THE INFLUENCE OF WATERLOGGING ON THE DISTRIBUTION OF TRACE METALS IN SOIL

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SUMMARY

The thesis is devoted to a consideration of soil factors influencing the availability of trace metals for plant uptake, with particular reference to waterlogging. Chapter 1 includes a brief survey of the roles of trace metals in biological systems and the forms which exist in soil fractions. The forms of trace metals influenced by environmental factors are also discussed. The importance of waterlogging in influencing the distribution of trace metals is discussed in more detail, as are the relevant chemical changes taking place in the soil with various waterlogging times and depths. The objectives of the thesis are to investigate the effects of waterlogging on the redistribution of trace metals in soil and the implications for plant uptake. The case is made out for this study.

Chapter 2 lays emphasis on specific extractants for the removal of metals held by different mechanisms in the soil, and the analytical techniques adapted in the thesis. As only small amounts of some metals will be present in the extracts, the analytical method used will be important. Atomic absorption spectrometry is a commonly used analytical method for soils and is used in this work. Great care is necessary, however, interferences such as matrix effects and multiple peaks are met when analysing the complex solutions which are obtained by soil extraction. Variation of ashing time, use of deuterium background correction, addition of equal volumes of 10%(v/v) nitric acid to the injected sample,

heating of the extract to dryness with a HNO_3-H_2SO_4 acid mixture and redissolution of the residue in hydrochloric acid, all had no effect in eliminating the interferences. After this lack of success by standard techniques, a method was devised to overcome these problems. It was found that interferences in the analysis of acetic acid, pyrophosphate and acid oxalate extracts of soil for Cd, Co, Cu, Ni and Pb can be overcome by extraction with dithizone in chloroform. Treatment of the extracts with hydroxyl ammonium chloride to reduce iron(III) to iron(II) increased the percentage recovery by up to 25%. The detection limits obtained by dithizone extraction method were (in ug cm^{-3} of the extract): Cd, 0.001; Cu, 0.05 ; Co, 0.004; Ni, 0.02; Pb, 0.02.

Chapter 3 investigates the influences of waterlogging on the distribution of trace metals in soil and assesses this by means of specific extractants. Calcium chloride, acetic acid, pyrophosphate and acid oxalate were applied to extract metals associated with exchange sites, specific sorption sites, organic complexes, and oxide materials, respectively. Results showed that the patterns of redistribution were dependent on the properties of the metals; Mn, Fe and Co all increased in water, acetic acid or calcium chloride fractions due to waterlogging. There were corresponding decreases in the pyrophosphate and oxalate fractions. This has been thought of as decomposition of organic matter and dissolution of oxide materials. Zn and Cu in soil have rather different responses to waterlogging, in that their pyrophosphate and calcium chloride fractions decreased at the point

at which the oxalate fraction increased. The increases of oxalate extractable Zn and Cu may be attributed to increases of active oxide surfaces which can adsorb more cations. Addition of organic matter to waterlogged soil promoted the rate of redistribution, while the patterns of metal changes were roughly the same. Addition of lime also quickened the reaction, but relations between different fractions and metals might change in some cases.

In Chapter 4 the waterlogging effect is illustrated in relation to trace metal uptake by French beans and maize. It was found that waterlogging effects persisted in soils after one month of free draining. Increases of water, calcium chloride and acetic acid extractable Mn, Fe and Co in soils due to previous waterlogging are reflected by plant uptake. However, reduction of Zn and Cu levels in water and calcium chloride extractable fractions, during waterlogging, did not necessarily reduce plant uptake. Under the identical soil condition, beans and maize showed opposite responses to uptake of Zn and Cu, on soil with previous waterlogging. This indicates that beans and maize have rather different selection abilities in relation to extraction of metals from the soil fractions. Further experiments on waterlogging during the growth period of beans showed increases of uptake of Mn, Fe, Co, Cu and Zn. This implied that plants could affect the status and distribution of metals in soil.

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CHAPTER 1 INTRODUCTION

1.1 Trace metals in plants and animals

In many instances, trace metal control over life processes is dramatically reflected in animal health and success of agriculture in a region. This may be traced back to the earlier part of this century. A dramatic response due to a trace metal was found in plants in Australia 1927; adding 84 kg/hectare manganese sulphate increased the grain yield of oats from nil to 2960 kg/hectare on volcanic ash soil (Donald and Prescott 1975). With the development of advanced analytical techniques, more and more trace metals have been found to be essential to life forms. At the present time, iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, vanadium and arsenic are generally accepted as essential trace metals. In this study, manganese, iron, cobalt, copper and zinc have been chosen for further discussion, because they are well known as components of enzyme complexes operating biological functions, and studies on these metals are comparatively extensive in literature. The following are summaries of the biological roles of these metals.

Manganese (Mn), atomic No.25, valence 1,2,3,4,6 or 7. Manganese is an important element for both plants and animals. The biologically active form of manganese is the Mn^{+2} ion. Manganese uptake differs considerably between plant species. It is believed that manganese is involved in the oxidation-reduction process in the photosynthetic electron transport system. It is also known that manganese substitutes for magnesium in many of the ATP-dependent enzymes of glycolysis. For animals, the requirements of manganese have been varied. It is involved with mucopolysaccharide synthesis, carbohydrate and lipid metabolism. A variety of bone disorders, retarded growth, and reproductive failure could be found in manganese-deficient animals.

Iron (Fe), atomic No.26, valence 2,3,4 or 6. Iron is an essential component of plants and animals. The important oxidation states of iron are Fe^{+2} and Fe^{+3}. At least more than 50 known proteins contain iron. The most well-known function of iron in enzyme system is as haem and nonhaem-iron protein. The haem enzyme systems include catalase, peroxidase, cytochrome oxidase and the various cytochromes. The nonhaem-protein includes ferredoxin, which is important for N_2 fixation, photosynthesis, electron transfer enzymes and hormone biosynthesis. The human body contains about 4-5 g of iron, and especially, iron in haemoglobin accupies a dominant role in humans.

Cobalt (Co), atomic No.27, valence 2 or 3. There is as yet as no evidence of cobalt essentiality to higher plants, however, low concentrations of cobalt can have a favourable effect on plant growth and there is more indication that cobalt may in some way promote some steps in oxidative metabolism, so that energy is made available for growth. It is well established that cobalt is essential for

N_2 fixation by blue-green algae, and some free living bacteria. The unique characteristic of cobalt among trace metals is that it is biologically active only when incorporated in the corrin ring as vitamin B_{12} or as one of the cobalamide derivatives.

Copper (Cu), atomic No.29, valence 1 or 2. Copper is a vital component of plants and animals. Copper is essential to photosynthesis as a protein complex, relatively high concentrations of copper occurring in chloroplasts. Recent research may also suggest that copper is involved with the synthesis or the stability of chlorophyll and other plant pigments. Copper in animals is an essential trace metal because of several copper-dependent enzymes involved with iron metabolism, collagan formation, melanin production, and integrity of the central nervous system.

Zinc (Zn), atomic No.30, valence 2. In every stage of the life cycle, zinc is required for normal growth, development and function in plants and animals. Zinc in plants is a special element to activate carbonic anhydrase, the enzyme which regulates CO_2 formation and is important to the process of photosynthesis. Zinc is also closely involved in the nitrogen metalolism of the plant. In animals, the carbohydrate, lipid, protein and nucleic acid metabolisms are examples of functions in which zinc is involved.

The requirement of trace metals for plants and domestic animals is given in Table 1.1.1, and some examples of the biological functions of trace metals are given in

Table 1.1.2. More details of the functions of trace metals in plants and animals appeared in Boardman (1975), Nicholas (1975), Underwood (1975, 1977), Mengel and Kirkby (1978) and Young (1979).

Less or more than the adequate amounts of essential trace metals in plants or animals would cause deficiency or toxicity effects that have been widely reported in the literature. The adequate amount of metal to plants and animals is generally confined to a narrow range, however, itis much dependent on the given species. The deficiency and toxicity of metals ultimately results in growth failure. Such effects have been widely reported (Russell and Duncan 1956, Lucas and Knezek 1972, Smith and Gawthorne 1975, Foy 1978, Mengel and Kirkby 1978, Mills 1979, and National Research Council 1980).

Table 1.1.1 The requirement of trace metals for plants and domestic animals

Metals	Plant (ppm)	Domestic animal (ppm)
Mn	20-500	20
Fe	25-500	80
Co	0.1-0.67	0.25
Cu	5-20	60
Zn	25-150	50

Table 1.1.2 Some enzymes and biological functions of Mn, Fe, Co, Cu, and Zn (extracted from Mengel and Kirkby 1978, Underwood 1975)

Metals	Enzyme	Function
Mn	IAA oxidases	IAA oxidation
	Phosphotransferase	Activating phosphorylation
	Haemoglobin	O_2 transport
	Arginase	Urea formation
	Pyruvate carboxylase	Pyruvate metabolism
Fe	Ferredoxin	Photosynthesis
	Cytochromes	Electron transfer
	Succinate dehydrogenase	Aerobic oxidation of carbohydrates
	Catalase	H_2O_2 decomposition
Co	Cobamide coenzymes	N_2 fixation, N-metabolism
Cu	Plastocyanin	Photosynthesis
	Cytochrome oxidase	Principal terminal oxidase
	Lysyl oxidase	Lysine oxidation
	Tyrosinase	Skin pigmentation
	Cytocuprein	Superoxide (O_2^-) dismutation
Zn	Carbonic anhydrase	CO_2 formation; regulation of acidity
	Carboxypeptidase	Protein metabolism
	Alcohol dehydrogenase	Alcohol metabolism
	Cytocuprein	Superoxide (O_2^-) dismutation

1.2 Trace metals in soils

For most plants and animals, their source of trace metals is directly or indirectly from soil, therefore, the understanding of chemistry of soil is important. Generally, soils contain large amounts of iron, of which only trace amounts are used biologically. The work described here extends the term "trace metals" to include iron deliberately in the following text. Manganese, iron, cobalt, copper and zinc occasionally occur native; however, these forms are rarely important forms from an agricultural point of view. The concentration of trace metals in soils is related to the rocks from which they are derived. The concentration of trace metals in certain rocks and soils is given in Table 1.2.1.

Table 1.2.1 Trace metals in rocks and soils (in ppm)

Metal	Earth's Crust	Basic Rocks	Acid Rocks	Sedimentary Rocks	Soils
Mn	1,000	2,000	600	670	850
Fe	50,000	86,000	27,000	33,000	38,000
Co	40	45	5	23	8
Cu	70	140	30	57	20
Zn	80	130	60	80	58

after Hodgson (1963)

Distribution of trace metals in rocks and soils has been well examined by Hodgson (1963) and Mitchell (1964). The total concentrations of trace elements in nine typical Scottish soils on parent materials derived from different rock types showed soils could have a wide range of metal concentrations (Mitchell 1964). Not all the metal in a soil can be used by plants. It has commonly been found that total metal concentrations in soil have little correlation with plant uptake. Eventually, the forms of metal which exist in soil and factors which influence the available state are more important to plants. Estimation of availabilities of trace metals in soil demands some knowledge of the forms in which metals may occur. Based on Hodgson (1963), there are many ways in which a metal can be bound in soils: (I) It can be in ionic and complexed forms in the soil solution, (II) It can be adsorbed on organic or inorganic exchange sites; (III) It can be adsorbed on specific sorption sites (McLaren and Crawford, 1973b); (IV) It can be precipitated or coprecipitated with oxide materials; (V) It can be associated into biological systems and their residues in the soil; (VI) It can be in the lattice structure of primary and secondary minerals. The equilibria and reactions between these forms are fundamental to an understanding of the soil chemistry of trace metals.

At present, chemical extraction methods are commonly used for the distinction of separated forms of metals by their defined extractabilities. However, this is only on an experimental basis and the distinction between these forms of metals is not always clear cut. More details concerning extraction methods will be discussed in Chapter 2.

1.2.1 Metals in soil solution

Only a small fraction of soil metals exist in this form; however, they are vital to plant uptake. Quoted by Loneragan (1975), ranges of metal concentrations in soil solution are: Mn 1.1-3740 ppb, Fe 22-280 ppb, Co 0.4-16 ppb, Cu 0.6-38 ppb, and Zn 1.9-196 ppb. In acid soils the inorganic ions of Mn, Fe, Co, Cu, and Zn are mostly the divalent cations M^{+2}, although Fe^{+3} may exist. In neutral and alkaline soils the monovalent hydroxy cations $M(OH)^{+}$ would also be important. It is not uncommon that large amounts of total soluble metals exist in complexed forms in some soils. About 84-99% of Mn, 8-50% of Co, 89-99.8% of Cu and 28-99% of Zn were complexed in the solution (requoted from Loneragan 1975). Fe in solution was reported about 10-30% (Pao, Liu and Yu 1978) and 15-40% (Sims and Patrick 1978) in complexed form under anaerobic condition; however, 100% complexing has also been reported (Olomu, Racz and Cho 1973).

Control mechanisms for trace metals in soil solution are involved with various complex reactions. Metal ions in solution are buffered by exchange sites and surface adsorption, and presence of organic matter also affects the equilibrium. An important factor which limits solubilities of metals in solution is formation of related mineral compounds. The regulating factors of minerals on trace metals are presented in an excellent form by Lindsay (1979).

1.2.2 Metals on exchange sites

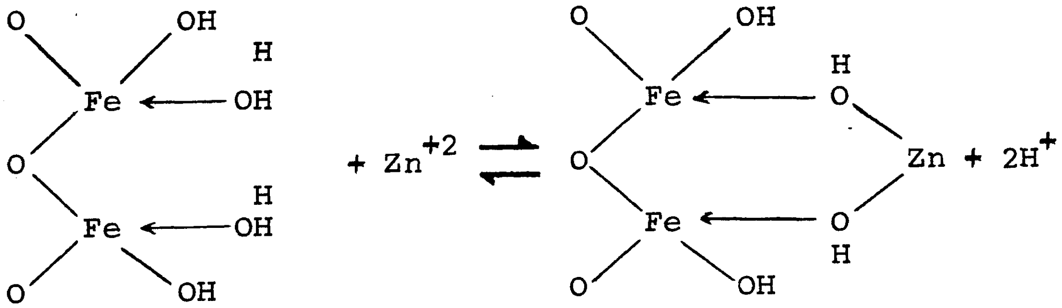
Soil clays, humus and oxides can carry electrical negative charges on their surfaces which may be neutralised by simple cations. These cations which are adsorbed onto the surfaces of particles can be replaced by others. If a neutral salt solution is applied to extract the soil, for example, ammonium chloride, then some original adsorbed cations would be replaced by ammonium ions. The sites that hold ammonium are called exchange sites. Exchange sites can regulate the metal concentration in soil solution, and also the metal ions they absorb are readily exchangeable, so they are vital to the uptake by plants. The fraction of soil metals adsorbed on these sites varies and depends on the cation exchange capacity of a soil.

Amounts of manganese and iron held on exchange sites are affected considerably by pH and redox potential. At low pH and Eh, more exchangeable manganese and iron exist. In a silty clay loam soil, manganese and iron decreased from 121 ppm and 1295 ppm at pH 4.5 to 96 ppm and 1 ppm at pH 7.5, respectively. From Eh +500 mV to -150 mV, manganese and iron increased from 22 ppm and 1 ppm to 169 ppm and 1226 ppm (Sims and Patrick 1978). The same report showed that exchangeable copper and zinc had less dramatic changes with pH and Eh, however, 100% differences in values could still be found.

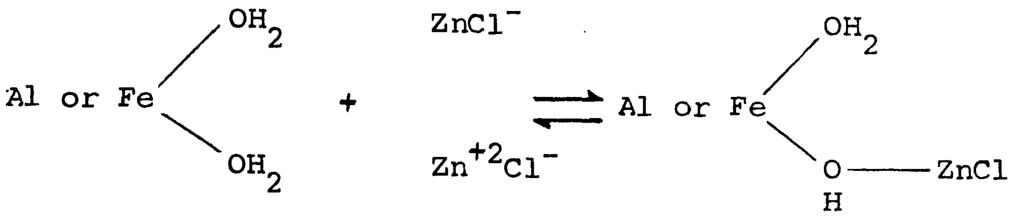
1.2.3 Metals on specific sorption sites

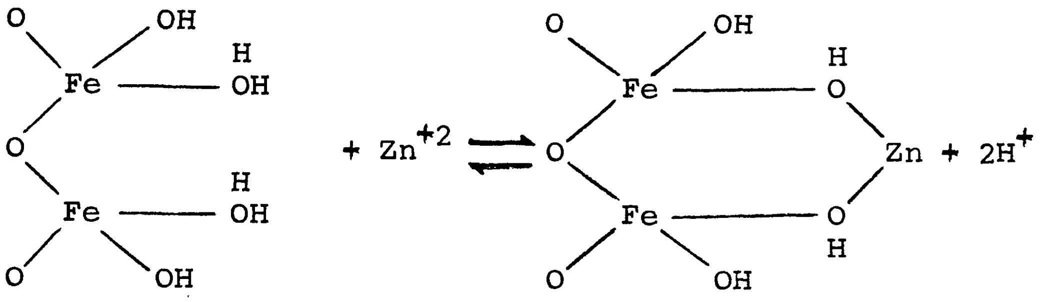
Specific sorption sites are adsorption sites on soil particle surfaces, which adsorb cations that can not be removed by reagents nomally used for determining the exchangeable cations (McLaren and Crawford 1973b).

In the 1940's, soil scientists found that a portion of metals added to some soils could not be recovered by neutral salt solution, for example Cu and Zn (Hibbard 1940, Peech 1941). These strongly bound metals could be extracted by using acid extractants. Elgabaly and Jenny (1943) explained that the strongly bound metals had entered the octahedral layer of the crystal lattice. But more recent research may provide a better explanation. Quirk and Posner (1975) proposed that, the adsorption of Zn (II) at an oxide surface may be represented as a bridging ligand between two neutral sites:



They considered such adsorption can be viewed as a growth or an extension of the surface and its properties would accord with the lack of complete reversibility of such an adsorption. Later Kalbasi, Racz and Loewen-Rudgers (1978) proposed two possible mechanisms for zinc adsorption by the aluminum and iron oxides surface:

(I) 

(II) 

They further concluded that these mechanisms may be involved with the frequently reported fixation and unavailability of added Zn to soil. Other metal ions may have similar adsorption. A study of specific adsorption of trace metal ions with an iron oxide (goethite) found the affinities for the oxide surface in the order Cd < Co < Zn < Pb < Cu (Forbes, Posner and Quirk 1976).

McLaren and Crawford (1973b) found the adsorption maxima of copper on various soil constituents were in the following order:

manganese oxides > organic matter > iron oxides > clay minerals.

According to Mitchell (1964), acetic acid could extract as high as the order of 10-fold of manganese, iron, cobalt, copper and zinc than ammonium acetate. This may suggest specific adsorption is fairly intensive in soils.

1.2.4 Metals associated with organic matter

Hodgson (1963) mentioned three classes of systems for the reactions between organic matter and metals: (1) Association between metals and high molecular weight organic compounds which are essentially immobile, such as lignins. (2) Association between metals and short-chain organic acids and bases which promote the solubilities and movements of metals. (3) Forming insoluble salts of metals with other complexing agents which appear to be soluble themselves.

Organic matter participates in metal ion binding by the functional groups on their surfaces. Prominent functional groups are the carboxyls (-COOH), and the hydroxyls (≡COH) as aliphatic alcohols and reactive phenolic OH-groups, followed by the carbonyl (=C=O) groups. The importance of organic matter and its functional groups in relation to metals has been reviewed by Schnitzer and Khan (1972).

As mentioned in Section 1.2.1, metal ions can be strongly bound with organic matter. In general, copper is the most strongly bound. The stabilities of metal-organic complexes increase as pH increases up to the point where the complexes break up. A large fraction of total copper in organic forms implies that it would play an important role in the soil (McLaren and Crawford 1973a). Lindsay (1978) suggests that the lower prevalence of copper deficiencies than zinc deficiencies is due to the greater ability of copper to form complexes, although Zn^{+2} solubility is much higher than Cu^{+2}. This again shows the importance of organic complexes.

1.2.5 Metals occluded in oxide materials

Manganese and iron released from weathering primary materials are largely precipitated as oxides. They are ubiquitous in clays and soils, and have great abilities to adsorb other trace metals. Le Riche (1963) found oxides were the fractions richest in trace elements and contained large proportions of the total copper and cobalt.

Besides the normal ion exchange, one of the mechanisms of scavenging by oxides is specific adsorption, which had already been mentioned in Section 1.2.2. There has also been found an exchange of metal ions within the lattice structure during adsorption. Studies on adsorption of cobalt, copper and zinc onto manganese oxide surfaces, showed that the metals penetrated into the crystal lattice, and manganese was released into solution (Loganathan and Burau 1973, McKenzie 1975).

Coprecipitation is another factor which binds other trace metals into the oxide lattice. Ng and Bloomfield (1961) reported some metals in fermentation solutions are stable to atmospheric oxidation, but precipitate in the presence of ferrous iron when aeration occurs. The order of ease of metal coprecipitation with iron oxide appears to be zinc > copper > cobalt.

1.2.6 Precipitates of metals

When metal concentrations in soil solution reach the saturation point with respect to any mineral, certain anions are likely to be associated with the metal to form precipitates, for example, oxides, hydroxides, carbonates, phosphates and sulphides. Because of their solubilities and chemical equilibria in response to changes in pH, oxidation potential and the presence of soluble chelating agents, they are important to the movement and availabilities of metals to plants.

Some common minerals are listed as follows:

Mn: pyrolusite (MnO_2), hausmannite (Mn_3O_4), manganite (MnOOH), rhodochrosite ($MnCO_3$), and rhodonite ($MnSiO_3$).

Fe: olivine $(Mg, Fe)_2SiO_4$, pyrite (FeS_2), siderite ($FeCO_3$), haematite (Fe_2O_3), goethite (α-FeOOH), lepidocrocite (γ-FeOOH) and magnetite (Fe_3O_4).

Co: cobaltite (Co, Fe)AsS, linnaeite (Co_3S_4) and erythite $Co_3(AsO_4)_2$.

Cu: chalcocite (Cu_2O), covellite (CuS), chalcopyrite ($CuFeS_2$), cuprite (Cu_2O), tenorite (CuO), malachite $Cu_2(OH)_2CO_3$.

Zn: sphalerite (ZnS), smithsonite ($ZnCO_3$) and hemimorphite $Zn_4(OH)_2Si_2O_7 \cdot H_2O$

The solubilities of above minerals in relation to pH and Eh have been compiled by Lindsay (1979).

1.3 Factors affecting the availability of trace metals in soil to plants

Availability of trace metals is determined both by the forms of metals in soil and the plant's ability to absorb metals. Many factors in the natural environment could affect the uptake of metal by plants, such factors affect metals both in soil and plant. General factors which would affect the availability of metals are pH, organic matter, redox potential, microbial activity, rhizosphere activity and antagonistic reaction of metals.

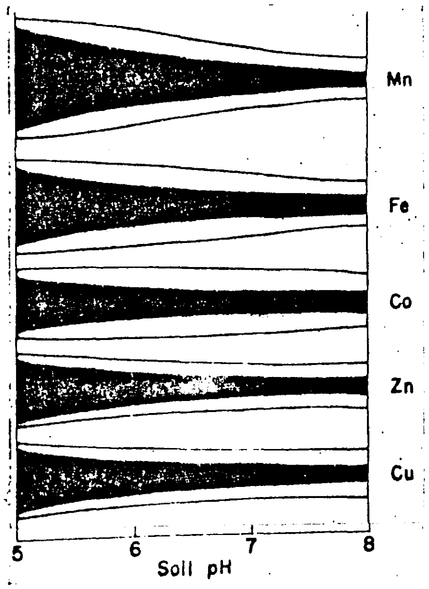
1.3.1 pH

Change of pH greatly affects the solubilities of minerals in soils. The effect on solubilities of minerals in soil has been well compiled by Lindsay (1979). He indicated Cu^{+2} and Zn^{+2} decreased 100-fold for each unit increase in pH. Manganese, iron and cobalt have also been found to decrease in solubilities as pH increases. Adsorption sites on oxides and organic matter are pH-dependent (Randhawa and Broadbent 1965, Shuman 1975, Bache 1976, Loganathan, Buran and Fuerstenau 1977). Their adsorption capacities increase as pH increases, however, solubilities of metals may increase at some pH's due to solublized complex-metals increasing eg. zinc (McBride and Blasiak 1979).

Microbial activities and chelate stabilities are subject to change with pH. Galloway (1978) reported that liming caused a rapid decomposition of organic litter in an acid lake, and suggested bacterial activity had been inhibited at low pH. Schnitzer and Skinner (1967) listed the order of stabilities of soil fulvic acid-metal complexes. At pH 3.5, the order is $Cu^{+2} > Fe^{+2} > Co^{+2} > Zn^{+2} > Mn^{+2}$; and at pH 5.0, the order is changed to $Cu^{+2} > Fe^{+2} > Mn^{+2} \approx Co^{+2} > Zn^{+2}$.

In addition to the above effect on soil, pH may also alter the plant uptake of metal through changing the ability of plants to absorb metal ions. A generalized relative effect of pH on the availability of five metals as determined by chemical extractions of the soils and by plant uptake is shown in Figure 1.3.1, which was composed by Truog (1946).

Figure 1.3.1 The effect of pH on the uptake of trace metals by plants (outer limit of enclosed areas) and on trace metals extractable by various solvents (darkly shaded areas)



1.3.2 Organic matter

The reaction of trace metals with organic matter has been mentioned on Section 1.2.5. Because of the great ability of organic matter to associate with trace metals, the forms and amount of organic matter in soil can induce a large change in the availability of trace metals.

Metals associated with high molecular weight organic matter are essentially immobilized. Such metals associated with low molecular weight and short chain organic complexes, however, tend to be increased in solubility. For example, although inorganic Zn^{+2} activity is higher than Cu^{+2}, copper deficiencies are less prevalent than zinc deficiencies in alkaline soils. This is attributed to strong complexation of Cu^{+2} with soluble organic matter which increases copper solubility (Lindsay 1978). Loneragan (1975) stated that a mixture of humic and fulvic acids complexed Mn from Mn^{+3} and Mn^{+4} oxides and occluded Mn^{+2} hydroxides, this is a reaction important in keeping Mn in soluble forms in alkaline soils. This may confirm the earlier finding that added Mn in calcareous soil only could be used by plants in the presence of organic matter (Wain, Silk and Wills 1943).

The effect of organic matter on the mobility of metals is rather different under waterlogging. Extractable manganese and iron further increased with organic matter and waterlogging (Prasad, Prasad and Singb 1976), but extractable zinc and copper decreased (Halder and Mandal 1979). Recently, it has been found that addition of organic matter could reduce the severity of iron toxicity on rice plants under waterlogged condition (IRRI 1976).

1.3.3 Redox potential

The state of oxidation or reduction in a soil is important to plant growth. Gillespie (1920) observed that 'good' soils are more oxidizing than 'bad' soils. In aerated soils, the process of oxidation is mainly that of addition of oxygen. When there is a shortage of oxygen, the process can be considered as loss of electrons. Similarly the process of reduction can be considered as gain of electrons. The tendency of the solution to gain or lose electrons, to reduce or oxidize, a substance added to it, can be measured by redox potential (Eh). Redox potential (Eh) is given by

$$Eh = Eo + \frac{RT}{nF} \ln \frac{a(ox)}{a(red)}$$

where Eo is the standard oxidation-reduction potential, R is the gas constant, T is the absolute temperature, n is the number of electrons involved, F is the faraday (96,500 coulombs) and a refers to activities of oxidized and reduced species. Eh is pH dependent, but the effect of a unit change in pH on the potential is not the same for all redox reactions. For convenience, to make comparisons between different media, -59/pH unit is generally used as the correction factor.

Under waterlogging, with the presence of organic matter, microorganisms can use a number of substances as electron acceptors to serve the respiration purpose, and change the oxidation condition of a soil from oxidized to reduced. Nitrate is the next oxidant to be reduced following oxygen depletion, it is followed by manganic compounds, ferric iron, sulphate, carbon dioxide and organic acids. The reduced counterparts are NH_4^+, Mn^{+2}, Fe^{+2}, H_2S and CH_4 (Ponnamperuma 1972). There seems to be a critical reduction potential for individual redox components although some of the reduction reactions may overlap (Gambrell and Patrick 1978). The critical redox potential (assuming pH 7.0) for nitrate, manganic manganese, ferric iron, sulphate and carbon dioxide are +220mV, +200mV, +120mV, -150mV and -250mV, respectively. The measurement of the Eh can, therefore, indicate the intensity of reduction of a soil and predict the chemical transformation.

Cobalt, copper and zinc are unlikely to be involved with redox transformations, however, as redox potential of a soil is lowered, manganese and iron oxides become unstable and more soluble (Gotoh and Patrick 1972, 1974), then metals associated with these oxides are expected to be influenced by the process (Sims and Patrick 1978). More details will be discussed in Section 1.4.3.

1.3.4 Microbial activity

Recycling of trace metals from organic matter depends on microbial activity which decomposes the organic matter and releases metals held in it. Without this activity trace metals would be exhausted by vegetation. Microbial activity could affect the availability of metals through reduction or oxidation of metal under different conditions, such as manganese and iron oxidation. The earlier reports for oxidation and reduction of metals by microorganisms have been reviewed by Hodgson (1963). Microbes may also markedly influence the maximum binding ability characteristics of the naturally occurring soil organic matter (Zunino and Martin 1977) and therefore influence the metal movement in soil.

During and after waterlogging, microbial activity differs greatly from the aerobic condition (Tate 1979). Less energy is released by decomposition of organic matter under waterlogging, and the end products of organic degradation are different. Results of Ng and Bloomfield (1961, 1962) indicate that anaerobic decomposition of organic matter produces greater amounts of soluble organic complexes, and it is also likely that the residual humic materials are of higher molecular weight and more structurally complex (Gambrell and Patrick 1978).

Another effect of microbial activity under waterlogged conditions is that it uses organic or inorganic constituents as hydrogen acceptors when oxygen is depleted. The reduced condition of a soil is almost entirely dependent on these reactions.

In waterlogged soil, microbial metabolism takes place consecutively as follows: (i) dissolved oxygen is consumed (ii) NO_3^- and NO_2^- to N_2 (iii) Mn^{4+} to Mn^{2+} (iv) Fe^{3+} to Fe^{2+} (v) SO_4^{2-} to S^{2-} (vi) H_2 and CH_4 are produced. A consequence is that redox potential drops rapidly and metal availability is affected.

1.3.5 Rhizosphere activity

Roots can modify their immediate environment by excreting chemical substances. These substances can alter the availability of metals through direct reaction with soil constituents or indirectly through their stimulation of micro-organisms. It has been found that the solution obtained by washing oat roots can dissolve manganese from insoluble oxides (Bromfield 1958a,b). The reductive capacity of roots has further been shown by reducing Fe^{3+} to Fe^{2+} in solution (Brown, Holmes and Tiffin 1961). Stimulation of root exudates on microorganisms has also been shown to be of importance to availability of metals. For example, some organisms in the soil rhizosphere can release zinc from insoluble silicates (Webley, Duff and Mitchell 1960).

1.3.6 Antagonistic reaction of metals

Although the above mentioned factors are probably more important to the changes of available metals, antagonistic reactions between metal ions can also be important. Higher concentrations of foreign ions could affect metal uptake, such as calcium salts inhibiting manganese absorption in nutrient solutions containing 0.3 uM Mn^{2+} (Robson and Loneragan 1970). Interference may also exist between trace metals. Bowen (1969) has shown that Zn^{2+} and Cu^{2+} have mutually competitive activities on absorption by sugarcane leaf tissue.

The mechanisms which carry ions through membranes to plant cells may have great effect on metal competition. Bowen (1969) reported Fe^{3+} significantly inhibited Zn^{2+} and Cu^{2+} absorption but not the absorption of Mn^{2+}. He then concluded Mn^{2+} was absorbed through a second independent mechanism other than that for Zn^{2+} and Cu^{2+}.

1.4 Soil waterlogging

Waterlogging a soil would cause most of the pore spaces to fill with water and consequently block gaseous diffusion through a porous medium. In such condition, oxygen supplies are limited and easily exhausted by microorganisms when decomposable organic matter exists. The shortage of oxygen would cause facultative or obligate anaerobic microorganisms to carry out a number of chemical reductions which may affect plant growth very considerably. In addition, the availabilities of soil trace metals can be influenced by subsequent changes of oxidation-reduction state.

Ponnamperuma (1972) pointed out the chemical changes in waterlogged soils led to series of influences on the ecosystem. They included: (a) the character of the sediment or soil that forms; (b) the suitability of wet soils for crops, (c) the distribution of plant species around lakes and streams and in esturaies, deltas, and marine flood plains (d) the quality and quantity of aquatic life, and (e) the capacity of lakes and seas to serve as sinks for terrestrial wastes.

1.4.1 Kinds of waterlogged soils

Considering different waterlogging times and depths, there are several kinds of waterlogged soil (Hesse 1971):

(1) Soils permanently submerged under fresh or salt water of various depths; for example, river, lake and ocean sediments.

(2) Soils periodically waterlogged by submergence; for example, coastal regions and river banks.

(3) Soils completely saturated and partly covered by surface water, such as swamps and bogs.

(4) Soils with an impeded layer or a high and fluctuating water table, which are thus periodically waterlogged as the season changes, such as some low-land soils.

(5) Soils periodically and artifically waterlogged by irrigation; for example, paddy soils.

1.4.2 Chemical changes in waterlogged soils

The distinguishing character of waterlogged soils is that reducing conditions prevail, mainly brought about by microbial activity. When soil surfaces are separated by water from the soil atmosphere, oxygen supply is limited and trapped oxygen soon will be exhausted by microbial respiration. Facultative and obligate anerobic microbes then become dominant in decomposition processes and utilize oxidized substances in the soil as hydrogen acceptors. The oxidized substances following oxygen to be used in soil reduction, in sequence, are NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2 and organic acids. Their reduced counterparts are N_2, Mn^{2+}, Fe^{2+}, S^{2-}, CH_4 and H_2. The redox systems of these oxidants have been well stated by Ponnamperuma (1972).

During soil waterlogging, the soil pH may also change. The overall trend is to increase the pH of acid soils and depress the pH of sodic and calcareous soils to a fairly stable pH value at 6.7-7.2. The increase in pH of acid soils is due to soil reduction (Ponnamperuma, Martinez and Loy 1966), and decrease of alkaline soil pH is due to accumulation of CO_2.

1.4.3 Waterlogging effect on soil metals

Besides the poor aeration and reducing conditions which would affect the crops, subsequent metal changes in waterlogged soils may also be important, for example iron toxicity and zinc deficiency have already been found in rice soils (IRRI 1976, Moormann and Breemen 1978).

Manganese and iron are subjected to oxidation-reduction reactions, at lower redox potentials, more insoluble manganese and iron oxides become soluble and easily exchangeable (Gotoh and Patrick 1972, 1974). Other metals associated with those oxides may probably be released by the reaction. For example, cobalt is found in association with manganese oxides (McKenzie 1975), and tends to be more extractable by acetic acid in poorly drained soil (Mitchell 1964). The large amounts of manganous and ferrous ions in waterlogged soil can take part in cation exchange, that may release other metals which are already adsorbed on exchange sites into solution.

Accumulation of products of sulfide, however, will limit the mobility of some metals. It has been found that metal sulfides of manganese, iron, zinc and copper were stable in a waterlogged soil (Engler and Patrick 1975). The decreases of solubilities of copper and zinc in solution have been attributed to formation of metal sulfide (IRRI 1976).

Metal mobility is further influenced by changes of pH which are caused by soil reduction. Regulatory processes like precipitation and dissolution of minerals, adsorption and desorption of ions, are profoundly affected by pH value. The influences of pH on these subjects have already been mentioned in Section 1.3.1.

The consistent findings are that the net result of soil waterlogging on trace metals is an increase of availabilities of manganese, iron (Clark and Resnicky 1956, Mandal 1961, Olomu, Racz and Cho 1973) and cobalt (Ng and Bloomfield 1962, Adams and Honeysett 1964). The net result of waterlogging on copper and zinc rather depends on soil conditions and may show an increase or decrease in availability (Ng and Bloomfield 1962, Halder and Mandal 1979).

Addition of organic matter or lime in waterlogged soil promotes the reduction reaction. Redox potential drops more sharply and lower with organic matter (IRRI 1976), or lime (Mukhopadhyay, Fisher and Smith 1967). Soluble and exchangeable manganese and iron also build up faster and higher (Clark and Resnicky 1956, Mandal 1961, Graven, Attoe and Smith 1965, Ramos and Standifer 1977). For zinc and copper, addition of organic matter may decrease the ammonium acetate-extractable zinc and copper faster and further in a lowland paddy soil (Haldar and Mandal 1979).

1.5 Aims of the project

In addition to the importance of rice growing as a major food crop in temperate and tropical areas, waterlogged soils occur in almost any climatic zone and affect all plants grown on them. Such wide spread influence is without doubt important and it is essential to understand the consequences which follow. Waterlogging has been artifically tried to cure a fungus disease of banana (Stover, Thornton and Dunlap 1953), to improve manganese deficiency (Mulder and Gerretsen 1952), and,the most well-known, to improve the growing conditions for rice cultivation (Moormann and Breemen 1978).

Recently the importance of trace metals has been recognized and attracted the attention of soil scientists. It has been found that waterlogging may have positive or negative effects on trace metal uptake; such as zinc (Pavanasasivam and Axley 1980), and in some extreme cases, deficiencies or toxicities of metals may occur (IRRI 1976). Although extensive data have been published for waterlogging effects on available trace metals in soil, little information is available on the redistribution of trace metals among different forms in soil. It is believed that an increased understanding of such redistribution of trace metals in waterlogged soil may contribute to effective crop management. The project is designed to determine qualitative and quantitative changes of trace metals between various forms in a waterlogged acid soil by using selective chemical extractants.

Because it has been widely reported that plants grown on waterlogged soils or soils of impeded drainage can have higher metal contents, such as cobalt (Mitchell, Reith and Johnston 1957), and certain detrimental effects due to high uptake of metal, such as manganese (Graven, Attoe and Smith 1965), plant growth experiments are used for studying waterlogging effects on metal uptake and their relations with soil metals.

As only small amounts of some metals will be involved in the redistribution between various forms, the analytical method used will be important. Atomic absorption spectrometry is a commonly used analytical method for soils and is used in this work. Great care is necessary however; interferences such as matrix effects and multiple peaks are met when analyzing the complex solutions which are obtained by soil extraction. Methods for overcoming such interferences will be considered.

