

THE TRANSFER OF HEAVY METALS THROUGH TROPHIC
LEVELS AND THEIR TOXICITY EFFECTS ON ORGANISMS
INCLUDING HUMANS

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Abstract

This research has investigated a number of topics pertaining to the effects of metalliferous mining. The combination of these has shown that past mining activities and their resultant waste have led to the accumulation of metals through trophic levels.

During visits to Blanchdown Wood, Devon, UK and Snailbeach, Shropshire, UK, samples were collected to examine the effects of the spoil tips on the surrounding ecosystem. Samples of vegetation, soil and animal materials were extracted and analysed by inductively coupled plasma-optical emission spectrometry for the presence of heavy metals and were found to contain varying concentrations of lead, copper, arsenic, tin, tungsten and zinc.

Laboratory investigations followed to determine the toxicity effects of lead, copper, and tungsten on *Pandorina morum*. This demonstrated that although single elements have effects on population growth, a combination of the three cations had pronounced and cumulative effects. It was found that the algae reduced the concentration of lead in the nutrient media, but it was not possible to determine whether lead was accumulated by the algae or adhered to the surface of the cells. In either case it is hypothesized that lead could then be transferred to the succeeding trophic levels.

Further samples examined included archaeological finds, such as Anglo-Saxon human skeletons from a non-mining area. The concentrations of lead in these samples indicated that the population had been exposed to lead, and this is further explored. Since the population resided in a non-mining area, it is suggested that the contaminant was ingested via trophic level inputs; thus, providing further evidence certain metals are available to be transferred through the trophic levels to be stored in the human skeleton.

Keywords: Bioaccumulation; human; trophic levels; remediation

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Chapter 1: Thesis aims

1.1. Introduction

The transfer of heavy metals through trophic levels has caused concerns due to the possible increase in these toxins in the human food chain. The introduction of contaminants into the environment, and therefore the food chain, can occur through anthropological sources. Heavy metals are located in veins in the ground, which are extracted and purified through mining and smelting processes. These activities have introduced the metals into the environment, which leads to the possibility of their accumulation into plants and the metals transfer through the trophic levels.

During the undertaking of this current research, it was determined that the subject area was substantial and therefore the research was divided into smaller studies: the dispersal of heavy metals through the terrestrial environment, the accumulation of heavy metals in plants and the accumulation of heavy metals in humans and other animals.

The metals which will be studied during this research are: arsenic, copper, lead, tin, tungsten and zinc.

1.2. The dispersal of heavy metals through the terrestrial environment

The initial stages of the uptake of heavy metals occur through the terrestrial environment. The presence of heavy metals in soil can lead to its uptake by plants as well as the transfer of the metals through atmospheric weathering. The contamination of the soil is not restricted to the immediate site of initial deposition. The aims for this portion of the study are:

- To evaluate the extent to which heavy metals have contaminated selected sites
- To determine the effect of lateral and vertical movement of heavy metals through soil
- To examine the success of remediation techniques

One site which was researched, Blanchdown Wood (Devon, UK), covered a large area and has not been managed for the removal of toxins, therefore it is possible that movement of the metals through the site can occur by various techniques (i.e. wind dispersal, leaching). Where as Snailbeach, in Shropshire, has had significant remediation techniques applied. This research project will examine the concentrations of metals in soils throughout the sites, and will therefore be able to give an indication as to the extent of the past mining activities on the present environment, and to what significance remediation techniques have had on the presence of the heavy metals.

As a method of transport, atmospheric pollution can have a devastating effect on the environment. Because of the mode of transport, the pollution is not restricted in the vicinity of the mine or smelting works.

1.3. The accumulation of heavy metals in plants

The existence of mining waste has meant that there is a large concentration of heavy metals remaining in the environment. Although there are high concentrations of the metals present in the environment, they are not always in available forms. The availability of the metals is dependent upon factors such as the pH and characteristics of the soil. Plant species such as *Cardaminopsis halleri* and *Agrostis tenuis* are known as metallophytes and these plants are capable of surviving in areas of high metal contamination. *Cardaminopsis halleri* has been considered for use in the remediation of a metal contaminated area where the plants can be used to remove metals from the soil through accumulation, the plants are then harvested, containing small amounts of the unwanted metals and therefore removing small quantities from the terrestrial environment.

At both sites that were investigated (Blanchdown Wood, Devon, UK and Snailbeach, Shropshire, UK), it was noted that a number of plants were growing in highly toxic areas. It was decided to use these plants to determine the extent of the accumulation of heavy metals. The aims for this portion of the study are:

- To determine if heavy metals are accumulated by the collected plants
- To determine if partitioning occurs within the plant structures
- To determine the likelihood of heavy metals being transferred to the next trophic level through the edible portions of the plant

Additionally, the effects of heavy metals on algae will also be considered. A number of laboratory experiments with algae (specifically *Pandorina morum*) as a scientific model, will be used. As the primary producers of the majority of food chains, algae are an integral part of ecosystems and therefore the contamination at this level could have detrimental affects for consumers.

The aims for this portion of the study are:

- To determine the effects of heavy metals on *Pandorina morum*.
- To determine if the alga can accumulate heavy metals
- If accumulation occurs, to determine if they could be used as a remediation tool
- To determine if the alga can transfer the heavy metals to the next level

1.4. The accumulation of heavy metals in humans and other animals

In this section of the research a number of animal (including human) samples will be examined to determine if heavy metals have been accumulated.

The accumulation of heavy metals in humans and other animals both ancient and modern could be dependent on different factors. From previous examination of human remains it has been found that heavy metals were accumulated in the skeletal structure (Baranowska *et al*, 1995; Gonzalez-Reimers *et al*, 2003), but the level of accumulation could be dependent on the sex, and the age of the individual. Another factor could be the pathway of accumulation, whether the metals were inhaled, ingested or absorbed through the skin. The aims of this section are:

- To determine if heavy metals are accumulated in human skeleton
- To determine if the accumulation occurs pre or post mortem
- To determine if partitioning occurs in the skeleton

During site visits, a number of samples were found and collected. These included foodstuffs sold for human consumption and animal remains. These samples will provide further evidence as to the extent of heavy metal accumulation in trophic levels. The aims of this section are:

- To determine if heavy metals are present in plant material sold for human consumption
- To determine if heavy metals were accumulated in animals
- To determine if heavy metals are represented in animal remains, reproductive products and excretions

Chapter 2: Heavy metals and the environment

2.1. Origins of elements

It is believed that mineral formation occurred millions of years ago when small cracks (fissures) appeared below the surface of the earth (Pirajno and Smithies, 1992). The formation of the mineral rich sites occurred through a process of hydrothermal mineralization, where the combination of water, high heat and high pressures, minerals and metals were dissolved; the dramatic decrease in the temperature resulted in the minerals and metals being deposited and the fissures becoming filled. The deposition order depends largely on the solubility of the element (Gregory, 1980).

The hardened deposits created veins of minerals which contained a high level of purity of the minerals and metals, more than one mineral or metal can often be found together. Because of the deposition process metals of similar solubility were found together. For example lead and zinc are commonly found in the same vein (Evans, 1993; Bishop *et al*, 2005).

2.2. Heavy Metals

In many research projects, the term “heavy metal” is used to describe some of the elements which have been extracted through mining. Although the term is frequently used, there is no one definition (Duffus, 2002).

The Periodic Table comprises 104 elements and their position in the Table depends on their properties and characteristics; those elements with similar properties are grouped together. The Table is made up of four main blocks: *s*, *d*, *p*, and *f* block, metals considered as heavy metals are located in the *p* and *d* block of the Periodic Table (Burton *et al*, 1994).

Upon the discovery of new metals, their properties and possible uses were determined. The extraction of the metals has been achieved through mining operations, technology for which has improved over time. The volume of metal produced from the mines allowed for the metals widespread use, depending on its properties (Robb and Pierpoint, 1983). Elements considered in this current research are outlined; giving their properties and known uses as well as some hazards that has arisen from the elements use. Table 2-1 displays some of the properties of the elements that are considered in this research.

Table 2-1: Some properties of the metals used in the study (modified from elemental data sheets supplied by Fisher Scientific UK)

	Arsenic	Copper	Lead	Tin	Tungsten (Wolfram)	Zinc
Symbol	As	Cu	Pb	Sn	W	Zn
Atomic number	33	29	82	50	74	30
Atomic Weight	74.921	63.546	207.2	118.710	183.8/4	65.39
Oxidation States	3	3	2	2	9	1
Natural isotopes	1	2	4	10	5	5
Melting point	817	1083	327	232	3422	419.5
Boiling point	614	2570	1751	2623	~5500	907

2.2.1. Arsenic

Arsenic (As) is number 33 in the Periodic Table and resides in group 5 of the *p*-block. It is not strictly a metal, but a metalloid, (a metallic element with some non-metallic characters). The bonding involved between elements depends on the oxidation state. Covalent bonding results in the compound having an oxidation state of +3 or -3. Using dative bonding gives the compound an oxidation state of +5.

2.2.1.1. Uses

Arsenic (Arsenic trioxide – odourless, tasteless and highly potent) is known as one of the great poisons (Azcue and Nriagu, 1994). Arsenic exists naturally in many forms e.g. arsenopyrite, realgar. Arsenic is usually produced as a by-product of smelting copper, lead, cobalt and gold. The smelting process results in the production of arsenic trioxide which is recovered from flue dust in a pure form (Azcue and Nriagu, 1994)

In the Middle Ages, the sulphide of arsenic was known as auropigmentum and was a common ingredient in most of the colours used in painting and writing (Thompson, 1956). Realgar was also a popular colourant in paints for ornamental and cosmetic purposes (Forbes, 1964). In modern day, arsenic is used widely, as an ingredient in pesticides and glassware (Dickerson, 1980) although presently its use in pesticides has decreased.

2.2.1.2. Hazards

Arsenic is one of the best known poisons both for use as pest control and for murder. Clinical effects of poisoning in humans can vary and depends on the state of arsenic exposed to as well as age, and medical health of the victim. There is also a time-dose relationship. In history there have been many cases where a murderer has slowly poisoned a person, through small quantities, over an extended period of time. Symptoms of acute poisoning include abdominal cramps, vomiting, cardiovascular collapse, coma and death. Symptoms of chronic poisoning can be difficult to diagnose as they are similar to a number of disorders. These symptoms include loss of appetite, nausea and vomiting, diarrhea and jaundice (Dickerson, 1980).

Although arsenic is a potent poison, few people during the 19th century, who worked in the manufacturing processes, died. It was believed that they had built up a tolerance to the arsenic and were therefore immune to its affects (Stewart, 2003)

2.2.2. Copper

Copper is found in the *d*-block of the Periodic Table and is number 29. It is a member of the transition metals, with the transition metals being defined as elements that form at least one ion with a partially full *d*-subshell (Burton *et al*, 1994). Copper can exist in three oxidation states. The most common are +2 and +1, +3 also occurs but it is uncommon and is easily reduced (Greenwood and Earnshaw, 1998).

Copper is commonly found in the form of copper pyrite (chalcopyrite), CuFeS_2 . It is estimated that this accounts for approximately 50% of all copper deposits (Greenwood and Earnshaw, 1998).

2.2.2.1. Uses

Copper is one of the first metals known to man, and was generally used in the production of coins. The Old Testament leads to the belief that copper was regarded as precious as gold:

“Ezra 8:27 Also twenty basons of gold, of a thousand drams;
and two vessels of fine copper, precious as gold”

Although the exact date of copper discovery is not known it is estimated that by 3500BC in the Middle East, copper was being produced by reducing the ore with coke (Greenwood and Earnshaw, 1998). Through the ages, copper has been used as a metal on its own for coinage, as well as in alloys for ornamental and other uses. The most common alloys used were bronze (copper and tin) and brass (copper and zinc) (Greenwood and Earnshaw, 1998).

Additionally, copper is an essential nutrient for plants including algae. The protein plastocynin contains copper and is found in the chloroplast. Plastocynin is a vital component in photosynthesis since it assists in the donation of electrons in the electron transfer chain (Robb and Pierpoint, 1983).

2.2.3. Lead

Lead is number 82 in the Periodic Table and resides in group 4 of the *p*-block. It can exist in two states: +2 and +4. This allows the metal to form complexes with a number of other elements. For example lead can combine with two oxygen molecules to produce PbO₂. Lead can also combine with one oxygen molecule to form PbO. The differences in the properties of compounds are dependent on the oxidation state. +2 oxidation states can cause the compound to have basic properties whereas +4 oxidation state of lead can have either basic or acidic properties (Burton *et al*, 1994).

Lead is one of the most abundant metals, and is found naturally in the Earth's crust as galena (PbS) (Ratcliffe, 1981).

2.2.3.1. Uses

Through the discovery of its properties it has been used for thousands of years. Although in its natural state the galena has limited uses, through refining processes, (melting and smelting) the number of uses has increased (Ratcliffe, 1981).

Uses of lead through history have been for glazes of pottery and for colouring paint. It was not until the Romans discovered that lead was resistant to deterioration that it was used in vast quantities. They used lead for water pipes and because of the increase in the demand for lead, many of the mining sites were exploited to the maximum (Ford, 1983).

Although lead is thought to have been used extensively since the Roman Period, it is known to have existed long before this; the Ancient Egyptians used the metal in the production of statues and in cosmetics. It was common for the men and women to use lead powder (white) as eye shadow (Ratcliffe, 1981). One of the earliest mentions of lead was in the book of Exodus in the Old Testament:

“Exodus 15:10 Thou didst blow with thy wind, the sea covered them: they sank as lead in the mighty waters.”

2.2.3.2. Hazards

Lead is considered to be a heavy metal poison. The active site of lead includes inhibition of enzymes such as ATPase, and also inhibits the production of haem, a major component of blood. Typical symptoms of lead poisoning are anaemia,

headaches, convulsions, kidney failure, brain damage, central nervous system disorders and death (Greenwood and Earnshaw, 1998).

There are a number of ways in which a person can be exposed to lead; these can be occupationally related as well as the consumption and inhalation of lead. In historical times, the consumption of contaminated foodstuffs was more frequent, as lead was used in the making of food utensils and for pipe work carrying water. Additional method of lead intake occurred through the use of lead powder for cosmetics. The powder was applied directly onto the skin and could then be absorbed through the skin (Ratcliffe, 1981).

2.2.4. Tin

Tin has an atomic number of 50 and resides in the *p*-block of the Periodic Table, directly above lead. It is naturally occurring in many different ores; however the most important ore is that of cassiterite (SnO₂). Tin has the largest number of stable isotopes (10) and because of this it is capable of forming complexes with the majority of the Periodic Table elements (Smith, 1998).

2.2.4.1. Uses

As with lead, tin is one of the oldest metals known to man, further more it was also mentioned in the Old Testament.

“Numbers 31:22-23 Only the gold, and the silver, the brass, the iron, the tin, and the lead, everything that may abide the fire, ye shall make it go through the fire, and it shall be clean: nevertheless it shall be purified with the water separation: and all that abideth not the fire ye shall make go through the water.”

The production of tin goes back to approximately 3500BC, when it was alloyed with copper to produce bronze and defined beginning of the Bronze Age. Bronze was used for tools as well as weapons and statues (Smith, 1998).

2.2.5. Tungsten

Tungsten is found in the *d*-block of the Periodic Table and is given an atomic number of 74. It is placed in the 6th period and occurs in the same group as molybdenum and chromium. There are five naturally occurring states, +2, +3, +4, +5 and +6. Tungsten occurs naturally as scheelite (CaWO₄) and wolframite (Fe, Mn (WO₄)) (Greenwood and Earnshaw, 1998).

2.2.5.1. Uses

Tungsten was one of the later metals to be discovered. In 1781, Swedish chemists, Scheele and Bergman isolated an oxide from the mineral now known as scheelite. The oxide was named as tungsten. Two years later two Spanish brothers (J. J. and R. d'Elhuyar) showed that the same oxide was a component of wolframite, and named the oxide "wolfram". It is this name that gives the metal its symbol (W) in the Periodic Table. But both names are widely used (Greenwood and Earnshaw, 1998).

Approximately 50% of the world's production of tungsten is in the form of carbide (WC). In this form tungsten is extremely hard and wear resistant and because of this, it is commonly used as tool tips. The most well known use of pure tungsten is in electric light bulbs (Greenwood and Earnshaw, 1998).

2.2.6. Zinc

Zinc is another of the transition metals in the *d*-block. It has an atomic number of 30 and is situated next to copper on the top row of the *d*-block. The most common zinc ore is that of sphalerite (ZnS). But the most commonly used ore is zincite (ZnO) due to its high percentage (80.3%) compared to sphalerite (67%) (Cammarota, 1980)

2.2.6.1. Uses

Pure zinc metal is difficult to obtain, since the ore needs to be reduced in the presence of charcoal at approximately 1000°C, at which point the metal becomes vaporised and is collected in a condenser without oxygen. Because of this, the pure form of zinc was not used until the 13th Century. However the alloy was used as it was easier to smelt with other metals. Palestinian Brass (between 1400 and 1000BC) was created alloying zinc and copper together. Since the percentage of zinc in the brass was approximately 23%, it is unlikely that this was a result of accidental contamination, but was done deliberately (Greenwood and Earnshaw, 1998).

Chapter 3: Mining history and associated problems

3.1. Mining history

The earliest evidence of metallurgy was found from a statue discovered in the temple of Abydos in Upper Egypt (Lead Development Association International, 2001). The statue is believed to be approximately 6000 years old, and suggests that the Egyptians had the knowledge to mine, extract and mould lead, as well as other metals. Gold and other precious metals were also used and consequentially it confirms that the Egyptians had developed the skills to mould different metals, with varying properties, as well as separation and purification. However, additional evidence of mining suggests that this activity began in the Palaeolithic period, approximately 100,000 years ago (Gregory, 1980).

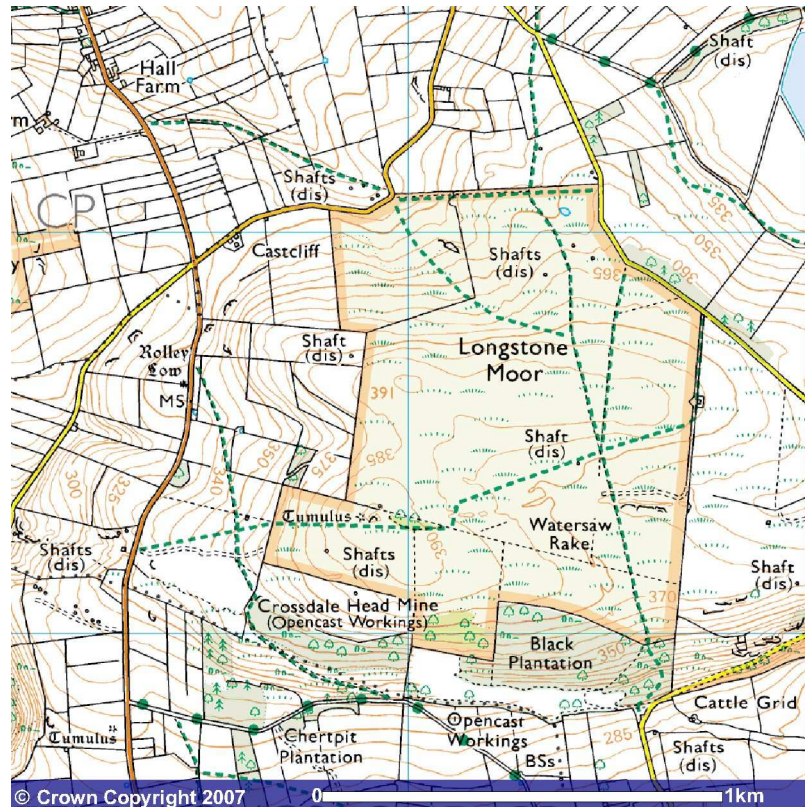
Throughout the history of mining, it has been evident that for the operation to be profitable, a high concentration of the mineral is required. Therefore, the miners developed ways of recognising where rich mineral veins were located. In the case of metals, the miners found that by examining the surrounding area and the presence of certain plant species it could be determined if there was a metal vein. For example, in the Peak District the presence of Spring Sandwort/Leadwort (*Minuartia verna*) and Mountain Pansy (*Viola lutea*) indicated a lead vein (Peak District National Park Authority, 2005). Once a vein was discovered, the area was mined extensively, however not all the metal could be extracted and a small proportion remained in the waste rock; the removal of which would have been unprofitable. Through improved technology, more of the metal could be removed (Gregory, 1980). The mining industry created a large volume of waste and since not all of the metal deposit was removed, the waste contained varying concentrations of the metal. The waste itself was deposited in the area, and these can still be seen in the form of spoil tips (Morgan, 1988).

3.2. Mining sites in Britain

As discussed previously, the mining industry has existed for over 6000 years and evidence of these activities can be seen in the form of abandoned mine tunnels, buildings and spoil tips. In Britain, it is believed that the underground mining of minerals and rocks began over 4500 years ago (British Geological Survey, 2007), with many counties becoming the most profitable in the world during their peak production.

The remains of mining in Britain can be seen on ordnance survey maps, and gives an indication as to counties where mining activity occurred. Through a combination of geological surveys and the examination of historical records, the individual mines were located. Figure 3-1 shows an example of an ordnance survey map illustrating the presence of shafts and mines. The map also shows the presence of a lead vein (Watersaw Rake) that would have been worked in the past.

Figure 3-1: An example of an OS Map – Longstone Moor, Derbyshire, UK
(SX195 730)



Throughout mining history, the discovery of metals and minerals properties led to the complete exhaustion of local reserves, consequently resulting in attempting to find other reserves in other countries. It is believed that this is one of the reasons the Romans invaded Britain (Anon, 2006). The Romans required lead for various uses including water pipes, and once mixed with tin for, pewter food utensils. It was determined that Britain had rich reserves of lead in a number of counties (For example Shropshire, Derbyshire and Somerset), and were mined for extensively. Evidence of which is limited, as there is little archaeological remains other than mining tunnels and pure lead ingots (pure lead blocks), which were dated with the year and place of production.

The mining sites designated on maps do not distinguish between the elements that were extracted. But through historical records, it has been determined that certain counties were more well known for certain elements compared to others. For example, Cornwall is famous for its production of tin, Derbyshire for its production of lead and Anglesey for its production of copper.

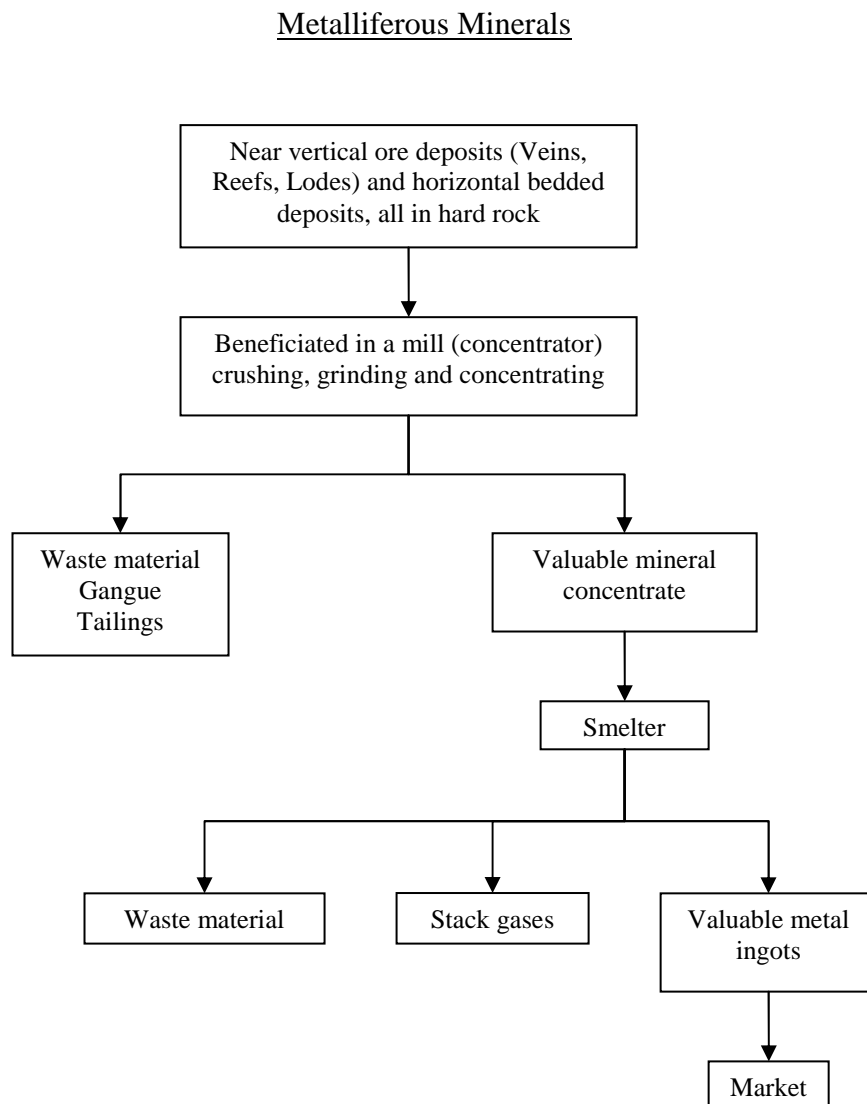
3.3. The mining process

There are a number of mining activities which take place, and these are dependent on the mineral that is being extracted:

- Metalliferous – The extraction of metallic minerals
- Industrial – The extraction of industrial minerals
- Fuel – The extraction of minerals needed for fuel (Gregory, 1980).

The process of metallic mining is depicted in Figure 3-2, and shows that the main stages of this process are mining, treatment and wastage.

Figure 3-2: The process of metal mining from extraction to sale (modified from Gregory, 1980)



The process involves several stages, and at each of the stages the mineral is increasingly concentrated. The first stage was beneficiating the ore, where the ore is crushed and ground by a mill; the lumps of ore are then removed leaving the unwanted minerals. During this stage, some of the ore is unavoidably lost. The lumps of ore that remains are then concentrated further, at this point another small fraction is lost. The remaining product is then placed in a smelter, which is usually found on the site of the mine, but other main sites were also used, depending on the distance from the mine. In the smelter, the ore is mixed with fluxing agents, fuel and are fed to the blasting furnace. The ore is heated to such a temperature that the metal melts and the molten metal is collected at the bottom of the hearth. Impurities collect and float above the molten metal as fluid slag (Gregory, 1980).

The molten metal is poured into moulds to create ingots, which once cooled, are sold on the open market (Gregory, 1980). It is apparent that throughout the mining and refining processes a large amount of waste is produced. It is this waste which is causing concerns as it still contains high concentrations of metals. Because it is inefficient to extract all the metal, an unknown concentration is deposited in the waste. Most of the waste is usually disposed of in the vicinity of the mine. The larger pieces of rock that have been extracted can be used for other purposes for example to build houses, or to prop up the ceiling in the mine shafts. Miners were not encouraged to waste any material.

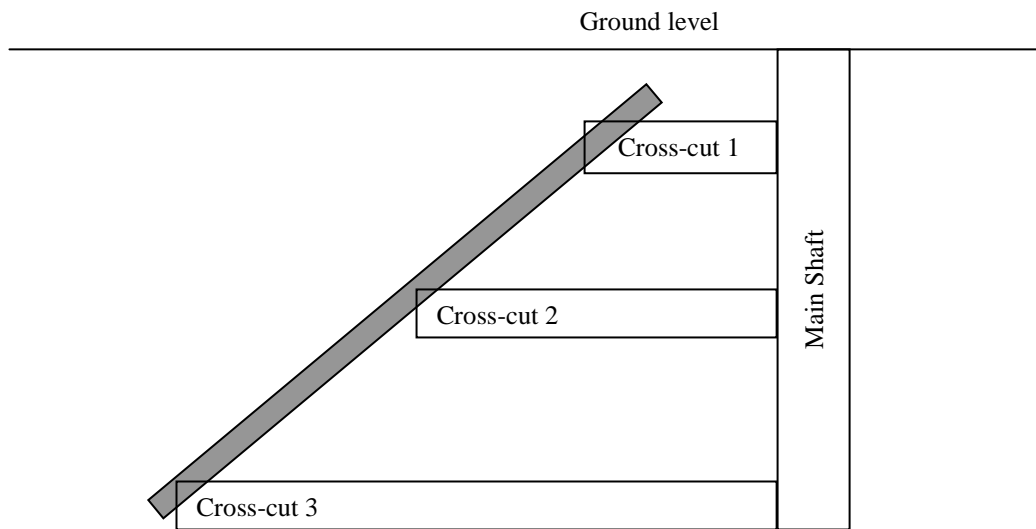
Currently there are four main classes of mining: underground, surface, alluvial, and non-entry (Thomas, 1973), which have largely been developed over the last century. In this research project only underground mining is considered. The other methods generally use modern technologies that were not available during the 1800s or earlier.

Underground mining was generally used when vertical or horizontal veins or lodes were found. During the 1800s these would have been worked by hand, using non-powered tools (shovels, pick axes). Explosives were employed to remove large volumes of rock, but were considered extremely dangerous. During the latter part of the 19th Century, pneumatic drills were introduced to increase the speed of the mining process, but unfortunately, was responsible for a number of deaths (Riewerts, 1973).

For vertical lodes, the main entrance to the mine was always the main shaft. This was a vertical shaft running through the ground. At points along the shaft, cross

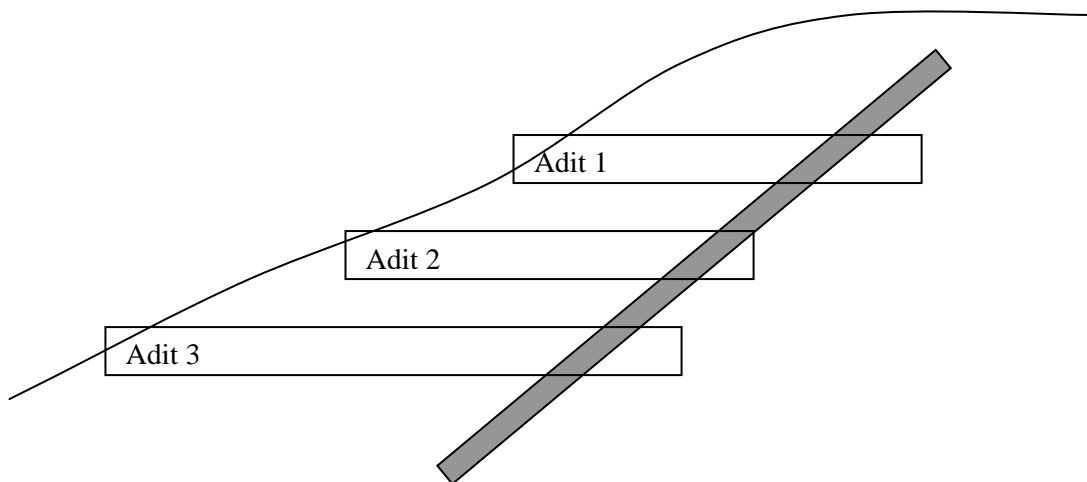
cuttings could be made to reach the vertical vein. These cross cuttings would be made at increasing depths down the shaft (See Figure 3-3)

Figure 3-3: Cross-cuts through a vertical vein of mineral (modified from Thomas, 1973)



If the mining site was found to be on a hillside, an easier method of reaching the lode would be to use horizontal shafts (adits) to extract the mineral (See Figure 3-4).

Figure 3-4: Mining for a near vertical vein of lead on a hillside using adits (modified from Thomas, 1973)



In both cases of mining, the depth of the mine that could be dug would be determined by the water table. If the vein ran through the water table, it was difficult to follow (Stokes, 1996). As the mine continues, the extraction of rock would be replaced by water. During the latter part of mining history, pumps were introduced to extract the water, and therefore enable the miners to continue mining at greater depths. However this only succeeded so long as the pump was successful in water extraction; in some cases the ground water would seep into the tunnels faster than the pump could remove the water and therefore the tunnel became flooded and as a consequence became unworkable. In the event of the complete depletion of the mines resources, the mine would be closed (Stokes, 1996).

Many times, throughout the history of mining, the same area would be mined several times or re-worked. As new technologies improved the extraction of smaller quantities of the metals from the ground and in some cases the spoil tips, mines were reopened. Smaller lodes that were not mined previously could now be worked (Kirkham, 1968). Because of this it is difficult to determine when the first mining attempts were made. Evidence for mining in an area can be found in historical documents and archaeological evidence. From this information it has been determined that certain mines in Derbyshire, UK, were worked by the Romans, and that these same mines were re-worked during the last century (Stokes, 1996).

3.4. Associated mining problems

In 1556, the first mining textbook was written by Geogius Agricola titled *De Re Metallica*. The author outlined the devastation caused in Germany because of mining activities. It was noted that the mining industry devastated the woodlands surrounding the mine through the removal of trees for various uses, including machinery and smelting of metals. The author also describes the impact on the freshwater environment:

“When the ores are washed, the water which has been used poisons the brook and streams, and either destroys the fish or drives them away.” (Agricola quoted in Down and Stocks, 1977)

Although written in 1556, it has been said that the book could have been written in recent times (Down and Stocks, 1977)

Problems surrounding the mining industry have always included visual impacts of mines and spoil tips as well as their physical effect on the environment. These impacts can include pollution of air, water and soil (Down and Stocks, 1977). The most notable impact, both physical and visual, is that of the remaining spoil tips, often centuries after the mine has closed. Visual impact is usually associated with the abandoned buildings falling into disrepair and the presence of the remaining waste tips. The existence of spoil tips in the environment has caused great concern as to their effect on the surrounding area (Shi and Erickson, 2001; Gabler and Schneider, 2000).

3.4.1. Atmospheric pollution

There are five main atmospheric pollutants, these are:

- Carbon monoxide – Generated by the incomplete combustion of hydrocarbons
- Hydrocarbons – Arises from the partial combustion of fossil fuels
- Oxides of nitrogen and sulphur – Resultant from burning fossil fuels
- Particulates – Solid or liquid particulates from combustion or disturbance (Down and Stocks, 1977: pp 59)

Atmospheric pollution is a major concern, as pollutants do not always reside in the area of discharge. Certain pollutants are transported and cause damage to a site that is markedly distant from the source (Aberg *et al*, 1999). For instance: Britain's production of sulphur dioxide during the last 50 years and its effects on the Netherlands's forests. However the extent of the transport of the pollutant depends on many factors including: type of pollutant (gaseous or particulate), inversions, wind speed and direction, climate and topography. In the case of particulates, the size of the particulate is also important; the smaller the particulate the further it can travel (Pyatt, 2004).

The settling of particulates causes the contamination of soils, water and vegetation and can result in long term issues. For soils, in high concentrations, the pollutant can move through the soil matrix. It can then either be taken up into plants, or move vertically through the soil into the water table (Cances *et al*, 2003). Settling of particulates in water bodies can result in the pollutants introduction into the food chain and being bioaccumulated (Schneider, 1984). Vegetation can either take up the pollutant from its deposition on the soil or block the stomata on the leaves preventing gas exchange and altering the health of the plant (Robb and Pierpoint, 1983).

3.4.2. Water pollution

The pollution of water courses and bodies arises mainly from the introduction of substances into natural waters; this then causes a physical and/or chemical change. This can result in the water being unsuitable for human consumption or for industrial use (Down and Stocks, 1977). This contamination of the hydrosphere, results in ecological damage, where changes occur including a decrease in biodiversity (Ward *et al*, 1984).

Pollution of water occurs through the direct contact with pollutants, as a result the water contains residual quantities of un-recovered minerals, large quantities of waste mineral matter and dissolved chemical constituents arising from these and traces of any reagents which have been used in the mining processes (Down and Stocks, 1977).

In abandoned mines, water pollution usually occurs through the movement of minerals. Runoff is considered a large problem since after rain fall, disturbed land is more susceptible to erosion and therefore the minerals are easily transported (Pyatt and Birch, 1994). Leaching also causes some concern as this involves the movement of minerals through the soil. As the rainfall moves through the soil toward the water

table, the toxic ions are dissolved into solution and therefore the movement of the contaminants occurs more readily (Yukselen and Alpaslan, 2001).

3.4.3. *Soil pollution*

Studies have been carried out to determine the extent of the contamination from spoil tips (Yukselen and Alpaslan 2001; Clark, Walsh and Smith 2001; Dahmani *et al* 2000). Maskall *et al* (1995) concluded that metals such as lead and zinc are leached vertically down through the soil at a rate of approximately 0.28 and 0.29 cm yr⁻¹ respectively. Although the figures are quite low, over a period of hundreds of years, the metals can reach ground water levels and therefore pollute water supplies. However they also concluded that the rate of migration was heavily dependent on the soil itself. Since it has been shown that vertical downward movement of metals can occur, so too can lateral movement (Davies and Balinger, 1990). This would mean that the surrounding area around the spoil tip can also be contaminated.

Chapter 4: Remediation of contaminated land

4.1. Reclamation or Restoration?

Since the British industrial revolution, the remains of a thriving mining industry can still be seen throughout the UK. However, it is not just the remaining buildings that have provided some concern, the by products of the mining activities have left a legacy of contaminated land, and in some cases the by-products are toxic to animals (including humans) and plants. Because of this it has been a priority to reduce the concentrations of certain contaminants to prevent any adverse effects. There are two ways remediation of contaminated land can occur; reclamation and restoration. Reclamation is by definition the reclaiming of land that was previously unsuitable for use. This involves the removal or containment of the contaminants to prevent their further movement and damage to the environment, whether it is plant and animal or future building works.

Where reclamation of the land is not possible, local authorities and historical societies work together to restore the old mining works and their spoil tips to its previous condition, and information centres have been set up to learn about the past mining activities.

Because of the volume of contaminated land that is remaining the task is ongoing and the process of assessing contaminated land is discussed here, along with some of the various reclamation techniques currently in use.

4.2. Investigating contaminated land

The presence of industrial archaeological at various sites around the UK, are signs of an industrial past, however these are not the only remains. As a consequence of the booming industry during the past two hundred years, acres of land have been left contaminated with various pollutants. In 1997, the suggested estimate of contaminated land was between 100,000 and 220,000 ha, equating to approximately 0.4% and 0.8% of the total UK land area (Young *et al*, 1997). Although all the “pollutants” are naturally occurring, with these concentrations being referred to as background concentrations, the extracting and smelting processes have altered the compounds in two ways, firstly by increasing the concentrations and secondly by altering the compound to such an extent where they were once harmless and are now potentially hazardous to human health.

To prevent any confusion as to what constitutes contaminated land, the Environment Protection Act (1990) stated that the definition of contaminated land is:

“Any land which appears to the local authority in whose area it is situated to be in such a condition, by reason of substances in, on, or under land, that –

- a) Significant harm is being caused or there is a significant possibility of such harm being caused; or
- b) Pollution of controlled waters is being or is likely to be caused.”

Where harm is later defined as:

“harm to the health of living organisms or other interference with the ecological systems of which they form part and, in the case of man, includes harm to his property” (Bell and McGillivray, 2000: pp. 533)

This definition has meant that if there is contamination of soil and/or water, but is not causing harm then it is possible to consider leaving it alone until the land needs to be developed or reclaimed (Young *et al*, 1997).

The investigation of contaminated land involves a risk assessment to determine the level of contamination and whether further steps should be taken to reduce these risks. When decided that an area of land has been contaminated, a series of risk assessments are performed to determine if the land is in need of remediation. During this

assessment of a site, the area is evaluated by gathering and interpreting information concerning the characteristics of the contaminant(s) including their source, pathways (a route in which a receptor can be exposed to the contaminant) and receptors (an organism which is being harmed or affected by the contaminant). The characteristics of the contaminants are used to determine their distribution, fate and transport and are thus used to determine:

- a) Suitability of chemical analyses during the site investigation
- b) Exposure assessment
- c) Screening of remedial technologies (Young *et al*, 1997)

4.3. Reclamation Methods

Once the risk assessment has established that the investigated site is likely to have a detrimental affect on the environment and organisms, the site is recommended for remediation. Remediation methods differ, depending on the extent of the contamination and the size of the area that is affected, but the objective is to remove the potential hazardous material to reduce the risk of harm to the environment and to organisms (Wood, 1997).

4.3.1. Containment

The containment method of remediation is achieved through the use of low permeable barriers to either isolate or prevent the spread of contaminants. These include the use of cover systems or in-ground barriers.

4.3.1.1. Cover systems

The use of a cover system is common in the UK for the reclamation of land that was previously used and now contains harmful contaminants, including sites for waste disposal, gasworks and metal mining. It consists of a layer or a number of layers of uncontaminated material covering the contaminated area which prevents these contaminants from being exposed to the vegetation, animals (including humans) and the environment. The layers can be composed of different materials such as natural clays, soils, concrete and non-contaminated waste materials (e.g. sewage sludge) (Wood, 1997).

Although a simple solution, it is not advisable for long term problems as the contaminants remain at the site and therefore the potential to cause harm still exists.

4.3.1.2. In-ground Barriers

In-ground barriers are physical barriers that can be used to isolate the contaminants at the site of deposition. The barriers are designed to prevent the movement of contaminants vertically or laterally and are placed either above, below or around the contaminated site and therefore isolating the site. Problems arise through the construction of the barrier as it is difficult to ensure the continuity of the barrier, especially where there are obstructions present, gaps in the barrier allow for movement of the contaminants (Purves, 1985).

The success of the barrier depends greatly on the types and nature of the contaminants and the physical condition of the site. Like the cover system, the barrier is only a short term solution and does not remove the contaminants from the site; also over time the barrier may need replacing due to degradation, and therefore constant monitoring is required.

Used in conjunction with cover systems, the contaminants can be contained to a certain degree, however long term monitoring is needed to ensure the containment methods are still performing up to expectations.

4.3.2. Removal

The most common and often used method of remediation is that of removal, the contaminated material is removed from the area and is disposed of in specially prepared land fill sites. This is a fast, low costing and effective method; however the problem is not altogether solved but transferred to a new site. Although the new site is specially prepared, there is no guarantee that the contaminants will be isolated; therefore there is a long term problem of exposure. The removal of modern spoil tips can prevent the release of the contaminants, but it is the old tips that cause the concern, since these have existed in the environment for various amounts of time and therefore some of the contaminants could already have an effect on the environment.

4.3.3. Physical processes

Physical processes are used to separate the contaminated from the uncontaminated material using their physical properties (e.g. density and particle size) either by exerting an external force or by altering a physical characteristic to allow separation to occur. By using these methods the contaminated material can become concentrated in a reduced volume.

4.3.4. Biological processes

Biological processes include the use of micro-organisms naturally present in the soil to decompose the contaminants. The process is based on the actions of the microbes to oxidise organic compounds and to therefore reduce them to less harmful forms (Syms, 1997). This method can involve maintaining the optimum environment for the microbes, including the addition of nutrients and the introduction of water and

oxygen but the eventual degradation of the contaminants is slow even under optimum conditions (Purves, 1985).

The use of microbes is limited since they can only be used for organic contaminants; however plants can be employed as a method for removing certain contaminants (e.g. heavy metals) from the soil through bioaccumulation (Wood, 1997). The contaminants can be absorbed into the plant with nutrients and water, but are then locked away in to the plant tissues and can not then be expelled from the plant, after a period of time the plant can be harvested and then disposed of safely. Ideally the plant is highly tolerant and is unaffected by the contaminant (Purves, 1985).

4.3.5. Chemical Processes

Chemical treatment of contaminated soil involves the conversion of the contaminants to less harmful compounds through a range of processes including oxidation-reduction, dechlorination and hydrolysis. Chemical agents are applied to the contaminated area to induce the chemical change, the agent applied and the subsequent chemical change depends on the contaminant (Wood, 1997). The use of chemical treatments can be problematic. They may be able to reduce the concentration of some contaminants, however, any unused chemical treatment remains in the soil, and long term effects of this is not known. Also during the chemical conversion, heat and mixing may be required to assist in the reaction and in the field this can be difficult (Wood, 1997).

4.4. Restoration

Currently a number of mining sites have been, or are in the process of being, restored to their original working conditions; these include Snailbeach in Shropshire, UK and Morwellham Quay in Devon, UK. Both have been restored through the dedication of volunteers and are now places where tourists can learn about the mining industry. It is also an important site from an industrial archaeological point.

Morwellham Quay was a copper mine in use during the 1800s, and because of its position on the Tamar River, it was one of the main sites for the transportation of minerals by boat from the local mines in the area. The area has now been transformed into an extensive open air and indoor museum that also allows visitors to ride the tramway into the main adit of the mine. Figures 4-1, 4-2 and 4-3 are photographs taken at Morwellham Quay, showing the near complete restoration of the Quay.

Figure 4-3 shows the outside of one of the restored miner's cottages. The building has been restored and even allows access to the upper floors for wheelchair users. The displays allow any visitor to see the kinds of conditions the miners lived in during the height of the mines activity.

Figure 4-1: The restored water wheel at Morwellham Quay that extracted water from the lower levels



Figure 4-2: Partly restored rail tracks at Morwellham Quay previously used to carry minerals from local mines to transport by boat



Figure 4-3: Restored miners cottages at Morwellham Quay, Devon, UK



Another restoration example is that of Hafnôr Mine, near Betws-y-coed in Wales, UK (see Figure 4-4); the restoration is very different from Morwellham Quay as the buildings have not been restored to their original condition but have been made safe for visitors to explore. Notice boards with information have been placed at significant locations, and the area is now a popular place to visit for walkers. The area around the mine where spoil tips once existed has been revegetated with grass.

Figure 4-4: Remains of the mine buildings at Hafnôr Mine, Betws-y-coed, Wales, UK



Chapter 5: Methodology

5.1. Selection of sites and metals

A number of skeletal samples were made available to the research project. To incorporate these samples in the project, it was decided to investigate the transfer of lead through trophic levels. To study this further a site was required to determine the flow of lead from the environment.

The process of examining the transfer of lead through trophic levels should begin with the origins of the metal. Therefore, it was decided to investigate sites that were known for the mining and smelting of lead. Through literature research it was determined that sites previously mined for lead included areas of Derbyshire, Devon and Wales.

When deciding on a research site, a number of criteria were defined:

- Accessibility of site
- Health and safety considerations around the site
- Presence of spoil tips
- Presence of vegetation
- Present activities occurring at the site (if remediation techniques are currently being attempted)

Research surrounding possible sites was retrieved from a number of sources including the internet. Although, internet sources can be unreliable, much of the information was published by mining enthusiasts, who had visited the sites for historical research. After confirming the information through library resources, visits to a number of sites were made. During the visits, each site was assessed on the possibility of conducting further research. Sites that were visited were: Derbyshire; Matlock, Bakewell and Stoney Middleton; Anglesey and North Wales; Devon and Cornwall.

Although Devon and Cornwall are not well known for their lead, an enthusiast had compiled a CD of mines in Devon and Cornwall (Coleman, 1999). The author had

included information on the metals and minerals that were extracted from the mines as well as grid references.

Many of the sites that were visited were found to be unsuitable as they had been maintained by the local authorities. In some cases the area had been made safe for tourists. Other sites had restricted access as the area was unsafe due to the unknown locations of mine shafts.

One of the main sites selected was Blanchdown Wood, Devon. The site was found through conversations with locals and was an area that was popular with recreational users. Blanchdown Wood was a confirmed mining site on the CD, but was not initially considered as it was not a major producer of lead, but a copper and arsenic mine. Regardless of this, the site was visited and it was determined that the site was suitable for the research project. The site was located on private property managed by Tavistock Woodlands Estate, after conversations with the manager, Mark Snellgrove; permission was given to visit the site and to conduct the research. The site met all the criteria that were initially laid.

The second site that was decided on was that of Snailbeach, Shropshire, UK. This site was a major lead producing mine and its history spanned several centuries. This site was chosen as there were clear signs of remediation activities. The examination of this site meant that there could be a direct comparison between Blanchdown Wood, with no remediation activities, and Snailbeach, with numerous remediation techniques used.

At both sites soil samples were taken to determine the concentration of metals in the soil. Also a number of vegetation samples were taken, to determine the concentration of the metals accumulated from the soil.

Initially the metals that were researched were those that the mine extracted. Blanchdown Wood was a copper and arsenic mine and Snailbeach was a lead and zinc mine. Through researching the sites, it was found that secondary analytes were extracted; mostly as by-products these included tungsten, tin and lead (Blanchdown Wood). Therefore the research centred on: copper, arsenic, tungsten, tin and lead, at Blanchdown Wood and lead and zinc at Snailbeach. Although the analytes differed between the sites, comparisons could be made as to the extent of the pollution from the mining activities and how remediation techniques affect the pollution.

5.2. Collection

During the research project, a number of different types of material will be used; these include bone, plant and soil. For each type of material, different collection methods were used and these are outlined.

As discussed previously, Blanchdown Wood was the site of mining and smelting activities, and as a result abandoned building remains, spoil tips and waste sites are still present. The presence of these obstacles meant that the sampling of soil and vegetation was restricted. After examining the site, a sampling strategy was determined based on areas that were easily accessed, with minimal risk.

Other samples that were included in the research were: animal remains, bird of prey pellets, foodstuff sold for human consumption, and reproductive products. These were opportunistic samples that were not originally in the sampling strategy

5.2.1. Soil

Soil material was collected from the sites using a clean stainless steel trowel and was sampled 5cm below the surface layer of soil. These samples were then placed in bags and were labeled and sealed, to prevent the samples from accidental damage, the samples were then placed in another bag. All the samples were kept in a sealed container during the transport back to the laboratory.

On returning to the lab, the samples were air dried for 7 days, and then dried in an oven at 105°C for 3 hours (Radojevic and Bashkin, 1999). The samples were then ground up using an acid washed pestle and mortar. The ground material was then sieved through a stainless steel 2mm sieve. The sieved samples were placed in a clean HDPE bottle and stored until analysis.

5.2.2. Plant material

Plant material was collected from the site using clean stainless steel secateurs and was kept whole as much as possible. The plant material was stored in bags to prevent cross contamination between the samples in some cases this meant using bin liners. These too were then placed in a sealed container for the journey back to the laboratory.

On returning to the laboratory, the plant material was thoroughly washed with deionised water. Using gloves, different parts of the plants were removed, i.e. the leaves, berries and flowers (where applicable) were separated from the stem, these

were then left to air dry separately for 7 days. After this period the samples were dried in an oven at 70°C for 24 hours. The samples were cooled and either ground up in an acid washed pestle and mortar or placed in a stainless steel blender for 5 minutes. The samples were then stored in clean HDPE bottles until analysis.

5.2.3. *Bones*

A number of bone samples will be used during this research, from various locations. All samples of bones were handled using gloves and protective laboratory coats. In the laboratory, the bones were photographed and where possible, they were identified. The bones that were found at sites were washed and dried in a closed cabinet. Samples of the all the bones were taken using a hand held saw, in a fume cupboard with the sash lowered as far as possible. Protective equipment was also used including gloves, eye shields, face mask and laboratory coat. The sub-samples were then oven dried for 3 hours and then stored in clean HDPE bottles until analysis.

5.3. Analysis: Heavy metals

During the research different types of materials will be sampled; this means that there will be varying degrees of difficulty in extracting the metals. Methods that have been used are listed below with corresponding literature.

- Nitric acid / Sulphuric acid / Perchloric Acid with microwave digestion, Palumbo *et al*, 2000
- Aqua Regia, Kim *et al*, 2001
- Nitric acid / Hydrochloric acid, heated, Eades *et al*, 2002
- Nitric Acid, Davies and Balinger, 1990
- Perchloric acid / Hydrofluoric acid / Hydrochloric acid, Dahmani-Muller *et al*, 2000

Each of the methods extracts the heavy metals into acid solution and are then analysed with atomic absorption spectrophotometer (AAS), or inductively coupled plasma – optical emission spectrometer (ICP-OES). The successfulness of metal extraction is dependent on the metal that is being extracted and the method used. The most effective method for heavy metal uses Perchloric and Hydrofluoric acids. However this method requires specialist equipment that was not available in the laboratory (Radojevic and Bashkin, 1999).

In many of the soil laboratories in the UK, Aqua regia is quickly becoming the standard method as the method is relatively easy to use, and can be used to analyse multiple samples.

5.3.1. Acid digestion – Soil, Plant, Bone

Chemicals

- Concentrated Hydrochloric acid (32%)
- Concentrated Nitric Acid (70 %)

Method

All samples were analysed using the Aqua Regia method (Radojevic and Bashkin, 1999). The amount of sample that was analysed depended on the type of material. For soil samples, 1.0g was used, but for the plant and bone material, 0.5g were analysed.

This is for two reasons: plant material is believed to accumulate higher concentrations of heavy metals and so smaller samples of the plant material were used. Also there was a limited supply of the material, for both the bones and the plant material. Each of the samples were analysed in replicate. Ideally 10 replicates were used but in some cases this was not possible and so as many replicates as possible were performed.

The Aqua Regia was made up using 150mL of 32% Hydrochloric Acid added to 50mL of 70% Nitric Acid. 10ml of this solution was added to each of the samples and left over night. The samples were then gently heated until they were simmering for 1 ½ hours. The samples were then cooled. After the cooling period a further 5mL of the Aqua Regia was added and the samples were boiled again for another 30 minutes. The samples were left to cool.

Due to the extreme variability of the hot plate, (+/- 10°C) the method of digestion was altered to ensure the variability did not affect the final results. The temperature was adjusted to approximately 70°C (a beaker with water and a thermometer were used to determine the temperature) but since part of the hotplate was either above or below this, the flasks were randomly placed on the hot plate and this ensured that the replicates were not all in the same area. After the hot plate had reached temperature the flasks rearranged every 30 minutes to ensure the samples were digested evenly. The flasks were left with only a few mL of solution. The samples were then removed from the hotplate and left to cool. A further 5mL of Aqua Regia is added and boiled again for a further 30 minutes. The samples were then cooled and filtered through Whatman No 541 or similar. The solution is made up to 50mL with ultra pure water. The final samples are stored at 4°C and then examined for various elements.

Using an inductively coupled plasma – optical emission spectrometer (ICP-OES), the samples were analysed for their concentrations of metals. This allowed for the concentrations of multiple analytes to be determined at the same time.

The calculation for the concentration of analyte in the sample is as follows:

$$\text{Concentration in sample (mg/kg)} = C * V/M \dots\dots\dots (1)$$

Where C is the concentration of metal in the extract (mg/L), V is the volume of extract (mL) and M is the weight of the sample (g).

5.4. Analysis: Nutrient media

During the algae experiment it was necessary to monitor the concentration of nitrates and phosphorus in the growing media. This can give a good indication as to the successful growth of the algae. A large decrease in either of nitrate or phosphorus compared to the initial concentrations can indicate that the nutrients are being depleted by the algae.

5.4.1. Nitrate

Chemicals

- 1000ppm nitrate (1.6306g Potassium nitrate in 1L distilled water)
- Ionic Strength Adjuster (ISA – 2M ammonium sulphate)

Method

A Nitrate electrode from Orion Research Incorporated, model 93-07 was used to determine the concentration of nitrate. The method used was modified from the original operating instructions. Nitrate standards of 100ppm and 10ppm are prepared using the stock nitrate solution. 20mL of each standard is placed in a beaker and 0.4mL ISA is added. A stirring bean is also added to ensure the solutions are thoroughly mixed. The probe is first lowered into the 10ppm Nitrate solution and the ISO/STD PT control is used to alter the display, until it reads 10ppm. The probes are then rinsed thoroughly with distilled water and then lowered into the 100ppm solution. As before the ISO/STD PT control is altered until the display is reading 100. The probes are then rinsed and placed in the 10ppm to ensure the display has not changed. This procedure may have to be repeated several times until the probes are calibrated. Once calibrated the probes can be rinsed and lowered into the samples. As with the standards, 20mL of sample is placed in a beaker with a stirring bean and 0.4mL ISA. Each sample is read in triplicate and the final results are averaged.

To express the reading as Nitrate (N) the following equation is used:

$$\text{Nitrate (N) mg/L} = ((\text{Reading} * 5) * (14/62) * \text{dilution}) \dots\dots\dots (2)$$

5.4.2. *Phosphorus (orthophosphate)*

The phosphorus content of water can be present as organic phosphorus, orthophosphate (in the form of inorganic PO₄) or as condensed (solid) phosphorus (US EPA, 2006). Since the primary source of phosphorus for algae is orthophosphate (Chapman and Chapman, 1981), its concentrations were monitored during the experiments.

In the past, the analysis of phosphorus was done using large amounts of sample solution. But the method has been scaled down by Victoria Surrage and William Carlile of the Nottingham Trent University (unpublished PhD thesis). The method now only uses a fraction of the sample (12.5µL instead of 0.5mL).

Chemicals

- 1.5M Sulphuric acid (2.5mL concentrated sulphuric acid made up to 150mL with distilled water)
- Ammonium molybdate (15g Ammonium molybdate dissolved in 750mL distilled water, 75mL concentrated sulphuric acid, and made up to 1L with distilled water)
- 1.5% Ascorbic acid (1.5g Ascorbic acid in 100mL distilled water)
- Phosphorus standard (0.44g potassium di-hydrogen orthophosphate in 1L distilled water (100ppm phosphorus))

Method

Depending on the expected concentrations of the samples, dilutions are prepared for the calibration curve and the appropriate amount of phosphorus standards are added to wells in the microplate. The total volume of standards in each well is 12.5µL. Initially two curves will be prepared, the first in the range of 0 – 25ppm of phosphorus and the second will be in the range of 0 – 100ppm of phosphorus. On each of the plates, both standard curves will be prepared in replicate.

For the samples, 12.5µL is added into the wells, and these too are done in replicate. The following outlines the order and quantities of solutions added to the wells:

- 12.5µL Sample or standard or control
- 12.5µL 1.5 M Sulphuric Acid
- 62.5µL Ammonium Molybdate reagent
- 62.5µL Ascorbic Acid

The final addition of ascorbic acid must be done within 3 minutes as this is the initiator of the reaction. The samples are left to be developed for 30 minutes and are then read on a plate reader at 620nm. The calibration curves are plotted and the equation of the line determined. The sample concentrations are then determined using the equation of the line. If dilutions of the samples have been made, the values are multiplied accordingly.

5.5. Inductively coupled plasma-optical emission spectrometer

The concentration of heavy metals was determined through the inductively coupled plasma – optical emission spectrometer (ICP-OES). The recent purchase of the Perkin Elmer Optima ICP-OES, allowed for the analysis of multiple elements in a relatively short period of time. Previously this would have been done using Flame Atomic Absorption Spectrometer; however the spectrometer needs to be calibrated with each element individually. This would increase the volume of sample required and be highly time consuming.

The ICP-OES was fitted with an AS-90 plus autosampler, capable of holding 106 samples, including calibration standards. Table 5-1 shows the standard operating parameters of the ICP-OES used in the research.

Table 5-1: Standard processing data used for the analysis of the elements

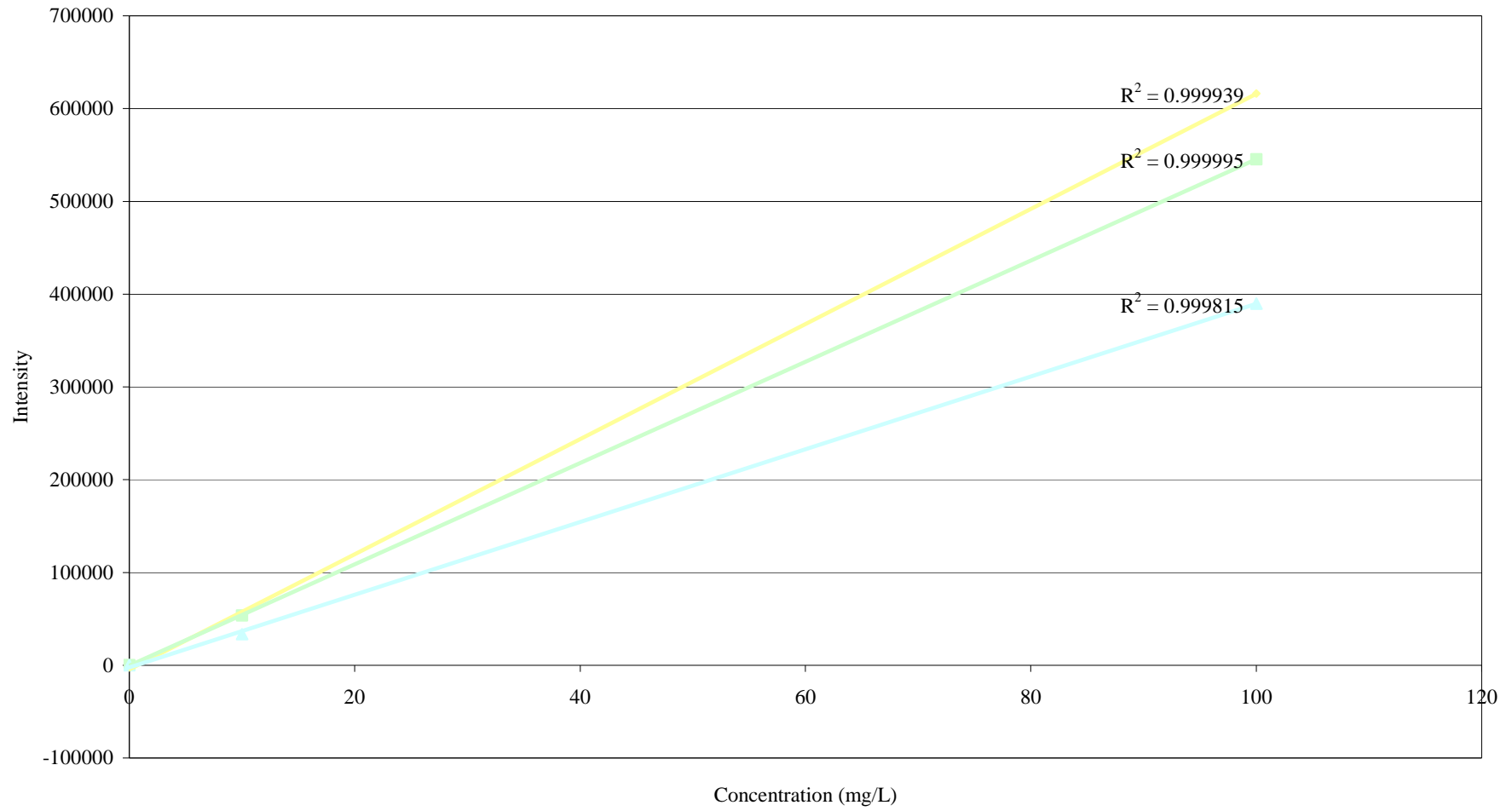
Plasma L min ⁻¹	Auxiliary L min ⁻¹	Nebulised L min ⁻¹	View dist. mean	Plasma View	Pump flow rate L min ⁻¹	Autosampler	
						Flow rate	Time
15.0	0.2	0.8	15.0	axial	1.5	1.5 L min ⁻¹	30 secs

Throughout the research, the equipment was calibrated using 3 standards. These were 0, 10 and 100ppm of the elements tested (copper, lead, tungsten, tin, arsenic, zinc, phosphorus, calcium, iron). Where concentrations were lower or higher than this range, the calibration standards were altered accordingly. To ensure the ICP-OES was producing accurate results, a number of standards were randomly placed in the autosampler.

The calibration standards were produced using certified solutions, from Fisher Scientific UK, diluted to the required concentration using blank solutions from the acid digestion process. Figure 5-1 shows an example of the calibration curves produced by the ICP-OES along with the corresponding correlation coefficients.

For all samples and standards, the readings were measured 3 times by the instrument, and an average was given.

Figure 5-1: Calibration curves for lead, tin and tungsten



5.6. Statistical Analysis

The use of statistics is essential when examining data as it enables us to reduce the number of data points down to manageable levels. With most if not all research, the replication of samples is used, to give an accurate view of the results.

In this research a number of statistical methods were used to analyse the data including standard deviation, 95% confidence intervals and ANOVA. Explanations of the statistical methods can be found in Dytham (2006) and Fleming and Nellis (2000).

5.6.1. Limit of detection and limit of quantification

With all instrumental equipment, there is a limit to its detection of analyte concentrations; this can vary not only between different types of instrumentation but also between instruments of the same type. The limit of detection (LOD) is defined as the concentration which gives an instrumental signal significantly different from the blank. The limit of quantification (LOQ) is the lower limit for precise quantitative measurements (Miller and Miller, 2000). To determine this limit a minimum of five standards, with known concentrations of the analyte, are used to create a calibration curve and from this the limit of detection and quantification can be determined. The limits are determined using the equations:

$$\text{Limit of detection} = Y_B + 3S_B \dots\dots\dots (3)$$

$$\text{Limit of quantification} = Y_B + 10S_B \dots\dots\dots (4)$$

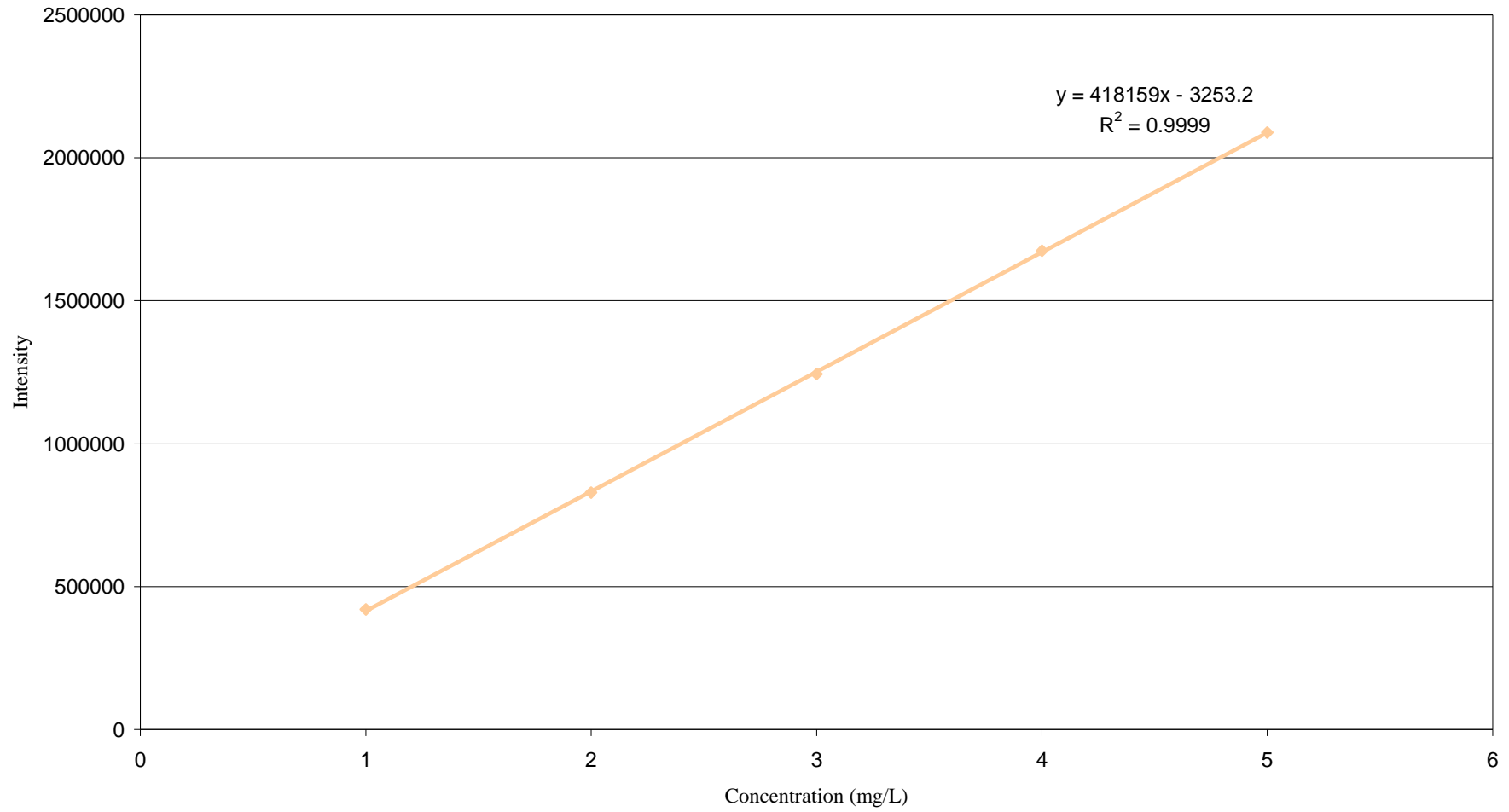
The values used to determine the limits are derived directly and indirectly from the calibration curve. Where Y_B is the concentration of the analyte that produces a signal equal to the blank (intercept) and S_B is the calculated gradient of the line. Figure 5-2 shows a calibration curve for copper concentration giving the equation of the line and the R^2 value. S_B is calculated using real intensity values and from calculated values using the linear equation. The calculation has resulted in an intensity value for LOD and LOQ being 82780.88 and 248439.8 respectively. These values can then be used in the linear equation and an LOD and LOQ in mg/L can be determined.

Table 5-2 gives the LOD and LOQ for the analytes used in this research (Miller and Miller, 2000).

Table 5-2: The LOD and LOQ values for analytes used in this study

Analyte	LOD Intensity	LOD mg/L	LOQ Intensity	LOQ mg/L
Arsenic	814.858	0.229	3045.66	0.765
Calcium	121759.475	0.606	380294.85	2.02
Copper	82780.88	0.174	248439.8	0.580
Lead	2830.982	0.110	8300.74	0.367
Phosphorus	1145.98	0.137	3636.3	0.457
Tin	1209.683	0.068	3593.61	0.226
Tungsten	6768.174	0.527	22328.18	1.756
Zinc	7021.916	0.077	22148.72	0.256

Figure 5-2: A Calibration curve for copper



Chapter 6: Research sites

6.1 The Geology of Britain – A brief description

The geology of the Earth varies considerably due to its history. Over billions of years the surface of the Earth has changed dramatically, primarily due to extensive continental drift. The movement of the tectonic plates, and land masses, has resulted in their current locations (Read and Watson, 1972). The plate boundaries are considered to be regions of active geological areas, where earthquakes and volcanic activity occur (McAlester, 1973).

The combination of volcanic activity, earthquakes and continental drift has led to the formation of differing rock types which are represented Britain (British Geological Survey, 2007). To understand the geology of an area, rock definitions are needed. There are three major types of rock; Igneous, Sedimentary, and Metamorphic (Ager, 1975). Igneous rock is formed from the cooling of molten solutions. The molten solution (magma) comes from relatively shallow depths in the Earth's mantle. The magma can come to the surface through the eruption of a volcano or it can rise up into the crust and hardens to form granite. Sedimentary rock is derived when areas of the Earth's crust is exposed as land are eroded by either water (rivers and seas) wind or ice (Bishop *et al*, 2005). The material that is produced is small particles called sediment. The sediment can be transported away from the source and laid down in layers. These can occur on the sea bed, in lakes and rivers, or on land to form greater thicknesses of sediment. Over a period of time the laid down sediment is hardened to form sedimentary rocks. Plate tectonics processes can cause folds being raised above sea level to form new continental areas. Some of the best sedimentary rocks are Sandstone and Limestone. Heat and pressure close to the plate boundaries can cause the rocks to become both chemically and physically different from its original state. The alteration can occur through the collision of large land masses. For instance the Alps and the Himalayas contain large quantities of metamorphic rocks resulting from the underlying plates coming together (Redfern, 2000).

Through the geology history of Britain there are different periods where metamorphic rocks have been created. The dates of these have been determined by the fossil content (Rodgers, 1977). From knowing the history of the Earth, with

corresponding information concerning the different species present, the metamorphic rocks can be aged. More recent methods have involved dating using chemical analysis (Ager, 1975). Using radioactive decay of certain elements, the age of the rocks can be determined more accurately. Essentially there are three major periods, Palaeozoic, Mesozoic and Caenozoic literally meaning ancient, middle and new life respectively, the division of the timeline is illustrated in Table 6-1.

