine site remediation has therefore stimulated interest in finding additional uses for readily available by-products that might otherwise simply be discarded; in this chapter the utility of using water treatment residuals (WTRs) in the remediation of mining impacted soils is tested.

The most common method of clarifying raw water sources for drinking is to add metal salts (usually aluminium or iron based) to coagulate and flocculate impurities and remove them via settling and sedimentation. This process generates a sludge or residue material (water treatment residuals, WTRs) that comprise the removed impurities and the reaction/precipitation products of the added metal salts (Howe et al., 2012).

When aluminium or iron coagulant adds to the water, they react with natural or supplemented alkalinity of the water and producing aluminium or iron hydroxide respectively. Typical constituents and properties of partially dried water treatment residuals (WTRs) are shown in Table 5-1, compiled from (Babatunde and Zhao, 2007, Castaldi et al., 2014, Lombi et al., 2010, Ulén et al., 2012, Wang et al., 2012)

Table 5-1: Typical constituents and properties of partially dried water treatment residuals (WTRs) (compiled from (Babatunde and Zhao, 2007, Castaldi et al., 2014, Lombi et al., 2010, Ulén et al., 2012, Wang et al., 2012)).

Al %	Fe %	Ca %	Mn %	Pb mg/kg	Zn mg/kg	Organic matter %	pH
4 – 18	1 - 10	0.4 – 2	0.04 - 1	3 - 16	5 - 60	20 – 30	6.5- 8.0

Millions of tons of WTRs are produced every year all over the world (Basibuyuk and Kalat, 2004). According to Henderson et al (2009) the United Kingdom uses 138,000 tonnes of aluminium-based salts (generating what can be referred to as Al-WTR) and 181,000 tonnes from ferric-based salts (Fe-WTR), while the USA generates more than 2 million tons of WTRs every day (Prakash and SenGupta, 2003). WTRs are considered as an inert waste under the European Union Council Directive 99/31/EC (1999), as cited in Keeley et al. (2014), and can be used as construction materials such as for ceramics and bricks (Goldbold et al., 2003) and for geotechnical works materials (Carvalho and Antas, 2005). Another use that has been proven successful is as the main substrate in constructed reedbeds for waste water filtration (Zhao et al., 2011). A portion of the generated WTRs can also be recycled as coagulant (Keeley et al., 2014).

Other studies have demonstrated that WTRs can be used as an inexpensive adsorbent for the removal of As, Co, Hg and perchlorate from water and wastewater (Jiao et al., 2017, Ociński et al., 2016, Makris et al., 2006, Hovsepyan and Bonzongo, 2009) and to remove Cr, Cd and Pb from landfill leachate (Mohammed et al., 2016). Chiang et al (2012) found that adsorption of Cd, Co, Ni, Pb, and Zn in contaminated sediments using WTRs performed significantly better than goethite. Disposal of WTRs via application to soil has also become more common, and Zhao et al. (2015) found that WTRs can be used as a suitable soil amendment to avoid glyphosate contamination of marine ecosystems by improving the glyphosate retention capacity in soils. The most common soil application of WTRs reported in the literature to date has been as a general amendment and as a means to limit P mobility in over-fertlised or otherwise over-enriched soils (Oliver et al., 2011).

Beneficial use of WTRs is therefore an attractive option that offers financial advantages and facilitates development of a more circular economy with greater levels of materials recycling. The use of WTRs in the remediation of mining and metal contaminated soils has yet to be comprehensively investigated. Moreover, while a number of studies have investigated their effects on soil microbes following soil amendment with WTRs (e.g., Garau et al. (2014)), very few, if any, have examined the influence of WTR application on plants, earthworms, and soil solution chemistry in mining-affected soils. This is a gap in current understanding of the risks and benefits of using these materials in mining-affected soils, especially considering that plants and earthworms are widely recognized as essential ecosystem factors.

Soil ecotoxicology deals with toxicological impacts on soil ecology caused by chemicals or conditions. Assessment of contaminated sites, long term monitoring programs, remedial action plans, assessment of the success of remediation processes, and approving registration requests of a new chemical substance all require, or can be enhanced by, the use of soil toxicity (ecotoxicity) and bioaccumulation tests. Furthermore, soil limit values designed to protect ecology and ecosystems (so-called ecological soil screening levels values) for some chemicals have been derived by some environmental agencies such as those in the United States, Australia and the Netherlands and the derivation of the soil screening level values were based on ecotoxicology tests. A potential risk is posed by a metal (or other substance) when the soil contains higher levels than its ecological soil screening level value. Soil ecotoxicity tests can include assays for soil microorganisms, terrestrial plants, terrestrial invertebrates and terrestrial vertebrates (Plants et al., 2009)

5.1.1 Aims and objectives

The aims of the study, therefore, were: 1) to investigate the adsorption and retention by two types of WTRs (one generated at a plant using Al salts, Al-WTRs, and the other generated from use of Fe salts, Fe-WTRs) of two important metals, Pb and Zn, that are often present as contaminants in waters and wastes, and 2) to assess the remediation of mining contaminated soil using two types of WTRs from central England, UK.

A set of objectives were therefore set to achieve the aims. 1) Adsorption and retention of Pb abd Zn were tested both singularly and in combination, the latter in order to assess effects of cation adsorption competition. Adsorption isotherms were also fitted to the data in order to describe and examine the adsorption processes; 2) examining improvements in soil and soil porewater chemical properties as well as improvements in plant growth and earthworm survival in mining influenced soil amended with WTRs as a remediation measure.

5.2 Methods

5.2.1 Water treatment residuals and soils – source and preparation

Water Treatment Residuals (WTRs) from two water treatment plants in Staffordshire, England, were provided by Severn Trent Water. One plant primarily uses Al salts and the other Fe-based salts, generating what is designated here as Al-WTRs and Fe-WTRs, respectively. According to the results from a previous project carried out on the same materials by Howells et al. (2018), the properties of Al-WTRs once dry were pH 7.34 ± 0.06, Al content 11.64 ± 1.08% w/w, organic matter (OM) content 28.0 ± 0.1% w/w, Fe 0.91 ± 0.08% w/w and those of Fe-WTRs were pH 7.37 ± 0.01, Fe 17.69 ± 0.19% w/w, OM 25.9 ± 0.2% w/w, and Al 0.71 ± 0.12% w/w. The WTRs were dried at 30°C until stable mass and were ground to pass a 2 mm sieve.

Soil samples were collected from a restored coal mining site in Staffordshire, England (Figure 5-1), that is now used partly as a nature reserve and partly for cattle grazing. The site is in the heart of the coal mining area of the English Midlands, a region in which hundreds of small mines and several large ones had been worked over the centuries. At various points around the site there are large patches of bare soil where no, or very little, vegetation grows (Figure 5-1), suggesting that the mining legacy at the site still impacts the soil either through remaining contamination hotspots or through upwelling or percolation of mining affected near-surface groundwater. Soil samples from the top 15 cm depth (~8 kg total) were collected from each of 3 such bare points (or ‘seeps’) that were ~200 m to 300 m apart. The samples were stored in sealed plastic containers and transported to the laboratory where they were air dried and ground to pass a 2 mm sieve. Samples collected from within a particular seep point were bulked and homogenised for use in the experiments.

Figure 5-1: Soil sample collection general location in Staffordshire, England (inset maps) and example of the bare soil ‘seep’ points (main image).

Loss on ignition (≥4h at 450°C) was used to determine organic matter content (Nelson and Sommers, 1996) , and pH was determined in 0.001M $CaCl_2$ extracts (1:5 solid: solution) using a Jenway 3510 pH meter and probe.

5.2.2 Drinking water treatment residuals (WTRs) for adsorption and retention of lead and zinc both singularly and in combination

5.2.2.1 Determining adsorption capacity for Zn and Pb

Following commonly employed batch exchange procedures for determining adsorption capacity of a substance, 2.0 g WTR samples were equilibrated with 20 mL solutions with Pb or Zn concentrations of 10, 50, 100, 200, 300, or 400 mg/l (3 replicates for each WTR type at each concentration). Samples were equilibrated via end-over-end shaking (40 rpm; 20°C) for 24h, which is generally recognised as sufficient time to establish equilibrium (e.g. (Coles and Yong, 2006). Equilibration was immediately followed by centrifugation, filtration using 0.45 µm syringe filters, acidification with a drop of trace analysis grade concentrated nitric acid (PrimarPlus) and then analysis for metals using ICP-OES (e.g. as per (Dada et al., 2012). Concentrations determined in the equilibrium solutions were used to calculate amounts of metal adsorbed to WTRs and the data were further examined through constructing Henry's, Langmuir, Freundlich, Temkin, Dubinin-Radushkevich adsorption isotherms models, as outlined below in Table 5-2. Equation 5-1, which was reported by Vanderborght and Van Griekenm (1977), was used to calculate the quantity of sorbate retained by a unit of mass of the sorbent Q_e^{ads} (mg/g).

$$Q_e = [(C_0 - C_e^{ads}) * V]/m \qquad \text{Equation 5-1}$$

Where C_0 = the initial solution concentration before adsorption; C_e^{ads} = the concentration at adsorption equilibrium; V = Volume of the adsorbate, and m = the mass of WTRs in kilograms.

The adsorbed amount of Zn or Pb from each of Al-WTR and Fe-WTR can be expressed as adsorption percentage, see Equation 5-2 which is ratio of the mass of adsorbed ions at adsorption equilibrium from each WTR to initial mass of adsorbate ions (OECD, 2000).

$$\text{Adsorption \%} = \frac{(\mathrm{C}_0 - \mathrm{C}_e^{ads}) * \mathrm{V}}{(C_0 * \mathrm{V})} * 100\% \qquad \text{Equation 5-2}$$

At its simplest, adsorption of a metal (or other substance) from solution into solid can be expressed as Henry's adsorption isotherm, with K_H typically denoted as Henry's adsorption constant (units of L/g). However, adsorption onto a solid across a wide concentration range is not typically constant. In order to understand some of the adsorption characteristics of Zn and Pb into WTRs, the experimental data were fitted to Henry's, Langmuir, Freundlich, Temkin and Dubinin-Radushkevich (D-R) linerised equations. Those adsorption isotherm equations were described elsewhere (Dada et al., 2012, Yildirim, 2006).

From a fitted Langmuir isotherm one can calculate the Q_m value, which refers to the maximum monolayer coverage capacity (mg/g) onto the adsorbent assuming that the binding energy of the sites are homogenous, and can also determine the b value which is the term used for the Langmuir isotherm constant (L/mg) (Dada et al., 2012, Bonilla-Petriciolet et al., 2017, Foo and Hameed, 2010).

According to the Temkin isotherm, the heat of the adsorption for all adsorbates decreases linearly as the amount of the adsorbed materials on the sorbent increases. An important calculated parameter for this isotherm is often designated the B value, which is the constant related to heat of sorption (J/mol); the AT value is the Temkin isotherm equilibrium constant (l/g) (Dada et al., 2012, Bonilla-Petriciolet et al., 2017, Foo and Hameed, 2010).

The adsorptions into a heterogeneous surface with a Gaussian energy distribution can also be described by applying DR model. The DR isotherm is based on the theory of volume filling of micropores. The Q_s value determined by this isotherm model is the theoretical isotherm saturation capacity (mg/g), and parameter Ɛ (kJ) is equal to the difference between the chemical potentials of the adsorbate in the state of normal liquid and the adsorbed state at the same temperature. In the Dubinin–Radushkevich constant, adsorption free energy is related to the derived Kad constant (Dada et al., 2012, Bonilla-Petriciolet et al., 2017, Foo and Hameed, 2010).

For adsorptions onto adsorbents for which the distribution of heat of adsorption of binding sites is not or cannot be assumed to be uniform, the Freundlich isotherm model is often used. It is a widely applied isotherm for describing adsorption characteristics and generates the following parameters and constants: 1/n, which is dimensionless and is a function of the strength of the adsorption; and K_f is the Freundlich isotherm constant (with units of mg $^{1-(1/n)}$.g^{-1}.L$^{(1/n)}$) (Dada et al., 2012, Bonilla-Petriciolet et al., 2017, Foo and Hameed, 2010). Although widely used for the purpose, determining characteristics of adsorption processes based on comparison of Freundlich K_f values can be problematic when 1/n values are not the same or $C_e^{ads} \neq 1$ because the units of K_f will be different (Chen et al., 1999). To address this, Chen et al. (1999) proposed a unified adsorption variable (K_u^{ads}) to unify the unit of K_f^{ads} to be L/g. The K_u^{ads} is the slope of the isotherm at any value of C_e^{ads} or Q_e^{ads}, and can be calculated over a range of C_e^{ads} or Q_e^{ads} using K_f^{ads} and 1/n using Equation 5-3 or Equation 5-4. Therefore K_u^{ads} was also calculated in the present study.

$$\mathrm{K}_{u}^{ads} = \mathrm{K}_{f}^{ads} / C_{\mathrm{e}}^{ads\,(n-1)/n} \qquad \text{Equation 5-3}$$

$$\mathrm{K}_{u}^{ads} = \mathrm{K}_{f}^{ads\,n} / Qe^{ads\,n-1} \qquad \text{Equation 5-4}$$

Thermodynamic data such as the standard adsorption energy can be obtained from Langmuir and Temkin equation, see Equation 5-5.

$$\mathrm{K} = e^{-\Delta G_{ads}^{0}/RT} \qquad \text{Equation 5-5}$$

Table 5-2: Linear and non-linear isotherm equation as described in (Dada et al., 2012, Yildirim, 2006).

Model	Non-linear equation	Linear equation
D-R	$Qe=(Q_S)*\exp(-Kad*\varepsilon^2)$	Ln(Qe)=ln(Q_S)- Kad*Ɛ^2
Freundlich	Qe=Kf*(Ce)^(1/n)	Qe=Kf*(Ce)^(1/n)
Henry's	------	Qe= K_H *Ce
Langmuir	Qe=(b*Qm*Ce)/(1+b* Ce)	1/Qe=(1/Qm))+[(1/(Qm *b)*(1/ Ce)]
Temkin	Qe=B*ln(AT*Ce)	Qe= B*ln(AT)+B*ln(Ce)

(D-R) refers to Dubinin-Radushkevich

5.2.2.2 Desorption of Pb and Zn

The desorbability of Zn and Pb bound to Al-WTR and Fe-WTR was determined in batch desorption experiments to determine the degree of reversibility of adsorption by the Al-WTR and Fe-WTR materials. That is, to determine how well they retain the pollutant metals after exposure to clean solution.

Desorption experiments were conducted on the 2 g samples after removal of the supernatant following the initial equilibration process described above for the adsorption experiment. In order to facilitate accurate desorption measurements, i.e. to allow any metals remaining entrained in the WTRs after solution removal to be fully accounted and adjusted for, the mass of each tube had been recorded (for WTRs and tube) before adding Pb or Zn solution in the sorption experiment and was recorded again at the end of the batch adsorption equilibrium experiments (i.e. after centrifugation and solution removal). The difference in mass enabled calculation of the remaining volume of solution within each tube and this, together with the measured concentrations of metals in the removed solution, allowed calculation of the amount of entrained metals in that remaining solution.

For desorption, the removed supernatant was replaced by fresh 0.001 M $CaCl_2$ solution and the samples were shaken (40 rpm; 20°C) again for 24 hours, which was immediately followed by centrifugation, filtration using 0.45 µm syringe filters and then analysis for metals using ICP-OES. Concentrations measured in the 0.001 M $CaCl_2$ desorption supernatant solutions are C_e^{des} values and were adjusted for the calculated amounts of entrained metals remaining from the initial sorption solution.

The desorbed amount of Zn or Pb from each of Al-WTR and Fe-WTR can be expressed as desorption percentage, see Equation 5-6 which is ratio of the net of desorbed ions concentration from each WTR to concentration of adsorbed ions on each WTR at adsorption equilibrium (OECD, 2000).

$$\text{desorption } \% = \frac{(C_e^{des} * V)}{(C_o * V) - (C_e^{ads} * V)} * 100\% \qquad \text{Equation 5-6}$$

5.2.2.3 The competitive adsorption of Zn-Pb ions

In addition to separate adsorption experiments with Zn and Pb individually, adsorption experiments with Zn mixed with Pb were conducted to examine competitive adsorption. The experiments were carried out as described above but with solution concertation of 10 mg/l (both Pb and Zn) and 50 mg/l (both Pb and Zn).

5.2.3 Remediation of contaminated soils using water treatment residuals

5.2.3.1 Plant growth trial

Soil from each of the three seep collection points were amended separately with 10% Al-WTR w/w or 10% Fe-WTR w/w, or 10% agricultural lime w/w (total solid mass 250 g). Controls, i.e. with no amendment, were also established. All treatments and controls were prepared in triplicate (n=3). Every individual replicate was prepared separately (as opposed to large batch mixing and splitting) to ensure a consistent addition rate was achieved across treatments and replicates. Once prepared, each soil sample was placed into a separate plastic plant pot (~0.25 L). The addition rate of 10% w/w was selected as it was thought to represent the upper limit of what might realistically be used in a remediation scenario but still avoid the worst of the reductions in available soil phosphorus reported at rates of WTR application above this level by Dayton and Basta (2001). The agricultural lime treatment was included to allow an assessment as to whether any improvements observed were mainly attributable to pH increases anticipated to be brought about by WTR addition.

Ultrapure water was added (60% of water holding capacity; determined by soaking and allowing draining and calculating water retained) to all samples and moisture was maintained every day by watering to mass using ultrapure water. 1.5 g of Perennial ryegrass (*Lolium perenne*) seeds that had been moistened were added to the top of the soil followed by adding vermiculite (1 g) in order to decrease loss from evaporation. To allow full access to natural light, the pots were placed in an un-heated incubator next to a large window. The growth periods were 4 weeks in November and January in the UK. The humidity inside the incubator was maintained by placing 100 ml of ultrapure water in a glass beaker inside the incubator. At 28 days after sowing, shoots were removed by cutting at the surface with scissors and were dried at 65-70 °C in an oven for 48 hours followed by recording mass of dry weights.

Once dry mass was recorded, samples were cut into small pieces with scissors and digested in nitric acid (Yilmaz, 2007). In this method about 0.4 g of dry plant material is placed in the digestion vessel then 10 ml of high purity concentrated (16 M) HNO_3 is added and the digestion is carried out using a CEM-MARS 6 microwave (CEM Corporation Mathews, NC, USA). This procedure achieved complete digestion and dissolution of the plant samples (i.e. no visible residue). The digest solutions were transferred to centrifuge tubes, made up to 20 ml with 0.1% HNO_3, and filtered through a 0.45 µm syringe filter into plastic vials. Nutrients and heavy metals concentrations were analysed using ICP-MS/OES. In each batch of analyses a blank sample was carried out for quality control, and certified reference solutions were used for calibration standards. All acids used were of high purity grade (i.e. Primar Plus trace analysis).