CHAPTER 2 EXTRACTION AND ANALYTICAL METHODS

2.1 Introduction

Studies on the correlations of plant uptake of trace metals with soil content and the transformation of trace metals in soil depend on the ability to analyse different forms in which metals are held in soil and metals in plant tissues. At the present time, no method is better than chemical extraction to separate different phases of metals in soil (Gambrell, Khalid, Verloo and Patrick, 1977) and it has been widely used in soil science (Le Riche and Weir. 1963, Avery 1973). For the analysis of metals in plant tissue, the destruction of the organic matter before elemental analysis is necessary. The most popular methods are dry ashing and acid digestion (Gorsuch 1959).

Once the metals are in solution, they can be determined by many analytical methods, eg, colorimetric methods, atomic absorption spectrometry, ion selective electrodes and neutron activation analysis, etc. For judging the acceptability of analytical methods, which must be sufficiently precise and accurate, if results are to be sensible and unbiased, McFarren, Lishka and Parker (1970) suggested methods with total errors less than 25% are excellent, and with total errors less than 50% are acceptable. In the report they concluded that atomic absorption spectrometry was acceptable for the determination of Zn, Cr, Cu, Mg, Mn, Fe and Ag. Whitney and Risby (1975) pointed out that methods of analysis should be judged on seven factors (1) required sensitivity, (2) accuracy, (3) presence of interferences, (4) time per sample, (5) number and technical skill of laboratory personnel required, (6) require use of standard or reference methods, (7) cost per sample. They developed a system of factor weighing to compare methods for first row transition metals. Results covering colorimetry, spectrophotometry, atomic absorption spectrometry, atomic fluorescence spectrometry, X-ray fluorescence spectrometry, polarography anodic stripping voltammetry, ion selective electrodes, neutron activation analysis and chromatography, showed atomic absorption spectrometry to be very successful.

Recently, flameless atomic absorption has been developed (L'vov 1961, West and Williams 1969) and investigated (Woodriff, Stone and Held 1968, L'vov 1969, Donega and Burges 1970). Its high sensitivity can determine the trace metals at very low level using microliter samples. This makes atomic absorption spectrometry more popular for trace metals analysis.

Work in this thesis mostly concentrates on selective extractants and ashing techniques as a means of bringing metals into solution before analysis by atomic absorption. More detailed descriptions of the methods used are given in the following sections.

2.2 Soil analysis

2.2.1 Selective extractants

In general, the total metal level in soil is not directly correlated to nutritional aspects, because just a small portion of metal may be used by plants, and different soils have different proportions of metals held in various forms (Mitchell 1963). Metals in soil, from the standpoint of availability can be divided into (1) readily available (2) easily available (3) potentially available and (4) unavailable forms. Of the ways in which metals are held in soil (Hodgson 1963, Viet and Lindsay 1973), the forms may include (1) in true solution with ionic or complexed forms, (2) on exchangeable sites (3) held in biological residues and living organisms, (4) occluded in soil oxide materials, (5) as a component of the lattice structure of primary and secondary minerals.

Not only the forms of metals in soil influence plant uptake, different species of crops would have different sensitivities to the available supply of metals (Brown 1953, Mitchell 1964). As the availability of metal and plant uptake both affect the movement of soil metal into the plant, knowledge of the forms of metal in soil is more desirable.

To investigate the relationship between particular forms of metals in soil and metals in plants, selective extractants have been developed in an attempt to remove specific fractions of metal from soil. The following examples are some commonly used selective extractants and their expected functions.

selective extractant	metals fractions removed
Ammonium Acetate, 1 M at pH 7.	Exchangeable forms
Acetic Acid, 0.5 M or 2.5%.	
Calcium Chloride, 0.05 M.	
Tamm's Oxalate Soln, pH 3.3.	Oxide forms or metal held by oxide materials
Sodium Dithionite	
Hydroxylamine Hydrochloride.	
Ethylenediaminetetra-acetic Acid, (EDTA) 0.05 M at pH 7.	Metals chelated or held by biological residues
Tetra-Sodium Pyrophophate, 0.1 M at pH 10.	
Hydrogen Peroxide.	

Recently, specific extractants were used in sequential extraction procedures, and have been applied to study forms of metal in soil and sediment (McLaren and Crawford 1973a, Brannon and Engler 1976) and the metal transformation in soil and sediment (Gambrell, Khalid, Verloo and Patrick JR. 1977, Sims and Patrick JR. 1978). These ideas were adapted for the studies on transformation of metal in waterlogged soil.

For this study as large numbers of samples have to be dealt with in certain time periods, a time saving and easy handling procedure is preferred. After a preliminary test to modify McLaren and Crawford's method using six soils, the results shown in Table 2.2.1 were obtained. This method was used for the project. The following are the details of the procedure.

2.2.2 Extraction procedure

Soil samples were collected at regular intervals from the soil container using a glass tube (soil preperation on Section 3.2, Chapter 3) and transferred to a Buchner funnel with a Whatman No.1 filter paper. If the sample contained excess water, the top of the Buchner funnel was sealed by double layer of parafilm to prevent air re-oxidizing soil sample) and a high vaccum pump was used to suck out the excess water. The exact amount of soil from the Buchner funnel was weighed into the centrifuge bottle, and the extractant was added as soon as possible. Extraction procedures are given in the following flow diagram, Figure 2.2.1.

Extractants:

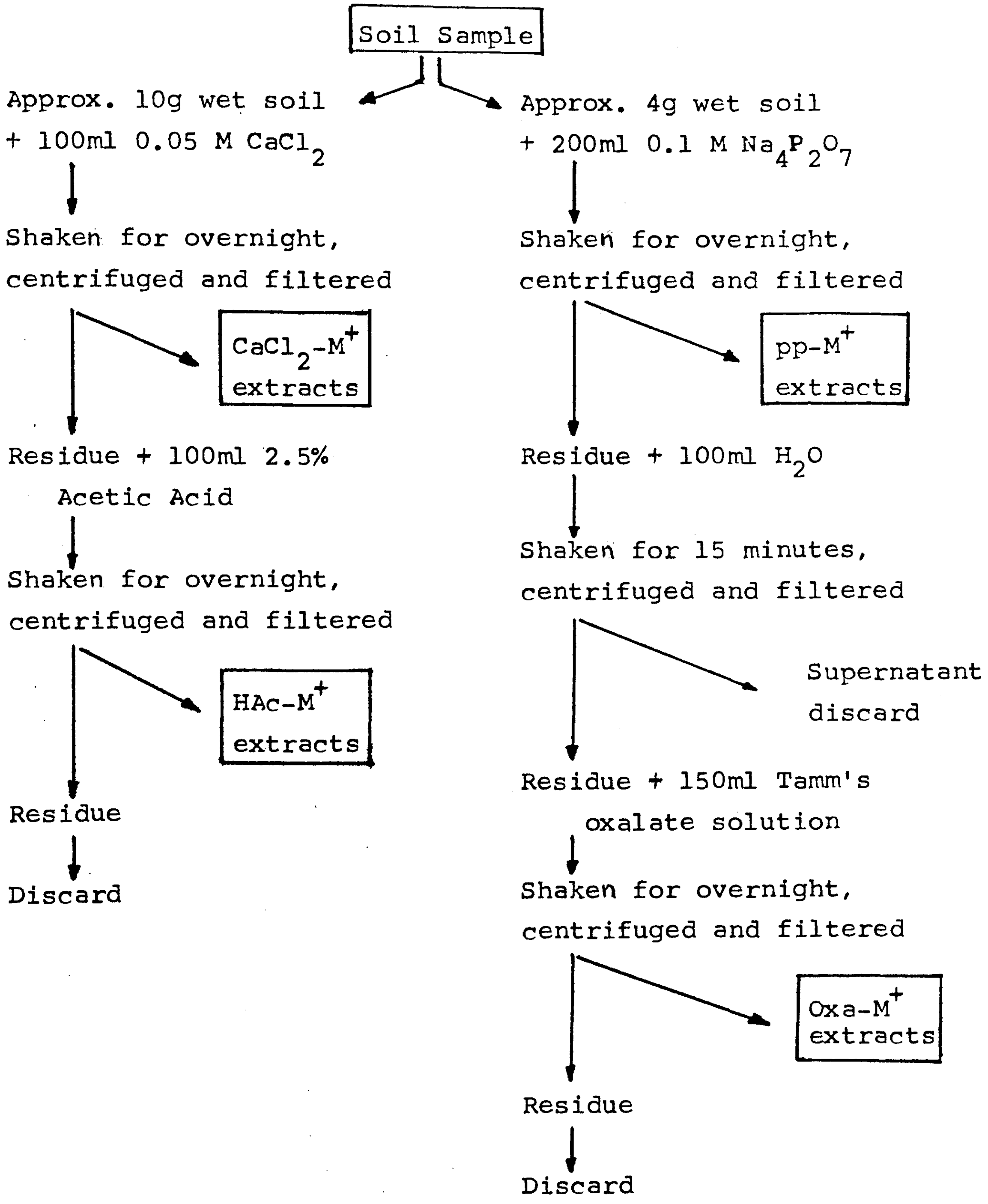
0.05 M Calcium chloride — exchangeable metals

Amount of soil roughly equal to 5 g oven-dried soil was extracted by 100ml of 0.05 M $CaCl_2$ overnight using an end-over-end shaker. The suspension was centrifuged at 2000 r.p.m. for 15 minutes and filtered through a Whatman No.1 filter paper. The solution was preserved for metal analysis in a polypropylene bottle. Soil residue was

Table 2.2.1 Amounts of extractable metals in soils (in ppm)

	Soil	$CaCl_2$	HAc	$CaCl_2$ + HAc	Single Extraction HAc	pp	Oxa	pp + Oxa	Single Extraction Oxa
Fe	DALBUY 7A	5.01	5.77	11.78	13.42	4656.	4904.	9550.	8775.
	" 7B	3.05	10.32	13.37	15.05	2257	3687	5944	5427
	" 7C	1.93	17.57	19.50	37.48	4231	4338	8569	7723
	DALBUY uA	7.41	11.63	19.04	26.74	4297	1789	6086	5630
	" uB	1.89	4.63	6.52	45.93	2322	1509	3831	4447
	" uC	2.14	24.22	26.36	85.71	4178	3699	7877	5022
Mn	DALBUY 7A	51.50	47.85	99.35	77.97	64.00	97.47	161.47	207.21
	" 7B	12.81	12.97	25.78	22.16	13.50	18.77	32.27	66.24
	" 7C	1.98	2.01	3.99	37.48	4.04	11.26	15.30	17.20
	DALBUY uA	100.50	74.20	174.70	166.38	115.70	228.41	344.11	486.51
	" uB	53.22	50.34	103.56	165.75	71.42	196.63	268.05	474.69
	" uC	21.74	25.63	47.37	71.92	30.96	162.90	193.86	214.70
Zn	DALBUY 7A	5.68	4.51	10.19	8.06	15.00	9.75	24.75	16.80
	" 7B	2.43	1.51	3.94	3.44	9.51	6.76	16.27	9.45
	" 7C	1.27	1.71	2.98	2.08	5.00	6.76	11.76	7.85
	DALBUY uA	5.06	1.95	7.01	24.48	14.02	7.51	21.53	13.26
	" uB	3.92	1.90	5.82	23.96	7.99	5.61	13.60	9.96
	" uC	2.53	4.35	6.88	69.69	13.98	8.23	22.21	7.80
Al	DALBUY 7A	7.74	127.40	135.10	144.72	2174.	637.33	2811.	1719.
	" 7B	11.65	84.24	95.89	89.55	3654	600.75	4254	1494
	" 7C	2.21	54.23	56.44	43.33	3255	525.84	3781	1121
	DALBUY uA	184.00	263.17	447.17	433.64	1928	413.24	2341	1690
	" uB	181.47	329.41	510.88	560.00	1922	449.54	2371	1699
	" uC	84.78	230.61	315.40	355.35	3095	1048.50	4143	1902

Figure 2.2.1 Flow diagram for the sequential extraction method



carried through the acetic acid extraction.

2.5% Acetic Acid — exchangeable and specific adsorption site's metal

The soil residue from the above extraction was extracted by 100 ml acetic acid overnight. The suspension was centrifuged at 2000 r.p.m. for 15 minutes and filtered through a Whatman No.1 filter paper. The solution was preserved and the soil residue discarded.

Aqueous 0.1 M sodium pyrophosphate (pH 10) — Insoluble organic chelated complexes

Soil roughly equal to 2 g of oven-dried soil weight was shaken overnight with 200 ml sodium pyrophosphate solution. After separation, the solution was preserved for metal analysis. Soil residue was carried through for subsequent extraction.

Tamm's oxalate (pH 3.25) — free oxide material

Residue from pyrophosphate extraction was washed with 100 ml of water, suspension was shaken for 15 minutes and separated. The washing was discarded and residue was then extracted by 150 ml of Tamm's oxalate solution overnight. The suspension was centrifuged and filtered. The solution was preserved for metal analysis and the residue discarded. Soil extracts were analysed by flame or flameless atomic absorption spectrometry.

2.3 Plant analysis

2.3.1 Ashing of plant tissues

One of the major problems of plant analysis for trace metals is the procedure used for destruction of the organic matrix, even though some metals can be extracted into solution without complete oxidation of plant material (Baker and Greweling 1967, Leggett and Westermann 1973).

The oxidation process to destroy the organic matrix of plant tissue before elemental determination involves either combustion at a temperature about 500°C or acid digestion using various combinations of HNO_3, $HClO_4$ and H_2SO_4 and H_2O_2. These procedures are also called dry ashing and wet ashing respectively. Reasons for choosing dry ashing or wet ashing are different, and will depend on elements and materials of interest. A number of reviews and discussions have dealt with ashing procedures (Middleton and Stuckey 1953, Gorsuch 1959).

Following are the general essential differences between dry ashing and wet ashing:

dry ashing	wet ashing
(1) No addition of reagents	(1) Oxidizing acid is used
(2) Large sample can be handled	(2) Small sample is easier to control
(3) Higher temperature, about 500°C	(3) Comparatively low temperature
(4) Complete oxidation is under dry conditions and slow	(4) Procedure is maintained in liquid phase and fairly rapid

(5) Crucible and muffle furnace used	(5) Tall form beaker and hot plate or kjeldahl flask

2.3.1.1 Dry ashing

The first 2 points are the advantages of the dry ashing method, it can reduce the blank value and concentrate elements in the sample solution. Disadvantages are involved with high temperature that could cause losses of volatile elements (Middleton and Stuckey 1953, Gorsuch 1959), and low recoveries of specific elements (Doshi, Sreekumaran, Mulay and Patel 1969). Ashing residues and crucible used in dry ashing method probably cause the retention losses, for example, the loss of copper in dry ashing organic material appears to be retention of the copper on the silica of the crucible (High 1947).

2.3.1.2 Wet ashing

The main danger when using wet ashing methods is concerned with the use of strong oxidizing agents, especially perchloric acid which can be hazardous because of the explosive nature of some perchlorate. The safety aspects of perchloric acid can be consulted in the Society for Analytical Chemistry (1959, 1960).

Large sample weights are not easily handled, so the method uses a smaller scale if possible. Against these inconveniences, wet ashing has its merit points. Low temperature prevents losses of volatile metals. Having the process under wet conditions can prevent low recoveries caused by retention. However, because of the simplicity of apparatus and rapid complete oxidation, the method is easy to adapt for a routine analysis.

2.3.2 Ashing procedure

0.3 to 0.5 g of oven-dried ground plant tissue were transferred into a 250 ml tall form beaker and followed by 5 ml of conc. HNO_3. The beaker was swirled gently and the sample allowed to soak with HNO_3. The mixture was covered by a watch glass and digested slowly at a moderate heat on a hot plate, the temperature being increased later. After the brown fumes disappeared inside the beaker, 2 ml of perchloric acid were added. Through the process, the temperature of hot plate should not be too high ($<200^\circ C$) and the sample never allowed to dry out. The oxidation was completed when solution was clear. The sample was allowed to cool and diluted into 25 ml volumetric flask. Blanks were run on same procedure. Metals in solution were analysed by atomic absorption spectrometry.

2.4 Atomic absorption spectrometry

Atoms whose electrons are in the ground state can absorb the radiation of their own characteristic wavelengths, the intensity of absorbance being proportional to the population of atoms. Atomic absorption spectrometry is based on this principle and can be simply defined as absorption of radiant energy by atoms. The theoretical basis for atomic absorption spectrometry has been covered in the literature (Thompson and Reynolds 1978).

An atomic absorption spectrometer usually consists of the following components.

(1) A light source, emitting the resonance line of the element to be determined.

(2) Atomization system, which can vaporize and decompose analyte into atoms.

(3) A monochromator to isolate the resonance line.

(4) A photomultiplier that detects the intensity of light energy.

Details on application of atomic absorption spectrometry have been increased in recent years (Thompson and Reynolds 1978). Despite the wide application of atomic absorption spectrometry for analysis of metal, however, interference problems can easily be overlooked. Interferences of atomic absorption spectrometry have concerned many workers (Wineforder and Vickers 1964a,b, 1965, Childs and Gaffke 1974, Thompson 1977), so interferences and methods of compensation are mentioned here.

2.4.1 Interferences

Interferences in atomic absorption spectrometry are typically of five types; chemical interference, matrix interference, ionization interference, spectral interference and background interference. Generally, the interferences are easier to control in flame atomic absorption. In flameless atomic absorption, physical differences of analyte on atomization system can cause various difficulties. The following are brief details of interferences in flame and flameless atomic absorption spectrometry, and possible compensation methods;

(i) Chemical interferences - when foreign elements exist and combine with the element of interest, which may affect the atomization efficiency or change the production of neutral atoms in flame. Chemical interferences are also referred as condensed phase interferences. The same type of interferences are observed in photometry, as has been reported by Alkemade (1966). Chemical interferences can normally be overcome by addition of a releasing agent to form a more stable compound with the interfering substance, or the use of a higher temperature flame providing more energy to break down the compound formed. An example of the use of releasing agent is the addition of lanthanum or EDTA into calcium solutions to overcome the effects of phosphate (air-acetylene flame condition). The use of a higher temperature flame (nitrous oxide-acetylene) can reduce some chemical interferences, for example, phosphorus interference with calcium is not observed with a nitrous oxide-acetylene flame.

(ii) Matrix interferences - these may be caused by the different physical characteristics of sample and standard solutions. Sample containing a high concentration of dissolved salt or acid will affect viscosity, surface tension of solution and uptake which may influence the aspiration rate. Matrix interferences can be controlled by diluting the sample solution, matching the standard solution, or in cases where it is not possible to match the standard, the use of method of additions is applicable, ie. standard amounts of the element being analysed added to samples. For example, in the determination of metals in seawater, the standards are made to contain approximately the same concentration of the major dissolved solids as are normally found in seawater.

(iii) Ionization interferences - it can happen when the flame contains higher energy to ionize an appreciable fraction of the neutral atoms from the ground state. The absorbance may be affected since the number of neutral atoms in the flame is reduced by ionization. To overcome the ionization interferences, a large excess of an easily ionized element (Cs, Rb, K, Na, etc.) can be added to sample and standard solutions. For example, even refractory elements such as aluminium, are ionized in nitrous oxide-acetylene flame (Amos and Willis 1966). 1000 to 2000 ug/ml of alkali metal can be added into sample and standard solution to remove the interferences.

(iv) Spectral interferences - when some elements present in the sample absorb at a wavelength which falls within the width of the absorption line of the element of interest, then spectral interference occurs. These types of interferences are serious in the flame emission method but are rather rare in atomic absorption analysis. Jawarowaki and Weberbing (1966) have experienced spectral interferences when analysing

Ni with a multielement hollow cathode lamp, but the interferences were eliminated when they used a single element nickel lamp.

(v) Background absorption - the existence of gaseous molecular species, salt particles, smoke or other causes in the flame may result in background absorption. Some effects of flame absorption, molecular absorption and light scattering are also included in background absorption interferences. These types of background absorptions can be distinguished from the special elemental absorption, because the element can absorb only the very narrow line emitted by the source lamp, and background absorption is less specific and extends over a broader wavelength band than the elemental absorption does. Elimination of the background absorption interferences can be obtained by using a deuterium background corrector, which is designed to automatically correct for nonatomic absorption, or compensating by using a nearly non-absorbing wavelength.

2.4.2 The problem of multiple peaks in flameless atomic absorption

The levels of certain metals in soil solution and extracts are often below the concentrations which can be detected by flame atomic absorption spectrometry, but can be measured by the graphite furnace procedure.

However, not just the above mentioned interferences (Section 2.4.1) may happen in flameless atomic absorption.

For example, the matrix effects of the extracts, which arise both from the high content of solute in the original extractant and from high levels of elements extracted form soil, lead to substantial interferences during the analysis (Cruz and VanLoon 1974, Regan and Warren 1976). The characteristics of the graphite furnace could cause some pronounced interferences, like the problem of multiple peaks, which was not experienced in flame atomic absorption.

The effects on trace metal analysis of three soil extractants, acetic acid, Tamm's oxalate and aqueous sodium pyrophosphate, which have been mentioned on Section 2.2.2, are described in this section.

When aliquots of the soil extracts were injected directly into the graphite furnace, multiple peaks were obtained in some cases, especially with pyrophosphate extracts, and have been reported by many workers, eg. Dudas (1974).

Various instrumental and chemical techniques employed to remove the interferences proved unsuccessful. Variation of ashing time, use of deuterium background correction, addition of equal volumes of 10% (v/v) nitric acid to the injected sample, heating of the extract to dryness with a HNO_3-H_2SO_4 acid mixture and redissolution of the residue in hydrochloric acid, all had no effect in removing the multiple peaks. The use of mini-flow, i.e. a reduced argon flow maintained during the atomization step, produced a single peak but with greatly reduced sensitivity.

After this lack of success by other methods, attempts were made to overcome the interferences by using solvent extraction. A similar technique based on chelation by ammonium pyrrolidinedithiocarbamate (APDC) and extraction into methyl isobutyl ketone (MIBK) has been previously used for Co, Ni, Cu, Pb and Zn (Chao and Sanzolone 1973), and such extracts have been used for cadmium with graphite furnace atomization (Dudas 1974). Chelation by dithizone (diphenylthiocarbazone) is commonly used in colorimetric techniques and has been used for atomic absorption determinations of cadmium and lead in soils and plant tissue (Williams, David and Iismaa 1972, Ure and Mitchell 1976; Ure, Hernandez-Artiga and Mitchell 1978) and for trace elements in rocks and sediments (Armannsson 1977).

The APDC-MIBK method was rejected for two reasons: (a) the metal-APDC complexes became unstable after a few hours, as has also been observed by other workers (Brooks, Presley and Kaplan 1967; Dudas 1974; Eller and Haartz 1977), (b) an emulsion formed at the aqueous-organic interface during extraction into MIBK, especially with pyrophosphate extracts. Neither of these aspects proved to be a problem in dithizone-chloroform extractions.

The major problem encountered in dithizone extractions has been interference from iron (Armannsson 1977, Ure, Hernandez-Artiga and Mitchell 1978). Sandell (1959) attributed this to the oxidation of dithizone by iron (III) and recommended the use of hydroxylammonium chloride to remove the interference during the colorimetric determination of metals. The same reagent was recommended by Saltzman (1953) to eliminate the formation of water-soluble products which prevented complete extraction. Both the oxalate and pyrophosphate reagents are capable of extracting high levels of iron from soils (Bascomb 1968; Gamble and Daniels 1972; Webber, Mckeagne, Raad, DeKimpe, Wang, Haluschak, Stonehouse, Pettapiece, Osborne and Green 1974).

The method described here attempts to provide maximum recoveries of five metals present in soil extracts by using a combination of techniques previously employed for other types of analysis.

2.4.2.1 Experimental reagents

Where possible analytical-grade reagents were used; deionized water was used for all solutions unless other wise specified.

Dithizone in chloroform (0.1% and 0.05% w/v) --- Appropriate amounts of dithizone were dissolved in 500 cm^3 of chloroform. This reagent should be stored in a refrigerator in a dark bottle and made up fresh after 3-4 days.

Ammonium citrate --- Citric acid (50 g) was dissolved in not more than 30 cm^3 of water, and 60 cm^3 of ammonia liquor was added. The solution was left until cool and all crystals of citric acid had dissolved, and then made up to 100 cm^3 with water.

Hydroxylammonium chloride --- Hydroxylammonium chloride (20 g) was dissolved in 30 cm^3 of ammonia liquor, and then made up to 100 cm^3 with water.

2.4.2.2 Procedure

The sample solution (10 cm^3) was pipetted into a 100-cm^3 separating funnel, followed by 5 cm^3 of ammonium citrate solution and 2 cm^3 of hydroxylammonium chloride solution. In the case of acid oxalate extracts, water (10 cm^3) was added to prevent the formation of a precipitate. The contents of the separating funnel were well mixed and the pH adjusted to between 9 and 10 with hydrochloric acid (1+4) or ammonia solution (1+1). Dithizone in choroform (5 cm^3 of 0.1%) was then added; the funnel was shaken and the pressure was released before shaking on a reciprocating shaker for 5 min. When the two phases had separated, the organic phase was collected in a 10-cm^3 volumetric flask. The pH of the aqueous phase was then adjusted to between 8 and 9 with hydrochloric acid (1+4) and a second extraction was carried out with 5 cm^3 of 0.05% (w/v) dithizone in chloroform. The shaking procedure was repeated as before and the organic phase added to the 10-cm^3 volumetric flask. If necessary, the flask was filled to the mark with chloroform. This organic phase is suitable for injecting directly into the graphite furnace.

2.4.2.3 Interferences

The effect of increasing iron concentrations (0, 100, 200 and 500 ug cm^{-3}) and the effectiveness of the hydroxylammonium chloride treatment were tested for standard 0.2 ug cm^{-3} solutions of each trace metal studied, made up in each of the three extractants. The percentage recoveries of the metals were tested in actual samples by adding 2 ug of the metal ion to aliquots of each of the soil extracts containing known quantities of each trace metal, determined by the above method.

2.4.2.4 Instrumentation

All analyses were done with a Perkin-Elmer 306 spectrophotometer and a HGA-74 graphite furnace.

To optimize the reproducibility, the sample was injected into the furnace by holding the syringe in the vertical position. The standard error of the mean based on six replicate injections of the sample adopting this method was less than ±1.5%.

2.4.2.5 Results and discussion

Table 2.4.1 shows the influence of increasing concentrations of iron on the analysis of standard 0.2 ug cm^{-3} solutions of the five metals studied, made up in the three extracting agents, and the increased recoveries obtained in

Table 2.4.1

Effect of the hydroxylammonium chloride treatment on the percentage recovery of added metal (0.2 $\mu g\ cm^{-3}$) in the three soil extractants with differing iron contents added.
(The numbers in parentheses are the standard deviations based on 3 analyses)

Fe content of solution ($\mu g\ cm^{-3}$)	Cd		Co		Cu		Ni		Pb	
	A[a]	B[b]	A	B	A	B	A	B	A	B
Acetic acid										
0	100(1.8)	100(3.2)	100(4.8)	100(4.4)	100(4.3)	100(3.2)	100(2.2)	100(2.3)	100(1.1)	100(1.0)
100	100(1.6)	100(2.0)	80(3.4)	98(7.5)	98(4.0)	103(7.8)	100(3.9)	100(1.4)	95(6.6)	100(1.5)
200	102(2.1)	100(4.3)	83(6.3)	98(5.2)	103(6.6)	105(5.6)	100(2.0)	98(4.2)	80(4.3)	100(3.0)
500	100(2.4)	102(3.7)	93(14)	100(4.3)	103(11)	103(3.7)	100(1.5)	100(7.9)	75(4.4)	97(4.5)
Oxalate										
0	100(1.0)	100(3.4)	100(4.3)	100(4.9)	100(2.4)	100(1.5)	100(7.5)	100(1.1)	100(5.9)	100(1.4)
100	107(1.9)	100(4.1)	119(7.6)	100(7.1)	105(4.0)	100(1.9)	110(1.7)	95(2.8)	105(7.6)	103(3.7)
200	105(5.4)	103(4.9)	113(4.6)	108(5.5)	105(4.5)	95(5.2)	105(4.4)	103(4.7)	120(4.5)	100(4.9)
500	97(2.8)	103(2.2)	100(4.3)	108(6.5)	110(5.5)	95(8.7)	98(4.7)	108(6.6)	150(3.9)	100(5.3)
Pyrophosphate										
0	100(3.2)	100(3.9)	100(4.6)	100(3.3)	100(6.0)	100(5.7)	100(1.9)	100(4.3)	100(5.7)	100(3.3)
100	100(2.1)	100(8.0)	100(1.6)	102(7.1)	80(4.8)	100(8.8)	100(6.3)	100(2.4)	90(5.6)	98(2.1)
200	100(2.8)	100(3.2)	103(0.4)	100(9.5)	75(4.7)	100(2.6)	100(6.8)	100(5.6)	90(4.8)	93(4.7)
500	100(1.7)	100(2.9)	95(5.1)	105(2.0)	83(3.9)	100(13)	100(3.4)	100(6.8)	93(7.6)	93(6.2)

[a] A: Without hydroxylammonium chloride [b] B: With hydroxylammonium chloride.

certain cases by inclusion of treatment with hydroxylammonium chloride.

2.4.2.5.1 Acetic acid extracts

Without the hydroxylamine treatment, recoveries of cobalt and lead were low. It was during analyses for these two metals in the acetic acid soil extracts before treatment with dithizone in chloroform that multiple peaks were normally produced. Treatment of the acetic acid extracts by the full procedure gave recoveries of Cd, Co, Cu, Ni and Pb in the range 97-103% in solutions containing up to 500 ug Fe cm^{-3} (Table 2.4.1).

2.4.2.5.2 Acid oxalate extracts

Extraction of acid oxalate solutions by dithizone -chloroform resulted in single peaks. Inclusion of a hydroxylamine treatment did not have such an obvious effect as in the acetic acid extracts, but did, in general, result in an improvement in recovery. This diminished effect may be due to the combination of complex formation and reduction of iron by oxalate. The signal for lead was enhanced when more than 100 ug Fe cm^{-3} was present and no hydroxylamine treatment was included.

2.4.2.5.3 Pyrophosphate extracts

The multiple peaks produced during the analyses for all the metals studied in pyrophosphate extracts were removed by the extraction with dithizone in chloroform. Inclusion of a hydroxylamine treatment resulted in a substantial increase in the percentage recoveries for copper and lead. Overall, the treatment with hydroxylammonium chloride resulted in an improvement in the recoveries (within the ranges 100-105% for Cd, Co, Cu and Ni and 93-100% for Pb).

Table 2.4.2 Percentage recoveries of 2.00 ug of metal ion added to 10-cm^3 soil extracts

Metal	Metal found in soil extract (ug)	Total recovery (ug)	Recovery (%)
Acetic acid			
Cd	0.04	2.00	98
Co	0.40	2.40	100
Cu	1.25	3.50	108
Ni	0.50	2.45	98
Pb	0.70	2.50	93
Acid oxalate			
Cd	0.04	2.00	98
Co	0.90	3.00	103
Cu	0.50	2.60	104
Ni	0.80	3.00	107
Pb	2.30	4.40	102
Pyrophosphate			
Cd	0.02	2.00	99
Co	0.05	1.95	95
Cu	1.70	3.60	97
Ni	0.50	2.30	92
Pb	0.30	2.25	98

Table 2.4.2 shows the percentage recoveries for each metal after 2 ug had been added to aliquots of soil extracts. The recoveries lay in the range 93-108%.

The method has proved suitable for routine analysis of trace metals in soils. The detection limits obtained were: 0.001 ug Cd cm^{-3}, 0.05 ug Cu cm^{-3}, 0.004 ug Co cm^{-3}, 0.02 ug Ni cm^{-3} and 0.02 ug Pb cm^{-3}.

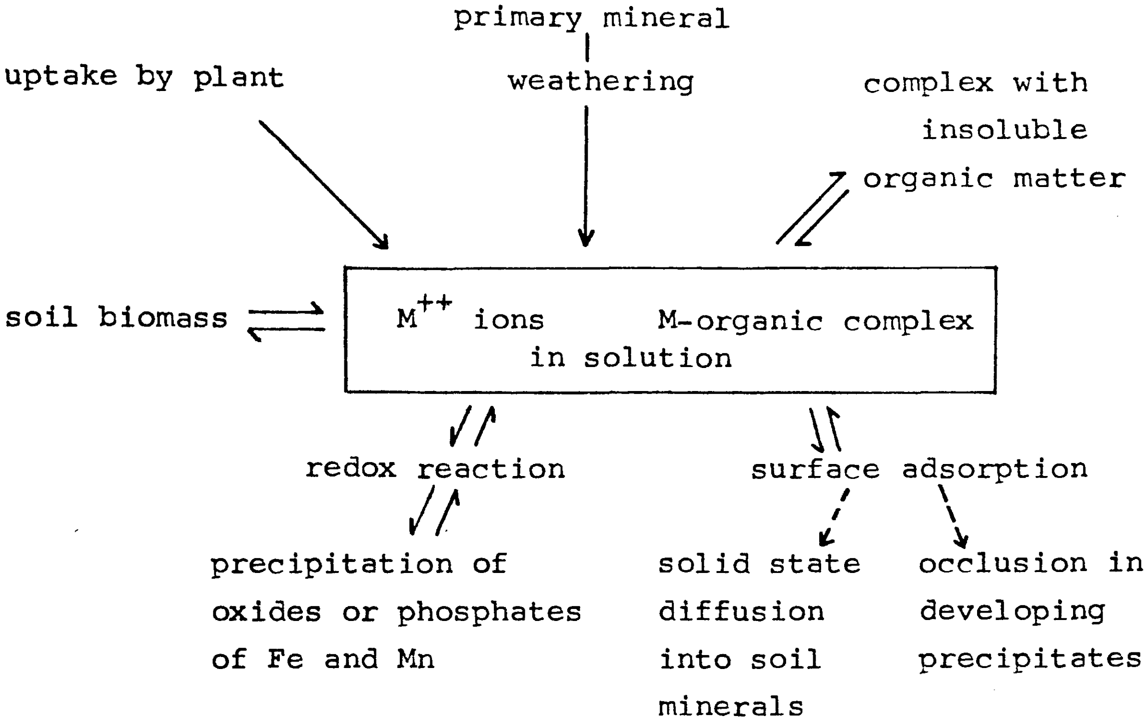
CHAPTER 3 EFFECTS OF WATERLOGGING ON THE BEHAVIOUR OF TRACE METALS IN SOILS

3.1. Introduction

Plant uptake is effectively related to the forms of metals in soil. Relations between each form of metal are summarized in Fig 3.1.1, in which it is shown that each form is related directly or indirectly to the soil solution, through some pseudo equilibrium distribution (Hodgson 1963). In most soils, soluble and plant immediately available metal ions are maintained at a low proportion of the total concentration, and are governed by various factors; e.g. oxide material and organic matter.

Soil moisture has a pronounced effect on the forms of metal. It has been noticed that soil under poorly-drained conditions would have higher plant available metals than under well drained conditions (Mitchell, Reith and Johnson 1957, Prasad, Prasad and Singh 1976). These results can be attributed to changes in the regulatory mechanisms which influence the release and uptake of metals in soil. The aqueous diffusion of dissolved oxygen is ten thousand times slower than gaseous diffusion through a porous medium (Greenwood 1961). Once there is free water in much or all of the soil pore space, due to impeded drainage or flooding, gaseous diffusion will be cut off, and as the chemical and microbial demand for oxygen exceeds the rate of oxygen supply, anaerobic conditions develop.

Fig 3.1.1 Possible changes in the chemical form of a metallic cation in soils (Hodgson 1963)



Under anaerobic conditions, facultative anaerobic and anaerobic organisms predominate in soil, and utilize either inorganic or organic substrates as terminal electron acceptors. A sequential reduction of inorganic constituents from weakly oxidized to strongly reduced conditions is noted and reactions are listed in Table 3.1.1.

On reduction of a waterlogged soil, nitrate is the next oxidant to be reduced following oxygen depletion; and then insoluble Mn(IV) and Fe(III) are mobilized in waterlogged soil as Mn^{2+} and Fe^{2+} by reduction as decribed in Table 3.1.1. Some metals present in soil are probably not involved in oxidation-reduction reactions; however, their mobilities may be affected by instability of colloidal hydrous oxides on which they are adsorbed or coprecipitated.

Another metal regulatory mechanism influenced by submergence is complex formation with insoluble organic matter. A change in microbial ecology would markedly change the metal binding ability of organic matter in soil (Zunino and Martin 1977). Aerobic and anaerobic respiration differ in the amount of energy released by decompostion of organic substrates and produce rather different end products. In a well drained soil, the main end products of organic matter degradation are carbon dioxide, water, nitrate and sulphate plus some residual humic materials; in submerged soil, they are carbon dioxide, methane, hydrogen, ammonia, amines, mercaptans, hydrogen sulphide and partially humified

Table 3.1.1 Reactions of some redox systems operating in waterlogged soil

Systems	Eh(mV) at pH 7
$1/4\ O_2(gas) + H^+ (soln) + e \rightleftharpoons 1/2\ H_2O$	300
$1/5\ NO_3^-(soln) + 6/5\ H^+ (soln) + e \rightleftharpoons 1/10\ N_2(gas) + 3/5\ H_2O$	225
$1/2\ MnO_2(solid) + 3H^+(soln) + e \rightleftharpoons 1/2\ Mn^{2+}(soln) + H_2O$	200
$Fe(OH)_3(solid) + 3H^+(soln) + e \rightleftharpoons Fe^{2+}(soln) + 3\ H_2O$	120
$1/8\ SO_4^{2-}(soln) + 5/4\ H^+(soln) + e \rightleftharpoons 1/8\ H_2S(gas) + 1/2\ H_2O$	-150

residues (Ponnamperuma 1972).

It has been suggested that the residual humic material resulting from anaerobic respiration is likely to have a larger and more complex structure, which tends to increase the metal retention capacity and the metal binding ability of insoluble humic material (Gambrell and Patrick 1978). In addition, an optimum pH or pH range usually exists for the stability of a given metal complex (Schnitzer and Skinner 1967) and oxidation-reduction potential exerts a similar effect on certain metals. Further types of reaction in submerged soils are the formation of metal carbonates (Ponnamperuma, Loy and Tianco 1969) or sulfide (IRRI 1972).

As a result of the various regulatory processes mentioned above, the net result of soil submergence is found to be increasing availability of iron and manganese (Mandal 1961), cobalt and copper (Adams and Honeysett 1964) and depression of zinc availability (IRRI 1971, 1976). However, it is believed that an increased understanding of the forms, amounts and movements of metals in waterlogged soil may contribute in developing effective crop management and human health, thus, the expriments described in the following sections were planned to answer such points.

