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Monitoring the mineral status and health of sheep grazing on a rehabilitated bauxite residue stockpile and on alkaloam amended pasture

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Monitoring the Mineral Status and Health of Sheep Grazing on a Rehabilitated Bauxite Residue Stockpile and on Alkaloam Amended Pasture

Gerard M. Smith, Agriculture Western Australia, October 1998
(Alcoa Contract 1311546)

Contents

EXECUTIVE SUMMARY

This is the final report on the study **"Monitoring the mineral status of sheep grazing pasture established on a rehabilitated bauxite residue stockpile (96HA5)"**.

Outline of Trial

Sheep were grazed for up to nearly a year on a rehabilitated bauxite stockpile (Rehabilitation Area, see appendix 1). There were three sites grazed; a dryland (medic and ryegrass) and an irrigated site (clover and kikuyu), both on the Rehabilitation Area, and a site on adjacent farmland. Sub-groups of sheep were removed from the Rehabilitation Area for tissue sampling at various times during the trial. At the end of the trial all remaining sheep on the Rehabilitation Area, as well as a sub-group of sheep from the farm area, were removed for tissue sampling. Mineral concentrations were measured in blood and tissue samples collected over the trial as well as in samples of pasture.

Results of the Rehabilitation Area trial were;

- Sheep maintained good health and grew well over the trial.

- The statuses of essential minerals investigated in sheep were adequate, except for low cobalt (vitamin B12) status in sheep on the Irrigated site. Pasture cobalt levels were low on both Rehabilitation Area sites, but deficiency may not have developed in the Dryland sheep because of involuntary soil intake. Cobalt availability to pasture plants might be poor because of the alkalinity of bauxite residue. Cobalt deficiency is not uncommon and can be readily treated by either top dressing pasture with cobalt salts or the use of intraruminal cobalt pellets (Underwood 1977).

- There was no evidence of accumulation of the minerals investigated to harmful levels or levels that exceeded regulatory limits (maximum permissible concentrations, MPC).

- There was no evidence of contamination of lung tissue with any of the minerals investigated, even in sheep on the Dryland site which had a high exposure to the dust.

- Molybdenum and iron levels were high in pasture. This could lead to health effects in ruminants, principally through induced copper deficiency. The use of copper top dressing at the trial sites most likely prevented this. Copper supplementation recommendations for animals grazing on bauxite residue need to be developed.

Supplementary Reports on Alkaloam Studies

This document also contains supplementary final reports on two Alkaloam studies. In the first study we investigated the effect of Alkaloam on the mineral composition of capeweed grown in pots. Treatments were; Alkaloam applied to the surface of the potting soil, Alkaloam mixed into the potting soil and potting soil only (control). Results showed that alkaloam decreased manganese, zinc and cadmium and increased iron in the capeweed. However these results would have been greatly influenced by

the fact that the potting mix was already alkaline before the application of the Alkaloam.

In the second study we investigated the mineral status of sheep grazing for 142 days on capeweed dominant pasture grown on soil amended with Alkaloam (at 20 t/ha). Sheep were also grazed on unamended pasture (controls). Blood samples taken during the experiment and tissue samples at the start and end of grazing were used to monitor mineral statuses.

Results of the Alkaloam grazing experiment were;

- Sheep maintained good health and grew well.
- The statuses of essential minerals in sheep were adequate.
- There was no evidence of accumulation of the minerals investigated to harmful levels or levels that exceeded regulatory limits (MPC).
- Alkaloam greatly increased the availability of selenium. Selenium is required for good health and so supplements are often given to farm animals. Therefore this effect would be seen as beneficial. However there is the possibility that use of Alkaloam in conjunction with generous selenium supplementation could lead to tissue levels of selenium greater than the MPC.
- Alkaloarn treatment decreased the concentration of manganese in capeweed. The manganese status in ruminants grazing Alkaloam amended pasture may need to be monitored. Monitoring of manganese levels in different pasture types on the Rehabilitation Area might also be considered.
- As with the rehabilitation site, pasture molybdenum concentrations were increased by Alkaloam amendment. As discussed above this has implications for animal health.
- As with the rehabilitation sites, pasture iron was increased, either directly or through contamination. As discussed above this has possible implications for animal health.

INTRODUCTION

This is the 'final report on the study **"Monitoring the mineral status of sheep grazing pasture established on a rehabilitated bauxite residue stockpile (96HA5)"**. This work was carried out by Agriculture Western Australia under Alcoa contract 13 11546.

This document also contains supplementary final reports on two Alkaloam studies:

1) **The effect of Alkaloam on the mineral composition of capeweed; a pot study (94PE50).**

2) **The mineral status of sheep grazing on capeweed dominant pasture grown on soil amended with Alkaloam (95VA3).**

Background to the Rehabilitation Area Study

Bauxite residue is the by-product resulting from the extraction of alumina and consists of a fine silt called "red mud", and a tine to medium sand called "red sand". Red sand is predominantly silica and contains about 5% red mud. The residue sand is initially strongly alkaline (pH 10 to 11) mainly due to the presence of sodium carbonate. However after amendment with gypsum and organic amendments and subsequent leaching under vegetation, the surface alkalinity quickly reduces to around pH 8 to 9.

Over 14 million tonnes of bauxite residue are produced by refineries in WA per year. A large proportion of this residue is stored permanently on land surrounding the refineries. Storage of the residue leads to a number of management issues, all of which are highly relevant to the local community. One key concern is whether these residue storage areas can eventually be used for productive agricultural land use. In 1994 Alcoa of Australia established a Rehabilitation Area on stockpiled bauxite residue at its refinery at Pinjarra. The area has been divided into several sites onto which different pastures have been established (see appendix 1 for a description of the Rehabilitation Area).

The major constituents of red mud are iron oxide (25-40%), aluminum oxide (15-20%), calcium oxide (2-4%), titanium oxide (2-4%) and sodium oxide (1.5% to 3.0%) (Summers and Bradby 1993). In red sand these concentrations would be expected to be about one twentieth.

The trace minerals that make up 3% of red mud are listed in Table 1, along with their estimated concentrations in red sand, which for several of the minerals are comparatively low. To some degree pasture mineral composition will reflect this mineral composition. However the alkalinity of bauxite residue will have large effects on the availability of a range of minerals to plants. For example, manganese, zinc, iron and copper usually become less available with an increase in soil pH, while other minerals such as molybdenum usually become more available (Introduction; Figures 1 and 2). Besides possible inadequacies or excesses in pasture minerals, imbalances can occur because of complex mineral interactions in grazing animals.

With this in mind a trial was designed to monitor the mineral status in sheep grazed for nearly a year on pastures established on the Rehabilitation Area at the Pinjarra refinery.

Background to Supplementary Alkaloam Studies

Red mud, also called Alkaloam, is used as a soil amendment, and can significantly reduce the phosphorus losses to the environment by soil absorption mechanisms (Vlahos *et al* 1989). In addition it is a cost effective means of increasing pasture production (Summers 1994). The beneficial effects of Alkaloam (e.g. increased soil pH and phosphorus retention) occur immediately and may continue for 5 years or longer (Rob Summer pers. comm.; Summers 1994). Because of its alkalinity, Alkaloam will affect the availability of certain minerals to pasture plants, as well as directly affecting composition because of its own mineral content (Table 1). These changes will obviously be reflected in the mineral status of grazing ruminants. Two studies were carried out to investigate these aspects of Alkaloam. Firstly a pot experiment which examined the effect of Alkaloam, either mixed through the soil or applied to the surface, on the mineral composition of capeweed. Because of changes in the mineral content of capeweed seen in that study, especially a decrease in cadmium (an economically important pollutant), a field experiment was then carried out. This study investigated the effect of Alkaloam amendment on the mineral status of young sheep grazing capeweed dominant pasture. Both studies used capeweed as it has a high propensity to absorb minerals such as cadmium (Tracey 1991).

Table 1: Concentration of minerals in bauxite residue and background concentrations in soil.

Element	Background concentrations in soil (mg/kg)#	Mean concentrations in red mud (mg/kg)†	Estimated concentrations in red sand (mg/kg)‡
Antimony	4-44	0.3	0.02
Arsenic	0.2-30	29	1.4
Barium	20-200	121	6
Cadmium	0.04-2	4.5	0.2
Chromium	0.5-l 10	314	16
Cobalt	2-170	22	1
Copper	l-190	27	1.4
Lead	<2-200	10	0.5
Manganese	4-12	165	8.2
Mercury	0.001-0.1	< 0.05	< 0.0025
Molybdenum	<1-20	1.9	0.1
Nickel	Z-400	< 5	< 0.2
Selenium	0.6-1.6*	< 10	< 0.5
Tin	l-25	8.7	0.4
zinc	2-180	32	1.6
Boron	1-75	< 20	< 1
Fluorine	200	1130	56

Australian and New Zealand Environment and Conservation Council - National Health and Medical Research Council (1992). Australian and New Zealand guidelines for the assessment and management of contaminated sites (January 1992).

† Summers R and Bradby R *Use of Bauxite Residue in the Peel-Harvey Coastal Plain Catchment.* Public Environmental Review. April 1993 Department of Agriculture Western Australia.

‡ Based on the assumption that red sand is predominantly silica with a 5% red mud content

* Fujii R and Deverel SJ In: *Selenium in Agriculture and the Environment* ed. Jacobs LW Soil Science Society of America Special Publication #23, ASA Madison, Wisconsin (1989) pp23

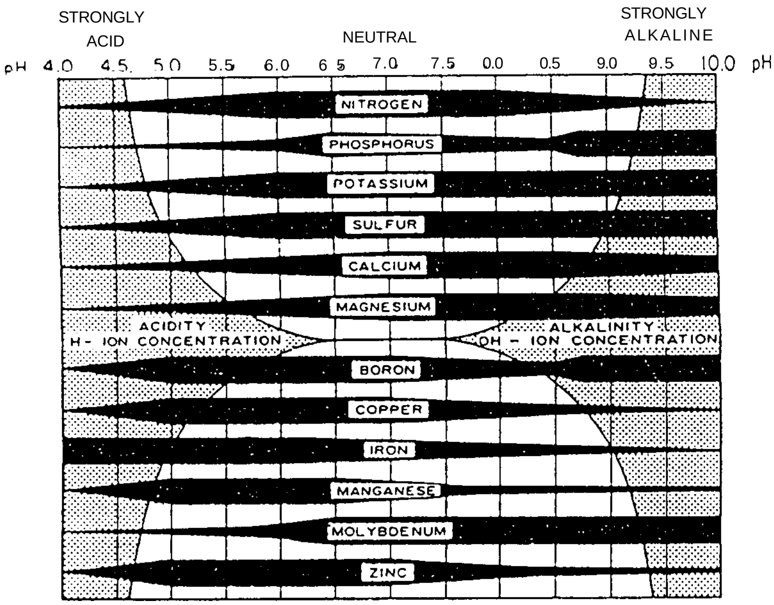


Fig 1. Influence of pH on the availability of plant nutrients in mineral soils; widest parts of the shaded areas indicate maximum availability of each element.
Adapted from L.B. Nelson (Ed.), *Changing Patterns in Fertilizer Use,* Soil Science Society America, Madison, WI (1968)

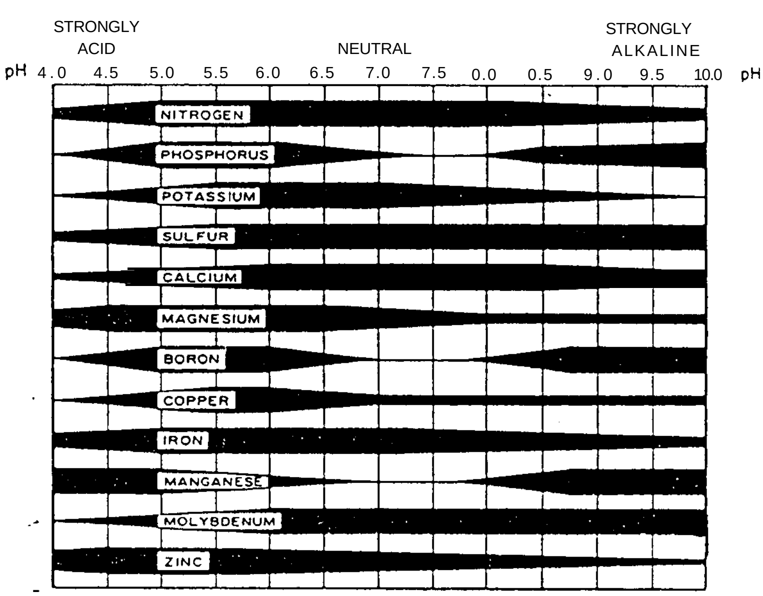


Figure 2. Influence of pH on the availability of plant nutrients in organic soils; widest parts of the shaded areas indicate maximum availability of each element.
Adapted from R.E. Lucas and J.F. Davis. Relationships between pH values of organic soils and availability of 12 plant nutrients, Soil *Science 92:* 177-l 2 (1961)

Monitoring the Mineral Status of Sheep Grazing Pasture Established on a Rehabilitated Bauxite Residue Stockpile

Outline

Sheep were grazed for up to nearly a year on a rehabilitated bauxite stockpile. There were three sites grazed; a dryland (medic and ryegrass) and an irrigated site (clover and kikuyu), both on the Rehabilitation Area, and a site on adjacent farmland. Sub-groups of sheep were removed from the Rehabilitation Area for tissue sampling at various times during the trial. At the end of the trial all remaining sheep on the Rehabilitation Area, as well as a sub-group of sheep from the farm area, were removed for tissue sampling. Mineral concentrations were measured in blood and tissue samples collected over the trial as well as in samples of pasture.

Material and Methods

Experimental site

Sheep were grazed (from July 1996 to May and July in 1997) on two pasture sites located on the Residue Rehabilitation Area 1 at the Alcoa refinery in Pinjarra, as well as on an adjacent farm paddock (about 30 m from the Rehabilitation Area; Appendix 1). The Rehabilitation Area consisted of a mound of red mud onto which a two metre layer of red sand had been placed. Pastures had been established in 1994.

One pasture site consisted of 2.5 ha of predominately kikuyu/clover pasture that was irrigated by fixed overhead sprinklers during the dry months, while the other site consisted of 3.2 ha of mostly medic/ryegrass pasture. These sites are referred to, hereafter, as the "Irrigated site" and the "Dryland site", respectively. At various times sheep on the Dryland site had access to two adjoining pasture areas, both of which had the same pasture composition as the Dryland site (see Appendix 1). However the majority of grazing was on the Dryland site itself and testing of pasture samples from the adjoining pasture areas indicated no major differences in mineral composition from pasture in the Dryland site.

Grazing on the Dryland site was managed so that by autumn pasture was short and some areas of red sand were exposed (see photograph 1). This was done to maximise exposure of the sheep to the residue through inhalation of dust and involuntary ingestion of soil (Underwood 1977). Issues on animal welfare (i.e. weight loss) as well as dust control were taken into account in grazing management. In the Irrigated site there was little opportunity for direct exposure of the sheep to the residue (see photograph 2, also taken in autumn). Small areas of exposed red sand adjacent to the Visitors Information area were fenced off soon after sheep were placed onto the site.

Sheep on the adjacent farm paddock (hereafter referred to as the "Farmland site") grazed on pasture of mixed grasses established on "normal" soil. Each site had a single water trough which was fed off scheme water.

Photograph 1: **Dryland** site, autumn **(18/4/1997).**

Photograph 2: Irrigated site, autumn **(18/4/1997).**

The Farmland, Irrigated and Dryland sites, as well as the two adjacent grass pasture areas to the Dryland site, were top dressed with super-phosphate containing 0.6% copper and 0.6% zinc at the rate of 250 kg/ha on 9/9/96. The same top dressing had occurred annually.

Sheep

Merino wethers, 12 months old, were transported from Wongan Hills Research Station (Agriculture WA) on the 24/7/96 (day 0 of the trial) to the refinery at Pinjarra. They had not been given selenium or cobalt supplements. On arrival the sheep were weighed and then allocated from within live weight strata to 4 groups consisting of 35, 35, 25 and 15 sheep. A group of 35 sheep was placed onto each of the Dryland and Irrigated sites, and the group of 25 sheep onto the Farmland site. The remaining group of 15 was transported back to the Animal Health Laboratories at South Perth and placed onto a predominately chaff diet. Twelve days later these sheep were killed and tissue samples taken so as to establish levels of minerals and heavy metals in the sheep at the start of the grazing period. These sheep are hereafter referred to as the "Baseline" group.

