AL, CU, FE AND MO CONTENT OF *PHALARIS AQUATICA* AND *TRIFOLIUM SUBTERRANEUM* GROWN ON A TAILINGS DAM AT CADIA HILL GOLD MINE NEAR ORANGE, NSW

by

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DECLARATION

I certify that this dissertation does not incorporate, without acknowledgement, any material previously submitted for degree or diploma in any university. It does not contain any material previously published or written by another person except where due reference is made in the text.

This dissertation does not exceed 15,500 words.

Signed:

Vanessa Connick

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Abstract

Pasture samples from *Phalaris aquatica* (phalaris) and *Trifolium subterraneum* (sub clover), growing on various soil substrates in a tailings rehabilitation site at a gold—copper mine in Central NSW, were analysed to determine their content of Al, Cu, Fe and Mo. Soil samples taken from the various soil substrates were also tested for these metals as well as other soil properties.

Results from plant samples reveal that there was greater content of all metals in sub clover than phalaris, and that metal content in both sub clover and phalaris was significantly higher when growing on the tailings substrate than the amended substrates for all the metals tested The results also revealed higher content of metals found in the winter samples compared to the spring samples. Regression analysis detected associations between Cu, Fe and Mo content in sub clover and Al content of soil. A significant association was also found between Al content in sub clover and K, Na and sulphur levels in the soil. No association was found between soil conditions and metal content in phalaris.

The most significant anticipated problem in regards to livestock health was the Cu:Mo ratio of sub clover growing on the tailings substrate being 1:2, while the minimum recommended ratio for livestock health is 2:1. Cu levels were on or below the minimum recommended levels for livestock health for all substrates, and Mo levels were significantly higher on the tailings and biosolids substrates. The most likely implication of these results is Mo toxicity (molybdenosis) or Cu deficiency in livestock.

1 Introduction

Metal and mineral mining is one of Australia's largest export earners, earning around $116 billion in 2007—2008 (ABARE, June 2008). Cadia Valley Operations (CVO) is the largest gold mining operation in NSW and one of Australia's largest gold producers, producing 715,588 oz of gold and 60,687 oz of copper in 2007—2008 (Newcrest Mining Ltd, 2008).

However, with a growing level of environmental consciousness in many countries, it is no longer acceptable for mining companies to leave behind residues that contaminate and pollute the environment upon the closure of the mine. Historically, mines have left massive levels of contamination mess all over the world, costing governments of those countries billions of dollars to clean the pollution up. Most countries, in consequence, require mine operators to generate an Environmental Impact Assessment (EIA) and Environmental Impact Statement (EIS) of their operations, and they are, by law, obligated to fulfil any rehabilitation obligations identified within those documents (Australian Mining Act 1993; Bengson, 2000).

A mandatory requirement of the CVO's Environmental Impact Statements (EIS) dated 1995 is that CVO rehabilitate the area under the tailings dams to be "returned to the pre-mining land use of good quality grazing and pasture land" (Cadia Holdings Pty Ltd, 1995). CVO's EIS states that the tailings surface will be rehabilitated by adding topsoil which is then direct seeded. Low areas of the tailings dam could be filled with a 'veneer' of rock before topsoil is added and the area seeded. Rehabilitation of the tailing dams is a project that will be carried out upon the completion of mining activity in 15—25 years, and this commitment is reiterated with all subsequent mining leases and development consents (Newcrest Mining Ltd, 2008; Jeff Burton, 29.2.08 pers.comm.).

1.1 Metal uptake by plants

There are 16 elements currently known to be essential for plant health, being C, O, H, N, K, Mg, Ca, P, S, Fe, Cl, Mn, Zn, B, Cu and Mo (Singer & Munns, 1991). All metals behave differently in plants, and can generally be divided into those who accumulate primarily in the shoots of plants (for example, Fe and Mo), and those who accumulate primarily in the roots (for example, Cu and Al) (Menzies et al, 2007). Where a metal accumulates in a plant will affect the plants' toxicity to grazing animals, and needs to be taken into consideration when assessing the impact of metals on grazing livestock. This study will assess the plant content of four metals, namely Al, Cu, Fe and Mo, in introduced pastures growing on the CVO tailings rehabilitation site.

1.1.1 Uptake of Al by plants

Al is not considered an essential micronutrient in plants, however, this is still debated (Barker & Pilbeam, 2007). High Al levels are usually strongly associated with acidic soils, with Al in its water-soluble form (Al^{3+}) increasing in availability as pH drops below 5.0 (National Research Council, 2005; Barker & Pilbeam, 2007; Singer & Munns, 1991; Department of Agriculture and Food WA, 2008). The following reaction (equation 1) shows the dissolution of aluminium hydroxide, which results in a thousandfold increase of available Al^{3+} in the soil for every one-unit drop in pH (Singer & Munns, 1991):

$$Al(OH)_3 = Al^{3+} + 3\ OH^- \quad (1)$$

In soils with a pH above 5.0 Al is less available for plant uptake and can precipitate as $Al(OH)_3$ (Barker & Pilbeam, 2007; Singer & Munns, 1991; Department of Agriculture and Food WA, 2008). Crop plants, such as grasses and legumes, generally exclude Al from uptake by their roots (Barker & Pilbeam, 2007). Al^{3+} is toxic to plants and, as it does not easily cross the plasma membrane, it generally accumulates outside the endodermis of plant roots (Barker & Pilbeam, 2007). Levels of Al in the soil solution exceeding 20 mg/litre is toxic to most pastures, resulting in immobilised phosphorous, damaged plant roots, and restricted ability of plant roots to access other nutrients and water (DPI NSW, 2009; Department of Agriculture and Food WAl, 2008).

1.1.2 Uptake of Cu by plants

Cu is an essential micronutrient of plants, yet its uptake is usually quite low and it can be toxic when present in high quantities (Barker & Pilbeam, 2007). It is most available for plant uptake at a soil pH of 6.0 or below, as it binds strongly to soil minerals and organic matter at a pH above 7.0 (NSW DPI, 2009; Barker & Pilbeam, 2007). Cu is more available for plant uptake in the form of Cu^{2+}, however, absorbed Cu is not easily transported through the plant, and therefore usually accumulates in plant roots, with decreasing concentrations accumulating in the flowers, then leaves and then stems (Barker & Pilbeam, 2007). The levels of Cu in the shoots of plants are likely to be at their highest during periods of intense growth (Barker & Pilbeam, 2007). Legumes generally absorb greater levels of Cu than grasses (National Research Council, 2005; Board of Agriculture, 2000; Merck Veterinary Manual, 2008; Saunders Comprehensive Veterinary Dictionary, 2007; Barker & Pilbeam, 2007), however, the study by McBride ((2005) mentioned below in point 1.4) showed grasses absorbing more Cu than legumes. The optimum level of available Cu in the soil is between 2 to 50 mg/kg dry plant matter (NSW DPI, 2009).

1.1.3 Uptake of Fe by plants

Fe is an essential micronutrient for plants. In the soil Fe is generally present in the insoluble ferric (Fe^{3+}) form, and becomes more soluble (as Fe^{2+}) as the pH decreases, being most available for plant uptake at a pH of less than 4.0 (Barker & Pilbeam, 2007; Peverill et al, 1999; Magdoff & Weil, 2004). From pH 4.0 to 8.0 the availability of Fe decreases, only to become more available again as pH becomes greater than 9.0 (Barker & Pilbeam, 2007). Fe uptake is generally lower in calcareous soils, and its uptake is positively influenced by increasing the level of S in the soil (Astolfi et al, 2006). Fe can become toxic under acidic soil conditions, though this usually only happens in flooded soils (such as irrigated rice fields) (Dimkpa, 2007; International Rice Research Institute, 2003). Fe toxicity is likely to occur to plants growing in a soil containing > 300 mg Fe/L soil, or with a K:Fe ratio below 18:1 (International Rice Research Institute, 2003).

Grasses are more efficient than other plant species in absorbing Fe from the soil, with grasses and legumes adopting different methods of absorption, and legumes are more sensitive to all the above factors affecting uptake of Fe (Barker & Pilbeam, 2007). In a healthy plant, 60% of leaf Fe is stored in the chloroplasts of rapidly-growing leaves, with lesser concentrations found in the roots, xylem, phloem and seeds (Yadav and Singh, 1988; Barker & Pilbeam, 2007).

1.1.4 Uptake of Mo by plants

Mo is an essential micronutrient for plant growth, however, it is required in only very small amounts (Barker & Pilbeam, 2007). Generally, metals within soils are immobilised when the pH is above 5.0 (Bobrowsky, 2002; Bengson, 2000), however, Mo in the soil becomes more easily available for plant uptake with increased alkalinity. Uptake of Mo by plants is enhanced by soluble phosphorus and nitrate, and inhibited by available sulphur (Reid, 2001; Pasricha et al, 1977; Barker & Pilbeam, 2007). In the soil Mo is absorbed as molybdate and is easily translocated throughout the plant via the xylem and phloem, where it is stored in greatest concentrations in Mo-containing enzymes, as well as in the vacuoles of peripheral cell layers of the plant (Barker & Pilbeam, 2007). Mo is found in greatest concentrations in seeds, followed by legume root nodules, leaves and stem, in decreasing order (Barker & Pilbeam, 2007). Legumes generally require Mo in higher levels than do other plant species, especially during times of rapid growth (Barker & Pilbeam, 2007). The optimum level of Mo in the soil is 2 mg/kg dry plant matter (NSW DPI, 2009).

1.2 Process of metal uptake by plants

The uptake of metals by plants is similar for all elements and plant species; metal ions dissolved in soil water, diffuse through the cell wall of the root hairs to the outer cell membrane, which can control the uptake of an ion depending on the ion's concentration in the surrounding cells (Singer & Munns, 1991; Rost et al, 1984). Some plants contain special transfer enzymes which are able to absorb or exclude certain ions from passing through the plasma membrane (Singer & Munns, 1991; Rost et al, 1984). Once within the plant, metals are translocated via the xylem to the growing leaves and shoots of the plant. This movement of metal ions into and within the plant involves two forces; firstly, water will always flow from areas of high ion concentration to areas of low ion concentration, with the difference between these concentrations expressed as the water potential (Rost et al, 1984) and is usually how metals enter the plant via the roots. Secondly, water is pulled up through the xylem of the plant due to forces arising from leaf transpiration and osmosis (Rost et al, 1984). Metals are usually then assimilated into the growing leaves and shoots of the plant. As the leaves and shoots age, the assimilated metals are released and re-translocated via the phloem to young growing leaves, roots, fruits or storage organs (Singer & Munns, 1991). However, generally micronutrients (including metals) do not re-translocate with ease (Singer & Munns, 1991), resulting in them remaining in greater concentrations in older plant tissues when the soil-availability of such metals is limited.

1.3 Factors affecting metal uptake by plants

Complex interactions within the soil affect metal uptake by plants and these include:

- presence and levels of metals in the soil
- soil chemical properties (mineralogy) and cation-exchange capacity
- soil pH
- other soil properties, including permeability and moisture-holding capacity, soil organic matter content, and soil micro-organism activity
- surrounding environment and climate

(Singer & Munns, 1991; Wilkinson et al, 2003; National Research Council, 2005; Barker & Pilbeam, 2007; Peverill et al, 1999; Magdoff & Weil, 2004).