The object of the first study in this chapter was to obtain further information about changes in distribution of 5 metals between aerobic, alternating waterlogged and waterlogged treatments within the B_2 and B_{3g} horizon of a

Scottish acid brown earth soil.

Since liming and adding organic matter are common techniques in crop management, the second study was undertaken to examine the different changes brought about by these treatments in waterlogged soil. The chemical fractionation procedure mentioned in Chapter 2 was used to determine the quantity of metals in various extractable forms.

3.2 The influence of continuous and alternating waterlogging on the trace metals held in Carbeth B_2 and B_{3g} horizon soils

3.2.1 Introduction

Many Scottish soils are derived from impermeable parent material or parent rock close to the surface (Fitzpatrick 1964). As a result gleys ie. soils in which there is evidence of iron reduction and reprecipitation within the profile, are a common soil type. The water content of these soils fluctuates on either side of their field capacity, depending on the degree of precipitation. In winter, it is quite common to see large areas of agricultural land at or above field capacity, as the poor drainage does not allow rapid loss of water. Such conditions may persist over long periods. Many of these soils have an acid pH, of the order of 4 to 6, especially those used for rough grazing or long-term grass leys. There appears to be little information on the effect which waterlogging of this type of soil can have on the form and availability of trace elements (Graven, Attoe and Smith 1965). This is of particular relevance on areas used for grazing ruminants, which require adequate levels of certain trace elements in the herbage. More information is available on the effect of waterlogging on trace elements in other soil types, for example, rice soils. Such soils however tend to be of high pH (greater than 7) and low organic matter content (Olomu, Racz and Cho 1973, IRRI 1971, 1977; Halder and Mandal 1979). Little information is available concerning the distribution of trace elements in waterlogged soil between the various reservoirs in which they are held (Mandal 1961, Sims and Patrick 1978).

Using a sequential extraction method to monitor the changes of metal fractions in the soils under waterlogging, the trial may provide valuable results for understanding the ways in which the behaviour of metals changes in a soil containing excess water.

3.2.2 Materials and methods

3.2.2.1 Soil condition

A suitable site for this study was selected at Carbeth, which is located 10 Km north of Glasgow (NS 523785). This site is typical of many found in Western Scotland. In the winter, it suffers from cold and wet weather. It is undisturbed and readily accessible, much background information is available as other members of the section are carrying out field studies on bracken eradication, herbicide degradation and the influence of chelating agents on metal uptake from soil.

Carbeth soil was developed from basalt parent material. Top soil was covered by bracken or grasses, soil profile, B_2 and B_{3g} horizon have high organic content. B_2 horizon is from 5 cm to 21 cm depth, B_{3g} horizon is from 21 cm of depth. Underground water was flowing through the B_{3g} horizon in the winter, giving the B_2 and B_{3g} different drainage conditions for some period of a year.

Some details of B_2 and B_{3g} horizon soil are shown in Table 3.2.1.

Table 3.2.1 Physical properties of B_2 and B_{3g} horizon in Carbeth

	pH[a]	O.M.[b](%)	Sand(%)	Silt(%)	Clay(%)	C.E.C. (me/100g)
B_2	4.35	13.04	42.32	30.35	27.33	46.7
B_{3g}	4.67	9.06	41.68	35.83	22.49	45.4

a: pH value based on 1 : 2.5 soil and water ratio.

b: based on Walkley-Black method and multipled by factor 1.72 (Hesse 1971).

Carbeth B_2 and B_{3g} horizons have significant differences in drainage conditions in the winter season. Since saturated and waterlogged condition could affect the regulatory factors of the available metals in soil, it was expected that the forms of metals in the two horizons would show considerable differences brought about by this particular field condition.

In the following trials, because soil pre-treatment could change the behaviour of macro and micro-element in soil, fresh soil samples were used throughout the experiments.

3.2.2.2 Procedure

Carbeth B_2 and B_{3g} soils were held at three different moisture levels.

(i) Soil at 60% field capacity (aerobic control).

(ii) Soil alternately submerged for one week and drained for one week (one cycle lasting two weeks).

(iii) Soil under waterlogged condition.

Fresh soil was passed through a 2 mm sieve. The moisture content of sifted soil was determined and 2 kg of soil on oven-dried basis, were weighed out.

A sprayer was used to apply a calculated weight of water evenly to adjust soil moisture to 60% field capacity, and the soil was transferred into 21 cm diameter plastic containers.

Submerged conditions were achieved by adding water slowly into the container until there were 4-5 cm over the soil surface. Drainage of the alternating waterlogged soil was brought about by drilling a hole in the side of the container from which water could be removed at the scheduled time.

B_2 and B_{3g} soils received the same treatment and were incubated at 26°C ± 2° in a temperature controlled cabinet and left for 15 weeks. The soil moisture was maintained at 60% by periodic spraying of a small amount of water onto the soil surface, and the waterlogged water level was kept at 4-5 cm above the surface by adding water directly.

Subsamples were removed from the containers using a hollow glass tube. All samples were transferred to a Buchner funnel, and in the case of waterlogged samples suction was applied to remove the excess water. The contents of the Buchner funnel were then further sampled for moisture determination and for sequential extraction. Details of extraction procedures have been described in Section 2.2.2. The pH value of the soils was determined on a 1 to 2.5 soil water ratio using a pH meter.

3.2.2.3 Analysis

Soil solution and soil extracts were analysed for cobalt, copper, iron, manganese and zinc, because these elements may be associated with plant and animal nutrition problems in either dificiencies or excesses. All metals were analysed by flame atomic absorption except some of the cobalt and copper determinations, which were analysed by flameless atomic absorption using the dithizone extraction method described in Chapter 2.

3.2.3 Results and discussion

There had been some visual differences between the soil samples through the experiment. In the B_2 soil, cloudy standing water with a brown colour had been observed in the waterlogged treatment after 9 weeks incubation. At that stage, the standing water on the alternating waterlogged soil was still quite clear, although the drainage from that treatment was light brown in colour. After that, standing water on the waterlogged B_2 soil started to form brown clods, after which a layer of brown material covered the water surface. Thereafter, standing water on the alternating waterlogged soil also showed a similar phenomenon. None of these changes were observed with B_{3g} soil samples. The development of a brown coloured precipitates in waterlogged B_2 soil might have been caused by a large amount of easily decomposable organic matter which could have accentuated the reducing conditions and subsequently increased the mobilized iron.

As on purpose to compare the metal changes in waterlogged and aerobic soils, metals in chemical extraction analyses are expressed as the difference in value between the waterlogged treatment and the aerobic one (see Chapter 2). Thus ΔHAc-Mn equals waterlogged HAc-Mn minus control (aerobic) HAc-Mn. Positive values therefore indicate a higher level of extractable metal in the waterlogged soil than in the control, and <u>vice versa</u>.

Figure 3.2.1 to 3.2.22 show the pH changes and the changes in the various fractions of metals between the treated and control soils over 15 weeks. Data of original extracts' analyses are shown in appendix 1.

3.2.3.1 B_2 horizon

3.2.3.1.1 pH

The pH values of Carbeth B_2 soil plotted against time are shown in Figure 3.2.1. The pH of the aerobic control soil was not influenced by incubation and remained at approximately pH 4.35. Meanwhile, alternating waterlogged and waterlogged treatments both showed an increase in pH. According to Ponnamperuma, Martinez and Loy (1966), the subsequent increase in pH of waterlogged acid soils is due to soil reduction. The B_2 soil in the alternating waterlogged treatment had a lower pH than the waterlogged, and could have been caused by periodic re-oxidation delaying the soil reduction.

3.2.3.1.2 Redistribution of Manganese

ΔH_2O-Mn and ΔHAc-Mn in the waterlogged soil increased with incubation, Δpp-Mn increased in the first three weeks then stayed constant, until week 11, it then started to decrease, reaching negative values. Δoxa-Mn decreased after a week of incubation and became stable in week 9 (Figure 3.2.2).

It was found that the sum of positive "Δ" values were balanced by the sum of negative "Δ" values over the four extracts. Thus the increase of ΔH_2O-Mn and ΔHAc-Mn

Figure 3.2.1 Variation in pH over 15 weeks waterlogging.

X——X aerobic control
△——△ continuous waterlogging
O——O periodic waterlogging

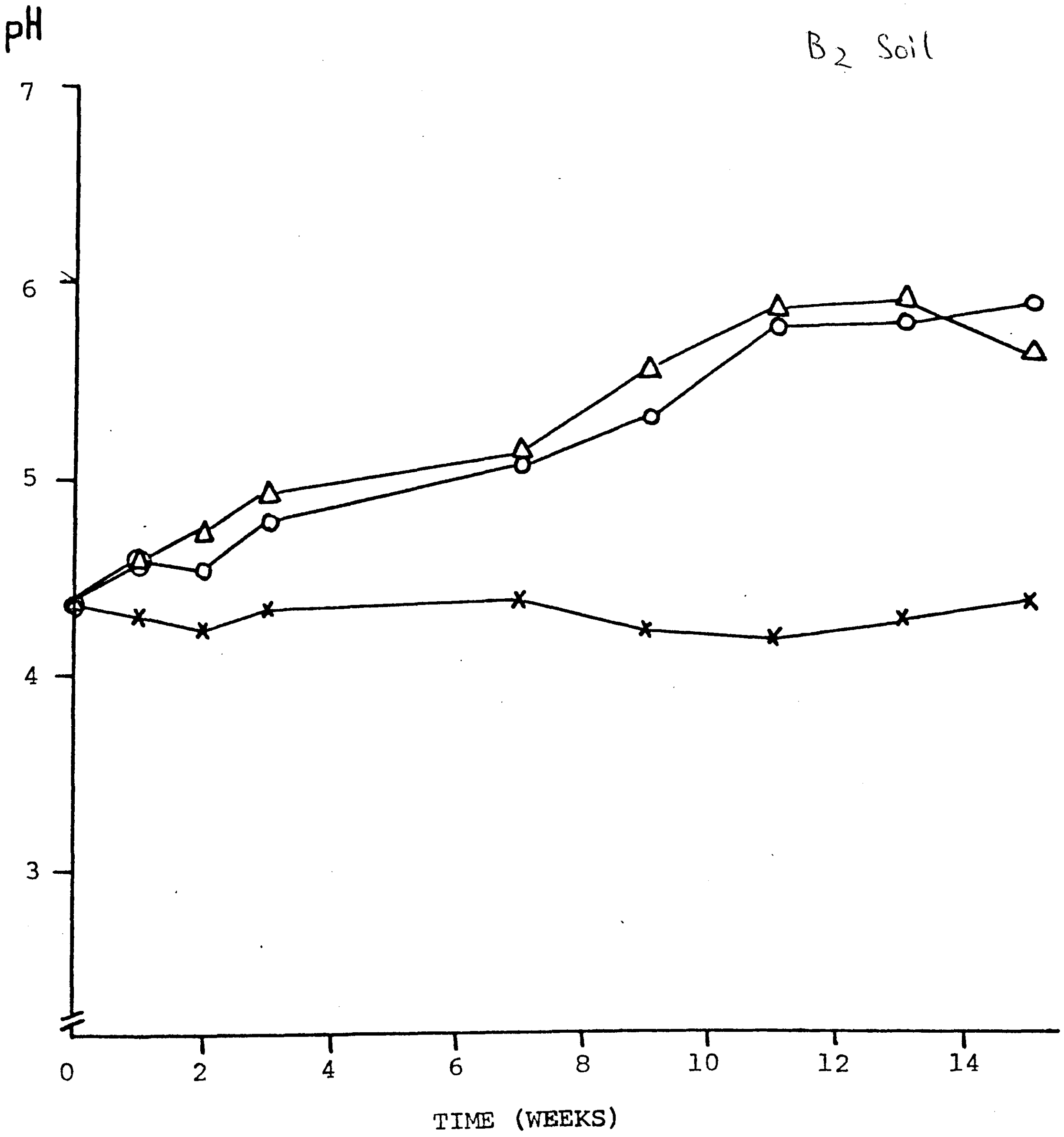


Figure 3.2.2 Variation in the levels of manganese removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 continuously waterlogged.

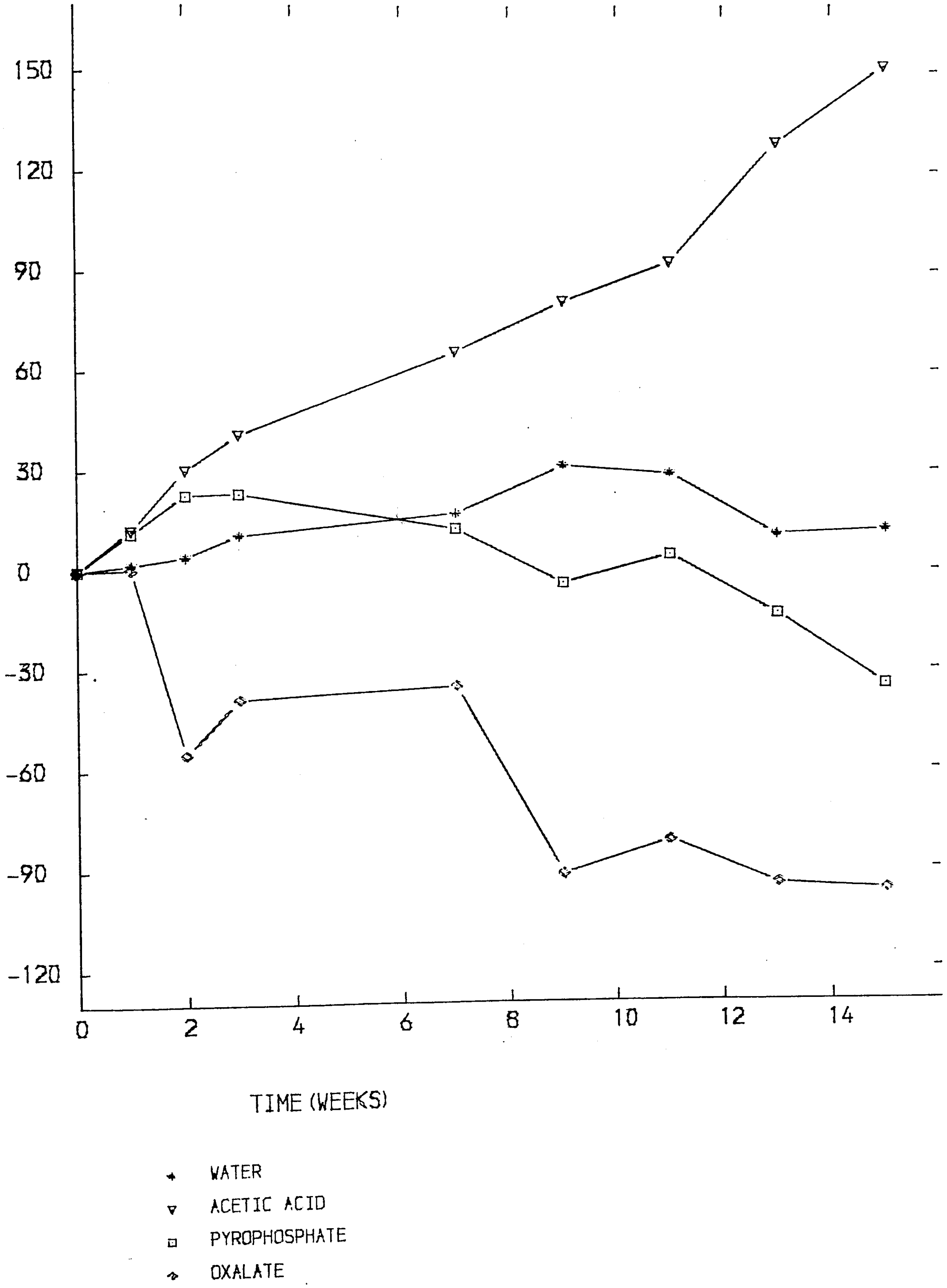
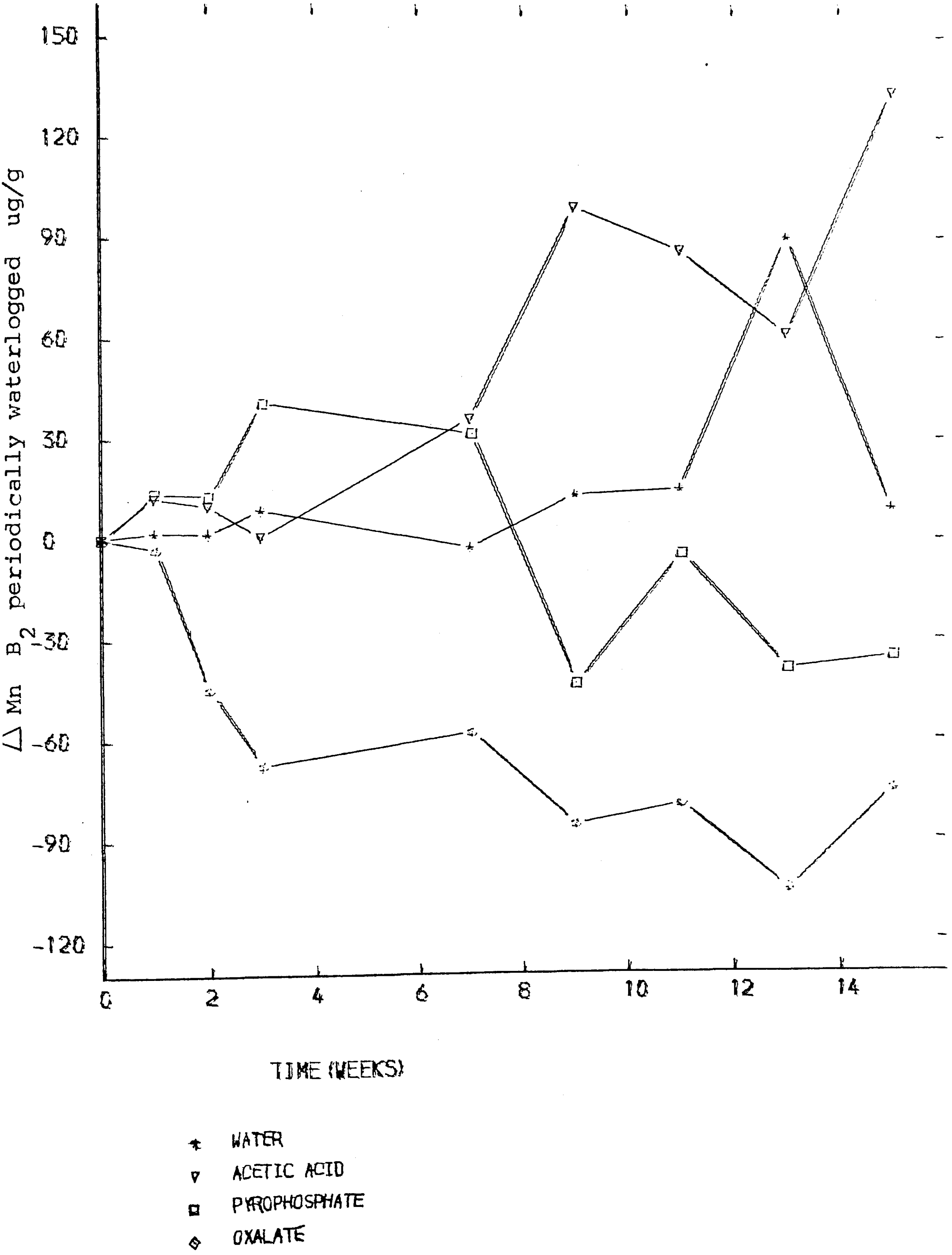


Figure 3.2.3 Variation in the levels of manganese removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 periodically waterlogged.



may explain the decrease of Δoxa-Mn, and later the decrease of Δpp-Mn.

Alternating waterlogging had the same effect on soil manganese as continuous waterlogging, and its changes are shown in Fig 3.2.3. The fluctuations of ΔHAc-Mn and Δpp-Mn values in alternating waterlogging compared with continuous waterlogging were perhaps mainly caused by alternating drying and wetting. However, the sum of the positive and negative values were still kept approximately in balance, so the explanation for changes of manganese in continuous waterlogging may apply here.

3.2.3.1.3 Redistribution of iron

Effects of waterlogging and alternating waterlogging on iron are shown in Fig 3.2.4 and Fig 3.2.5. These two treatments had similar patterns of changes although the ranges were different, ΔH_2O-Fe and ΔHAc-Fe increased. Continuous waterlogging generally had higher ΔHAc values than alternating waterlogging. Δpp-Fe showed initial decreases, then increases to +1300ppm and +900ppm at week 11 for alternating and continuous waterlogging respectively. Thereafter, Δpp-Fe had different degrees of falling between the two treatments. The reason for the changes of Δoxa-Fe were not understood at that stage. The second experiment's results, see later (Section 3.3.3.3), elucidated this point.

Figure 3.2.4 Variation in the levels of iron removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 continuously waterlogged.

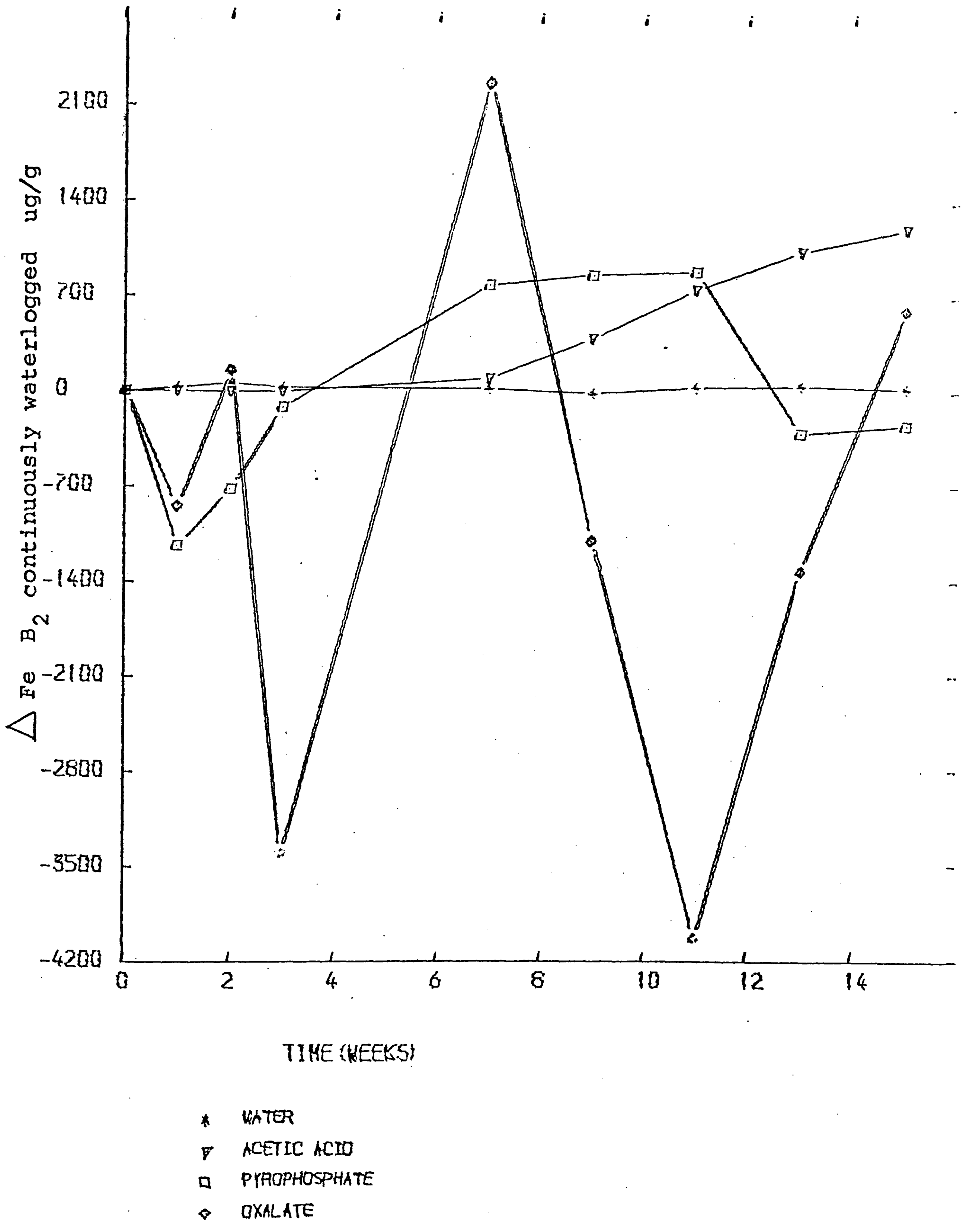
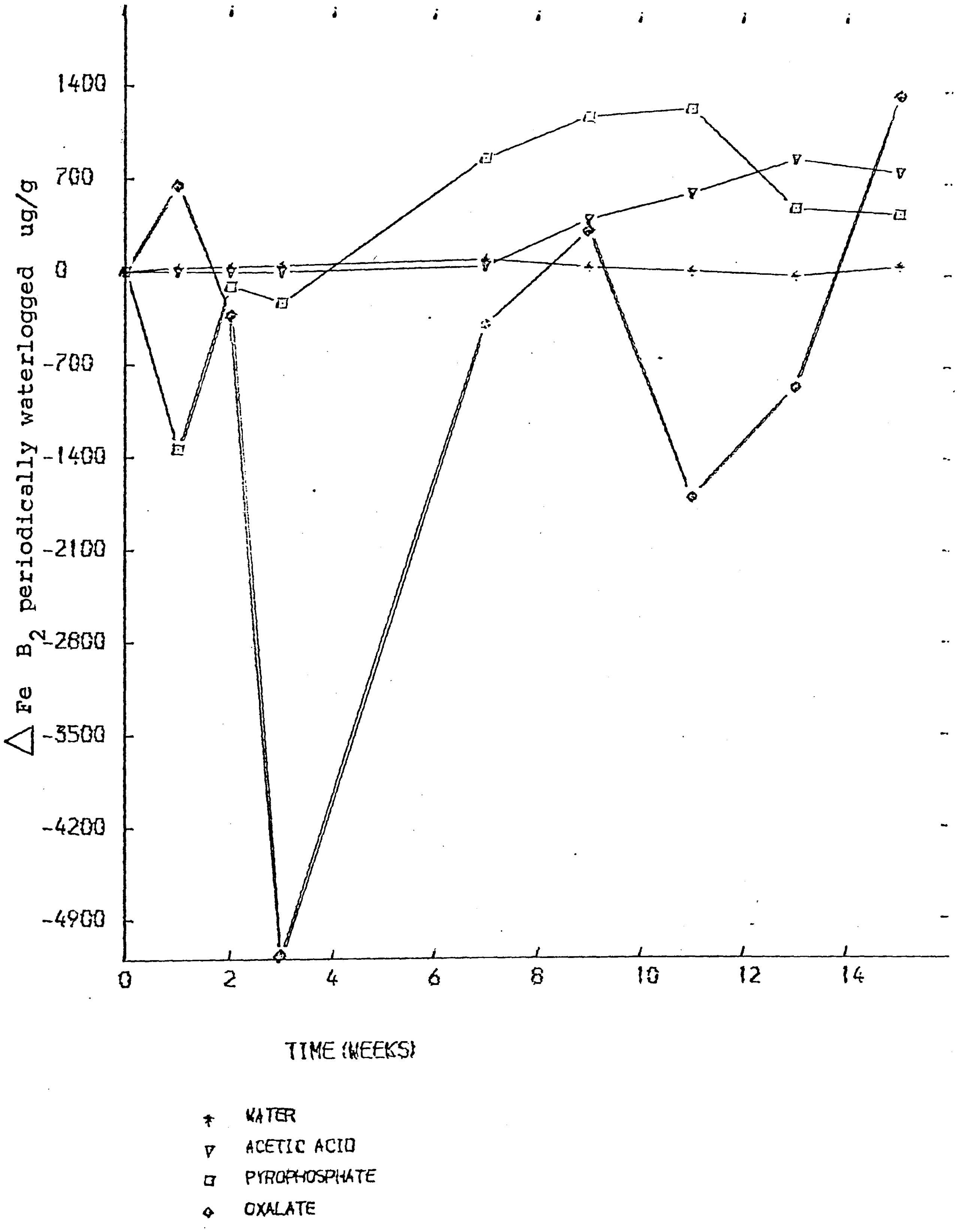


Figure 3.2.5 Variation in the levels of iron removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 periodically waterlogged.



3.2.3.1.4 Redistribution of zinc

Rather different characteristics of zinc redistribution compared with manganese and iron in waterlogged soil were observed. ΔHAc and Δoxa-Zn between waterlogged and control soil were increased by incubation, and these were accompanied by decreases of ΔH_2O-Zn and Δpp-Zn (Fig 3.2.6). Increase of Δoxa-Zn, which coincided with decreases of ΔH_2O-Zn and Δpp-Zn, may be responsible for the reported deficiency of zinc in waterlogged soils (Kalbasi, Racz and Loewen-Rudger 1978).

A similar reaction happened on the alternating waterlogged soil (Fig 3.2.7), but the trends of zinc changes were not so clearly marked.

3.2.3.1.5 Redistribution of copper

Changes of copper in waterlogged B_2 soil were similar to zinc. Fig 3.2.8 suggests that ΔHAc-Cu and Δoxa-Cu were increasing but Δpp-Cu was decreasing. The sudden fluctuations of Δpp-Cu at the later stage of the experiment were not understood at this stage (see Section 3.3.3.5).

The copper changes in alternating waterlogged soil are shown in Fig 3.2.9. ΔHAc-Cu and Δoxa-Cu increased as in continuous waterlogging, however, Δpp-Cu was changing too erratically to allow any judgement to be made.

Figure 3.2.6 Variation in the levels of zinc removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 continuously waterlogged.

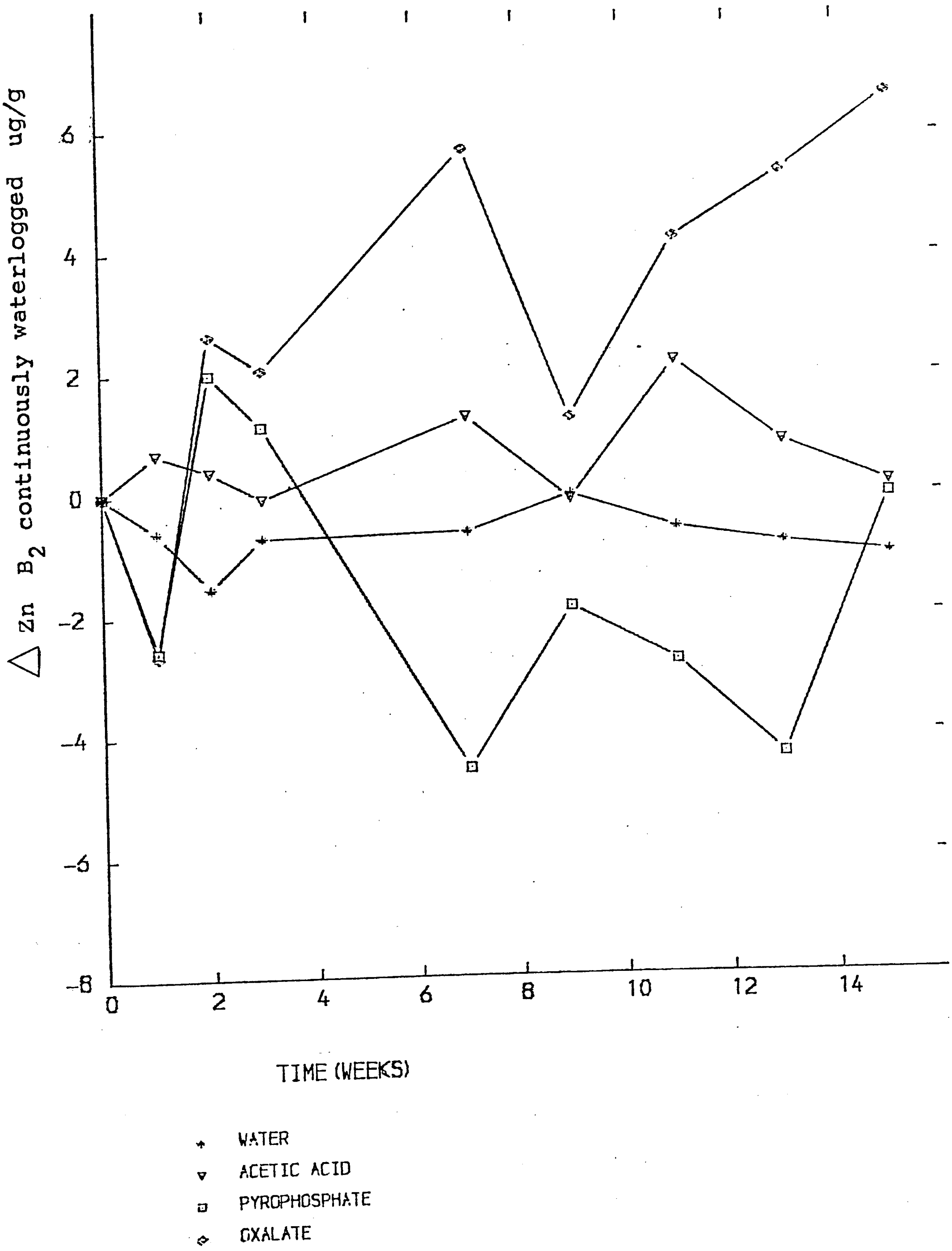


Figure 3.2.7 Variation in the levels of zinc removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments.
B_2 periodically waterlogged.

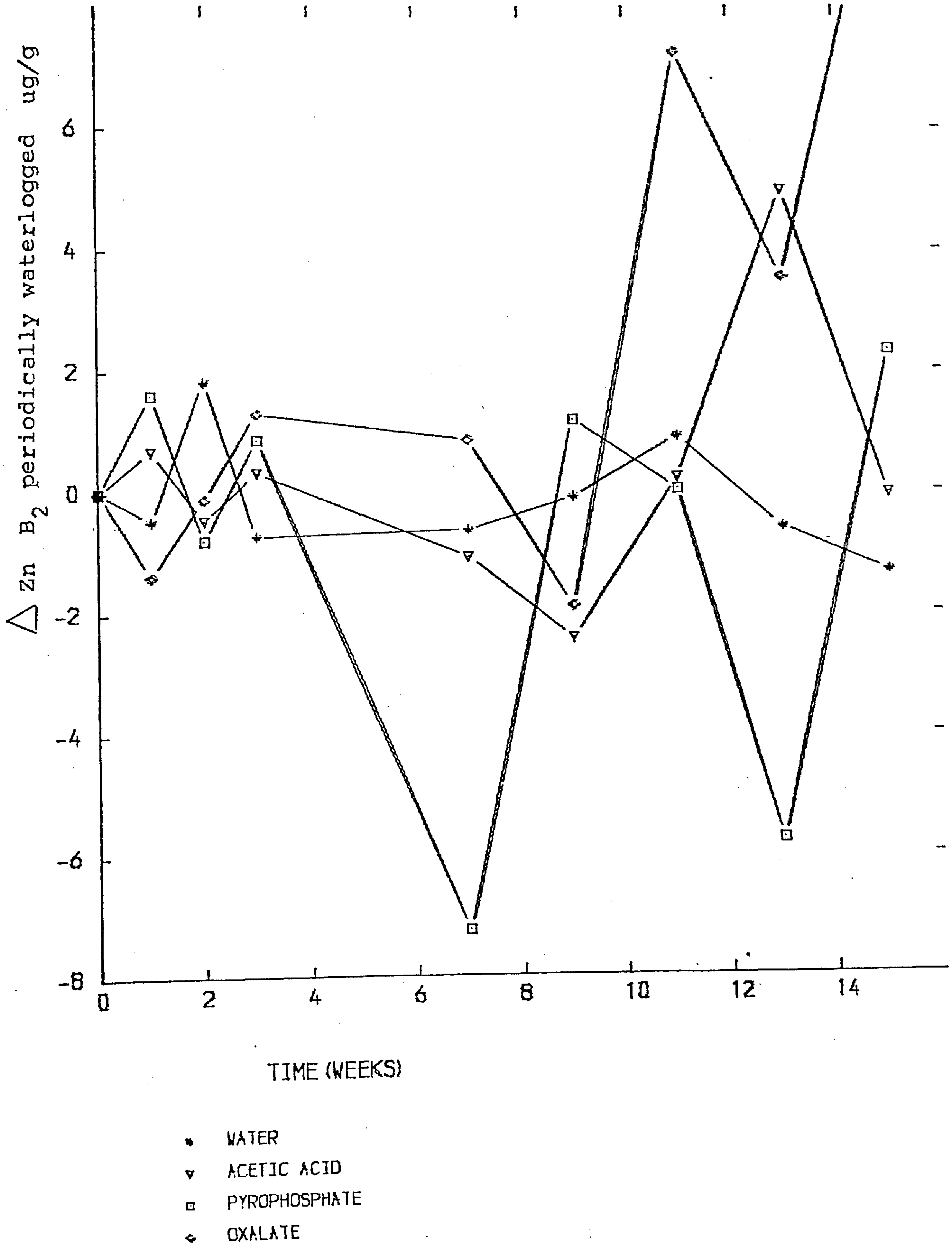


Figure 3.2.8 Variation in the levels of copper removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 continuously waterlogged.