Sheep on the Dryland site were grazed for a maximum of 308 days (until 28/5/97) while sheep on the Irrigated and the Farmland sites for a maximum of 349 days (until 8/7/97). Sheep at all sites were shorn and jetted over days 112 to 114 (13 to 15/11/96) and given a worm drench (Ivomectin®) on day 140 (11/12/96) and a repeat dose on day 279 (29/4/97). No other treatments were given.

Some sheep on the Dryland site were removed in spring and in late summer, with the last of the sheep removed in early winter, at the end of grazing. Similarly, some sheep on the Irrigated site were removed in spring and the remainder at the end of grazing in mid-winter. Numbers of sheep removed from both sites over the trial, together with dates, are listed in Table 1. All sheep removed during the trial were randomly selected. From the Farmland site, two sheep were removed on day 104 (5/11/96) because of flystrike, and ten were removed at the same time the last group were taken from the Irrigated site (Table 1). Live weights of sheep from the Dryland and Irrigated sites were recorded on days given in Table 1.

Samples

Pasture samples were taken from the Dryland and Irrigated sites at various times over the trial period as indicated in Table 1. They were collected as grab samples taken approximately every ten to fifteen paces along a diagonal transect of the site and then pooled. Samples were not taken from the Farmland site because of the diversity of plants and the large area that the sheep had access to.

Blood samples were collected into 10 ml lithium heparin treated tubes from randomly selected sheep on the Dryland and Irrigated sites over the trial period. The numbers of sheep sampled together with sampling dates are listed in Table 1. Blood samples were not collected from sheep on the Farmland site during the trial.

Sheep removed from the sites were transported to South Perth and killed by stunning and exsanguination the following day. Blood and the following tissue samples were collected at slaughter from all animals; liver (dorsal lobe), kidneys, lung (bottom of large lobe), muscle (vastus lateralis) and rib bone (number 11), with the exception that

lung tissue was not collected from the Baseline group. All blood samples were centrifuged within 4 hours of collection and the plasma stored at - 40°C, and red blood cells at 4°C, prior to analysis. Tissue samples were stored at -20°C prior to analysis.

Table 1: Days when live weights were recorded and blood and tissue samples were taken from sheep grazing on either the Dryland or Irrigated sites, as well as when pasture samples were taken from these sites. Numbers of sheep selected for blood sampling, or removed for slaughter and tissue collection, from each site, are also listed.

Date	Day of Trial	When Live Weights Recorded	Type of Samples Taken	Number of Animals Blood Sampled and /or Removed for Slaughter from each Site	
				Site	
				Dryland	*Irrigated*
2407196	0	D I*	blood	18	18
01/08/96	8		pastures		
24/09/96	62	DI	pastures, bloods	20	20
08111/96	107		bloods, tissues (R)	10	10
28/11/96	127	DI	bloods	12	12
02112196	131		pastures		
16/01/97	176	DI	pasture, bloods	12	12
11/02/97	202	D			
28/02/97	219		bloods, tissues (R)	11	
02/04/97	252	DI	pasture, bloods	14	15
18104197	268	D			
13/05/97	293	DI	pasture, bloods	10	10
29/05/97	309		bloods, tissues (R)	14	
09/07/97	350	I	pasture, bloods, tissues (R)		25

* D = weights recorded on sheep from the Dryland site; I = weights recorded on sheep from the Irrigated site. R = blood and tissue samples collected at slaughter from sheep removed from pasture the day before.

Anaiyses

Pasture samples were dried at 80" C for two days before analysis. In most cases samples were not washed prior to drying, however during summer two consecutive samples (January and April) from the Dryland site were split and one half washed (rinsed in tap water then three times with distilled water) to determine the contribution of contaminating dust on mineral content. Pasture samples were analysed for manganese, zinc, copper, boron, iron, phosphorus, potassium, sodium, calcium, magnesium and sulfur using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) after wet acid digestion (McQuaker *et al* 1979). Pasture molybdenum concentrations were determined colorimetrically by the method of Bingley (1963). Pasture cobalt concentrations were determined by graphite furnace atomic absorption after wet acid digestion. All pasture results are expressed on a dry weight basis.

Blood samples were analysed for red blood cell glutathione peroxidase activity, an indicator of selenium status (Paynter *et al* 1985). Plasma samples were analysed for concentrations of copper and zinc (by trichloroacetic acid treatment of plasma and then flame atomic absorption spectroscopy) and calcium and magnesium (Roche

Diagnostic kit). Samples collected on the 16/1/97 were also analysed for glutamate dehydrogenase, creatinine, urea, aspartate aminotransferase and creatine phosphokinase, as indicators of liver, kidney or muscle damage, using commercial kits.

Liver and lung samples were analysed for copper, zinc iron, manganese, lead and cadmium, whilst kidney cortex samples were analysed for cadmium (all by flame atomic absorption spectroscopy after wet acid digestion). Lung samples were also analysed for chromium by graphite furnace atomic absorption spectroscopy after wet acid digestion. The concentration of cadmium in the whole kidney was estimated from the cortex concentration by using the conversion equation; ln [cadmium whole] = - 0.4162 + 0.9747 ln [cadmium cortex] (Masters and Petterson 1991). This value together with the weight of the whole kidney tested was then used to calculate whole kidney cadmium. Samples of liver and lung taken from five sheep in the final collections from each for each of the three sites, were analysed for aluminium, vanadium and arsenic. Methods used were ICPAES and Inductively Coupled Plasma Mass Spectrometry. Samples of rib bone from the same 15 sheep were analysed for fluorine concentrations (Chemistry Centre of Western Australia).

Normal Ranges

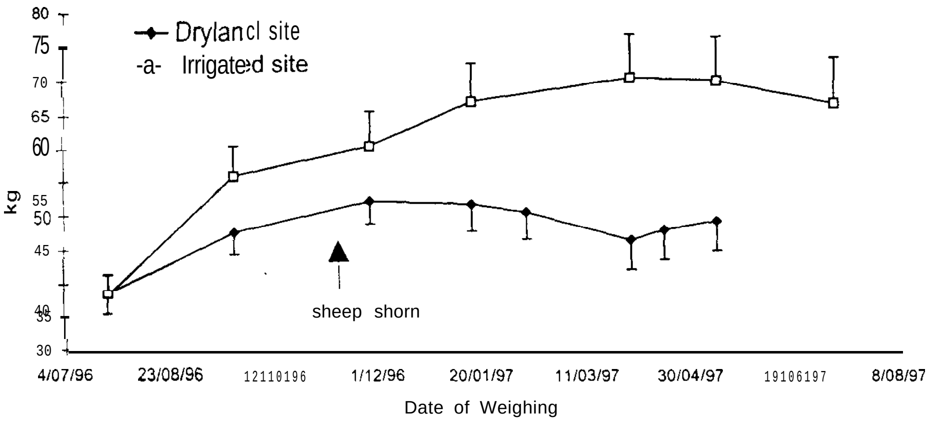
Unless otherwise stated, the normal ranges used in the “Results and Discussion” section are those routinely used by this laboratory. Ranges are given in Appendix 2, along with maximum permissible concentrations.

Results and Discussion

Sheep Health and Pasture Growth

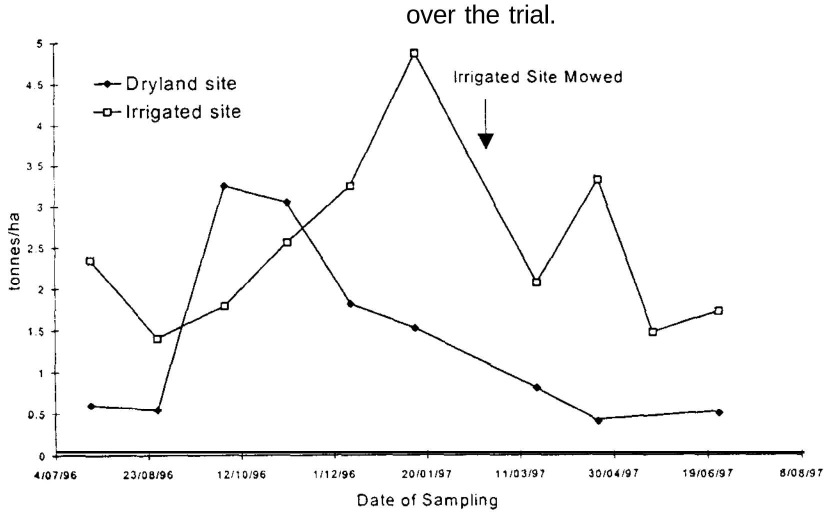
All sheep grazing on the two pasture sites at the Rehabilitation Area and on the Farmland site maintained good health over the trial, which ran for 349 days. Clinical biochemistry tests carried out on sheep on the Rehabilitation Area sites in January, about mid-way through the trial, indicated that kidney, liver and muscle were healthy. Problems that did occur, such as parasitic worm burdens and fly strikes, were minor and not considered unusual. Average growth rates for sheep on the Dryland and Irrigated sites were respectively, 32 and 77 g/day. These gains are what would be expected of healthy sheep grazing on these types of pastures (Standing Committee on Agriculture 1990). Liveweights of sheep over the periods of grazing at these two sites are shown in Figure 1. At necropsy, no gross abnormalities were seen, providing further evidence that the sheep were healthy.

Figure 1: Mean (± SD) live weights of sheep grazing on either the Dryland or the Irrigated site.



Food on offer (FOO) values for the two Rehabilitation Area sites over the trial are shown in Figure 2. As expected FOO values on the Dryland site were at their lowest in late autumn, with pasture very short and some areas of soil exposed (Photograph 1). The drop-off in FOO values on the Dryland site over summer/autumn resulted in a decrease of around 10% in mean liveweight of the sheep. In contrast, pasture on the Irrigated site remained lush over the entire grazing period (Photograph 2). The drop in FOO values after January was as a result of the site being mowed.

Figure 2: Estimated Food on Offer (FOO) on the Dryland and Irrigated Sites over the trial.



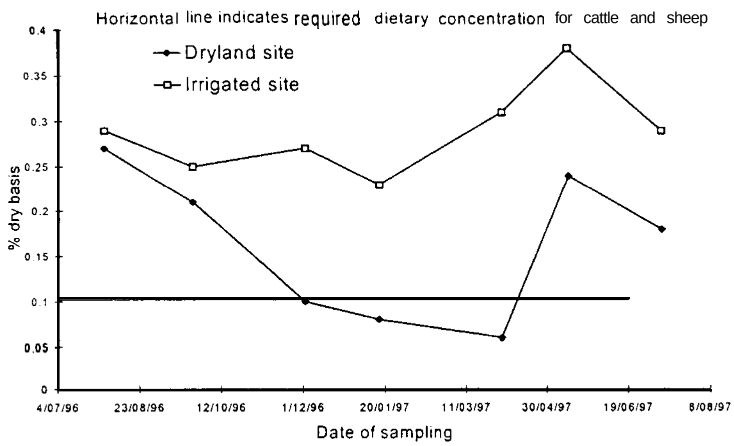
Macro-Minerals

Magnesium in Pasture

Concentrations of magnesium in pasture from the Irrigated site remained above 0.2 % over the trial (Figure 3). In comparison, concentrations in pasture from the Dryland site were much lower, with concentrations over the summer months falling to between 0.1 and 0.06%. The minimum needs of sheep and cattle for growth can generally be met by pastures containing 0.07% magnesium (Underwood 1981). However, a higher proportion, about 0.1% magnesium, is considered necessary for lactating ewes

and cows (Blaxter and McGill 1956). High potassium levels in pasture can greatly reduce the absorption of magnesium (Underwood 198 1). However the potassium concentrations in pasture at both sites (means of 1.4 and 2.8 mg/kg in the Dryland and Irrigated sites, respectively) would have been unlikely to interfere with the magnesium status of the sheep (Mayland and Grunes 1979).

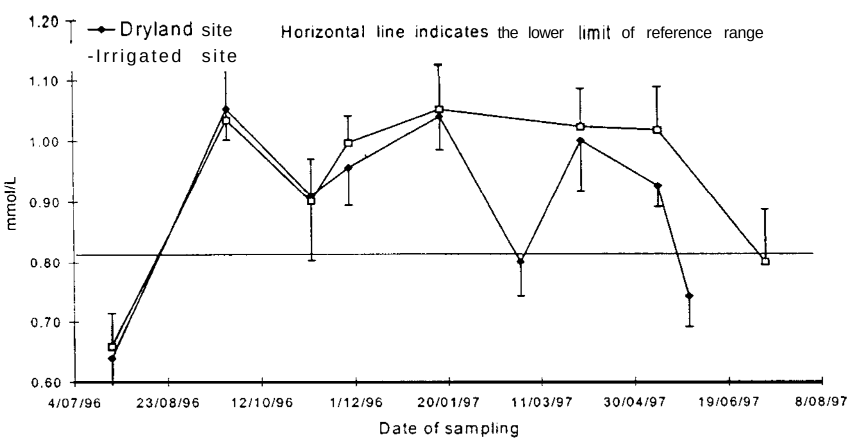
Figure 3: Concentrations of magnesium in pasture from the Dryland and Irrigated sites over the trial.



Magnesium in Plasma

Plasma magnesium concentrations were generally similar in sheep on the two Rehabilitation Area sites over the trial (Figure 4). The normal range for sheep is 0.82 to 1.44 mmol/L, and at nearly all times concentrations in sheep on both sites fell within this range. This supports the finding of generally adequate magnesium levels in the pastures. The lower levels seen at the start and end of the trial in sheep on both sites, and in sheep on the Dryland site on sample dates 8/11/96 and 28/2/97 and in sheep on the Irrigated site on the 8/11/96, were probably as a result of stress caused by transport (Ellison 1994).

Figure 4: Mean (±SD) concentrations of magnesium in plasma of sheep grazing on either the Dryland or Irrigated site.

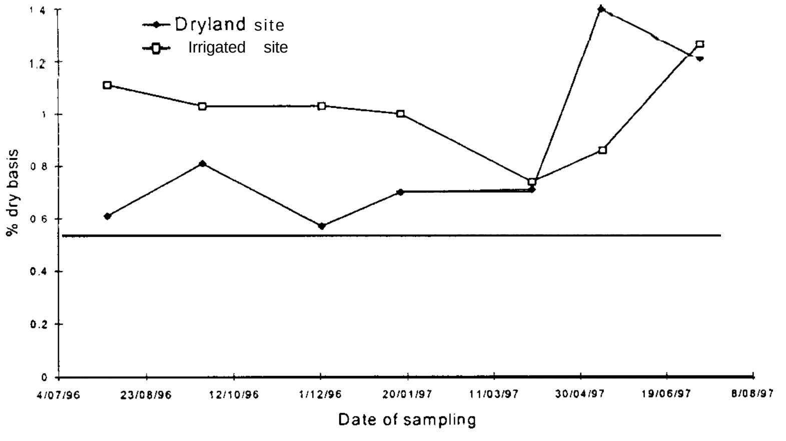


Calcium and Phosphorus in Pasture

Calcium concentrations in pasture from the Irrigated site remained above 0.7 % over the trial, while concentrations in pasture from the Dryland site remained above 0.57% (Figure 5). Grasses usually have lower calcium levels than clover pastures (Jolley and Leaver 1974). These concentrations would meet the growth requirements of cattle and sheep (Underwood 198 1), and probably those of lactating cows (Temouth 1990a).

Figure 5: Concentrations of calcium in pasture from the Dryland and Irrigated sites over the trial.

