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**THE INFLUENCE OF ZINC AND COPPER FERTILIZER
APPLICATION ON ZINC, COPPER AND CADMIUM
CONCENTRATION IN MIXED PASTURE**

**A Thesis presented in partial fulfillment of the requirements for
the degree of Master of Applied Science in Soil Science at
Massey University, Palmerston North, New Zealand.**

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ABSTRACT

There has been considerable debate about the accumulation of cadmium (Cd) in agricultural soils and its subsequent uptake by pasture plants due to phosphate fertilizer application. Ruminants grazing pastures absorb a small fraction of this Cd, and some of this is subsequently accumulated in the liver and kidney. Although tissue accumulation of Cd in grazing livestock is generally small ($< 1 \text{ mg Cd kg}^{-1}$ fresh tissue), but any reduction in plant uptake is beneficial in reducing such accumulation further, especially in the kidneys. Uptake of Cd by pasture may be affected by the concentration of other nutrient cations, such as zinc (Zn) and copper (Cu). In addition, since Zn and Cu are complexed by the same metal binding protein (metallothionein) as Cd, a change in the ratio of these nutrients in pasture may also reduce Cd accumulation rates by interfering with Cd accumulation.

In order to assess the effects of Zn and Cu on Cd uptake by pasture, a field experiment was conducted, using three pairs of pasture plots with low ($0.2 \text{ mg Cd kg}^{-1}$) and high ($0.6 \text{ mg Cd kg}^{-1}$) background Cd status. Twelve sub-plots (1.44 m^2) were laid out in each plot and increasing levels of Zn (0, 5, 15 and 40 kg ha^{-1}) and Cu (0, 2, 5 and 10 kg ha^{-1}) were added as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ and $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ respectively. Pasture samples were collected at regular intervals and analysed for dry matter yield, botanical composition and Zn, Cu and Cd uptake. Soil samples were extracted with 0.01M CaCl_2 and 0.1M HCl solution to measure the plant available Zn, Cu and Cd.

It was found that the plots with a high background Cd status in the soil resulted in a higher Cd concentration in mixed pasture ($0.22 \text{ mg Cd kg}^{-1} \text{ DM}$) than those with a low background Cd status ($0.10 \text{ mg Cd kg}^{-1} \text{ DM}$) at the first harvest (after 73 days). The Cd concentration in the mixed pasture was higher during the summer (December) period than in the early spring (September).

Application of Zn fertilizer increased the Zn concentration in pasture from 37 to $150 \text{ mg kg}^{-1} \text{ DM}$ at the first harvest. Excessive amounts of Zn lead to a decrease in DM yield. The growth of pasture was controlled principally by the amount of plant available Zn,

which depended on the amount of both added Zn and added Cu. The effect of the added Cu was to increase the toxicity of the added Zn.

Application Cu fertilizer increased the Cu levels from 9 to 16 mg kg⁻¹ DM at the first harvest. The Cu concentration in pasture continued to decrease with time following the addition of fertilizers. The legumes are more tolerant of Cu than grass. The Cu concentration in harvest 4 (after 159 days) ranged from 6.9 to 7.0 mg kg⁻¹ DM in grass and 8.9 to 9.9 mg kg⁻¹ DM in legumes.

The Cd concentration in the pasture decreased with increasing Zn concentration in the pasture at the first harvest. The effect of Zn on Cd uptake was more pronounced on plots with a high background Cd status in the soil. The effect of Zn on Cd concentration depends on the external Zn concentration levels.

There was no consistent effect of Cu concentration on Cd concentration. The effect of the addition of Cu and Zn in fertilizer was to lower the Cd:Cu and Cd:Zn ratios in the herbage.

There was a good relationship between soil available Zn as extracted by 0.1M HCl and Zn concentration in the herbage. A similar observation was obtained for Cu. But there was no consistent relationship between 0.01M CaCl₂ extractable Cd and the Cd concentration in pasture.

The results indicated that pasture and soil analysis for Cd and Zn may provide useful guides to situations where Cd concentrations in pasture may be decreased by Zn applications.

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TO MY

BELOVED PARENTS

TABLE OF CONTENTS

	Page
ABSTRACT	i
ACKNOWLEDGMENTS	iii
TABLE OF CONTENTS	v
LIST OF TABLES	xi
LIST OF FIGURES	xii
LIST OF APPENDICES	xiv
 CHAPTER ONE: 	
INTRODUCTION	
1.1 Background	1
1.2 Objective	2
 CHAPTER TWO: 	
REVIEW OF LITERATURE	
2.1 Introduction	3
2.2 Sources of cadmium	3
2.2.1 Cadmium in fertilizer	4
2.2.2 Cadmium in sewage sludge	6
2.3 Availability of soil cadmium	7
2.4 Cadmium in plants	8
2.5 Cadmium in farm animals	10

2.6	Factors influencing the accumulation of Cd in plants, soil and animals	12
2.6.1	Plant uptake of fertilizer Cd from soil	12
2.6.1.1	Time of contact and form of Cd	12
2.6.1.2	Effect of fertilizer application	13
2.6.1.3	CEC and soil organic matter effects	15
2.6.1.4	Effect of Zn and Cu on Cd concentration in plants, soil and animals	17
2.6.1.4.1	Zinc and Cd interactions	17
2.6.1.4.2	Copper and Cd interaction	19
2.6.1.4.3	Copper and Zn interaction	19
2.6.1.5	Effect of soil pH and liming on Cd concentration	20
2.7	Effect of plant species on Cd concentration	23
2.8	Factors that influence the uptake of Cd in animals and humans	24
2.9	Seasonal influences on DM yield, concentration of Zn, Cu and Cd	26
2.9.1	Effect on growth rate, botanical composition and DM yield	26
2.9.2	Effect on Cu concentration	28
2.9.3	Effect on Zn concentration	29
2.9.4	Effect on Cd concentration	30
2.10	Effect of Zn and Cu fertilizers on Zn and Cu concentration	30
2.10.1	Effect on Cu concentration	30
2.10.2	Effect on Zn concentration	32
2.11	Toxicity effect	33
2.11.1	Toxicity effect of Cu	33
2.11.2	Toxicity effect of Zn	33

CHAPTER THREE:
MATERIALS AND METHODS

3.1	Location and fertilizer management	35
3.2	Fertilizer treatment	37
3.3	Field preparation	37
3.4	Experimental design	38
3.5	Fertilizer rate and application	38
3.6	Collection of soil and pasture samples	39
	3.6.1 Soil sampling	39
	3.6.2 Pasture sampling	39
3.7	Botanical composition	40
3.8	Soil and herbage sample preparation	40
	3.8.1 Initial soil for chemical analysis	40
	3.8.2 Soil samples for pseudo-total Cd analysis	40
	3.8.3 Soil samples for available Zn, Cu and Cd analysis	41
	3.8.3.1 0.01M CaCl ₂	41
	3.8.3.2 0.1M HCl	41
	3.8.4 Pasture samples for Zn, Cu and Cd analysis	41
3.9	Chemical analysis	42
3.10	Statistical analysis of analytical data	42

CHAPTER FOUR:
RESULTS AND DISCUSSION

4.1	Reliability of the data in pasture and soil samples	43
4.2	Initial chemical properties	44
4.2.1	Initial soil chemical properties	44
4.2.2	Initial pasture chemical composition	46
4.3	Dry matter yield of pasture	47
4.3.1	Dry matter yield of mixed pasture	47
4.3.1.1	Effect of treatment	47
4.3.1.2	Effect of Cu level	49
4.3.1.3	Effect of Zn level	51
4.3.1.4	Effect of background Cd	52
4.3.2	Dry matter yield of pasture species	53
4.3.2.1	Effect of treatment	53
4.3.2.2	Effect of Cu level	53
4.3.2.3	Effect of Zn level	54
4.3.2.4	Effect of background Cd	56
4.4	Pasture growth rate	57
4.4.1	Growth rates in mixed pasture	57
4.4.1.1	Effect of treatment	57
4.4.1.2	Effect of Cu level	58
4.4.1.3	Effect of Zn level	58
4.4.1.4	Effect of background Cd	61

4.4.2	Growth rates of pasture species	61
4.4.2.1	Effect of treatment	61
4.4.2.2	Effect of Cu level	62
4.4.2.3	Effect of Zn level	63
4.4.2.4	Effect of background Cd	64
4.5	Botanical composition	65
4.6	Copper content	67
4.6.1	Copper content in mixed pasture	67
4.6.1.1	Effect of treatment	67
4.6.1.2	Effect of Cu level	68
4.6.1.3	Effect of Zn level	70
4.6.1.4	Effect of background Cd	71
4.6.2	Copper content in pasture species	72
4.6.2.1	Effect of treatment	72
4.6.2.2	Effect of Cu level	72
4.6.2.3	Effect of Zn level	74
4.6.2.4	Effect of background Cd	75
4.7	Zinc content	75
4.7.1	Zinc content in mixed pasture	75
4.7.1.1	Effect of treatment	75
4.7.1.2	Effect of Cu level	75
4.7.1.3	Effect of Zn level	76
4.7.1.4	Effect of background Cd	77

4.8	Cadmium content	78
4.8.1	Cadmium content in mixed pasture	78
4.8.1.1	Effect of treatment	78
4.8.1.2	Effect of Cu level	79
4.8.1.3	Effect of Zn level	82
4.8.1.4	Effect of background Cd	87
4.8.2	Cadmium content in pasture species	88
4.8.2.1	Effect of treatment	88
4.8.2.2	Effect of Cu level	89
4.8.2.3	Effect of Zn level	90
4.8.2.4	Effect of background Cd	91
4.9	Seasonal influences on dry matter yield, Cu, Zn and Cd concentrations in pasture	92
4.9.1	Effect on DM yield	92
4.9.2	Effect on Cu	93
4.9.3	Effect on Zn	94
4.9.4	Effect on Cd	95
4.10	Available Cu, Zn and Cd content in soil	96
4.10.1	Available Cu	96
4.10.2	Available Zn	99
4.10.3	Available Cd	100
4.11	Total Cd concentration in soil	104
	CONCLUSIONS	105
	REFERENCES	108
	APPENDICES	128

LIST OF TABLES

Table 2.1	Concentration of cadmium in sewage sludge.	7
Table 2.2	Cadmium concentration in some plants.	10
Table 2.3	Effect of different P sources on Cd concentrations in plants.	15
Table 2.4	Dry weight and Cd concentration of oat shoots and exchangeable soil Cd as affected by soil cation exchange capacity.	17
Table 2.5	Effect of soil pH on the Cd concentration in different crops.	22
Table 2.6	Cadmium concentration in liver and kidney samples of cattle and sheep in various countries.	26
Table 2.7	Annual herbage yields and seasonality of yields of ryegrass-clover pastures.	27
Table 2.8	Effect of season on Zn concentration of annual pastures in SW Australia.	29
Table 3.1	Treatment combinations of the field experiment.	37
Table 4.1	Initial soil chemical properties.	45
Table 4.2	Chemical composition of the initial mixed pastures	46
Table 4.3	Botanical composition of the mixed pasture.	66
Table 4.4	Available soil Cu, Zn and Cd concentrations.	98
Table 4.5	Total Cd concentration in soil after 73 days from fertilizer application.	102

LIST OF FIGURES

Fig. 2.1	The relationship between Cd and P contents of phosphatic fertilizers (Williams and David, 1973).	6
Fig. 3.1	Experimental layout in the field.	36
Fig. 4.1	Effect of Cu levels on DM yield of mixed pasture.	48
Fig. 4.2	Effect of Zn levels on DM yield of mixed pasture.	50
Fig. 4.3	Effect of background Cd status on DM yield of mixed pasture.	52
Fig. 4.4	Effect of Cu levels on DM yield of pasture species.	54
Fig. 4.5	Effect of Zn levels on DM yield of pasture species.	55
Fig. 4.6	Effect of background Cd status on DM yield of pasture species.	56
Fig. 4.7	Effect of Cu levels on pasture growth rates of mixed pasture.	59
Fig. 4.8	Effect of Zn levels on pasture growth rates of mixed pasture.	60
Fig. 4.9	Effect of background Cd status on pasture growth rates of mixed pasture.	61
Fig. 4.10	Effect of Cu levels on growth rates of pasture species.	62
Fig. 4.11	Effect of Zn levels on growth rates of pasture species.	63
Fig. 4.12	Effect of background Cd status on growth rates of pasture species.	64
Fig. 4.13	Effect of Cu levels on Cu concentration in mixed pasture.	69
Fig. 4.14	Effect of Zn levels on Cu concentration in mixed pasture.	70
Fig. 4.15	Effect of background Cd status on Cu concentration of mixed pasture.	71
Fig. 4.16	Effect of Cu levels on Cu concentration in pasture species.	73
Fig. 4.17	Effect of Zn levels on Cu concentration in pasture species.	74
Fig. 4.18	Effect of Cu levels on Zn concentration in mixed pasture.	76
Fig. 4.19	Effect of Zn levels on Zn concentration in mixed pasture.	77

Fig. 4.20	Effect of background Cd status on Zn concentration in mixed pasture.	78
Fig. 4.21	Effect of Cu levels on Cd concentration in mixed pasture.	80
Fig. 4.22	Effect of Cu concentration on Cd:Cu ratio.	81
Fig. 4.23	Relationship between pasture Zn concentration and pasture Cd concentration.	82
Fig. 4.24	Effect of Zn levels on Cd concentration in mixed pasture	83
Fig. 4.25	Effect of Zn concentration on Cd:Zn ratio.	84
Fig. 4.26	Effect of Zn+Cu concentration on Cd:(Zn+Cu) ratio.	84
Fig. 4.27	Effect of background Cd status on Cd concentration in mixed pasture.	87
Fig. 4.28	Effect of Cu levels on Cd concentration in pasture species.	89
Fig. 4.29	Effect of Zn levels on Cd concentration in pasture species.	90
Fig. 4.30	Effect of background Cd status on Cd concentration in pasture species.	91
Fig. 4.31	Effect of days following the addition of fertilizers on DM yield.	92
Fig. 4.32	Effect of days following the addition of fertilizers on Cu concentration in mixed pasture.	94
Fig. 4.33	Effect of days following the addition of fertilizers on Cd concentration in background Cd status.	95
Fig. 4.34	Relationship between available soil Cu and pasture Cu in harvest 1.	97
Fig. 4.35	Relationship between available soil Zn and pasture Zn in harvest 1.	99
Fig. 4.36	Relationship between available soil Cd and pasture Cd in harvest 1.	101
Fig. 4.37	Relationship between total soil Cd content and available soil Cd.	103

LIST OF APPENDICES

Appendix 1	Rates of Zn and Cu fertilizer additions in the field experiment.	128
Appendix 2	Dry matter yield of pasture at different harvests.	129
Appendix 3	Growth rates of pasture for different harvests.	130
Appendix 4	Copper concentrations in pasture for different harvests.	131
Appendix 5	Zinc concentrations in pasture for different harvests.	132
Appendix 6	Cadmium concentrations in pasture for different harvests.	133

CHAPTER ONE

INTRODUCTION

1.1 Background

Cadmium (Cd) was identified as an element in 1817 and its large scale use by humans began in the 1940s. However, the serious consideration has given to Cd as a food contaminant only in the last 30 years (Sherlock, 1984). Cadmium is naturally present in all parts of the environment including sea and fresh water, soils, sediment, and the air. Consequently all food, whether of plant or animal origin, is exposed to and contains Cd.

Most recently, interest in Cd has been directed at progressive accumulation in biological systems at the low levels at which Cd generally occurs in the environment. Continued exposure to small amounts of Cd leads to accumulation, in human and animal liver and kidney tissue, as a metallo-protein cadmium-thionein, resulting in damage and dysfunction to these organs (Webb, 1975). Most ruminants are born with low Cd burdens and progressively accumulate the element in their kidneys and to a lesser extent livers and other organs (Underwood, 1977).

Cadmium concentration in kidneys increases with the age of animal, though the accumulation rate has recently been shown to decrease with age (Lee *et al.*, 1994). The Cd accumulating in tissues of grazing animals is derived from naturally occurring background Cd in soils, plants and from that added through farming practices. One such farming practice that is a source of Cd is the application of phosphatic fertilizers (Williams and David, 1973; Williams and David, 1976; Rothbaum *et al.*, 1986; Roberts *et al.*, 1993; Loganathan *et al.*, 1994 and 1995).

In ruminants, ingested Cd is transported to and stored in the animal's kidney as part of the natural detoxification process. It appears that Cd metabolism in animals is closely associated with Zinc (Zn) and Copper (Cu) metabolism and with metallothionein (MT), a heavy metal-protein complex.

The MT allows the storage of Cd, and gives protection to the animal from its toxic effects. Zinc, copper and cadmium share the same basic absorption systems (Underwood, 1977), and hence compete with each other for absorption into gastrointestinal mucosa. Zinc is also a powerful stimulant of MT in the mucosal cells, and high Zn can therefore lead to the trapping of Cd as Cd-MT within those cells, blocking Cd absorption. The extent to which this interaction is significant in practice will depend on the relative amounts of the elements in the diet.

Application of Zn has been shown to have an antagonistic effect (Abdel-Sabour *et al.*, 1988; Choudhary *et al.*, 1994), no effect (White and Chaney, 1980) or a synergistic effect (Williams and David, 1976) on Cd uptake by plants. In contrast, little is known of the effect of added Cu on Cd uptake. Fertilizer additions may be manipulated in an attempt to minimize Cd concentration in herbage. Copper is used in New Zealand to overcome the Cu deficiency in cattle and sheep. A field experiment was conducted to determine the overall effect of Zn and Cu fertilization on the uptake of Cd by pasture plants.

1.2 Objectives

The specific objectives of this study were to examine:

- a. The effect of Zn and Cu fertilizers on Zn and Cu uptake by pasture.
- b. The effect of Zn and Cu fertilizers on Cd uptake by pasture and the ratio of Cd:Zn and Cd:Cu in pasture.
- c. The seasonal variation in the uptake of Cd by different pasture species.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Introduction

The accumulation of Cd in the agricultural environment increases the potential for Cd to enter food products and ultimately affect human health. Continual Cd exposure to small amounts of Cd present in the pastoral environment leads to an accumulation by the grazing animal in tissues, such as the liver and kidney. The degree of Cd uptake by plants is known to be influenced by factors such as soil pH, soil organic matter, fertilizer practices, plant species and seasonal influences. The subsequent absorption of Cd in the ruminant is likely to be affected by the concentration of other nutrient cations, such as zinc (Zn) and copper (Cu) in the herbage. Cadmium can enter the agricultural food chain through uptake by plants of Cd naturally present in soil, from anthropogenic inputs via the atmosphere, the disposal of sludge on agricultural land, or from the application of phosphate fertilizers (Williams and David, 1976).

The objective of this review is to describe the current understanding of the soil, pasture, and fertilizer management factors that affect Cd concentration in plants, soils and animals and to summarize the literature on the effects of added Zn and Cu in fertilizer on Cd concentration and Cd:Zn and Cd:Cu ratios of pasture and grazing animal.

2.2 Sources of Cadmium

In nature, Cd occurs mainly in association with zinc ores, of which sphalerite (zinc sulfide) forms the main commercial source of Cd. The concentration of Cd in igneous rocks, sandstones and limestone is generally low (mean of 0.2 mg kg^{-1}), but the mean values of Cd concentration in rocks derived from lacustrine sediments, marine black shales and in phosphorites are considered to be high (11 to 25 mg kg^{-1}) (Peterson and Alloway (1979). None of the latter occur to any great significance in New Zealand, where soil parent materials are derived primarily from igneous, metamorphic or other sedimentary rocks (Suggate *et al.*, 1978).

Prior to the development of industry, Cd entered the agriculture systems at a low rate as a result of the weathering of rocks and volcanic activity. Cadmium is released into the atmosphere through various industrial processes which include: the smelting of metals, combustion of coal, oil and wood, incineration of wastes and also the use of phosphatic fertilizers and the disposal of sewage sludges. Much of the published work on Cd and its occurrence in soil relates to the use of municipal sewage sludge as a soil amendment (Dijkshoom and Lampe, 1975; Anderson and Nilsson, 1976; Pepper *et al.*, 1983; Bidwell and Dowdy, 1987; Ross *et al.*, 1991; Xiu *et al.*, 1992; Beckett, 1992 and Candelaria *et al.*, 1995).

Roberts *et al.* (1994) examined the Cd status of non-agricultural and agricultural pasture soils in New Zealand and found that in non agricultural soils ($n=86$) Cd concentrations (0 to 7.5 cm depth) ranged from 0.02 to 0.77 mg kg⁻¹, with a mean value of 0.20 mg kg⁻¹ and in pastoral soils ($n=312$) the range was 0.04 to 1.53 mg kg⁻¹, with a mean value of 0.44 mg kg⁻¹. The Cd enrichment of pastoral soils has been attributed to the continued use of phosphate fertilizers.

2.2.1 Cadmium in fertilizers

Schroeder and Balassa (1963) were the first to warn that fertilizers are implicated in raising Cd concentrations in food crops and since then much work has been performed to investigate the impact of impurities in fertilizers on crop uptake of potentially toxic elements (Mclaughlin *et al.*, 1996).

Many reports in the literature indicate that phosphate fertilizers form the main source of Cd in agricultural soils (Williams and David, 1976; Rothbaum *et al.*, 1986; Mortvedt *et al.*, 1987; Roberts *et al.*, 1993; Loganathan *et al.*, 1994). Williams and David (1976) stated that the plants take up only 1-5% of soluble Cd added to the soil. They also found that in soils which had received phosphate fertilizers for 20 years, at least 85% of the fertilizer Cd had remained in the cultivated depth of the soil.

Application of ammonium nitrate has been found to increase the Cd uptake by as much as 50% although they could not explain this result; however $\text{Cd}_3(\text{PO}_4)_2$ is more soluble in a solution of ammonium salts than in water (Weast, 1972).

From analyses of diammonium phosphate (DAP) fertilizers given by Mortvedt *et al.* (1981) the Cd concentration in the western USA phosphate rock is calculated to be up to $760 \text{ mg Cd kg}^{-1} \text{ P}$.

It has been observed that the range in Cd input values are consistent with measured increases in Cd concentrations in surface soils due to fertilizer application, e.g. up to 0.14 and 0.18 mg kg^{-1} soils for cereal and pasture soils, respectively (Williams and David, 1973) and about 0.10 mg kg^{-1} for pasture soils (Merry and Tiller, 1991).

The Cd contamination of phosphate fertilizers has been shown to present a considerable problem with regards to the accumulation of Cd in agriculture and its potential transfer into higher food chains. The relationships of Cd and P content in various phosphatic fertilizers (Williams and David, 1973) are shown in Figure 2.1.

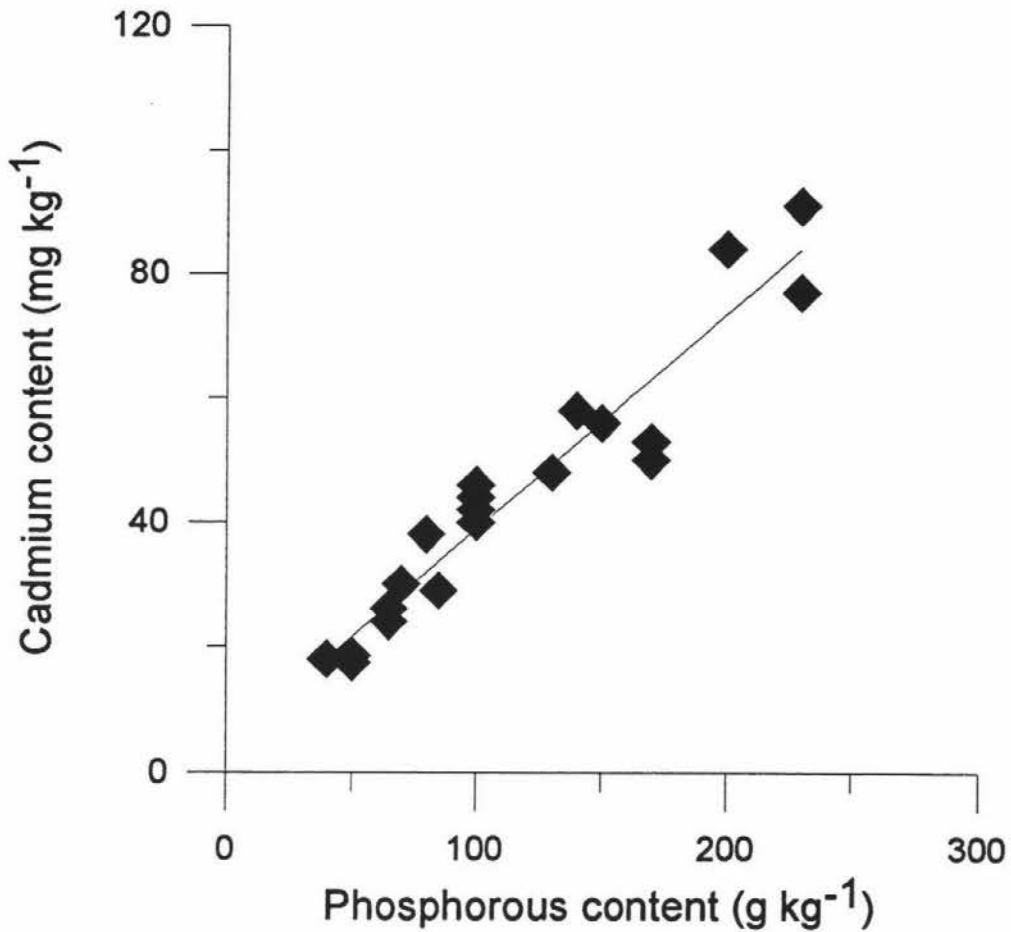


Fig. 2.1 The relationship between cadmium and phosphorus contents of phosphatic fertilizers (Williams and David, 1973).

2.2.2 Cadmium in sewage sludge

Land application of municipal sewage sludges is becoming more popular because of constraints placed on alternative disposal methods, such as ocean dumping and incineration. In the United Kingdom, about 45% of the sludge produced annually is utilized in agriculture (Davis and Coker, 1979). This is equivalent to 510×10^3 dry tones per year, with a median Cd concentration of 22.6 mg kg^{-1} . Hansen and Tjell (1982) reported that, on a national basis, sewage sludge contributes about 5% of the Cd burden in UK agriculture; the remainder results from aerial deposition (70%) and inorganic fertilizers (25%).

Utilization of sewage sludge on agricultural lands is often associated with a potential risk of contaminating the food chain, with Cd posing the greatest concern. Most of the Cd in sewage sludge is derived from industrial sources. Because of the variable Cd contents of sewage sludges, the Cd concern appears to be more local than national, with big cities generating the greatest amounts and also those sludges with the highest Cd concentrations. Tiller *et al.* (1994) have recently estimated potential sludge-Cd loading to Australian agricultural soils to be about 2-4 tons year⁻¹. The Cd concentration of sewage sludge in New Zealand is low compared to other countries (Table 2.1).

Table 2.1 Concentrations of cadmium (mg kg⁻¹) in sewage sludges.

Country	Mean	Range	References
USA	104	7 - 444	Furr <i>et al.</i> (1976)
UK	<200	<60 - 1500	Berrow and Webber (1972)
Ireland	1	<1 - 90	O'Riordan <i>et al.</i> (1986)
Sweden	13	2 - 171	Page (1974)
Canada	38	-	Oliver and Cosgrove (1975)
New Zealand	4.5	-	Wells and Whitton (1977)

2.3 Availability of soil cadmium

Chaney and Hornick (1977) identified the factors affecting the activity of Cd in soils and its availability for plant uptake. These include: soil pH; the amount of Cd present; the metal sorption capacity; the hydrous metal oxide content; the presence of micro elements, such as zinc; the presence of macro nutrients, such as phosphate; soil temperature, moisture and soil aeration. The Cd sorption capacity of the soil is influenced by the organic matter content and the nature and number of cation exchange surfaces.

Many authors (Bramley and Rimmer, 1988) have noted that the solubility and hence the availability of heavy metals increases with a decrease in pH. Tiller (1988) stated that the affinity of Cd for soil surfaces depends on the pH and the type of surface. The affinity of Cd for absorption surfaces increases with pH and decreases with the concentration of added Cd.

The importance of organic matter in binding Cd appears uncertain in view of the conflicting comments of Haghiri (1974) and Peterson and Alloway (1979). One aspect of Cd-organic matter interactions, which has been much neglected in literature is the role of micro-organisms. However, Chanmugathas and Bollag (1987) investigated the microbial mobilization of Cd under both aerobic and anaerobic conditions in two sands and found that the amount released was greater under anaerobic conditions. They further established that the mobilization of strongly bound or fixed Cd was a microbially mediated process as Cd release only occurred in the presence of microorganisms and nutrients.

2.4 Cadmium in plants

Cadmium is a non essential element for both plants and animals, so there are no critical concentrations below which deficiency occurs (Van Bruwaene *et al.*, 1984). The literature clearly indicates that increasing the amount of Cd in soil leads to an increase in plant Cd uptake (Javris *et al.*, 1976; Van Bruwaene *et al.*, 1984).

Maclean (1976) investigated the Cd uptake and availability as influenced by organic matter, the addition of lime, P, Cd and Zn in a range of species grown on an unpolluted loam (pH 7.1), with a background Cd content of 0.65 mg kg⁻¹. With no additions, the Cd concentration in dry matter ranged from 0.18-0.99 mg kg⁻¹. Addition of 5 mg Cd kg⁻¹ to the soil led to an increase in plant Cd, for timothy roots, from 0.61 to 15.28 mg kg⁻¹ (Table 2.2).

Javris *et al.* (1976) obtained similar results with perennial ryegrass exposed to a solution containing Cd, plant uptake increased with increasing solution concentration. Lund *et al.* (1981) found that the Cd concentration in swiss chard, radish and pepper increased as its concentration in the soils increased.

Liming in most cases increased the Cd adsorption and reduced the Cd availability to plants in soils (Street *et al.*, 1978; Eriksson, 1989). However increasing soil pH does not always reduce plant uptake of Cd (Eriksson, 1989). The pH-Cd uptake relationship is dependent on soil types and plant species.

Several workers have noted the interaction between Cd and Zn and also other ions. Turner (1973) and Haghiri (1974) found that high Zn levels enhanced the Cd uptake into radish leaves, Peterson and Alloway (1979) concluded that Cd concentrations will generally be higher in plants grown on high Zn soils than in plants grown on soils low in Zn. In soils, however Zn may compete with Cd for binding sites, thereby increasing the Cd concentration in the soil solution.

The results of the Tiller *et al.* (1984a and 1984b) provide some evidence for the difference in Cd-Zn interaction between soils; in soils where exchange surfaces are dominated by 2:1 layer clays, Zn is preferentially adsorbed over Cd, but soils with high organic matter, the reverse occurs. He and Singh (1995) found that application of Cd-containing NPK fertilizers, ranging from 2.7 to 12.52 mg Cd kg⁻¹, increased the Cd concentration in plant species (Table 2.2) as well as the extractable Cd in the soil.

Table 2.2 Cadmium concentration in some plants.

Crops	Mean Cd concentration (mg Cd kg⁻¹)	References
Alfalfa, top	1.113	Macleane, 1976
Alfalfa, root	6.34	
Timothy, tops	1.41	Macleane, 1976
Timothy, roots	15.28	
Carrot, root	0.48	Guttormsen <i>et al.</i> 1995.
Carrot, leaves	0.91	
Rye grass	0.10	He and Singh, 1995
Oats, grain (1991)	0.136	
Oats, grain (1992)	0.093	

2.5 Cadmium in farm animals

Cadmium concentrations increase with age because ruminants are born with a low Cd burden, and much of the Cd ingested and adsorbed there after is retained as Cd-metallothionein in the liver and kidney. It is postulated that differences in Cd levels between geographical regions, species and stocking rate are partially due to the consumption of Cd derived from fertilizer containing Cd.

Soil Cd distribution in sheep grazed hill pasture is strongly influenced by phosphate fertilizer history, soil depth and land slope (Loganathan *et al.*, 1995). Van Bruwaene *et al.* (1984) stated that of the Cd ingested, 0.3-0.4% is retained by goats and 0.75% is retained by cattle; no figure was available for sheep.

Assuming that sheep retain similar amounts of Cd as goats (0.35%), the amount of Cd accumulated annually by sheep and cattle would be 0.27 mg and 3.9 mg, respectively. The results of Langlands *et al.* (1988) suggested that the likely partitioning of the retained Cd between kidney, liver and muscle would be 70, 27.5, and 2.5% in sheep and 73, 24, and 3% in cattle respectively.

Typical values for the carcass weights of sheep and beef cattle are 20 and 250 kg respectively, of which 45% is muscle (Dalgarno, 1980). Typical kidney fresh weights are 95 g for sheep and 430 g for cattle, and the fresh weight of the liver of these animals is typically 620 g and 2.8 kg respectively.

Thus assuming that the amount of Cd in other organs and the blood is negligible (Dalgarno, 1980; Sharma *et al.*, 1982), of the total Cd retained in a sheep, 0.19 mg yr⁻¹ accumulates in the kidneys, 0.075 mg yr⁻¹ in the liver, and 0.007 mg yr⁻¹ in muscle. In cattle, the amounts of Cd accumulated in each year will be 2.8, 0.9, and 0.1 mg (Cd kidney: Cd intake ratio of 1:100; Dalgarno, 1980) in the kidneys, liver and muscle respectively. These calculations are based on the following assumptions:

The likely annual accumulation of Cd in New Zealand sheep and cattle is respectively 2.01 and 6.5 mg kg⁻¹ fresh weight in the kidneys, 0.12 and 0.32 mg kg⁻¹ in the liver, and 7×10⁻⁴ and 9×10⁻⁴ mg kg⁻¹ in muscle. Therefore the values of kidney Cd are significantly higher than the allowable limit of 1 mg kg⁻¹.

Morcombe *et al.* (1994) conducted an experiment as a part of residue survey by taking 4973 kidneys of sheep stratified by age and shire of origin within Western Australia, for the analysis of Cd. The geometric mean of Cd concentration in the kidney of hogget ewes, 4 tooth ewes and adult ewes were 0.9 mg kg⁻¹, 1.47 mg kg⁻¹ and 3.34 mg kg⁻¹ on wet weight basis, respectively. The annual increase in Cd concentration of kidney from hogget sheep was estimated to be 0.65 mg kg⁻¹. They have found that the higher Cd concentration resulted from the establishment of some volunteer species (capeweed: *Arctotheca calendula* L.) of winter annual pastures.