1.3.1 Presence and levels of metals and minerals in the soil

The types of metals present, and their concentration in the soil, will influence metal uptake by plants. Some metals, such as Cu and Mo, have an antagonistic relationship (that is, compete with each other for plant uptake), resulting in high levels of Mo in the soil reducing Cu uptake by plants. High Al levels in the soil will affect uptake of nutrients and metals in the following ways:

- inhibition of root elongation, suppressed root and shoot biomass and abnormal root morphology all interfere with nutrient uptake
- decrease uptake and translocation of P, Ca, Mg and K, which may result in deficiencies in these nutrients in the plant and in grazing livestock
- restricts water uptake and movement in plants
- suppresses photosynthesis
- inhibits root symbiosis with Rhizobia bacteria; a study has found the percentage of plant nitrogen declines in sub clover as foliar Al levels increased (Panford et al, 1993; Barker & Pilbeam, 2007).

1.3.2 Soil chemical properties (mineralogy) and cation exchange capacity

Tailings soils are typically high in salts (such as Na, Ca and Mg (US EPA, 2000)), and the types of salts present, and their concentration in the soil, will influence metal uptake by plants. High levels of salts within the soil (saline soil) will compete with metal ions for uptake by plants. Sodic soils contain high levels of exchangeable Na (in excess of 6%) within the soil, which will also compete for metal ions for uptake by the plant, but also has the effect of physically disrupting the soil structure. Sodic soils may become impermeable due to Na causing the colloidal particles to disperse into individual particles, and the soil pores clog up (Singer & Munns, 1991).

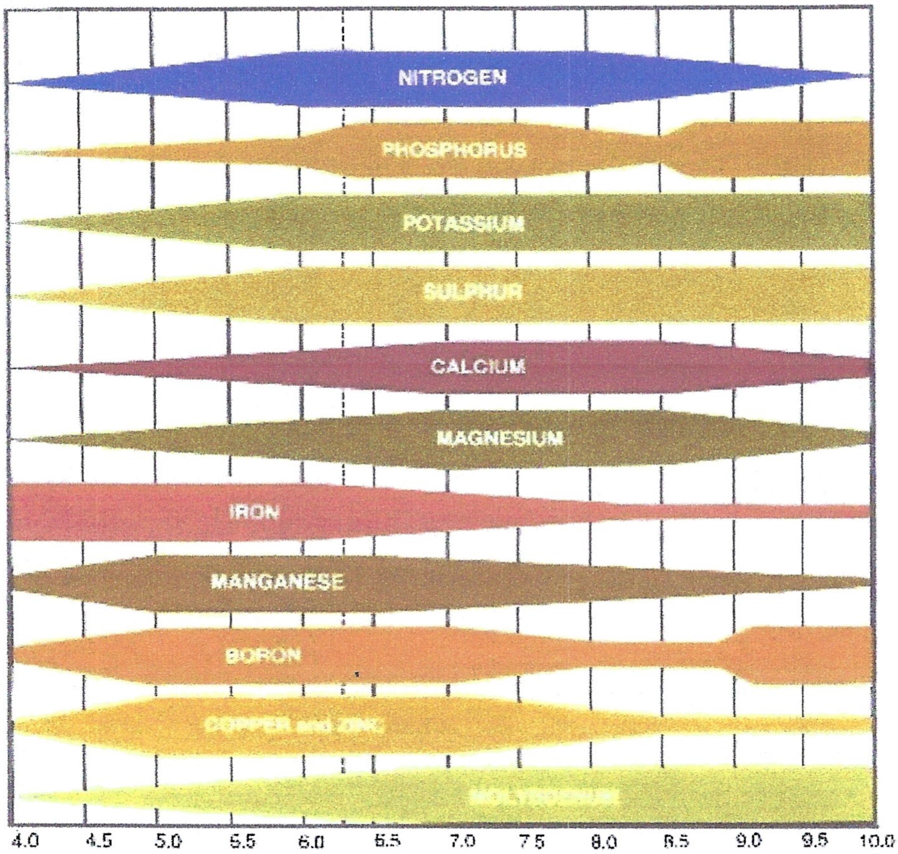
Electrical conductivity (EC_e) of a soil solution measures the salt content of the soil, and a soil is considered saline when it has an EC_e value greater than 4dS/m (Singer & Munns, 1991; Dairy Extension Centre, 2005). Saline soils are generally alkaline, however, can be acidic where soils are high in sulphides which produce sulphuric acid when oxidised (characteristic of tailings soil) (Singer & Munns, 1991). High EC_e values in the soil affect metal uptake by plants as the high concentrations of salts in the soil make it more difficult for water to enter the roots of plants due to salt concentrations of the roots being less than that of the surrounding soil.

Cation exchange within the soil is where positively-charged ions (Ca, Mg, Na, K and Al) may be released or attached to the colloidal soil particles from the soil water solution, and is greatly influenced by the amount of organic matter in the soil, and the type (and how finely divided they are) of clay particles within the soil (Singer & Munns, 1991; Rost et al, 1984; NSW DPI, 2009). Its measurement, and relevant percentages of cations present, gives a good indication of soil fertility (Singer & Munns, 1991; Rost et al, 1984; NSW DPI, 2009). Generally, tailings soil consists of very little organic matter, however, the cation exchange capacity (CEC) may still be very high, usually because the clay particles within the soil have been crushed to a fine powder, offering large amounts of negatively-charged surface area on which the positively-charged ions may attach.

1.3.3 Soil pH

To maintain a chemical balance within the roots, the uptake of cation and anion (negatively-charged ions) must be balanced by the exporting of H+ and OH- or HCO3- respectively, which accordingly affects the pH of the rhizosphere (Singer & Munns, 1991; Department of Agriculture and Food, WA, 2008). Therefore, in acid soils H+ ions replace cations on the negatively-charged clay surfaces, allowing the metals to be leached with the soil water (Rost et al, 1984). Al and Fe are variable-charged ions, with their positive or negatively charged ions dependent upon soil pH, and becoming more positive and increasing in solubility as pH drops below 5.0 and 4.0 respectively (Rost et at, 1984; Barker & Pilbeam, 2007). Figure 1 shows the availability of macronutrients and some metals over a range of pH values.

Figure 1: Availability of macronutrients and metals over pH range (www.organicgarden.org.uk)



Alkalinity in tailings is associated with the pH of the parent rock, together with inadequate drainage of bases and arid conditions. Acidity in tailings soil is generally associated with the oxidation of pyrite, producing sulphuric acid.

1.3.4 Other soil properties including permeability and moisture-holding capacity, soil organic matter content, soil texture, and soil micro-organism activity

Soils that have poor soil structure, such as tailings soils, generally have poor permeability, poor moisture-holding capacities and are susceptible to waterlogging. The poor permeability is due to the dispersed clay particles at the surface of the soil forming a crust that impedes the infiltration of water into the soil and results in water-stress for the plants, reduced microbial and plant growth, and reduced metal uptake. The lack of organic matter within the soil and the lack of pore spaces between the soil particles results in poor moisture-holding capacity, with the same results as above. Waterlogged soils result in less oxygen being available for the plant roots, as well as for the soil micro-organisms. Lower soil oxygen levels will result in a reduction of other substances required (instead of oxygen) for plant and micro-organism respiration (Singer & Munns, 1991). An example is the reduction of Fe from iron oxide to the more absorbable Fe^{2+} is shown in the following reaction (2):

$$Fe(OH)_3 + 3H + e^- = Fe^{2+} + 3H_2O \quad ^{(2)}$$

Two common forms of symbiotic relationships between plant roots and soil micro-organisms are mycorrhizae and bacteria nodules (Rost et al, 1984). Mycorrhizae are short roots of a plant which form a symbiotic relationship with soil fungus, in which the plant provides vitamins and carbohydrates in exchange for mineral nutrients from the fungus (Rost et al, 1984). Mycorrhizae are found in 85% of all plant species, and occur in most crops and grasses (Wang and Qiu, 2006). Bacteria nodules are found on the roots of many legumes and are formed by the bacterium *Rhizobium* which change ('fix') the nitrogen in the soil from N_2 to NH^{4+} (ammonia) which is usable by the plant. The bacteria infects the hairs of the plant roots, creating an infection thread through the epidermal cells into the cortical cells and allowing easy passage for the ammonium to pass into the plant (Rost et al, 1984). Both these symbiotic relationships are believed to enhance water, nutrient and metal uptake by plants. However, some studies have shown that the presence of mycorrhizal fungi may enhance the root/shoot barrier, preventing toxic levels of metal uptake by the plant (Dehn & Schuepp, 2003; Takacs et al, 2001).

Soils with low levels of organic matter (such as tailings soil) may have lower levels of metal uptake by plants due to reduced microbial activity and chelating agents present in the organic matter in the soil, greater soil compaction resulting in decreased plant root growth, and (as low organic matter levels and soil compaction tend to result in soils which retain moisture) can be anaerobic (Peverill et al, 1999; Barker & Pilbeam, 2007).

1.3.5 Effect of surrounding environment and climate

Temperate inland areas in the lower latitudes of South Eastern Australia typically display a Mediterranean climate, with distinct seasons and generally higher rainfall in the cool to cold winter months. The energy required by plants to maintain a more concentrated solution of elements, including metals, within the roots than in the surrounding soil, in order for water intake to continue, comes from photosynthesis. Photosynthesis is therefore reliant on day length and temperature, and cold, overcast winters result in less plant growth, and low soil temperatures can also reduce metal uptake by slowing down microbial activity in the soil (Barker & Pilbeam, 2007).

Microbial activity in the soil—root zone is believed to affect the availability of plant nutrients and metals, either by assisting their uptake by plants (Singer & Munns, 1991), or by reducing metal toxicity by making the metals less bioavailable for plant uptake, depending upon the type of soil microbe present (Pepper & Gerba, 2004). Microbial activity is generally sensitive to extremes of temperature and lack of moisture, with low soil temperatures generally reducing metal uptake by slowing down microbial activity in the soil (Barker & Pilbeam, 2007).

A clear understanding of the behaviour of metals within the soil, and the variation within plant species as to their mineral and metal requirements and their ability to uptake or exclude metals is required when predicting the implication of livestock grazing pastures growing on metal-rich soils. When assessing impact on grazing livestock, it is also important to understand where the mineral is stored in the plant tissue; the roots, shoots or seeds and flowers – testing shoot tissue for mineral levels will not reflect mineral levels in the soil or roots if that mineral is stored in the roots of the plant (Menzies et al, 2007), which are unlikely to be eaten by livestock.

1.4 Variation of metal content between plant species

There is great variation among plant species in the ability to take up and store metals, with some species of plant able to tolerate higher metal levels, and some can even hyper-accumulate certain metals (Mertens et al, 2005). The level of metals found in plant tissue is dependent upon the ability of the plant to uptake the metal, or to exude such metal from the roots, or to prevent the movement of the metal within the plant (Wilkinson et al, 2003). Generally, the uptake or exclusion of metals by plant roots is specific to that plant species and is the result of low molecular-weight root exudates, composed of sugars, amino acids, phenolic compounds and organic acids (Wilkinson *et al*, 2003). Specific preferences of the transfer enzymes found in plant roots differs among the species, and these enzymes can discriminate between closely related metal ions (Singer & Munns, 1991), allowing high levels of some metals to be absorbed, whilst excluding others.

Studies have shown that legumes growing on a sludge-amended site in New York absorb higher levels of Mo from the soil when compared with grasses growing on the same soils, while the grasses absorbed higher levels of Cu than the legumes (McBride, 2005).

1.5 Mineral and metal requirements and tolerances of sheep and cattle

Many minerals (inorganic elements) found in the earth's crust are essential to the health of livestock, while others are non-essential. The essential minerals, being Ca, Cl, Mg, P, K, Na, S, Co, Cu, I, Fe, Mn, Mo, Se and Zn, have well-defined nutritional and biochemical roles, yet others, including Al, appear to be required in trace amounts yet their role in livestock nutrition is still debated and not completely understood (National Research Council, 2005; Judson & McFarlane, 1998). Table 1 below gives the recommended Cu, Fe and Mo requirements of sheep and cattle grazing Australian pastures.