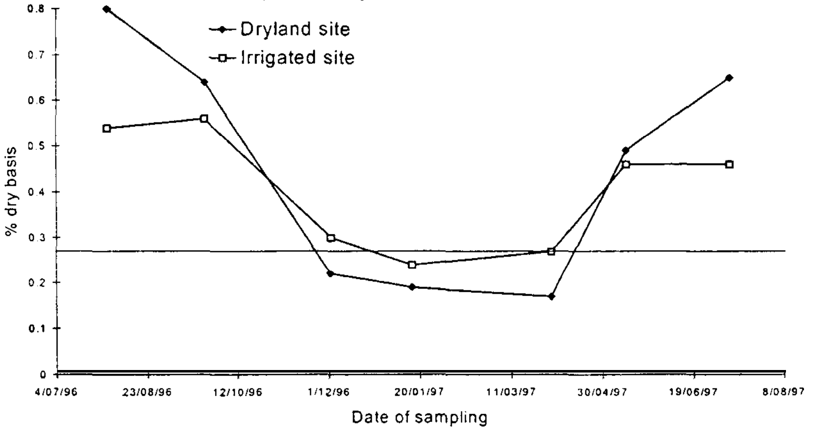
Horizontal line indicates required dietary concentration for lactating ewes and dairy cattle



Phosphorus concentrations in pasture at both sites were similar and far lower in summer compared to winter (Figure 6). This is a normal pattern for pasture phosphorus under Mediterranean conditions (Underwood 1977). The recommended minimal dietary phosphorus concentration for sheep and cattle ranges from 0.11 to 0.27 %, depending on factors such as growth rate, pregnancy and lactation (Temouth 1990b). Based on this, pastures from these sites would generally meet the requirements of grazing ruminants. The marginal concentrations over summer are not unusual in pasture in Western Australia (Underwood 1981). An important indicator of satisfactory calcium and phosphorus nutrition in grazing animals is the ratio of these two minerals in the diet, with calcium:phosphorus ratios of between 1 and 7 considered suitable for sheep and cattle (Underwood 1981). For pasture on the Dryland site the ratio ranged from 0.8 to 4.2 and on the Irrigated site, from 1.9 to 4.2, providing further evidence that the calcium and phosphorus requirements of sheep and cattle would be met by pasture from both sites.

Figure 6: Concentrations of phosphorus in pasture from the Dryland and Irrigated sites over the trial.

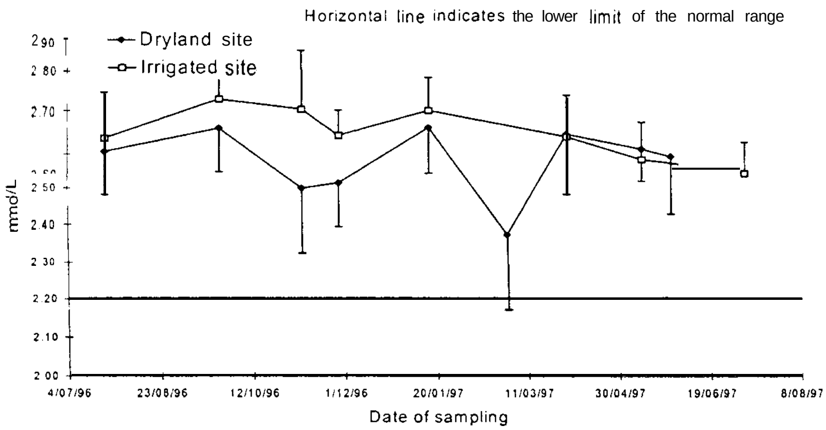
Horizontal line indicates required dietary concentration for sheep and cattle under all conditions



Calcium in Plasma

Plasma calcium concentrations in sheep on both the Dryland and Irrigated sites were similar and within the normal range over the trial (Figure 7). The normal range for sheep is 2.2 to 3.0 mmol/L. While plasma calcium is under control of homeostatic mechanisms, concentrations will drop within weeks if sheep are placed onto a diet frankly deficient in calcium (Underwood 1981). Based on pasture and plasma calcium concentrations, it would appear that calcium nutrition was adequate in sheep on both sites.

Figure 7: Mean (± SD) concentrations of calcium in plasma from sheep grazing on either the Dryland or the Irrigated site.



Phosphorus in Plasma

Phosphorus concentrations in plasma in sheep on both sites remained relatively constant and similar over the grazing period, with an overall mean (± SEM) of 2.0 ± 0.12 mmol/L in sheep on the Irrigated site and 2.0 ± 0.07 mmol/L in sheep on the Dryland site. All concentrations fell well within the normal range of 0.9 to 2.5 mmol/L indicating that the sheep had adequate phosphorus intake over the trial. Plasma phosphorus is reported to be a good indicator of phosphorus intake (Underwood 1981), however plasma concentrations did not decrease in sheep on the Irrigated site in spite of the decrease in pasture phosphorus.

Sulfur in Pasture

Sulfur concentrations decreased in pasture on both sites during summer, although the decrease in the Dryland site pasture (to a low of 0.13%) was greater than that in the Irrigated site pasture (to a low of 0.26%). During winter concentrations averaged around 0.55% in the Dryland site pasture, and 0.36% in the Irrigated site pasture.

Pasture sulfur concentrations for both Rehabilitation Area sites generally remained within the range considered adequate for ruminants (Underwood 1981). The concentration of sulfur in pasture in Western Australia is usually 0.3 % to 0.4% in winter and about 0.1 % in summer (Purser 1979), and so the range of concentrations reported here would be considered normal.

Sodium and Potassium in Pasture

Sodium concentrations in pasture at both sites were also generally adequate for ruminants. Mean concentrations over the entire trial for the Dryland and Irrigated sites were 0.26 and 0.34% respectively. Underwood (1981) states that 0.08 to 0.10% of the dry diet is adequate for sheep. In the Dryland site pasture potassium concentrations were higher in winter (average of 3.5 mg/kg) and lower in summer (average of 0.4 mg/kg). A similar pattern occurred in pasture on the Irrigated site, but differences between seasons were not as great (average of 3.7 mg/kg in winter and 2.2 mg/kg in summer). Concentrations of 0.8 g/kg should meet the requirements of sheep and cattle (Standing Committee on Agriculture 1990). While concentrations in Dryland pasture over summer were below this level, this is most likely a situation that occurs commonly under normal grazing in Western Australia.

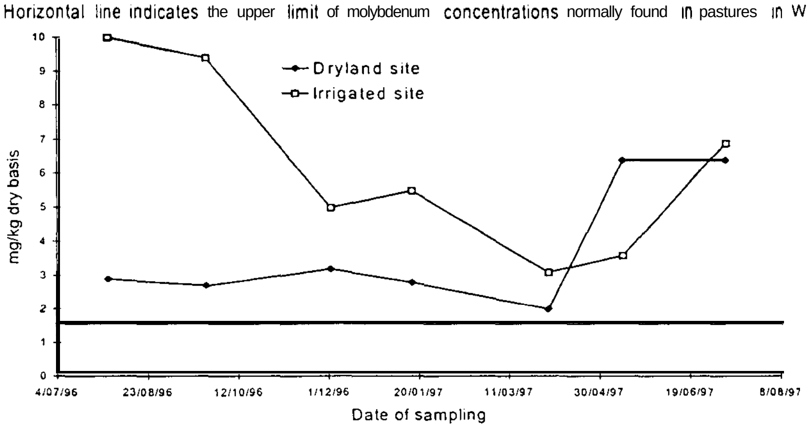
Trace Elements

Molybdenum in Pasture

Molybdenum concentrations in pasture from the Irrigated site were higher over winter (maximum of 10 mg/kg) and lower over summer (Figure 8). Concentrations in pasture from the Dryland site remained relatively constant (between 2 to 3 mg/kg) from the start of the trial until April, after which they increased over two fold (Figure 8). The much higher concentrations seen in the clover pasture compared to the grass pasture is not unexpected (Underwood 1977). The fact that molybdenum concentrations were similar between the two sites in the latter part of the trial may have been due to the increasing percentage of kikuyu grass in the Irrigated site pasture. In Western Australia pastures usually contain molybdenum concentrations in the range of 0.2 to 1.5 mg/kg , with concentrations as high as 5 mg/kg being occasionally recorded (Gartrell 1979). The concentrations recorded here must therefore be considered unusually high. The increased availability of molybdenum would stimulate symbiotic nitrogen fixation with resultant yield increases in pasture (Fleming 1980b). Clover growth on the Irrigated site would probably have been increased because of the high molybdenum levels.

Figure 8: Concentrations of molybdenum in pasture from the Dryland and Irrigated sites over the trial.

Horizontal line indicates the upper limit of molybdenum concentrations normally found in pastures in W A



The elevated pasture molybdenum levels at both Rehabilitation Area sites is most likely due to the alkalinity of the bauxite residue (Fleming 1980a). This is in spite of the comparatively low concentrations of molybdenum in bauxite residue (Introduction; Table 1). High soil pH is reported to have a stronger effect on molybdenum content in clover than in grass (Underwood 1977). Top dressing of the Rehabilitation Area sites in September could also have influence molybdenum levels (Fleming 1980a). However the overall effect is difficult to predict because of a number of interacting factors such as soil type and the presence of certain minerals. For example copper in the superphosphate could have reduced pasture molybdenum because of a copper-molybdenum antagonism, whereas the phosphate may have had the opposite effect (Fleming 1980a).

While molybdenum is required in extremely low concentrations in the diet for animal health, concentrations too high can be detrimental (Underwood 1981). For example, molybdenum in a diet at 5 mg/kg has been shown to delay puberty in cattle and cause a lower conception rate (Phillippo et *al* 1985) and to reduce growth (Phillippo *et al* 1987). Health problems due to increased intake of molybdenum is referred to as

molybdenosis. However most health effects due to high molybdenum intakes (5 mg/kg or higher in the diet) are as a result of an induced copper deficiency (Underwood 1981). Molybdenum binds copper in the rumen and so prevents its absorption. For this reason adequacy of dietary copper is best described in terms of the dietary copper:molybdenum ratio. Blood and Radostits (1989) have suggested that the minimum ratio in animal feeds for acceptable copper availability is around 2:1. Molybdenosis can be prevented to some degree by increasing the level of dietary copper which reduces the amount of molybdenum absorbed.

Copper in Pasture

Concentrations of copper in pastures from the Dryland and Irrigated sites are shown in Figure 9. On the Dryland site, pasture copper concentrations were lowest during the summer months. Copper concentrations in pasture from the Irrigated site was at a maximum soon after the fertiliser application, and then tending to decrease thereafter. Copper pasture concentrations are usually less than 10 mg/kg (Peter 1979). Long-term ingestion of pastures with high copper concentrations can lead to copper toxicity in sheep (Howell and Gooneratne 1987). However because of the high molybdenum levels a significant amount of this copper would have been made unavailable. In terms of copper deficiency, the copper:molybdenum ratio remained greater than the critical value of 2:1, except in December in the Dryland site pasture, and in July in the Irrigated site pasture (Figure 10).

Figure 9: Concentrations of copper in pasture from the Dryland and Irrigated sites over the trial.

Horizontal line indicates the upper limit of copper concentrations normally found in W A

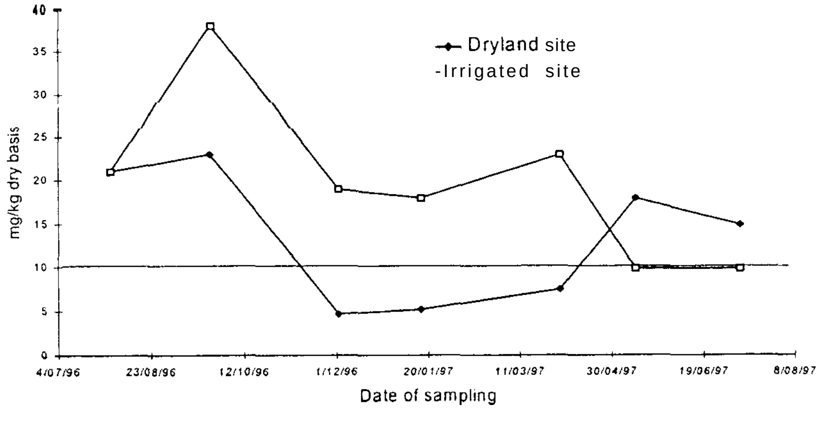
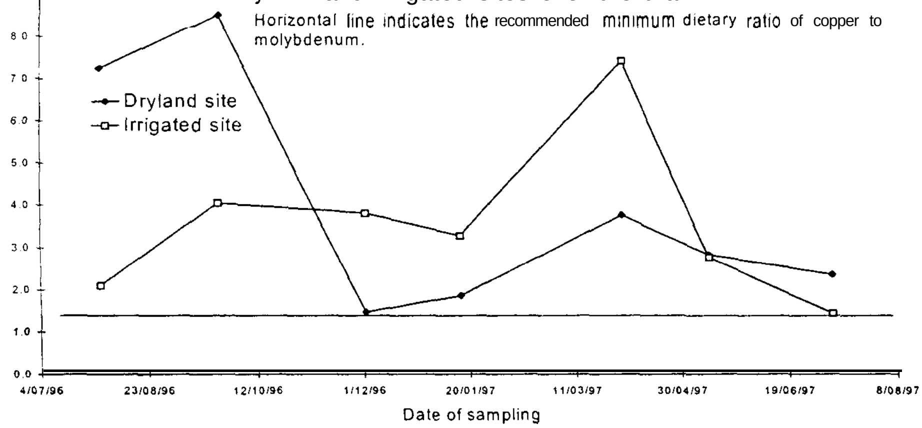


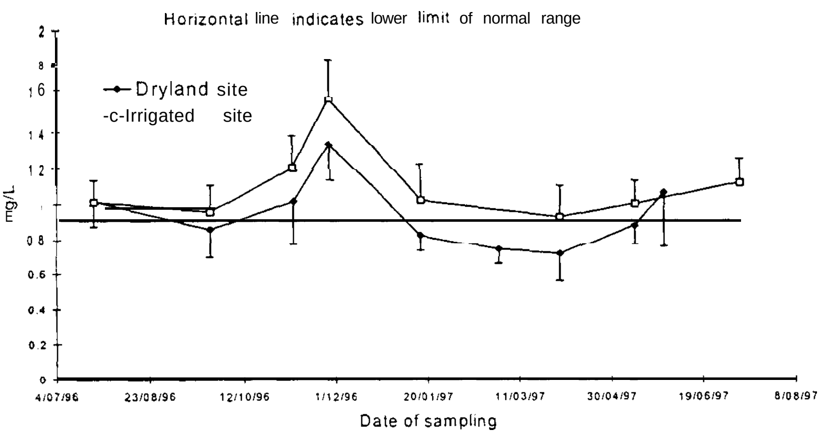
Figure 10: Ratio of copper to molybdenum in pasture from the Dryland and Irrigated sites over the trial.



Copper in Plasma

Plasma concentrations of copper in sheep on the Irrigated site tended to be higher than in sheep grazing the Dryland site, with concentrations falling below the lower limit of the reference range between January and April (Figure 11). This fall may not have been related soley to the decrease in pasture copper concentrations that occurred over summer (the copper:molybdenum ratio indicates that availability of copper should have been satisfactory; Figure 10), since plasma copper can also be influenced by factors such as the level of nutrition (Masters 1979). The normal range for plasma copper is 0.9 to 1.4 mg/L.

Figure 11: Mean (± SD) concentrations of copper in plasma of sheep grazing either the Dryland or the Irrigated site.



Copper in Liver

Mean concentrations of copper in livers of sheep from all three sites over the trial are given in Table 2. Concentrations in all sheep, and at all times, indicated adequate copper status, with concentrations well above the lower limit of the normal range (30 mg/kg). Concentrations were also less than 400 mg/kg which would indicate that copper toxicity was unlikely (Suttle 1995). Liver copper apparently decreased in sheep on the Rehabilitation Area over the first 107 days, but thereafter increased (Table 2). At the end of grazing all sheep had similar liver copper concentrations to that at the start of the trial (Table 2). These results indicate that the potentially

harmful concentrations of molybdenum and copper in the pasture, especially on the Irrigated site, effectively neutralised each other.

Table 2: Mean (± SD) concentrations of copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) and vitamin B12 in livers of sheep killed at the start of the trial (Baseline) and in livers of sheep grazing on the Dryland, Irrigated and Farmland sites over the trial. Minerals on a dry weight basis, and vitamin B12 on wet weight basis.