Table 6-1: The different periods in Earths' history not including the Precambrian era (Ager, 1975)

Eras	Periods	Millions of years ago	
Caenozoic	Quaternary	2	
	Tertiary	65	
Mesozoic	Cretaceous	135	
	Jurassic	190	
	Triassic	225	
Palaeozoic	Upper	Permian	280
		Carboniferous	345
		Devonian	395
	Lower	Silurian	430
		Ordovician	500
		Cambrian	570

6.2 Tamar Valley and its geology

The Tamar valley lies along the county divide between Devon and Cornwall, in western England. The Tamar River begins approximately 6km from the sea, north of Bude in Cornwall and then stretches southwards and finally out to sea through Plymouth. The Tamar valley contains regions of Carboniferous and Devonian metamorphic rock, but it is the Granite region in the centre of the valley that has caused most interest (Perkins, 1972). The Tamar mineral lodes are associated with the reappearance of granite in the central valley. The river itself has managed to avoid the granite as far as possible, but it is at Blanchdown Wood that the river isolates a small area of Granite on the Devon Bank and giving rise to mineral lodes covering an estimated area of 20km wide and 6km to the north and south.

The predominant minerals found in the Tamar valley are tin and copper, but a number of associated minerals can also be found, for instance lead, arsenic (Booker, 1967). The lodes run mainly in an east-west direction through highly metamorphosed slate. The concentration of the minerals in the east-west lodes were minor compared with the north-south lodes. These are referred to as cross-courses and these were formed at a later date compared to the east-west lodes. Miners of the Tamar valley found that these cross-courses split rich east-west deposits (Goodridge, 1964).

6.2.1 Blanchdown Wood, Tamar Valley, Devon, UK: History

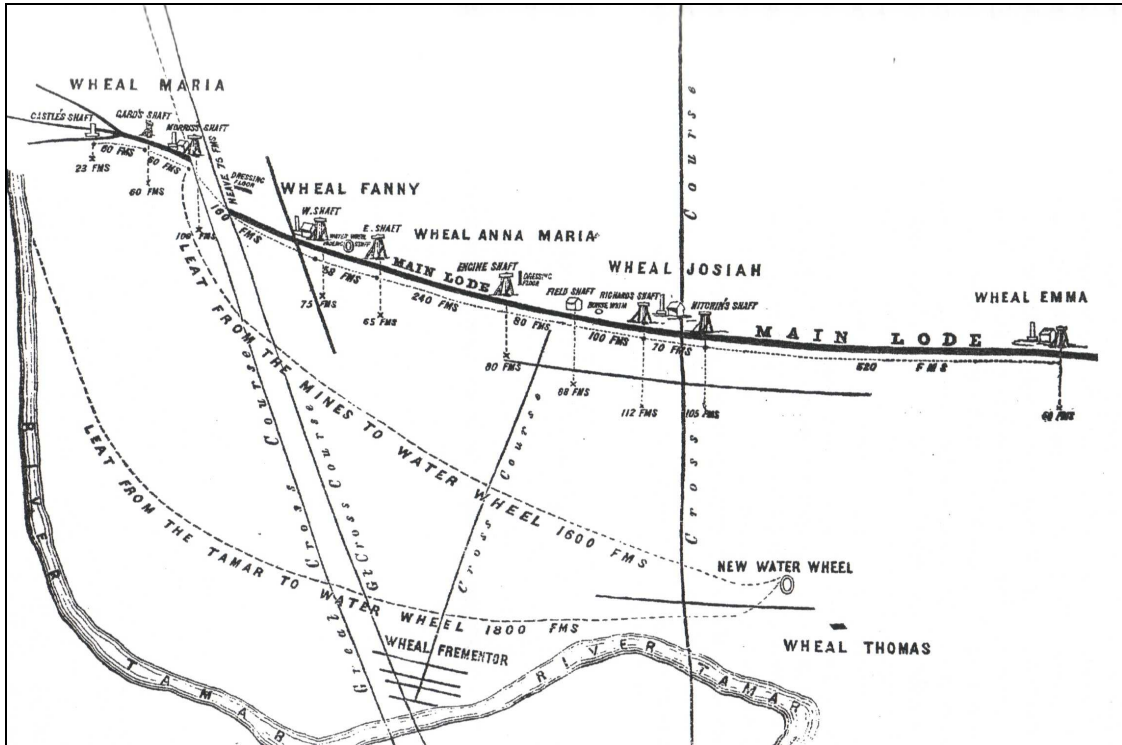
On March 25th 1844, Josiah Hitchens was granted the lease for Blanchdown Wood by the Duke of Bedford. Together with five other gentlemen, the Devonshire Great Consolidated Copper Mining Company was founded. The company had a joint stock of 1024 shares (Goodridge, 1964). The initial board of directors consisted of T. Morris, R. Gard, and W. Thomas. In 1846, the board was expanded to include W. Morris and J. Thomas.

An abandoned and neglected shaft, believed to have been sunk by ancestors of Josiah Hitchens, which had previously been sunk to a depth of 14 Fathoms (approximately 25.6m), was extended a further 4.5m to find a rich copper lode. It was determined that the lode was approximately 9m wide and contained 17% of copper (Stewart, 2003). At that time the average yield of copper ore sold between July 1844 and July 1845 in Devon and Cornwall was 7¾ % (mining Journal 26th July 1845). The high percentage yield for copper ore meant that this was the richest lode found in the UK.

After the discovery of the richness of the ore, production expanded rapidly. The lode was followed eastwards for approximately 30m before it was dislocated by the Great Cross Course, the fault line that shifted the lode 140m to the right. However the lode was easily located and Wheal Fanny was soon established. Following the course of the lode Wheals Anna Maria, Josiah, and Emma were soon established and in May 1846 the group of mines were collectively renamed as the Devon Great Consol Mines (DGC). At this stage the main lode had extended eastwards for nearly 3km (Jenkins, 2005). Figure 6-1 shows a sketch of the principal works, dated September 1850 (Stewart, 2003). It can be seen that the main lode extended almost continually from the initial workings of Gard's shaft to Wheal Emma. It was also determined that there were minor lodes found parallel to the main lode. These were not worked as the main lode was considered more important. However by the time work was closing on the main lode, DGC were finding it difficult to remain open.

During 1850 the works was so successful that over 1000 people were employed by DGC, including 342 young boys and girls (Stewart, 2003).

Figure 6-1: A sketch of the surface workings at Blanchdown Wood, September 1850
(modified from Stewart, 2003)



In 1864, the first signs of copper decline were seen in Devon and Cornwall. The production levels had fallen by 20%. Competition from mines in South Australia and America forced the price of copper down from £111 to £87 per ton. The new sources of copper were often found in shallow deposits, meaning the extraction of the ore was achieved more cheaply compared to the deep shafts of Devon and Cornwall. In 1867 it was decided to set up reduction works which would use the low grade ores, which were unsellable at the time, to make arsenic. Previously the presence of arsenic was a mere nuisance, but after the decline in copper it was decided to expand the workings to include the production of arsenic. By December 1868, 50 tons per month of arsenic was being produced, it was decided that the production was to be increased through expansion of the works to produce 150 tons per month. Blanchdown Wood was once again the largest works in the UK, but this time it was for arsenic production. The works covered an area of 8 acres and consisted of 5 calcining ovens and 43 refineries with 4805 ft of flues. A mill for grinding the refined arsenic was driven by water

power and in 1891, 3500 tons of arsenic was being produced per year (Goodridge, 1964).

Production of arsenic and copper continued until approximately 1903, small amounts were produced until 1913, but on a smaller scale. During its activity a total of over 1 million tons of copper ore was produced, to an approximate value of £3.4 million (The data was collated from Burt, 1984). This equated to approximately 17.69% of the total amount of copper ore produced in the UK. The total amount of arsenic produced was 75,000 tons and was sold for £880,000. This equated to approximately 42.27% of UK total production of arsenic ore.

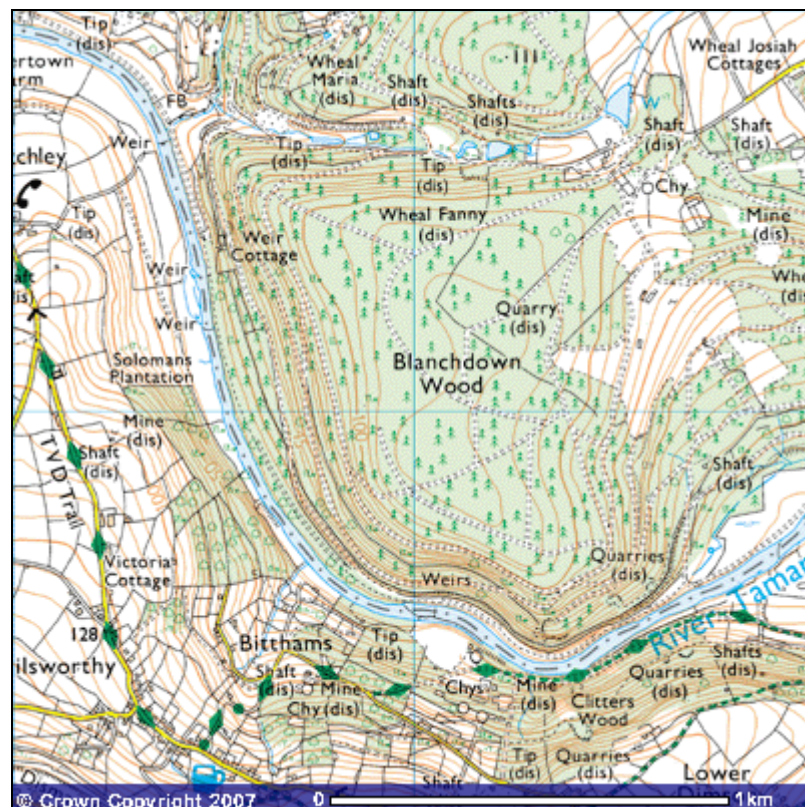
Between 1844 and 1913, DGC was one of the largest mineral producers in the UK. But it was in May 1903, that the mines were abandoned and sold off after a failed attempt to save the mine was proposed by the then director Peter Watson. He proposed that since there were still ore reserves of the value of £57,140, by better application of the enormous water power potential and provision of improved haulage gear, the mine could remain active. Unfortunately due to politics within the shareholders the scheme was defeated by the influential minority (Goodridge, 1964). After nearly 60 years the great prosperity to the area, the mines at Blanchdown Wood closed. What once was a disused mine shaft became the most noted achievements of Victorian mining enterprise.

6.2.2 *Blanchdown Wood, Devon, UK: The site*

It is evident that the heavy metals from mining operations cannot be destroyed, and so become redistributed in the environment indefinitely (Nriagu, 1978). The question remains as to what effect these metals cause. In this study an area of land will be monitored for the concentration of copper and other metals in plants, animals and soil.

The area that was studied is in Blanchdown Wood (See Figure 6-2) currently managed by the Tavistock Estate Management. With their permission the area was explored to gain information as to the viability of the site for study.

Figure 6-2: A map of Blanchdown Wood, Devon, UK and the surrounding area (OS map: SX 426 735)



Wheal Fanny was one of the main sites for copper (and later arsenic) mining and smelting (Jenkins, 2005). It was common for some of the metals to be transported elsewhere for purification (Kirkham, 1968). Because of the expense of building the smelting sites, it was common practice for the stack to be used by several mines in the area which lead to the concentration of waste in the immediate vicinity.

Blanchdown Wood was the site for mining and smelting, this meant that there was waste from both the mining activities and the smelting. In the immediate area there are a number of well used mines, e.g. Wheal Josiah, Wheal Maria, Wheal Fanny and Wheal Anna-Maria (Coleman, 1999).

Although the mine shafts in the area have been blocked and made safe for the public there are other signs to indicate the presence of past mines. As with most villages and towns, the names provide information as to the history of the area. For example, many small villages have adopted the names of the mines.

The cottages of the villages are unique as they were built from the larger portions of rock that were removed from the mines and gives the cottages a distinctive look that can be recognized, for example Figure 4-3 (p28) shows miners cottages at Morwellham Quay. Morwellham Quay was the site of mining and transport activity during the 1800 for copper and arsenic. The buildings have been restored and conserved by the Morwellham and Tamar valley trust. The restoration was based on original plans and photographs (Morwellham and Tamar Valley Trust, 2003).

The mining area in Blanchdown Wood covers approximately 3.5km². Although the area is managed, the majority of the spoil tips have remained intact. It can also be seen that there is a large amount of industrial archaeology that has remained. Figure 6-3 shows the buildings used for the arsenic treatment. Figure 6-4 is an aerial photograph of the area and shows the extent of the spoil tips.

Figure 6-3: Examples of industrial archaeology at Blanchdown Wood, Devon, UK



Figure 6-4: An aerial photograph of Blanchdown Wood, Devon, UK: Scale–2cm:25m
(Modified from the millennium map (getmapping, 2000))



6.3 Shropshire and its Geology

Of the 12 recognised geological periods, 10 are represented in Shropshire making the area one of the most geologically diverse areas. In comparison the Snowdonia region only contains three geological periods (Toghill, 2004).

Between 545 and 418 million years ago, Shropshire lay almost continuously under the sea; with the only landmasses above the water level were areas such as Long Mynd and the Stiperstones. During this period the rocks which were formed are now recognised as the Stiperstone-Shelve area and contained rich deposits of lead ore and barytes (Toghill and Chell, 1984). The rock that contains the rich lead ore is known as Mytton Bed rock, consisting of hard flagstone and grit stone over 300m thick. The beds exist in two oval shaped islands; one to the west of the Stiperstone ridge of quartzite and the other along the eastern flank of the Hope Brook. Because of the underlying rocks, the rich lead ore is confined to a small area approximately in a 5km radius surrounding Shelve (Toghill, 2004). The lead veins run generally south-west to north-east, but the geology differs significantly between the sites of the mines.

6.3.1 *Snailbeach: History*

Snailbeach was reported to be one of the richest lead mines in Europe, and its history covers many centuries. Although for part of its active history, documents were not kept, and only archaeological finds suggests dates of its first use. The first mining activity is estimated to be by the Romans, although the exact dates are not known. The evidence of mining by the Romans includes lead pigs (large blocks of lead) and discarded tools found in the vicinity of the mines. One such find, was a lead pig that was discovered in Linley near Stiperstones and was stamped with the name of Emperor Hadrian (AD 117-138) (Pearce, 1995). Romans used basic methods of extracting the lead ore using hand tools from open trenches and shafts reaching lower levels, these methods did not change for hundreds of years until the invention of water extraction tools since the groundwater flooding was a limiting factor of mine depth.

Another suggested date is during the 12th Century; when in 1181 Hugh Pentalf (Sheriff of Shropshire) accepted £55 from the Kings lead mines at Shelve (Brook and Allbutt, 1973). Other dates mentioned are from the 11th and 13th Century although there has been no evidence confirming this, only speculation.

The first documented evidence of mining at Snailbeach is in 1676, when a group of Derbyshire miners leased the mine but there are no records as to how successful the mine was at that time. The most successful mining operator is considered to be Thomas Lovett and his descendents, when in 1782 he leased the mine from the land owner (Marquis of Bath) for initially 21 years (Brook and Allbutt, 1973). In 1783 Lovett had determined that the mine could be extremely profitable but needed finances in order to achieve this, he then entered into a partnership in which the monetary values of the investments is not known. Originally there were ten partners with Lovett controlling the highest allocation of shares.

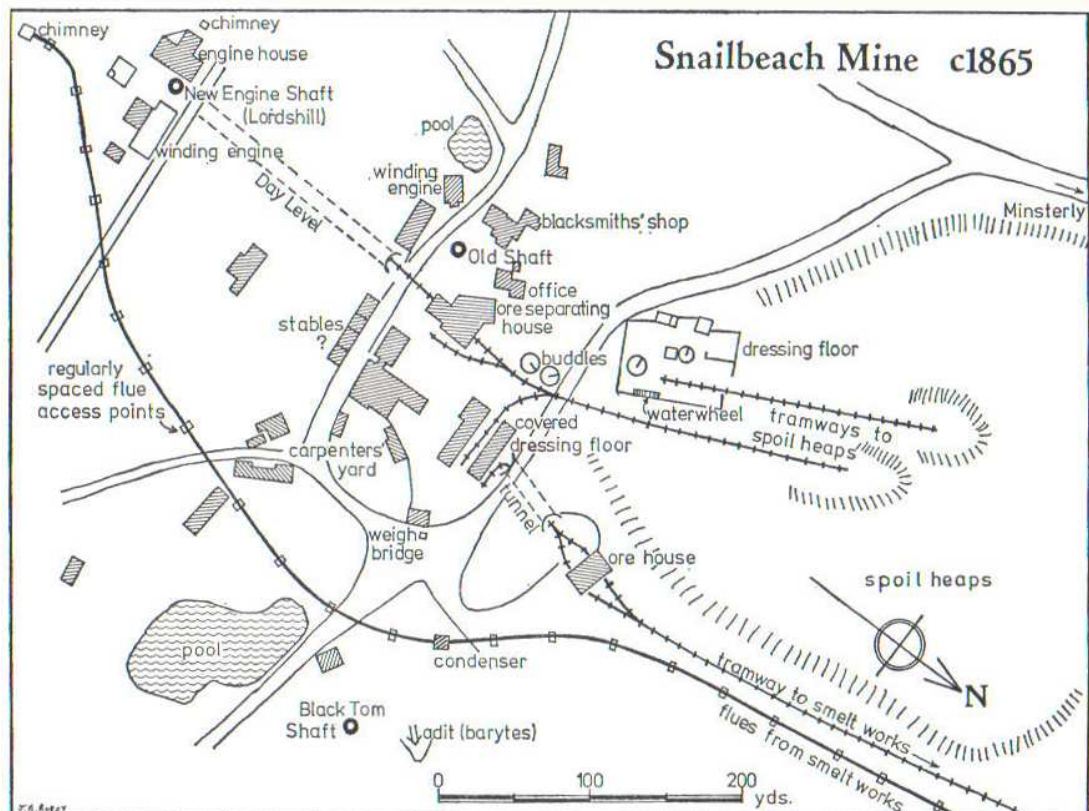
For 73 years, the equipment first set up by Lovett was in constant use, producing regular profits, but in 1857 a father and son management team (Stephen and James Ray Eddy) were employed to increase in the mines prosperity. The new management instigated changes and improvements that would increase the profits ten fold (Brook and Allbutt, 1973). These changes included the abandonment of one of the lowest levels, the level was constantly being flooded and the use of men to drain the level by hand was considered as uneconomical. Instead new veins were sought through crosscutting as well as new levels driven off the main lode; other improvements to the

site included the implementation of a new 61” Cornish engine and the remodelling of the dressing floors.

A further expansion of the mining company was to lease land adjoining the Pontesbury to Pontesford road on the outcrop of the coal measures; this allowed the company to sink several shafts with the aim to extract coal for use in the smelting works. Lovett also built a smelting mill next to the colliery, also in the same year; and was used until 1873 when a new mill was built and in 1873 the new mill took over as the primary smelting mill. The new mill was closer to the mine and used water from the nearby Pontesford hill to feed the water wheel that worked the bellows for hearths at the smelting mill (Brook and Allbutt, 1973).

Figure 6-5 shows the plan of the surface works at Snailbeach in 1865 during the most productive time of its history.

Figure 6-5: Snailbeach surface works c1865 (Modified from Brook and Allbutt, 1973)



Under the Eddys' management the mine was given the reputation of being the richest mine per acre in Europe, with regular returns of £14,000 per annum that were shared out amongst the shareholders. The volume of ore produced at Snailbeach was

approximately 131969 tons equating to about 55.8% of Shropshires total production of ore (Burt *et al*, 1990).

The mines at Snailbeach were also producers of other metals and minerals including zinc and barytes. During 1856 and 1913, Snailbeach extracted approximately 1.5% of the total zinc ore and 20% of the total baryte produced in Shropshire.

The need to maintain this level of prosperity was overwhelming, and in 1870 a Cornish engineer was employed to modernise the site and so maintain the good returns. The resultant modernisation of the underground levels and the surface workings meant that the work force could be reduced from nearly 400 to just 130 in 1878 (Brook and Allbutt, 1973). The reduction in the workforce enabled the company to continue with the remarkable returns; however the pressures of foreign lead producers had meant that the price of lead fluctuated. Increased pressure from the Marquis of Bath also facilitated the first company loss in 1884 to the sum of £3000; because of this the company was forced into liquidation on 28th August 1884. However the liquidation allowed the company to break free of certain aspects of the lease hold where a sum of money was set aside for the investigations of new lodes, this enabled the company to reform in 1885, as Snailbeach Lead Mining Company, without this stipulation and the company continued to work the mine but on a reduced scale (Brook and Allbutt, 1973). Because of the reduced works, the mine was not producing enough ore to be smelted in the Snailbeach mill, therefore in 1895 the mill was closed and ore was transported elsewhere for processing. In 1899 the mine reached a depth of 552yds (Brown, 1976), but although sinking the mine to this depth meant that profits were still being made, the world price and the now depleting source of lead in the mine lead to the Snailbeach Lead Mining company to abandon all works at the site in 1910. In the following year the Cornish engine on Lordshill, removing the water from the deepest levels of the mine, was stopped and the mine was allowed to flood to the 112yd adit. After the abandonment by the Snailbeach Lead Mining Company, small workings continued by the Gravels Trading Company (an offshoot of the Dennis Empire) in some of the shallower levels of the mine but it was the barytes that proved to be more successful; however the quality declined and in 1955 the mine was forced to close after nearly 200 years of constant activity.

6.3.2 *Snailbeach, Shropshire, UK: The site*

Snailbeach lies 16km to the south-west of Shrewsbury near Minsterly (SJ 375 822). The area is currently owned by the local council. The mine itself once covered a much larger area, with a number of shafts and other mining works. Figures 6-6 and 6-7 are maps show maps of the area at different periods, the first is a modern day map and the second is a map published in 1881.

Figure 6-6: A map of Snailbeach and the surrounding area (OS map: SJ 370 020)

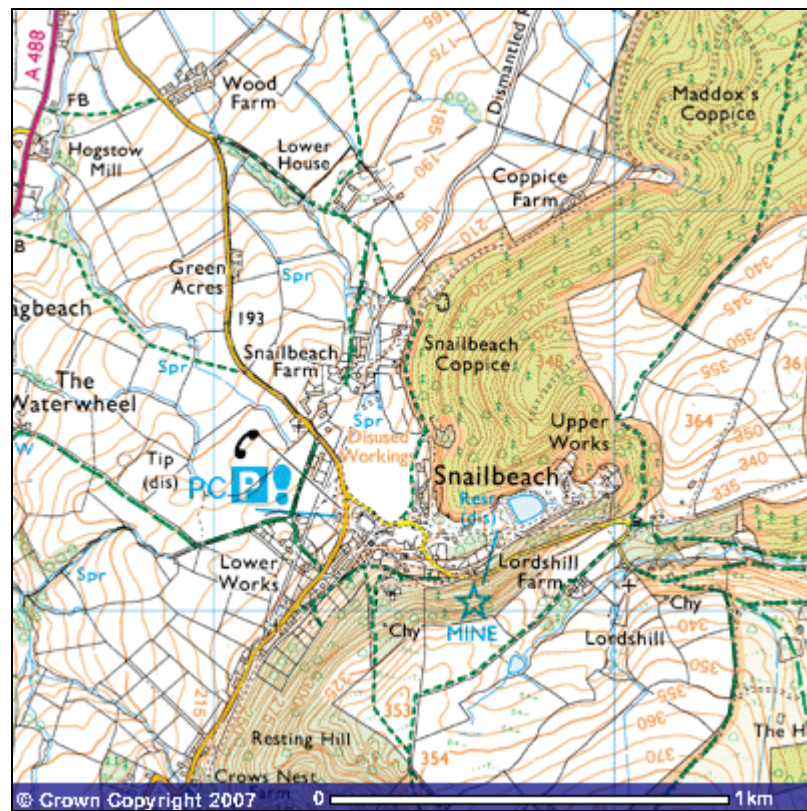
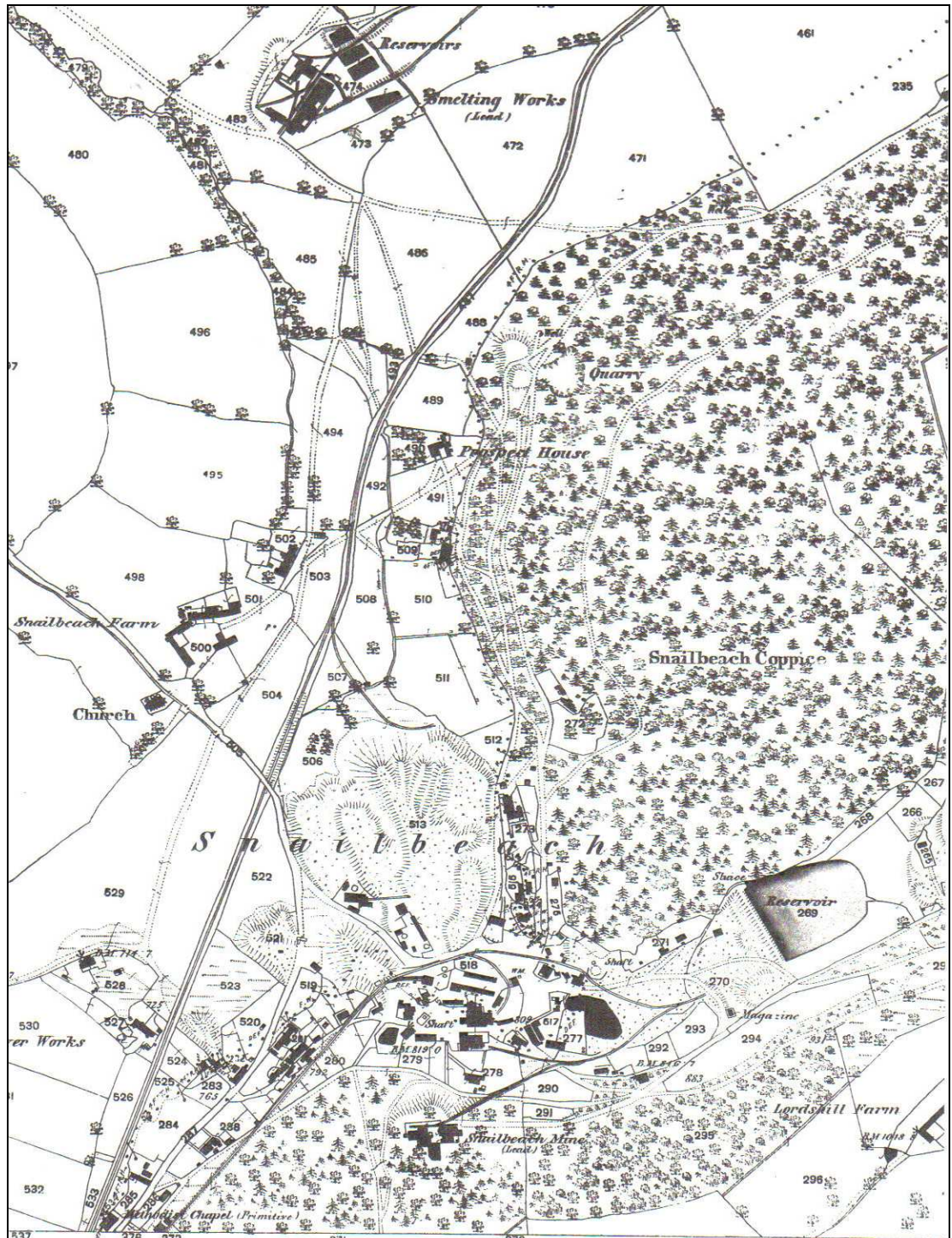


Figure 6-7: An 1882 map of the Snailbeach area (OS map: sheet XLVIII.2)



At present there are works carried out to restore the majority of the buildings to their original state. In conjunction with the council, the Shropshire Mines Trust has turned Snailbeach into an information site which allows visitors to view the area as it would have been a hundred years ago. The Trust also conducts tours into Perkins shaft to give an indication as to the conditions the miners endured during their working life (Shropshire Mines Trust, 2006). Figure 6-8 is a photograph of the compressor house at Snailbeach and is currently being restored. Throughout the site, by joining the Shropshire Way, it is possible to view many of the remaining structures and to get an idea as to the size of the site. In the active days of the mines the landscape would have been very different with additional mines in the area each mining for lead.

Figure 6-8: A photo of the current state of the compressor house at Snailbeach, Shropshire, UK



There are two main spoil tips that are partially visible, and it is possible to explore these to some extent. Figure 6-9 shows one of the remaining spoil tips. From the photograph it is difficult to make out the tip itself as some of the area has been treated. A mesh barrier was placed over the lower region of the tip and a layer of top soil and grass was added possibly to restrict the movement of contaminants from the waste. Along the far side of the tip a row of shrubs were planted and it is evident that

these have been growing successfully for a number of years. The second tip contains finer particles from the mine, possibly tailings from the smelting mill. Over the years this area has become colonised by plants (e.g. *Sarothamnus spp*, *Betula pendula* and *Alnus glutinosa*).

Figure 6-9: One of the rubble spoil tips at Snailbeach, Shropshire, UK



In the past, Snailbeach has been subjected to disruption caused by the mining and smelting industry. The impact of which can be seen in the form of spoil tips. However, less obvious impacts need to be considered: the possible transfer of metal ions through the ecosystem. The ion transfer could affect the flora and fauna in the area. To achieve this, extensive research is required to determine the extent the past activities have had on the ecosystem.

Chapter 7: Dispersal of heavy metals through the terrestrial environment

7.1 Soil Contamination

The remains of mining activities, as discussed earlier, have resulted in the contamination of the terrestrial environment. The minerals and metals that remain in the abandoned waste sites cause great concern as these are not restricted to where they were initially left. In most cases the minerals and metals are mobile and can move throughout the terrestrial environment increasing the area of contamination. Remediation has been used to prevent this, but the issue of transfer through the environment has only been studied closely during the last 50 years and it is evident that the mining waste tips have been present for longer than this. This leads to the conclusion that the tips could have had a large effect on the terrestrial environment over a prolonged period. Although some sites are being treated to prevent the movement of metals, not all waste sites are being treated and therefore the problem persists and this could have potentially devastating effects on the environment.

The process of the movement of elements through the environment is under study by many researchers (Maskall *et al*, 1995; Sterckeman *et al*, 2000; Wilson *et al*, 2005) as there are many variables that can affect whether the transfer actually occurs, and if so the rate. Soil characteristics, such as soil pH and soil type, play an important role as they can either accelerate or restrict the transfer rate of metals through the soil.

Blanchdown Wood (as discussed in Chapter 6) was the site of the largest copper and arsenic producing mines, and as a result has left numerous volumes of waste that have an affect on the surrounding environment. During this study an appraisal of the impact this has had on the environment will be conducted.

7.2 Aims and Objectives

The aims and objectives of this study are to determine the effects of mining waste on the terrestrial environment, including the extent of lateral and vertical flow through the soil.

- To determine if the metals examined show different concentrations around the site
- To determine if vertical distance from the surface affects the concentration of the metals

7.2.1. Experimental Procedure

During repeated visits to Blanchdown Wood, a number of soil samples were taken at varying distances from the spoil tip. At each site three samples were taken in a 2m quadrat. The 2m quadrat was used as this would enable soil to be sampled from different areas, but were still representative of the sampling site.

The vertical samples were taken using a soil auger, with samples being removed at different distances from the surface.

An extra sample of vertical samples was also taken in the form of “pod soils”. When preparing to place pitfall traps for invertebrates, two samples of soils were removed from the sampler. A modified planter was used to remove soil to be replaced by a small cup to capture crawling insects. The planter removed a sample of soil and this was found to be intact and vertical samples could be sampled.

All samples were placed in sealed bags and stored in air tight containers to prevent any contamination. Upon return to the laboratory, the soil samples were prepared for analysis as discussed in Chapter 5.

7.3. *Results*

During visits to Blanchdown Wood, three sampling regimes of the soil were undertaken: lateral dispersal samples, vertical sections of soil without vegetation and vertical sections with vegetation. Figure 7-1 shows a map of Blanchdown Wood with the sampling sites numbered.

7.3.1. *Lateral Dispersal*

Sampling at Blanchdown Wood produced a number of samples taken at individual sites. In most cases there were three soil samples taken, and these are shown in Figures 7-2 to 7-6. These demonstrate that for all metals detected in the soil samples, there were intra site variations. The concentration of analytes tested show wide variations within the site, including arsenic which shows the largest variation with concentrations ranging from 1101.408mg/kg to 18408.463mg/kg at DVS16/LS/6 (See Figure 7-6). Although the remaining analytes have similar variations, the concentrations are not as great. The ranges of the concentrations are for copper: 668.381 to 7840.488mg/kg (DVS17/LS/7), lead: 34.380 to 1307.467mg/kg (DVS16/LS/6) tungsten: 19.757 to 222.478mg/kg (DVS14/LS/4) and tin: 47.425 to 7772.471mg/kg (DVS16/LS/6) (See Figures 7-2 to 7-5 respectively).

Figures 7-7 and 7-8 show the mean results for copper and arsenic (Figure 7-7) and lead, tungsten and tin (Figure 7-8) for each of the sites sampled at various distances from the spoil tip. Included on the graphs are the 95% confidence intervals, indicating the variation of the sample means. It can be seen that for all the analytes, the site with the lowest concentrations was that of DVS12/LS/2.

Interesting results for tin can be seen in Figure 7-8, where the highest concentrations can be seen at DVS11/LS/1, DVS13/LS/3 and DVS14/LS/4. These sites are located on the south, south-west and south side of the small mound.

Both figures show that there is a wide variation in the concentration of arsenic, lead, copper, tungsten and tin throughout the site. Although there is a wide variation in the analyte concentration, it can be seen from Figure 7-9 that the pH of the soil from around Blanchdown Wood does not vary to a great extent, with a range between 4.1 and 4.75.

Figure 7-1: A map of Blanchdown Wood, Devon, UK and designated sampling sites © Crown Copyright, an Ordnance Survey supplied service (Based on OS map sheet SX 47 SW, 1:10,000)

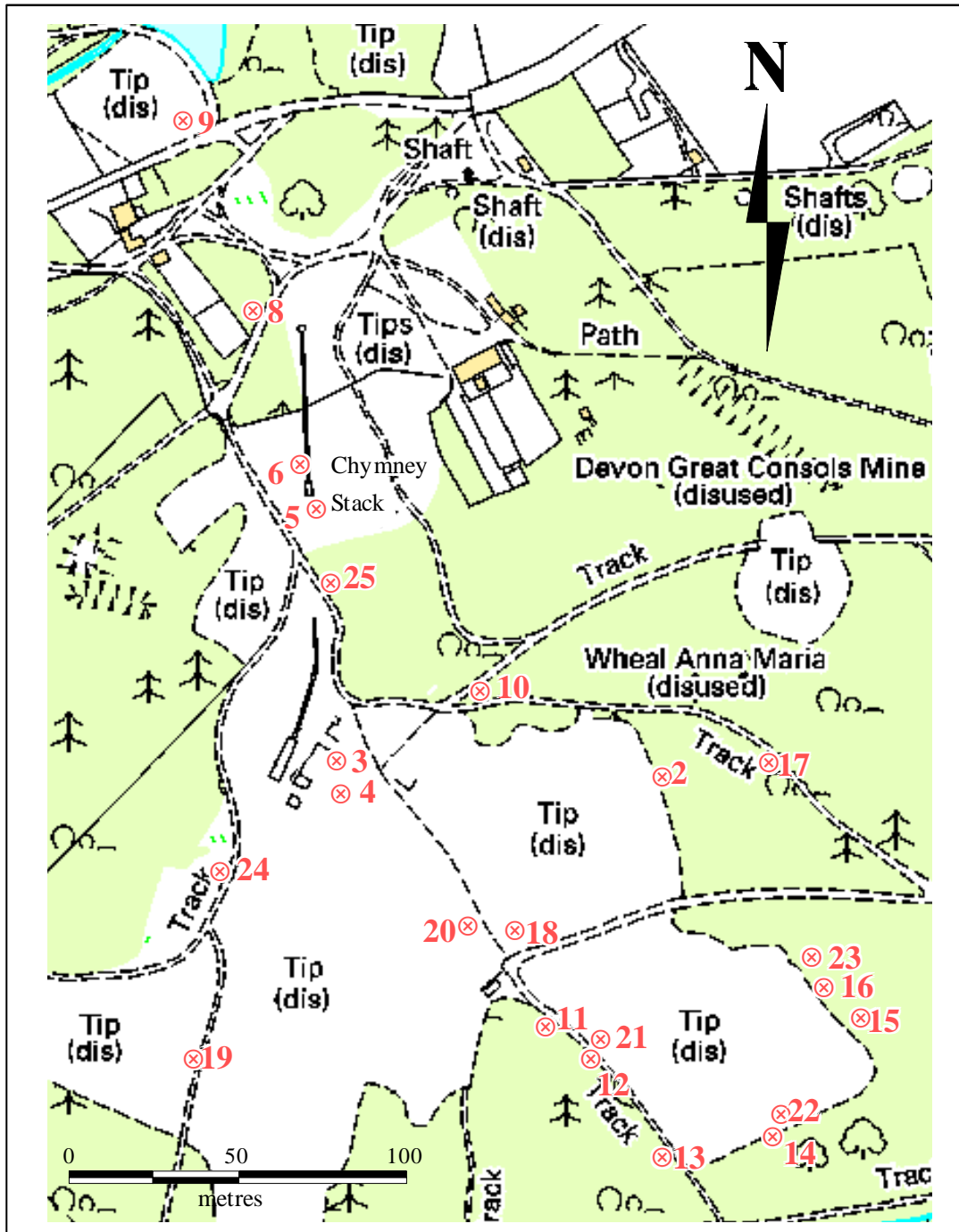


Table 7-1: A key for code definitions

Code	Definition
DVS	Devon soil
DV	Devon
CO	Cotoneaster Plant
VS	Vertical Soil Samples
PS	Picea Plant
LS	Lateral Soil Samples
HI	Deer Bone
FS	Frog Spawn
PP	Pop Profile
PE	Owl Pellet
ES	Egg Shell

Sample names follow the convention:

LOCATION - SITE NUMBER / TYPE OF MATEIAL / SAMPLE NUMBER

E.g. DVS14/LS/4: Devon soil – site 14 / lateral soil sample / Number 4

Site 1 is not located on the map as this was a control sample of *Cotoneaster microphyllus* taken from a site not in the Blanchdown Wood area. Site 7 is also absent from the map as this was a site sampled for invertebrates that were not used in the final thesis.

Table 7-2: The locations of sites and the corresponding samples taken

Site	Sample	Description	Site	Sample	Description
1	DV1/CO/1	Cotoneaster Plant	18	DVS18/LS/8	Lateral Soil
2	DVS2/VS/1	Vertical Soil	19	DVS19/LS/9	Lateral Soil
	DVS2/VS/2	Vertical Soil		DV19/HI/1	Deer Bones
	DVS2/VS/3	Vertical Soil		DV19/HI/2	Deer Bones
	DVS2/CO/2	Cotoneaster Soil		DV19/HI/3	Deer Bones
	DV2/CO/2	Cotoneaster Plant		DV19/HI/4	Deer Bones
3	DVS3/CO/3	Cotoneaster Soil		DV19/HI/5	Deer Bones
	DV3/CO/3	Cotoneaster Plant		DV19/HI/6	Deer Bones
	DVS3/PS/4	Picea Soil		DV19/HI/7	Deer Bones
	DV3/PS/4	Picea Plant		DV19/HI/8	Deer Bones
4	DVS4/CO/4	Cotoneaster Soil		DV19/HI/9	Deer Bones
	DV4/CO/4	Cotoneaster Plant		DV19/HI/10	Deer Bones
5	DVS5/VS/4	Vertical Soil		DV19/HI/11	Deer Bones
	DVS5/VS/5	Vertical Soil		DV19/HI/12	Deer Bones
	DVS5/CO/5	Cotoneaster Soil		DV19/FS/1	Frog Spawn
	DV5/CO/5	Cotoneaster Plant	20	DVS20/PP/1	Pod Profile
6	DVS6/CO/6	Cotoneaster Soil		DVS20/PP/2	Pod Profile
	DV6/CO/6	Cotoneaster Plant	21	DVS21/PS/1	Picea Soil
8	DV8/CO/7	Cotoneaster Plant		DV21/PS/1	Picea Plant
9	DVS/VS/6	Vertical Soil	22	DVS22/PS/2	Picea Soil
10	DVS10/CO8	Cotoneaster Soil		DV22/PS/2	Picea Plant
	DV10/CO/8	Cotoneaster Plant	23	DVS23/PS/3	Picea Soil
11	DVS11/LS/1	Lateral Soil		DV23/PS/3	Picea Plant
12	DVS12/LS/2	Lateral Soil	24	DV24/PE/1	Pellet Bone
13	DVS13/LS/3	Lateral Soil		DV24/PE/2	Pellet Material
14	DVS14/LS/4	Lateral Soil		DV24/PE/3	Pellet Material
15	DVS15/LS/5	Lateral Soil		DV24/ES/1	Egg Shell
16	DVS16/LS/6	Lateral Soil	25	DVS25/PS/5	Picea Soil
17	DVS17/LS/7	Lateral Soil		DV25/PS/5	Picea Plant

Figure 7-2: The copper concentration of soil sampled, in replicate, at various locations around Blanchdown Wood, Devon (see Tables 7-1 and 7-2 for key to sample names)

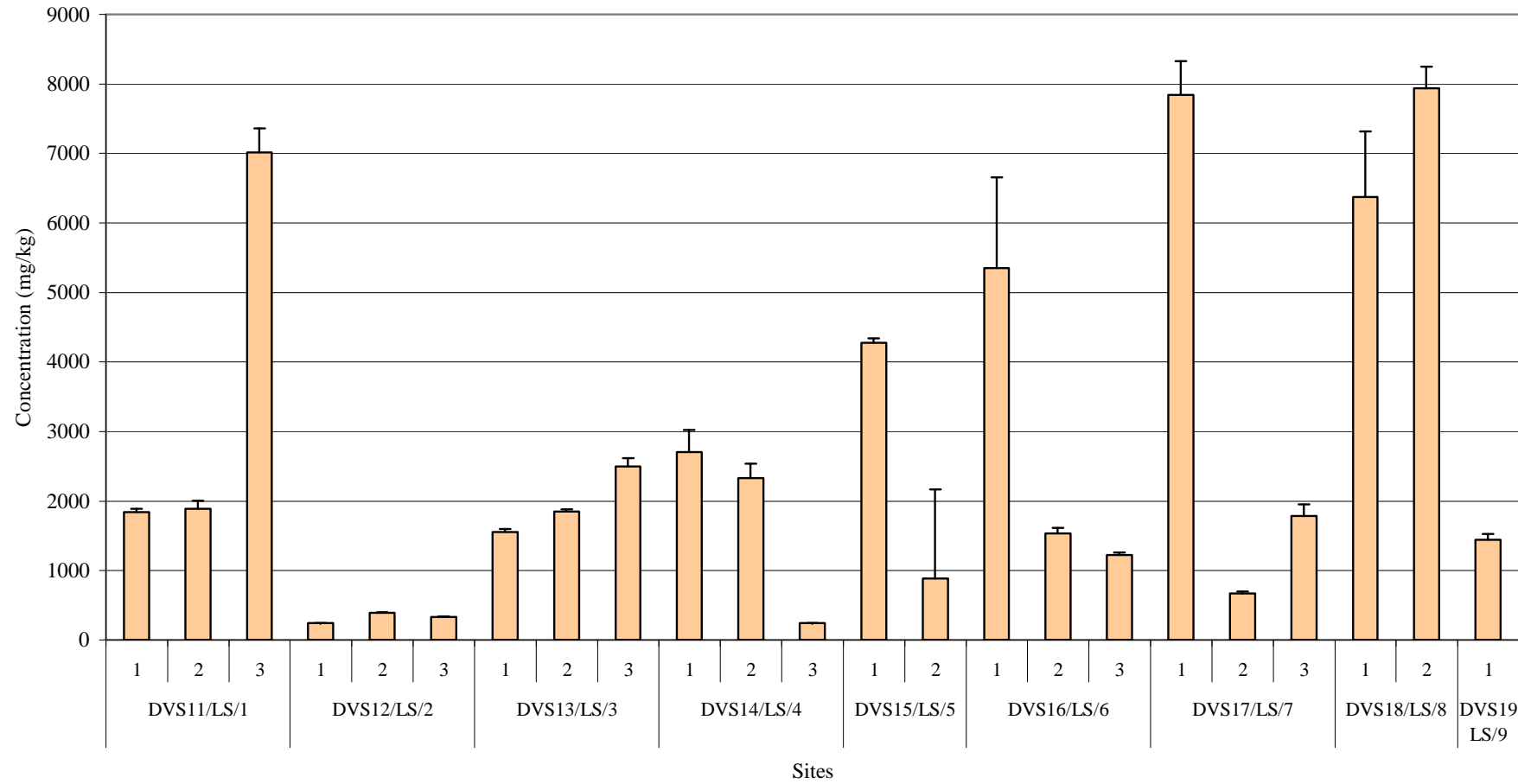


Figure 7-3: The lead concentration of soil sampled, in replicate, at various locations around Blanchdown Wood, Devon
 (see Tables 7-1 and 7-2 for key to sample names)

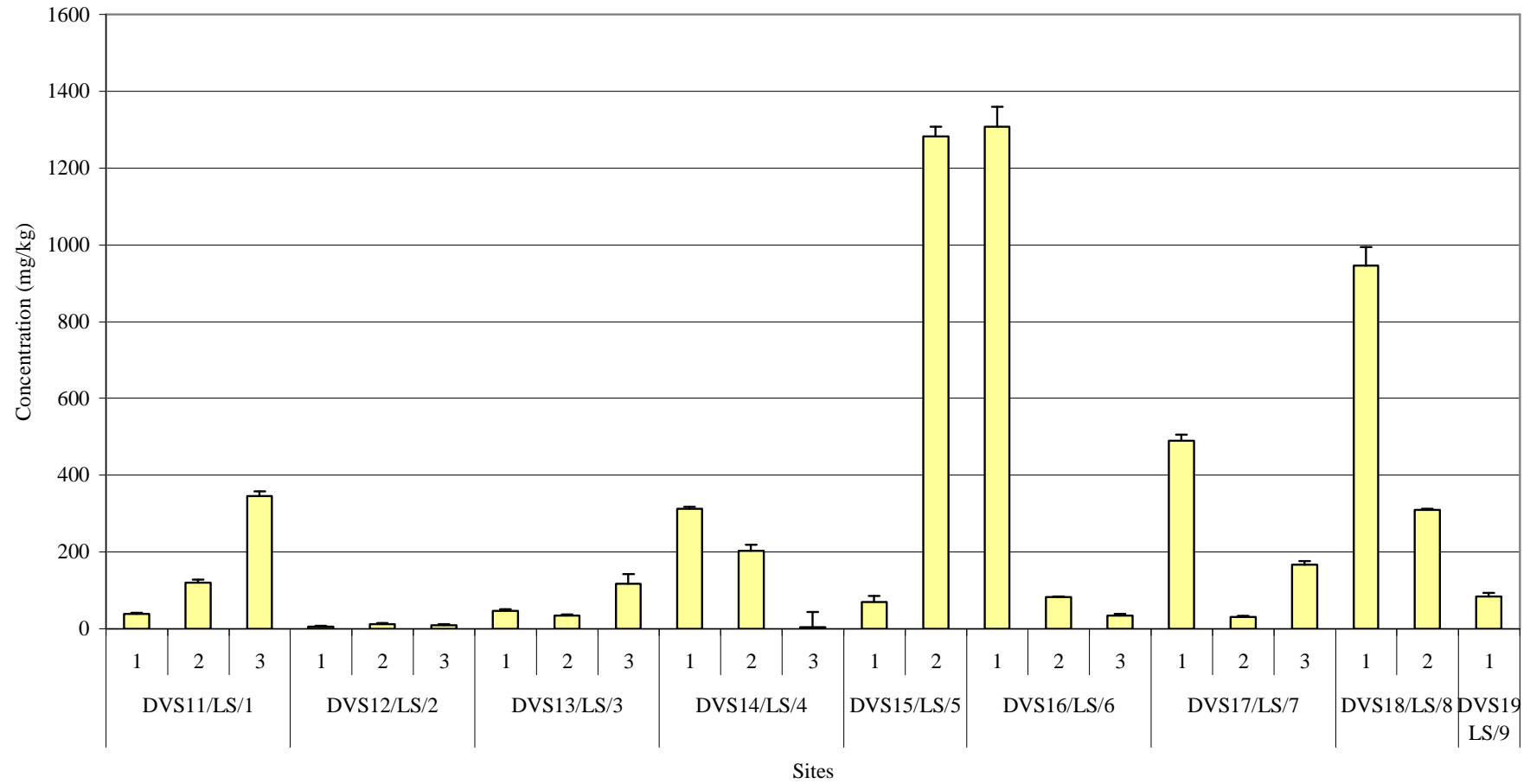


Figure 7-4: The tungsten concentration of soil sampled, in replicate, at various locations around Blanchdown Wood, (see Tables 7-1 and 7-2 for key to sample names)

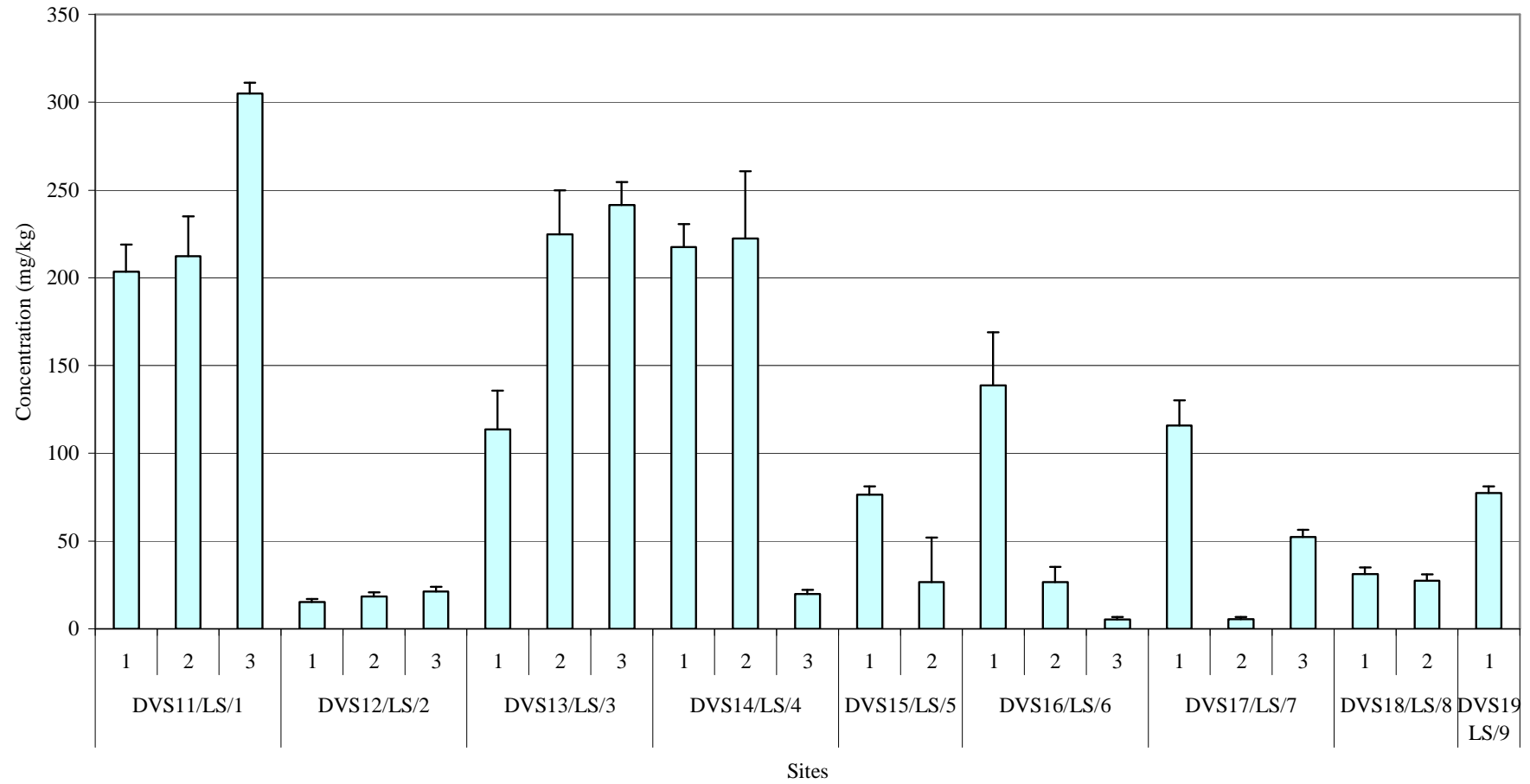


Figure 7-5: The tin concentration of soil sampled, in replicate, at various locations around Blanchdown Wood, Devon
 (see Tables 7-1 and 7-2 for key to sample names)

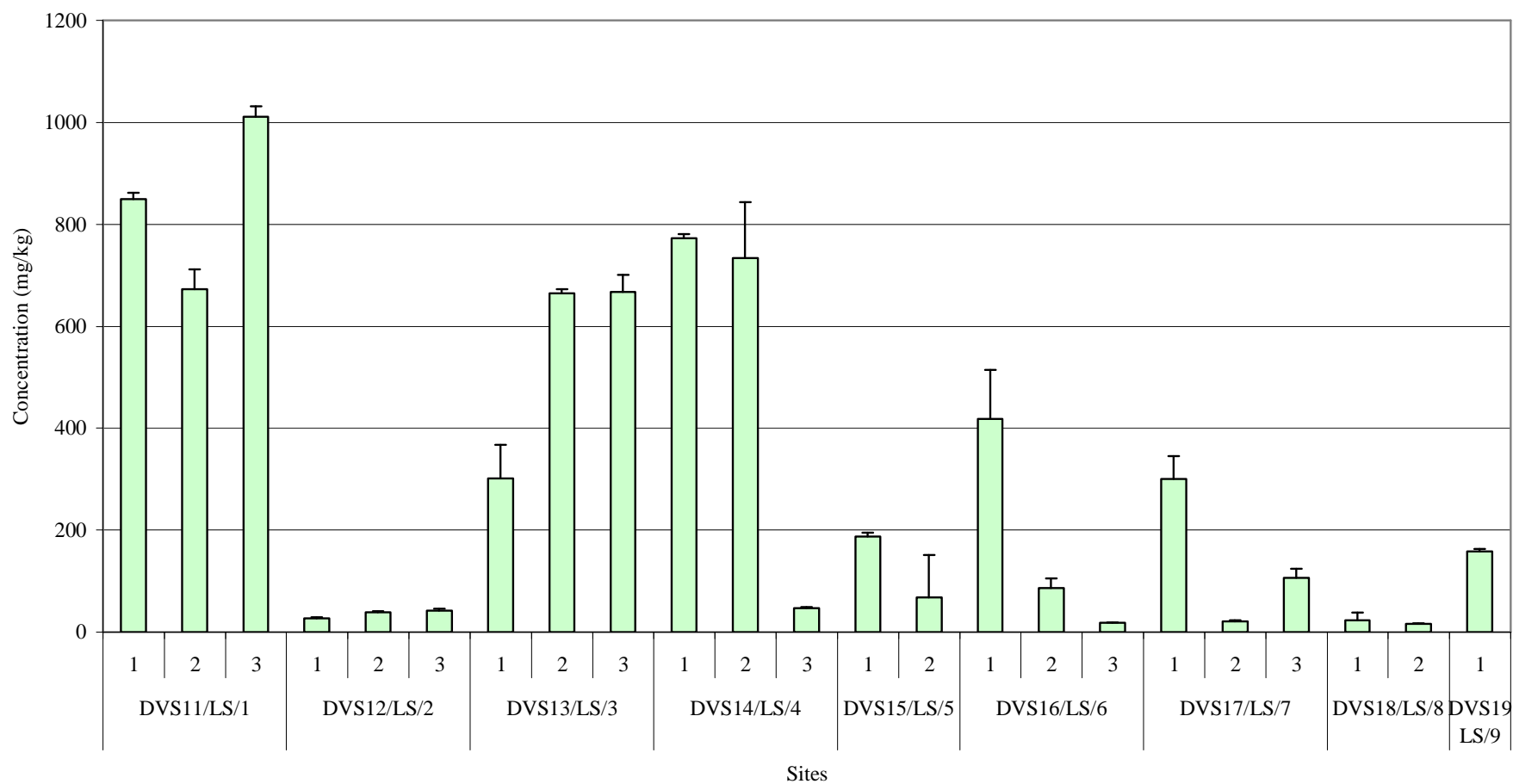


Figure 7-6: The arsenic concentration of soil sampled, in replicate, at various locations around Blanchdown Wood, Devon (see Tables 7-1 and 7-2 for key to sample names)

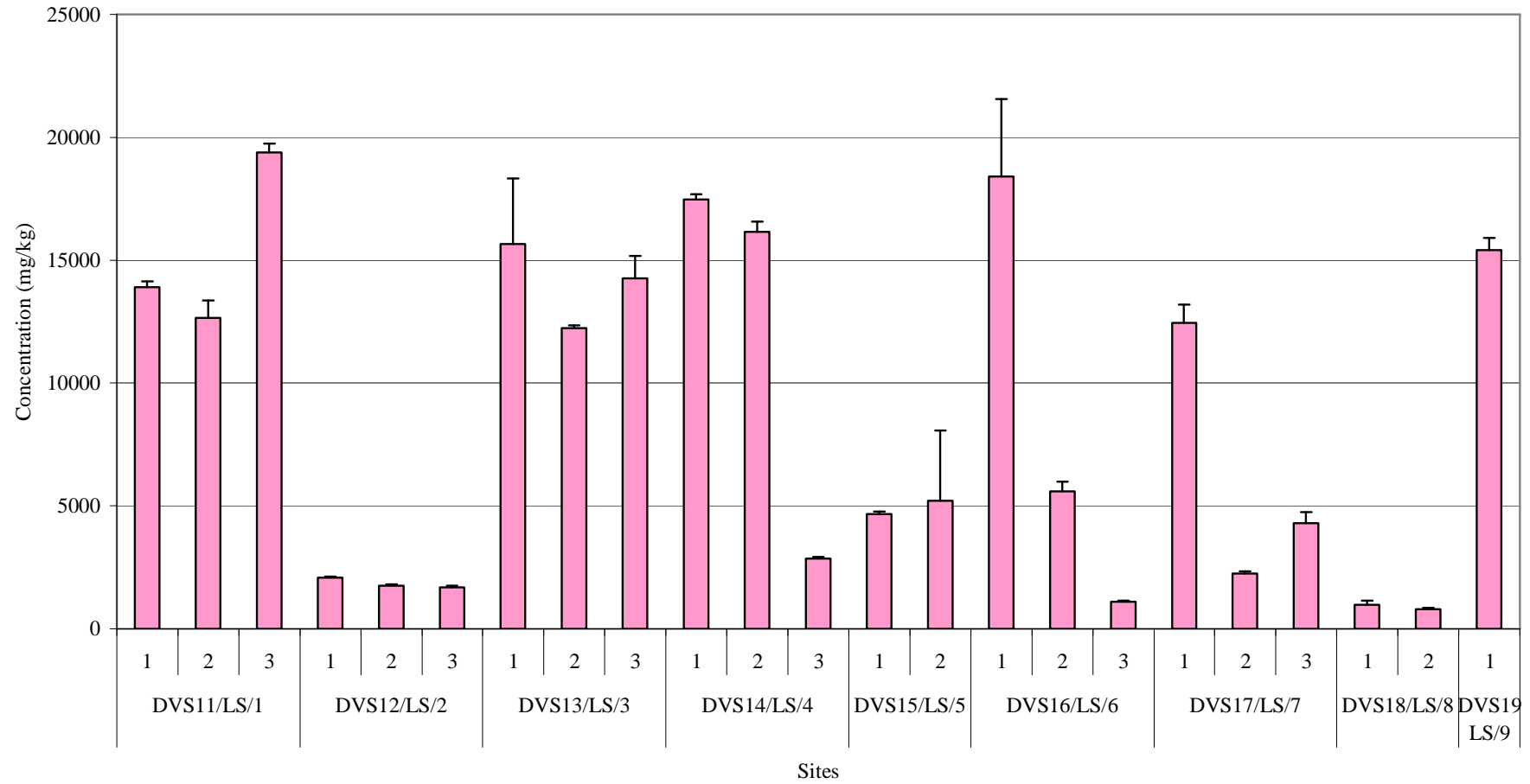


Figure 7-7: The concentration of copper and arsenic in soil from various locations around Blanchdown Wood, Devon
(see Tables 7-1 and 7-2 for sample details)

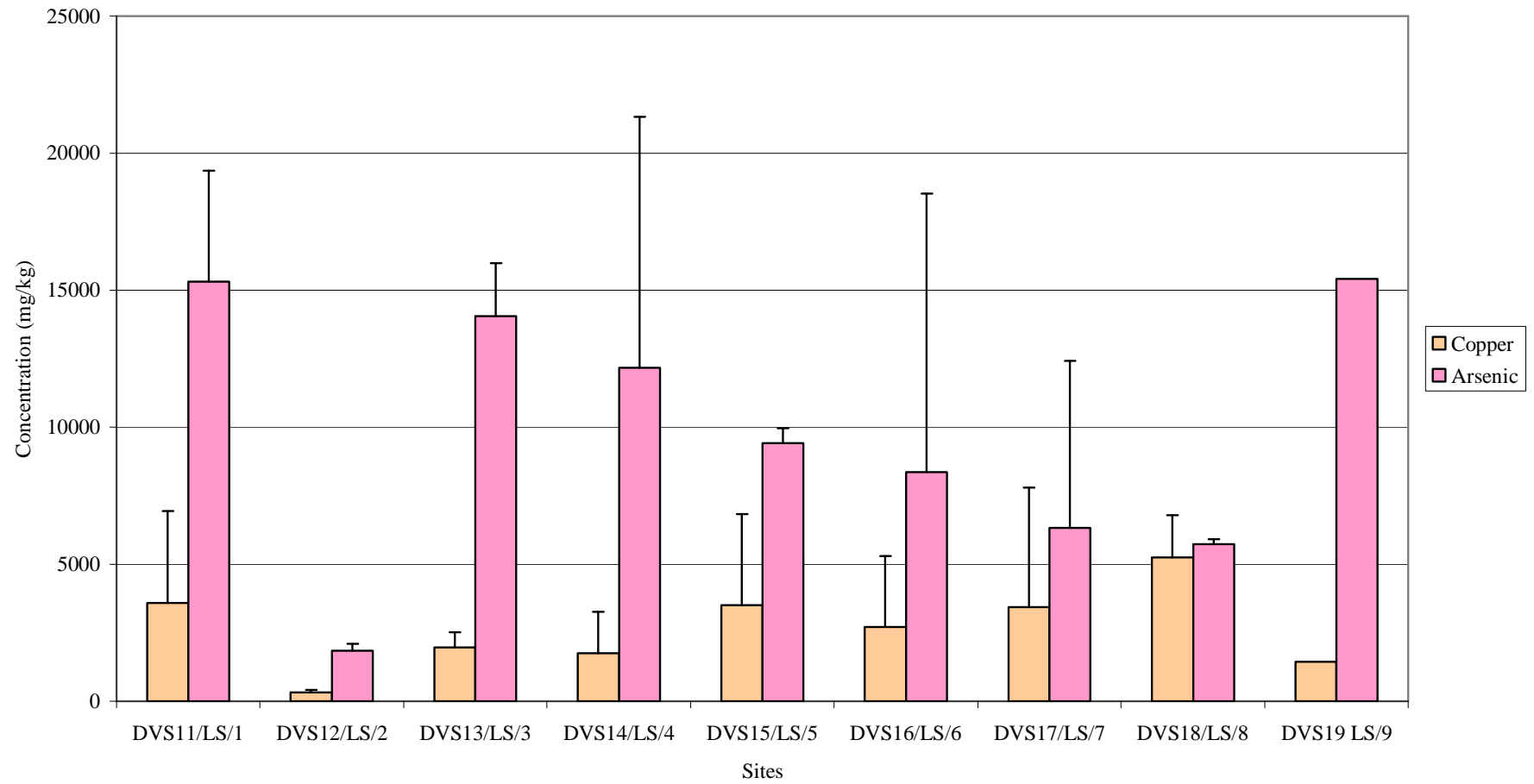


Figure 7-8: The concentration of lead, tungsten and tin in soil from various locations around Blanchdown Wood, Devon
 (see Tables 7-1 and 7-2 for sample details)

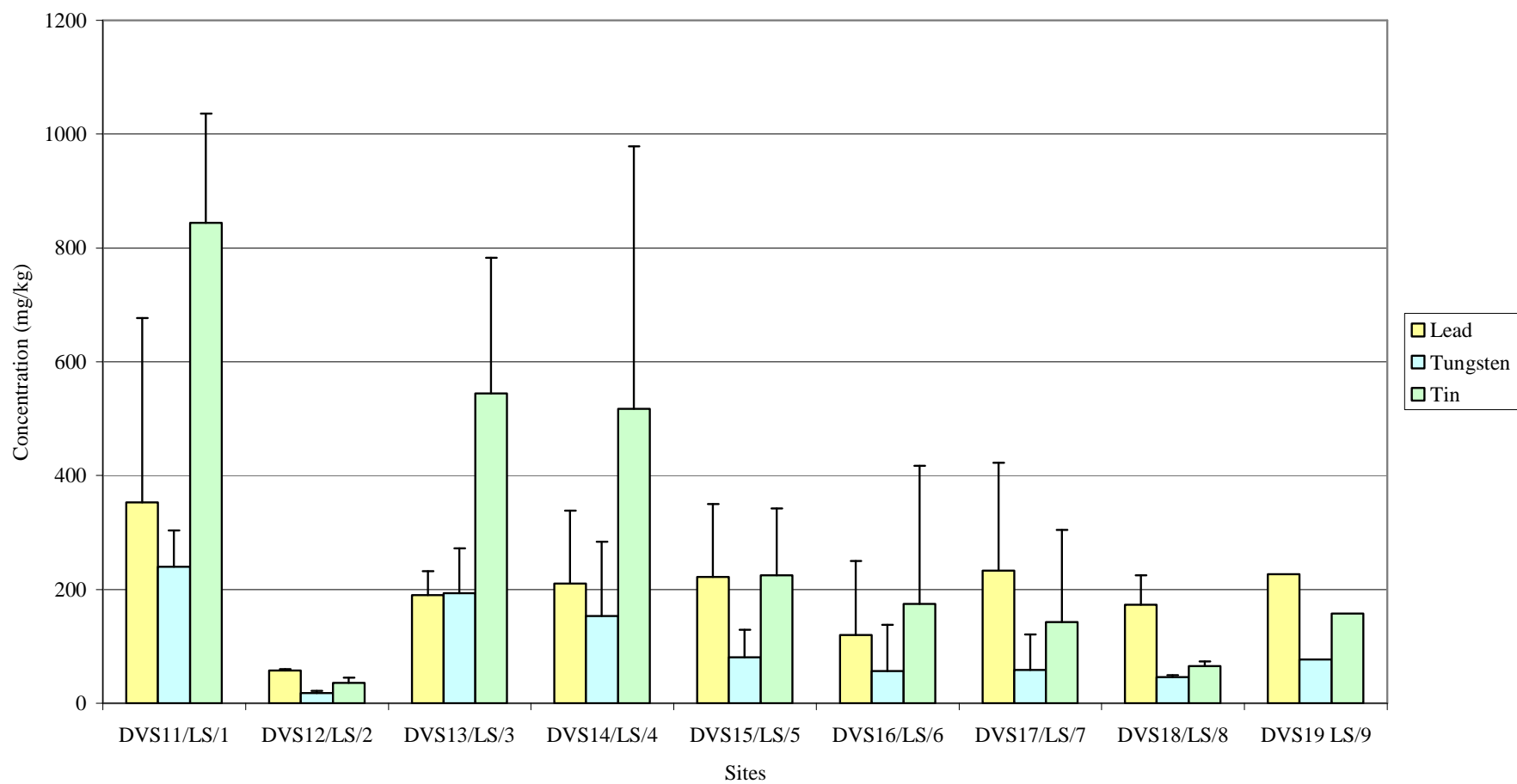
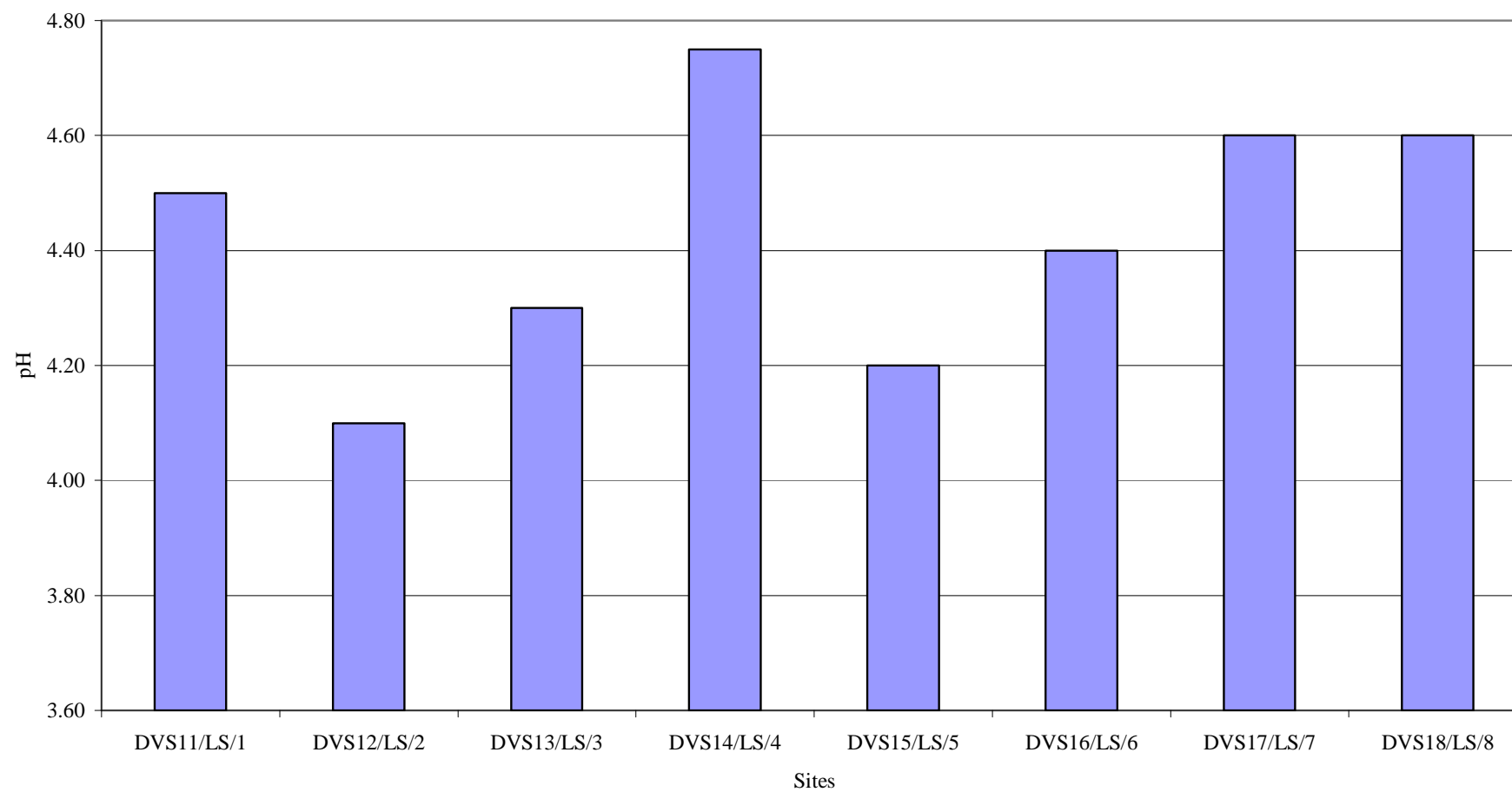


Figure 7-9: The pH values of soil sampled from different sites around Blanchdown Wood, Devon, UK
(see Tables 7-1 and 7-2 for key to sample names)



7.3.1.1. Statistical Analysis

The null hypothesis for the analysis was: there is no significant difference between the concentrations of copper, lead, tungsten, tin and arsenic within the replicates at each site. Table 7-3 shows the summary of the statistical data and shows that there is a significant difference between the concentrations for the replicates of the sites

Table 7-3: A summary of the statistical analysis for differences between the concentrations of the analytes within the sites

Site	Analyte	F	Fcrit	P-Value	H ₀
(DVS11/LS/1)	Copper	752.102	5.6136	<0.01	Reject
	Lead	4097.875	5.6136	<0.01	Reject
	Tungsten	45.909	5.6136	<0.01	Reject
	Tin	155.220	5.6136	<0.01	Reject
	Arsenic	213.880	5.6136	<0.01	Reject
(DVS12/LS/2)	Copper	5.194	5.6136	<0.01	Accept
	Lead	239.840	5.6136	<0.01	Reject
	Tungsten	6.624	5.6136	<0.01	Reject
	Tin	36.147	5.6136	<0.01	Reject
	Arsenic	53.789	5.6136	<0.01	Reject
(DVS13/LS/3)	Copper	109.020	5.7804	<0.01	Reject
	Lead	322.154	5.7804	<0.01	Reject
	Tungsten	38.578	5.7804	<0.01	Reject
	Tin	86.244	5.7804	<0.01	Reject
	Arsenic	3.355	5.7804	<0.01	Accept
(DVS14/LS/4)	Copper	62.547	5.7804	<0.01	Reject
	Lead	159.665	5.7804	<0.01	Reject
	Tungsten	244.455	5.7804	<0.01	Reject
	Tin	11936.886	5.7804	<0.01	Reject
	Arsenic	9047.600	5.7804	<0.01	Reject
(DVS15/LS/5)	Copper	220.371	8.8616	<0.01	Reject
	Lead	10712.715	8.8616	<0.01	Reject
	Tungsten	540.868	8.8616	<0.01	Reject
	Tin	1401.208	8.8616	<0.01	Reject
	Arsenic	184.264	8.8616	<0.01	Reject

Site	Analyte	F	Fcrit	P-Value	H ₀
(DVS16/LS/6)	Copper	317.188	5.7804	<0.01	Reject
	Lead	73.997	5.7804	<0.01	Reject
	Tungsten	415.548	5.7804	<0.01	Reject
	Tin	174.401	5.7804	<0.01	Reject
	Arsenic	598.942	5.7804	<0.01	Reject
(DVS17/LS/7)	Copper	613.376	6.3589	<0.01	Reject
	Lead	336.356	6.3589	<0.01	Reject
	Tungsten	166.816	6.3589	<0.01	Reject
	Tin	94.631	6.3589	<0.01	Reject
	Arsenic	217.652	6.3589	<0.01	Reject
(DVS18/LS/8)	Copper	12.102	10.044	<0.01	Reject
	Lead	122.969	10.044	<0.01	Reject
	Tungsten	1.279	10.044	<0.01	Accept
	Tin	1.327	10.044	<0.01	Accept
	Arsenic	35.745	10.044	<0.01	Reject

Table 7-3 showed that for all but four results the null hypothesis is rejected. The results where the hypothesis is accepted are: Copper at site DVS12/LS/2, arsenic at site DVS13/LS/3, and tin and tungsten at site DVS16/LS/6. Although the copper at site DVS12/LS/2 was accepted at P=0.01, the null hypothesis could be rejected at P=0.05. For the remaining accepting results (Site DVS13/LS/3 – arsenic, Site DVS18/LS/8 – tin and tungsten), they would still be rejected at P=0.05.