5.2.3.2 Earthworm survival trial

The earthworm survival assay was conducted in accordance with OECD protocol 222. Treatments and controls (i.e. 0 %, 10% Al-WTR, 10% Fe-WTR or 10% lime, n=3, replicates mixed individually) were prepared separately but in an identical manner to those described above for the plant test. Once prepared, control or treatment soils were placed in plastic containers (approximately 0.35L) for the earthworm assay. Four ‘procedural controls’ were also prepared from a pristine soil collected from pasture land on Keele campus, in order to verify that the earthworm assay design and conditions met the validity criteria of the OECD 222 protocol (viz. adult mortality ≤10% in negative controls; this criterion was achieved and thus the assay was validated). Ultrapure water was added (60% of water holding capacity) to all samples and maintained every day (using ultrapure water). Then 5 adult *Eisenia fetida* earthworms were weighed and added to each container. Oatmeal, about 2 g, was added as a food source. Cling film was used to cover the containers to prevent escape and was pierced to facilitate air flow. Oatmeal was added every week. After 4 weeks survival was determined and living worms were allowed to depurate for a day then rinsed with ultrapure water, then patted dry, weighed and frozen. To determine element contents, the worms were dried at 70-100 °C for 48 hours in borosilicate glass beakers, weighed and then digested in 10 ml of HNO_3 (Primar Plus trace analysis). The digest solutions then were transferred to a centrifuge tube and made up to 20 ml with 0.1% HNO_3. The solutions were filtered through a 0.45 µm syringe filter into a plastic vial. Nutrients and heavy metals concentrations were analysed using ICP-MS/OES.

5.2.3.3 Simulated soil solution investigation

After the earthworm survival experiment, the soils were saturated with ultrapure water and allowed to stand for ~ 3 days in order to generate simulated soil porewater (Ardestani and van Gestel, 2013, Ma et al., 2006). Soil solution was then extracted from each pot by centrifugation for 20 minutes at 3500 rpm. The resulting extracted solutions were filtered using 0.45 µm syringe filters then acidified with 0.1 mL concentrated HNO_3 and kept cold at 4°C until the solutions were analysed using ICP-MS/OES.

Examinations of the effects of a wetting-drying cycle on plant yield, earthworm survival, and element uptake in amended and non-amended soils were also carried out. After completion of the plant and earthworm trials and the simulated soil solution generation, all of the samples were subjected to a wet-dry cycle to investigate the effects of such on plants and earthworms in the treated soils (i.e. simulating longer term effects in the field). Each individual replicate was dried at 25 °C in an oven for one week followed by re-grinding and homogenisation and determination of remaining water content (a small subsample was also removed for determination of soil pH following treatments). Ultrapure water was added to re-establish moisture content at 60% of water holding capacity and samples were allowed to equilibrate for a few days before the plant and earthworm trials were repeated following the same protocols described above.

5.2.3.4 Statistical analyses

Statistical assessment of differences amongst treatments and controls were conducted via T-tests and ANOVA, when underlying assumptions of the tests were met (i.e. normality of distribution), or via Mann-Whitney tests if necessary. All statistical assessments were conducted using GraphPad Prism software.

5.3 Results

5.3.1 Drinking water treatment residuals (WTRs) for adsorption and retention of lead and zinc both singularly and in combination

5.3.1.1 Adsorption Capacity of WTRs for Zn and Pb

Adsorption of both Pb and Zn was very high at all concentrations tested and on both types of WTRs, with the maximum amounts adsorbed at the highest solution concentrations imposed (400 mg/l) equating to 4025 mg/kg for Pb and 3579 mg/kg for Zn on Al-WTRs (Table 5-3 and Figure 5-2). For Fe-WTRs it was 3980 mg/kg for Pb and 3579 mg/kg for Zn (Table 5-3 and Figure 5-2). Indeed, in all tested concentrations adsorption was >92% (thus explaining the very similar maximum adsorption values). Moreover, the realised Zn and Pb adsorption capacities of the Al-WTR and Fe-WTR increased lineary with increasing initial concentration of adsorbate (C_0), as shown in Figure 5-2, indicating that adsorption maxima had not been reached for either WTR type (Table 5-3 and Figure 5-2).

Adsorption isotherms of Zn and Pb ions in a mono-metal system on Fe-WTR and Al-WTR were created (see Figure 5-3). The shapes of isotherms for adsorption of Zn into both WTRs, as well as Pb into Fe-WTR, are similar to the type I isotherm (i.e. asymptotic towards a maximum, suggesting adsorption is limited to a single monolayer on the surface) while the shape of Pb adsorption isotherm into Al-WTRs is similar to the Henry isotherm (i.e. linear) (Lowell et al., 2012).

Table 5-3: Range of adsored amount Q_e (mg/kg) and range of adsorption % for Zn and Pb ions in a mono-metal system on Fe-WTR and Al-WTR.

Test	Range Q_e (mg/kg)	Range of adsorption %
Zn on Al-WTR	86.92- 3579.28	97-93
Zn on Fe-WTR	86.92-3579.77	97-92
Pb on Al-WTR	104.90-4025.50	97-100
Pb on Fe-WTR	103.20-3980.20	96-99

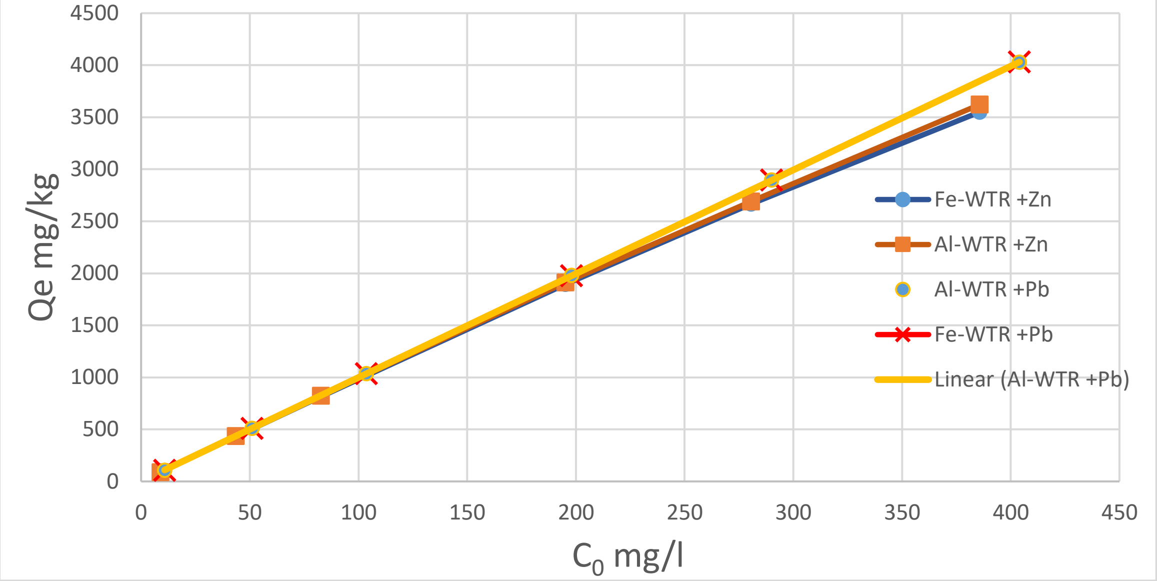

Figure 5-2: Effect of initial (C_0) concentration on Zn, Pb adsorption capacity of Al-WTR and Fe-WTR.

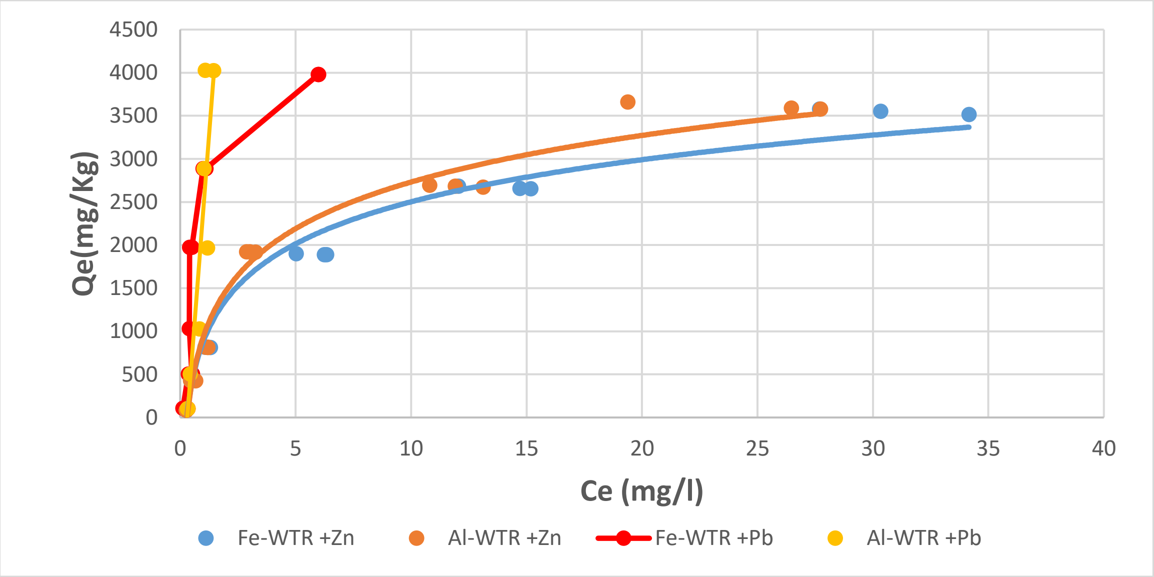

Figure 5-3: Adsorption isotherms in a mono-metal system for: Al-WTR with Zn, Al-WTR with Pb, Fe-WTR with Zn and Fe-WTR with Pb. Isotherms constructed using Equation 5-1. Qe is quantity of sorbate retained by a unit of mass of the sorbent; Ce is the concentration at the equilibrium. The ICP-OES detection limit for Zn was 0.27 (mg/l) and for Pb was 0.1 (mg/l).

The experimental data reveal that the maximum monolayer coverage capacity (Q_m) of the Zn adsorption onto Fe-WTR and Al-WTR from Langmuir Isotherm models were 4.38mg/g and 4.68 mg/g, respectively, and that the Langmuir isotherm constants (b) were 0.13 L/mg for Zn adsorption into both Fe-WTR and Al-WTR Table 5-4. Adjusted R-squared, R^2(adj), values were 0.92 and 0.86 for Zn+Fe-WTRs and Zn+Al-WTRs respectively, indicating that the adsorption data fitted well to the Langmuir Isotherm model. However, the experimental data of the Pb adsorption onto Fe-WTR and Al-WTR did not fit the Langmuir Isotherm model (see Table 5-5).

From Freundlich isotherms, the 1/n values of Zn adsorption onto Fe-WTR and Al-WTR were 0.68 and 0.74 respectively (Table 5-4), while 1/n values for Pb adsorption onto Fe-WTR were 0.87 while the model did not fit the equilibrium adsorption data for Pb onto Al-WTR. The values of R^2(adj) indicate that the Freundlich isotherm did describe the majority of the data accurately, having ranged between 0.86-0.87 (see Table 5-4 and Table 5-5) while for Pb adsorption onto Fe-WTR it was 0.56. In order to facilitate better comparison of the Kf values when the 1/n values are different, Ku values (its unit being L/kg) were calculated over a range of Ce values, see Equation 5-3, to unify the unit of Kf (Chen et al., 1999). The Ku values (l/g) of Zn adsorption onto Fe-WTR and Al-WTR ranged 0.70-0.15 and 0.69-0.21 respectively, from lower to higher initial concentration, respectively. Ku values (l/g) of Pb adsorption onto Fe-WTR ranged 1.65-1.06 a, from lower to higher initial concentration, see Table 5-6.

From the Temkin model, the Timken equilibrium constant AT values of Zn adsorption onto Fe-WTR and Al-WTR were 3.50 l/g and 3.32 l/g respectively and the adjusted R-squared was 0.97 for both, while AT values of Pb adsorption onto Fe-WTR and Al-WTR were 6.50 l/g and 2.99 l/g respectively and the adjusted R-squared was 0.77 and 0.74 respectively (Table 5-4 and Table 5-5). The standard Gibbs free energy of the adsorption can be calculated using Equation 5-5. Based on the Temkin constant AT, the standard Gibbs free energy of Zn adsorption onto Fe-WTR and Al-WTR were -13.24 and -13.11 kJ/mol, respectively; and of

Pb adsorption onto Fe-WTR and Al-WTR were -17.56 and -15.67 kJ/mol respectively. These results indicate that Pb adsorption with more negative values is more easily adsorbed on WTRs than Zn.

Based on D-R equation, theoretical isotherm saturation capacity values were 2.34 and 2.31 (mg/g), for Zn adsorption onto Fe-WTR and Al-WTR respectively, while values were calculated as 2.82 and 6.08 (mg/g) for Pb adsorption onto Fe-WTR and Al-WTR respectively. The values of the adjusted R-squared indicate that the D-R isotherm did describe the data accurately having ranged between 0.91-0.94 (see Table 5-4 and Table 5-5), except that for Pb adsorption onto Fe-WTR was only 0.60.

Table 5-4: Isotherms constants and correlation coefficient for adsorption of Zn with Fe-WTR and Zn with Al-WTR.

Zn adsorption into Fe-WTR			Zn adsorption into Al-WTR		
D-R isotherm			D-R isotherm		
Q_s (mg/g)	K_{ad} (mol^2/kJ^2)	R^2(adj)	Q_s(mg/g)	K_{ad} (mol^2/kJ^2)	R^2(adj)
2.34	0.23	0.94	2.31	0.24	0.93
Freundlich			Freundlich		
K_f($mg^{1-(1/n)}.g^{-1}.L^{(1/n)}$)	1/n	R^2(adj)	K_f($mg^{1-(1/n)}.g^{-1}.L^{(1/n)}$)	1/n	R^2(adj)
0.46	0.68	0.87	0.49	0.74	0.86
Henry’s isotherm:			Henry’s isotherm:		
K_H (l/g)	R^2 (adj)		K_H (l/g)	R^2(adj)	
0.1046	0.85		0.13	0.81	
Langmuir isotherm:			Langmuir isotherm:		
Q_m (mg/g)	b (L/mg)	R^2(adj)	Q_m (mg/g)	b (L/mg)	R^2(adj)
4.38	0.13	0.92	4.68	0.13	0.86

Temkin isotherm:			Temkin isotherm:		
AT(l/g)	ΔG°=-RT*lnAT	R^2 (adj.)	AT(l/g)	ΔG°=-RT*lnAT	R^2 (adj)
3.50	-13.24	0.98	3.32	-13.11	0.98

Table 5-5: Isotherms constants and correlation coefficient for adsorption of Pb with Fe-WTR and Pb with Al-WTR.

Pb adsorption into Fe-WTR			Pb adsorption into Al-WTR		
D-R isotherm			D-R isotherm		
Q_s (mg/g)	K_{ad} (mol^2/kJ^2)	R^2(adj)	Q_s (mg/g)	K_{ad} (mol^2/kJ^2)	R^2(adj)
2.82	0.13	0.60	6.08	0.336	0.93
Freundlich			Freundlich		
K_f(mg$^{1-(1/n)}$.g^{-1}.L$^{(1/n)}$)	1/n	R^2(adj)	K_f(mg$^{1-(1/n)}$.g^{-1}.L$^{(1/n)}$)	1/n	R^2(adj)
1.34	0.871	0.56	N.F	N.F	N.F
Henry's isotherm:			Henry's isotherm:		
K_H (l/g)	R^2 (adj)		K_H (l/g)	R^2 (adj)	
0.541	0.60		3.469	0.77	
Langmuir isotherm:			Langmuir isotherm:		
Q_m (mg/g)	b (L/mg)	R^2(adj)	Q_m (mg/g)	b (L/mg)	R^2(adj)
N.F	N.F	N.F	N.F	N.F	N.F
Temkin isotherm:			Temkin isotherm:		
AT(l/g)	ΔG°=-RT*lnAT	R^2(adj)	AT(l/g)	ΔG°=-RT*lnAT	R^2(adj)
6.50	-17.56	0.78	2.99	-15.67	0.74

N.F the experimental data does not fit the linear equation.

Table 5-6: K_u (l/g) for adsorption of Zn on Al-WTR and on Fe-WTR and Pb on Fe-WTR; calculated over a range of C_e values, see Equation 5-3.

Fe-WTR +Zn		Al-WTR +Zn		Fe-WTR +Pb	
Ce mg/l	Ku L/g	Ce mg/l	Ku L/g	Ce mg/l	Ku L/g
0.07	0.70±0.00	0.01	0.69±0.00	0.23	1.65±0.16
0.39	0.59±0.01	0.06	0.57±0.03	0.45	1.49±0.05
1.18	0.44±0.01	0.31	0.47±0.01	0.45	1.49±0.03
5.87	0.26±0.01	3.85	0.37±0.01	0.46	1.48±0.03
13.98	0.20±0.01	11.40	0.26±0.01	1.06	1.33±0.01
30.73	0.15±0.00	23.54	0.21±0.01	5.99	1.06±0.00

Ku values for adsorption of Pb on Al-WTR could not be calculated because whose experimental data did not fit the Freundlich linear equation.

5.3.1.2 The competitive adsorption/ desorption of Zn-Pb ions

The removal efficiency (% adsorption) of Pb and Zn when in a competitive adsorption situation is given in Table 5-7. The removal efficiency was 100% for Pb and almost 100% (>98%) for Zn, showing that the WTRs had a high capacity for adsorbing both metals when present together but demonstrating that the Pb ions presented a marginally higher affinity for the cationic adsorption sites of the Al-WTR and Fe-WTR than that for Zn ions Table 5-7. A similar pattern was evident in the desorption findings in which no Pb was detectably released from either WTR while only a very small percentage of adsorbed Zn (0.03% - 3.92%) was released, thus also indicating a higher affinity and/or binding of Pb for the cationic adsorption sites of the Al-WTR and Fe-WTR than that for Zn ions Table 5-8.

Table 5-7: Adsorption percentage (%) of Zn(II) and Pb(II) when in solution together.

Solution concentration (mg/l)	Pb + Al-WTR	Zn + Al-WTR	Pb+ Fe-WTR	Zn + Fe-WTR
10Pb+10 Zn	100±0.00	98.35±0.22	100±0.00	99.89±0.01
50Pb+50 Zn	100±0.00	99.72±0.04	100±0.00	98.87±0.02

Table 5-8: Desorption percentage (% of adsorbed metal released) of Zn(II) and Pb(II) when in solution together.

Solution concentration (mg/l)	Pb + Al-WTR	Zn + Al-WTR	Pb+ Fe-WTR	Zn + Fe-WTR
10Pb+10 Zn	0.00±0.00	3.92±0.01	0.00±0.00	0.44±0.00
50Pb+50 Zn	0.00±0.00	2.57±0.00	0.00±0.00	0.03±0.00

5.3.1.3 Desorption of Pb and Zn (single element)

The desorption percentage (based on the percentage of adsorbed metal extractable using fresh 0.001M $CaCl_2$) of both Pb and Zn were very low at all concentrations tested and on both types of WTRs. The desorption percentages from WTRs originally exposed to 400 mg/l metal solutions, and from which had adsorbed 4029 mg/kg for Pb and 3579 mg/kg for Zn in the case of Al-WTRs, were 0.23 ±0.06% and 1.26±0.03% for Pb and Zn respectively for the Al-WTRs. Samples of Al-WTRs subjected to the lowest initial concentration of Pb and Zn, i.e. 10 mg/l, and which had adsorbed 105 mg/kg for Pb and 87 mg/kg for Zn, had desorption percentage values of 6.24 ±1.23% and 7.10±2.77% for Pb and Zn respectively (Table 5-9).

For the Fe-WTRs, desorption percentages for samples subjected to initial concentration of Pb and Zn of 400 mg/l (which had adsorbed 3980 mg/kg for Pb and 3580 mg/kg for Zn) were 0.00 ±0.00 % and 0.36±0.06% respectively. For Fe-WTR samples subjected to the lowest initial concentration of Pb and Zn, i.e. 10 mg/l, which produced adsorbed amounts of 103 mg/kg for Pb and 87 mg/kg for Zn, the desorption percentages were 0.02 ±0.00% and 2.24±1.56% for Pb and Zn respectively (Table 5-9). This again shows that both types of WTRs have high capacity for binding and retaining metals.