	Date of Sampling *(Day of Trial)*				
Site/ Mineral	**5/08/96 (12)**	**8/11/96 (107)**	**28/02/97 (219)**	**29/05/97 (309)**	**9/07/97 (350)**
Baseline	n= 15				
CU	229 ± 64				
Zn	128 ± 17				
Fe	306 ± 124				
Mn	6.5 ± 1.54				
Vitamin B 12	1.24 ± 0.38				
Drvland		n= 10	n = 11	n= 14	
cu		144 ± 62	207 ± 59	239 ± 99	
Zn		135 ± 17	114112	147 ± 25	
Fe		197 ± 63	588 ± 134	367 ± 139	
Mn		7.1 ± 0.98	8.4 ± 0.75	10.0 ± 1.69	
Vitamin B 12		0.79 ± .17	1.00 ± 0.14	1.19 ± 0.21	
Irrigated		n= 10			n = 25
cu		159 ± 41			254 ± 138
Zn		137 ± 10			121 ± 20
Fe		248 ± 52			419 ± 121
Mn		7.6 ± 1.08			7.7 ± 1.30
Vitamin B12		0.91 ± .17			0.10 ± 0.08
Farmland		n = 2#			n = 10
cu		172			170 ± 98
Zn		254			127 ± 10
Fe		355			276 ± 76
Mn		11.4			11.9 ± 1.46
Vitamin B12					1.21 ± 0.26

Actually killed 5/1 1/96

Selenium in Pasture

Selenium concentrations in pasture from the Dryland site were lower in winter (0.03 to 0.05 mg/kg) and higher in summer (around 0.1 mg/kg). Similarly, selenium concentrations in pasture from the Irrigated site were 0.06 to 0.08 mg/kg in winter and 0.10 to 0.18 in summer. The concentration of selenium, which is not an essential element for plants, tends to be at its lowest during rapid pasture growth due to a 'dilution' effect (Fleming 1980b). A dietary intake of 0.1 mg/kg, according to Underwood (1977) should provide more than adequate selenium for grazing sheep and cattle, however he does cite evidence that sheep can grow normally on pasture containing only 0.03 mg/kg selenium. I would expect the availability of selenium

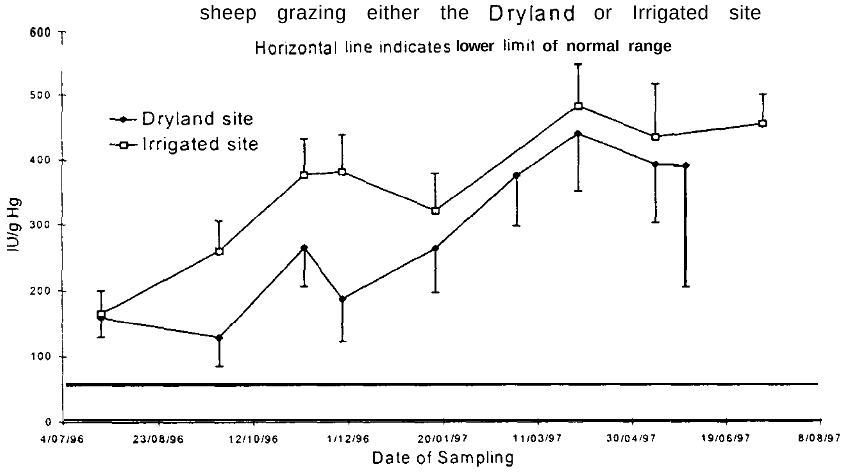
from bauxite residue to be good since the solubility of selenium is highest, and therefore its availability, when soil pH is above neutral. (Fleming 1980b).

Glutatlzione Peroxidase

Measurement of glutathione peroxidase activity in red blood cells is a useful indicator of selenium status in sheep (Underwood 1981), with activities greater than 50 IU/g Hb indicative of adequate selenium status (Paynter et *al 1985).* Sheep on the Rehabilitation Area sites began the trial with adequate selenium status, and even though no selenium supplements were given, the status increased steadily over the trial in sheep on both sites (Figure 12).

Figure 12: Mean (± SD) red cell glutathione peroxidase activity in sheep grazing either the Dryland or Irrigated site

Horizontal line indicates lower limit of normal range



Selenium in Liver

The increases in glutathione peroxidase activity were also reflected in increases in liver selenium. Sheep began the trial with a mean (± SD) concentration of 0.45 (± 0.04) mg/kg, and this increased by the end of grazing on the Dryland, Irrigated and Farmland sites to mean concentrations of 0.79 (± 0.025), 0.91 (± 0.037) and 0.51 (± 0.028) respectively. The normal range for liver selenium is 0.5 to 3.0 mg/kg. The glutathione peroxidase and liver selenium results therefore confirmed that pasture selenium levels were adequate.

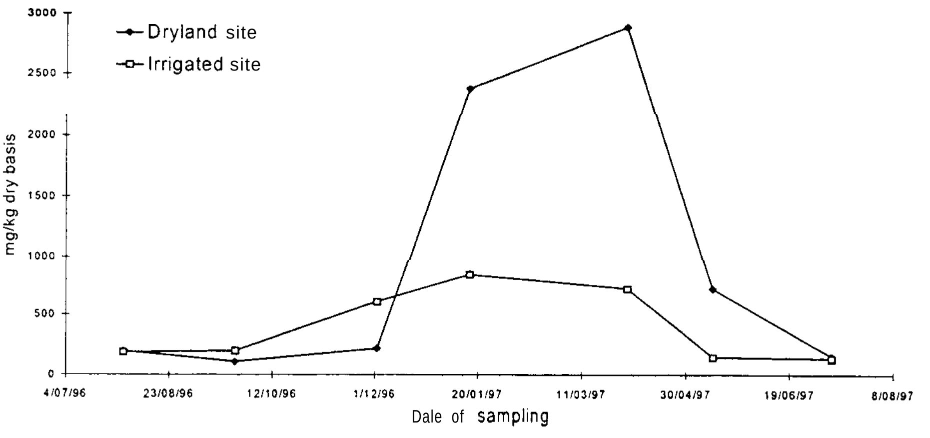
Iron in Pasture and Liver

Concentrations of iron in pasture from the Dryland site exceeded 2,400 mg/kg in January and April, but then concentrations decreased by over ten fold during the winter months (Figure 13). Likewise concentrations in pasture from the Irrigated site were higher during summer, however they did not exceed 840 mg/kg (Figure 13). Leguminous pasture plants usually contain 200-400 mg/kg, although values as high as 800 mg/kg have been recorded for luceme and as low as 40 mg/kg for grasses grown on sandy soil (Underwood 1977). Concentrations shown in Figure 13 are for unwashed pasture. When sub-samples of pasture collected in January and April were washed then the concentrations were reduced to 730 and 840 mg/kg, respectively, indicating that surface contamination was contributing a large percentage of the iron (about 70%). Alkaloam has a very high iron content (Summers and Bradley 1993), so contamination, in spite of the washing , may still have been a factor. The alkalinity of

the Rehabilitation Area soil would be expected to reduce the availability of iron (Bernard and Ellis 1980). Iron requirements for sheep and cattle (a dietary level of 40 mg/kg) would have been easily met by pasture on either site (Underwood 1981). In fact the iron concentrations may have been sufficiently high to affect animal health. The maximum tolerable concentration of iron for cattle has been estimated at 1,000 mg/kg (National Research Council 1996) and concentrations as low as 250 mg/kg have caused copper depletion in ruminants (Bremner *et al* 1987). Excessive iron intake can cause clinical signs such as diarrhoea, acidosis, reduced feed intake and liveweight gain (National Research Council 1996).

Liver iron concentrations vary greatly in healthy grazing sheep (normal range of 200 to 800 mg/kg), with high concentrations resulting in late summer because of ingested soil. In this trial liver iron concentrations all remained within the normal range, with no indication of accumulation of high levels (Table 2). This is probably because iron present in Alkaloam is in the largely insoluble form of ferric oxide (Fe203) which has been reported as having poor availability to the ruminant (Underwood 1977). However ferric oxide has been shown to inhibit copper absorption when added to the diet of sheep (Suttle and Peter 1985). In conjunction with the high pasture molybdenum levels this could place additional pressure on the copper status of the sheep.

Figure 13: Concentrations of iron in unwashed pasture from the Dryland and Irrigated sites over the trial.



Cobalt in Pasture and Vitamin B12 in Liver

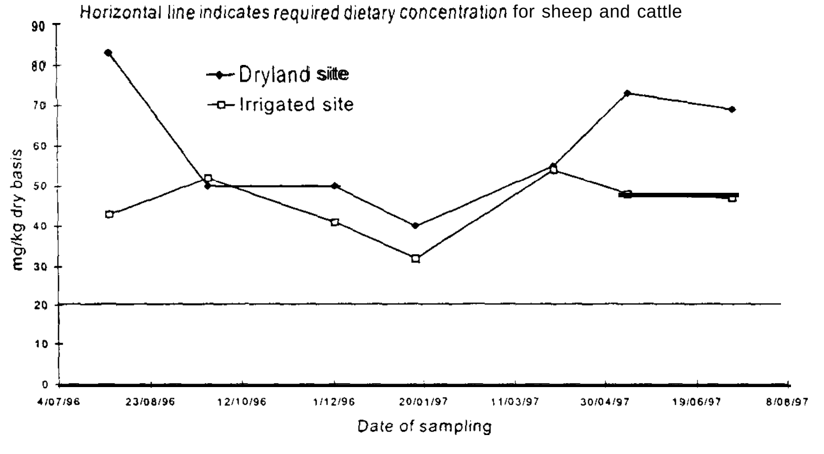
The vitamin B12 requirements of sheep and cattle are met through production of the vitamin in the rumen by micro-organisms, a process that requires dietary cobalt (Underwood 1977). Pasture cobalt levels at both sites were lower in winter and higher in summer, which is considered a normal pattern. On the Dryland site pasture cobalt averaged 0.089 mg/kg over the summer months and 0.05 mg/kg over the winter months, while on the Irrigated site pasture cobalt averaged 0.071 mg/kg over the summer months and 0.02 mg/kg over the winter months. The lower concentrations in the Irrigated pasture is most likely due to a 'dilution' effect since cobalt is not an essential mineral for higher plants (Smith 1990). Requirements for cobalt are highest in lambs, followed by mature sheep, calves and mature cattle in that order (Underwood 1977). Underwood (1977) suggests that minimum requirements for sheep lay between 0.08 to 0.11 mg/kg, which would indicate that pasture levels at the

two sites would be considered generally inadequate. Uptake of cobalt by plants is a function of the concentration of cobalt in the soil and the amount of 'available' cobalt. Increasing soil pH decreases 'available' cobalt and so reduces uptake by plants. For example in a survey of pasture cobalt in Scotland, low cobalt levels (less than 0.08 mg/kg) were only found when soil pH was greater than 6.0 (Mitchell *et al* 1957). Because of the high pH of bauxite residue, as well as its high iron content, the availability of cobalt would be expected to be relatively poor (Smith 1990). Cobalt concentrations in red sand are estimated to be about 1 mg/kg (based on data given by Summers and Bradby 1993) which should be more than adequate since concentrations of 0.25 mg/kg or more are likely to produce pastures containing sufficient cobalt (Blood, Henderson and Radostits 1979). Vitamin B 12 concentrations in liver remained adequate (greater than 0.4 ug/g on wet basis) in sheep on the Dryland and the Farmland sites (Table 2). However, in sheep on the Irrigated site, liver concentrations indicated that animals had inadequate vitamin B 12 status at the end of the trial (Table 2). The reason why sheep on the Dryland site maintained a comparatively good vitamin B12 status may have been due to involuntary soil intake which would have occurred far less in sheep on the Irrigated site. Soil cobalt is readily available to the ruminant (Underwood 1977).

Zinc in Pasture

Zinc pasture concentrations at both sites were generally lower in summer and higher in winter, especially on the Dryland site (Figure 14). In pasture from this site, zinc concentrations ranged from 40 to 83 mg/kg, while concentrations in pasture from the Irrigated site varied from 43 to 54 mg/kg. Pasture zinc concentrations greater than 20 mg/kg DM meet both growth and reproductive requirements of sheep and cattle (Underwood 1977). The application of fertiliser in September would have increased pasture zinc concentrations (Underwood 198 1).

Figure 14: Concentrations of zinc in pasture from the Dryland and Irrigated sites over the trial.

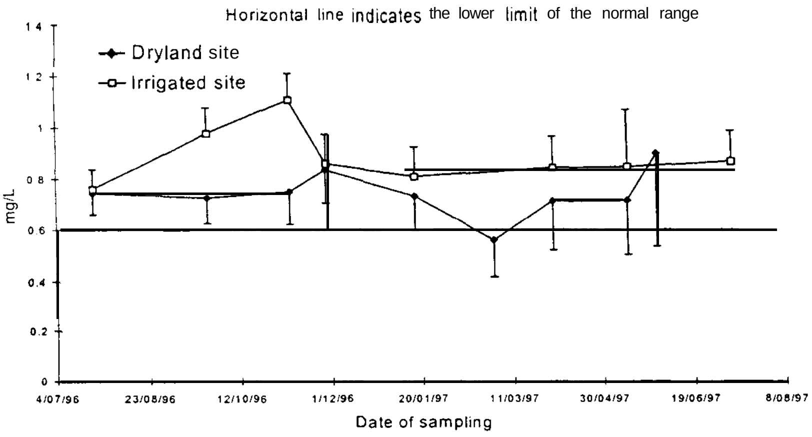


Zinc in Plasma

Mean plasma zinc concentrations in sheep on the Irrigated site remained above 0.8 mg/L over the trial. Concentrations in sheep on the Dryland site remained above 0.7 mg/L, except in late February when the mean concentration fell to 0.56 mg/L (Figure 15). Concentrations consistently less than 0.6 mg/L indicate a zinc deficiency. While measurement of zinc concentrations in plasma is widely used for measuring zinc status, it can be influenced by several factors such as stress and level of nutrition

(Underwood 1981). However concentrations in liver are far less influenced by such factors.

Figure 15: Mean (± SD) concentrations of zinc in plasma from sheep grazing either the Dryland or the Irrigated site.



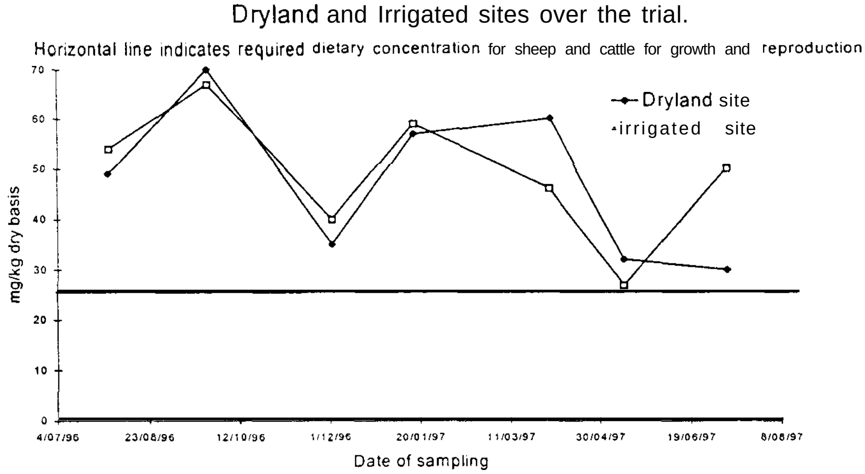
Zinc in Liver

Mean concentrations of zinc in livers of sheep from all three sites over the trial are given in Table 2. In all cases, concentrations indicated adequate zinc status (greater than 100 mg/kg) and with no evidence of zinc accumulation over the trial. The zinc status of the sheep as determined by plasma and liver zinc concentrations confirmed the adequacy of pasture zinc at both sites.

Manganese in Pasture

Concentration of manganese in pasture from the Rehabilitation Area sites were similar over the trial, with values in the range of 25 to 70 mg/kg (Figure 16). Experimental evidence suggests that 10 mg/kg manganese DM is adequate to support growth of sheep and cattle, while concentrations of 20-25 mg/kg are adequate for reproduction (Masters 1990). Concentrations of 400 to 500 mg/kg have been shown to adversely affect growth of cattle (Underwood 198 1).

Figure 16: Concentrations of manganese in pasture from the Dryland and Irrigated sites over the trial.



Manganese in Liver

Manganese concentrations in livers of sheep were lower than expected at the start of the trial (Table 2). Typical manganese concentrations of normal sheep and cattle livers are 8-10 mg/kg (dry basis) (Underwood 1977). However liver concentrations increased in sheep on the Dryland and Farmland sites, so that by the end of grazing liver concentrations were apparently normal. Liver concentrations also increased in sheep on the Irrigated site but not to the same extent, suggesting that the availability of manganese from the Irrigated pasture was less than from the Dryland pasture. This may also be a reflection of reduced involuntary soil intake. The liver manganese concentrations confirmed the adequacy of the pasture.

Lead in Liver, Kidney and Lung

Lead was not detected in liver, kidney or lung tissue in sheep from any of the three sites. This means that concentrations were less than 0.07 mg/kg wet weight, the limit of detection of the assay. Survey data from the Bureau of Resource Sciences gives a median value of 0.05 mg/kg for sheep liver (Branford 1997), which would suggest that the non-detection of lead using the method here is not unexpected.