For those results where the null hypothesis was rejected a further Tukey Test was done. The results of which are displayed in Table 7-4.

As only one sample was taken at DVS19/LS/9 (pond soil), there is no statistical data.

Table 7-4: The results of the Tukey Test for the differences between analytes within sites

Table 7-4a: DVS11/LS/1

Copper critical value: 382.701			Tin critical value: 48.090		
	2	3		2	3
1	0	5169.700	1	177.150	161.680
2		5129.600	2		338.830
Lead critical value: 15.744			Arsenic critical value: 863.382		
	2	3		2	3
1	74.708	453.270	1	1261.100	5467.900
2		527.980	2		6729
Tungsten critical value: 29.310					
	2	3			
1	0	101.620			
2		92.674			

Table 7-4b: DVS12/LS/2

Copper critical value: 17.164			Tin critical value: 4.684		
	2	3		2	3
1	149.670	89.316	1	11.703	11.245
2		60.354	2		0
Lead critical value: 3.754			Arsenic critical value: 105.393		
	2	3		2	3
1	0	4.803	1	331.820	413.430
2		0	2		0
Tungsten critical value: 4.213					
	2	3			
1	0	6.139			
2		0			

Table 7-4c: DVS13/LS/3

Copper critical value: 89.757			Tin critical value: 80.586		
	2	3		2	3
1	284.920	881.800	1	368.620	355.150
2		596.880	2		0
Lead critical value: 10.180			Arsenic critical value: 3183.387		
	2	3		2	3
1	14.873	57.225	1	3257.200	0
2		42.353	2		0
Tungsten critical: 41.125					
	2	3			
1	110.640	133.370			
2		0			

Table 7-4: (cont)

Table 7-4d: DVS14/LS/4

Copper critical value: 385.178			Tin critical value: 13.860		
	2	3		2	3
1	0	2461.100	1	0	727.930
2		2231.600	2		737.280
Lead critical value: 51.333			Arsenic critical value: 308.236		
	2	3		2	3
1	83.838	224.430	1	1064	14687
2		140.590	2		13623
Tungsten critical value: 27.184					
	2	3			
1	0	195.180			
2		214.690			

Table 7-4e: DVS16/LS/6

Copper critical value: 909.572			Tin critical value: 54.560		
	2	3		2	3
1	4308.900	4620.100	1	373.490	441.730
2		0	2		68.240
Lead critical value: 21.897			Arsenic critical value: 1216.846		
	2	3		2	3
1	218.500	227.110	1	14367	18802
2		0	2		4435.100
Tungsten critical value: 11.888					
	2	3			
1	125.610	148.590			
2		22.981			

Table 7-4f: DVS17/LS/7

Copper critical value: 778.380			Tin critical value: 50.227		
	2	3		2	3
1	7183.700	6177.800	1	259.860	180.220
2		1006.000	2		79.641
Lead critical value: 24.450			Arsenic critical value: 1374.201		
	2	3		2	3
1	328.930	141.550	1	10405.000	8419.300
2		187.380	2		1985.900
Tungsten critical value: 16.852					
	2	3			
1	117.830	70.663			
2		47.170			

The results in Table 7-4 give an indication as to where the significant differences are. The critical values are also displayed and where a '0' has been put in the table, there is no significant difference between the values.

Through statistical analysis it has been determined that there is a significant difference between the concentration of replicates at different sites. With this in mind, an analysis was performed to determine if there is a significant difference between the mean concentrations of the sites. The null hypothesis was: there is no significant difference between the mean concentrations of copper, lead, tungsten, tin and arsenic and the sites. The results of the analysis are displayed in Table 7-5.

Table 7-5: The statistical results of the differences between the sites

Analyte	F		P-Value	H ₀
Copper	11.406	2.767	<0.01	Reject
Lead	5.716	2.767	<0.01	Reject
Tungsten	23.795	2.767	<0.01	Reject
Tin	34.998	2.767	<0.01	Reject
Arsenic	22.943	2.767	<0.01	Reject

Since the results of the analysis shows that the hypothesis was rejected for each of the analytes, there is a significant difference between the concentrations of the metals and the sites. A Tukey Test was then performed to determine where the differences were and are displayed in Table 7-6.

Table 7-6: The results of the Tukey Test for the differences between sites

Table 7-6a: Copper critical value: 2384.58

	2	3	4	5	6	7	8	9
1	2853.14	0	0	0	0	0	3490.57	0
2		0	0	0	3134.8	3110.93	6343.71	0
3			0	0	0	0	4669.19	0
4				0	0	0	4800.64	0
5					0	0	4397.77	0
6						0	3208.91	0
7							3232.78	0
8								5161.40

Table 7-6b: Lead critical value: 137.425

	2	3	4	5	6	7	8	9
1	261.84	0	0	120.64	168.13	0	159.60	0
2		130.08	165.18	141.19	0	189.7	0	175.16
3			0	0	0	0	0	0
4				0	0	0	0	0
5					0	0	0	0
6						0	0	0
7							0	0
8								0

Table 7-6c: Tungsten critical value: 70.523

	2	3	4	5	6	7	8	9
1	211.44	0	0	185.20	157.99	165.44	199.44	151.32
2		176.70	141.27	0	0	0	0	0
3			0	150.46	123.25	130.70	164.70	116.58
4				115.03	87.82	95.27	129.27	81.14
5					0	0	0	0
6						0	0	0
7							0	0
8								0

Table 7-6d: Tin critical value: 216.616

	2	3	4	5	6	7	8	9
1	808.24	295.6	305.45	737.39	601.13	702.31	821.74	681.98
2		512.64	502.79	0	0	0	0	0
3			0	441.79	305.53	406.72	526.15	386.38
4				431.94	295.67	396.86	516.29	376.52
5					0	0	0	0
6						0	220.61	0
7							0	0
8								0

Table 7-6e: Arsenic critical value: 5427.778

	2	3	4	5	6	7	8	9
1	13151.28	0	0	10778.82	0	8678.1	14074.32	0
2		12521.3	10354.8	0	8525.12	0	0	13671.7
3			0	10148.86	0	8048.14	13444.37	0
4				7982.313	0	5881.59	11277.82	0
5					6152.66	0	0	11299.2
6						0	9448.17	0
7							0	9198.48
8								14594.7

The results of the Tukey Test indicate that there is a large variation in the concentrations of the metals between the sites. This shows that there is a mosaic effect of the metals (see section 7.4 for discussion).

7.3.2. *Vertical Dispersal*

During the sampling procedure at Blanchdown Wood, a number of vertical samples were taken at varying distances from the spoil tips. It was not possible to take more samples as there was a layer of rock under the surface of the soil. It is likely that these are large extracted rock from the mines. The samples that were taken were situated at sites 2, 5 and 9 (See Figure 7-1).

Figures 7-10 to 7-12 show the results of the analysis for copper, lead, tin, tungsten and arsenic. They show that there is a difference between the vertical samples and the sites.

There appears to be no correlation between the distance from the surface and the concentration of copper (Figure 7-10). The second and third samples, taken at DVS2/VS/3, show similarities to one another as they show that the concentration of copper decreased, at the second vertical section, and then increased with the third section. The pH values in Figure 7-13 also shows a similar pattern in the way that the values decrease and then increase. All three samples were taken at similar depths from the surface. For DVS5/VS/4, two vertical sections were taken, and these show that for copper there appears to be a significant difference in the values between the sites and between the vertical sections.

Figure 7-11 shows that for all but one of the samples taken, the concentrations of lead were similar for each of the vertical sections in the samples. DVS5/VS4 showed a large difference in lead concentration between the 14cm and 21cm sections (93.238 to 197.003mg/kg respectively). The remaining sites showed little or no difference in lead concentration between the sections. The results for tungsten show that for site DVS2/VS/3 the concentration decreased from 62.608 to 39.290mg/kg. Samples DVS2/VS/1 and DVS2/VS/2 show that the concentration increased slightly but at the final section (38cm) had similar concentrations as the second sections (26 and 23cm respectively). For the remaining samples, the concentration of tungsten decreased as the distance from the surface increased. The tin results also shown in Figure 7-11, showed that for samples DVS2/VS/1, DVS2/VS/3, DVS5/VS/5 and DVS9/VS/6 there was an increase in tin concentration as the distance from the surface increased. DVS5/VS/4 results show that the concentration of tin dramatically decreased with increasing distance from the surface (327.110 to 80.139mg/kg), and these were mirrored by the results for lead, tungsten and copper. These results correspond to the highest values of pH shown in Figure 7-13, possibly restricting the movement of the

ions through the soil at a pH of over 4. The results for DVS9/VS/6 are interesting as these samples contained the highest concentration of tin, but also the lowest pH. Arsenic concentrations are shown in Figure 7-12, and show that for DVS2/VS/1 and DVS2/VS/2 there was an increase and then a decrease in the concentration of arsenic. For samples DVS2/VS/2 and DVS9/VS/6 there was a decrease in arsenic concentration. As with tin, the concentration of arsenic is highest in sample DVS9/VS/6, where the pH of the soil was lowest, this could be due to the lower pH increasing the flow of the tin and arsenic through the soil.

Figure 7-10: The copper concentration in vertical sections of soil from various locations around Blanchdown Wood, Devon (see Tables 7-1 and 7-2 for sample details)

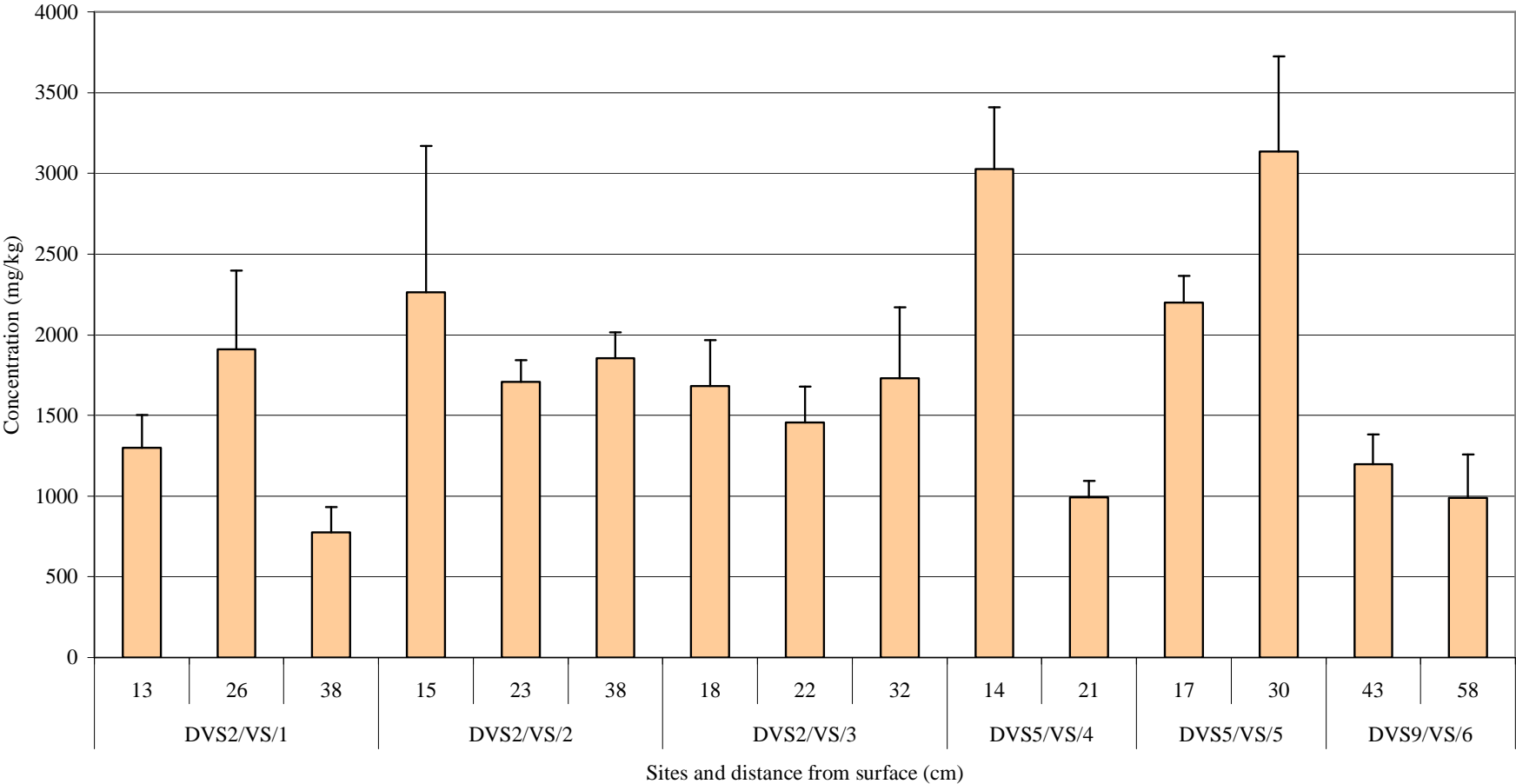


Figure 7-11: The concentrations of lead, tungsten and tin in vertical sections of soil from various locations around Blanchdown Wood, Devon (see Tables 7-1 and 7-2 for sample details)

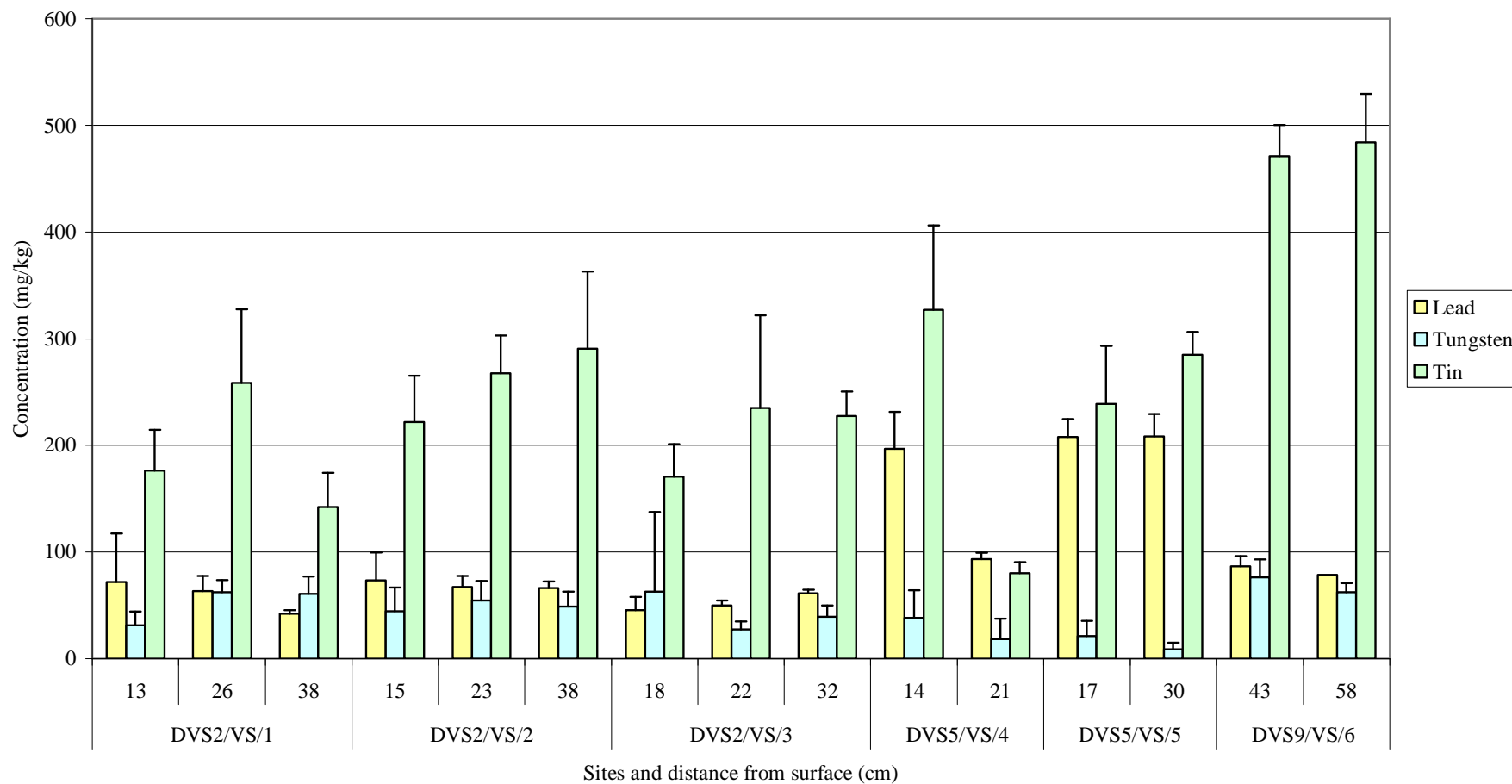


Figure 7-12: The arsenic concentration in vertical sections of soil from various locations around Blanchdown Wood, Devon (see Tables 7-1 and 7-2 for sample details)

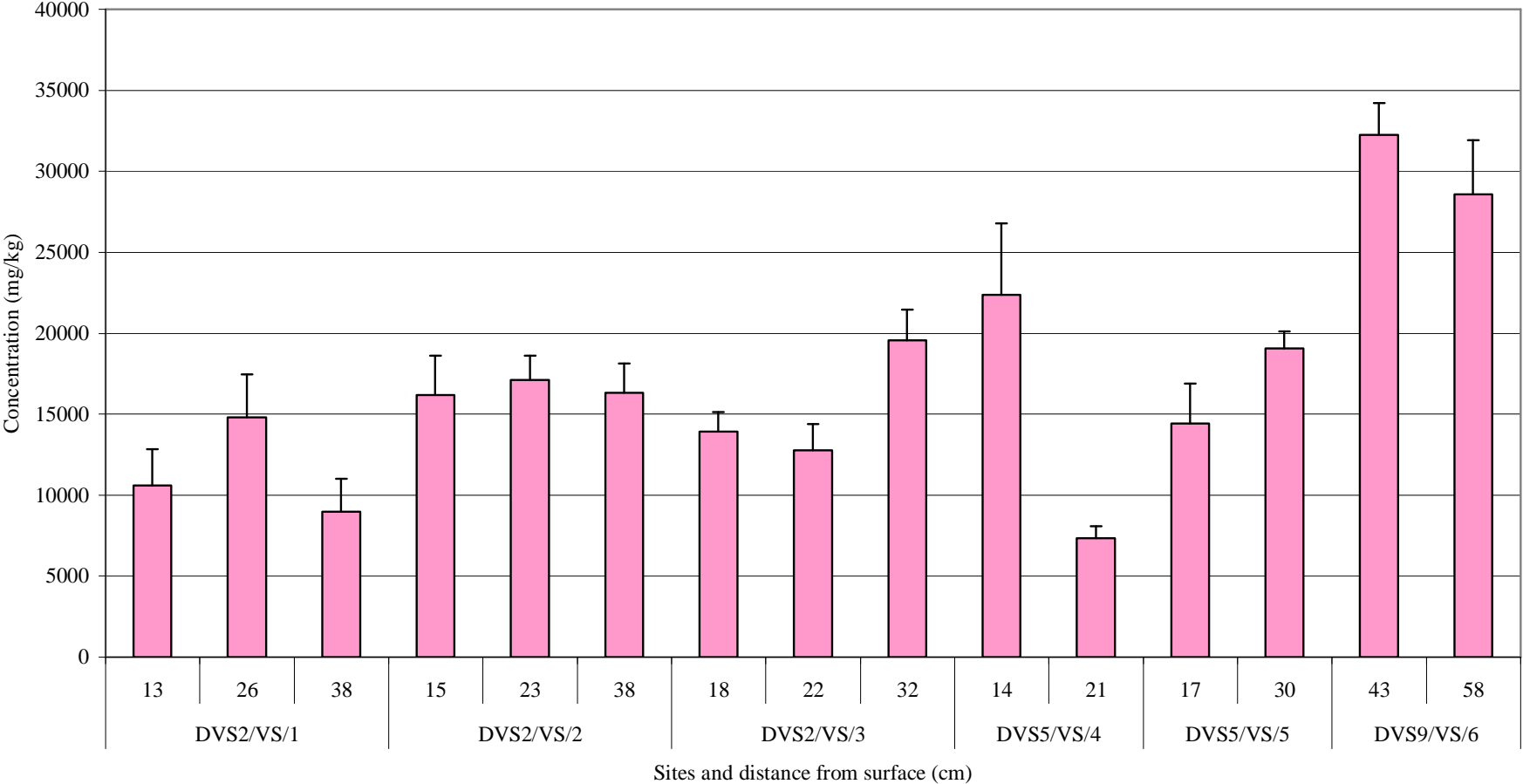
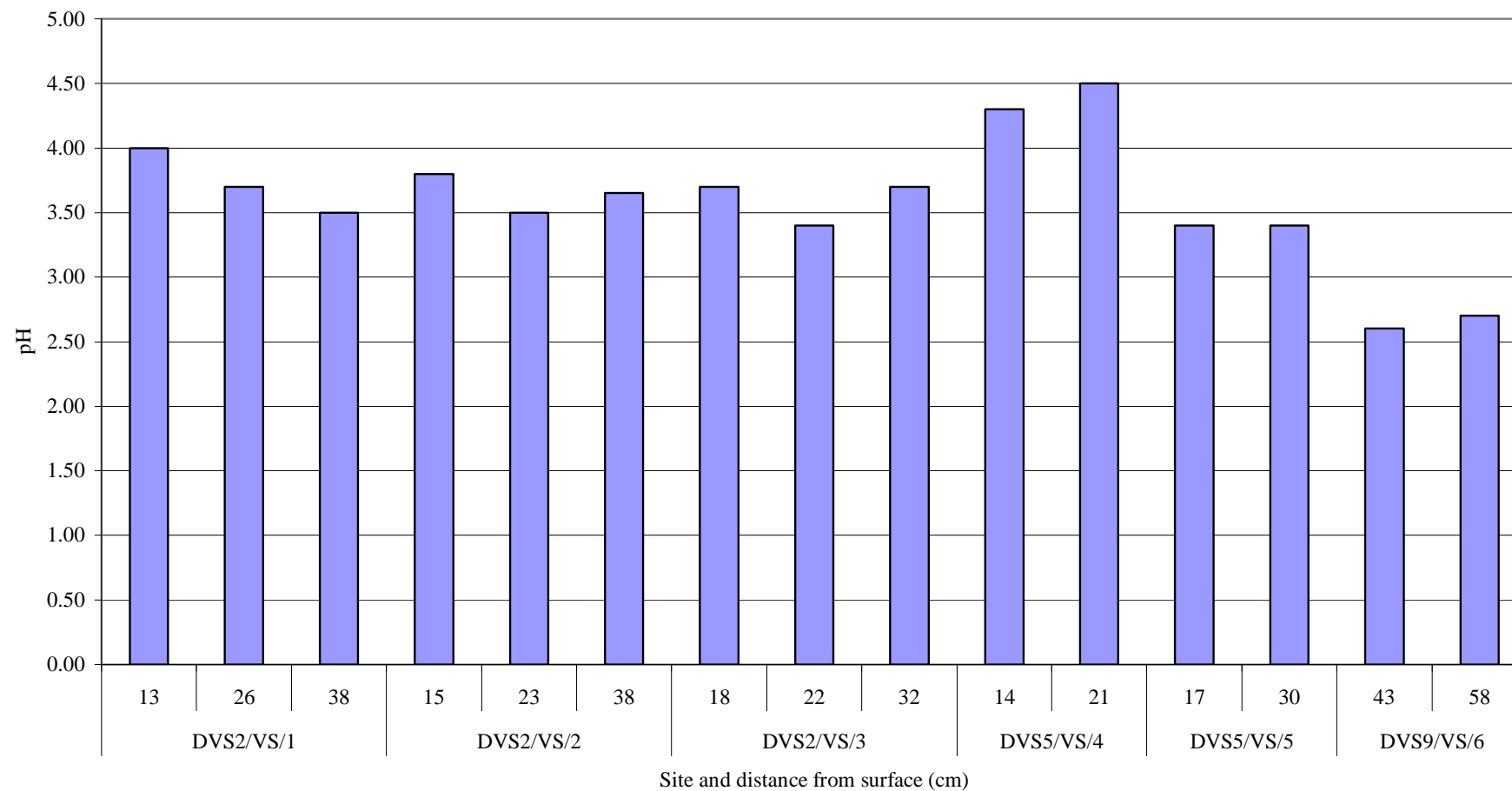


Figure 7-13: The pH values of soil in vertical sections from various locations around Blanchdown Wood, Devon (see Tables 7-1 and 7-2 for sample details)



7.3.2.1. Statistical Analysis

The null hypothesis for the analysis is: there is no significant difference between the concentrations of copper, lead, tungsten, tin and arsenic in vertical sections of soil profiles.

Table 7-7: The statistical results of differences between the vertical sections for the tested analytes

Site	Analyte	F	F crit	P-Value	H ₀
DVS2/VS/1	Copper	13.313	5.6136	<0.01	Reject
	Lead	5.394	5.6136	<0.01	Accept
	Tungsten	6.212	5.6136	<0.01	Reject
	Tin	5.717	5.6136	<0.01	Reject
	Arsenic	6.492	5.6136	<0.01	Reject
DVS2/VS/2	Copper	1.106	5.6136	<0.01	Accept
	Lead	0.222	5.6136	<0.01	Accept
	Tungsten	0.294	5.6136	<0.01	Accept
	Tin	1.725	5.6136	<0.01	Accept
	Arsenic	0.244	5.6136	<0.01	Accept
DVS2/VS/3	Copper	1.438	6.0129	<0.01	Accept
	Lead	3.620	6.0129	<0.01	Accept
	Tungsten	0.563	6.0129	<0.01	Accept
	Tin	1.964	6.0129	<0.01	Accept
	Arsenic	16.920	6.0129	<0.01	Reject
DVS5/VS/4	Copper	87.296	10.044	<0.01	Reject
	Lead	33.773	8.8616	<0.01	Reject
	Tungsten	1.229	8.8616	<0.01	Accept
	Tin	36.820	8.8616	<0.01	Reject
	Arsenic	46.440	8.8616	<0.01	Reject
DVS5/VS/5	Copper	6.255	8.8616	<0.01	Accept
	Lead	0.016	8.8616	<0.01	Accept
	Tungsten	1.968	8.8616	<0.01	Accept
	Tin	2.812	8.8616	<0.01	Accept
	Arsenic	10.726	8.8616	<0.01	Reject

Table 7-7: (cont)

DVS9/VS/6	Copper	1.636	8.8616	<0.01	Accept
	Lead	1.640	8.8616	<0.01	Accept
	Tungsten	1.991	8.8616	<0.01	Accept
	Tin	0.207	8.8616	<0.01	Accept
	Arsenic	3.471	8.8616	<0.01	Accept

For the results where the null hypothesis was rejected (Table 7-7), a Tukey Test was done to determine where the differences were. For sites DVS5/VS/4, DVS5VS/5 and DVS9VS/6, only two samples were taken, therefore where the null hypothesis is rejected there is a significant difference between the two sections in the vertical profile. The results for the Tukey Test are shown in Table 7-8. Where a 0 has been entered in the table, there was no significant difference between the samples.

Table 7-8: The Tukey Test results for the differences between vertical sections

Table 7-8a:
DVS2/VS/1

Copper critical value: 572.27			Tungsten critical value: 24.77		
	2	3		2	3
1	609.441	0	1	31.1926	29.3085
2		1136.727	2		0
Tin critical value: 88.381			Arsenic critical value: 4158.218		
	2	3		2	3
1	0	0	1	4189.43	0
2		116.4735	2		5817.949

Table 7-8b:
DVS2/VS/3

Arsenic critical value: 3170.922		
	2	3
1	0	5689.77
2		6702.76

As with the dispersal results, there is little correlation between the concentrations of the metals and the site. The mosaic effect seems to occur through the profile of the soil. However the pH may play an additional role and therefore restrict or enhance the movement of the metals through the profile.

7.3.3. Pod Profiles

The samples of pod profiles were taken in the same area, approximately 3m apart. Both samples contained vegetation on the surface and these were separated and analysed. For DVS20/PP/1 (which was closest to the spoil tip), the surface vegetation contained moss spp and *Festuca spp*, but DVS20/PP/2 only contained moss spp. Because both samples contained vegetations, the first layer (DVS20/PP/1) and the first two layers (DVS20/PP/2) were excluded from the statistical analysis as they contained both vegetation and soil material. It was not possible to completely remove all the soil from the plants roots and therefore the concentrations are the total for that layer.

The site was visited on a number of occasions, and it was found that the area was prone to flooding after heavy rain. On one visit, it was found that the area was flooded to a depth of approximately 3-5cm. This would suggest that the area becomes waterlogged and takes time for water to be absorbed through the soil.

Figures 7-14 and 7-15 show that there is a difference in the concentrations of the metals within both samples and that there is a variation between the samples. It can be seen that the concentration of arsenic is higher than that of all the other metals, in some cases reaching over 15000mg/kg. The results for DVS20/PP/1 show that for copper and arsenic, the values increased with depth and then decreased, where as the lead and tungsten remained at similar concentrations for each layer, however it should be noted that the statistical analysis indicates that there was a significant difference between the layers. The tin results for DVS20/PP/1 indicate that the concentration increased through the pod profile.

All the metals analysed for in DVS20/PP/2 showed a decrease in the concentration with increasing distance from the surface. The first 2cm contained plant material and was therefore excluded.

The pH of the soil (See Figure 7-16) shows that there is little variation between the layers of soil, however there was a significant difference between the two pod soils.

Although the results for the two pods differed, it can be seen that they had the same order of concentrations for the metals with the highest concentration first:

- DVS20/PP/1: As>Cu>Sn>Pb>W
- DVS20/PP/2: As>Cu>Sn>Pb>W

Figure 7-14: The concentrations of copper, lead, tungsten and tin in different sections of a soil pod
 (see Tables 7-1 and 7-2 for sample details)

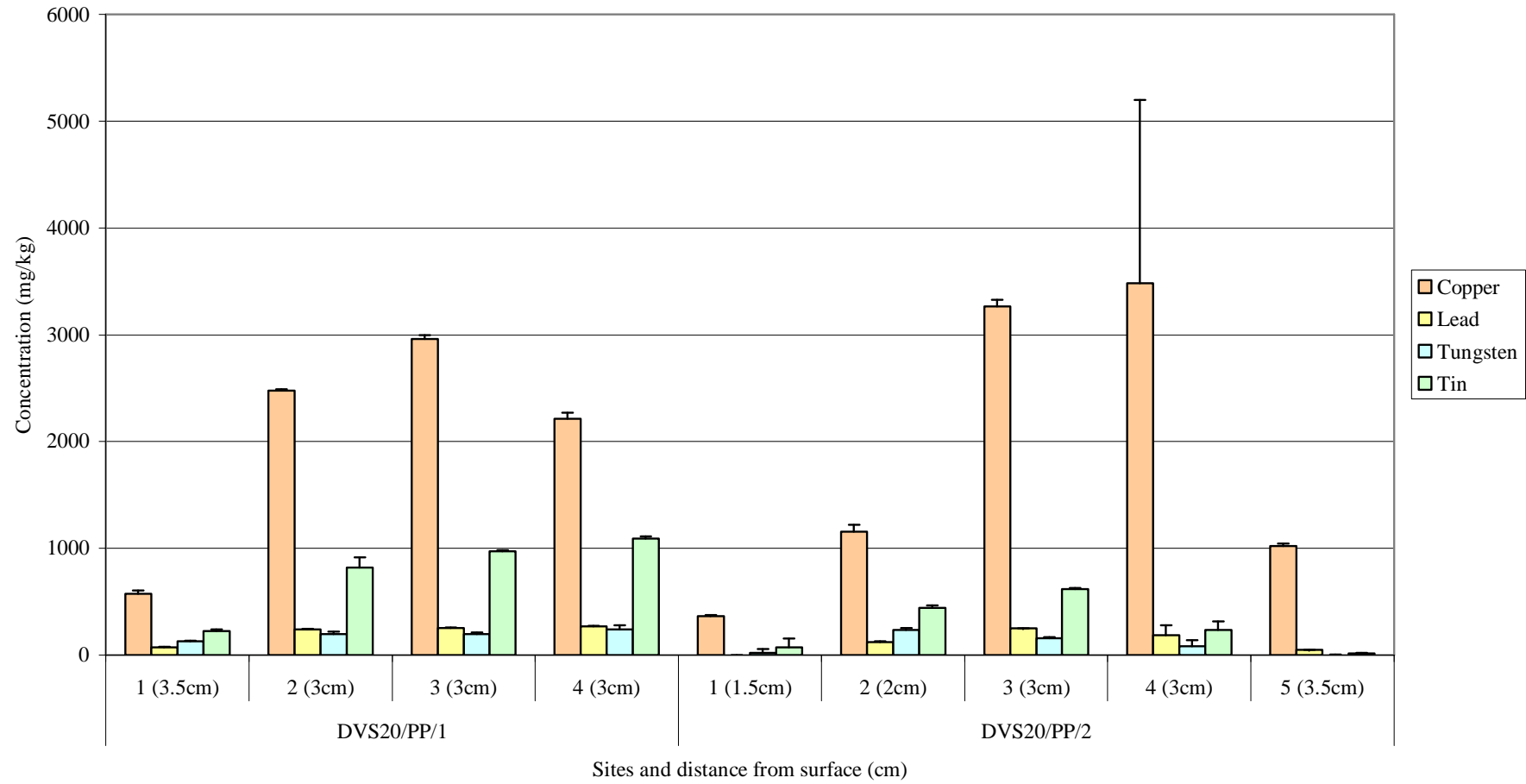
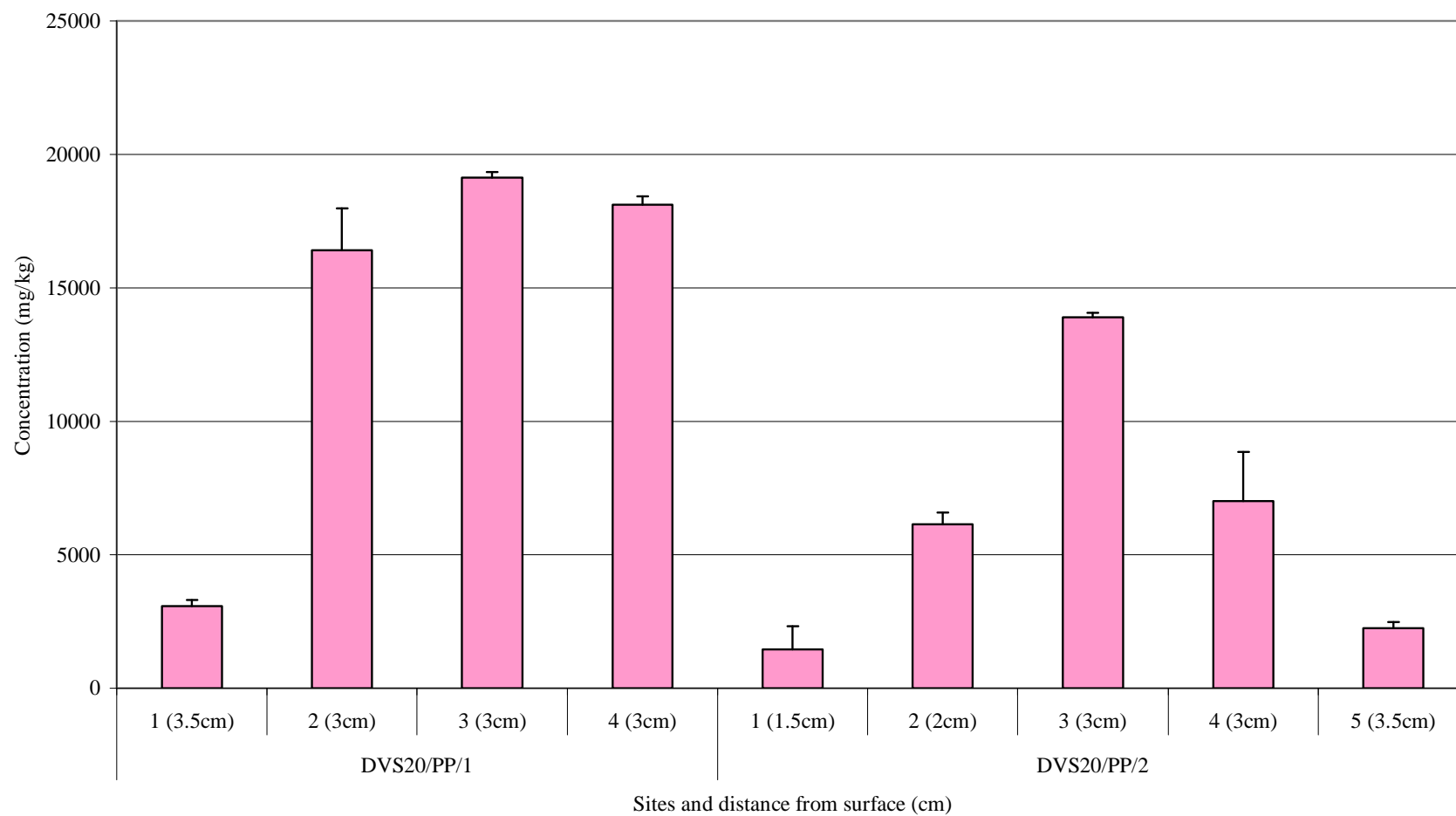


Figure 7-15: The concentration of arsenic in different sections of a soil pod
(see Tables 7-1 and 7-2 for sample details)



7.3.3.1. Statistical Analysis

The null hypothesis is: there is no significant difference between the vertical sections of the pod sample. The layers that contained plant material and soil were not included in the analysis as whether the ions were found in the plant or the soil could be determined.

Table 7-9: The statistical results for differences between the pod samples

	Analyte	F	F crit	P-Value	H ₀
DVS20/PP/1	Copper	256.064	5.7804	<0.01	Reject
	Lead	50.547	5.7804	<0.01	Reject
	Tungsten	4.834	5.7804	<0.01	Accept
	Tin	21.338	5.7804	<0.01	Reject
	Arsenic	8.185	5.7804	<0.01	Reject
DVS20/PP/2	Copper	6.471	5.6136	<0.01	Reject
	Lead	13.108	5.6136	<0.01	Reject
	Tungsten	18.777	5.6136	<0.01	Reject
	Tin	131.441	5.6136	<0.01	Reject
	Arsenic	93.219	5.6136	<0.01	Reject

Table 7-9 shows the results from the statistical analysis, and it concludes that there is a significant difference between the concentrations of the ions depending on the distance from the surface. To determine where the differences were, a Tukey Test was done. The results of which are shown in Table 7-10.

For DVS20/PP/1, the tungsten null hypothesis was accepted at $P < 0.01$, however if using $P < 0.05$, the hypothesis would be rejected indicating that there was a significant difference between the layers concentration of tungsten at this level of probability

Table 7-10: The Tukey Test results for differences between the pod samples

Table 7-10a:
DVS20/PP/1

Copper critical value: 84.434			Tin critical value: 107.291		
	2	3		2	3
1	484.26	259.2915	1	153.231	276.331
2		743.5512	2		123.1
Lead critical value: 7.639			Arsenic critical value: 1719.081		
	2	3		2	3
1	22.0022	29.0931	1	2707.02	1761.13
2		0	2		0

Table 7-10b:
DVS20/PP/2

Copper critical value: 1965.438			Tin critical value: 97.551		
	2	3		2	3
1	0	2245.157	1	377.4895	603.9052
2		2618.345	2		226.4158
Lead critical value: 100.392			Arsenic critical value: 2138.115		
	2	3		2	3
1	0	198.4369	1	6741.769	11647.94
2		146.9025	2		4906.174
Tungsten critical value: 64.436					
	2	3			
1	75.05975	158.1274			
2		83.06763			

7.4. *Discussion*

The dispersal of metals in the environment occurs through various pathways, including atmospheric deposition and leaching. The atmospheric deposition can occur through washout of pollutants that have been emitted from a point source, or from erosion caused by the weathering of contaminated material. Blanchdown Wood is a site where the mining and smelting operations ceased several decades ago. Therefore the deposition of pollutants from a point source is no longer an issue. But however the volume of pollution produced by the smelting stack during its activity would introduce contaminants over a large area. Although the area is no longer subjected to the constant pollutants from the smelting stack, the area is still subjected to contaminants from the spoil tips that remain. Figure 7-16 shows a photograph of Blanchdown Wood taken from approximately 1.5km away.

Figure 7-16: A photograph of Blanchdown Wood from a distance



It can be seen that Blanchdown Wood is situated in a small valley; the mound in the centre consists of tailings from the smelting stack. The smelting stack can also be seen in the distance. The photograph shows the area the mining activities have covered, although most of the area has been planted with pine trees. Although the volume of waste is evident, this is probably only a small percentage of what was

produced. The waste would have been deposited in convenient places, and over the years these have been overgrown and are no longer visible additional sites may have been used. Even though the waste materials are no longer visible, it is probable that they are having an affect on the environment. Where the waste is exposed to the elements, particles are transported by the weather to be deposited elsewhere. Because of the plantation that has occurred, this has had limited effects on the environment out of the boundaries of the wood. The trees have provided a small barrier preventing further areas from being contaminated, although this is not a conclusive solution, as depending on the weather the particles can still the transported.

Once in the soil, the contaminants are unable to be destroyed and are either taken up into plants or are leached further into the environment. The transfer into the environment occurs either through lateral or vertical dispersal:

- Lateral dispersal - occurs through the transfer of the contaminants through a lateral plane. This can then contaminate increasing areas.
- Vertical dispersal - arises when the contaminants diffuse vertically through the soil, and eventually reaching increasing depths and possibly the water table.

Vertical dispersal causes concern as the water from the water table can become contaminated and if ingested by animals (including humans) or used for providing water for crops, can introduce the contaminants directly into the food chain.

The results from the lateral dispersal analysis show that there is very little correlation in the concentration between the sites. There is also a large variability between the sites. The variation of the concentrations of metals, whether between sites or within, has a number of factors that need to be considered. The physical, chemical and biological properties are important as these can determine if the contaminants are mobile or immobile and therefore if they are able to pass through the terrestrial environment.

Soil permeability (a physical property) allows for the diffusion of liquid to pass through the soil, and this is dependent on the spaces between the soil particles. Soils with large spaces between soil particles have a high drainage capability and therefore water is diffused quickly through the soil, e.g. sandy soils. An increase in the soil permeability can be achieved through the presence of worms and plants, as these produce channels that can encourage the downward movement of water.

The chemical properties of the soil can either encourage or restrict the movement of the contaminant and these include the pH and the adsorption capabilities of the soil. Adsorption is the method of contaminants becoming bound to soil particles. In turn the adsorption capability of the contaminant can also predict the bioavailability of the contaminants and therefore whether it is a hazard to plant and animal life.

The large number of factors affecting the dispersal of contaminants through the soil indicates that a mosaic effect is produced. Blanchdown Wood consists of a large variation of soil throughout the site, whether the variation is brought about by the natural properties of the soil or via induced chemical changes from the mining activities. Chemical changes can arise through the combination of a number of contaminants present in increased concentration, altering the properties of the soil. This can lead to an increase or decrease in the mobility of the contaminants.

Although a number of research papers (Fakayode and Onianwa, 2002; Pichtel *et al*, 2000; Clark *et al*, 2001; Pyatt *et al*, 2000) have examined the metal contents of soils from different sites, it is difficult to compare the results as there are a number of properties of the soils mentioned previously that could affect the concentration of the metals. Because of the variations that can arise through the soil properties, it should be assumed that there would also be a variation in the behaviour of the metal ions, although generalisations can be made. Additionally, a synergistic or additional effect could be created by the presence of different ions, whether occurring naturally in the soil or as a contaminant.

The results showing the vertical changes in the soil concentration show that there is a difference in the concentrations, but this too is mosaic like. There are a number of factors that could be controlling the flow of the ions vertically through the soil, including the pH and volume of organic matter. The vertical profiles showed that there was a large variation of pH values ranging from 4.5 to 2.6. The pH of the soil can affect the mobility of different elements in different ways. For example a decrease in the pH can increase the mobility of lead and zinc (Korte *et al*, 1976). The vertical profile results show that there is a large concentration of all the metals found at the different depths through the soil. However, it can not be conclusively said that a flow of ions has occurred as the soil material was most likely mixed with tailings. The soil texture was sandy, indicating that there would be a high permeability, therefore the ions are more likely to have adsorbed to the surface of the particles. However, it must be assumed that only a certain number of metal ions can be adsorbed to the surface

and a possible affect of this is that the ions move further down the profile until it has become bonded to the next available particle. Although the soil has a high permeability, there is still a high concentration of each of the analytes in the soil, indicating that there is either a continual deposition of contaminants or that the contaminants were in much higher concentrations that the flow of the analytes have occurred, but the volume of ions has restricted the rate and are unable to move through the profile. It should also be considered that the pH of the sampled soil is considered to be low and could therefore restrict the movement of the ions through the profile.

Since their initial deposition, high concentrations are still present and it could be assumed that these values should have decreased significantly. However estimates have been made to suggest the movement rate of certain metals (Maskall *et al*, 1995; Sterckerman *et al*, 2000), but this can be slightly unreliable as unless the soil conditions are equal to those of the experiments, the rates could be quicker or slower. Additionally, these calculated values do not include the possible increase in the analyte concentration due to increased deposition.

The pod profiles were of different material compared to the vertical profiles, as it could be seen from the texture that there was a higher volume of organic material in the soil. However as mentioned previously, the area where the samples were taken was prone to flooding, indicating that the soil had a low permeability, either through compaction or by the underlying clay content. The low permeability would restrict the movement of the metal ions. However from the results there is a clear change in the concentrations of the metals through the soil. As the soil is not composed of tailing material, it is likely that the contaminants have been deposited from the atmosphere or from leaching from the main spoil tip situated close to the sample site. During heavy rain contaminants in the spoil tip could be dissolved into the rainwater and washed out onto the sample site. The presence of the plant material on the surface of the pod samples could slow down the movement of the atmospheric deposited contaminants, by acting as a covering

As with the vertical dispersal samples, the contaminants could be adhered to the surface of the soil particles, and therefore, effectively, locking away the contaminants. It is proposed that the concentration of the metals would have decreased further from the surface of the soil. Essentially only the topsoil was examined and therefore further studies of the soil beneath this layer could produce lower concentrations.

Chapter 8: Accumulation of metals in plants

8.1. Plants as accumulators

The problem of metals in the terrestrial environment has been well documented and as discussed in Chapter 4, the use of plants (phytoremediation) has proven to be successful in the treatment of metals. Phytoremediation involves the identification of plants tolerant to metal concentrations in the soil, and their consequential uptake into the plant tissues. The plants can then be harvested and the metals can be removed from the area and disposed of in a controlled manner thus reducing the concentration of the metal in the soil. However there are problems that arise from this solution; it is difficult to determine which plants are capable of doing this and it is unknown as to how successful the plants can be. The use of plants is a long term solution and is time consuming and therefore the results may take many years to have a significant affect on the environment. Phytoremediation can be divided into many types (Terry and Banuelos, 2000):

- Phytoextraction (hyperaccumulator) – The use of plants to extract contaminants from the environment. When the plants have reached a saturation point, the plants are harvested and removed from the site.
- Phytodegradation – The use of plants to degrade organic pollutants through their metabolic processes
- Rhizofiltration – The use of plant roots to absorb contaminants from soil and ground water, similar to phytoextraction.
- Phytostabilisation – The use of soils and plants to reduce the bioavailability of contaminants and therefore restrict or prevent migration of contaminants
- Phytovolatilisation – The use of plants to volatilise contaminants (solvents) from soil or water

A number of research studies have been performed to confirm that members of the brassicae family are extremely successful hyperaccumulators (Zhu *et al*, 2003; Yanqun *et al*, 2004), but many species found wild have been found to tolerate the toxic metals and could therefore be used as phytoremediation (Pratas *et al*, 2004). In nature, the use of wild plants as accumulators have a number of concerns as in the

food chains they provide sustenance for wildlife, this can lead to the transfer through the food chains and a possible biomagnification of the toxic metals.

During numerous visits to Blanchdown Wood and Snailbeach a number of plants were noted to be growing in areas where it was considered as inhospitable. These plants were identified and sampled. The plants were identified as *Cotoneaster microphyllus*, *Picea sitchensis* (Blanchdown Wood, Devon, UK) and *Viburnum opulus* (Snailbeach, Shropshire, UK). An additional sample of unknown fungi was also collected at Snailbeach. Of the plants only the *V. opulus* was planted intentionally, and the reasoning behind this was possibly that the plants would be able to reach a height to prevent dust from the spoil tip being transported further.

8.1.1. Aims and Objectives

The aims and objectives of this section of the study was to determine if any of the mentioned plants (and fungi) accumulate the metals from the environment and if so do they show any indication of partitioning. All these plants provide sustenance to a number of birds and animals and therefore the contamination of the foodstuffs at this level could introduce the contaminants into food web.

8.2. *Cotoneaster microphyllus*

There are a number of species within the genus *Cotoneaster*, with a number of species being created by horticulturists.

On the site of Blanchdown Wood, a number of wild *Cotoneaster microphyllus* shrubs have been found to be growing successfully with little indication of harm from the toxic metals in the terrestrial environment. The shrub is known for its ability to grow in varied environments, and as such has no definitive environment in which it prefers. The plant exhibits enhanced levels of tolerance for soil condition especially in well drained, and can grow in areas of either high or low nutrients. The plant can grow flat on the ground or as a small shrub (Lang, 1987).

It is reported to be slow growing and provides food for a number of animals and birds. Flowering occurs during May and June and these then develop into berries that can remain throughout the winter. Figures 8-1 and 8-2 show the leaves, flowers and fruit of *C. microphyllus*.

Figure 8-1: Photograph of *C. microphyllus* leaves and flowers (Wikipedia, 2006)



Figure 8-2: A photograph of *C. microphyllus* leaves and fruit (Wikipedia, 2006)



The plant is of some interest as it appears to be extremely tolerant. The site at Blanchdown Wood is highly contaminated by the remains of mining and smelting activities over the last century (See Chapter 6). There is a possibility that the plant could accumulate the contaminants, and therefore could have uses as a hyper accumulator. There is also the possibility that the plant cannot accumulate the contaminants and must therefore have some kind of mechanism to prevent the contaminants entering the plant.

Figure 8-3: *C. microphyllus* growing amongst other vegetation at Blanchdown Wood, Devon



Figure 8-4: *C. microphyllus* growing around building remains at Blanchdown Wood, Devon



8.2.1. Experimental procedure

At 6 different sites, at various distances, around the spoil tip, the *C. microphyllus* were sampled and the sampling sites were recorded using a GPS. To sample the plant material, a pair of secateurs was used to cut a sample; between sampling the secateurs were cleaned to prevent any cross contamination. The section that was cut contained stem, leaves, flower buds and berries; where possible the roots were extracted but this was not possible for all samples. The samples were stored in individual sealed bags. A control sample was obtained from an area with no known metal pollution. Upon return to the laboratory, the plant material was prepared as discussed in Chapter 5.

8.2.2. Results

During the analysis of samples from Blanchdown Wood, arsenic, copper, tungsten, tin and lead were identified as being present in high concentrations in the soil; therefore these were the elements that were looked for in the *C. microphyllus* samples. Figures 8-5, 8-6 and 8-7 show the concentrations of lead, tungsten, tin (Figure 8-5), copper (Figure 8-6) and arsenic (Figure 8-7) in the soil where the plant was found, and it should be noted that copper and arsenic were in high concentrations (arsenic up to 20,000mg/kg and copper up to 2000mg/kg). It can also be seen that the concentrations were high for the remaining analytes, but all were less than 200mg/kg. It should also be noted that one site had a reduced concentration of the analytes compared to the other sites. This was DV6/CO/6, and using the map in Figure 7-1 it can be seen that it is located behind the chimney stack, where the area is nearly completely sheltered. Not all the sites have a soil sample; DV1/CO/1 and DV8/CO/7 were in areas where the soil could not be accessed due to unsafe conditions.

The results obtained from the analysis showed that only copper and tungsten was present in parts of the *C. microphyllus*, and is shown in Figure 8-8 with corresponding 95% confidence intervals. The control sample (DV1/CO/1) showed small amounts of copper in all parts of the plant material. But in the *C. microphyllus* sampled at Blanchdown Wood, the copper results were more varied, with some parts of the plant showing little or no accumulation of copper. It can be seen that where tungsten was found, copper was also present. All the stem samples collected were found to only contain copper.

When comparing the concentrations of tungsten in the soil and in plant material, there appears to be only a slightly higher concentration in the soil compared to plant material. Sites DV3/CO/3 and DV4/CO/4 were found that there was a higher concentration of tungsten in the soil compared with the leaves (29.342mg/kg in soil and 12.233mg/kg in leaves; 91.874mg/kg in soil and 11.021mg/kg in leaves, respectively). Sites DV2/CO/2 and DV6/CO/6 contained higher concentrations in the leaves than the soil (14.149mg/kg in soil and 16.513mg/kg in leaves; 0.0mg/kg in soil and 10.120mg/kg in leaves, respectively)

Figure 8-8 also shows that the highest concentration of copper was found in the stem of DV4/CO/4 (118.552mg/kg). The soil also contained the highest concentration of copper (10526.770mg/kg), when compared to other *C. microphyllus* soil samples. The leaves also contained one of the highest concentrations of copper, as did leaves

from DV2/CO/2 and DV3/CO/3. These two sites were located close to the remains of buildings, and were in areas that were exposed. However DV4/CO/4 was found in a wooded area on the eastern side of the large mound containing tailings.

Because of the high concentrations, the results for the roots are displayed in Figure 8-9 and 8-10. It can be seen that copper, arsenic (Figure 8-9) tin and lead (Figure 8-10) were found in the roots, but tungsten was not represented. However, not all the sites had root samples, as some of the roots were inaccessible.

Figure 8-5: The concentrations of lead, tungsten and tin in soil at sites with *C. microphyllus*
(see Tables 7-1 and 7-2 for key to sample names)

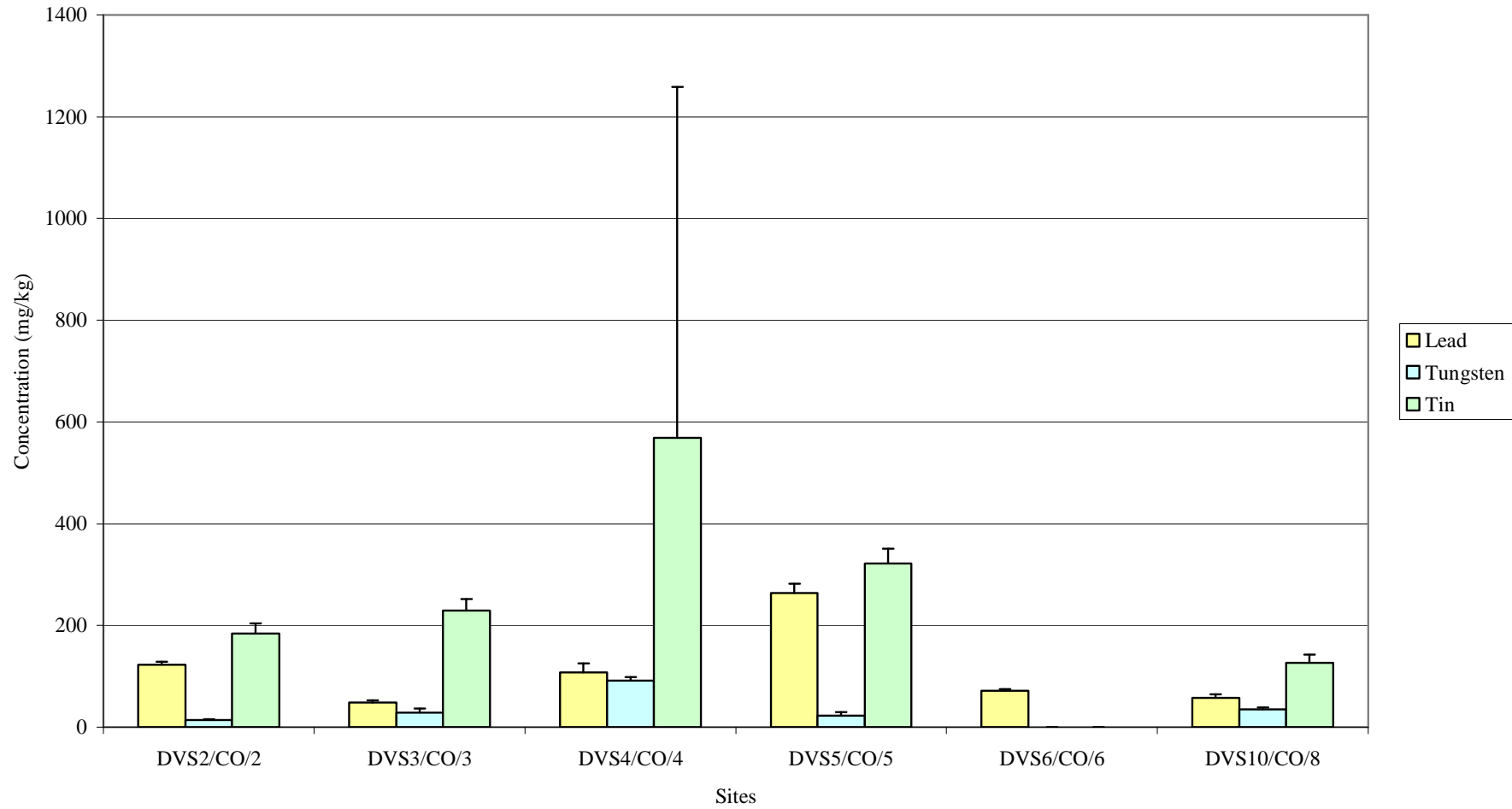


Figure 8-6: The concentration of copper in soil at sites with *C. microphyllus*
(see Tables 7-1 and 7-2 for key to sample names)

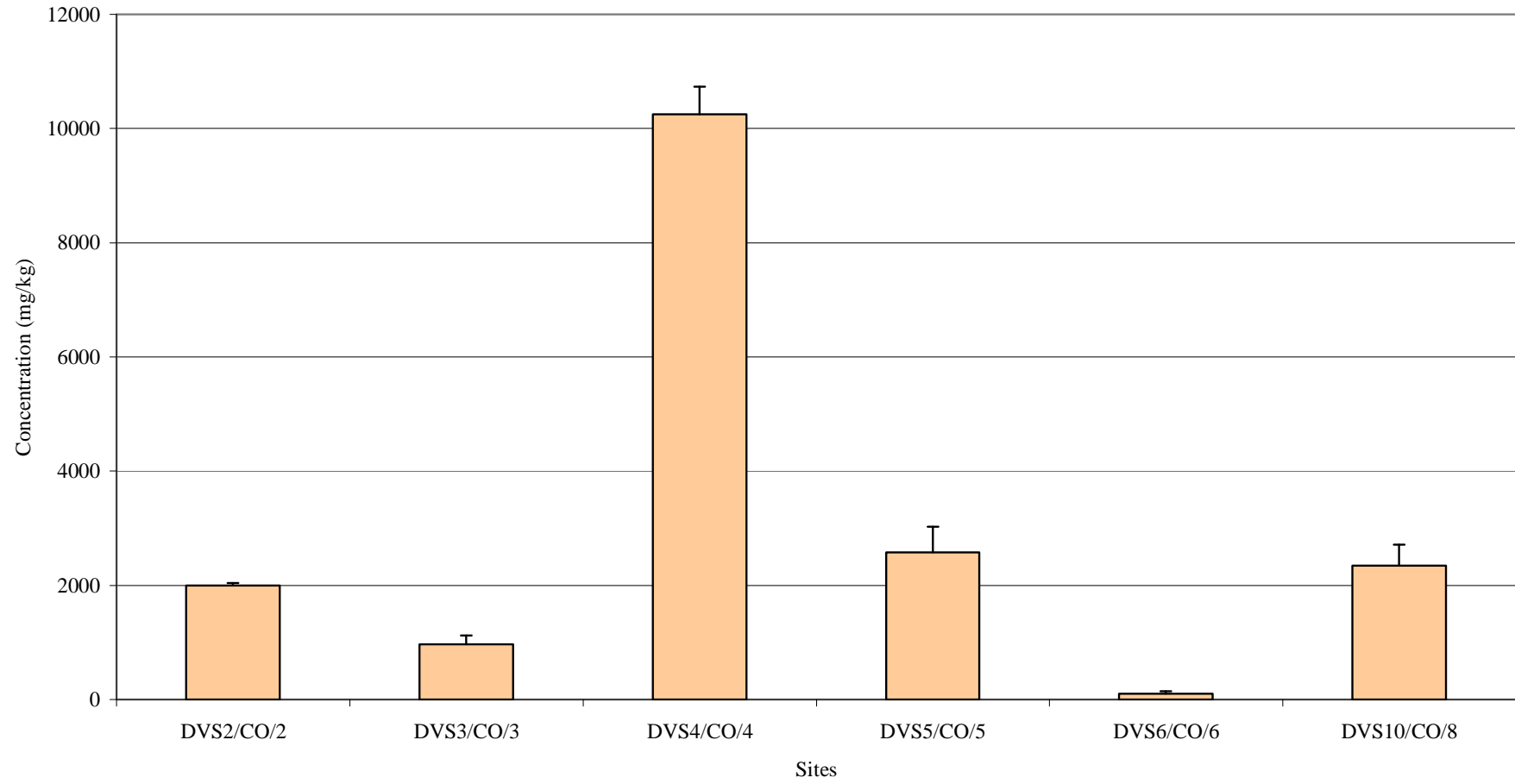


Figure 8-7: The concentration of arsenic in soil at sites with *C. microphyllus*
(see Tables 7-1 and 7-2 for key to sample names)

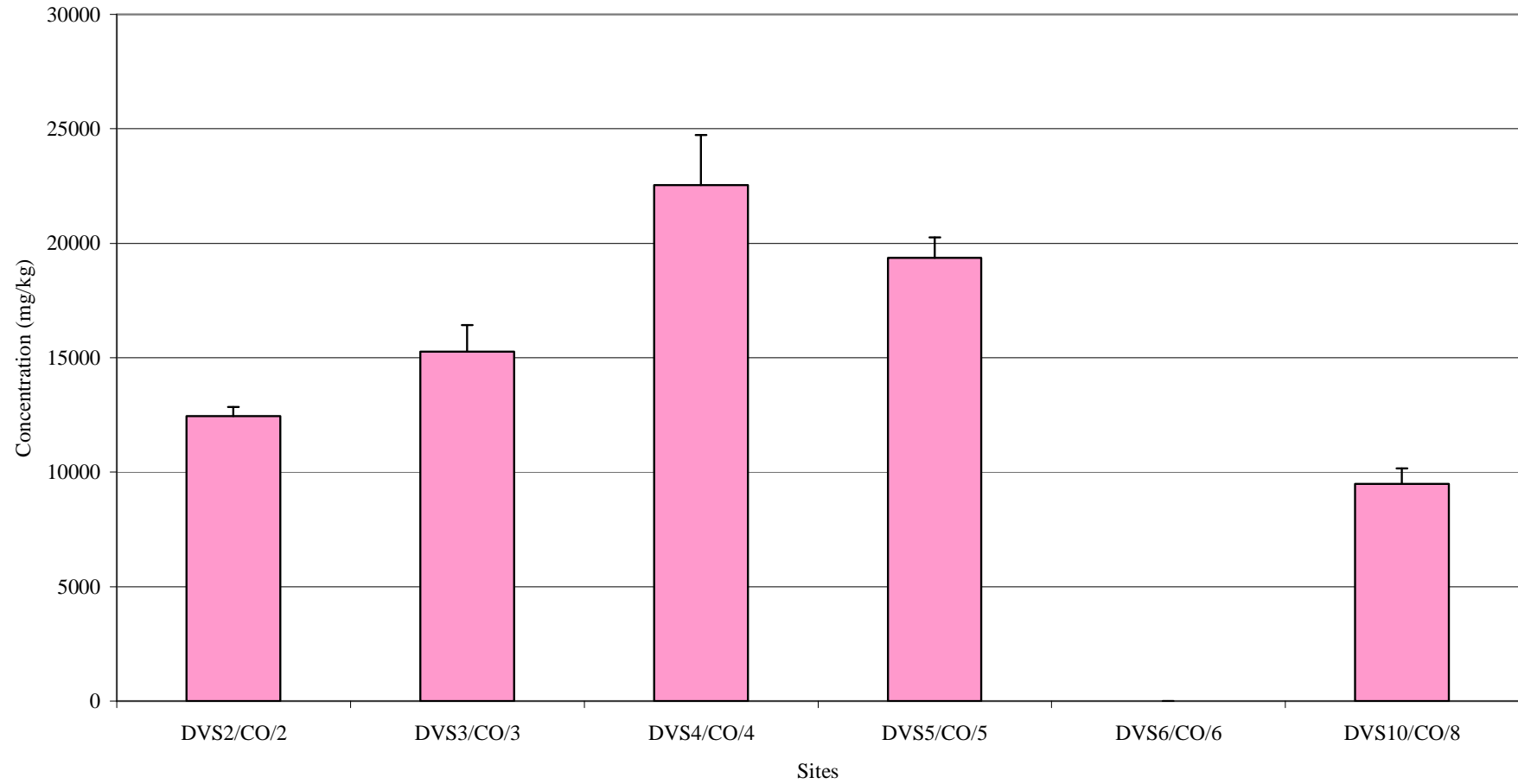


Figure 8-8: The concentrations of copper and tungsten in various parts of *C. microphyllus*
 (see Tables 7-1 and 7-2 for key to sample names)

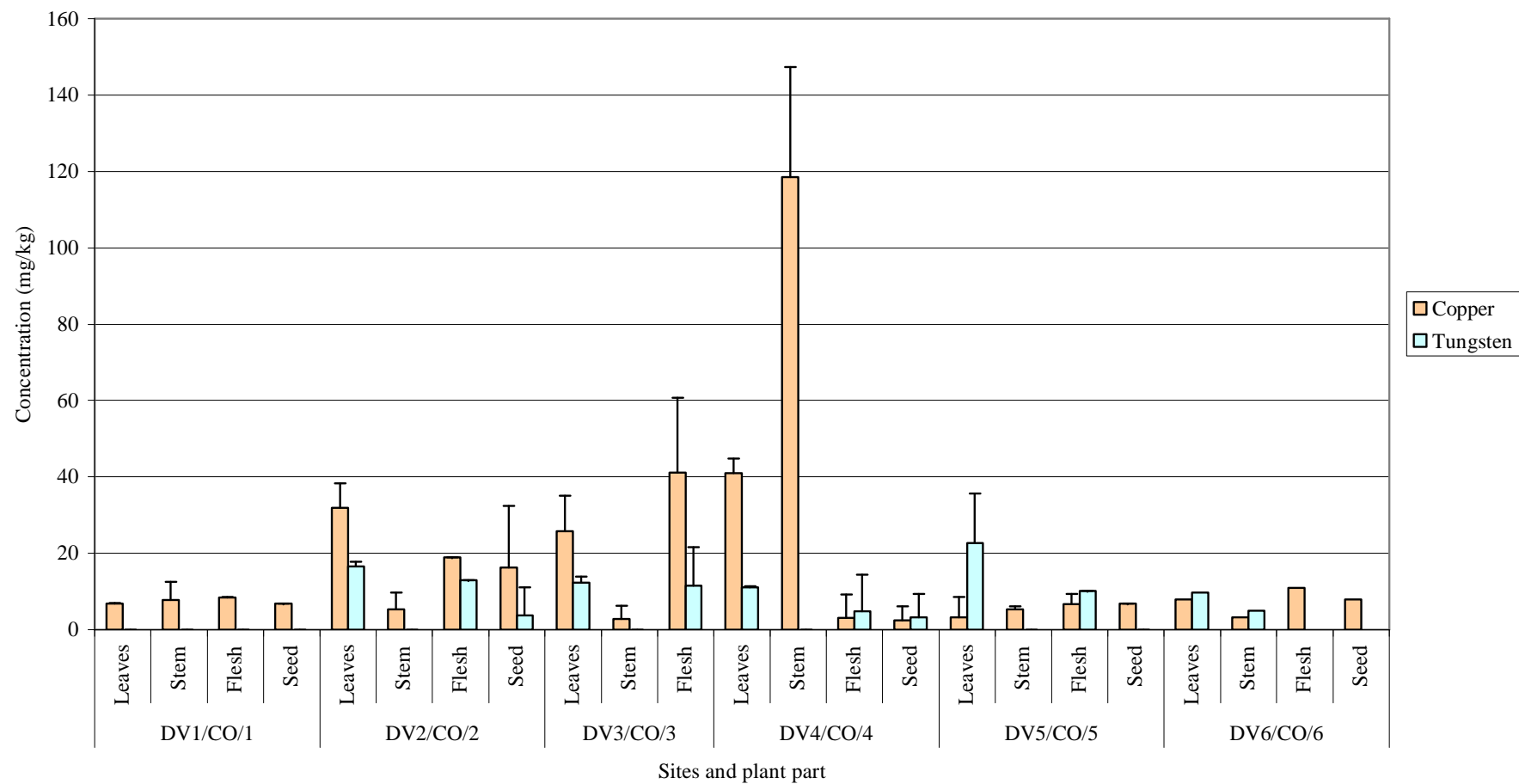


Figure 8-9: The concentration of copper and arsenic in roots of *C. microphyllus*
(see Tables 7-1 and 7-2 for key to sample names)

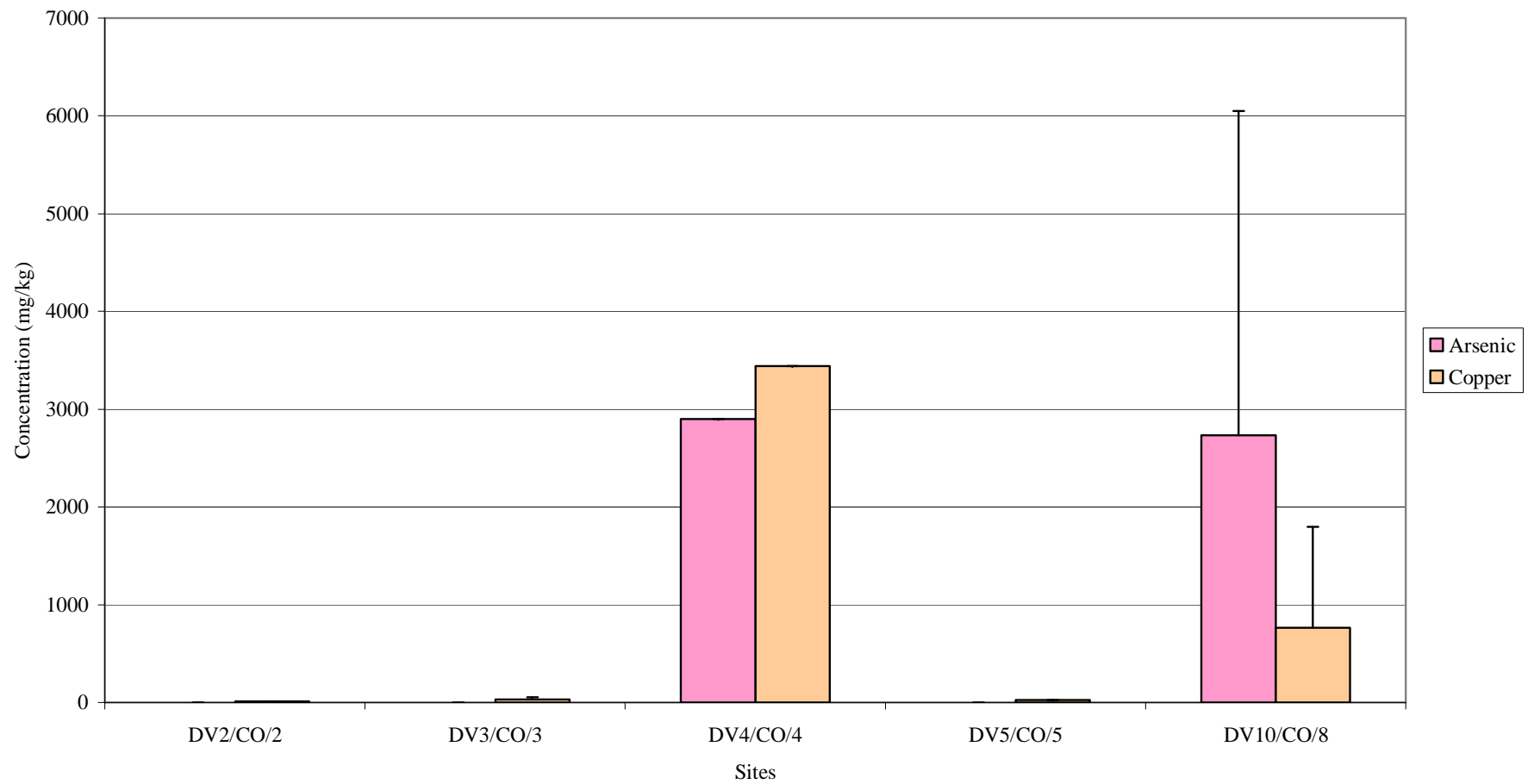
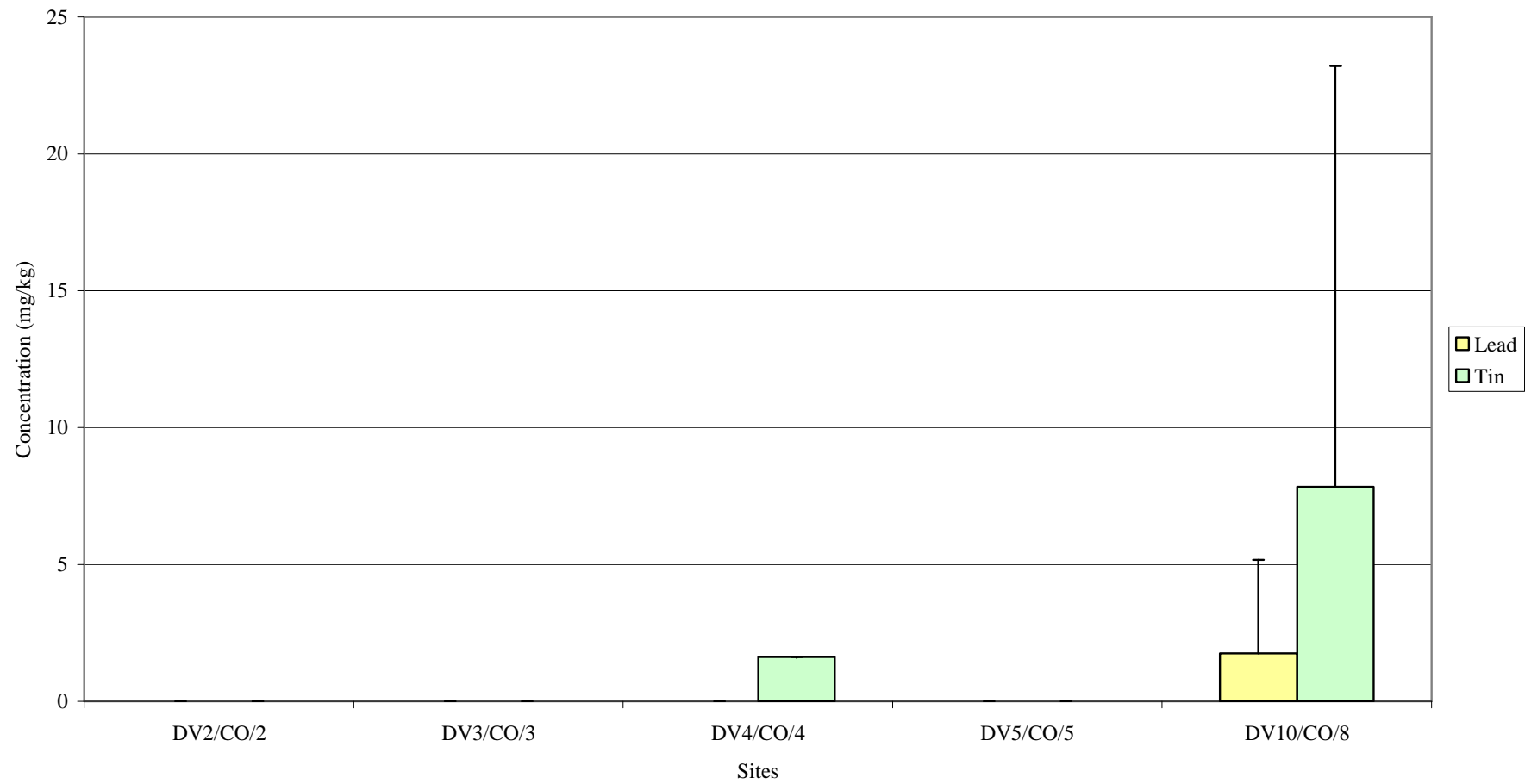


Figure 8-10: The concentration of lead and tin in roots of *C. microphyllus*, sampled at Blanchdown Wood, Devon, UK
(see Tables 7-1 and 7-2 for key to sample names)



8.2.2.1. *Statistic analysis*

The null hypothesis is: there is no significant difference between the samples tested. A summary of the statistical results is displayed in Table 7-10.