Table 5-9: Desorption percentages (% of adsorbed metal released) for Zn and Pb ions in a mono-metal system on Fe-WTR and Al-WTR.

Initial solution conc. in adsorption step (mg/l)	Fe-WTR +Zn		Al-WTR +Zn		Fe-WTR +Pb		Al-WTR +Pb	
	Desorbed mean %	SD	Desorbed mean %	SD	Desorbed mean %	SD	Desorbed mean %	SD
10	2.24	1.56	7.10	2.77	0.02	0.00	6.24	1.23
50	0.86	0.19	1.31	0.56	0.00	0.00	1.35	0.01
100	0.45	0.08	0.77	0.16	0.00	0.00	0.82	0.21
200	0.26	0.03	1.40	0.12	0.00	0.00	0.45	0.06
300	0.26	0.03	1.22	0.01	0.00	0.00	0.31	0.03
400	0.36	0.06	1.26	0.03	0.00	0.00	0.23	0.06

5.3.2 Remediation of contaminated soils using water treatment residuals

5.3.2.1 Soil pH and organic matter (OM) effects

Despite the high organic matter content of the WTRs (typically 28% in the Al-WTR and 26% in the Fe-WTR), their addition at 10% w/w had a negligible impact on the total organic matter percentage of the soils determined by loss on ignition as shown in Table 5-10. This is likely due to the mining-impacted soils still having the presence of small coal fragments that were combusted during the organic matter content measurement. Addition of lime did decrease the overall organic matter content of the soil as the lime contained no organic matter. However, WTR treatment had a profound effect on soil pH, see Table 5-10, with statistically significant increases from pH<4 in the untreated controls to generally pH>4 in treated soils. Both Al-WTRs and Fe-WTRs significantly increased the pH, with no consistent pattern as to which raised the pH more. The pH buffering capacity of the soils, calculated based on response to lime addition, were shown to vary (Table 5-11), and this enabled examination of the lime equivalent value of WTRs in the various soils. The lime equivalent of WTRs was calculated using slope of the line equation shown in Table, with the slope of the WTRs equation divided by the slope of the corresponding lime equation. The WTR/lime equivalent values were different among the seep soils.

Table 5-10: Mean of organic matter content (%) and pH of untreated and treated soils, plus buffering capacity of the soils calculated from response to lime addition.

Soil	Non-amended (control)	Al-WTR amended	Fe-WTR amended	Lime amended	Buffering capacity#
	---------------------------- Organic matter (%) ----------------------------				
Seep 1	32.09±0.31	32.88±0.18	32.18±0.26	8.17±0.19*	n/a
Seep 2	27.92±0.23	26.62±0.27*	24.95±0.88	22.48±0.4*	n/a
Seep 3	8.11±0.3	8.99±0.1	8.23±0.09	5.14±0.06*	n/a
	------------------------------------- pH -------------------------------------				
Seep 1	3.28±0.02*	4.38±0.06*	3.80±0.02*	6.36±0.04*	21400±0.00
Seep 2	3.83±0.06*	5.67±0.10*	6.01±0.05*	6.88±0.03*	32700±0.00
Seep 3	3.40±0.01*	5.23±0.06*	5.42±0.02*	7.15±0.06*	26600±0.00

*Significantly different from non-amended (α 0.05); #based on lime addition and measured pH, with units: mg $CaCO_3$/kg soil/pH unit; n/a = not applicable.

Table 5-11: The approxmate WTR/lime equivalent calculated from pH measured in the 0% addition and 10% addition of WTR treatments.

Soil label	Amendment type	Soil pH		line equation	Slope	WTR/lime Equivalent
		0% addition	10% addition			
Seep 1	Al-WTR	3.28 ±0.02	4.38±0.06*	pH = 11*Al-WTR% + 2.85	11	0.24
	Fe-WTR	3.28 ±0.02	3.80±0.02*	pH = 7.5*Fe-WTR% + 2.85	7.5	0.17
	Lime	3.28 ±0.02	6.36±0.04*	pH = 45.2*lime% + 2.85	45.2	1.00
Seep 2	Al-WTR	3.83 ±0.06	5.67±0.10*	pH = 18.7*Al-WTR% + 3.89	18.7	0.61
	Fe-WTR	3.83 ±0.06	6.01±0.05*	pH = 21.7*Fe-WTR%+ 3.89	21.7	0.71
	Lime	3.83 ±0.06	6.88±0.03*	pH = 30.7*lime% + 3.81	30.7	1.00
Seep 3	Al-WTR	3.40 ±0.01	5.23±0.06*	pH= 18.9*Al-WTR% + 3.41	18.9	0.50
	Fe-WTR	3.40 ±0.01	5.42±0.02*	pH = 20.4*Fe-WTR% + 3.39	20.4	0.54
	Lime	3.40 ±0.01	7.15±0.06*	pH = 37.5*lime% + 3.41	37.5	1.00

5.3.2.2 Plant yield and element concentrations

The increase in dry mass of plant growth in all amended soils was statistically significant in comparison with that of non-amended control soil in both the initial plant growth trial and the trial following a wet-dry cycle Table 5-12. In the initial trial the highest masses were found at Fe-WTR amended soils which were 238%, 136% and 215% of that in non-amended control soils in seep 1, seep 2 and seep 3, respectively. The increase in dry mass of plant growth in Al-WTR and lime amended soils was almost the same. In the trial following the wet-dry cycle, the yield increases achieved with Al-WTR, Fe-WTR and lime treatments were similar Table 5-12.

Table 5-12: Mean of dry plant mass per pot in grams (g)± standard devation (SD) of the mean, at original plant trial yield and following wet-dry cycle.

Site	Non-amended	Al-WTR	Fe-WTR	Lime
--------------------------------------Original plant trial yield---------------------------------------				
Seep 1	0.18±0.02	0.34±0.00*	0.43±0.00*	0.35±0.00*
Seep 2	0.36±0.00	0.47±0.00*	0.49±0.00*	0.42±0.00*
Seep 3	0.20±0.01	0.35±0.01*	0.43±0.01*	0.36±0.00*
--------------------------------------Yield following wet-dry cycle----------------------------------				
Seep 1	0.35±0.01	0.45±0.01*	0.48±0.01*	0.58±0.01*
Seep 2	0.38±0.00	0.46±0.02*	0.51±0.01*	0.47±0.00*
Seep 3	0.32±0.02	0.42±0.01*	0.43±0.00*	0.43±0.01*

*Significantly different from non-amended (α 0.05)

The elements content in plant tissues from the initial plant growth trial is shown in Table 5-13, there is significant decrease in Al assimilation in all seeps after applying Fe-WTR and Al-WTR and lime. As shown in Table 5-13, there were significant decreases in arsenic assimilation by all amendments addition in seep 3, while the decreases in arsenic assimilation by all amendments addition were not significant in seep 2. There were non-significant increases in arsenic content in plants grown in amended soil from seep 1 compared with that grown in non-amended seep 1 soil. It can be seen from Table 5-13 that Ca, Mg and K assimilation increased significantly in all study sites after applying each of the used amendments. Analysis of Cd concentrations reveals detectable amounts only in the plant grown in non-amended soil from seep 2 and seep 3, that were 0.06±0.05 mg/kg and

0.07±0.05 mg/kg respectively, while there were no detectable levels of Cd in the plant grown in amended soils from seeps 2 and 3.

The statistical tests did not reveal a significant difference in the Co assimilation between the groups. What is interesting about Cr content in the plants is that Fe-WTR, Al-WTR and lime significantly decreased Cr uptake only in seeps 3, while the differences are not significant in seeps 2 and 1 after applying any of the used amendments. It is apparent from Table 5-13 that Cu assimilation increased significantly in all study sites after applying each of the used amendments. Regarding Fe assimilation, for seep 1 and 2 there was no significant difference, however, the mean of Fe assimilation was highest in the control compared with all treatments. In seep 3 there was a statistically significant reduction after applying the treatments.

What stands out in the data of Mn content in the plant is that Mn assimilation was increased in seep 1 by a factor of almost 7 by Al-WTR and Fe-WTR treatments, while not being increased in lime amendment. Ni assimilation was significantly decreased in seeps 2 and 3 by Fe-WTR addition and lime addition, while, only in seep 2 there was a significant decrease in Ni assimilation by applying Al-WTR. It seems clear that P contents in ryegrass leaves grown in amended soils from seeps 1 and 3 were significantly or almost significantly greater than that at non-amended soil, whereas, significant decreases were observed in P content after applying the amendments for the soil from seep 2. It can be seen that both Fe-WTR and Al-WTR significantly decreased Pb assimilation in all seeps. S assimilation was significantly increased by adding Al-WTR and lime in all sites except in seep 3 for which the increase is not statistically significant. Conversely, there were significant decreases of S uptake from all seep soils using Fe-WTR amendment. Zn assimilation was significantly decreased by each of the amendments in all seep sites except seep 1, where no significant differences were identified.

Table 5-13: Mean of element content in plant tissue (mg/kg) or % w/w ± SEM, from the initial plant growth trial.

Element	Soil	Non-amended control	Al-WTR	Fe-WTR	Lime
Al	Seep 1	176.02±6.64	44.02±1.38*	51.81±5.98*	78.85±1.22*
	Seep 2	67.79±7.02	38.52±3.23$^{\#}$	33.23±0.58$^{\#}$	35.42±1.39$^{\#}$
	Seep 3	602.69±24.15	30.05±0.07*	32.49±0.06*	37.03±4.13*
As	Seep 1	0.14±0	1.31±1.12	0.98±0.65	0.28±0.05
	Seep 2	0.34±0.05	0.32±0.04	0.29±0.03	0.28±0.07
	Seep 3	0.27±0.09	0.10±0.01*	0.08±0.00*	0.09±0.01*
Ca%	Seep 1	0.15±0.00	0.78±0.02*	1.17±0.01*	0.89±0.00*
	Seep 2	0.30±0.00	0.55±0.00*	0.67±0.01*	0.64±0.05*
	Seep 3	0.27±0.00	0.85±0.00*	1.31±0.06*	1.26±0.09*
Cd	Seep 1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	Seep 2	0.06±0.05	0.00±0.00	0.00±0.00	0.00±0.00
	Seep 3	0.07±0.05	0.00±0.00	0.00±0.00	0.01±0.01
Co	Seep 1	0.55±0.01	0.20±0.00*	0.51±0.02	0.1±0.00*
	Seep 2	9.2±0.33	1.02±0.00*	1.3±0.09*	1.19±0.08*
	Seep 3	9.54±0.43	0.45±0.00*	0.63±0.01*	0.74±0.00*
Cr	Seep 1	1.37±0.24	1.4±0.13	1.32±0.15	3.87±2.7
	Seep 2	1.03±0.14	1.2±0.10	1.2±0.00	1.03±0.17
	Seep 3	2.47±0.30	1.31±0.12*	1.03±0.15*	0.75±0.06*
Cu	Seep 1	11.61±0.67	15.62±0.11*	13.5±0.42*	14.15±0.11*
	Seep 2	11.85±0.2	15.85±0.32*	14.54±0.09*	13.57±0.53*
	Seep 3	10.81±0.41	13.05±0.36*	12.41±0.61*	13.11±0.16*
Fe	Seep 1	522.19±37.11	179.24±22.02*	402.85±62.03	230.48±12.76*

	Seep 2	205.91±29.87	135.75±2.97	132.07±3.75	102.17±3.29
	Seep 3	1446.76±59.51	112.64±1.82*	112.82±1.39*	104.5±4.34*
K%	Seep 1	0.56±0.03	0.79±0.02*	0.94±0.02*	1.57±0.35*
	Seep 2	2.39±0.03	3.52±0.01*	3.13±0.01*	3.68±0.01*
	Seep 3	1.44±0.00	2.04±0.05*	2.31±0.14*	2.95±0.15*
Mg%	Seep 1	0.16±0.00	0.30±0.01*	0.32±0.00*	0.20±0.00*
	Seep 2	0.34±0.00	0.38±0.00*	0.36±0.00*	0.24±0.00*
	Seep 3	0.32±0.01	0.36±0.00	0.42±0.00#	0.24±0.01
Mn	Seep 1	45.3±0.43	339.45±8.32*	286.02±11.36*	60.17±3.26*
	Seep 2	372.05±0.51	283.03±3.45*	190.21±5.69*	101.89±2.05*
	Seep 3	298.41±4.85	342.40±4.48*	247.23±1.92*	125.34±7.93*
Ni	Seep 1	9.59±0.82	7.10±0.31	7.24±0.16	5.33±0.10
	Seep 2	37.33±2.17	28.24±1.66*	13.71±1.13*	6.59±0.17*
	Seep 3	28.98±5.54	14.92±2.5	7.55±0.35*	7.09±0.34*
P%	Seep 1	0.80±0.05	1.14±0.01*	0.95±0.01#	0.99±0.03
	Seep 2	0.97±0.01	0.79±0.01*	0.72±0.01*	0.74±0.01*
	Seep 3	0.61±0.03	0.88±0.02*	0.77±0.04*	0.82±0.00*
Pb	Seep 1	0.71±0.08	0.65±0.20*	0.58±0.03*	1.33±0.48
	Seep 2	0.65±0.17	0.26±0.02*	0.27±0.03*	0.32±0.05
	Seep 3	0.39±0.06	0.09±0.02*	0.09±0.01*	0.35±0.23
S%	Seep 1	2.61±0.03	3.65±0.06*	2.39±0.03*	4.12±0.13*
	Seep 2	4.82±0.13	5.89±0.05*	2.73±0.15*	5.17±0.09*
	Seep 3	4.44±0.09	4.57±0.23	2.71±0.05*	4.97±0.07#
Zn	Seep 1	74.22±4.89	65.05±4.79	53.6±1.28	45.91±0.24#
	Seep 2	72±1.09	59.99±0.31*	53.9±1.24*	61.88±0.58*
	Seep 3	79.54±1.67	52.66±0.40*	48.12±1.55*	49.42±0.31*

*Significantly different from non-amended (α 0.05) and # almost significantly different from non-amended (P less than 0.06).

The elements content in plant tissues in the trial following the wet-dry cycle (Table 5-14) reveal that again there was significant decreases in Al assimilation in all seeps after applying Fe-WTR and Al-WTR and lime. One-way ANOVA revealed that there was a significant decrease in As assimilation by Al-WTR addition, Fe-WTR addition and lime addition in all seeps, unlike in the initial plant test in which As concentrations were only decreased for seep 3. Consistent with the first plant trial, it can be seen from Table 5-14 that Ca, Mg and K assimilations increased significantly in all study sites after applying each of the used amendments, except Ca assimilation was not significantly increased in seep 3 after lime addition. The statistical tests did not reveal a significant difference in the Co assimilation between the groups but the mean of Co assimilation was highest in the non-amended compared with all treatments in seeps 2 and 3. Cr content in plant growth in Fe-WTR, Al-WTR and lime amended soils was significantly decreased in seeps 3, while the differences are not significant in seep 2 and 1 using Al-WTR and Fe-WTR amendments. Lime addition significantly decreased Cr uptake only in seeps 1 and 3.

It is apparent that Cu assimilation increased significantly in plants grown in Seep 1 and 3 soils amended with any and all of the amendments, but in seep 2 the increase was only significant with Fe-WTR treatment. One-way ANOVA indicated that there was no significant difference among the mean of Fe assimilation among groups, except that in seep 1 amended by Al-WTR and in seep 3 amended by lime were significantly less than the non-amended groups. Mn assimilation was significantly increased in seep 1 and seep 3 amended by Al-WTR and Fe-WTR, but conversely was significantly decreased in seep 2 when amended with either. There was a significant decrease in Mn uptake in plant growth in lime amendment soil in all seep soils.

Ni assimilation was significantly decreased by Al-WTR addition, Fe-WTR addition and lime addition in all seeps except in seep 1 with Al-WTR addition. Unlike in the first plant test, P contents in ryegrass leaves grown in amended soils from seep 1 and 3 were not significantly incresed from that at non-amended soil and instead there was no difference. However, consistent with the first test, significant decreases were observed in P content after applying the amendments for the soil from seep 2. Al-WTR and lime treatments significantly decreased Pb assimilation in seep 1 and 3 relative to untreated soils. Fe-WTR treatment significantly decreased Pb assimilation in seep 1, while, the decrease is not significant in seeps 2 and 3. The mean of Pb assimilation was highest in the non-amended compared with all treatments.

S assimilation was increased by adding Al-WTR but this was not statistically significant. S assimilation was increased by adding Fe-WTR, this increase was almost significant in seep 1, not significant in seep 2 and significant seep 3. There was no statistically significant difference in S assimilation in the lime amended group in seeps 2 and 3 whereas S assimilation in the lime amended group in seep 1 were significantly greater than that in non-amended. It seems that Zn assimilation in Ryegrass plant in Fe-WTR amended soil were significantly increased in seep 1 and 3 while the increase was not significant in seep 2. There was a significant increase in Zn assimilation only in seep 1 by adding Al-WTR, conversely, on seep 1 the Zn assimilation was decreased significantly by adding lime. There were no significant differences in Zn assimilation in seep 2 and 3 amended by Al-WTR and lime.

Table 5-14: Mean of element concentrations in plant following a wet/dry cycle (mg/kg)±SEM, in the trial following the wet-dry cycle.