2.6 Factors influencing the accumulation of Cd in plants, soil and animals

2.6.1 Plant uptake of fertilizer Cd from soil

It is generally thought that the chemical form of Cd taken up by plants is the free uncomplexed Cd²⁺ ion present in soil solution (Chaney, 1988). Thus any treatments or changes in soil conditions which affect the concentration of the Cd²⁺ ion will affect plant accumulation Cd. The soil and plant factors controlling Cd uptake from soils have been extensively studied and reviewed by many authors (Chaney, 1988; Page *et al.*, 1987; Alloway, 1990; Bramley, 1990; Singh *et al.*, 1995 and Mclaughlin *et al.*, 1996).

2.6.1.1 Time of contact and forms of Cd

The effect of time on Cd retention may have an important implication for Cd uptake by plants and hence Cd accumulation in the food chain. In soils as Cd moves into less available forms with time there will be less danger of its uptake by plants (Brams and Anthony 1988; Bell *et al.*, 1991). There is some evidence that Cd availability to plants decreases with time (Street *et al.*, 1978). Mann and Ritchie (1994) found that an increase in the time of contact of Cd with soil decreased the soluble and exchangeable forms of Cd in all the soils of Western Australia except the siliceous sand. They have concluded that the rate and extent of the decrease in soluble and exchangeable forms of Cd with time depend upon the type and relative proportion of the absorption surfaces present in soil, pH and the rate of Cd addition.

The amounts of soluble and exchangeable Cd are indications of the labile or bioavailable forms in the soil and hence, their transformation to other forms with time suggests a decrease in the availability of Cd. This is in agreement with the findings of Street *et al.* (1978) who found that increasing the incubation time decreased the availability of Cd to plants. These changes have the potential to decrease the risk of Cd loss to ground water by leaching and the risk of Cd uptake by plants.

2.6.1.2 Effects of fertilizer application

Fertilizer applications may also affect the uptake of heavy metals. Additions of fertilizer cations such as K^+ and NH_4^+ affect the solubility of heavy metals in soil. Eriksson (1990), and Willaert and Verloo (1992) reported that Cd availability in two soils increased after the application of acidifying N fertilizers. Busch (1985) also found that the increasing applications of N fertilizer increased Cd uptake and toxicity in several vegetable crops grown in pots with two different soils previously treated with sewage sludge.

Lorenz *et al.* (1994) have found that the excess of K^+ and NH_4^+ not only increased the concentration in soil solution of added K^+ , but was also closely related to changes in the concentration Ca, Mg, Cd and Zn in solution. Concentration of all cations in soil solution increased 5 to 15 fold from day 0 to day 35, corresponding to the increasing excess of added K^+ and NH_4^+ in the soil. The increase in the concentration of ions in solution and the decrease in soil pH can mainly be explained by ion exchange mechanisms.

The fertilizer cations, K^+ and NH_4^+ , are likely to shift the soil chemical equilibrium desorbing a quantity of those ions that are adsorbed, on to exchangeable sites on soil colloids, including major elements, heavy metals and H^+ . The resulting ion concentrations in solution were much larger than the number of moles of fertilizer ions added, implying that nitrification and uptake by plants of the added NH_4^+ may have contributed to the lowering of soil pH and the desorption of major and heavy metal ions.

It is also possible that desorption is not the only mechanism responsible, but that dissolution of carbonates also occurred at acidic pH values. Busch (1985) suggested that increased Cd availability after the application of N fertilizer could be attributed to a decrease in pH and an increase in salt concentrations in the soil.

The role of P in Cd uptake by plants may be important when considering the possibility of accumulation of Cd from phosphatic fertilizers. According to Williams and David (1973), granules of superphosphate when placed in contact with moist soil for 8 days lost all of their water-soluble P, but still retained 60% of their original Cd. These investigators further showed that Cd in the water extracts was approximately proportional to Ca extracted, indicating that Cd was associated with Ca in both the phosphate and sulphate components of the fertilizer.

Williams and David (1977) found that the addition of phosphate fertilizer to the surface of P deficient soils led to the proliferation of roots in the phosphate treated layer which resulted in enhanced Cd uptake from it, especially at low levels of P application. When Cd was also concentrated in the same layer, increased uptake of Cd occurred, although this "priming" effect was less evident in soils with high P status.

Phosphatic fertilizers contain varying amounts of Cd as an impurity depending on the source of the rock phosphate used (Williams and David, 1973). It has been shown that the Cd content of plants increases with the amount of superphosphate applied (Williams and David, 1973) and with a decrease in soil pH (Williams and David, 1973; Tiller, 1988 and Whitten and Ritchie, 1991). Anderson and Siman (1991) reported increasing levels of Cd in plants with increasing rate of P fertilizer containing 2.5 mg Cd kg⁻¹ over a 20 years.

Guttormsen *et al.* (1995) conducted a field experiment in which NPK fertilizers containing 1, 30, 90 and 400 mg Cd kg⁻¹ P were applied at the rates of 0.07, 2.1, 6.3 and 28 g Cd ha⁻¹ yr⁻¹. The amounts of Cd added through phosphate rock also ranged from 0.1 to 28 g ha⁻¹ yr⁻¹. The increased Cd application rates through NPK fertilizers increased the Cd concentration in Chinese cabbage (0.7 g ha⁻¹ yr⁻¹) and carrots (1.3 g ha⁻¹ yr⁻¹).

Recent studies by He and Singh (1994 and 1995) gives clear evidence for the accumulation of Cd in plants due to different Cd containing fertilizers (Table 2.3).

Table 2.3 Effect of different P (30 mg P kg⁻¹ soil) sources on Cd concentrations in plants.

Crops	P sources	Mean mg Cd kg ⁻¹	References
Oat grain (1992)	CaH ₄ (PO ₄) ₂ H ₂ O	0.036	He and Singh, 1995.
	CaH ₄ (PO ₄) ₂ H ₂ O+CdCl ₂ H ₂ O	0.181	
	Low-Cd NPK	0.040	
	High-Cd NPK	0.097	
	Phosphate rock	0.076	
Ryegrass (1992)	CaH ₄ (PO ₄) ₂ H ₂ O	0.058	He and Singh, 1995.
	CaH ₄ (PO ₄) ₂ H ₂ O+CdCl ₂ H ₂ O	0.124	
	Low-Cd NPK	0.100	
	High-Cd NPK	0.128	
	Phosphate rock	0.058	

2.6.1.3 Cation exchange capacity and organic matter effects

In sorption studies, Kuo and Baker (1980) found that CEC played an important role in the sorption of Cd by soils. It should be noted that the pH buffering capacity of soils increases with CEC. Thus, the potential for increased Cd uptake by plants in acidic soils is less with high CEC than with low CEC.

Haghiri (1974) examined the effect of soil CEC on Cd content and the yield of oat shoots, and on the amount of Cd extracted by 1N NH₄Ac (Table 2.4). The Cd concentration in oat shoots decreased with increased soil CEC. The increased ability of the soil to adsorb Cd with increasing CEC resulted in a decrease in Cd availability as judged by the reduction in the Cd concentration of the soil extract. A significant positive correlation ($r = 0.78$) was obtained between the exchangeable soil Cd and the

concentration of Cd in oat shoots. The increase in CEC by the addition of organic matter also resulted in increased growth of oat shoots. This is due to the reduction in Cd availability which in turn diminished the deleterious effect of Cd on the growth of oat plants. In addition to high CEC, soil organic colloids have a chelating ability and certain heavy metals have a tendency to combine with certain chelating groups and become fixed.

In many cases fertilizers, containing Cd as a contaminant, are applied to the surface layers of a soil, which usually have the highest organic matter content. Hence, it is to be expected that the organic matter would adsorb Cd until all the sites were saturated. Any excess Cd would be in the exchangeable or soluble form and so be prone to leaching to the next soil depth or be taken up by plants.

Loganathan *et al.* (1995) found that the Cd concentration per unit of soil volume was highest on the low slopes and declined with increasing slope. This is due to the high organic matter content of the lower slopes when compared to steeper slopes. They also found that the accumulation of Cd in pasture was less on lower slopes than on steeper slopes. This was attributed, in part, to the high organic matter content of the soils, which reduced the bioavailability of Cd from the lower slopes when compared to that of steeper slopes (He and Singh, 1993).

Table 2.4 Dry weight and Cd concentration of oat shoots and exchangeable soil Cd affected by soil CEC (Haghiri, 1974).

CEC(meq100g ⁻¹)	(Cd/CEC)×10 ⁻⁵	Dry wt(g)	Plant Cd (mg kg ⁻¹)	Exch. soil Cd (mg kg ⁻¹)
17.1	208	0.99c *	13.7a	2.94a
18.9	189	1.4b	12.9a	2.90a
23.1	154	1.34b	11.0b	2.55b
26.9	132	1.66a	9.5c	2.34b
30.5	117	1.76a	7.9d	1.95c

*Means followed by the same letter are not significantly different at the 5% level.

2.6.1.4 Zinc and copper effects on Cd concentration in plants, soil and animals

2.6.1.4.1 Zinc and Cd interactions

The elemental interaction most commonly observed is Zn with Cd. Zinc applications have the following effects on Cd:

- a) Competes for adsorption sites in soils and therefore desorb more Cd, thus increasing the availability.
- b) Competes for uptake sites in roots, so decreasing the uptake of Cd.
- c) In Zn deficient soils increases plant growth, causing more dilution of Cd in the root zone.

In recent studies, several researchers (Smilde *et al.*, 1992; Moraghan, 1993 and Choudhary *et al.*, 1994 and 1995) found mainly antagonistic effects between Zn and Cd, in which applied Zn reduced the plant uptake of Cd in a range of crop plants grown in soils. Mckenna *et al.* (1993) found a similar effect for lettuce and spinach grown in nutrient solutions.

Haghiri (1974) observed that the addition of Zn from 5 to 50 mg L⁻¹, apart from raising the Zn concentration, significantly increased the concentration of Cd in soybean shoots as compared to the control. The addition of Zn depressed the yield of soybean shoots. The reduction in Cd concentration relative to the control began to occur at the 100 mg L⁻¹ level of added Zn. The increase in Cd concentration in plant tissue, by the addition of 5 to 50 mg L⁻¹ Zn, was due to decreased plant growth and possibly increased displacement of Cd into the soil solution from the soil exchange complex. The depression in the Cd concentration of soybean shoots grown in nutrient solution with 100 to 400 mg L⁻¹ Zn levels probably was caused by the dilution of Cd (Cd/Zn ratio) in the soil solution due to the excess amounts of Zn being present. Soil application of Zn as an agent in reducing the uptake of Cd by soybean tops did not appear to be practical since the suppression of Cd occurred only when the large amounts of Zn were added.

Lagerwerff and Biersdorf (1972) found that at 0.1 mg L⁻¹ Cd in solution culture, the increase in Zn from 0.02 to 0.4 mg L⁻¹ increased the concentration of Cd in radish leaves by 10%.

Villarroel (1993) found that with an increased yield of swiss chard, the uptake of Cd also increased by 50%, and the uptake of Zn remained unchanged. He also found that the plant uptake of Cd and Zn in a sludge treated soil was limited by the rate of Cd and Zn desorption from solid phase.

McLaughlin *et al.* (1993) conducted an experiment with potatoes on Zn adequate soils which indicated a reduction in potato tuber Cd concentrations with the addition of Zn, but the magnitude of the effect was small (approximately 10-15% reduction).

Oliver *et al.* (1994) observed that the applications of low rates of Zn fertilizer (up to 5 kg Zn ha⁻¹) markedly decreased the Cd concentration in wheat grain. They also concluded that the effectiveness of applied Zn on grain Cd concentration decreased with time following Zn application.

Choudhary *et al.* (1994 and 1995) found that the application of Zn to soil with N plus P fertilizers increased tissue Zn concentration to sufficiency levels and decreased plant Cd concentration of the two durum wheat lines, DT 627 and Medora, categorized as a low and high Cd accumulator respectively.

The decrease in Cd concentration in mixed pasture and in species affected by the addition of increased soil Zn supply is due to the direct competition between Zn and Cd, either externally at the root surface or within the plant (Robson and Pitman, 1983).

Cakmak and Marschner (1988) have shown that Zn deficiency causes the loss of membrane integrity in the root cells. Application of Zn fertilizer to the soil to alleviate the deficiency could restore the integrity of the root membrane and hence, the amount of Cd accumulated by the plant would decrease.

A further possibility is the mobilization of Cd by phytosiderophores, which are released by cereal roots in response to nutrient deficiency (Crowley *et al.*, 1987). Thus, in gramineous species either Fe or Zn deficiency may also enhance solubility and mobility of Cd in the rhizosphere due to the release of phytosiderophores. The most likely explanation is a combination of loss of membrane integrity and Cd mobilization by phytosiderophores.

2.6.1.4.2 Copper and Cd interactions

There has been no work published on the effect of Cu on Cd concentration.

2.6.1.4.3 Copper and Zn interactions

Studies of Lidon and Henriques (1992b and 1993) using rice (*Oryza sativa* L.) seedlings showed a decrease in net uptake of Cu by the roots due to an increase in the translocation to the shoots of Cu, and other elements, including Zn, Fe and Mn, with an increase in the external concentration of Cu.

In contrast, Beckett and Davis (1978) working with nutrient solutions and found that Cu had little effect on the amount of Zn uptake in barley or vice versa.

Kabata and Pendias (1984) described that the Zn-Cu interaction as an antagonism effect and also observed that addition of large amounts of Zn decreased the uptake of Cu in the plant top.

2.6.1.5 Effect of soil pH and liming on Cd concentration

Soil pH is considered one of the major factors controlling solubility, mobility and bioavailability of Cd in soils (Alloway, 1990; Witter, 1989). In variable charge soils increasing soil pH tends to increase the number of exchange and specific adsorption sites for cations. Soluble and exchangeable forms of Cd are considered to be the most labile and available pools for leaching and uptake by plants (Harrison *et al.*, 1981; Hickey and Kittrick, 1984). Hence the amount of Cd in these forms will be indicative of the potential for Cd accumulation in plants or for Cd contamination of ground waters via leaching.

Adsorption of heavy metals on to the clay minerals and organic matter is increased by increasing soil pH (Kiekens, 1984). The sharp increase in Cd sorption with small changes in soil pH was observed by Brummer *et al.* (1988) and Naidu *et al.* (1994).

Thus the availability of trace elements for uptake by plants is generally greater at low pH than at high pH and the net effect of an increase in soil pH value, by liming of the soil for example, is a reduction in metal absorption by plants (Davis and Coker, 1980).

He and Singh (1995) observed that application of Cd-containing fertilizers generally had a more pronounced effect on Cd concentrations in plants, when grown at lower pH than when grown at higher soil pH levels. The plant tissue concentration of Cd generally decreases with increasing soil pH provided that other soil properties remained unchanged (Table 2.5). This inverse relationship between soil pH and plant uptake of Cd is well established by several investigators (Narwal *et al.*, 1983; He and Singh 1993).

Page *et al.* (1981) reported that the Cd concentration of swiss chard leaves increased by factors of between 2 and 3.9 when the soil pH was reduced from 7.4 to 4.5. The uptake Cd by rice decreased when the pH was increased from 5.5 to 7.5 (Bingham *et al.*, 1986).

Smith (1994) and Singh *et al.* (1995) found that the Cd concentration in plants grown in two soils (loam and clay loam) generally decreased with increasing soil pH especially above pH 6.5 (Table 2.5).

Guttormsen *et al.* (1995) conducted a field trial over a three year period with chinese cabbage and carrots grown in a sandy soil with the pH adjusted to 5.5 and 6.5. They found that the Cd uptake by both the chinese cabbage and the carrots was significantly ($p < 0.01$) higher at pH 5.5 than at 6.5. At pH 5.5, Cd concentrations in the two crops, based on a three year average, were 23 and 46% higher than at pH 6.5.

Maclean (1976) found that the addition of lime raising the soil pH from 6 to 7, reduced plant Cd. And in unlimed soil the addition of P also greatly reduced plant Cd, although this effect was absent in soils of neutral pH. An explanation for this might be that the addition of phosphate to soils will increase the total surface negative charge (Bolan *et al.*, 1988) with the result that the concentration of Cd in soil solution is reduced.

Table 2.5 Effect of soil pH on the Cd concentration (mg Cd kg⁻¹) in different crops.

Soil type/ location	Crops	pH Levels	Mean (mg Cd kg ⁻¹)		References
			Grain	Straw	
Clay loam, Norway	Wheat (1994)	5.5	0.72	0.71	Singh <i>et al.</i> (1995)
		6.5	0.20	0.17	
		7.0	0.30	0.32	
		7.5	0.20	0.18	
Loam soil, Norway	Lettuce (1994)	5.5	0.51	6.32	Singh <i>et al.</i> (1995)
		6.5	0.20	2.73	
		7.0	0.13	2.15	
		7.5	0.19	1.69	
Swinton, UK.	Potato	5.0	0.26	0.96	Smith, 1994.
		5.5	0.23	0.79	
		6.0	0.21	0.63	
		7.0	0.16	0.40	

The difference in Cd accumulation by plant species was reported first by Merry and Tiller (1978). Roberts *et al.* (1993) found that low fertility pasture species and weeds accumulate Cd.

Capeweed (*Arctotheca calendula*) accounted for 35 to 50% of pasture in some of the agricultural regions of Western Australia (Arnold *et al.*, 1985). It has, on average, 5 times the Cd concentration of subterranean clover plants (Merry, 1988) which, in turn, is 2-3 times higher than the Cd concentration of Phalaris (Williams and David, 1973).

Roberts *et al.* (1994) found that the overall mean Cd content of weeds from pastoral sites was significantly greater than the mean for native site weeds. Differences in Cd uptake by plant species are generally related to their genetic characteristics and physiological state of development.

Williams and David (1973) showed that the concentration of Cd in legumes is several times higher ($0.155 \text{ mg Cd kg}^{-1}$) than the associated grasses ($0.031 \text{ mg Cd kg}^{-1}$). The increased concentration of Cd in legumes is related to the acidification of rhizosphere and the release of Cd.

Roberts *et al.* (1994) however found that there were no consistent differences in mean Cd contents of legumes between native and pastoral sites with the overall mean Cd content for native and pastoral site legumes being similar at 0.07 and 0.06 mg kg^{-1} , respectively.

Relative differences in Cd accumulation between cultivars are often significant enough to warrant inclusion of Cd as a selection factor in breeding programs. Tiller *et al.* (1994) and Mclaughlin *et al.* (1994) have found in field screening studies that site and soil factors play a dominant role in determining absolute plant Cd concentrations, so that selection of germplasm low in Cd may be of little use.

Reduced dietary intake of Ca may enhance Cd toxicity and accumulation (Washko and Cousins 1977; Hamilton and Smith 1978). When the level of dietary Ca is low, the parathyroid gland increases the production of Ca binding protein (CaBP) resulting in an increased absorption of Ca. However this protein also readily binds with Cd. Thus when levels of Ca are low and Cd concentration is enhanced. Cadmium readily associates with CaBP, leading to increased Cd absorption, and so intensifying its toxic effects (Washko and Cousins, 1977).

Fitzgerald *et al.* (1985) conducted an experiment where cattle were allowed to graze pastures, variously treated with anaerobically digested sludge (ADS) for up to 8 years. This resulted in higher concentrations of heavy metals and some organic compounds, in some tissues than cattle not exposed to ADS. They found that the mean Cd in kidneys of cattle in the control and those exposed to ADS was 9 and 44 mg kg⁻¹ on a dry weight basis, respectively.

Most of the Cd accumulated by grazing animals is derived from pasture intake. Bramley (1990) estimated that annually approximately 55 and 275 mg Cd is ingested per head by sheep and cattle, respectively, through the intake of herbage.

Roberts *et al.* (1994) have shown that even though sheep ingest 36-46 kg soil per year, this only contributes 5 to 8% of the total Cd intake for lax and hard grazed flocks, respectively.

Ruminants do not have a homeostatic control mechanism for regulating Cd absorption or excretion, which is affected by the level of dietary Cd content. Although intestinal uptake of Cd has been estimated to account for over 90% of the total Cd absorbed, most of the Cd ingested is discarded. About 80 to 90% of the total ingested Cd is excreted in the faeces and only 0.05% excreted in the urine. Most dietary Cd is bound to metallothionein and is absorbed intact into the circulation. Over 50% of Cd ultimately accumulates in the kidney and liver. In animals, kidney and liver Cd accounts for 50-70% of the total Cd with kidney having a higher Cd concentration than the liver.

In blood Cd is associated with an albumin like protein which is transported to the kidney, where it is filtered through the glomerulus and reabsorbed by the proximal tubules (Friberg *et al.*, 1985).

More recent testing of animal offal in New Zealand has indicated that some 22-28% of sheep and 14-20% of cattle between 1988-1991 had kidney Cd content greater than the permissible level of 1 mg Cd kg⁻¹ (Roberts *et al.*, 1993). In general, older animals had a higher kidney Cd content as these animals had had longer exposure to Cd, in their environment, and hence greater opportunity to consume and retain Cd. The Cd concentration in liver and kidney samples of cattle and sheep in various countries is shown in the Table 2.6.

Langlands *et al.* (1988) attributed following reasons to the higher concentration of Cd in the liver and kidney of sheep than cattle in Australia:

- Sheep graze in areas within states containing more Cd than those grazed by cattle.
- The differences in the composition of the diet selected.
- The differences in the absorption and storage of ingested Cd
- The greater consumption of soil by sheep particularly at higher stocking rates.

Chowdhary and Chandra (1987) reported that in animals, the effect of prolonged exposure to abnormal levels of Cd include testicular necrosis, placenta destruction, abortion, teratogenic malformations, renal damage, osteomalacia, immunosuppression, pulmonary oedema and emphysema. In humans, severe Cd exposure can result emphysema, bronchitis, ulceration of nasal mucosa, renal dysfunction, liver necrosis, anaemia, hypertension, skeletal deformities, prostrate and lung cancer and teratogenesis.

Table 2.6 Cadmium concentration in liver and kidney samples of cattle and sheep in various countries.

Country	Animal	Cd concentration (mg Cd kg ⁻¹ fresh weight)		References
		Liver	Kidney	
Australia	Cattle	0.18	0.30	Langlands <i>et al.</i> (1988)
	Sheep	0.30	0.96	
New Zealand	Cattle	0.10	0.25	Solly <i>et al.</i> (1981)
	Sheep	0.10	0.25	
Netherlands	Cattle	0.11	0.36	Vos <i>et al.</i> (1987)

2.9 Seasonal influences on growth rate, DM yield, concentration of Zn, Cu and Cd in pasture.

2.9.1 Effect on growth rate, botanical composition and DM yield

There is considerable literature on pasture yield, botanical composition, fertilizer responses and the seasonality of pasture growth for different regions in New Zealand. Table 2.7 shows the average total annual yield of ryegrass-clover pastures under sheep grazing, the coefficient of variation (CV) of annual yields, and the percentage of pasture growth in each of the four seasons for different regions in New Zealand.

Table 2.7 Annual herbage yields and seasonality of yields of ryegrass-clover pastures (Radcliffe *et al.*, 1974-1978).

Site	Rainfall (mm)	Yield (tDM ha ⁻¹)	CV (%)	Percentage yield			
				Spring	Summer	Autumn	Winter
Dargaville	1250	17.2	21	30	33	22	15
Hamilton	1260	10.2	28	44	25	18	13
Masterton	1050	10.9	22	48	16	20	16
Hindon, Otago	630	8.8	38	41	39	18	2

Temperature and soil moisture conditions are favourable during spring resulting in maximum pasture growth rates. Whereas in winter with temperatures falling below 10°C slow pasture growth rates results.

Lambert (1967) observed that the temperature effects on grass species are specially noticeable in Northland, New Zealand where two species, paspalum (*Paspalum dilatatum* Poir) and kikuyu (*Pennisetum clandestinum* Hochst), become important pasture ingredients. At Kaikohe paspalum and kikuyu were compared with Ruanui ryegrass.

Suckling (1975) found that a series of pure grass swards at Te Awa, a hill site in the Manawatu contained 63% of ryegrass, 21% cocksfoot and other grasses.

Corkill *et al.* (1980) stated that much of the disparity between the species contribution to production is explained by the different environmental factors which affect pastures in various farming regions in New Zealand.

Lancashire (1984) found that though perennial ryegrass and white clover are by far the most commonly sown herbage species in New Zealand, farm pastures contain a large number of other species, often in significant amounts.

Lambert *et al.* (1986) found that the poa and ryegrass have maximum growth rates in spring, most low fertility adapted grass produce maximum growth in late spring to early summer.

Del Pino Machado (1994) observed that the high fertility status soil comprises of 89.7% grass and 8.5% legume and in low fertility status soil comprises 75.5% grass, 4.2% legume and the rest were weed and dead materials. She also found that the pasture growth rates ranged from 108 kg DM ha⁻¹ day⁻¹ in early summer to less than 2 kg DM ha⁻¹ day⁻¹ in late winter.

2.9.2 Effect on Cu concentration

The seasonal pattern of pasture Zn, Cu and Cd concentration may differ with location, soil type, climate, pasture species and management practices.

Reuter *et al.* (1981) recorded that the Cu concentration in subterranean clover declined from 3.9 mg kg⁻¹ at 26 days after sowing to 1.6 mg kg⁻¹ at 98 days.

Sherrell and Rawnsley (1982) found that an application of 2 to 4 kg Cu ha⁻¹ as copper sulphate increases herbage Cu concentration from 5 to 12 mg kg⁻¹ within 4 weeks and then it decreases markedly to 8 mg kg⁻¹ over the next 9 to 10 months. He also observed that the clovers tend to take up larger amounts of Cu and its persistence is greater than grasses.

McLaren *et al.* (1990) found that initially less than 10% of the Cu adsorbed by the soil was desorbed and after three months of soil contact, only a negligible amount (<1%) of the adsorbed Cu could be desorbed.

Willimott (1995) found that an application of 0, 5, 10 kg Cu ha⁻¹ as copper sulphate increased the herbage Cu concentration up to three months after Cu application and then decreased back to the initial levels nine months after application.

2.9.3 Effect on Zn concentration

Zinc deficiency is more prevalent in cool and wet seasons. Soil temperature effects appear to be largely on rate of mineralization of Zn in soils.

Schwartz *et al.* (1987) found that barley grown at a root temperature of 10⁰ C had decreased root growth with thicker, shorter roots, but accumulated higher concentration of Zn in roots when supplied at adequate levels.

Marschner and Cakmak (1989) and Moraghan and Mascagni (1991) found that high light intensity and long day length are the major factors in promoting Zn stress in plants.

The effect of season on Zn concentration of annual pastures is summarised in the Table 2.8. It indicates that the Zn concentration in pasture samples in South West Australia can fall by 50% between spring and summer (White *et al.*, 1991). It is therefore evident that plant material, which is adequate in Zn with respect to plant growth can become deficient with respect to animal health and production.

Table 2.8 Effect of season on Zn concentration (mg Zn kg⁻¹) of annual pastures in South West Australia.

Season	Growth stage	Masters and Somers (1980) ^A		White <i>et al.</i> (1991) ^B
		Minimum	Maximum	
Winter	Slow growth	20	51	-
Spring	Active growth	24	34	20
Summer	Mature, mainly dry	12	39	12
Autumn	Dry, dead	15	27	10

^AMean of 6 properties.

^BSingle site.

2.9.4 Effect on Cd concentration

Tiller (1988) found that an increase of soil temperature from 10^o C to 18^o C can give rise to a four fold increase in Cd uptake and Cd recovered from fertilizer by subterranean clover. He further stated that species differences can lead to several fold differences in Cd concentration in plants.

Roberts *et al.* (1994) observed that the weeds in grassland have high concentration of Cd due to the high proportion of weeds harvested during autumn. The lowest pasture growth rate was observed in autumn.

Loganathan *et al.* (1996) have found in all three sampling years that Cd concentration was highest during autumn and lowest in spring. When clover growth rate was high during summer, the Cd concentration of clover become lower due to a dilution effect but Cd uptake is high. The clover production was very small, and therefore, the seasonal pattern of herbage Cd concentration and uptake was mainly controlled by the grass Cd concentration and uptake. They have also stated that the differences could be due to changes in temperature, soil moisture and species between seasons.

2.10 Effect of Zn and Cu fertilizers on Zn and Cu concentration

2.10.1 Effect on Cu concentration

Copper concentration in forage and pasture crops depends on soil availability of Cu, plant species, stage of growth, time of year, and lime and fertilizer applications. Legumes tend to take up larger amounts of Cu than grasses. In some cases, non-crop species or weeds may also contribute to the increased dietary intake of Cu by grazing livestock.

Kubota (1983) found that mixed pasture herbage rarely contains more than 20 mg Cu kg⁻¹ of dry matter and usually less than 10 mg Cu kg⁻¹.

Excessive levels of Cu in soils are not expected to cause forage levels above 20-30 mg Cu kg⁻¹, so the forage is safe for sheep and cattle when the soil level of Mo is in the range of 2-5 mg kg⁻¹ and the soil pH is 6 or above. However, grazing animals may ingest up to 10 times more Cu in the form of soil than in the herbage. Soil ingestion commonly ranges from 1-10% of the dry matter intake of grazing cattle and upto 30% of sheep (Thornton, 1979).

Wells (1957) recorded that the pasture Cu concentration ranged from 3.5 to 18 mg Cu kg⁻¹. He also found that pasture species had a marked differences in Cu concentration. The Cu concentration in white clover, red clover and ryegrass was 10.6 mg kg⁻¹, 19.1 mg kg⁻¹ and 4.0 mg kg⁻¹, respectively when these plants were grown on the same soil type.

Gilkes and Lim-Nunez (1979) observed that increasing levels of Cu promoted Cu uptake in wheat. Jarvis (1978) found that with increasing concentrations of Cu in solution, the Cu concentration in roots of most species increased much more rapidly than that of shoots in ryegrass. He also found that in most species Cu concentrations seldom exceeded 30 mg Cu kg⁻¹ when very large additions of Cu (953 mg Cu kg⁻¹ soil or 10 mg Cu L⁻¹ solution) have been made to either soils or solution cultures.

Rasheed and Seeley (1966) found that the Cu concentration in legume shoots increases more rapidly than grass.

Davis and Beckett (1978) estimated that the upper critical tissue concentration of Cu in rye grass was 21 mg kg⁻¹. Similarly Davis and Carlton Smith (1984) recorded that the upper critical foliar concentration of Cu in ryegrass was 22 mg Cu kg⁻¹.

Reuter *et al.* (1981) noted that the increasing rates (ranged from 0 to 533 mg Cu kg⁻¹) of Cu application increased the Cu concentration in plant tops and roots.

Plenderleith and Bell (1984) found that with increasing addition of Cu (4 to 600 mg Cu kg⁻¹ soil) the Cu concentration in tropical grasses increased from 17 to 27 mg kg⁻¹.

Alva and Chen (1995) observed that the concentration of Cu in shoots increased linearly both in *Cleopatra mandarin* and *Swingle citrumelo* citrus rootstock seedlings with an increase in external Cu concentration.

Minnich *et al.* (1987) measured Cu^{2+} activity on soil saturation extracts and related it to the accumulation of Cu in young snap bean (*Phaseolus Vulgaris* L.). They found that the Cu concentration in both shoots and roots increased with measured Cu^{2+} activity.

2.10.2 Effect on Zn concentration

Ruano *et al.* (1987) observed that the Zn concentrations of the stems increased from 105 to 347 mg kg^{-1} in bush beans grown with increasing levels of Zn (ranging from 0.13 to 0.75 mg Zn L^{-1}).

Singh and Jeng (1993) concluded that the concentration of Zn increased linearly with increasing Zn application rates in ryegrass.

Davis and Beckett (1978) estimated that the upper critical tissue concentration of Zn in rye grass was 201 mg kg^{-1} . Davis and Carlton Smith (1984) measured the upper critical foliar concentration of Zn in ryegrass was 140 mg Zn kg^{-1} .

Plenderleith and Bell (1984) found that the Zn concentration in tropical grasses ranged from 475 to 1925 mg kg^{-1} by addition of 4 to 1000 mg kg^{-1} of Zn.

According to Chaney, Lee and Murray (1990), Perennial ryegrass and Canadian bluegrass contained 1040 and 898 mg Zn kg^{-1} respectively.

Australian Food Information Centre (1987) recorded that the vegetative part of perennial ryegrass contained 57 to 138 mg Zn kg^{-1} .

2. 11 Toxicity effect

2.11.1 Toxicity effect of Cu

Copper (Cu) is required in trace amounts for various metabolic processes in plants, at higher concentrations Cu is toxic to plant growth. Published reports on Cu toxicity to plant includes the effects on photosynthesis (Lidon and Henriques, 1991) and nitrogen metabolism (Weber *et al.*, 1991).

Heavy metals such as Cu tend to accumulate in the roots and in turn, affect the growth of the whole plant. Lidon and Henriques (1992a and 1993) observed that the Cu concentration in roots increased linearly with increasing external Cu concentration. Decreased translocation of Cu to the above ground parts from the roots has been suggested as a mechanism to withstand Cu toxicity.

Beckett and Davis (1977) found that the high levels of Cu depressed plant growth in barley. Alva and Chen (1995) observed that with an increase in external Cu concentration, the shoot and root dry weight of citrus plants decreased significantly.

Yongming and David (1995) observed Cu and Zn interactions on barley plant growth and concluded that the growth of barley was controlled principally by the amounts of both added Zn and Cu. The effect of the added Cu was to increase the toxicity of the added Zn.

2.11.2 Toxicity effect of Zn

Boawn and Rasmussen (1971) found that the tissue Zn concentrations in a range of crops are associated with a 20% yield decrease. Response was evaluated in terms of dry matter yield decrease and Zn concentration in tops. Zinc concentration that decreased growth by 20% ranged from 295 mg kg⁻¹ for alfalfa to 770 mg kg⁻¹ for spinach. They also found that the increasing levels of Zn up to 500 mg Zn kg⁻¹ increased the Zn concentration in clover tops upto 252 mg kg⁻¹ and decreased the yield by 9%. They observed that crops that underwent a significant yield reduction because of

excess Zn were stunted but showed no discoloration, malformation, or necrosis indicative of a direct metal toxicity.

White *et al.* (1979a) found that leaf dry weight of a number of soybean cultivars was decreased by the higher Zn addition (393 mg kg^{-1}) and tissue Zn concentration increased by addition of increasing levels of soil Zn.