Table 1: Livestock metal requirements and tolerances and the critical concentrations of these metals in the whole shoot for plant growth (National Research Council, 1980; Standing Committee on Agriculture, 1990; Reuter and Robinson, 1997). Source: Judson & McFarlane, 1998.

Mineral	**Min. Concentration in diet**^{A}		**Max. tolerable concentration in diet**^{B}		**Min. adequate concentrations in whole plant shoots**	
	Sheep	Cattle	Sheep	Cattle	Subterranean Clover	Phalaris
			Trace Elements (mg/kg dry matter)			
Aluminum	?	?	1000	1000	?	?
Copper	5	7-10	25^{D}	100	4-5	2-4^{C}
Iron	40	40	500	1000	50-75^{C}	40-60^{C}
Molybdenum	0.1?	0.1?	10	10	0.1-0.2	0.1-0.15^{C}

[A] Where a range is given, the lower value is for maintenance and the higher value is for growing and lactating animals.

[B] Where a range is given, the lower value is for lactating animals.

[C] Young tissue.

[D] The maximum tolerable concentration for Cu depends upon the presence of other dietary elements and values as low as 10 mg/kg can be toxic (see text).

? Figure is still debated

The maximum tolerable level of a dietary mineral is the level that should not induce or inflict any health impairment on an animal when fed over a period of time (National Research Council, 2005). The maximum tolerable limits of minerals in livestock diets varies according to the relative concentration and availability of other minerals in the diet. For example, Cu and Mo act antagonistically towards each other, so high levels of Mo will reduce the availability of Cu (Judson & McFarlane, 1998; National Research Council, 2005). The main factors affecting the absorbance and accumulation of metals by livestock are:

1. the animal's age, gestational status, metal levels, and general health
2. conditions in the gastrointestinal tract
3. the type and amount of metals ingested
4. duration of exposure to the contaminated soil and pasture
5. other components of the diet which may interact with the metals to either enhance or inhibit absorption (Gupta, 2007; Wilkinson *et al*, 2003).

Mineral deficiencies and toxicities can either cause specific known disorders, such as swayback in lambs due to Cu deficiency, or result in general ill-health and lower production outputs (Judson & McFarlane, 1998). Diagnosing minor deficiencies and ill-health in livestock is difficult, as there may be more than one mineral deficiency, or there may be other complicating factors such as gastrointestinal parasites, which can affect the animal's tolerance, need or uptake of minerals (Judson & McFarlane, 1998).

Grazing cattle can consume up to 100g soil/kg dry matter herbage, and sheep up to 300 g soil/kg dry matter herbage, with this figure varying seasonally with the height and abundance of pasture (Judson & McFarlane, 1998). Accordingly, the concentration of minerals in the soil needs to be taken into consideration when predicting the implications of metals in livestock's' diet. Studies on Cu levels of livestock have shown that ingestion of soil can raise or lower their Cu status, and therefore both pasture and soil analysis is required to accurately predict the level of Cu ingested by livestock (Campbell et al, 1974; Suttle et al, 1975; Mayland et al, 1977; Langlands et al, 1982; Grace et al, 1996).

1.5.1 Al requirements of grazing livestock

Al is the third most abundant mineral in the earth's crust (Allen, 1984; National Research Council, 2005), and while there is no evidence showing Al as being an essential nutrient in livestock diets, it's role is still poorly understood and there is some indirect evidence showing that it may be essential, if only in extremely small amounts (Barker & Pilbeam, 2007; Allen, 1984). Current evidence suggests that Al affects livestock health indirectly; by affecting the absorption and role of other nutrients within livestock. Barker and Pilbeam (2007) state that the maximum tolerable level of Al in cattle and sheep is 1000 mg/kg dry feed, with this value being for highly soluble forms of Al, and the maximum limit can be increased with less soluble forms.

There is a strong link between high Al levels in the soil and acidic soils (Barker & Pilbeam, 2007). Levels of Al up to 1000 mg/kg dry feed do not appear to affect cattle and sheep, however as levels increase there is a proportional increase in altered metabolism of fluoride, P, Ca and Mg within the livestock (Allen, 1984).

Al is believed to react with P in the gastrointestinal tract to form non-absorbable Al-P compounds which are then excreted in the faeces (Allen, 1984). High levels of Al in the diet are thus associated with livestock deficiencies in P (Allen, 1984; Barker & Pilbeam, 2007). High levels of Al in the livestock diet also inhibits Mg absorption, which increases the likelihood of animals developing grass tetany, a metabolic disorder of ruminants (Dennis, 1971; Allen and Robinson, 1980; Barker & Pilbeam, 2007). Allen (1984) states that ingestion of dry feed containing 2,000 ppm Al could result in lowered serum Mg levels and, coupled with increased Ca loss (such as pregnancy and lactation), may result in outbreaks of grass tetany. The National Research Council US (2005) reported that lambs fed a diet containing 2000 mg Al/kg in the form of Al chloride displayed depressed growth, feed intake, plasma P levels, and P absorbance.

Al forms insoluble complexes with fluoride, inhibiting the toxic effects of fluoride on livestock, which can cause the mottling of teeth (fluorosis) (National Research Council US, 2005).

1.5.2 Cu requirements of grazing livestock

Cu is a micronutrient, essential for growth and as a component of a number of enzymes, including lysyl oxidase, cytochrome oxidase, superoxide dismutase, ceruloplasmin, and tyrosinase in livestock (McDowell, 1992; National Research Council, 2005). These enzymes are an integral part of energy metabolism, maturation and stability of collagen and elastin, pigmentation, the antioxidant defense system, iron metabolism and other biological processes (National Research Council, 2005). Cu deficiency in livestock can result in diaorrhea, cardiovascular disorders, depigmentation of hair, loss of crimp in wool, anemia, reduced growth and poor weight gains, neonatal ataxia, bone abnormalities (including swollen and painful joints) and impaired immune responses (National Research Council, 2005; UC Davis Veterinary Medicine Extension, 1997).

Cu absorption in ruminants is low (1-10%) compared to non-ruminants, and it is greatly affected by complex interactions with molybdenum and sulphur (National Research Council, 2005; Board of Agriculture, 2000; Merck Veterinary Manual, 2009). Cu absorption occurs in the rumen and is adversely affected by the presence of ruminal microflora, resulting in greater absorption of Cu (up to 75%) in young ruminants with less established ruminal microflora (National Research Council, 2005). Once Cu is absorbed in the blood, it travels to the liver where it is used for synthesis of Cu-dependant enzymes, stored or excreted in bile (National Research Council, 2005).

Most Cu deficiencies in ruminants are caused by the antagonistic relationships between Cu, Mo and S in the rumen (Board of Agriculture, 2000; Judson & McFarlane, 1998; The National Research Council, 2005; UC Davis Veterinary Medicine Extension, 1997). Mo levels of 4-5 mg/kg dry matter and S levels of 0.3% of diet may cause Cu deficiency in ruminants (National Research Council, 2005). When Mo and S are high in the diet, the molybdate and sulfide interact to form thiomolybdates, which then react with the Cu in the rumen to form insoluble complexes (Board of Agriculture, 2000; National Research Council, 2005). The main consequences of thiomolybdates on Cu metabolism are: 1) increased excretion of Cu in the bile; 2) removal of Cu from Cu-dependant enzymes; and 3) binding of Cu to plasma albumin which reduces the transport of available Cu for biochemical processes.

When the Cu:Mo ratio in the livestock diet falls to 2:1 or less, Cu absorbance by the ruminant will be severely impaired and Cu deficiency is likely (UC Davis Veterinary Medicine Extension, 1997; McBride, 2005).

1.5.3 Fe requirements of grazing livestock

Fe is an essential micronutrient of livestock, being a component of proteins involved in oxygen transportation or utilisation (Board of Agriculture, 2000). More than 50 percent of the Fe in the body is present in haemoglobin, with smaller amounts present in other Fe-requiring proteins and enzymes, and in protein-bound stored Fe (Board of Agriculture, 2000). The minimum requirement for Fe is 40 mg/kg diet in sheep and beef cattle (Judson & McFarlane, 1998).

Under normal dietary conditions, Fe is poorly absorbed and excreted by livestock; with only 5-15% of Fe ingested absorbed into the body of the animal (Gupta, 2007). Fe is most easily absorbed in the small intestine (specifically, the duodenum) of livestock in the form of ferrous sulphate, followed in decreasing order of absorption by ferrous carbonate and ferric oxide, which is difficult to absorb (National Research Council, 2005; Board of Agriculture, 2000). In the ferrous state (Fe^{2+}), Fe is absorbed by mucous cells of the small intestine, and is then transferred to the serum where it reacts with hydrogen peroxide to produce the ferric form of Fe (Fe^{3+}), which is then stored on the transferrin and ferritin proteins (Cornell University, 2008; Gupta, 2007; National Research Council, 2005). The Fenton Reaction (shown in equation 3 below) is the reduction of hydrogen peroxide (HOOH) when reacting with ferrous Fe to produce the hydroxyl ion (OH^-) and the extremely reactive and dangerous hydroxyl radical (OH^+) (Gupta, 2007).

$$\longrightarrow \mathrm{HOOH} + \mathrm{Fe}^{2+}\ \mathrm{Fe}^{3+} + \mathrm{OH}^- + \mathrm{OH}^+ \quad ^{(3)}$$

Free radicals contain one or more unpaired electrons in their outer orbital shell, and are highly reactive; damaging and destroying proteins and DNA by causing cross-linkage, and causing the spontaneous degeneration of molecules such as lipids (Gupta, 2007). The free radicals produced in the Fenton Reaction can normally be controlled by antioxidants, however, if the Fe levels in the diet are too high it will result in a build-up of free radicals (Cornell University, 2008; Gupta, 2007). Excess Fe is stored in ferritin in the major organs, including the liver, spleen, bone marrow, pancreas and heart, and too much Fe will cause excess Fe build-up and tissue destruction in these organs (Cornell University, 2008; Gupta, 2007; National Research Council, 2005).

The maximum tolerable amount of Fe is 500 mg/kg diet for sheep and 1,000 mg/kg diet for beef cattle (Board of Agriculture, 2000; Judson & McFarlane, 1998; National Research Council, 2005). Livestock suffering Fe toxicity will display diaorrhea, metabolic acidosis, hypothermia, and reduced weight gain and feed intake (National Research Council, 2005). Soil ingestion can also contribute largely to the Fe levels in livestock's diet (Board of Agriculture, 2000).

1.5.4 Mo requirements of grazing livestock

Mo is listed as an essential micronutrient for livestock, however the minimum concentration required is still uncertain, and at present is listed as 0.1 mg/kg dry matter (Judson & McFarlane, 1998). Mo functions as a component of the enzymes xanthine oxidase, sulfite oxidase, and aldehyde oxidase (Mills and Davis, 1987), and it's believed that it enhances microbial activity in the rumen (Board of Agriculture, 2000). Cattle and sheep are more sensitive to Mo toxicity than other ruminants and non-ruminants, and cattle are generally more susceptible than sheep (National Research Council, 2005; Merck Veterinary Manual, 2005).