Summers et *al* (1996) studied the effect of Alkaloam amendment on lead in pasture plants and found that concentrations remained less than 5 mg/kg. They also measured thorium, uranium and mercury, but all were below the level of detection.

Cadmium in Kidney and Liver

Kidney cadmium concentrations in sheep grazing all three sites changed little over the experiment (Table 3). However, when expressed as total cadmium in the kidneys, a small accretion of cadmium was evident in sheep on the Dryland and Irrigated sites (Table 3). In contrast, liver concentrations decreased noticeably. Sheep grazing on the Dryland site had liver cadmium concentrations at the end of grazing 76% lower than at the start (after 308 days), while concentrations in sheep on the Irrigated and Farmland sites were 62% and 72% lower, respectively (after 349 days). Dilution of liver cadmium by an increase in the size of the organ over the trial would have resulted in a reduction of only about 25%. The sheep has a slow elimination of cadmium from the liver so these results are surprising (Han *et al* 1994). The estimated cadmium concentration in red sand is about 0.2 mg/kg (based on data given by Summers and Bradby 1993), and soils with similar concentrations have been associated with the accumulation of cadmium in the offal of grazing sheep (Williams and David 1976). The poor availability of cadmium from bauxite residue could be due to a number of factors. Firstly, the cadmium could be absorbed onto hydrous oxides of iron and aluminium or bound into insoluble organometallic complexes (Alloway 1990). The ability of red mud to bind heavy metals from waste materials such as municipal compost has previously been demonstrated (Hofstede and Ho 1991). Secondly the high pH of bauxite residue would be expected to reduce cadmium availability. For example, applying lime to pasture reduces the cadmium concentration of the pasture (Williams 1977). Finally the relatively high calcium content of bauxite residue (2 to 4% as calcium oxide in red mud) could also reduce availability of cadmium to the pasture (Haghiri 1976). High pasture molybdenum levels in pastures on the Rehabilitation area site could also reduce cadmium absorption in sheep (Smith and White 1998).

Table 3: Mean (± SD) concentrations (mg/kg wet weight) of cadmium in kidney and liver and total amounts of kidney cadmium (Cd; mg in one kidney) in sheep killed at the start of the trial (Baseline) and in sheep grazing on the Dryland or Irrigated sites over the trial.

	Date of Sampling (Day of Trial)				
	5/08/96 (12)	**8/11/96 (107)**	**28/02/97** (219)	**29/05/97** (309)	**9/7/97** (350)
Baseline	*n* = **15**				
Kidney	1.28 ± 0.027				
Total Cd kidney	0.041 ± 0.0086				
Liver	0.68 ± 0.03				
Drvland		*n = 10*	*n = 11*	*n* = 14	
Kidney		1.22 ± 0.29	1.42 ± 0.151	1.2 ± 0.38	
Total Cd kidney		0.045 ± 0.010	0.04 ± 0.013	0.05 ± 0.016	
Liver		0.33 ± 0.03	0.36 ± 0.03	0.16 ± 0.03	
Irrigated		*n* = **10**			*n = 25*
Kidney		0.86 ± 0.219			1.22 ± 0.414
Total Cd kidney		0.038 ± 0.010			0.050 ± 0.018
Liver		0.10 ± 0.02			0.26 ± 0.02
Farmland					*n = IO*
Kidney					1.02 ± 0.31
Total Cd kidney					0.043 ± 0.01
Liver					0.19 ± 0.04

Aluminum in Liver and Lung

The aluminium concentrations of liver and lung samples were measured in five sheep sampled at the end of grazing at each site. All concentrations in liver and lung were below 10 mg/kg wet weight, the limit of detection of the assay. Underwood (1977) cites two studies reporting lung aluminium concentrations in dogs and humans; ranges were, respectively, 20-60 mg/kg and 18.2 ± 9.7 mg/kg, both on wet weight. This would suggest that contamination through dust inhalation did not occur. Ingested aluminium is very poorly absorbed and it has low toxicity (Underwood 1977).

Vanadium and Arsenic in Liver

Liver vanadium and arsenic concentrations were measured in the same sheep as tested for aluminium. For both elements, all concentrations were less than 1 mg/kg wet weight, the detection limit of the assay. Vanadium concentrations in a variety of vertebrate tissues range from 0.02 to 0.3 ppm, with a mean of 0.1 mg/kg (Underwood 1977), so it would seem unlikely that sheep had any significant exposure to vanadium. Underwood (1977) states that farm animals usually have tissue arsenic concentrations of less than 1 mg/kg.

Chromium in Lung

In spite of their much greater exposure to the bauxite residue via soil and dust, lung chromium was not noticeably increased in sheep grazing on the Dryland site relative to sheep on the Irrigated and Farmland sites (Table 4). Furthermore, concentrations in

lung were similar to concentrations measured by these laboratories in the kidneys (mean of 0.135 mg/kg) and livers (mean 0.198 mg/kg) of healthy, penned sheep. Therefore these results would suggest that there had been no significant exposure to chromium in any of the sheep. Chromium in soil exists in two predominate forms, chromium (III) and chromium (VI). It is the (VI) form which has the higher toxicity, however because it is such a strong oxidising agent it is rapidly converted, when in contact with organic matter, to the far less dangerous (III) form (McGrath and Smith 1990). And at soil pHs above 5.5, chromium (III) becomes completely insoluble and precipitates out (McGrath and Smith 1990). For these reasons it is suspected that chromium in bauxite residue would not pose a risk to grazing ruminants.

Other Minerals in Lung

Concentrations of minerals in lung tissue at the end of the grazing periods from sheep grazing on the three sites are listed in Table 4. There is no indication that sheep on the Dryland site, which potentially had a much higher exposure to bauxite residue dust, had noticeably higher concentrations of copper, zinc, iron, manganese or cadmium in lung tissue compared to sheep on the Farmland or Irrigated site.

Table 4: Mean (± SD) concentrations (dry weight basis) of copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cadmium (Cd) and chromium (Cr) in lungs of sheep killed at the end of grazing on the Dryland, Irrigated and Farmland sites.

Site/Mineral	
Dryland n = 14	
CU	18 ± 4
Zn	92 ± 8
Fe	492 ± 89
Mn	0.49 ± 0.21
Cd	0.16 ± 0.06
Cr	0.134 ± 0.084
Irrigated n = 25	
CU	19 ± 5
Zn	84 ± 4
Fe	526 ± 62
Mn	0 ± 0
Cd	0 ± 0
Cr	0.112 ± 0.078
Farmland n = 10	
CU	12 ± .5
Zn	84 ± 8
Fe	355 ± 197
Mn	0.28 ± 0.19
Cd	0 ± 0
Cr	0.117 ± 0.037

Fluorine in Bone

Mean concentrations of fluorine in rib bone from sheep on the Dryland, Irrigated and Farmland sites were 688 ± 99, 358 ± 33 and 532 ± 83 mg/kg (fat-free), respectively. In normal adult farm animals not unduly exposed to fluorine, the concentrations in whole, dry fat-free bones usually lie within the range of 300-600 mg/kg, while the toxic thresholds for sheep are in the range of 4,000-6,000 mg/kg (Underwood 1977). The higher concentrations measured in the sheep from the Dryland site compared to the Irrigated site suggests that these animals had greater exposure to fluorine, probably through dust-contamination of pasture or ingestion of soil, since most plant species have a very limited capacity to absorb fluorine (Underwood 1977).

Contribution of Dust Contamination of Pasture to Mineral Concentrations

As already discussed iron concentrations were greatly reduced, by an average of 70% over the January and April collections, by washing the Dryland pasture prior to testing. Other minerals reduced were potassium (average of 30%) and manganese (average of 36%). Differences in the other minerals were close to or less than 10%, which would be close to the coefficient of variation for the testing method. Without knowing the formulation of the minerals, it can only be assumed that the potassium and manganese were available to the sheep.

The Effect of Alkaloam (Red Mud) on the Mineral Composition of Capeweed

Outline

The aim of this experiment was to determine the effect of Alkaloam treatment of soil on the mineral composition of capeweed grown in pots. A potting soil was prepared by treating an acidic sandy soil with cadmium and with a mineral fertilizer. Treatments were; Alkaloam applied to the surface of the potting soil, Alkaloam mixed into the potting soil and potting soil only (control).

Material and Methods

Preparation of potting soil

Cadmium was added to the potting soil so as to ensure that concentrations would be sufficiently high to detect any effect of the Alkaloam. Cadmium, as a solution of the acetate salt, was added to 60 kg of soil (Karrakatta sand, pH(H2O) 6.7, with no history of fertiliser application and therefore with a low cadmium level) so as to give a concentration of 2.0 mg/kg dry weight. Following its initial application to soil, cadmium continues to react slowly with sorbing surfaces. In order to decrease time-dependent changes in the plant availability, the moistened potting soil was incubated at 60°C for 24 h (Bramley and Barrow 1993). A mineral fertiliser was then applied to the potting soil at the rates (g/kg) of; nitrogen (0.1), phosphorus (0.05) potassium (0.1) , calcium (0.07), zinc (0.01), magnesium (0.006), manganese (0.004), molybdenum (0.001), copper (0.001) and cobalt (0.0001).

Treatments

This soil was then used for producing the following three treatments, each consisting of 20 pots (13 cm diameter, at 0.9 kg experimental soil/pot);

1) “Control”; No Alkaloam

2) “Surface”; Alkaloam applied onto the surface of the soil in the pots and then lightly mixed into the top two cm of soil. Rate of application was approximately 20 t/ha.

3) “Mixed”; Alkaloam mixed thoroughly into approximately 18kg of soil, at the same rate as used in Surface treatment. This soil was then placed into pots.

Three capeweed seedlings, which had been germinated on clean sand, were planted in each pot. Pots in the three treatments were place randomly onto trays and kept in a glass house for the duration of the experiment (65 days). Pots were lightly watered daily.

Samples and Analyses

At the end of the experiment soil was sampled from pots in each treatment and these samples then pooled and soil pH (H_20) measured. In the case of the Surface treatment, samples were taken from the surface as well as from mid-depth in the pots. At the end of the experiment one whole plant was randomly selected from each pot and weighed. Samples of capeweed leaf were analysed for cadmium, copper, zinc,

iron, manganese, phosphorus, potassium, sodium, calcium, magnesium, sulfur, molybdenum and aluminum by inductively coupled plasma atomic emission spectroscopy after acid (nitric/perchloric) digestion (McQuaker et ***al*** 1979). Prior to testing, capeweed leaf samples were rinsed three times with distilled water and then dried at 80°C over 3 days. Treatment means were compared by analysis of variance, with P values less than 0.05 considered significant.

Results and Discussion

Soil pH

Soil pH values for Control, Mixed, Surface (top and lower) treatments were 8.2, 8.6, 8.6 and 7.8, respectively. It is most likely that the addition of the fertilizer mix was responsible for the high pH of the potting soil. The pH of the soil before treatment with the fertilizer was 6.7.

Capeweed Growth

The Alkaloam treatments did not affect whole plant wet weights, which had an overall mean weight of 2 1.4 g. There is anecdotal evidence that Alkaloam retards the growth of capeweed.

Mineral Concentrations in Capeweed

Mean concentrations of minerals in leaf material are given in Table 1. The Alkaloam treatments had no significant effect on calcium, potassium, molybdenum, sulfur, sodium, magnesium or copper. The lack of effect on molybdenum and copper was unexpected since increasing alkalinity has been shown to, respectively, increase and decrease the availability of these minerals to pasture plants (Fleming 1980a; Bernard and Ellis 1980; Summers *et al* 1996). However this may be because both the Control soil and the Alkaloam treated soils were well within the alkaline range, and so the pH effect was already at a maximum (Introduction; Figure 1). The very high leaf molybdenum levels certainly support this (Table 1). In contrast, Alkaloam significantly reduced cadmium, phosphorus (but only when mixed), manganese and zinc, with the reductions being greater in the Mixed treatment. There was tendency (P = 0.06) for the Alkaloam treatments, when combined for statistical analysis (t-Test), to increase aluminum, which is not unexpected because of its high aluminum content. Interestingly, Alkaloam treatment increased iron when applied to the surface but not when mixed in. This may be because contamination of the plant material with Alkaloam, which is very high in iron, was more likely to occur when the Alkaloam was on the surface of the soil.

Table 1: Mean (± SEM) concentrations (mg/kg dry weight) of minerals in capeweed grown in soil either treated with Alkaloam (applied to the surface or mixed through the soil, both at a rate of about 20 t/ha) or untreated (control).

	Treatment		
Element	**Control**	**Surface**	**Mixed**
Calcium	1.32 ± 0.03	1.12 ± 0.07	1.43 ± 0.04
Potassium	1.36 ± 0.03	1.23 ± 0.07	1.22 ± 0.03
Sulfur	0.14 ± 0.002	0.13 ± 0.001	0.14 ± 0.003
Phosphorus	0.1 ± 0.006”	0.324 ± 0.01”	0.224 ± 0.001^{b}
Sodium	1.14 ± 0.098	1.76 ± 0.138	1.52 ± 0.072
Magnesium	0.23 ± 0.002	0.19 ± 0.011	0.22 ± 0.004
Copper	39 ± 0.66	33 ± 1.61	35 ± 1.544
Zinc	247 ± 19.52”	127 ± 8.94^{b}	63 ± 4.17^{c}
Molybdenum	21 ± 0.87	23 ± 1.22	21 ± 1.42
Manganese	157 ± 8.23”	104 ± 4.01^{b}	41 ± 3.22”
Iron	82 ± 1.72”	149 ± 7.4^{b}	107 ± 5.3”
Aluminium	242 ± 18.9	374 ± 19.1	412 ± 63.6
Cadmium	44.7 ± 2.389^{a}	27.6 ± 1.452^{b}	15.6 ± 1.001”

Within rows, values with different superscripts are significantly different ($P < 0.05$).

The Mineral Status of Sheep Grazing on Pasture Grown on Soil Amended with Bauxite Residue

Outline

This experiment investigated the effect of amending soil with Alkaloam (at 20 t/ha) on the mineral status of sheep grazing capeweed dominant pastures over a period of 142 days. Blood samples taken during the experiment and tissue samples at the start and end of grazing were used to monitor mineral statuses.

Material and Methods

Experimental site

The site was located 230 km south of Perth on the Swan Coastal Plain. It was of uniform soil type (medium to fine acidic sand with a low capacity to retain nutrients) and had been capeweed dominant in the previous growing season. This site had been top dressed with selenium (Agsel®, Pitman-Moore, Bringelly, NSW), cobalt sulfate and copper sulfate at the rates of 1, 0.3 and 5 kg/ha, respectively, in May of the previous year. In early March 1995 the site was separated into 6 plots of 1 hectare each. Around the same time, 30 core soil samples (2 cm in diameter and 10 cm deep) were taken from each plot over a zigzag pattern and then pooled into a single sample. Soil pH ($CaCl_2$) and total cadmium concentrations were measured in these samples and were found to be uniform across the plots (overall mean ± SD of 4.5 ± 0.3 and 0.091 ± 0.008 mg/kg, respectively).

Treatments

Alkaloam (supplied from the Alcoa alumina refinery, Pinjarra, Western Australia) was applied at the rate of 20 tonnes/ha on the 24 March to 3 randomly selected plots. The remaining 3 plots are hereafter referred to as the "control plots". The break of season was in mid May, and soon after germination all the plots were sprayed with selective herbicides to ensure that capeweed was the dominant species.

Experimental Procedure

Weaner merino wethers (about 6 months old) and which had not been given selenium or cobalt pellets were used in the experiment. On day 0 (1 1/7/95, and 109 days since the application of the Alkaloam), 105 sheep were allocated by liveweight stratification to 7 groups (15 sheep per group). Six of these groups were drenched with an anthelmintic (Ivomec®, Merck Sharp & Dohme, Granville, NSW) and then one of each placed onto each of the 6 plots. Sheep in the seventh group were transported to South Perth and killed on day 2. These sheep were used to establish baseline mineral statuses, and hereafter are referred to as the "Baseline" group. The experiment ended on day 142 (30/1 1195) when feed was becoming limited. Over the experiment groups within treatments (amended or control) were rotated monthly across the plots to minimise plot effect.