White *et al.* (1979b) conducted an experiment to evaluate the variation of tolerance among cultivars of soybean to phytotoxic levels of added soil Zn at two pH levels (pH 5.5 and 6.5). They found that soil Zn addition significantly reduced the yield of all parts except the primary leaves at pH 5.5 and also recorded a positive linear relationship between soil Zn addition and leaf Zn content at both pH levels. The soybeans with leaf Zn concentrations of 400 to $730 \text{ mg Zn kg}^{-1}$ in plants suffered yield reductions of 10-33%. They also stated that addition of Zn salts such as ZnSO_4 normally strongly acidify soils as the Zn displaces protons from the adsorption surfaces.

Chaney *et al.* (1975) showed the interaction of soil pH and total soil Zn (added ZnSO_4) in a pot study with soybeans. As soil Zn was increased, the critical pH for alleviation for Zn phytotoxicity rose.

Plenderleith and Bell (1984) conducted an experiment to evaluate the growth response of 12 sub-tropical grasses with addition of Cu and Zn. They observed that depending on the species the Cu and Zn concentration was associated with a 50% yield reduction ranging from 17 to 27 mg kg^{-1} and 475 to 1925 mg kg^{-1} , respectively.

CHAPTER THREE

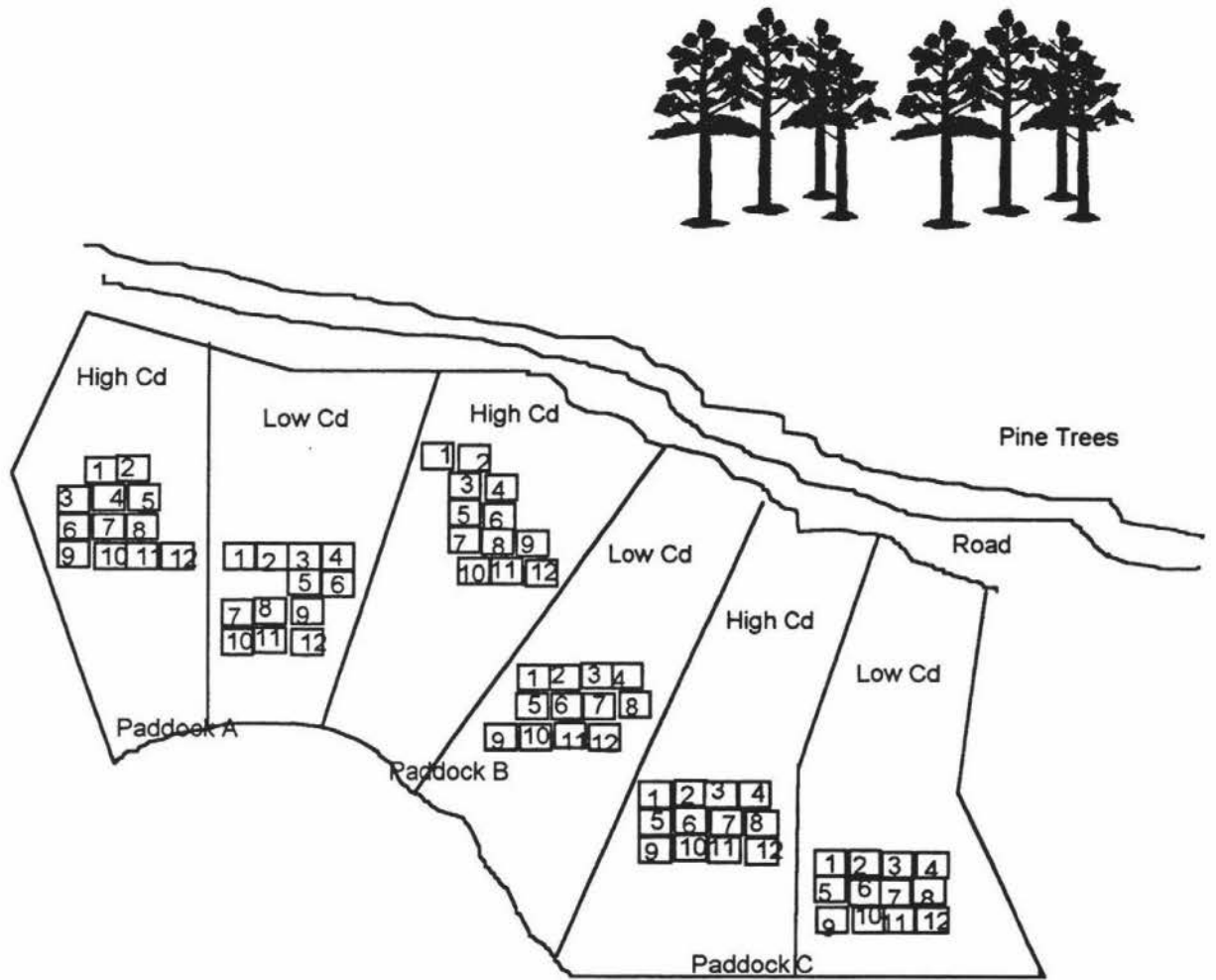
MATERIALS AND METHODS

3.1 Location and fertilizer management

The study area was at the AgResearch Hill Country Research Station, Ballantrae, located in the foothills (250-350 m altitude) of the Southern Ruahine Range near Woodville, 20 km north-east of Palmerston North, New Zealand. The average annual rainfall is 1200 mm.

The soils are yellow brown/yellow grey earth intergrades and related steepland soils. This trial site is typical of much of the moist hill pastures in the Southern North Island of New Zealand. Superphosphates used over the past 20 years on the station were made from blends of phosphate rocks having different concentrations of Cd (ranging from 5 to 71 mg Cd kg⁻¹) and P (ranging from 11.9% to 16.4%) (Loganathan *et al.*, 1995).

Three pairs of plots (paddocks) with either low (0.2 mg Cd kg⁻¹) or high (0.6 mg Cd kg⁻¹) background soil Cd status were selected (Figure 3.1). A small field experiment was laid down in each of the six plots, with different levels of Zn and Cu fertilizers added as ZnSO₄.7H₂O and CuSO₄.5H₂O, respectively.



Slope categories of the plots

- Paddock A: High background cadmium Plot (14-18⁰)
Low background cadmium Plot (15-17⁰)
- Paddock B: High background cadmium Plot (18-22⁰)
Low background cadmium Plot (15-18⁰)
- Paddock C: High background cadmium Plot (5-10⁰)
Low Background Cadmium Plot (5-10⁰)

Figure 3.1 Experimental layout in the field (the numbers correspond to the treatment numbers outlined in Table 3.1).

3.2 Fertilizer treatments

There were 12 treatment combinations of Zn and Cu (kg ha^{-1}) fertilizers on each plot. The combinations were Zn_0Cu_0 , Zn_0Cu_2 , Zn_0Cu_5 , $\text{Zn}_0\text{Cu}_{10}$, Zn_5Cu_0 , Zn_5Cu_2 , Zn_5Cu_5 , $\text{Zn}_{15}\text{Cu}_0$, $\text{Zn}_{15}\text{Cu}_2$, $\text{Zn}_{15}\text{Cu}_5$, $\text{Zn}_{40}\text{Cu}_0$ and $\text{Zn}_{40}\text{Cu}_{10}$. The treatment combinations are shown in Table 3.1.

Table 3.1 Treatment combinations of the experiment.

Cu levels (kg ha^{-1})	Zn levels (kg ha^{-1})			
	0	5	15	40
0	1	5	8	11
2	2	6	9	*
5	3	7	10	*
10	4	*	*	12

* Denotes this treatment combination was not used in the experiment.

3.3 Field preparation

Six plots of a size approximately 36 m^2 were selected in three pairs of paddocks. The plots were mown and fenced on July 7, 1995, to exclude the grazing animal. Twelve sub-plots ($1.2 \text{ m} \times 1.2 \text{ m}$) were marked using wooden pegs. The sub-plots and the surrounding area, within each plot, were mown to allow equal pasture growth. The sub-plots were mown by grass mower after each harvest.

3.4 Experimental design

The experiment was laid out as a split plot design. The whole experimental area was divided into three pairs of paddocks. Each pair consisted of paddocks with low and high background soil Cd status. These two were considered as the main plots in the design. An area 6 m × 6 m was selected within each main plot and the treatments (Table 3.2) were laid out in each of them. All the 12 sub-plots in each main plot were of a uniform size (1.44 m²). Thus there were two main plots (low and high background soil Cd status) and 12 split plots (12 treatments). The main plots have 3 replications and the sub-plots have 6 replications.

3.5 Fertilizer rate and application

In New Zealand, generally 2-5 kg Cu is applied per hectare annually to correct Cu deficiencies in pasture. Zinc is not normally applied to New Zealand pastures. In the present experiment, we used a range of levels of Zn (0, 5, 15 and 40 kg Zn ha⁻¹) and Cu (0, 2, 5 and 10 kg Cu ha⁻¹) to examine the effect of increasing Zn and Cu levels on pasture Zn and Cu concentration and their subsequent effects on Cd uptake.

The amounts of Zn and Cu fertilizers required per sub-plot were calculated from the fertilizer rates per hectare (Appendix 1). Zinc sulfate (ZnSO₄·7H₂O) and copper sulfate (CuSO₄·5H₂O) were used as a source of Zn and Cu, respectively. In addition, single superphosphate was used as a carrier fertilizer at the rate of 40 kg P ha⁻¹, which was mixed with dry river sand at the ratio of 1:10. The river sand contained negligible amounts of Zn (56 mg kg⁻¹). The fertilizers were mixed thoroughly and broadcast on to the individual sub-plot (Figure 3.1) on July 12, 1995.

3.6 Collection of soil and pasture samples

3.6.1 Soil sampling

A first set of soil samples was collected using a corer (25 mm diameter) at 0-75 mm depth from each plot in the month of July, 1995 (before the application of fertilizer). Each sample was a composite of 10 cores taken randomly. A second set of soil samples was collected from each individual sub-plot containing 5 cores of soil taken randomly in the month of September, 1995.

Soil samples were dried (65⁰ C) and soil aggregates were crushed using a porcelain mortar and pestle. Plant roots were ground in Cyclotec 1093 sample mill with a tungsten-carbide grinding ring and chamber and the soil and root samples were then mixed thoroughly. Sub-samples were taken and were again ground using the porcelain mortar and pestle.

3.6.2 Pasture sampling

A first set of pasture samples was collected from each plot before fertilizer application in July, 1995. Subsequent pasture samples were collected at 73, 119, 139, 159 and 187 days after fertilizer application. The pasture samples were collected from each sub-plot with electrical shears from a quadrat area of 0.0976 m². The pastures were cut to a height of approximately 0.5 cm.

The samples were weighed and washed with reverse osmosis (RO) water and dried using a freeze-drier for the initial samples. Further samples were dried at 70⁰ C in a forced air oven. The dry weights of the pasture samples were recorded and the samples were then ground using Cyclotec 1093 sample mill, and kept in air tight polyethylene bags for chemical analysis.

3.7 Botanical composition

At the third harvest (139 days) the botanical composition (legume, grass and weed) of the pasture was ascertained by direct harvesting of the pasture with shears followed by hand separation and drying of each component (Brown, 1954). The dry weight of the different species was recorded. At the fourth harvest (159 days) the grass and legume species were separated by hand and the samples were washed with RO water and dried at 70^o C in a forced air oven. The grass and legume samples were then ground using Cyclotec 1093 sample mill and kept separately in air tight polyethylene bags for chemical analysis.

3.8 Soil and herbage sample preparation

3.8.1 Initial soil for chemical analysis

Soil pH (1:2.5 H₂O), cation ion exchange capacity by 1M NH₄OAc (pH 7) extraction, Olsen P by 0.5 M NaHCO₃ extraction, sulphate by 0.04 M Ca(H₂PO₄)₂ extraction, organic carbon by 1N K₂Cr₂O₇ extraction (Black *et al.*, 1965), pseudo-total Cd by HNO₃ and HCl extraction and exchangeable K, Ca, Mg and Na were determined by standard methods (Blackmore *et al.*, 1987).

3.8.2 Soil samples for pseudo-total Cd

A known weight (0.5 g) of finely ground soil samples were placed into 50 ml acid-washed conical flasks. About 10 ml of (1:4) Aristar grade HNO₃ (69%) and Aristar grade HCl (37%) was added. A reflux funnel was placed on top of the conical flask and left overnight under a fumehood. The sample was then digested for at least 4 hours at 150^o C (until brown fuming stopped). The funnel was then removed and the temperature was increased slowly to 250^o C and the digest was boiled down for 2-3 hours on a hot plate, until first dry spots appeared. 10 ml of 1% HNO₃ was added to the dry (digested) samples and left for 16 h. The flask was then swirled and was left to settle for another 6 h. The supernatant was poured into 10 ml auto storage tubes. A one ml aliquot was taken from the storage tube and the volume made up to 10ml with 1% HNO₃ for the determination of pseudo-total Cd.

3.8.3 Soil samples for available Zn, Cu and Cd

3.8.3.1 0.01M CaCl₂ extractant

A known weight (4.0 g) of the finely ground soil samples were placed into 50 ml polyethylene centrifuge tubes with 20 ml of 0.01M CaCl₂ (Whitten and Ritchie, 1991). The suspension was then shaken in an end-over-end shaker for 16 h at 25⁰ C and then centrifuged for two minutes at 5000 rpm in the Sorvall RC 5C automatic superspeed refrigerated centrifuge and the supernatant was filtered through Whatman No. 41.

3.8.3.2 0.1M HCl extractant

A known weight (8.0 g) of the finely ground soil samples were placed into the 50 ml polyethylene centrifuge tubes with 20 ml 0.1M HCl (Haynes and Swift, 1985). The suspension was then shaken in an end-over-end shaker for 2 h at 25⁰ C and then centrifuged for two minutes at 5000 rpm in the Sorvall RC 5C automatic superspeed refrigerated centrifuge and the supernatant was filtered through Whatman No. 42.

3.8.3 Pasture samples for Zn, Cu and Cd

A known weight (0.4 g) of the finely ground pasture (mixed, grass or legume) samples were poured into 50 ml acid-washed conical flasks. About 10 ml of Aristar grade HNO₃ (69%) was added each conical flask. A reflux funnel was placed on top and left for 16 h under a fumehood. The samples were then digested for at least 4 h at 150⁰ C (until brown fuming stops). The reflux funnel was removed and the temperature was increased slowly upto 250⁰ C. The sample was boiled until just dry and then mixed with a further 10 ml 1% HNO₃ (69%) to make the volume upto 10 ml. The extractant was then stored in storage tubes for chemical analysis.

3.9 Chemical Analysis

Cadmium in the soil and plant digests, and in the soil extract (0.01M CaCl₂) was analysed by Zeeman graphite furnace atomic absorption Spectrophotometry.

Zinc and copper in the plant digests and in the soil extract (0.1M HCl) were analysed using an Inductively Coupled Plasma (ICP) analyser. Also the Cd in the soil extract (0.1M HCl) was analysed using the ICP.

All glassware were acid washed, rinsed with deionized water, and oven dried before use. To check the reproducibility of the analytical procedure, three blanks were included in each batch of 42 samples of pasture and soil. Certified soil and herbage samples were also analysed in parallel with the unknown samples. All results are expressed on an oven-dry weight basis.

3.10 Statistical analysis

Statistical analysis of all data was carried out using the 6.10 program (SAS Institute Inc., 1994) on an IBM computer. The dry matter yield, Zn, Cu and Cd concentration of mixed pasture and individual species, botanical composition, available Zn, Cu and Cd content in soil were subjected to analysis of variance to determine the statistical significance of the effects of the Zn and Cu treatments, background soil Cd status and the treatment interactions. The statistical parameters (LSD, CV) in the model were estimated by the Least Significant Difference Test (LSD). In the discussion 5% significance level is denoted by * and 1% level of significance is denoted by **.

CHAPTER FOUR

RESULTS AND DISCUSSION

The effect of different levels of Cu and Zn fertilizers on dry matter (DM) yields of pasture as well as Zn, Cu and Cd content in mixed pasture was measured at 73, 119, 139, 159 and 187 days following the addition of fertilizers. The dry matter yields of species (grass and legume) as well as Zn, Cu and Cd content in species were determined at 159 days after fertilizer application. The pseudo-total Cd content of the soil was measured before and after fertilizer application. The available Zn, Cu and Cd content of the soil was also measured for soil samples collected at 73 days after addition of fertilizer. The results are presented and discussed below for all the agronomic and chemical attributes, firstly the effect of the Zn and Cu treatment combinations is discussed followed by the effects of Cu and Zn levels and the background Cd status. The seasonal effect on DM yield, Cu, Zn and Cd concentration will be discussed in a separate section (4.9).

4.1 Reliability of the data of Zn, Cu and Cd concentration in soil and pasture samples

Standard soil and herbage samples were used in every batch of samples analysed and both the experimental and the standard samples were digested and analysed simultaneously. There was close agreement between the measured concentration of Cu, Zn and Cd and the values given for the standard herbage and soil samples. These results suggest that there was a negligible error in the digestion and analysis of the samples. Herbage samples were thoroughly washed with RO water before digestion. Further the data on Fe and Al in the herbage samples indicate that there was a negligible contamination by soils of the herbage samples.

Care was taken to avoid contamination during drying and grinding by using the same drier and grinder for all harvests except harvest 5, where samples were dried in a different drier.

The Cu and Cd concentrations in the herbage repeated in this study were within the range obtained by Grace *et al.* (1995) and Loganathan *et al.*(1995), respectively for the site on the hill country research station. However the Zn concentrations were high in harvests 2 to 5, and particularly harvests 3 and 5.

Since we were not able to identify the reasons for the high levels of Zn concentration in herbage, Zn data from harvests 2 to 5 are not reported or discussed in this section. Zn data for harvests 2 to 5 are reported in the Appendix 5 only. These data should not be used for drawing conclusions.

4.2 Initial chemical properties

4.2.1 Initial soil chemical properties

The initial soil properties are presented in Table 4.1. Except for the pseudo-total Cd, there was no significant difference in other properties between the low and high background Cd status paddocks. The pH value of the soil samples obtained from the low and high background Cd status was 5.4 and 5.5 respectively. The CEC of soil samples was 22 (high background Cd) and 23 cmoles kg⁻¹ (low background Cd). The organic matter content of the soil was 5.9% (high background Cd) and 6.16% (low background Cd). The Olsen P obtained was 28.4 mg kg⁻¹ in low background Cd and 34 mg kg⁻¹ in high background Cd. The pseudo-total Cd content of the soil was higher (0.093 mg kg⁻¹) in the high background Cd status plots than in the low background Cd status plots (0.042 mg kg⁻¹). The amounts of exchangeable cations were at sufficient level.

To maintain a balance between the major cations it is suggested that the soil test values of Ca, K and Mg be in the ratio of 1.6-2.0:1:2 (Clarke *et al.*, 1986). Due to the nature of cation measurement and the mineralogy of the clay fraction in New Zealand soils there are major difficulties associated with achieving the so called “optimum ratio” based on this measurement. As a consequence of this the percent saturation of each of the elements Ca, Mg and K will be artificially low, implying that additional fertilizers are required if strict adherence to the optimum ratios is being followed.

Table 4.1 Initial soil chemical properties

Characteristics	Paddock A		Paddock B		Paddock C	
	H	L	H	L	H	L
Soil pH	5.6	5.4	5.4	5.4	5.5	5.6
Olsen P (mg kg⁻¹)	33.3	21.4	30.0	28.6	38.6	35.7
SO₄ (mg kg⁻¹)	18.0	9.5	8.5	10.0	11.0	11.0
Exch. K (c moles kg⁻¹)	1.08	1.04	0.66	0.64	0.86	1.33
Exch. Ca (c moles kg⁻¹)	9.6	7.7	5.8	7.8	6.0	6.3
Exch. Mg (c moles kg⁻¹)	1.57	1.32	1.20	1.43	1.21	1.97
Exch. Na (c moles kg⁻¹)	0.16	0.15	0.16	0.17	0.15	0.19
CEC (c moles kg⁻¹)	26	23	21	24	19	22
Organic carbon(%)	5.74	5.55	6.22	6.6	5.78	6.35
Total Cd (mg kg⁻¹)	0.0926	0.0422	0.0801	0.0472	0.1076	0.0379

H, represents high background Cd status soil.

L, represents low background Cd status soil.

Table 4.2 Chemical composition of the initial mixed pastures.

Site	Cd status	Cu (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cd (mg kg ⁻¹)
Paddock A	H	12.7	31.5	0.2802
	L	7.8	26.0	0.1550
Paddock B	H	10.4	25.0	0.1843
	L	4.0	15.0	0.0574
Paddock C	H	16.3	43.5	0.3642
	L	14.6	41.75	0.2155

H, represents high background Cd status soil.

L, represents low background Cd status soil.

4.2.2 Initial pasture chemical composition

The initial pasture chemical composition is presented in Table 4.2. There was a significant difference in pasture Cd concentration between the low Cd status (0.142 mg kg⁻¹) and high Cd status (0.276 mg kg⁻¹) soils. In general the pasture from the low background Cd status soils contained less Cu and Zn than those from the high background Cd status soils. The mean Cu concentration of the pasture samples obtained from the low and high background Cd status soils was 8.8 and 13.3 mg kg⁻¹ respectively. Similarly the mean Zn concentration was 27.5 mg kg⁻¹ (low background Cd status) and 33.3 mg kg⁻¹ (high background Cd status).

4.3 Dry matter yield of pasture
4.3.1 Dry matter yield of mixed pasture.
4.3.1.1 Effect of treatments

Dry matter yield of the pasture was recorded after each harvest. Results presented in Appendix 2 indicate that the treatment effect of Zn and Cu fertilizer application on DM yield was significant in harvests 1, 2 and 3.

At harvest 1, the dry matter yields ranged from 405 kg ha⁻¹ in Zn₅Cu₀ to 919 kg ha⁻¹ in Zn₀Cu₀. The treatment Zn₀Cu₀ produced the highest pasture yield of 919 kg ha⁻¹ which was similar to DM yields for treatments Zn₀Cu₂, Zn₅Cu₅, Zn₄₀Cu₀ but significantly higher than the yields recorded in the rest of the treatments.

The DM yields ranged from 1963 kg ha⁻¹ in Zn₅Cu₀ to 2905 kg ha⁻¹ at Zn₀Cu₀ treatment at harvest 2. The treatment Zn₀Cu₀ produced the highest pasture yield of 2905 kg ha⁻¹, which was similar to DM yields due to Zn₀Cu₂, Zn₀Cu₅, Zn₀Cu₁₀, Zn₅Cu₂, Zn₅Cu₅, Zn₁₅Cu₅, Zn₄₀Cu₀, but significantly higher than the yields recorded in the rest of the treatments.

The dry matter yields ranged from 693 (Zn₀Cu₂) to 1015 (Zn₁₅Cu₂) kg ha⁻¹ at harvest 3. The treatment Zn₁₅Cu₂ produced the highest dry matter yield, which was similar to all treatments except the treatment Zn₀Cu₂.

The dry matter yields ranged from 1514 (Zn₁₅Cu₅) to 2336 (Zn₀Cu₅) kg ha⁻¹ and 951 (Zn₁₅Cu₂) to 1459 (Zn₀Cu₀) kg ha⁻¹ for harvests 4 and 5 respectively. The treatment effect of Zn and Cu fertilizers on dry matter yield was not significant in harvest 4 and 5.

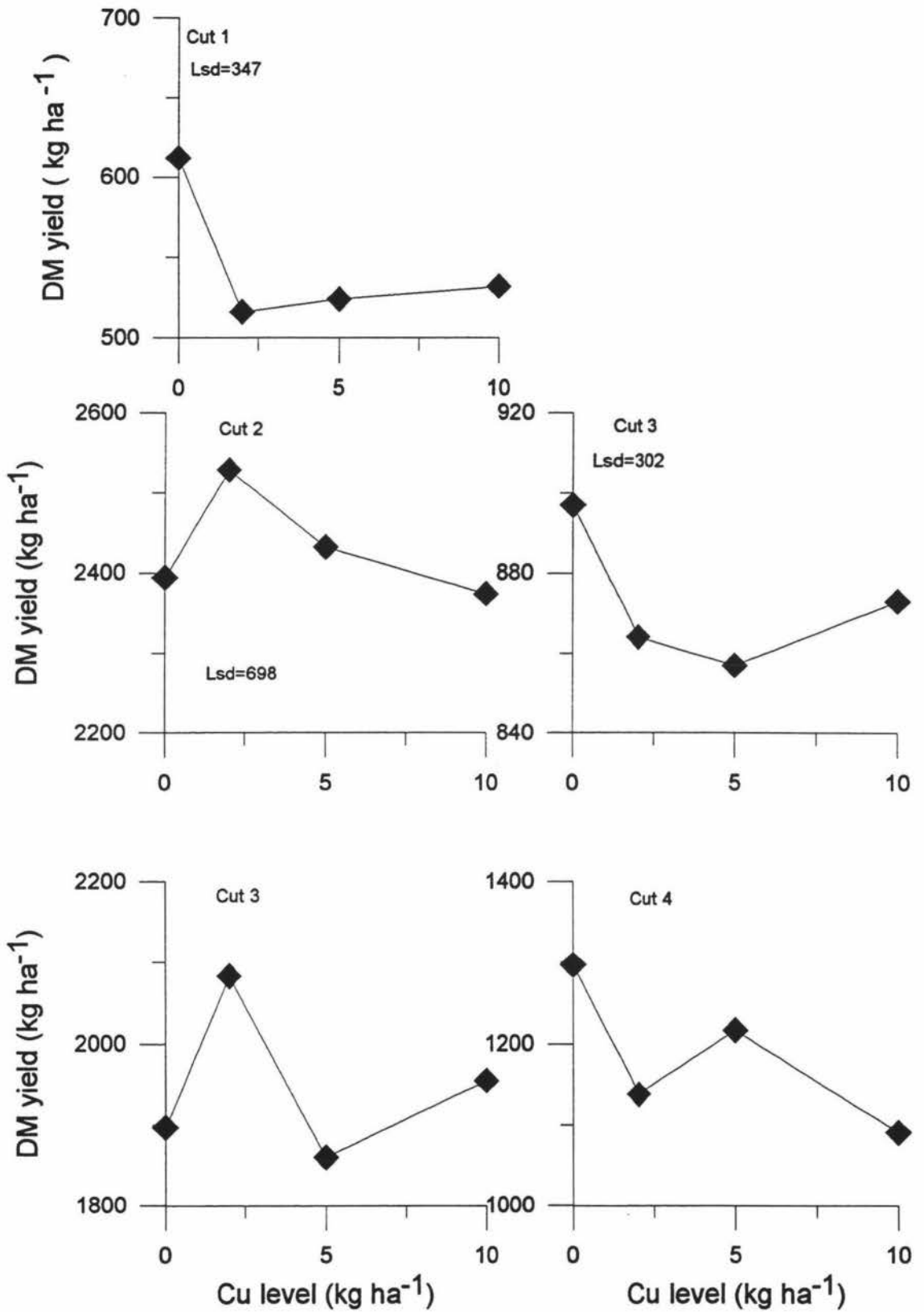


Fig. 4.1. Effect of Cu on DM yield of mixed pasture.

4.3.1.2 Effect of Cu level

The range of mean DM yields of mixed pasture for the different Cu levels was 516 to 612 kg ha⁻¹, 2394 to 2529 kg ha⁻¹, 857 to 897 kg ha⁻¹, 1860 to 2083 kg ha⁻¹ and 1091 to 1297 kg ha⁻¹ at harvests 1, 2, 3, 4 and 5 respectively. It was observed that the Cu₀ level produced the highest DM yield of mixed pasture at harvests 1, 3, 5 and Cu₂ level produced the highest dry matter yield at harvests 2 and 4 respectively (Fig. 4.1).

Dry matter yield was affected by Zn additions, also by Cu additions and by a Zn-Cu interaction effect. This can be interpreted in two ways: a) Zn alone was not toxic (this appeared to be the case for the smaller Zn additions), but in combination with Cu it became toxic; b) Cu was toxic and its toxicity was affected by the addition of Zn.

Copper is required in trace amounts for various metabolic processes in plants, at higher concentrations Cu is toxic to plant growth. Copper toxicity in plants has been shown to affect photosynthesis (Lidon and Henriques, 1991) and nitrogen metabolism (Weber *et al.*, 1991).

Heavy metals tend to accumulate in the roots and in turn, affect the growth of the whole plant. Lidon and Henriques (1992b and 1993) observed that the Cu concentration in roots increased linearly with increasing external Cu concentration. Decreased translocation of Cu to the above ground parts from the roots has been suggested as a mechanism of withstand Cu toxicity.

Alva and Chen (1995) found that the shoot and root dry weight reduced significantly in *Cleopatra mandarin* and *Swingle citrumelo* citrus rootstock seedlings with an increase in external Cu concentration.

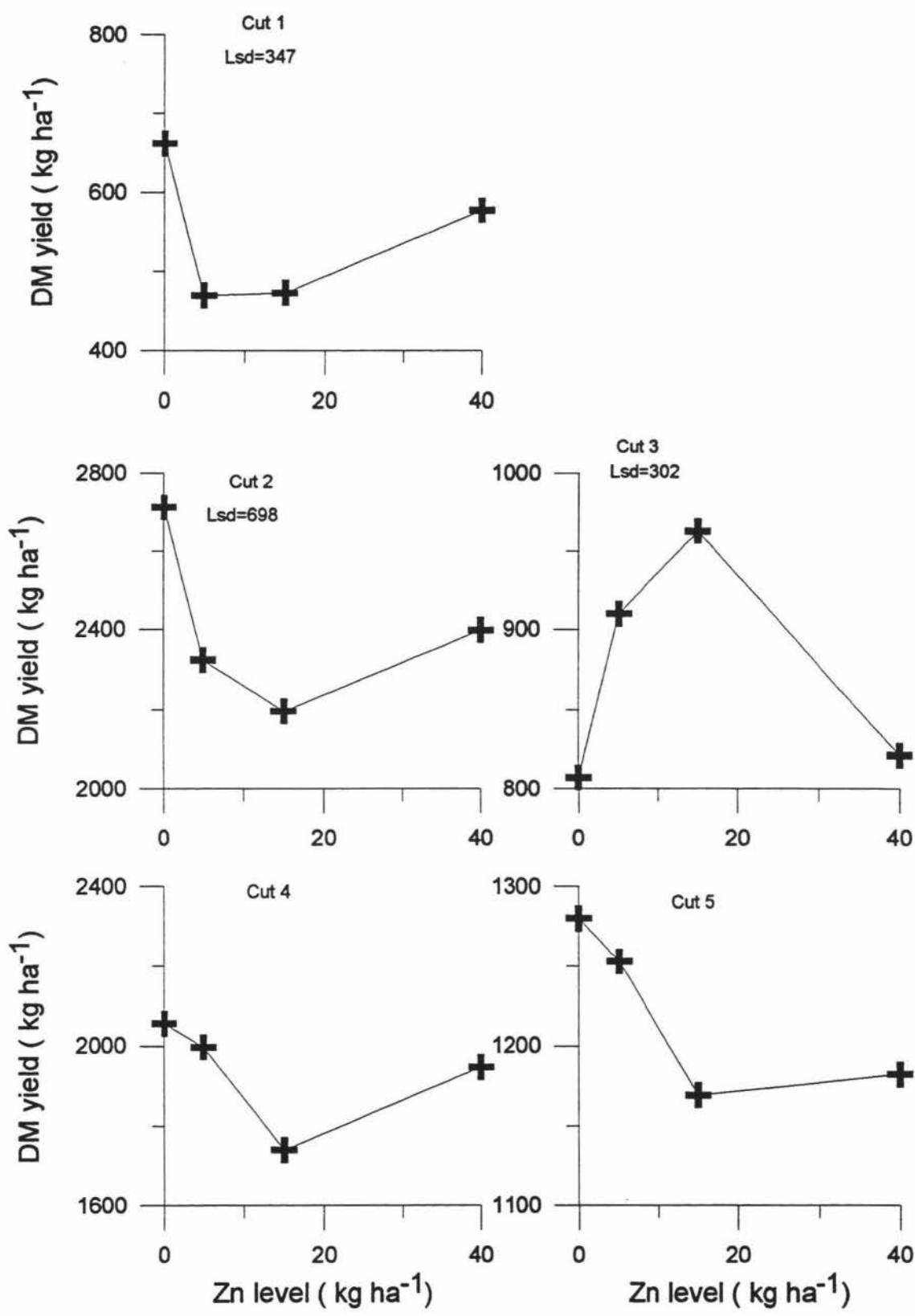


Fig. 4.2 Effect of Zn level on DM yield of mixed pasture.

4.3.1.3 Effect of Zn level

The DM yields of mixed pasture for different Zn levels ranged from 469 to 662 kg ha⁻¹, 2195 to 2712 kg ha⁻¹ and 807 to 963 kg ha⁻¹ at harvests 1, 2 and 3 respectively. It was found that Zn₁₅ level produced the highest DM yield of mixed pasture at harvest 3 but Zn₀ level produced the highest DM yields at harvests 1, 2, 4 and 5 (Fig. 4.2). There was no significant effect of Zn levels on DM yields recorded at harvest 4 and 5.

There was a yield decrease resulting from the addition of Zn fertilizers, when averaged over all treatments. A similar result was observed by Maclean (1976) with addition of Zn (5 mg kg⁻¹) to alfalfa.

Chaney *et al.* (1975) concluded that increasing the concentration of soil Zn, the critical pH for alleviation for Zn phytotoxicity increased.

White *et al.* (1979a) found that the retardation of leaf dry weight in soybean cultivars caused by a higher Zn addition (393 mg kg⁻¹) and tissue Zn concentration increased with increasing levels of soil Zn.

White *et al.* (1979b) observed that the Zn concentration associated with 10-33% yield reduction in soybeans ranged from 400 to 730 mg Zn kg⁻¹ of plants.

The toxicity to Zn was the most likely explanation for the reduction in yields of mixed pasture with increasing Zn levels, both in low and high background Cd status.

4.3.1.4 Effect of background Cd

The mean DM yields for the low and high background Cd status plots were 586 and 518 kg ha⁻¹ at harvest 1, 2377 and 2480 kg ha⁻¹ at harvest 2, 814 and 934 kg ha⁻¹ at harvest 3 and 1199 and 1207 kg ha⁻¹ at harvest 5 (Fig 4.3). It was observed that except for harvest 4 there was no significant difference in DM yields of mixed pasture between the low and high background Cd status plots. At harvest 4, the low background Cd status plots produced lower DM yields of mixed pasture (1526 kg ha⁻¹) than did the high background Cd status (2362 kg ha⁻¹) (Appendix 2) (Fig 4.3).