Element	Soil	Non-amended	Al-WTR	Fe-WTR	Lime
Al	Seep 1	61.69±4.2	28.47±6.68*	10.68±0.05*	38.34±7.47
	Seep 2	20.02±2.31	11.12±1.09*	3.57±0.60*	5.24±0.45*
	Seep 3	153.7±4.69	9.65±0.83*	7.98±0.32*	8.09±1.76*
As	Seep 1	0.06±0.01	0.03±0.00*	0.03±0.00*	0.04±0.00*
	Seep 2	0.25±0.01	0.12±0.02*	0.06±0.00*	0.07±0.00*
	Seep 3	0.1±0.00	0.07±0.00*	0.06±0.00*	0.08±0.00*
Ca%	Seep 1	0.13±0.00	0.65±0.00*	0.69±0.00*	0.76±0.03*
	Seep 2	0.26±0.01	0.55±0.02*	0.49±0.02*	0.57±0.01*
	Seep 3	0.21±0.00	0.65±0.00*	0.68±0.00*	0.07±0.00*
Co	Seep 1	0.31±0.00	0.13±0.00*	0.53±0.00*	0.04±0.00*
	Seep 2	5.22±0.16	0.14±0.00*	0.58±0.03*	0.58±0.01*
	Seep 3	3.29±0.03	0.02±0.01*	0.28±0.01*	0.29±0.03*
Cr	Seep 1	1.24±0.21	1.05±0.20	0.97±0.05	0.76±0.05*
	Seep 2	1.25±0.17	0.98±0.10	1.01±0.05	1.15±0.10
	Seep 3	1.97±0.14	1.53±0.20*	1.31±0.18*	1.08±0.06*
Cu	Seep 1	8.85±0.32	12.11±0.38*	15.36±0.1*	10.53±0.28*
	Seep 2	10.73±0.32	11.43±0.13	11.87±0.14*	10.88±0.00
	Seep 3	8.03±0.25	11.32±0.14*	11.94±0.01*	9.85±0.37*
Fe	Seep 1	349.56±40.16	131.61±18.23*	133.41±8.53*	132.63±1.92*
	Seep 2	91.61±14.26	91.54±3.74	65.38±1.01	57.01±0.21
	Seep 3	79.4±2.34	71.8±3.19	77.21±0.56	62.09±0.75*
K%	Seep 1	1.00±0.02	1.65±0.00*	1.82±0.03*	2.82±0.26*
	Seep 2	1.84±0.10	3.65±0.12*	3.74±0.10*	3.67±0.03*
	Seep 3	1.44±0.04	2.16±0.11*	2.62±0.01*	1.66±0.34

Mg%	Seep 1	0.15±0.00	0.26±0.00*	0.31±0.00*	0.17±0.00*
	Seep 2	0.29±0.01	0.32±0.01	0.23±0.00#	0.19±0.00#
	Seep 3	0.17±0.00	0.27±0.00*	0.26±0.00*	0.20±0.00*
Mn	Seep 1	52.27±2.37	286.86±0.26*	327.51±11.33*	34.17±2.2*
	Seep 2	274.84±5.33	274.21±11.87	153.35±3.67*	76.48±2.15*
	Seep 3	104.7±0.06	243.71±2.4*	234.15±0.67*	71.48±0.27*
Ni	Seep 1	7.1±0.31	5.33±0.1*	6.23±0.28	3.51±0.34*
	Seep 2	28.24±1.66	6.59±0.17*	6.58±0.46*	6.82±0.54*
	Seep 3	14.92±1.77	7.09±0.24*	6.57±0.08*	5.77±0.27*
P%	Seep 1	1.14±0.01	1.21±0.05	1.15±0.02	1.06±0.04
	Seep 2	1.38±0.02	1.01±0.04*	0.86±0.01*	0.87±0.01*
	Seep 3	1.07±0.02	1.12±0.02	1.07±0.02	0.92±0.01
Pb	Seep 1	0.37±0.02	0.14±0.03*	0.08±0.00*	0.08±0.01*
	Seep 2	0.11±0.01	0.07±0.01	0.06±0.01	0.03±0.00
	Seep 3	0.11±0.00	0.05±0.00*	0.08±0.02	0.04±0.00*
S%	Seep 1	2.17±0.04	2.93±0.04	3.57±0.16$^{\#}$	4.11±0.35*
	Seep 2	3.69±0.05	4.07±0.22	4.10±0.11	3.31±0.05
	Seep 3	2.92±0.06	3.19±0.05	3.75±0.11*	2.92±0.21
Zn	Seep 1	42.51±0.34	51.22±1.31*	64.45±1.53*	27.7±1.47*
	Seep 2	54.16±1.36	54.47±1.01	55.63±1.51	53.12±1.19
	Seep 3	48.3±0.32	47.5±0.54	58.77±0.43*	43.14±1.7

*Significantly different from non-amended (α 0.05)

5.3.2.3 Earthworm trial (survival and element uptake)

Survival percentages of earthworms were high in the procedural control soil samples (91±4%), meeting the validity criteria of the protocol (data not shown). In the initial earthworm trial, there was no survival (100% mortality) of earthworms in the non-amended seep soils (Table 5-15). The highest survival percentages (100%) were observed in lime amended soils in all seep soils. Survival rates were significantly increased in Al-WTR and Fe-WTR treatments of seep 1 soil, with values of 80% and 40% respectively. In seep 2, the mean of survival percentage by Al-WTR addition and Fe-WTR addition also increased, to 53.33±29.05 and 20.0±11.54 respectively. A high survival rate of 85%±9.57, with significant differences from untreated control (ANOVA $p>0.05$), was observed in Fe-WTR treated seep 3 soil, but no improvement was found for seep 3 soil amended with Al-WTRs.

In the second trial, i.e. following the wet-dry cycle, it seems that overall the general pattern was similar in that treatment with WTRs or lime greatly increased survival of earthworms. The exception is that in the post wetting-drying cycle trial all worms died in Seep 1 soil, the reason for which is not clear and warrants further investigation. There is a possibility that the wet/dry cycle (and possibly the re-grinding that followed), triggered the release of a toxic component and/or otherwise altered the conditions in seep 1 soils rendering them too hostile for the earthworms.

Table 5-15: Earthworm survival percentage (mean±SE).

Site	Non-amended	Al-WTR	Fe-WTR	Lime
----------------------Survival percentages of earthworms in Original trial--------------------				
Seep 1	0.00±0.00	80±8.16*	40±8.16*	100±0.00
Seep 2	0.00±0.00	53.33±29.05	20±11.54	100±0.00
Seep 3	0.00±0.00	0.00±0.00	85±9.57*	100±0.00
-------------Survival percentages of earthworms in the trial after wet-dry cycle----------				
Seep 1	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Seep 2	13.33±13.33	80±20.00≠	100±0.00*	93.33±6.66*
Seep 3	0.00±0.00	60±20.00	100±0.00*	100±0.00*

*Significantly different from non-amended P value < 0.05; ≠ P value = 0.06.

Table 5-16 shows the water content of worms recovered from the soil samples from the initial trial. It seems clear that the surviving earthworms were in good health condition with rather consistent water contents, which can be another good indicator that adding these amendments enhances the soil condition.

Table 5-16: Water content (% w/w) of worms from the initial trial.

Treatment	Al-WTR	Fe-WTR	Lime
Seep 1	82%*	85%	88%
Seep 1	86%	83%	87%
Seep 1	84%	82%	86%
Seep 2	N.A#	N.A	85%
Seep 2	87%	80%	87%
Seep 2	86%	86%	84%
Seep 3	N.A	71%	86%
Seep 3	N.A	93%	86%
Seep 3	N.A	84%	84%
Mean	Mean 85.0%	Mean 83.2%	Mean 86.4%

*Note that individual replicate values are shown because the non-survival in some replicates could otherwise distort the data presentation. # N.A. represents not available. Not determinable because there were no surviving earthworms.

Elemental analysis of earthworms was, by definition, restricted to those treatments from which earthworms were recovered. There were no surviving earthworms in the non-amended seep soils and therefore there are no values for the measured elements from those samples. This means it is not possible to compare element uptake in treated vs non-treated seep soils. Table 5-17 therefore shows element concentrations in earthworms from the amended samples only.

Analysis of Variance was tried between the three amended groups within each trial, where data were sufficient and met the background assumptions of normality, etc. However, the amount of viable data limited this approach, as did the variability across replicates, and so most of the results of the analysis of variance between amended groups were not significant except in a few cases. Nevertheless, some patterns appeared; in both trials the addition of Al-WTRs resulted in higher mean Al concentrations than were observed in the other treatments (where data is available to directly compare, Table 5-17 and Table 5-18), indicating that a portion of the Al in the WTRs was bioavailable. The increase in Fe in Fe-WTR amended samples was much less distinct, and only apparent in the tissues of earthworms from the trial post wetting / drying. Unsurprisingly, in both trials, the addition of lime increased the Ca concentrations in earthworms.

Table 5-17: Mean of element contents in earthworm tissue (mg/kg or µg/kg, as indicated) ± SEM.

Element	Site	Al-WTR	Fe-WTR	Lime
Al mg/kg	Seep 1	290.20±181.381	33.55±8.5	121.18±27.44
	Seep 2	603.78±178.56	380.14±350.26	207.64±34.56
	Seep 3	N.R	431.02±60.62*	227.96±75.37
As µg/kg	Seep 1	B.D	3.69±3.69	4.16±2.2
	Seep 2	11.14±3.84	B.D	B.D
	Seep 3	N.R	B.D	4.24±3.03
Ca mg/kg	Seep 1	1693.03±144.99	1890.22±258.86	6166.19±698.69
	Seep 2	1989.83±231.28	10148.77±9234.85	8106.78±1048.33
	Seep 3	N.R	2796.43±386.15	6776.81±2625.36
Co µg/kg	Seep 1	6.19±1.49	18.63±7.85	1.92
	Seep 2	1.24	2.01±0.42	2.86±1.75
	Seep 3	N.R	4.16±1.41	1.11±0.49

Cr µg/kg	Seep 1	4.79±2.88	27.19±8.36	B.D
	Seep 2	B.D	B.D	4.29
	Seep 3	N.R	2.77±1.18	B/D
Cu µg/kg	Seep 1	19.9±1.87	40.22±12.45	15.23±2.96
	Seep 2	12.15±2.29	6.12±2.62	10.27±0.27
	Seep 3	N.R	9.59±1.64	4.53±1.14
Fe mg/kg	Seep 1	6585.79±4833.78	1252.11±274.93	10250.5±2544.85
	Seep 2	3722.8±722.61	7767.2±N.R	6317.71±714.82
	Seep 3	N.R	8657.76±779*	3387.83±461.58
K mg/kg	Seep 1	7045.24±111.77	7283.72±651.26	6417.23±389.89
	Seep 2	6613.93±583.44	9074.32±4198.43	6364.92±449.61
	Seep 3	N.R	6087.23±614.85	4995.38±1066.11
Mg mg/kg	Seep 1	462.09±55.9	319.04±83.49	402.33±7.96
	Seep 2	427.44±18.73	797.64±651.12	613.38±48.3
	Seep 3	N.R	663.44±56.87	499±125.48
Mn mg/kg	Seep 1	28.78±10.11	31.59±7.48	5.55±1.68
	Seep 2	17.56±3.5	19.16±9.05	21.13±1.98
	Seep 3	N.R	40.69±4.46*	13.94±0.46
Ni µg/kg	Seep 1	1.91±0.88	8.3±4.18	1.55±0.92
	Seep 2	0.93±0.93	B.D	2.99±0.97
	Seep 3	N.R	3.22±1.37	0.64±0.34
Pb µg/kg	Seep 1	4.26±1.6	2.86±1.95	4.16±0.5
	Seep 2	1.21±0.33	9.4±3.45	10.64±2.88
	Seep 3	N.R	4.71±1.33	3.68±1.88
Zn µg/kg	Seep 1	75.63±6.54	105.7±29.35	55.77±5.33
	Seep 2	56.79±9.2	133.67±9.3	66.95±6.22
	Seep 3	N.R	72.51±10.84	49.04±7.46

*significantly different ($p<0.05$) from Al-WTR. B.D refers to below detection, N.R represents not reported because no earthworms survived hence element analysis was not possible.

Table 5-18: Mean of element concentrations in earthworm tissue following a wet/dry cycle (mg/kg or µg/kg, as indicated)±SEM

Element	Site	Al-WTR	Fe-WTR	Lime
Al mg/lkg	Seep 1	N.R	N.R	N.R
	Seep 2	1329.26±616.13	574.27±36.27*	218.4±8.28*
	Seep 3	723.61±602.8	499.96±167.28	217.56±53.55
As µg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	0.50±0.17	0.58±0.06	0.46±0.25
	Seep 3	0.46±0.04	0.5±0.18	0.63±0.10
Ca mg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	4382.54±147.7	4721.19±335.89	6652.13±663.48
	Seep 3	3015.1±510.68	3276.77±330.02	5410.34±478.9
Cd µg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	6.21±1.99	0.58±0.58	6.2±5.05
	Seep 3	1.58±1.58	0.87±0.87	0.00±0.00
Co µg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	2.43±2.43	0.00±0.00	4.05±4.05
	Seep 3	1.58±1.58	0.87±0.87	0±0
Cr µg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	2.88±1.44	0.00±0.00	7.07±5.5
	Seep 3	5.77±2.96	3.64±2.30	2.80±0.61
Cu µg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	18.15±1.42	9.96±0.67	1.4±1.4*
	Seep 3	1.77±1.77	2.08±2.08	0.00±0.00

Fe mg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	9057.67±2929.46	19533.93±1566.92*	6112.58±724.09*
	Seep 3	2263.22±1511.04	6572.04±973.25	2398.69±445.07
K mg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	5963.13±515.11	5727.94±182.75	63387.02±29159.54
	Seep 3	103125.32±19210.66	95517.02±7713.3	80982.49±3675
Mg mg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	720.48±35.26	654.05±35.24	608.54±49.09
	Seep 3	659.04±9.72	823.98±39.18	630.43±39.39
Mn mg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	59.29±10.38	98.47±5.38*	30.37±5.82*
	Seep 3	32.92±4.37*	48.41±7.24	12.85±1.61*
Ni µg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	7.72±2.16	1.83±0.59	3.5±3.5
	Seep 3	1.58±1.58	0.87±0.87	0.00±0.00
Pb µg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	10.12±2.27	7.21±1.39	7.44±5.58
	Seep 3	2.39±2.06	1.58±1.39	0.73±0.73
Zn µg/kg	Seep 1	N.R	N.R	N.R
	Seep 2	N.R	N.R	N.R
	Seep 3	N.R	N.R	N.R

*significantly different ($p<0.05$) from Al-WTR

5.3.2.4 Simulated soil solutions–element concentrations

Element concentrations in simulated soil solutions are shown in Table 5-19. It can be seen that all treatments significantly reduced the soil solution Al concentration, with Fe-WTRs and lime being the most effective. The As concentrations were marginally increased in seep

1 soil following Al-WTRs and Fe-WTRs amendment, but were more substantially increased by lime treatment. The other seep soils did not have significantly different As concentrations in their soil solutions following treatment. The WTR treatments significantly reduced Cd concentrations in Seep 3 solutions, from ~11 µg/l to <0.25 µg/l, but had negligible influence on the other seeps (which had much lower initial Cd concentrations). The concentrations of Co were generally greatly reduced by all amendments, except in the case of seep 1 amended with Fe-WTRs which had a significant increase from ~100 to 330 µg/l. Fe, Ni and Zn concentrations were all generally reduced by each of the treatments whereas K was increased in every case, with the degree of change rather variable amongst the treatments and amongst the various seep soils (Table 15-9). Addition of the amendments generally decreased the concentration of Pb or had no effect, except for the lime treatment in seep 1 which resulted in a much higher concentration than in the untreated sample (i.e. ~161 µg/l vs < 1 µg/l).

Table 5-19: Mean of element concentrations (µg/l or mg/l) in simulated soil solutions,

Element	Site	Non-amended	Al-WTR	Fe-WTR	Lime
Al mg/l	Seep 1	154.39±44.36	54.07±8.32	6.96±2.93*	1.05±0.21*
	Seep 2	3.38±1.6	0.3±0.20#	0.07±0.00#	0.06±0.00#
	Seep 3	34.85±11.27	0.22±0.05#	0.07±0.00#	N.R
As µg/l	Seep 1	2.86±0.21	3.54±0.38*	4.09±0.2*	12.2±0.44*
	Seep 2	19.68±5.82	12.61±1.95	7.5±2.61	6.52±0.87
	Seep 3	12.24±1.56	23.63±6.94	9.98±2.1	N.R
Ca mg/l	Seep 1	201.21±12.5	240.54±3.86	236.57±1.16	231.88±0.25
	Seep 2	247.4±0.44	253.53±1.78*	252.09±1.78*	268.72±0.94*
	Seep 3	251.37±1.27	249.96±8.46	269.41±0.25*	N.R
Cd µg/l	Seep 1	5.89±2.61	2.78±0.28	7.35±3.87	0.02±0.15

	Seep 2	1.57±0.98	0.00±0.00	0.00±0.00	0±0
	Seep 3	11.21±3.98	0.22±0.16*	0.00±0.00*	N.R
Co µg/l	Seep 1	99.76±7.68	48.45±4.03*	331.76±58.07*	0.36±0.17*
	Seep 2	550.27±66.48	10.07±2.04*	40.37±9.32*	0.02±0.01*
	Seep 3	538.89±63.46	4.39±0.9*	47.7±2.36*	N.R
Cr µg/l	Seep 1	3.54±0.67	4.99±0.91	7.37±1.82	0.29±0.16
	Seep 2	1.24±0.07	0.00±0.00	0.00±0.00	0.00±0.00
	Seep 3	17.19±2.89	2.18±0.41	0.58±0.02	N.R
Cu µg/l	Seep 1	48.52±16.21	28.31±2.04	39.23±11.73	81.42±21.19
	Seep 2	8.48±2.49	3.47±0.92	2.85±0.61	1.44±0.37*
	Seep 3	24.58±11.12	3.01±0.79	1.94±0.5*	N.R
Fe mg/l	Seep 1	913.32±170.23	96.87±16.85*	670.15±289.76	205.83±76.54$^{\#}$
	Seep 2	327.42±53.91	61.15±10.27*	65.79±2.67*	0.00±0.00*
	Seep 3	395.29±77.6	42.92±15.15*	95.95±12.85*	N.R
K mg/l	Seep 1	8.32±1.68	555.48±45.13*	156.91±43.92*	5546.39±131.27*
	Seep 2	254.96±18.65	446.62±41.31*	408.81±21.69*	327.97±14.73*
	Seep 3	51.57±2.42	151.84±21.42*	142.12±9.94*	N.R
Mg mg/l	Seep 1	93.96±5.07	135.79±2.51*	159.31±6.54*	98.81±8.98
	Seep 2	261.17±1.73	198.12±12.67*	209.06±13.46*	124.05±5.09*
	Seep 3	231.68±0.92	140.76±14.93*	158.72±1.56*	N.R
Mn mg/l	Seep 1	9.99±0.76	60.7±2.85	114.25±16.69	0.23±0.04
	Seep 2	68.9±2.61	17.97±2.98*	27.66±4.21*	0.17±0.01*
	Seep 3	45.49±1.75	18.18±7*	32.43±0.62*	N.R
Ni µg/l	Seep 1	962.35±73.95	124.46±13.33*	501.59±176.86*	6.06±1.65*
	Seep 2	2425.22±248.02	22.03±2.79*	53.76±10*	11.06±0.29*
	Seep 3	2289.93±261.57	36.55±6.7*	76.06±6.44*	N.R
Pb µg/l	Seep 1	0.54±0.2	0.83±0.57	0.79±0.35	161.05±89.51

	Seep 2	4.86±1.01	2.67±1.59	0.47±0.14*	0.8±0.32*
	Seep 3	5.69±1.03	1.09±0.34*	0.31±0.13*	N.R
Zn µg/l	Seep 1	1182.15±133.4	415.46±43.32*	1128.03±342.36	26.76±1.57*
	Seep 2	1453.84±341.3	25.2±2.19*	50.12±12.78*	6.19±0.9*
	Seep 3	2420.86±471.37	27.39±2.48*	46.97±3.62*	N.R

≠ P value = 0.06, * P value < 0.05 (indicating significant difference from non-amended control), N.R. = not reported due to sample loss.

5.4 Discussion

5.4.1 Adsorption and desorption

The first step in this study sought to determine the adsorption characteristics of Zn and Pb on WTRs, in the context of prior studies such as Chiang et al. (2012) and Hua et al. (2015) who had noted the potential utility and importance of WTRs in immobilising some metal contaminants. Successful immobilisation of potentially toxic elements in contaminated soil would limit their ecological impacts and thus addition of WTRs could be a cheap and effective way to ameliorate mining contaminated soils, therefore determining their sorption and retention capacity is important to assess this beneficial use.

The maximum realised sorption under the conditions imposed in the experiment were 4025 mg/kg and 3980 mg/kg for Pb on Al-WTRs and Fe-WTRs respectively while for Zn the maximum was 3579 mg/kg on each of Al-WTRs and Fe-WTRs. This did not reflect maximum potential sorption capacities (see theoretical sorption capacity calculations and related discussion below), but does provide definitive values of directly measured sorption that adds to the growing literature on WTR sorption (e.g. (Chiang et al., 2012, Zhou and Haynes, 2011, Silvetti et al., 2015), all of which points toward strong Pb and Zn sorption. It is interesting that the Al-WTRs and the Fe-WTRs both performed equally at sorbing Zn, whereas the study by Chiang et al. (2012) found that Fe-WTRs outperformed Al-WTRs in this regard. This might be due to other differences between the plants from where Chiang and partners obtained their WTRs, i.e. different raw water characteristics and/or different supporting electrolytes or levels of lime added during water treatment, which could have made a greater difference between their WTRs beyond just that created from Al vs Fe coagulants.