Samples

Blood samples were collected into heparinised tubes from all sheep on day 0, and then from 10 randomly selected sheep on each plot on the following days; 37(17/8/95), 72 (21/9), 107 (26/10) and 142 (30111). All sheep were weighed and samples of capeweed taken from each of the plots on the same days that blood samples were taken. Capeweed samples consisted of grab samples which were taken every 10 to 15 paces (depending on the amount of growth) along a diagonal transect of the plot, and then pooled for the plot. Estimations of food on offer (FOO) were also made on the same days by the rising plate technique. Towards the end of the experiment FOO had fallen to a level too low (less than 500 kg/ha) to be accurately assessed. At day 101 (20/10/95) clean fleece weights were recorded in all sheep. At the end of the experiment, on day 142, all sheep were killed at a nearby abattoir and samples of liver and whole kidneys taken. Because the sheep were commercially processed there were restrictions on what samples could be collected. The same samples were also taken from the Baseline sheep. Soil samples were taken, as described above, from the plots at the end of the experiment.

Analyses

Concentrations of calcium and magnesium were measured in plasma using commercial kits (Trace Scientific, Clayton, Victoria). Plasma copper concentrations in plasma were determined by trichloroacetic acid treatment of plasma and then flame atomic absorption spectrophotometry. Red blood cells were assayed for glutathione peroxidase (GSHPx) activity using an automated modification of the method of Paynter *et al* (1985). Measurement of GSHPx activity is an effective means of determining selenium status in sheep (Paynter *et al* 1985). Concentrations of cadmium, copper, zinc, iron and manganese were measured in liver and kidney cortex samples by flame atomic absorption after wet ashing the sample in concentrated nitric and perchloric acids (12:3 v/v). Liver and capeweed selenium concentrations were measured using the method described by Koh and Benson (1983). Concentration of liver vitamin B 12 was determined by the method of Hutner *et al* (1956). Vitamin B 12 status in ruminants is directly dependent on the dietary intake of cobalt (Underwood 1977). Samples of capeweed were also analysed for cadmium, copper, zinc, iron, manganese, phosphorus, potassium, sodium, calcium, magnesium, sulfur and boron by inductively coupled plasma atomic emission spectroscopy after acid (nitric/perchloric) digestion (McQuaker *et al* 1979). Prior to all testing, capeweed samples were rinsed three times with distilled water and then dried at 80°C over 3 days. Capeweed molybdenum concentrations were determined colorimetrically by the method of Bingley (1963).

Statistical Analysis

Effects of the Alkaloam treatment on liveweight, the concentrations of plasma copper, calcium and magnesium, the activity of GSHPx in red cells, and the mineral concentrations in capeweed were analysed using repeated measures analysis of variance. In some cases means of mineral concentrations in capeweed at certain dates were compared by t-Test. Clean fleece weights and pasture FOO values were also compared by t-Test. Differences in all other means were determined by analysis of variance. Analyses were performed using the program Superanova (Abacus Concepts). In all tests probabilities of < 0.05 were considered significant.

Normal Ranges

Unless otherwise stated, the normal ranges cited are those routinely used by this laboratory. Ranges are listed in Appendix 2, along with maximum permissible concentrations.

Results and Discussion

Sheep Health and Pasture Growth

All sheep appeared healthy over the experiment, and all carcasses at the end of the experiment past abattoir inspection. There were no significant differences in liveweights between sheep grazing on the Alkaloam amended plots and those on the control plots over the experiment (Figure 1). The mean growth rate of sheep on the Alkaloam amended plots was 135 g/head/day and 142 g/head/day for sheep on the control plots. Sheep on the amended plots tended (P = 0.055) to have lower clean fleece weights (mean ± SEM; 3.9 ± 0.06 kg) compared to sheep on the control plots (4 ± ***0.06*** kg).

Mean (± SEM) FOO values for amended and control plots over the experiment are shown in Figure 2. There was evidence that Alkaloam retarded the growth of the capeweed as the control plots had significantly high FOO values at the start of the experiment and at day 37. However this difference became non-significant as the growth rate of the capeweed increased over the warmer months of September and October. Late rains in October ensured that FOO values remained high. However in November the capeweed went into senescence and FOO values rapidly fell to under 500 tonnes/ha by the end of the experiment.

Soil pH

At the end of the experiment, mean soil pH (CaC12) was 4.4 and 5.2 in the control and the amended plots, respectively. This pH increase was similar to that reported by Summers *et al* (1996).

Figure 1: Mean (± SEM) liveweights of sheep grazing on plots either amended or unamended with Alkaloam.

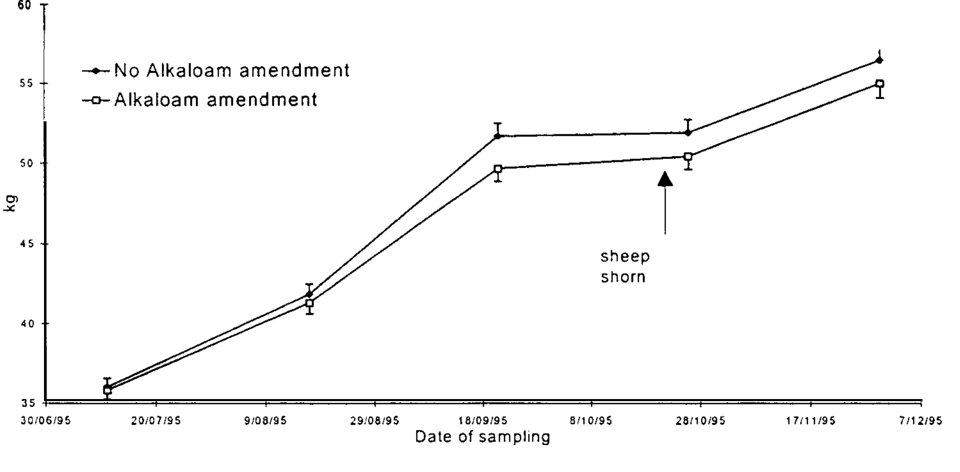
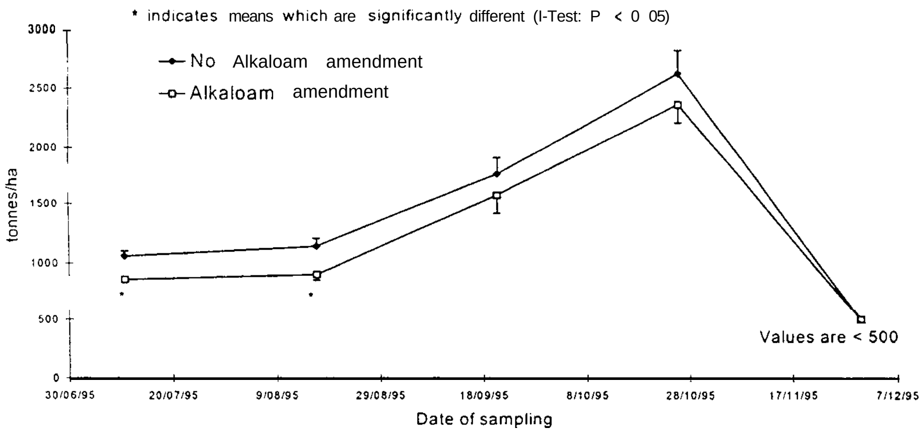


Figure 2: Mean (± SEM) Food on Offer (FOO) values for the Alkaloam amended and unamended plots over the experiment.



Macro-Minerals

Capeweed

Mean concentrations of macro-minerals in capeweed from the amended and control plots over the experiment are given in Table 1. Alkaloam amendment had no significant effect on phosphorus, calcium, magnesium or sulfur in capeweed, but it did significantly reduce potassium and increase sodium. The higher sodium is probably a reflection of the high sodium content of Alkaloam (Summers and Bradley 1993). Interestingly the potassium concentrations in capeweed grown on the amended plots stayed relatively constant over the experiment, whereas the concentration in capeweed grown on the control plots decreased. Generally phosphorus, calcium and sulfur requirements of sheep and cattle would be met by the concentrations of these elements in capeweed grown on either the amended or control plots. None of these minerals were in concentrations that would be considered excessive (Standing Committee on Agriculture 1990; Underwood 1981). Availability of magnesium to grazing animals is dependent on pasture potassium. The ratio of potassium/(calcium + magnesium) is a good predictor of the likelihood of magnesium deficiency (as expressed as grass tetany), with ratios less than 2.2 being desirable (Underwood 1981). Ratios in capeweed from the amended plots were less than 2 at all times, while ratios in the control plots were initially high (mean of 2.9) but decreased steadily over the experiment to a final mean value of 1.2. These values, together with the absolute magnesium concentrations, suggest that magnesium intake would be adequate (Underwood 198 1).

In agreement with these findings, Summers *et* al (1996) reported that Alkaloam had no effect on plant (clover and annual ryegrass) concentrations of calcium, magnesium or sulfur, as well as nitrogen. In contrast to results here they found no effect on potassium and a decrease in phosphorus. Effects on mineral composition reported here have been determined 109 days after the application of the Alkaloam and so earlier effects would have been missed.

Table 1: Mean (± SEM) concentrations (% on a dry weight basis) of phosphorus (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg) and sulfur (S) in capeweed grown on plots either amended or unamended with Alkaloam (at 20 t/ha).

		Date of Sampling (Day of Experiment)				
Mineral	**Alkaloam**	**11/7/95 (0)**	**17/8/95 (37)**	**21/9/95 (72)**	**26/10/95 (107)**	**30/11/95 (142)**
P	+	.38 ± .06	.42 ± .02	.39 ± .01	.28 ± .01	.18 ± .01
		.44 ± .02	.46 ± .02	.39 ± .02	.3 ± .01	.18 ± .005
K*	+	2.7 ± .55	2.8 ± .005	2.97 ± .07	2.90 ± .02	2.60 ± .21
		4.37 ± .20	3.8 ± .05	3.93 ± .12	3.63 ± .40	2.77 ± .14
Na*	+	2.13 ± .43	2.3 ± .08	2.27 ± .09	1.67 ± .03	1.2 ± .05
		1.83 ± .09	1.9 ± .09	1.67 ± .03	1.27 ± .17	1.13 ± .20
Ca	+	1.15 ± .09	1.06 ± .02	1.24 ± 05	1.5 ± .09	1.76 ± .14
		1.12 ± .03	1.09 ± .03	1.27 ± .02	1.6 ± .04	1.81 ± .07
Mg	+	.31 ± .01	.34 ± .02	.34 ± .01	.46 ± .02	.56 ± .04
		.34 ± ,005	.39 ± .02	.36 ± .01	.47 ± .03	.55 ± .02
S	+	.29 ± .02	.3 ± .02	.27 ± .02	.24 ± .02	.18 ± .01
		.31 ± .01	.3 ± .005	.24 ± .005	.21 ± .005	.18 ± .005

* Alkaloam amendment had significant effect as determined by repeated measures analysis

Calcium and Magnesium in Plasma

Plasma calcium and magnesium concentrations, in agreement with findings in the capeweed, were not affected by the application of Alkaloam (Figures 3 and 4, respectively). Furthermore all sheep maintained concentrations that indicated an adequate status of both minerals. The lower values recorded on day 0 for calcium and magnesium were most likely a result of transport stress and or feed restriction (Ellison 1994).

Figure 3: Mean (± SEM) concentrations of calcium in plasma from sheep grazing on plots either amended or unamended with Alkaloam.

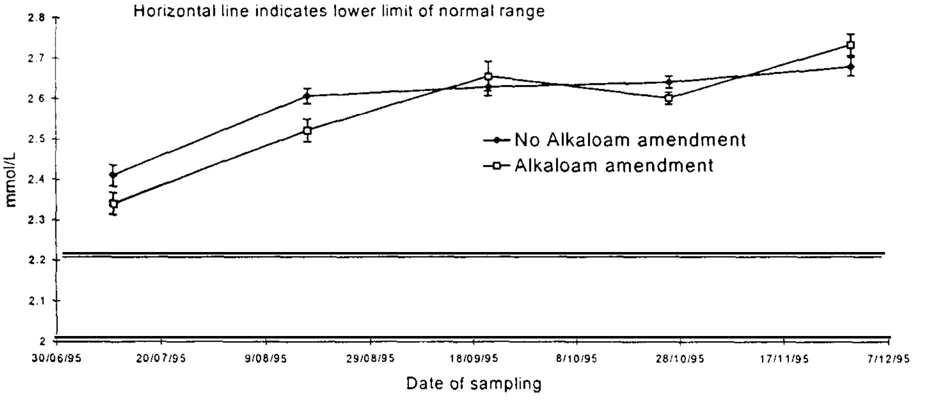
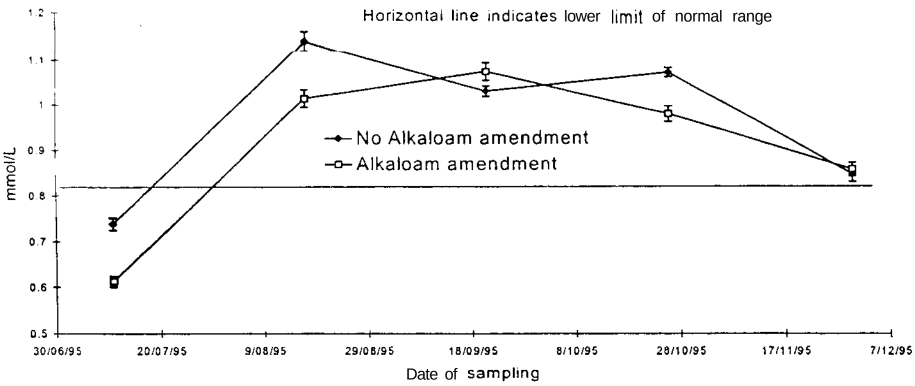


Figure 4: Mean (± SEM) concentrations of magnesium in plasma from sheep grazing on plots either amended or unamended with Alkaloam.

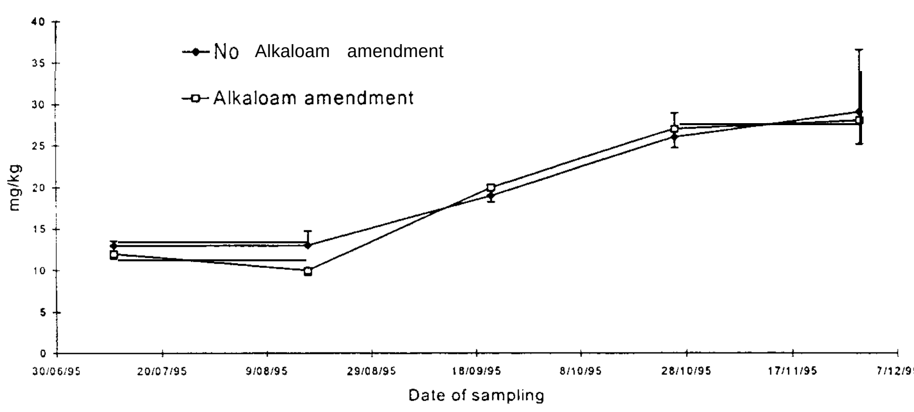


Trace Elements

Copper and Molybdenum in Capeweed

Alkaloam amendment had no significant effect on concentrations of copper in capeweed (Figure 5). This result is surprising since increasing the alkalinity of soil would be expected to decrease the availability of copper (Bernard and Ellis 1980; Summers *et al* 1996). The recent copper top dressing of the site may have masked any effect. Over the experiment copper concentrations increased markedly from around 12 mg/kg at the start, to around 28 mg/kg at the end of the experiment. Copper pasture concentrations are usually less than 10 mg/kg (Peter 1979), so these levels would be considered high. A likely explanation is the copper top dressing and the fact that capeweed is an effective accumulator of copper (Robson and Gartrell 1979).

Figure 5: Mean (± SEM) concentrations (mg/kg on dry weight basis) of copper in capeweed grown on plots either amended or unamended with Alkaloam.



Over most of the experiment molybdenum concentrations were far higher in the capeweed from the amended plots (Figure 6), with concentrations highest at the start of the experiment and decreasing steadily thereafter. Concentrations in capeweed from the control plots changed little over the experiment (Figure 6). Because it reduces the availability of copper, dietary molybdenum is an important determinant of copper

status in grazing ruminants (Underwood 1981). For this reason the adequacy of dietary copper is best described in terms of the dietary copper:molybdenum ratio. Blood and Radostits (1989) have suggested that the minimum ratio in animal feeds should be 2: 1. Mean ratios in capeweed from the amended and control plots are shown in Figure 7. Up to day 37 (17/8/95) the ratio in capeweed from the amended plots was close to 2, but increased as capeweed copper increased and molybdenum decreased. These ratio values would suggest that copper deficiency was unlikely. However without the recent copper top dressing the probability of deficiency would have been high. In contrast, sheep on the control plots might have been at risk from copper toxicity. For example long-term ingestion of pastures with 10-15 mg/ kg copper and less than 0.2 mg/kg molybdenum can lead to copper toxicity. (Howell and Gooneratne 1987). Excess molybdenum intake can also directly lead to health effects (Phillippo *et al* 1987), but this can be prevented to some degree by increasing the level of dietary copper which reduces the amount of molybdenum absorbed.