As stated above in point *1.5.2*, there is an antagonistic relationship between Mo and Cu and S, affecting the metabolism of these minerals (Board of Agriculture, 2000; Merck Veterinary

Manual, 2008; National Research Council, 2005; NADIS, 2004; Ward, 1978; UC Davis Veterinary Medicine Extension, 1997). Excess Mo in the diet results in decreased intestinal absorption and increased excretion of Mo in faeces or milk, which could pose a risk to calves at foot (Mills and Davis, 1987; National Research Council, 2005). Mo toxicosis (molybdenosis) can be caused by high levels of Mo in the diet or, alternatively, low Cu levels (< 5 mg/kg dry matter), or a low Cu:Mo ratio (< 2:1) in the diet (National Research Council, 2005; UC Davis Veterinary Medicine Extension, 1997). For example, when cattle are fed 100 mg Mo/kg dry matter they will display symptoms of Mo toxicity regardless of the dietary Cu and S levels. Diets containing 25-50 mg Mo/kg dry matter may also produce Mo toxicity, but diets containing less than 25 mg Mo/kg dry matter were unlikely to produce Mo toxicity in cattle unless accompanied by low levels of dietary Cu (National Research Council, 2005).

Mo can also react with other minerals, such as Fe, further implicating livestock's diet.

1.6 By-products of gold—copper mining

The contamination that results from gold and copper mining activity usually includes extremely disturbed landforms, massive waste rock dumps, and one or more tailings dams, depending on the size and magnitude of mining activity. Tailings are a slurry consisting of the waste crushed rock, together with additives used during processing to extract the metals from the ore (Newcrest Mining Limited, 2008). Tailings generally contain high levels of cyanide, sulphides and heavy metals, as well as having poor soil structure, low water-holding and drainage capacity, low organic matter levels and micro-organism activity, low nutrient levels and an acidic pH (US EPA, 2000).

Tailings provide a hostile environment for plant growth, and metal uptake by vegetation growing on tailings soil will be influenced by a variety of factors, including the levels and combinations of metals present in the soil, soil pH, organic matter level, soil structure and moisture holding capacity, rainfall and the species of vegetation growing. CVO require information about the levels of Al, Cu, Fe and Mo found in the soil substrates on their tailings rehabilitation site, as well as the levels of these same metals in the introduced pasture species growing on such soil. Armed with this knowledge under known soil and climate conditions, predictions are able to be made concerning the availability of Al, Cu, Fe and Mo to plants in the future and the consequences of livestock grazing such plants.

1.7 Objectives of the study

Therefore, the objectives of this project are:

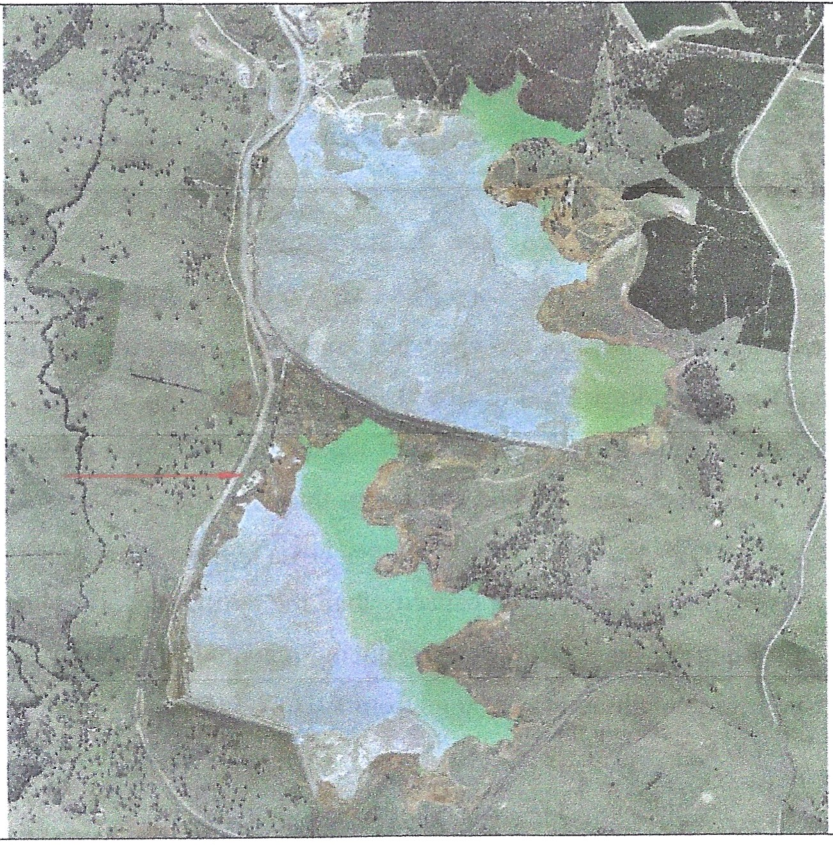
- to determine the levels of Al, Cu, Fe and Mo in *Phalaris aquatica* and *Trifolium subterraneum* growing on the three various amended soil substrates on the tailings rehabilitation site in winter and spring
- to determine the levels of Al, Cu, Fe and Mo in the various soil substrates, and to also test for other soil conditions which may affect metal uptake
- to examine the relationship between soil metal content and soil characteristics and plant metal content
- to compare metal content in plants between the seasons
- to compare metal content between the pasture species
- to compare metal content in plants between the amended soil substrate treatments
- to extrapolate the results as to whether the metal content in plants are within tolerable limits for grazing sheep and cattle.

2 Materials and Methods

2.1 Cadia Hill Gold Mine, Orange

This research project was carried out in a designated rehabilitation experiment site located at Cadia Hill Gold Mine, one of Australia's largest gold mines, operated by Cadia Valley Operations (CVO) for Newcrest Mining Limited. CVO operate two mines co-jointly: Cadia Hill Gold Mine (CHGM), located 25 km south-west of Orange (central tablelands of New South Wales), and Ridgeway Mine (RM), located 3km north-west of CHGM. Cadia Hill Gold Mine has been operating since August 1998 and is an open pit mine, whereas the Ridgeway Mine has been operating since April 2002 and is an underground mine (Newcrest Mining Ltd, 2008). Both CHGM and RM extract gold and copper from the ore using the flotation and gravity processing method (Newcrest Mining Ltd, 2008).

Figure 2: Aerial view of the Cadia Hill Tailings Storage Facility (top), Southern Tailings Storage Facility (bottom) and the tailings rehabilitation site (arrow).



With both the open cut and underground mining, rock is blasted using explosives, with unprocessed waste rock removed and stored in massive waste dumps, and ore sent to the processing plant situated at CHGM. Gold-copper concentrate is then piped to Blayney, where it is dewatered and freighted by rail to Port Kembla, and subsequently shipped to Japan. Waste-ore material (tailings) is piped from the processing plant and stored in one of the two tailings dams at Cadia – the Cadia Hill Tailings Storage Facility and the Southern Tailings Storage Facility (Figure 2, above).

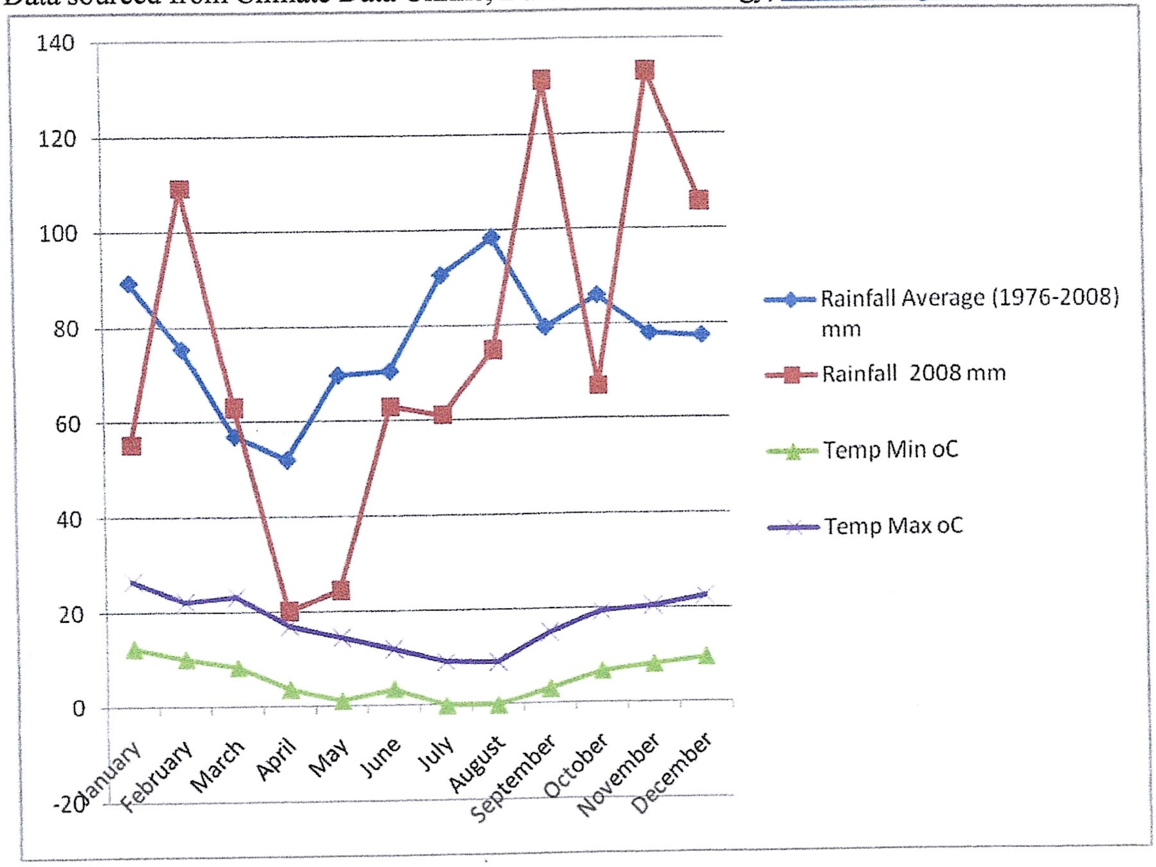
Both the rock-waste dumps and tailings dams are highly disturbed landforms which will require massive resource inputs to ensure restoration back to useable and sustainable systems upon the completion of mining activity in 15-25 years (Newcrest Mining Ltd, 2008; Jeff Burton, pers.comm. 29.2.08).

The physical and chemical characteristics of tailings vary depending on the parent-rock material and methods used in mineral extraction and tailings deposition (Reid, 2004). The CHGM and RM gold-copper mineralisation is hosted by 'sheeted quartz veins' and 'sheeted quartz sulphide veins' occurring in Ordovician volcanics and sediments (Newcrest Mining Ltd, 2008). The processing plant equipment at CHGM crushes the ore to a fine sediment. Copper cleaners, pyrite cleaners and tailings thickeners are used in the flotation plant to extract the gold-copper concentrate from the ore (Cadia Holdings Pty Ltd, 1995). Compared to other contemporary gold-copper mines, CHGM tailings are generally lower in sulphur, copper, lead, cadmium and mercury levels (Cadia Holdings Pty Ltd, 1995). CVO produces four types of tailings ore, with tailings derived from pyritic ore (less than 5% of the total ore at CVO) containing higher levels of copper, zinc, manganese, sulphur and chromium than the other types of tailings ore (Cadia Holdings Pty Ltd, 1995). The other three ore types at CVO have lower sulphur levels (<0.2%) and therefore have a higher neutralising capacity than the pyritic ore. By blending the four ore types the resulting tailings have a low acid-producing potential (pH of soil samples taken May 2008 from the tailings rehabilitation site range from 5.0 to 7.3 ($CaCl_2$)).

2.2 Climate history of site in 2008

Figure 3 shows total rainfall received at Orange during 2008 as being 906.6 mm, close to the average rainfall for the area of 922.7 mm. Orange receives a typically Mediterranean climate, with cold, wet winters and warm, dry summers. Frequent frosts and occasional snow occurs over winter.