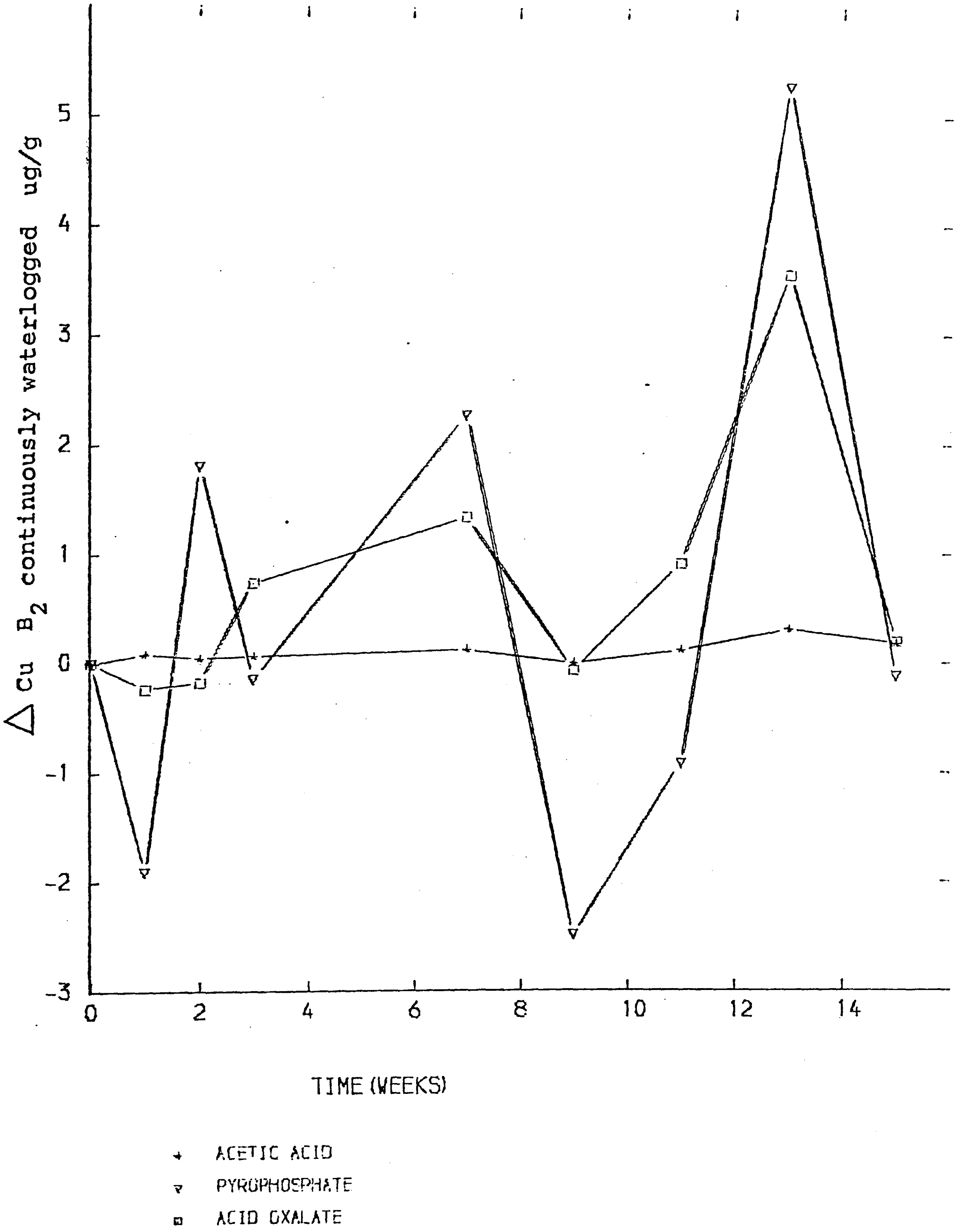
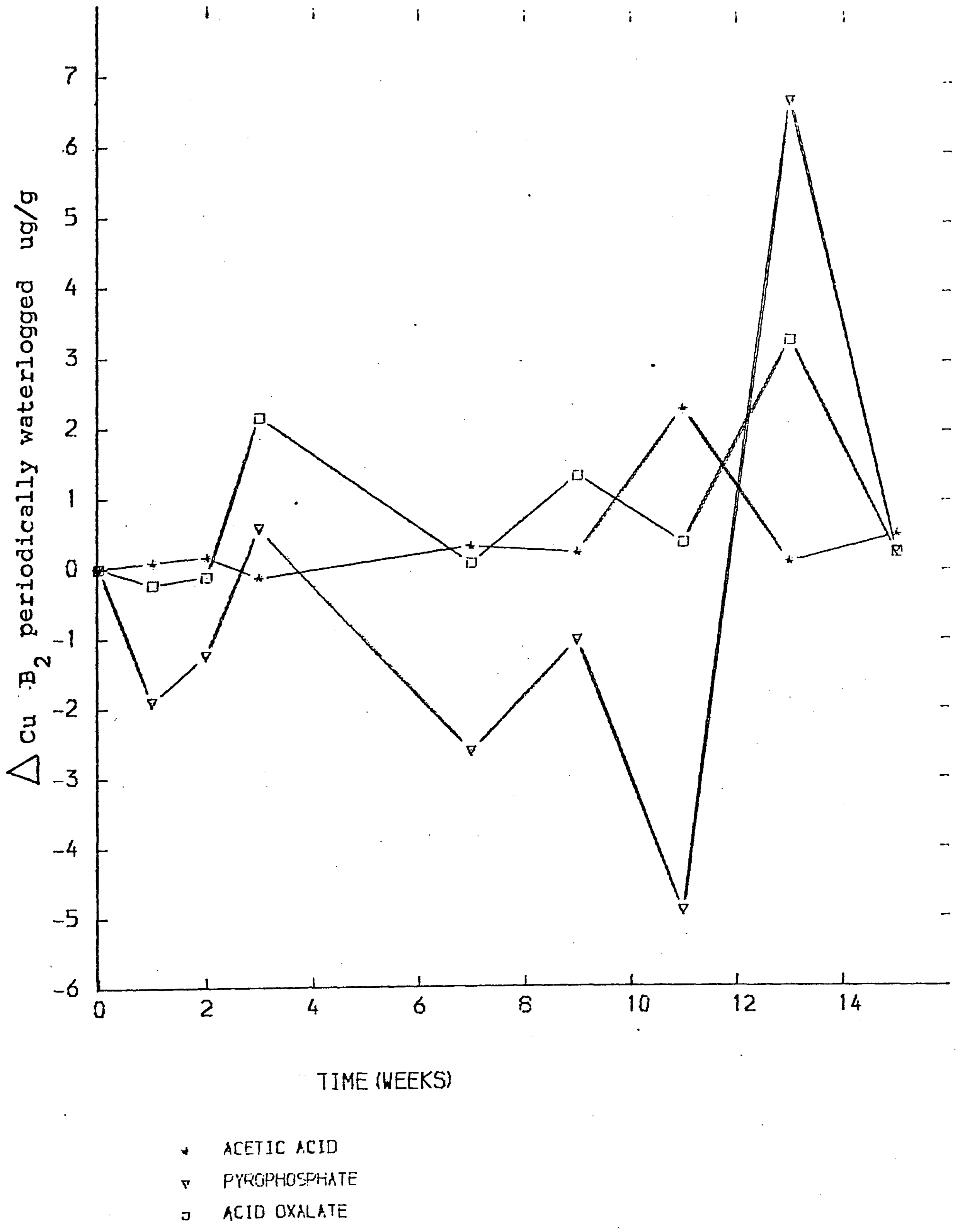


Figure 3.2.9 Variation in the levels of copper removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 periodically waterlogged.



3.2.3.1.6 Redistribution of cobalt

Figure 3.2.10, show ΔH_2O-Co and ΔHAc-Co increased in continuous waterlogging and reached a peak at week 9, after the peak value there was some decrease. Δoxa-Co decreased and the lowest value was observed at week 9. When the experiment ended, Δoxa-Co was back to positive value. Mitchell, Reith and Johnston (1957) found acetic acid extracted more cobalt than EDTA (unlike other metals). In this experiment, cobalt was also found to be higher in acetic acid than pyrophosphate extracts. Thus, normal modification of "Δpp-Co", was not included in the modified calculation. Nevertheless, Δpp-Co was increasing but compared with scales of ΔHAc-Co and Δoxa-Co, it was nonsignificant.

The alternating waterlogging result is shown in Figure 3.2.11. It is very much like that of continuous waterlogging. The increases of ΔH_2O-Co and ΔHAc-Co may be attributed to the decrease of Δoxa-Co.

3.2.3.2 B_{3g} horizon

3.2.3.2.1 pH

Waterlogging did not affect the pH in the B_{3g} soil. With or without waterlogging treatment, B_{3g} soil samples were in the range pH 4.7 to 4.9 over 15 weeks incubation (Figure 3.2.12).

Figure 3.2.10 Variation in the levels of cobalt removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 continuously waterlogged.

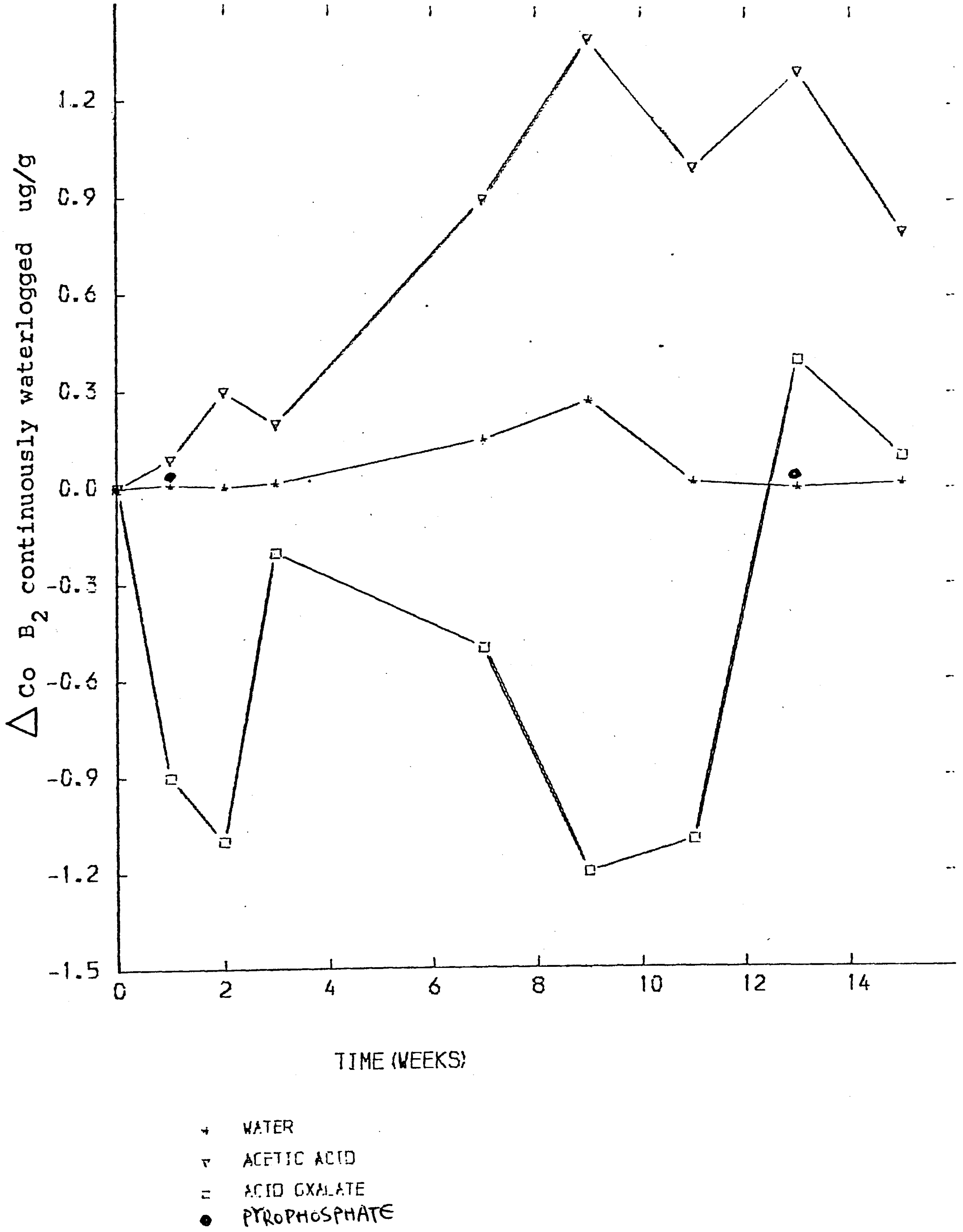


Figure 3.2.11 Variation in the levels of cobalt removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_2 periodically waterlogged.

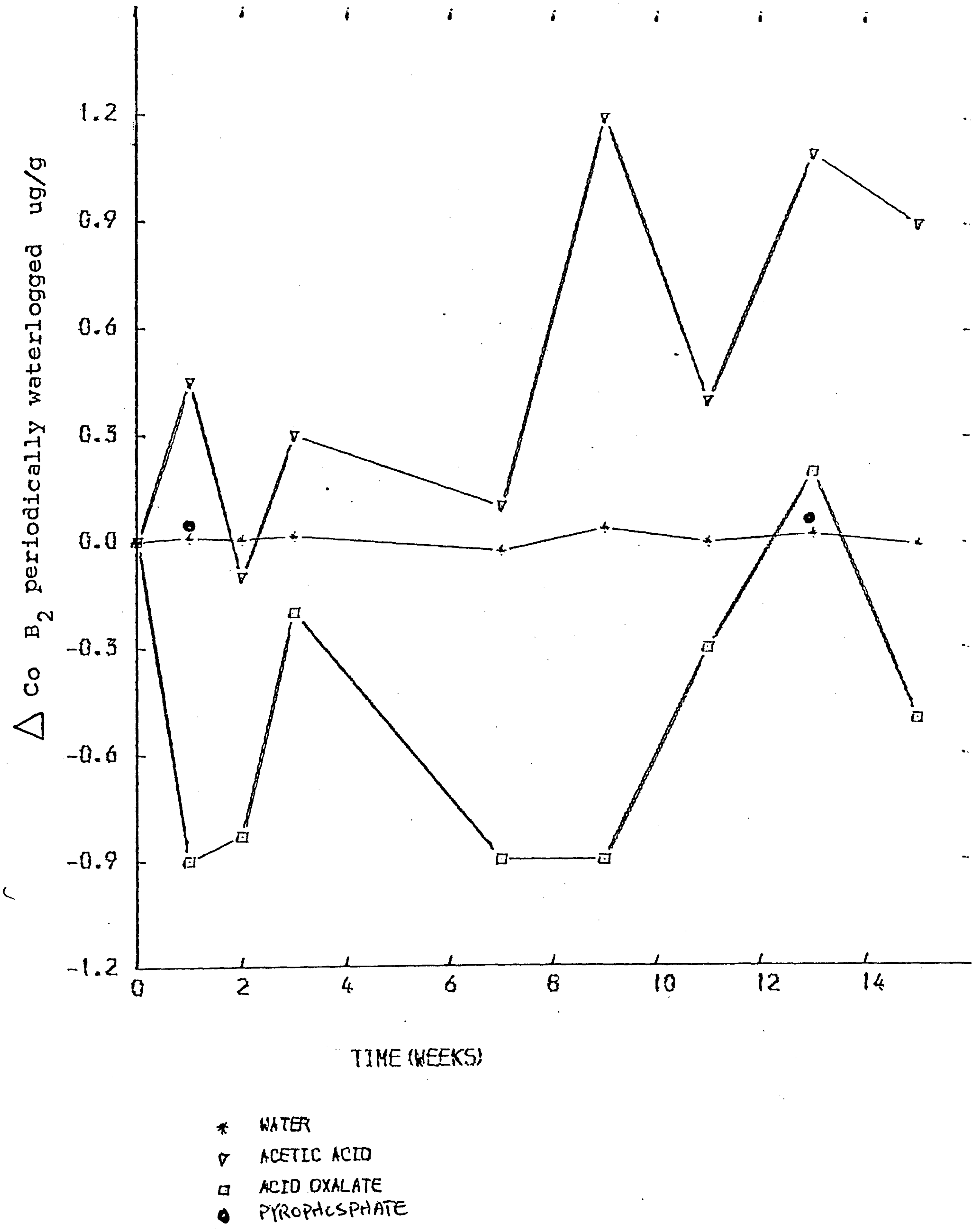
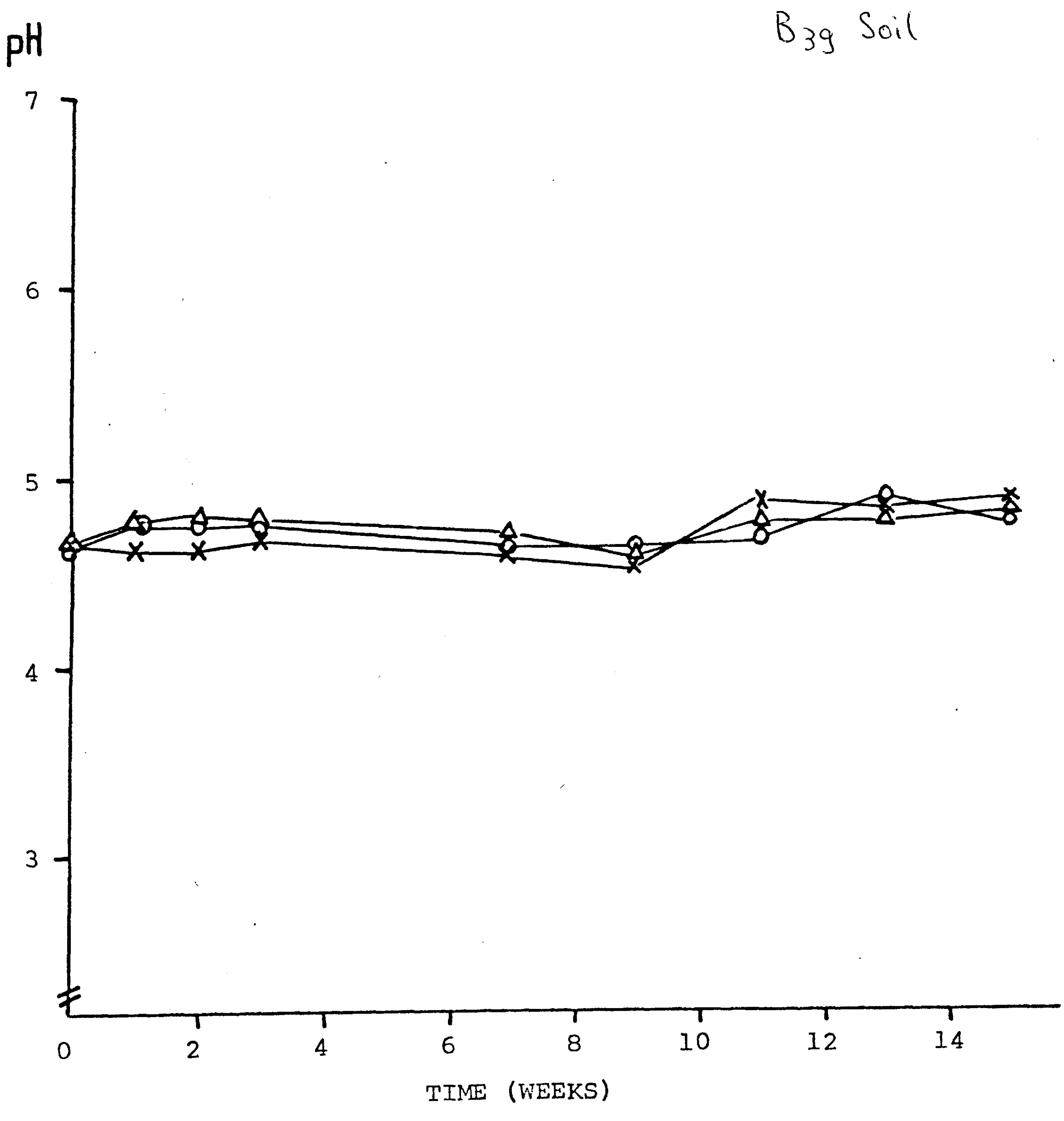


Figure 3.2.12 Variation in pH over 15 weeks waterlogging.

X——X aerobic control
Δ——Δ continuous waterlogging
O——O periodic waterlogging



It has been proposed that the wet dry cycles result in the solubilization or release of previously resistant compounds(Sorenson 1974, Davidson 1975). Many years of water flowing through the B_{3g} horizon in winter would accentuate the losses of easily decomposable organic matter. The unchanged pH in waterlogged B_{3g} soil may be accounted for by removal of easily decomposable organic matter.

3.2.3.2.2 Redistribution of manganese

The changes in ΔH_2O-Mn in B_{3g} soil were similar to the B_2 soil, however waterlogging had different effects on the other soil manganese fractions of the B_{3g} and B_2 horizons. Results in the previous section showed, ΔHAc-Mn in the B_2 soil was increased by waterlogging, however, the ΔHAc-Mn of the B_{3g} soil decreased with the same treatment. In addition, the Δpp-Mn did not change much (e.g. Fig 3.2.2) and Δoxa-Mn actually increased (Fig 3.2.13). The B_{3g} horizon was influenced by underground water in winter, soluble Mn and easily decomposable organic matter may have been taken away by the flowing water. pH of the B_{3g} soil was not influenced by waterlogging, and the much slower rate and lower values of manganese in the standing water compared with B_2 soil, confirmed this difference.

ΔHAc-Mn between alternating waterlogging and control decrease quite clearly (Fig 3.2.14). At the end of the experiment, ΔHAc-Mn in alternating waterlogging was

Figure 3.2.13 Variation in the levels of manganese removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} continuously waterlogged.

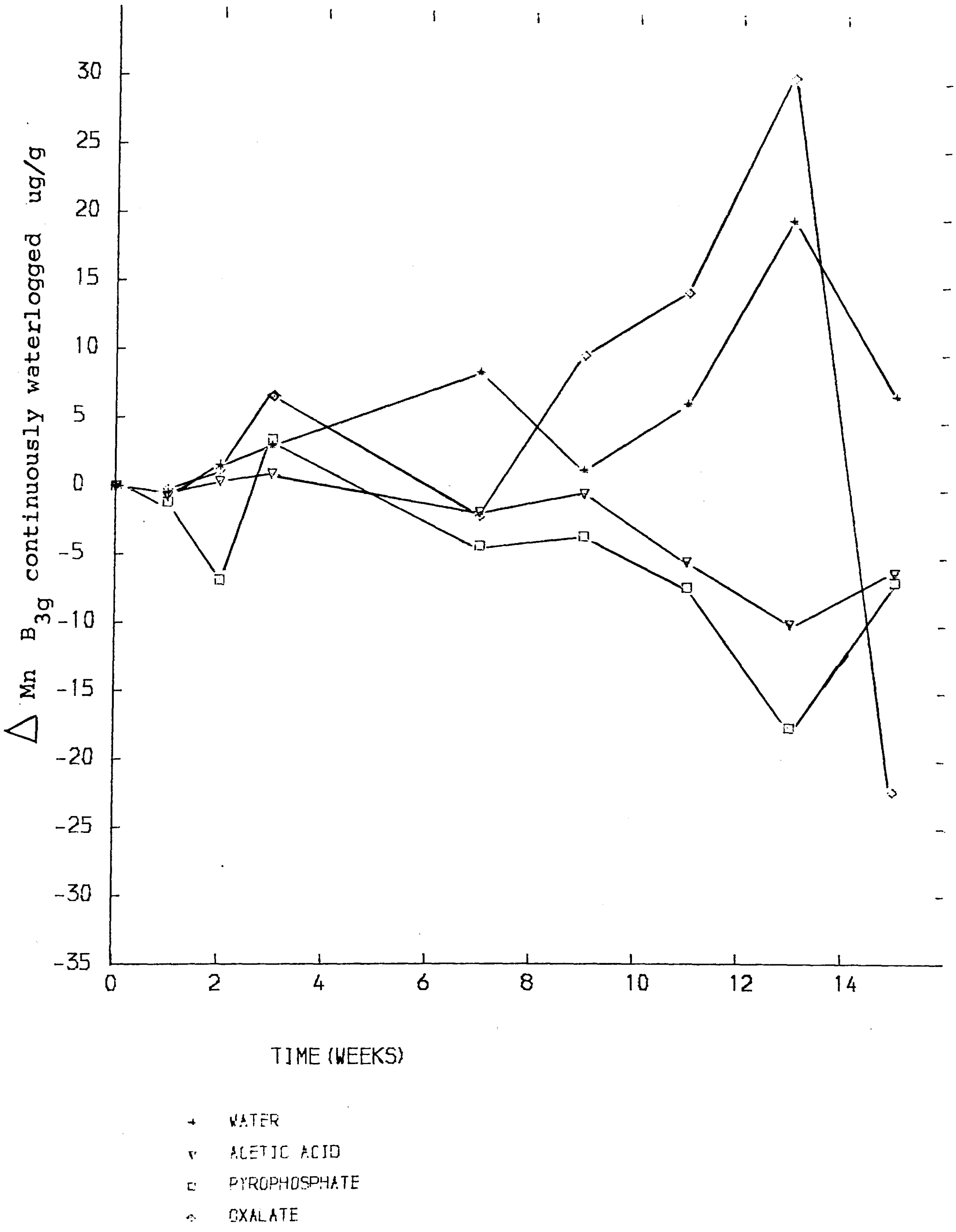
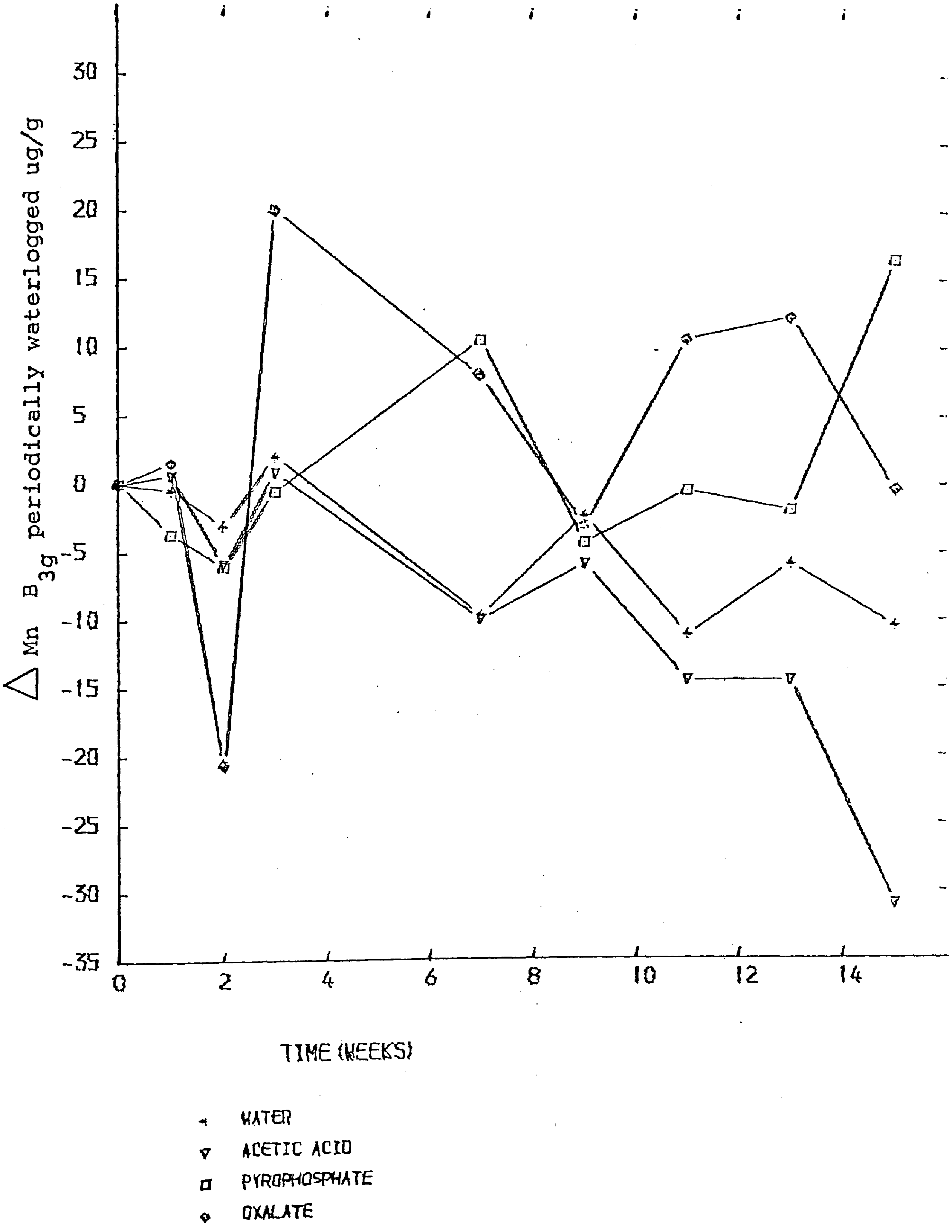


Figure 3.2.14 Variation in the levels of manganese removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} periodically waterlogged.



-31ppm. Overall changes of Δoxa-Mn increased, but showed erratic changes.

3.2.3.2.3 Redistribution of iron

Water extractable iron was very low or undetectable in B_{3g} soil samples. Acetic acid extractable iron in waterlogged and control B_{3g} soil was virtually the same, Δpp-Fe showed an initial increase followed by a decrease to -1100ppm (Fig 3.2.15). Δoxa-Fe again showed erratic changes, and seemed to have no effect on ΔHAc-Fe.

Fig 3.2.16 illustrates that alternating waterlogging had similar effects as waterlogging on iron in the B_{3g} soil.

3.2.3.2.4 Redistribution of zinc

More extractable zinc was found in waterlogged than control B_{3g} soil (Fig 3.2.17). ΔHAc-Zn, Δpp-Zn and Δoxa-Zn had positive values in most of the samples. The effects of alternating waterlogging on zinc redistribution in B_{3g} soil resembled the trends for continuous waterlogging, but in different concentration ranges.

3.2.3.2.5 Redistribution of copper

Waterlogging increased the extractable copper in B_{3g} soil during the first 7 weeks of incubation (Fig 3.2.19). After that period, HAc-Cu maintained higher values in waterlogged soil than control, but Δpp-Cu and Δoxa-Cu were decreased sharply.

Figure 3.2.15 Variation in the levels of iron removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} continuously waterlogged.

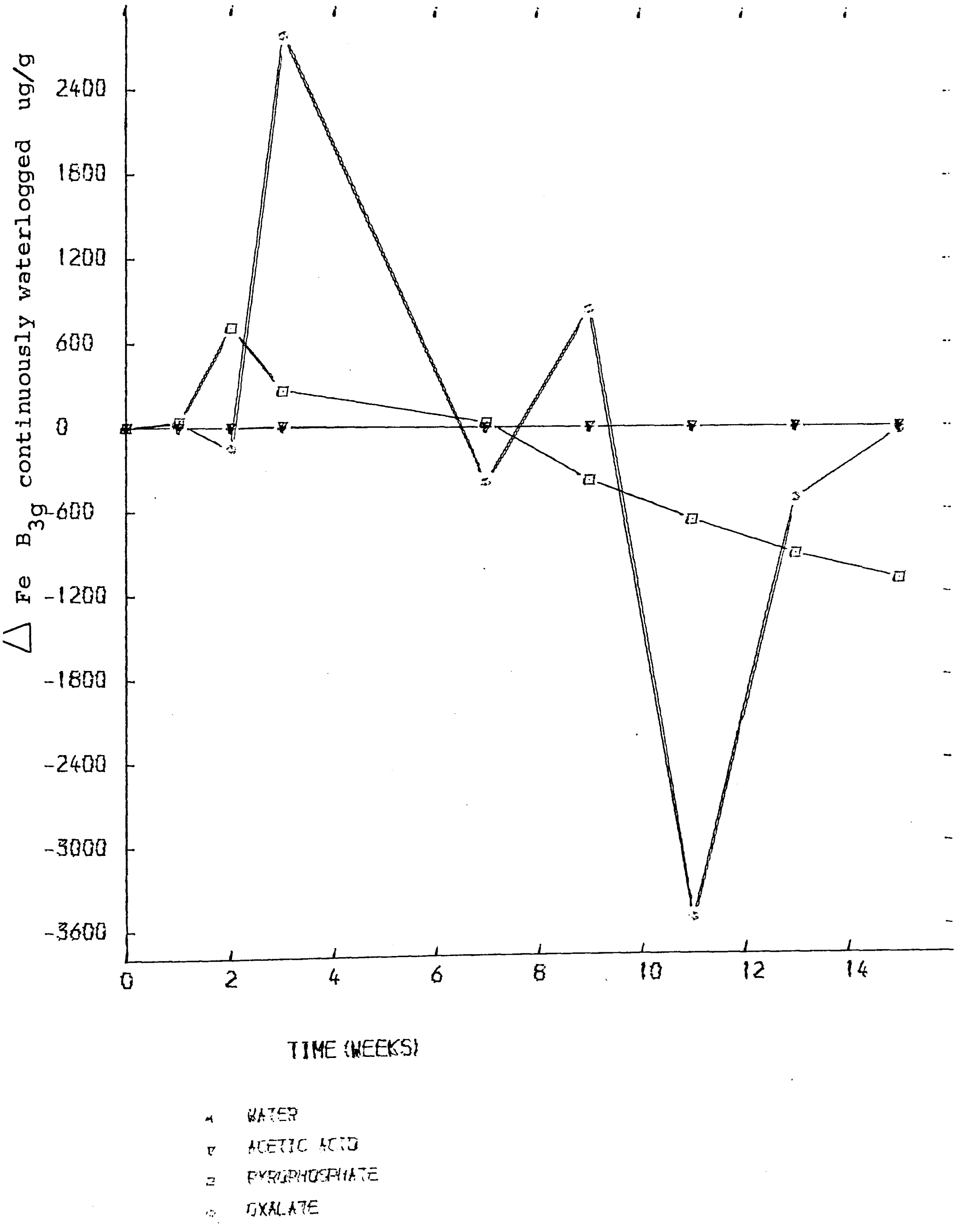


Figure 3.2.16 Variation in the levels of iron removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} periodically waterlogged.

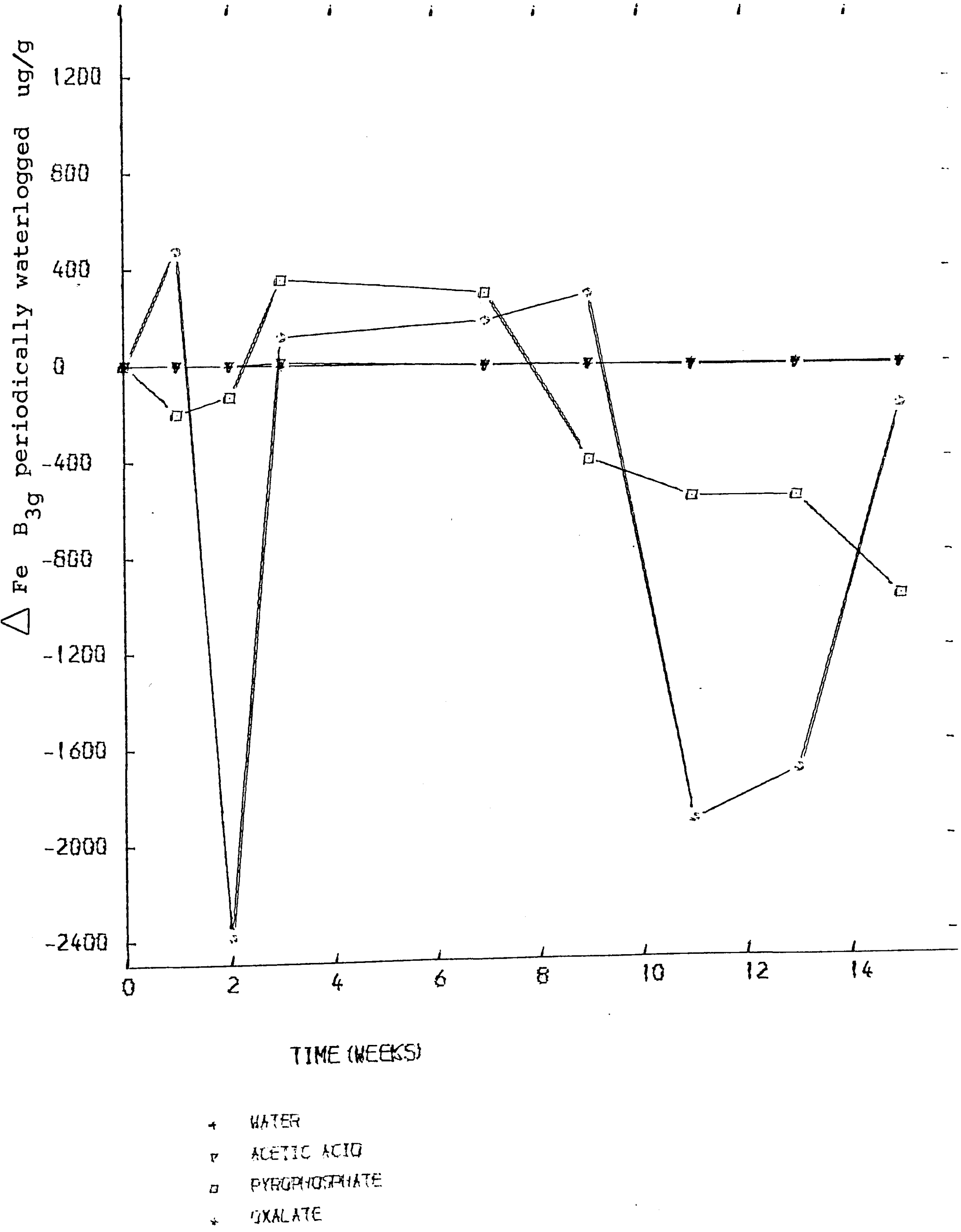
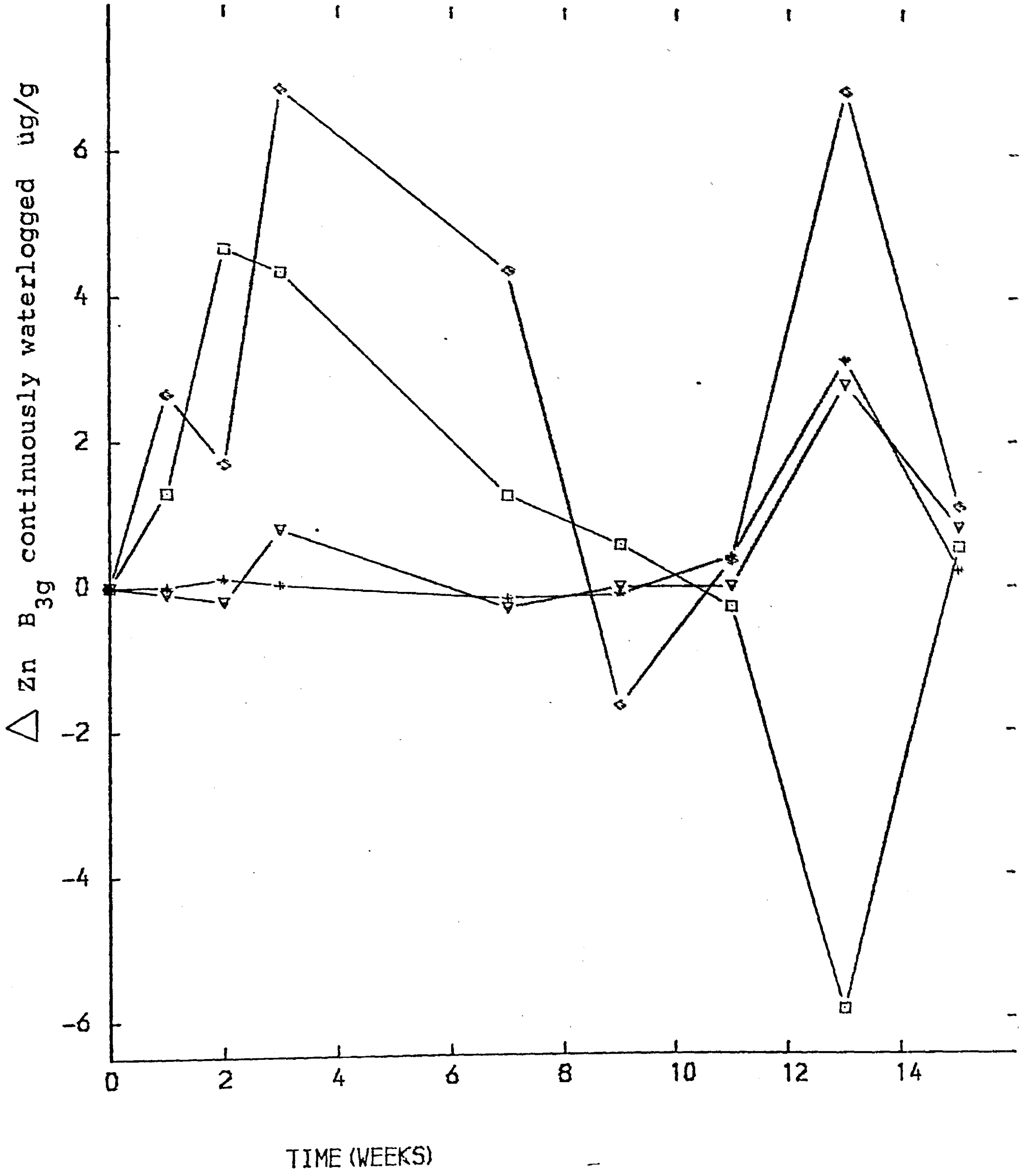


Figure 3.2.17 Variation in the levels of zinc removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} continuously waterlogged



+ WATER
▽ ACETIC ACID
□ PYROPHOSPHATE
◇ OXALATE

Figure 3.2.18 Variation in the levels of zinc removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} periodically waterlogged.