Table 8-1: The statistical results for the differences between plant and soil samples

Material	Analyte	F	P-Value	H ₀
Soil	Copper	625.627 _{6,57}	<0.01	Reject
	Lead	59.447 _{6,57}	<0.01	Reject
	Tungsten	67.766 _{6,57}	<0.01	Reject
	Tin	2.001 _{6,57}	0.08	Accept
	Arsenic	513.090 _{6,57}	<0.01	Reject
Stem	Copper	40.575 _{7,20}	<0.01	Reject
Seeds	Copper	1.440 _{6,8}	0.31	Accept
	Tungsten	0.305 _{6,6}	0.91	Accept
	Arsenic	1.227 _{5,7}	0.39	Accept
Roots	Copper	7.15 _{4,7}	0.01	Reject
	Tungsten	1.458 _{4,7}	0.31	Accept
	Lead	1.41 _{4,7}	0.32	Accept
	Tin	1.459 _{4,7}	0.31	Accept
	Arsenic	3.239 _{4,7}	0.08	Accept

The table gives the F value and the corresponding P value. The critical p-value is 0.05, and results greater than this indicates that there is a chance that the hypothesis is correct and is therefore accepted (there is no significant difference between the sample means).

Where the null hypothesis was rejected, a further test was done to determine where the differences were. The Tukey Test gives a result indicating where the differences are. Each of the individual mean is compared and a score is given (Significant difference = 1, No significant difference = 0). Table 7-10 shows the summary of the results indicating where the differences were found. Because there were uneven numbers of replicates, the critical value was determined using a modified equation:

$$T_{ij} = \frac{q}{\sqrt{2}} * \sqrt{\text{within variance} * \left(\frac{1}{n_i} + \frac{1}{n_j} \right)} \dots\dots\dots(5)$$

Table 8-2: The Tukey Test results for the differences between plant and soil samples

Table 8-2a: The concentration of lead in soil samples

	DVS3/CO/3	DVS4/CO/4	DVS5/CO/5	DVS6/CO/6	DVS10/CO/8
DVS2/CO/2	1	0	1	0	0
DVS3/CO/3		1	1	0	0
DVS4/CO/4			1	0	0
DVS5/CO/5				1	1
DVS6/CO/6					0

Table 8-2b: The concentration of arsenic in soil samples

	DVS3/CO/3	DVS4/CO/4	DVS5/CO/5	DVS6/CO/6	DVS10/CO/8
DVS2/CO/2	1	1	1	1	1
DVS3/CO/3		1	1	1	1
DVS4/CO/4			1	1	1
DVS5/CO/5				1	1
DVS6/CO/6					1

Table 8-2c: The concentration of copper in soil samples

	DVS3/CO/3	DVS4/CO/4	DVS5/CO/5	DVS6/CO/6	DVS10/CO/8
DVS2/CO/2	1	1	0	1	0
DVS3/CO/3		1	1	1	0
DVS4/CO/4			0	1	1
DVS5/CO/5				1	1
DVS6/CO/6					1

Table 8-2d: The concentration of tungsten in soil samples

	DVS3/CO/3	DVS4/CO/4	DVS5/CO/5	DVS6/CO/6	DVS10/CO/8
DVS2/CO/2	0	1	0	0	0
DVS3/CO/3		1	0	1	0
DVS4/CO/4			1	1	1
DVS5/CO/5				1	0
DVS6/CO/6					1

Table 8-2e: The concentration of copper in stem samples

	DV2/CO/2	DV3/CO/3	DV4/CO/4	DV5/CO/5	DV6/CO/6	DV8/CO/7
DV1/CO/1	0	0	1	0	0	0
DV2/CO/2		0	1	0	0	0
DV3/CO/3			1	0	0	0
DV4/CO/4				1	1	1
DV5/CO/5					0	0
DV6/CO/6						0

Table 8-2: Cont

Table 8-2f: The concentration of copper in roots samples

	DV3/CO/3	DV4/CO/4	DV6/CO/6	DV10/CO/8
DV2/CO/2	0	0	0	0
DV3/CO/3		1	0	0
DV4/CO/4			0	0
DV6/CO/6				0

8.3. *Picea sitchensis*

At Blanchdown Wood a number of different species of trees were identified. These included *Pinus sylvestris* (Scots Pine), *Picea abies* (Norway spruce) and *Picea sitchensis* (Sitka Spruce). The coniferous trees are believed to have been planted around the site over the last 50 years after mining works stopped (Snellgrove, M: personal communication). On visiting the site a number of young (less than 5 years old) *P. sitchensis* were found and were sampled whole to determine if heavy metals had been accumulated and if so in which parts of the plant the metals were found.

The *P. sitchensis* trees are large coniferous tree that can grow to between 50 and 70m tall, it is a member of the *Pinaceae* family and is related to the pine species. It is the largest of the Spruce trees and is native in North America and is known for its rapid growth and therefore age can not be determined by the size of the tree alone. Because of its rapid growth, the *P. sitchensis* is capable of growing in a wide range of environments such as nutrient deficient soils and exposed areas and therefore is widely planted around the world especially in areas where other species of trees are incapable of growth (White and More, 2003).

The tree has various uses in forestry for timber and is widely used in musical instrument manufacture (Hora, 1981).

Figure 8-11: A photograph of a mature *Picea sitchensis* (Wikipedia, 2006)



8.3.1. Experimental Procedure

Five young (estimated less than 5 years old) *P. sitchensis* trees were located at various locations around Blanchdown Wood. The trees were extracted whole from the soil and were placed in large bags to prevent any contamination. Where possible, as much of the soil was removed from around the roots and was stored separately from the plant material. Upon return to the laboratory, the plant material was separated and was prepared as discussed in Chapter 5.

8.3.2. Results

Figures 8-12 and 8-13 show the concentrations of lead, tin, tungsten (Figure 8-12), copper and arsenic (Figure 8-13) in soil where *P. sitchensis* trees were sampled. It can be seen that the concentrations of copper and arsenic are extremely high with maximum values of 2244mg/kg for copper at DVS21/PS/1 and 25492mg/kg for arsenic at DVS22/PS/2. Lead, tin and tungsten also had high concentrations but these did not exceed 765mg/kg. Notable results are those of DVS22/PS/2 and DVS25/PS/5, where the values of lead, tin and tungsten exceed those of the other sites.

Like the *C. microphyllus*, only copper and tungsten were present in the plant parts. From Figure 8-14 it can be seen that sites DV21/PS/1, DV22/PS/2 and DV23/PS/3 have the highest concentrations of both copper and tungsten. When comparing the results with the location map (Figure 7-1) it can be seen that these three sites are west, East and South of the small mound. It was found that these sites were exposed to the spoil from the small mound tip, and were in fine particle form.

When comparing the concentration of copper and tungsten in the upper plant portions to the roots, it can be seen that there was an increase in the root concentration. These were plotted on separate graphs to illustrate the significance of this. Root concentrations of copper ranged from 23.78mg/kg at DV3/PS/4 to 688.38mg/kg at DV25/PS/5. The tungsten concentrations ranged from 0mg/kg at DV21/PS/1 to 42.94mg/kg DV25/PS/5

Figure 8-12: The concentration of lead, tin and tungsten in soil at sites where *P. sitchensis* was sampled (see Tables 7-1 and 7-2 for key to sample names)

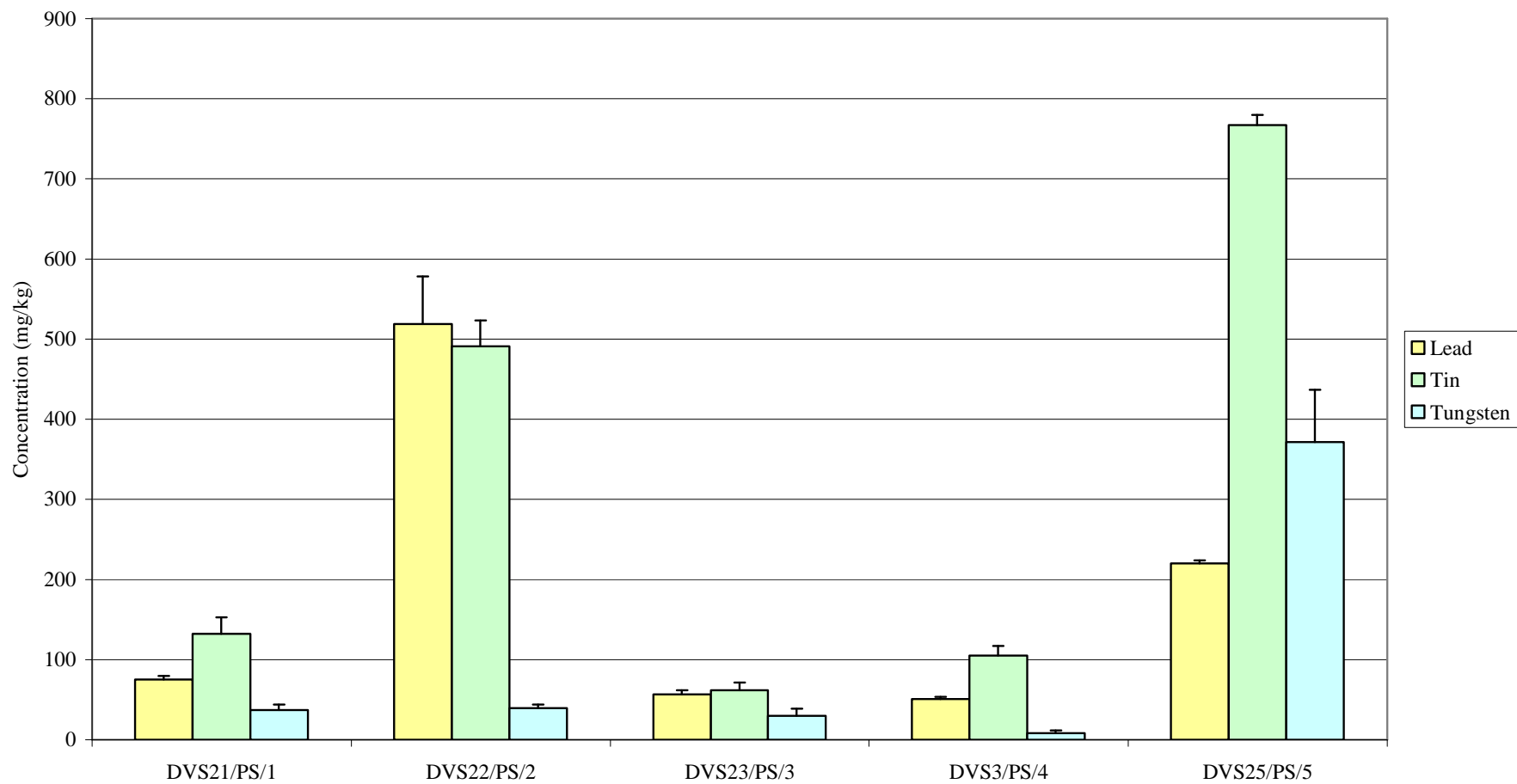


Figure 8-13: The concentration of copper and arsenic in soil at sites where *P. sitchensis* was sampled (see Tables 7-1 and 7-2 for key to sample names)

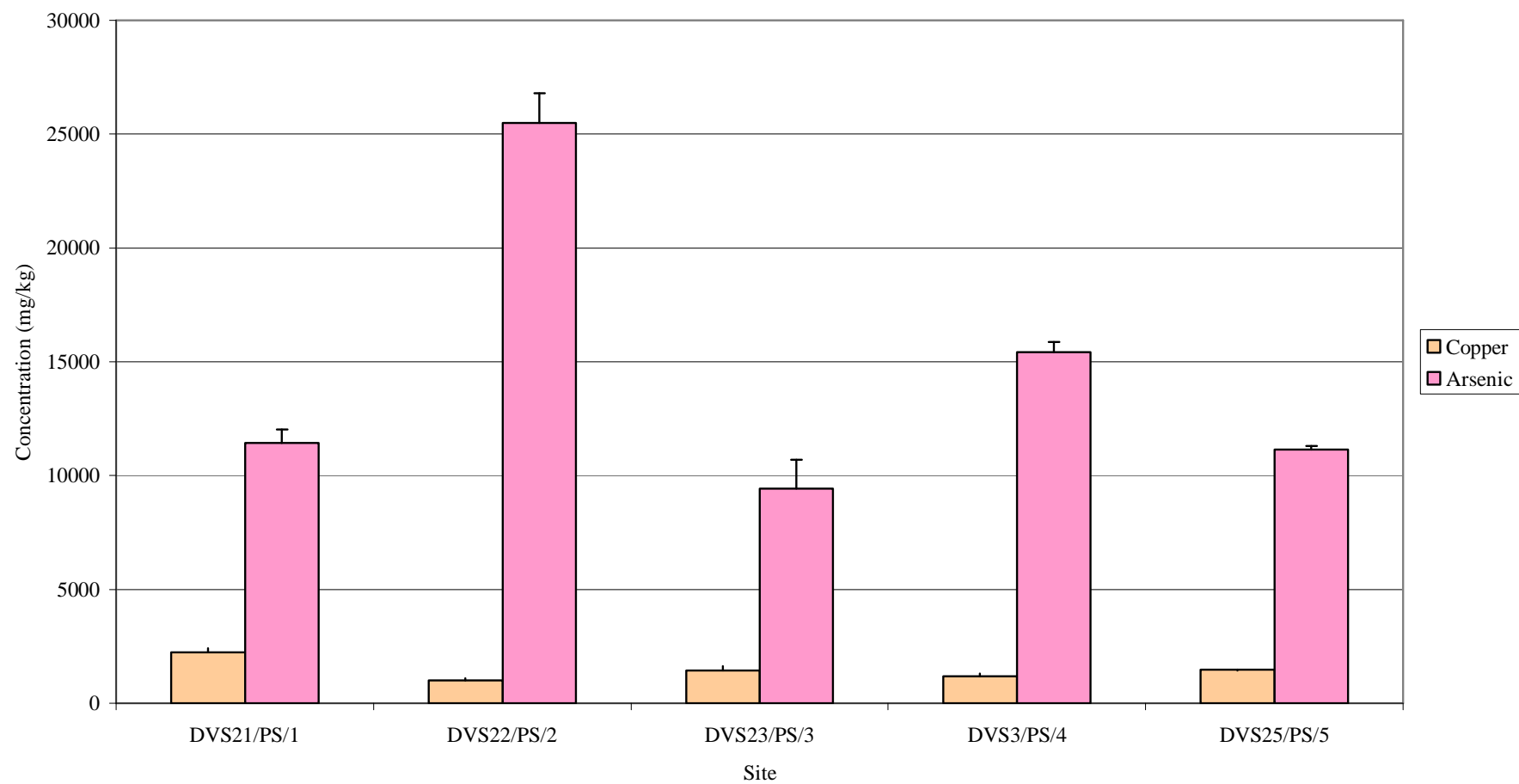


Figure 8-14: The concentration of copper and tungsten in various parts of *P. sitchensis*
 (see Tables 7-1 and 7-2 for key to sample names)

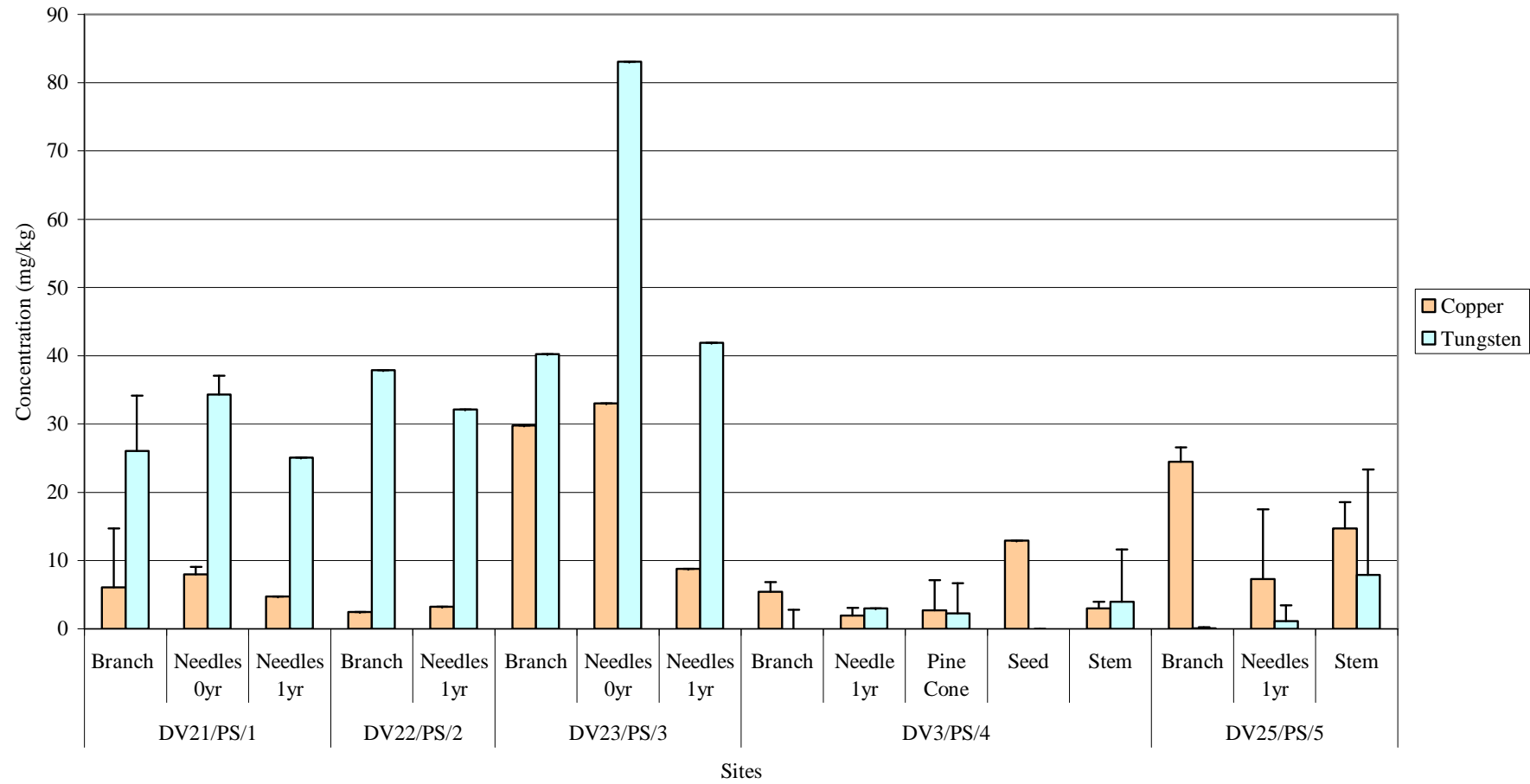


Figure 8-15: The concentration of copper in the roots of the *P. sitchensis* at different sites
(see Tables 7-1 and 7-2 for key to sample names)

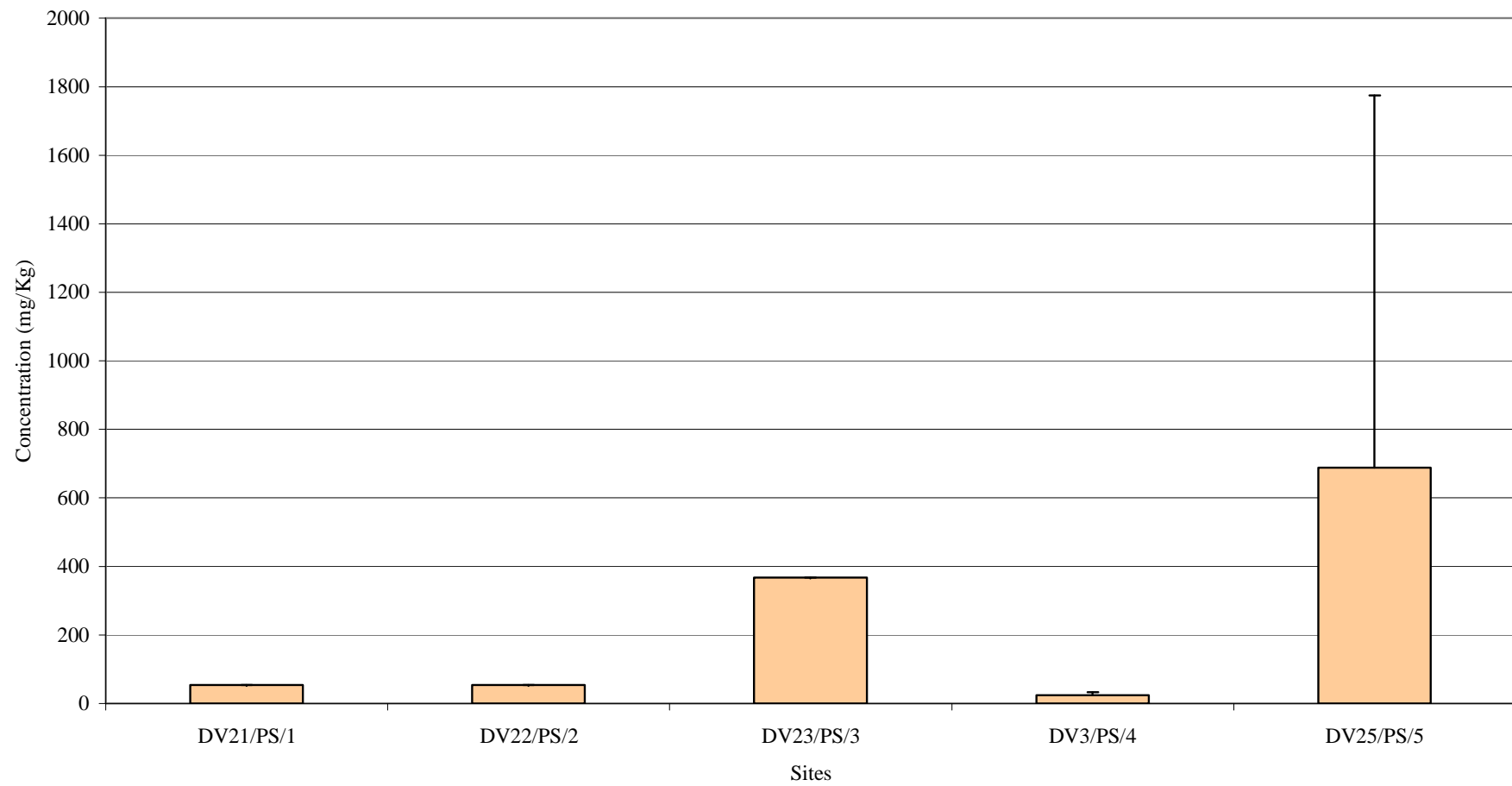
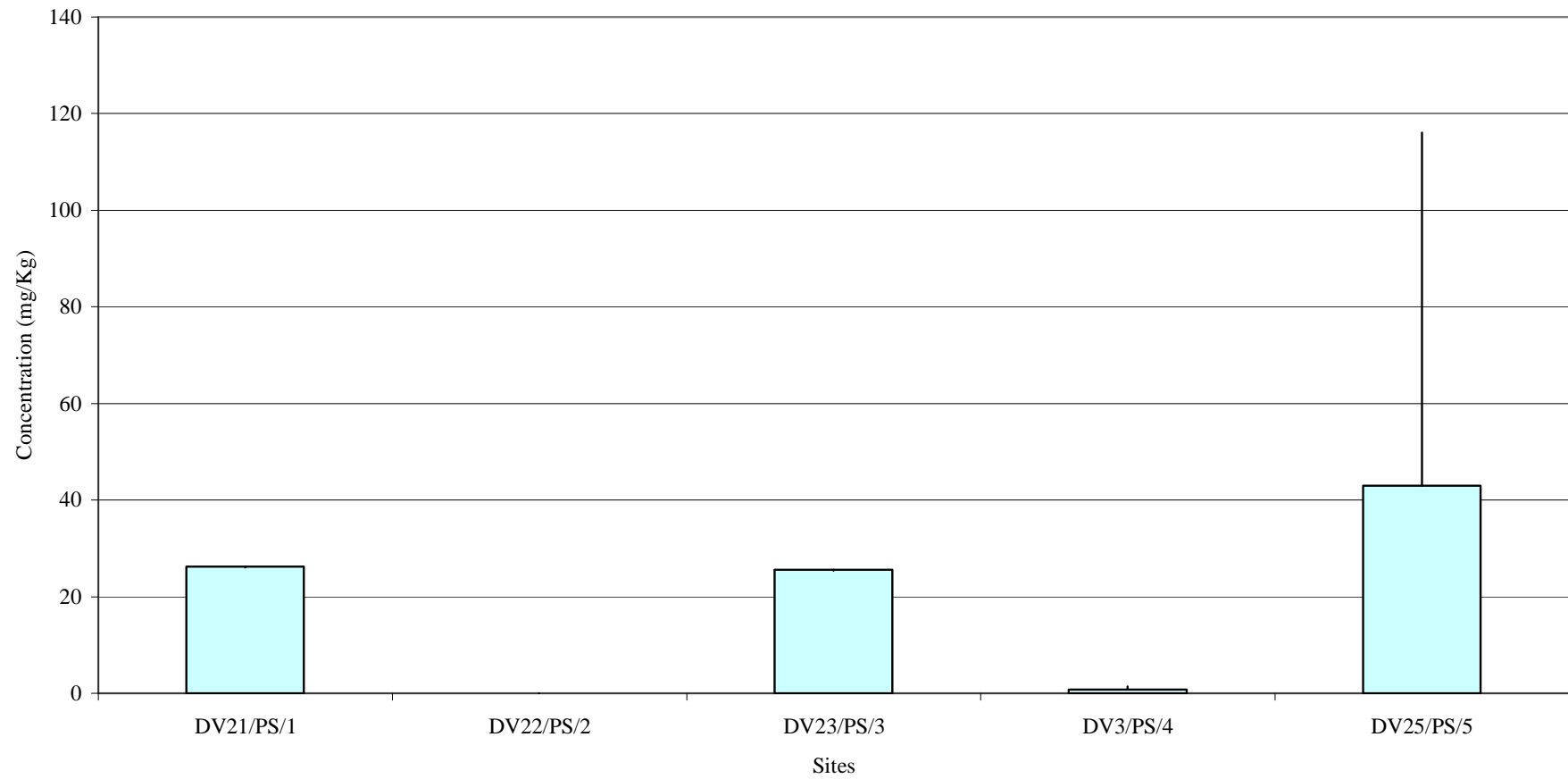


Figure 8-16: The concentration of tungsten in *P. sitchensis* roots
(see Tables 7-1 and 7-2 for key to sample names)



8.3.2.1. *Statistical analysis*

The null hypothesis was: there is no significant difference between the samples. The critical p-value is 0.05, and results greater than this indicates that there is a chance that the hypothesis is correct and is therefore accepted (there is no significant difference between the sample means). The summary of the statistical results are displayed in Table 7-12.

Table 8-3: A summary of the statistical analysis for the differences between samples

Material	Analyte	F	F _{crit}	P-Value	H ₀
Soil	Copper	43.083 _{4,48}	2.584	<0.01	Reject
	Tungsten	101.485 _{4,48}	2.584	<0.01	Reject
Branch	Copper	36.919 _{4,12}	3.837	<0.01	Reject
	Tungsten	537.518 _{4,12}	3.837	<0.01	Reject
Needle 1yr	Copper	0.753 _{4,8}	6.388	0.604	Accept
	Tungsten	102.274 _{4,8}	6.388	<0.01	Reject
Root	Copper	2.14 _{4,11}	4.120	0.17	Accept
	Tungsten	2.09 _{4,11}	4.120	0.186	Accept

The results show that there is no significant difference between concentrations of copper and tungsten in the roots at the different sites. There is also no significant difference between the concentrations of copper in first year needles between the sites.

Where the null hypothesis was rejected a Tukey Test was performed to determine where the differences were. Each of the individual means are compared and a score is given (Significant difference = 1, No significant difference = 0). Table 7-13 shows the summary of the results indicating where the differences were found.

Table 8-4: The Tukey Test results for the differences between samples

Table 8-4a: The concentration of copper in soil samples

	DVS22/PS/2	DVS23/PS/3	DVS3/PS/4	DVS25/PS/5
DVS21/PS/1	1	0	1	1
DVS22/PS/2		0	0	0
DVS23/PS/3			0	0
DVS3/PS/4				0

Table 8-4b: The concentration of copper in branch samples

	DVS22/PS/2	DVS23/PS/3	DVS3/PS/4	DVS25/PS/5
DVS21/PS/1	1	0	1	1
DVS22/PS/2		0	0	0
DVS23/PS/3			0	0
DVS3/PS/4				0

Table 8-4c: The concentration of tungsten in soil samples

	DVS22/PS/2	DVS23/PS/3	DVS3/PS/4	DVS25/PS/5
DVS21/PS/1	0	0	0	1
DVS22/PS/2		0	0	1
DVS23/PS/3			0	1
DVS3/PS/4				1

Table 8-4d: The concentration of tungsten in 1 yr needles samples

	DVS22/PS/2	DVS23/PS/3	DVS3/PS/4	DVS25/PS/5
DVS21/PS/1	0	1	1	1
DVS22/PS/2		0	1	1
DVS23/PS/3			1	1
DVS3/PS/4				0

Table 8-4e The concentration of tungsten in branch samples

	DVS22/PS/2	DVS23/PS/3	DVS3/PS/4	DVS25/PS/5
DVS21/PS/1	1	1	1	1
DVS22/PS/2		0	1	1
DVS23/PS/3			1	1
DVS3/PS/4				0

8.4. *Viburnum opulus*

There are approximately 150 species of the genus *Viburnum*, and is common throughout the northern hemisphere with a number of species reaching as far as South America and Asia. Most of the species of *Viburnum* are popular cultivation shrubs and are common place in gardens and landscapes; they can grow up to a height of 4m and can be grown in a variety of soils with no particular preference. *Viburnum opulus* is more commonly known as Guelder Rose and flowers in May to June producing fruit, varying in colour from red to purple, containing a single seed. The fruit is eaten by birds and other wildlife and is also edible to humans when cooked (Phillips and Rix, 2002). Photographs of *Viburnum* leaves, flowers and fruit are shown in Figures 8-17 and 8-18.

It is believed that the long shoots of the *Viburnum* were used for arrow shafts, and an example of these arrow shafts were found with Otzi the Iceman (Dickson, 1995).

Figure 8-17: A photograph of *Viburnum opulus* leaves and flowers (Wikipedia, 2006)



Figure 8-18: A Photograph of *Viburnum opulus* leaves and fruit (Wikipedia, 2006)



8.4.1. Experimental procedure

A number of *V. opulus* shrubs had been planted on the edge of the rubble spoil tip at Snailbeach. Figure 8-19 shows a photograph of the row of *V. opulus*, and it can be seen that there is an incline. Parts of the *V. opulus* were sampled with site 1 located at the lower portion of the tip and site 4 at the top. Figure 8-20 shows a map of the Snailbeach with the sampling sites marked on in green. Using a stainless steel pair of secateurs, branches were cut so that samples of leaves and berries could be taken; the samples were placed in bags to prevent any contamination between sites. As well as plant samples, soil samples were also taken and upon return to the laboratory the plant material and soil were prepared as discussed in Chapter 5.

Figure 8-19: A photograph of the sampling area at Snailbeach, Shropshire, UK



Figure 8-20: A map of Snailbeach with designated sampling sites © Crown Copyright, an Ordnance Survey supplied service (Based on OS map sheet SJ 30 SE, 1:10,000)

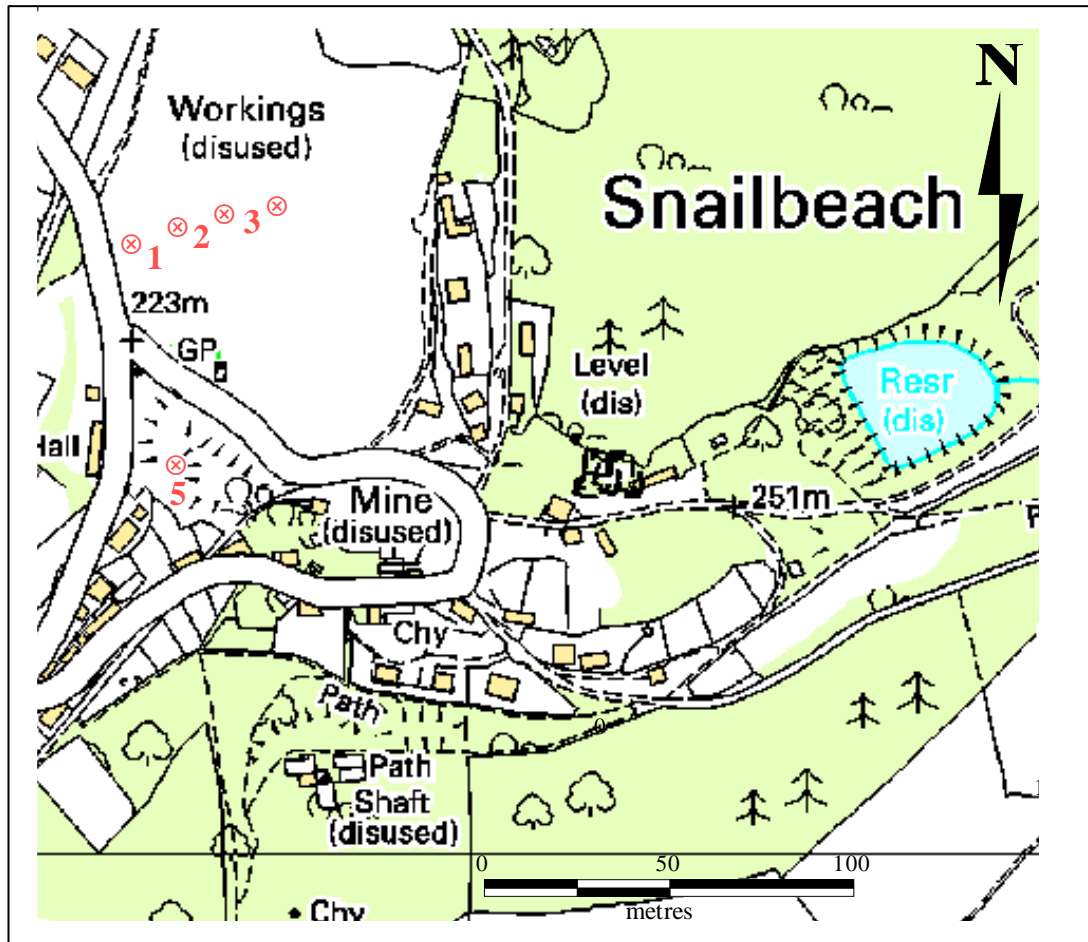


Table 8-5 A key to the sample codes

Code	Definition
SBS	Snailbeach Soil
SB	Snailbeach
VO	Viburnum
FU	Fungi

Sample names follow the convention:

LOCATION - SITE NUMBER / TYPE OF MATEIAL / SAMPLE NUMBER

E.g. SB3/VO/3: Snailbeach – site 3 / Viburnum sample / Number 3

Table 8-6: Locations of sites and the corresponding samples taken

Site	Sample	Description
1	SBS1/VO/1	Viburnum Soil
	SB1/VO/1	Viburnum Plant
2	SBS2/VO/2	Viburnum Soil
	SB2/VO/2	Viburnum Plant
3	SBS3/VO/3	Viburnum Soil
	SB3/VO/3	Viburnum Plant
4	SBS4/VO/4	Viburnum Soil
	SB4/VO/4	Viburnum Plant
	SB4/FU/1	Fungi Sample 1
5	SB5/FU/2	Fungi Sample 2

8.4.2. *Results*

As the samples were taken, there appeared to be no adverse affects of growing on the spoil tip. Upon investigation around the area a number of piles of discarded seeds were found where the fruit had been removed and presumably eaten. Figure 8-21 illustrates the concentration of lead and zinc in the soil at Snailbeach; it shows that the highest concentrations of lead and zinc were found at the top of the spoil tip. There appears to be an increase in the concentration of both analytes going up the slope.

During the analysis one anomalous result was found with the skin of the berries found at SB3/VO/3. Although ten replicates were analysed, only one replicate was found to contain high concentrations of zinc (1591.45mg/kg). When considering all the sites investigated there were only two skin samples that contained zinc, the other sample contained 3.3mg/kg and comparing these values would lead to the possibility that there had been some contamination. Because of this the value has been removed from Figure 8-22.

It can be seen that zinc was found in all the leaf samples, with the highest concentration being found at SB4/VO/4 (37.334mg/kg). Overall, SB3/VO/3 was found to contain the least amount of zinc in all parts of the plant, with only leaves and seed containing small concentrations (1.8mg/kg and 0.249mg/kg respectively)

Figure 8-23 shows the results of the concentrations of lead found in the *V. opulus* parts. It can be seen that there appears to be little correlation between the parts of plants concentration and the site location. It should also be noted that there was a high level of variability between the replicates during the chemical analysis. This can be seen by the large confidence intervals.

When analysed it was found that the seeds, stalks and flesh did not contain lead, but zinc was found to be accumulated in leaves, seed, skin, stalk and stem. There was no zinc found in the flesh of the berries. When examining the soil lead results it can be seen that lead was only present in the soil at sites SBS2/VO/2, SBS3/VO/3 and SBS4/VO4, however lead was not found in all parts of the plant. Only the leaves, skin and stem were found to contain small concentrations (less than 1mg/kg).

Whilst sampling, the distance between the sampling sites was measured, and the distance between the site SB1 and site SB4 was approximately 54.5m.

Figure 8-21: The concentration of lead and zinc in soil samples where *V. opulus* were sampled (see Tables 8-5 and 8-6 for key to sample names)

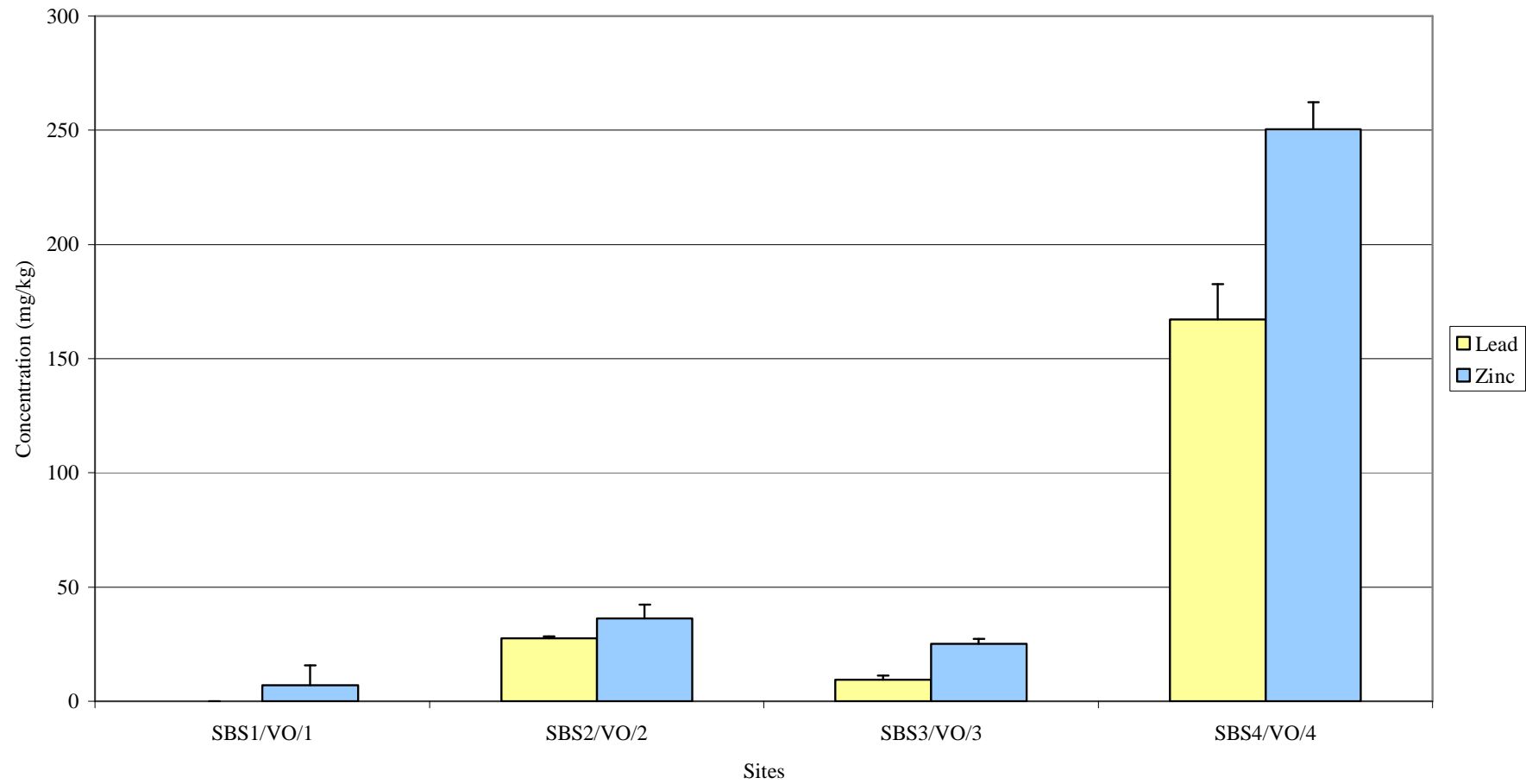


Figure 8-22: The concentration of zinc in different parts of *V. opulus*
 (see Tables 8-5 and 8-6 for key to sample names)

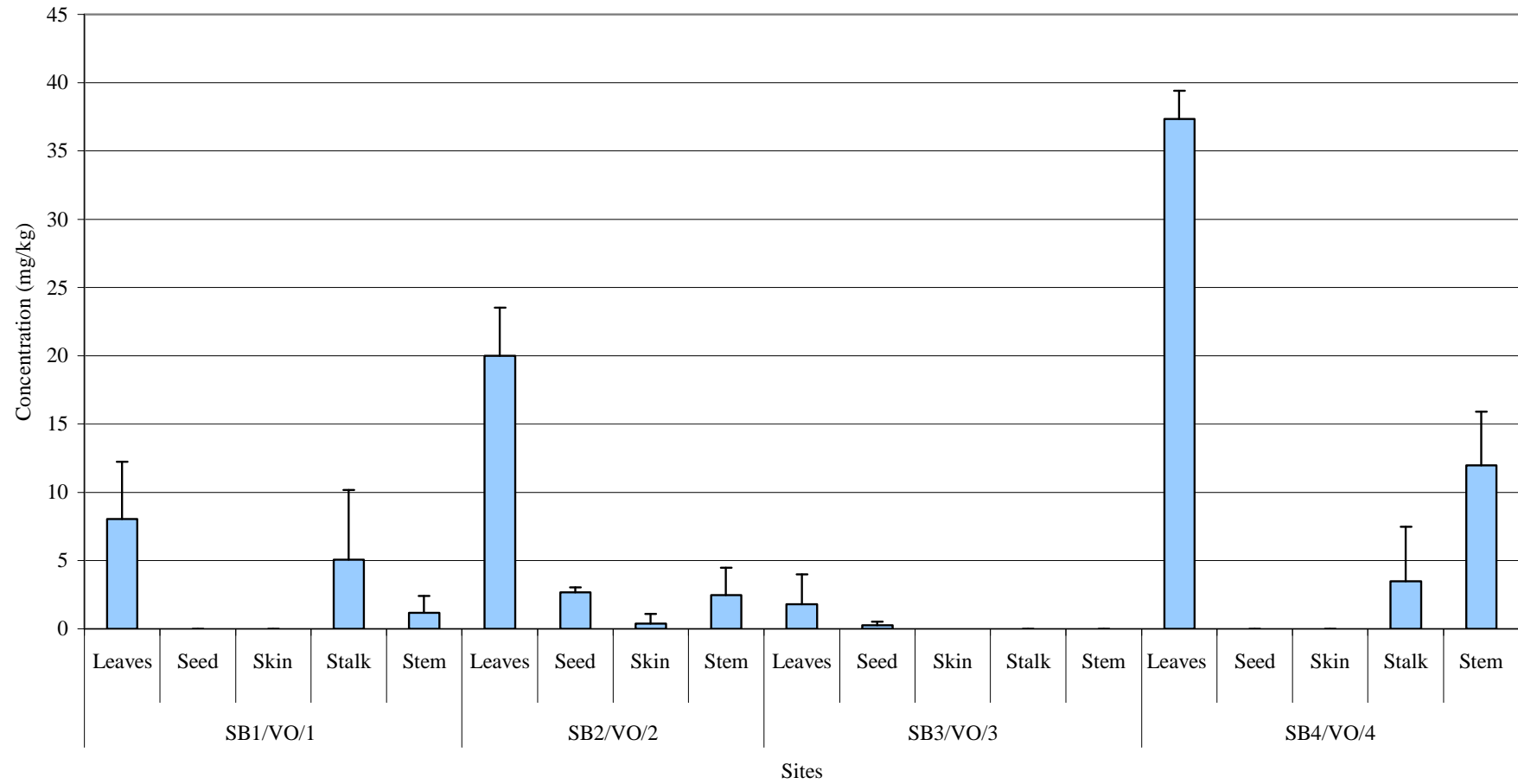
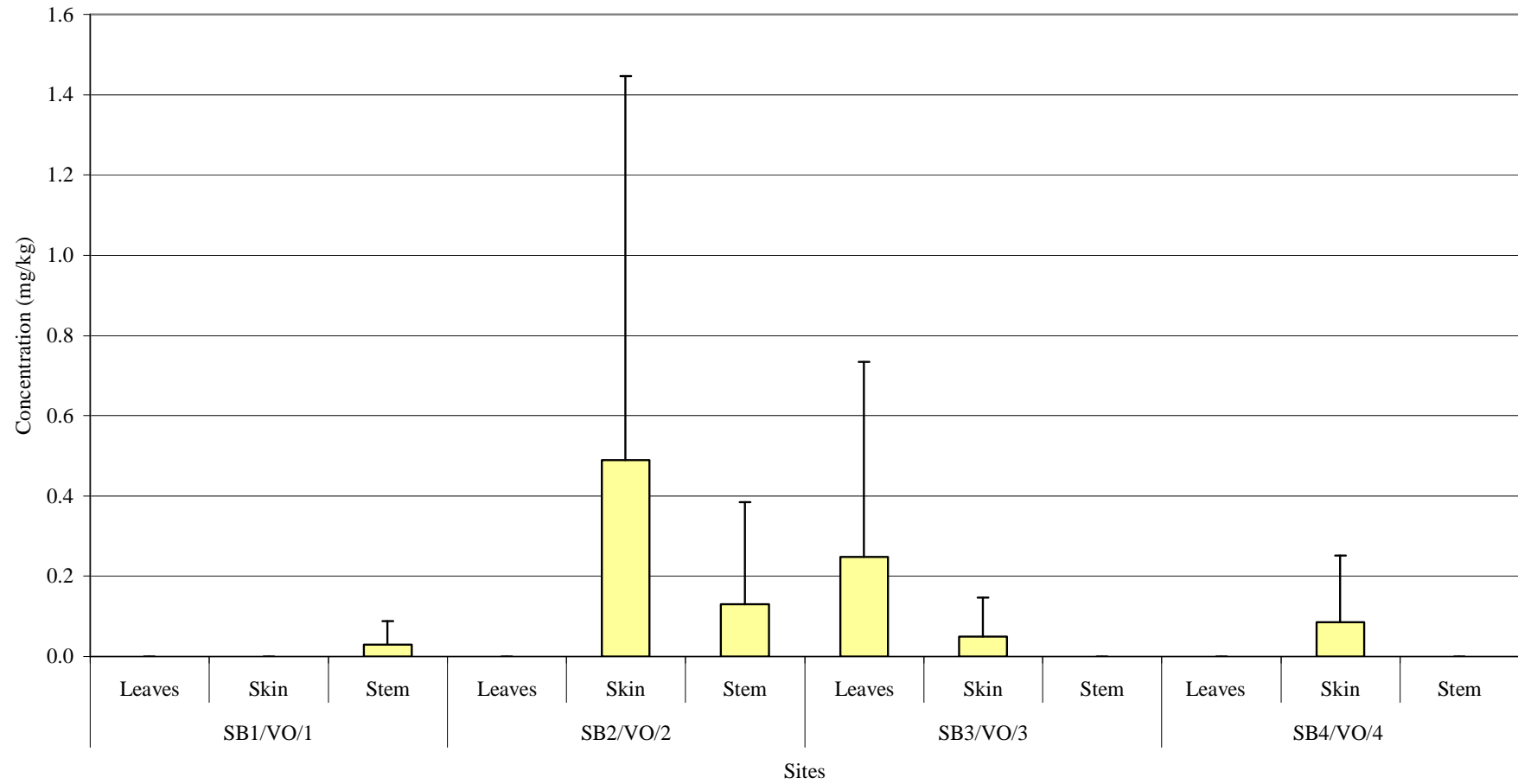


Figure 8-23: The concentration of lead in different parts of *V. opulus*
 (see Tables 8-5 and 8-6 for key to sample names)



8.4.2.1. Statistical analysis

The null hypothesis was: there is no significant difference between the samples. Table 8-6 gives a summary of the statistical analysis.

Table 8-6: A summary of the statistical analysis results

Material	Analyte	F	F _{crit}	P-Value	H ₀
Soil	Zinc	789.275 _{3,37}	2.883	<0.01	Reject
	Lead	492.539 _{3,37}	2.883	<0.01	Reject
Leaves	Zinc	95.710 _{3,19}	3.239	<0.01	Reject
	Lead	1 _{3,19}	3.239	0.418	Accept
Seed	Zinc	115.983 _{3,11}	4.066	<0.01	Reject
	Lead	N/A			
Skin	Zinc	0.999 _{3,35}	2.901	0.406	Accept
	Lead	0.794 _{3,35}	2.901	0.506	Accept
Stalk	Zinc	1.202 _{3,7}	6.591	0.416	Accept
	Lead	N/A			
Stem	Zinc	22.030 _{3,39}	22.03	<0.01	Reject
	Lead	0.854 _{3,39}	2.866	0.473	Accept

The null hypothesis used was that there was no significant difference between the samples. The results shown in Table 8-6 indicate that for zinc, there was a difference between the sites for samples of soil, leaves, seeds, and the stem. However for lead there was only a significant difference between the sites for the soil, indicating that there is no significant difference between the sites and the concentration of lead in the *V. opulus* plant.

Where the null hypothesis was rejected a further Tukey Test was completed to determine where the differences were and these are summarised in Table 8-7.

Table 8-7: The Tukey Test results for the differences between samples

Table 8-7a: The concentration of zinc in soil samples

	SBS2/VO/2	SBS3/VO/3	SBS4/VO/4
SBS1/VO/1	1	0	1
SBS2/VO/2		0	1
SBS3/VO/3			1

Table 8-7b: The concentration of zinc in leaf samples

	SB2/VO/2	SB3/VO/3	SB4/VO/4
SB1/VO/1	1	0	1
SB2/VO/2		1	1
SB3/VO/3			1

Table 8-7c: The concentration of zinc in stem samples

	SB2/VO/2	SB3/VO/3	SB4/VO/4
SB1/VO/1	0	0	1
SB2/VO/2		0	1
SB3/VO/3			1

Table 8-7d: The concentration of zinc in seed samples

	SB2/VO/2	SB3/VO/3	SB4/VO/4
SB1/VO/1	1	0	0
SB2/VO/2		1	1
SB3/VO/3			0

Table 8-7e: The concentration of lead in soil samples

	SBS2/VO/2	SBS3/VO/3	SBS4/VO/4
SBS1/VO/1	1	0	1
SBS2/VO/2		1	1
SBS3/VO/3			1

When the null hypothesis is rejected the Tukey Test distinguishes which samples were significantly different. From the results in Table 8-7, it can be seen that the samples at site 4 were significantly different from the other sites. This applies to all but the seed results, these show that when comparing with samples from site 1 and site 3 there was not a significant difference.

8.5. *Discussion*

For survival, all plants require nutrients and these are classified as macro or micronutrients depending on their relative concentrations in the plant tissue. Examples of macro and micronutrients are displayed in Table 8-8 along with the concentrations that may be required by plants. These are generalisations only, and variations may occur depending on the species

Table 8-8: Concentrations of elements required by plants (modified from Taiz and Zeiger, 1998)

Element	Chemical Symbol	Conc. in dry matter ($\mu\text{mol/g}$)
Macronutrients:		
Nitrogen	N	1000
Potassium	K	250
Magnesium	Mg	80
Phosphorus	P	60
Micronutrients:		
Iron	Fe	2.0
Zinc	Zn	0.30
Copper	Cu	0.10
Molybdenum	Mo	0.001

The examples in the table are only a selection of the elements required by plants; therefore the mineral uptake system needs to be able to remove the necessary minerals from the environment efficiently and transport them to areas of the plant where they are needed most. There are two mechanisms in which plants obtain nutrients: through the roots and through foliar uptake. The uptake through the roots occurs by removing the nutrients from the surrounding medium via absorption, and transporting them through the xylem to different parts of the plant. Foliar uptake occurs when the nutrients are taken up through the leaf surface. This is considered to be more efficient than the root absorption as the nutrients do not have to be transported a great distance.

Root absorption use two methods:

- Bulk flow - nutrients are carried through the soil to the root by water
- Diffusion - nutrients are diffused through the root membrane from a region of high concentration to a region of low concentration.

A lack of water can restrict the flow of nutrients into the roots, where as during optimum water conditions, the absorption of nutrients can occur at an unrestricted rate.

Since it is clear that most elements can be absorbed into the root system, it is highly likely that a number of metals can be accumulated into plants. Because of the existence of metallophytes ecotypes and pioneer species, it is evident that some plants are capable of growing successfully on toxic soils. Peterson (1978) divides tolerant species into three basic types:

- Exclusion plants are those which use a restrictive transport system, they prevent the metal from being transported else where in the plant.
- Index plants are species that reflect the soil concentrations and have previously been named as indicator species as they were used to identify areas where ores were found.
- Accumulator plants are those that have developed a tolerance for toxic elements and which are capable of absorbing the toxic metal into parts of the plant effectively removing the element from the environment.

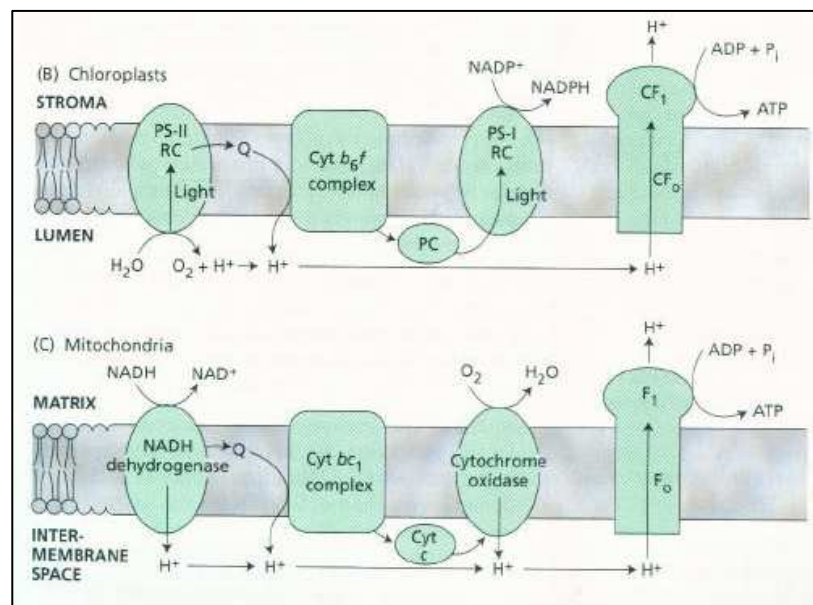
Different plants could be defined using all three terms depending on the element involved. Peterson (1978) discusses findings that the accumulation of lead in plants was restricted to certain parts of the plant. An example of this can be seen in *Triodia pungens* where the highest concentration of lead was located in the leaves (Nicolls et al, 1965) and could therefore be described as accumulator species, however if the plant is known to grow in a lead environment, it could also be termed as an index species. Because of these definitions it is not ideal to determine which plant belongs to which category.

Although certain heavy metals are considered to be toxic to plants, a small number are required in relatively low concentrations, these include copper and zinc. Since

copper and zinc are required in small concentrations it is concluded that these could be absorbed into the roots with no hindrance.

Copper is an important component required in plants for the electron transfer chain during photosynthesis (Robb and Pierpoint, 1983). The copper ions can be bound to organic molecules in the mitochondria and chloroplasts. Figure 8-24 is a simplified diagram of the photosynthesis process in the chloroplasts and the mitochondria. In the chloroplasts the copper ions are bound to plastocyanin (PC), and connects photosystem II (PS-I) and photosystem I (PS-II) by transferring electrons through the intermediate cytochrome b6f complex. This process results in the splitting of water to produce H^+ ions that are used to reduce $NADP^+$ to NADPH, the final result is the production of ATP which is used in cellular processes. In the mitochondrial membrane water is reformed through the addition of O_2 and H^+ , the process involves the activity of cytochrome oxidase which has a major component of copper, again the copper ions assist in the transference of electrons resulting in the production of ATP.

Figure 8-24: Simplified diagram of the photosynthesis processes in the chloroplast and mitochondria of plants (Modified from Taiz and Zeiger, 1998)



C. microphyllus has shown that there are indications of accumulation of copper and tungsten. It has been documented that copper is a known micronutrient and could

theoretically be freely taken up in to the plant. However, plants generally only absorb nutrients when they are needed but Baker (1983) describes how in some cases the excess nutrients can be absorbed and stored in the vacuoles of cells.

The samples of *C. microphyllus* show that copper and tungsten were present in high concentrations compared to the control samples. The control samples contained a small concentration of copper in all parts of the plant that were sampled. Unfortunately it was not possible to sample the soil from which the plant was growing, but it is assumed that the plant was growing successfully and in a well watered area. The growing media at Blanchdown Wood, when present, was sandy in composition and therefore would have difficulty maintaining the ideal conditions for nutrient uptake for all plant life. Optimum water conditions would occur after a period of rain, but because of the soil type, the water would easily be drained away or evaporated, restricting the nutrient uptake. Therefore the uptake of nutrients would occur at a rapid rate when water was present. The root system absorbs the required nutrients relatively rapidly to maintain the successful growth of the plant, and then the excess nutrients are stored in the vacuoles of the cells for later use.

Because the concentration of copper in the soil was higher than that of the plant it is likely that the diffusion mechanism could increase the copper concentration within the plant. The control sample would have a constant supply of water and therefore nutrients would be readily available and therefore only the required concentration of copper would be absorbed into the plant, with a small amount being stored. Taiz and Zeiger (1998) discuss the effects of mineral deficiencies on plants and stated that during periods of deficiency, certain minerals are found to be mobile while others are immobile. Copper is considered to be immobile, therefore when there is a decrease in the copper concentration in the plant; it cannot be removed from one part of the plant to another. Because of this there would be a relatively constant concentration in all areas of the plant, depending on their requirements. The control plant contained similar concentrations of copper throughout the plant, but plants sampled from Blanchdown Wood contained variable concentrations. Another observation is that *C. microphyllus* samples from Blanchdown Wood located at sites 5 (DV5/CO/5), 6 (DV6/CO/6) and 8 (DV8/CO/7) were not statistically different from the control samples. Examining the locations of these sites on Figure 7-1 it can be seen that these were sampled in areas sheltered with a number of other plants and trees. These could provide the growing media with added organic material on top of any spoil present

and therefore the heavy metals may not be available in the same concentrations as those found at the remaining sites, also the organic matter may be able to retain the water better and thus providing an ideal situation for nutrient uptake preventing any nutrient deficiencies. The remaining samples were found in areas where the plants were exposed directly to the spoil both from the growing media and the atmosphere, as particles can be transported from the spoil tip and deposited on the plants. This deposition can also increase the concentration of the metals in the plants as after deposition on the leaves the metals can then be taken in either through absorption through the cuticle or particles can enter through the stomata. Absorption through the cuticle can be increased when the dust containing the metals is mixed with rain water; the metals become dissolved in the water and are then absorbed.

Unfortunately it was not possible to sample a control *P. sitchensis* of the same age as those sampled at Blanchdown Wood. From the results it can be seen that samples DV21/PS/1, DV22/PS/2, DV3/PS/4 and DV25/PS/5 have a lower concentration of copper in the needles and stem compared to DV23/PS/3. By examining the locations of these sites it can be seen that DV23/PS/3 is positioned on the eastern side of the small mound, with a prevailing south-westerly wind, particles from the small mound could be blown directly onto the plant. Therefore the accumulation of copper through the leaves would be greater therefore increasing the concentration of copper within the plant. *P. sitchensis* are known for their fast growth, therefore to maintain the growth high levels of nutrients are needed. In the early stages of the trees growth copper would be needed in higher concentrations than a more mature tree.

The results for both the *C. microphyllus* and the *P. sitchensis* showed that there was an increased concentration of tungsten in the plants. For the *C. microphyllus* plants, it was found that two parts of the plant did not contain any tungsten: roots and stem. This would indicate that the tungsten is not being taken up through the roots and is not transported through the stem. It is possible that the tungsten is not being absorbed by the roots but by the leaves. Comparing the results with those of the control, the control contained no tungsten, but because there were no soil samples it can only be suggested that the growing media for the control contained no tungsten. A notable result is that of DV6/CO/6; it can be seen from the soil concentrations that there was no tungsten present, but there was in the plant material. This further suggests that the predominant absorption of tungsten occurs through the leaves. Through examination of the *C. microphyllus* plants it was seen that the roots were

shallow and were easily extracted from the soil. But it is possible that the majority of the nutrients were absorbed through the leaves, as growth appeared to be normal with little visual affects of the harsh environment.

The *P. sitchensis* samples showed that there was a small concentration of tungsten in the roots from sites DV21/PS/1, DV23/PS/3, DV3/PS/4 and DV25/PS/5. This indicated that there was an uptake of tungsten through the roots. It would appear that there is a significant difference between the uptakes of tungsten in the *C. microphyllus* compared to *P. sitchensis*. This could possibly be attributed to the genetic make up of the plants, and would be worth exploring further.

As mentioned previously, nutrients are absorbed by the roots through diffusion where the elements are absorbed from a region of high concentration to a region of low concentration. DV25/PS/5 shows that there is the highest concentration of tungsten in the roots (42.937mg/kg), because this site also contained the highest concentration of tungsten in the soil (371.687mg/kg) it is probable that tungsten was absorbed but not distributed from the roots to other parts of the plant, therefore it is also probable that the roots are capable of storing tungsten.