Figure 3: Climate history of Orange, NSW,
Data sourced from Climate Data Online, Bureau of Meterology, www.bom.gov.au



2.3 Tailings Rehabilitation Site

The tailings rehabilitation site (with an area of 0.5 ha) is adjacent to the Southern Tailings Storage Facility, situated immediately below the Cadia Hill Tailings Storage Facility (both facilities total approximately 1,000 ha). The tailings rehabilitation site completed filling in October 2003 (Reid, 2004) and has been used as an experimental research site ever since. This study involved sampling and analysing introduced pasture vegetation growing on the tailings rehabilitation site.

The CVO tailings are a stratified loam with poor soil structure and no organic matter, which results in poor drainage, poor hydraulic properties and poor aeration of the soil, which is highly susceptible to erosion (Jeff Burton, pers.comm. 29.2.08). The tailings substrate has soil characteristics of typical tailings soil; very low organic matter content, high density, poor water-holding and draining capacity, and is saline. It is more alkaline than a typical tailings dam, though, and this is due to a high content of carbonates in the parent rock material, as well as lime being added during processing and water used during processing being alkaline (Reid, 2004). Since the initial testing in 2003, the EC_e value and pH of all soil substrates on the tailings rehabilitation site has steadily decreased. These characteristics affect air and water movement within the soil, plant-available water, root penetration, and seedling and plant growth – all of which affect metal uptake by plants.

As the tailings soil is a harsh environment to establish vegetation, previous studies made at the tailings rehabilitation site examined the effects of various soil treatments on the tailings chemical characteristics and vegetation growth. Of these various soil treatments, this study examined pasture-potential plants growing on three different soil substrates:

2.3.1 Tailings substrate

The unamended tailings substrate had NPS (14.3:12:10.5) fertiliser broadcast at a rate equivalent to 160 kg/ha (or 385 g/4 x 6 m^2 plot). Muriate of potash (50% potassium) was also broadcast at a rate equivalent to 60 kg/ha (Reid, 2008). Pasture growth covered approximately 50% of the surface area and was shorter than in the other substrates.

2.3.2 Topsoil substrate

Stockpiled topsoil had been added to the tailings at a depth of 15 cm. To this substrate fertiliser had been added (as above). The EC_e and pH of the stockpiled topsoil fell within satisfactory levels for pasture growth, and represents typical soil characteristics of soils found in the area.

Composition of pastures was balanced in this substrate, with good healthy growth, although not as vigorous as in the biosolids substrate.

2.3.3 Biosolids substrate

Dewatered biosolids, at a rate of 30 t/ha, had been incorporated into the tailings soil to a depth of up to 30 cm. To this substrate fertiliser had been added (as above). The EC_e and pH of the biosolids soil fell within satisfactory levels for pasture growth, and represents a low-quality soil with a slightly greater organic matter content to the topsoil substrate. The growth of grasses dominated the pastures in this substrate, allowing the Sub clover to grow in gaps of vegetation or along the edge of the plots. Pasture growth was vigorous and healthy.

2.4 Selection of plant species

Two introduced pasture species commonly grown for forage in the central west of NSW are a winter-growing annual legume, *Trifolium subterraneum* (sub clover) and a perennial grass, *Phalaris aquatica* (phalaris) (Figures 4.1 and 4.2 below). Sub clover is a hardy plant, able to grow well on poor-quality soils and able to withstand a range of conditions. It is especially suited to Mediterranean climates, with their cold, wet winters and hot, dry summers. Sub clover flowers from March to May and matures from April to June (UC SAREP Online, 2009). It is known to tolerate a wide range in soil pH, however, low pH (below 4.5) and Al levels greater than 10 micromoles, will prevent root nodulation, affecting plant growth (Richardson et al, 1988). Phalaris is a tough and drought-hardy temperate perennial grass. It grows to about a metre in height and flowers in early summer, and it's growth will also be adversely affected by the soil pH dropping below 5.0 (NSW DPI, 2009).

Both sub clover and phalaris are sensitive to soluble Al, and are affected at levels above 150 mg/kg dry matter (Bouma et al, 1981) or at more than 10% of the cation-exchange capacity within the soil (DPI NSW, 2009). Their sensitivity to Al will affect the pasture's root development and uptake of other nutrients and metals.

Figure 4.1: *Trifolium subterraneum*
Source: Oregon State University

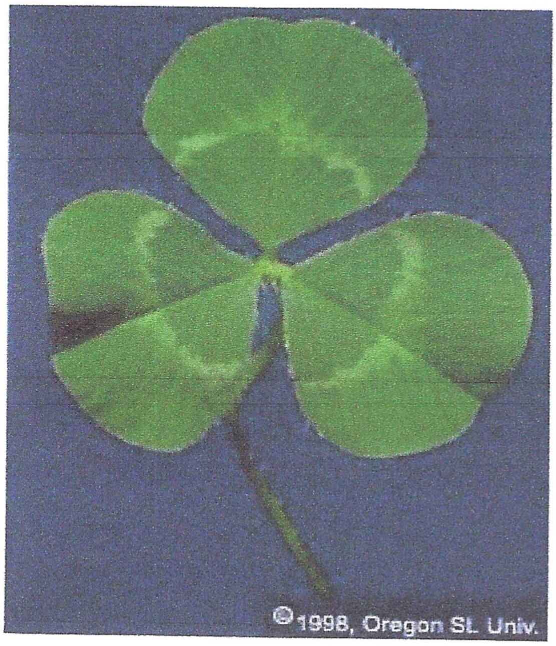


Figure 4.2: *Phalaris aquatica*
Source: NRCS Plant Materials Center

2.5 Experimental design

The experimental design utilised the existing tailings rehabilitation site. The design used was a split-block design with three factors for the soil – tailings, topsoil and biosolids; and two factors for plants – grasses and legumes. Two blocks were used and soil and plant samples were taken from each plot (Table 2 below).

Observational pasture samples of phalaris and sub clover were also taken at five locations in the hills surrounding Cadia Hill Gold Mine, on a property called 'Tunbridge Wells' (TW). These samples were all taken within 6.5 km of the tailings rehabilitation site, and all came from paddocks with a history of being grazed by livestock, and of having no cropping or mining activity. These samples were taken to provide comparative data with the samples taken from the tailings rehabilitation site, and to give an indication of the levels of metals found in pastures growing naturally in the area. TW samples were only taken in spring (October) 2008.

2.6 Soil analysis

2.6.1 Sampling

Soil samples to a depth of 15 cm were taken within each plot at the same time as the initial winter plant sampling in May 2008. Two samples were taken in each plot, close to the base of the sampled phalaris and sub clover plants.

2.6.2 Analysis

The soil samples were analysed using an ICP-AES by ALS Laboratory Group, Environmental Division, Sydney Australia.

2.7 Plant analysis

2.7.1 Sampling

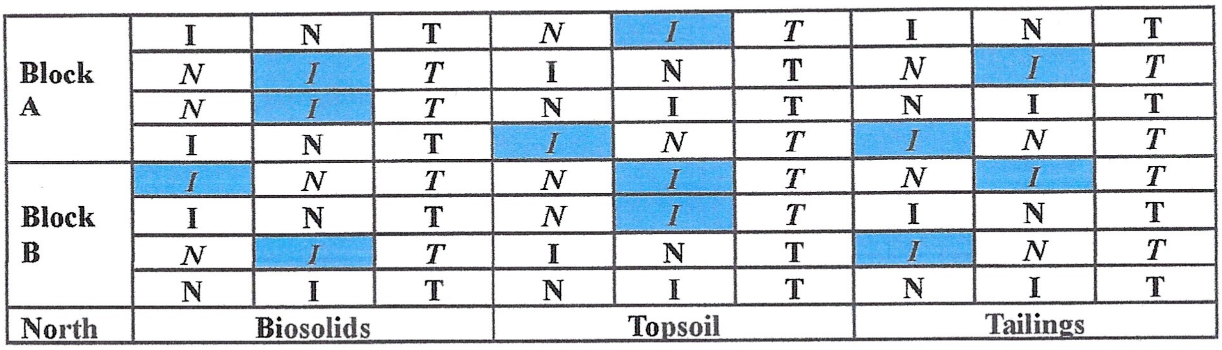
Standard plant tissue sampling techniques were used, with samples being taken using a random sampling method. From each plot containing introduced pastures and fertilised soils (*I*), one (or two small) typical phalaris and sub clover plants were randomly selected and cut at ground level. The plant samples were placed in paper bags and dried in a drying oven at 60°C for at least seven days. The samples were not washed before drying as the aim of this research is to analyse the metal content in plant samples in relation to the implication to livestock health. Livestock would be ingesting the plant material, along with dust and soil from the site, and this should be taken into consideration in this research. It is worth noting that the samples had no soil attached to them, although there may have been some dust.

Table 2: Cadia Tailings Revegetation Site experimental design

Key: I = introduced pasture
N = native pasture
T = trees and shrubs
italics = nutrient
🟦 = samples taken randomly within these plots

Source: Reid, 2004.

South	**Substrate**		
	Topsoil	**Tailings**	**Biosolids**



Block A	**I**	**N**	**T**	***N***	***I***	***T***	**I**	**N**	**T**
	N	***I***	***T***	**I**	**N**	**T**	***N***	***I***	***T***
	N	***I***	***T***	**N**	**I**	**T**	**N**	**I**	**T**
	I	**N**	**T**	***I***	***N***	***T***	***I***	***N***	***T***
Block B	***I***	***N***	***T***	***N***	***I***	***T***	***N***	***I***	***T***
	I	**N**	**T**	***N***	***I***	***T***	**I**	**N**	**T**
	N	***I***	***T***	**I**	**N**	**T**	***I***	***N***	***T***
	N	**I**	**T**	**N**	**I**	**T**	**N**	**I**	**T**
North	**Biosolids**			**Topsoil**			**Tailings**		

2.7.2 Analysis

200 mg (± 5mg) of the ground dried plant samples were weighed and placed in 50 ml plastic tubes. Three repeats were done of each sample (a, b and c). The two-step wet-oxidation procedure (Corbett et al, 1996) was used to digest plant samples. This involved adding 4 ml nitric acid (69%) to each sample, which were then placed in an incubator set at 30°C for at least 12 hours. The samples were then removed and 4 ml hydrogen peroxide (3%) was added, with the samples placed back in the incubator set at 30°C for at least another 12 hours. The samples were then removed and were diluted to 20 ml with distilled water.

All plant samples were analysed using the argon-supported inductively coupled plasma atomic emission spectrometer (ICP-AES) Varian at the Environmental & Analytical Laboratory at CSU, Wagga Wagga. An ICP-AES is able to subject samples to temperatures high enough to cause not only dissociation into atoms, but to cause significant amounts of collisional excitation (and ionisation) of the sample atoms to take place (Boss & Fredeen, 1989). The intensity of the light emitted at specific wavelengths is measured and used to determine the concentrations of the elements at interest (Boss & Fredeen, 1989).

The high temperature sources in the ICP-AES can record a number of different energy levels for several different elements at the same time, enabling the user to analyse several different elements at once. The ICP-AES is calibrated and set up for the elements to be tested by making a blank and two or three (depending on the metal tested) standard solutions, and then analysing these to create a calibration curve for each element tested. The standard solution concentrations used for each metal were as follows (Table 3):

Table 3: Standard solution concentrations used for metal analysis on ICP-AES

	Standard 1	**Standard 2**	**Standard 3**	**Standard 4**
Al mg/L	0.1	0.5	5.0	10.0
Cu mg/L	0.01	0.05	0.50	1.00
Fe mg/L	0.1	1.0	10.0	20.0
Mo mg/L	0.01	0.05	0.50	1.00

The plant samples were then analysed on the ICP-AES with their results plotted according to their respective calibration curves. To ensure accuracy of the plant analysis, a reference plant sample (ASP43), with known metal concentrations, was digested and analysed along with the other plant samples.