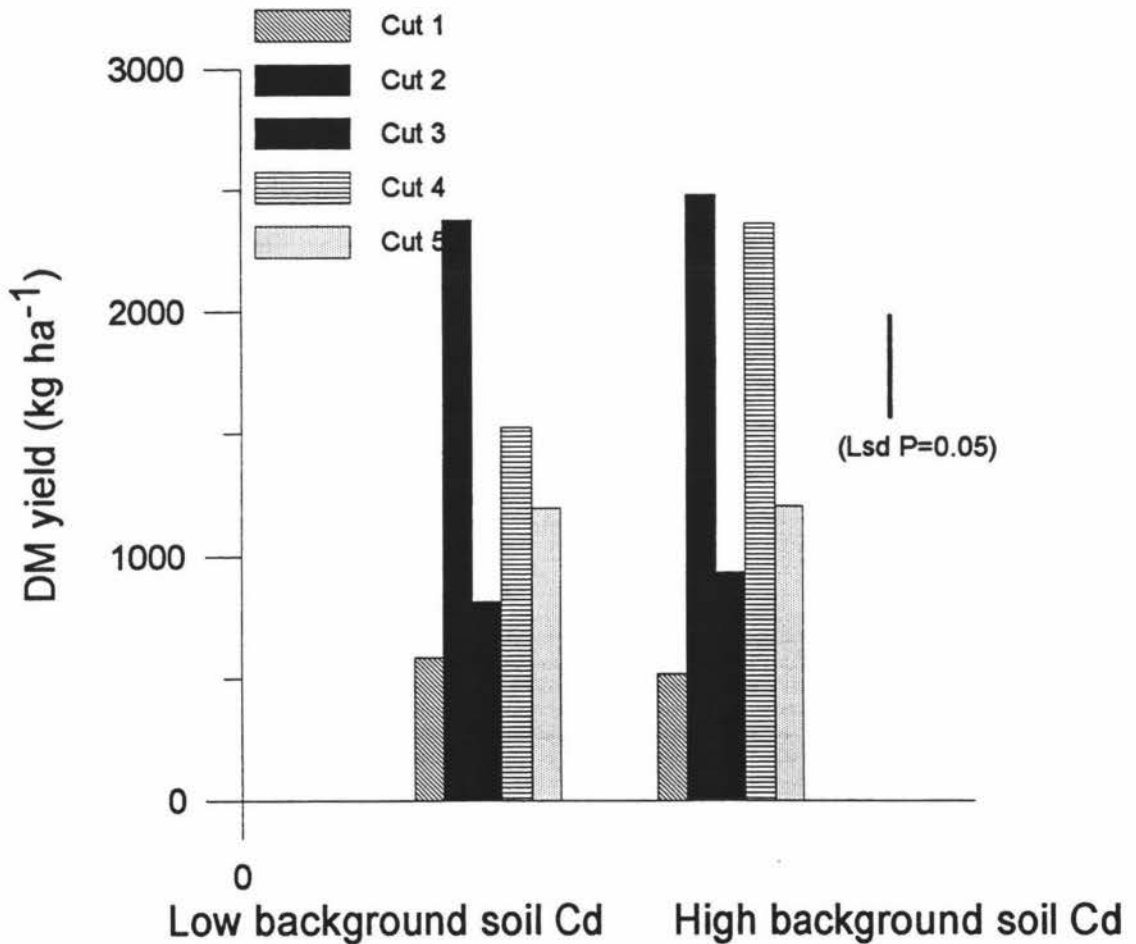


Fig. 4.3 Effect of background Cd on DM yield of mixed pasture.

4.3.2 Dry matter yield of pasture species

4.3.2.1 Effect of treatments

The results presented in Appendix 2 indicate that the treatment effect of Zn and Cu fertilizers on DM yields of grass and legume is not significant. It was recorded that treatment Zn_0Cu_5 produced the highest grasses (1940 kg ha^{-1}) and legumes (352 kg ha^{-1}) DM yield and treatment $Zn_{15}Cu_5$ produced the lowest DM yield of grasses (1303 kg ha^{-1}) and treatment $Zn_{40}Cu_0$ produced the lowest DM yield of legumes (105 kg ha^{-1}).

4.3.2.2 Effect of Cu levels

It was recorded that Cu_2 level produced the highest DM yield of grasses (1746 kg ha^{-1}) and Cu_5 level produced the lowest DM yield of grasses (1581 kg ha^{-1}). But the Cu_5 level produced the highest DM yield of legumes (243 kg ha^{-1}) and Cu_0 level produced the lowest DM yield of legumes (195 kg ha^{-1}) (Fig. 4.4). This indicates that the Cu toxicity is greater in grasses than in legumes. Legumes tend to take up larger amounts of Cu than grasses (Rasheed and Seeley, 1966; Sherrell and Rawnsley, 1982). Increasing Cu supply may also depress plant growth by interfering with the uptake of other nutrients.

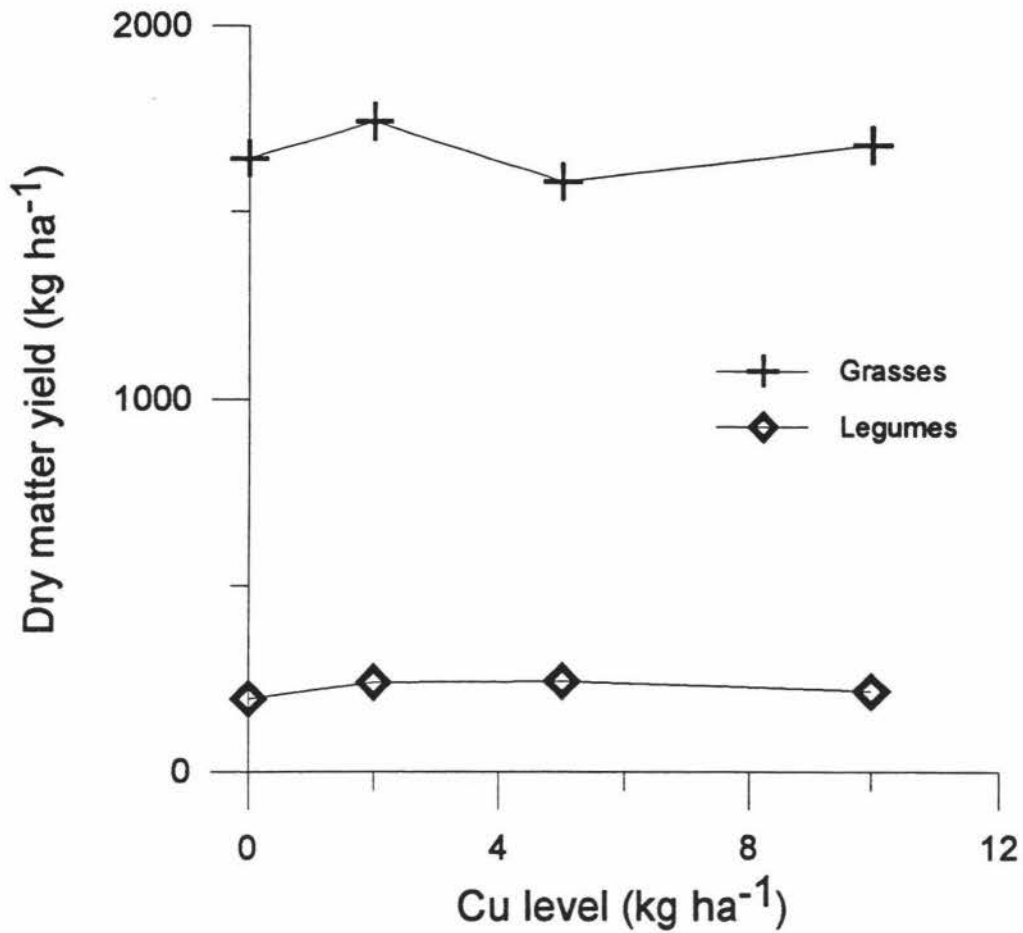


Fig. 4.4 Effect of Cu level on DM yield of pasture species.

4.3.2.3 Effect of Zn levels

The effect of different levels of Zn on DM yield of pasture species was not significant. It showed that Zn₄₀ level produced the highest DM yield of grasses (1777 kg ha⁻¹) but the lowest DM yield of legumes (112 kg ha⁻¹). The lowest DM yield of grasses was recorded at Zn₁₅ level (1546 kg ha⁻¹) and the highest DM yield of legumes was recorded at Zn₅ level (285 kg ha⁻¹) (Fig. 4.5). It shows that the grass species are more tolerant than legumes at higher levels of Zn.

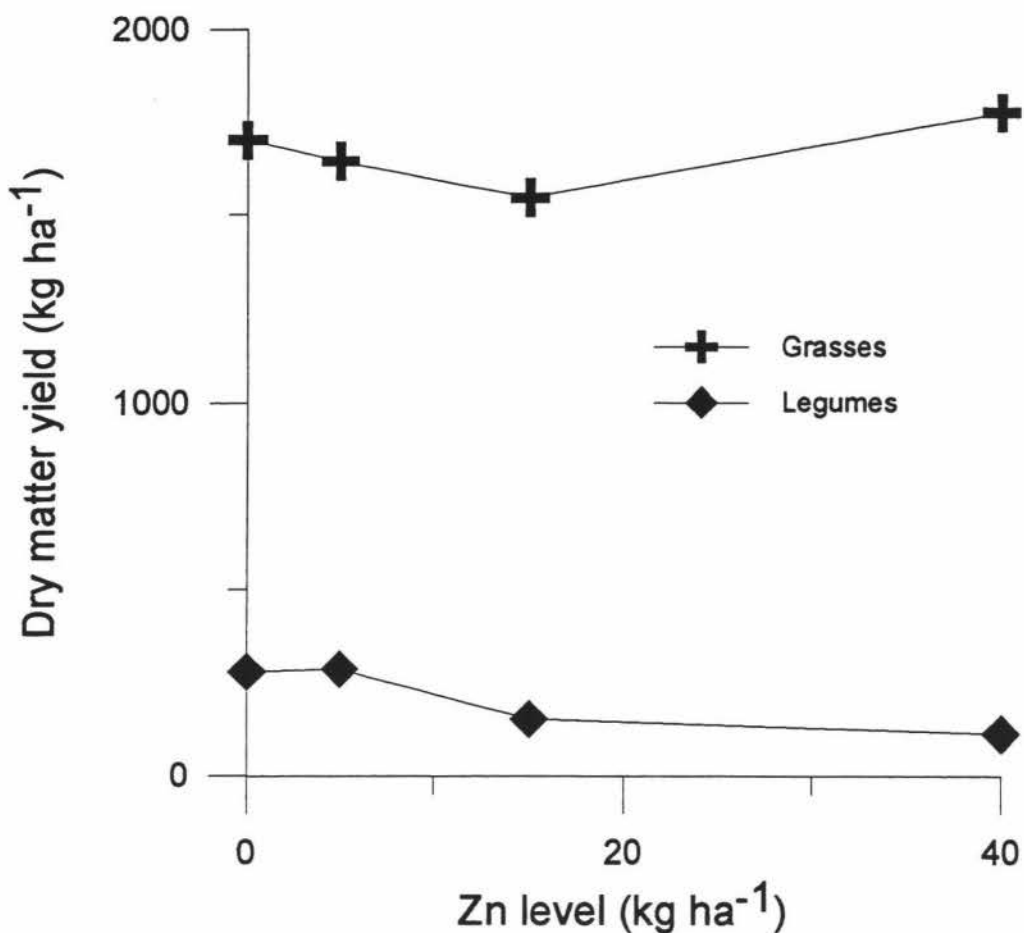


Fig. 4.5 Effect of Zn level on DM yield of pasture species.

Boawn and Rasmussen (1971) observed that the Zn concentration in shoots associated with a 20% yield reduction, ranged from 240 mg kg⁻¹ for field beans to 740 mg kg⁻¹ for sugar beet. They also found that increasing Zn concentrations in soils (up to 500 mg kg⁻¹) increased the Zn concentration in the tops of clover (252 mg kg⁻¹) and alfalfa (345 mg kg⁻¹) and reduced the yield by 9% and 22%, respectively.

4.3.2.4 Effect of background Cd

The effect of background Cd status on DM yield was significant for grasses but not for legumes. The low background Cd status produced lower DM yield both in grasses (1299 kg ha⁻¹) and legumes (183 kg ha⁻¹) than did the high background Cd status (grasses-2022; legumes-259 kg ha⁻¹) (Fig. 4.6).

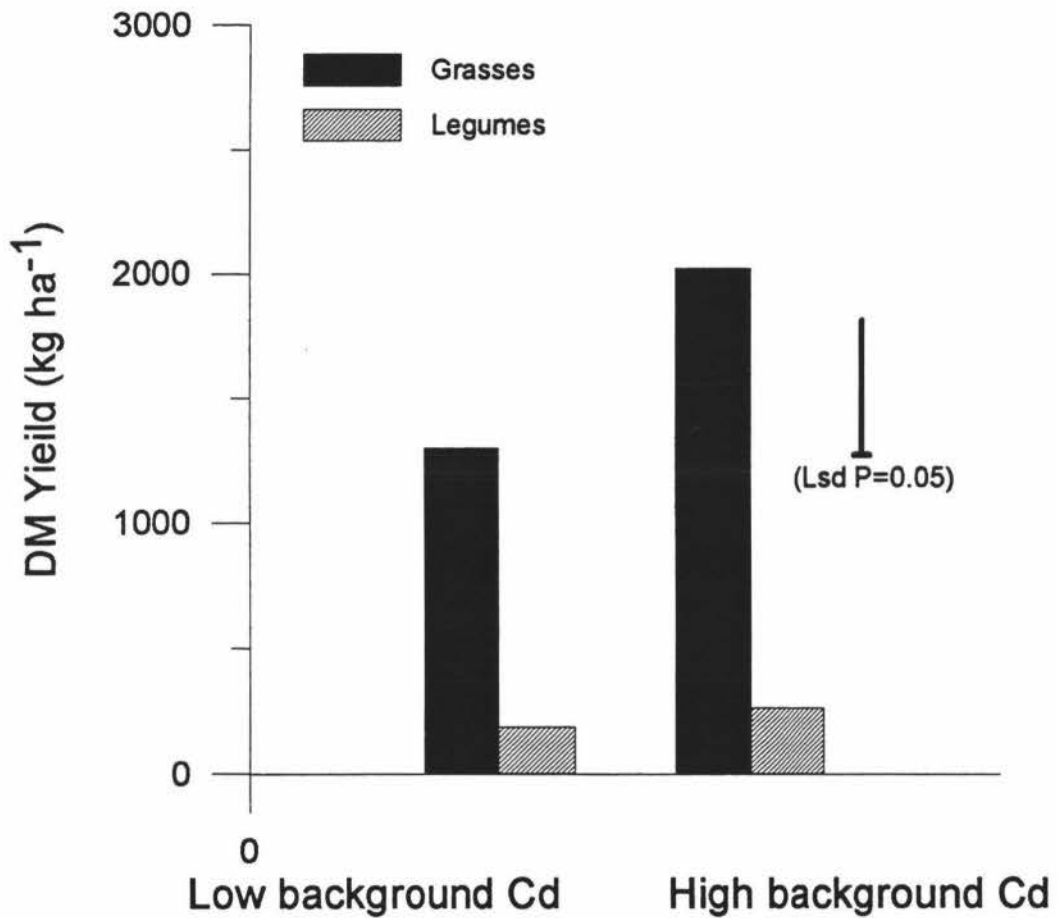


Fig. 4.6 Effect of background Cd on DM yield of pasture species.

4.4 Pasture growth rate
4.4.1 Growth rate in mixed pasture
4.4.1.1 Effect of treatment

Pasture growth rates for the various treatments were calculated for all harvest periods. Growth rate was calculated as the average dry matter yield per day over a harvesting period ($\text{kg ha}^{-1} \text{ day}^{-1}$). Pasture growth is influenced by the complex interactions of rainfall, wind, evaporation rate, temperature, sunlight, hill slope, fertilizer practice, pasture species and grazing management. Moisture deficit will have a greater influence on total production in summer when temperature is not limiting.

It was observed that the treatment effect of Zn and Cu fertilizers on the growth rate was significant only in harvests 1, 2 and 3 (Appendix 3). The growth rate ranged from 5.56 (Zn_5Cu_0) to 12.6 $\text{kg ha}^{-1} \text{ day}^{-1}$ (Zn_0Cu_0), 42.68 (Zn_5Cu_0) to 63.16 $\text{kg ha}^{-1} \text{ day}^{-1}$ (Zn_0Cu_0) and 34.66 (Zn_0Cu_2) to 50.79 $\text{kg ha}^{-1} \text{ day}^{-1}$ ($\text{Zn}_{15}\text{Cu}_2$) in harvest 1, 2 and 3 respectively. Zn_0Cu_0 treatment produced the highest growth rate of 12.6 and 63.16 $\text{kg ha}^{-1} \text{ day}^{-1}$ respectively in harvests 1 and 2.

At harvest 1, the growth rates were significantly lower in the Zn_0Cu_5 , $\text{Zn}_0\text{Cu}_{10}$, Zn_5Cu_0 , Zn_5Cu_2 , $\text{Zn}_{15}\text{Cu}_0$, $\text{Zn}_{15}\text{Cu}_2$, $\text{Zn}_{15}\text{Cu}_5$ and $\text{Zn}_{40}\text{Cu}_{10}$ treatments than in the Zn_0Cu_0 treatment.

At harvest 2, the Zn_0Cu_0 , Zn_0Cu_2 , Zn_0Cu_5 , $\text{Zn}_0\text{Cu}_{10}$, Zn_5Cu_2 , Zn_5Cu_5 , $\text{Zn}_{15}\text{Cu}_5$ and $\text{Zn}_{40}\text{Cu}_0$ treatments produced similar growth rates, which were higher than that for the Zn_5Cu_0 , $\text{Zn}_{15}\text{Cu}_0$, $\text{Zn}_{15}\text{Cu}_2$ and $\text{Zn}_{40}\text{Cu}_{10}$ treatments.

The treatment $\text{Zn}_{15}\text{Cu}_2$ produced the highest pasture growth rate, though it was similar in all treatments except the treatment Zn_0Cu_2 in harvest 3. In harvest 4, the treatment effect was not significant but it produced the highest growth rate of all the harvests, which ranged from 75.7 $\text{kg ha}^{-1} \text{ day}^{-1}$ in $\text{Zn}_{15}\text{Cu}_5$ to 116.8 $\text{kg ha}^{-1} \text{ day}^{-1}$ in Zn_0Cu_5 . Growth rates ranged from 33.98 ($\text{Zn}_{15}\text{Cu}_2$) to 52.12 $\text{kg ha}^{-1} \text{ day}^{-1}$ (Zn_0Cu_0) in harvest 5.

In our experiment pasture growth rates followed a strong seasonal pattern with the lowest growth rates in early spring (harvest 1), and the highest in early summer (harvest 4).

Lambert *et al.* (1986) found that poa and ryegrass have maximum growth rates in spring. But most of the low fertility grasses produce maximum growth in late spring and early summer.

Similar results were observed by Del Pino Machado, (1994) who found that the pasture growth rates ranged from 108 kg DM ha⁻¹ day⁻¹ in early summer to less than 2 kg DM ha⁻¹ day⁻¹ in late winter.

4.4.1.2 Effect of Cu levels

The range of mean growth rates of mixed pasture for the different Cu levels was 8.3 to 7.2 kg ha⁻¹ day⁻¹, 52.0 to 51.6 kg ha⁻¹ day⁻¹, 44.8 to 43.7 kg ha⁻¹ day⁻¹, 94.8 to 97.8 kg ha⁻¹ day⁻¹ and 46.3 to 38.9 kg ha⁻¹ day⁻¹ in harvests 1, 2, 3, 4 and 5 respectively. It was observed that the Cu₀ level produced the highest growth rate for mixed pasture in harvests 1, 3 and 5 and the Cu₂ level produced the highest growth rate in harvests 2 and 4 (Fig. 4.7).

Copper (Cu) is required in trace amounts for various metabolic processes in plants, at higher concentrations Cu is toxic to plant growth. Beckett and Davis (1977) found that high levels of Cu depressed growth of barley.

4.4.1.3 Effect of Zn levels

The growth rate of mixed pasture for different Zn levels ranged from 9.8 to 7.9 kg ha⁻¹ day⁻¹, 58.9 to 52.1 kg ha⁻¹ day⁻¹ and 40.4 to 41.06 kg ha⁻¹ day⁻¹ for harvests 1, 2 and 3. It was found that the Zn₁₅ level produced the highest growth rates of mixed pasture at harvest 3 but the Zn₀ level produced the highest pasture growth rate at harvests 1, 2, 4 and 5 (Fig 4.8). There was no significant effect of Zn levels on pasture growth recorded in harvests 4 and 5.

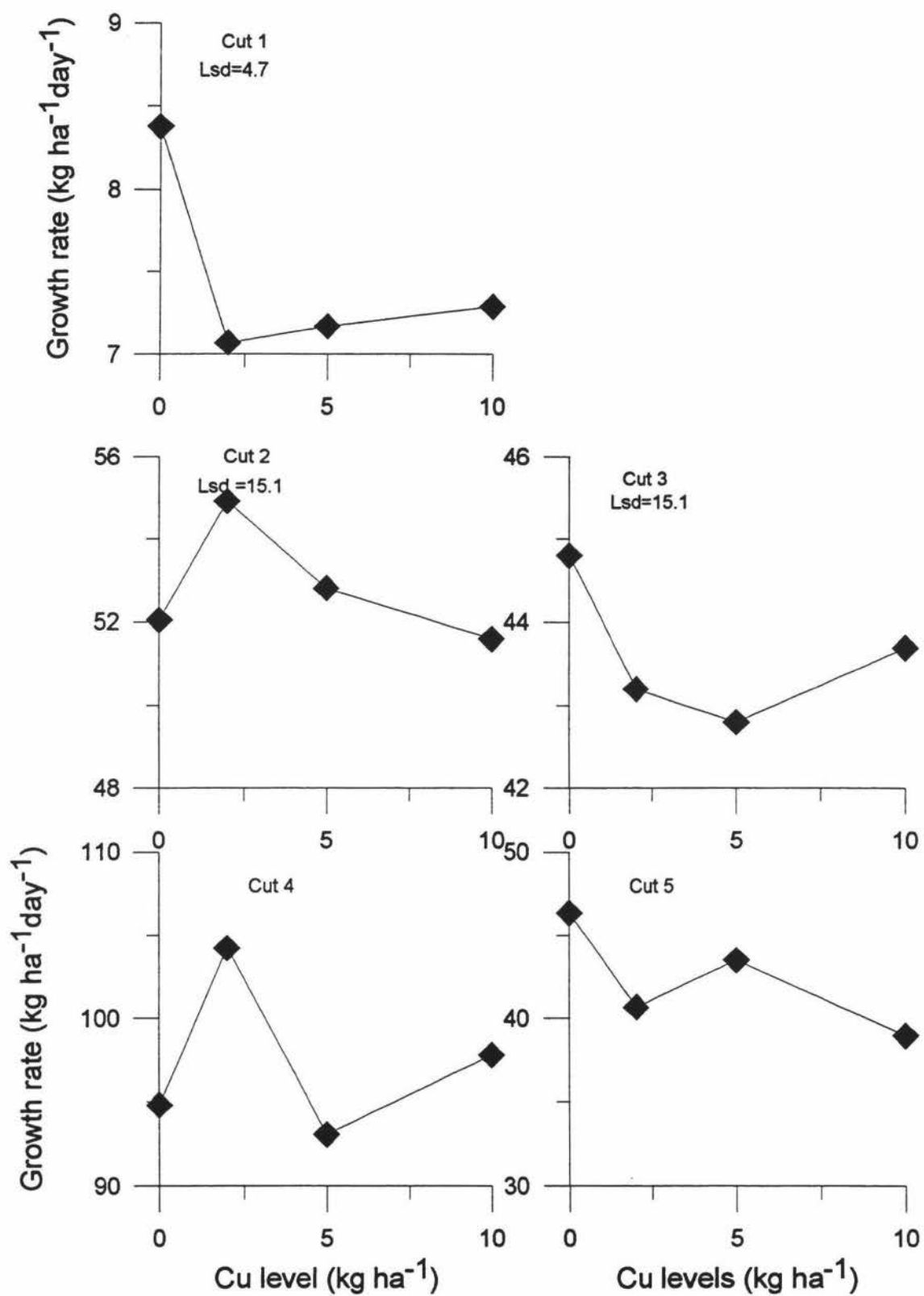


Fig 4.7 Effect of Cu levels on pasture growth rates in mixed pasture.

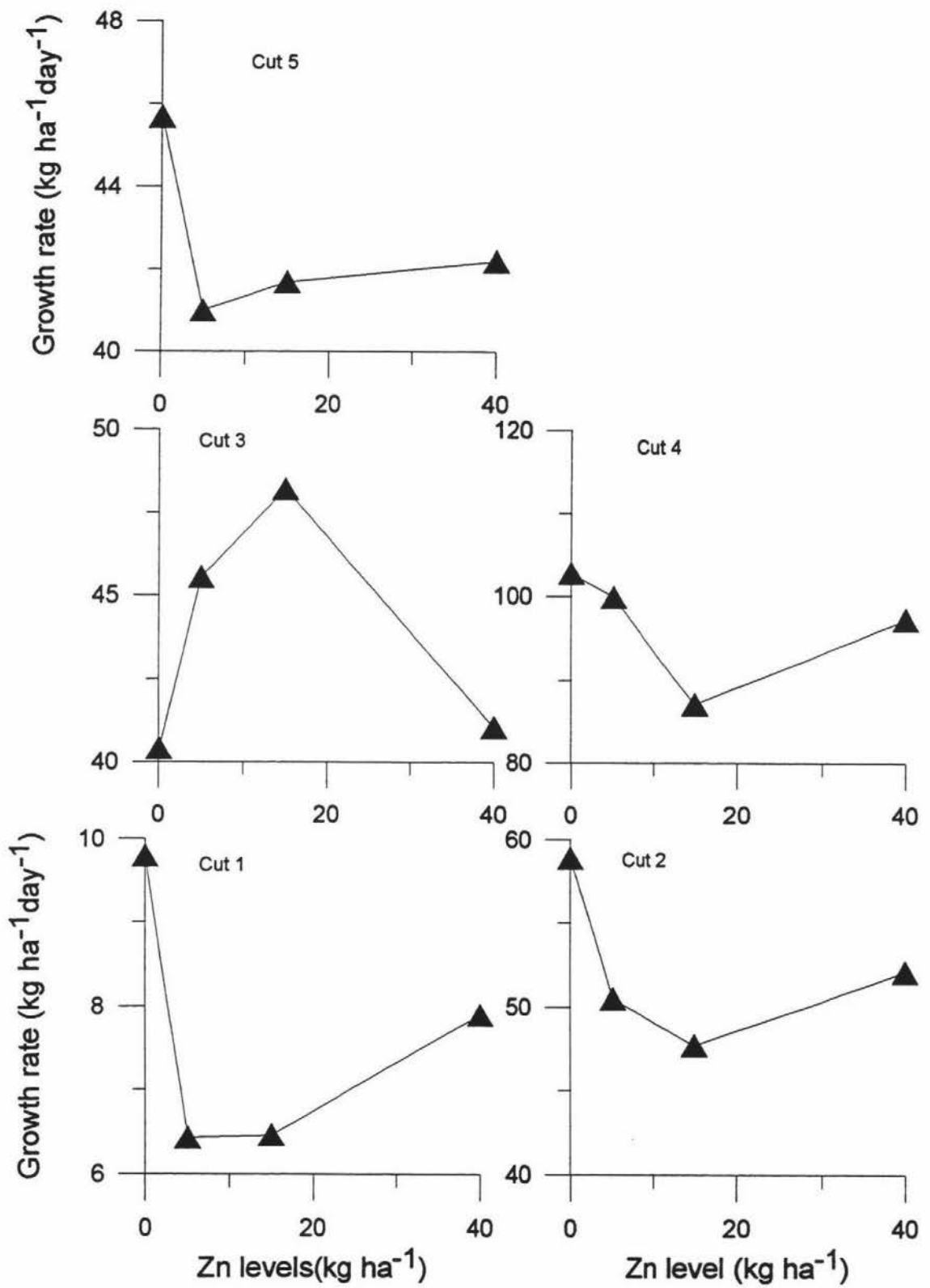


Fig. 4.8 Effect of Zn levels on pasture growth rates in mixed pasture.

4.4.1.4 Effect of background Cd

The effect of background Cd status on pasture growth rate was significant at harvest 4. The high background Cd status produced a higher growth rate ($118.13 \text{ kg ha}^{-1} \text{ day}^{-1}$) than did the low background Cd status ($76.34 \text{ kg ha}^{-1} \text{ day}^{-1}$) (Fig. 4.9).

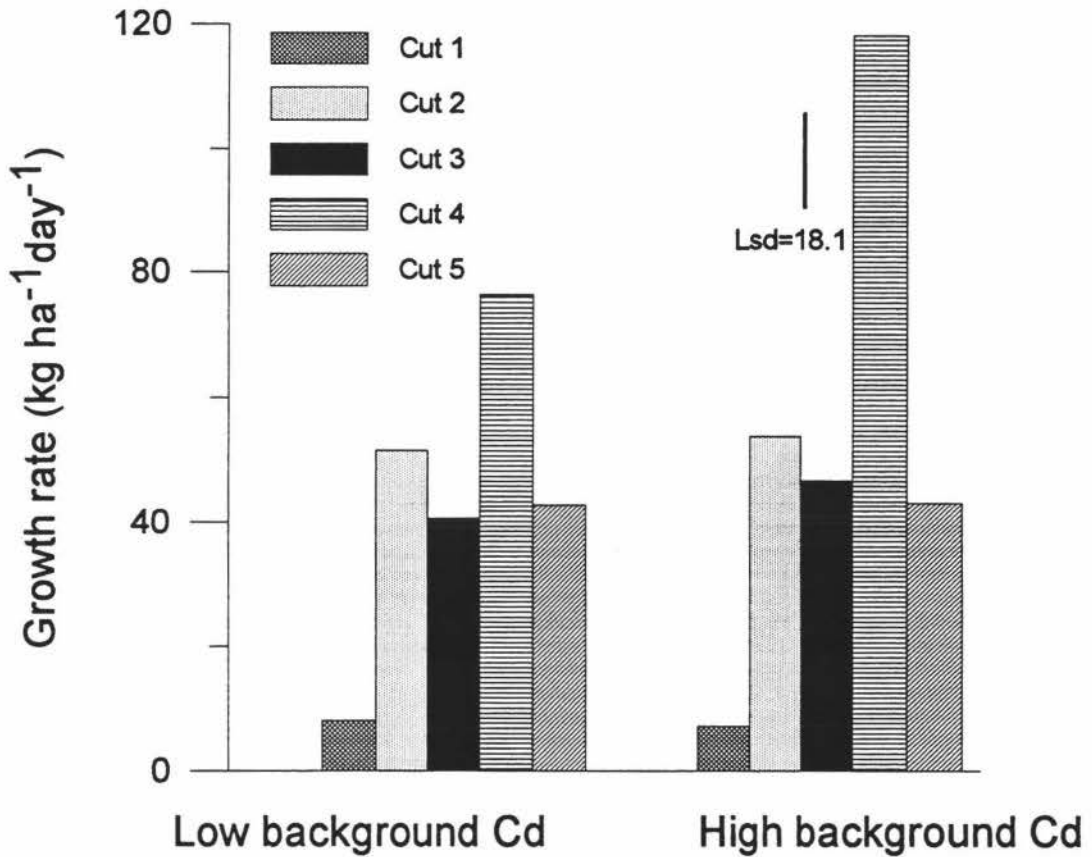


Fig. 4.9 Effect of background Cd on pasture growth rates in mixed pasture

4.4.2 Growth rates of pasture species

4.4.2.1 Effect of treatment

Growth rates ranged from $65.18 \text{ kg ha}^{-1} \text{ day}^{-1}$ ($\text{Zn}_{15}\text{Cu}_5$) to $97 \text{ kg ha}^{-1} \text{ day}^{-1}$ (Zn_0Cu_5) of grasses and $5.27 \text{ kg ha}^{-1} \text{ day}^{-1}$ ($\text{Zn}_{40}\text{Cu}_0$) to $17.61 \text{ kg ha}^{-1} \text{ day}^{-1}$ (Zn_0Cu_5) of legumes.

4.4.2.2 Effect of Cu level

It was observed that the Cu₂ and Cu₅ levels produced the highest (87.3 kg ha⁻¹ day⁻¹) and the lowest (79.0 kg ha⁻¹ day⁻¹) growth rates of grasses. But the Cu₅ and Cu₀ levels produced the highest (12.1 kg ha⁻¹ day⁻¹) and the lowest (9.75 kg ha⁻¹ day⁻¹) growth rates of legumes, respectively (Fig. 4.10). This indicates that Cu toxicity is greater in grasses than in legumes. Legumes tends to take up larger amounts of Cu than grasses (Rasheed and Seeley, 1966; Sherrell and Rawnsley, 1982).