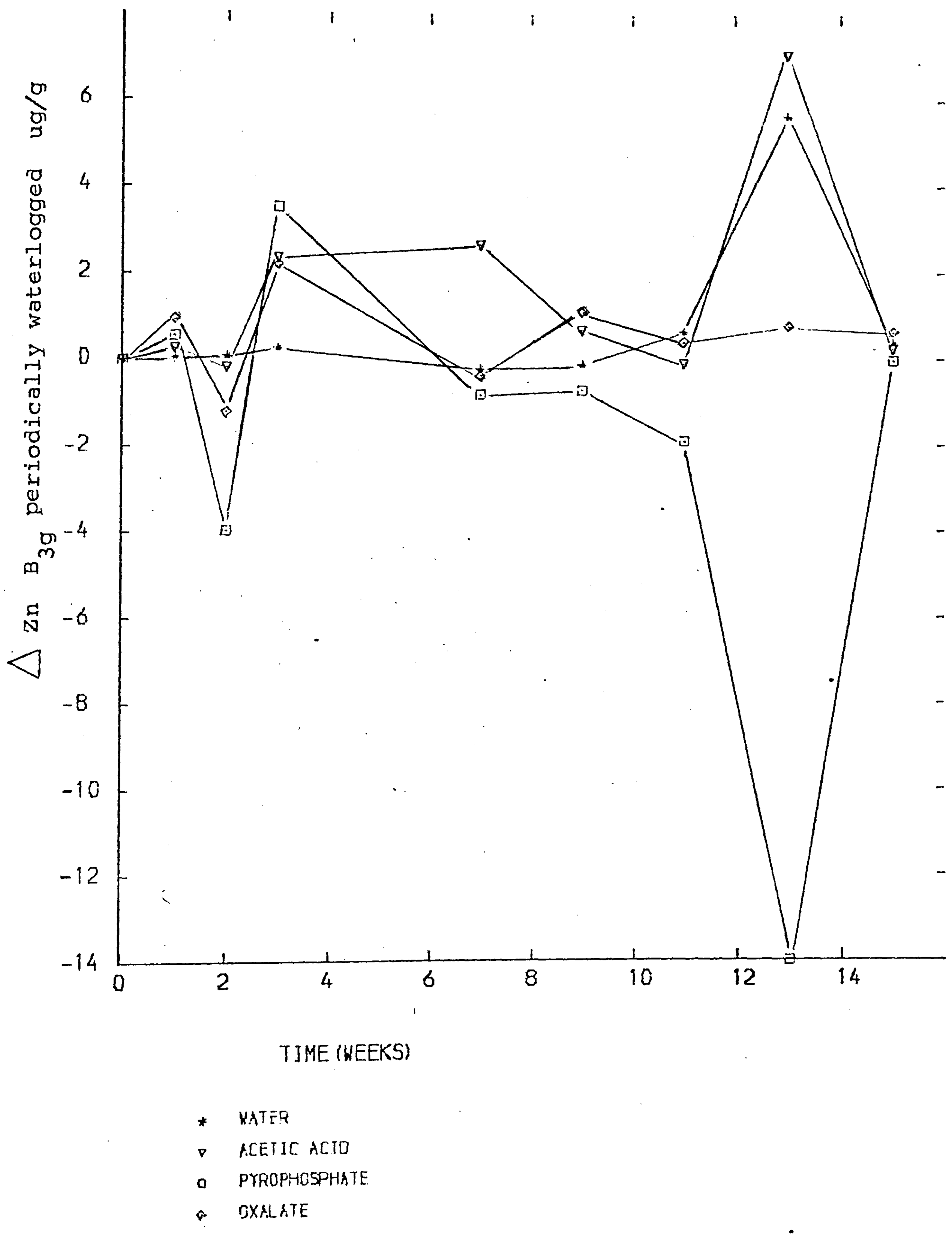


Figure 3.2.19 Variation in the levels of copper removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} continuously waterlogged.

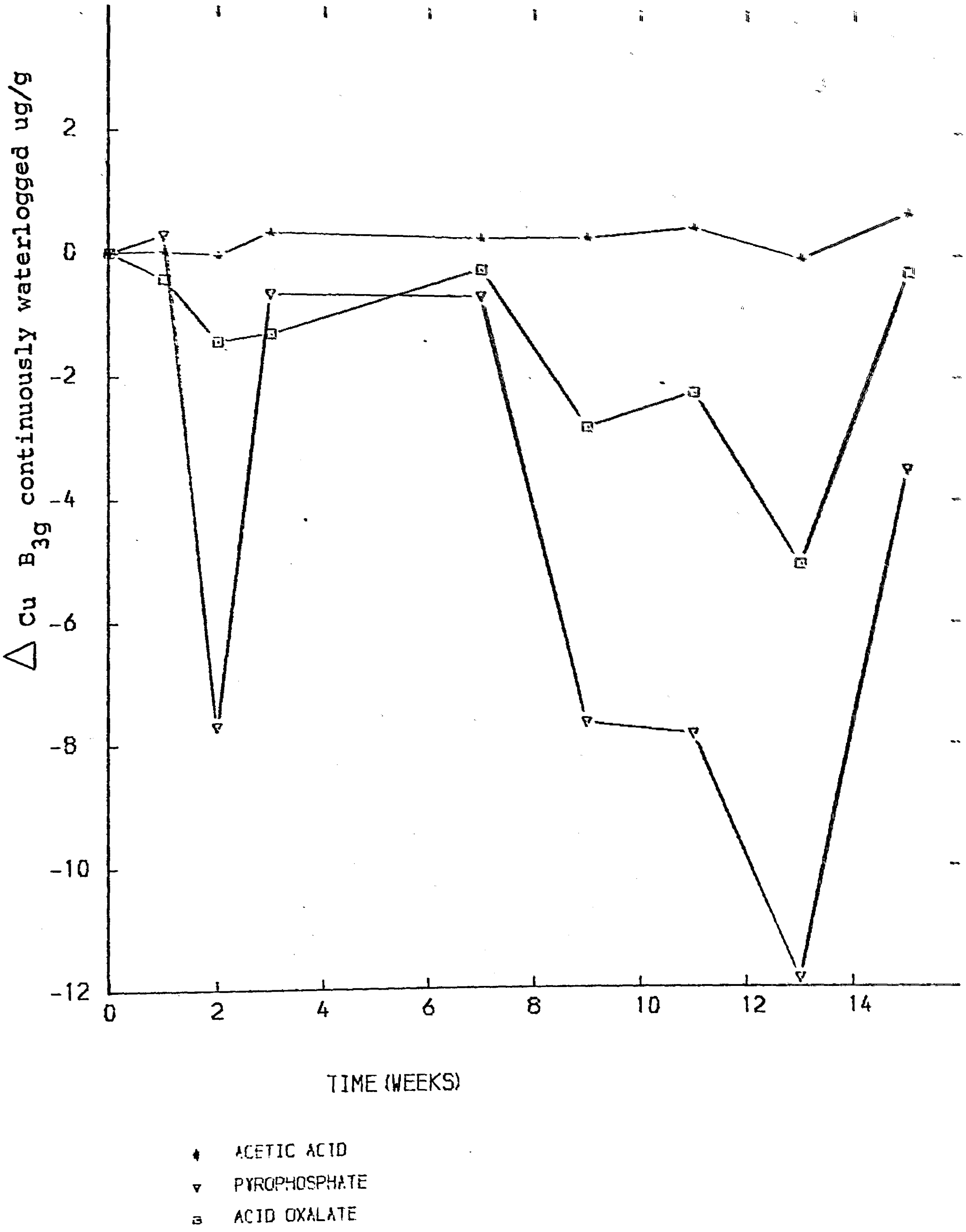
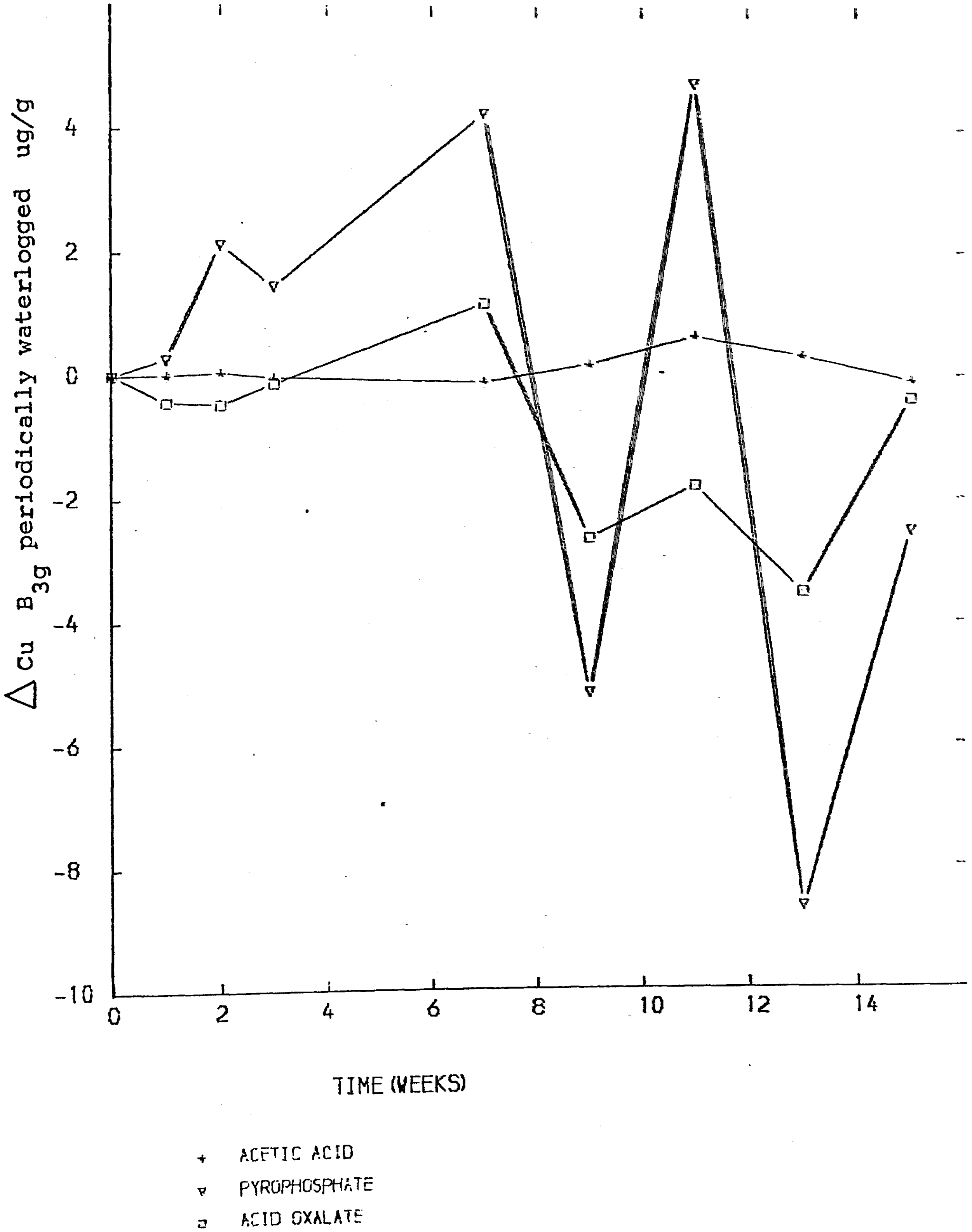


Figure 3.2.20 Variation in the levels of copper removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} periodically waterlogged.



The initial increases of Δpp-Cu and Δoxa-Cu in continuous waterlogging were not found in alternating waterlogging. Moreover, Δpp-Cu and Δoxa-Cu values were lower under alternating waterlogging (Fig 3.2.20).

3.2.3.2.6 Redistribution of cobalt

The changes in extractable cobalt between waterlogged and alternating waterlogged B_{3g} soils are shown in Fig 3.2.21 and Fig 3.2.22. ΔHAc-Co increased after 1-2 weeks incubation, but showed no significant differences at the end of the experiment whether in continuous or alternating waterlogged condition. Δoxa-Co in the continuous and alternating waterlogged soils increased but fluctuated. Pyrophosphate extractable cobalt again was lower than in acetic acid extracts and showed no significant changes.

The above results show that increases of certain fractions of metals in soils by waterlogging are caused by corresponding decreases of other fractions. The redistribution of metals is also dependent on the properties of metals. Increases of ΔH_2O-Mn and ΔHAc-Mn in waterlogged soils coincided with decreases of Δpp-Mn and Δoxa-Mn, and indicate release of manganese from organic-bound sites and oxide materials to more mobilized forms. Decreases of Δoxa-Mn and Δpp-Mn would be caused by reducing conditions in waterlogged soil and competition of other metals for organic-bound sites. More detailed discussions are presented in the next experiment. However, increases of ΔHAc-Zn and Δoxa-Zn, with

Figure 3.2.21 Variation in the levels of cobalt removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} continuously waterlogged.

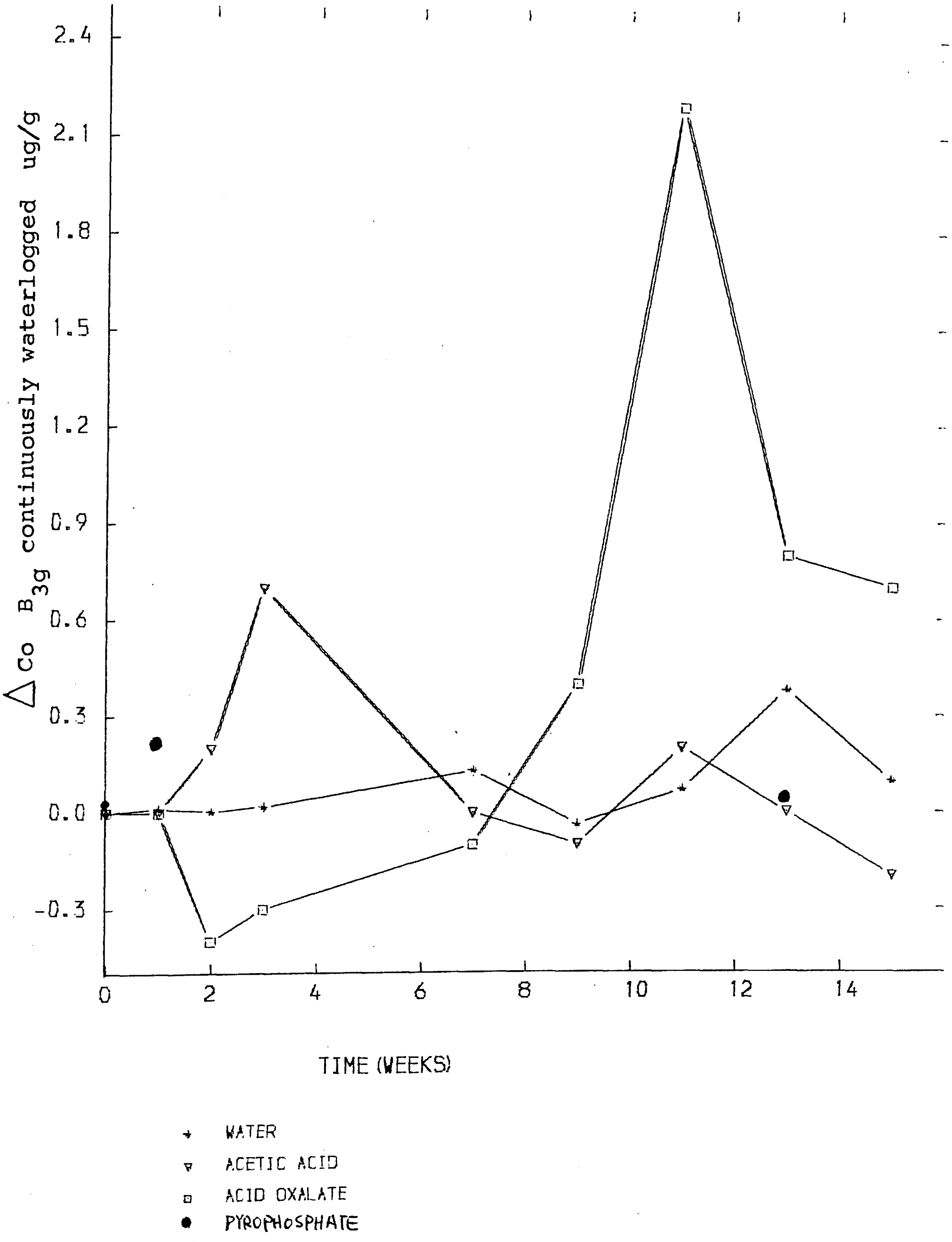
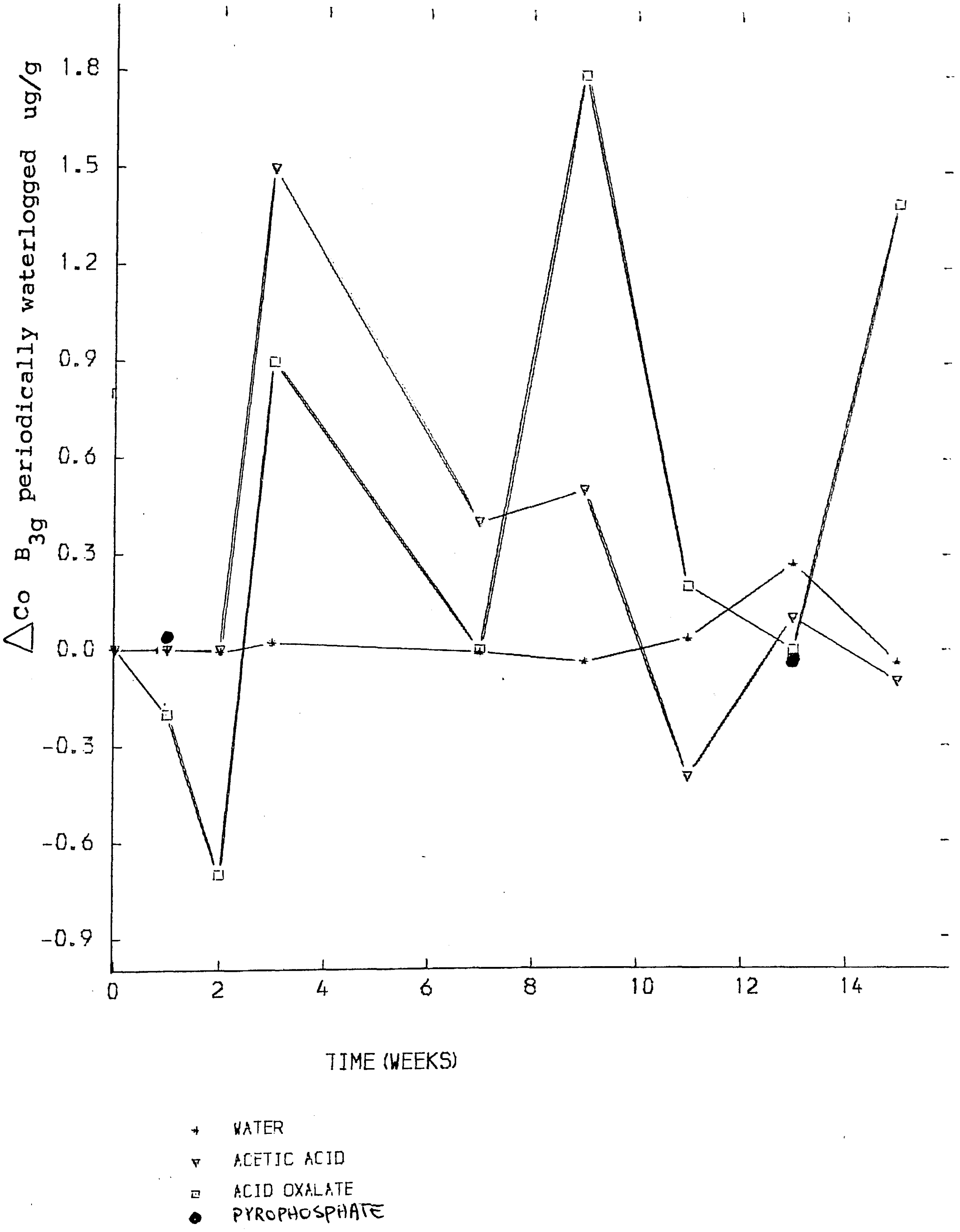


Figure 3.2.22 Variation in the levels of cobalt removed by the selective extractants over 15 weeks, expressed as the difference in content between the waterlogged and aerated treatments. B_{3g} periodically waterlogged.



corresponding decreases of ΔH_2O-Zn and Δpp-Zn, in the waterlogged B_2 soil show different behaviour to that of manganese. If the water-extractable metals represent the available metals, then in the waterlogged B_2 soil it means more available manganese but less available zinc for plants.

Of the other metals in the waterlogged B_2 soil, iron and cobalt tend to have similar behaviour to manganese, water and acetic acid extractable fractions increased, pyrophosphate and oxalate fractions decreased. Copper, however, behaved like zinc. Unfortunately, not all the metals mentioned showed the changes as clearly as manganese and zinc. These less clear-cut results may have been caused by expremental errors.

Samples were extracted at different temperatures and had a varied storage time before analysis, this would introduce some errors. Furthermore, the suction of excess water from waterlogged samples and only duplicates being analysed might not help to get a clear view of the metal changes. Attempts were made to eliminate such errors on the next experiment.

Redistribution of metals in B_2 and B_{3g} soils occurred in different ways under waterlogging, proving that the history of drainage conditions will influence the metal fractions in soil. For example, the decrease of zinc in water and pyrophosphate extracts of B_2 soil was not found in B_{3g} soil, but there was a total gain of extractable zinc.

The pH value of B_{3g} soil did not change on waterlogging, while pH of B_2 soil increased, further evidence for the drainage effect on these two soils. Metals in fractions which are easy to change to mobilized forms and dissolved by waterlogging would be expected to be exhausted by underground spring water. The smaller changes of manganese and iron in B_{3g} than in B_2 soil support this view.

Generally, the redistribution of metals in alternating waterlogged soil follows similar trends to the continuous waterlogged one. Ng and Bloomfield (1962) reported that short periods of re-aeration of soils did not greatly affect the extractabilities of metals. The changes in alternating waterlogged soils may be considered the same as in continuous waterlogging.

Using points from the present preliminary trial, a modified experiment was set up to investigate further details and to confirm the findings of this section. The changes in B_{3g} soil by waterlogging and alternating waterlogged samples are less remarkable, so the next trial (Section 3.3)focusses on the waterlogged B_2 soil and other additional conditions.

3.3 The influence of waterlogging, lime and organic matter on trace metals held in Carbeth B_2 horizon soil

3.3.1 Introduction

Previous experimental results showed that the mobilization of metals in soils by waterlogging is related to the various metal-holding fractions. Manganese in the B_2 soil, especially, showed qualitative and quantitative changes, indicating that the sequential extraction method could provide a good way of studying the changes in other metals in waterlogged soil, if the experimental errors can be reduced.

In the following experiment, analysis of four replicates was used to reduce the analytical errors; and re-oxidization of soil samples was reduced by sealing the Buchner funnel with a double-layer of paraffin wax film.

A common crop management technique to improve the soil condition for plant growth is the addition of lime or organic matter. These treatments may influence the rates and degrees of metal mobilization in the waterlogged condition (Graven, Attoe and Smith 1965; Prasad, Prasad and Singh 1976; Haldar and Mandal 1979). To compare and further investigate the Carbeth B_2 soil with various amendments under waterlogging, 1% organic matter or lime was added to study the effect of soil pH or organic matter addition, on the transformation of metals in waterlogged B_2 soil.

3.3.2 Materials and methods

3.3.2.1 Procedure

Freshly collected soil was passed through a 2 mm sieve and five duplicate samples of 800 g soil, on an oven-dry basis, were weighed out.

In all cases, the B_2 horizon soil was used, and the treatments were:

(i) soil at 60% field capacity (aerobic control)
(ii) waterlogged soil
(iii) waterlogged soil +1% organic matter
(iv) soil at 60% field capacity +1% lime
(v) waterlogged soil +1% lime

All soils were initially held at 60% field capacity by carefully spraying on the required volume of water. The amended soils were prepared by adding 8 g calcium carbonate or dried grass, which was thoroughly mixed into the soil. The soils were then transferred to 2 l beakers and allowed to equilibrate for 3 days. The waterlogged treatments were then prepared by slowly adding water until there was 5 cm of standing water above the soil surface. This was considered as day 0 and the soils held in a constant temperature room at $26^\circ C \pm 2^\circ$ for 16 weeks. Moisture levels were corrected as required.

Sub-samples were removed periodically from the beakers using a glass tube. They were transferred to a Buchner funnel, which was sealed with a paraffin wax film, and excess water removed by suction. The soil in the funnel was further sampled for moisture content determination and sequential extraction.

The extraction procedure used was similar to that described in Section 3.2.2.3, except that the H_2O extractant was substituted by 0.05M $CaCl_2$.

Soil Eh and pH values were both monitored regularly. Eh determination was by platinum electrode and calomel reference electrode with a volt-meter, reading at 2 inches under soil surface.

3.3.2.2 Analysis

See Section 3.2.2.3.

3.3.3 Results and discussion

Visual observation indicated that soils with additional lime or organic matter were changing much quicker than untreated soil in waterlogged incubation. Standing water on waterlogged soil with lime or organic matter addition become very cloudy and had an odour after only 4 weeks waterlogging. Soil without amendment did not show these changes until 10 weeks waterlogging. Limed soil tended to form black coloured precipitates and have grey-yellow coloured standing water. Other two waterlogged samples had brown coloured precipitates and standing water.

Sequential extraction results are discussed in the following sections. The extracted metal values are expressed as the difference in value between the waterlogged treatment and the aerobic one, as described in Section 3.2.3. Original data for analysis are shown in appendix 2.

3.3.3.1 pH and Eh

Eh values are plotted as direct measurements and have not been adjusted to pH 7.

Fig 3.3.1-3.3.3 show the pH and Eh changes in the waterlogged soils over a period of 16 weeks. Eh in all waterlogged samples were found to decrease on incubation. The decreasing rate of Eh was accentuated by application of organic matter or lime. This may be attributed to higher activities of micro-organisms, encouraged by the readily decomposable organic matter and/or near-neutral pH.

The pH of the untreated waterlogged soil and the organic matter treated waterlogged soil rose, whereas the pH of the limed waterlogged soil fell, with time, following the expected pattern (Ponnamperuma 1972). In all cases the pH stabilized in the range 5.6 to 6.4.

3.3.3.2 Redistribution of manganese

Fig 3.3.4-3.3.6 show the changes in the various

Figure 3.3.1 Variation in pH and Eh over 16 weeks waterlogging. O—O Eh, X—X pH Waterlogged, no amendment. B_2 horizon

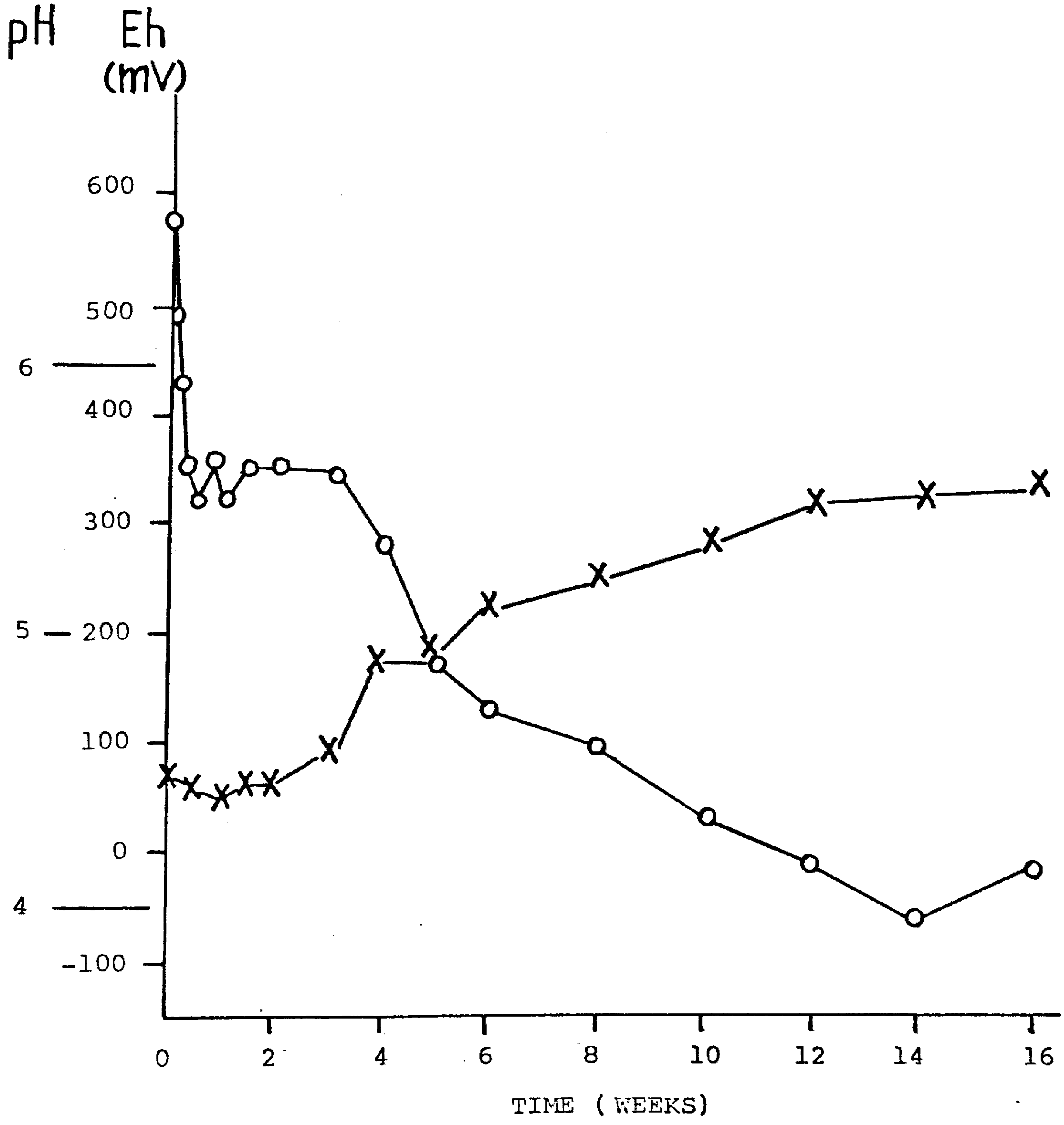


Figure 3.3.2 Variation in pH and Eh over 16 weeks waterlogging. O—O Eh, X—X pH Waterlogged + 1% dried grass. B_1 horizon

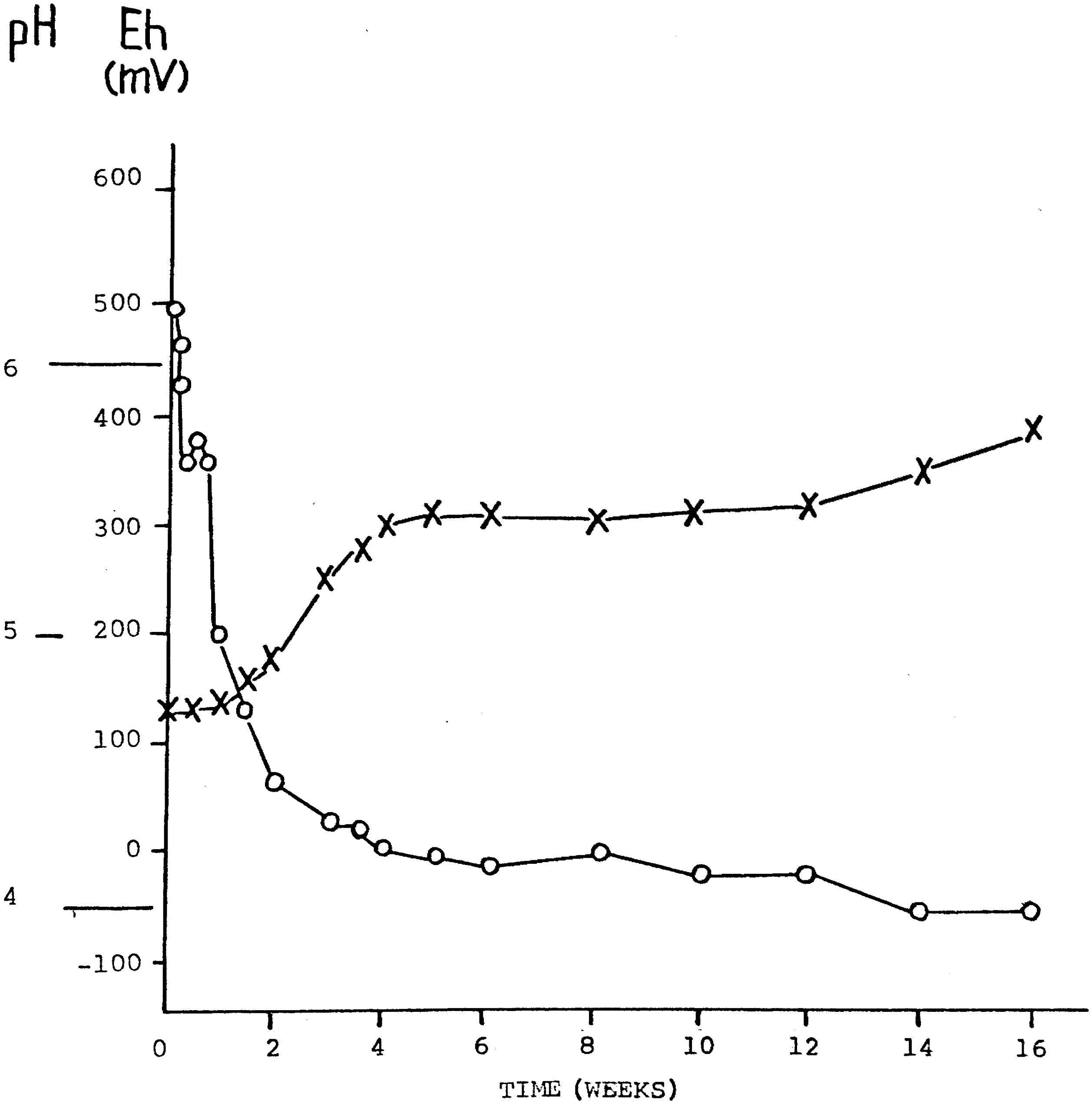
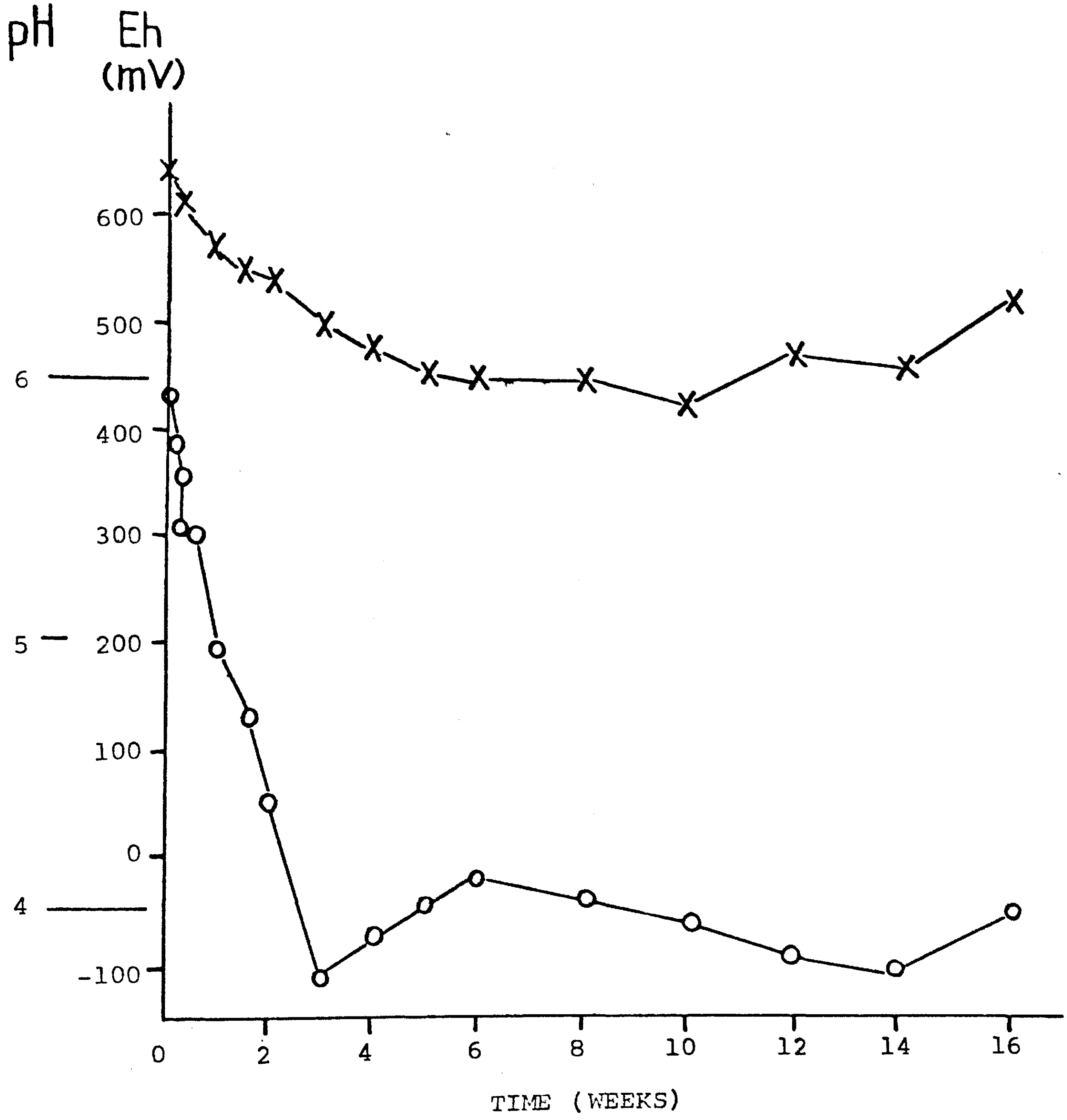


Figure 3.3.3 Variation in pH and Eh over 16 weeks waterlogging. O—O Eh, X—X pH
Waterlogged + 1% lime . B_2 horizon



fractions of manganese between the waterlogged and control soils over 16 weeks. These changes are shown in relation to the Eh changes in Fig 3.3.7-3.3.9.