The Zn adsorption isotherms (Figure 5-3) clearly indicated a high affinity of both WTR materials for this metal. However, although there was a very high adsorption efficiency across the entire range of concentrations imposed (always >93%,Table 5-3), there was a relative decrease in the adsorption efficiency at higher concentrations which, based on the Temkin model, might be attributed to a process in which the heat of adsorption of all the molecules in the layer decreases linearly with increase in the coverage of WTRs surface due to adsorbent–adsorbate interactions (Bonilla-Petriciolet et al., 2017). It could also be simply due to, presumably, a decrease in the reaming available adsorbing sites as the amount of surface-bound Zn increases. It is also possible that a combination of these factors was at work.

The shapes of the isotherms for adsorption of Zn onto both WTRs, as well as Pb onto Fe-WTRs, are similar to the type I isotherm. This behaviour is similar to that of microporous materials with small width pores which prevents multilayer adsorption (Lowell et al., 2012), as this consequently limits the amount adsorbed. The nature of the pores within WTRs was examined in part by research done by Makris et al. (2004), who noticed that the carbon dioxide gas adsorption (232 picometers) was greater than that of N_2 (370 picometers) suggesting steric restriction of N_2 diffusion by narrow micropore openings. If WTRs typically have micropores of this scale, steric restriction might be the reason that multilayer adsorption is potentially prevented on WTRs because zinc hydrated ionic radius was calculated to be 430 picometers (Oter and Akcay, 2007) and lead hydrated Ionic radius was calculated to be 401 picometers, see Table 5-21 (Petrella et al., 2017).

The shape of Pb adsorption isotherm onto Al-WTRs is similar to the Henry (linear) isotherm which is valid for low surface saturation coverages (Q_e/Q_m) (e.g. at (Q_e/Q_m)less than 0.1). This suggests that for Pb the Al-WTRs had a great reserve capacity for further adsorption. It is known that Pb has more affinity for organic matter than that of Zn, based on a typical affinity sequence of organic matter for metals (at pH 5) (McBride, 1989), however the organic matter (OM) contents of the two WTRs were similar (28% and 26% by mass) and so it seems unlikely any differences in the OM% can account for the different shaped isotherms. A chemical precipitation / fixation process might have been involved, as has been indicated in studies with rock surfaces employing scanning electron microscope with Energy Dispersive X-Ray Spectroscopy (Németh et al., 2016). It was noticed that heavy metals were precipitated (as hydroxide form) from test solutions on the surface of host rock minerals (calcite, volcanic glass) and within pores. Such a process may similarly happen on the surfaces of WTRs and the degree to which it occurs might be influenced by their constituents (i.e. Al-WTRs vs Fe-WTRs). However, as high adsorption was observed with both WTRs used in the present study it is possible that a chemical precipitation might contribute to the high removal of both Pb and Zn in the case of both WTR types in addition to the adsorption processes. In relation to that, it is worth noting that based on values of standard electrode potential E° for Pb and Al in an electrochemical series, reduction reactions of the Pb could possibly occur in the presence of Al. Such a process would result in tight binding of Pb to the surface that would not be easily dislodged (i.e. strong retention).

The experimental data for adsorption of Zn onto WTRs did fitt D-R model better than Langmuir model, and the experimental data for adsorption of Pb onto WTRs did not fit Langmuir model, therefore, the maximum adsorption capacity will be calculated based on D-R equation. As alluded to above, theoretical isotherm saturation capacity values were greater for Pb (viz. 2.82 mg/g on Fe-WTRs and 6.08 mg/g on Al-WTRs) than for Zn (2.34 and 2.31 mg/g, respectively). Hydrated cationic radius for the metal ions play an important role in the adsorption capacity; for example, the radius of the hydrated ions can limit its passing to the pores of the adsorbent if it is bigger than the radius of pores. Oter and Akcay (2007) found that the adsorption capacity of Pb onto a Zeolite is greater than that of Zn and they had attributed their findings to that Pb has the smaller hydrated cationic radius than hydrated cationic radius for Zn. If that was applied to the present study, it may be that Pb can pass more easily through the pores of Al-WTRs than Zn and that might be part of the explanation for the higher adsorption maximum. Both WTR presumably are microporous material as were inferred form the adsorption isotherms of Zn into both WTR types.

There might be another explanation linked to differences in surface areas, even though WTRs in general are considered to have high specific surface areas. For example, as Mohammed et al. (2016) found in a survey of WTRs from 14 plants in the UK, the mean specific surface areas were 327.9 ± 115.50 m^2/g and 184.8 ± 95.21 m^2/g for Al-WTR and Fe-WTR respectively. The specific surface area affects the number of binding sites (Segalini et al., 2012) and so the differences in the theoretical sorption capacity and the shape of the isotherm for Pb onto Al-WTRs might be linked to a higher specific surface area.

The maximum capacity determined from D-R Isotherm model in the present study were compared with those for Zn and Pb adsorption capacities of some other comparable adsorbents reported in the literature (see Table 5-20). It is interesting to note that Fe-WTR and Al-WTR have Q_m values for Zn greater than that of chemically modified *Pinus Pinaster* bark, *Chlorella minutissima,* Carbon aerogel, Hazelnut shells, tannic acid immobilized activated carbon, Low rank Turkish coal, and sugar beat pulp, but Fe-WTR and Al-WTR have Q_m values for Zn that were less than that of Low-grade phosphate and Fly ash. Al-WTR has Q_m for Pb greater than that of Activated carbon and chemically modified *Pinus Pinaster* bark and Fe-WTR has Q_m for Pb greater than that of chemically modified *Pinus Pinaster* bark. Both Al-WTR and Fe-WTR have Q_m for Pb less than that of Goethite, Hematite, and *Chlorella minutissima*. These comparisons reveal the potential of WTRs for environmental applications because the WTRs are in great abundance and have adsorption capacities better than some renewable materials such as pine bark and have values approaching those (if slightly lower) of important finite geological resources such as goethite which require much extraction and processing.

Table 5-20: The maximum monolayer coverage capacity (mg/g) for Zn using Langmuir Isotherm model for this study in compared with that of others in the literatures. Q_m calculated using Langmuir Isotherm; 1/n calculated using Freundlich isotherm model.

Sorbent material	Reference	Zn		Pb	
		Q_m	1/n	Q_m	1/n
Low-grade phosphate	(Kandah, 2004)	10.32	0.57		
Sugar beat pulp	(Pehlivan et al., 2006)	0.18	0.99		
Fly ash	(Pehlivan et al., 2006)	11.11	0.94		

Low rank Turkish coal	(Karabulut et al., 2000)	1.66			
Tannic acid immobilized activated carbon	(Üçer et al., 2006)	1.23	0.36		
Hazelnut shells	(Cimino et al., 2000)	1.78	0.52		
Carbon aerogel	(Meena et al., 2005)	1.18	0.67		
Activated carbon	(Teles de Vasconcelos and Gonzalez Beca, 1994)	-	-	2.95	-
Chlorella minutissima	(Roy et al., 1993)	0.78		9.74	
Goethite	(McKenzie, 1980)			10.57	
Hematite	(McKenzie, 1980)			11.19	
Rice hulls	(Roy et al., 1993)			11.4	
Chemically modified *Pinus Pinaster* bark	(Vazquez et al., 1994)	1.18	0.58	1.59	0.27
Fe-WTR	This study	2.34*		2.82*	
Al-WTR	This study	2.31*		6.08*	

*calculated using D-R Isotherm.

Generally, adsorption is accompanied by a decrease in the residual forces of the surface onto which something is being adsorbed. This implies that some energy is released when the adsorbate bonds to the surface and that the change in enthalpy is negative for each adsorption process. The degree of freedom of the adsorbate decreases upon adsorption which means that the change in the entropy is also negative for the process. It is known that for any thermodynamic process to be spontaneous the Gibbs free energy must be negative: $\Delta G=\Delta H-T\Delta S$. The value of ΔG can be negative if ΔH is sufficiently negative since $-T\Delta S$ is positive for the adsorption process (Dada et al., 2012, Yildirim, 2006). Bearing this in mind, using the Temkin model (which was the best fitting model for adsorption of Zn onto both WTRs as well as for Pb onto Fe-WTRs), the Free energy of the process can be calculated. That is, applying the Temkin adsorption constant in the classical Van’t Hoff equation can calculate standard Gibbs free energy for the process. Standard Gibbs free energy values for adsorption of Zn onto Fe-WTR and Al-WTR were -13.24 and -13.11, respectively, while for adsorption of Pb onto Fe-WTR and Al-WTR were -17.56 and -15.67, respectively. Generally, the ΔG (NOT ΔG°) value of physisorption is in the range of 0 to −20 kJ/mol (Húmpola et al., 2013) and this does not necessarily indicate that the processes are physiosorption because the calculated values represent standard Gibbs free energy value not the Gibbs free energy, see Equation 5-7 to Equation 5-9. These findings also indicate that the processes were spontaneous and the processes were thermodynamically favourable.

$$\Delta \mathrm{G} = \Delta \mathrm{G}^{\circ} + \mathrm{RT} * \ln(X) \qquad \text{Equation 5-7}$$

$$\Delta \mathrm{G} = -\mathrm{RT} * \ln\left(K_{eq}\right) + RT * \ln(X) \qquad \text{Equation 5-8}$$

$$\Delta \mathrm{G} = \mathrm{RT} * \ln(X/K_{eq}) \qquad \text{Equation 5-9}$$

Where X refers to mass action quotient (the values of products and reactants that are present before the adsorption reaction) before equilibrium has been reached.

The removal efficiency (% adsorption) of the competitive adsorption of Zn-Pb ions test (as was presented in Table 5-7) demonstrated that the Pb ions presented a higher affinity for the cationic binding sites of the Al-WTR and Fe-WTR than that for Zn ions. The difference in adsorption percentages was not a big difference but nevertheless it might be related to the metal ions' properties. For example, difference in electronegativity, free energy of hydration and hydrated radii of the metal ions, the differences between which were discussed above, might be responsible for the small difference in selectivity observed in this study, see Table 5-21. For example, Üçer et al., (2006) observed a difference in adsorption of Cu^{2+} compared with that for Cd^{2+} on tannic acid activated carbon and they attributed those findings to the difference in electronegativity of the metal ions. Lo et al. (2012) found a similar trend for adsorption of Cu and Cd onto bamboo activated carbon.

Table 5-21: Data on ionic radii, hydrated radii, hydration energy and electronegativity.

Metal	Ionic radius (Pecometer)[a]	Hydrated radius (Pecometer)[b]	Hydration energy (kJ/mol)	Electronegativity
Pb^{2+}	119	405	-1481	2.33
Zn^{2+}	74	430	-2046	1.65
Ca	106	348	-1577	1.00

[a] From (Shannon, 1976). [b] From (Nightingale Jr, 1959). [c] From (Lide, 2016).

It may be that the ions with the greatest charge density (ratio of charge to ionic radius) or the most electronegative is first adsorbed and if there are still available sites, then the lower charge density or lower electronegative ion is adsorbed in sequence. The order of the electronegativity for the studied metal ions was Pb(2.33)> Zn(1.65), see Table 5-21, and this might also be part of the reason for the adsorption of the Pb being more than that for Zn. Allen and Brown (2000) thought that the competition of metal ions were a function of one or all of the following parameters: electronegativity, ionic radii and hydration capacity.

When a hydrated ion is subjected to electrostatic interactions, the rate of the solvent exchange between the hydration shells of an ion and the bulk of the water is determined by two factors. They are the ligand field stabilization and the electric field of the ion. The electric field is the charge density around the ion which is the ratio of the charge of the ion to its radius. The electric field determines the rate of the solvent exchange in a way that the larger the field strength, the slower the exchange (Marcus, 1988, Burgess, 1999). Based on the data in Table 5-21 the charge density around Zn^{2+} is greater than that around Pb^{2+}, therefore, the exchange of solvent (H_2O) around Pb is faster than that along to Zn, and this is another potential explanatory factor for the adsorption of the Pb being slightly more than that for Zn.

Desorption of Pb and Zn

As alluded to previously in chapter 1, metal cations contact with adsorbent surface sites either through an electrostatic bond of low energy called outer-sphere complex or through a stronger ionic or covalent bond directly with a surface functional group energy called inner-sphere complex. The latter can be monodentate when a ligand e.g. oxygen on the surface donate a single pair of electrons to a metal atom and can be bidentate when two ligands on the surface are bound to the metal (Sparks, 2003). Quantitatively important inner sphere complexes have been directly noticed for adsorption of Pb to Al, Fe and Mn (hydr)oxides as well as for Zn to Al, Fe and Mn (hydr)oxides in the literature, as shown in Table 5-22. Therefore, the very low percentages of desorption ($CaCl_2$-extractable) observed might be attributed to the formation of inner-sphere Zn and Pb complexes that tightly bind them.

In the presence of a ligand such as SO_4^{2-}, CO_3^{2-}, can occur in which the ligand is between the surface functional group and the metal, acting as a bridge in a so-called ternary complex. When this occurs the solubility of metals and ligand drop below those expected from either adsorption or precipitation alone (Roberts et al., 2005), thus the ligands present in the WTRs would also enhance the sorption and retention. Adsorption of Pb onto Goethite in presence of SO_4^{2-} at pH 3–7 were researched by Ostergren et al. (2000) (2000a). Based on X-ray absorption fine structure (XAFS), Attenuated total reflection Fourier-transform infrared (ATR-FTIR) data, they found inner-sphere bidentate binding due to ternary complex formation. Such a process may similarly happen here and might be a reason for very low percentages of desorption ($CaCl_2$-extractable).

Inner-sphere bidentate was noticed Zn to Alumina powders as well as mixed metal–Al hydroxide surface precipitates were observed at 7–8.2 using XAFS (Trainor et al., 2000). Such a process may similarly happen here and might be a reason for very low percentages of desorption ($CaCl_2$-extractable).

Table 5-22: Mechanisms of Pb and Zn adsorption to Al, Fe and Mn (hydr)oxides

Metal-Adsorbent	Adsorbent mechanism	PH	Analytical techniques.	Reference
Pb to γ-Al_2O_3	Inner-sphere monodentate mononuclear	6	XAFS	(Chisholm-Brause et al., 1990)
Pb to γ-Al_2O_3	Inner sphere bidenate, Surface polymers	6.5	XAFS	(Strawn et al., 1998)
Pb to Al_2O_3 powders	Inner-sphere bidentate mononuclear and Dimeric surface complexes	6 and 7	XAFS	(Bargar et al., 1997a)

Pb to Goethite and hematite	Inner-sphere bidentate	6–8	XAFS	(Bargar et al., 1997b)
Pb to Goethite	Inner-sphere	Variable	XAFS	(Roe et al., 1991))
Pb to Ferrihydrite	Inner-sphere bidentate	5	XAFS	(Scheinost et al., 2001)
Pb to Birnessite	Inner-sphere mononuclear	3.5	XAFS	(Matocha et al., 2001)
Pb to Manganite	Inner-sphere mononuclear	6.7	XAFS	Matocha et al. (2001)
Pb to Montmorillonite	Inner-sphere	6.77	XAFS	Matocha et al. (2001)
Zn to Alumina powders	Inner-sphere bidentate Mixed metal–Al hydroxide surface precipitates	7–8.2	XAFS	(Trainor et al., 2000)
Zn to Manganite -	Multinuclear hydroxo complexes or Zn-hydroxide phases	6.17–9.87	XAFS	(Bochatay and Persson, 2000)
Zn to Al, Fe and Mn (hydr)oxides oxides	Inner-sphere adsorption complexes	3.2	XAFS, XRD and EM	(Roberts et al., 2002)
Zn to Al_2O_3	inner-sphere mode (mononuclear bidentate complexes) to edges of AlO_6 octahedra	6 and 7	XAFS	(Bargar et al., 1997a)

5.4.2 Effects of WTR addition on soil characteristics

Addition of the WTR amendments resulted in negligible or marginal differences in total organic matter (OM) content of the tested soils as determined by loss on ignition (LOI).

However, the LOI method does not indicate the ecological importance of the OM determined and does not distinguish ecologically active OM from combustible materials left behind from a coal mining history. Therefore, total OM is not the only consideration, for example, humic acids can immobilise Cr, Pb, Cd, and Cu by binding them (Alvarenga et al., 2009, O'Dell et al., 2007, Song and Greenway, 2004, Walker et al., 2004) while other organic forms may mobilise them. Therefore, alteration of the distribution and availability of metal(loid)s in soil can result from addition of organic amendments directly or indirectly. This can depend on the type of soil, metal(loid) or on amendment properties e.g pH, CEC, EC, and moisture content (Walker et al., 2004, Bernal et al., 2007, Shuman, 1999).

In this study the WTRs raised soil pH by 0.5 to >2 units. The liming effect of Al-WTRs were different from that of Fe-WTRs and this might be related to some chemical properties such as bicarbonate concentration, hydroxide content, surface reactivity and cation exchange capacity. The soil pH neutralizing capacity of Al-WTR and also Fe-WTR were different in each of the seep soils, which can be attributed to differing buffering capacities of the seep soils but might also reflect variation in the buffering capacity of WTRs themselves, as Howells et al. (2018) found. Heil and Barbarick (1989) found that neutralizing capacity of Fe-WTR was different from that of Al-WTR as well as their effectiveness as liming agent were different within different soils. The soil's ability to resist the change in pH (soil buffering capacities) is the key factor. This finding is corroborated by that of Van Rensburg and Morgenthal (2003) in which WTRs were effectively utilised as a neutralizing agent for acid-generating mine waste.

5.4.3 Plant yield and element concentrations

The premise of this part of the study was that WTRs have the ability to be an adsorbent for some chemical components present in contaminated soil that can have a stressing effect on the plants, and also that WTR addition could raise pH in acidic soils. The WTRs indeed raised soil pH, consequently increasing the phytoavailability of certain essential macronutrients (Üçer et al., 2006) as well as decreasing the bioavailable amounts of potentially toxic elements such as Al (see Table 5-13). All amendments raised plant yields, with a fair degree of consistency. Interestingly, the yields in the non-treated control samples were higher in the second trial (after wet/dry cycle) than in the first, raising the possibility that the wet/dry step may have leached out some contaminant or acidic components and made the seep soils more acceptable for plant growth. Such a process has itself been used in some commercial remediation efforts, where it is referred to as soil washing. This is more widely done for soils contaminated with industrial solvents, petroleum products and other organic chemicals rather than for soils contaminated with metals, and it involves using water and various additives (depending on the main contaminants present) to scrub excavated soil and replace it after washing and sieving, often with removal of the fine fraction where much of the contaminants are retained. Of course, this remediation strategy requires soil excavation and treatment and so is very expensive, and the removal of the fine fraction can also change the texture of the soil which may limit some future uses. It also leaves the removed, contaminated fine fraction as a waste material which then requires secure disposal. These aspects limit its appeal as a remediation method. A good review of cases where metal contaminated soils were treated with soil washing was presented by Dermont et al. (2008).

A mining soil remediation trial was carried out by Alvarenga et al. (2009) using sewage sludge, municipal solid waste compost and garden waste compost on a very comparable, highly acidic, pH 3.7-4.1, sandy loam soil. A comparison of the plant growth increases observed in the present study, as shown in Table 5-12, with those reported in Alvarenga et al. (2009), Table 5-23, indicates that the WTRs achieved better plant yield increases than that by sewage sludge and garden waste compost in all sites except at seep 2, while plant growth percentage increase reached using municipal solid waste compost was greater in that study compared to that reached by WTRs in the present study except for plant growth achieved using Fe-WTR at seep 1, which was the same as that using municipal solid waste compost. This indicates that WTRs are equally as good as, and in some cases even more effective than, these other readily available recovered wastes/resources in terms of restoring soils to a condition where plant growth is possible and is enhanced.