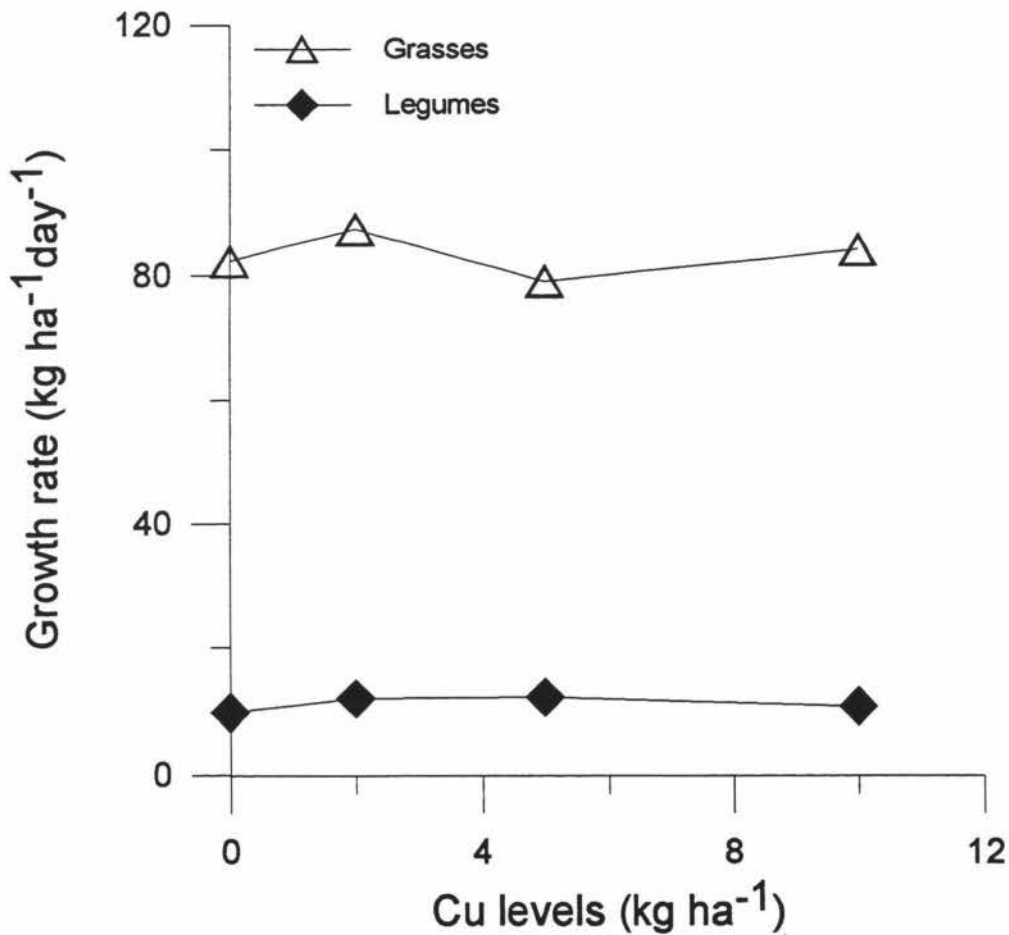


Fig. 4.10 Effect of Cu levels on growth rates of pasture species.

4.4.2.3 Effect of Zn level

Figure 4.11 shows that the Zn_{40} level produced the highest pasture growth rates for grasses ($88.8 \text{ kg ha}^{-1}\text{day}^{-1}$), but the lowest pasture growth rate of legumes ($5.6 \text{ kg ha}^{-1}\text{day}^{-1}$). The lowest growth rate of grass was recorded at the Zn_{15} level ($77.3 \text{ kg ha}^{-1}\text{day}^{-1}$) and the highest growth rate for legumes was recorded at the Zn_5 level ($14.8 \text{ kg ha}^{-1}\text{day}^{-1}$). This shows that grass species are more tolerant than legumes at higher levels of Zn.

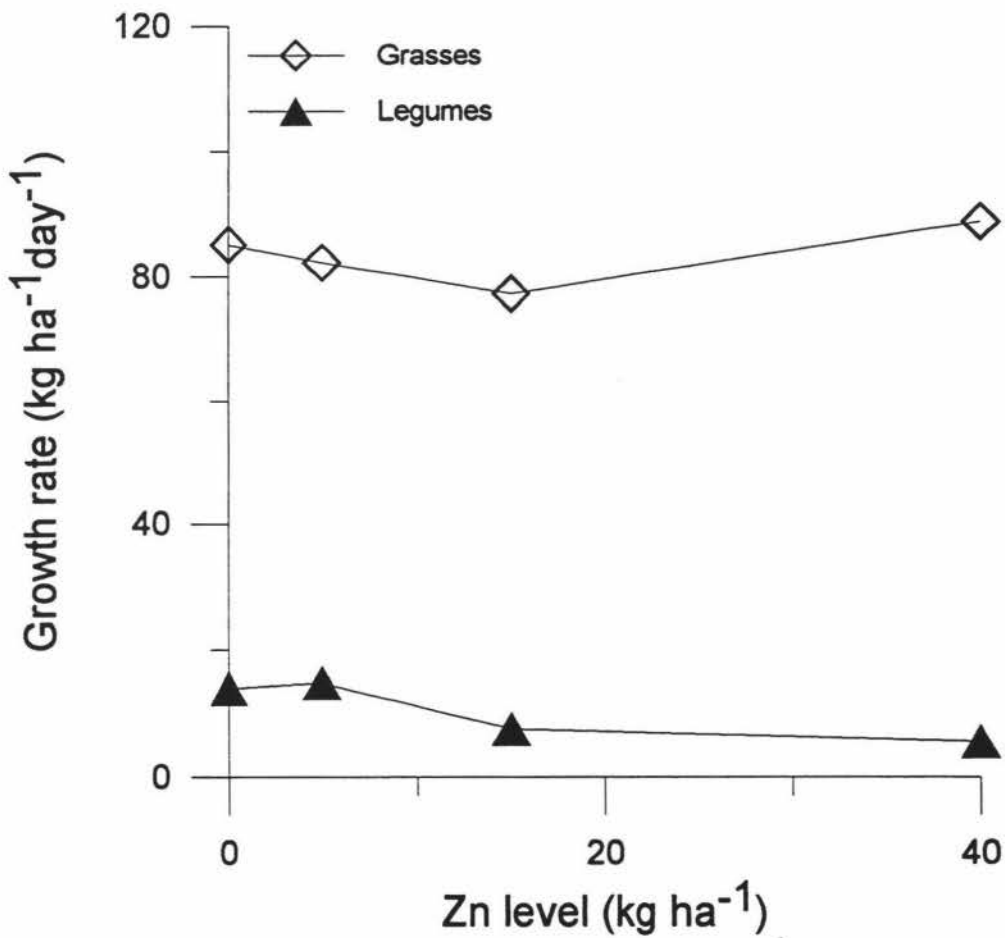


Fig. 4.11 Effect of Zn levels on growth rates in pasture species.

4.4.2.4 Effect of background Cd

Results presented in Appendix 3 indicate that the treatment effects were not significant in the growth rates of pasture species. But the effect of background Cd status on growth rates was significant for grasses, where high background Cd status produced a higher growth rate ($101.13 \text{ kg ha}^{-1} \text{ day}^{-1}$) than did the low background Cd status ($64.98 \text{ kg ha}^{-1} \text{ day}^{-1}$). A similar trend was observed in the case of legumes, where high background Cd status has the higher growth rate of $12.95 \text{ kg ha}^{-1} \text{ day}^{-1}$ than the low background Cd status of $9.17 \text{ kg ha}^{-1} \text{ day}^{-1}$ (Fig. 4.12).

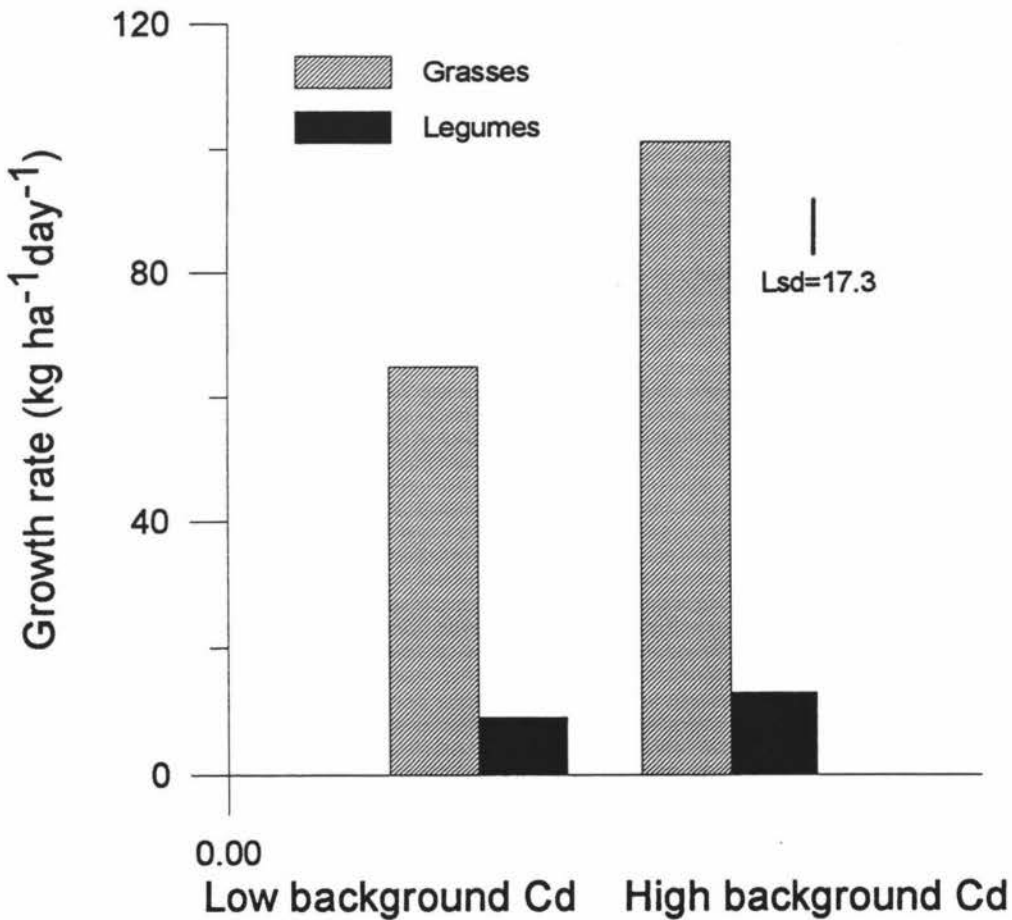


Fig. 4.12 Effect of background Cd on growth rates of pasture species.

4.5 Botanical composition.

Results reported in Table 4.3 indicate that the treatment effects and the effect of background Cd status on botanical composition of the mixed pasture was not significant. It was observed that grass was the dominant component in all treatments and the grass percentage ranged from 76.9 in Zn_0Cu_2 to 91.4 in $Zn_{40}Cu_{10}$ and the legume percentage ranged from 6.1 in $Zn_{40}Cu_0$ to 16.3 in Zn_5Cu_0 .

Grass is considered to be the dominant component of all the pastoral scene in New Zealand. Different pasture species have different agronomic characteristics in terms of annual DM yield, seasonality of yield, tolerance to heavy stocking and frequent defoliation, tolerance of high and low temperatures, insect resistance and feeding value.

Suckling (1975) observed that the yields of pure grass swards comprised of 63% ryegrass, 21% cocksfoot and 16% other grasses.

Corkill *et al.* (1980) and Lancashire (1984) concluded that most pastures in New Zealand are mainly perennial ryegrass and white clover.

Lambert *et al.* (1986) found that poa and ryegrass have maximum growth rates in spring. But most of the low fertility grasses produce maximum growth in late spring to early summer.

These results are in agreement with the findings of Del Pino Machado (1994), who has found that high fertility status soils contained 89.7% of grasses and 8.5% legumes, and low fertility status soils produce 75.5% grasses and 4.2% legumes and the rest are weeds and dead material.

Table 4.3 Botanical composition of the mixed pastures¹.

Treatments	Grass (%)	Legume (%)
Zn₀Cu₀	89.16	8.35
Zn₀Cu₂	76.92	16.03
Zn₀Cu₅	83.78	14.27
Zn₀Cu₁₀	79.71	16.29
Zn₅Cu₀	80.50	16.30
Zn₅Cu₂	81.94	13.61
Zn₅Cu₅	87.19	10.94
Zn₁₅Cu₀	88.44	8.87
Zn₁₅Cu₂	90.31	6.65
Zn₁₅Cu₅	87.83	9.94
Zn₄₀Cu₀	90.69	6.18
Zn₄₀Cu₁₀	91.42	6.22
CV%	16.51	92.29
Low Cd	85.77	11.26
High Cd	85.55	11.02

¹The dead material and weed content was not included.

4.6 Copper content
4.6.1 Copper content in mixed pasture
4.6.1.1 Treatment effect

Results reported in Appendix 4 indicate that the treatment effects of Zn and Cu fertilizers on Cu concentration in the mixed pastures was significant at all harvests. It was found that the treatment Zn_0Cu_{10} in all harvests contained the highest Cu concentration.

In harvest 1 the Cu concentration ranged from 9.45 mg kg^{-1} ($Zn_{40}Cu_0$) to 16.13 mg kg^{-1} (Zn_0Cu_{10}). The Cu concentration in treatment Zn_0Cu_{10} was significantly different from that in treatments $Zn_{40}Cu_0$, $Zn_{15}Cu_0$, Zn_5Cu_0 and Zn_0Cu_0 but similar to the rest of the treatments.

In harvest 2, the Cu concentration ranged from $8.1 \text{ mg Cu kg}^{-1}$ (Zn_0Cu_0) to $10.43 \text{ mg Cu kg}^{-1}$ (Zn_0Cu_{10}). The treatment (Zn_0Cu_{10}) was significantly different from the treatments Zn_0Cu_0 and $Zn_{40}Cu_0$, but similar to the rest of the treatments.

In harvest 3, the Cu concentration ranged from $6.02 \text{ mg Cu kg}^{-1}$ ($Zn_{15}Cu_0$) to 7.93 mg kg^{-1} (Zn_0Cu_{10}). The highest concentration of $7.93 \text{ mg Cu kg}^{-1}$ obtained in Zn_0Cu_{10} treatment was significantly different from the treatments $Zn_{15}Cu_0$, $Zn_{15}Cu_2$ and $Zn_{40}Cu_0$ but similar to the rest of the treatments.

In harvest 4, the Cu concentration ranged from $6.7 \text{ mg Cu kg}^{-1}$ (Zn_0Cu_0) to 8.8 mg kg^{-1} (Zn_0Cu_{10}). The treatment Zn_0Cu_{10} contained the highest amount of Cu ($8.86 \text{ mg Cu kg}^{-1}$), which was significantly different from that in Zn_0Cu_0 ($6.74 \text{ mg Cu kg}^{-1}$) and Zn_5Cu_2 ($7.21 \text{ mg Cu kg}^{-1}$), but similar to the rest of the treatments.

In harvest 5, the Cu concentration ranged from 6.10 mg kg^{-1} ($Zn_{15}Cu_0$) to 7.17 mg kg^{-1} (Zn_0Cu_{10}). The Cu concentration in treatment Zn_0Cu_{10} ranked the highest (7.17 mg kg^{-1}), which was significantly different from $Zn_{15}Cu_0$, $Zn_{15}Cu_2$ and $Zn_{40}Cu_{10}$, but similar to the rest of the treatments.

Wells (1957) concluded that pasture Cu concentration varies from 3.5 to 18 mg Cu kg⁻¹. Kubota (1983) observed that mixed pasture herbage rarely contains more than 20 mg Cu kg⁻¹ of dry matter and usually less than 10 mg Cu kg⁻¹.

In our experiment, we have found less than 10 mg Cu kg⁻¹ in all harvests except in harvest 1. In harvest 1, it was more than 10 mg Cu kg⁻¹, this is probably due to the quick dissolution of Cu fertilizers in soils. It may also be due to a slow growth rate of pasture in harvest 1.

4.6.1.2 Effect of Cu level

It was observed that increasing Cu levels have influenced the Cu concentration in the mixed pasture of all harvests. The Cu concentration for different Cu levels ranged from 9.98 to 15.89 mg kg⁻¹, 9.17 to 10 mg kg⁻¹, 6.47 to 7.46 mg kg⁻¹, 7.73 to 8.14 mg kg⁻¹ and 6.51 to 6.69 mg kg⁻¹ at harvests 1, 2, 3, 4 and 5 respectively. There was a general trend that the Cu concentration increased with an increasing level of Cu in all harvests (Appendix 4) (Fig. 4.13). The highest Cu concentration was found in Cu₁₀ level.

Jarvis (1978), Gilkes and Lim-Nunez (1979) and Reuter *et al.* (1981) recorded that the increasing rates of Cu application increased the Cu concentration in ryegrass, wheat and subterranean clover tops, respectively.

Sherrell and Rawnsley (1982) found that the Cu concentration in herbage ranged from 5 to 12 mg kg⁻¹ within 4 weeks by an addition of 2 to 4 kg Cu ha⁻¹.

Alva and Chen (1995) observed that the concentration of Cu in shoots increased linearly both in *Cleopatra mandarin* and *Swingle citrumelo* citrus rootstock seedlings with an increase in external Cu concentration.

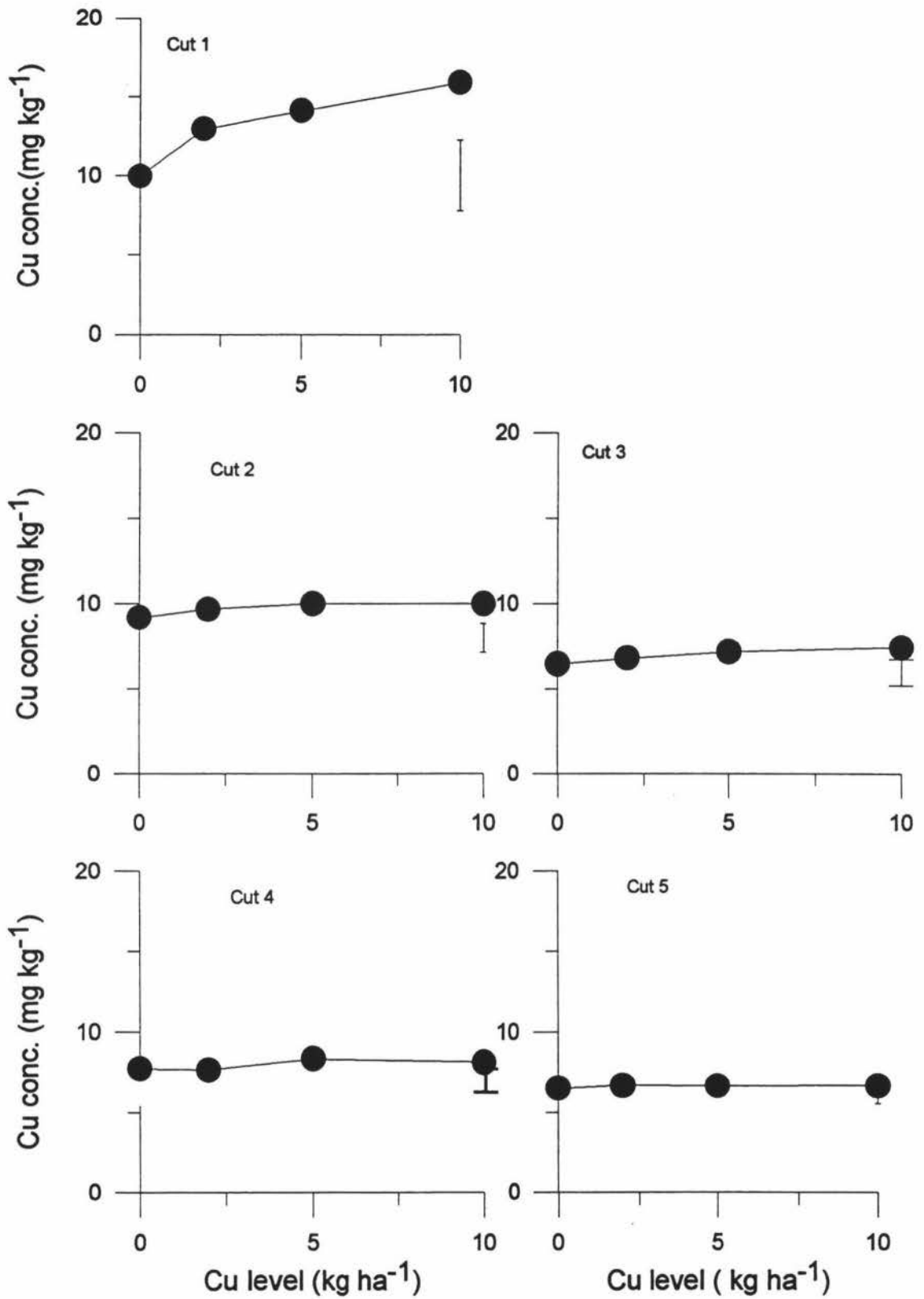


Fig. 4.13 Effect of Cu level on Cu concentration in mixed pasture.

4.6.1.3 Effect of Zn level

For the different levels of Zn addition, the Cu concentration ranged from 13.37 to 12.54 mg kg⁻¹, 9.58 to 9.11 mg kg⁻¹, 7.34 to 6.6 mg kg⁻¹, 7.96 to 7.57 mg kg⁻¹ and 7.03 to 6.22 mg kg⁻¹ in harvests 1, 2, 3, 4 and 5 respectively. It was observed that the increasing level of Zn tends to decrease the Cu concentration in harvests 1, 3 and 5. The effect of Zn level on Cu concentration was more pronounced only at the Zn₄₀ level (Fig. 4.14). This effect may be due to an excessive level of Zn suppressing the Cu uptake antagonistically in mixed pasture.

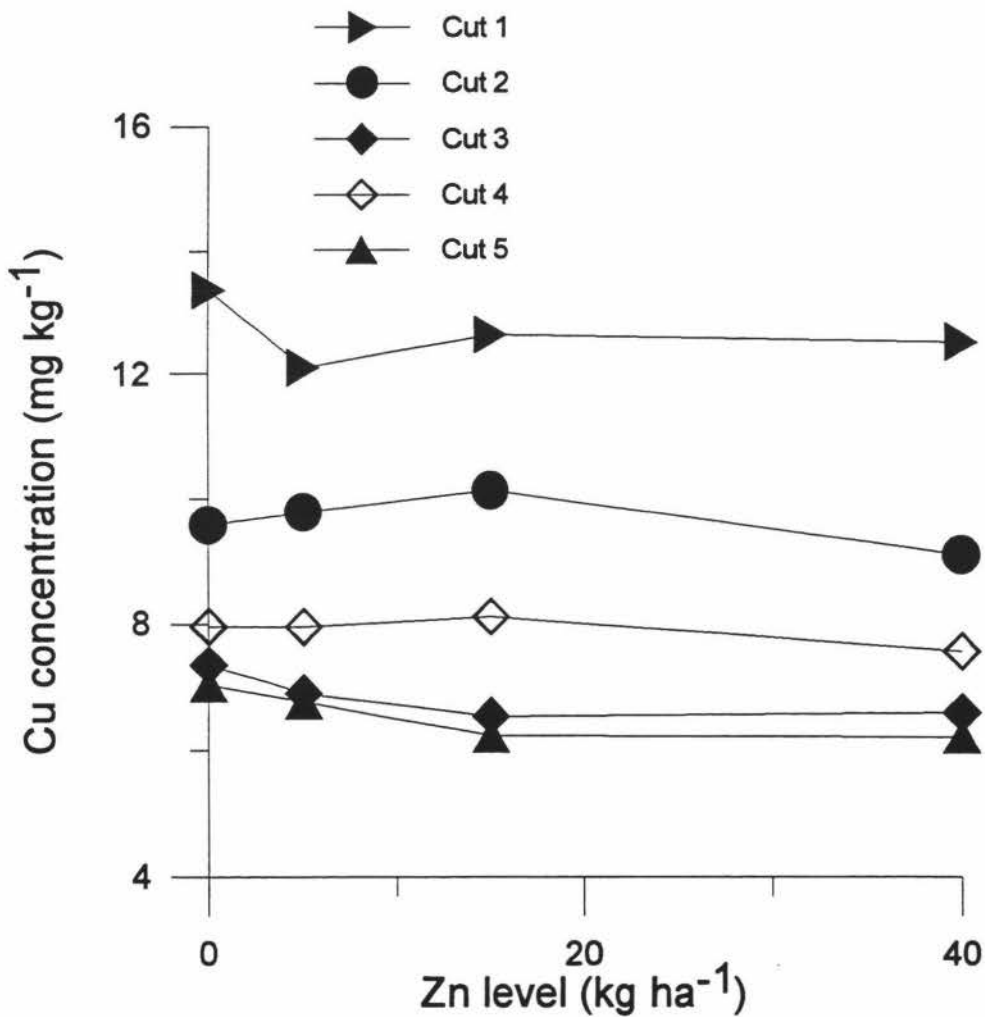


Fig. 4.14 Effect of Zn on Cu concentration in mixed pasture

Beckett and Davis (1978) observed an antagonistic effect between Zn and Cu in a nutrient solution study of young barley plants.

Kabata and Pendias (1984) described the Zn-Cu interaction as an antagonism effect, and observed that large Zn additions decreased the uptake of Cu in the shoot material.

4.6.1.4 Effect of background Cd

The Cu concentration was higher in low background Cd status than in the high background Cd status except in harvest 1. The Cu concentration was similar (12.7 mg kg^{-1}) in both low and high background Cd status for harvest 1.

It was recorded that the mean Cu concentration for low and high background Cd status was 10.28 and 8.98 mg kg^{-1} in harvest 2, 7.89 and 5.91 mg kg^{-1} in harvest 3, 8.54 and 7.33 mg kg^{-1} in harvest 4 and 7.19 and 6.06 mg kg^{-1} in harvest 5 (Appendix 4) (Fig. 4.15).

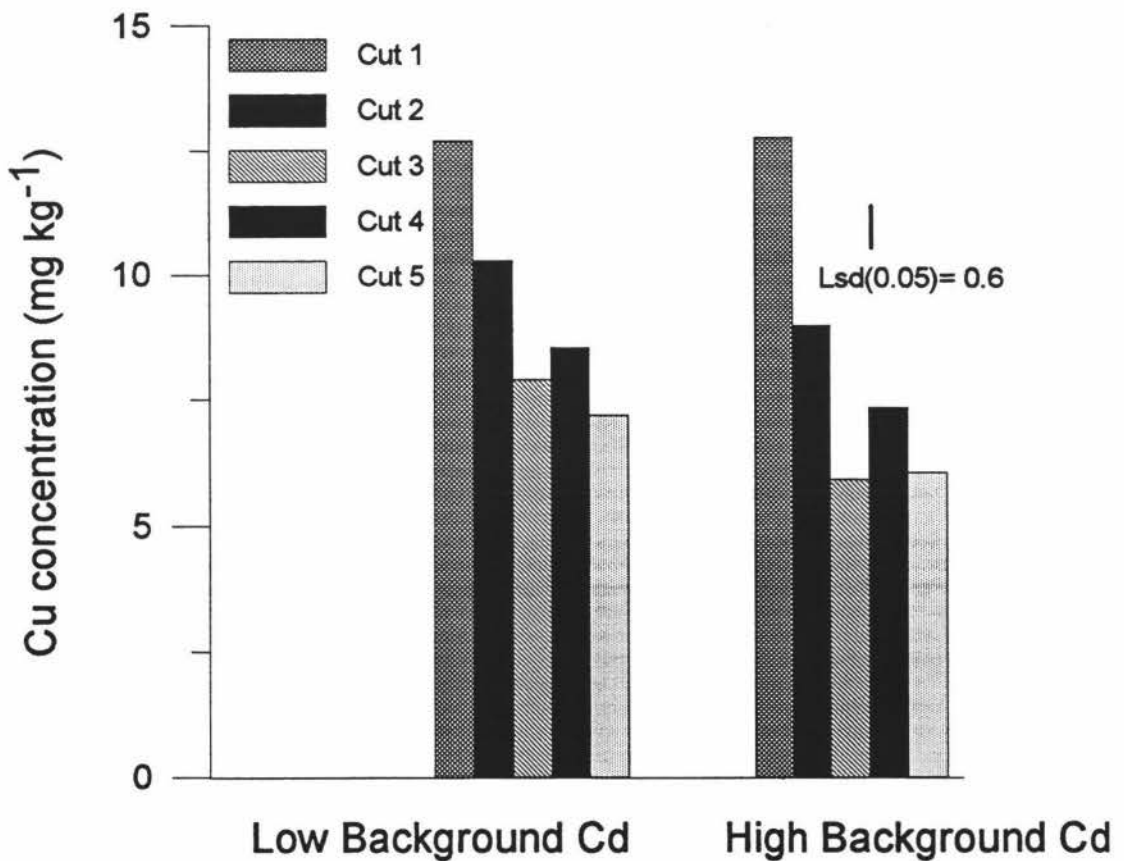


Fig. 4.15 Effect of background Cd status on Cu concentration.

It was recorded that the initial pasture samples contained a lower (8.8 mg Cu kg⁻¹) Cu concentration in low background Cd status than in the high background Cd status (13.3 mg Cu kg⁻¹) (Table 4.2). From this we can conclude that the higher initial rates of Cu fertilizers may have a greater effect in minimising the Cu deficiencies in low background Cd status than in the high background Cd status.

4.6.2 Copper content in pasture species

4.6.2.1 Treatment effect.

It was observed that the treatment effect of Zn and Cu fertilizers on Cu concentration in pasture species was significant in legumes but not in grasses (Appendix 4). The Cu concentration in legumes ranged from 8.53 mg kg⁻¹ (Zn₀Cu₀) to 10.88 mg kg⁻¹ (Zn₁₅Cu₅). The treatment Zn₁₅Cu₅ contained the highest amount of Cu, which was significantly different from the treatments Zn₀Cu₀, Zn₀Cu₂, Zn₅Cu₀, Zn₅Cu₂, Zn₁₅Cu₀, Zn₄₀Cu₀ and Zn₄₀Cu₁₀, but similar to rest of the treatments.

Copper in forage and pastures crops depends on soil availability of Cu, plant species, stage of growth, time of year, lime and fertilizer applications. Clovers tend to take up larger amounts of Cu and the persistence of Cu is greater than in grasses (Sherrell and Rawnsley, 1982). In some cases, non-crop species or weeds may also contribute to the increased dietary intake of Cu by grazing livestock.

Wells (1957) found that pasture species had a marked differences in Cu concentration. He reported that Cu concentrations were 10.6 mg kg⁻¹ in white clover, 19.1 mg kg⁻¹ in red clover and 4.0 mg kg⁻¹ in ryegrass when these plants were grown on the same soil.

4.6.2.2 Effect of Cu level

The Cu concentration for different levels of Cu ranged from 6.92 to 7.04 mg kg⁻¹ in grasses and 8.79 to 9.94 mg kg⁻¹ in legumes but the difference was not significant. It was observed that the Cu concentration in legumes increased with increasing levels of Cu up to 5 kg ha⁻¹, but the grasses did not show any response with increasing level of Cu (Fig. 4.16).

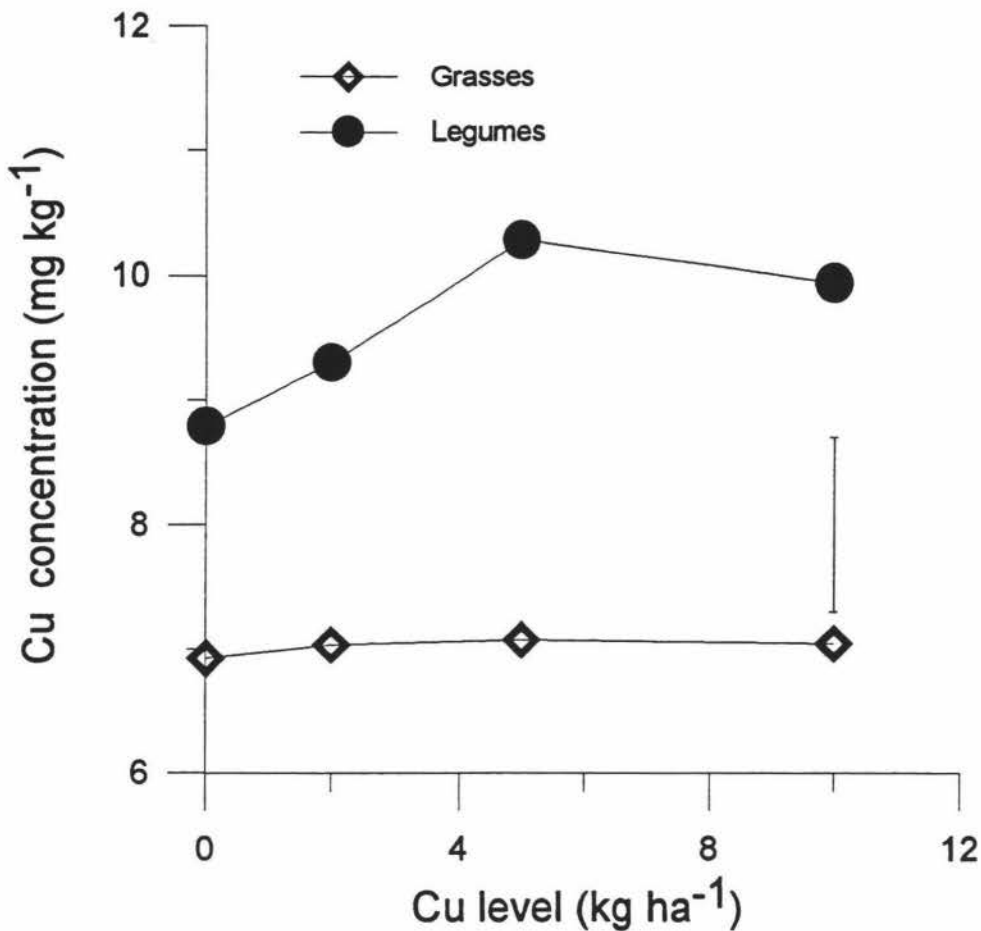


Fig. 4.16 Effect of Cu level on Cu concentration of pasture species.

Jarvis (1978) found that with increasing concentrations of Cu in solution, the Cu concentration in roots of the most ryegrass species increased much more rapidly than that of shoots. He also concluded that in most species Cu concentrations seldom exceeded 30 mg Cu kg⁻¹ when large amounts of Cu had been added to either soils or solution cultures.

Rasheed and Seeley (1966) observed that the Cu concentration in legume shoots increases more rapidly than in grasses.

Davis and Beckett (1978) estimated that the upper critical tissue Cu concentration was 21 mg kg⁻¹ in ryegrass. In a later study with ryegrass, upper critical foliar concentration measured by Davis and Carlton Smith (1984) was 22 mg Cu kg⁻¹.

Plenderleith and Bell (1984) found that the Cu concentration in tropical grasses ranged from 17 to 27 mg kg⁻¹ with addition of 4 to 600 mg kg⁻¹ of Cu to soil.

In our study with increasing levels of Cu (0, 100, 250, 500 mg Cu kg⁻¹), the Cu concentration in grasses and legumes ranged from 6.92 to 7.04 mg kg⁻¹ and 8.79 to 9.94 mg kg⁻¹, respectively.

4.6.2.3 Effect of Zn level

At different levels of Zn application, the Cu concentration ranged from 6.8 to 7.1 mg kg⁻¹ in grasses and 9.6 to 9.8 mg kg⁻¹ in legumes. It was observed that the increasing level of Zn tends to slightly increase the Cu concentration in grass. The effect was more pronounced only at the Zn₁₅ level (Fig. 4.17). It is however interesting to point out that the Cu concentration in the mixed pasture slightly decreased with increasing Zn levels.