In the first three weeks of incubation, there were no differences between waterlogged and controlled soil extracts, except a small increase in the $CaCl_2$-extract (Fig 3.3.4). Meanwhile, the Eh value of waterlogged soil was maintained at about +350mv (Fig 3.3.1). If this value is corrected by -59mv per pH unit to pH 7, then its value is about +220mv. After appropriate correction to pH 7, nitrate reduction was reported to occur around +220mv (Bohn 1971) and would tend to poise redox potential around this level until nitrate reduction is nearly complete (Bailey and Beauchamp 1971). Judging by the data in Fig 3.3.1, the good poise of the soil in the first three weeks under waterlogged incubation might be attributed to nitrate reduction, which would prevent the release of Mn^{2+} (Ponnamperuma and Casto 1964).

After three weeks incubation, Eh dropped sharply, $\Delta CaCl_2$-Mn started to increase significantly, and correspondingly, Δpp-Mn and Δoxa-Mn decreased. This indicates release of the mobile Mn^{2+} from organic bound and oxide bound fractions. The report of Gotoh and Patrick (1972) agreed with part of the present result. They observed an increase in mobile forms of manganese at lower Eh levels, which was accompanied by a decrease in the reducible Mn levels, indicating that

Figure 3.3.4 Variation in the levels of manganese removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatment. Waterlogged, no amendment. B_2 horizon

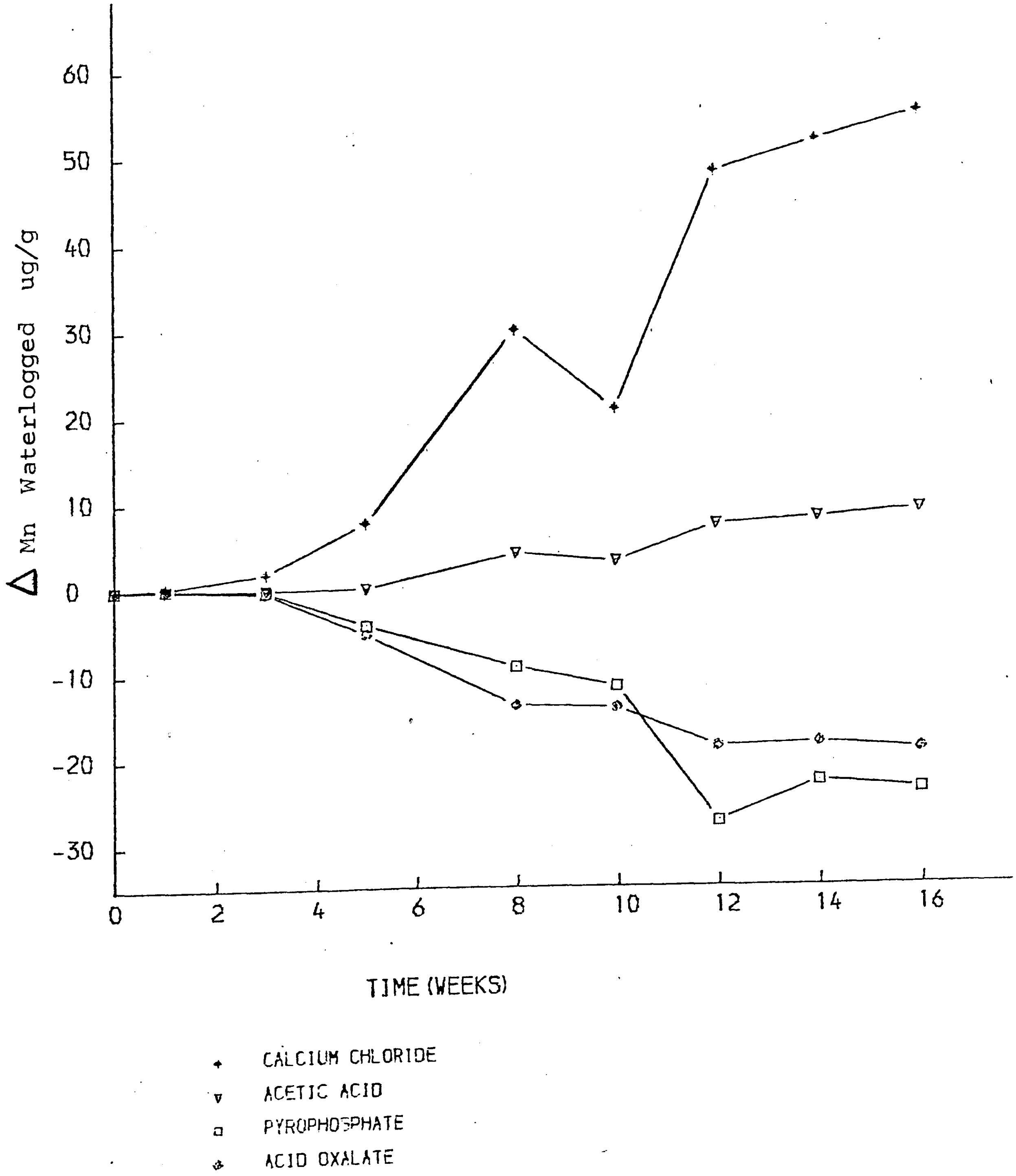


Figure 3.3 5 Variation in the levels of manganese removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% dried grass. B2 horizon

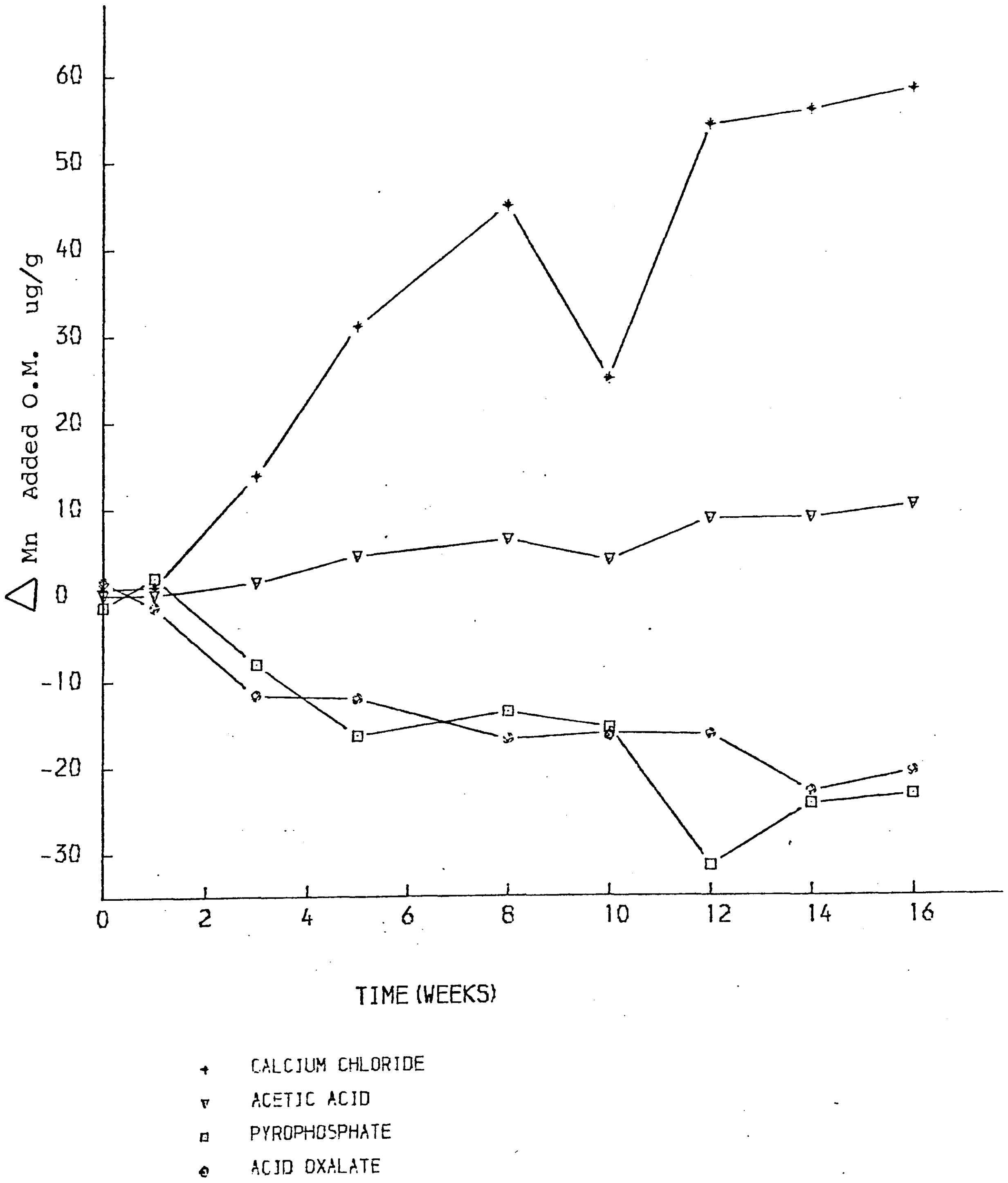


Figure 3.3.6 Variation in the levels of manganese removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% lime . B_2 horizon

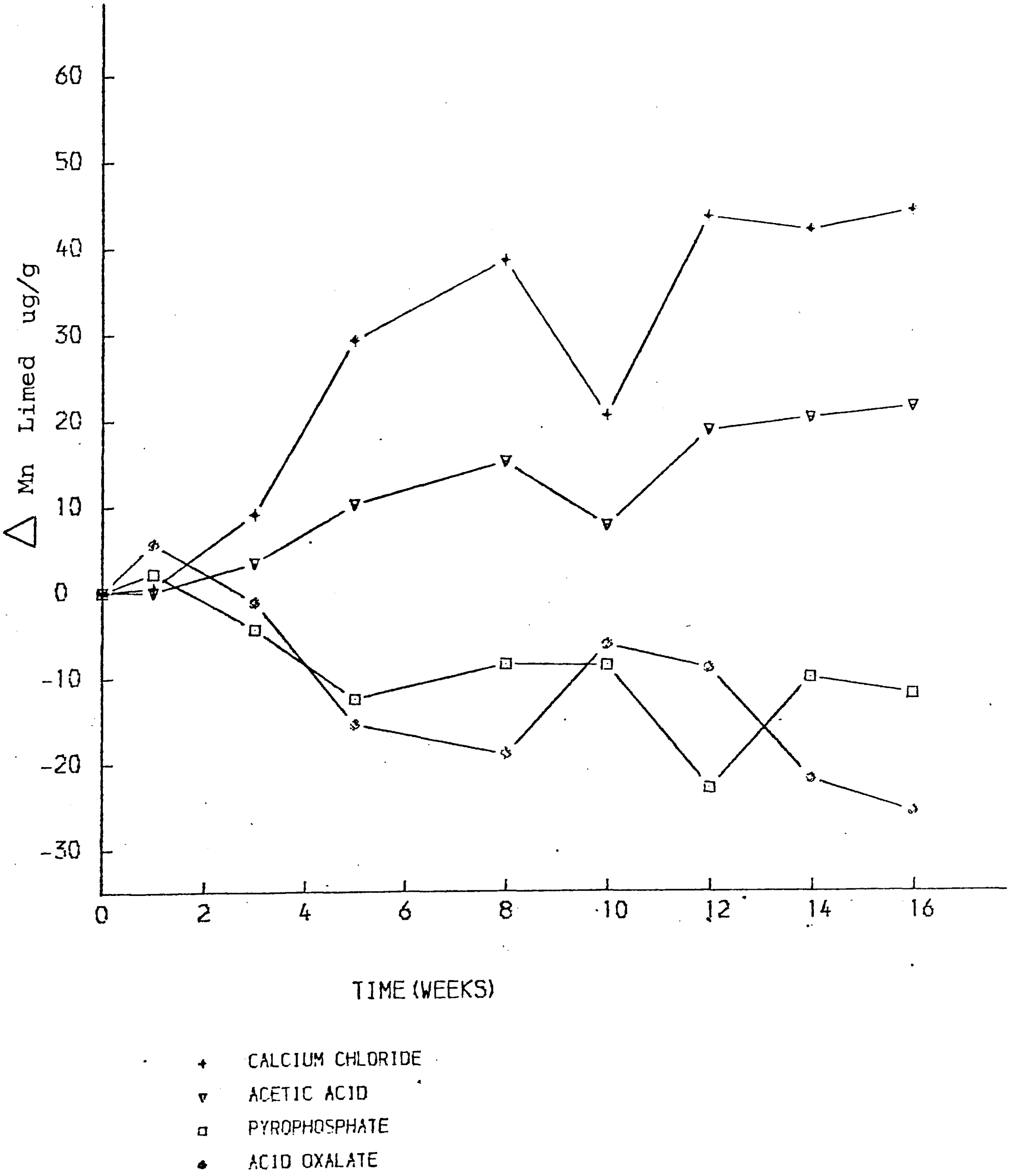


Figure 3.3.7 Changes in manganese removed by the selective extractants with decrease in Eh. Waterlogged, no amendment. B_2 horizon

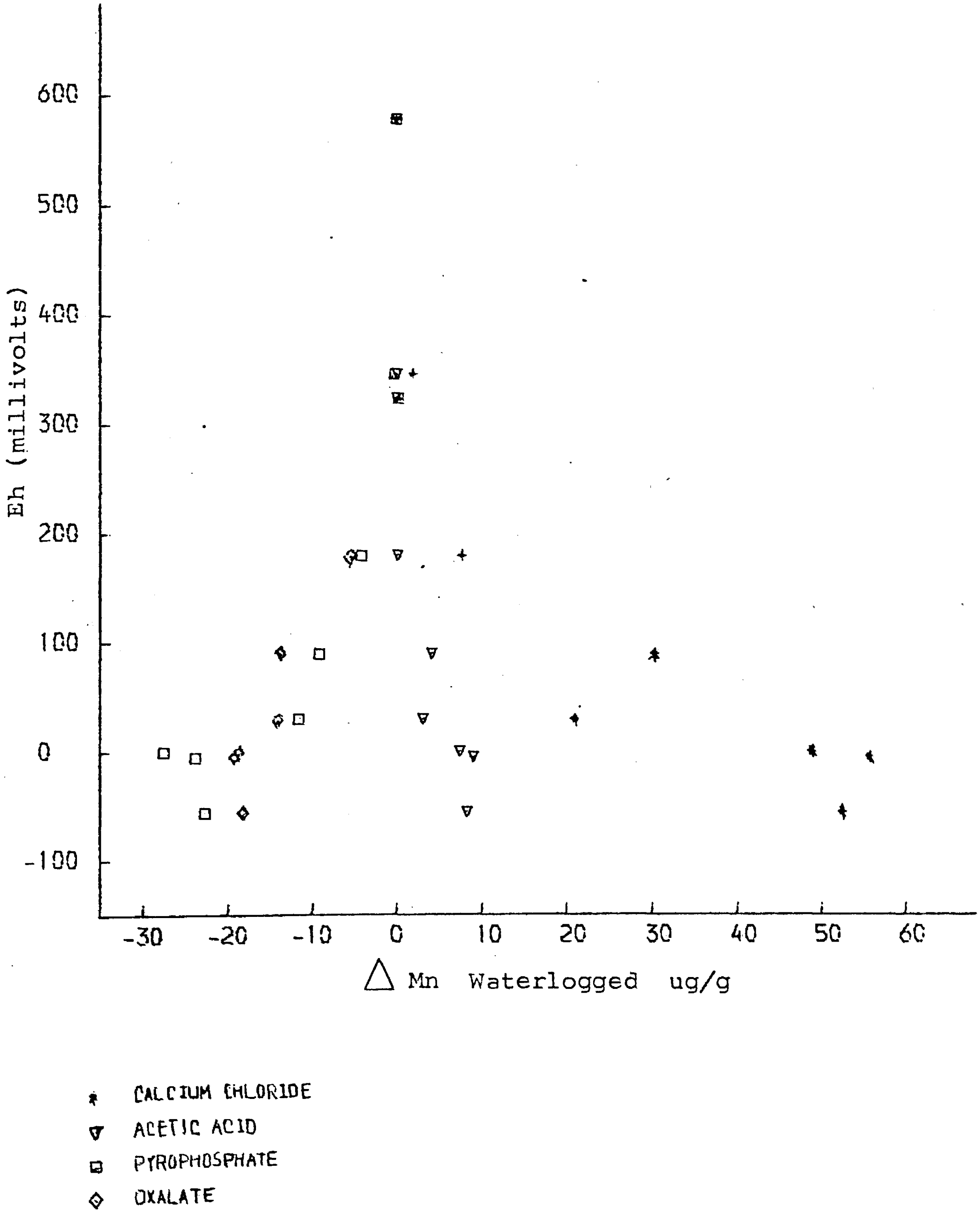


Figure 3.3.8 Changes in manganese removed by the selective extractants with decrease in Eh. Waterlogged + 1% dried grass. B_2 horizon

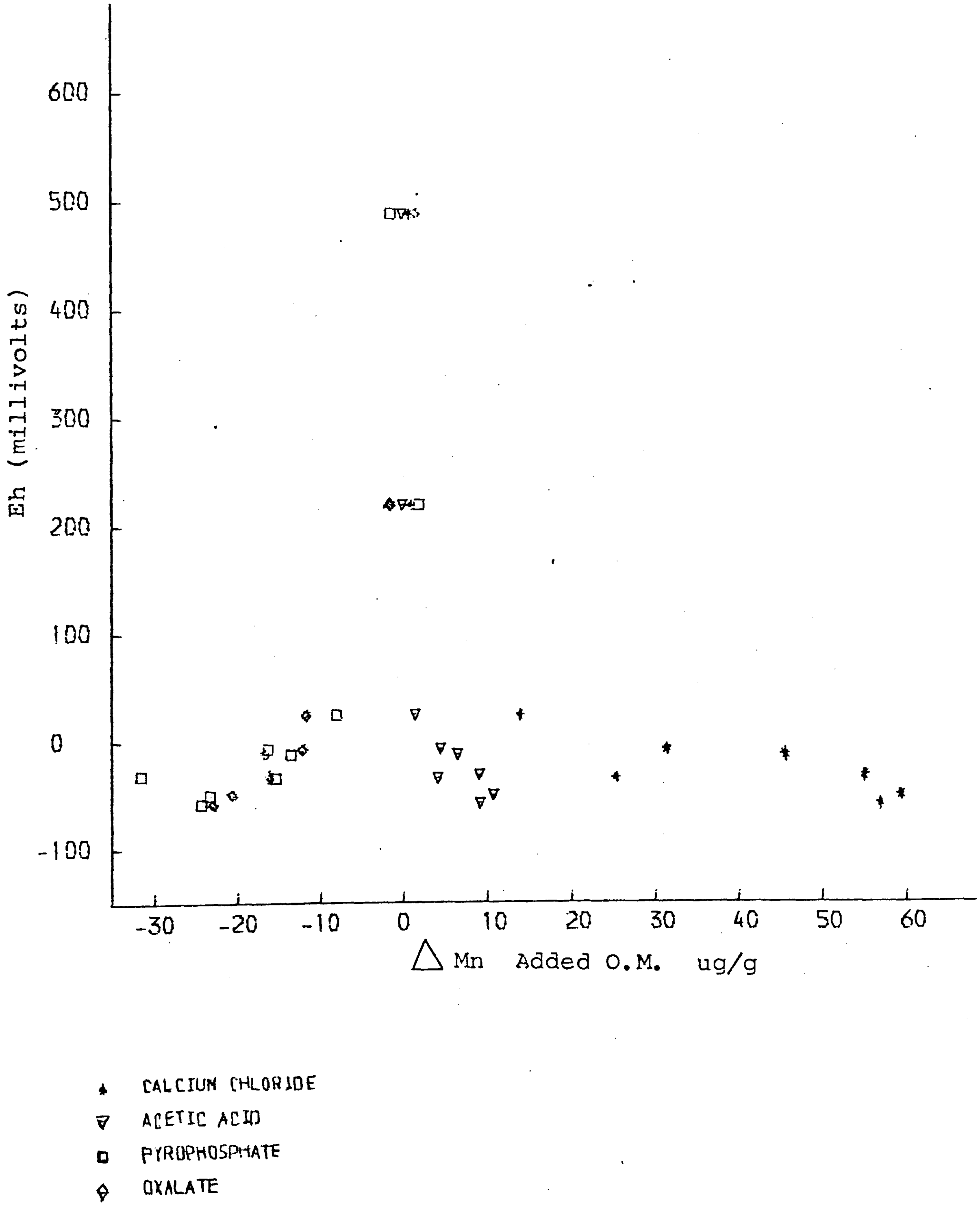
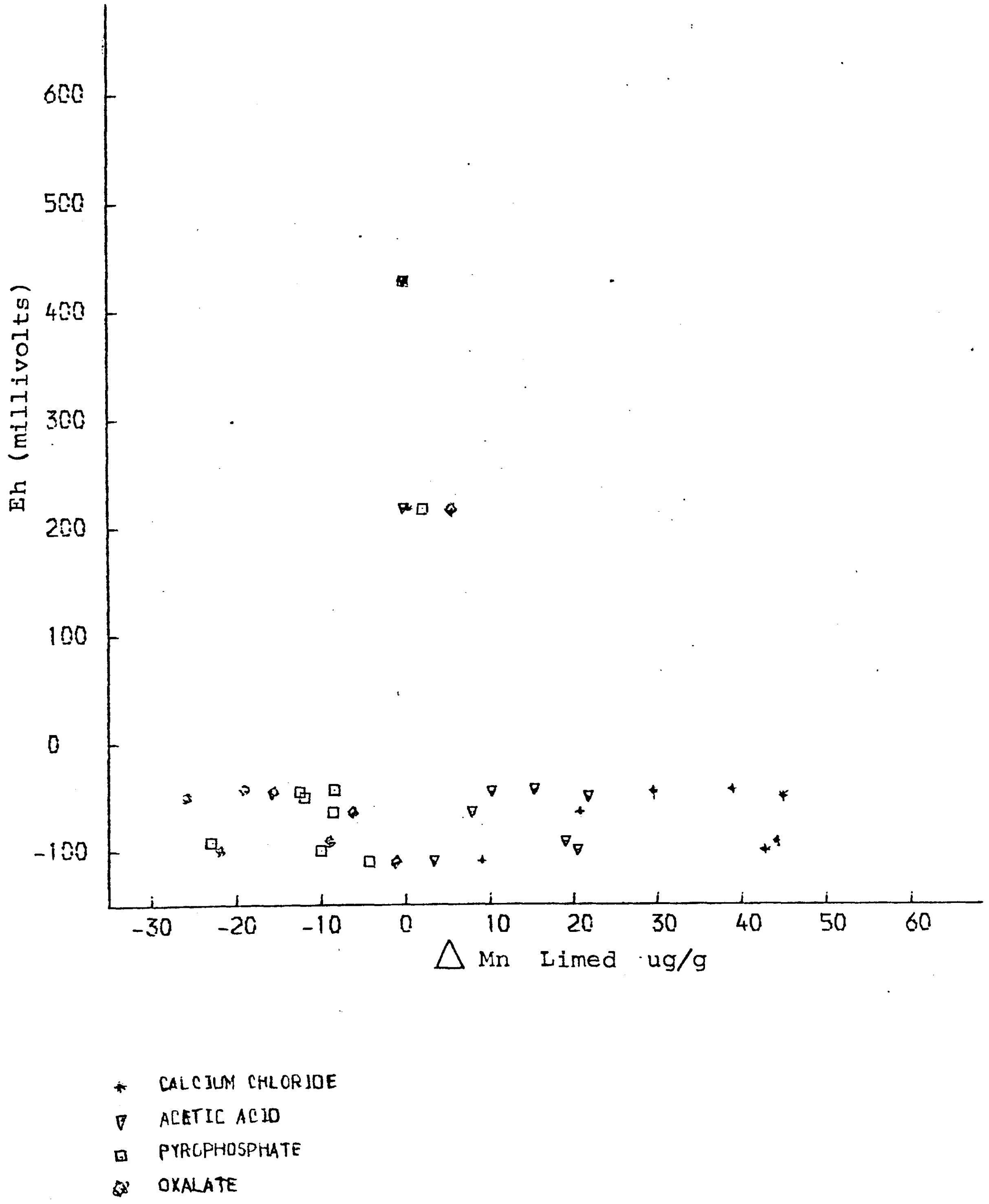


Figure 3.3.9 Changes in manganese removed by the selective extractants with decrease in Eh.
Waterlogged + 1% lime B_2 horizon



some of the insoluble oxides and hydroxides became unstable and dissolved under reduced conditions. However, Mandal (1961) found the organic fraction of manganese was not involved with the increase of mobile manganese in waterlogged soil. Results found in this experiment have shown that organic bound manganese also decreased due to waterlogging and thus a large amount of mobile manganese came from organic fractions by the end of the incubation time. It showed even more mobile manganese was released from organic forms than from oxide forms. The nature and amount of the organic material incorporated in the soil seems to be of great importance, and high organic matter content in this soil might be the reason for having this result.

Organic matter has long been found in association with soil manganese (Beckwith 1955), but it is not known what the most favourable condition is for the formation of this organic manganese. Nevertheless, manganese was found either not complexed or only weakly complexed with the soil organic fraction under reducing conditions (Olomu, Racz and Cho 1973, Gambrell, Khalid and Verlos 1977, Sims and Patrick 1978). The soil used in this experiment had a high organic content (13%) and high proportion of organic bound manganese. In the aerobic soil pyrophosphate extracts contained 28% of a single extraction of oxalate extractable manganese; a single extraction of acetic acid or, $CaCl_2$ extractable plus sequential acetic acid extractable manganese, contained only 1.9%, by comparison (Table 3.3.1).

Table 3.3.1 Levels of manganese removed by the various extractants

	Single extractions			Sequential extractions	
Extractant	oxa	pp	HAc	$CaCl_2$	HAc
Extractable Mn (ppm)	123.8	34.7	2.33	1.74	0.56
Mn extracted as % of oxalate extraction	100.0	28.0	1.90	1.40	0.40

In the past, most of the soils used for waterlogging experiments were low in organic content (Mandal 1961, Gotoh and Patrick 1972), and the great differences of binding ability of organic matter to manganese between aerobic and anaerobic conditions tended to be ignored. The results shown here indicate the organic bound manganese played an important role and may be of great importance to mobilization of manganese under anaerobic conditions.

When 1% dried grass was added to the soil, as a source of actively decomposing organic matter, reduction of the waterlogged soil was faster (Figure 3.3.2). Whereas Eh was stabilized at +350 mv for 3 weeks with no organic addition, this period was reduced to 3-4 days by addition of the dried grass. This accelerated change in Eh is reflected by the faster increase in $\Delta CaCl_2$-Mn and fall in Δpp-Mn and Δox-Mn (Figure 3.3.5). The magnitude of each of these values was greater in the organic-treated soil than in the soil with no grass addition up to 12 weeks incubation. Thereafter the values in both treatments are similar. Apart from the differences in rate of change and magnitude, the changes in the various manganese fractions are similar to the previous treatment and can be accounted for in the same way.

The rate of fall of Eh was also accelerated by liming (Fig 3.3.3), agreeing with previous findings (Olomu, Racz and Cho 1973, Pavanasasivam and Axley 1980), and being similar to the soil treated with dried grass. $\Delta CaCl_2$-Mn and ΔHAc-Mn increased quickly up to 12 weeks incubation, but showed no significant increase thereafter. Conversely Δpp-Mn and Δoxa-Mn decreased with time. Compared with the other two waterlogged treatments, the limed soil had at 16 weeks the lowest $\Delta CaCl_2$-Mn value, highest ΔHAc-Mn value and the least decrease of Δpp-Mn.

3.3.3.3 Redistribution of iron

The changes in the various fractions of iron between waterlogged and control soils are shown in Fig 3.3.10-3.3.12 in relation to time and in Fig 3.3.13-3.3.15 in relation to Eh.

The erratic changes of Δoxa-Fe shown in Section 3.2.3 are not found here. Fig 3.3.10 illustrates overall changes of iron in the untreated soil are similar to those found with manganese; $\Delta CaCl_2$-Fe and ΔHAc-Fe increased, Δpp-Fe

Figure 3.3.10 Variation in the levels of iron removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged, no amendment. B_2 horizon

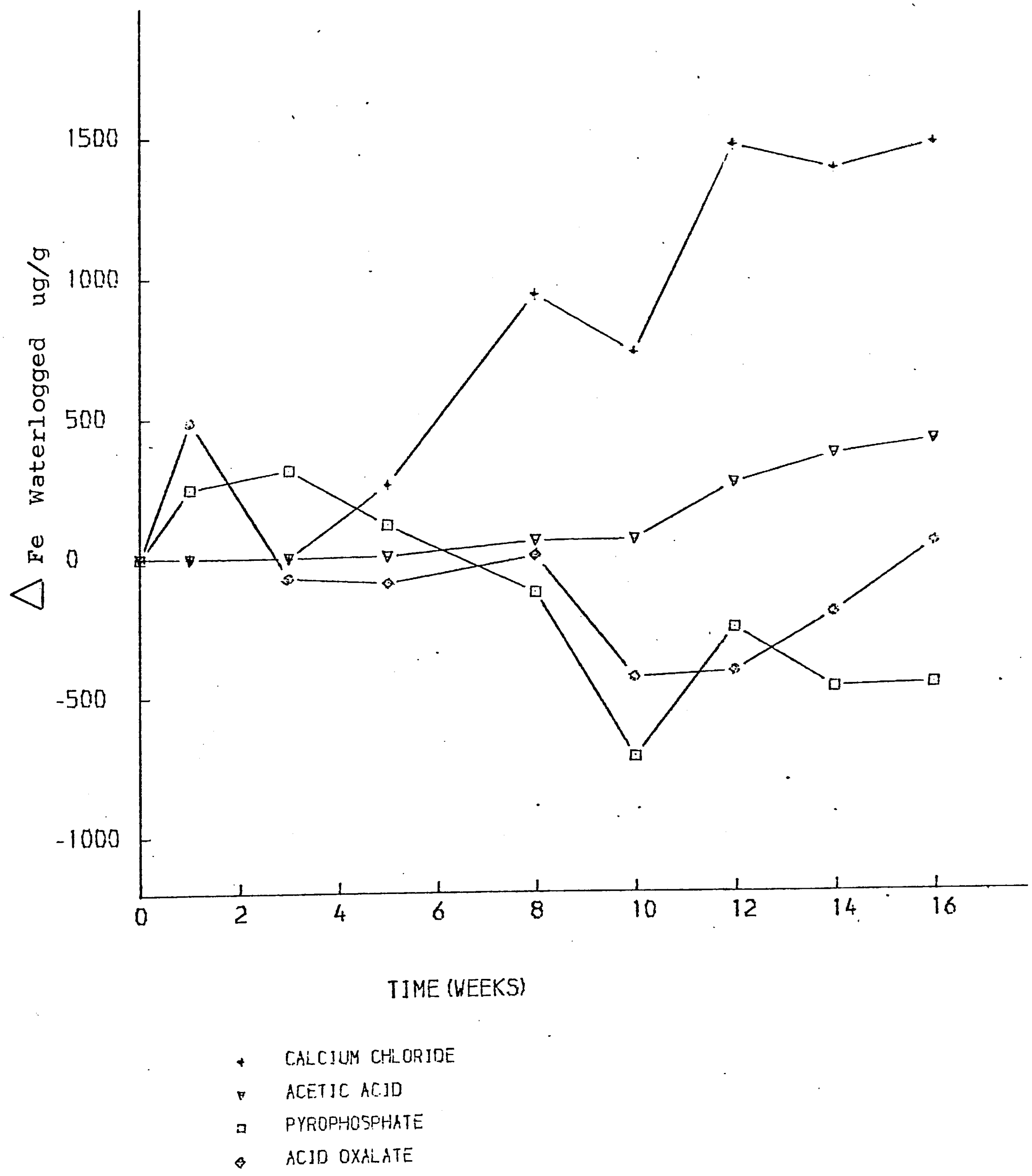


Figure 3.3.11 Variation in the levels of iron removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% dried grass B_2 horizon

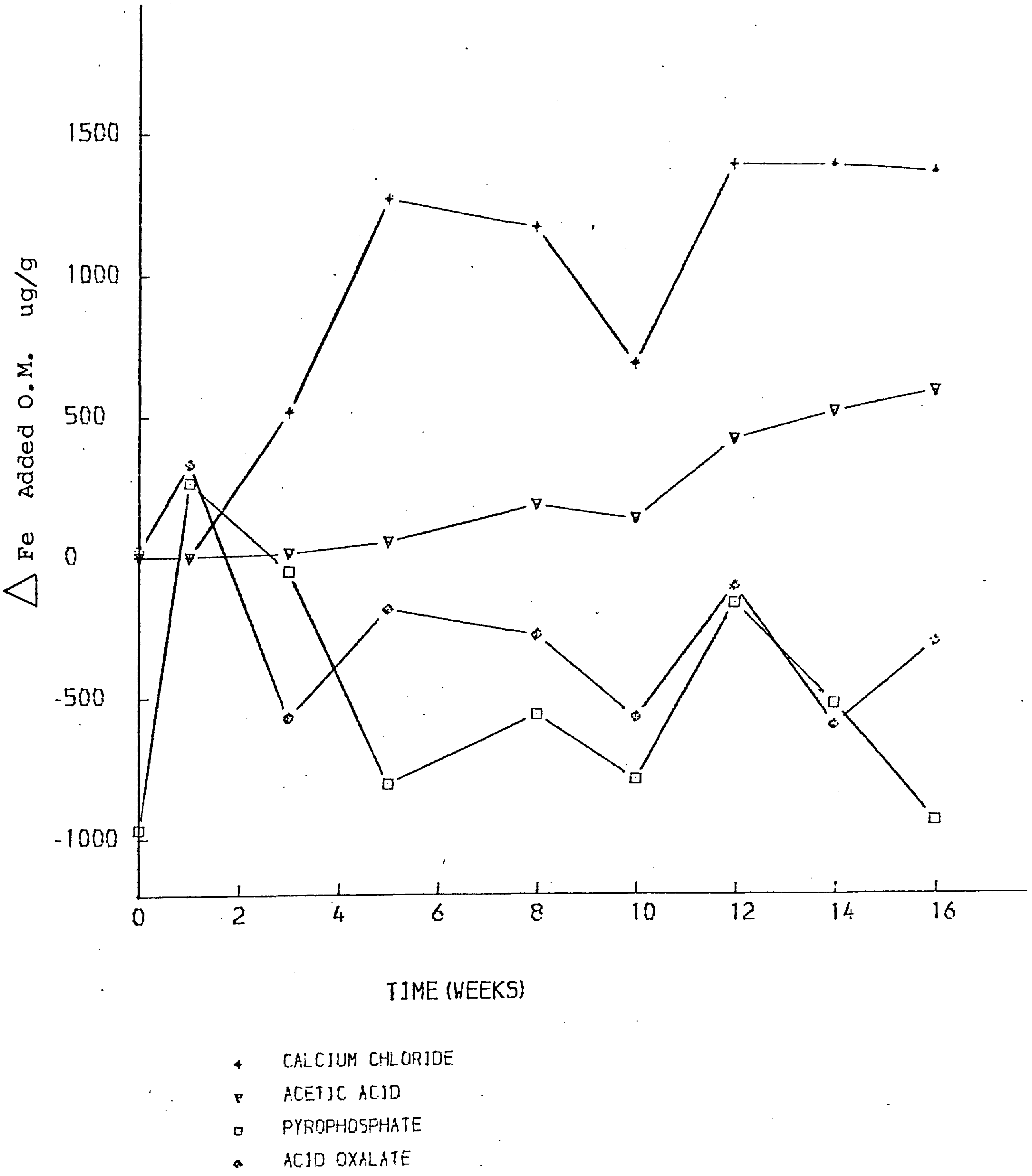


Figure 3.3.12 Variation in the levels of iron removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments.
Waterlogged + 1% lime B_2 horizon