Molybdenum concentrations were measured again in October 1996. Mean concentration in capeweed from the amended plots was 2.30 mg/kg, compared to 0.95 mg/kg in the control plots. This would indicate that Alkaloam was still having a similar effect on capeweed molybdenum. Copper concentrations on the same samples were 30 mg/kg and 26 mg/kg, respectively, and so similar to concentrations measured in the previous year.

Figure 6: Mean (± SEM) concentrations (mg/kg on dry weight basis) of molybdenum in capeweed grown on plots either amended or unamended with Alkaloam.

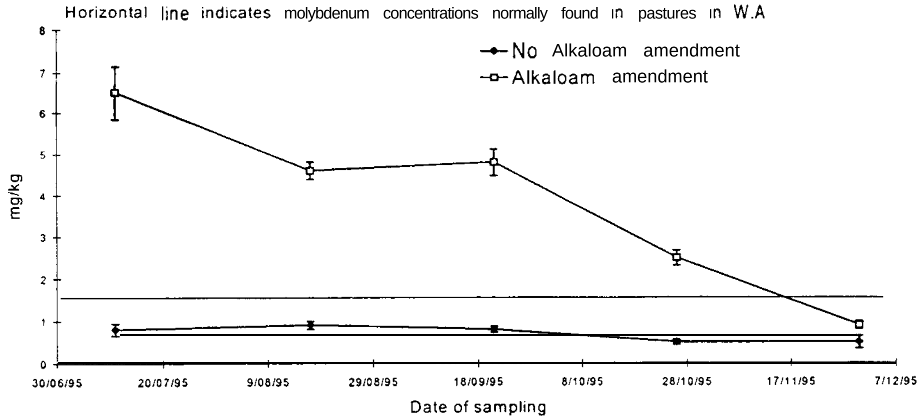
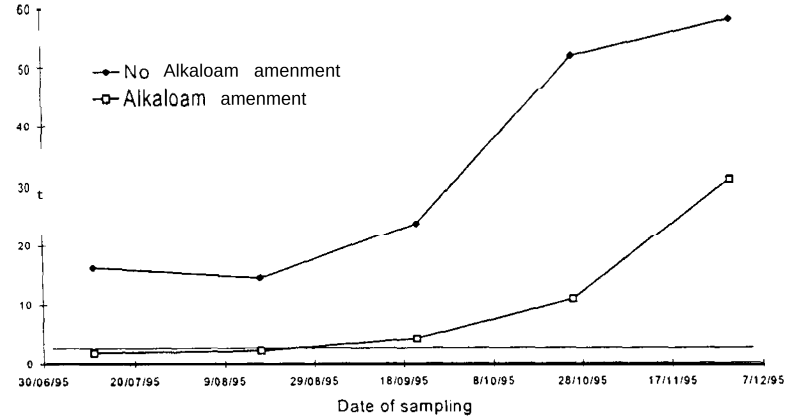


Figure 7: Ratio of copper to molybdenum in capeweed grown on plots either amended or unamended with Alkaloam.

The horizontal line indicates the recommended minimum dietary ratio of copper to molybdenum.

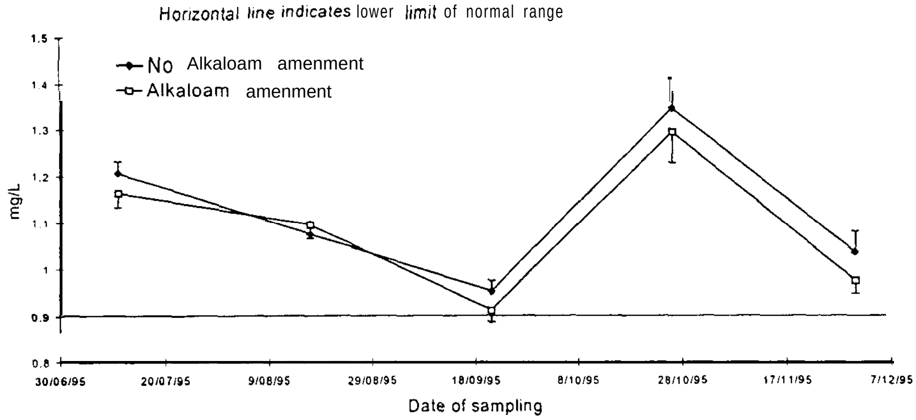


Copper in Plasma

Plasma copper concentrations were unaffected by Alkaloam amendment, and indicated that all sheep had adequate copper status over the experiment (Figure 8).

Figure 8: Mean (± SEM) concentrations of copper in plasma from sheep grazing on plots either amended or unamended with Alkaloam.

Horizontal line indicates lower limit of normal range



Copper in Liver and Kidney

Liver copper concentration increased in all sheep over the experiment, however concentrations were significantly less in sheep grazing on the amended plots (Table 2). The lower concentrations in these sheep was most likely due to the much lower dietary copper:molybdenum ratios (Figure 7). While copper status was adequate in all sheep (liver concentrations greater than 30 mg/kg dry weight), the mean concentrations in sheep on the control plots may have been excessively high. For example concentrations greater than 400 mg/kg dry weight have been associated with the early stages of copper toxicity in sheep (Suttle 1995).

Alkaloam also reduced kidney copper concentrations, but by very little (Table 3). All concentrations fell within the normal range in all sheep (Table 3). Copper preferentially accumulates in the liver so this result is not unexpected (McC Howell 1979). There was no change in kidney copper in the control group over the experiment and only a small decrease in sheep on the amended plots (Table 3).

Table 2: Mean (± SEM) concentrations of copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), cadmium (Cd), selenium (Se) and vitamin B12 (cobalt) in livers of sheep killed on day 0 (Baseline group), or which have grazed for 142 days on capeweed grown on plots either amended or unamended with Alkaloam (at 20 t/ha). Concentrations are expressed as mg/kg dry weight, except for Cd and vitamin B12 which are expressed on a wet weight basis. Normal ranges and Australian maximum permissible concentrations are given in appendix 2.

	Liver Element						
Alkaloam	**Cu**	**Zn**	**Fe**	**Mn**	**Cd**	**Se**	**B12**
20 t/ha	229 ± 15 [a]	144 ± 4 [a]	303 ± 12 [a]	9 ± 0.21 [a]	0.46 ± .02 [a]	1.79 ± .04 [a]	1.12 ± .02
none	412 ± 19 [b]	144 ± 3 [a]	297 ± 15 [a]	10 ± 0.24 [a]	0.59 ± .02 [a]	0.78 ± .02 [b]	1.11 ± .03
Baseline group	141 ± 16 [c]	115 ± 4 [b]	224 ± 18 [b]	6 ± 0.41 [b]	0.17 ± .02 [b]	0.38 ± .03 [c]	**0.94** ± .06

Within an element, values with a different superscript are significantly different ($P < 0.05$)

Table 3: Mean (± SEM) concentration of copper (Cu), zinc (Zn), iron (Fe) and cadmium (Cd) in kidneys of sheep which have grazed for 142 days on capeweed grown on plots either amended or unamended with Alkaloam (at 20 t/ha). Concentrations are expressed as mg/kg dry weight, except Cd which is expressed as mg/kg wet weight. Normal ranges and Australian maximum permissible concentrations are given in appendix 2.

Alkaloam	**Element**			
	Cu	**Zn**	**Fe**	**Cd**
20 t/ha	17 ± 0.4 [a]	148 ± 4 [a]	664 ± 197	1.06 ± .04 [a]
none	19 ± 0.4 [b]	164 ± 4 [b]	656 ± 160	1.22 ± .05 [b]
Baseline group	19 ± 0.9 [b]	133 ± 7 [a]	242 ± 16	0.5 ± 0.04'

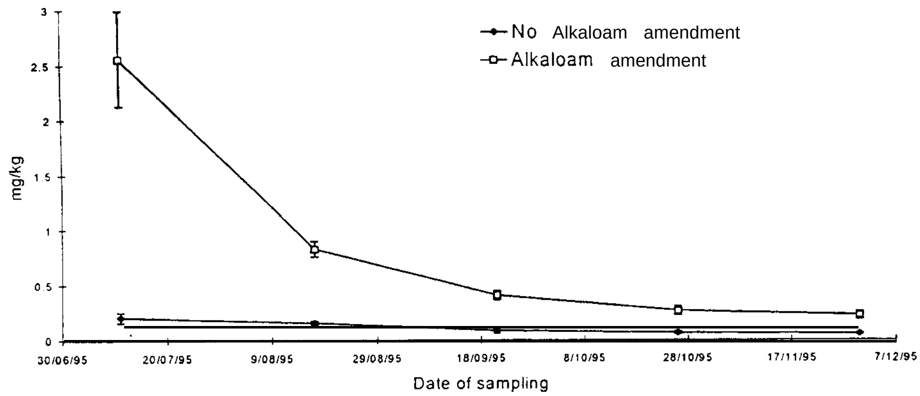
Within an element, values with a different superscript are significantly different ($P < 0.05$)

Selenium in Capeweed

Alkaloam amendment produced much higher selenium concentrations in the capeweed, however concentrations decreased steadily over the trial (Figure 9). It is most likely that the alkalinity of Alkaloam, together with the fact that the site had been recently top dressed with a selenium fertiliser were responsible for the much higher concentrations (Flemin g 1980b). Selenium is toxic, but these concentrations

would be unlikely to be harmful since levels of more than 5 mg/kg in the diet must be sustained for long periods before signs of toxicity appear (Peter 1990).

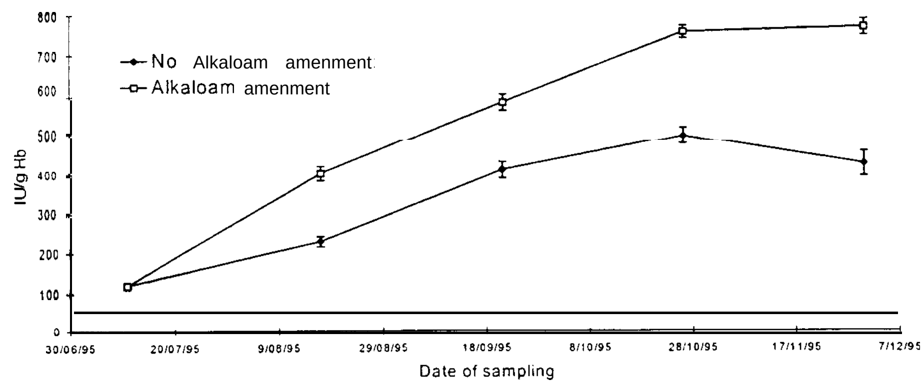
Figure 9: Mean (± SEM) concentrations (mg/kg on dry weight basis) of selenium in capeweed grown on plots either amended or unamended with Alkaloam.



Glutathione Peroxidase

Activities of GSHPx in red blood cells increased in sheep on the Alkaloam amended and control plots over most of the experiment, but, as would be expected, activities were significantly higher in sheep on the amended plots (Figure 10). In all sheep GSHPx activities indicated an adequate selenium status over the trial.

Figure 10: Mean (± SEM) red cell glutathione peroxidase activities in sheep grazing on plots either amended or unamended with Alkaloam.
Horizontal line indicates lower limit of normal range



Selenium in Liver

In agreement with GSHPx activities, selenium concentration in the liver increased in all sheep over the experiment (Table 2), with higher concentrations in sheep grazing on the Alkaloam amended plots. Concentrations in these sheep (mean of 0.63 mg/kg; adjusted to wet weight) were still less than the Australian maximum permitted concentration (MPC) for offal of 2.0 mgikg wet weight (Branford 1997).

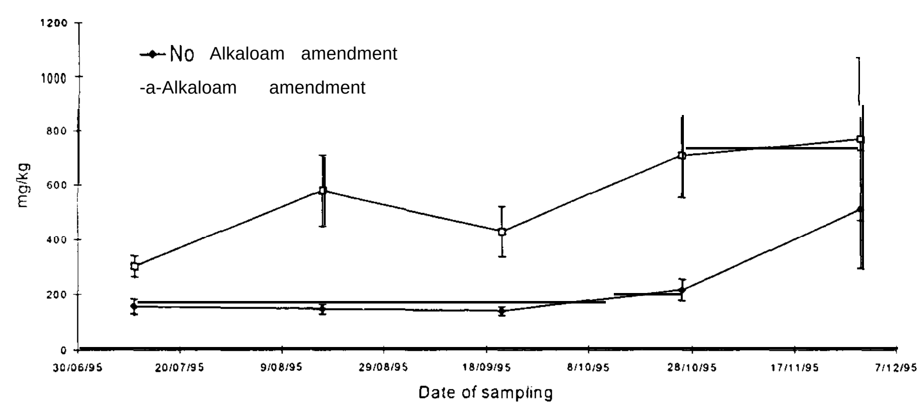
Iron in Capeweed

Capeweed grown on the amended soil had significantly higher iron concentrations, which tended to increase over the experiment (Figure 11). The availability of iron

would be expected to be reduced by Alkaloam amendment because it increases the alkalinity of the soil (Bernard and Ellis 1980). There is a possibility that surface contamination by the Alkaloam, which has a very high iron content (Summers and Bradley 1993), may have been a factor, even though samples were washed prior to testing. Iron requirements for sheep and cattle (a dietary level of 40 mg/kg) would have been met by capeweed grown on either the amended or control plots (Underwood 1981). In fact the iron concentrations may have been sufficiently high to affect animal health. Concentrations as low as 250 mg/kg (on dry weight basis) have caused copper depletion in ruminants (Bremner et *al* 1987). Excessive iron intake (greater than 1,000 mg/kg on dry weight basis) can cause clinical signs such as diarrhoea, acidosis, reduced feed intake and liveweight gain (National Research Council 1996). Iron present in Alkaloam is in the largely insoluble form of ferric oxide (Fe_2O_3) which probably has poor availability to the ruminant (Underwood 1977). For this reason its toxicity should be low, however it has been shown to inhibit copper absorption when added to the diet of sheep (Suttle and Peter 1985).

It may be that molybdenum provided the dominant pressure on copper status in the first half of the experiment and iron in the second. The decrease in plasma copper in the last collection, in spite of the increasing capeweed copper, would support this. In contrast to the results here Summers *et al* (1996) reported no effect on pasture plant iron.

Figure 11: Mean (± SEM) concentrations (mg/kg on dry weight basis) of iron in capeweed grown on plots either amended or unamended with Alkaloam.



Iron in Liver and Kidney

The higher iron levels in capeweed on the Alkaloam amended plots were not reflected in the liver concentrations (Table 2). This would suggest that the iron was possibly present as a poorly available ferric oxide contaminant (Underwood 1977). Liver iron increased in sheep on both the control and amended plots over the experiment.

High intakes of cobalt, zinc, cadmium, copper and manganese can all reduce iron uptake by ruminants, however none of these was apparently elevated. Kidney iron was also unaffected by Alkaloam amendment (Table 3), and concentrations in liver and kidney both fell within the normal ranges. The range in kidney iron in all sheep was very large (Table 3). As a result iron concentrations in the kidneys from the basal sheep, while appearing lower, were not significantly different from sheep in the other two groups (Table 3).

Vitamin B12 (Cobalt) in Liver

The main function of cobalt in ruminants is the formation of vitamin B12, and so low dietary levels of cobalt leads to vitamin B12 deficiency. Alkaloam treatment did not affect liver concentrations of vitamin B12 (Table 2). The availability of cobalt would be expected to be relatively poor from the amended plots because of the increased alkalinity (Smith 1990). However recent top dressing with cobalt might have masked any effect. Based on liver vitamin B12 concentrations, all sheep were adequate in this vitamin.

Zinc in Capeweed

There was no effect of Alkaloam amendment on zinc concentration in capeweed. Mean concentrations remained close to 40 mg/kg over most of the experiment, which would meet growth and reproductive requirements (dietary levels of 20 mg/kg) of sheep and cattle (Underwood 1977). In contrast to the results here Summers *et al* (1996) reported a decrease in pasture plant zinc.