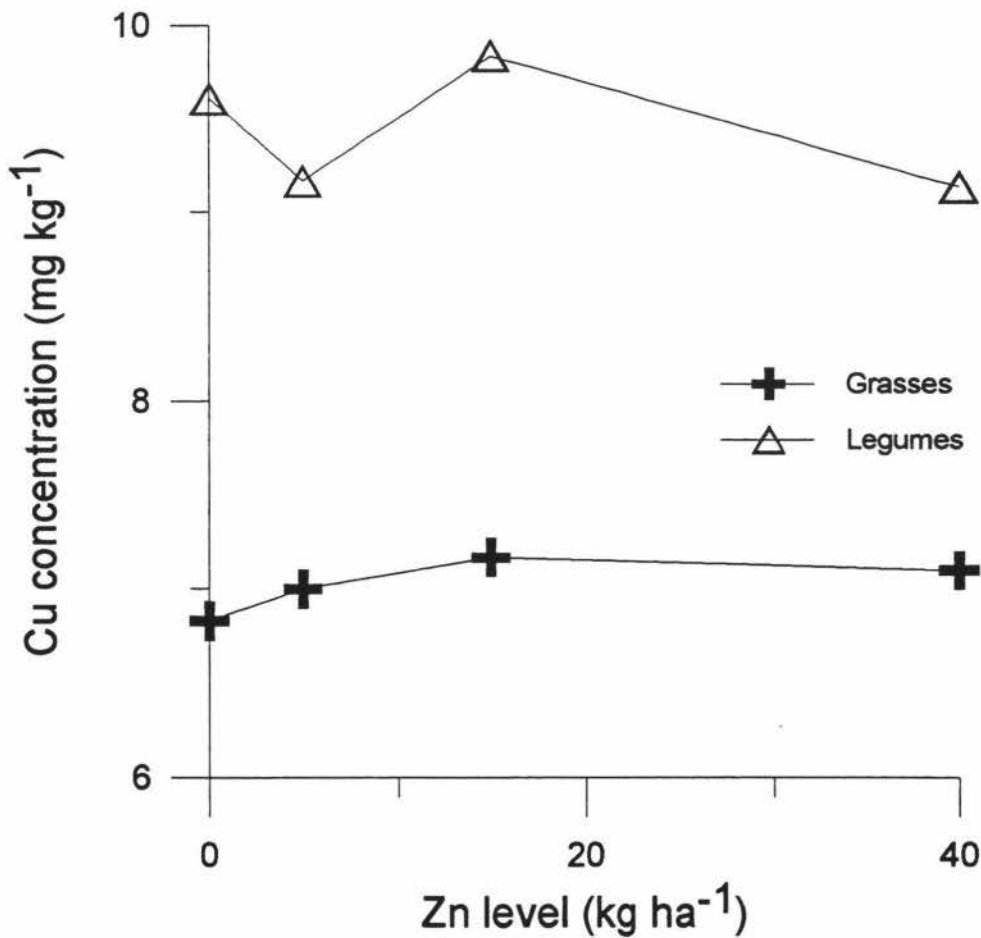


Fig. 4.17 Effect of Zn level on Cu concentration in species.

4.6.2.4 Effect of background Cd

Results reported in Appendix 4 indicate that the effect of background Cd status on Cu concentrations in grasses and legumes was not significant, and it was similar to the Cu concentration in mixed pasture.

4.7 Zn content

4.7.1 Zn content in mixed pasture

4.7.1.1 Treatment effect

Results presented in Appendix 5 show that the treatment effect of Zn and Cu fertilizers on Zn concentration was significant at the harvest 1. Data for Zn concentration in herbage for harvests 2 to 5 are not presented because of the higher than expected Zn levels in the pastures (the data are presented in Appendix 5). Care must be taken in the use of Zn data in harvests 2 to 5.

The Zn concentration ranged from 36.7 mg kg⁻¹ in Zn₀Cu₀ to 174.5 mg kg⁻¹ in Zn₄₀Cu₀ in harvest 1. The Zn₄₀Cu₀ treatment contained the highest amount of Zn, which was significantly different from rest of the treatments.

4.7.1.2 Effect of Cu level

The effect of different Cu levels on Zn concentration was not significant (Fig 4.18). But the Cu₅ level has an effect on Zn concentration in harvest 1. The Cu concentrations are depressed in their activity by the antagonism of disproportionately high amounts of Zn.

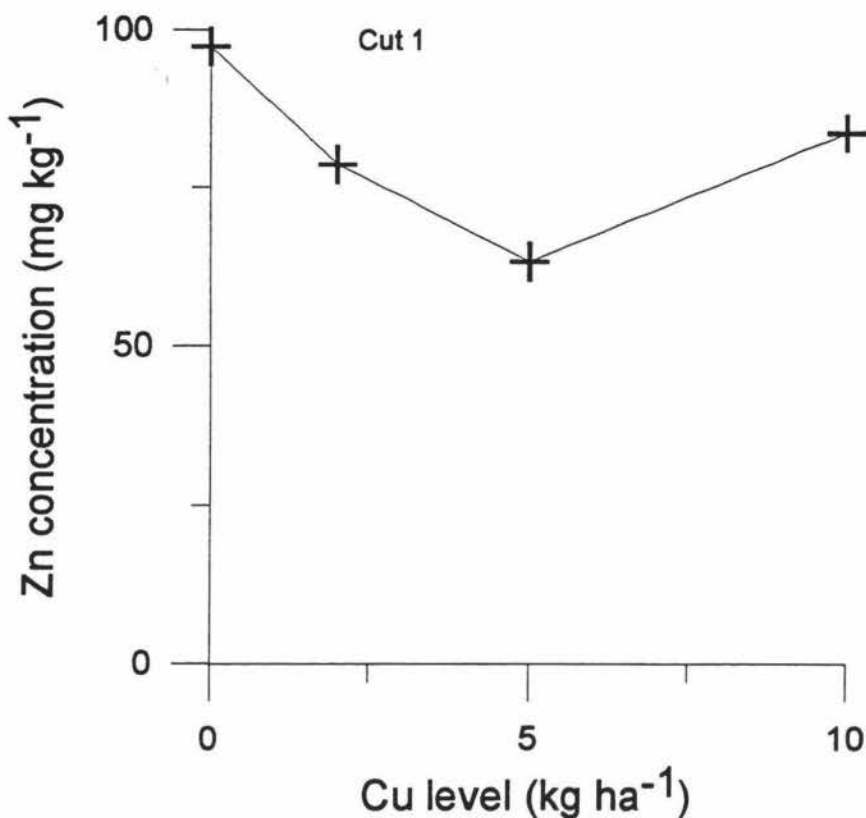


Fig. 4.18 Effect of Cu level on Zn concentration in mixed pasture.

4.7.1.3 Effect of Zn level

At different levels of Zn addition, the Zn concentration ranged from 45 to 150 mg kg⁻¹ in harvest 1.

It was observed that Zn levels have a greater influence on Zn concentration than Cu in harvest 1 (Fig. 4.19). There was a general trend showing the Zn concentration increases in the mixed pasture with increasing levels of Zn. Large amounts of Zn would lead to luxury uptake and accumulation of Zn in pasture leading to the toxicity, which decreased the DM yield.

Maclean (1976) found that the concentration of Zn in the plants increased with addition of Zn to the soils.

Ruano *et al.* (1987) found that the Zn concentrations in bush beans ranged from 105 to 347 mg kg⁻¹ with increasing levels of Zn addition (ranging from 0.13 to 0.75 mg Zn L⁻¹).

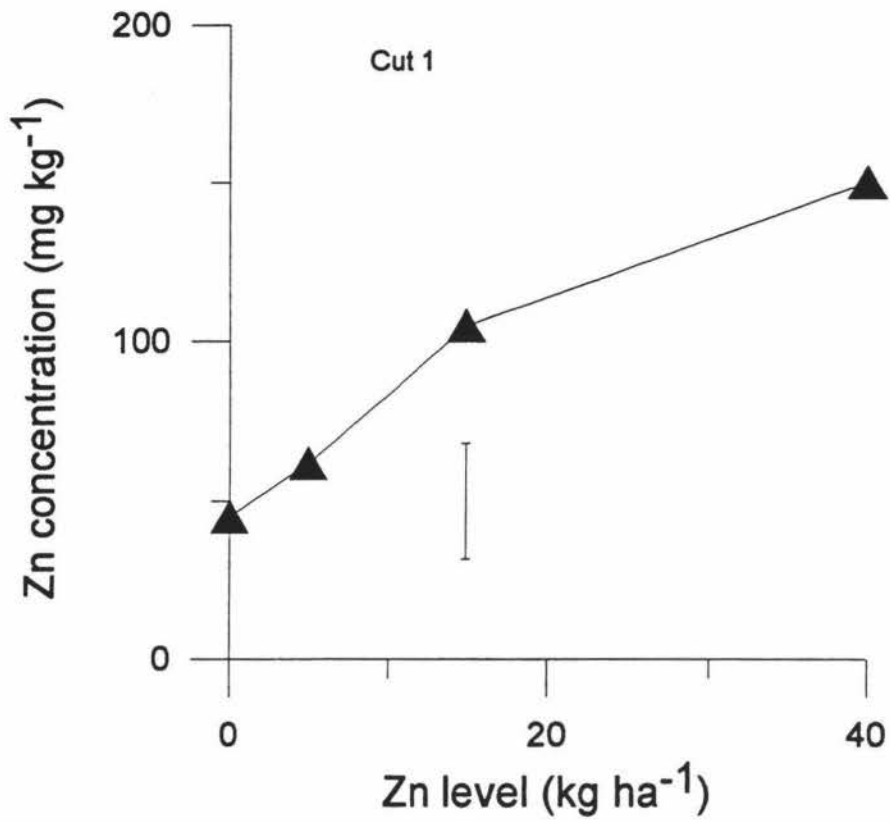


Fig. 4.19 Effect of Zn levels on Zn concentration of mixed pasture.

4.7.1.4 Effect of background Cd

Results reported in Appendix 5 show that the background Cd status has no significant effect on Zn concentration. Similar Zn concentration (81 mg kg⁻¹) was recorded both in low and high background Cd status for harvest 1 (Fig. 4.20).

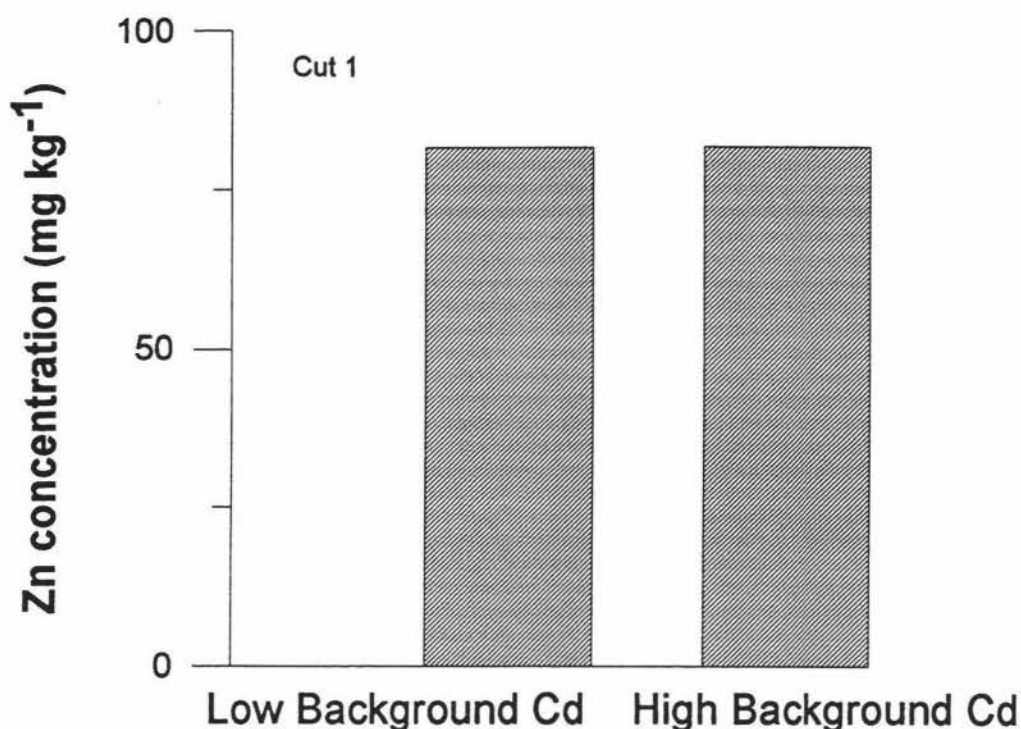


Fig. 4.20 Effect of background of Cd status on Zn concentration.

4.8 Cd content

4.8.1 Cd content in mixed pastures.

4.8.1.1 Treatment effect

Results presented in Appendix 6 indicate that the combined effect of Zn and Cu fertilizers on Cd concentration was not significant at the 5% level except at harvest 2. It was observed that the Cd concentrations in harvest 1 ranged from 0.113 mg kg⁻¹ in Zn₄₀Cu₁₀ to 0.207 mg kg⁻¹ in Zn₅Cu₀.

In harvest 2, Cd concentrations ranged from 0.144 mg kg⁻¹ (Zn₄₀Cu₁₀) to 0.322 mg kg⁻¹ (Zn₅Cu₅). The treatment Zn₅Cu₅ contained the highest Cd concentration, which was similar to Zn₀Cu₀, Zn₀Cu₅, Zn₀Cu₁₀, Zn₅Cu₀, Zn₅Cu₂, Zn₁₅Cu₀, Zn₁₅Cu₂ and Zn₄₀Cu₀ but significantly different from the rest of the treatments.

The Cd concentration was highest in Zn₅Cu₀ (0.287 mg kg⁻¹) and was lowest in Zn₄₀Cu₁₀ (0.171 mg kg⁻¹) in harvest 3.

In harvest 4, Cd concentrations ranged from 0.219 mg kg⁻¹ in Zn₄₀Cu₁₀ to 0.411 mg kg⁻¹ in Zn₅Cu₅. The Cd concentrations ranged from 0.224 mg kg⁻¹ (Zn₅Cu₀) to 0.292 mg kg⁻¹ (Zn₅Cu₅) in harvest 5.

4.8.1.2 Effect of Cu level

It was observed that the different Cu levels had no significant effect on Cd concentration in the mixed pastures (Fig. 4.21). The Cd concentrations of mixed pasture recorded for different levels of Cu ranged from 0.144 to 0.168 mg kg⁻¹, 0.167 to 0.171 mg kg⁻¹, 0.216 to 0.226 mg kg⁻¹, 0.246 to 0.256 mg kg⁻¹ and 0.251 to 0.252 in harvests 1, 2, 3, 4 and 5 respectively. Only in harvest 1, did large amounts of plant available Cu lead to an increased uptake and accumulation of Cu in pasture tissue, leading to decreased Cd uptake.

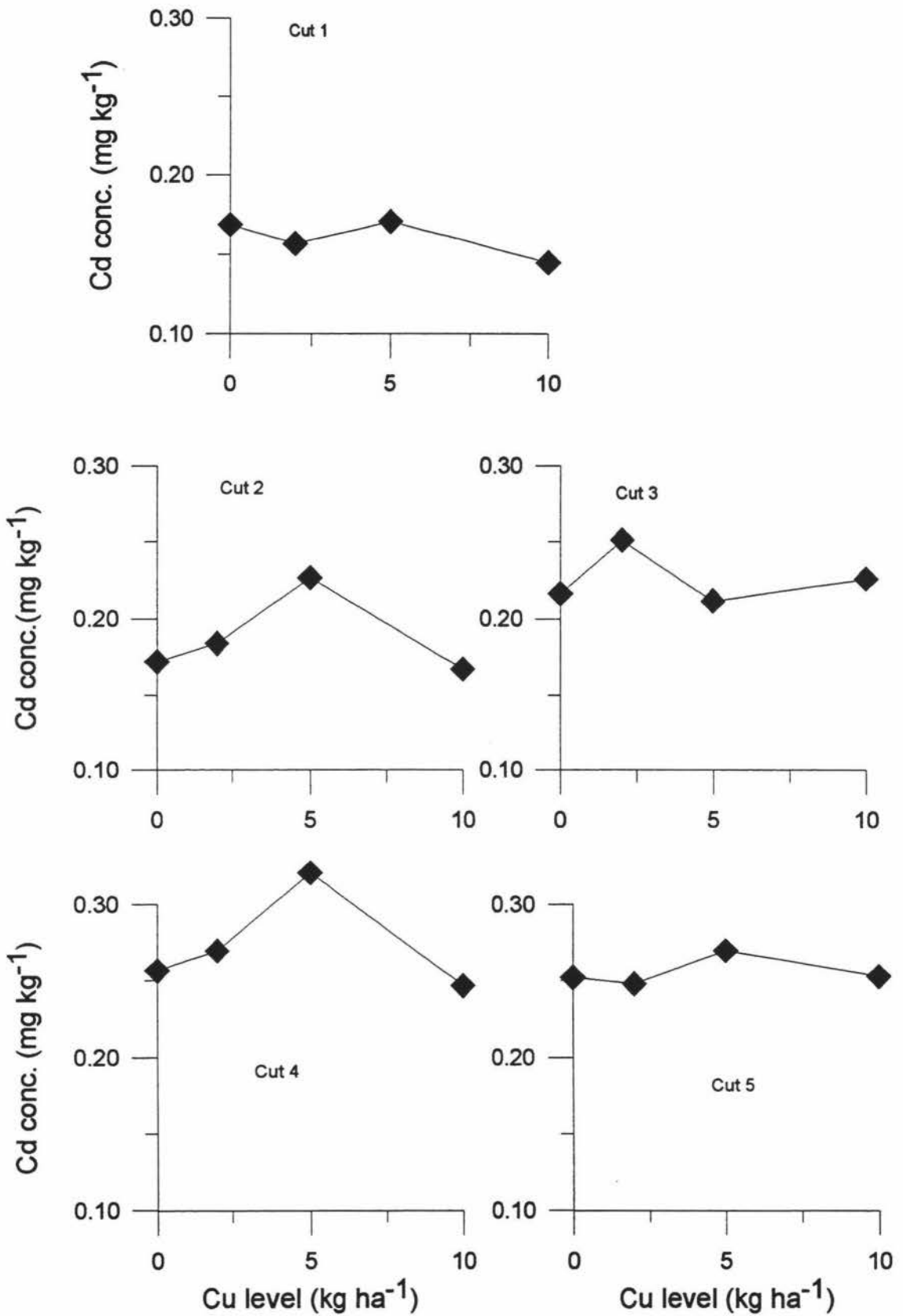


Fig.4.21 Effect of Cu levels on Cd concentration in mixed pasture.

It was found that increasing Cu concentration alone showed only a slight decrease in Cd:Cu ratios in harvest 1 and 4. (Fig. 4.22).

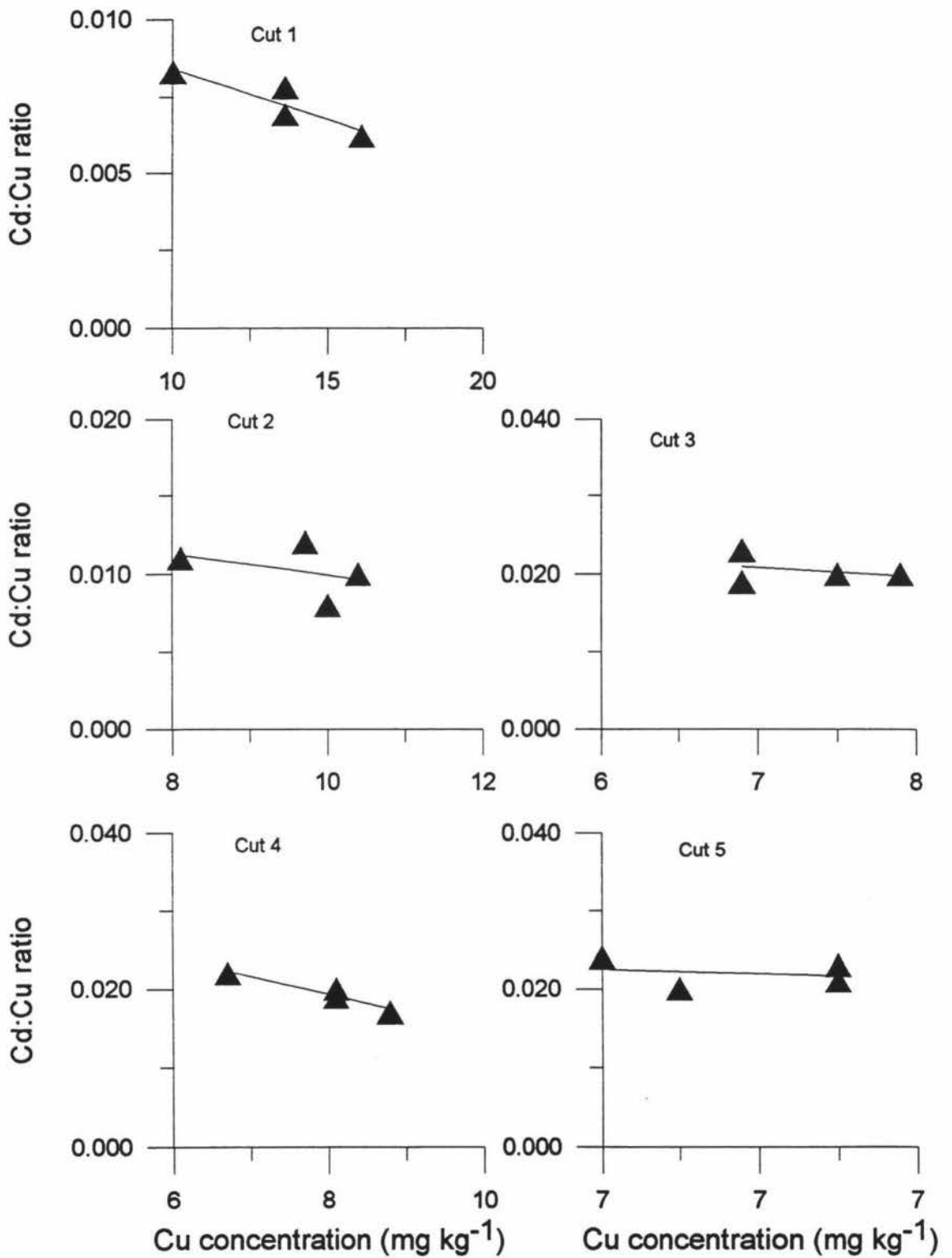


Fig. 4.22 Effect of Cu concentration on Cd:Cu ratio.

4.8.1.3 Effect of Zn level

It was found that the Zn levels have an effect on Cd content in the mixed pastures at all harvest (Fig. 4.24). In Zn₄₀ level markedly reduced the Cd concentration in the mixed pastures in comparison to all other levels. The Cd concentrations recorded for different Zn levels ranged from 0.117 to 0.168 mg kg⁻¹, 0.150 to 0.177 mg kg⁻¹, 0.176 to 0.268 mg kg⁻¹, 0.222 to 0.273 mg kg⁻¹ and 0.230 to 0.271 mg kg⁻¹ for harvest 1, 2, 3, 4 and 5 respectively.

Results reported in Appendix 6 and Fig. 4.23 showed a negative correlation of pasture Zn concentration with pasture Cd concentration in harvest 1.

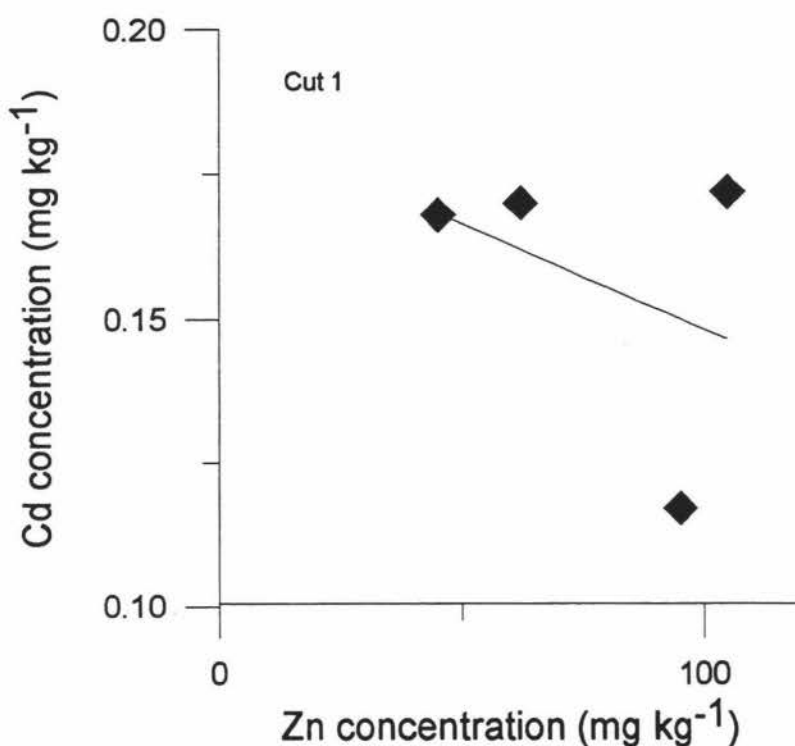


Fig. 4.23 Relationships between pasture Zn concentration and pasture Cd concentration.

It was observed that the Zn concentration alone showed a marked decrease in pasture Cd:Zn ratio in harvest 1 (Fig. 4.25).

Effect of Zn+Cu concentration on Cd:(Zn+Cu) ratio was similar to that for Zn alone on Cd:Zn ratio (Fig. 4.26).

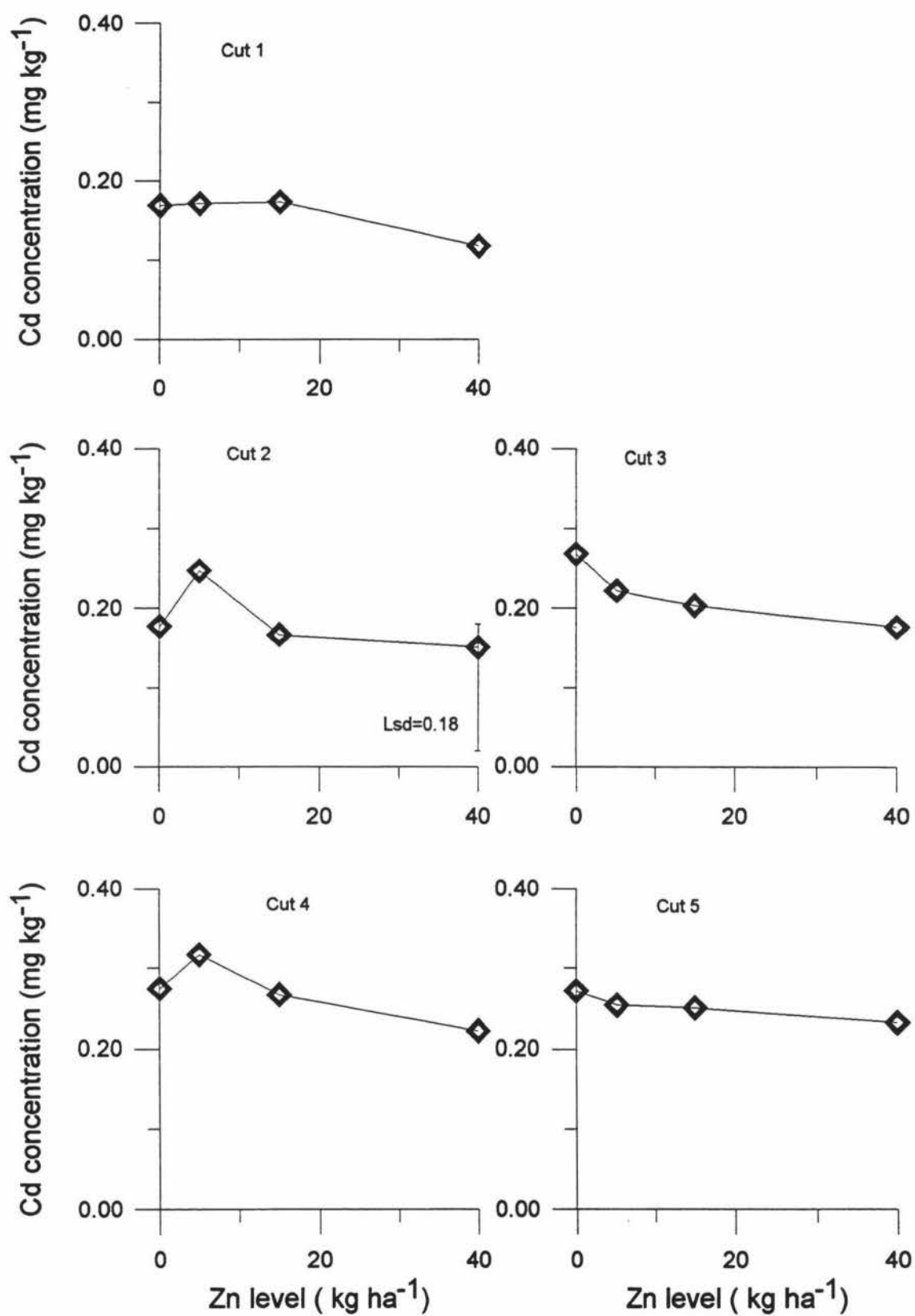


Fig. 4.24 Effect of Zn level on Cd concentration in mixed pasture.

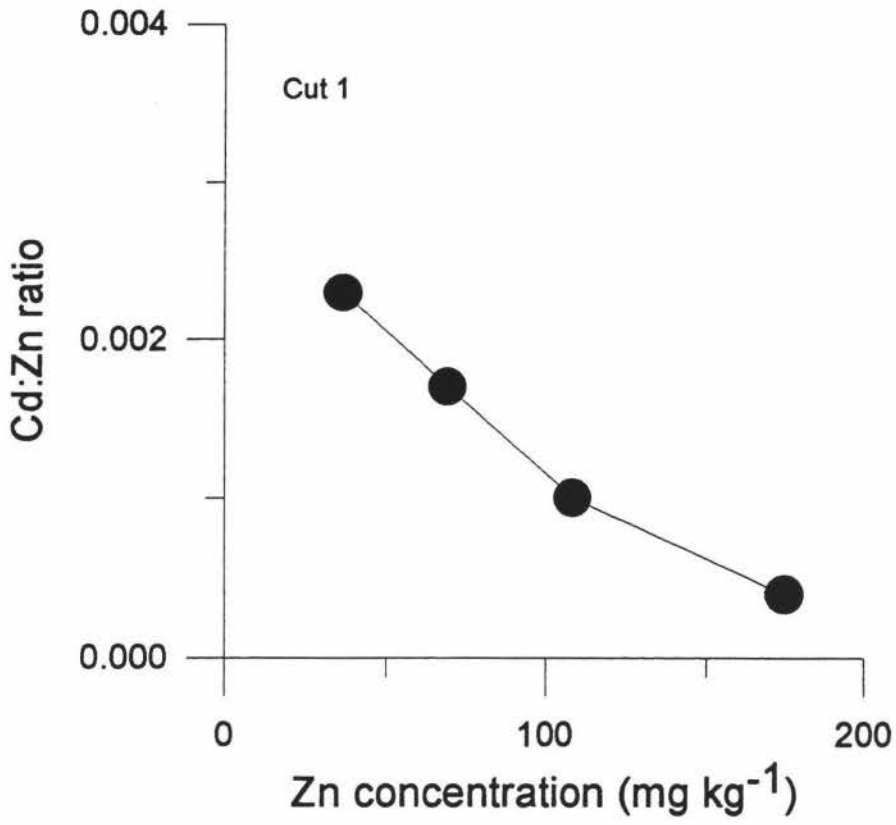


Fig. 4.25 Effect of Zn concentration on Cd:Zn ratio.

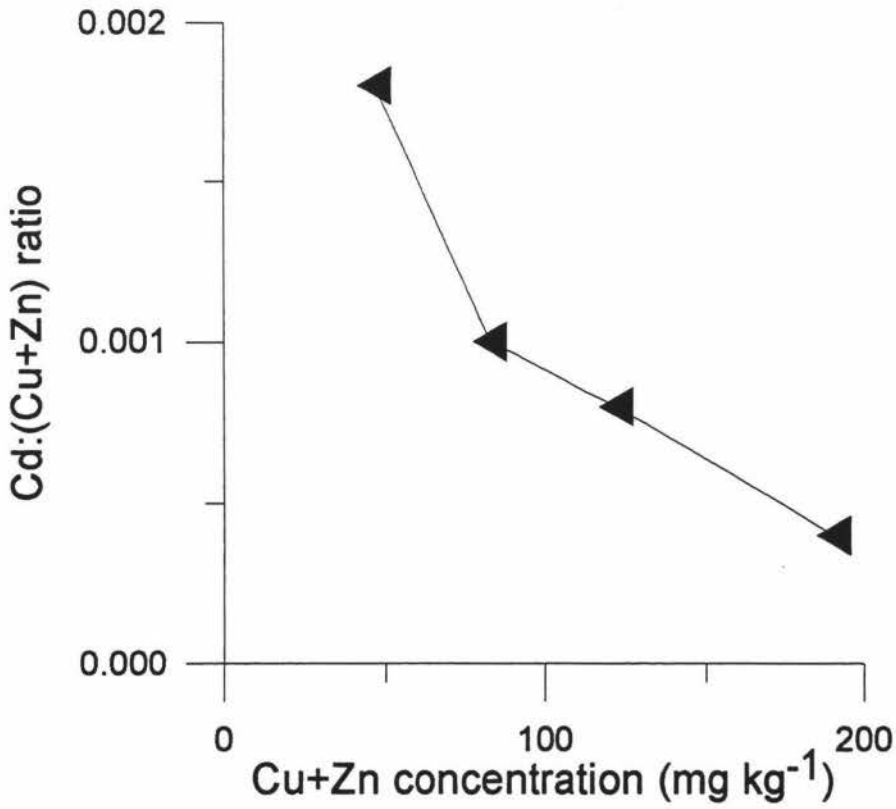


Fig. 4.26 Effect of Cu+Zn concentration on Cd:(Cu+Zn) ratio.

Haghiri (1974) observed that the initial increase in Zn addition (5 to 50 mg L⁻¹), increased both the Zn and Cd concentration in soybean shoots as compared to the control. The reduction in Cd concentration occurred at the 100 mg Zn L⁻¹ level. The increase in Cd concentration in plant tissue, by the addition of 5 to 50 mg Zn L⁻¹, was due to decreased plant growth and a possibility of increased displacement of Cd into the soil solution from the soil exchange complex. The reduction in the Cd concentration in soybean shoots at the 100 to 400 mg Zn L⁻¹ levels was caused by the dilution of Cd (Cd/Zn ratio) in the soil solution due to excess amounts of Zn being present. He also stated that application of Zn to soils to achieve a reduction in Cd uptake by soybean tops did not appear to be practical since the suppression of Cd occurred only when large quantities of Zn were added.