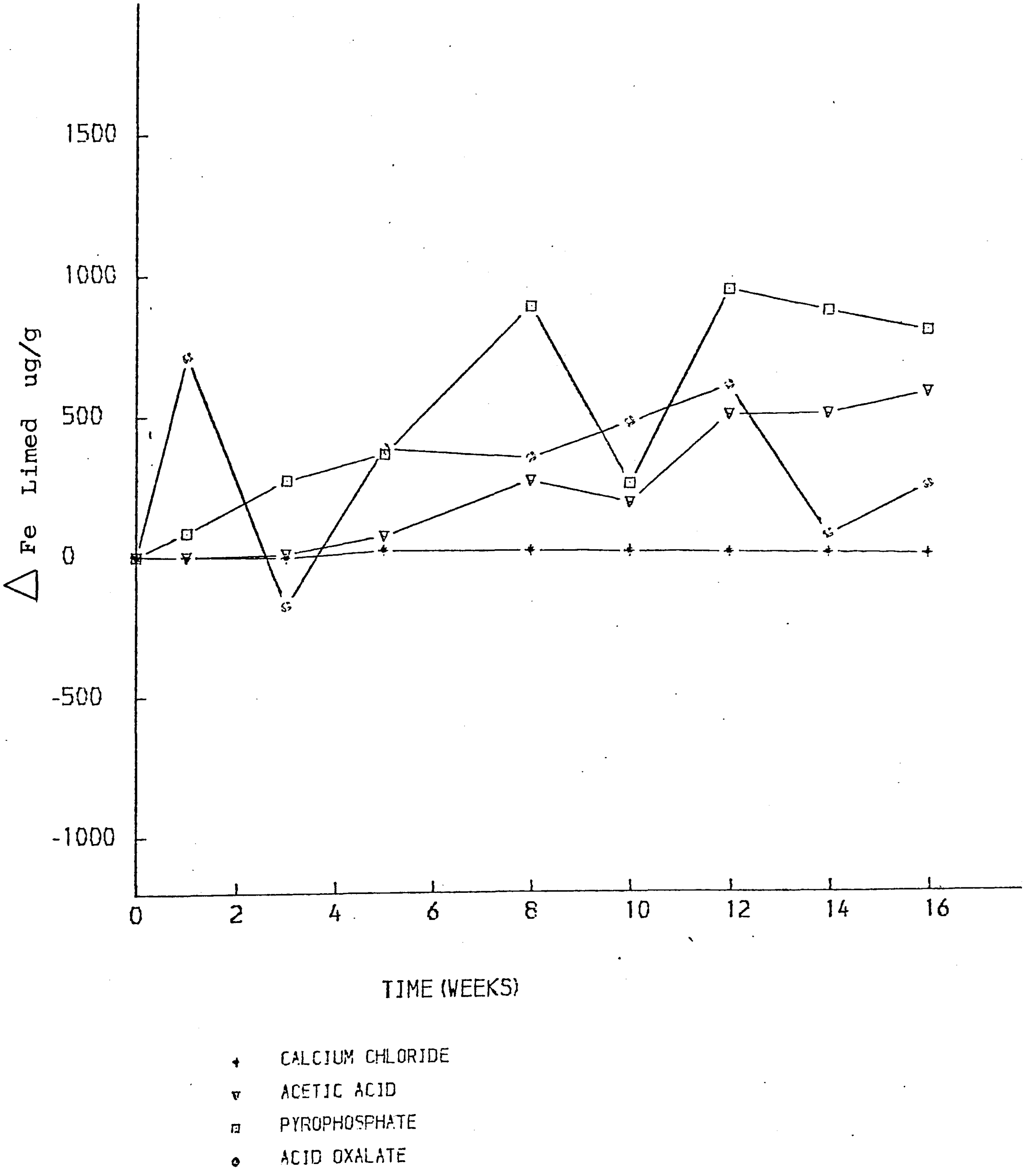


Figure 3.3.13 Changes in iron removed by the selective extractants with decrease in Eh. Waterlogged, no amendment. B_2 horizon

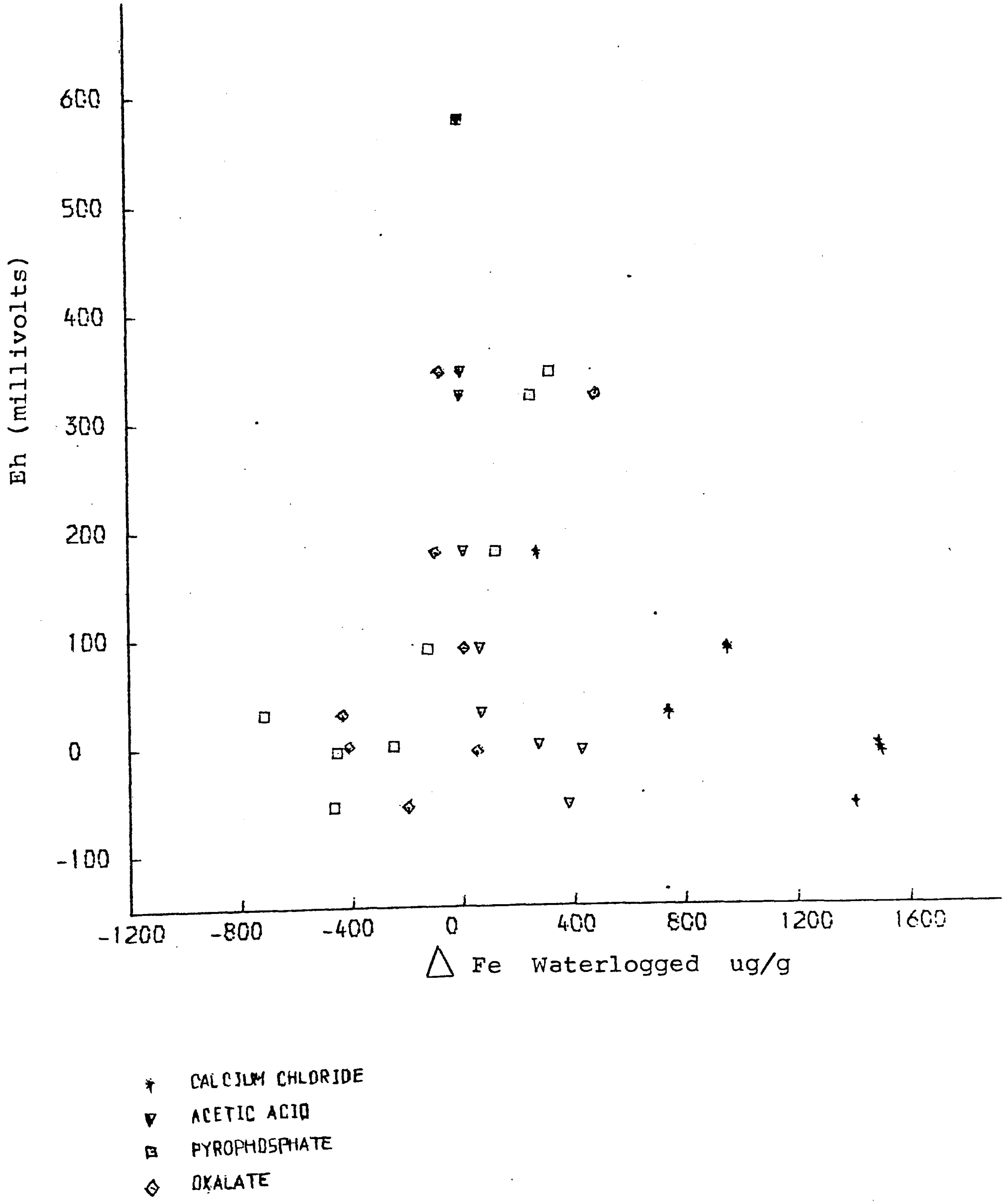


Figure 3.3.14 Changes in iron removed by the selective extractants with decrease in Eh. Waterlogged + 1% dried grass. B_2 horizon

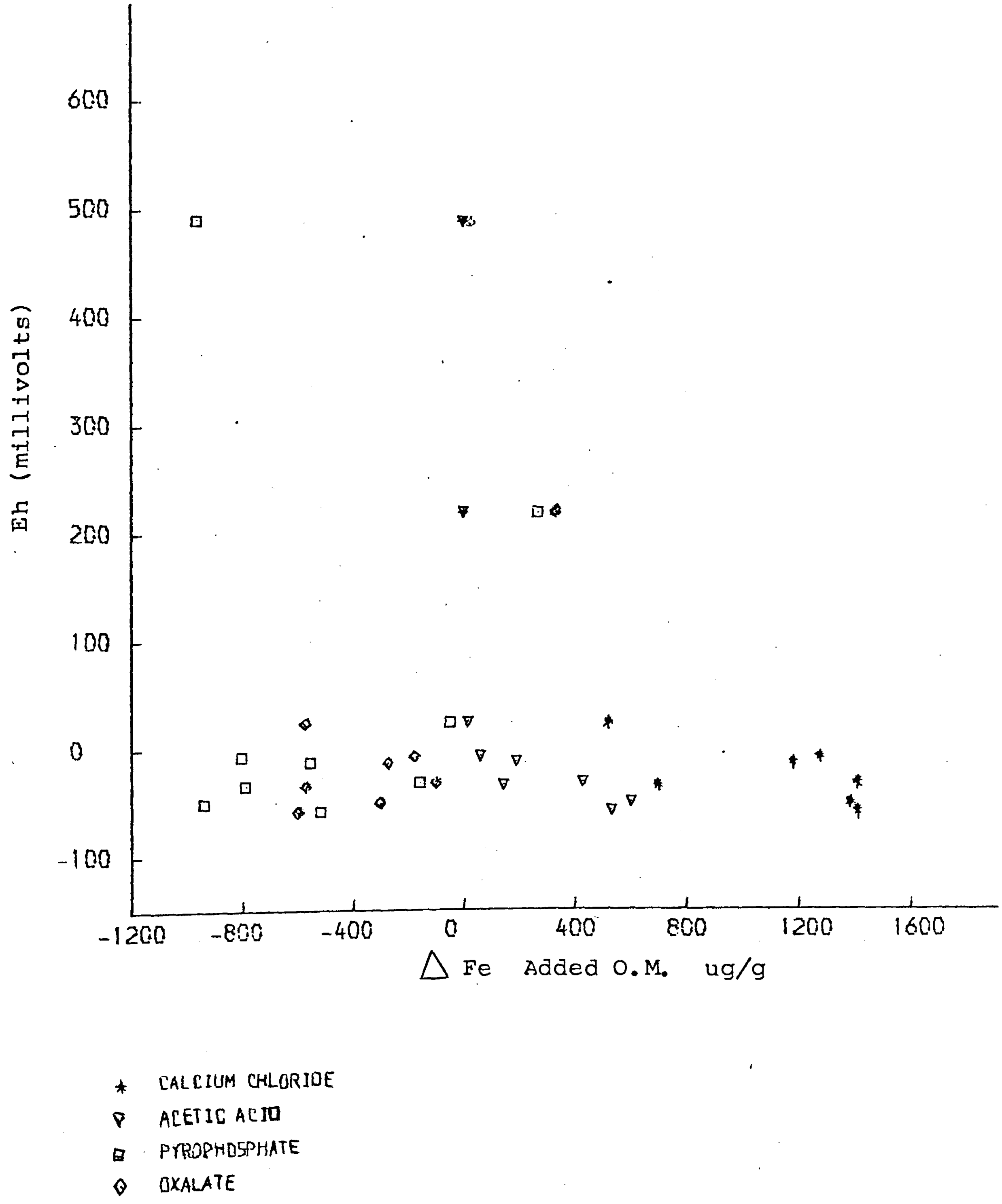
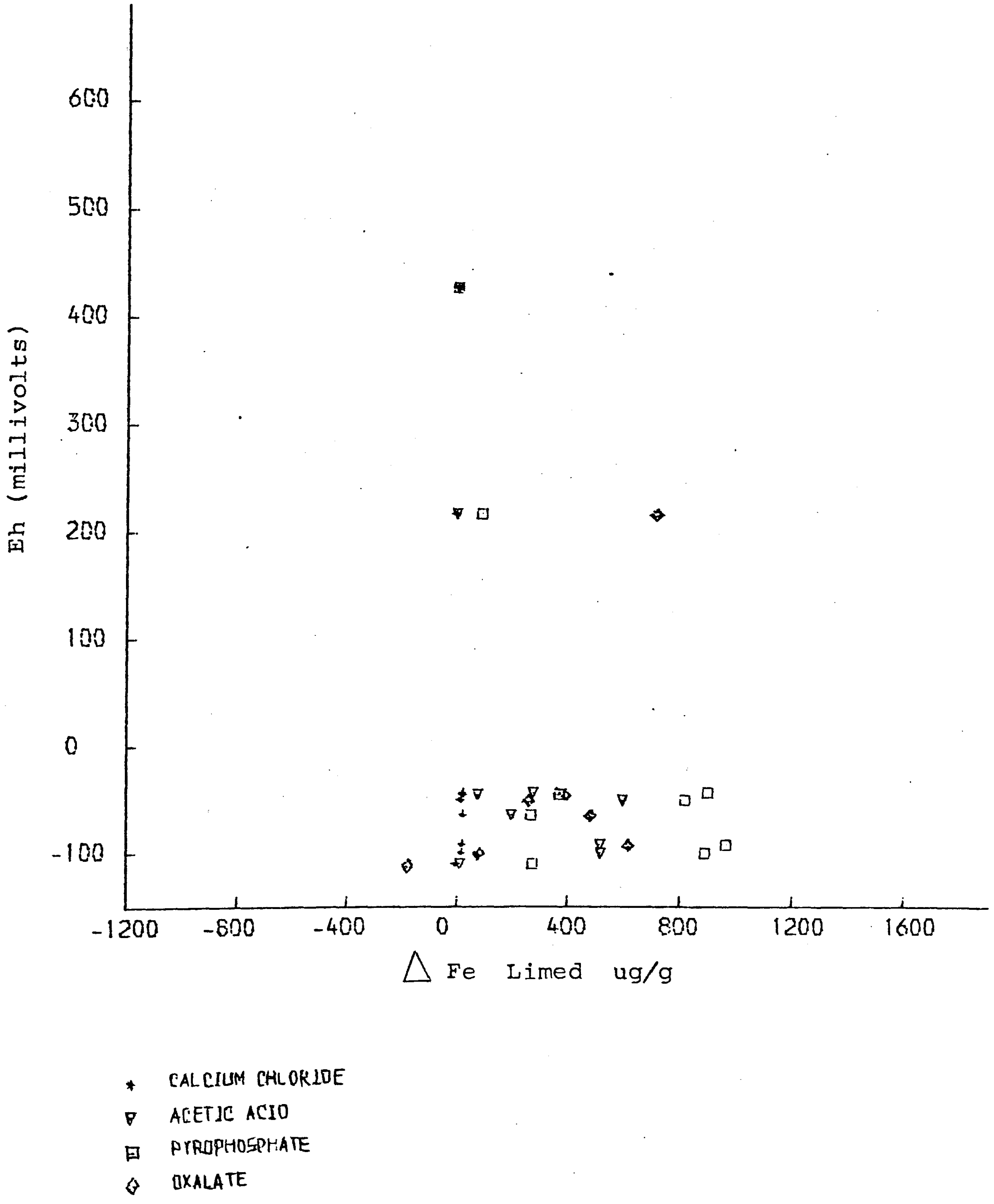


Figure 3.3.15 Changes in iron removed by the selective extractants with decrease in Eh. Waterlogged + 1% lime B_2 horizon



and Δox-Fe decreased. A major difference was an initial increase in the values of Δpp-Fe and Δoxa-Fe. Furthermore, there appears to be a net gain in iron over the period of the experiment. For manganese, at week 16 the sum of Δpp-Mn plus Δoxa-Mn approximated the sum of $\Delta CaCl_2$-Mn plus ΔHAc-Mn. As these values were of opposite sign, this accounted for all the manganese being studied in this system. With iron however there is an imbalance at week 16 of $\Delta CaCl_2$-Fe plus ΔHAc-Fe over Δpp-Fe plus Δoxa-Fe; an apparent gain of iron. This is probably accounted for by release of iron from well crystallized iron oxides or silicate iron, which would not be extracted by acid oxalate in the absence of ultra-violet light.

When 1% dried grass was added to the soil, the increases in $\Delta CaCl_2$-Fe and ΔHAc-Fe with time were faster, as with manganese. Similarly Δpp-Fe and Δoxa-Fe fell more rapidly after an initial rise (Fig 3.3.11).

The behaviour was different in the limed soil however, there being an increase in iron in the waterlogged soil throughout the incubation period. It is suggested that this may be due to two factors; release of iron from well crystallized oxides or silicate lattices and adsorption of mobilized iron by calcium carbonate, which could be extracted by pyrophosphate and oxalate.

The results show an overall similarity in the behaviour of manganese and iron in waterlogged soil. As both elements can easily undergo oxidation and reduction this may be expected.

3.3.3.4 Redistribution of zinc

Changes with time in the various fractions of zinc between waterlogged and control soils for the three treatments are shown in Figure 3.3.16-3.3.18, Figure 3.3.19 -3.3.21 show these changes in relation to the fall in Eh. The redistribution of zinc is qualitatively similar for all treatments, but is faster on addition of 1% organic matter or lime. In all cases there were increases in acetic acid extractable zinc (ΔHAc-Zn) and oxalate extractable zinc (Δoxa-Zn) on waterlogging, and corresponding decreases in calcium chloride extractable zinc ($\Delta CaCl_2$-Zn) and pyrophosphate extractable zinc (Δpp-Zn).

It is well known that zinc availability can be decreased by waterlogging soil (IRRI 1971, 1976, Giordano and Mortvedt 1972, Haldar and Mandal 1979), although contradictory results have been reported (Ng and Bloomfield 1962). The unavailability of zinc has been attributed to the formation of insoluble compounds (sulphide, hydroxide, carbonate or organic chelates) microbiological immobilization or antagonistic effects of other cations (Yoshida and Tanaka 1969, Ponnamperuma 1972, Haldar, and Mandal 1979).

$\Delta CaCl_2$-Zn started to decrease at the onset of waterlogging, at which time the Eh was too high ($>$ 300 mv)

Figure 3.3.16 Variation in the levels of zinc removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged, no amendment. B_2 horizon

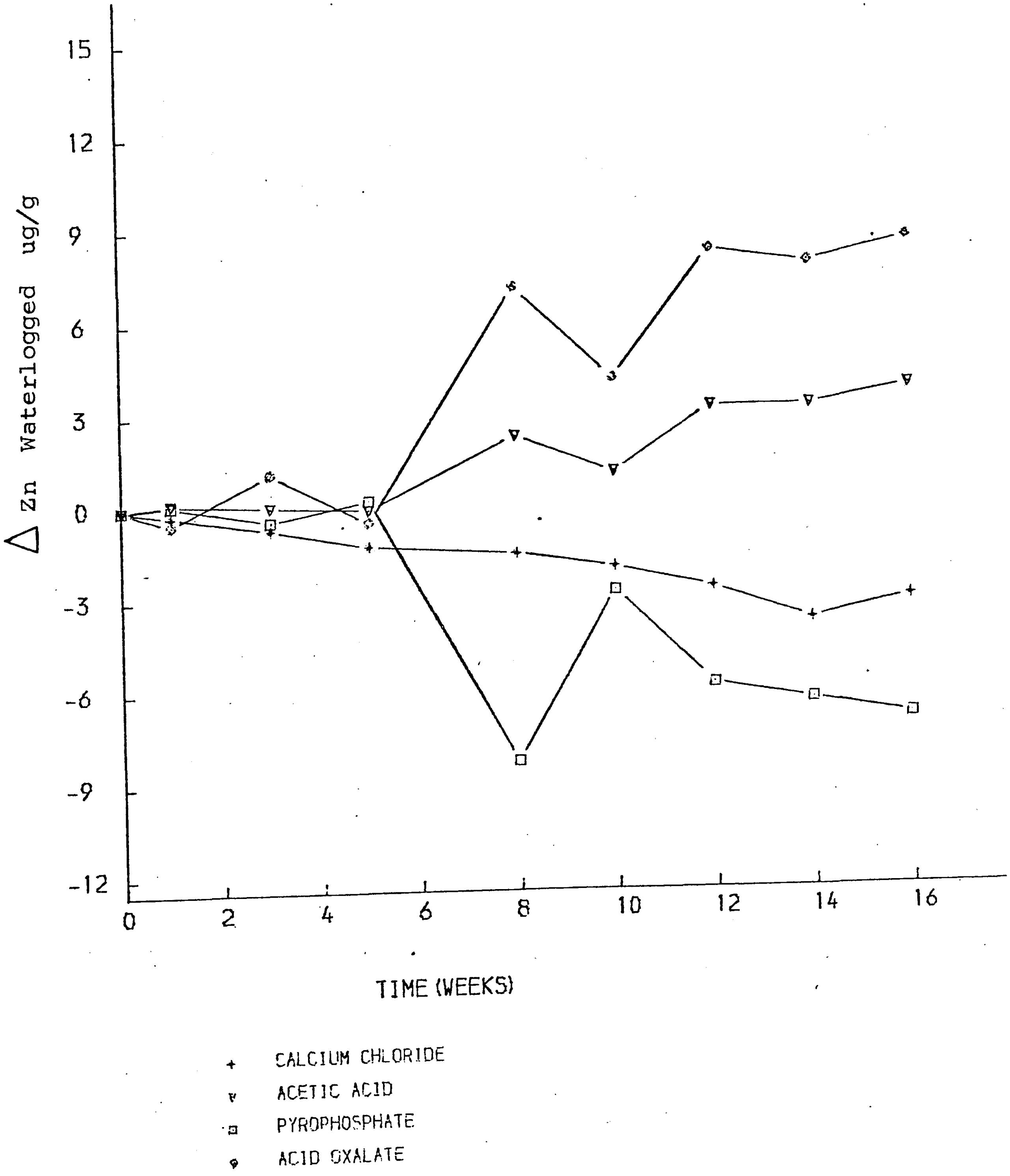


Figure 3.3.17 Variation in the levels of zinc removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% dried grass. B_2 horizon

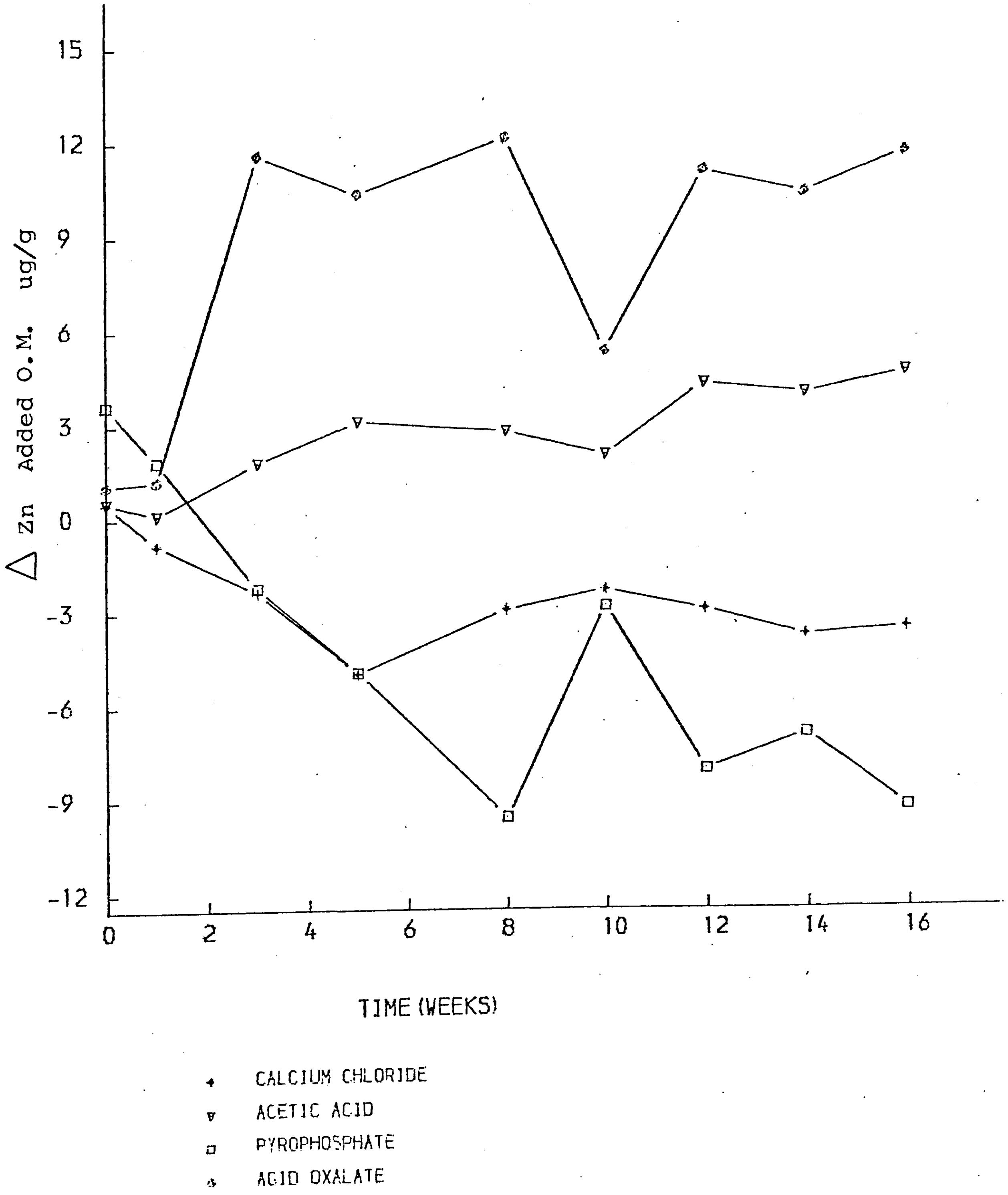


Figure 3.3.18 Variation in the levels of zinc removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% lime. B_2 horizon

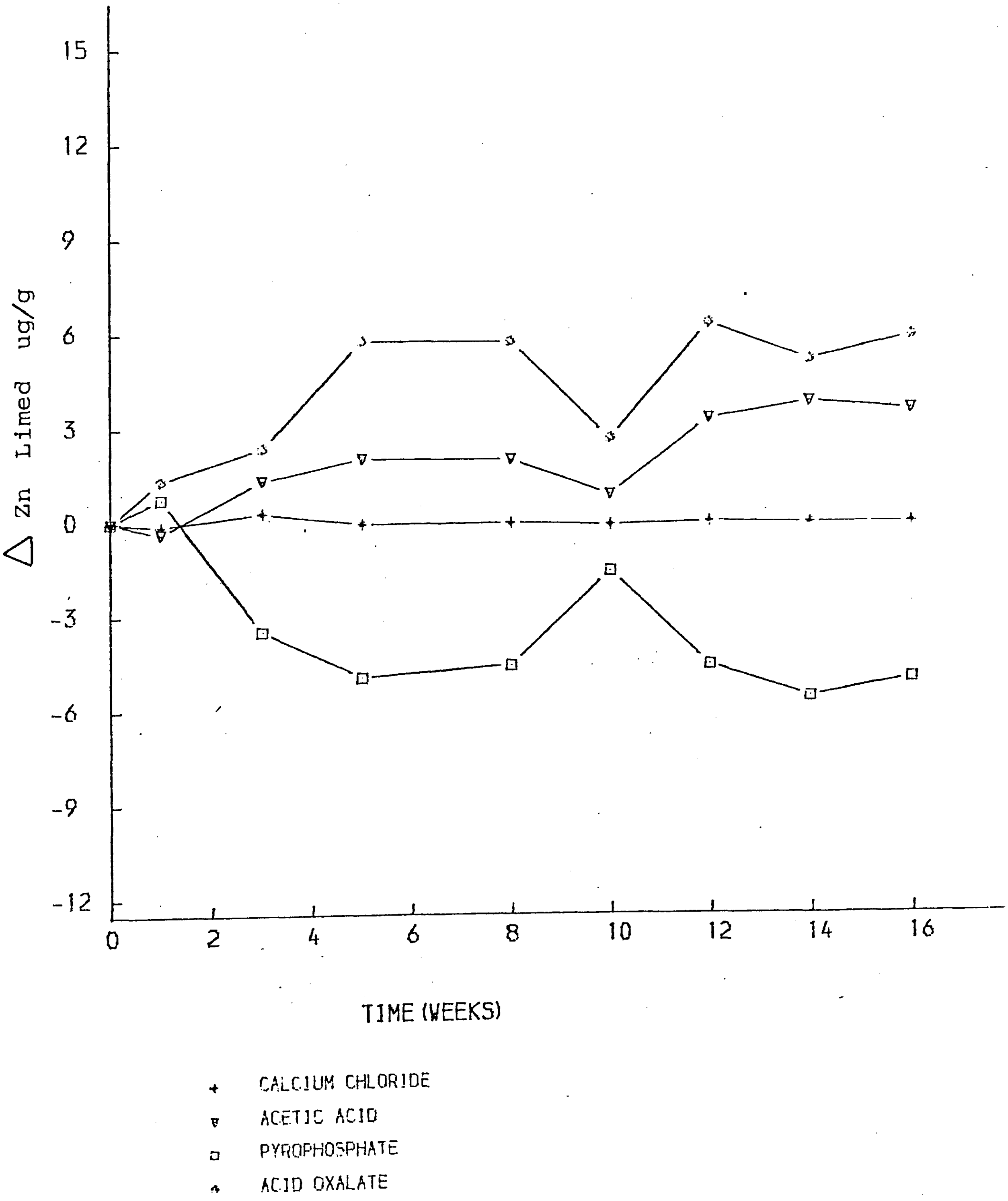


Figure 3.3.19 Changes in zinc removed by the selective extractants with decrease in Eh. Waterlogged, no amendment. B_2 horizon

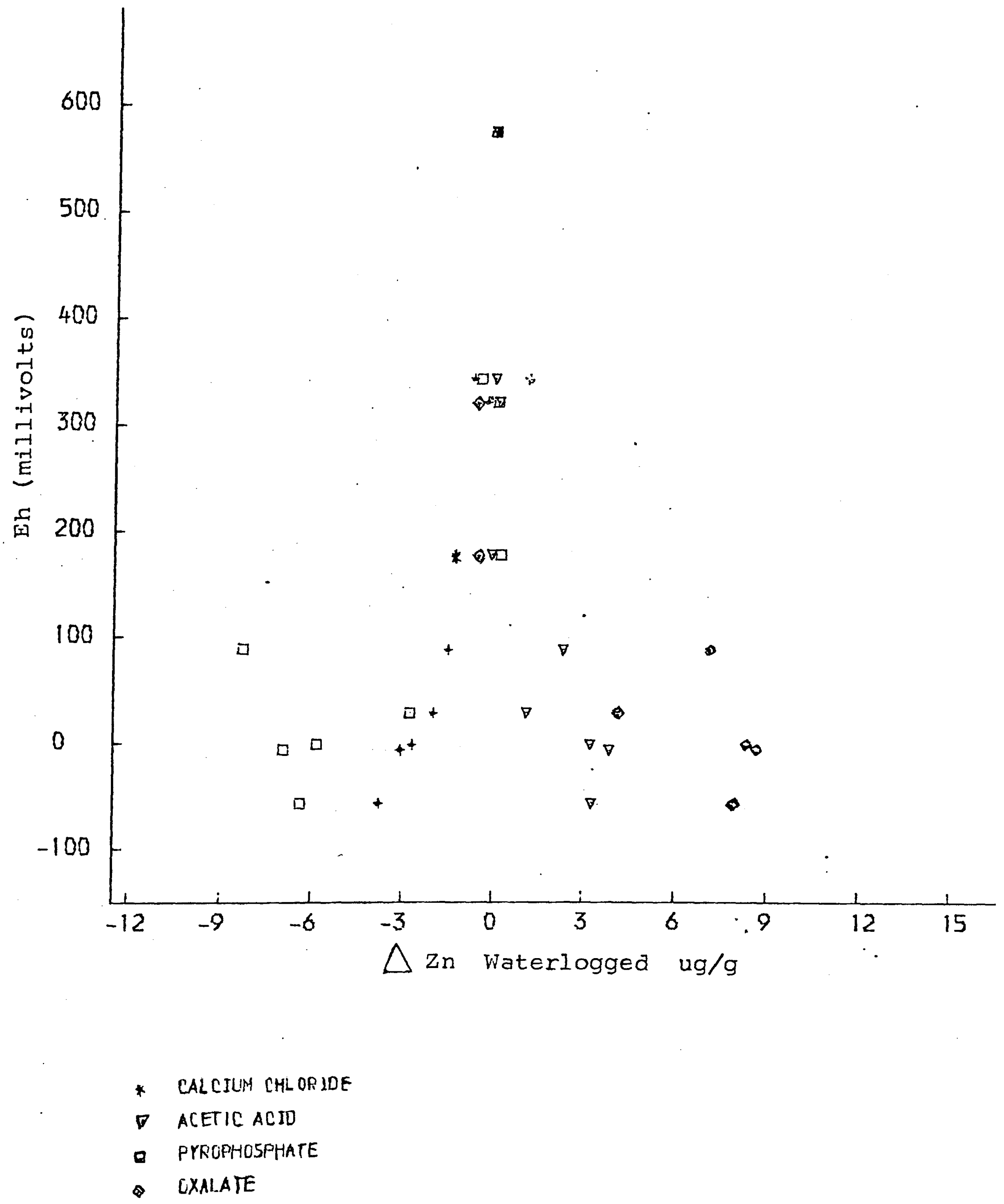
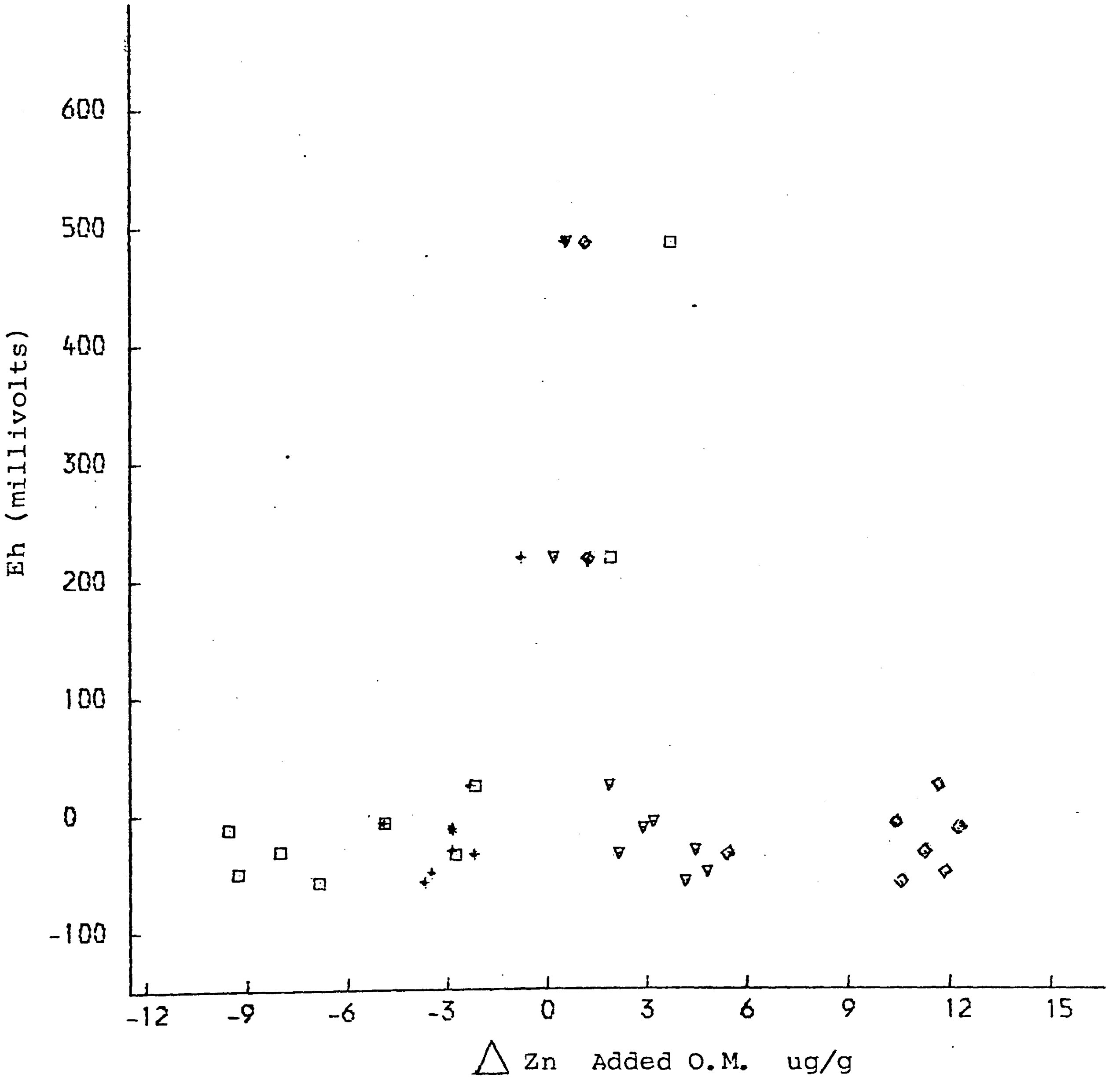
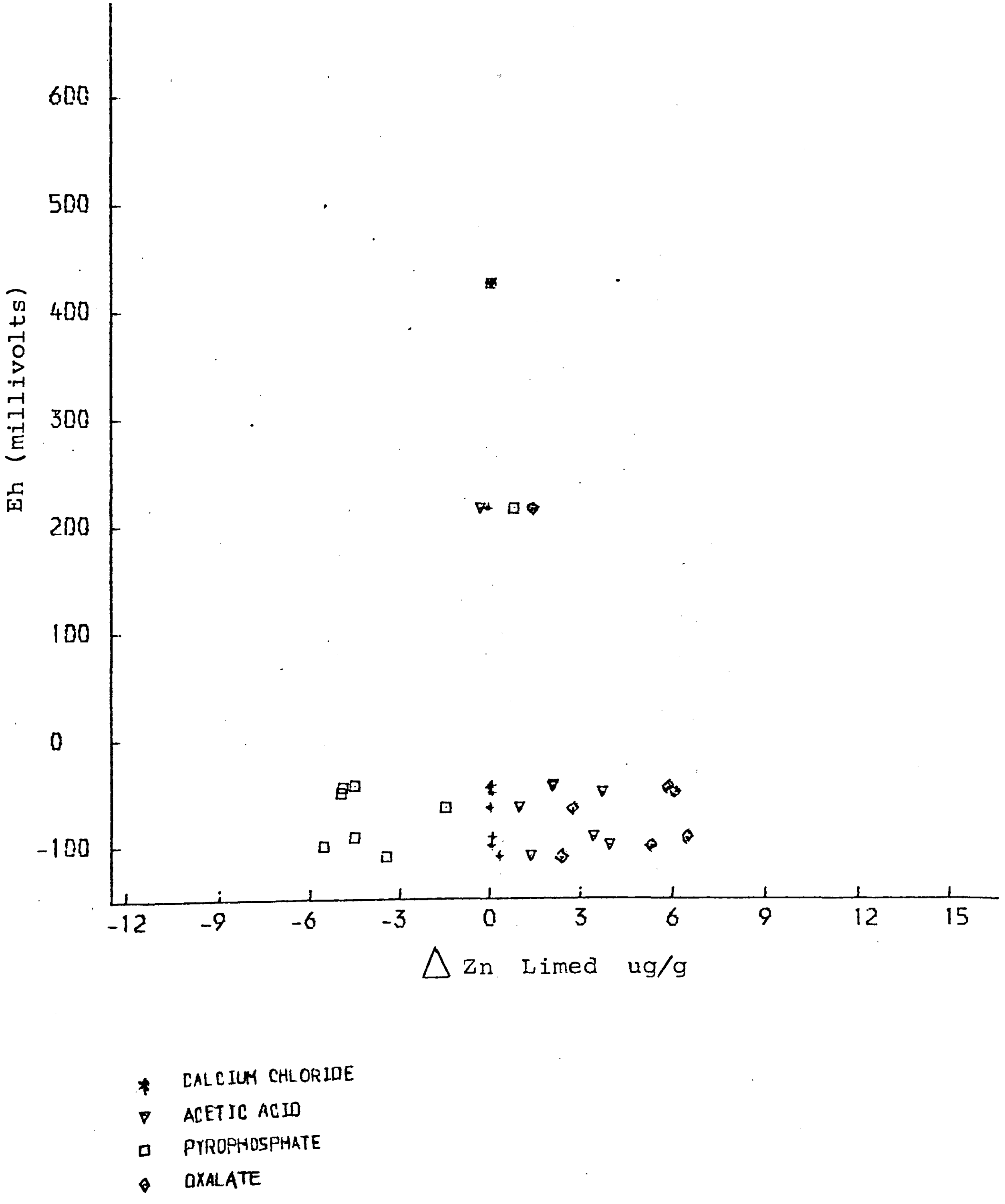


Figure 3.3.20 Changes in zinc removed by the selective extractants with decrease in Eh. Waterlogged + 1% dried grass. B_2 horizon



+ CALCIUM CHLORIDE
▽ ACETIC ACID
□ PYROPHOSPHATE
◇ OXALATE

Figure 3.3.21 Changes in zinc removed by the selective extractants with decrease in Eh. Waterlogged + 1% lime. B2 horizon



to allow sulphide formation. Furthermore, the solubility of zinc hydroxide is too high at the pH of the soil being studied to allow precipitation (Chester 1965). These mechanisms can therefore be discounted as means of reducing zinc availability in this soil. The decrease in Δ pp-Zn indicated that the reduction in available zinc was not caused by formation of insoluble organic chelates. The amount by which $\Delta CaCl_2$-Zn decreased could be accounted for by the acetic acid extraction of the residue from the $CaCl_2$ extraction. Removal of this zinc by a relatively mild extractant (2.5% acetic acid) would tend to discount the possibility of microbiological immobilization. It may be possible to explain the decrease in $\Delta CaCl_2$-Zn and Δpp-Zn by competition effects of other cations, but this does not account for the fate of the missing zinc.