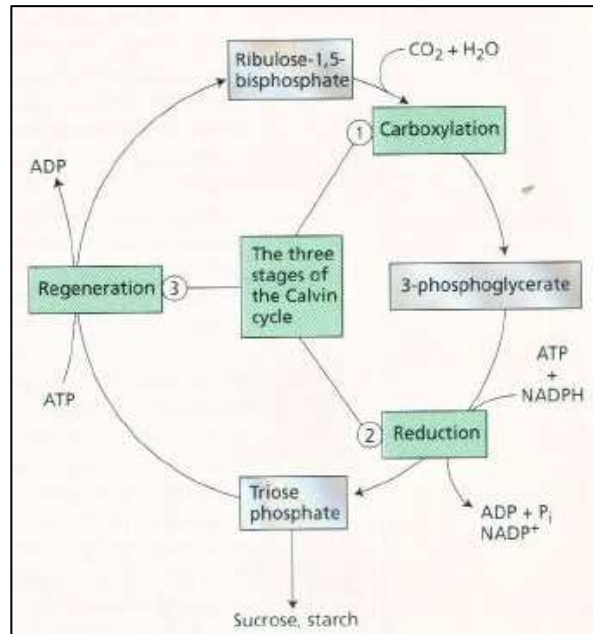
Although tungsten is not a required nutrient by plants, high concentrations were present. Koutsospyros *et al* (2006) stated that the accumulation of tungsten differs between genotypes and that some plants act as excluders and others as accumulators. Other authors (Gough *et al*, 1979) have indicated that there is evidence that tungsten encourages growth in some plants, Strigul *et al* (2005) have suggested that in other plants tungsten becomes toxic (Strigul *et al*, 2005). Koutsospyros *et al* (2006) also commented on the replacement of molybdenum in nitrogen reductase by tungsten, producing a toxicity affect, because of the already established differences in the reactions to tungsten, depending on the genotype it is probable that in some species the replacement of tungsten for molybdenum could not have an adverse affect on the plant. In conclusion it is likely that *P. sitchensis* and *C. microphyllus* are capable of accumulating tungsten with no adverse affects to their growth, but are accumulated by different mechanisms.

In total five elements were tested for in all soil and plant samples. It was found that although at most of the sites there were high concentrations of arsenic, tin and lead in the soils, these were not represented in the plant material. Therefore the *C. microphyllus* and the *P. sitchensis* could be termed as excluders of these elements. When examining the root data for the *C. microphyllus* it can be seen that there were

high concentrations of arsenic and copper in the roots of plants taken from DV4/CO/4. This site contained the highest soil concentrations of copper and arsenic, also it was noted that the plant seemed to be of a different growth type to the other samples. The plant was still identified as *C. microphyllus* by the flowers and leaf shape; the stem and roots were flattened and grew along the ground whereas the other samples had stems that were cylindrical and grew upright, however it may be likely that it was a different subspecies. When looking at the results it can be seen that the stem of this sample had the highest concentration of copper up to five times the concentration as the other samples. To confirm differences between the samples, their DNA could be extracted and examined.

As a micronutrient, zinc is an important component of many enzymes in plants including several dehydrogenases, ribulose biphosphate carboxylase and carbonic anhydrase. A deficiency in zinc within the plant can lead to a dramatic decrease in photosynthetic activity, partially due to its role in the transportation of carbon dioxide during photosynthesis. During PS-I and PS-II ATP and NADPH are produced and are then utilised in the formation of carbohydrates. This is known as the Calvin Cycle and not only uses the products from both photosystems but also fixes carbon dioxide. Figure 8-25 illustrates a simplified diagram of the Calvin Cycle and it can be seen that there are three major stages. The cycle is continuous providing there are adequate concentrations of nutrients, light, water and carbon dioxide. The first step in the fixation of carbon dioxide involves the reaction between ribulose-1,5-bisphosphate, catalysed by the enzyme ribulose biphosphate carboxylase/oxygenase (Rubisco). Zinc has been found to be a major component of rubisco and therefore the deficiency of zinc would lead to a reduction in the Calvin Cycle and would have an adverse affect on other systems requiring the products of the cycle. Because of the importance of the mineral and although it is only required in small concentrations, it is possible that plants absorb increased concentrations when it is available, and store the excess in the vacuoles of the cells.

Figure 8-25: A simplified diagram of the Calvin cycle (modified from Taiz and Zeiger, 1998)



Currently, Snailbeach is in the process of remediation by Shropshire County Council. Unfortunately due to confidentiality reasons, information concerning remediation methods used at the site is unavailable. But upon visiting the site it can be seen that the area where the *V. opulus* were sampled, a layer of top soil had been added and then a layer of grass. It is not known when these were put in place but it is estimated to have occurred over the past five years. Since it is assumed that the *V. opulus* were planted at the same time. It is also estimated that the plantation of the *V. opulus* were part of the remediation, but again details are not known. It is assumed that the topsoil that was used was uncontaminated and was probably enriched with fertilisers to encourage growth of the grass and *V. opulus*. If the soil was uncontaminated, there are clear signs that the soil has become contaminated with both lead and zinc, with an increase in their concentrations nearer the summit of the spoil tip.

Three possible contamination methods could be responsible for this:

- Wind erosion - Through wind erosion, small dust particles from the contaminated site can be transported to where the samples were taken.
- Water erosion - The ions can be picked up from the exposed region of the tip and carried down the slope. This would lead to an increase in the ions being passed down the tip. At present the highest concentrations are at the top of the tip, and reducing in concentration towards the base.
- Reverse capillarity - Could arise through the absorption of water from the lower levels of the soil. As the water on the surface of the soil is either taken up by plants or evaporated into the environment, the water from further below the surface could be drawn up through the soil. If this has occurred the water would also draw up the metals and thus transporting them closer to the surface of the soil.

The soil content of zinc is believed to range between 10-300ppm (Shuman, 1980) but since background concentrations lie in this region it could be determined that the zinc concentrations found at Snailbeach could be attributed to the background concentrations. However this is unlikely as there is a dramatic difference between the sites. If, as believed, the soil is applied it is more likely that the soil would have a much lower and universal concentration of zinc throughout the site, and therefore there would not be a significant difference between the sampled sites.

Since it has already been established that zinc is a micronutrient, it is clear that traces of zinc would be found in most, if not all, plants. Table 8-8 indicates the general concentration of zinc required by plants. It can be seen that the concentration is approximately 0.30 μ mol/g; concentrations found which exceed this value can be termed as significant. In the majority of the samples, the zinc values exceeded this value; plants generally store minerals in vacuoles, in order to maintain supply when there is a deficiency. A deficiency of zinc can lead to a reduction in the photosynthesis process, which could cause the plant to become stunted or even lead to plant death. Without the products of the photosynthesis, such as glucose and amino acids, the plant would not be able to increase in size or possibly to produce fruit for reproduction.

The results show that the majority of the zinc found in the *V. opulus* plant, was contained within the leaves. Since photosynthesis occurs primarily in the leaves, this would be the obvious site of nutrient storage. Apart from the leaves, there is no conclusive evidence to suggest that there is a form of zinc partitioning occurring in the *V. opulus*. However there is some evidence to suggest that the plants that were sampled at the top of the spoil tip contained a higher concentration of zinc. Although not found in every part of the plant it could be that the predominant site of excess zinc uptake is through the leaves from wind erosion. Sites SB1/VO/1, SB2/VO/2 and SB4/VO/4, all contained relatively high concentrations of zinc in the stem and seed stalks. This would indicate that zinc can be freely moved within the plant as it was present in the transportation parts of the plant.

The concentrations of lead found in the plant tissue were extremely low, and showed no indications of partitioning. It is possible that the plant could have absorbed the lead from the soil or from the atmosphere through the leaves. The soil samples show that at SB1/VO/1, the lead concentration was below the concentration detection limit. Therefore if there were concentrations of lead found within the plant, it is likely that they were accumulated from the atmosphere rather than the soil. Because of the size of the *V. opulus* it was not possible to sample the roots. This would have provided information as to whether they were the site of the uptake of either lead or zinc.

8.6. *Fungi*

Once classified with plants, fungi were determined to be significantly different from plants and now reside in a kingdom of their own. The fungi kingdom is subdivided into three major groups, determined by the differences in reproduction; these are Zygomycota (e.g. black bread mold: *Rhizopus stolonifer*), Ascomycota (e.g. Truffle: *Tuber melanosporum*) and Basidiomycota (e.g. Common puffball: *Lycoperdon gemmatum*) (Jennings, 1999).

All fungi are heterotrophic which depend on autotrophs, such as green plants, to acquire all their necessary nutrients (Taiz and Zeiger, 1998). The nutrients are absorbed after they have been broken down; the fungi secrete a hydrolysing enzyme to decompose the complex molecules into simpler compounds which can then be absorbed and used. Fungi derive their nutrients from different material and it is the differing material which can be used to differentiate between fungi within the major groups (Deacon, 1988):

- Saprobiotic – absorb nutrients from nonliving organic material, such as fallen trees, animal corpses and wastes of living animals.
- Parasitic – absorb nutrients from the cells of living host
- Mutualistic – absorb nutrients from another organism but benefits the partner organism

Zygomycota and ascomycota will not be discussed further.

Within the basidiomycota division there are approximately 30,000 fungi (Kirk *et al*, 2001) and are important decomposers of wood and other plant material, and therefore play an important role in the carbon cycle. The basidiomycota are extremely variable and can be unicellular or multicellular, sexual or asexual and can be terrestrial or aquatic.

In general, fungi that reproduce sexually are only visible during the sexual reproduction stage where the basidiocarps become visible. The familiar mushrooms (basidiocarp) provide support and protection to the large surface area of basidia (cells where the sexual spores are produced) on the gills. Each basidiocarp is capable of releasing a billion spores from the basidia (Elliott, 1994).

The basidiocarps are constructed with minute tubular threads, hyphae that combine to produce a feeding network called a mycelium (Campbell, 1996).

Figure 8-26: A generalised fungal mycelium (Campbell, 1996)

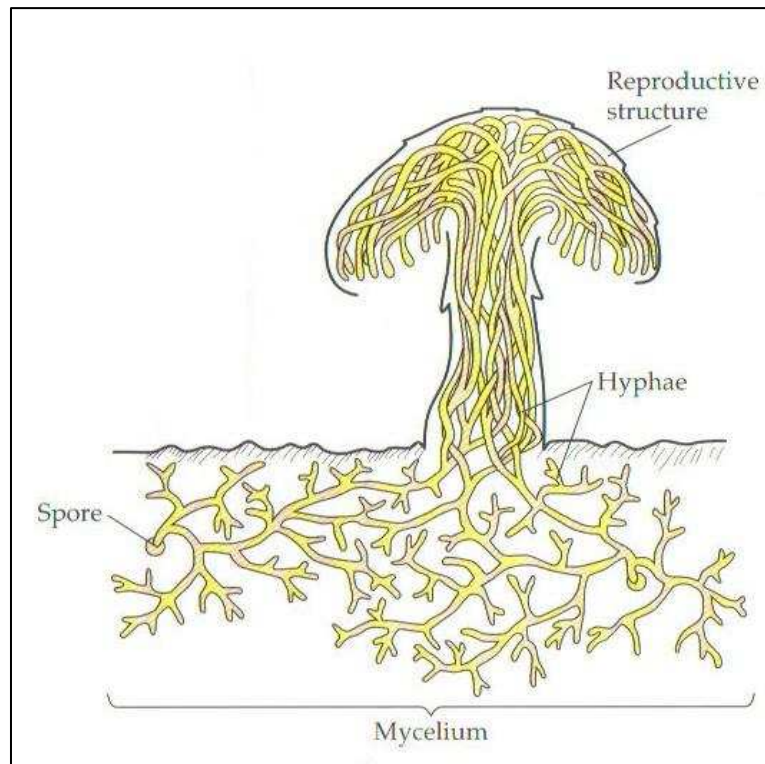


Figure 8-27: A generalised fungal hyphae, (a) cell structure of hyphae, (b) Haustoria penetrating living plant cells (Campbell, 1996)

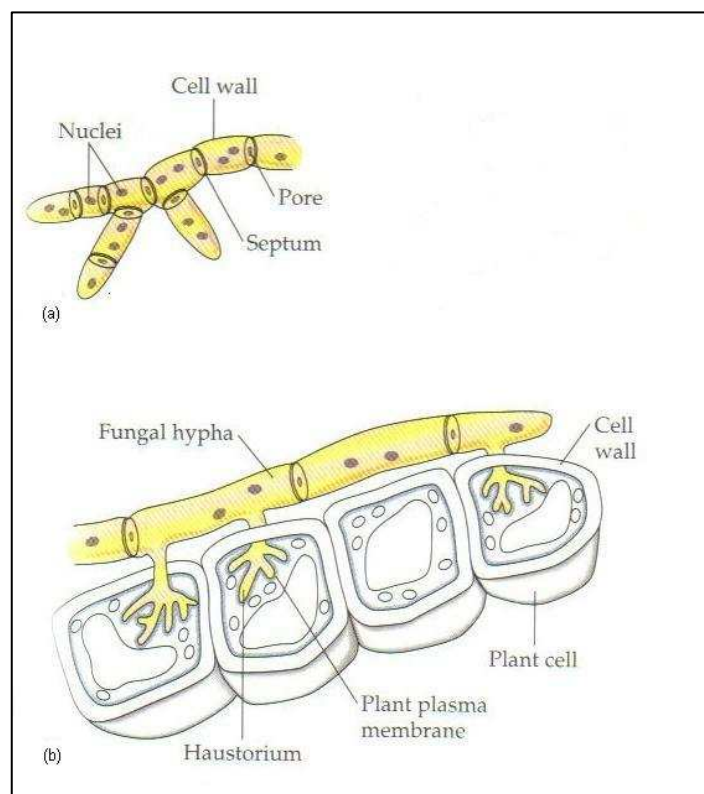
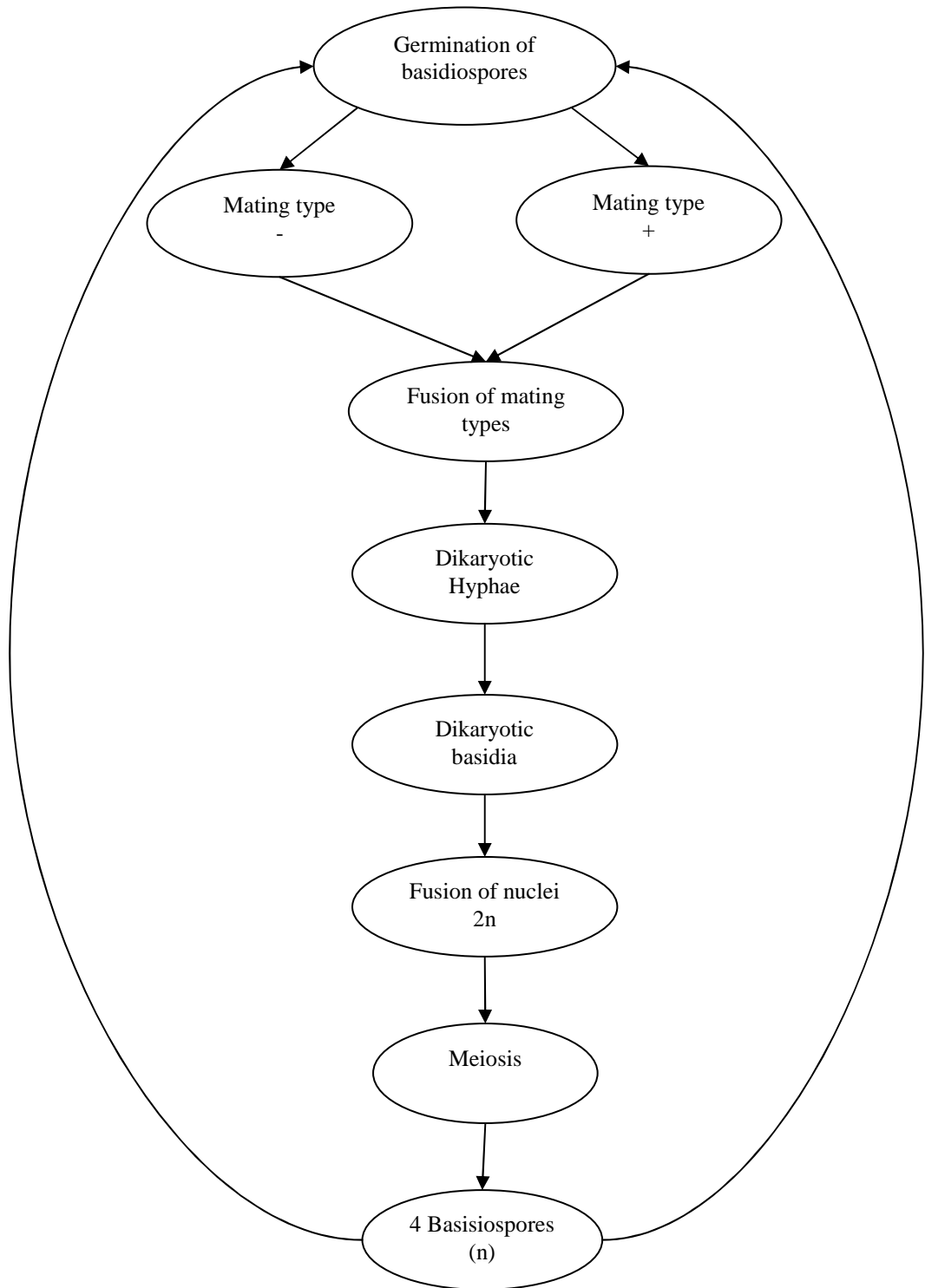


Figure 8-26 shows the basic structure of the fungi and illustrates the way the hyphae can spread throughout the substrata.

The general structure of the hyphae is given in Figure 8-27a, it shows that the hyphae are divided into cells by a septum, which generally have pores large enough to allow the movement of nuclei, ribosomes and mitochondria through into adjacent cells. Figure 8-27b illustrates how parasitic fungi have adapted their hyphae to have haustorium, which penetrates the tissue of the host cell to allow for the absorption of nutrients.

Reproduction in basidiomycota can occur either asexually or sexually. Asexual reproduction occurs through budding, where any cell in the organism becomes separated from the parent and develops into a new cell. Sexual reproduction occurs in the fruiting body (basidiocarp) and is illustrated in Figure 8-28. The basidiospores germinate and opposite mating types are fused together; the resultant fused hyphae are termed as dikaryotic as they contain two different nuclei, one from each of the parent. After the growth of the fruiting body, the nuclei can then fuse together to produce diploid cells (basidia). The diploid basidia cells are short lived as they quickly undergo meiosis, resulting in four haploid nuclei. These nuclei then migrate to the end of the basidium and form projections which are then separated by cell walls and develop into basidiospores, which are then released and the cycle begins again.

Figure 8-28: The sexual life cycle of basidiomycota (modified from Campbell, 1996)



Whilst visiting Snailbeach, Shropshire, UK, a number of fungi samples were found and sampled whole. These were of interest as they were capable of growing in a hostile environment. The fungi were tentatively identified as basidiomycotina, and were photographed in the field. Upon return to the laboratory the fungi had deteriorated to such an extent that identification could not be made. From the photograph, the first sample was identified as *Laccaria spp.* The second is still to be identified. The photographs of the samples are given in Figures 8-29 and 8-30. Although not identified it is clear that the two fungi sampled are not of the same species.

Figure 8-29: A photograph of the fungi found at SB4/FU/1



Figure 8-30: A photograph of the fungi found at SB5/FU/2



8.6.1. Experimental Procedure

The fungi samples were removed from the field carefully and where possible any soil or plant material found on the fungi were removed. The samples were placed in clean plastic bags and sealed until preparation for the analysis. Upon return to the laboratory, the samples were placed in a drying oven for 24 hours at 60°C to remove any excess water. The samples were then weighed and digested in the same way as the other plant material as discussed in Chapter 5. Because of the size of the samples it was not possible to separate the head from the stalk, or to repeat the analysis in replication.

8.6.2. Results

Table 8-9: The concentration of lead and zinc found in two species of fungi

Site	Weight (g)	Zinc (mg/kg)	Lead(mg/kg)
SB4/FU/1	0.0775	0	0
SB5/FU/2	0.2464	252.2321	370.1299

From the results in Table 8-9, it can be seen that there was no evidence of lead or zinc accumulation in the fungi found at SB4/FU/1. The sample taken at SB5/FU/2 showed a great increase in zinc and lead concentration when compared with the sample taken at SB4/FU/1. It gives evidence that lead and zinc are accumulated by these particular species of fungi.

8.6.3. *Discussion*

From the results given in Table 8-9, there is evidence to suggest that certain types of fungi can accumulate lead and zinc. However because of the size of the samples it was not possible to determine if the fungi showed partitioning. Ideally it would be preferred to have separated the stalk from the cap and therefore determine if the metals were restricted to the reproduction cells or the growing hyphae. Additionally replication could not be performed, because there was insufficient sample.

The lack of zinc and lead found in the fungal sample at SB4/FU/1 was interesting, as the sample was found on top of one of the main spoil tip sites. It would be believed that the concentration of lead and zinc would be accumulated into the fungal body. The lack of metal accumulation could indicate that this particular species does not absorb the metals with the nutrients. It is also possible that the ground near the surface has little or no concentrations of lead and zinc. It is possible that the lead and zinc have decreased in concentration through leaching or diffusion to the lower levels. Another note is that the size of the sample was very small (0.0775g) and could possibly be below the detection limit. To determine if this is the case, a number of samples should be taken with a minimum weight greater than this 0.0775g. In the original method the acid digestion solution was diluted to 50mLs, however, because of the size of the sample the solution was diluted to 25mLs. Decreasing this value could improve the results, but as the volume is decreased the level of accuracy deteriorates.

The second sample taken at SB5/FU/2 showed that there was a significant increase in the concentration of lead and zinc accumulated in the fruiting body of the fungi. Again, the size of the sample restricted the analysis and therefore partitioning could not be determined, or replication of the sample. However the large concentrations indicate that the fungi could accumulate lead and zinc, and showed little signs of adverse effects on the organism. This could therefore lead to its possible use as a remediation method. The fungi could be used to hyperaccumulate lead and zinc and therefore remove some lead and zinc from the surface of soils.

Chapter 9: Accumulation of heavy metals in algae

9.1. Introduction

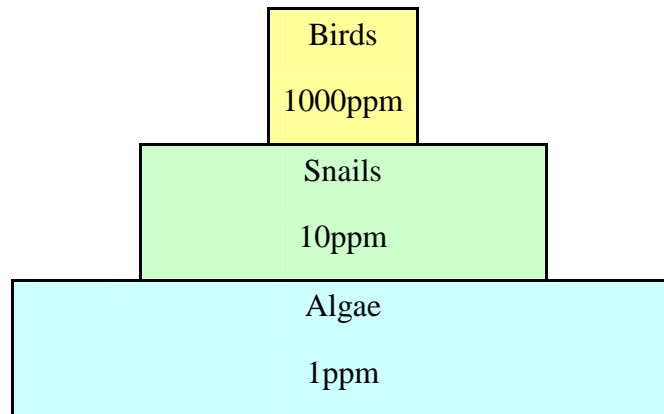
In the environment, waste water is not restricted to one pollutant, but a multitude. In addition variables such as the climate, nutrient availability as well as the number of pollutants present and their concentrations can affect the behaviour of organisms in the ecosystems towards the pollutant(s), therefore these factors can affect the successfulness of remediation (Hester and Harrison, 1997).

As part of monitoring plans, algae are used to indicate a change in the quality of freshwater (US EPA, 2006). The input of polluted water can induce different effects, depending on the contaminants (Whitton, 1984). In water bodies, the presence of certain algae can indicate that the aquatic environment has had an input of nutrient enriched water. An increased concentration of nutrients (e.g. sewage waste) increases the number of algae species and their population. The addition of poisonous waste (e.g. mining waste) can decrease the number of species and their populations. The more tolerant species of algae can become dominant, and it is this identification of the more tolerant species that can lead to the remediation of the water environment. The tolerant species can be used in one of two ways: as hyper-accumulators or as an indicator species. Algae that can tolerate the contaminated water can act as hyper-accumulators by accumulating the metals into their structure, and can therefore remove the contaminants from the water. As a tolerant species, the presence could indicate that a contaminant is present and can therefore be termed as an indicator species.

There has also been some speculation as to whether algae can accumulate metal ions from the aquatic environment (Whitton, 1984). This can lead to their possible use in remediation of water bodies. However the possibility of accumulation also means that the metal ions can become transferred through the trophic levels and therefore become available in food webs. In terms of food webs, algae are the primary producers. The algae provide food for consumers e.g. zooplankton and invertebrates. Due to the size of the algae, the quantity required by the consumer is vast. Therefore if algae have accumulated just 1ppm of copper, by consuming 1000 algae, 1000ppm of copper could have been consumed. Figure 9-1 illustrates the possible increase of copper, and other metal, ions through the trophic levels.

However, all organisms excrete waste. In some cases the majority of the pollutant can be excreted and therefore is not available to be passed on to the next trophic level.

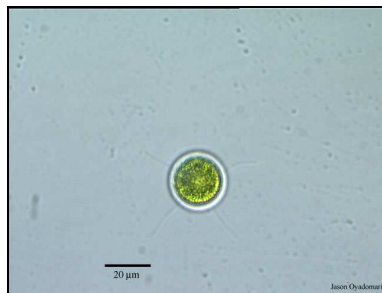
Figure 9-1: A food pyramid illustrating the increased concentration of metal ions through trophic levels



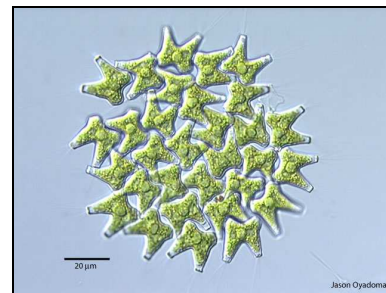
9.1.1. Algal structure and reproduction

The basic structure of the majority of algae consists as a single cell, unicellular, and in some cases these individual cells can combine together to form colonies. There appears to be some degree of organisation, but this can vary between groups. The colonial cells are capable of separation; at this point the separated cell can reproduce itself to form a new colony (Lund, 1996). The arrangement of the cells gives rise to different types of colonies, by forming threadlike chains, filaments can be formed. These filaments can exist as solitary threads or in groups, and can be branched or unbranched (Bold and Wynne, 1985). Figure 9-2 illustrates the different types of algal growth found throughout the plant kingdom (Oyadomari, 2001).

Figure 9-2: Examples of algal cells (a) unicellular, (b) colonial, (c) unbranched filamentous, d) and branched filamentous algae



a) *Carteria* spp



b) *Pediastrum* spp



c) *Oedogonium* spp



d) *Chaetophora* spp

Unicellular, colonial, and filamentous algae are capable of various types of motility, with motility being defined as swimming or creeping, pushing or by floatation devices. Some species that are non-motile reproduce cells that are motile (Lund, 1996).

Algal reproduction can occur both asexually and sexually and the complexity of the reproduction process is dependent on the species of the algae (Bold and Wynne, 1985).

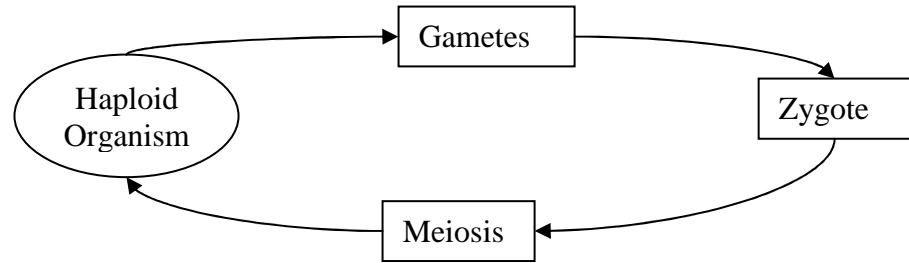
Asexual reproduction in unicellular algae occurs through simple cell division, giving rise to two or more motile (zoospore) or non-motile (autospores) cells. Filamentous algae and other multicellular algae can reproduce via fragmentation or by the liberation of zoospores from ordinary vegetative cells or from specialised structures (sporangium) (Bold and Wynne, 1985). When asexual reproduction occurs, the daughter cells are identical to the parent cell (Chapman and Chapman, 1981).

Sexual reproduction can be generalised into three basic types (Bold and Wynne, 1985):

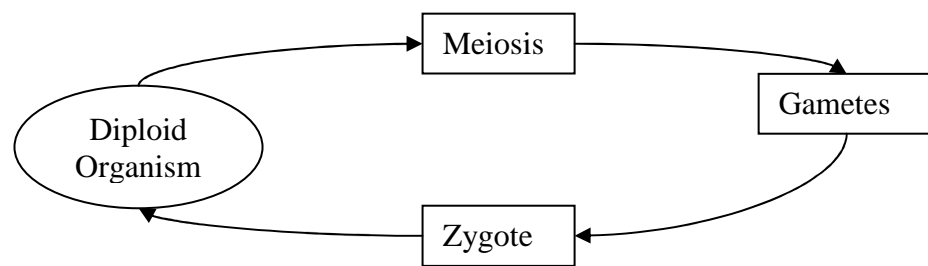
- Zygotic meiosis - two haploid gametes are united to form a diploid zygote. Upon germination, the zygote divides by meiosis to produce haploid cells resembling the parent (see Figure 9-3a).
- Gametic meiosis - gametes are released from the diploidic parents and fuse together forming a diploid zygote (see Figure 9-3b).
- Sporic meiosis - diplobiontic (having two free living organisms in the life history); a haploid gametophyte and a diploid sporophyte. The haploid gametes are united and fuse together to produce a diploid zygote. The zygote then matures into a sporophyte. As the sporophyte matures and form spores, meiosis occurs and the resultant cells develop into gametophytes (Figure 9-3c).

For unicellular algae, the algae themselves can act as the gametes.

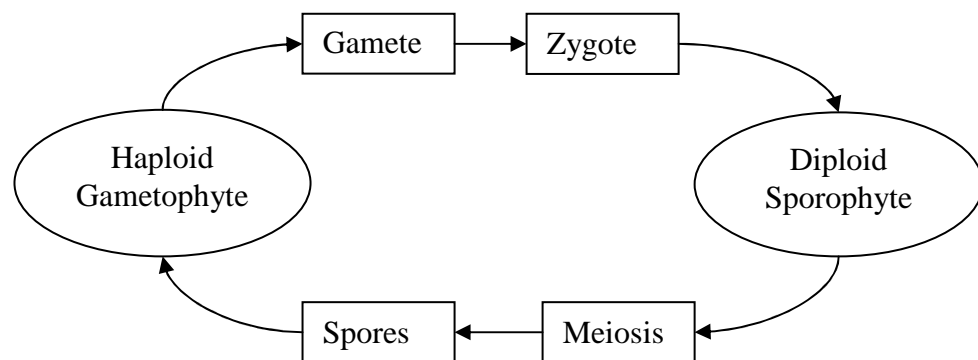
Figure 9-3: A generalised diagrammatic illustration of algal sexual reproduction
 (modified from Bold and Wynne, 1985)



(a) Zygotic meiosis



(b) Gametic meiosis



(c) Sporic meiosis

9.1.2. *Pandorina morum*

During this experiment the effects of copper, tungsten and lead have on the alga *Pandorina morum* will be monitored. Figure 9-4 is a photograph of *P. morum* used in the experiments. The photograph was taken through a microscope at a magnification of x400.

Figure 9-4: A microscopic photograph of *Pandorina morum* a) 21.2 μ m, b) 12.1 μ m



This species was chosen as little research has been conducted concerning the effects of metals on colonial algae. The species was also readily available commercially. *P. morum* has a relatively rapid reproduction cycle, and therefore any change caused by a pollutant can be seen over a relatively short period of time.

P. morum occurs in 4, 8, 16, or 32 cells aggregated into a colony of a spherical shape, the colonies possess a number of flagellates which assist in the movement, and are embedded in mucilage surrounding the colony (Round, 1973). *P. morum* generally move by spinning and vibrating around and colliding with other cells or structures.

The colonies can reproduce asexually or sexually. Asexual reproduction arises through the exclusion of a mature cell from the colony; the cell can then either use sexual or asexual reproduction to increase the number of cells within the colony. Sexual reproduction occurs when two mature cells come together and exchange nuclear material. In *P. morum*, the two cells are only different in the size and motility. The larger of the two is less motile and attracts the smaller more motile cell, termed as anisogamy (Chapman and Chapman, 1981)

9.2. Aims and objectives

The experiment is designed to examine the effects the metal ions have on the algae. A number of concentrations of metals will be examined as well as the effect a number of metals can have on the algae. The specific aims are outlined below:

- To determine the threshold limit value (TLV) of copper, lead and tungsten to *Pandorina morum*
- To determine if copper, lead and tungsten affect reproduction in *Pandorina morum*
- To determine if *Pandorina morum* bioaccumulate copper, tungsten and/or lead, and to determine the concentration accumulated from the growing media
- To determine if a combination of copper, lead and tungsten have a synergistic effect on *Pandorina morum*

9.3. Experimental Procedure

For this experiment the following list identifies the equipment that will be used:

- Conical Flasks
- Bungs – allowing for gas exchange
- Incubator
- Growing media
- Cultures of *Pandorina morum*
- Microscope
- Haemocytometer
- Lead nitrate
- Copper nitrate
- Tungsten solution

Before the start of the experiment, all glassware with accompanying bungs, and media were autoclaved to ensure complete sterilisation. The equipment was sterilised at 121°C, 15 lb pressure for 15 minutes (Belcher, 1982). Because of the number of flasks needed, the experiment has been divided into stages. After each stage all the glassware will be autoclaved again to ensure no contamination. The algal cultures were supplied by Sciento based in Manchester. Upon arrival, the cultures were examined and identification was confirmed. Sub samples from the original cultures were used to inoculate flasks containing 150mL of nutrient media. The samples were then stored in an incubator set at 21°C with alternating light and dark periods of 12 hours. The original samples were kept and nutrients were added at regular intervals. This is to ensure the culture is viable for use in the subsequent experiments. The metal solutions were made up using copper nitrate, lead nitrate and tungsten solution. A concentration of 1000ppm was made up of each metal and sterilised.

The nutrient media was also supplied by Sciento, and was based on Bold's Basal medium (Bischoff and Bold, 1963). Table 9-1 outlines the components of the medium.

Table 9-1: The components of Bold's Basal Medium used in the experiment

	Stock Solution:	Per 400mL
1	NaNO ₃	10.0g
2	MgSO ₄ .7H ₂ O	3.0g
3	NaCl	1.0g
4	K ₂ HPO ₄	3.0g
5	KH ₂ PO ₄	7.0g
6	CaCl ₂ .2H ₂ O	1.0g
7	Trace Elements solution:	Per L
	ZnSO ₄ .7H ₂ O	8.82g
	MnCl ₂ .4H ₂ O	1.44g
	MoO ₃	0.71g
	CuSO ₄ .5H ₂ O	1.57g
	CoNO ₃ .6H ₂ O	0.49g
8	H ₃ BO ₃	11.42g
9	EDTA – KOH Solution:	Per L
	EDTA	50.0g
	KOH	31.0g
10	FeSO ₄ .7H ₂ O	4.98g
	H ₂ SO ₄	1.0mL

Final solution: Stock solutions 1-6: 10mL each

Stock solutions 7-10: 1mL each

The final solution was then made up to 1L with distilled water.

Sciento provided this solution in sealed bottles, and prior to algal inoculation was diluted (100mL of raw nutrient media was made up to 1L with distilled water) and autoclaved, as discussed earlier.

The metal solutions were also made up with distilled water. A total volume of 150mL (of nutrient media and metal solution) was placed in each of the conical flasks, the components and volumes can be seen in Table 9-2.

Table 9-2: Volumes of components for the algal experiments

Component	Volume (mL)
Undiluted nutrients	15
Distilled water	130
Algae	5
Metal solutions (varying concentrations)	5
Total:	155

To make the varying concentrations a mixture of 1000ppm metal solution and distilled water was used to get the required metal concentration in the flask. Before addition to the media, the number of algal cells in the stock solution was determined. Using a haemocytometer a drop of the algae solution was counted and multiplied up to determine the population of algae in 1mL. This was repeated 5 times and averaged. Figure 9-5 shows an example of algal cells on a haemocytometer.

Figure 9-5: A view of algal cells on a haemocytometer grid at a magnification of x100

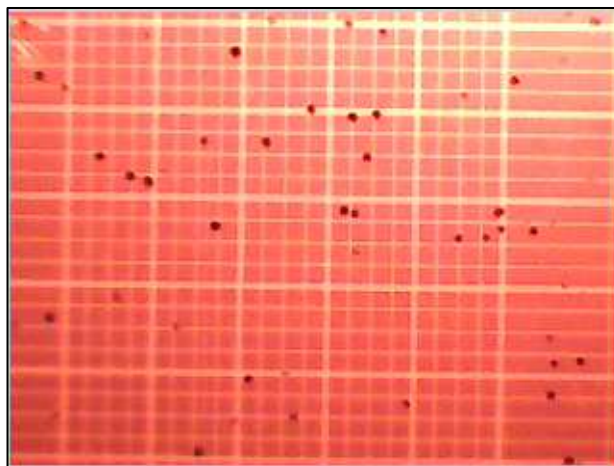


Figure 9-6: A view of the different sized grids used to perform counts of cells

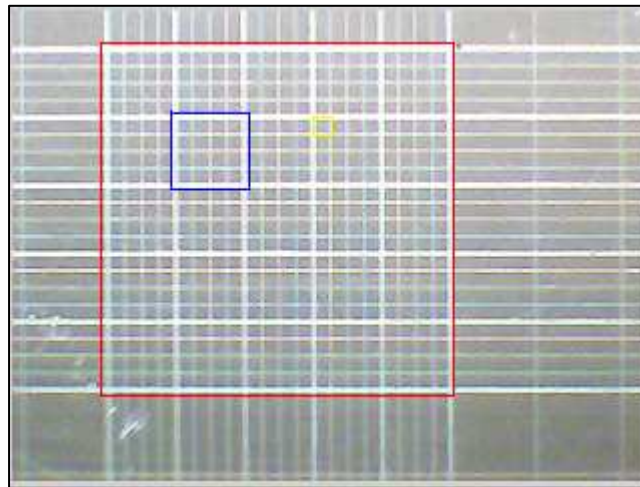


Figure 9-6 shows the centre grid of a Haemocytometer. It shows that the centre grid (red) comprises 5x5 squares and each of these smaller grids (blue) are divided into a further 16 squares coloured yellow. The large red square measures a total area of 0.1mm^3 ; the medium blue square has an area of $6.25 \times 10^{-4}\text{mm}^3$, and the smallest grid in yellow measures $2.5 \times 10^{-4}\text{mm}^3$.

Depending on the size and number of cells in the solution to be counted the different sized grids can be used. The haemocytometer can be used under all magnifications, to allow for easier counting of the smaller cells e.g. red blood cells. Where a solution contains a high number of cells, the smallest of the three grids can be used. In these cases a number of grids are used to count the number of cells in the squares. The squares which are counted are chosen at random and the final figure is multiplied up, using the following calculation:

$$\left[\frac{\text{Number of cell}}{\text{Number of squares}} \right] * \left[\frac{1}{\text{area of squares}} \right] * \text{Dilution} = \text{cells per mm}^3 \dots\dots(6)$$

For example: for the entire grid (red grid) 21 colonies with no dilution

$$\text{Number of colonies per mm}^3 = 21/1 * 1/0.1 = 210 \text{ per mm}^3$$

Therefore the number of colonies per mL = 210,000

For the first experiment the range of metals used were between 5ppm and 25ppm. At higher concentrations e.g. 1000ppm, the nutrient media became cloudy and precipitation was observed, and was therefore not used.

Because of space limitations, the experiments were divided into stages. The first stage of the experiments was to determine the TLV for each of the metals, beginning with lower values (0-25ppm) and if necessary this will be expanded to cover a larger range.

Tables 9-3, 9-4, 9-5 and 9-6 show the concentrations of the individual metals used in stages 1, 2, 3 and 4 respectively.

Table 9-3: The concentrations of copper, tungsten and lead used in stage 1

Copper Concentration (ppm)	Tungsten Concentration (ppm)	Lead Concentration (ppm)
0 (control)	0 (control)	0 (control)
5	5	5
10	10	10
15	15	15
20	20	20
25	25	25

During the first experiment it was found that copper was toxic at concentrations greater than 10ppm, therefore testing at higher concentrations was not necessary.

Table 9-4: The concentrations of tungsten and lead used in stage 2

Tungsten Concentration (ppm)	Lead Concentration (ppm)
0 (Control)	0 (Control)
15	75
45	90
60	125
75	150
	200

Table 9-5: The concentrations of copper, tungsten and lead used in various combinations for stage 3

Copper and Lead Concntrations (ppm)		Copper and Tungsten Concentration (ppm)		Lead and Tungsten Concentration (ppm)	
Copper	Lead	Copper	Tungsten	Lead	Tungsten
0 (control)	0 (control)	0 (control)	0 (control)	0 (control)	0 (control)
5	5	5	5	5	5
5	15	5	15	15	15
5	30	5	30	30	30

Table 9-6: The concentrations of copper, tungsten and lead used in combination for stage 4

Copper, Tungsten and Lead concentration (ppm)		
Copper	Tungsten	Lead
0 (Control)	0 (control)	0 (control)
5	5	5
5	15	15
5	30	30

The experiments were repeated in duplicate. The use of incubators was restrictive as the concentration of light needed to be maintained. Therefore the same incubators were used throughout the experiments. The flasks were also placed in the same positions, to ensure that each of the flasks received the same amount of light throughout the experiments.

9.4. Results: Stage 1 Effects of individual metals on algal populations

9.4.1. Copper

Figure 9-7 shows the percentage of nitrate and phosphorus used by *P. morum* and also the percentage change in the population of the culture. It can be seen that for concentrations exceeding 8.406ppm, the population decreased. It also showed that the percentage of phosphorus used decreased. When comparing with control, there was a lower percentage of nitrates used, indicating that nitrates were removed from the nutrient media, although the population decreased. Figure 9-8 shows the pH of the nutrient before and after the experiment. It showed that there was an increase (more alkaline) in the pH for samples containing 3.625 and 3.9ppm of copper, but the remaining samples showed decreases in the pH.

Since it was not necessary to examine the effects of higher concentrations of copper, it was determined that the estimated threshold limit value (TLV) lies between 8.406 and 11.61ppm

9.4.2. Tungsten

There was a high percentage increase in the population of *P. morum*, because of this the population results are shown on a separate graph (Figure 9-9). Compared to the control samples, there was a dramatic increase in the population with the addition of tungsten. The highest value was that at a concentration of 7.997ppm, indicating that this could be the optimum concentration of tungsten for increased population growth. After the initial examination of the samples, it was clear that the tested concentrations of tungsten had no short term effects on the algae, it was decided to begin two samples with higher concentrations of tungsten: 120.5 and 127.5ppm. These results showed that there was a decrease in the population for both concentrations, although the lower of the two only produced a small decrease in the population. This indicates that the concentration of tungsten was too high and that the algae were unable to continue growth at these concentrations. Figure 9-10 shows the nitrate and phosphorus results and showed that at these high concentrations there was still a small percentage of nitrates being removed from the media. Although, there is still a percentage of the nitrates being used by *P. morum*, the value is lower than that of the control. The phosphorus results show that there were similar amounts used in all the samples, but

towards the higher range, this value decreased slightly when compared to the control. The pH values given in Figure 9-11, show that for the lower range of concentrations (0-9.063ppm) there was a increase in the pH, concentrations above this range also produced increases but not to the same extent. Concentrations of 25.79 and higher showed a decrease in the pH values.

At this stage it could only be determined that the TLV for tungsten lies between 35.91 and 120.5ppm. To narrow this range, concentrations between 35 and 120ppm should be examined.

9.4.3. Lead

Like the tungsten results, the population data was plotted on a separate graph due to the high percentage increase produced when lead was added to the nutrient media. Figure 9-12 indicates that the concentration of lead of 6.219 and 12.54ppm produced population results that were much higher than the control. At the higher concentrations (above 19.09ppm) there was still an increase but these were equivalent to those of the control. The nitrates and phosphorus results in Figure 9-13 show that the results were similar, irrespective of the concentration of lead that was added to the nutrient media. The results did fluctuate, but this could be attributed to the population growth and the positioning in the incubator. Where possible the flasks were removed from the incubator and replaced in the same positions. However, the incubator light strips were not universal in the quality of light that was produced.

The pH results (see Figure 9-14) showed that there was an increase in the pH and that this was the same for all the results.

Also like the tungsten results a definitive value for the TLV could not be determined. Provisionally it can be said that the TLV is above 28.21ppm but unlike the tungsten, an extreme value could not be done at this stage due to space constraints. Therefore experiments examining the effects of concentrations higher than 28ppm need to be performed.

Because it could not be determined the TLV for tungsten and lead, it was decided to repeat the experiments using greater concentrations of tungsten and lead. These were done in stage 2.

Figure 9-7: The percentage of nitrate and phosphorus used by *P. morum* and the associated change in population for varying concentrations of copper

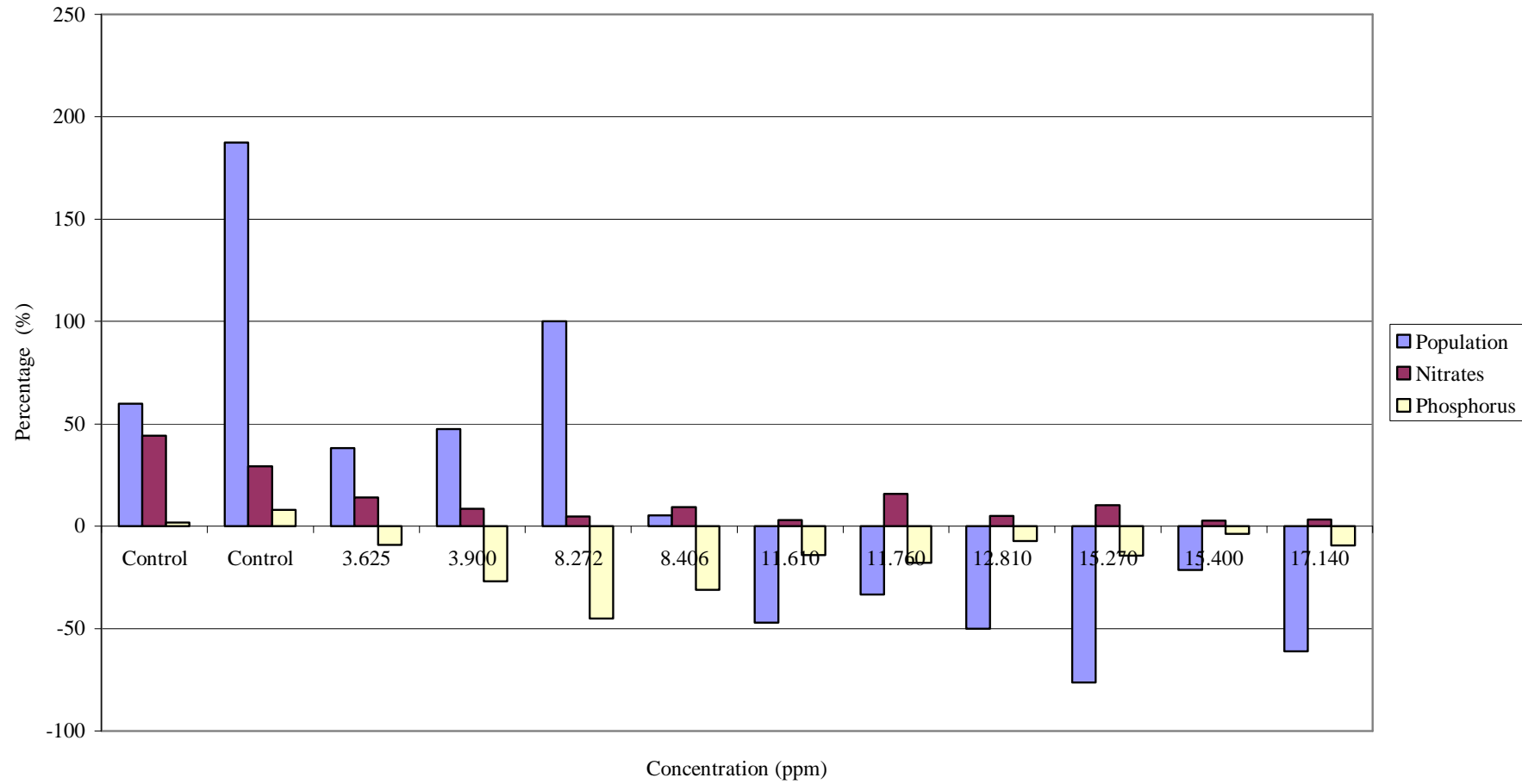


Figure 9-8: The pH of nutrient media for *P. morum*, contaminated with various concentrations of copper

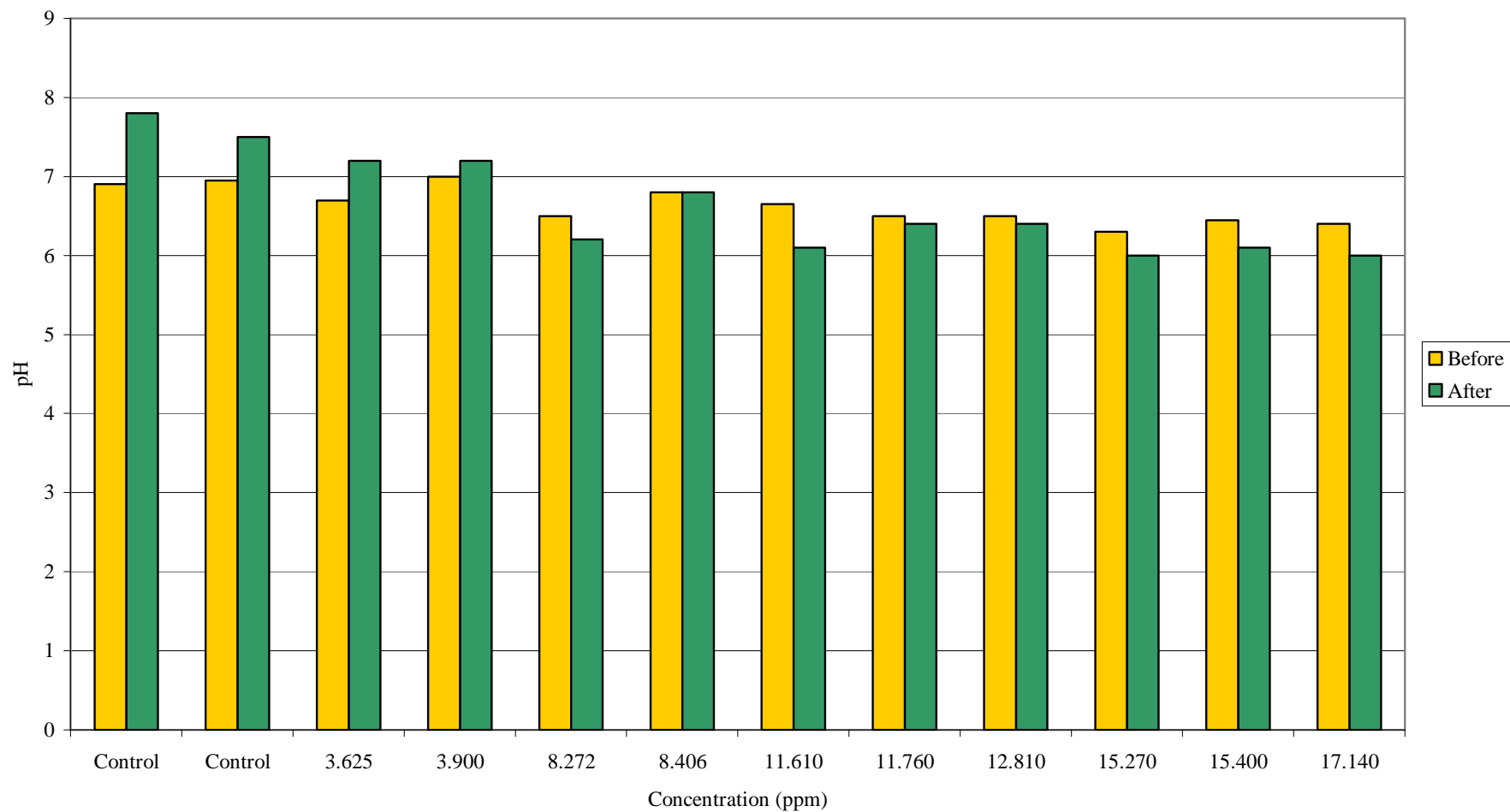


Figure 9-9: The change in the population of *P. morum* when incubated in nutrient media contaminated with tungsten

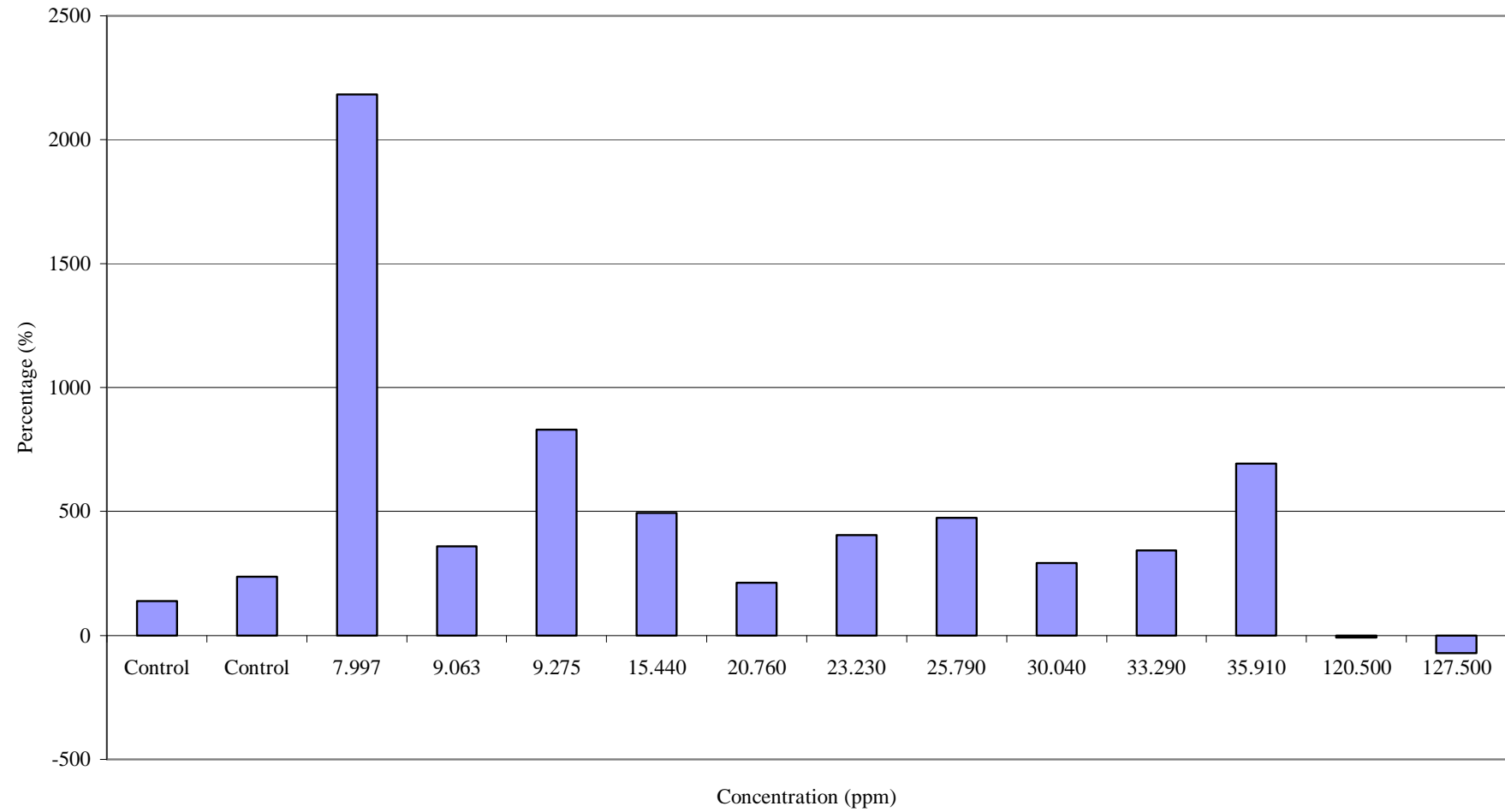


Figure 9-10: Percentage of nitrate and phosphorus used by *P. morum* for varying concentrations of tungsten

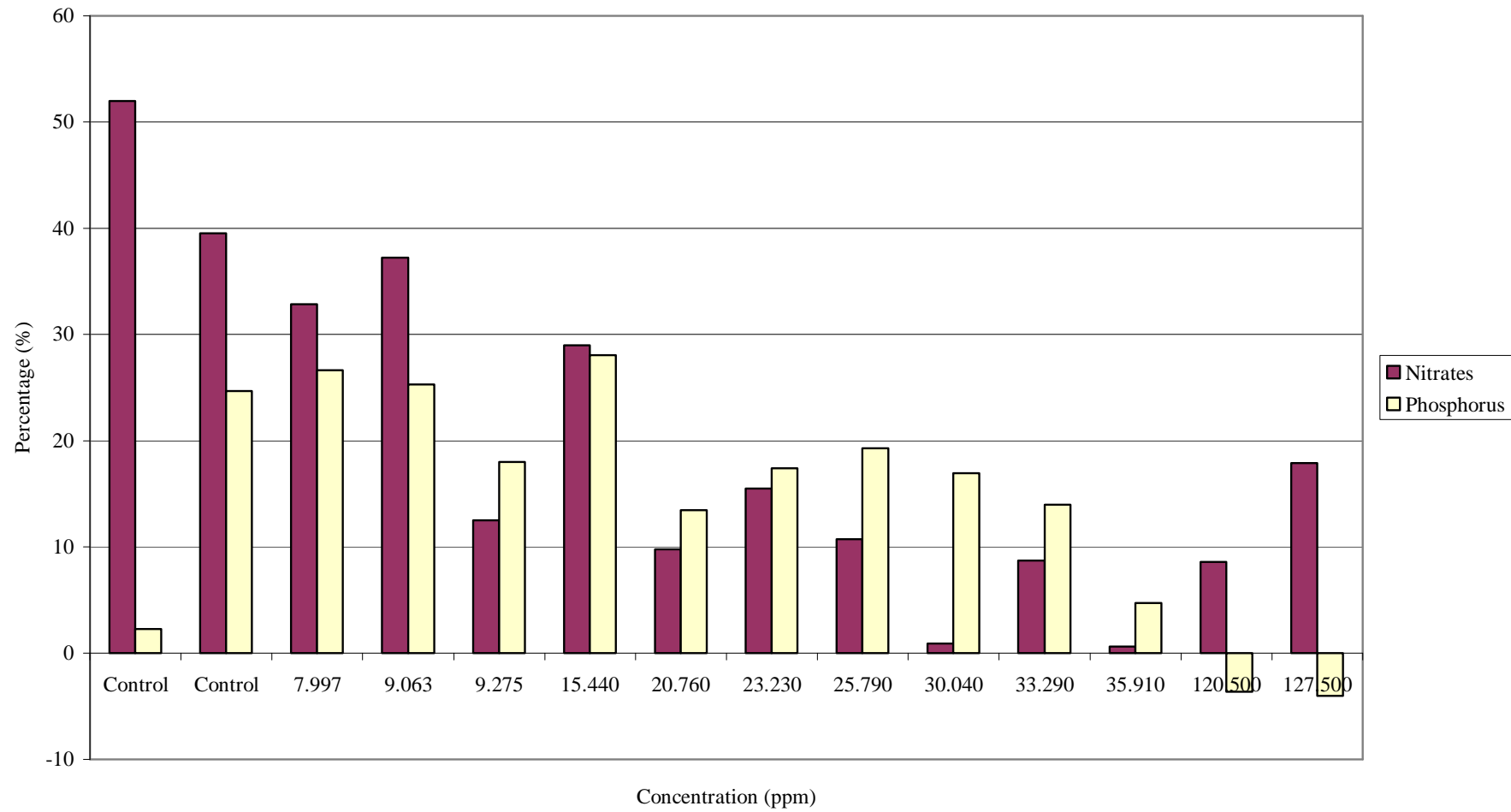


Figure 9-11: The pH of nutrient media for *P. morum*, contaminated with various concentrations of tungsten

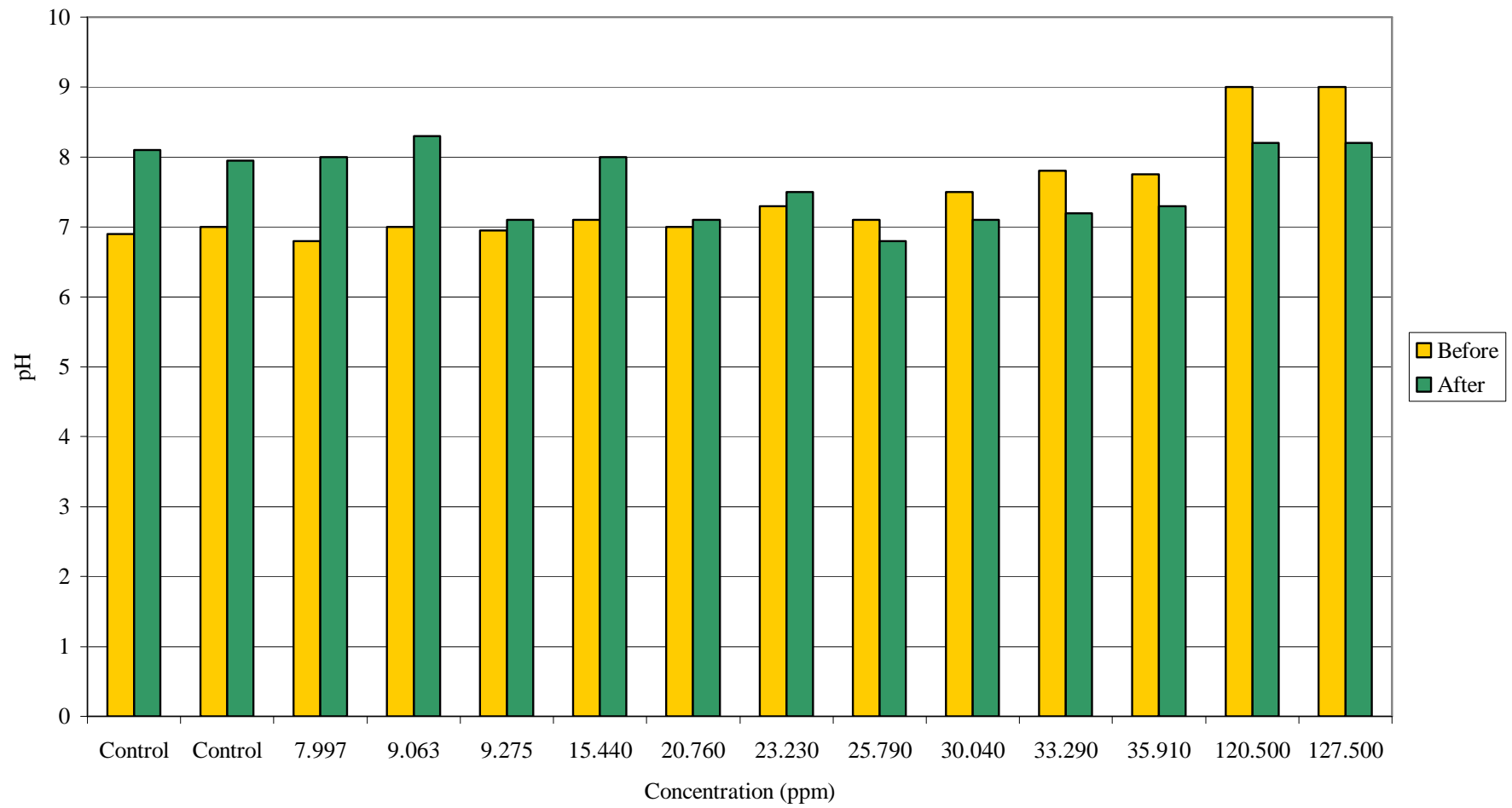


Figure 9-12: The change in population of *P. morum* when incubated in nutrient media contaminated with lead

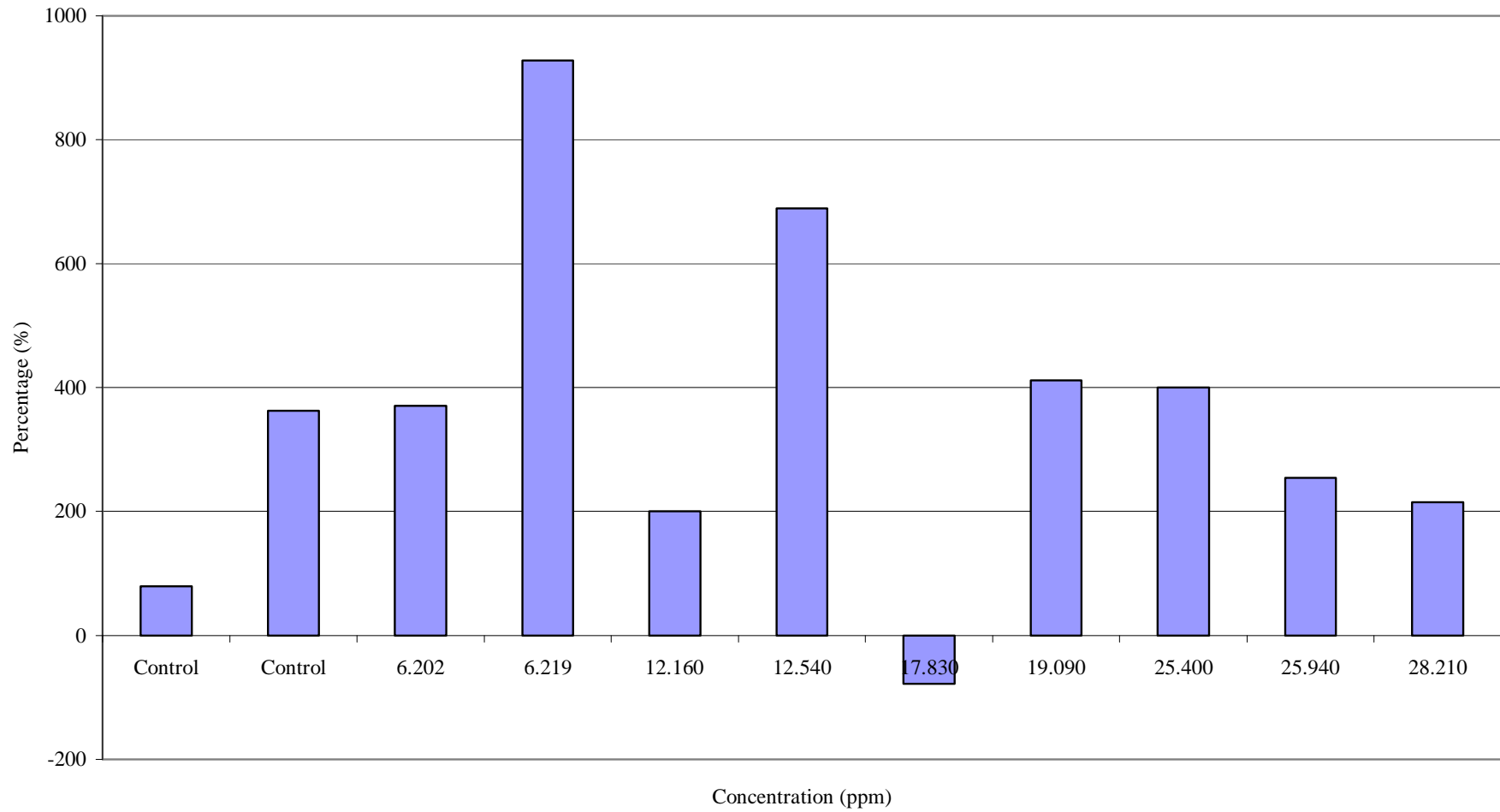


Figure 9-13: The percentage of nitrate and phosphorus used by *P. morum* incubated in nutrient media contaminated with varying concentrations of lead

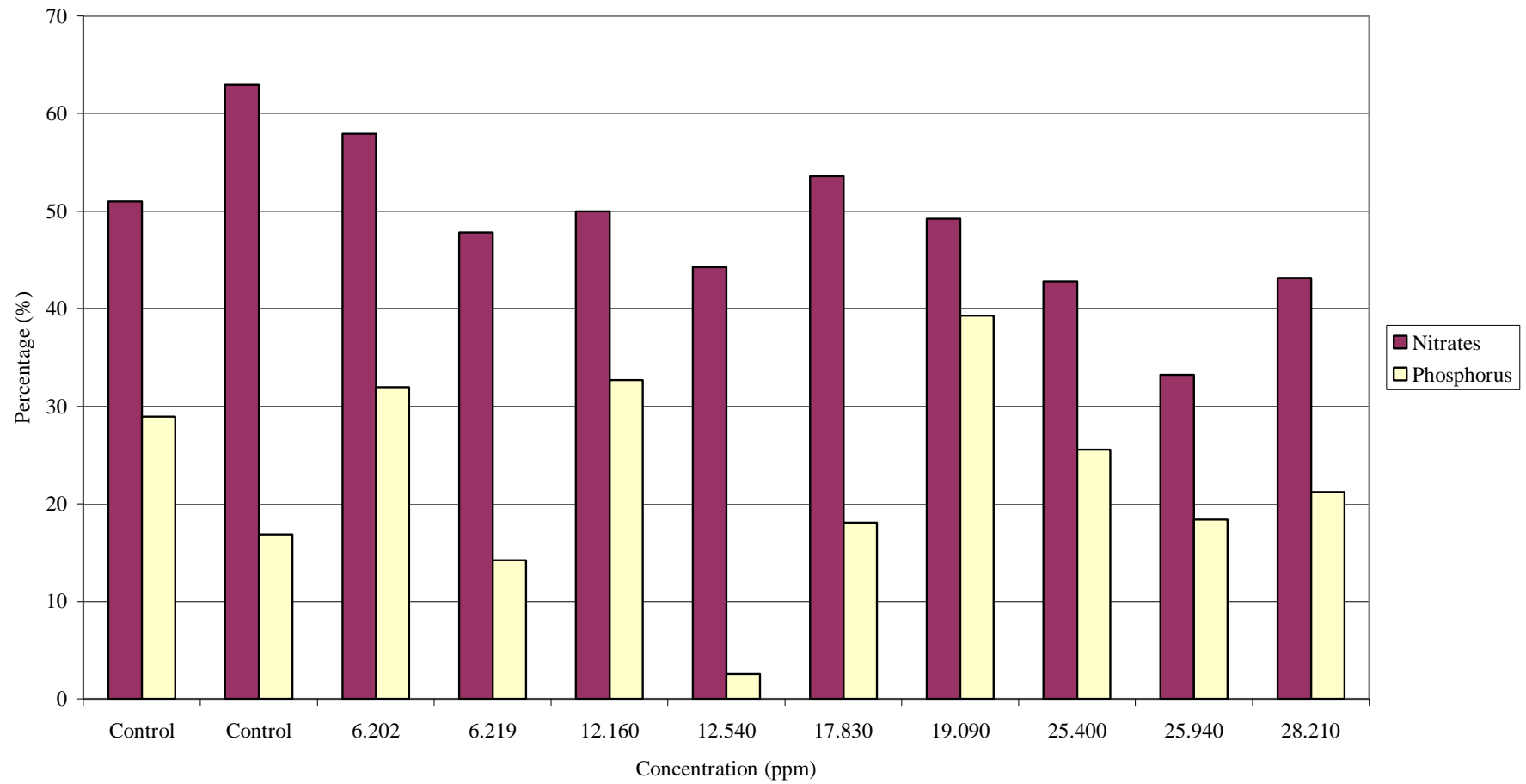
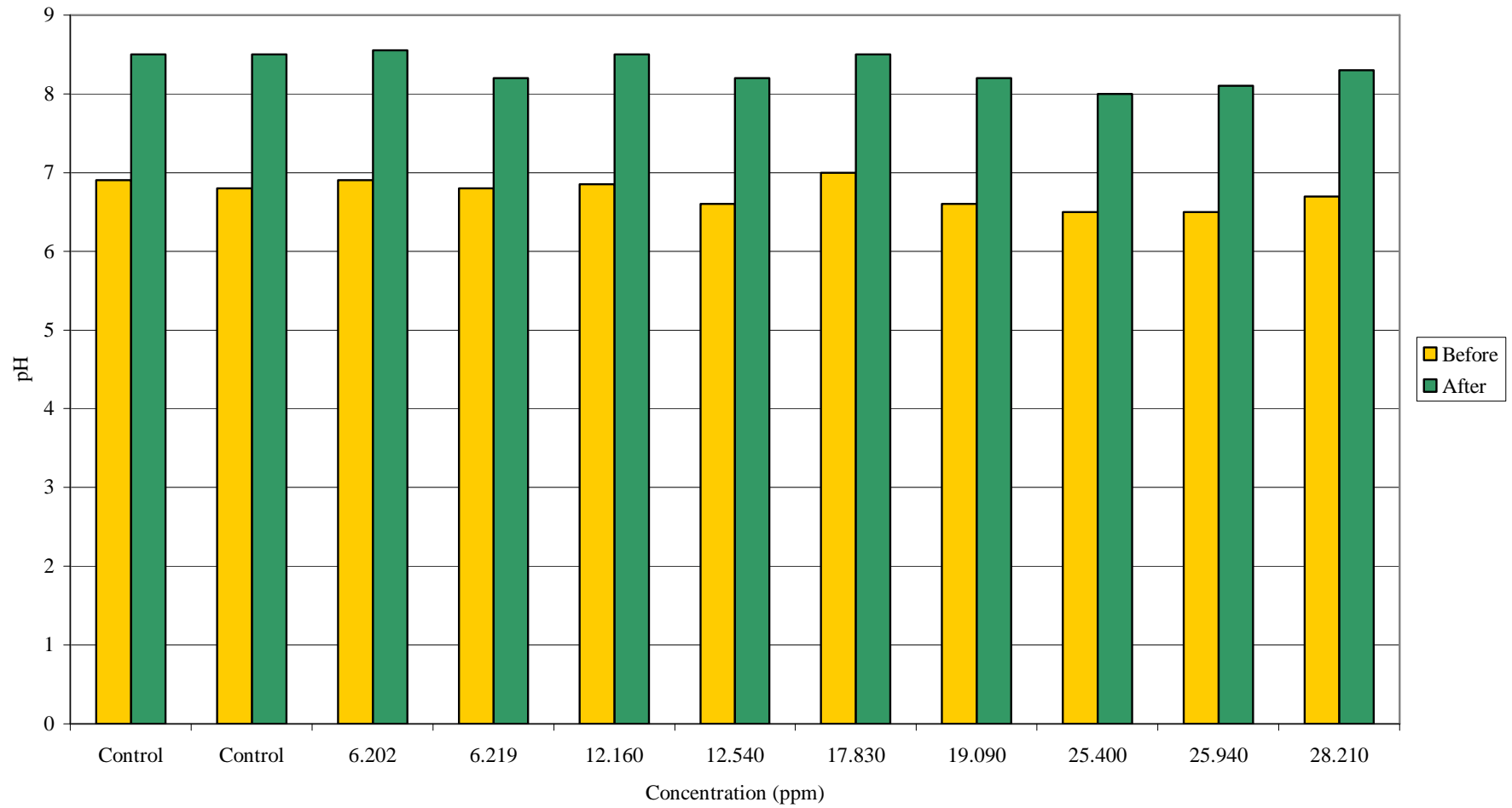


Figure 9-14: The pH of nutrient media for *P. morum* contaminated with various concentrations of lead



9.5. Results - Stage 2: Higher concentrations of tungsten and lead

9.5.1. Tungsten

The population results (Figure 9-15) for the increased concentration of tungsten showed that even at the higher concentrations, *P. morum* was still able to reproduce. The nitrate and the phosphorus results (Figure 9-16) show that both were being removed from the nutrient media, however the removal of nitrates were somewhat reduced compared to the control. The phosphorus did not begin to decrease until about 74.43ppm. This would indicate that phosphorus was still being removed from the nutrient media possibly for uses within the cell structure.