Zinc in Liver and Kidney

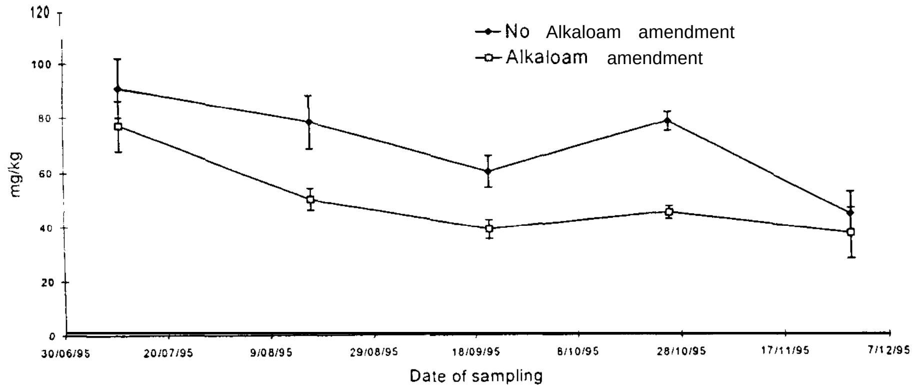
Alkaloam amendment had no significant effect on zinc concentrations in liver (Table 2). Liver zinc increased in all sheep over the experiment, and all concentrations were within the normal range.

In contrast, Alkaloam reduced kidney zinc however all concentrations still fell within the normal range (Table 3). Sheep on the control plots increased in kidney zinc over the trial, whereas there was no such increase in the sheep on the amended plots (Table 3).

Manganese in Capeweed

Amendment of plots with Alkaloam significantly decreased the concentration of manganese in capeweed (Figure 12), and this was most likely a result of increased alkalinity (Bernard and Boyd 1980). However concentrations were still more than adequate to meet the requirements (dietary levels of 20-25 mg/kg) of sheep and cattle (Masters 1990). Summers *et al* (1996) also reported a decrease in pasture plant manganese with Alkaloam treatment, and that concentrations fell to below 20-25 mg/kg. When capeweed samples were taken in the following year (October 1996), mean manganese concentration in capeweed plants from the Alkaloam amended plots was 18 mg/kg, and 49 mg/kg in the control plots, suggesting that the Alkaloam might decrease manganese concentrations to inadequate levels for sheep and cattle in the longer term.

Figure 12: Mean (± SEM) concentrations (mg/kg on dry weight basis) of manganese in capeweed grown on plots either amended or unamended with Alkaloam.



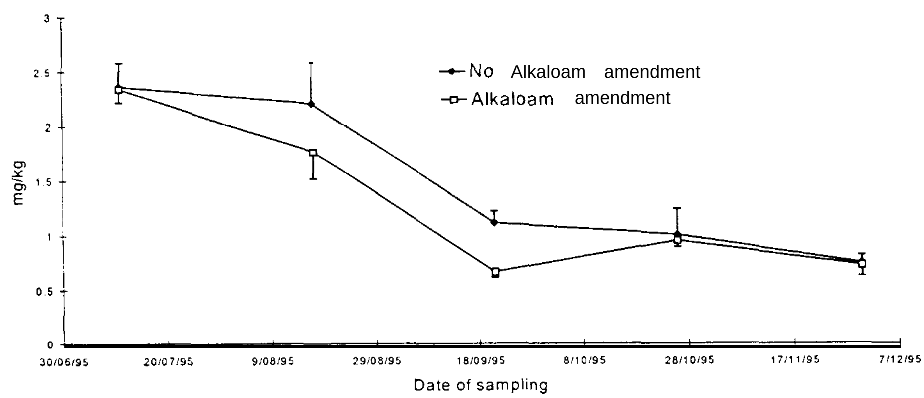
Manganese in Liver

All sheep increased in manganese status, as measured by liver manganese concentration, over the experiment (Table 2). Differences in capeweed manganese content were not reflected in the liver concentrations, probably because manganese was present in amounts excessive to requirements. All liver concentrations fell within the normal range.

Cadmium in Capeweed

Overall Alkaloam had no significant effect on cadmium concentration in capeweed, although on day 72 (21/9) cadmium concentrations were significantly lower (t-Test, P = 0.001) (Figure 13). Concentrations of cadmium in capeweed reported here are similar to those reported elsewhere (Tracey 1991). In contrast Summers *et al (1996)* reported that cadmium concentrations in clover/rye grass pasture fell with Alkaloam amendment.

Figure 13: Mean (± SEM) concentrations (mg/kg on dry weight basis) of cadmium in capeweed grown on plots either amended or unamended with Alkaloam.



Cadmium in Liver and Kidney

Sheep grazing on the amended plots had significantly lower kidney cadmium concentrations (Table 3) and tended to have lower liver cadmium concentrations (P =

0.077; Table 2). One possible explanation is that the higher molybdenum intake by the sheep on the amended plots may have reduced the absorption of cadmium (Smith and White 1997). All concentrations in liver and kidney were below the Australian MPCs for this heavy metal (1.25 mg/kg and 2.5 mg/kg wet weight, respectively). These MPC values are based on whole kidney whereas in this experiment kidney cortex was analysed. Adjusting the values here to whole kidney cadmium concentration would reduce them by about 33% (Masters and Petterson 1991). Sheep on all plots accumulated cadmium over the experiment (Tables 2 and 3). Morcombe et ***al*** (1994) identified capeweed in pasture as a risk factor for elevated tissue cadmium levels in sheep in Western Australia.

Boron in Capeweed

The requirement of pasture plants for boron has been well established, but only recently has it been identified as an essential mineral for animals, with an important role in the maintenance of normal bones and hormone function (Nielsen 1990). Requirements and normal ranges in ruminants have not yet been established. Alkaloam had no influence on boron concentration in capeweed, (which remained between 33 and 38 mg/kg on all plots over the experiment) even though alkalinity is usually associated with reduced availability, especially in the presence of calcium (Fleming 1990a).

Conclusions

Rehabilitation Area study

1. Sheep grazing for about a year on pasture on the Rehabilitation Area at the Pinjarra refinery maintained good health and grew well.

2. The statuses of essential minerals in sheep were adequate, taking into account fluctuations in mineral status that occur naturally under a Mediterranean climate.

3. Low vitamin B 12 status did develop but only in sheep on the Irrigated site. This was most likely due to several factors. Firstly pasture cobalt levels were low. This would have resulted from the rapid plant growth and probably because of poor availability of cobalt to the pasture (due to the high soil pH and iron content). Secondly, involuntary soil intake would have been minimal because pasture growth was so luxurious. Soil ingestion provides a good a source of cobalt for ruminants, as was probably the case with sheep on the Dryland site. The cobalt content of pastures grown on bauxite residue warrants further investigation. Cobalt deficiency is not uncommon and can be readily treated by either top dressing pasture with cobalt salts or the use of intraruminal cobalt pellets (Underwood 1977).

4. There was no evidence of contamination of lung tissue with minerals, even in sheep on the Dryland site.

5. In the minerals investigated, there was no evidence of accumulation of minerals to harmful levels in tissues or to levels that exceeded maximum permissible concentrations.

6. Sheep on the Dryland site did have higher bone fluorine concentrations than sheep on the Irrigated site. However the levels would still be considered normal and are far below toxic levels. In addition they were similar to levels in sheep on the Farmland site. The possibility of an increase in fluorine intake, albeit small, through dust contamination should be considered in future monitoring.

7. Molybdenum levels were high in pastures. This is most likely due to a pH effect since the level of molybdenum in the residue is comparatively low (Introduction; Table 1).

    - A high molybdenum intake from pastures on Rehabilitation Areas could cause health effects in grazing animals either directly or through induced copper deficiency. Practical means of field control involve top dressing pasture with copper salts such as copper sulphate. Copper top dressing is a common and economical practice for maintaining adequate copper status in grazing ruminants (Underwood 1977). Agriculture Western Australia recommends that one application of 400 kg/ha of 0.6% copper and 0.6% zinc super-phosphate will supply adequate copper for at least 20 years (Gartrell 1994). However because of the elevated molybdenum levels a specific recommendation for rehabilitated areas would need to be developed.

    - Because of this increased availability, molybdenum levels in the rehabilitation soil may become exhausted over several years, leading to poor pasture growth

and an increased possibility of copper toxicity. This would impact on recommendations on copper supplementation of livestock.

- Increased molybdenum availability will stimulate nitrogen fixation, and so growth of pasture plants such as clover.

8. Levels of iron in plants were high, probably, to a large degree, because of surface contamination. This may lead to health problems either directly or through induction of copper deficiency. It is suspected, based on tissue iron levels, that the iron as a contaminant is poorly available, but this should be verified.

Alkaloam studies

Pot study

Alkaloam in the pot study decreased manganese and increased iron content in capeweed, which was also seen in capeweed grown on the amended plots in the field study. However in contrast to the field study, zinc and cadmium were decreased and molybdenum unchanged in capeweed grown in the Alkaloam treated pots. These differences between the Alkaloam pot and field studies are most likely due to the fact that pH changes caused by the Alkaloam in the pot study happened over an already very alkaline range. For example, the pH effect on availability of molybdenum might already have been at a maximum prior to the Alkaloam treatment (Introduction; Figures 1 and 2).

Field study

1. Sheep grazing for about 140 days on capeweed pasture amended with Alkaloam (20 tonnes/ha) maintained good health and grew well.

2. There were no trace mineral deficiencies or toxicities observed in the sheep.

3. The greatly increased availability of selenium with Alkaloam amendment means that the application rates of selenium fertilisers, and the use of other selenium supplements, would need to be reassessed, especially in terms of violative tissue levels. This would very likely apply to the Rehabilitation Area as well. Selenium supplements are often given to grazing ruminants so this effect may be beneficial.

4. Alkaloam treatment decreased the concentration of manganese in capeweed. The manganese status in ruminants grazing Alkaloam amended pasture may need to be monitored. Monitoring of manganese levels in different pasture types on the Rehabilitation Area should also be carried out.

5. As with the rehabilitation site, pasture molybdenum concentrations were increased by Alkaloam amendment. As discussed above this has possible implications for animal health.

6. As with the rehabilitation site, pasture iron was increased, either directly or through contamination. As discussed above this has possible implications for animal health.

Influence of soil type on Alkaloam effects

Increases in soil alkalinity by Alkaloam is probably the dominant factor influencing the availability of minerals to pasture plants. The effect of Alkaloam amendment on

the mineral status of grazing sheep will therefore be greatly influenced by soil characteristics such as pH, buffering capacity and level of organic matter (Introduction; Figures 1 and 2). The differences between the results of the capeweed pot study and the Alkaloam field study, as well as the findings of Summers *et al* (1996), exemplifies this. For the above reasons, effects on mineral content of pasture plants would be expected to be far more consistent on rehabilitation sites.

Overview of Studies

Because of copper top dressing, copper pasture levels were high in both the Rehabilitation Area and Alkaloam field studies. However Alkaloam amendment has been shown to reduce copper and zinc status of pasture plants (Summers *et al 1996).* It would therefore be expected that copper concentrations in pasture grown on a Rehabilitation Area, in the absence of copper supplementation, would be comparatively low to that of many agricultural soils. This, combined with the high pasture molybdenuin and iron levels observed in these studies, could precipitate a copper deficiency and or molybdenosis in grazing animals. This is the most serious concern related to the use of rehabilitated areas, as well as Alkaloam. There is a need to develop a copper supplementation strategy for sheep and cattle under such circumstances.

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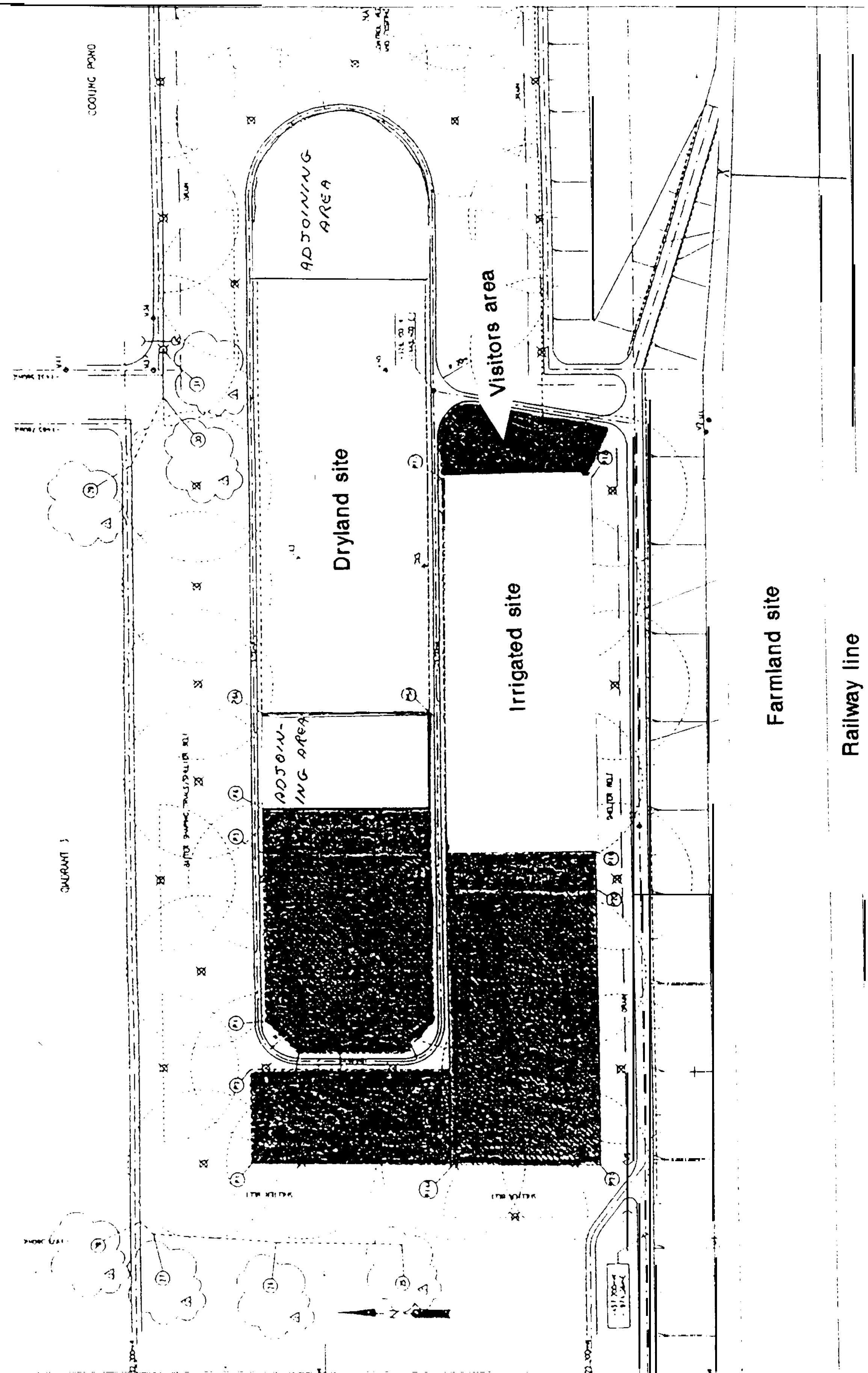
Appendix 1.

Plan of Land Uses : Residue Rehabilitation Area 1



APPENDIX 2:

Normal Ranges for minerals routinely used at the Animal Health laboratories, Agriculture Western Australia.

Plasma:

Calcium	2.2-3.0 mmol/L
Magnesium	0.82-1.44 mmol/L
Phosphorus	0.9-2.5 mmol/L
Copper	0.9-1.4 mg/l
Zinc	> 0.6 mg/L
Red Blood Cell Glutathione Peroxidase (GSHPx)	> 50 IU/g Hb

Liver*.

Copper	30-700 mg/kg
Zinc	100-200 mg/kg
Iron	200-800 mg/kg
Selenium	0.5-3.0 mg/kg
Vitamin B 12	> 0.4 ug/g

Kidnev

Copper	15-25 mg/kg
Zinc	100-180 mg/kg
Iron	< 1000 mg/kg

Australian Maximum Permissible Concentrations (wet weight): (Branford 1997)

Arsenic	1.0mg/kg
Chromium	none proclaimed
Copper	200 mg/kg in ovine liver (about 610 mg/kg dry weight)
Selenium	2.0 mg/kg in offal (about 6 mg/kg dry weight)

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