Table 5-23: Relative growth (% relative to control) in the literature for similar mining soil remediation trials using sewage sludge, municipal solid waste compost and garden waste compost (Alvarenga et al., 2009).

Organic amendment	Relative growth (%)
10% (100 Mg/ ha) of sewage sludge	118±7a
10% (100 Mg/ ha) of municipal solid waste compost	238±49cd
10% (100 Mg/ ha) of garden waste compost	158±13ab

Increases in the plant yields are likely to mean that there is an increase in the amounts of C, H, N and O, as these elements constitute about 99% of the total composition of plants (Prasad, 2013). It is useful to compare the measured concentration of elements in the tested plants with the concentrations generally considered to be at a deficient level and at a toxic level for Perennial ryegrass (*Lolium perenne*) (Reuter and Robinson, 1997).

The critical value of P for Ryegrass is 0.34 %w/w, and all treatments and non-amended controls had P concentrations above this hence P deficiency would not likely have been a problem in any of the plants. It was noteworthy that all amendments increased P in seep 1 and seep 3 plants (e.g. from 0.8% to 1.14% w/w for Al-WTRs in seep 1; Table 5-13), but all amendments decreased P in seep 2 plants (e.g. from 0.97% to 0.72% w/w for Fe-WTRs). In the second trial, after wetting/drying, a similar result was obtained in that P was only decreased (and only by a small amount) in the seep 2 treated soils. This decrease in seep 2 plant P is in agreement with what has been reported on occasions (and what has sometimes been the main aim in some situations) following application of WTRs, i.e. to immobilise excess P in soils by ligand exchange mechanisms (Makris et al., 2004). This immobilisation has caused excessive restriction of P availability in some studies (e.g. Elliott and Dempsey, 1991, Lucas et al., 1994, Lombi et al., 2010), but has not been observed universally. This inconsistency of effect on soil P is likely intertwined with the effects WTRs have on soil pH, because altering soil pH itself has an impact on P availability with maximum plant available P usually occurring within the pH range of ~6.3-7.5 (Weil, 2016). Added to this, the chemistry of mining-affected soil might be different from that which have been studied previously (i.e. mostly agricultural soils) and therefore the increase in the P assimilation noticed at seep 1 and 3 might be related to other aspects associated with mining soils. There is a need for further examination of the P assimilation in plants grown in mining-affected soil.

At all seeps and in both the first and second plant trial, K uptakes in amended groups were higher than that in non-amended soils. This is important because K is a key plant nutrient vital for many aspects of plant physiology. Even so, despite the increase, plants in seep 1 still had K levels within the deficiency range of less than 1.7% w/w (Reuter and Robinson, 1997). For seep 3, all amendments resulted in plants moving from within the K deficiency range into the K sufficient range, while all seep 2 plants were also in the sufficient range (including controls). Ca uptake in all amended groups in the first trial and all amended groups except seep 3 lime treated soil in the second trial was higher than that in non-amended groups; this might be attributed to increases in the Ca phytoavailability as a result of enhancing soil pH. Lower than 0.2 % of Ca is reported as deficient value, thus Ca deficiency effects might occur in non-amended seep 1 soil. Ca is not considered to have a directly toxic effect on plants so the considerable amounts of Ca often present in WTRs should note pose any risks of toxicity.

The deficiency range for Mg in grass tissues is considered to be anything below 0.13% w/w (Reuter and Robinson, 1997), a value above which all plants had in the trials (including non-amended controls). For the first plant trial, WTR amendments increased plant Mg further into the sufficient range for all seeps, while lime only resulted in an increase for seep 1 plants and to a decrease in seep 2 plants. This would seem to indicate that the WTRs provide some plant available Mg that is not present in the lime. The second trial had a more variable pattern, but differences were rather minor.

Sulphur uptakes in Al-WTR amended groups were higher than that at non-amended groups. A ligand exchange between SO_4^{2-} and OH^- has been detailed when hydration of Al-WTR occurs, see Equation 5-10 (Upadhyayula et al., 2009). This might be the reason for increasing of S assimilation as due to increasing the S phytoavailable level of the element. In contrast, S uptakes in Fe-WTR amended groups were lower than that at non-amended groups. Adsorption of sulphide on Fe-WTR has been expected to occur due to ligand exchange and oxidization mechanisms (Wang and Pei, 2012). Lower than 0.22% w/w of S is reported as deficient value for ryegrass (Reuter and Robinson, 1997), so even with the variable effects on S concentrations observed an S deficiency effect is not likely.

$$\equiv Al)_2 - SO_4 + 2HO - H \rightleftharpoons 2 \equiv Al - OH + SO_4^{2-} + 2H^+ \qquad \text{Equation 5-10}$$

The Cu concentration in plant tissues generally increased by a modest amount in all treatments and seeps in the first plant trial, and in the second trial a similar patter emerged except that increases were not observed for seep 2 in the Al-WTRs and lime treatments. An increase in plant Cu being observed across all treatments and not just with WTRs is potentially indicative of the increase being partly associated with the pH increase in the soils following application of the amendments because, although Cu^{2+} ions are more mobile at lower pH (Cavallaro and McBride, 1980, Sauvé et al., 1997), it is known that organic matter induced mobilisation of Cu is maximised at more neutral pH. However, a number of the samples showed greater increases in plant Cu following WTR treatments than lime treatments, which suggests that the WTRs might act as a source of this micronutrient.

Shahin et al. (2019) have studied adsorption of copper ions onto WTRs and related processes and they suggested that Cu associated with low molecular weight organic components can be released during decay of biota residues in WTRs and so that process may greatly increase the availability of Cu to plants. This supports the idea that WTRs might be a source of Cu micronutrient and may give part of the reason for the increase of Cu assimilation at the amended groups. It should be noted that greater than 21 mg/kg of Cu is reported as being the toxic threshold value for ryegrass (Reuter and Robinson, 1997), so even with the increases observed in the treatments Cu toxicity is not likely to arise when WTRs are used to treat soils in this way.

At all seeps in the first plant trial, Fe uptake in amended groups was less than that in non-amended soils, this suggests that the soil pH governs the Fe mobility, however, plant metabolism controls on Fe uptake might be also a reason for the decrease (i.e. healthier plants can better regulate their Fe uptake). The second trial had a little variable pattern, but differences were rather minor. Importantly, lower than 40 mg/kg of Fe is reported as a deficient value and so it is not likely that the decreases in plant Fe observed here pose any risk of Fe deficiency (Reuter and Robinson, 1997).

Mn uptake increased for seep 1 plants in both trials amended with both WTRs. This would seem to indicate that the WTRs provide some plant available Mn. Variable patterns were observed in seep 2 and 3 in both trials. This inconsistency of effect on Mn is likely intertwined with interactions between Mn and other trace metals, for examples, antagonism interaction between Mn and iron (Alvarez-Tinaut et al., 1980), and antagonism between Mn and Ca, K, Mg, Na, N, and P (Kabata-Pendias, 2010). Added to this, other factors such as redox processes (Marschner, 1988) and interactions between roots and microorganisms (Marschner and Rengel, 2005) might also impact Mn phytoavailability. Greater than 1110 mg/kg of Mn is toxic value, which is much higher than the concentrations observed here, so Mn toxicity is not expected in these soils following WTRs application.

In the first trial, Zn assimilations in all amendments soils were decreased in all soils, this was expected as a result of decreasing the Zn availability due to enhancing the soil pH and also to the ability of WTR to adsorb Zn. Interestingly, Zn uptake in nonamended soils samples was decreased in the second trial (after wet/dry cycle) compared with that in the first trial, raising the possibility that the wet/dry step may have leached out Zn from the control soils. A similar trend for Zn assimilations was observed in lime amended treatments in the second trial (after wet/dry cycle). While, in the second trial, Zn assimilations were increased in most WTRs amended soil, this suggests that WTRs contain Zn which would be available as a result of wet/dry cycle. Lower than 10 mg/kg of Zn is reported as deficient value, therefore, Zn deficiency effect is not likely in the cases of decreasing Zn content to the levels observed here. Metabolic and/or nonmetabolic process within plants, which are affected by plant health status, might also contribute to increases (or decreases) in uptake because that process can moderate and control Zn uptake. There is a need for further examination the impact of the wet/dry cycle on the Zn assimilation.

The Pb concentration in plant tissues is shown in Table 5-13. The significant decreasing in Pb assimilation (by applying Al-WTR in seep 1 and 3 and by applying Fe-WTR in seep 1) were expected because the high affinity of Al-WTR and Fe-WTR for adsorption of Pb, as shown in adsorption percentage values in Table 5-7. Binding Pb to the oxyhydroxide or organic components in WTRs might contribute in decreasing the phytoavailable concentration of Pb which consequently could decrease the Pb plant uptake.

There was not significant difference in Cr uptake in seeps 1 and 2, while, Cr uptake decreased in seeps 3 which might be attributed to an ability of WTRs to adsorb Cr (Mohammed et al., 2016). Added to this, Wang et al. (2014) found that the Cr leachability from WTR was less than 0.01 mg/l in pH greater than 4.

In both trials, there were significant decreases in arsenic assimilation following Al-WTR addition, Fe-WTR addition and lime addition in seep 3, in agreement with a previously published study (Sarkar et al., 2007). That study demonstrated the effectiveness of WTRs in immobilizing As in As-contaminated soils. The differences were not significant in seep 1 and 2 in all amendments in the first trial, however, after the wet/dry process, the decreases were significant in all seeps in all amendments. This suggests that the wet/dry process might be the reason for that, perhaps through exposing fresh surfaces on the WTRs or the lime that could better sorb As.

In both trials, the observed decrease in Ni assimilation in all soils which were amended with WTRs might be attributed to immobilised Ni through adsorption onto WTRs, as has been detailed by Chiang et al (2012). Enhancing the soil pH would seem the reason for decrease Ni assimilation in lime amended soils in both trials, Table 5-13. Ma et al. (2013) found that increased soil pH lead to increases in the rate and extent of aging reactions of Ni in soil consequently decreasing Ni availability to plants. This might also partly explain decreases in the Ni uptake after wet/dry cycle.

5.4.4 Earthworm survival and element uptake

Survival of earthworms in non-amended control soils was zero in all seeps across both trials (pre- and post-wetting/drying cycle), with the exception of 13% survival in seep 2 control soil in the second test. This shows that the seep soils were very hostile to earthworms, with the low pH (~3.3-3.8) and likely associated Al toxicity being an important factor. Other studies have shown that *Eisenia fetida* can survive at pH 4 and are content at pH 5 if other soil factors are favourable (Spurgeon and Hopkin, 1996, Dominguez and Edwards, 2011), but here the soil was too acidic without amendment. It may also be that the soil had toxic components that needed neutralisation for earthworms to survive. The amendments, in general, greatly increased survival and this is likely to be primarily linked to the pH increase they brought about. A similar finding was reported previously for acidic forest soils (Homan et al., 2016) in which soils at pH 3.1 had zero survival of species *Lumbricus terrestris* in laboratory tests but when the pH was raised with lime an increase in survival was achieved even with a modest increase to pH 3.7. The same study also reported field observations that included zero earthworms in the soil at pH 3.6 but an abundance of them in areas that had been limed so that the soil pH had reached 4.4. One investigation that examined remediation of Zn-Pb smelter contaminated soil found that only when biosolid amendments were mixed with lime did earthworm survival occur (100% mortality in non-amended and biosolids only amended soil, but <10% mortality in biosolids+lime amended soil; (Conder et al., 2001). This illustrates the importance of pH and liming effect (either from lime or with amendments that have liming capacity). However, in the present study, despite good improvements in survival in treated seep 1 soil in the first trial, all seep 1 soils in the post wet/dry cycle test had zero survival. This raises the possibility that, with the chance of experimental artefacts or errors aside, the wet/dry cycle (and possibly the re-grinding that followed), triggered the release of a toxic component in this seep soil and/or otherwise altered the conditions rendering them too hostile again for the earthworms. It is unlikely that the amendments caused the toxicity because it was not observed in the other seep soils and it has been shown that *E. fetida* are not affected by WTR application even at up to 20% w/w addition (Howells et al., 2018) .

The treatments enabled earthworm survival in most cases and the water contents of the surviving earthworms closely approximated the 82% ± 7.7% that Hartenstein et al. (1980) identified as typical for the *E. fetida* species. This suggests that the treatments not only facilitated survival, but also allowed the earthworms to be in a healthy condition in terms of moisture retention.

Element contents of earthworms were highly variable between treatments, trials and, in some instances, amongst replicates. This makes interpretation challenging, as does the 100% mortality in the non-treated seep soils which prevents evaluation of whether the treatments reduced the assimilation of potentially toxic elements (i.e. with no values for non-treated seep soils there is no baseline to compare with). However, concentrations observed can be evaluated using the wider literature. The Al concentrations determined in the surviving earthworms in the first trial in the present study were, in all cases except one, at or below 437 mg/kg, which was the concentration reported by Hartenstein et al. (1980) for *E. fetida* maintained in uncontaminated soils (the exception having been seep 2 soil treated with Al-WTRs, which had a mean Al concentration of 603 ± 181 mg/kg). The second trial, following the wet/dry cycle, generated higher earthworm Al concentrations in the Al-WTR and the Fe-WTR treatments (~500 – 1330 mg/kg; Table 5-18), suggesting that the wet/dry and re-grinding process may have brought more Al into the bioavailable pool. However, although these values are above those reported as typical by Hartenstein et al. (1980) , even these values are below the ~1600 mg/kg value reported by Zhang et al.(2013a) for *E. fetida* maintained in a clean garden soil (a latosol) with a comparable pH of 4.3. This would indicate that the Al concentrations observed here in the treated soils were not too far removed from what might be expected in a normal soil with low pH.

The greatest As concentration observed was ~11 µg/kg, having occurred in the first trial in earthworms from the seep 2 soil amended with Al-WTRs, however even this value is much lower than the 35 - 40 mg/kg reported as the level at which cocoon production was decreased by 10% in *E. fetida* (Bustos et al., 2015). The As concentrations in the present study are also all lower than those reported for *E. fetida* earthworms subjected to experiments using contaminated soils from an old gas works (viz. ~20 mg/kg; (Gomez-Eyles et al., 2011). It would therefore seem that As is not at high toxicity levels in the surviving earthworms from the treated soils of the present study.

For Cu, there was variation from near zero to 40 µg/kg in the first experiment but little evident pattern. In the second (post wetting/drying) experiment the earthworm tissues had generally lower Cu levels but again there was no clear pattern. The concentrations were within or below the typical Cu concentrations reported for compost-bred *Eisenia fetida* (i.e. 22 – 812 mg/kg; Gunya et al. (2016) and therefore no toxicity from Cu is likely to have occurred. The Fe concentrations in earthworm tissues varied very widely, across treatments and across seep soils. Concentrations were equally high in lime and Fe-WTR treatments in the first experiment (up to 10000 mg/kg and 8600 mg/kg, respectively), but differed in the second experiment where the highest concentrations were noted in the Fe-WTR treatment (19534 mg/kg for seep 2 soil). The concentrations in earthworms from all treatments were high compared to typical Fe values reported elsewhere for clean soils or composts (i.e. <1500 mg/kg; Gunya et al. (2016); Hartenstein et al. (1980), but it is difficult to determine whether the measured body burdens would have any negative effects and therefore this could be a direction for future research. The Mg, Mn, Ni and Pb concentrations were all within typical ranges observed in earthworms from clean environments (Langdon et al., 2005, Howells et al., 2018, Gunya et al., 2016), with e.g. typical Pb concentrations reported at up to 16.4 mg/kg for a related *Eseinia* species (Langdon et al., 2005). This indicates that the treatments did not facilitate assimilation of these metals to unusual or dangerous levels. The concentrations of Zn, where measured, were also consistent with values reported for control soils (e.g. 80 mg/kg; Howells et al 2018) and thus excessive Zn uptake was not a problem experienced by the earthworms.

The earthworm results were useful and allow evaluation of general earthworm and wider ecosystem health if these amendments were used for remediation of mining impacted soils, but the wide variability across the results do limit how definitive conclusions can be. This possibly reflects the variability of element uptake generally for earthworms and may indicate effects of other factor on the elements uptake in earthworms such as their digestion of soil and the effectiveness of depuration. It is also important that future studies examine metal uptake on earthworms using more replicates which can help in the power of statistical tests.

5.4.5 Simulated soil solutions

The premise of this part of the study was to examine the idea that WTRs have the ability to be an adsorbent for some chemical components present in the solution of contaminated soil and also that WTR addition could raise pH in acidic soils which in turn would modify (and generally decrease) the solubility of some elements. Together these processes would lead to reduced mobility and bioavailability for contaminants and therefore to improvements in the soil environment for biota. All treatments greatly reduced the soil solution Al concentration, often by orders of magnitude, and this was likely due to the increases in pH the amendments brought about. These decreases also matched up with the decreases in plant tissue Al observed in the treated seep soils, indicating that the amendments did reduce both mobility and bioavailability as was the aim. The soil solution Al concentrations observed in the untreated seeps was highest (154 mg/l) in seep 1, with the values being similar to those observed in the subsoil of acid sulphate soils (pH 3.8 – 4.2) in Finland (e.g. 113 – 159 mg/l; Virtanen et al. (2014). The successful reduction of the Al concentrations observed following treatment, particularly with Fe-WTRs, might suggest that acid sulphate soils remediation measures could also benefit from incorporating addition of Fe-WTRs.

Reduced root length effects for *Lolium perenne* (ryegrass) were observed by Hackett (1965) at 25 mg/l of Al solution at pH 3.5. Pavan and Bingham (1982) observed slight injuries on roots of coffee plants at Al concentration in solution of 1 mg/l, and what they termed medium or severe injuries at a concentration of 4 mg/l. Comparing the Al concentrations in the soil solutions of the untreated seep soils here (i.e. ~3.4 mg/l to 154 mg/l) with those levels determined by Pavan and Bingham would therefore lead to a conclusion that injuries to plant roots would likely occur to some degree in all the seeps (confirming the plant yield results discussed above) whereas seep 2 and 3 soils, once treated, would have Al concentrations in their soil solutions that were below the level causing injury. While seep 1 soil did have a drastic reduction in soil solution Al concentrations following all types of treatment imposed, the levels appear to still be near to or above those that could start to cause plant root injury. This suggests that the treatments were only partially successful in seep 1 soil in relation to completely lowering the Al in the soil solution to ecologically unimportant levels.

One estimate for an average natural abundance of As in soil solution is 0.75 µg/l (Wolt, 1994), while concentrations reported to cause a 10% inhibition (EC10) in growth of cucumber (*Cucumis sativa*) ranged 2.2 µg/l to 697 µg/l across a selection of soils from Australia (Lamb et al., 2016). An example of increased arsenic in pore water of arsenic-contaminated soil is 430 µg/l from land impacted by mining activities at Mina Mónica (Madrid, Spain) (Beesley et al., 2013). In some cases, despite high total concentrations of As (131–202 mg/kg), pore water concentrations of As remained close to the limit of detection (ICP-MS) (Moreno-Jimenez et al., 2011), showing that other soil factors affect As mobility. Applications of Fe-WTRs (2.5% w/w) were found to decrease As in the porewaters of an As contaminated soil in Denmark (Nielsen et al., 2011), however, in the present study, concentrations were only reduced by the treatments in seep 2 and were actually increased in seep 1 (from 2.9 µg/l to 12.2 µg/l). A similar increase in porewater As was reported for soils amended with biochar, with the effect attributed to mobilisation by increases in dissolved organic carbon and pH (Beesley et al., 2010). Because As occurs as an oxyanion in the porewater environment (i.e. typically as arsenate or arsenite), its solubility can increase when the pH is brought up from acidic conditions to more neutral conditions as was the case when the treatments were added. Desorption (mobilisation) of arsenic can also occur in the presence of nutrient anions such as PO_4^{2-},CO_3^{2-},$SO_4^{2-}$and Cl^- (Violante et al., 2008), because of competition for binding places and displacement, so a similar case might be in effect here as the treatments can contribute nutrients to the soil. Nevertheless, the porewater As concentrations observed in the treated soils were very much at the lower end of the EC10 thresholds noted by Lamb et al. (2016).