The pH results (Figure 9-17) show that the control results had increases in value as expected, but the higher concentrations of tungsten reduced the pH value. 74.43 and 90.3ppm produced pH values that were significantly lower than the before results.

A definitive result could not be determined but it could be stated that the TLV does lie in the range of 90.3 and 120.5ppm

9.5.2. Lead

Because the extreme values of lead could not be tested previously it was decided to use a wide range for this experiment. The values tested were between 70.6 and 203.2ppm. After preliminary experiments it was found that concentrations of lead higher than this range induced the production of precipitation. Therefore it was decided that those values would be too high to be used.

During this experiment it could be seen from the population results in Figure 9-18 that there was some population growth at a concentration of 151.4ppm but at 203.2ppm there was a decrease in the population. This would indicate that these concentrations are too high for *P. morum* to maintain a constant growth. The nitrate and the phosphorus results in Figure 9-19 showed that there were large quantities being removed from the nutrient media, equivalent to and in some cases exceeding the results given for the controls. There appears to be little or no effects on the growth of the algae at these concentrations. The pH values in Figure 9-20 also confirm these results, as there was an increase in the pH values indicating that there was a continued unaffected growth of the alga. The only result that did not increase was that of 203.2ppm. Although there was a population decrease, small values of nitrates were

still used; also there was an increase in the phosphorus concentrations in the media. The result for the sample containing 203.2ppm of lead shows a change in the pH values. The initial pH value for this concentration was around 1.8, and would indicate that the solution was too acidic to promote cell growth of the algae.

As with the tungsten, only a range could be determined for the TLV. This was decided to be between 151.4 and 203.2ppm.

To confirm the concentration of the metal added into the media, samples were analysed by ICP-OES. After the completion of the experiment the samples were again analysed through the ICP-OES to determine if there was a change in the concentration. It was found that only lead showed a difference between the before and after values. Figure 9-21 illustrates the changes in the concentration of lead for all the samples; it shows that for all, except one, there was a dramatic reduction in the lead concentration. The 203.2ppm had no decrease in the concentration. This would indicate that *P. morum* is actively absorbing the lead, or lead has become adhered to the cell surface removing it from the solution.

Figure 9-15: The change in population of *P. morum* when incubated in media contaminated with various concentrations of tungsten

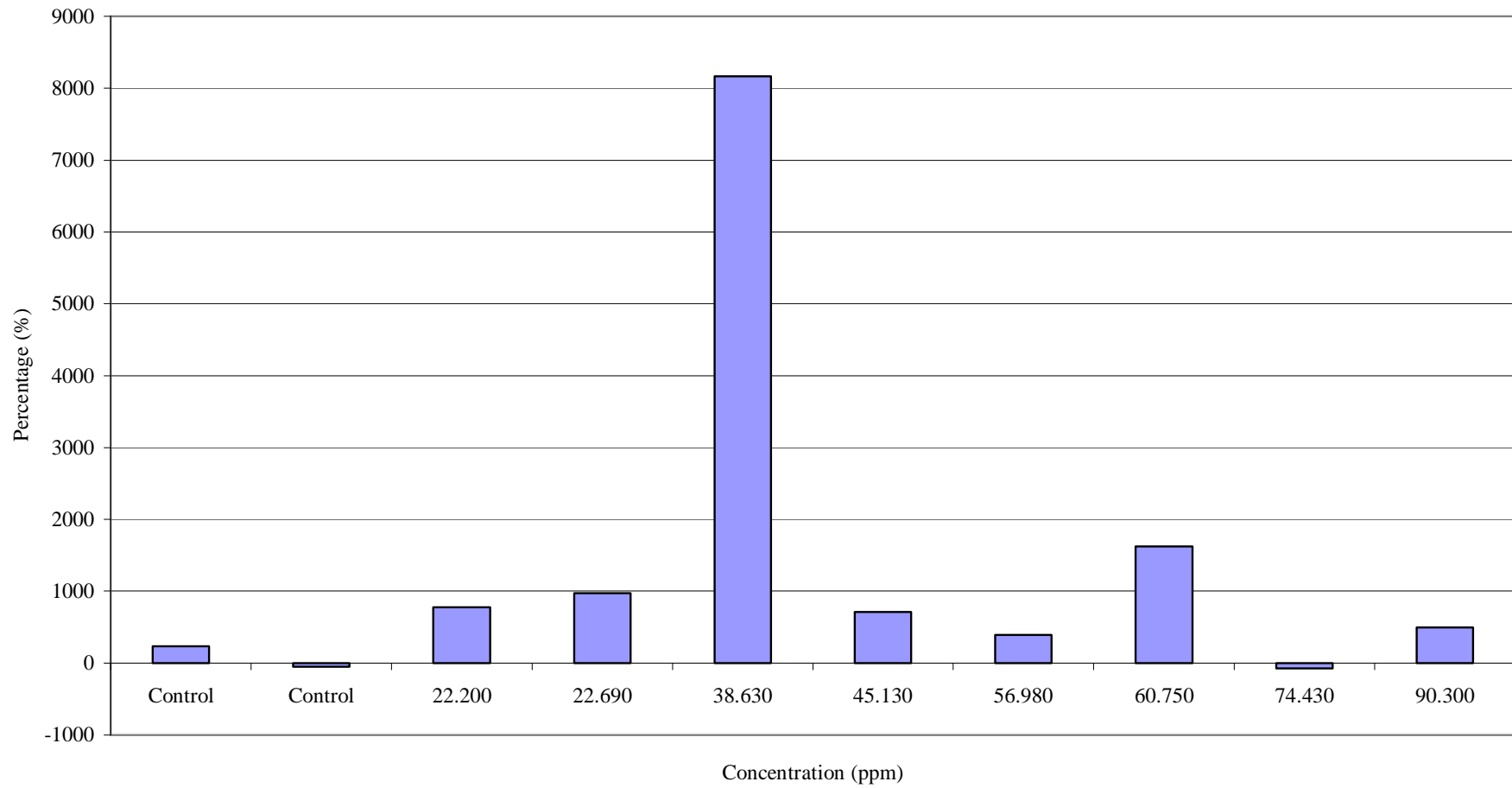


Figure 9-16: The percentage of nitrate and phosphorus used by *P. morum* for varying concentrations of tungsten

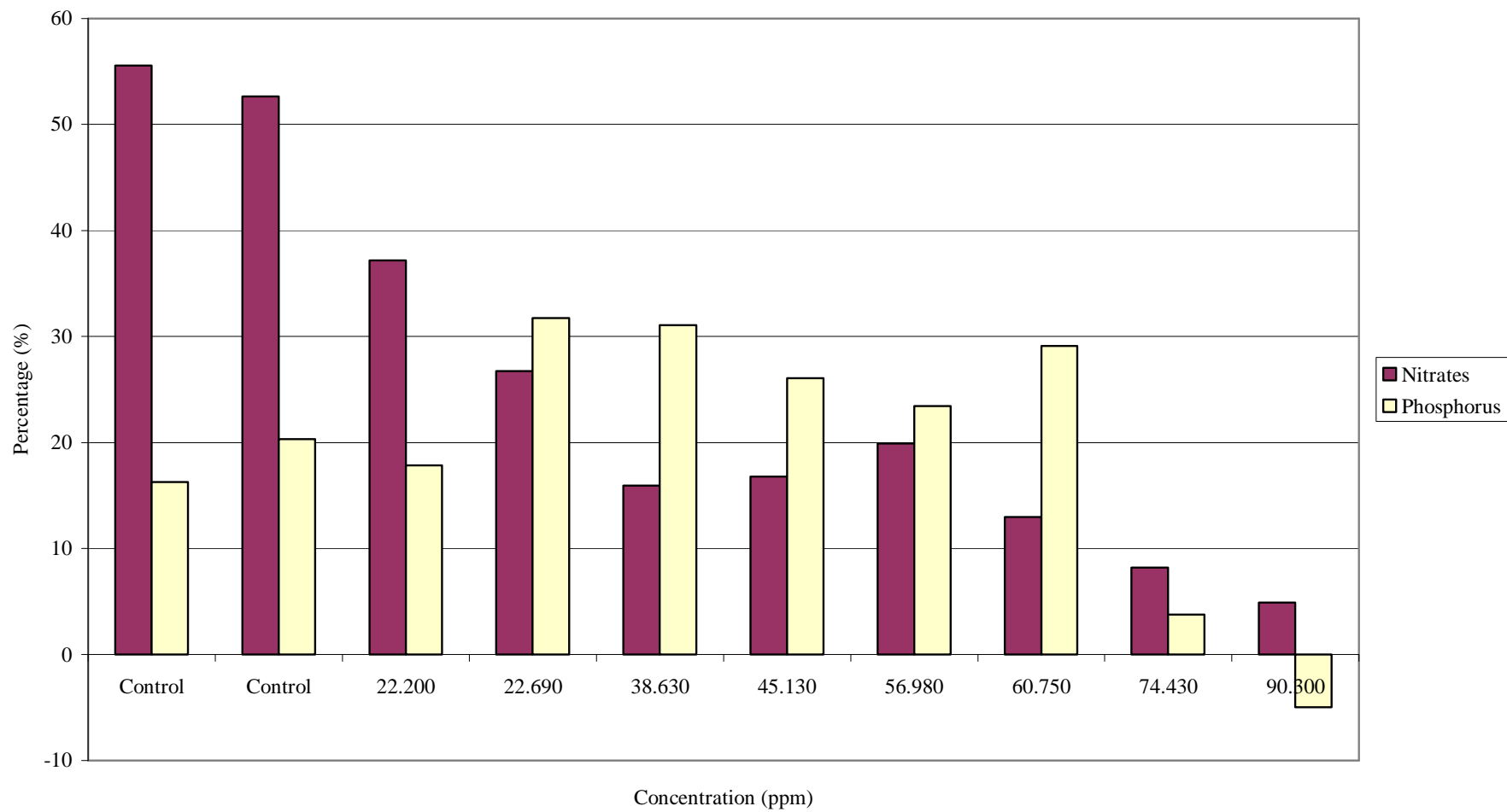


Figure 9-17: The pH of nutrient media for *P. morum*, contaminated with various concentrations of tungsten

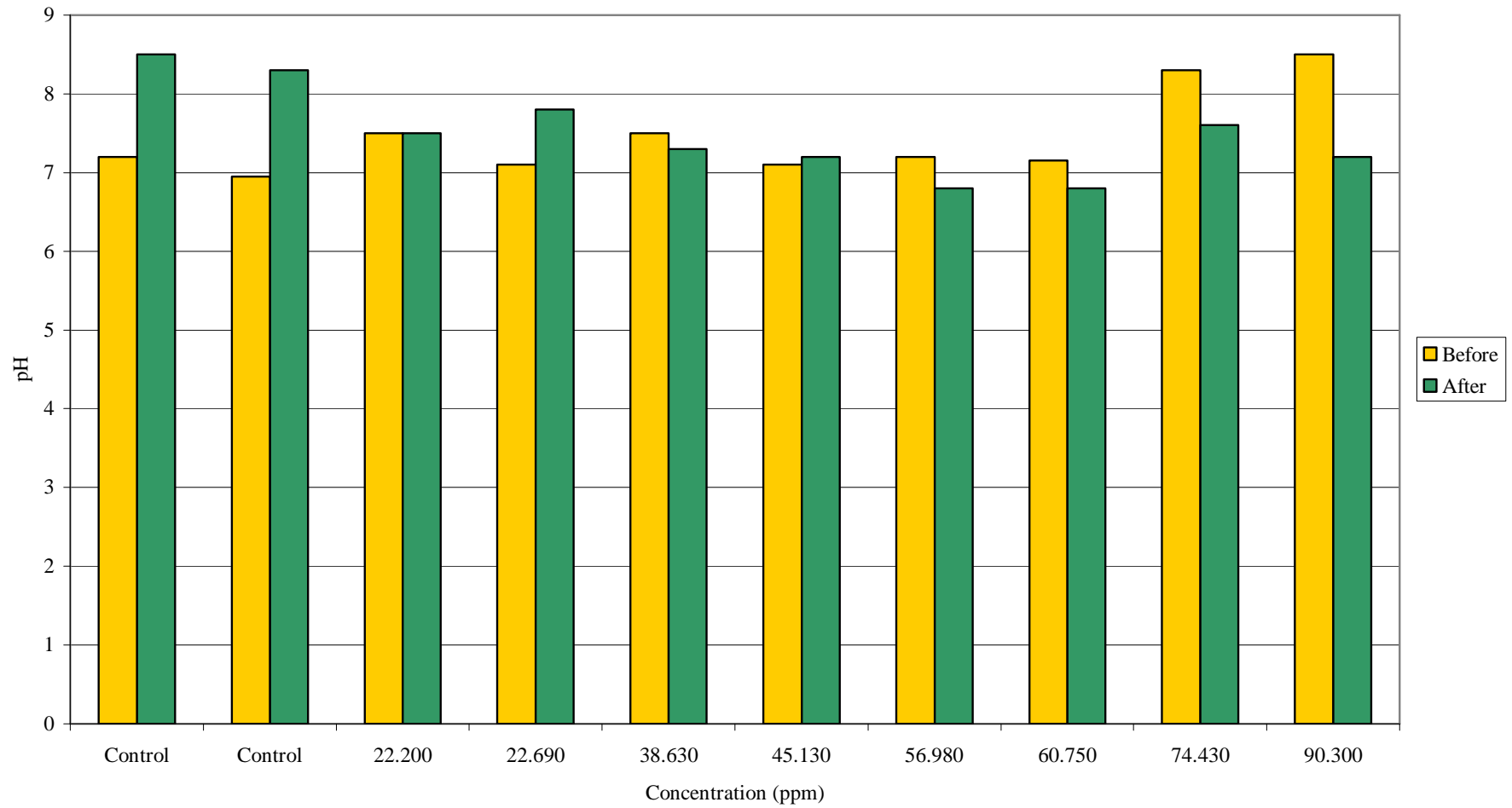


Figure 9-18: The change in population of *P. morum* when incubated in nutrient media contaminated with various concentrations of lead

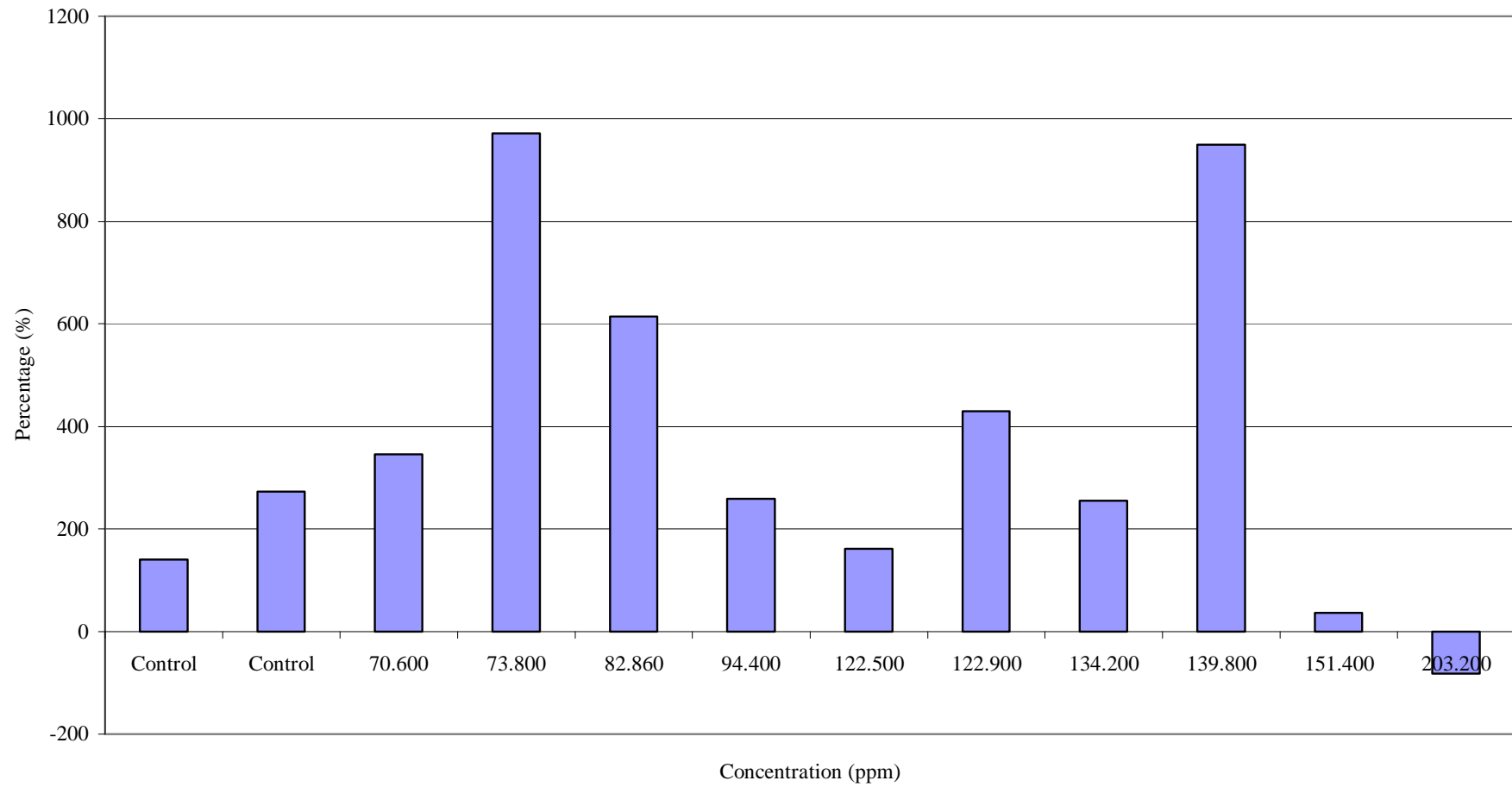


Figure 9-19: The percentage of nitrate and phosphorus used by *P. morum* for various concentrations of lead

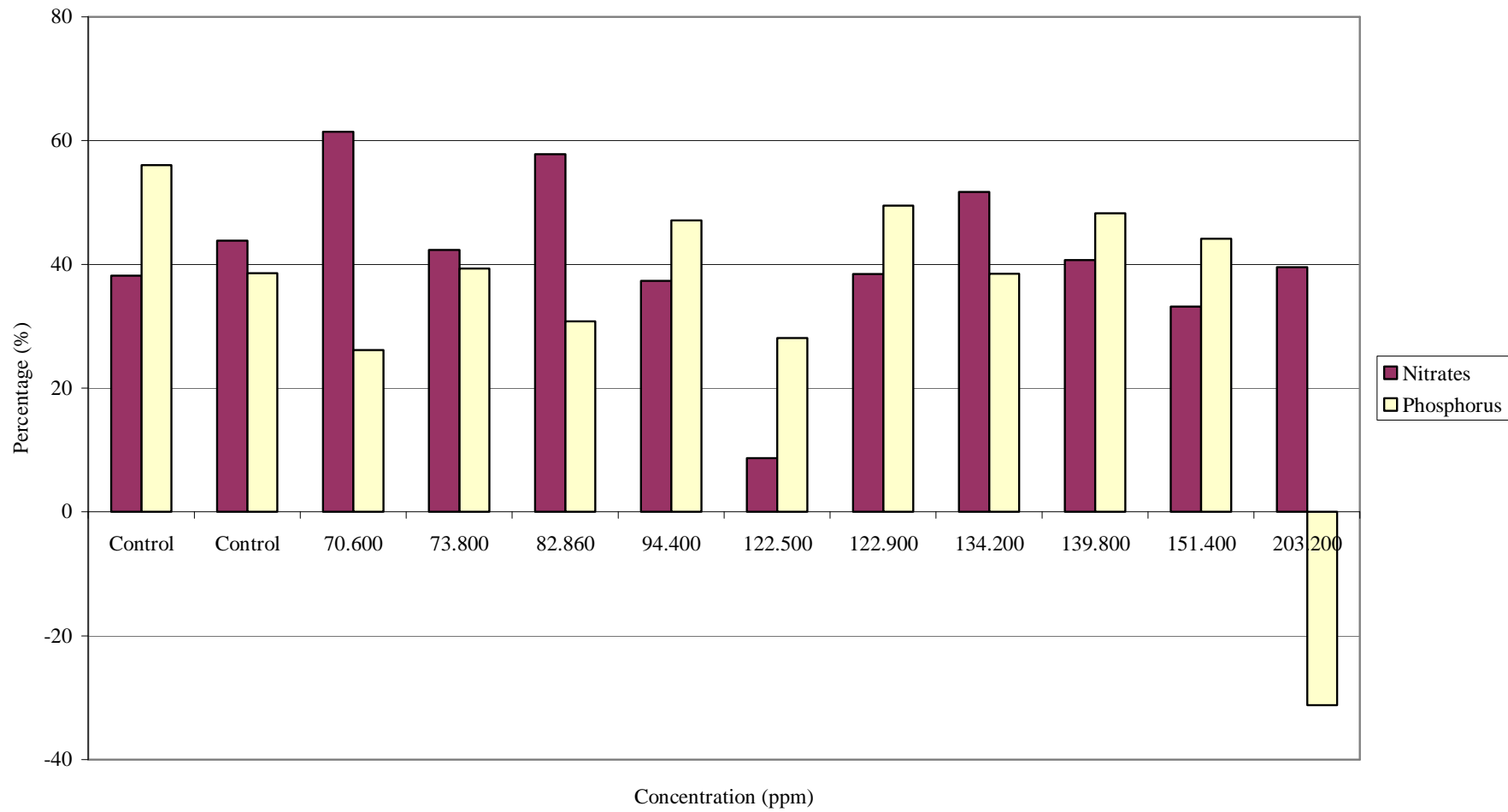


Figure 9-20: The pH of nutrient media for *P. morum* contaminated with various concentrations of lead

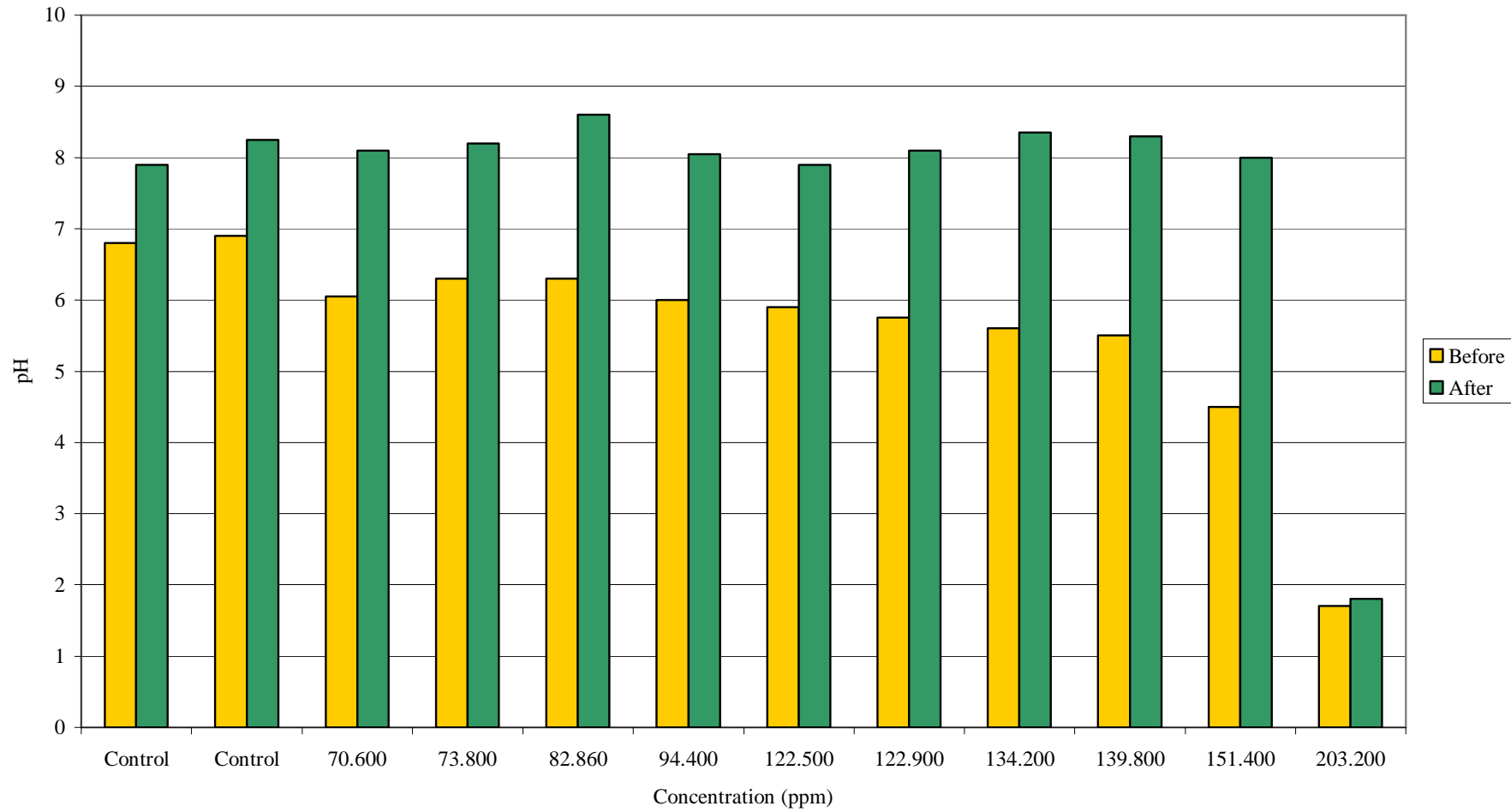
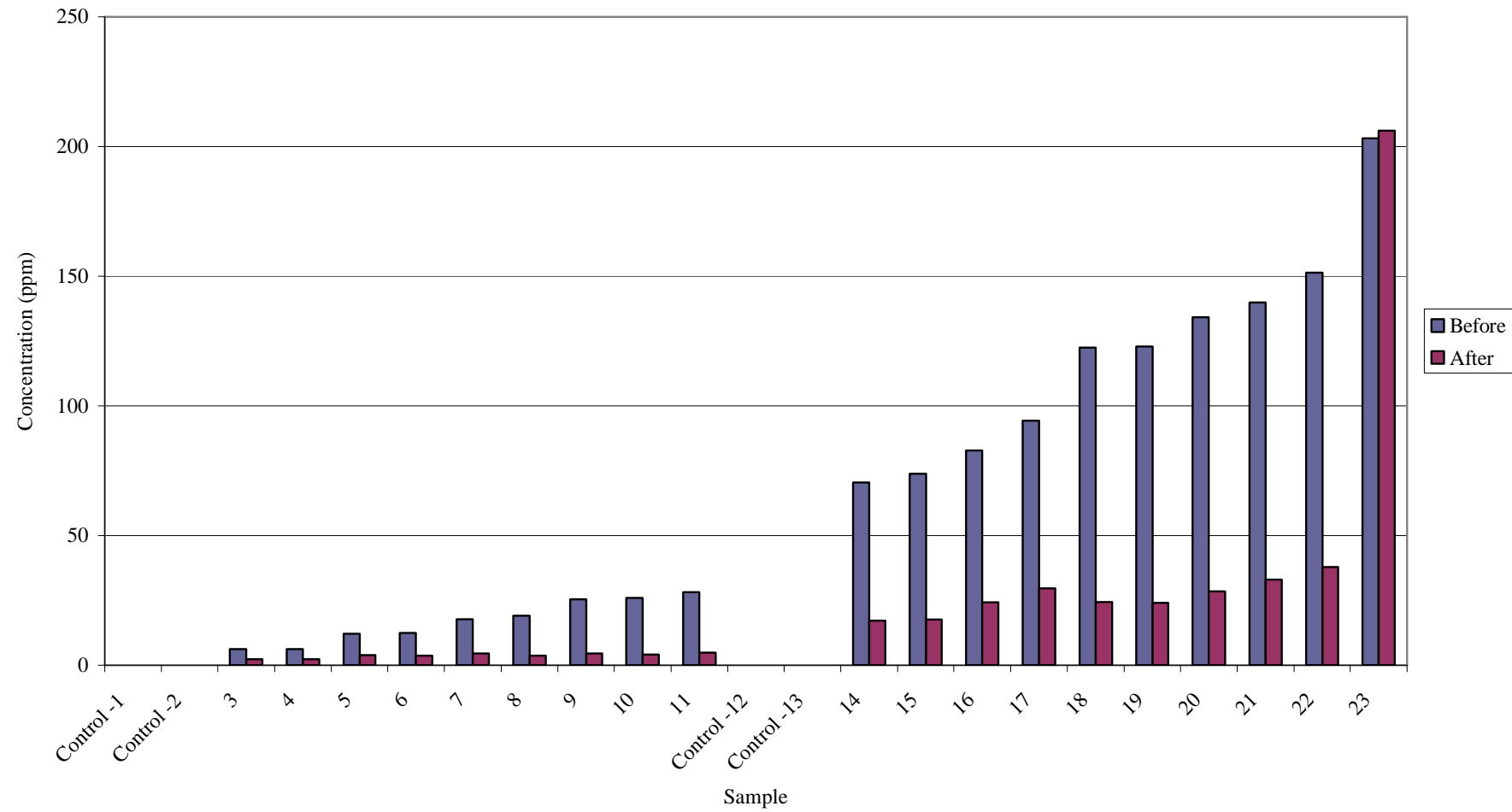


Figure 9-21: The changes in the concentration of lead in the nutrient media for *P. morum*



9.6. Results – Stage 3: Combinations

It was decided to use concentrations of metals that did not previously have an adverse effect on the algae. For copper this was found to be 5ppm, in previous experiments, copper at this concentration did not affect the algae. For tungsten and lead there were a range of concentrations that did not affect the algae, so the concentrations were those at the lower end of the range. This was to determine if it was the combinations of the metals that affected the algae and not the individual metals.

9.6.1. Copper and tungsten

The results in Figure 9-22 show that only the control samples had normal growth. All the samples with copper and tungsten were affected by the metals. The nitrate and the phosphorus results produced negative results, indicating that nitrates and phosphorus were being introduced to the media, assumedly by the algae. However there were three samples that showed an increase in the population, but not to the same extent as the controls: 5/15 and 5/30. The pH values showed that only the values for the control increased (Figure 9-23).

Because *P. morum* could grow at copper concentrations of 5ppm, and at tungsten concentrations of 5-30ppm, the results would suggest that the combination of the two metals has caused considerable damage to the algae.

9.6.2. Copper and lead

The combination of copper and lead do not appear to have a lethal affect, and this is shown in Figure 9-24. The population results show that there is either an increase in the population or no population growth. This does not suggest that there is a decrease in the population but that the death of the cells is equal to the reproduction rate. However, the phosphorus and the nitrate results show that there is an increase in their concentration in the nutrient media for all the samples with metals except one, 5/5. This suggests that the nutrients are not being taken up for cell processes. The pH results in Figure 9-25 show that there are increases in the pH values but compared to the control they are not as great.

In the individual experiments, *P. morum* grew successfully in the media with lead, but in this combination the population growth has been inhibited.

9.6.3. Lead and tungsten

The combination of lead and tungsten does seem to have an effect on *P. morum*. The results in Figure 9-26 show that although there is normal population growth, the percentage of phosphorus used by the algae decreases at the higher concentrations and actually increases in the media at the highest concentration (15/25ppm). There was a small percentage of the nitrate removed from the media at the higher concentrations but for the highest concentrations, the nitrate in the media increased. With the increase in the population it was expected that the pH of the media would increase as it has done before, however the higher concentrations pH value decreased quite dramatically (see Figure 9-27).

The addition of tungsten to the media in previous experiments produced an increase in the population of *P. morum*, this also occurred when lead was added. However the combination of the two metals appears to have hindered the population growth.

Figure 9-22: The percentage of nitrates and phosphorus used by *P. morum* and the associated change in population for media contaminated with copper and tungsten

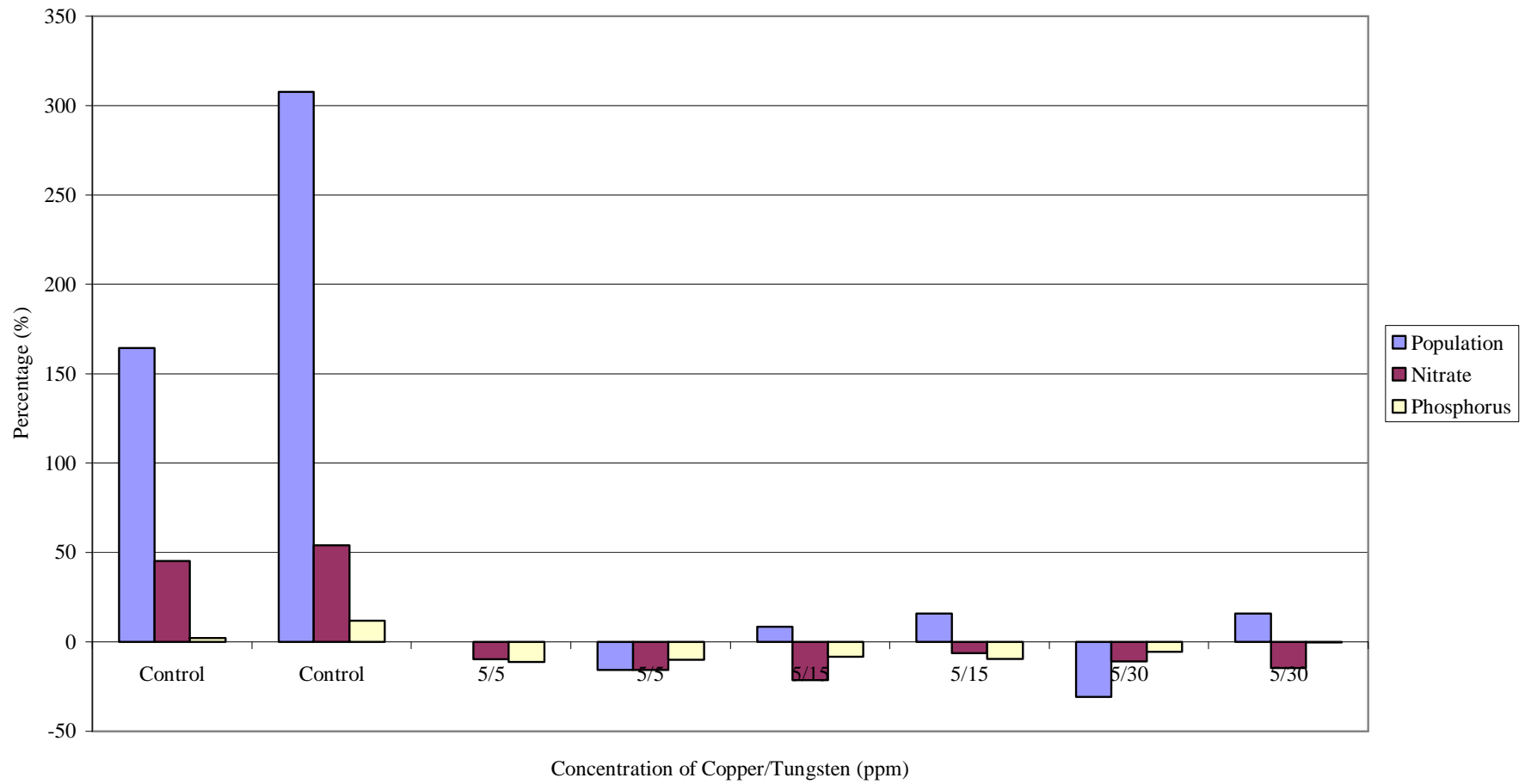


Figure 9-23: The pH of nutrient media for *P. morum* contaminated with copper and tungsten

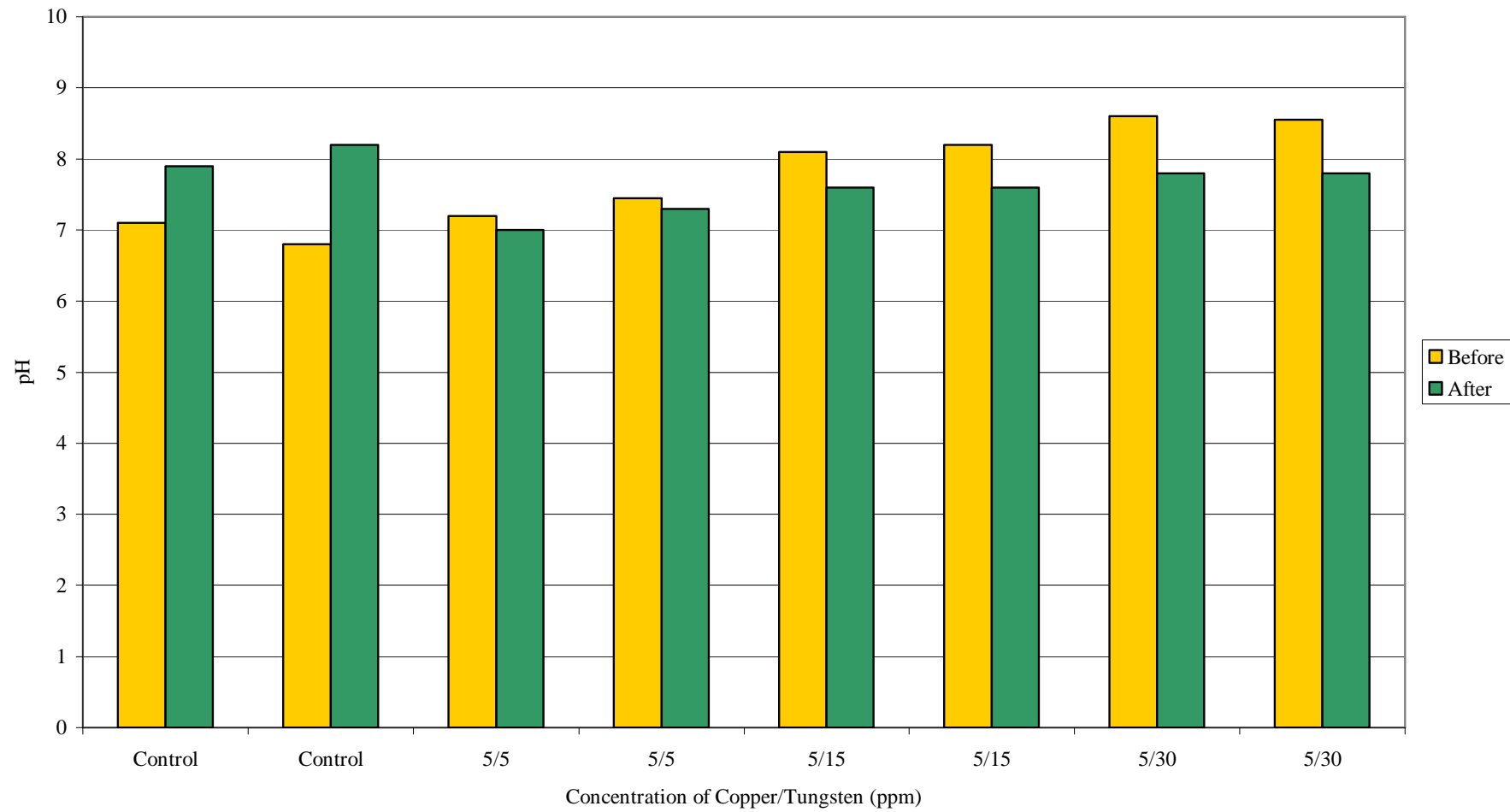


Figure 9-24: The percentage of nitrate and phosphorus used by *P. morum* and associated change in population for nutrient media contaminated with copper and lead

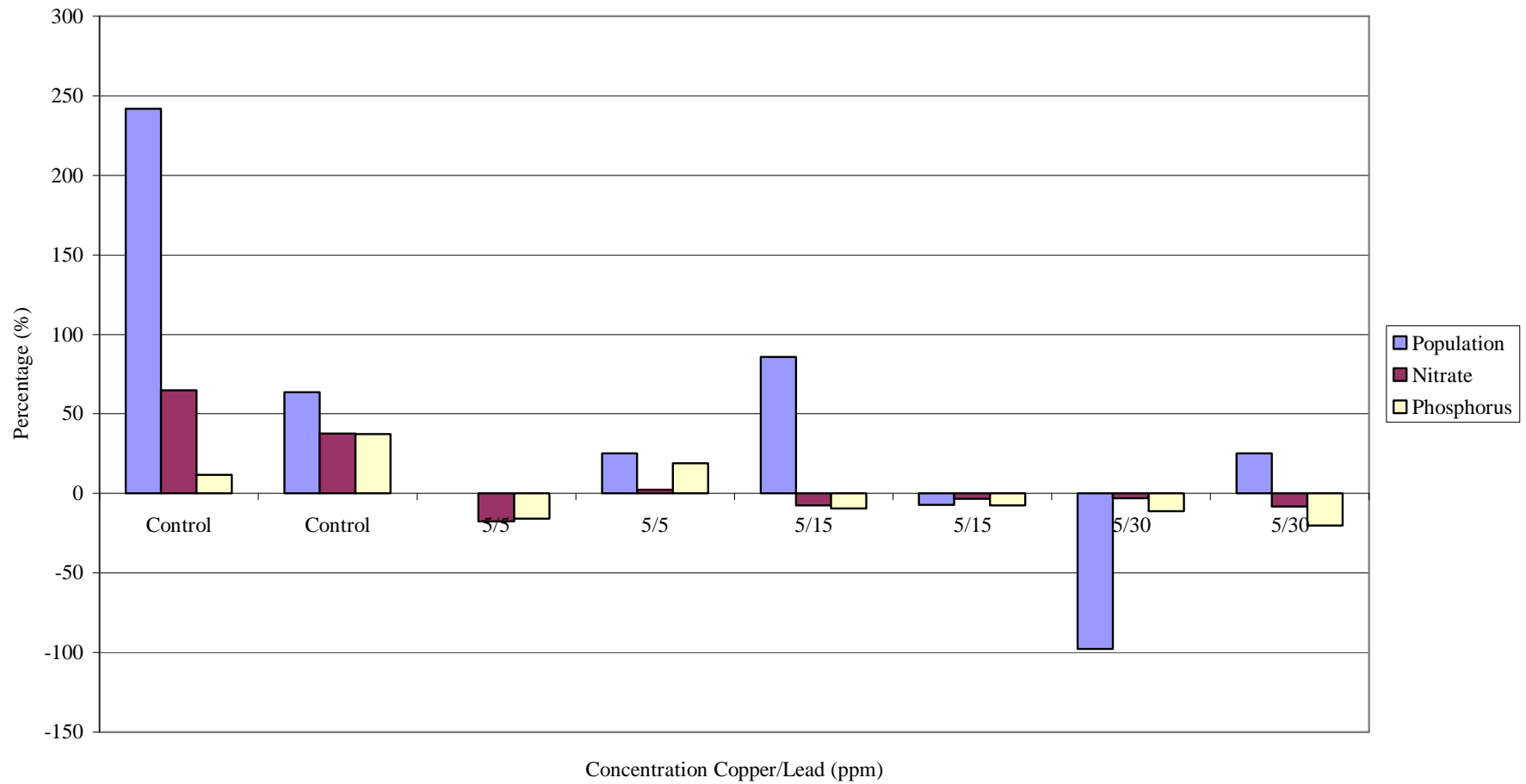


Figure 9-25: The pH of nutrient media for *P. morum*, contaminated with copper and lead

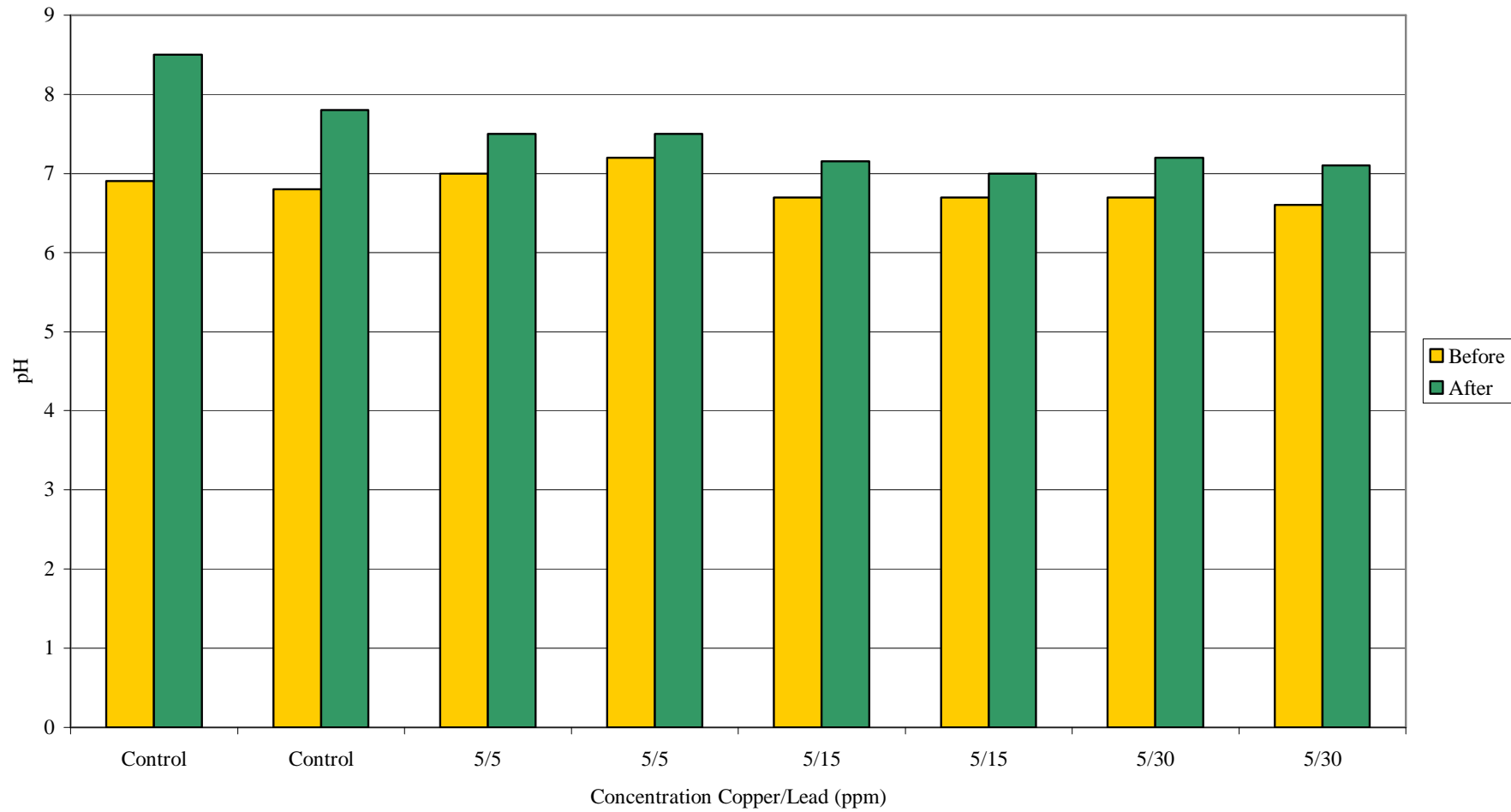


Figure 9-26: The percentage of nitrate and phosphorus used by *P. morum* and associated change in population in nutrient media contaminated with lead and tungsten

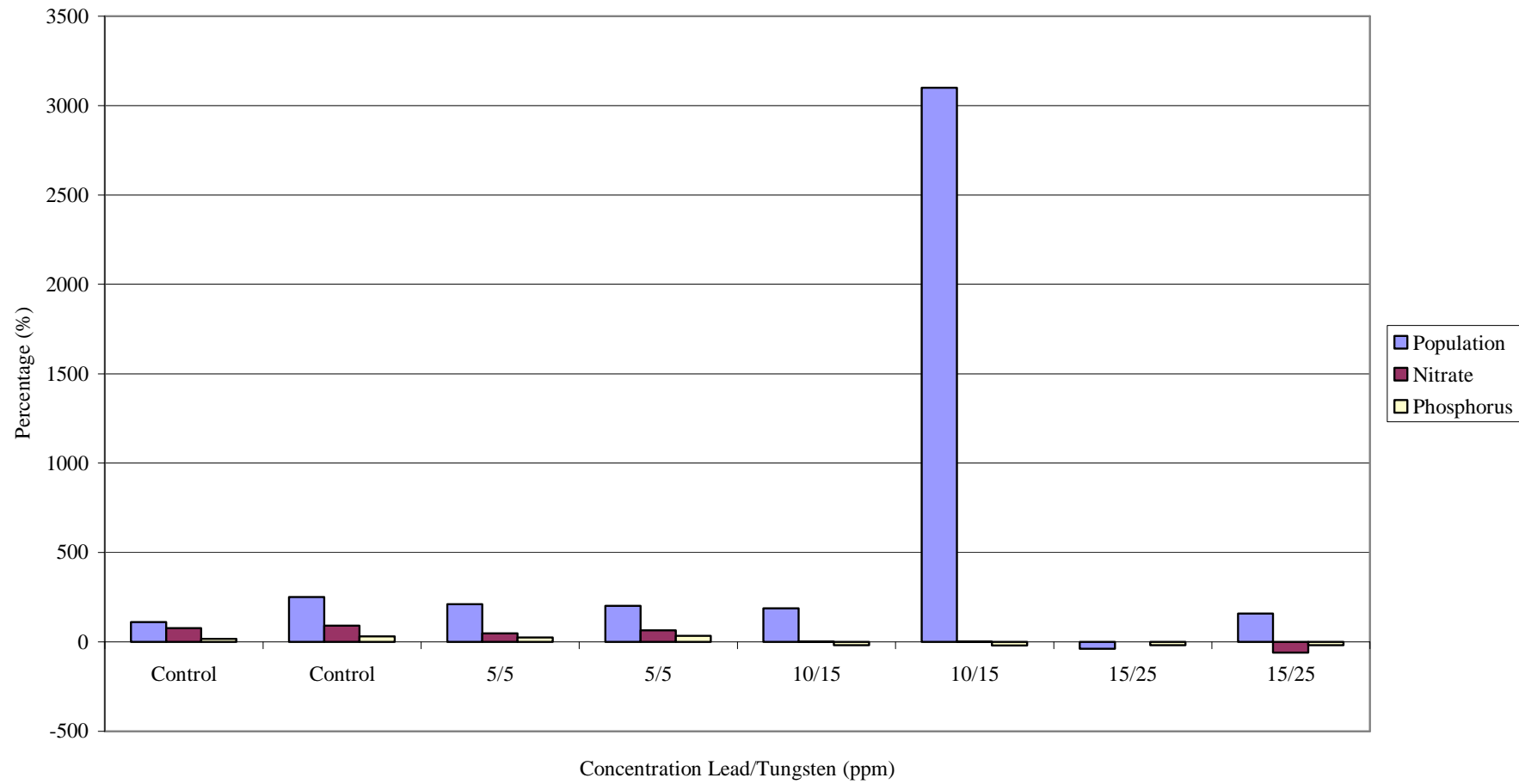
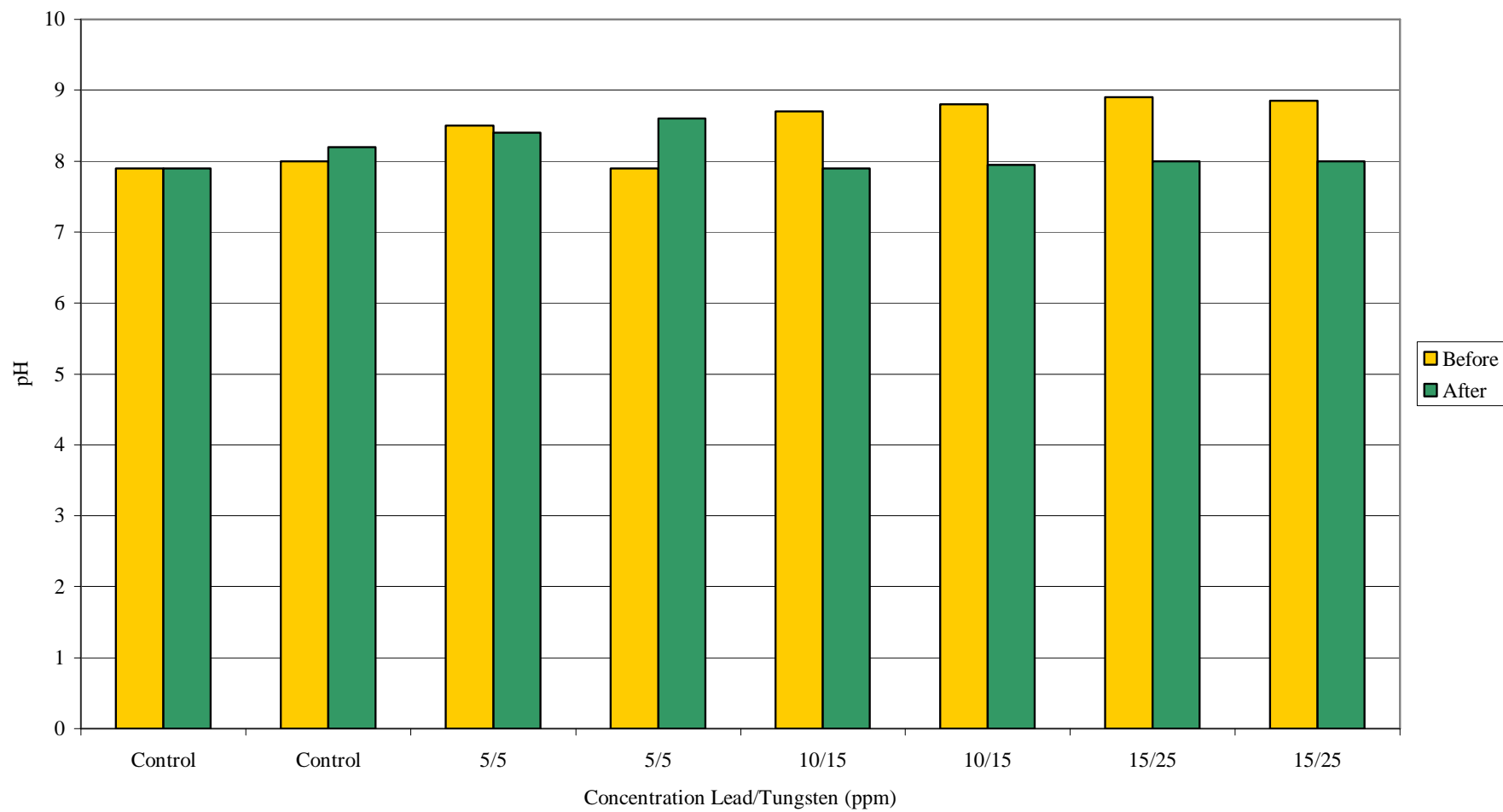


Figure 9-27: The pH of nutrient media for *P. morum*, contaminated with lead and tungsten



9.7. Results – Stage 4: The effects of a combination of copper, tungsten and lead.

The final experiment was to determine the effect of copper, tungsten and lead, in combination, has on the algae. It could be seen in Figure 9-28 that even at the lower concentrations, there was no population growth, and there was also no removal of nitrates or phosphorus from the nutrient media. The pH values given in Figure 9-29 shows that there was a slight decrease in the values. These results would indicate that the combinations of all three metals are lethal to the *P. morum*.

Figure 9-28: The percentage of nitrate and phosphorus used by *P. morum* and associated change in population for nutrient media contaminated with copper, tungsten and lead

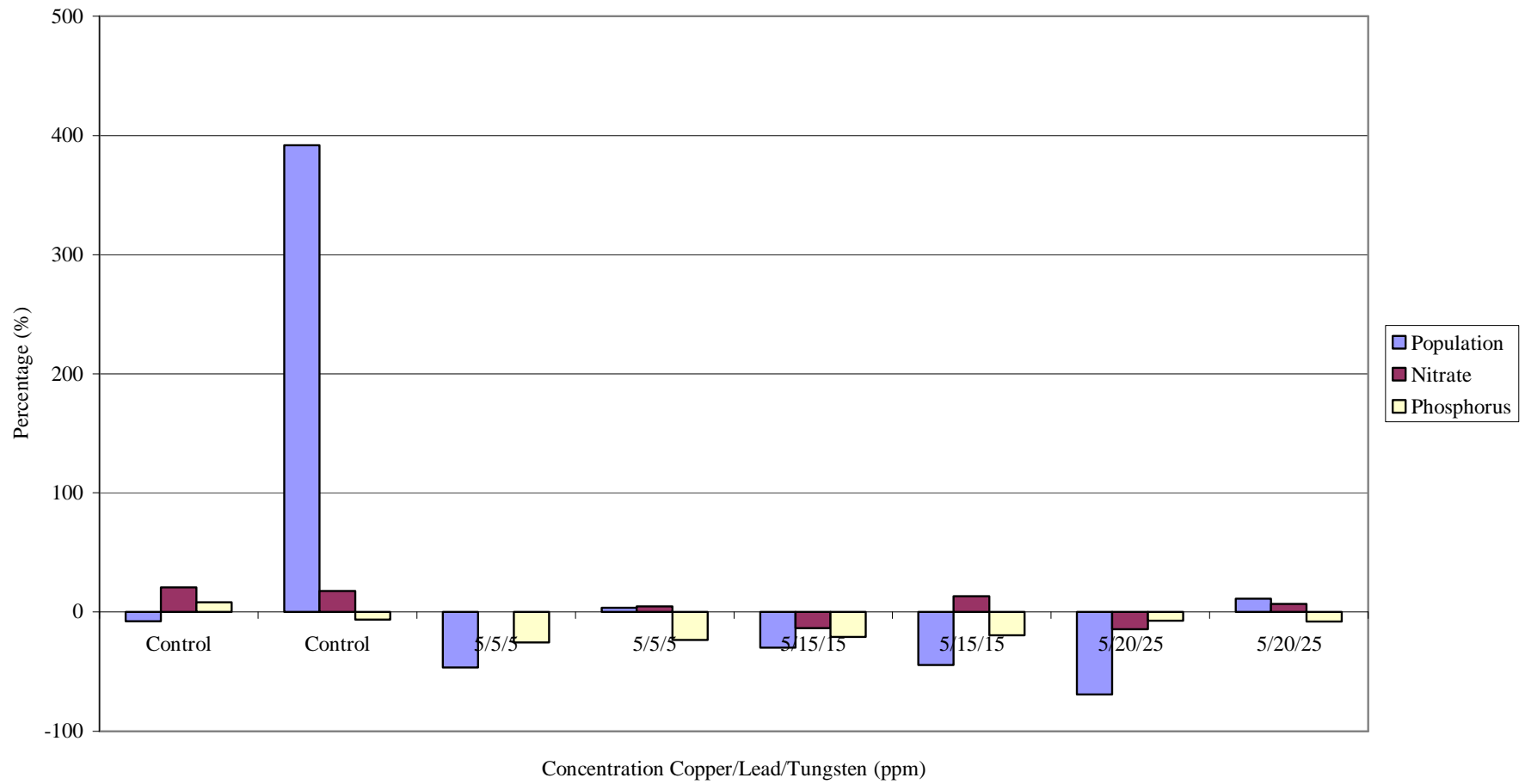
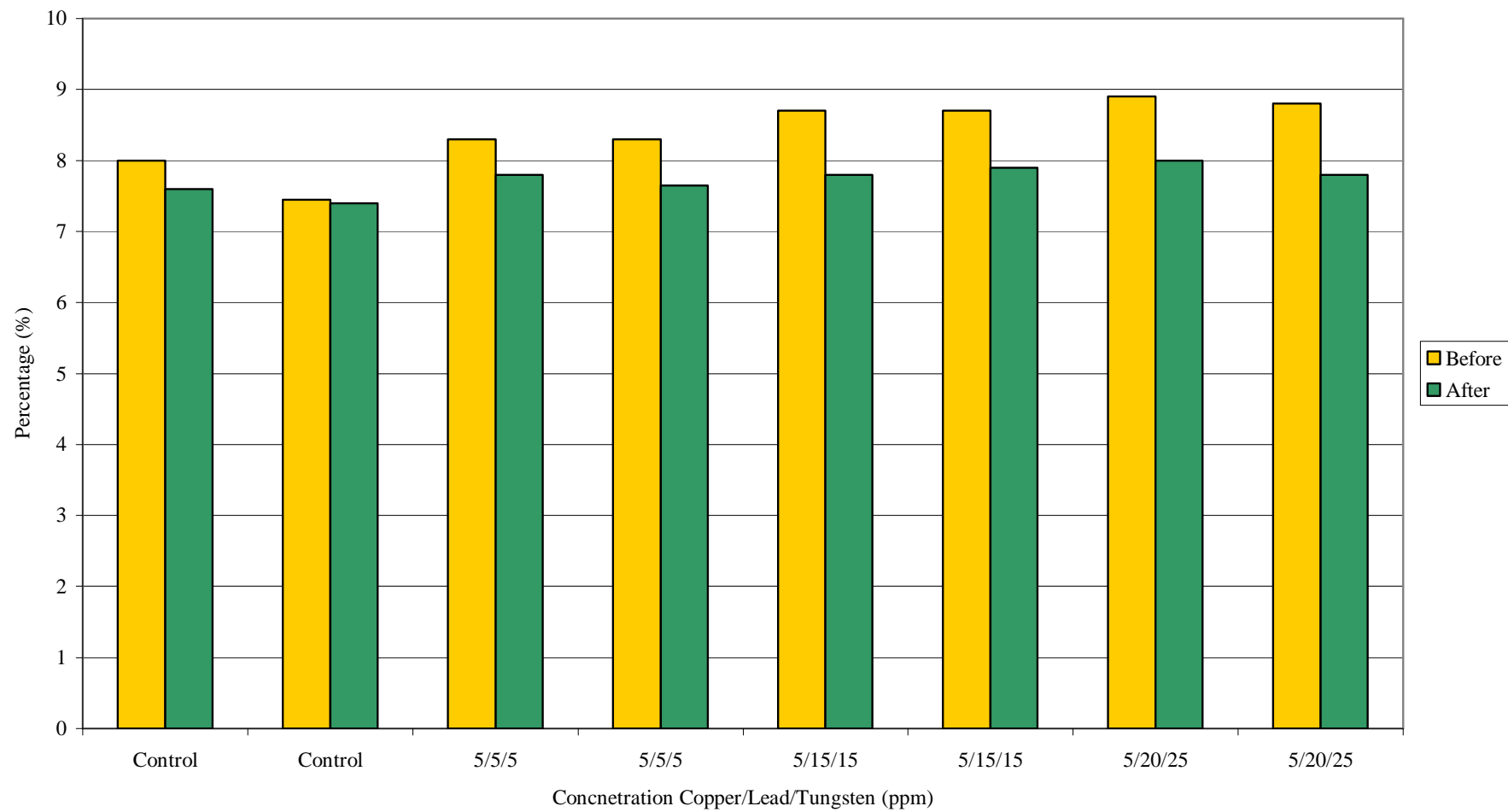


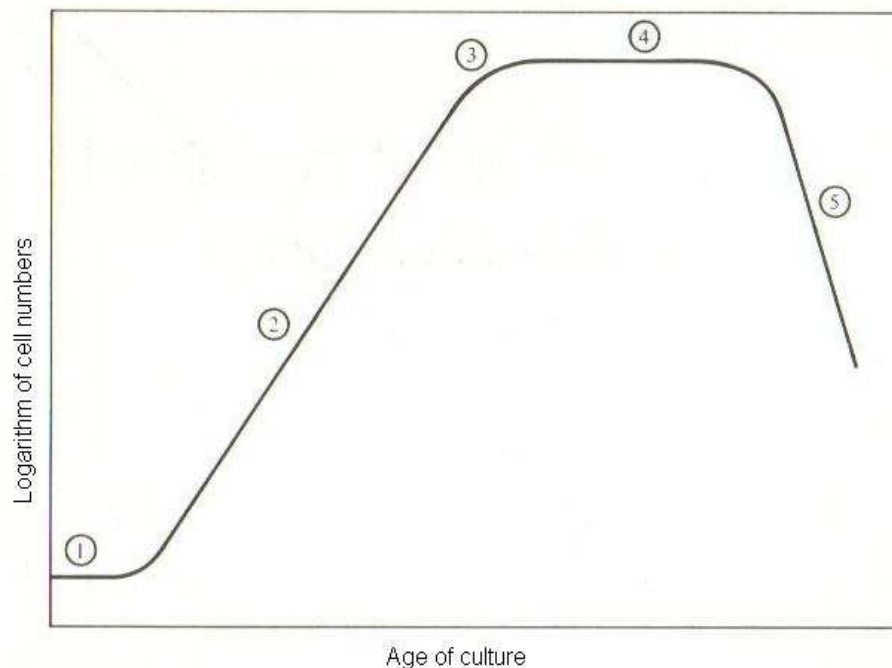
Figure 9-29: The pH of nutrient media for *P. morum* contaminated with copper, lead and tungsten



9.8. Discussion

Within a closed experiment a number of factors need to be considered, including the length of the experiment. When using a limited volume of media, the algae increase in population is limited to the concentration of nutrients, light availability and space. Restrictions of any of these can inhibit reproduction. Figure 9-30 illustrate the growth stages of a unicellular alga in a limited volume of media. It can be seen that during the first stages the population increase at an exponential rate (2), but after this phase the increase declines and the alga enter into a stationary phase (4) where there is no increase or decrease in the population numbers. During the stationary phase the nutrient concentrations become limited and the culture enters into the final death phase (5) where the limiting factors become apparent.

Figure 9-30: The growth stages of a unicellular alga grown in a limited volume of culture medium (Fogg, 1975)



1: Lag phase, 2: Exponential phase, 3: Phase of declining relative growth rate, 4: Stationary phase, 5: Death phase

During the early stages of these experiments it was found that leaving the experiment for longer than 4 weeks, allowed the algal culture to enter into the death phase. It was therefore decided to limit the length of the experiment to 3 weeks. This

allowed the algae to complete the first three stages of growth and to partially enter the stationary phase. At this point it could be determined if it was the added metal ions affecting their growth and not any of the other limiting factors.

Since the experiments were each to run for 3 weeks, space issues became a problem. It was not possible to run a number of different experiments at the same time. When different incubators were used, there was an additional variable that had to be considered when examining the results. The different incubators would have different strengths of light and therefore could alter the rate of growth for the algae. It was decided to use percentage increase/decrease in the analysis.

Each of the experiments used 150mL of media and this proved to be not enough to complete the analysis. When the nutrient media was examined before the application of metals, it was not possible to extract the ideal volume of solution to monitor the heavy metal (10mL), phosphorus (1mL), nitrate (20mL), and ammonia (50mL); a total of 81mLs of solution would be preferred before and after the experiment. Although not ideal, the volume extracted before the experiment was reduced to 40mL, and it was decided not to monitor the ammonia levels. In a further expansion of the experiment, it would be preferred to use 250mL of nutrient media. The increased volume would allow for the analysis of the media before and after the experiment, leaving sufficient media for growth of the algae. However the increase in the volume of nutrient media would increase the size of container and therefore decrease the number of containers used in the incubators.

Towards the end of the experiments, it was found that a number of rotifers had contaminated the algal stock solution. Upon discussion with the suppliers, it was determined that the eggs of the rotifers had contaminated the stock and that this was considered an ongoing problem with the algal stock solution, and as yet has been an extremely difficult to problem to resolve. Therefore the later stages of the experiments could have been affected by the presence of the rotifers, since they too would require phosphorus and nitrates from the medium. However the numbers of rotifers found in the samples did not exceed 1 per mL, and thus their effect on the overall results of the experiment would be negligible. It should be noted that the rotifers were found in samples which had been contaminated by metals.

In algae experiments, it is difficult to determine whether the algae have died. It can only be determined for certain when the cells begin to breakdown. However, in this experiment the lack of movement over a period of time lead to the opinion that the

algae were dead. But this is not conclusive as it has been determined that algae can enter into a state of hibernation. Therefore the results only consider the increase in the population numbers.

From the nutrient components it can be seen that there was already a small concentration of copper, and because it is also a required micro nutrient the algae are capable of surviving in media containing copper. However, excess copper becomes toxic as can be seen in Figure 9-7. Concentrations higher than 8.406ppm reduced the population numbers, and could therefore be concluded that this concentration was high enough to kill the algae.

For both the lead and tungsten experiments, higher concentrations were required as the algae continued to increase in population numbers. It was found that, for lead, a concentration of 151.4ppm still allowed the algae to increase in population numbers, but not as quickly as the other concentrations. The final concentration of 203.2ppm appeared to prevent any population increase. Therefore it can be stated that the TLV for lead would reside in the range between 151.4ppm and 203.2ppm. Further experiments would be able to narrow the figure down, but due to time constraints this was not possible.