Lagerwerff and Biersdorf (1972) found that an increase in Zn from 0.02 to 0.4 mg L⁻¹ in nutrient solution increased the Cd concentration in radish leaves by 10%.

Jarvis *et al.* (1976) observed a depression in short-term uptake of Cd by living roots due to competition with Ca, Mn and Zn ions.

Smeyers-Verbeke *et al.* (1978) observed in solution culture that a fraction of the Cd adsorbed by the roots involved metabolically mediated processes that were inhibited by Zn and Cu.

Mclaughlin *et al.* (1993) observed a reduction in potato tuber Cd concentrations with addition of Zn, but the magnitude of the effect was negligible (approximately 10-15 % reduction).

Oliver *et al.* (1994) found that the Cd concentration in wheat grain markedly decreased with the application of Zn fertilizer (upto 5 kg Zn ha⁻¹). No further significant decreases in Cd concentration in grain occurred at higher rates of applied Zn. They also concluded that the effectiveness of applied Zn on grain Cd concentration decreased with time after Zn application.

Choudhary *et al.* (1995) have found that the application of Zn (20 mg Zn kg⁻¹) to soil with N and P fertilizers increased tissue Zn concentration to sufficiency level and decreased the Cd concentration of wheat plants.

The results presented in this experiment do not allow conclusions to be drawn whether the limiting effect of Zn on Cd concentration is a process related to the physiology of the plants or to the chemical behaviour of these elements in the soil. The decrease in Cd concentration, in mixed pasture and in species, as affected by addition of increased soil Zn supply may have been due to direct competition between Zn and Cd, either externally at the root surface or within the plant (Robson and Pitman, 1983).

Cakmak and Marschner (1988) observed that Zn deficiency caused the loss of membrane integrity in root cells. Application of Zn fertilizer to the soil to alleviate this deficiency could restore the integrity of the root membrane and hence, the amount of Cd accumulated by the plant would decrease.

A further possibility is the mobilization of Cd by phytosiderophores, which are released by cereal roots in response to nutrient deficiency (Crowley *et al.*, 1987). Thus, in gramineous species either Fe or Zn deficiency may also enhance the solubility, and the mobility of Cd in the rhizosphere, due to the release of phytosiderophores. The most likely explanation is a combination of loss of membrane integrity and Cd mobilization by phytosiderophores.

In the present study the application of Zn to the soil decreased the Cd concentration in mixed pasture. The study confirmed that the application of Zn to the soils at levels suitable for fertilizer amendments is likely to reduce the pasture Cd concentration, and increase the pasture Zn concentration to sufficiency levels. The results are in general agreement with the findings of Choudhary *et al.*(1995).

Our experiment and the above literature indicate that the effects of Zn on Cd concentration might depend on external Zn concentration levels and depends also on the plant species.

In our experiment, we observed that the soil application of Zn as an agent in reducing the Cd concentration of mixed pasture was not seen to be significant since the suppression of Cd occurred only when large amounts of Zn were added.

4.8.1.4 Effect of background Cd

The background Cd status had a significant effect on the Cd concentration of mixed pasture in all harvests. The Cd concentrations recorded for background Cd status was 0.104 and 0.219 mg kg⁻¹, 0.109 and 0.265 mg kg⁻¹, 0.142 and 0.307 mg kg⁻¹ and 0.166 and 0.344 mg kg⁻¹ in low and high background Cd status in harvests 1, 2, 3 and 5 respectively. The highest Cd concentrations were recorded in harvest 4 (Appendix 6) for both the low background Cd status (0.176 mg kg⁻¹) and the high background Cd status (0.373 mg kg⁻¹) (Fig. 4.27).

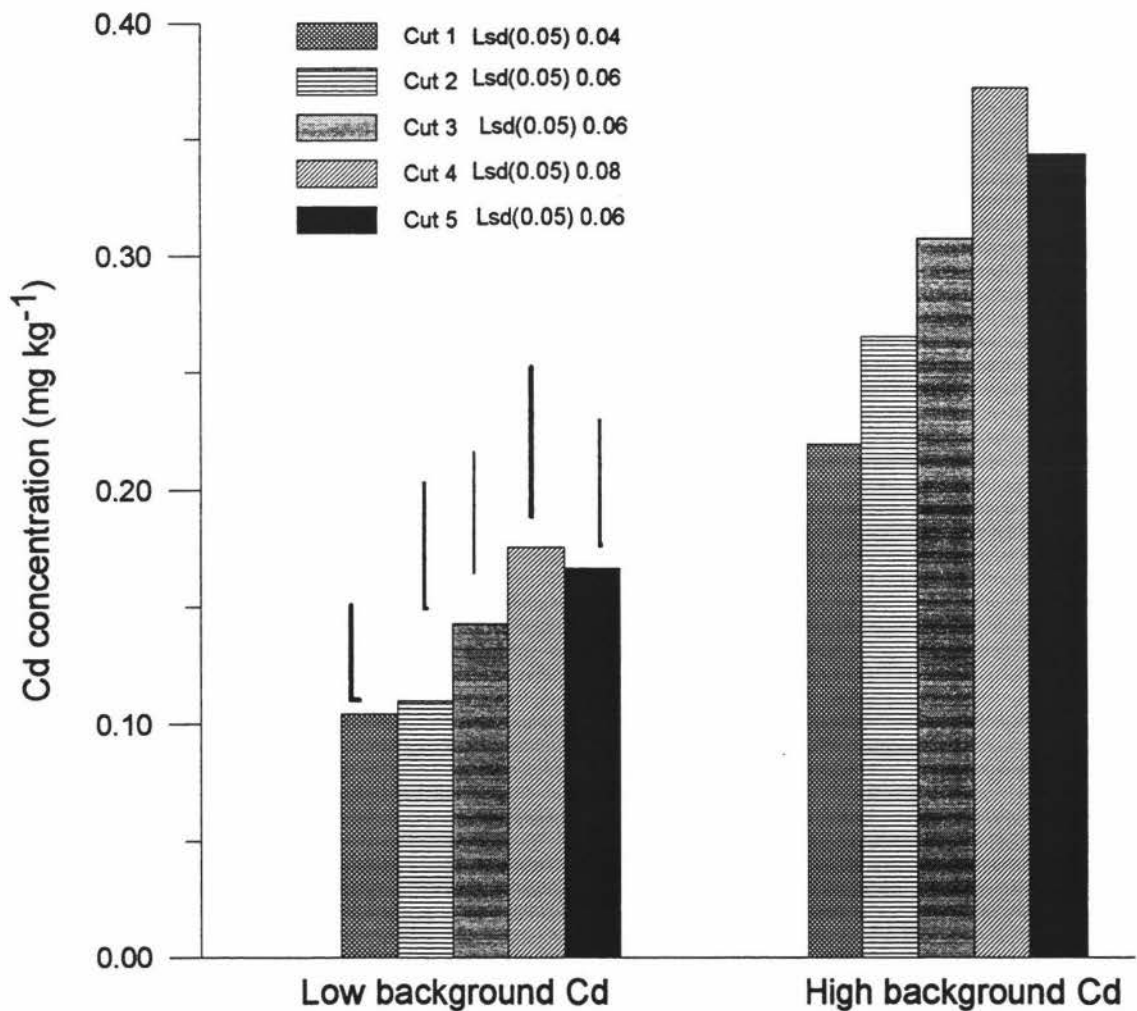


Fig. 4.27 Effect of background Cd on Cd concentration in mixed pasture.

4.8.2 Cd content in pasture species

4.8.2.1 Treatment effect

The effect of Zn and Cu fertilizers on Cd content both in grasses and legumes was significant (Appendix 6). It was noted that the Cd concentrations in grasses ranged from 0.204 mg kg⁻¹ with Zn₁₅Cu₂ to 0.394 mg kg⁻¹ with Zn₅Cu₅. The treatment Zn₅Cu₅ contained the highest Cd concentration, which was similar to all treatments except Zn₁₅Cu₂. In the case of legumes, it ranged from 0.136 mg kg⁻¹ in Zn₄₀Cu₁₀ to 0.399 mg kg⁻¹ in Zn₀Cu₅.

It was found that the Cd concentration of legumes in Zn₄₀Cu₁₀ was significantly different from Zn₀Cu₅, Zn₅Cu₅, Zn₁₅Cu₀ and Zn₄₀Cu₀, but similar to the rest of the treatments.

Williams and David (1973) show that the concentration of Cd in legumes is several times higher (0.155 mg Cd kg⁻¹) than in the associated grasses (0.031 mg Cd kg⁻¹).

Maclean (1976) recorded that alfalfa and timothy tops contained 1.11 mg Cd kg⁻¹ and 1.41 mg Cd kg⁻¹ with the addition of 5 mg Cd kg⁻¹ to the soil.

He and Singh (1994 and 1995) observed that the highest Cd concentrations in ryegrass ranged from 0.058 to 0.128 mg Cd kg⁻¹ when a high-Cd NPK fertilizers was applied (Cd ranging from 2.7 to 12.52 mg Cd kg⁻¹).

Roberts *et al.* (1994) found that there were no significant differences in mean Cd contents of legumes between native (0.07 mg Cd kg⁻¹) and pastoral (0.06 mg Cd kg⁻¹) sites.

Loganathan *et al.* (1996) concluded that low Cd concentration in clover may be related to high clover growth during summer; the Cd concentrations of clover become lower due to a dilution effect, but Cd uptake is high.

4.8.2.2 Effect of Cu level

The Cd concentration at different levels of Cu ranged from 0.171 to 0.259 mg kg⁻¹ (legumes) and 0.262 mg kg⁻¹ (grasses). It was observed that the Cu₁₀ level decreased the Cd concentration in legumes only (Fig. 4.28). This may be due to the depression of Cd uptake by excessive Cu.

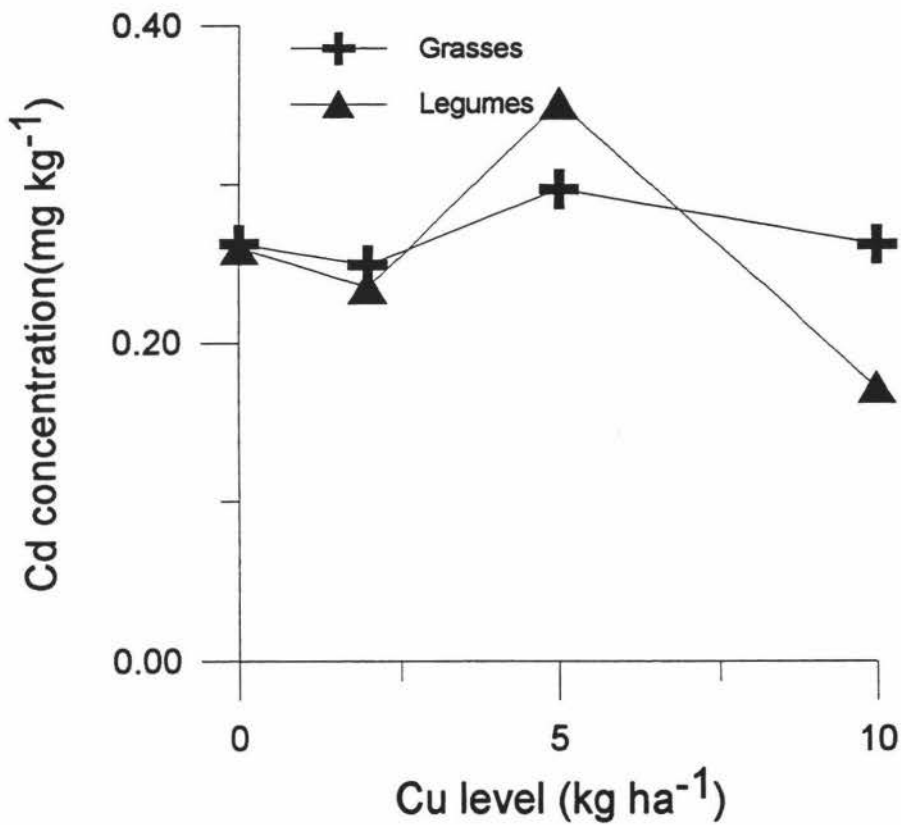


Fig. 4.28 Effect of Cu level on Cd concentration of species.

4.8.2.3 Effect of Zn level

The data for herbage Cd concentration in various Cu and Zn treatment combinations indicated that there was a slight decrease in Cd concentration with increasing levels of Zn (Appendix 6). However there was no significant effect of Zn levels in the mean Cd concentration in the pasture species. The mean Cd concentration at different Zn levels ranged from 0.230 to 0.276 mg kg⁻¹ in grasses and 0.260 to 0.325 mg kg⁻¹ in legumes (Fig. 4.29).

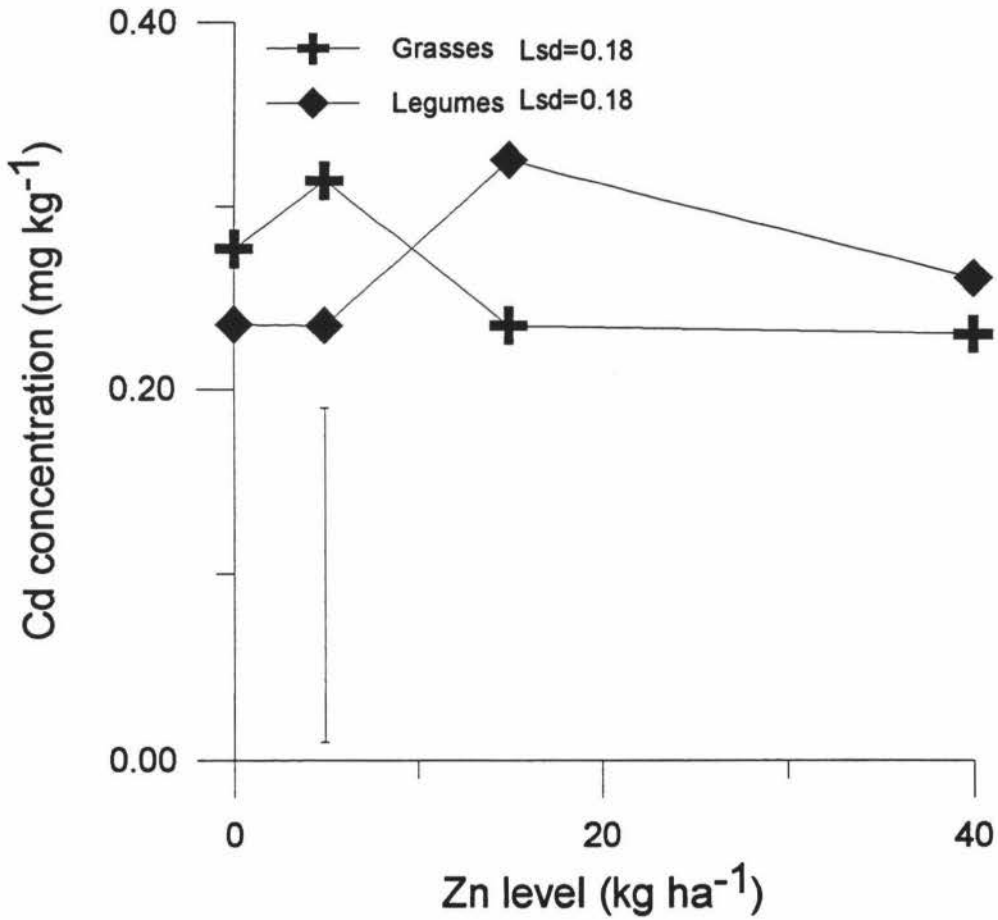


Fig. 4.29 Effect of Zn level on Cd concentration in species.

4.8.2.4 Effect of background Cd

It was seen that the background Cd status has a significant effect on Cd concentration both in grasses and legumes (Appendix 6). The Cd concentrations in low and high background Cd status was 0.170 mg kg⁻¹ and 0.364 mg kg⁻¹ (grasses) and 0.192 mg kg⁻¹ and 0.334 mg kg⁻¹ (legumes) (Fig. 4.30).

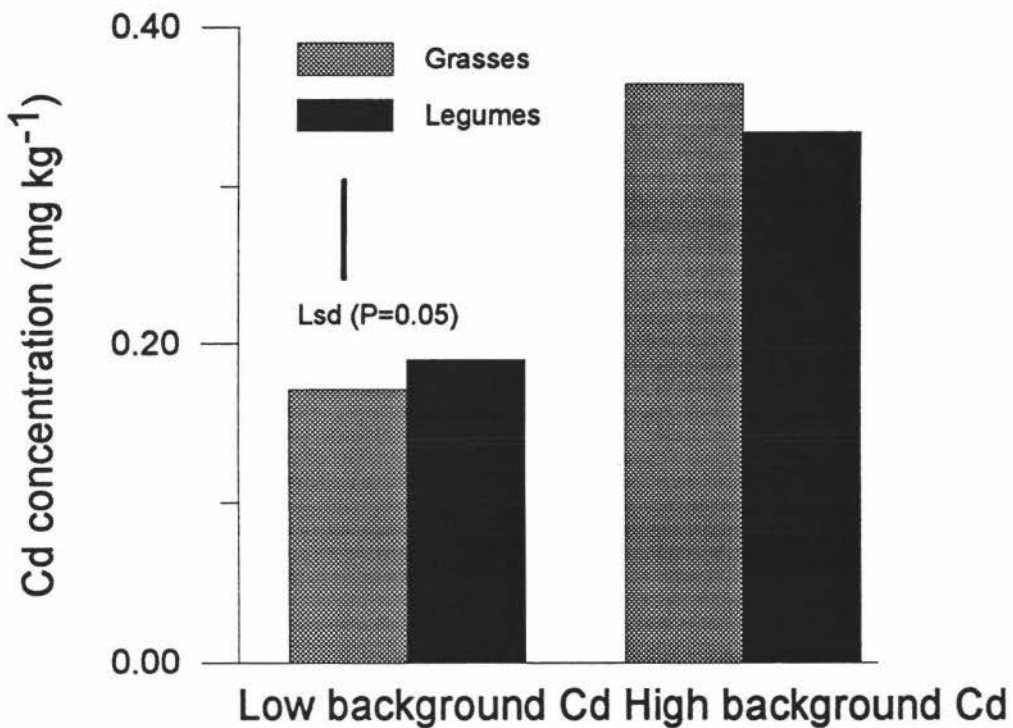


Fig. 4.30 Effect of background Cd on Cd concentration in pasture species.

4.9 Seasonal influences on dry matter yield, Cu, Zn and Cd concentrations in mixed pasture.

4.9.1 Effect on DM yield

Pasture dry matter yields followed a strong seasonal pattern with the lowest growth rates for early spring (harvest 1), and the highest for early summer (harvest 4) (Fig. 4.31).

The seasonal pattern of pasture yield and elemental concentration may differ with locations, soil type, pasture species and management practices. Temperature and soil moisture conditions are favourable during spring resulting in maximum pasture growth. Whereas in winter as the temperature falls below 10°C slow pasture growth rates result.

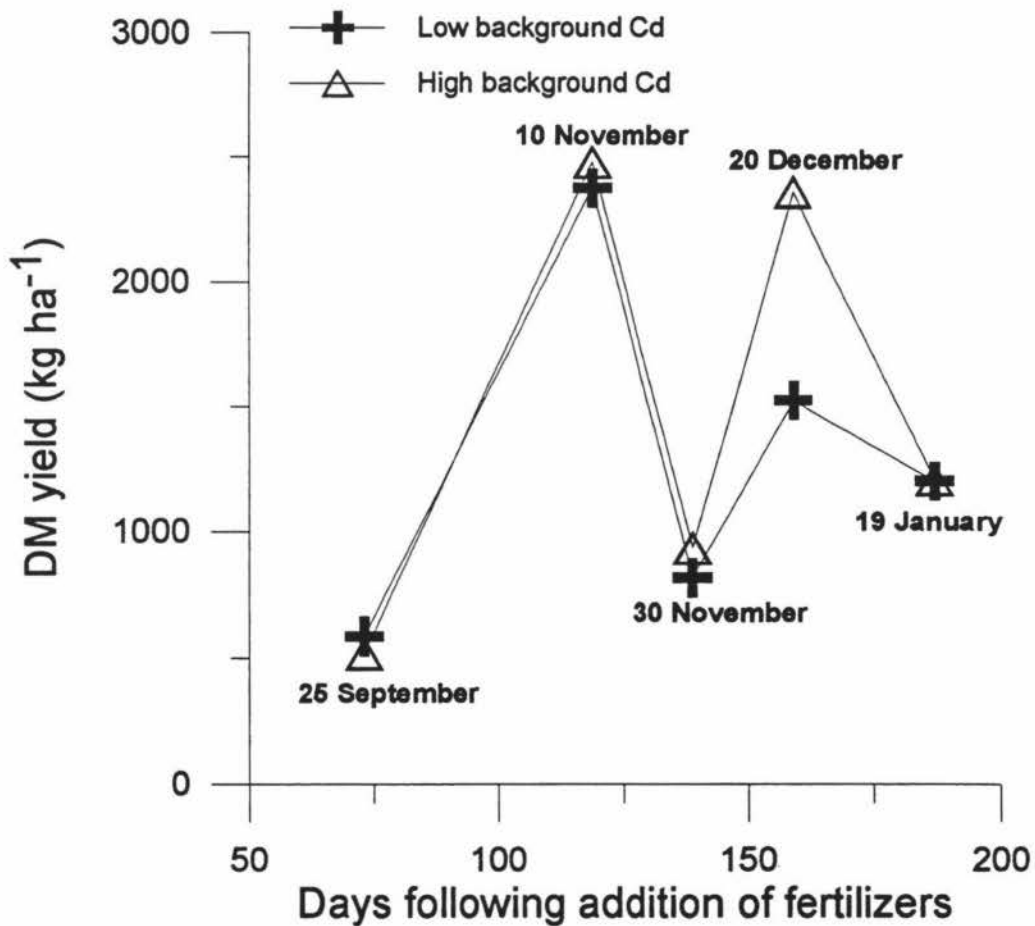


Fig. 4.31 Effect of days following the addition of fertilizer on DM yield.

Lambert (1967) found that the temperature effects on grass species are specially noticeable in Northland, New Zealand where two species, paspalum and kikuyu become important pasture components.

Lambert *et al.* (1986) observed that the poa and ryegrass have maximum growth rates in spring, most low fertility grasses produce maximum growth in late spring to early summer.

In our present study, we found that DM yield over the harvesting periods followed a strong seasonal pattern, the lower growth rates in early spring and the higher in early summer.

4.9.2 Effect on Cu

It was observed that the Cu concentration in the mixed pasture and in separate species decreased with time after fertilizer application (Fig. 4.32). This is due to adsorption/desorption processes which control the amount and rate of release of Cu for plant uptake. This applies both to native soil Cu and Cu applied as fertilizers.

Reuter *et al.* (1981) found that the Cu concentration in subterranean clover declined from 3.9 mg kg⁻¹ at 26 days from application to 1.6 mg kg⁻¹ at 98 days.

Sherrell and Rawnsley (1982) found that with an application of 2 to 4 kg Cu ha⁻¹ as copper sulphate the Cu concentration in the herbage increased from 5 to 12 mg kg⁻¹ within 4 weeks. The Cu concentration, however decreased to 8 mg kg⁻¹ over the next 9 to 10 months.

McLaren *et al.* (1990) found that initially less than 10% of the Cu adsorbed by the soil was desorbed. After three months of soil contact only a negligible amount (<1%) of the adsorbed Cu could be desorbed. Desorption of native soil Cu into soil solution decreased with increasing soil pH and was affected by temperature. The desorption of Cu added to soils showed similar trends to those observed for native Cu.

Willimott (1995) found that an application of 0, 5, 10 kg Cu ha⁻¹ as copper sulphate increased the herbage Cu concentration upto three months after addition of the Cu fertilizer, and had returned to close to initial levels nine months following addition of fertilizer.

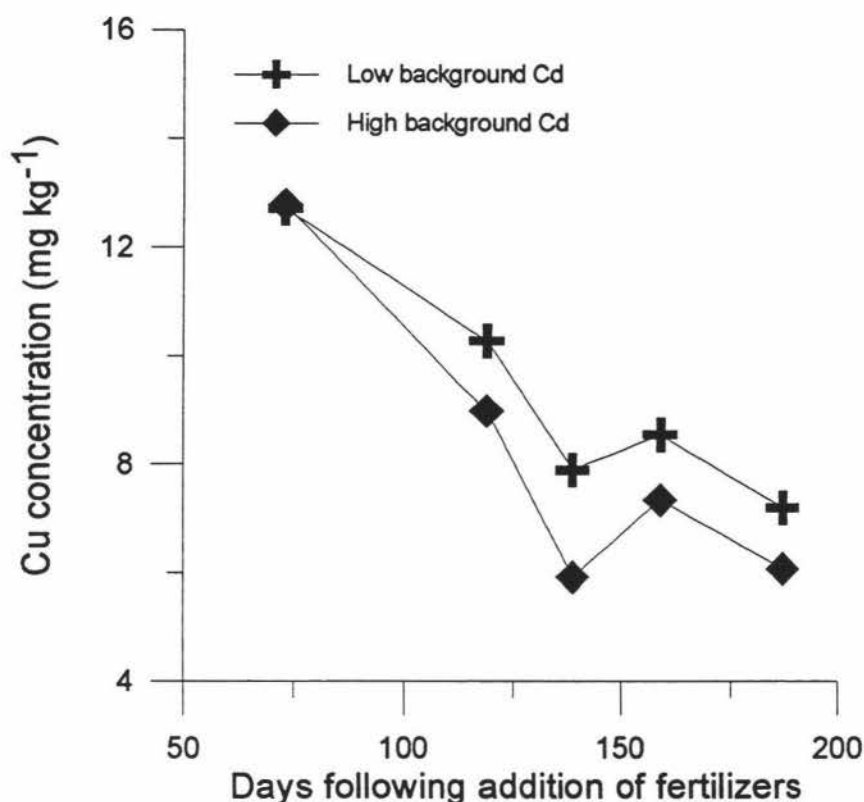


Fig. 4.32 Effect of days following addition of fertilizers on Cu concentration in mixed pasture.

4.9.3 Effect on Zn

Since the Zn concentration in the herbage for harvests 2 to 5 was not included in the discussion, it was not possible to examine the seasonal effect on Zn concentration.

Zinc deficiency is found to be more prevalent in cool and wet seasons. Soil temperature effects appear to be largely on the rate of mineralization of Zn.

Schwartz *et al.* (1987) found that barley grown at a root temperature of 10^o C had decreased root growth with thicker, shorter roots, but accumulated higher concentrations of Zn in roots when supplied at adequate levels.

Marschner and Cakmak (1989) and Moraghan and Mascagni (1991) found that high light intensity and long day length are major factors in promoting Zn stress in plants.

White *et al.* (1991) found that the Zn concentration in pasture samples can fall by 50% between spring and summer.

4.9.4 Effect on Cd

The Cd concentration of mixed pasture increased gradually both in low and high background Cd status (Fig. 4.33).

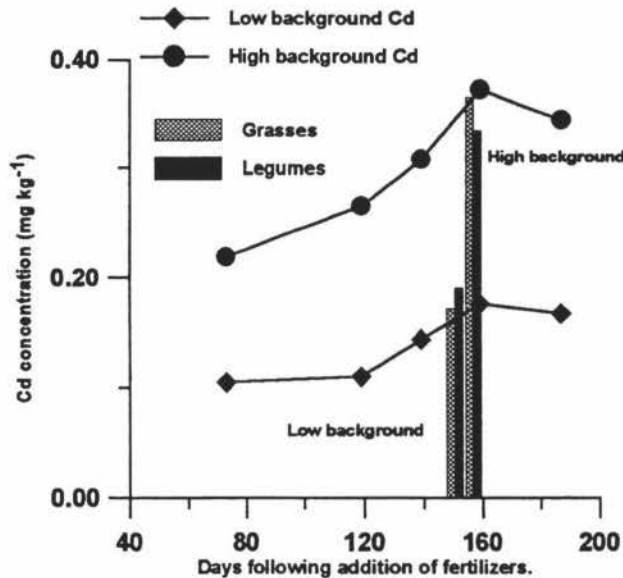


Fig. 4.33 Effect of days following the addition of fertilizers on Cd concentration in background Cd status.

Tiller (1988) recorded that an increase in Cd uptake and Cd recovery from fertilizer by subterranean clover with an increase in soil temperature from 10^o C to 18^o C. He further stated that species differences can lead to several fold differences in Cd concentration in plants.

Roberts *et al.* (1994) found that the weeds in grassland contained high concentration of Cd mainly due to slow pasture growth rates in autumn.

Loganathan *et al.* (1996) observed a strong seasonal effect on grass Cd. The Cd concentration and uptake was highest during autumn and lowest in spring. They related the seasonal differences in Cd concentration to the differences in the growth rates.

During summer when clover growth rates were high, the Cd concentration of clover become lower due to a dilution effect. The clover production was very small and therefore the seasonal pattern of herbage Cd concentration and uptake was mainly controlled by the grass Cd concentration and uptake. They have also stated that the differences could be due to changes in temperature, soil moisture and species between seasons.

4.10 Available Cu, Zn and Cd content in soil

4.10.1 Available Cu

It was recorded that the effect of Zn and Cu fertilizers on available Cu concentration (0.1M HCl extractant) in soil was significant (Table 4.4). The available Cu content in soil ranged from 0.00 (not detectable) mg kg⁻¹ in Zn₀Cu₀ to 0.83 mg kg⁻¹ in Zn₄₀Cu₁₀. The treatment Zn₄₀Cu₁₀ contained the highest amount of available soil Cu, which was similar to Zn₀Cu₁₀, but significantly different from the rest of the treatments. The treatment effects on available soil Cu concentration closely parallel the behaviour observed for Cu concentration in the mixed pasture.

Results presented in the Table 4.4 and Fig. 4.34 showed a positive correlation ($r=0.71$) of soil available Cu concentration with pasture Cu concentration in harvest 1 (Appendix 4).

Minnich *et al.* (1987) measured the Cu^{2+} activity in soil saturation extracts and related it to the accumulation of Cu in young snap beans (*Phaseolus Vulgaris* L.). They found that the Cu concentration in both shoots and roots increased with measured Cu^{2+} activity.

It was noted that the effect of background Cd status on available soil Cu was not significant. The mean Cu concentration in low and high background Cd status soils was 0.26 mg kg^{-1} and 0.29 mg kg^{-1} , respectively.

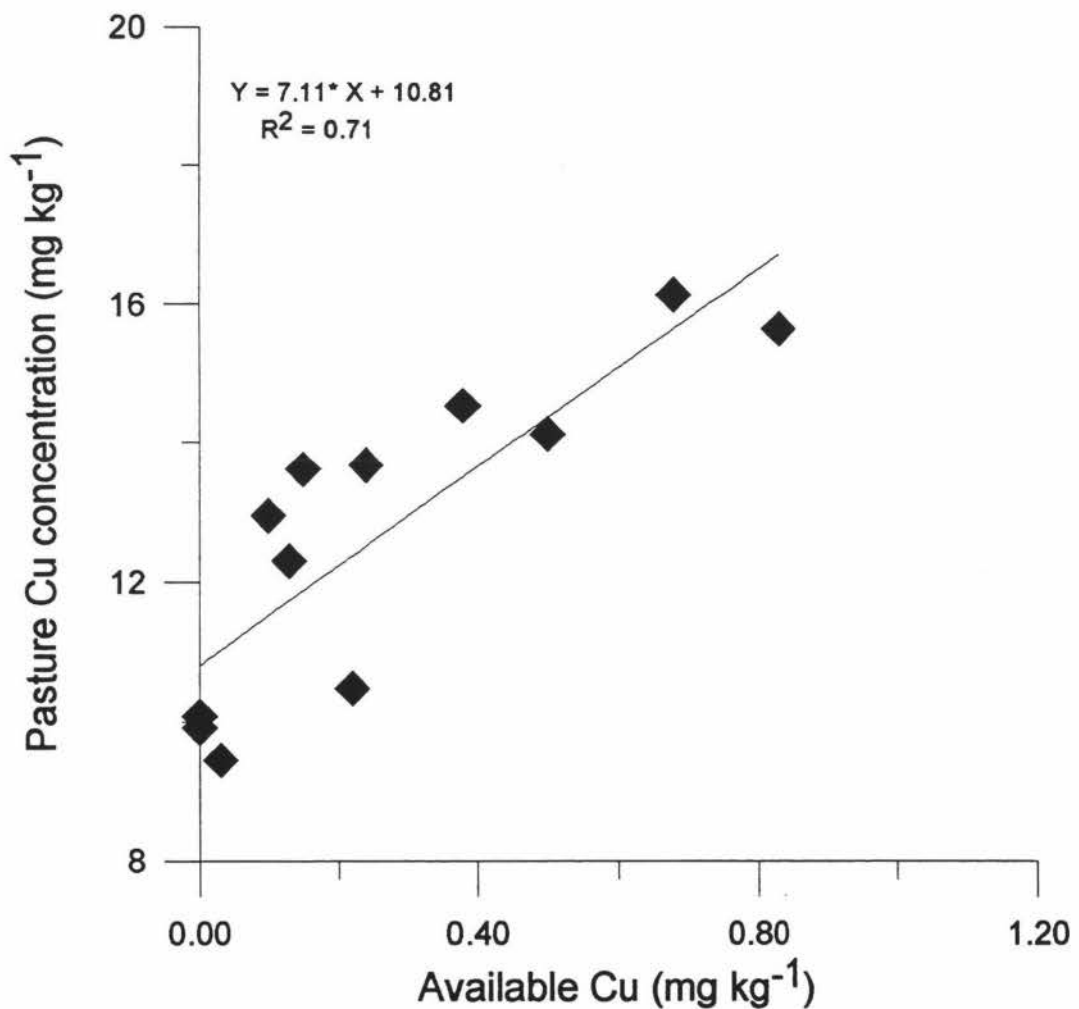


Fig. 4.34 Relationship between soil available Cu and pasture Cu (harvest 1).

Table 4.4 Available soil Cu, Zn and Cd concentrations.