The increases in Ca in all treatments in all seeps suggest that the amendments provide Ca to the soil. The increases were within typical Ca levels in soil solution in acid soils which have been reported to range from 15.2 to 372 mg/l (Kamprath, 1978). The increase in element Ca in the soil solution has a corresponding increase in element Ca in the plants in all seeps in both trials.

The increase in Mg in both WTR treatments in seep 1 suggests that the WTR amendments can provide or release soluble Mg to the soil. Contrastingly, decreases in soil solution Mg were noted in all treatments in seep 2 and 3, showing that the effects on Mg are variable. Differences in the chemistry of seep 2 and 3 soils from seep 1 soil might led to Mg fixation , for example, formation of Mg-silicates, immobilization of Mg in the interlayer of aluminous chlorites (Gransee and Fuhrs, 2013). Plant available Mg concentrations in the soil solutions have been reported to vary between 3 mg/l and 204 mg/l even in non-contaminated soils (Barber, 1995), so the Mg contents observed in this study do not appear to present an environmental problem.

Big increases in K in the soil solutions following all treatments in all seeps suggests that the amendments provide readily mobile K to the soil. The increase in element K in the soil solution has a corresponding increase in element K in the plants in all seeps in both trials. Considering that K is a very important element that is often added as a fertilizer component, this is likely to be viewed as another positive associated with these treatments.

Wolt (1994) reported that the natural abundance of Cd in soil solution can be up to 4.5 µg/l, while the predicted no effect concentration for Cd (i.e. the amount at or below which there is no anticipated negative effects) in soil solution was determined to be in the range 1.3 to 3.2 µg/l (de Vries et al., 2007). Statistical tests indicated that treatments did not significantly decrease the Cd in soil solution for seep 1 soil, but significant decreases were observed in seeps 2 and 3 and the resulting concentrations were less than 1 µg/l or below the limit of detection using ICP-MS in those soils. This means that the amendments lowered soil solution Cd in seeps 2 and 3 to levels considered to be unlikely to impact soil biota. It is also in agreement with the plant growth results, which found effectively zero bioavailable

Cd in the soils. The reduced pore water Cd in the treated soils is consistent with the high sorption capacity WTRs have shown for Cd, e.g. ~18000 mg/kg (Chiang et al., 2012). Silvetti et al. (2015) found that adsorption of Cd ion forms inner-sphere surface complexes with the inorganic and organic phases of WTRs, meaning that the sorption binding is likely to be strong and long lasting. This would suggest adsorption of Cd onto WTRs did occur here. Added to this, the increasing pH would likely result in increased adsorption of Cd by SOM (Kabata-Pendias, 2010).

Generally in soils, Co is only slowly mobilised and low concentrations are expected in soil solution with the concentration ranges typically between 0.3 and 87 µg/l (Kabata-Pendias, 2010). In the present study, soil solution Co in non-treated seep 2 and 3 was much elevated above this typical range, with both having >500 µg/l. All treatments reduced the solution Co concentrations in these soils to <50 µg/l. Seep 1 soil had ~100 µg/l in the untreated state and this decreased to <50 µg/l following Al-WTR and lime treatments but increased to 331 µg/l following Fe-WTR treatment. The results of the two plant growth trials discussed above showed a related pattern, with the Co in the plant tissues having decreased significantly in treated soils compared to untreated seep soils except in one case which was again seep 1 soil treated with Fe-WTRs (for which there was no significant difference from the untreated seep soil in the first plant trial and a small relative increase in the second trial). The reason for the difference in Co mobility in this seep soil + Fe-WTR combination is unclear and it is difficult to provide a speculative explanation for other than perhaps something linked to a possibly different organic matter component present in the Fe-WTRs that is released when mixed with the seep 1 soil, as organic matter differences have been reported to have the potential to mobilise soil Co (Lange et al., 2016). This warrants further investigation on the mechanisms involved because it would be anticipated that Co in solution would be decreased following treatment and that the amendments would immobilise Co in the soil solution since WTRs have been shown to have the ability to adsorb Co (Chiang et al., 2012). Nevertheless, in all cases in the present study, the soil solution Co concentration was well below the No Observed Effect Concentration (NOEC, i.e. the highest concentration imposed in a toxicity test that shows no negative effect) reported previously for the springtail species *Folsomia candida*, which was stated as 62200 µg/l and 9440 µg/l respectively in the two soils in which it was determined (Lock et al., 2004).

The average natural abundance of Cr in soil solution is 0.52 µg/l (Wolt, 1994) and all the untreated seep soils had concentrations above this typical amount. All treatments produced substantial decreases in seeps 2 and 3, which is likely to be related to adsorption of Cr onto the WTRs, which is in agreement with their Cr sorption capacity previously noted (Mohammed et al., 2016, Nielsen et al., 2011). In addition to this, the solubility of Cr(III) is known to decrease as solution pH is raised (Bartlett and Kimble, 1976). It is known that Cr is non-essential for plants (Hayat et al., 2012), so the decrease in the soil solution Cr concentration observed when seeps 2 and 3 were treated with the amendments is a good result. When seep 1 was treated no differences in soil solution Cr concentration resulted, the reason for which is unclear.

Concentrations of Cu in soil solution commonly range from 0.5 to 135 µg/L, depending on measurement techniques used and on soil types (Kabata-Pendias, 2010), and therefore the concentrations observed here were not remarkable other than a partial trend towards decreasing with treatments in seeps 2 and 3. Any decreasing trend in soil solution Cu was not however sufficient to adversely affect plant uptake, because that had remained unchanged or increased with the amendments as discussed above. Kabata-Pendias and Sadurski (2004) found that mobility of Cu is especially reduced by oxyhydroxide particles of Al, Mn, Fe, and by OM, so exposed surfaces of WTRs bearing those may have been responsible for the small decreases observed in some treatments.

In very acid soils, Fe concentration in soil solution can exceed 2 mg/l (Kabata-Pendias, 2010) and it is the soil pH that typically controls Fe content in soil solutions (Willard, 1979). Therefore, the observed decrease in Fe in the solutions of all amended seep soils is most likely related to enhancing the soil pH through the addition of the amendments. The decrease in Fe in the soil solutions had a corresponding decrease in element Fe content in the plants of some treated seeps, which is consistent with the well understood process of Fe being obtained by plants via the soil solution. However, because no evidence of induced chlorosis was visible (i.e. yellowing of tissues), the decreases in soil solution Fe were not linked to any negative effects.

The complicated chemistry of Mn, which has common valence states of +2, +3, +4, +6 and +7, means that mobility of the element is heavily influenced by Eh–pH conditions as well as sorption processes which therefore effects the Mn content of the soil solution (Willard, 1979). Therefore, the observed decrease in Mn in all amended soil solutions in seeps 2 and 3 is most likely related to enhancing the soil pH due to the addition of the amendments despite the possibility that the WTRs might be a source of available Mn (i.e. they have considerable Mn content). The decrease in Mn in some of the soil solutions did not have any consistent relationship with either increases or decreases in Mn in the plants, reflecting the many complex processes that control Mn availability that include reduction of MnO_2 forms and complexing by root exudates (Hodgson et al., 1965) coupled with Fe oxides interactions and microbiological soil activity that also have impacts on redox of Mn compounds (Zajic, 1969). Because of this it is difficult to draw any firm conclusion on how the amendments will affect Mn in soil solutions.

The concentration of Ni in solutions of untreated seep soils was 962 µg/l in seep 1, 2425 µg/l in seep 2 and 2290 µg/l in seep 3, which are within the range of those reported for Ni contaminated soils from a Ni ore smelting region in Canada (mostly 120 – 28700 µg/l; Nolan et al. (2009) and for a set of European soils that had been deliberated dosed to a total Ni concentration expected to cause 10% reduction in plant growth (Ma et al., 2013). This would suggest that the levels of Ni in the solutions of the untreated seep soils would have probable negative effects on soil biota if not addressed. All treatments reduced the soil solution Ni, with lime achieving the greatest level of decrease which would indicate that the soil pH enhancement was the primary reason for the changes. Of the two WTR types, Al-WTRs achieved a greater reduction in the Ni concentrations. Previous work had shown the capacity of Fe-WTRs to sorb Ni from solution, with an addition rate in sediment of 250 mg/g of Fe-WTR achieving a sediment porewater Ni decrease of more than 80% (Chiang et al., 2012). The decrease in Ni in the soil solution did have a corresponding decrease in element Ni in the plants for all amendments in all seep in both trials, indicating that the amendments were able to control the excess Ni in solution and render it less bioavailable. This is an important and a positive outcome in these seep soils which, in the untreated state, had soil solution Ni in the potentially toxic range.

Pb is not known as an essential or as a beneficial element for any living organisms and therefor Pb deficiency is not a concern. The average natural abundance of Pb in soil solution has been estimated at 1.04 µg/l (Wolt, 1994) and a number of parameters such as soil pH, CEC, clay content, $CaCO_3$, organic matter content and Pb concentration govern Pb sorption-desorption processes in agricultural soils (Hooda and Alloway, 1998). The untreated seep soils had soil solution Pb concentrations (all <6 µg/l) that were below or marginally above the estimated typical background levels. One study (Zhang et al., 2019) showed that even in a very sensitive soil from Germany the concentration causing just a 10% reduction in the reproduction (i.e. the reproduction EC10) of the *Enchytraeus crypticus* worm was 6 µg/l, while the EC10 vales in the other soils tested range 21 to 90 µg/l. This would suggest that the Pb soil solution concentrations in the seep soils would not pose any substantial risk to invertebrates. All amended seep 2 and 3 soils had lower Pb concentrations in soil solution than that of non-amended soil, which suggests that the amendments immobilized the Pb either directly by sorption or by their effect on pH. This was in agreement with the findings of the ability of Al- and Fe- WRTs to adsorb Pb from solution as it was found earlier in section 5.3.1.1. An exception to the decreased solution Pb was observed in seep 1 soil amended with lime, in which a large increase was observed. However, there was great variability amongst the replicates for that soil-treatment combination and so the result for it is questionable. It is possible that a contaminant was introduced during the analysis or that a particle of Pb had been incorporated into the portion of lime added.

The average natural abundance of Zn in soil solution has been estimated at 7.3 µg/l (Wolt, 1994) but it varies greatly and, generally, soil solution Zn concentrations rise fivefold per unit pH decrease. For example, a heathland soil from Belgium with a pH of 3.5 had a Zn soil solution concentration of 330 µg/l (Degryse et al., 2003) while a grassland soil from Rhydtalog in the UK with pH 4.8 had 1200 µg/l (Smolders et al., 2004b). Adsorption of Zn onto pH-dependent binding sites of oxyhydroxides minerals and organic matter, as well as ion-exchange reactions on clay minerals at high Zn concentrations, are the main processes governing the Zn content in soil solution (Alloway, 2012). The effects of Zn in solution on soil biota can vary widely, with the EC10 values determined for microbial processes having been found to span 100 to 296000 µg/l in a broad set of European soils (data from Smolders et al. 2004). The Zn concentrations in the solutions of the untreated seep soils (1182 - 2420 µg/l) were within this very wide range. The findings of the Zn adsorption onto WTRs test discussed above (section 5.3.1.1) were reflected again here in the soil solution results in that there was a clear decrease in Zn in the soil solution of amended soils with the exception of seep 1 amended with Fe-WTRs for which there was no significant change. Too much of a decrease in the soil solution Zn concentration would be undesirable because Zn is an important plant nutrient however, as discussed in previous sections, in the plant trial the amounts of Zn assimilated by plants was in the healthy range (i.e. no deficiency).

5.5 Chapter conclusions

The aims of the chapter:

In this investigation, the aim were to investigate the adsorption and retention by two types of WTRs (one generated at a plant using Al salts, Al-WTRs, and the other generated from use of Fe salts, Fe-WTRs) of two important metals, Pb and Zn, that are often present as contaminants in waters and wastes, also to assess the remediation of mining contaminated soil using two types of WTRs from central England, UK.

Main findings:

The investigation has shown that the two WTRs tested, one Al- and one Fe- based, had high sorption capacity for Pb and Zn both separately and in combination. Moreover, the WTRs retained the vast majority of the sorbed metals even through a desorption process. It was also shown that application of the WTRs to acidic soils impacted by former mining activities led to significant improvements in pH, plant yield and earthworm survival that were comparable to, or in some cases better than, those achieved by liming.

The implications of the study:

These findings indicate that the WTRs could be used successfully as soil amendments to immobilise contaminants and raise pH and could therefore be used as a cheap alternative to lime.

The significance of the findings or contribution:

The liming aspect may be of particular benefit to acidic soils, but only if the current regulations were adjusted to allow applications to land with soil pH less than 6. In general, the addition of WTRs also resulted in soil solution element concentrations being adjusted to and/or maintained at desirable or tolerable levels and therefore would enhance plant growth. The remediation method used for this mining-affected soil may be applied to other mining-affected soils elsewhere in the world.

The findings will be of interest to environmental protection agencies and trade companies of removals Zn or Pb from contaminated water, sediments and soils. Using water treatment residuals for removal Pb and Zn contamination offers financial advantages and facilitates development of a more circular economy with greater levels of materials recycling.

Limitations:

In the ecotoxicological test of soils using earthworms, the small sample size did not allow to increase statistical power and tease out more subtle effects. It is unfortunate that the study did not include assessment of the impact of adding WTRs on the soil microbiome and/ or soil microbiological functions, as these are also important aspects of soil health. Notwithstanding these limitations, the study suggests that WTRs can be used not just as a general soil amendment, but also as a low-cost alternative to agricultural lime for treating low pH soils.

Recommendations:

Further work is needed to fully understand the implications of adding WTRs on the soil microbiome and/ or soil microbiological functions as these are also important aspects of soil health. A natural progression of this work is to conduct field-scale trials of soil remediation with WTRs in the open environment and maintain them over a longer time frame, to further test the conclusion that current regulations restricting WTRs to soils with pH above 5 or 6 are overly conservative. The findings strongly recommend a reconsideration of the current regulations about applications of WTRs to land with soil pH less than 6.

6 General conclusions, study implications, study limitations, recommendations and future directions

6.1 General conclusions

In this thesis several environmental approaches have been considered and the main findings, of the analysed problems, will be briefly discussed in the following sections.

6.1.1 Seep soils and stream water problems

6.1.1.1 Soil acidity

The result of soil properties evaluations indicates low soil pH in seep 1, seep 2 and seep 3 soils. This has negative impacts on the soils, most importantly via probable Al toxicity to biota, low effective cation exchange capacity, and being highly sensitive to other metals toxicity and metal(loid)s dissolution. The soil ecotoxicological tests verify this hostility to life, with zero earthworm survival rate and very low plant yield in all seep soils. This acidity is likely generated as a result of sulphide minerals oxidation, a legacy of mining activity and exposed ores and wastes, which was described in chapter one. This means that the bare soils in this area are in need of remediation if they are to become productive and useful and to avoid being a potential source or flow path for pollution to the stream.

6.1.1.2 Element toxicity to soil and water dwelling organisms

In seep 1, seep 2 and seep 3 soils, the measured concentrations of easily mobile Al and Fe revealed by $CaCl_2$-extractation were high, while the $CaCl_2$-extractable amounts and soil solution concentrations of As, Cr, Cu, Cd, Co, Fe, Ni, Pb and Zn (i.e. mobile amounts) were all possibly in the toxic range based on comparison with known soil contamination sites reported in the literature. The exchangeable fraction (BCR fractionation) also indicated potential toxicity in some seep soils from mobile As and Co as well as these other elements. Comparison of the measured pseudo-total contents with their predicted soil-specific ecotoxicological threshold concentrations or with general ecotoxicological soil thresholds also indicated potential risk from some of the assessed metal(loid)s in some seep soils, e.g. Fe, Mn, Ni and Zn. The poor plant yields and 100% mortality of earthworms in untreated seep soils in the remediation trials (covered further below) confirm that the seep soils are very hostile to biota.

The elevated soil element concentrations, and more importantly their high mobility, are not only concerning to soil biota but also to organisms living in stream water and stream sediment, which would be the likely destination of the mobilised elements. Evidence for this risk was found in the high contents of both dissolved and particulate forms of certain metals in stream water and elevated total content in sediment samples collected from the crossing point and downstream sites. Low *Daphnia* survival percentages in the crossing point and downstream samples confirm that the water there is hostile to crustaceans, and it is likely that Al, Fe, and Mn toxicity, along with turbidity, are a part of the problem. Dissolved (0.45 µm filtered) concentrations of Co, Fe, Mn, Ni, and Zn in the stream here were all higher than regulatory EQS or other guidelines, further confirming that water quality here is posing a serious potential threat to ecological integrity. Besides, the data of particulate form of some metal(loid)s in both crossing point, and downstream suggests that Al, As, Cu, Fe, and Pb has implications for any filter-feeding organisms and contribute to the deterioration of sediments quality when deposited. Therefore, adverse effects on stream water and sediment organisms are likely.

6.1.1.3 Deficiency of Ca, K and Mg in soil due to leaching

The BCR fractionation data has shown low amounts of mobile (and so available) Ca and Mg in the seep sites compared with Ref.1 and Ref.2 soils, which indicated a potential for plant deficiencies in these elements (K also for seep 1). The plant (ryegrass) trials supported this possibility, with the ryegrass tissues grown in some of the seep soil sites being in the deficiency range for Ca and K. The interpretation for that is that as a consequence of sulphide minerals oxidation and creation of acidic conditions, the carbonate rocks or components in soil are dissolved and leached out from the seep soils through rainwater or/and seepage water, such that H^+ and Al^{3+} eventually replace exchangeable base cations Ca^{2+}, Mg^{2+}, K^+ and Na^+.

6.1.2 Using Water Treatment Residuals to amend mine impacted soils

The adsorption study demonstrated the strong capacity for WTRs of both types to sorb and retain metals (Pb and Zn), even against a desorption step, and thus gave encouragement for their use in soil remediation from immobilising contaminants and rendering them less ecologically important. The remediation trial took that further and assessed the capacity for using water treatment residuals to ameliorate the contaminated soil to make it more fit for plant growth and other biological activity.