From the stage 1 and stage 2 experiments it can be seen that the TLV for tungsten lies between the range of 90.3ppm and 120.5ppm. At 90.3ppm there was still an increase in the population numbers, greater than that of the control, but at 120.5, there was a decrease in the population numbers.

As with all plants, nutrients are required for the continuation of life. Without reproduction the organism will die and become extinct. Any chemical that interferes with the reproduction cycle can have detrimental affects. When the concentrations of tungsten were added to the media, there appeared to be little or no effects. However after a period of incubation it was clear that there was a dramatic increase in the population numbers. These increased and surpassed the populations of the control. Throughout the experiments, the control flask showed an increase in population 100 fold, but when tungsten was added to the media, the increase was 1000 fold. When tungsten is present in the media there is a clear effect on the reproduction rate. However this was not found to occur when copper or lead were also present.

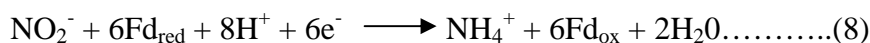
It has been suggested that tungsten substitutes for molybdenum (Hille, 2002; Williams and da Silva, 2002), if this is the case, then the increase in the tungsten could mimic the effect of increased levels of molybdenum. However the concentration of

molybdenum in the nutrient media is approximately 4.732×10^{-4} mg/L, therefore the concentration difference between before and after would be negligible and assuming that tungsten could substitute for the molybdenum, a change in the tungsten concentration would not necessarily be detected. In plants (and algae) molybdenum is essential for the assimilation of nitrogen. Molybdenum is a major component of the enzyme nitrate reductase.

The process of nitrogen assimilation involves a number of steps; nitrate to nitrite to ammonia. These are achieved indirectly with photosynthesis through its products (Round, 1973). NADPH produced during the light reactions of photosynthesis is used by the enzyme nitrate reductase to convert nitrate to nitrite:



The ferredoxin (also from the light reactions) is then used by nitrite reductase to convert nitrite to ammonia.



During the conversions, the products are then recycled back into the photosynthesis processes. Because the assimilation is indirectly related to photosynthesis, it is also controlled by some of the factors affecting photosynthesis, such as light availability, oxygen and carbon dioxide concentration and nutrients.

As a by-product of nitrogen assimilation, ammonia is produced and is then used in the Calvin Cycle. The ammonia could not be determined for the experiments, due to the volume of solution required; however it can be assumed that the ammonia production is related to the pH. Because ammonia is used in the production of amino acids it would therefore be assumed that the concentration in the nutrient media should not increase. However, when there is an ample supply of nitrate it could possibly encourage the conversion of nitrate to ammonia regardless of the rate of ammonia being used in metabolic processes; the unused ammonia would then be transported out of the cell and into the media, thus increasing pH.

Throughout the experiments, where there was successful growth of the algae, there was an increase in the pH of the medium and this corresponds to results from previous experiments (Round, 1973). The initial pH values varied depending on the individual

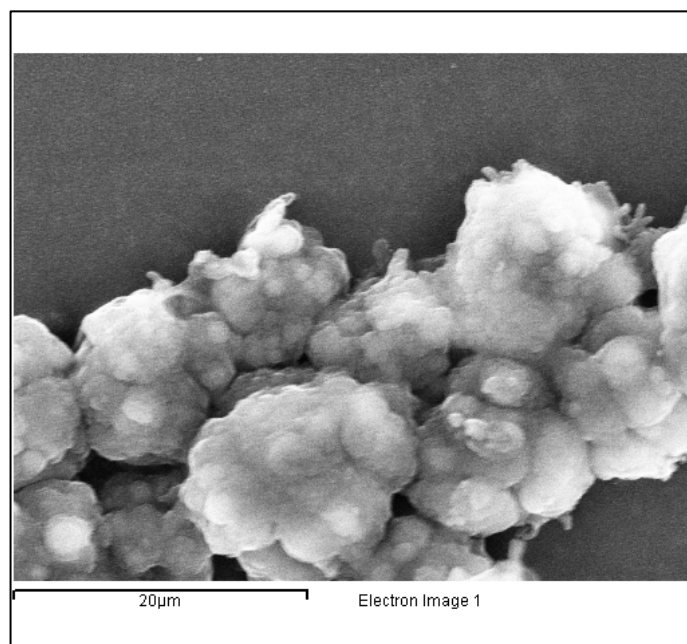
metal and the concentration that was added. Where there was little or no increase in population numbers the pH decreased. This indicates that the nitrogen assimilation was not occurring and therefore there was no production of ammonia.

When examining the results from the tungsten applications, it can be seen that the pH of the media decreased after the application of 23.23ppm of tungsten. This suggests that ammonia was not being produced. However the population increased to numbers exceeding that of the control. It is also interesting that although the population was increasing at the higher concentrations of tungsten, the percentage of nitrates used was less than that of the controls. This corresponds to the possible lack of ammonia being produced. It should also be noted that the percentage of phosphorus used was at similar levels when compared to the controls and this would indicate that photosynthesis and reproduction was still occurring but nitrogen assimilation was not.

It is possible that other metabolic processes have been altered, for instance the production of the mucous layer that surrounds the colonies. During this process, products from photosynthesis and nitrogen assimilation are used to form sugars and proteins. Any interference of this process could reduce the layer surrounding the colony. If tungsten is interfering with the production of ammonia as is theorised, it would occur during the early stages of nitrogen assimilation, that is, with the conversion of nitrate to nitrite. Therefore it is likely that the tungsten is altering the molybdenum based enzyme nitrate reductase, either through substituting for the molybdenum or acting on the enzyme reducing its activity. Because the nitrates are still being used, it is more likely that the former is the case, since some normal activity was still occurring. Because the number of colonies has increased dramatically when compared to the control, reproduction is occurring at a faster rate. Observations noted that the colonies appeared to be more numerous and smaller when compared to the controls. During the life cycle of the alga, a mucilage layer surrounds the colonies. A possible explanation for the increased number in algal colonies is that the mucilage layer is inhibited by the presence of tungsten. In normal circumstances the layer protects and provides some structure to a number of colonies. However the presence of tungsten could alter the properties of the layer and therefore not containing the colonies. More of the individual cells enter into asexual reproduction after being excluded from their original colonies. The number of cells per colony would decrease and but the number of overall colonies would increase. Experiments to determine this would include extensive testing for the mucilage surrounding the colonies.

The presence of lead on its own seemed to have little or no effect on the population of the algae. However, there is a change in the concentration of the lead ion in the media. There was a definite decrease in the concentration of lead when comparing the before and after experiments. This did not occur for either the copper or tungsten, it also did not occur when the metals were applied in combinations. It is possible that the lead was accumulated into the algae or that the lead was adhered to the surface of the algae. It was attempted to determine which by using a scanning electron microscope but this was unsuccessful. A photograph from the microscope is shown in Figure 9-31. The results were inconclusive, but the method could be improved by embedding the algal samples in resin and then thinly slicing sub samples for examination under the electron microscope. It would then be possible to examine the infrastructure of the algal cells as well as the surface. Theoretically, it would be possible to determine if the metals were accumulated in the cells or adhered to the cell surface.

Figure 9-31: An electron microscope photograph of *P. morum*



During the photosynthesis cycle, the production occurs through the use of ATP. ATP becomes reduced to ADP or AMP. To recycle the basic molecule phosphorus is taken up from the surrounding media and added to the reduced state ADP and AMP to again become ATP. This can then be used in the cycle. Plants in general (Including

algae) utilise phosphorus in the form of orthophosphate (OP) (Fogg, 1975). Some of the results from the experiments showed that there was an increase in the concentration of phosphorus in the medium; this is possibly due to the breakdown of the cell structure thus releasing the phosphorus from molecules such as ATP. The increase in the phosphorus concentrations corresponds to experiments where it was found that the algae had not survived the concentrations of the metal ions.

When the elements were applied together they produced a toxic effect. Therefore in a natural environment, the presence of more than one contaminant would be sufficient to kill the algae. Even when there is small concentrations of copper (~5ppm), the combinations of lower concentrations of lead or tungsten is enough to kill the algae. In freshwater habitats, more than one contaminant is released. This would lead to the conclusion that the algae would not be able to survive in a situation where more than one contaminant was present. This could lead to the conclusion that in an environment where the algae were found naturally, their absence could be used to determine that there were a number of contaminants present. The algae could be used as bio indicators rather than hyper-accumulators. However, in the unlikely event that only lead is found in a freshwater system, the algae could be used to extract some of the lead from the water. Although it has been stated that the combination of lead/copper, lead/tungsten, copper/tungsten and lead/copper/tungsten has a detrimental effect on the algae, other combinations may not have the same effect. For example, it was not determined if combination of lead and zinc could have a detrimental effect on the algae. The next stage of the experiment would be to test the algae with contaminated water taken from a polluted site.

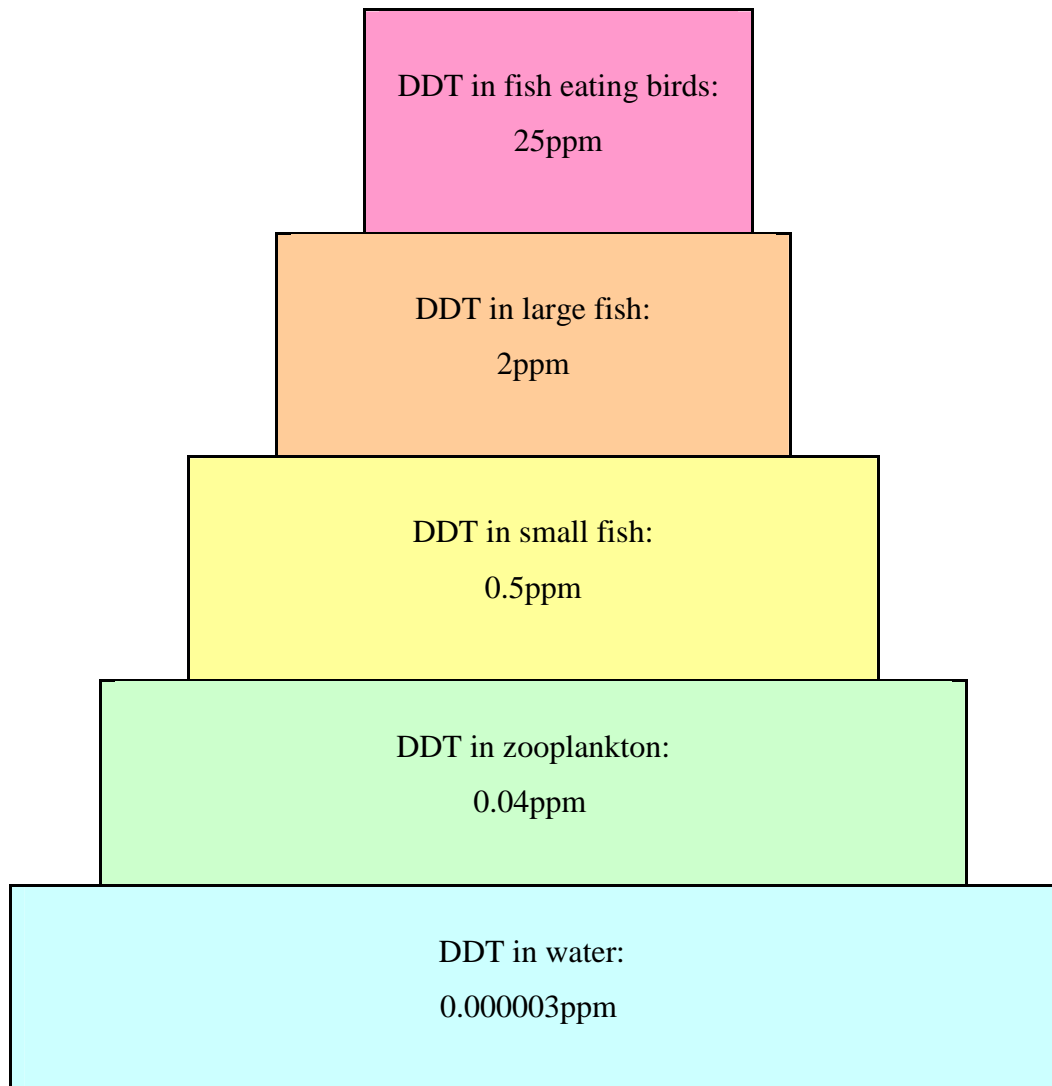
Chapter 10: Accumulation of metals in humans and other animals

10.1. Metal Accumulation – An introduction

The accumulation of metals through the trophic levels has presented a number of issues, including the possible biomagnification of contaminants through the levels. Biomagnification occurs when the concentration of the contaminant increases with the higher trophic levels. An example of this was seen when the effects of dichloro diphenyl trichloroethane (DDT) were shown to increase through the trophic levels including detectable levels in humans (Trojanowska *et al*, 1972; Rudd *et al*, 1981).

During the World War II DDT was used as an insecticide for the control of insects such as mosquitoes and agricultural pests. DDT was found to be an effective insecticide but was too persistent in the environment and easily transported by water over great distances. After the publication of *Silent Spring* by Rachel Carson (1962), there was increased pressure to remove the insecticide from use as it was believed to cause cancer in humans. By the early 1970s it was found that the insecticide was causing damage to bird populations including *Haliaeetus leucocephalus* (Bald Eagles), where numbers had dropped (Grier, 1982). The decision was made to remove the insecticide from use and was banned in most developing countries. Figure 10-1 shows the concentration increase that was found in animals of different trophic levels.

Figure 10-1: The biomagnification of DDT through trophic levels (modified from Campbell, 1996)



Although heavy metals are not considered as contaminants in the same sense as DDT, it is possible that they could have similar biomagnification effects on the trophic levels. With increasing concentrations of heavy metals in the environment, the long term effects are causing great concern. The metals cannot be destroyed and therefore have persistent effects on the environment. In cases where the metal is capable of imitating nutrients within plants and animals, there is an increase in the concentration that can become accumulated. For instance both lead and tungsten can imitate nutrients that are required and can replace these nutrients. Lead is chemically similar

to calcium and could therefore substitute for calcium in bones of animals; tungsten is chemically similar to molybdenum and can replace it in plant processes.

In most cases the effect of the metal is dependent on the availability and its concentration in the environment. If the metal is considered as unavailable to either the plant or animal, uptake will not occur thus accumulation is not possible. The concentration of the metal is important as if the concentration is very small; it is likely to be removed from the plant or animal naturally. In plants, when there is a presence of an unwanted substance, it can be stored in the vacuole where it cannot affect the plant. In animals the same partitioning can occur, but more likely the unwanted substance can be passed through the system and is removed with the waste. However, when high concentrations enter the body, the contaminants cannot be excreted effectively and can then be stored in parts of the body. It is this that causes the concern as the contaminants are not “locked” away, and can be removed from the stores and affect body systems. Where the contaminants are chemically similar to components required in processes throughout the body, the contaminants can be removed from the stores instead of the component. This can affect the processes, and can also cause the processes to stop. Examples of this are the substitution of lead for calcium in structural processes (bone manufacture) and neurological function.

10.2. *Human consumption of contaminated foodstuff*

In today's climate, the presence of metals in foodstuffs has been found to be a regular occurrence. Because of this, the European Community (and other governing bodies) has described limits of certain contaminants permitted in food and water. Table 10-1 gives examples of these limits for lead and cadmium, taken from the Commission Regulation EC 466/2001 (Official Journal of the European Communities, 2001).

Table 10-1: The concentration of lead and cadmium limits in food for human consumption

Food Item	Lead (mg/kg)	Cadmium (mg/kg)
Cows Milk	0.02	-
Meat of bovine animals, sheep, pig and poultry	0.1	0.05
Cereals	0.2	0.1
Fruit	0.1	0.05

The concentration that is present in the food and water is dependent on the concentration of the contaminants found at the site of origin. For instance, vegetables grown in an area close to mining activities are likely to accumulate contaminants from the soil. However, where the contaminants are in a chemical form that is unavailable for plant uptake, the process of accumulation cannot occur and it could be termed safe for the growing of edible vegetation.

During visits to Devon, a number of market stalls were found to be selling local produce. Selections of these products were purchased to examine the concentration of heavy metals in the food. There were two vegetables (kale and cabbage) and some cheese (Cornish Yarg). The kale was grown around Gunnislake; approximately 4km from Blanchdown Wood, the remaining samples were only described as locally produced.

Because these products were locally produced, it is likely that there may be a small concentration of heavy metals present. If this was proven correct, then it would mean that there the heavy metals could enter into the food chain.

10.2.1. Aims and objectives

The aim of this section was to determine if heavy metals had been accumulated into food that was sold for human consumption.

10.2.2. Experimental procedure

During a sampling visit to Blanchdown Wood, some cabbage, kale and Cornish yarg were purchased from a nearby village. It was advertised that the kale had been grown locally in Gunnislake, 8km from Blanchdown Wood. The Cornish yarg and cabbage were described as being produced locally, but with no further details. The samples were stored in sealed plastic bags and upon return to the laboratory were washed thoroughly and dried. The samples were then digested as discussed in Chapter 5.

10.2.3. Results

The results of the digestion analysis are shown in Figure 10-2 and 10-3. It shows that although copper, tungsten, lead, arsenic and tin were analysed for, only copper and tungsten were found. All three samples were found to contain small concentrations of copper (Figure 10-2). The Cornish yarg and the kale were found to contain an average of 7.689 and 7.248mg/kg respectively. The cabbage however contained an average of 28.677mg/kg of copper. The tungsten concentrations, shown in Figure 10-3, for all samples did not exceed 1.5ppm. It was found that a number of the replicates did not contain a detectable concentration of tungsten.

Figure 10-2: The concentration of copper in foodstuffs collected from Devon, UK

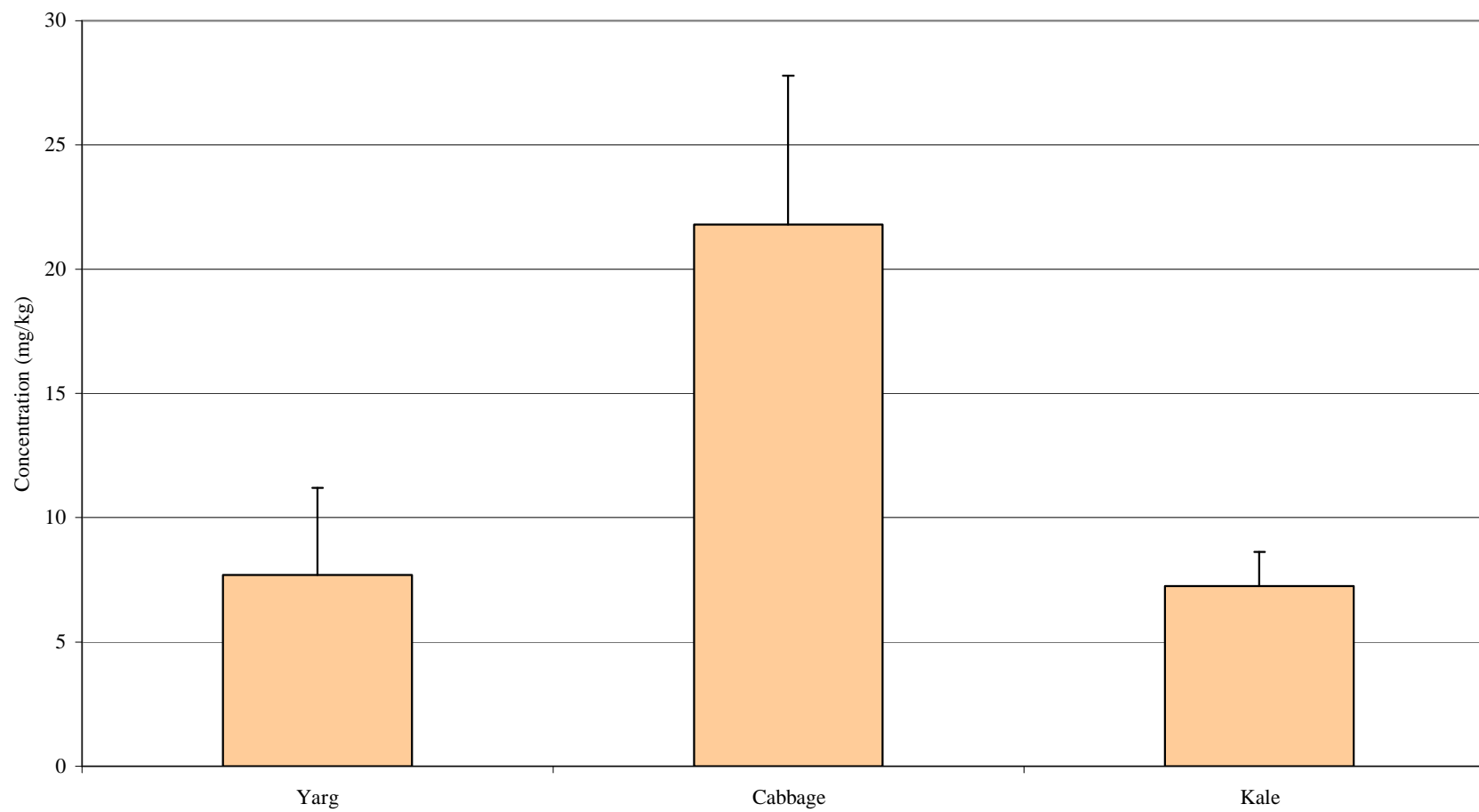
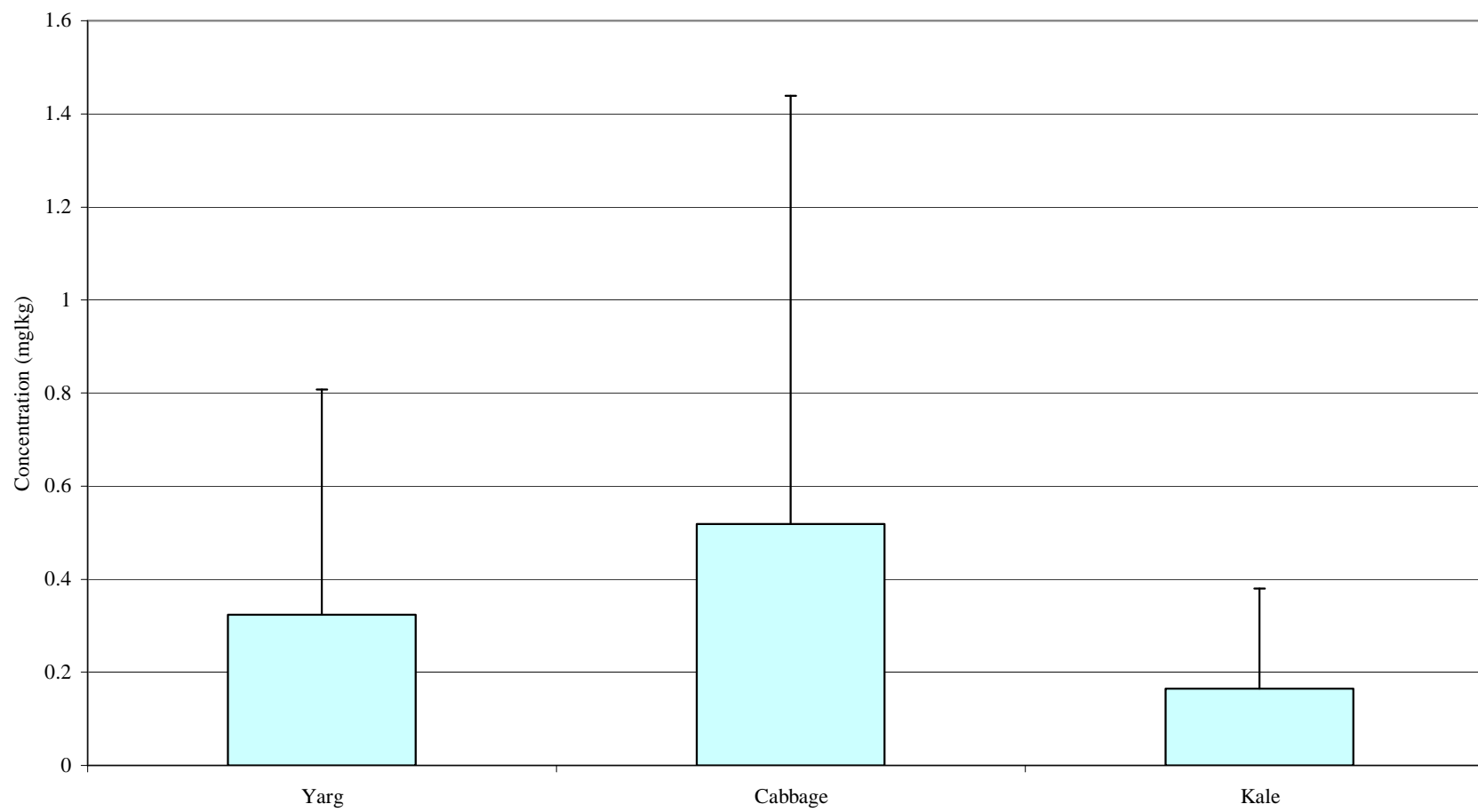


Figure 10-3: The concentration of tungsten in foodstuffs collected from Devon, UK



10.2.4. Discussion

During the analysis it was found that the concentration of tungsten varied between the replicates for all samples. Table 10-2 shows the results of the replicates. It can be seen that a number of the replicates did not contain any tungsten; therefore the average would be affected. Ideally control samples should have been used to compare the results to, however the cabbage and kale could have been grown anywhere in the country, and therefore it would be impossible to determine if the growing site was in an area of contaminated land.

Table 10-2: The concentration of tungsten in the replicates for cabbage, kale and Cornish Yarg

Cabbage (mg/kg)	Kale (mg/kg)	Cornish Yarg (mg/kg)
0.099	0.794	2.483
0	0	0
4.267	0	0.599
0	0	0
0.298	0	0.150
0	0.851	0
0	0	0
0	0	0
0	0	0
0	0	0

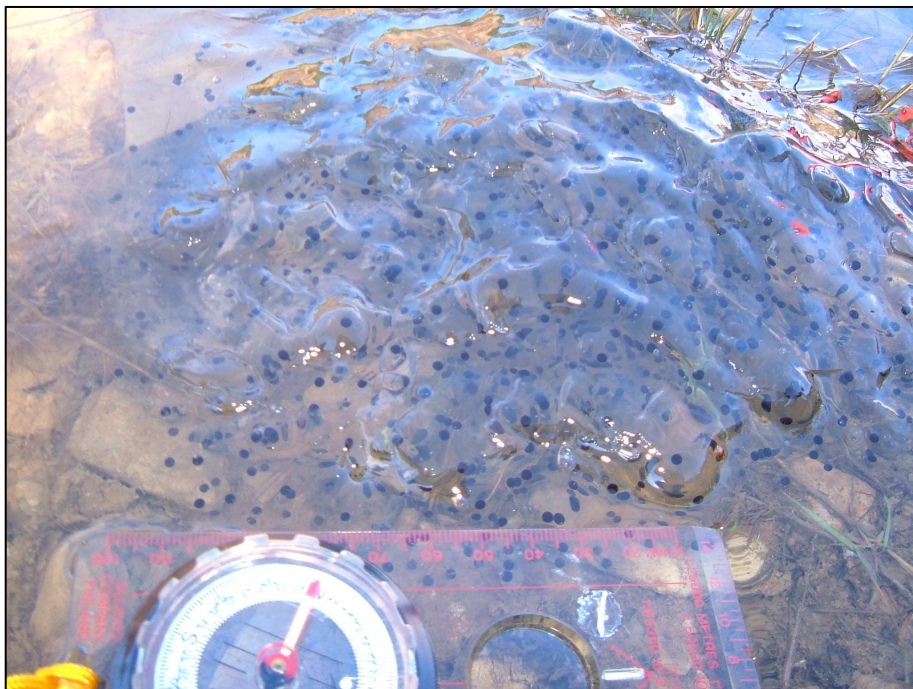
Since the Cornish yarg is a cheese delicacy that is predominantly made in Cornwall, it would be difficult to obtain a control sample. It cannot be determined if the pasture sites was uncontaminated. For the production of the yarg, the cows are believed to have been pastured on the edge of Bodmin Moor (Lynher Dairies, 2006), but it is not known whether this site was contaminated. Many of the waste sites of mining activities were not always documented. Therefore it could be possible that the animals were feeding in an area that was contaminated. The yarg is made from pasteurised cow milk and is wrapped in nettle leaves to give its unique flavour. The

origin of the nettle wrapping is also unknown, and could therefore be a source of contaminated. From the results it can be seen that there was a small concentration of copper and tungsten found in the cheese, but it is unclear as to where the contamination originated. However, it does demonstrate how copper and tungsten can be introduced into the human food chain, either through the consumption of vegetation (nettle wrapping) or from the transfer through the trophic levels (vegetation-cow-human).

10.3. Metal content in reproductive products

In the classification of the animal kingdom there are more than 33 phyla described. The most commonly known phylum is that of chordate, containing the animals with backbones. These can be further divided into classes including birds (Aves), reptiles (Reptilia), mammals (Mammalia) and amphibians (Amphibia). The reproduction process of these can be divided into those that produce live young and those which lay eggs. In most cases where the young are produced via eggs, the egg structure provides some nutrients for the young in the early days of life. All the eggs have one thing in common, the fact that the embryo is surrounded by a protective layer enclosing fluid containing nutrients. For amphibians (e.g. Frogs) the embryo is surrounded by a jelly like substance. The jelly is produced after the fertilisation process where water is absorbed to produce a jelly like substance which surrounds the embryo providing protection. In the early stages of life, after the embryo has developed, the tadpole eats a portion of the jelly, before starting to feed on algae within the water environment. Figure 10-4 shows a sample of frog spawn that was found at Blanchdown Wood.

Figure 10-4: A photograph of frog spawn at Blanchdown Wood, Devon, UK



Bird eggs are produced after mating has occurred, but eggs do not necessarily hold fertilised embryos. The protective shell is composed primarily of calcium carbonate and its condition can determine the successful hatching of the young. If the shell is of poor quality, it can break under the pressure of the adults incubating the egg, before the offspring is ready to emerge.

The calcium carbonate is formed by the female; factors that affect the shell formation include malnutrition. Where there is a possible chance of metal accumulation in the female bird, the shell quality can be affected. The shell can become thin and weak, causing it to break during the incubation period. Thus the quality of the shell can give an indication as to the quality of the female bird.

10.3.1. Aims and objectives

The aim of this section was to determine if concentrations of copper, lead, tungsten, tin and arsenic were accumulated in egg shells and frog embryos found at Blanchdown Wood, Devon.

10.3.2. Experimental procedure

During visits to Blanchdown Wood, samples of frog spawn were found in a small pond. Some of the spawn was collected and placed in a sterilised bottle containing 70% industrial strength methylated spirit (IMS) to prevent any deterioration of the sample. In the laboratory, the IMS was filtered off, leaving the spawn. The spawn was shaken for 1 hour on an automatic shaker and then filtered through a Whatman No 1 paper. The intention was to separate the jelly from the embryo. This would allow for the determination of the concentration of metals passed on to the offspring before it has had contact with the environment. Also as the jelly absorbs water from the environment there could possibly be contamination from the water incorporated into the jelly. The tadpoles were then digested as discussed previously in Chapter 5.

The egg shells that were found were stored in sealed plastic bags. Unfortunately during the transport process, the shells were broken and it could not be identified which species of bird the shells came from. When back in the laboratory the shells were carefully washed, in de-ionised water, to remove any contaminants on the surface. The samples were then digested as discussed in Chapter 5.

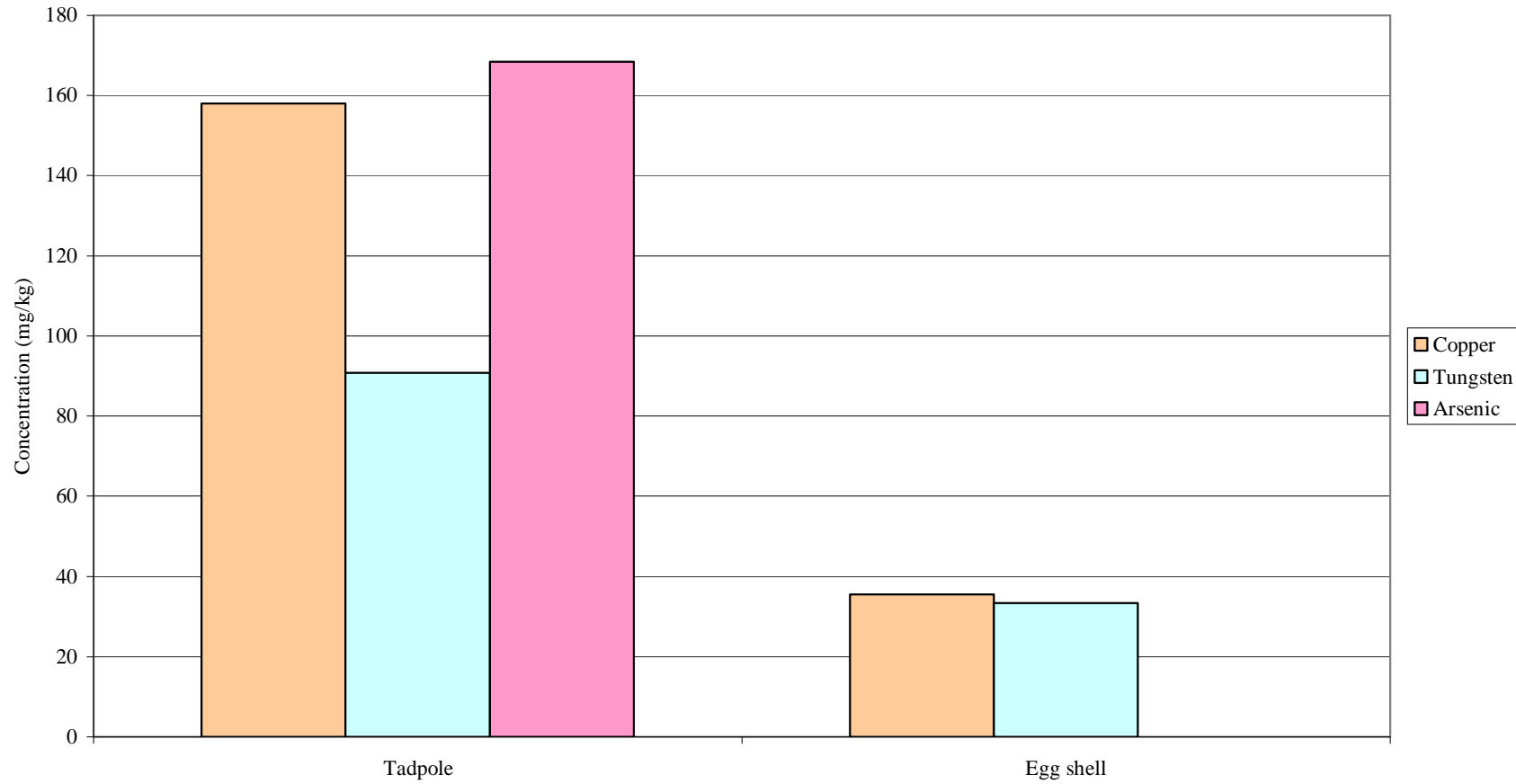
10.3.3. Results

Figure 10-5 illustrates the concentration of copper, tungsten and arsenic found in the frog embryos and egg shell found at Blanchdown Wood Devon. It can be seen that there is a relatively high concentration of copper (157.96mg/kg), tungsten (90.84mg/kg) and arsenic (168.41mg/kg) in the frog embryos.

The concentrations of copper and tungsten in the egg shell were 35.46mg/kg and 33.36mg/kg respectively.

During the analysis, concentrations of lead and tin were not found in either the egg shell or the frog embryo.

Figure 10-5: The concentration of copper, tungsten and arsenic in frog spawn and egg shell sampled at Blanchdown Wood, Devon, UK



10.3.4. Discussion

As a species, frogs generally eat small invertebrates such as insects, slugs, snails and worms. The analysis showed that the embryo of the frogs contained high levels of copper, tungsten and arsenic. Since the embryo is a product of the parents, material from the parents would be used to form the embryo. The jelly like protective shell is introduced around the embryo and then absorbs water from the surrounding environment and swells to form a protective layer. The soil from the pond, where the spawn was collected, was analysed and was found to contain high concentrations of copper, tungsten, lead, tin and arsenic. Because of the high concentrations in the soil, it is likely that the water would contain similar concentrations; therefore the protective jelly would absorb the contaminants from the water and become a component of the jelly. Once the embryo has developed into a tadpole, the protective jelly is consumed by the tadpole. When the tadpole is ready, it moves out of what is left of the protective jelly and begins to feed on algae in the water.

Because it was difficult to remove all of the protective jelly from the embryos, it is likely that there may have been a small concentration of the copper, tungsten, and arsenic, however if there was some contamination then there would also be small concentrations of lead and tin, but these were not found. It is more likely then that the embryo itself contained the contaminants.

The presence of the contaminants in the embryo indicates that the parent is likely to have passed on small quantities to the offspring. Because the egg is fertilised out of the female body by the male, it is likely that it is the female passing on the contaminants and that she is likely to have accumulated these contaminants. The contaminants are probably accumulated from the diet of the frog, feeding on insects and other small invertebrates, which are generally eaten whole. The invertebrates may not have accumulated the contaminants from the environment through their food, but may have had dustings from the soil. Particles of contaminated soil could have been on the ingested invertebrates and therefore the contaminants would have been consumed. The lack of lead and tin in the sample could indicate that they are being stored in areas of the body where their removal is difficult or that these two elements are not available to the stored in the frogs' body and are removed efficiently through excretions.

In the egg shell, only tungsten and copper were found. Recent studies by Skrivan *et al* (2006) demonstrated that in hen eggs, an increase in the dietary copper

concentrations increased the concentration of copper found in the egg shells,. They found that by increasing the concentration of copper in the birds diet from 9mg/kg to 35mg/kg increased the concentration of copper in the egg shell by 26%. Therefore although copper is necessary in the diet of birds, increased concentrations result in the removal of the excess copper through excretion or possibly, to some degree, during the egg laying process. However, Skrivan *et al* (2006) also showed that there was not a significant increase in the copper concentration in the embryo (yolk or white).

Because copper is required as a micronutrient, it is possible to find small concentrations in different parts of the body. Tungsten is not required by animals (including humans) or by the majority of plants, however previous evidence discussed in Chapter 9 has suggested that tungsten imitates molybdenum in certain processes. It is likely that during the formation of the egg shell, tungsten is supplemented for molybdenum and therefore can be found in the shell.

A clear indication of the transfer of these contaminants through trophic levels is shown by the presence of copper and tungsten in the egg shell. Although the species of the bird that laid the egg could not be identified, it does show that the parent bird had consumed the contaminants and a concentration of these contaminants was deposited in the egg shell.

10.4. Bird of prey pellets

The birds that eat prey are divided into two groups the *Falconiformes* (daytime hunters) and *Strigiformes* (owls). These are the only birds that eat meat, and so have developed special techniques to consume the prey. This includes the development of a strong beak and talons for catching and killing their prey. These birds cannot chew their food and so eat the animal whole. Where the prey is too large to be eaten whole, it is torn into smaller pieces before being swallowed. The food is passed directly into the digestive system and the digestion process begins in the first part of the stomach (proventriculus) where the lining produces enzymes, acids and mucus which breakdown some of the food. The partially broken down food is then passed to the second part of the stomach (gizzard). The gizzard does not contain any digestive glands, but acts as filter, retaining insoluble parts of the prey such as the bones, fur, teeth and feathers. The remaining soluble parts are then passed on further to the small and large intestines where digestion is completed through the absorption of nutrients through the linings of the intestines (Parry-Jones, 1998).

Several hours after eating, the indigestible parts of the prey are compressed into a pellet in the gizzard, once formed the pellet travels back to the proventriculus where it remains for up to ten hours before being regurgitated and expelled from the body. Regurgitation usually signifies that the bird is ready to feed again, as once a pellet is formed the bird can not feed until it has been expelled from the body (Parry-Jones, 1998).

10.4.1. Aims and objectives

During this part of the research there were two main aims:

- To determine if the carnivorous birds ingested concentrations of metals from the prey
- To determine if the prey had accumulated metals

10.4.2. Experimental procedure

During a visit to Blanchdown Wood, Devon, two pellets were found and placed in a plastic bag and sealed. Upon return to the laboratory, the pellets had dried out. The samples were placed in petri dishes and samples of hair and bones were removed. The species of owl the pellets came from, could not be determined; the bones were preliminary identified by Professor Brian Pyatt as a small rabbit. Figure 101 shows the bone fragments which were found in the pellets.

The remaining samples were sieved through a 1mm sieve and samples less than 1mm and greater than 1mm were placed in sealed containers with 200mL of distilled water and placed on a shaker for 30mins. The mixed samples were filtered through Whatman No 1 filter papers. The filter papers were then dried and the particles were removed from the paper into petri dishes. These were then dried for 7 days in a closed cabinet. The bones were washed thoroughly and dried. Once dried, all the samples were digested as discussed in Chapter 5.

Figure 10-6: Bone samples found in the owl pellet



It was not possible to determine which bird deposited the pellet, although during visits to the area sightings of buzzards were made. These were not what produced the pellet, as the pellets were too small. The pellets were estimated to be 4cm long and approximately 2.5cm wide. Buzzard pellets are approximately 6-7cm long and 3cm wide (Bang, 2004).

10.4.3. Results

Figures 10-7 and 10-8 show the concentration of arsenic, copper, tungsten, tin and lead found in different samples from the owl pellet. It can be seen that the bone samples contained high concentrations of lead (1000.34mg/kg), arsenic (28236.9mg/kg) and tin (197.23 mg/kg). The concentrations exceed the values that were found in the soil from all sites. It should also be noted that the concentration of copper found in the pellet material was 1718.59 (DV24/PE1 (<1mm)) and 1975.0mg/kg (DV24/PE2 (>1mm)).

During the preparation of the sample it could be seen that there were small particles of spoil present in the owl pellet material and a number of seeds and seed casings which could not be identified.

Figure 10-7: The concentration of arsenic in samples from owl pellet collected from Blanchdown Wood, Devon, UK
(see Tables 7-1 and 7-2 for key to sample names)

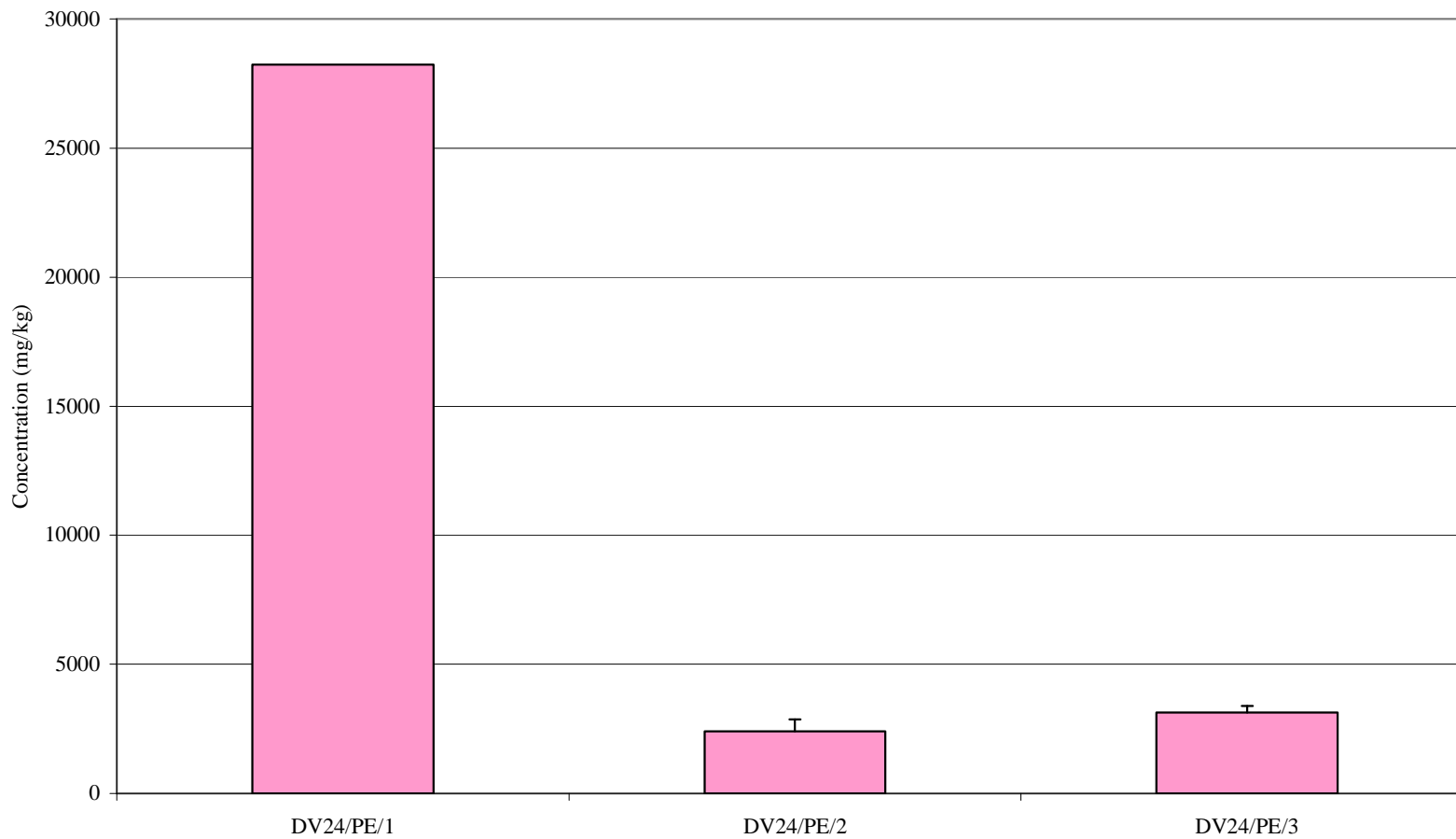
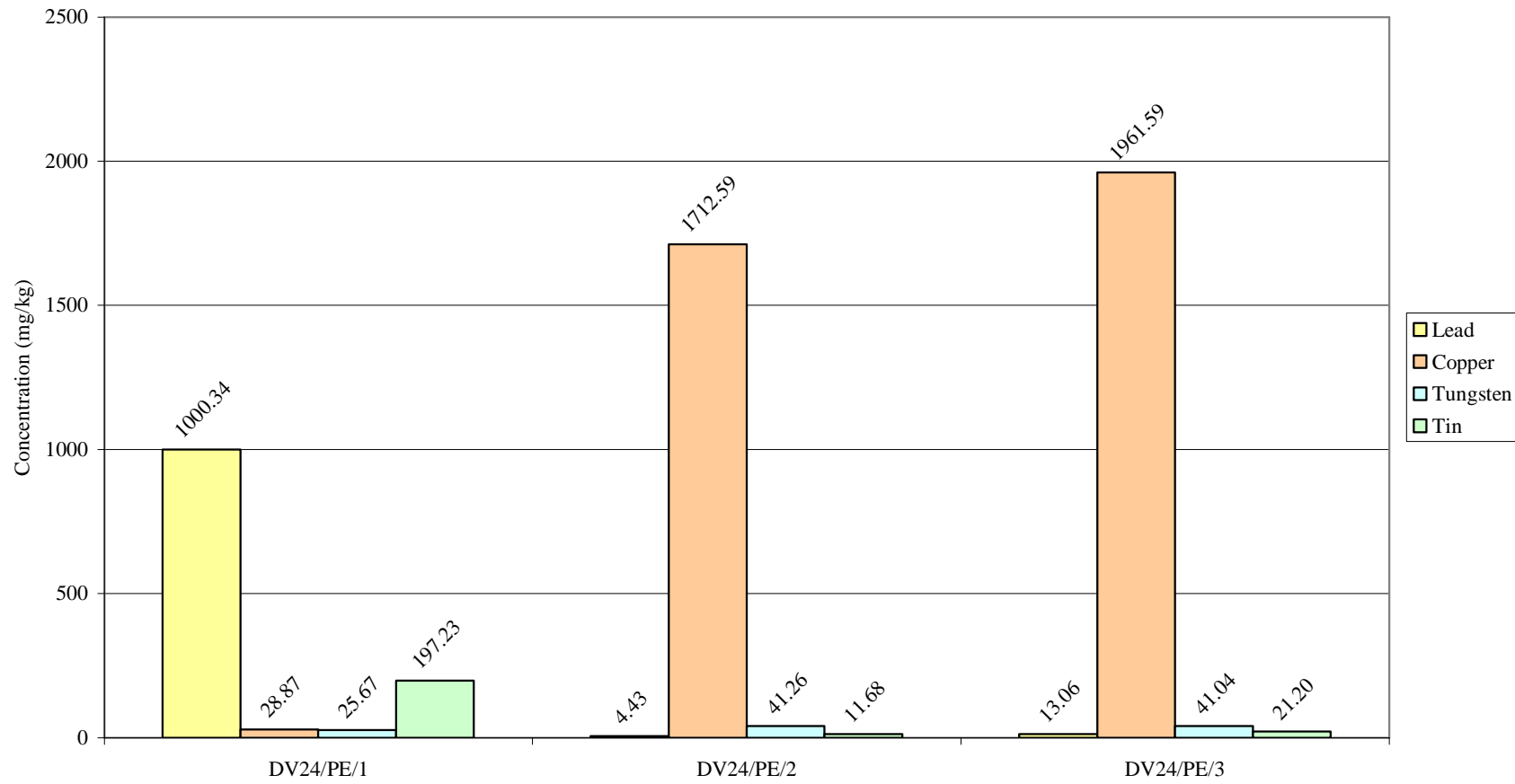


Figure 10-8: The concentration of lead, copper, tungsten and tin in samples from owl pellets collected from Blanchdown Wood, Devon, UK (see Tables 7-1 and 7-2 for key to sample names)



10.4.4. Discussion

As birds of prey, owls are in the same position of the food chain as humans. They are carnivores, by nature, and as so can be used as a model for the eventual transfer of metals into humans. The pellets that were found at Blanchdown Wood, Devon contained the bones and other indigestible material from a small animal. The analysis results showed that the concentration of lead, tin and arsenic in bone fragments were higher than that of the other material. The bone material is generally used for the storage of excess minerals in the body (discussed further, later in this chapter), therefore the concentrations of the contaminants in the bone are a good indication as to their concentration in the soft tissues. The soft tissues of the mammal are part of the digestible portion of the animal and therefore the contaminants present in the tissues would have been broken down and absorbed into the birds' body. Although the pellet contained small particles of spoil, these were regurgitated and removed from their body, but it does lead to the conclusion that some of the metals would be digested with the food by the birds.

10.5. The accumulation of metals in bone

A typical human skeleton consists of 206 bones and are linked together with cartilage and ligaments that form the joints. The structure is designed to support the weight of the body (Christiansen and Gryzbowski, 1993). The basic skeletal structure of newborns begins development during the gestation period. The skeleton is derived from mesoderm, one of the three basic embryonic tissues. When born the majority of the skeleton is still in the form of cartilage, and can therefore not support the weight of the child. The process of converting soft cartilage to bone occurs through ossification that begins whilst the child is still in the uterus and continues until the body is fully matured (Vaughan, 1981).

The conversion of cartilage to bone occurs through two methods of ossification. The type of bone that is resulted depends on the ossification method. Intramembranous ossification results in the formation of flat bones and some of the facial bones. Endochondrial ossification forms the long bones for example femur and tibia, and also plays a large role in the healing of fractured or broken bones.

The structure of the bone differs, depending on the function of the bone. There are two types of bone structure:

- Spongiosa – consists of a network of fine trabeculae, enclosing cavities that contain red or yellow marrow
- Compact – located in the shafts of the long bones, surrounding the marrow cavities

The spongy trabecular bone directly remodels the bone onto the surface of the trabeculae, and is considered to be more active than compact bone (Vaughan, 1981).

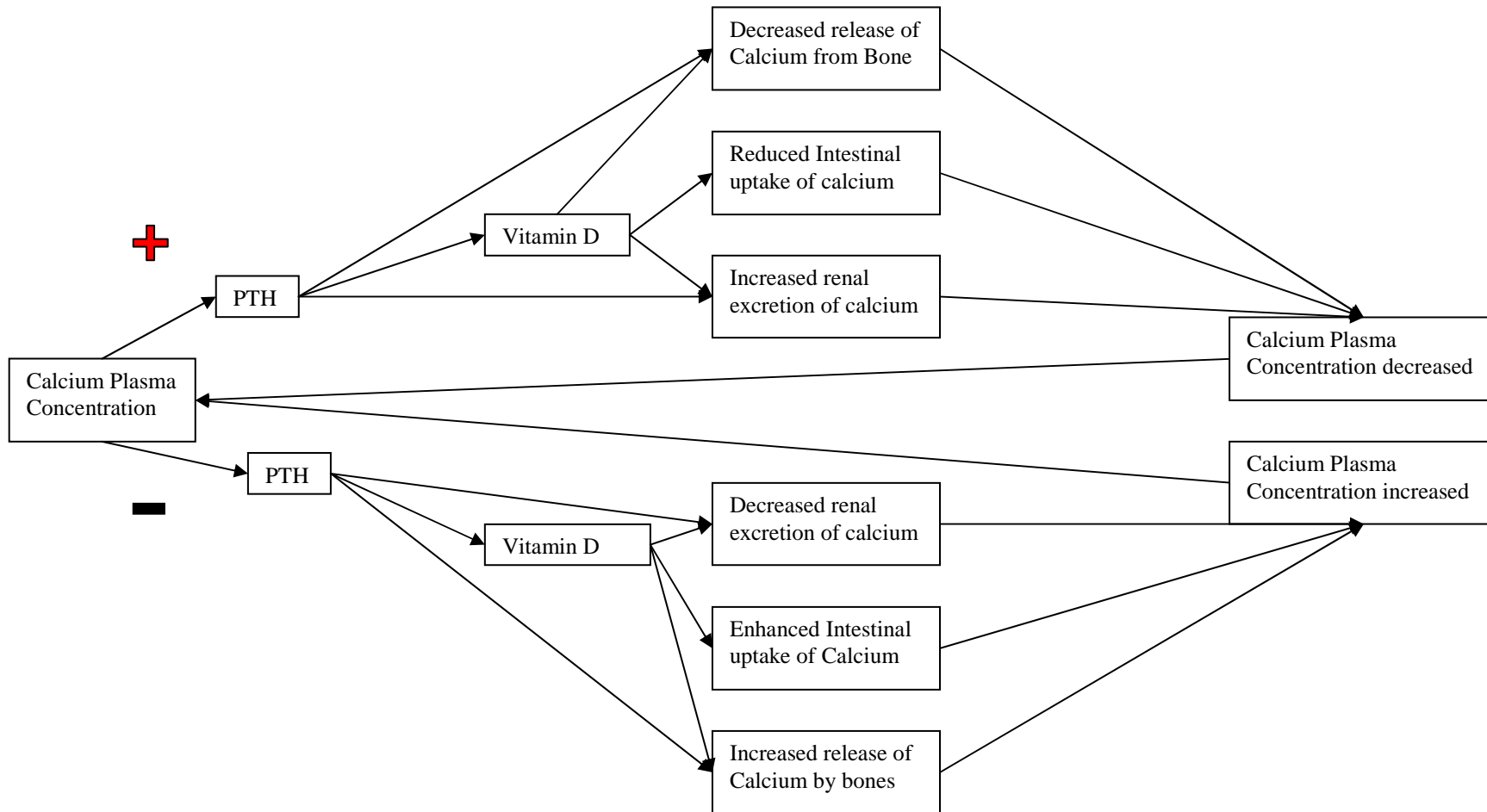
The skeleton's primary role is to provide support and protection to the body, however another vital role is to act as the main reservoir of calcium and phosphorus for the body, as well as additional ions (e.g. sodium, magnesium) (Cohn and Roth, 1996). It is believed that up to 99% of the body's calcium is stored within the bone matrix (Christiansen and Grzybowski, 1993). A mechanism of homeostasis is used to control the concentration of calcium released or absorbed by the matrix. Within the mechanism organs such as the kidneys and intestines play a vital role, as well as the parathyroid hormone and vitamin D (Cohn and Roth, 1996).

The homeostatic mechanism is controlled using PTH, vitamin D and calcitonin. Each has their specific effects on the absorption and resorption of calcium. They maintain a balance of calcium concentration in the blood. Calcium originates in the dietary intake and is absorbed into the blood plasma through the intestines. Calcium is used in cells and is vital to the body's function. Figure 10-9 illustrates the homeostatic control of plasma calcium by PTH and vitamin D. Calcitonin is not shown due to its minimal influence on blood calcium levels in humans, although in other species calcitonin has a significant effect on lowering calcium levels (Spence and Mason, 1987).

Past research, into lead accumulation from mining sites, has revealed that there is also an input of lead into animals and more importantly humans through the trophic levels (Pyatt *et al*, 2002; Pyatt *et al*, 2004). Lead contamination is believed to have originated from the workings of mines and smelting activities and has continued to modern day exposure from car fumes and other by-products of industry. As a result the surrounding land and water courses have become contaminated.

Because of the contamination of the land and water, lead has entered the food chain. As well as bioaccumulation, there is also the aspect of biomagnification of lead in animals including humans. The concentration of lead in individual species of edible plants may not be toxic in itself, but the ingestion of a number of the plants would increase the intake of lead.

Figure 10-9: The homeostatic control of calcium (modified from Cohn and Roth, 1996)



10.5.1. Aims and objectives

The primary aim of this study is to examine the extent of accumulation of heavy metals by humans. To achieve this other aims need to be considered:

- To determine if heavy metals are accumulated in bone
- If accumulation does occur, is there any indication of partitioning through the bone
- To determine if accumulation occurs before or after death

10.5.2. Experimental Design

A number of samples were made available to the project, from different sources. Selections of human femur bones were available to extract samples for analysis and the determination of heavy metal accumulation. Also selections of animal bones were found on site at Blanchdown Wood and were also analysed for heavy metal accumulation.

During the project it was determined that a controlled experiment could be used to identify the extent of metal accumulation after the death of the animal.

10.5.2.1. Laboratory experiment

The experiment was designed to determine if lead was accumulated after death. If lead had been accumulated from the soil after death, the concentration of lead in the experimental bones would be high. For this purpose pig bones were used as their skin texture and tissue is similar to that of humans. The bones were buried in soil, as this would imitate the conditions the human body encounters after death, as follows:

- Uncontaminated bones/tissue in uncontaminated soil (Control) – 1 (PB04-1)
- Uncontaminated bones/tissue in contaminated soil – 2 (PB04-2)
- Contaminated bones/tissue in uncontaminated soil – 3 (PB04-3)
- Contaminated bones/tissue in contaminated soil – 4 (PB04-4)

This provided data as to the direction of lead after burial, whether the lead was absorbed by the bones from the soil or if lead was released into the soil. It can therefore be determined if lead contaminates the bones or whether lead from the bones contaminates the soil. During the decomposition process, the tissue was broken down in the soil. After the complete decomposition of the tissue, the bones were removed. Several soil samples were extracted to determine the concentration of lead.

To prevent the release of lead into the surrounding area a container was used to hold the soil and the bone materials. Four containers were used, and sunk into the ground. In the bottom of the containers a layer of gravel was used to raise the soil to prevent water logging of the soil and to remove excess water in the bottom of the container. The container was then lined with a permeable mesh to allow for the free movement of water. The soil and turf were replaced together in the containers. These were left to allow for the soil to settle.

After a period of 4 weeks the containers were individually dosed with a lead solution. This occurred over a 4 week period; where by a number of applications were used. Using a small hand held sprayer a solution of lead nitrate (with a concentration of 1000ppm lead) was applied in 1L doses over the period. A total of 4 applications were given to the soils.

Throughout the initial set up of the containers, a hand pump was used to remove any excess water from the bottom of the containers to prevent water logging.

The pig's legs that were used were obtained from the local butchers. The four legs were whole and separated from the carcass at the joint, to prevent the bones from

being cut. A total of two carcasses were used. The skin and tissue was left on the bones and were submerged in water solutions. Two samples were submerged in a solution of approximately 1000ppm lead. This was made up using lead nitrate with a concentration of lead nitrate in the solution was 1.598g/L. 10L of this solution was used to submerge the legs. The remaining two legs were also submerged, but in 10L of distilled water. Both sets of legs were placed in sealed containers and were stored in a cold room for 6 weeks. The temperature of the cold room was set at 10°C.

When the pig's legs had been submerged for 6 weeks, they were then buried in the containers. During the burial process it was evident that there was an odour originating from the boxes. After only a few minutes, this odour had attracted two species of flies; *Lucilia spp* (Green bottle) and *Calliphora spp* (Blue bottle). Although there was a visible difference in the number of flies around the lead contaminated material compared to the non-contaminated material, it appeared that possibly there was a subtle difference in the odour that was attracting the flies. The flies preferred the non-contaminated material. However, there were a small number of flies around the contaminated material. Upon the removal and burial of the non-contaminated material, the flies began to increase in number around the contaminated material. This would suggest that the flies have a preference to the condition of meat, and that they could detect the differences. After both types of material had been buried, it was apparent that the flies had laid eggs, on both containers.

After 2 years the samples were removed from the ground and the samples of soil was taken. Figure 10-10 shows a photograph of the bones after their removal from the soil. It could be seen that the tissue had completely decomposed and only the bones remained. Unfortunately, only three of the four samples could be extracted, as the soil in the fourth sample had become compacted and could not be removed at the time.

Figure 10-10: The pig bones that were removed from the laboratory experiment



From the pig bones, three samples of the bones were taken and analysed for lead content. Figures 10-11 to 10-13 shows photographs of the individual bones and where samples were taken. In all cases similar amounts of samples were taken. The samples were cut from the bone using a stainless steel saw. During the sampling, eye protection, mask, gloves and laboratory coat were worn. All sampling of bone material was conducted in a fume cupboard.

Figure 10-11: Control: Uncontaminated bones/tissue in uncontaminated soil (PB04-1)



Figure 10-12: Uncontaminated bones/tissue in contaminated soil (PB04-2)



Figure 10-13: Contaminated bones/tissue in uncontaminated soil (PB04-3)



10.5.2.2. Cambridge archaeological samples

A number of archaeological samples were made available by Tim Reynolds, research fellow. These samples were excavated from a site near Cambridge, where a number of skeletons were found. The samples were dated by the excavators as approximately 900AD. Upon receipt of the bones, they were stored in a sealed container to prevent any contamination from other sources, also to preserve the samples. Five samples were taken from two of the femur bones, and were pictured shown in Figure 10-14 and 10-15. For both femur bones, samples were taken at different points along the bone. Although not clear from the photograph, a sample was taken from the hip.

Figure 10-14: Cambridge bone sample CA05/B-1



Figure 10-15: Cambridge bone sample CA05/B-2



10.5.2.3. Animal remains

During a visit to Blanchdown Wood a number of animal bones were found and collected. The samples were stored in sealed bags and on return to the laboratory were thoroughly washed and dried. Samples for analysis were cut from the bone and were digested as discussed in Chapter 5. Photographs of the bones were sent to Phil Piper at York University, and were preliminary identified as those of a small deer, possibly a Chinese deer. He confirmed that the remains were probably less than a year old. Figures 10-16 to 10-22, show photographs and identification numbers of each of the bones that were analysed.

Figure 10-16: Bone samples 1-3 of the animal remains found at Blanchdown Wood, Devon



Figure 10-17: Bone samples 4-6 of the animal remains found at Blanchdown Wood,
Devon



Figure 10-18: Bone samples 7-8 of the animal remains found at Blanchdown Wood,
Devon



Figure 10-19: Bone sample 9 of the animal remains found at Blanchdown Wood,
Devon



Figure 10-20: Bone sample 10 of the animal remains found at Blanchdown Wood,
Devon



Figure 10-21: Bone sample 11 of the animal remains found at Blanchdown Wood,
Devon



Figure 10-22: Bone sample 12 of the animal remains found at Blanchdown Wood,
Devon



10.5.3. Results – laboratory experiment

The results in Figure 10-23 show the concentration of lead in the bone samples were elevated only in the sample where the soil was contaminated. The remaining two samples show that there was no accumulation of lead from the water they were left in. The second sample (uncontaminated bone/tissue in contaminated soil) shows that there was an increase in the concentration of lead in the bone. The concentration of lead in the soil from around the bones proved to be above 250mg/kg, and it has shown that the lead has been absorbed from the surrounding soil.

Although there are small concentrations of lead present in both sample 1 (control) and 3 (contaminated bone/tissue in uncontaminated soil), this can be background concentrations, and not directly from the experiment.

During the analysis it was decided to determine the concentration of calcium and phosphorus, and to investigate the loss of these components of bone during the decomposition process. From Figure 10-24, it can be seen that the concentrations of the calcium and phosphorus were only slightly elevated in the soil and could be the result of background concentration. The concentration of phosphorus appears to be constant along the bone, but the calcium concentrations of the bone seem to differ slightly.

Figure 10-23: The concentration of lead in the bones and soil from the laboratory experiment

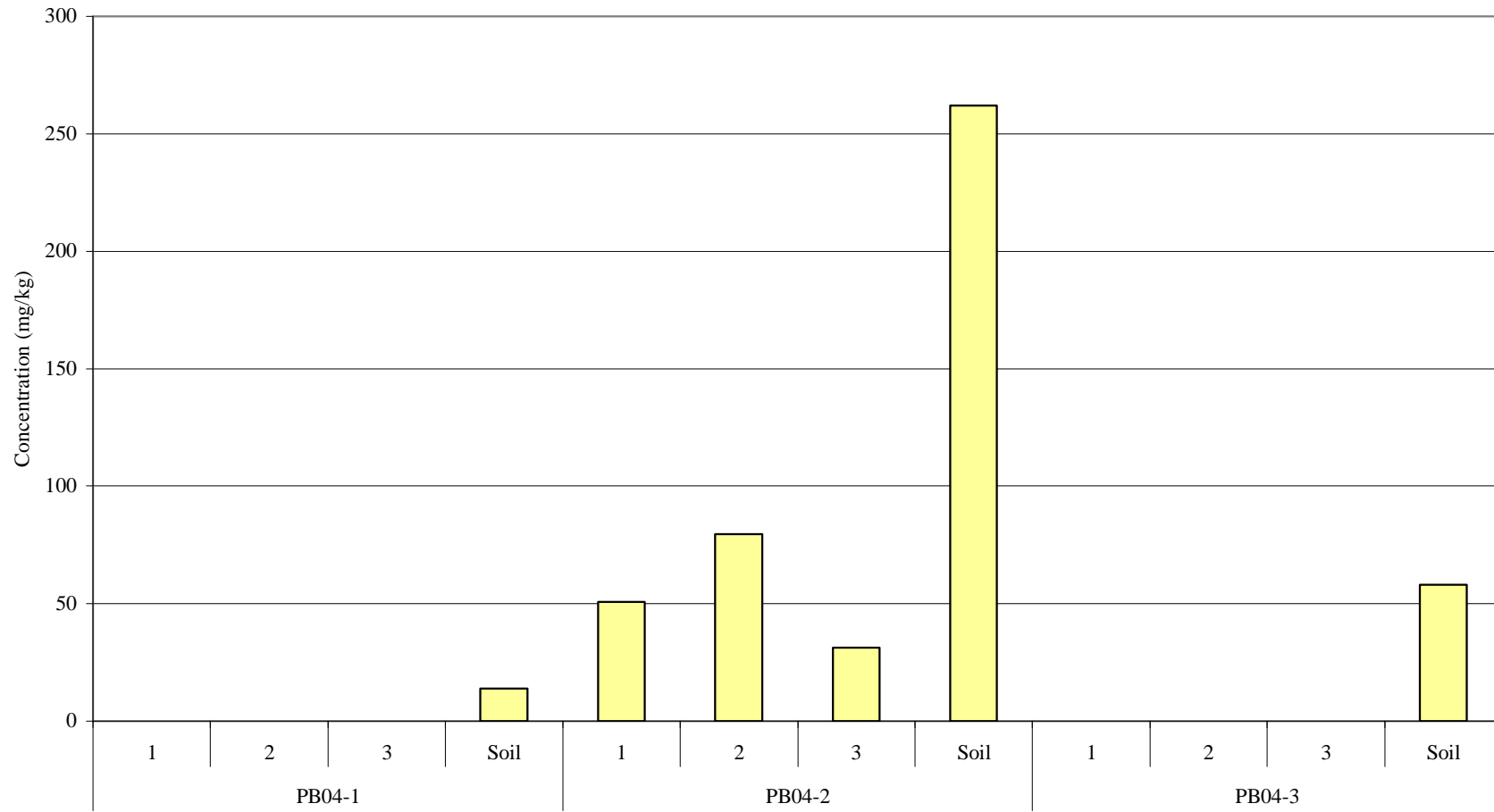
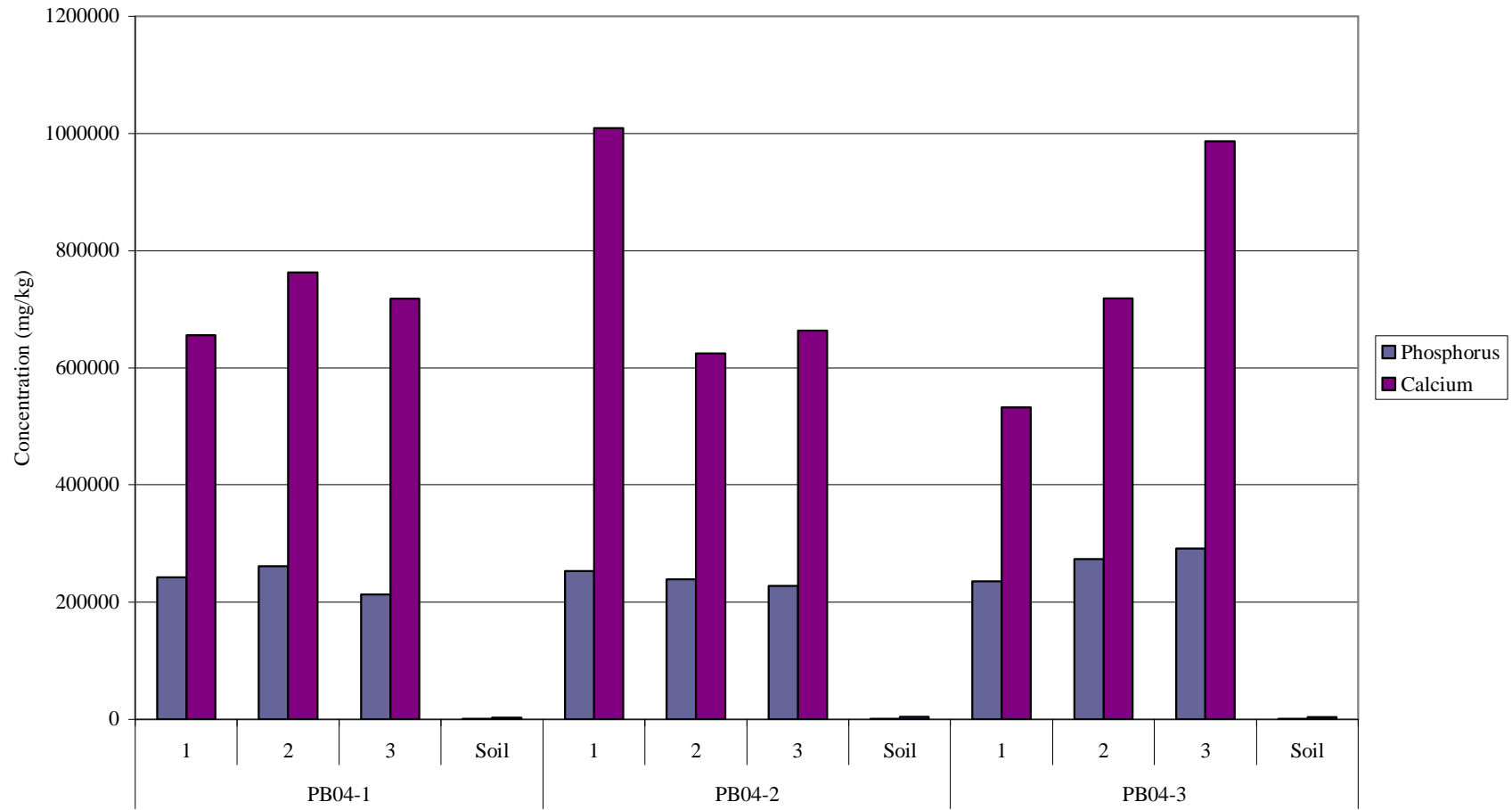


Figure 10-24: The concentration of calcium and phosphorus in bones and soil from the laboratory experiment



10.5.4. Results – Cambridge archaeological samples

Because of the lack of information surrounding the archaeological samples, it was decided to analyse for a number of elements, copper, lead, tungsten, tin, iron, calcium, phosphorus and arsenic. Of these, calcium, phosphorus and iron were expected to be present, but the remaining elements were not. It was found that out of the elements tested for, only lead was present. Figure 10-25 shows the concentration of lead along the bone samples. Sample 5 of both bones were taken from the hip, and in both cases the hip contained the highest concentrations of lead (CA05/B-1 – 138.59mg/kg and CA05/B-2 – 549.84mg/kg). It can also be seen that the concentrations of lead were higher in CA05/B-2 (ranging from 217.71 to 549.84mg/kg) compared to CA05/B-1 (ranging from 30.35 to 138.59mg/kg). Because replicates could not be performed, statistical analysis could not be completed.