Treatments	^A Cu (mg kg ⁻¹)*	^A Zn (mg kg ⁻¹)*	^A Cd (mg kg ⁻¹) ×10 ⁻³	^B Cd (mg kg ⁻¹)
Zn ₀ Cu ₀	0.00e	2.39d	0.165	0.0085
Zn ₀ Cu ₂	0.15de	2.75d	0.211	0.0116
Zn ₀ Cu ₅	0.24cde	4.11d	0.196	0.0106
Zn ₀ Cu ₁₀	0.68ab	4.43cd	0.185	0.0110
Zn ₅ Cu ₀	0.00e	5.43cd	0.173	0.0101
Zn ₅ Cu ₂	0.13de	5.60cd	0.176	0.0101
Zn ₅ Cu ₅	0.50bc	5.06cd	0.190	0.0115
Zn ₁₅ Cu ₀	0.22cde	11.69b	0.176	0.0101
Zn ₁₅ Cu ₂	0.10de	10.97b	0.173	0.0091
Zn ₁₅ Cu ₅	0.38cd	9.42bc	0.190	0.0113
Zn ₄₀ Cu ₀	0.03e	28.63a	0.163	0.0103
Zn ₄₀ Cu ₁₀	0.83a	24.55a	0.191	0.0111
CV%	96.55	46.39	42.55	34.16
LSD(0.05)	0.303	5.16	-	-
Low Cd	0.26	9.13	0.112b	0.0064b
High Cd	0.29	10.04	0.253a	0.0145a
LSD (0.05)	-	-	0.036	0.0017

*Treatment means followed by the same letter within a column are not significantly different at 5% level.

^A Denotes 0.1M HCl extraction and ^B Denotes 0.01M CaCl₂ extraction

4.10.2 Available Zn

The effect of Zn and Cu fertilizers on available soil Zn extracted by 0.1M HCl solutions was significant (Table 4.4). The Zn concentrations ranged from 2.39 mg kg⁻¹ in Zn₀Cu₀ to 28.63 mg kg⁻¹ in Zn₄₀Cu₀. The treatment Zn₄₀Cu₀ contained the highest amount of available Zn which was significantly different from the rest of the treatments except the treatment Zn₄₀Cu₁₀. These results exactly paralleled the treatment effects on Zn concentration of mixed pasture in harvest 1 (Appendix 5).

Results presented in the Table 4.4 and Fig. 4.35 showed a positive correlation ($r=0.84$) of soil available Zn with pasture Zn concentration in harvest 1.

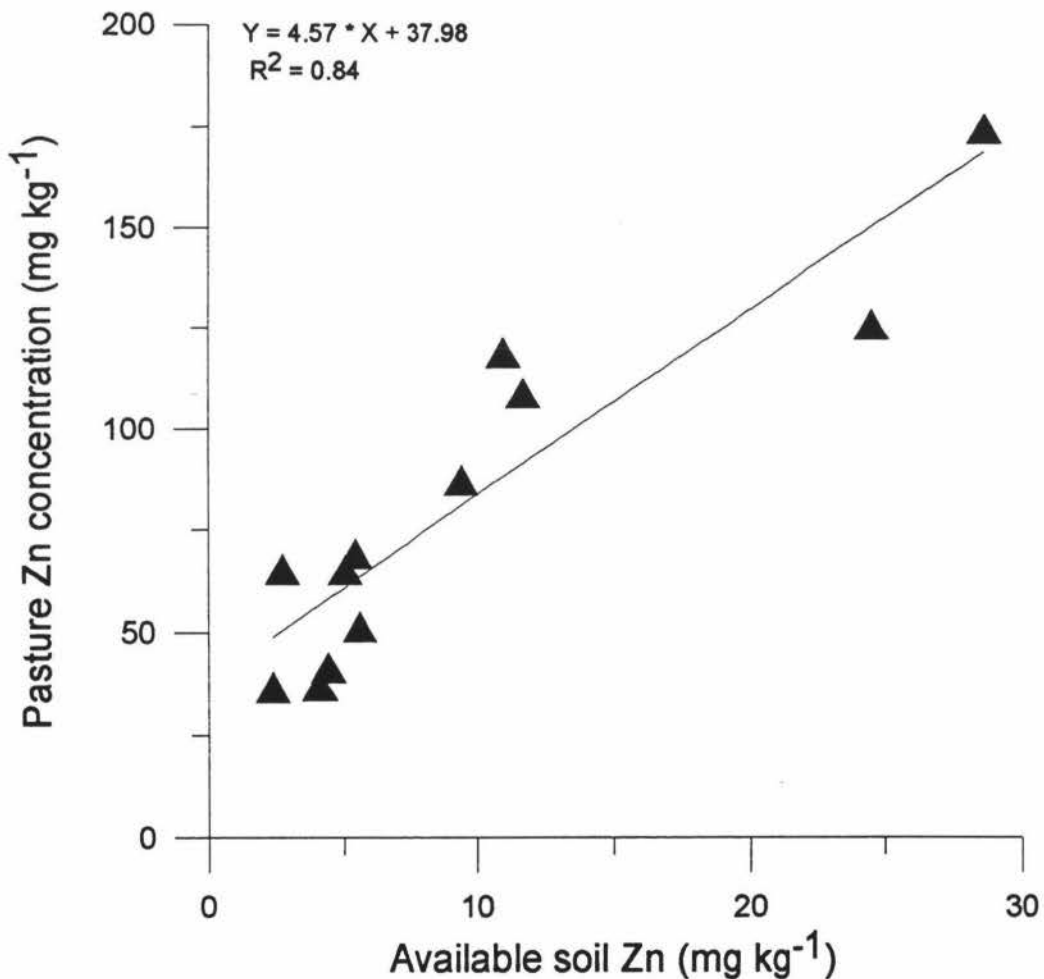


Fig. 4.35 Relationship of soil available Zn with pasture Zn concentration (harvest 1).

It was observed that the effect of background Cd status on available soil Zn was not significant. The mean Zn concentrations in the low and high background Cd status soils are 9.13 mg kg⁻¹ and 10.04 mg kg⁻¹, respectively

4.10.3 Available Cd

It was observed that the effect of Zn and Cu fertilizers on available soil Cd in both extractants was not significant. The Cd concentrations in the 0.1M HCl extract ranged from 0.163×10⁻³ mg kg⁻¹ in Zn₄₀Cu₀ to 0.211×10⁻³ mg kg⁻¹ in Zn₀Cu₂ and in the 0.01 M CaCl₂ extract and ranged from 0.0085 mg kg⁻¹ in Zn₀Cu₀ to 0.0116 mg kg⁻¹ in Zn₀Cu₂ (Table 4.4). The 0.1M HCl extract removed less Cd than did the 0.01 M CaCl₂ extract. The higher values in the 0.01 M CaCl₂ extracted soils were due to AAS measuring all forms of Cd in solution, whereas the 0.1M HCl extracted only from mineral surfaces and labile Cd-organic matter complexes in solution.

It was seen that the background Cd status has a significant effect on available Cd concentration both in 0.1M HCl and 0.01 M CaCl₂ extract. The low background Cd status contained lower amounts of available Cd than did high background Cd status. The Cd concentration in 0.1M HCl extract was 0.112×10⁻³ mg kg⁻¹ in low background Cd status and 0.253×10⁻³ mg kg⁻¹ in high background Cd status. It was observed that the Cd concentration in the 0.01 M CaCl₂ extract was 0.0064 mg Cd kg⁻¹ in low background Cd status and 0.0145 mg Cd kg⁻¹ in high background Cd status. Results presented in the Table 4.4 and Fig 4.36 show a positive correlation of soil available Cd (0.1M HCl extract) with pasture Cd concentrations in harvest 1.

The Cd concentration in the 0.01 M CaCl₂ extract was very close to the values observed for these soils (Andrews *et al.*, 1996). They have concluded that 0.01 M CaCl₂ may be suitable for assessing the plant availability of Cd in New Zealand soils with a wider range of CEC and organic matter contents. Whitten and Ritchie (1991) have also shown that 0.01 M CaCl₂ is a good indicator of Cd made available to subterranean clover by decreasing the pH of soils with low organic matter content from Western Australia.

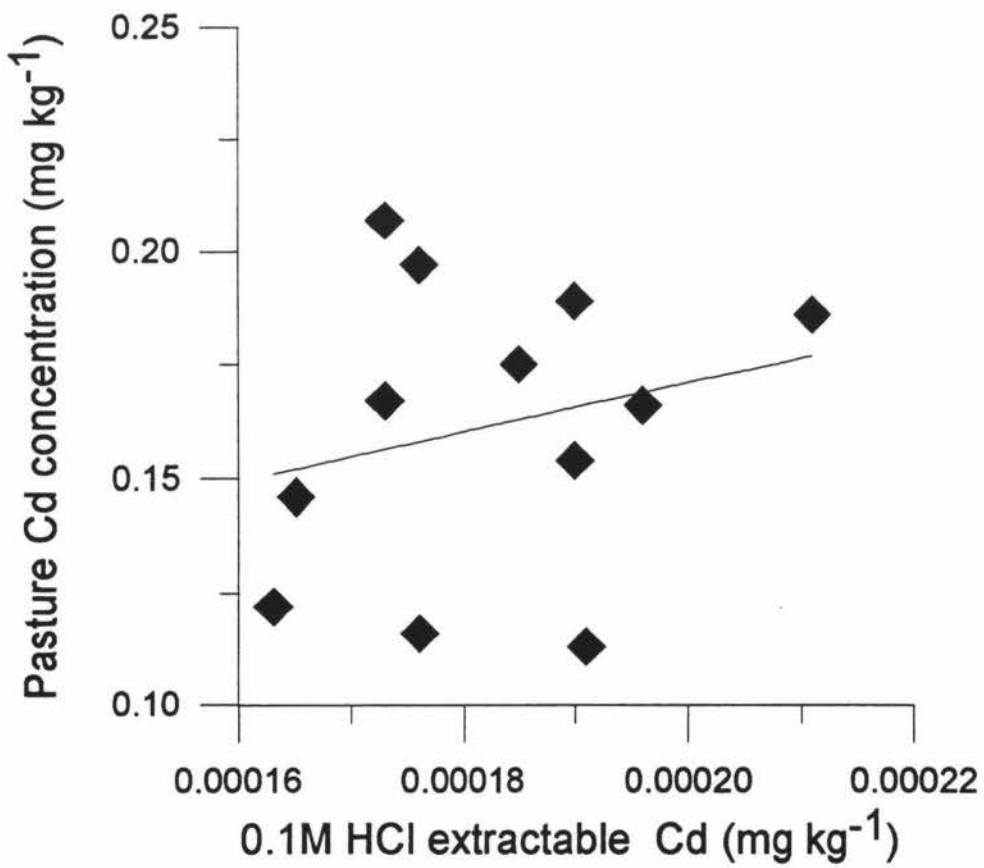
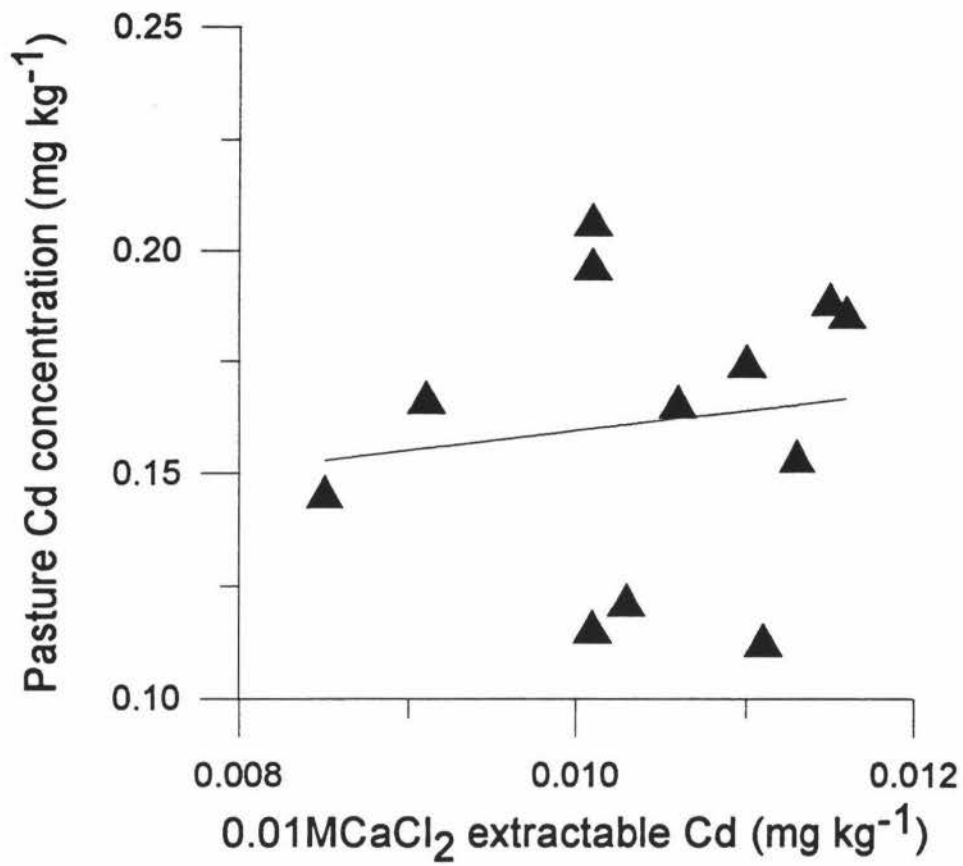


Fig. 4.36 Relationship between available Cd and pasture Cd concentration.

In our experiment since only two background levels of Cd were maintained it was not possible to obtain a good relationships between the available Cd and Cd uptake by pastures.

Table 4.5 Total Cd concentration in soil after 73 days from fertilizer application.

Treatments	Total Cd (mg kg ⁻¹)
Zn ₀ Cu ₀	0.0844
Zn ₀ Cu ₂	0.0848
Zn ₀ Cu ₅	0.0960
Zn ₀ Cu ₁₀	0.0787
Zn ₅ Cu ₀	0.0849
Zn ₅ Cu ₂	0.0875
Zn ₅ Cu ₅	0.1008
Zn ₁₅ Cu ₀	0.0867
Zn ₁₅ Cu ₂	0.0920
Zn ₁₅ Cu ₅	0.0939
Zn ₄₀ Cu ₀	0.0771
Zn ₄₀ Cu ₁₀	0.0927
CV(%)	37.37
Low Cd	0.058b
High Cd	0.118a
LSD(0.05)	0.015

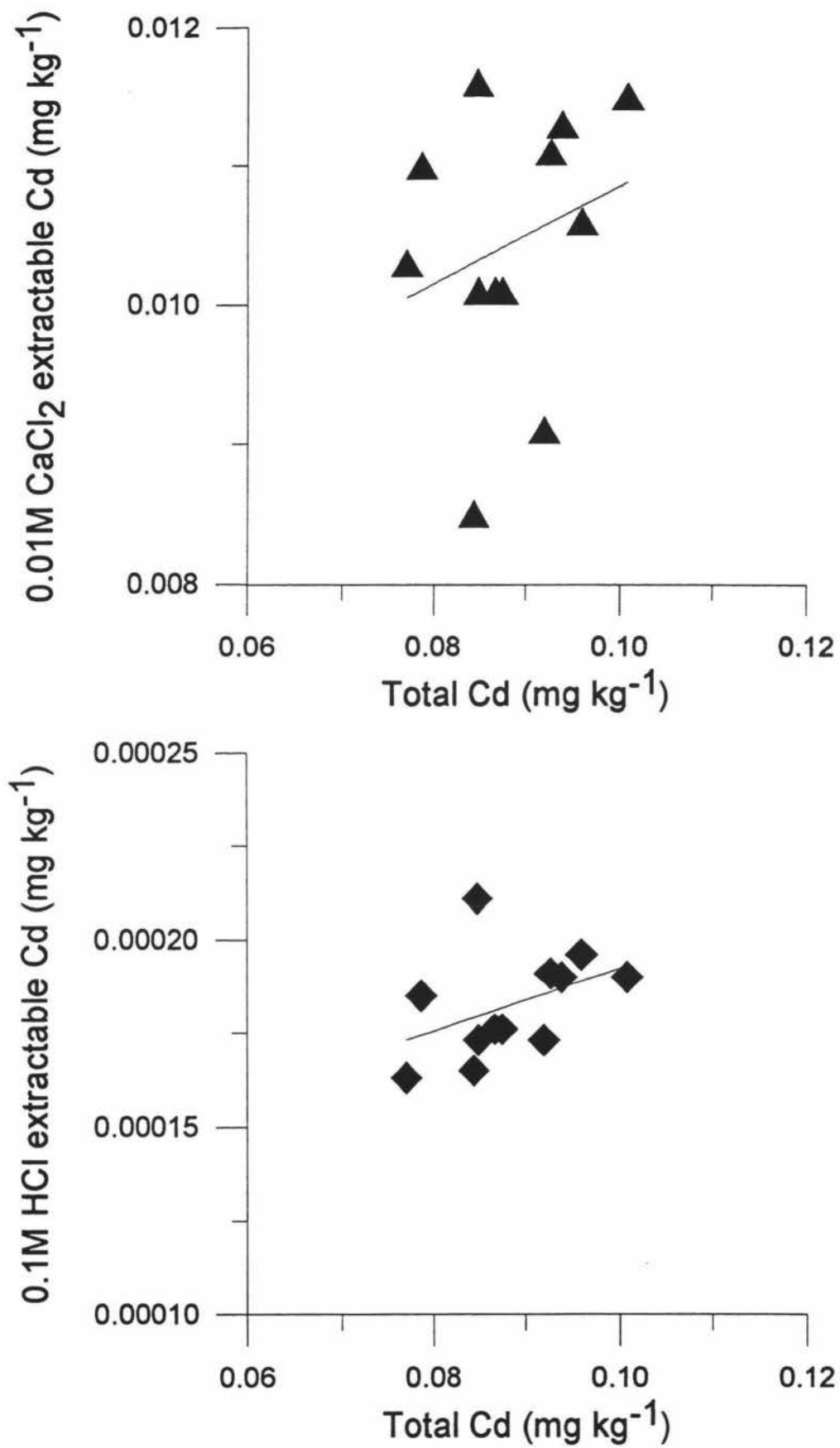


Fig. 4.37 Relationship of soil total Cd with available Cd.

4.11 Total Cd concentration in soil

Total Cd (pseudo-total) concentration was measured 73 days following the fertilizer application. Results presented in the Table 4.5 indicate that there was no significant effect of Zn and Cu fertilizers on soil total Cd concentration.

It was observed that the total Cd concentrations in the soil ranged from 0.0771 mg kg⁻¹ in Zn₄₀Cu₀ to 0.1008 mg kg⁻¹ in Zn₅Cu₅. These results are within the range reported by Loganathan *et al.* (1995) for Cd in surface soil, which averaged 0.40 ± 5 mg kg⁻¹ (range 0.18-0.6 mg kg⁻¹) on high fertility farmlets, whereas, on unfertilized farmlets, Cd concentration averaged 0.10 ± 0.01 mg kg⁻¹ (range 0.02 - 0.190 mg kg⁻¹).

Results presented in the Table 4.5 and Fig 4.37 show a good correlation of total Cd with soil available Cd (0.1M HCl extract), 73 days after fertilizer addition.

It was also observed that the effect of background Cd status has a significant effect on total Cd concentration. The Cd concentrations were 0.058 mg kg⁻¹ and 0.118 mg kg⁻¹ in low and high background Cd status respectively.

Merry and Tiller (1991) observed that the Cd concentration of pastoral soils in Australia was about 0.10 mg kg⁻¹.

Roberts *et al.* (1994) examined the Cd status of non-agricultural soils and agricultural pasture soils in New Zealand and found that in non agricultural soils (*n*=86) Cd concentrations (0 to 7.5 cm depth) ranged from 0.02 to 0.77 mg kg⁻¹, with a mean value of 0.20 mg kg⁻¹ and in pastoral soils (*n*=312) the range was 0.04 to 1.53 mg kg⁻¹, with a mean value of 0.44 mg kg⁻¹.

CONCLUSIONS

1 **DM yield response to Zn level**

The addition of 750 mg Zn kg⁻¹ to soil produced the highest DM yield only in harvest 3. The control (Zn₀) level produced the highest DM yield in all harvests. It was also found that grasses are more tolerant at higher levels of Zn than legumes. There was a yield decrease resulting from the addition of Zn fertilizers. Toxicity is the explanation for the reduction in yield of mixed pasture.

2 **DM yield response to Cu level**

The addition of 100 mg Cu kg⁻¹ to soil produced the highest DM yield only in harvests 2 and 4. It was observed that at Cu₀ level produced the highest DM yield in the rest of the harvests. There was a yield reduction with increasing levels of Cu. We have observed that grasses are more susceptible at higher levels of Cu than legumes. According to the published reports and the results of our experiment, we can conclude that at higher (>5 kg Cu ha⁻¹) Cu concentration is toxic to plant growth.

3 **Zinc concentration vs. Zn level**

The different Zn levels have a great influence on Zn concentration at harvest 1. Large amounts of Zn would lead to luxury uptake and accumulation of Zn in pasture leading to decreases the DM yield.

4 Copper concentration vs. Cu level

There was a general trend that the Cu concentration increased with increasing levels of Cu in all harvests. The addition of 500 mg Cu kg⁻¹ contained the highest Cu concentration in all harvests. The Cu concentration in legumes increased with increasing levels of Cu up to 250 mg Cu kg⁻¹ soil but the grass did not show any response with increasing levels of Cu. It was reported that the Cu concentration in the mixed pasture and in species decreased with time after fertilizer application.

5 Zinc concentration vs. Cd concentration

Results reported in this experiment show that the application of Zn to the soil decreased the Cd concentration in mixed pastures at harvest 1. The application of 2000 mg Zn kg⁻¹ soil showed the greatest effect in reducing the Cd concentration in mixed pasture. The effects of Zn on Cd concentration may depend on external Zn concentration levels and also on the plant species.

6 Copper concentration vs. Cd concentration

It was observed that the different Cu levels have no significant effect on Cd concentration in all harvests. Only in harvest 1, the addition of 500 mg Cu kg⁻¹ to soil led to a decrease in Cd concentration.

7 Zinc and Cu concentration on Cd:Cu and Cd:Zn ratios

The effect of the addition of Cu and Zn in fertilizers was to lower the Cd:Zn ratio in herbage, solely by increasing the Zn concentration in herbage.

The amount of Zn, Cu and Cd extractable from the soil in 0.1M HCl solution was affected by three factors; additions of Zn alone, Cu alone and an interaction between them. 0.1M HCl extractable Zn and Cu showed a positive correlation with Zn and Cu concentration in the mixed pasture (harvest 1).

The amount of extractable Cd both in 0.1M HCl and 0.01M CaCl₂ extractants did not show good relationships with Cd concentrations in the mixed pasture of harvest 1. It was also observed that the amount of extractable Cd for both extractants was well correlated with total Cd concentration in the soil.

It was noted that the background Cd status has a significant effect on available Cd for both extractants and also on total Cd concentration in the soil.

From this we can conclude that the levels of extractable Zn and Cu with 0.1M HCl appeared to be the most useful indicator of their availability to the pasture.

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Appendices

Appendix 1 Rates of Zn and Cu fertilizer additions in the field experiment.

Treatments	ZnSO ₄ ·7H ₂ O (g m ⁻²)	CuSO ₄ ·5H ₂ O (g m ⁻²)	SSP (g m ⁻²)	Sand (g m ⁻²)
Zn ₀ Cu ₀	0	0	40	400
Zn ₀ Cu ₂	0	0.8	40	400
Zn ₀ Cu ₅	0	2.0	40	400
Zn ₀ Cu ₁₀	0	4.0	40	400
Zn ₅ Cu ₀	2.27	0	40	400
Zn ₅ Cu ₂	2.27	0.8	40	400
Zn ₅ Cu ₅	2.27	2.0	40	400
Zn ₁₅ Cu ₀	6.81	0	40	400
Zn ₁₅ Cu ₂	6.81	0.8	40	400
Zn ₁₅ Cu ₅	6.81	2.0	40	400
Zn ₄₀ Cu ₀	18.18	0	40	400
Zn ₄₀ Cu ₁₀	18.18	4.0	40	400

Appendix 2 Dry matter yield (kg ha⁻¹) of pasture at different harvests.

Treatments	Mixed pasture					Pasture species	
	Harvest 1*	Harvest 2*	Harvest 3*	Harvest 4	Harvest 5	Grass	Legume
Zn ₀ Cu ₀	919a	2905a	860ab	1932	1459	1713	173
Zn ₀ Cu ₂	700ab	2788ab	693b	2030	1222	1613	283
Zn ₀ Cu ₅	486b	2540abcd	772ab	2336	1309	1940	352
Zn ₀ Cu ₁₀	546b	2618abcd	907ab	1924	1127	1540	309
Zn ₅ Cu ₀	405b	1963d	987ab	1939	1157	1537	336
Zn ₅ Cu ₂	413b	2629abcd	884ab	2323	1241	1896	313
Zn ₅ Cu ₅	588ab	2381abcd	861ab	1731	1051	1500	206
Zn ₁₅ Cu ₀	485b	2039cd	939ab	1812	1264	1606	164
Zn ₁₅ Cu ₂	434b	2169bcd	1015a	1897	951	1727	120
Zn ₁₅ Cu ₅	496b	2375abcd	938ab	1514	1291	1303	171
Zn ₄₀ Cu ₀	636ab	2667abc	801ab	1905	1308	1729	105
Zn ₄₀ Cu ₁₀	518b	2130bcd	840ab	1987	1056	1824	119
CV%	54.23	24.73	29.79	39.33	38.16	43.99	105.01
LSD(0.05)	347.96	698.97	302.7	-	-	-	-
Low Cd	586	2377	814	1526b	1199	1299b	183
High Cd	518	2480	934	2362a	1207	2022a	259
LSD (0.05)	-	-	-	362.52	-	346.35	-

*Treatment means followed by the same letter within a column are not significantly different at 5% level.

Appendix 3 Growth rates of pasture (kg ha⁻¹ day⁻¹) for different harvests.

Treatments	Mixed pasture					Pasture species	
	Harvest 1*	Harvest 2*	Harvest 3*	Harvest 4	Harvest 5	Grass	Legume
Zn ₀ Cu ₀	12.60a	63.1a	43.01ab	96.62	52.12	85.66	8.65
Zn ₀ Cu ₂	9.60ab	60.4ab	34.66b	101.54	43.67	80.70	14.18
Zn ₀ Cu ₅	6.66b	55.2abcd	38.61ab	116.85	46.78	97.03	17.61
Zn ₀ Cu ₁₀	7.49b	56.9abcd	45.37ab	96.21	40.26	77.01	15.46
Zn ₅ Cu ₀	5.56b	42.6d	49.36ab	96.98	41.35	76.86	16.85
Zn ₅ Cu ₂	5.67b	57.1abcd	44.24ab	116.18	44.35	94.83	15.67
Zn ₅ Cu ₅	8.07ab	51.7abcd	43.06ab	86.58	37.56	75.01	10.34
Zn ₁₅ Cu ₀	6.65b	44.3cd	46.95ab	90.63	45.16	80.34	8.24
Zn ₁₅ Cu ₂	5.95b	47.1bcd	50.79a	94.88	33.98	86.38	6.04
Zn ₁₅ Cu ₅	6.80b	51.6abcd	46.90ab	75.73	46.12	65.18	8.55
Zn ₄₀ Cu ₀	8.73ab	57.9abc	40.09ab	95.29	46.74	86.48	5.27
Zn ₄₀ Cu ₁₀	7.10b	46.3bcd	42.04ab	99.39	37.73	91.24	5.99
CV%	0.74	0.53	1.48	1.96	1.36	2.19	5.25
LSD(0.05)	4.76	15.19	15.13	-	-	-	-
Low Cd	8.04	51.69	40.71	76.34b	42.83	64.98b	9.17
High Cd	7.10	53.91	46.75	118.13a	43.12	101.13a	12.95
LSD (0.05)	-	-	-	18.12	-	17.31	-

*Treatment means followed by the same letter within a column are not significantly different at 5% level.

Appendix 4 Copper concentrations (mg kg⁻¹) in pasture for different harvests.

Treatments	Harvest 1*	Harvest 2*	Harvest 3*	Harvest 4*	Harvest 5*	Grass	Legume*
Zn₀Cu₀	10.0bcd	8.1c	6.9ab	6.7c	6.8ab	6.7	8.5d
Zn₀Cu₂	13.6abcd	10.0ab	7.5ab	8.1abc	6.9ab	7.1	9.3bcd
Zn₀Cu₅	13.6abcd	9.7ab	6.9ab	8.1abc	7.1a	6.5	9.8abcd
Zn₀Cu₁₀	16.1a	10.4a	7.9a	8.8a	7.1a	6.8	10.7ab
Zn₅Cu₀	9.9cd	9.6abc	6.7ab	8.1abc	6.8ab	6.8	8.6d
Zn₅Cu₂	12.3abcd	9.6abc	6.5ab	7.2bc	6.9ab	6.8	8.7d
Zn₅Cu₅	14.1abc	10.1ab	7.4ab	8.5ab	6.4ab	7.3	10.1abc
Zn₁₅Cu₀	10.4bcd	10.2ab	6.0b	8.3ab	6.1b	7.0	8.8cd
Zn₁₅Cu₂	12.9abcd	9.3abc	6.3b	7.6abc	6.1b	7.1	9.8abcd
Zn₁₅Cu₅	14.5ab	10.0ab	7.2ab	8.3ab	6.4ab	7.3	10.8a
Zn₄₀Cu₀	9.4d	8.6bc	6.2b	7.7abc	6.2ab	7.0	9.1cd
Zn₄₀Cu₁₀	15.6a	9.5abc	6.9ab	7.4abc	6.2b	7.1	9.1cd
CV%	30.1	14.8	19.2	17.2	12.1	10.6	12.7
LSD(0.05)	4.4	1.6	1.5	1.5	0.937	-	1.4
Low Cd	12.7	10.2a	7.8a	8.5a	7.1a	7.1	9.4
High Cd	12.7	8.9b	5.9b	7.3b	6.0b	6.8	9.4
LSD (0.05)	-	0.676	0.629	0.649	0.382	-	-

*Treatment means followed by the same letter within a column are not significantly different at 5% level.

Appendix 5 Zinc concentrations (mg kg⁻¹) in pasture for different harvests.

Treatments	Harvest 1*	Harvest 2	Harvest 3	Harvest 4*	^N Harvest 5*	Grass*	Legume*
Zn ₀ Cu ₀	36.7e	68.4	180.3	63.0b	253.3ab	54.0d	92.3bcde
Zn ₀ Cu ₂	65.3de	154.9	211.4	138.1ab	224.3ab	87.8bcd	60.5e
Zn ₀ Cu ₅	37.1e	153.2	159.1	129.3ab	146.7b	66.5cd	60.7e
Zn ₀ Cu ₁₀	41.5e	179.8	175.3	137.0ab	229.5ab	85.0bcd	75.2cde
Zn ₅ Cu ₀	69.2de	205.0	172.5	102.4ab	235.5ab	82.3bcd	74.4de
Zn ₅ Cu ₂	51.4de	159.0	206.0	124.7ab	305.1a	70.1cd	72.5de
Zn ₅ Cu ₅	65.3de	158.5	168.3	132.7ab	276.0a	74.6cd	96.4bcde
Zn ₁₅ Cu ₀	108.7bc	183.3	175.9	168.3a	246.1ab	96.8abc	131.5ab
Zn ₁₅ Cu ₂	118.6bc	163.8	198.5	111.6ab	251.3ab	75.1cd	154.6a
Zn ₁₅ Cu ₅	87.1cd	188.1	233.5	159.3a	196.1ab	128.0a	114.3abcd
Zn ₄₀ Cu ₀	174.5a	184.2	194.8	163.5a	214.1ab	111.5ab	156.9a
Zn ₄₀ Cu ₁₀	125.5b	184.8	224.4	153.0a	231.3ab	87.5bcd	121.5abc
CV%	38.6	80.5	59.6	51.7	45.3	35.0	39.7
LSD(0.05)	36.7	-	-	79.2	123.4	34.5	46.5
Low Cd	81.7	165.8	217.7	126.6	219.6	75.8b	103.4
High Cd	81.8	164.7	165.6	137.1	248.6	94.1a	98.4
LSD (0.05)	-	-	-	-	-	14.1	-

*Treatment means followed by the same letter within a column are not significantly different at 5% level.

^NPlease see the note in section 4.1 (page 43).

Appendix 6 Cadmium concentrations (mg kg⁻¹) in pasture for different harvests.

Treatments	Harvest 1	Harvest 2*	Harvest 3	Harvest 4	Harvest 5	Grass*	Legume*
Zn ₀ Cu ₀	0.146	0.157ab	0.287	0.261	0.288	0.261ab	0.140e
Zn ₀ Cu ₂	0.186	0.151b	0.264	0.286	0.242	0.278ab	0.210bcde
Zn ₀ Cu ₅	0.166	0.210ab	0.240	0.274	0.287	0.257ab	0.399a
Zn ₀ Cu ₁₀	0.175	0.189ab	0.280	0.273	0.266	0.309ab	0.207bcde
Zn ₅ Cu ₀	0.207	0.190ab	0.197	0.274	0.224	0.282ab	0.169de
Zn ₅ Cu ₂	0.116	0.229ab	0.269	0.263	0.245	0.265ab	0.184cde
Zn ₅ Cu ₅	0.189	0.322a	0.199	0.411	0.292	0.394a	0.350abcd
Zn ₁₅ Cu ₀	0.197	0.182ab	0.198	0.265	0.267	0.260ab	0.361abc
Zn ₁₅ Cu ₂	0.167	0.169ab	0.218	0.258	0.255	0.204b	0.310abcde
Zn ₁₅ Cu ₅	0.154	0.146b	0.193	0.275	0.228	0.239ab	0.303abcde
Zn ₄₀ Cu ₀	0.122	0.157ab	0.181	0.225	0.227	0.245ab	0.385ab
Zn ₄₀ Cu ₁₀	0.113	0.144b	0.171	0.219	0.238	0.214ab	0.136e
CV%	52.78	77.56	55.78	66.35	56.70	61.08	59.62
LSD(0.05)	-	0.169	-	-	-	0.189	0.182
Low Cd	0.104b	0.109b	0.142b	0.175b	0.166b	0.170b	0.192b
High Cd	0.219a	0.265a	0.307a	0.372a	0.344a	0.364a	0.334a
LSD (0.05)	0.040	0.068	0.059	0.086	0.068	0.077	0.074

*Treatment means followed by the same letter within a column are not significantly different at 5% level.