2.8 Data analysis

GenStat 11th Edition was used for the statistical analysis. Due to some soil samples being spilt, an unbalanced one-way ANOVA was used to determine differences in metal content of soils and soil properties between substrates treatments. Differences in metal content between substrate, species and season were determined by an unbalanced three-way ANOVA. Stepwise multiple regression was used to relate the content of each metal in the plant to the content of all metals in the soil substrate and soil characteristics.

3 Results

3.1 Soil samples

A summary of the soil results are shown in Table 4 below. The pH of the tailings and biosolids substrates have shown a gradual decline in pH from 8.3 in 2004 (Reid, 2004) to an average of 6.9 in May 2008. The topsoil substrate has remained the same. Al, Cu and Fe levels in the soil are all much higher than optimum levels in all soil substrates (Cu is at upper optimum level in topsoil).

Table 4: Mean metal levels (±SEM) and soil conditions per soil substrate as at May 2008. Means within rows followed by the same letter do not differ significantly by the Least Significant Difference test ($P < 0.05$). [1]Optimum levels Al and Fe taken from Dairy Extension Centre, 2005, [2]Optimum levels taken from NSW DPI website 2009

	Optimum levels	**Tailings**	**Topsoil**	**Biosolids**
Al mg/kg[1]	50	7632(±263.0)b	9158(±263.0)a	7838(±263.0)b
Cu mg/kg[2]	2-50	268(±9.95)a	64(±9.95)b	288.5(±9.95)a
Fe mg/kg[1]	410	20525(±1199.9)b	27250(±1199.9)a	22050(±1199.9)b
Mo mg/kg[2]	2	2.5(±0.289)a	2(±(.289)a	2(±0.289)a
pH ($CaCl_2$)[2]	4.8-6.5 sub clover 5.2-7.3 phalaris	6.9(±0.233)a	5.72(±0.233)b	7.03(±0.233)a
EC_e dS/cm at 25°C[2]	< 0.15	12.28(±2.51)a	1.54(±2.51)b	4.86(±2.51)b
Moisture content % (dried to 103°C)[2]		2.92(±0.99)b	6.73(±0.99)a	3.23(±0.99)b
Cation Exchange Capacity meq/100g[2]	>10	31.6(±2.15)a	15.7(±2.15)c	24(±2.15)b
Ca mg/kg (cation)[2]	65-80% CEC	1492(±297.1)a	102(±297.1)b	422(±297.1)b
Mg mg/kg (cation)[2]	10-15% CEC	110(±29.5)a	18(±29.5)a	38(±29.5)a
Na mg/kg (cation)[2]	0-1% CEC	142(±56.8)a	30(±56.8)a	38(±56.8)a
K mg/kg (cation)[2]	2-6% CEC	285(±66.5)a	45(±66.5)b	65(±66.5)b
Sulphate (SO_4^{2-}) mg/kg (anion)[2]	12	4568(±1052.5)a	90(±1052.5)b	1050(±1052.5)b

Results from ANOVA indicate that the mean EC_e ($P = 0.038$), CEC ($P = 0.002$), Ca ($P = 0.022$) and sulphate ($P = 0.034$) levels were significantly greater in the tailings substrate than the other substrates. Analysis showed that the mean pH ($P = 0.006$) and Cu ($P < 0.001$) levels were significantly greater in both the tailings and biosolids substrate than the topsoil substrate.

Analysis also showed moisture content ($P = 0.043$), Al ($P = 0.005$) and Fe ($P = 0.008$) was significantly greater in the topsoil substrate than the other substrates.

3.2 Experimental plant samples

Table 5: Mean concentrations (±SEM) of metal levels. Means within columns for each factor followed by the same letter do not differ significantly by the Least Significant Difference test ($P < 0.05$)

Factor	Treatment	Al mg/kg	Cu mg/kg	Fe mg/kg	Mo mg/kg
Season	Winter	70.86	8.84	150.7	11.92
		(±7.19)a	(±0.65)a	(±16.76)a	(±0.97)a
	Spring	41.66	6.92	88.3	8.47
		(±6.87)b	(±0.62)b	(±16.00)b	(±0.93)b
Species	Phalaris	43.41	5.68	93.4	3.38
		(±7.19)b	(±0.65)b	(±16.76)b	(±0.97)b
	Sub clover	66.82	9.83	140.8	16.30
		(±6.87)a	(±0.62)a	(±16.00)a	(±0.93)a
Substrate	Tailings	85.24	10.60	178.6	15.60
		(±8.70)a	(±0.79)a	(±20.28)a	(±1.18)a
	Topsoil	43.14	5.26	92.4	3.05
		(±8.70)b	(±0.79)c	(±20.28)b	(±1.18)c
	Biosolids	39.57	7.67	85.5	11.61
		(±8.41)b	(±0.76)b	(±19.61)b	(±1.14)b

3.2.1 Variation between the seasons

Unbalanced analysis of variance showed that the mean concentrations of all metals were significantly greater for the winter than the spring samples; Al ($P = 0.005$), Cu ($P = 0.026$), Fe ($P = 0.010$) and Mo ($P = 0.006$).

The results showed significant interaction between the winter season and the tailings substrate, positively affecting Al uptake ($P = 0.015$).

3.2.2 Variation between plant species

Results from analysis showed that the mean concentrations of all metals were significantly greater in sub clover than in phalaris; Al ($P = 0.018$), Cu ($P < 0.001$), Fe ($P = 0.038$) and Mo ($P < 0.001$).

The analysis also showed significant interaction between plant species and substrate for Mo plant content and the tailings substrate ($P < 0.001$).

3.2.3 Variation between soil substrates

Results from analysis showed that plants grown in tailings substrate had a significantly greater concentration of Al ($P < 0.001$), Cu ($P < 0.001$), Fe ($P = 0.003$) and Mo ($P < 0.001$) than those grown in topsoil or biosolids. There was also significantly greater concentration of Cu and Mo in biosolids than topsoil.

3.2.4 Regression analysis

Stepwise multiple regression analysis revealed the formula that best described the relationship between Al content in sub clover and soil properties was the constant (y) = 12.77 + 1.306 K – 1.062 Na – 0.015 sulphate (P = <0.001; adj. R^2 = 93.6). For Cu content in sub clover, the equivalent equation was y = 39.18 – 0.0034 Al (P =< 0.01 and adj. R^2 = 65.7). For Mo content in sub clover the equivalent equation was y = 122.8 – 0.0127 Al (P = 0.001 and adj. R^2 = 63.7). No significant effects occurred between metal content of phalaris and soil properties.

3.3 Observational plant samples

Table 6: Mean concentrations (±SEM) of metal levels in observational plant species. Means within columns followed by the same letter do not differ significantly by the Least Significant Difference test ($P < 0.05$).

Plant Species	Al mg/kg average	Cu mg/kg average	Fe mg/kg average	Mo mg/kg average
Phalaris	27.80 (±3.09)b	5.45 (±0.48)a	94.60(±8.22)a	0.98(±0.12)a
Sub clover	36.80 (±3.09)a	6.47(±0.48)a	60.80(±8.22)b	0.69(±0.12)a

General analysis of variance carried out on the observational samples showed that the mean concentrations for Al ($P = 0.048$) was significantly greater for sub clover than for phalaris, and that mean concentrations for Fe (P = 0.007) was significantly greater for phalaris than for sub clover. Concentrations of Cu and Mo did not differ significantly between the species.

4 Discussion

4.1 Effect of season on metal content

Results from this study show a significantly greater content of all metals in plants growing in winter compared to spring (Al P=0.005, Cu P=0.026, Fe P=0.010, Mo P=0.006 (Table 5)). This variation of results could have been due to the growth of the plants in winter (especially the sub clover) having not quite taken off at the time of sampling. Generally metals do not re-translocate with ease (Singer & Munns, 1991), resulting in them remaining in greater concentrations in older plant tissues.

Seasonal rainfall may have influenced the lower metal content in the spring sampling; the month before the spring sampling had above-average rainfall, which could have resulted in waterlogging in the poorly-drained tailings substrate, adversely affecting metal uptake. Studies have shown waterlogging to adversely affect root length and the association of plant roots with arbuscular-mycorrhizal fungi (Mendoza & Garcia, 2007), which would negatively affect metal uptake. Waterlogged soils have less available oxygen in the soil for mycorrhizae use, slowing their growth (Singer & Munns, 1991).

Mycorrhizae are able to enhance or inhibit metal movement into the roots and/or shoots of the host plant. Studies have shown that roots infected with mycorrhizal fungi generally absorb higher levels of metals when the metals are present in lower quantities in the soil, but restrict metal uptake when soil metal levels are higher (Dehn & Schuepp, 1990; Diaz et al, 1996; Weissenhorn et al, 1995). However, the effect of mycorrhizal fungi is extremely complex and is dependent upon the type of fungi present in the soil, soil metal levels and other soil conditions which may affect fungi growth (Weissenhorn et al, 1995; Diaz et al, 1996; Lin et al, 2007), so, without knowing the type or level of fungi present in the soil substrates, it is impossible to predict whether or not it is increasing or decreasing metal uptake

Temperature could have also influenced metal uptake; the winter samples were taken in May, before the ground had a chance to cool down, and therefore, mycorrhizae activity is more likely to have been highest then. Spring samples were taken in October, when temperatures were only starting to rise, so plant growth had occurred during five months of cold weather. Cold soil temperatures can stop mycorrhizae growth, without killing the organisms (Singer & Munns, 1991).

4.2 Effect of plant species on metal content

This study showed a significant difference in the metal content between sub clover and phalaris over all substrates. These findings support previous research that shows legumes generally take up Al, Cu and Mo in greater quantities than grasses (National Research Council, 2005; Barker & Pilbeam, 2007).

Levels of Al, Cu, Fe and Mo in sub clover fell within tolerable limits for plant growth on all substrates, in both seasons. The highest Al value recorded in this study (248 mg Al/kg in sub clover growing on tailings substrate), is slightly above the average figure for Al levels in herbaceous plants ((200 mg/kg) Barker & Pilbeam, 2007). Levels of Cu, Fe and Mo all occur at levels above the minimum adequate concentration in plant shoots for healthy plant growth, in both sub clover and phalaris.

Sub clover absorbed more Fe than grasses on all substrates. Fe is reportedly more easily absorbed by grasses (Barker & Pilbeam, 2007), however, Fe absorbance is highly dependent upon microbial activity in the soil, which may have been greatest in soil surrounding the legumes in this disturbed environment. Also, Fe uptake by gramineous plants (includes grasses) requires mugineic acids (MA) to complex with Fe^{3+} (Chang et al, 1998). It is possible that the extremely high Al levels in the substrates have depressed MA action, reducing Fe content in grasses.

Levels of Mo were significantly higher in sub clover than phalaris. This result was expected as legumes have a greater requirement for and ability to store Mo than grasses (Barker & Pilbeam, 2007). Mo content in sub clover was greatest in those plants growing on the tailings and biosolids substrate, whose pH values were more alkaline than the topsoil substrate, and therefore more suitable for Mo uptake.

4.3 Effect of soil substrate on metal content

This study showed significant variation of metal content between the three soil substrates. Plants growing on the tailings substrate had the greatest level of metal content for all metals tested.