The phosphorus and calcium concentrations are displayed in Figure 10-26. These show that for the phosphorus concentrations, there appears to be no difference between the partitioning samples, or between the individual samples. The calcium concentrations do appear to differ. Overall CA05/B-1 contained higher concentrations of calcium, and for both individual samples there was a fluctuation of the concentration along the bone.

Figure 10-27 shows the results of the concentration of iron in the femur samples. It can be seen that CA05/B-1 showed a fluctuation of iron along the shaft of the bone. Sample CA05/B-2 showed low concentrations compared to CA05/B-1 with a minimum of 342.55mg/kg at site 1. In both samples the highest concentration of iron was found in the femoral head region.

Figure 10-25: The concentration of lead in samples of femur bones

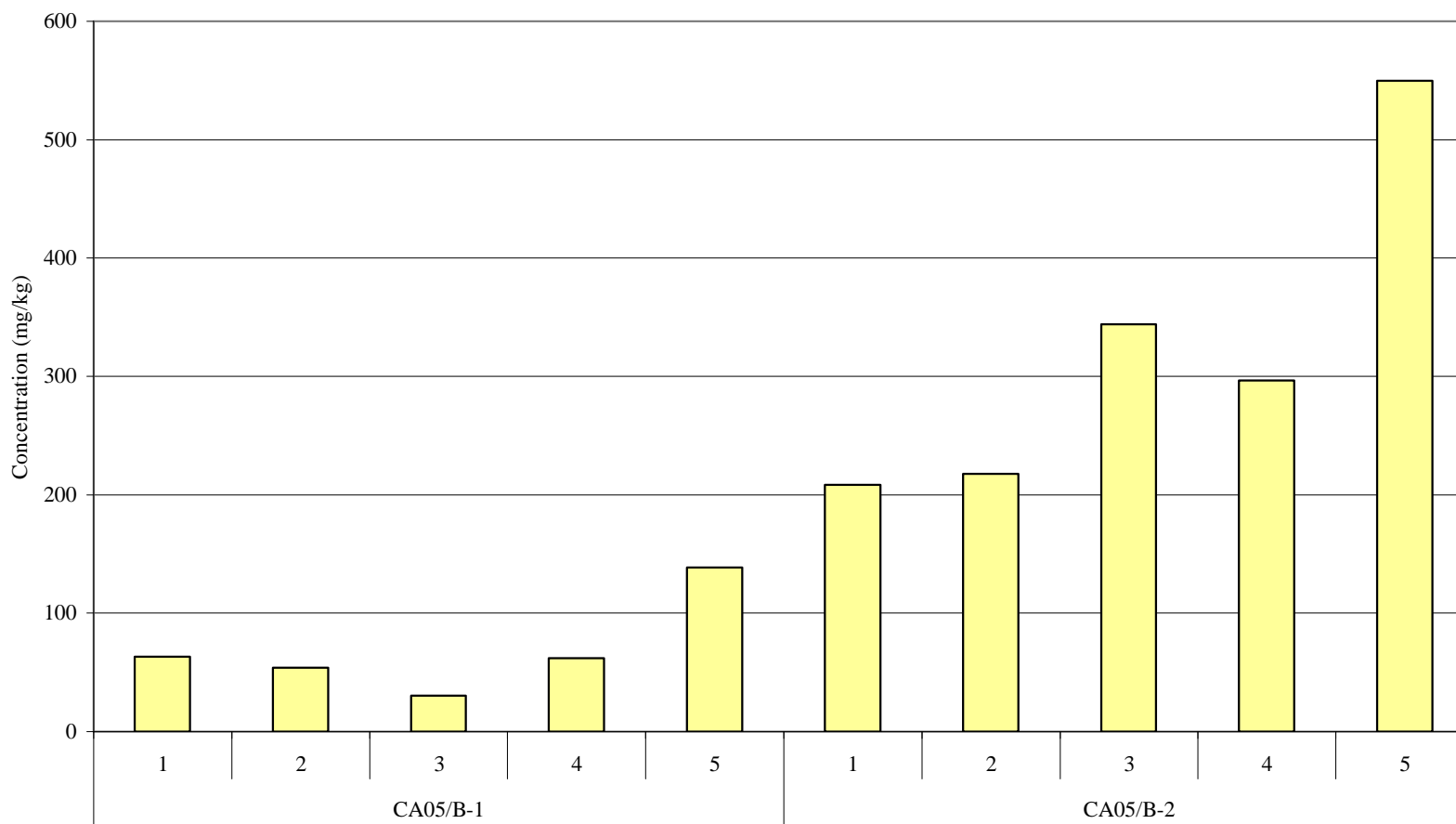


Figure 10-26: The concentration of calcium and phosphorus in samples of femur bones

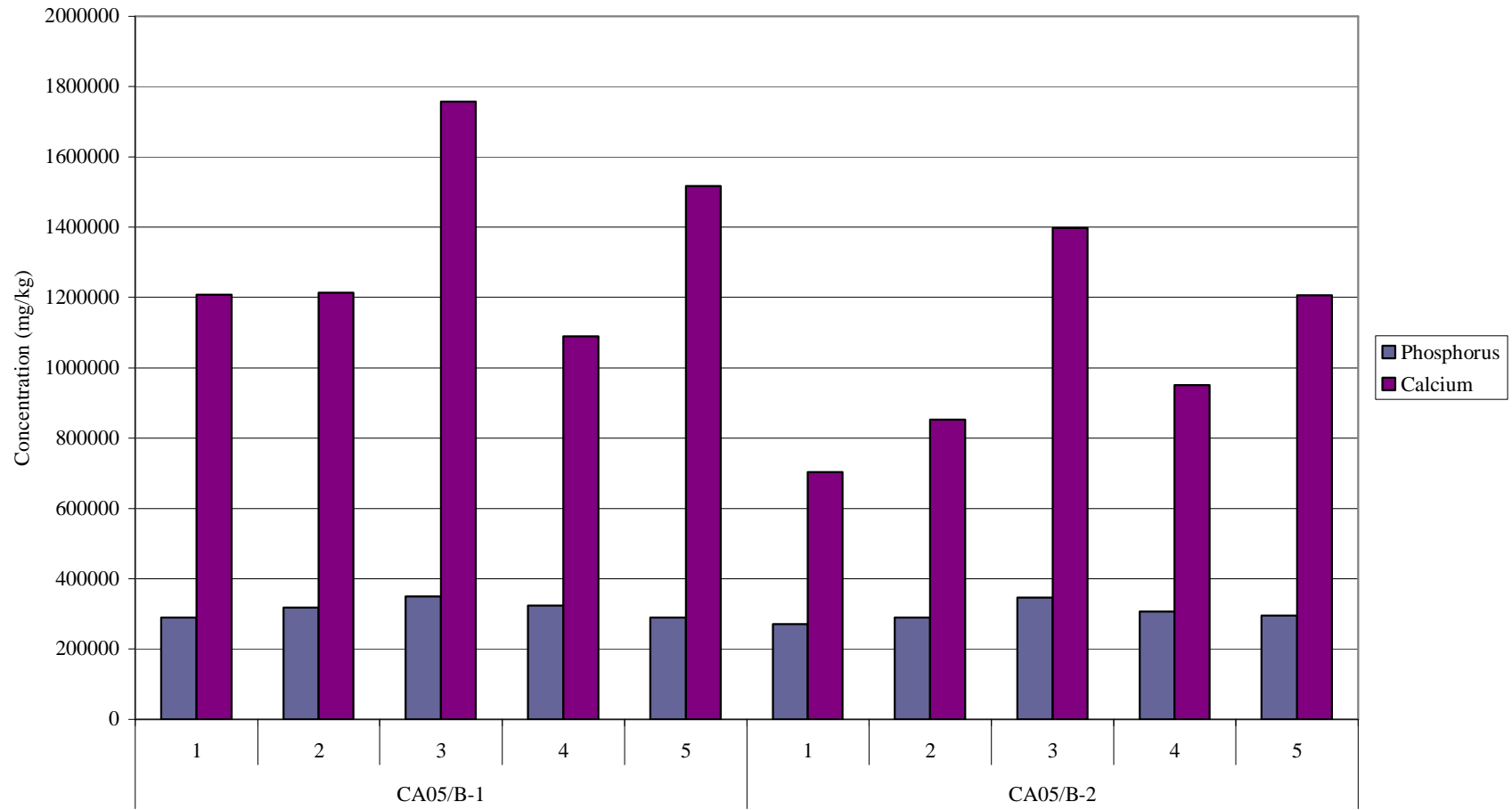
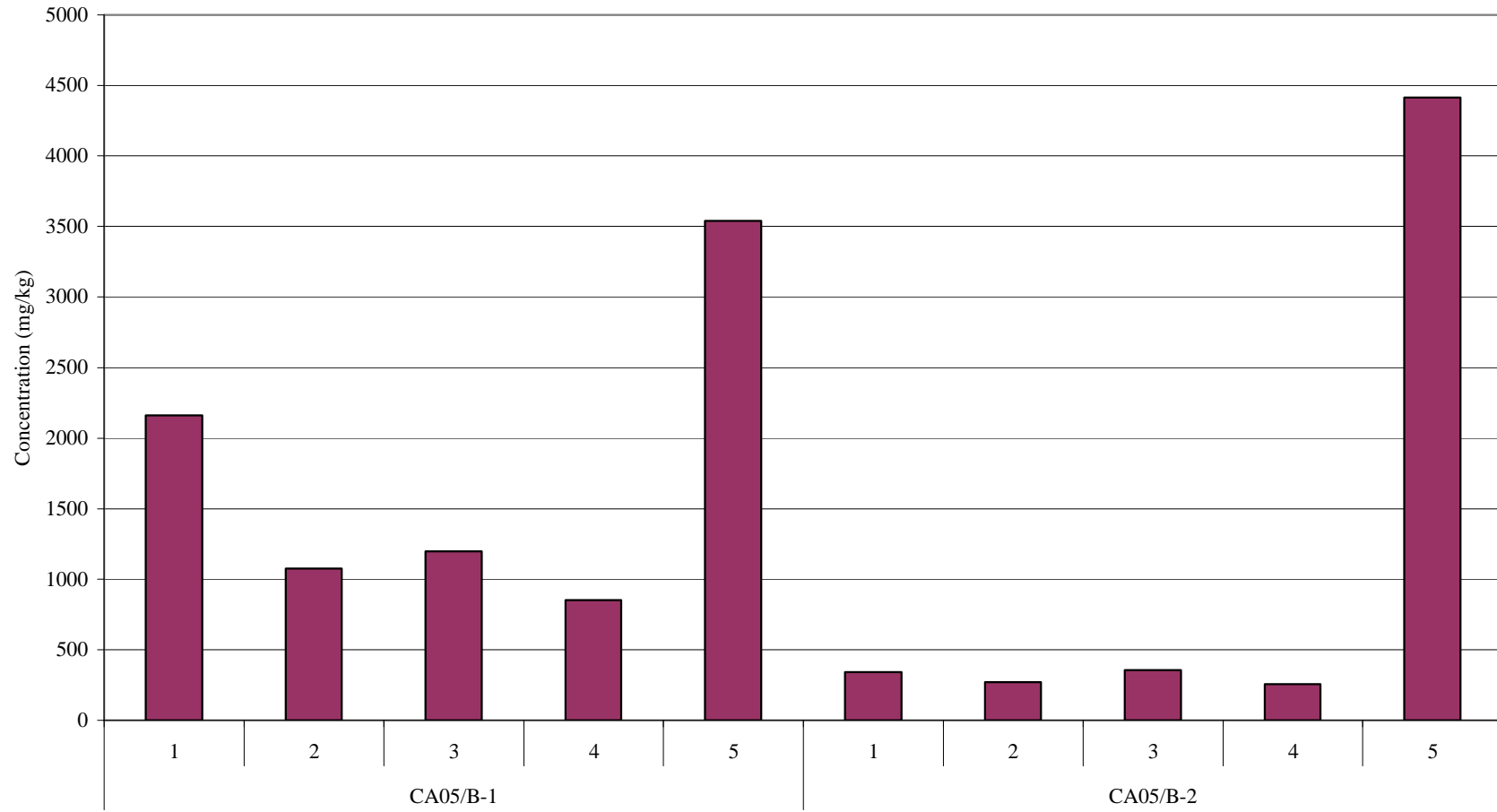


Figure 10-27: The concentration of iron in samples of femur bones

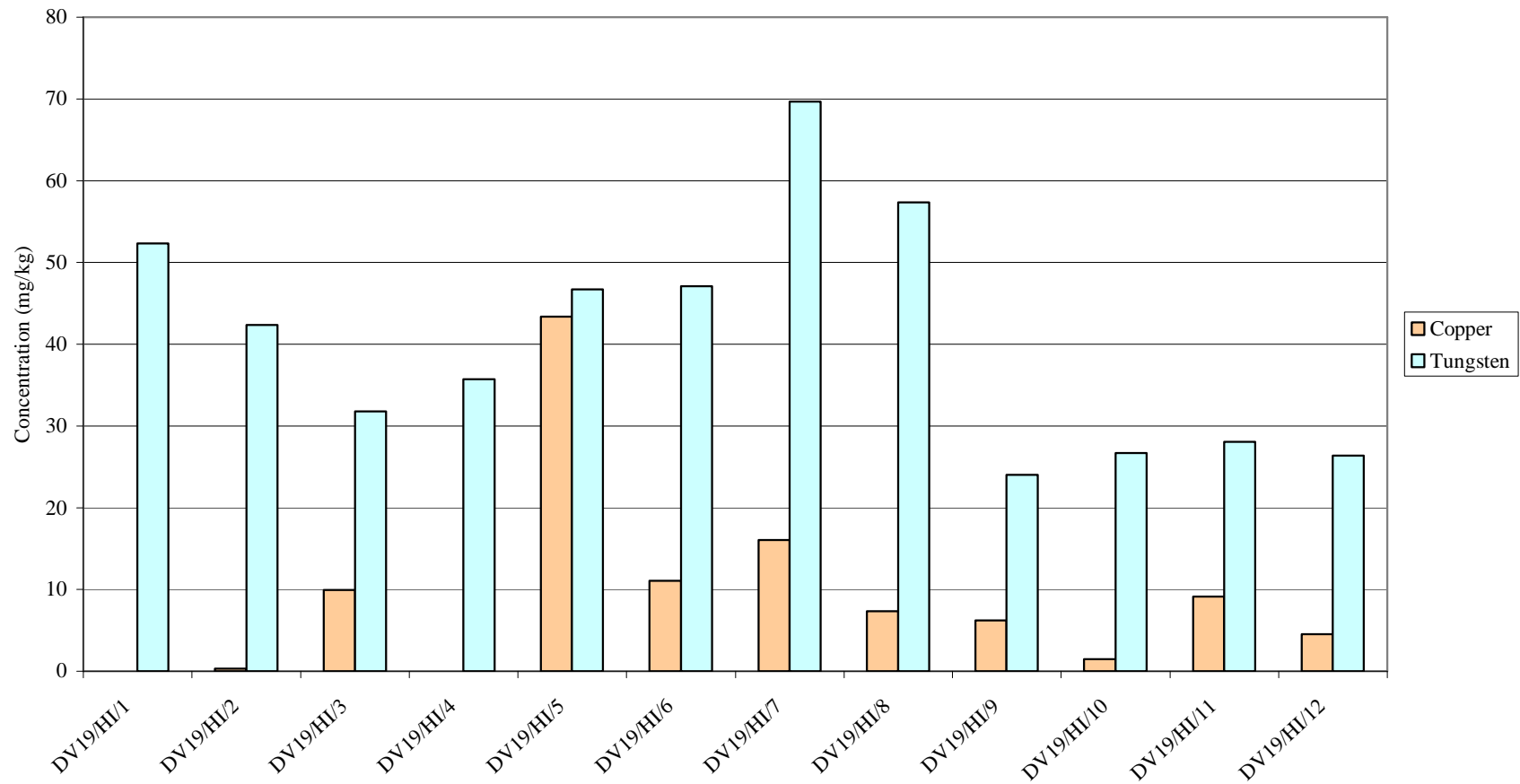


10.5.5. Results – Animal remains

Figure 10-28 display the results from the bone samples found at Blanchdown Wood. It was found that only copper and tungsten were found in the bone. Lead, tin and arsenic were not detected. It cannot be confirmed that the bones came from the same animal; also the food source of the animal could not be determined. The bones were believed to be that of a small deer, possibly a Chinese deer (*Inermis inermis*). These can cover great distances depending on the food availability. Therefore it can not be assumed that the primary feeding ground of the deer was at Blanchdown Wood.

The results show that there are varied concentrations of both copper and tungsten, with a maximum concentration of tungsten at 69.66 mg/kg (DV19/HI/7) and the maximum concentration of copper at 43.36 mg/kg (DV19/HI/5)

Figure 10-28: The concentration of copper and tungsten in bone samples from Blanchdown Wood, Devon, UK (see Tables 7-1 and 7-2 for key to sample names)



10.5.6. Discussion

During the analysis of the all the bone samples, it was found that lead, copper, tungsten and arsenic were represented in one or more of the samples. The accumulation of all these elements, except tungsten, in bone have been studied (Mateo *et al*, 2003; Sharma *et al*, 1977; Taggart *et al*, 2006; Gerhardsson *et al*, 2005; Martinez-Garcia *et al*, 2005).

In modern society, through the increased use of lead, the concentration of lead in the human body has also increased. Gonzalez-Reimers *et al* (2003) suggest that the daily intake of lead by Americans has increased by 100 times. The primary site for lead accumulation has been the skeleton and it is thought that up to 90% of the lead within the body has been stored in the bones (Drasch, 1982). The reasoning for this is believed to be lead's chemical similarity to calcium. This could result in the absorption of lead substitution for calcium. Because of the chemical similarity, lead and calcium could compete for the uptake of minerals in developing bones. There may not be a selective process, therefore the uptake of either lead or calcium could just depend on the concentrations and the close proximity to the osteoblasts. If the substitution of lead for calcium is based around the chemical similarity, it is also possible for lead to substitute for other chemically similar ions, for instance Iron (Fe^{2+}) in the haemoglobin complex (Ratcliffe, 1981).

Because of the wide use of calcium in the human body, it is essential that there is a permanent store. The human skeleton provides that store. Calcium is locked within the bone matrix in the form of a crystalline structure: Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$). The structure is such that there are three zones; crystal interior, crystal surface and hydration shell. Each zone presents surfaces available for exchanging ions. Therefore the substitution of calcium could occur in each zone.

In the analysis, the concentration of the heavy metals and the calcium and phosphorus concentrations were investigated. It would be assumed that an increase in the concentration of lead would decrease the calcium concentration in the bone material. The laboratory experiment was used to determine if there was an accumulation of lead after the death of the animal, and also to investigate the removal of calcium from the bone through the decomposition process. The results showed that there was a concentration of lead absorbed into the bone from the soil, but there was not an increase in the calcium concentration in the soil. Therefore over a short term, calcium is not lost from the bone, and lead is accumulated. However long term burial

of bones has led to an increase in the calcium concentration of soil, where the burial occurred approximately 1000 years ago (Wilson, B: personal communication). Because of the long periods to examine this further it is not possible to determine if over long periods lead (or other metals) is accumulated after death.

The archaeological samples from Cambridge indicated that there were high concentrations of lead accumulated in the femur bones. Information from the archaeologist involved with the excavations (Carroll, Q) identified CA05/B-1 as being male and approximately over 45, but CA05/B-2 could not be determined as male or female, as the bones were poorly preserved.

During the interpretation of the results it was suggested that the second sample could be from either an adult female, or from an elderly person (male or female). The reduced concentration of iron in the femur bone would lead to the conclusion that the person suffered from anaemia, and it would therefore be determined that the femur belonged to a female, since women are more likely to suffer from anaemia. The femur from the male had concentrations of iron higher than that of the other sample. If the second femur was male and of the same age, the concentrations of iron would be similar.

It was noted that there was an increase in the concentration of lead in the femur head samples compared to other samples taken from the bone shaft of the same bone. These also corresponded to a decrease in the calcium concentration at the same site.

The renewal rates of bone differ between parts of the skeleton. The rate also differs between the ages of the individual. During the early stages of life (up to adolescence) the absorption of calcium in the bone is greater than resorption. At about 30-35, the resorption and absorption are equal, however during the later stages of life the absorption rates decrease, and the loss of calcium from the bone structure is at its highest; this is associated with the onset of osteoporosis.

The concentration of lead found in the femoral head was highest in both bones and could be related to the fact that this region consists of spongy trabecular bone, which has an increased turnover rate of calcium. If the concentration of lead in the blood is reasonably high, it will be incorporated in higher concentrations in regions of bone where the absorption rates are highest, for instance the femur head and neck.

The iron concentrations found in the femur head, were determined to be of higher concentrations compared to samples taken from the shaft of the bone. This occurred in both femur samples, although CA05/B-2 contained lower concentrations from the

shaft when compared to CA05/B-1. Iron is a micronutrient required by humans and other animals for the transport of oxygen around the body. Iron is incorporated in the haemoglobin protein of the red blood cells and is obtained in small amounts in the food that is ingested. The body does not have a mechanism for the excretion of iron and therefore homeostasis is used to maintain the concentration through recycling. In general there is approximately 3-4g of iron in the body with 2.5g of this being attached to haemoglobin, 400mg being used in cell processes and 3-4mg in blood serum. The remaining iron is stored in various parts of the body, usually in sites where red blood cells are either formed or broken down (liver hepatocytes, spleen and bone marrow) (Hoffbrand *et al*, 2006).

Iron deficiency could lead to the excess removal from the stores in the bone marrow to compensate for the deficiency. The lack of iron in the body can be brought about by a number of reasons, the lack of iron in the diet, heavy bleeding and disorders such as Crohns' disease (Gasche *et al*, 1994).

CA05/B-2 has been suggested to be from a female, and could explain the differences in iron concentration between the two femur bones. A decrease in iron is more commonly brought about in women of child bearing years. Because both the femur samples were taken from the same site it is likely that both received similar diets, therefore a lack of iron in the diet would decrease the concentration of iron in both the femur bones. However, it is not possible to compare bones with the same variables, i.e. Age at death, health, diet, and period of time since death.

In the human body a number of metals are required as micronutrients. These include iron (discussed previously), molybdenum, copper, and zinc (Anon, 2005-2006). Micronutrients can not be manufactured and therefore must be ingested and to prevent excess loss, can be stored within the body. It was found that the animal remains found at Blanchdown Wood, Devon, contained small concentrations of copper in the bones, ranging from 0 to 43.36 mg/kg.

There was a wide range of the concentrations and as discussed earlier it can not be assumed that the remains were from the same animal, although the probability that two or more Chinese deer were killed and their remains discarded in the same place is extremely low. If the remains were from the same animal, it shows that there is large variability for the accumulation of copper in the rib bones. Copper is required as a micronutrient for enzymes which catalyse the production of the haem and therefore the iron incorporation to the molecule (Reeves and DeMars, 2006). Theil and Calvert

(1978) discuss the issues of copper deficiency and excess in the body. A decrease in the concentration of copper can induce anaemia, leading to the conclusion that iron is not being incorporated in the haem molecule and therefore the red blood cells are not being produced. However excess copper concentrations can induce kidney and hepatic damage. Because copper is required by the body for the incorporation of iron in the red blood cells, it is clear that concentrations would be found in the sites of red blood cell production.

As mentioned previously, molybdenum is also required as a micronutrient, and is also involved in iron metabolic processes. It is involved in metabolic reactions for carbon and nitrogen cycles. Molybdenum is attached to a number of enzymes collectively known as molybdoenzymes. An example is that of xanthine oxidase, which is involved in the purine degradation processes. Some researchers have found that molybdenum has been substituted by tungsten in plants and animals (Koutsospyros *et al*, 2006), but the precise mechanism has not been determined. The possible substitution of tungsten for molybdenum in biological processes could explain the concentrations found in the animal remains. There were high concentrations found, with a range of 24.01 to 69.66 mg/kg. Although these can not be compared to concentrations in control samples, Koutsospyros *et al* (2006) indicated that the concentration of tungsten in various organs and tissues of healthy individuals did not exceed 2600µg/kg. Thus it can be determined that the concentrations of tungsten in the bone samples were higher than the expected concentrations. It could therefore be determined that the animal consumed and accumulated increased concentrations of tungsten.

It has been identified that an excess of molybdenum has caused the excretion of copper from animals (Xiao-yun *et al*, 2006; Sievers *et al*, 2001). If this is applied to the samples that were found, the low concentrations of copper in some of the bones could be attributed to the high concentrations of tungsten substituted for molybdenum.

Although the bones were found in the open air, it is unlikely that there was absorption of metals from the environment as there would be other metals absorbed into the bones as well.

Chapter 11: Discussion and conclusions

11.1. Variations in soil concentration of metals

One of the main sources of heavy metals in the atmosphere is as a consequence of weathering. Weathering results in the removal of both cations and anions from the terrestrial environment either in the solid rock, soil or spoil remains, into the atmosphere. Atmospheric processes can transport the cations over both long and short distances; and the extent of dispersal is dependent on factors such as the velocity of the wind, the presence of any physical barriers, and the size of the particulates being transported. This means that although a spoil tip may be at a great distance, the atmospheric deposition of the contaminants can introduce them into a substantially increased area. The repercussions of this include an increased probability of metal accumulation into food chain following deposition of cations from the atmosphere to the terrestrial and aquatic environment.

There are a number of different methods of metal transport in the environment including leaching, weathering of soil and spoil, and run off. Because of the number of different methods of transportation no single method can be considered as responsible for the sole movement of the metals. A combination of the dispersal methods have resulted in the non-uniform transportation of the metals, resulting in the different concentration found between the samples as seen in the results of this current research.

11.1.1. Lateral dispersal

From the soil concentration results at Blanchdown Wood, Devon, UK, it can be seen that there was little or no relationship between the concentrations of copper, lead, tin, tungsten and arsenic in the soil within each of the sites examined during the course of this research program. By examining the results it can be seen that there were high concentrations of all the elements, with exceptionally high concentrations of copper and arsenic. These results showed that there was a high variability in the concentrations within each site. Additionally, during the analysis, 10 replicates of each of the soil samples were examined. These are represented in Figures 7-2 to 7-6. It is observed that there is little variation in the replicates as illustrated by the confidence interval bars.

The locations of the sample sites can be seen in Figure 7-1 (Page 68). It can be seen that sites 11-16 were located around the Southern waste tip. The remaining sites were located at various distances from the waste tip. With the exception of site 12, the remaining results showed a large variation in the concentration of each of the metals. Site 12 had similar concentrations in each of the samples that were extracted. Although site 12 was chosen at random, it was seen that there was an under layer of rubble with a layer of soil and organic material. Personal communication with the estate manager (Mark Snellgrove) provided information of the plantation of pine trees around Blanchdown Wood. Visual confirmation of tree felling was experienced at the Blanchdown Wood, and it is suggested that the removed trees are used for various purposes in industry. Although it was not considered as a remediation technique, they have provided the area with a small level of remediation. Although the plantation of trees has occurred throughout the site, it is not known whether during the plantation a layer of topsoil was added to aid the growth of the seedlings.

The movement of the cations can not be attributed to one method of transport. As discussed previously, the ions can be moved through leaching, runoff or atmospheric deposition. Currently all three can be considered as responsible for the transport of ions at Blanchdown Wood. The waste tips are composed primarily of spoil from the smelting activities. The fine particles can be deposited around the site depending on the direction and velocity of the wind. When sampling the spoil, it was seen that there were still high concentrations of the metals within the top 5cm. This would indicate that although the waste was deposited several decades ago, the contaminants are still present close to the surface, and are therefore subject to wind erosion and thus atmospheric deposition. However, atmospheric pollution is restricted by the presence of physical barriers, in this situation the presence of mature trees. The trees provide a barrier to prevent the movement of metals, although the movement still occurs to some extent.

When examining the results of the soil concentrations (Figures 7-7 and 7-8), it can be seen that there is a large variation throughout the site. The variations can be considered as a mosaic effect, where the effect seen in the soil indicates that the concentrations can vary depending on the dispersal mechanism, and the physical and chemical properties of the soil. Therefore where soil is analysed for the presence of heavy metals the results are not necessarily representative of the whole area and additional analysis is required. To accurately evaluate the concentration of metals in

the soil of an area, a number of samples are required. The numerous samples taken in an area can be used to map the concentrations of certain elements in an area. Additional information could also be used to illustrate the movement of the metals through the environment. Such information includes chemical and physical properties of the soil, presence of vegetation and animals and the presence of any other physical barriers in the vicinity of the contaminated site. Markus and McBratney (2001) studied the spatial distribution of lead in the environment. The authors summarised a number of research projects which examined the concentration of lead in soil. The authors concluded that to be able to predict the movement of lead in the environment, for risk assessment purposes, a spatial distributional map should be used.

Paton *et al* (2006) studied a nickel smelting complex located in the central part of the Kola Peninsula in Russia. The current activities have lead to the area being highly contaminated with copper and nickel. It was determined that the replicate sampling within a site was necessary and that variations of the copper and nickel concentrations were apparent for each of the replicates. Of the 7 sites sampled over a distance of 34km from the smelter, all soil contained increased levels of copper and nickel and contained significant variations at each site. The variation coincided with variations in the pH as seen in the results of this current research.

A common conclusion of many research papers is that there is a decrease in the concentration of contaminants from the source (Teutsch *et al*, 2001; Wilson *et al*, 2005; Wilson and Pyatt, 2007; Paton *et al*, 2006) However, it is difficult to locate the source of pollutants, as there could be a number of sources, both natural and anthropologic.

It is currently not possible to study the individual effect of mining operations. However, the past activities can be extrapolated through the examination of peat bogs and ice cores (Hong *et al*, 1996; Shotyk, 2002; Shotyk, 1996; Martinez Cortizas *et al*, 2002; Zheng *et al*, 2007). These provide a historic record of the extent of the atmospheric pollution produced during the height of mining and smelting activities. This allows researchers to suggest the contribution the mining and smelting activities can have on the present environment.

11.1.2. Vertical dispersal

The vertical soil and spoil cation concentrations are also varied and additionally indicate that there is a pronounced mosaic effect. In this case the method of dispersal is also an influence, as the atmospheric deposition of ions from the weathering can be deposited on the surface and then absorbed further down the vertical profile. Additionally the run off and leaching from other contaminated soil can also increase the concentration.

The flow of cations through the vertical profile of the soil has become increasingly important to monitor. The possibility of the ions reaching groundwater leads to the increasing probability of contaminated water being ingested by animals including humans. The results of this research show that at depths of 58cm, there was a significant concentration of all the metals. Although soil at greater depths was not sampled, it can be assumed that the concentrations penetrated further through the profile.

The mobility of cations has been studied with varying results (Sterckeman *et al*, 2000; Yukselen, 2002). But all have concluded that the mobility and thus the increase of a polluted area is dependent on a number of factors including physical and chemical properties of both the soil and the metal itself.

11.1.3. Movement of metals and soil properties

The movement of contaminants are dependent on the properties of the soil, physical, chemical and biological. The physical properties of the soil include the percentage of organic matter, soil composition and soil permeability. Chemical properties include the pH and adsorption capabilities of the soil. In the process of this research, the volume of soil that was collected at each site varied, and in some cases was limited to the quantity required for the heavy metals analysis. Therefore these parameters were not fully recorded. In further experiments, these would be monitored to determine the relationship between these parameters and the concentration of the heavy metals.

One of the main properties that dictate the mobility of the contaminants in soil is the pH. The pH of the soil can limit the movement of the ions through the soil. In general the lower the pH (more acidic) the more likely the contaminant can be mobile. In alkaline soil, the contaminants are less mobile and are not bioavailable. The results of this research showed that the pH of the soil sampled varied throughout the site. This

is not considered unusual as the average pH of soil in Britain is 6.0 (average of 6000 soil samples); however the range is between 3.1-9.2 (McGrath and Loveland, 1992). When considering the differences in the pH in the soil profiles it can be seen that the samples with the lowest pH also showed the highest concentrations of arsenic and tin. This leads to the conclusion that in this situation at a lower pH, tin and arsenic are more mobile compared to copper, tungsten and lead.

The adsorption and permeability properties can increase the concentration of the metals further down the profile. Soil adsorption occurs when the contaminant becomes adhered to the surface of the soil particles. The stronger the adsorption, the less likely the contaminants are transferred further down the soil profile. This also means that the contaminants have a lower bioavailability and are therefore not available for plant uptake.

The permeability of the soil allows for the movement between the soil particles. The larger the spaces between the soil particles, the more the contaminants can move through the soil profile. Soils are composed of a mixture of clay, sand and silt. The percentages of these can determine the size of the spaces between the soil particles. For instance soils composed of a high percentage of sand have large spaces between the particles. This leads to the increased movement of water and contaminants through the profiles. Where the soil has a high percentage of clay, the spaces between the particles are smaller and not capable of moving through the profile. In this situation, it is more likely that water and contaminants are moved through the environment by runoff on the surface of the ground.

Although the permeability of the soil was not quantifiably measured, a visual distinction could be made. The profile samples taken at Blanchdown Wood, consisted primarily of sand material. Therefore the permeability of the samples was high and thus the ions could move freely through the profile. However, the adsorption of the ions to the surface of the soil is not selective, and metal ions become adsorbed to particles in the upper portions of the soil. Sterkeman *et al* (2000) concluded that the increase in the distance travelled by the metals through the soil profile was related to the concentration in the upper most layers. The higher the concentration of the metals in this layer increased the distance the metals moved through the vertical profile, although this was only measured over a distance of 20-30cm. In the results of this research, it can be seen that the concentration of the metals in the upper most layers of the profiles was relatively high. Therefore using the conclusions of Sterkeman *et al*

(2000), it is likely that the metals could reach greater depths than was measured. Additionally the soil composition of the samples was primarily of sand; this property would also enhance the movement of the ions through the profile, and therefore increase the concentration at the lower levels.

The pod samples showed visible indications of a higher organic content compared to the other vertical profiles. The greatest depth of the pod samples was 12.5cm and 13cm. The pH values of these samples also varied with distance from the surface; however the lowest pH was 4.2. It is likely that the higher percentage of organic matter is maintaining the pH above 4.0 promoting the growth of the vegetation. The percentage of organic material contributes to the movement of contaminants through the profile with an increase in the organic matter decreasing the bioavailability of the heavy metals in soil. Therefore the introduction of vegetation on contaminated land, either as a remediation tool or as a natural coloniser, can decrease the bioavailability of the metals in the soil (Tordoff *et al*, 2000).

De Matos *et al* (2001) determined that the soil chemical properties were better at estimating the mobility of the heavy metals compared to the physical properties, although the physical properties should still be taken into account when considering the movement of the contaminants.

11.2. Failure of top soil application as a long term remediation technique

Whilst sampling at Snailbeach, Shropshire, UK, there was evidence of recent remediation techniques being employed. The site is currently subject to a legal dispute between a local farmer and the Shropshire County Council, the details of which are not, for legal reasons, available to this author. Consequently, information surrounding the current remediation practices was not available at the time of writing. However, whilst visiting the site, evidence of established soil coverage was seen as well as wire mesh restraining rubble on the spoil tip. In theory, the idea of using soil on top of spoil tips is to prevent “soil creep” and to limit the concentration of metals available for transport through mechanisms such as weathering and to protect surface organisms from upward mobility of heavy metals. Although the timescale of the remediation techniques is not known, it is clear from the soil results that there are still remains of concentrations of lead and zinc present in the soil. The metals were not however present, or in the same concentrations, at all of the sites which were sampled; this leads to the conclusion that the metals were not present in the soil before application of the soil overburden.

Although the presence of the metals cannot be disputed, the origins can. Thus, there is a possibility that the metals could be transported to the soil surface by atmospheric deposition as discussed earlier, from areas which are exposed to weathering. Additionally it is conceivable that the metals were transported up through the soil by reverse capillarity during particularly warm weather. The water nearer the surface becomes evaporated by the heat of the sun and this encourages the movement of water from lower levels with the implication of vertical transport of the cations.

Although it is not clear which method is increasing the concentration of lead and zinc in the soil, it has been determined that they are present, and that the remediation method of applying top soil is only effective over short periods of time, and that eventually concentrations will inevitably begin to increase.

11.3. Food web contamination

The contamination of food webs can be detrimental to the environment. An example of a simplified food web is illustrated in Figure 11-1, which demonstrates how a number of animals, e.g. hawks, can feed on different species. Therefore contamination of the web, even at the lower levels, can lead to a greater number of animals consuming contaminated material.

In most cases each animal feeds within a small habitat, the size of which depends on the number of breeding pairs of a species present, and the quantity and quality of food available in the habitat.

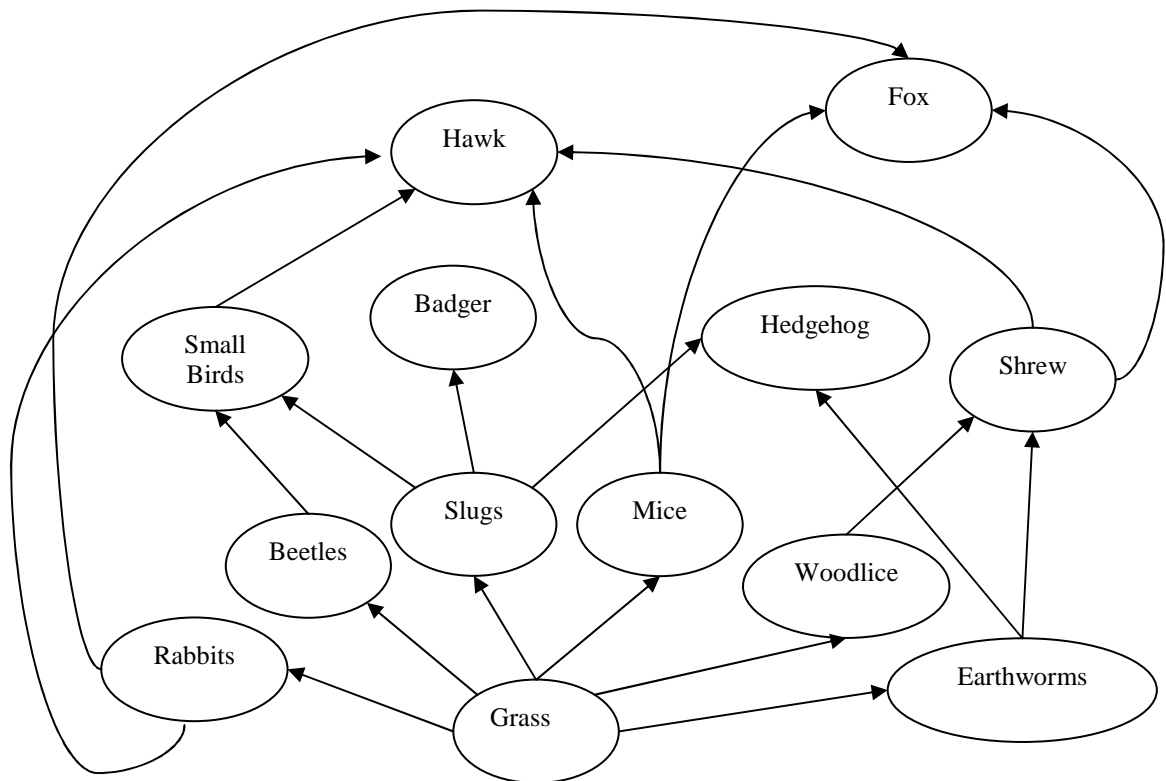
A contaminated site permits the transfer of heavy metals from the environment to enter into a food web and subsequently exhibit bioaccumulation. Transfer through the food web can begin at the lower levels by the consumption of plant material by primary consumers. Plant material grown in contaminated soil can accumulate heavy metals and consequently transfer these metals to the next trophic level. The intake of metals into animals can occur through the ingestion of contaminated material or by ingestion of contaminated particles from their fur. Most mammals (e.g. foxes, mice and shrews) clean their fur on a regular basis by licking their fur. If there are contaminated particles on the fur, these can be swallowed.

It has become apparent that the effects of the heavy metal accumulation are not restricted to a single trophic level, and therefore research studies concerning the implications of heavy metal accumulation in the lowest trophic level have become increasingly important. Notten *et al* (2005) studied the transfer of metals from the soil and plant tissue to a snail species (*Cepaea nemoralis*). The research concluded that there was an increase in the concentration of copper, zinc, cadmium and lead in *C. nemoralis*, compared to those residing in a non-contaminated area. The research demonstrated that the concentrations of these metals were transferred from soil and plant material to the next trophic level, *C. nemoralis*. It can also be seen that the concentration of the cadmium, copper and zinc was magnified in snail tissue, and therefore illustrated the biomagnification occurring in the food web.

In the freshwater environment, the same principles apply. Phytoplankton are the primary producers in the aquatic food webs and include a diverse species of algae. These in turn are either predated by zooplankton or by other organisms such as vertebrates. Phytoplankton, in common with terrestrial plants, can accumulate the heavy metals into their thalli. The phytoplankton are then consumed by animals

further up the food web such as fish. The fish, and other consumers, in the fresh or indeed salt water environment can also absorb metals from the water either through their skin or through their gills.

Figure 11-1: A generalised terrestrial food web (modified from Campbell, 1996)



11.3.1. Producers

In the research program, terrestrial and aquatic producers were examined. The terrestrial producers included vegetables sold for human consumption (cabbage and kale), and plants that would provide nutrients for small mammals and birds: *Viburnum opulum*, and *Cotoneaster microphyllus*. The results demonstrated that all of these plant species accumulated metals from their environment and possess the potential to transfer the contaminants to the next trophic level.

Both laboratory and field experiments have been conducted to investigate the accumulation of metals in edible portions of plants (An *et al*, 2004; Weber and Hrynczuk, 2000; Zhu *et al*, 2004; Almela *et al*, 2006). In these research studies, it was determined that concentrations of metals were accumulated in the edible portions of

the plants, demonstrating that these concentrations were available for transfer to the next trophic level. All the edible portions of *C. microphyllus* contained concentrations of copper and tungsten; additionally the stem portions also contained concentrations of copper, but not tungsten. This leads to the conclusion that during the formation of the fruit and leaves there was an increase in the concentration of tungsten. However, the flow is not maintained on a permanent basis, and it is conceivable that at the time of sampling, the plant was not transporting tungsten through the vascular bundle, and therefore not found in the stem regions. It also indicates that once accumulated into the fruit and leaves, it becomes immobile. Copper is required in small concentrations during the photosynthetic processes; therefore it would be conceivable that there would be a constant flow of the copper ion into the plants leaves and fruit, and this would explain the occurrence of copper in the stem, leaves and fruit at the time of sampling.

The presence of tungsten in *C. microphyllus* and the apparent non-toxic effect in *Pandorina morum*, suggests that they are tolerant to its presence. The substitution of tungsten for molybdenum has been theorised (Koutsospyros *et al*, 2006) and it has been established that further research is necessary. Molybdenum is an essential micronutrient and is necessary for the fixation of nitrogen by nitrate reductase in plants, including algae. Williams and da Silva (2002) discussed the involvement of molybdenum in life, and how it has become an essential micronutrient, despite the fact that there is not an abundant supply in the environment. They also discuss the fact that until 4.5×10^9 years ago, molybdenum was not present in the environment and that tungsten was possibly responsible for nitrogen fixation. It would therefore be conceivable that tungsten can still be used in the nitrogen fixation process of plants, when molybdenum is in limited supply, or when the concentration of tungsten is far greater.

Whilst examining the effects of copper and lead on the alga *Pandorina morum*, it was found that at low concentrations of copper (less than 10ppm); the algae were incapable of growth. Johnson *et al* (2007) determined that the microalgae *Nitzschia closterium* (a marine diatom) showed limited tolerance to copper. Lombardi *et al* (2007) also confirmed that copper has a detrimental effect on the populations of algae, although, lower concentrations (1×10^{-8} g/l) were tolerated during the laboratory experiments. Copper concentrations greater than 1×10^{-3} g/l, demonstrated that the fundamental metabolic processes were affected and that the experiments showed that

there was a decrease in the concentration of chlorophyll a. The reduction in the production of chlorophyll a suggests that the photosynthesis process was interfered with. During this research, similar results were found. A direct comparison cannot be made as different forms of copper contaminant and an alternative algae species was used. However in both experiments it was determined that at low concentrations, both algal species tolerated the presence of copper.

The algal experiments demonstrated that at concentrations less than 150ppm, there was little or no effect on the growth of the population. However there was a clear indication that the concentration of lead in the media decreased during the experiment. It has been suggested that algae can be used to reduce the concentrations of metals in the aquatic environment. Deng *et al*, 2005, monitored the removal of lead from wastewater and concluded that over short periods of time, *Cladophora fascicularis* was capable of extracting lead from the wastewater.

From Figure 11-1, it can be seen that contamination of the producer level can increase the concentration of the metals as the trophic levels increase. The effect of bio magnification can increase the concentration of the metals and thus contaminate the higher trophic levels. The results in this thesis, indicate that this has occurred to a small extent. However it was not possible to test whether the contaminants were passed on to the mammals or birds which consumed the contaminated material. In further research a number of small mammals, e.g. rabbits could be examined for contamination levels. Additionally, a series of tests could be carried out including tests on hair and blood samples progressing to a final dissection and examination of partitioning within the body.

11.3.2. Consumers

All animals require the consumption of material to acquire nutrients, such as minerals and vitamins for vital processes within the body. The consumption of contaminants is an unfortunate event that occurs when the consumed material is either grown in or has itself consumed contaminated material. As discussed, juvenile plants are capable of accumulating unwanted metals within their structure from the environment; this has a detrimental effect on the food chain.

The food chain concentration of the contaminants increases as many organisms (both plant and animal) are unable to expel the contaminant efficiently. In some cases the contaminant is substituted for another chemically similar element. For instance:

the substitution of tungsten for molybdenum in the alga *P. morum* and the plant *C. microphyllus*. In animals, similar substitutions can occur, and the substitution can have a detrimental effect.

During this current research, a number of animal products were analysed for the concentration of heavy metals. The products included frog spawn, egg shell, and bird of prey pellets.

11.3.2.1. Accumulation of metals in amphibians

In the case of the frog spawn there were significant quantities of copper, tungsten and arsenic present in the embryo. Amphibians have been employed as bioindicators of water quality (Ventrino *et al*, 2003). During the first stages of their lifecycle, the amphibians are susceptible to chemical changes in the water environment, and therefore monitoring of the amphibians' early lifecycle can be used to indicate contaminants in the aquatic environment. Karasov *et al* (2005) implemented a sampling strategy to monitor the effects of pollution on frog embryos and tadpoles. It was found that there was a positive correlation between the concentrations of heavy metals in the lake sediments and frogs tissue.

Soil was sampled from the sediment of the water body where the frog spawn was located. The results showed that there were high concentrations of arsenic, copper, lead, tungsten, lead and tin. Of these copper, tungsten and arsenic were present in the frog embryo. This indicates that the concentrations were present before the embryo was fully developed. The embryo was separated from the protective jelly, and therefore the concentrations of the copper, tungsten and arsenic were either absorbed from the protective jelly or transferred from the parents.

The concentrations of the copper, tungsten and arsenic in the embryo samples were: 157.958, 90.836 and 168.404mg/kg respectively. These concentrations are considered to be relatively high, and it could be assumed that these concentrations were obtained from the consumption of contaminated prey by the adults, most likely the female. The main diet of frogs is insects. Unfortunately the frog spawn could not be attributed to a species and therefore the specific diet could not be determined. However the consumption of insects at Blanchdown Wood, would lead to the direct consumption of spoil. In most cases, the insects would pick up spoil remains on their body, this would then be consumed by the frogs and a significant concentration of the metals would also be consumed. Animals are capable of removing unwanted waste

through excretions, but if this process is not efficient enough, metal remains would still be present and there would be no restriction of the transfer of the metals to the reproductive products. Where the frogs reside in a highly contaminated area, the consumption of insects dusted with spoil would occur on a daily basis. It is probable that the bodies' defenses are not efficient to remove all traces of the contaminants. An expansion of this would be to sample adult frogs and to dissect them to determine the extent to which they were contaminated with metals. It was unexpected to the author that no lead was found in the embryos. It is suggested that the lead that is consumed in the diet of the adults is accumulated into the bones and tissues of the adults and is thus not available for the transfer.

If the embryo was allowed to progress, it could be determined if metals could be accumulated from the jelly or from the environment. Berzins and Bundy (2002) conducted a research project to evaluate the uptake of lead in tadpoles from water and sediment. It was concluded that there was an increased concentration of lead in tadpoles with increased environmental concentrations of lead.

11.3.2.2. Accumulation of metals in birds

Although it could not be conclusively identified, the egg shell that was analysed did contain concentrations of both copper and tungsten. This suggested that the adult was accumulating concentrations of copper and tungsten and these were being deposited in the egg shell. Past research into the accumulation of metals in egg shells (Nam and Lee, 2006) suggested that the accumulation has not had an adverse effect on the population of the birds or the birth success rate of the organism. However, it was shown that the egg shells were thinner and consequently there is an increased probability of the eggs being broken during incubation. At Blanchdown Wood the presence of the spoil tips would mean that adult birds would be exposed to heavy metals derived from the metalliferous tips. Although a definitive research study would be required, it would be possible to conclude that the accumulation of the low concentrations of metals is unlikely to affect the viability of the eggs; however they would be more susceptible to breakage, due to the thinning of the shell. During this research the thinness of the shell could not be used in this study as the species of bird was not known and therefore control samples were not available.

A previous study (Ikemoto *et al*, 2005) has been successful in the monitoring of the trace metal content of the birds, through the examination of egg shells and the

extraction of blood. During further studies, this procedure could be used to determine the extent to which the spoil is having on the bird populations. This could include the monitoring of the endangered species *Caprimulgus europaeus* (Nightjars), as well as other birds residing in the area.

Humans and other animals are susceptible to the accumulation of metals due to the chemical similarity of contaminants with required elements. For example the chemical similarity of lead and calcium in bone structure and lead and iron in the haem portion of blood. This similarity can lead to the accumulation of lead throughout the body and also the substitution of these elements in vital processes.

11.3.2.3. Accumulation of metals in bone tissue

The accumulation of metals in bone is somewhat restricted to elements that are either already present in the bone matrix, or to those with chemical similarities to required nutrients. During this research, a number of bone samples, from different sources, were used to determine the concentration of the metals in the bone tissue. The material examined included ancient human femurs, small bones found in the bird of prey pellets and animal bone remains.

11.3.2.3.1. Bird-Of-Prey bone samples

Since birds-of-prey produce a pellet less than 24 hours after ingestion it is assumed that the bones were only exposed to the environment for a limited period of time. Therefore it is likely that any bioaccumulation of the metals by the animal occurs prior to death. However, this can only be concluded for lead, arsenic and tin as these were the only cations found following detailed analysis of the samples. When the results from the remaining pellet media were examined it could be seen that the concentrations of lead, arsenic and tin in the bone samples exceeded that of the pellet media (summarised in Table 11-1).

Table 11-1: A summary of metals found in the pellet samples (mg/kg)

Analyte	Pellet bone	Pellet material
Lead	1000.34	4.43
Arsenic	28236.9	2635.55
Tin	197.23	11.68
Copper	28.87	1712.59
Tungsten	25.67	41.26

Generally, the surrounding media contains a higher concentration of the metals when compared to the bones. Since the concentration of lead, arsenic and tin were greater in the bones, it is concluded that these were accumulated by the animal before death and that the copper and tungsten were more likely to have been absorbed from the environment, either in the stomach of the predator or after expulsion.

11.3.2.3.2. *Animal bone remains*

The animal remains were estimated to be less than a year old (Personal Communication: Phil Piper), and therefore would have been exposed to the environment for much of this period. Therefore it can be concluded that the concentration of copper and tungsten, that was determined, was likely to have originated from the environment. A laboratory experiment using lead demonstrated that over short periods of time pig bones can accumulate lead from surrounding contaminated soil. The animal bones that were collected were not buried; however they were exposed to the highly contaminated environment. The remains also showed a surprising lack of lead, tin and arsenic which would have been expected due to the results derived from the pellet bones. However, the bones were identified to be from a small Chinese deer (*Inermis inermis*), and it is possible that the animal did not consume contaminated material. Deer are herd animals and move around regularly, and consequently it is not possible to determine where the food was consumed. It is likely that the animal may have consumed some contaminated vegetation at grazing sites, and the contaminants were removed from the body efficiently.

11.3.2.3.3. Ancient human femurs

The ancient human bones that were included in the research originated from Cambridge. Unlike the bones that were sampled at Blanchdown Wood, Devon, the human bone samples were retrieved from an archaeological dig at Cherry Hinton (3 miles South-West of Cambridge). The archaeological dig found a number of human skeletons. These were estimated to be from the Anglo-Saxon period, approximately 1100 years old (Carroll, Q: Personal communication). Due to the approximate age of the skeletons, control samples were not available. Prior to sample analysis, it was not expected that the bones would contain any traces of lead, since their origin was not a lead mining region.

Although soil samples were not collected from the burial site, it can not be a definitive conclusion that lead was not absorbed from the soil. Studies have shown that bone tissue can accumulate lead from the soil (Waldron, 1983 and Waldron, 1981). During this research, an experiment was designed to determine if post mortem uptake of lead occurs over a short period of time. The results showed that lead was absorbed into the bone tissue after burial.

However, when all the results were considered it is probable that lead was accumulated through a diet of lead contaminated foodstuffs. The skeletons were buried in the same location, although it is now known the exact positions. During personal communications with one of the archaeologists, (Carroll, Q) it was determined that the skeletons were well preserved. A low pH is not conducive to skeletal preservation. For the movement of lead through soil, the pH of the soil should ideally be low. Because of this, it has been concluded that the skeletons did not accumulate a high concentration of lead from the soil due to the fact that if the soil had a low pH, the skeletons would not have been so well preserved. Although, the pH of the soil can change over time, a prolonged change in the pH would correspond to a deterioration of skeletal preservation. Therefore it is conceivable that small concentrations of lead could be absorbed during ideal conditions of lead movement. It has been concluded that the lead present in the femur samples primarily originate antemortem, although post-mortem concentrations of lead is possible. A research study by Carvalho *et al* (2004) also commented on the absorption of lead occurring ante and post-mortem.

As both the human skeletons were buried in the same area, it may therefore be assumed that if accumulation from the soil had occurred during ideal conditions, there

would be similar concentrations of lead represented in both femurs. But this did not occur, there was a large variation of lead concentration both between the bones and along the bone shafts (CA05/B-1: 30.35 – 138.59mg/kg, CA05/B-2: 208.21 – 549.84mg/kg). These results suggest that partitioning has occurred in the femur bone, because of the range of the results it is concluded that the partitioning occurred during the life of the individual. When studying the results (Chapter 10, Figure 10-25), it can be seen that the two different samples show similar patterns. Although great effort was used to sample at the same point on the bones, it is likely that there was some difference between the sample sites along the bones. Therefore a small amount of variation would be expected.

The results showed that the highest concentrations of lead were found in the femoral head region. This suggests that this region accumulates lead at a higher rate when compared to the shaft of the femur. As discussed in Chapter 10, this could be attributed to the femoral head being composed of spongy trabecular bone, which is responsible for an increased rate of calcium uptake when compared to compact one located in the femur shaft.

During the analysis it was determined that there were high concentrations of iron in the femur bone. Like the lead results, there was a high level of variation between the femur bones and along each of the bones. The highest concentration of iron was located in the femoral head. The concentrations for CA05/B-1 contained a larger variation of iron from 851.57 to 2160.74 mg/kg along the shaft of the bone, with a maximum of 3540 mg/kg in the femoral head. CA05/B-2 had little of no variation across the femur shaft, with concentrations of 254.28 – 354.26 mg/kg. Like CA05/B-1, the maximum concentration was located in the femoral head (4414.643 mg/kg). Carvalho *et al* (2004) also noted that there were high concentrations of iron found in human bones from the Middle Ages. Through examination of the bones by total reflection X-ray fluorescence, they determined that there was a decrease in iron concentration on the surface of the bone compared with inner samples. It was also noted that there was a high level of iron penetration from the soil into the bone, allowing for an increase in absorption from the environment. In reference to the results from this current research, the increased absorption is not likely to have affected the concentration of iron found in the bones. As mentioned before, soil samples were not available, therefore it could not be determined if there was a high

iron concentration in the soil. However, if there was a high iron concentration in the soil, it is likely that both samples would have exhibited increased concentrations.

It is suggested that the bone sample CA05/B-2, belonged to a female of child bearing years. It is also suggested that the woman had given birth to children, possibly quite recently before death. The concentration of iron that was found in the shaft and hip of the femur was at reduced levels compared to CA05/B-1. Although there are a number of disorders which cause iron deficiencies it is likely that such disorders could have contributed to death at an earlier date. Therefore it is suggested that the lack of iron would be due birth induced anaemia, where a mother is suffering from the lack of iron after giving birth.

Although, these conclusions are subjective and cannot be proved one way or the other.

11.4. Health problems associated with metal accumulation

Although this research has been unable to investigate the long term effects of metal accumulation, it has been evident through literature searches that there are a number of adverse health effects of heavy metals on the human body. These include neurological, and soft and hard tissue damage.

Of the metals examined in this study, only zinc and copper are essential micro-nutrients in the human body. These are required for various functions in the body including as components in enzymes and repairing and maintenance of soft tissues in the heart (Campbell, 1996). There are a number of research papers that have investigated the effects of copper deficiency (Oliver, 1975; Klevay, 1998; Hawk *et al*, 1998) indicating that copper is an essential nutrient, and deficiency leads to hypothyroidism, osteoporosis and cardiac defects in rat embryos. Although the studies were based on rats and mice it is likely that the same effects can be seen, to some extent, in humans. Further research has also been conducted regarding the effects of excess concentrations of copper in humans. These papers (Sokol *et al*, 1994; von Herbay *et al*, 1994; Carpenter *et al*, 1988) discuss the effects of excess concentrations of copper and how an overload of copper results in damage to liver cells in individuals with Wilson's disease (a hereditary disease preventing the excess copper from being removed from the body).

The total concentration of zinc in the human body is approximately 2g, and the majority of which is found in the brain. This leads to the conclusion that zinc is an essential nutrient that enables the brain to function normally. The concentration of zinc is believed to be related to disorders such as schizophrenia, Wilson's disease and Pick's disease (Pfeiffer and Braverman, 1982). Their research paper discusses how both schizophrenia and Wilson's disease can be treated with zinc and can therefore be managed. However Alzheimer's is a degenerative disorder of the brain which results in the person to suffer with dementia and it is believed that excess concentrations of zinc can contribute to the onset of the disease.

Tin is generally considered to be non-toxic, and sources on the internet have defined the metal as a possible essential trace element (Albion, 1996). However there seems to be some controversy as there has been little published information published to substantiate these claims. Research that has been done includes the high concentration of tin found in the thymus gland of a number of animals. Sherman and Cardarelli (1992) found the concentration of tin in selected tissues of dogs was

29.4ppm in the thymus gland compared with 12.8ppm in the spleen. Further research published by Cardarelli (1990) suggested that the thymus gland synthesised and secreted tin bearing factors that enhanced immune defenses. Currently, further research has not published by reputable journals or websites.

Like tin, there is little published information about the health effects of tungsten. However in 2006, Koutsospyros *et al* published a paper reviewing the importance of tungsten in the environment. It was stated that, in the USA, a number of leukaemia clusters in children was apparent in areas surrounding an industrial tungsten smelting plant, and it was hypothesised that it was the pollution from the plant which increased the number of leukaemia cases found in children. Although the authors could not definitively claim that it was the tungsten that was responsible, since there were a number of factors present which could also have contributed to the conditions. The paper recognised that more research is needed into the role of tungsten in the environment and its links to adverse human health.

One of the most toxic substances in the environment, arsenic is a well known poison, used previously in pesticides and most famously as a murder weapon. However the long term exposure from anthropogenic sources has caused animals and humans to experience chronic low doses of arsenic. These have been associated with the increased risk of cancer of the body to include: the skin, liver and kidney (Waalkes *et al*, 2004). The carcinogenic properties are brought about through the mutation of DNA; however arsenic can act as a co-mutagen with other DNA-damaging agents resulting in the increased risk of cancer (Reichard *et al*, 2006)

The health effects of lead on humans have been extensively studied. The health effects that are associated with chronic lead exposure include neurological, and soft and hard tissue damage. Hard tissue damage includes the increased risk of osteoporosis, which results in the loss of bone mineral density and can increase the risk of bone fractures. The sites that are more prone to the onset of osteoporosis are that of bones containing spongy bone, including vertebrae and the femur neck/head. Chronic exposure to lead can accelerate the onset of osteoporosis as lead is stored in bones. During the latter stages of life, the calcium is removed from the bone into the blood stream, when required, at a faster rate than is being absorbed back into the bone. The calcium that is extracted from the bone is used in processes around the body. However, if lead is extracted in place of calcium, it can then be substituted for calcium in the processes of the body. If these processes were highly specific and did not accept

the substitution of lead for calcium, more calcium would be required from the bone; therefore decreasing the bone density and thus increasing the risk of osteoporosis.

Some of the major organs in the body that are believed to be affected by lead include lungs, liver, kidney and stomach (Oktem *et al*, 2004; Fortoul *et al*, 2005; Weizsaecker, 2003). As an extension of this, a study by Lam *et al* (2006) was initiated to determine if there was a possible increased risk of cancer related to concentrations of lead. However the study was proved to be inconclusive due the lack of subjects and the limited study period of the subjects. Also there could be a synergistic effect where lead and another element have caused the cancer together, rather than individually. It is clear that further monitoring is needed over longer periods of time.

The possible renal damage caused by excess concentrations of lead in blood has been research by a number of different studies (Hernandez-Serrato *et al*, 2006; Landrigan *et al*, 1990; Staudinger and Roth, 1998; Chia *et al*, 1995). The common opinion is that higher blood concentrations of lead are related to an increased risk of renal damage. Landrigan *et al* (1990) and Staudinger *et al* (1998) suggest that concentrations in excess of 40mg/dL produce the highest risk of renal damage compared to subjects with a concentration less than 40mg/dL.

The causes of neurological disorders are varied and in some cases are still unknown, however research has concluded that lead can influence the neurological development of children who are exposed to lead at an early age (Patriarca *et al*, 2000; de Burbare, 2006; Vermeir *et al*, 2005). Other disorders, including degenerative disorders, are difficult to diagnose and the determination of the causes are also producing debates within the science community. However a common consensus is emerging that the exposure to environmental toxins, such as lead, mercury and cadmium can increase the risk of a degenerative neurological disorder such as Parkinson's disease (Bocca *et al*, 2004)

It is clear that from a medical point of view it is difficult to determine the exact cause of a medical condition. Although a number of research studies have been done concerning the effects of heavy metals on the human body, these can be inconclusive. Since many studies only investigate one element, it cannot be determined if multiple elements would produce the same results. Conversely the study of more than one element can produce results that are different from individual contaminants.

It is also clear, that no toxic element is found on its own, and therefore there could be an additional or synergistic effect between one and more of the elements. It is

therefore difficult to conclusively determine that one element is responsible for a certain medical condition. The effects of synergism are difficult to predict, in the environment and in the human body.

11.5. Critical Evaluation

During the analytical procedure, it was determined that a number of replicates were needed to confirm that the results were accurate. Where possible ten replicates were used as this meant that statistical analysis could be used to determine if the results were accurate. Unfortunately some samples, for instance bone samples, were unable to be analysed in replicate, as there was a limited supply of sample.

Because of the mosaic effect of the concentrations of the metals, there should be a number of samples taken in an area. In this research three replicates were taken in an area of 5m²; however it appears that this number should be increased, possibly to either five or ten samples in a small area. This would ensure that the average concentration values for a site would be more accurate and truly representative of the area.

As with most if not all analytical techniques there is an element of error that can be introduced. It was decided to use the aqua regia method involving the use of hydrochloric and nitric acid. For some samples the acids were strong enough to break down the samples without being left for a period of time, but in other cases, the samples needed to be left over night before progressing with the method. But in the interest of continuity, all samples were left in the acid solution over night. During the later stages of the method, the solutions were made up to a volume of 50mL with ultra pure water. At this point there is a possibility of an error due to inaccuracies with volumetric flasks. This inaccuracy can be increased with smaller volumetric flasks (25mL and 10mL). In some cases it was necessary to use the smaller flasks as there was only a small amount of samples for the analysis.

Although the errors cannot be avoided during the analysis, it would only affect the samples with the lower concentrations of the metals. To overcome these problems, a series of experimental dilutions of a known concentration could be used to determine the extent of the error. The smaller volumetric flasks would also be used. The manufacturers describe the flasks with a tolerance of 0.06mL (50mL), 0.04mL (25mL) and 0.025mL (10mL). This means that although the solution is made up to the line on the flask, the total volume could be plus or minus the tolerance.

The purchase of a new ICP-OES by the university was of great benefit to the research analysis. This allowed for up to 100 samples to be processed and a number of elements could be detected in these samples during the analysis. The convenience of the new equipment allowed for a large volume of samples to be analysed and for

multiple elements to be quantified. However the new equipment was also very sensitive and the use by a number of people meant that without careful maintenance results could be inaccurate. The ICP-OES works on the principle of the samples are sucked in through a tube, but depending on the sample, the tubes could become blocked. It was necessary to implement a booking system where information about the samples could be made available by all users. Further information on the use of the machine is available in the Perkin Elmer manual.

When examining the results for the metal concentration a number of times the results appeared to be wrong. This could be determined when the concentration of the samples is approximately known, for instance the concentrations of lead, copper and tungsten in the algal nutrient media. On one occasion it was found that the concentration of tungsten had increased 5 fold during the experiment. When this was found, the ICP-OES was examined and it was found that a previous sample run had clogged the tube giving wrong results. During the analysis it was possible to re-run the samples through the ICP-OES as there was still sample remaining. During the analysis excess solution was kept in the event of a problem with the ICP-OES.

Chapter 12: Further Research

During the undertaking of this research, a number of further research studies could be performed based on the results of this research. These include the expansion of the accumulation of algae, the difference in the concentration of metals through the soil and increased analysis of archaeological bone samples.

12.1. Vertical metal concentration differences

Previous research (Maskall *et al*, 1995) has been able to establish the rate of movement of metals through certain soils. In addition to this, it has been considered important to monitor the movement of metals through different types of soil, i.e. soil with different compositions. This can then be used as a guide to determine how quickly the metals move through the environment. Because a number of variables determine the rate of movement, in a laboratory based experiment these variables can be controlled. Such experiments can include a range of pH, organic material, concentration of metals and different combinations of metals. Experiments can be used to determine the rates of movement by placing soil with controlled values of organic matter, pH and concentration of the metal in a cylindrical pipe. The pipe will have a number of holes in the side to enable samples of soil to be extracted at different depths. Over a period of time small volumes of water can be added to simulate rain and the concentrations of the metal could be monitored. The experiment can then be varied to include different pH, organic material, concentration of metals and combinations of metals. When there are a number of different metals in the soil, this can have adverse effects on their movement. For instance the presence of one metal could restrict the movement of another or enhanced the movement. The data which would be produced from this could be used as a guide in remediation methods.

An additional method of vertical movement is possible and that is vertical upward movement. The heat of the sun can evaporate the water near the surface and draws water up through the soil. With this method it is possible that metals are also drawn up with the water. A laboratory experiment could be devised to determine if this does occur. Using a cylindrical pipe, as in the previous experiment with the addition of a tray of water underneath and a heat lamp above the pipe containing soil, known

concentrations of metals can be placed at different depths. The final concentration being determine by sampling through the side holes. During the experiment at different time intervals, samples will be taken through the small side holes. This would then be used to determine if there is movement of metals through the soil, whether it is upward or downward. In theory as the heat from the lamp evaporates the water in the top layers of the soil, water from the container will be drawn up through the soil.

12.2. Expansion of bone analysis

The Cambridge bone samples provided results suggesting that the an unknown sample was in fact female and of child bearing years. Further study of these skeletal remains could be used to confirm this. The skeletal remains that were discovered are assumed to be of the same population and therefore part of community. Within a community it is likely that the individuals would have similar diets, although the lower members of this community may have had a poorer diet they would not have been buried in the same place as those of a higher status. Therefore by examining more of the bones, especially those which have already been identified as male or female could support the conclusion of the unknown individual. However the examination of the bones is deemed as destructive as any of the sample that is used to determine elemental concentrations is destroyed. Also in some cases the extraction of the sample can result in the destruction of the bone.

In this current research, the bone samples were extracted using a saw; an adaptation of this would be to use a drill with a circular head. This would enable the sampler to retrieve a cylindrical sample. This would not destroy the bone completely and a number of tests would be able to be performed on the bone sample. The resultant disc could be divided into further sections for acid digestion, with the remaining bone being fixed in resin for preparation for use on an electron microscope. The electron microscope could identify the areas of the bone sample where certain elements were found and in what concentrations. The acid digestion of the sample would give a definitive indication as to the concentration of the metals. This method would leave the archaeological sample intact.

12.3. Expansion of the accumulation of metals in algae

This research has highlighted the fact that *Pandorina morum* is affected by tungsten, copper and lead. However, high concentrations of lead and tungsten did not produce a toxic effect on the algae. During this analysis it could not be determined if the metals were accumulated into the algae or were adhered to the surface. It was attempted to view the algae under an electron microscope, however the algal cells once removed from the nutrient media and dried, burst and an accurate picture could not be produced. A possibility to overcome this would be to embed the algae in resin and then examine thin slices using electron microscopy. This would allow the surface and the internal structures of the algal cells to be seen and therefore the determination of the location of the metals.

The results for algae grown in nutrient media contaminated with lead showed that there was a decrease in the concentration of lead in the solution. However the results for the tungsten did not show the same decrease. There was no significant difference between the concentration of tungsten before and after results. To determine if the metals have been removed out of the solution, it would be necessary to separate the algae from the nutrient media. This would involve filtering the algae from the media, however to be able to acid digest the algae, a significant amount of algae would have to be produced.

The results have shown that the metals have affected the population growth of the algae, but it was not determined if this could be passed on to the next trophic level. The next stage is to incubate a population of algae in nutrient media contaminated with metals at a concentration that is not toxic to the algae. The algae could then be filtered from the contaminated media and placed in a container with nutrient media and a population of primary consumers such as *Daphnia spp.* In most cases of metals, the toxic concentrations have been determined for *Daphnia spp.*, however it would be necessary to determine the toxicity levels for *Daphnia* in the testing system that would be used.

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