As the findings showed, adding Al-WTR and Fe-WTR to seep soils led to a significant improvement (increase) in soil pH. The liming effect of WTRs is possibly attributed to chemical properties such as bicarbonate concentration, hydroxide content, surface reactivity and cation exchange capacity. As a consequence, plant yield and earthworm survival rates (by both Al-WTR and Fe-WTR) improved significantly in a manner similar to, and in some cases better than, those achieved by liming. The element concentrations in soil solution and in plant tissues were adjusted to and/or maintained at desirable or tolerable levels following WTR application to soils, indicating an improvement in (and no negative effect on) ecological status of the soils. The plant and earthworm improvements were therefore probably achieved through a combination of inter-linked effects including soil pH increase (liming effect), reduction of contaminant mobility, and provision of nutrients by the WTRs. This is an important result and would logically lead to a recommendation that WTRs can be used not just as a general soil amendment for their organic matter content etc, but also as a low-cost alternative to agricultural lime for treating low pH soils. However, current regulations in the UK prohibit this, with restrictions in place that limit WTR application to soils with pH above 5 or 6 for fear of the Al content becoming mobilised. This study provides evidence to challenge that restriction because it has shown that mobile Al is reduced in low pH soils when treated with WTRs at quite high rates (10% w/w), and this reduced mobility remains the case even after a wetting and drying cycle. The next logical step would therefore be to assess this across a wider range of soils and over a longer time period (i.e. years to decades).

6.2 Implications and significance of findings

6.2.1 The implications of the refinement study of pseudo-total metal content

The results of methodological development in this study indicate that dilution of the digested solution of soil samples provides more precise data and is an environmentally friendly and time-saving alternative to drying down the digested solution of soil samples option that is commonly used in studies of soil and sediment. These findings of the refinement study will be of interest to all researchers who are analysing metals in soil samples and want to achieve more precise data with fewer steps. It is clear that if fewer steps are possible, without losing accuracy, then it is better because there are fewer points in the overall procedure where impurities can be inadvertently introduced and also because fewer laboratory consumables are used per sample (which means less waste generated by the laboratory).

6.2.2 Mine waste and impact characterisation

Characterisation of mine waste at an impacted site, whether it be in standing spoil heaps or spread within a contaminated soil, is critical when evaluating the current and likely future environmental impact. The mine waste can be an ongoing source of contamination that has profound implications for ecosystems and for human use of an area (e.g. for agriculture, recreation, conservation, industry or housing).

This research demonstrates that acid generation can occur, which leads to reduced pH and solubilising of metals in soils at mining-affected areas which could have significant adverse effects on the environment. A lowering of pH, as identified here by the low pH of the soils in seep areas, indicates the buffering capacity of any minerals or components within the soil itself became exhausted, leaving the soils in the area highly susceptible to further pH decreases. Such processes result in leaching out of the base metals in the soil, which are important nutrients required by biota including Ca, Mg, and K, and their replacement with Al, , Fe and H on the exchangeable sites. The latter elements become available to soil biota instead of the required nutrients and therefore the soil is further impacted by loss of fertility. Those eventually may leach into the soil solution, groundwater, and potentially surface discharge into sensitive receiving waters. It is therefore necessary to examine the mine waste material present at a site in order to gauge the risks of acidic discharges arising and also to assess the pH buffering capacity of other waste components or of the soil in order to determine the system's capacity to withstand acidic inputs. The examining of waste materials or soils they are mixed within should include determination of total element (or pseudo total element) concentrations, the distribution of those elements within the solid phase and the concentrations in soil pore water. This is important to understand the likelihood of metals leaching out and of their mobility in the environment. Comparison of these measured values can then be made with threshold values of likely impact or with studies of other locations at which certain levels of concentration have caused harm. This would provide a framework for assessing the mining waste and the soil at any contaminated site. However, in terms of environmental regulation of mining impact sites, more work is needed in the development of robust and enforceable environmental quality standards for impacted soils so that site assessments are not just comparative (i.e. with guideline values or measurements elsewhere) but can also be conducted with a definitve 'pass' or 'fail' outcome. Such soil quality standards should go beyond simple total element concentration thresholds and should incorporate bioavailability measurements such as those used in the present study. This is important because it is the bioavailable, or ecologically active, concentrations of metals (or other elements) that have environmental impact. There are many technical difficulties involved in developing such bioavailability based soil quality standards, but the site specific predicted no effect concentrations (PNECs) used in this study show that progress is being made in this area and that the approach can be made to work. Eventually, it may be possible to develop well established soil quality standards based on bioavailability analogous to those already in place for assessing certain metals (e.g. Cu, Mn and Zn) in river water [see the Water Framework Directive (Standards and Classification) Directions (England and Wales) 2015].

Future research:

The formation of acid or neutral mine drainage from a mining-affected area and its mixing with soils and surface or ground waters is a complex process and is one of the main potential mechanisms resulting in environmental impacts. It not only depends on chemistry but also depends on petrology and mineralogy, structural geology, geomorphology, surface-water and subsurface water hydrology, climatology, and microbiology. Therefore, for any site assessment where mining contamination is known or suspected, those factors should be included when assessing the contamination to get a better understanding of the situation and processes involved. Future research needs to build on the combined (but limited) approach employed here to develop a more comprehensive mine site and mine waste evaluation protocol that can be applied across sites.

Reconsideration of the mining wastes as potential exploitable resources instead of wastes, with a view to apply modern biotechnologies to reuse, recycle, or recover energy from those wastes, would also be a fruitful area for further work. Reusing the mining wastes as construction materials (e.g. for the basis of roads or in cement products) would seem a possibility with little barriers. It may also become possible to recover rare minerals or elements that were previously not possible to extract using conventional mine material processing, and this too would promote recycling and the idea of gaining wealth from waste.

6.2.3 Water quality

The wider contribution of this study:

The study has provided further strong evidence that former mining sites can have a continued negative impact on water quality in adjacent or nearby areas. This particular case study example demonstrated impacts on water turbidity, salinity, pH, ochre formation, elevated element concentrations and the toxicity all of this can have on aquatic biota (measured here directly by using Daphnia). The wider implications are that not only does this particular site need to be remediated to minimise future water quality impacts but that all of the other former coal mining sites throughout the English Midlands and elsewhere need to be assessed for their remediation needs. It is clear from this study that ongoing monitoring is required in order to understand the extent of the continued impact, justifying the expense of monitoring mining impacted streams even if they are below the size threshold that means monitoring is required under the Water Framework Directive. It would seem that a region or nationwide monitoring scheme is needed to specifically assess mining impacted rivers that are otherwise missed by existing schemes (e.g. the WFD). Any such scheme should adopt the multi-aspect approach employed in this study, i.e. chemical and physical characterisation of the water as well as biological assessments via crustacean or algae ecotoxicity assays or recording and assessment of any organisms observed in the water at the site (e.g. perhaps using methodology based on the biological monitoring working party score system, Hawkes, 1998 , that is used by some environmental regulatory organisations). That method would be a useful assessment approach to assess the contamination in water bodies around mine impacted areas elsewhere in the world.

Future research:

Development of a multi-component assessment protocol for mining impacted streams should include identification of mining-impact sensitive aquatic invertebrates. To date, the available aquatic invertebrate scoring systems are based on tolerance to organic pollutants and low oxygen levels and so, while these are good for general assessment of aquatic ecosystem health, a system that reveals mining impacts specifically would be a valuable tool to develop. Also, at this study site and elsewhere, it is apparent that to properly monitor and unserstand the water contamination in the mining-affected areas the interaction between surface water and groundwater, which in turn depends on physical geography, geology, climatic conditions, precipitation, and evapotranspiration, should be determined. This would more directly identify contaminant input pathways to the streams.

6.2.4 Sediment

The wider contribution of this study:

Both suspended and deposited sediments may pose a risk for *Daphnia* and other species of crustaceans as well as polychaetes, bivalves and other aquatic invertebrates because they may switch between suspension-feeding and deposit-feeding depending on the supply of suspended particles in the near-bottom water. The bed sediment also importantly provides living space and breeding habitat for many aquatic invertebrates and therefore impacts from mining legacy on sediment through increased element concentration or alteration to texture caused by ochre or other deposits is of concern. The sediments assessed in this study showed As levels more than three fold above the screening level benchmark recommended by the USEPA, demonstrating that coal mine pollution can include As contamination and accumulation in the sediments of nearby water bodies. This provides evidence to support urgent sediment assessment work in other closed and abandoned coal mines across the UK and other regions with similar geology where coal was previously mined. This study has also shown that while ochre (Fe oxide) deposits, which are well understood and frequently associated with coal mine pollution of rivers, occur near points of contaminant entry and so are readily identified, other elements such as Al, Cu and Mg may not be deposited immediately and therefore sediment pollution impacts from those elements may only arise at some distance downstream. This has important implications for assessment and management of mining impacted (or potentially impacted) rivers because monitoring points will need to include locations considerable distances downstream of suspected inputs.

Future research:

This study did not examine the associations of elements in stream sediment and so this should be done at this location in order to better understand the likelihood of release from the sediments. The same approach would apply at other locations because the possibility of the sediment acting as a soure of contaminant release would equally occur elsewhere. While toxicity of water was assessed with Daphnia experiments, no assessments of sediment toxicity were performed. Introducing a biotic toxicity assessment to investigations of this and other mining impacted stream sediments would be very beneficial as this would directly determine the threat to sediment dwelling organisms. Options would include the OECD 222 Sediment-Water Lumbriculus Toxicity Test, which exposes *Lumbriculus variegatus* (a sediment burrowing aquatic oligochaete worm) to contaminated sediment to determine impacts on growth and survival. Research should therefore focus on incorporating both chemical and biological assessments of this type into a systematic and consistently applicable framework of sediment toxicity assessment. Development of environmental quality standards for sediment would also be an area for future research, because at present these are lacking and there are many questions as to the utility and robustness of the limited number of guideline values that are sometimes used. Development of such standards requires the collection of evidence demonstrating damage to ecosystems and then a policy decision on acceptable levels of risk so that standards can be used for assessment of stream sediments at mining-affected areas.

6.2.5 Biota

The wider contribution of this study:

There are very few studies in the literature detailing investigations into the impacts of mining legacy on earthworms and other soil dwelling biota, therefore the evidence generated in the present study that shows earthworms can be used to examine toxicity of mining impacted soils is very useful and indicates an approach that can be used elsewhere. It also identifies wider problems in mining impacted soils; if earthworms are unable to inhabit them then all of the benefits and ecosystem services that earthworms provide (e.g. organic matter breakdown, nutrient release, soil mixing, aeration, creation of pore networks, etc) are lacking in these already damaged or impoverished soils. This suggests that earthworm surveys could form part of mining site evaluation and assessment protocols, i.e. to determine extent of impact and also to enable measurement of success of any soil remedation strategy subsequently implemented.

The effects of pollutants on earthworms might be as a result of absorption and/or uptake of the chemicals across the worm body, through either body wall or by ingestion.

Recognising this, it would also be possible to incorporate an earthworm survival protocol (either via laboratory assays or release to the field) as part of a site-specific screening level assessment of a particular contaminant or set of contaminants. If a battery of assays was developed, they should not just assess toxicity and survival of earthworms but also other important effects such as avoidance behavior, reproduction, and bioaccumulation; these points are also crucial in terms of establishment and viability of earthworm populations and thus overall soil health.

The stream sites were unable to sustain a population of fish, as deermined by chemical and physical parameter assessment (including turbidity, electrical conductivity, and total dissolved solids), hence this project serves as a case study for how mining impacts can render a site uninhabitable by fish and so prevent development of a more complex and diverse aquatic ecosystem. It also has implications for disruption to fish dispersion along minor tributaries, which in some instances can be important. The success of any remediation work on making the area suitable for fish would thus also serve as an excellent case study of restoration.

It is known *Daphnia,* and other species of crustaceans, polychaetes and bivalves, can switch between suspension-feeding and deposit-feeding depending on the supply of suspended particles in the water, therefore survivability of such aquatic invertebrates when exposed to mining impacted stream water gives a direct indication of toxicity. In addition, the dissolved and colloidal forms of contaminants in water can also be assimilated by these organisms and so assays utilising them as test subjects incorporates assessment of this exposure pathway too. Therefore, protecting those organisms and assessment of the risk of contamination of them should also include a combination of independent lines of evidence. For example, assess based on the content of dissolved metals and suspended particles metals as well as ecotoxicity test data. That is, as stated previously, a framework for assessing contamination and risks at mining impacted sites should include chemical parameter measurements and direct tests of toxicity using e.g. Daphnia for water compartment assessments. The present study's multi-aspect approach can serve as an example of how that type of framework might be developed.

Future research:

For the particular site of the study further biota assessments should be done to determine the extent of toxicity of the water, sediment and soil to a wider range of species. This would help with establishing priorities for remediation (i.e. identifying the most sensitive groups and therefore the blocks to system recovery). However, while intense assessment of one site is useful for understanding that site and developing a bespoke remediation strategy for it, in reality the development of a generic framework that would be useable for assessing all mining impacted sites requires a limited number of key measurements and tests that can be done within a reasonable budget. Therefore, future research should aim to determine what biota based tests give the most useful and informative results so that a consistent basic set of assessments can be established; for example, it should determine whether the Daphnia assays used here are best for providing an aquatic toxicity evaluation or whether a sediment dwelling organism test would be better. Similarly, for soils it should be determined whether an earthworm survival test and an avoidance test provide sufficient information or whether other tests such as those with springtails (e.g. *folsomia candida*) are more useful.

6.2.6 Remediation

The wider contribution of this study:

Remediation of former mining sites and wider mining impacted areas is necessary in order to restore functioning habitats and ecosystems and also to prevent further dispersion of mining related contaminants. If remediation can be done using low cost or even freely available waste materials then it is more likely to be done, particularly in less economically advantaged regions and countries. The use of drinking water treatment residuals (WTRs) for remediation fits squarely in that space. In general, the addition of WTRs to the mining impacted seep soils investigated in this study resulted in soil solution element concentrations being adjusted to and/or maintained at desirable or tolerable levels and, therefore, would enhance plant growth and so facilitate phytostabilisation of the soils. Moreover, the findings of the remediation study indicate that the WTRs could be used successfully as soil amendments to immobilise contaminants and raise pH and could, therefore, be used as a cheap alternative to lime for ameliorating soils with low pH. The liming capacity may therefore be of particular benefit to acidic soils, not only those that have become acidified through acid mine drainage inputs but also by any other means and so this study has shown that the remediation potential of WTRs goes beyond just improving mining impacted soils. However, this could only be put into practise in the UK if the current regulations were adjusted to allow applications of WTRs to land with soil pH less than 6. Results such as those of this study can contribute to a re-evaluation of reguations and polocies on WTRs use. Therefore the work here will be of real interest to policy makers as well as to land managers and mining companies with responsibility for mine closure and site restoration. Clearly, the remediation method used for this mining-affected soil may be applied to other mining-affected and otherwise metal and pH impacted soil elsewhere in the world.

The findings of the adsorption part of the study will also be of interest to environmental protection agencies and trade companies of removals Zn or Pb from contaminated water, sediments, and soils. Using water treatment residuals for removal Pb and Zn contamination offers financial advantages and facilitates the development of a more circular economy with higher levels of materials recycling.

Future research:

Further work is needed to fully understand the implications of adding WTRs on the soil microbiome and/ or soil microbiological functions as these are also important aspects of soil health and were not investigated here. It would be necessary to confirm that large scale applications of WTRs do not impede natural microbial processes in soil. Laboratory investigations have shown that WTRs can maintain microbial function (Oliver et al. 2011), but demonstrating this in large scale field trials would provide greater reassurance and remove uncertainties. Therefore, a natural progression of this work is to conduct field-scale trials of soil remediation with WTRs in the open environment and maintain them over a longer time frame, to confirm no harm occurs to microbial functions and to further test the conclusion that current regulations restricting WTRs to soils with pH above 5 or 6 are overly conservative and restrictive. The findings strongly recommend a reconsideration of the current regulations about applications of WTRs to land with soil pH less than 6.

6.3 Study limitations

As with any project, this study was subject to certain limitations. This study was the first scientific investigation ever conducted at the study site, and thus some aspects of the project were necessarily preliminary in nature. The approach was to characterise the soil, sediment, and water problems and to trial remediation of the soils.

6.3.1 The limitations of the evaluation of soil contamination

While the mining related contamination and its biological impacts were successfully identified, characterised and quantified in the impacted and reference soils, the precise mechanisms of contamination were not comprehensively determined. This was because examining subterranean sources and flow pathways (i.e. shallow and deeper groundwater upwellings and movement, and hyporheic zone exchanges, etc. , via wells and lysimeters) was beyond the capacity of the project. It would be necessary to exame these in order to quantify the discharge and acid solution generation rate below the surface and therefore enable a better understanding of the leaching of the contaminants from mining wastes and their total input to the soils, surface water and groundwater of the site. This is readily acknowledged and opens avenues for future research.

6.3.2 The limitations of the characterisation of water quality and sediment

In addition to the limitations regarding not characerising subterranean sources and flow pathways of water (see previous subsection), it is unfortunate that the study did not include measurement or estimation of flow rates or periodic flow volumes to make calculations of contaminant loads possible, which are useful for understanding the total scale of contamination transported through the system (this would in turn allow for a regional and national remediation prioritisation process to be undertaken). The speciation of contaminants in the stream water and distribution or association within sedment components were not determined and this too would be useful information for understanding toxicity risks to aquatic biota and the likelihood of element release from sediments and thus they have been identified as areas of future research. Notwithstanding these limitations, the study has definitively demonstrated that the mining-affected site continues to negatively impact the nearby stream and so warrants consideration for remediation.

6.3.3 The limitations of the trial of soil remediation

In the ecotoxicological test of soils using earthworms, the small sample size did not allow to increase statistical power and tease out more subtle effects. Unfortunately, the study did not include an assessment of the impact of adding WTRs on the soil microbiome and/ or soil microbiological functions, as these are also important aspects of soil health and so have been flagged (above) as future research priorities. Similarly, it would be useful to expand the range of test organisms utilised to include other earthworm species and species of other invertebrates such as springtails. The remediation trial must be concluded to have been successful, but it is acknowledged that it was conducted over a limited term period and in pots in a laboratory setting. A larger-scale field trial over a more extended period would add confidence to the conclusions. Notwithstanding these limitations, the study did show that WTRs can be used not just as a general soil amendment but also as a low-cost alternative to agricultural lime for treating low pH soils and for remediating minng impacted and contaminated soils.

6.3.4 Technical limitation

Another limitation to acknowledge is the capability of the ICP-OES equipment available; a great deal of effort was exerted to maximise its utility, but even so, its limit of detection was higher than desirable.

6.4 Recommendations Summary

The findings of the study have answered the research questions it aimed to address; however, the project has raised new questions that should be investigated. Also, to gain a deeper understanding of, and even greater confidence in, the results of the study, it is recommended to:

- Study the speciation of metal(loid)s as well as organic matters in stream water and soil solutions in the study area to define the ecotoxicity risks better.
- Reperform the aquatic ecotoxicological tests with a larger number of replicates, addition rates (e.g. 7) and species to increase statistical power and tease out more subtle effects that the stream water has on biota.
- Assess the distribution of contaminants in sediment components to determine the capacity for release back to water.
- Directly assess the toxicity of the sediment via ecotoxicology assays in order to determine whether dreging and disposal should be considered.
- Devise and implement a study to determine the hydrology of the site (using wells, lysimeters and related approaches) to define contaminant dispersion pathways from mine wastes to soil and to surface and ground water.

- Assess the impact of adding WTRs on the soil microbiome (i.e. assessing the suite of microbes present and their relative abundances) and/ or soil microbiological functions (i.e. decomposition rates, nitrification potential, and enzyme activity) in order to determine whether remediation with WTRs restores or enhances these in treated soil, as these are also important aspects of soil health.
- Use more than one species of earthworm, covering more than one ecological type (i.e. the anecic earthworm species *Lumbricus terrestris* as well as the epigeic species *Eisenia fetida*), and other invertebrate species in future tests to examine effects across a broader range of species and life habits and to facilitate broader comparisons with other studies.
- Conduct a policy and regulation review to determine whether remediation with WTRs of impacted soils with pH<6 should be permitted.

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