4.3.1 High Al and Fe content in soil

Stepwise multiple regression analysis showed significant relationships between metal content in sub clover and the soil conditions, however, it showed no relationships for phalaris. The regression analysis showed that the amount of Cu, Fe and Mo in sub clover plants was negatively affected by Al levels in the soil, with the adjusted R^2 value being 65.7, 23.0 and 63.7, respectively. An R^2 value of 70 (and above) is generally considered to indicate a high association between the subjects.

The CEC percentages in all substrates were strongly dominated by the presence of Al and Fe in the soil. Table 7 gives the optimum percentages for exchangeable cations present in the soil, together with those present in the soil substrates, revealing an average of 25% Al and 71% Fe in the substrates.

Table 7: CEC % per soil substrate
Optimum values from NSW DPI 2009
[1] Fe value from Spectrum Analytic (www.spectrumanalytic.com)

Cation	Optimum %	Tailings %	Topsoil %	Biosolids %
Ca	65-80	4.94	0.28	1.38
Mg	10-15 (20 max)	0.36	0.05	0.12
K	1-5 (10 max)	0.94	0.12	0.21
Na	0-2 (max)	0.47	0.08	0.12
Al	0 (ideal)-5 (max)	25.28	25.02	25.74
Fe[1]	0-3 (max)	67.99	74.45	72.41

Both sub clover and phalaris are reportedly sensitive to soluble Al, and are affected at levels above 150 mg/kg dry matter (Bouma et al, 1981) or at more than 10% of the cation-exchange capacity within the soil (DPI NSW, 2009). Al occupies 25% of the CEC in all substrates and high levels of Al in the soil affects uptake of nutrients and metals in the following ways: by inhibition of root elongation, suppressed root and shoot biomass and abnormal root morphology all interfere with nutrient uptake; a decrease in uptake and translocation of P, Ca, Mg and K, which may result in deficiencies in these nutrients in the plant and in grazing livestock; restrictions in water uptake and movement in plants; suppression of photosynthesis; inhibition of root symbiosis with *Rhizobia* bacteria (i.e. a study has found the percentage of plant nitrogen declines in sub clover as foliar Al levels increased) (Panford et al, 1993; Barker & Pilbeam, 2007).

The results of soil tests show high Fe levels in all substrates, and Fe dominant in the CEC percentage (68-74% CEC). Fe levels in the plant tissue was not at toxic levels, although Fe will become more available in the soil for plant uptake should the soil pH decrease. Fe levels in the soil usually only occur in levels toxic to plants in severely waterlogged or flooded soils, such as rice paddies, when it occurs at levels of 300-1000 mg/L in the soil (International Rice Research Institute, 2009). Fe is more likely to become toxic to plants growing in soils with unbalanced levels of nutrients, with low K levels (< 1% K) found in Fe-toxic plants (International Rice Research Institute, 2009). The K levels in all substrates is under 1% CEC, and is 0.12% CEC in the topsoil substrate.

Metal content was lowest for plants growing on the topsoil substrate, which was initially suprising considering it has a more ideal pH for Al, Cu and Fe uptake than the tailings and biosolids substrates. The regression analysis supports that the high Al levels negatively influenced Cu, Fe and Mo content in sub clover in all substrates, and it is probable that, as Al levels were highest in the topsoil substrate, this is why Cu, Fe and Mo uptake was lowest in this substrate, even though it had a more ideal pH.

The reason for the extremely high levels of Al and Fe in the topsoil substrate is unknown, and there is no data for natural soils in the area with which to compare. Topsoil was laid over the tailings surface at a depth of 15 cm in 2004, and soil samples were taken at a depth of 15 cm in May 2008. There is no obvious erosion at the site, however, the 2008 samples may have been contaminated by erosion of topsoil resulting in a shallow residual more easily mixed with underlying tailings and intrusion of wind or water borne contaminated material from adjacent tailings. There is no physical edging of the plots to contain the soil, so this scenario is possible. However, the coefficient of variation for Al levels in the substrates (tailings = 10.02%, topsoil = 4.57%, biosolids = 3.38%) are fairly homogenous, indicating that it is unlikely that there has been any sampling error or contamination of soil samples.

The adsorption of Cu^{2+} in a variably-charged soil (high Al and Fe levels) is strongly affected by the proportion of these metals in the soil (Barker & Pilbeam, 2007). Therefore, the dominant proportion of Al and Fe in the substrates is likely to be affecting the lower Cu content in pastures and resulting in the problematic Cu:Mo ratio occurring in the tailings substrate.

The ability of Al and Fe oxides to bind with Mo increases as soil pH falls below 6.0, which can explain the reduced Mo uptake in the topsoil substrate. Uptake of Mo is reportedly enhanced by available P and nitrate, and inhibited by available sulphur (Pasricha et al, 1977; Barker & Pilbeam, 2007). The regression analysis did not support this in the study; sulphate levels in tailings and biosolids substrates was extremely high (optimum = 12 mg/kg; tailings = 4228 mg/kg; biosolids = 1576 mg/kg), however, this did not significantly affect Mo content of sub clover growing in these substrates.

4.3.2 Soil K, Na and sulphate levels

The stepwise multiple regression analysis revealed a strong positive association between Al content of sub clover and soil K levels, and negative association with Na and sulphate levels. Levels of K were much higher in the tailings than other substrates, positively influencing Al uptake, while Na and sulphate were also higher in the tailings substrate.

The high sulphate levels in the tailings substrate may have also influenced Fe uptake in the form of ferrous sulphate, which is more easily absorbed by plant roots.

4.3.3 Other soil conditions

Al, Cu and Fe content were higher in plants growing in the tailings substrate, yet they all become more available when pH drops below 6.0, and less available above 7.0 (NSW DPI, 2009; Barker & Pilbeam, 2007), indicating the topsoil substrate should have been the most suitable for uptake.

The soil results show soil pH within the optimum range for phalaris in all soil substrates and for sub clover in the topsoil substrate, and above optimum level for sub clover in the tailings and biosolids substrates.

Metal absorbance by plants is greatly assisted by microbial activity and organic matter content, however the regression analysis did not support this. Soils with low levels of organic matter may have lower levels of metal uptake by plants due to reduced microbial activity and chelating agents present in the organic matter in the soil, greater soil compaction resulting in decreased plant root growth, and (as low organic matter levels and soil compaction tend to result in soils which retain moisture) can be anaerobic (Peverill et al, 1999; Barker & Pilbeam, 2007). The tailings are a stratified loam with poor soil structure and no organic matter, which is highly susceptible to erosion (Jeff Burton, pers.comm. 29.2.08), and therefore should have had plants with the lowest metal content, instead of the highest metal content. Therefore, this study has indicated the driving forces of Al, Cu and Fe content are not soil pH, microbial activity, organic matter content, soil compaction or waterlogging.

4.4 Metal content of observational plant samples

There is no soil data available for Tunbridge Wells (TW), therefore stepwise multiple regression analysis was not possible with these results. The metal content of plants growing on TW soils were only slightly less than those growing on the topsoil substrate for Al, Cu and Fe. These results were expected, given the topsoil used in the topsoil substrate originating less than 6.5 km away from TW soils.

The Mo content of pastures growing at TW, however, was much less than those from all the experimental substrates. The Cu:Mo ratio found in pastures at TW was between 5:1 and 9:1, which falls within the optimum range for livestock health. The Cu levels are borderline minimum levels for livestock (5 mg/kg sheep and 7-10 mg/kg cattle), being 5.45 mg/kg in phalaris and 6.47 mg/kg in sub clover. However, as the Mo levels are correspondingly low, it is unlikely that this will affect livestock health.

Al and Cu content was greater in sub clover than phalaris growing on TW, however phalaris contained higher levels of Fe and Mo.

4.5 Implications of livestock grazing these pastures

The purpose of this study was to investigate whether metal levels in the pastures on the rehabilitated tailings dams at Cadia would have an impact on cattle and sheep that graze these areas. These species of domestic livestock were chosen as they are typically grazed in the area of the study and are a logical choice for any future grazing management options.

The greatest potential problem likely to affect livestock grazing on pastures growing on the tailings rehabilitation dam is the Cu:Mo ratio found in plant tissue. For optimal livestock health, this ratio is meant to be at least 2:1 (UC Davis Veterinary Medicine Extension, 1997; McBride, 2005), however higher Cu values are preferable. Currently, the tailings substrate results from the winter sampling had an average Mo level of 28.9 mg/kg, and an average Cu level of 13.9 mg/kg, making the Cu:Mo ratio for sub clover in this substrate 1:2. Livestock grazing pastures at this ratio are very likely to experience molybdenosis (or Cu deficiency).

On the biosolids and topsoil substrates, the level of Cu only just makes the minimum requirements for livestock, especially in phalaris. Judson & McFarlane (YEAR) recommend that phalaris should contain a minimum of 2-4 mg/kg Cu, and Sub clover a minimum of 4-5 mg/kg for optimal livestock health. Mo levels in the diet of 4-5 mg/kg are likely to cause Cu deficiency in livestock when Cu levels in the diet are as low as was found in both sub clover and phalaris on all substrates. Cu requirements vary for the age and fertility status of the livestock, with cattle requiring higher levels of Cu than sheep, and older livestock also requiring higher levels.

There are no minimum recommended values for Al in livestock diets, however the maximum tolerable amount for both sheep and cattle is 1000 mg/kg. Al levels on all three substrates were below this figure, so it is unlikely that current Al levels in plants would pose any problem to livestock, such as phosphorous deficiency (or grass tetany). However, the high Al levels in the soil, and the extremely high Al percentage of CEC may decrease uptake and translocation of P, Ca, Mg and K (Barker & Pilbeam, 2007) which may affect the quality of livestock forage resulting in deficiencies.

Fe levels on all substrates are adequate for livestock health with Judson & McFarlane (1998) recommending phalaris to contain a minimum of 40-60 mg/kg and sub clover a minimum of 50-75 mg/kg. The high sulphate levels in the soil suggest that Fe in plants is likely to be in the form of ferrous sulphate, which is most easily absorbed by livestock.

Should the pH of the soil substrates continue its decreasing trend, there will be a subsequent increase in available Al, Cu and Fe for plant uptake, while inhibiting Mo uptake. Al and Fe oxides present in the soil have a pH-dependant surface charge which becomes more positive as soil pH decreases, and they bind to Mo, reducing its availability (Barker & Pilbeam, 2007). Higher levels of available Al in the soil (which will increase with falling pH) will have the following effect on plants:

- root nodulation in the sub clover plants will be adversely affected, resulting in less atmospheric nitrogen being fixed by the plant, and the quality of the pasture will decrease.
- growth and ability of roots to absorb nutrients will be adversely affected
- decrease uptake and translocation of P, Ca, Mg and K, which may result in deficiencies in these nutrients in the plant and in grazing livestock
- restricts water uptake and movement in plants
- suppresses photosynthesis
- inhibits root symbiosis with Rhizobia bacteria (Barker & Pilbeam, 2007).

With the increase in availability of Al, Cu and Fe, it is likely that it will be Al which may affect plant growth and nutrient quality of the pastures, which will indirectly affect livestock health and production. However, as Al uptake is generally restricted by both grass and legume roots, it is unlikely that Al content in the plant will be high enough to make it toxic for grazing livestock.

Soil ingestion can also contribute largely to the metal levels in livestock's diet (Board of Agriculture, 2000), so if is very possible that the very high levels of Al, Fe and Cu in the substrates will influence the diet of grazing livestock. All three soil substrates had very high levels of Al (ranging from 7425 to 8973 mg/kg) and Fe (ranging from 20150 to 29562 mg/kg), in similar quantities. The tailings and biosolids substrates had high levels of Cu (272 and 281 mg/kg respectively), and they all contained normal levels of Mo (<2 mg/kg). It is possible that the high levels of Cu in the soil may negate the detrimental Cu:Mo ratio occurring in sub clover plants growing on the tailings substrate. It is also possible that the high Al or Fe levels found in the soil could cause mineral imbalances within grazing livestock, resulting in toxicities of these metals, or deficiencies of others (such as P). Greater ingestion of soil is more likely on the tailings substrate, as the cover of vegetation on this substrate is not as dense as on the other substrates, and the vegetation is shorter.

To reduce the effects of metal deficiencies or toxicities to grazing livestock the following management practices could be implemented:

- limit grazing to shorter periods of time
- avoid fresh new plant growth which is often higher in metal content
- graze rehabilitated grasslands with cattle as they are slightly more tolerant than sheep to high Mo levels
- supplement livestock's diets with any deficient minerals
- avoid grazing by pregnant and young livestock
- fertilize the soil with any deficient minerals
- manage pastures to minimise legume growth
- ensure good drinking water quality, low in toxicants
- keep stocking rates suitable to the quantity of feed available to reduce ingestion of soil by livestock

4.6 Limitations of this study

- The selection of only two pasture species for analysis of metal content does not accurately predict the total diet consumed by grazing livestock, even when taking soil mineral levels into account. It can give an indication only.

- This study only assessed the levels of four metals; Al, Cu, Fe and Mo. These were chosen due to their high concentration in the soil tests (in the case of Al, Cu and Fe) and their ability to influence the content of other metals (in the case of Mo). Other minerals which occur in the tailings soil which may be present in quantities which could affect livestock health are As, Co, Cr, Pb and Zn. The uptake of minerals by plants is so complex, it makes predictions very difficult.
- This study only assessed metal content in the leaves and shoots of plants and did not include metal content of roots. Al, Cu and, to a lesser extent, Mo have a large proportion of absorbed metal stored in the roots.
- Even when knowing the dietary mineral intake of livestock, predicting the implications of such diet is difficult without knowing the amounts of those minerals already stored in the animals' body, which will affect their dietary need for such minerals (Judson & McFarlane, 1998).
- This study does not allow for the selective grazing behaviour of livestock, which varies greatly between species and depends on the quantity and quality of feed available.
- This study does not take into consideration the quality of the drinking water which would be consumed by livestock and can influence the total dietary mineral intake.
- Soil and plant testing can only partly explain an extremely complex biological system and predictions should be made with caution, and approached in a holistic matter. Extrapolation of the implications on grazing livestock using results from soil and plant analysis should be aware of the limitations of such analysis. There are many other factors which will contribute to the end overall health of the livestock, including breed and species of animal, age and gestational status, health, mineral reserves within the body, quantity and quality of feed available, drinking water quality and pasture composition.

5 Conclusions

It can be concluded that in pastures growing on rehabilitated the tailings dam it is likely that;

- Sub clover will contain higher levels of metals than phalaris regardless of the substrate.
- Not top dressing the tailings substrate with topsoil or incorporating biosolids will result in plants that contain more metals
- The high Al levels in the soil will negatively affect the content of Cu, Fe and Mo in sub clover, and this is likely to increase if soil pH continues to decline.
- The Cu:Mo ratio in sub clover will be the most immediate threat to grazing livestock health.
- The time period after soil amendation must be taken into consideration by CVO when proposing land uses for the site in the future. The pastures tested are growing on soil substrates amended four years previously, which have had time to accumulate some organic matter and soil microorganisms and decline in pH. It is highly possible that should these same pastures have been analysed three or four years previously, the Mo levels may have been even higher (due to the higher pH then), and the resulting threat to livestock even more acute.
- The implications for livestock will change over time. For example, if the current trend in pH continues to become more acidic, Mo will become less available to plants, while Al, Cu and Fe will become more available. It is likely then that the levels of these metals will increase in plant tissue, resulting in different implications for grazing livestock.
- Should the soil pH drop below 5.0, available Al^{3+} in the soil will increase by a thousandfold for every further one-unit drop. This will result in the complications associated with an acidic soil, including reduced root growth and nodulation, affecting plant growth and nutrient—metal uptake by plants.

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7 Appendix A

Winter (May) 2008 results

Sample No.	Al	Cu	Fe	Mo

	mg/kg	mg/kg	mg/kg	mg/kg
ASP43a	35.2	10.8	35.4	1
ASP43b	36.2	12.5	40	1.1
Phalaris				
1c	144.8	14.7	342.6	6.1
4a	37.8	3.7	92.5	3.8
5b	31.2	4	74.1	3.2
6a	26.7	5.6	68.9	3.5
6b	26.9	5.2	78.9	3
6c	29.9	5.2	75.5	2.2
7a	46	4.5	97.1	3.3
7b	45.8	4.1	88.3	3
7c	43.4	4.1	106.7	2.9
8a	86.9	6.1	177.4	2.7
8b	128.1	6.7	194.1	2.7
8c	84.3	6.9	183.3	3
9a	48.2	6.1	116	8.3
9b	49.4	5.3	103.7	9.3
10a	62.4	7.1	144.8	5.9
10b	75.9	8.5	145.1	6.5
10c	58.7	7.5	152	5.5
11a	40	7.4	95.5	3.3
11c	43.9	4.8	82.3	3.1
12a	33.2	8	85.3	2.5
Sub Clover				
1a	116.1	19.9	212.2	49.7
1b	97	15.2	180.3	47.1
1c	67.3	14.8	156.8	42.2
2a	248.2	15.9	584.2	29.4
2b	230.5	15	621.7	31
2c	276	19.3	620	33.5
3a	30.7	8.5	83.2	6.6
3b	31.8	7.6	80.7	6.9
4a	33.7	8.9	92	2.6
4b	34.6	8.2	90.8	3.3
4c	40.8	8	118.7	4.6
5a	29.2	12.5	77.1	21.1
5b	27.3	10.1	68.8	19.2
5c	24.2	10.5	70.8	18.7
6a	63.8	16.9	123.6	23
6c	51.4	11.3	116.8	22.5
7a	48.8	9.2	120	5.7
7b	48	6.3	108.1	5.6
7c	44.2	6.6	115.6	5.4
8a	52.7	7.6	137.1	1.5
9a	55.2	8.9	121.8	15.8
9b	51.3	7.8	115	15.1
10a	99.4	13.5	223	22

10b	122.2	14.6	238.4	23.6
11a	68.5	9.5	137.1	21.2
11b	57.5	8.7	131.1	21.7
11c	67.9	9.4	145.8	23
12a	45.6	9.3	110.7	29.2
12b	45.4	9	98.6	28
12c	47.2	9.1	95.6	27.4

Spring (October) 2008 results

Sample no.	**Al mg/kg**	**Cu mg/kg**	**Fe mg/kg**	**Mo mg/kg**
ASP43a	55.1	10.3	48.0	0.6
ASP43b	42.6	16.0	62.8	0.6
Phalaris				
1a	32.1	5.3	62.9	4.4
1b	40.4	12.5	128.3	4.3
1c	40.1	9.1	113.6	4.4
2a	41.5	19.9	220.4	4.7
2b	error			
2c	44.1	12.5	117.9	4.6
3a	32.4	3.0	37.4	1.4
3b	18.7	2.3	32.9	1.3
3c	26.8	3.4	42.0	1.1
4a	29.4	3.6	37.8	2.2
4b	19.3	2.5	30.3	2.1
4c	24.9	1.9	28.1	1.7
5a	18.0	3.0	33.7	2.7
5b	27.7	3.0	36.4	2.8
5c	29.8	2.9	42.2	2.9
6a	22.4	2.6	32.8	2.4
6b	34.6	3.2	44.4	2.6
6c	34.4	3.5	44.2	2.7
7a	18.4	3.8	38.0	2.3
7b	24.8	3.6	42.6	2.7
7c	23.8	3.7	38.4	2.6
8a	22.1	3.5	45.6	1.3
8b	26.4	3.2	45.2	1.1
8c	17.0	3.3	41.4	1.2
9a	28.9	3.3	36.4	3.2
9b	23.9	2.8	38.5	3.3
9c	27.4	2.8	34.1	3.2
10a	30.3	3.7	61.0	2.9
10b	33.3	3.4	54.5	3.0
10c	33.1	3.5	56.6	3.3
11a	25.2	2.2	36.4	1.3
11b	23.9	2.3	35.7	1.4
11c	17.4	2.5	33.8	1.3
12a	18.1	5.7	51.3	1.7
12b	19.3	6.2	54.8	2.0

12c	18.3	4.5	42.2	1.5
TW1a	21.2	4.9	56.7	0.7
TW1b	19.0	5.2	63.9	0.7
TW1c	22.8	5.2	62.4	0.5
TW2a	41.6	4.5	137.3	0.6
TW2b	43.4	6.0	154.0	0.9
TW2c	53.2	9.8	206.4	0.7
TW3a	32.7	7.3	96.4	0.7
TW3b	25.4	3.8	62.4	0.8
TW3c	19.4	3.8	57.3	1.0
TW4a	30.7	5.9	124.5	0.7
TW4b	25.3	5.8	95.0	0.5
TW4c	30.3	5.2	99.8	0.5
TW5a	16.3	4.3	63.2	2.2
TW5b	17.1	4.9	68.2	2.0
TW5c	18.1	5.1	71.9	2.2
Sub Clover				
1a	38.6	7.7	85.8	23.0
1b	62.3	13.9	145.8	24.8
1c	52.3	9.6	99.1	21.0
2a	76.6	9.6	159.7	20.6
2b	100.7	28.0	298.4	25.6
2c	77.3	10.9	173.0	20.7
3a	36.3	4.5	75.1	4.6
3b	35.9	7.3	86.1	4.2
3c	35.3	4.6	71.6	4.3
4a	80.4	5.5	159.4	0.5
4b	43.5	7.0	125.6	4.6
4c	53.9	4.7	97.5	5.0
5a	63.1	25.4	200.1	16.2
5b	68.4	10.1	126.6	16.4
5c	70.1	7.8	132.7	15.8
6a	40.9	7.4	95.0	11.4
6b	38.7	9.2	107.8	11.8
6c	54.1	6.7	112.7	11.9
7a	37.3	4.2	75.4	2.6
7b	40.4	4.8	76.9	2.7
7c	36.0	3.5	71.0	2.5
8a	67.7	9.3	170.3	1.8
8b	49.5	4.3	93.7	1.8
8c	52.7	4.2	98.7	1.9
9a	75.6	10.3	193.8	22.1
9b	57.6	5.0	96.7	19.3
9c	63.6	4.9	103.9	18.7
10a	50.8	9.2	108.2	18.7
10b	62.2	16.0	157.7	22.8
10c	45.4	8.7	99.2	21.1
11a	44.0	9.1	89.9	14.6
11b	44.4	6.0	74.7	14.6
11c	48.1	12.5	120.2	17.2
12a	39.0	7.6	74.3	23.7
12b	44.0	7.0	73.3	24.2

12c	50.8	13.7	113.8	27.8
TW1a	26.2	4.1	47.6	0.9
TW1b	27.4	5.9	61.5	0.6
TW1c	39.3	6.3	79.7	0.8
TW2a	68.1	6.3	67.0	0.6
TW2b	47.2	4.5	46.3	0.8
TW2c	53.6	4.1	51.3	0.5
TW3a	48.7	7.8	74.8	0.6
TW3b	35.0	7.9	60.9	0.6
TW3c	41.2	7.2	65.4	0.7
TW4a	29.9	13.0	73.2	0.3
TW4b	29.1	5.1	53.3	0.3
TW4c	23.7	6.3	53.3	0.6
TW5a	31.6	7.4	68.8	0.9
TW5b	23.7	5.7	50.1	1.0
TW5c	27.2	5.4	59.3	1.1