The trends observed in Δoxa-Zn suggest the importance of the poorly ordered soil oxides to the solubility of zinc in waterlogged soil. Gambrell, Khalid and Verloo (1977) found most of the potentially reactive zinc of sediments was associated with a hydroxide phase and lesser amounts were found with insoluble organic. Iron and manganese oxides have long been known as active 'scavengers' of trace metals in soils and sediments (Jenne 1968), the hydrated forms being more reactive than the dehydrated forms (Ponnamperuma, Tianco and Loy 1967). Moreover, the adsorption abilities of these oxides for trace metals are highly pH dependant (Loganathan and Burau 1973). When Eh decreases, oxides of iron and manganese become unstable (Lindsay 1978) and the crystallinity decreases (Willett 1979), with an increase in surface area and crystal imperfections.

Under waterlogged conditions a number of changes occur in soil; Eh is decreased by microbial respiration, the pH value of acid soils are increased, hydrated forms of iron and manganese oxides prevail, oxide surfaces are increased and crystallinity decreased. All of these changes will increase the ability of the iron and manganese oxides to adsorb zinc. It is also possible that ferrous and manganous ions reaching the oxygenated surface water will precipitate as colloidal oxides which will have high adsorptive capacities.

The addition of actively decomposing organic matter caused an increase in the rate of redistribution and greater values of Δpp-Zn and Δoxa-Zn (Figure 3.3.17). The waterlogged soil with addition of organic matter showed an immediate reduction in Δpp-Zn and an increase in Δoxa-Zn after 1 week's incubation. This faster redistribution of zinc between various fractions coincided with the accelerated rate of decrease of Eh and increase in pH (Figure 3.3.2).

Differences in values of extractable zinc between waterlogged and control limed soils are shown in Figure 3.3.18 and 3.3.21. $\Delta CaCl_2$-Zn showed no significant change throughout the experiment. Both Δpp-Zn and Δoxa-Zn showed quick responses to waterlogging in the same way as the organic matter treated soil. In the limed soils the ranges of Δpp-Zn and Δoxa-Zn are smaller than in the other two treatments, possibly due to the higher initial pH of the soils. ΔHAc-Zn increased, even though $\Delta CaCl_2$-Zn did not decrease. It may be that the specific inorganic adsorption sites preferentially take up zinc at the expense of the organic sites.

3.3.3.5 Redistribution of copper

Ammonium acetate-extractable copper in soil was reported to be decreased (Haldar and Mandal 1979) and the copper uptake by rice was reduced (Beckwith, Tiller and Suwadji 1975) by waterlogging. Copper deficiency has also been reported under such conditions (IRRI 1976). In the present work, copper was found to decrease in calcium chloride and pyrophosphate extracts.

The effects of waterlogging on the various fractions of copper are shown in Fig 3.3.22-3.3.24 in relation to time and Fig 3.3.25-3.3.27 in relation to Eh. Copper followed approximately similar trends to zinc, but the symmetrical pattern of the zinc changes was not repeated. $\Delta CaCl_2$-Cu was only slightly reduced, whereas ΔHAc-Cu increased steadily. The changes in Δpp-Cu and Δoxa-Cu were brought about faster than with zinc. This could be because copper can be adsorbed onto oxide surfaces at a lower pH than zinc (Kinniburgh, Jackson and Syers 1976, McBride and Blasiak 1979).

In the later stages of the incubation, only small amounts of copper which disappeared from the pyrophosphate

Figure 3.3.22 Variation in the levels of copper removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged, no amendment. B_2 horizon

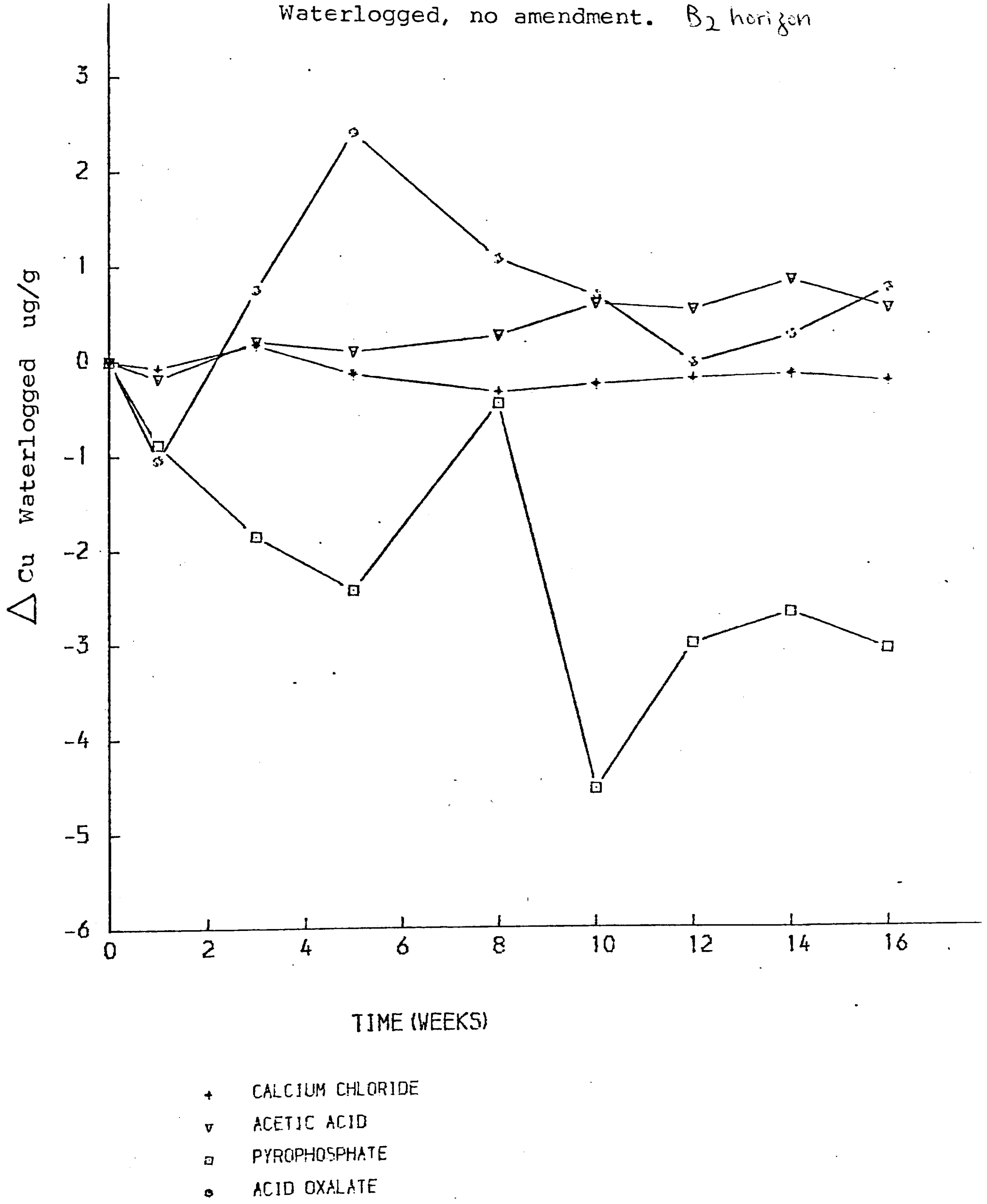


Figure 3.3.23 Variation in the levels of copper removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% dried grass. B_2 horizon

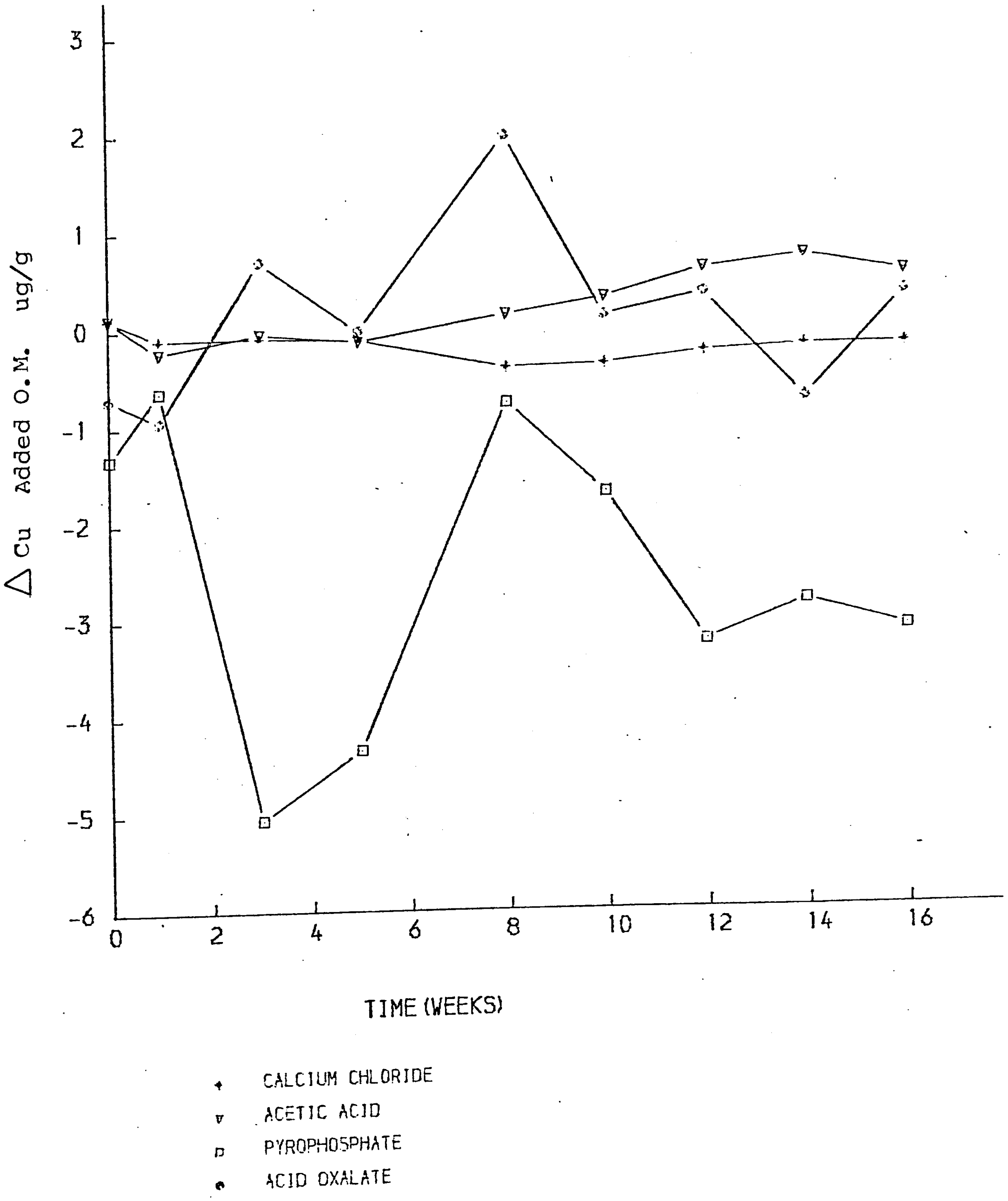


Figure 3.3.24 Variation in the levels of copper removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% lime . B_2 horizon

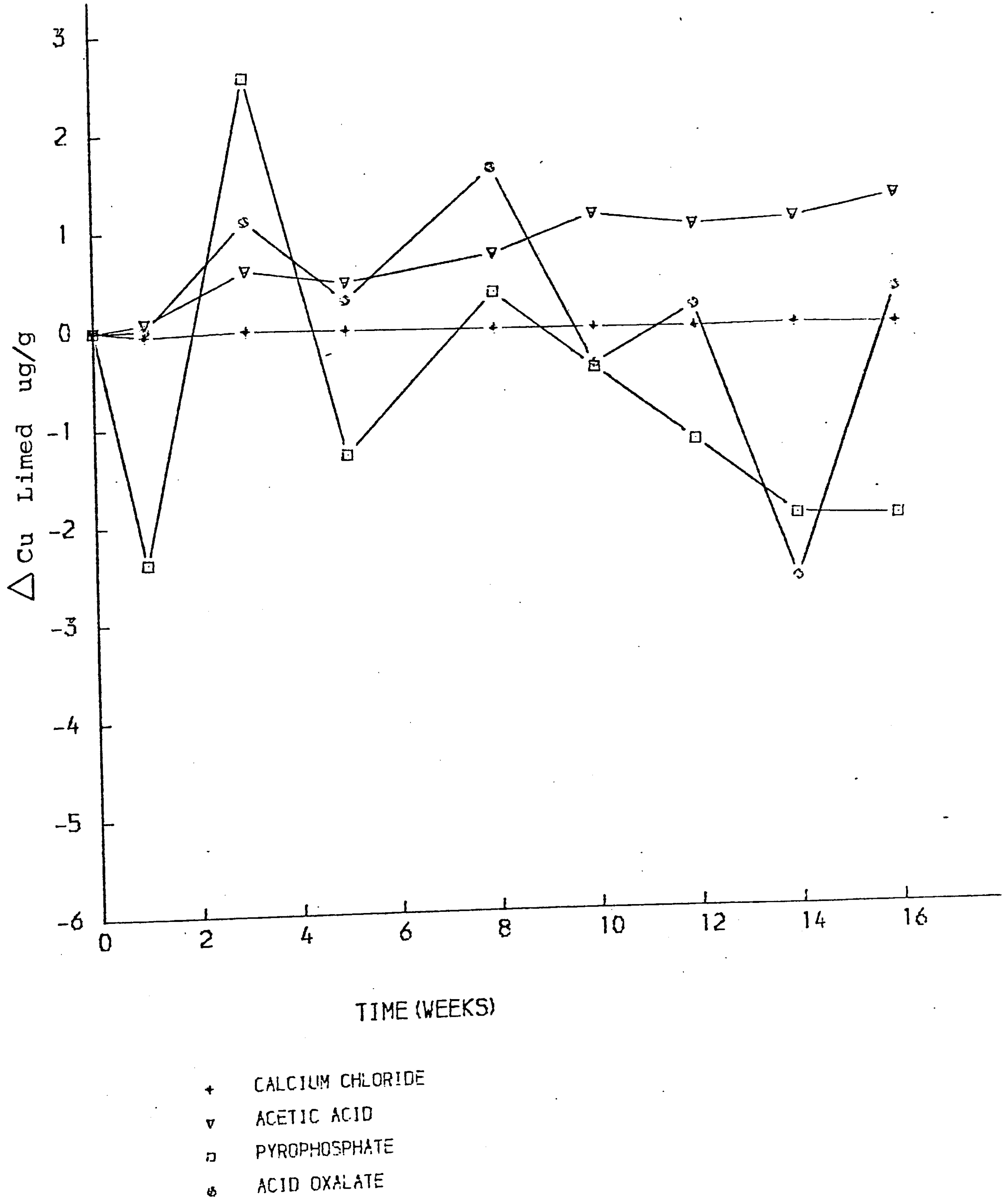


Figure 3.3.25 Changes in copper removed by the selective extractants with decrease in Eh. Waterlogged, no amendment. B_2 horizon

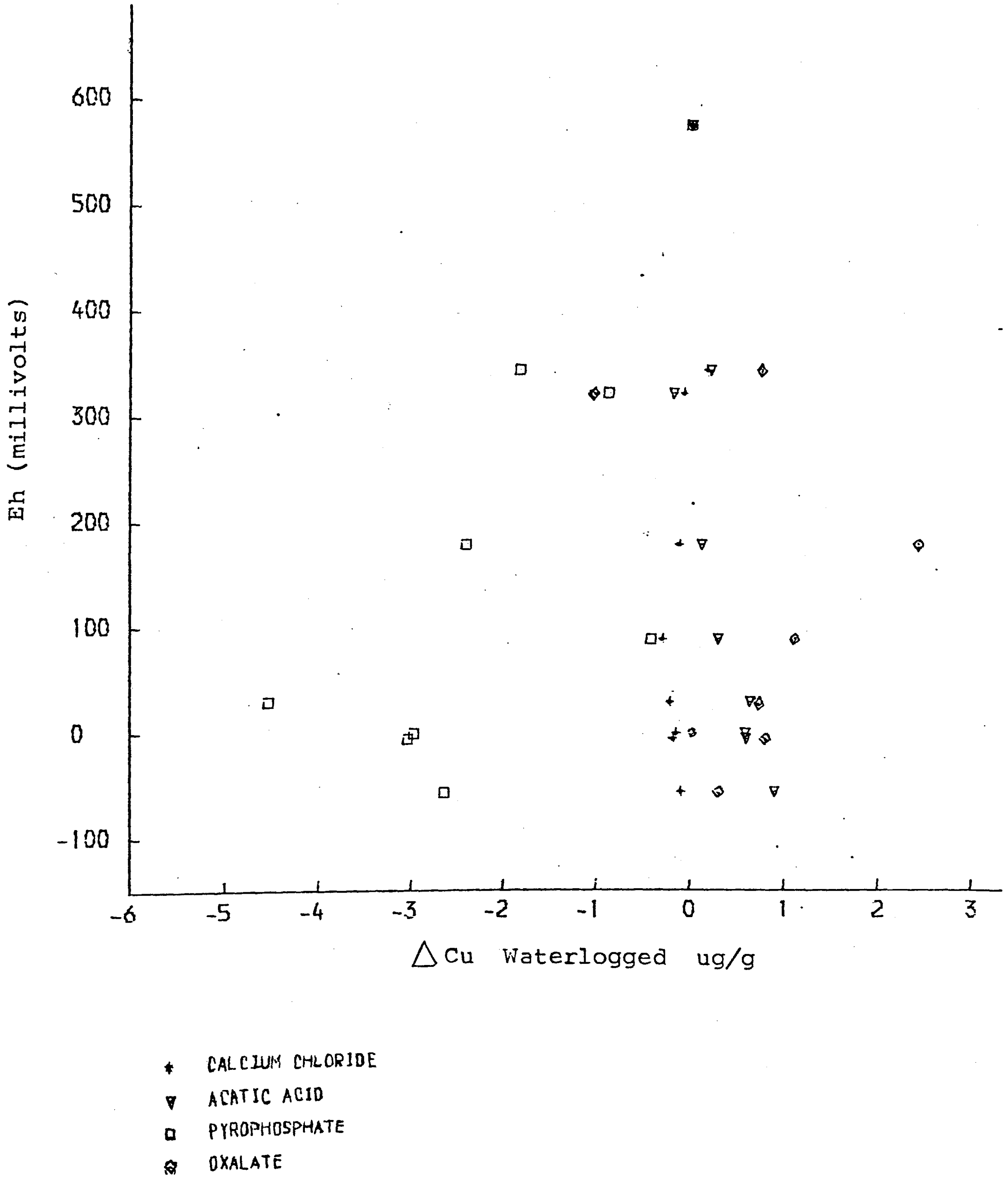


Figure 3.3.26 Changes in copper removed by the selective extractants with decrease in Eh. Waterlogged + 1% dried grass. B_2 horizon

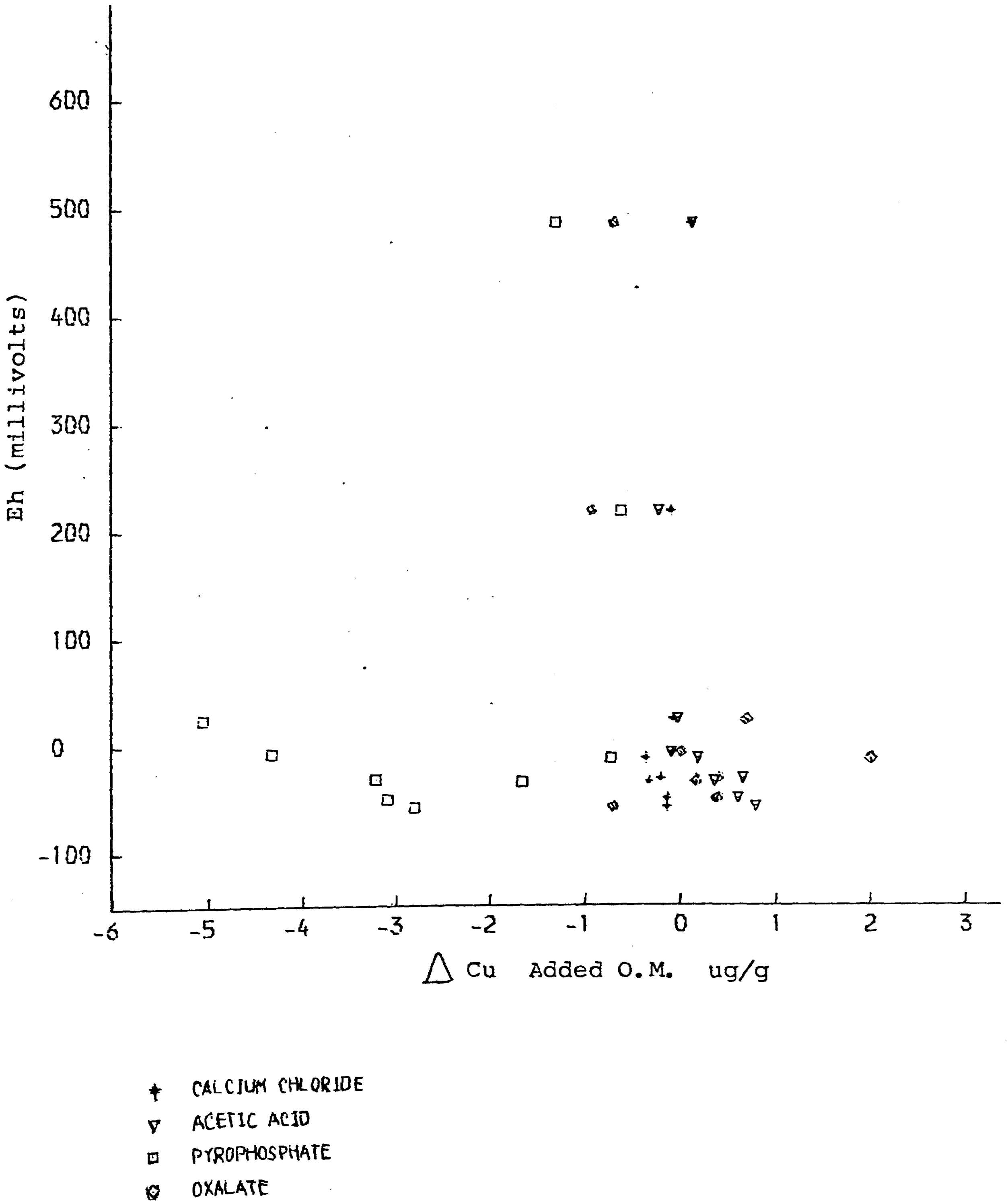
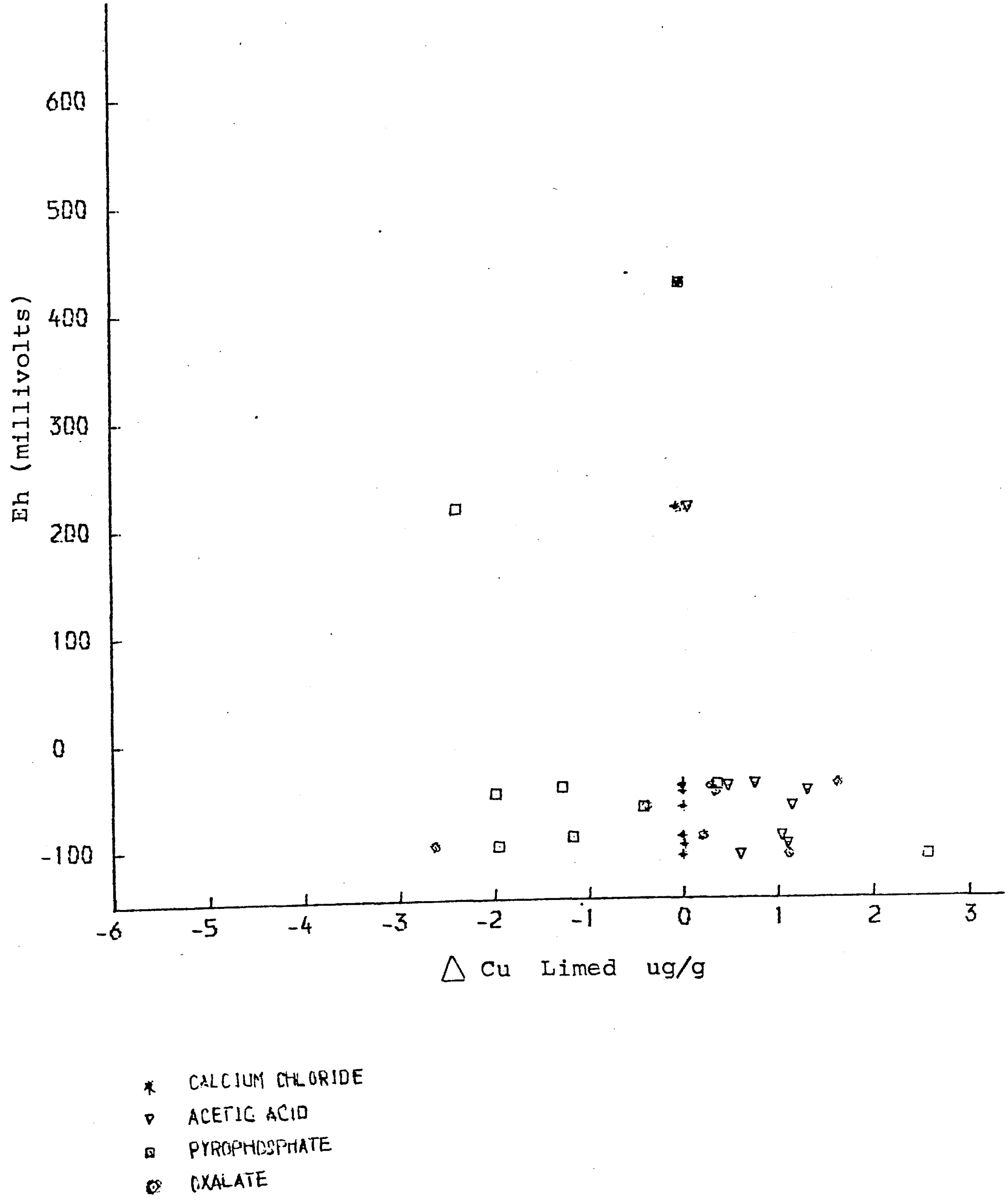


Figure 3.3.27 Changes in copper removed by the selective extractants with decrease in Eh. Waterlogged + 1% lime. B2 horizon



extracts were recovered by oxalate extraction. This may be accounted for by the different chemical characteristics of the two elements. In the standing water of the waterlogged treatments the concentration of copper increased, whereas the zinc concentration fell. Analysis of the water (previous results Section 3.2.) showed an increase in copper levels from 0.003 ppm at week 1 to 0.025 ppm at week 15, and a decrease in zinc levels from 0.045 ppm at week 1 to 0.01 ppm at week 15. It is known that copper may be present in soil solution at a higher concentration in a complexed form than zinc (Hodgson, Lindsay and Trierweiler 1966). As there would be a large amount of soluble organic matter in the soils used, the high affinity of copper to form complexes could account for the non-adsorption of copper onto oxide surfaces. The experimental design involved removal of excess soil water from the surface before extraction, hence an apparent decrease in pyrophosphate extractable copper. There does however seem to be some competition for copper between the adsorption sites on the oxides and soluble organic matter, presumably with chelating ability.

3.3.3.6 Redistribution of cobalt

It had been found that acetic acid extracted more cobalt than pyrophosphate in Section 3.2, but the experiment described in this section did not support this. The soil used in Section 3.2 was collected in summer, the present one was collected in winter. Field moisture and temperature condition at the time of collection might be the main factors to affect the changes. Because of relative higher pyrophosphate extract-

Figure 3.3.28 Variation in the levels of cobalt removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged, no amendment. B_2 horizon

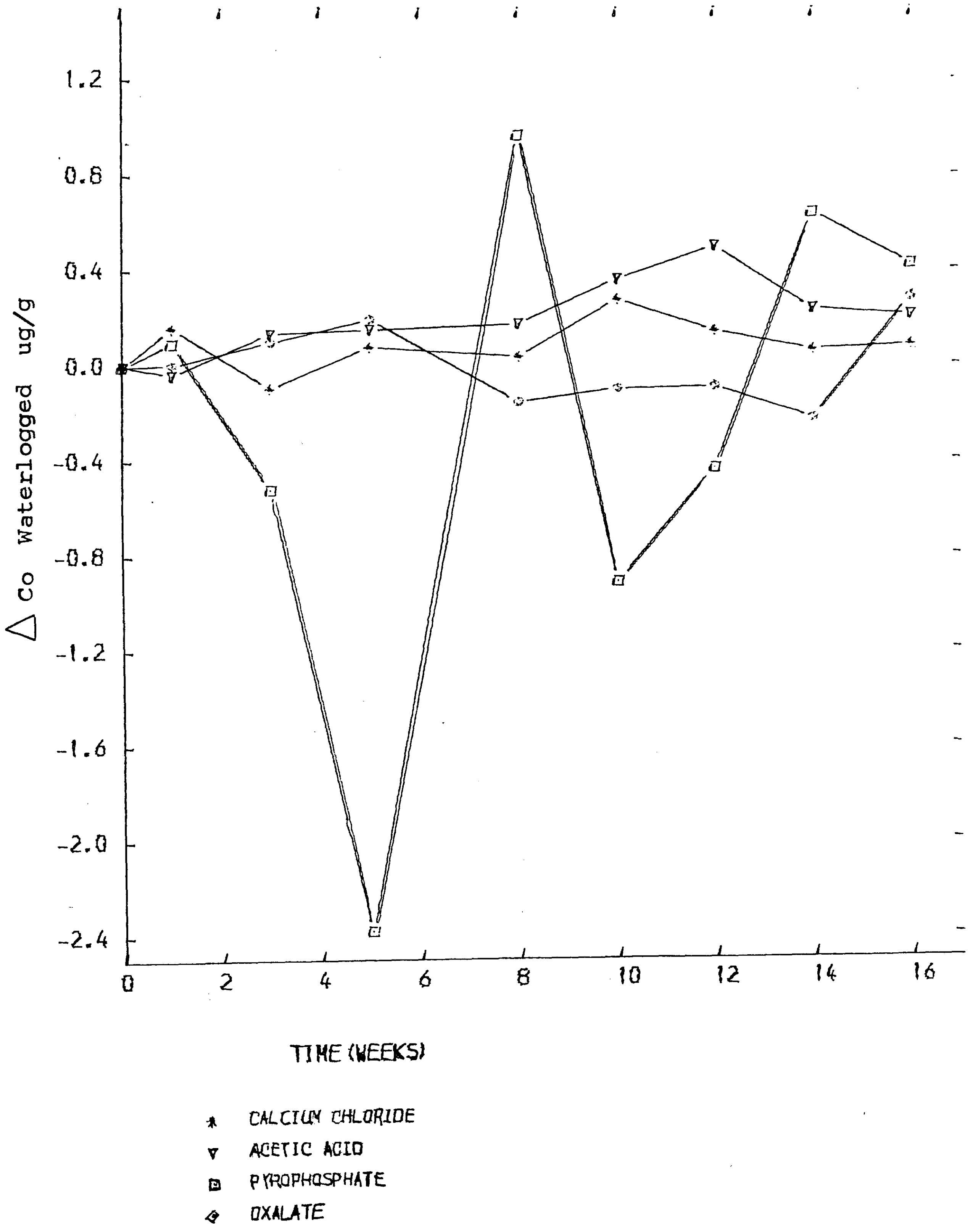


Figure 3.3.29 Variation in the levels of cobalt removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% dried grass. B_2 horizon

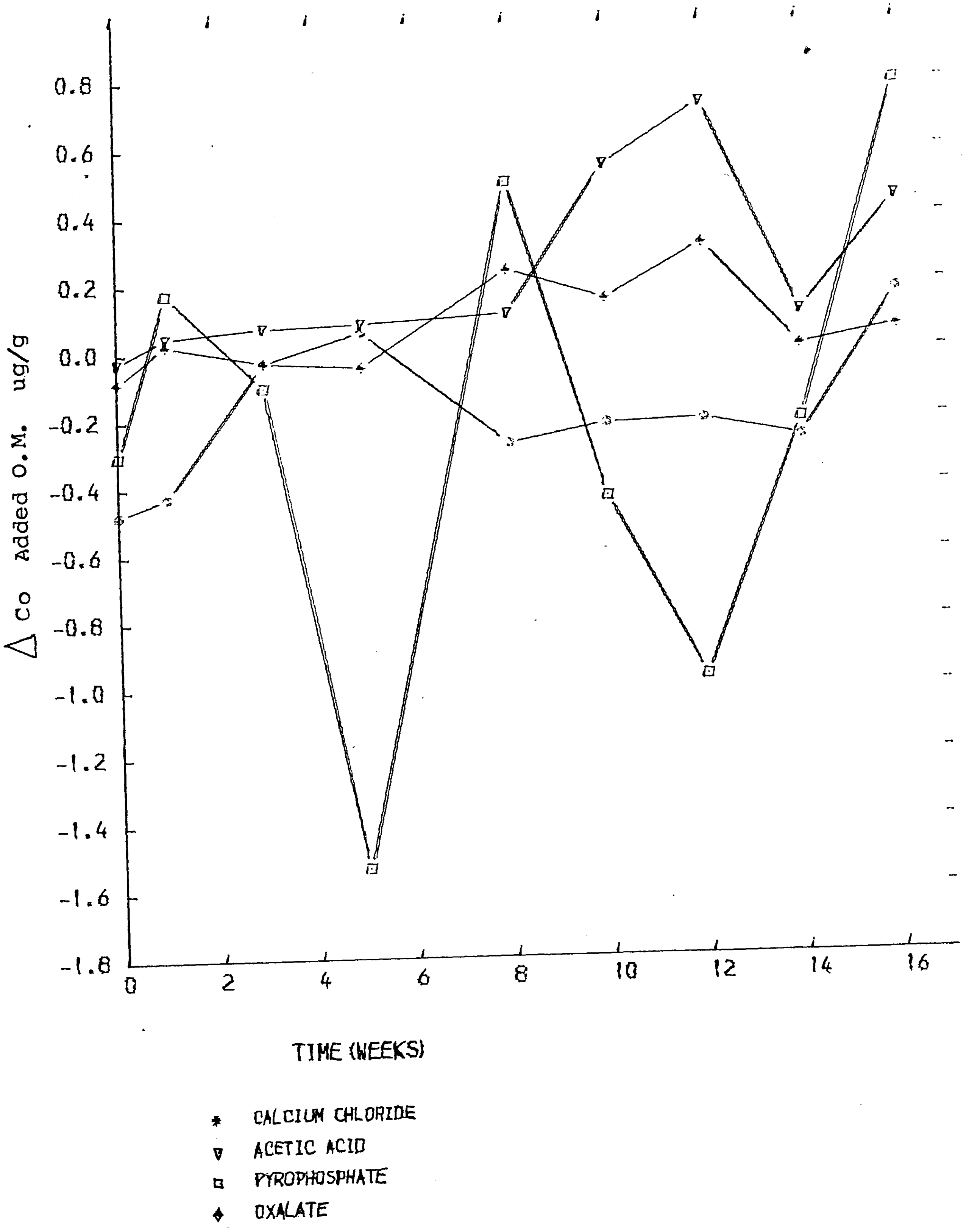


Figure 3.3.30 Variation in the levels of cobalt removed by the selective extractants over 16 weeks, expressed as the difference in content between the waterlogged and aerated treatments. Waterlogged + 1% lime. B_2 horizon

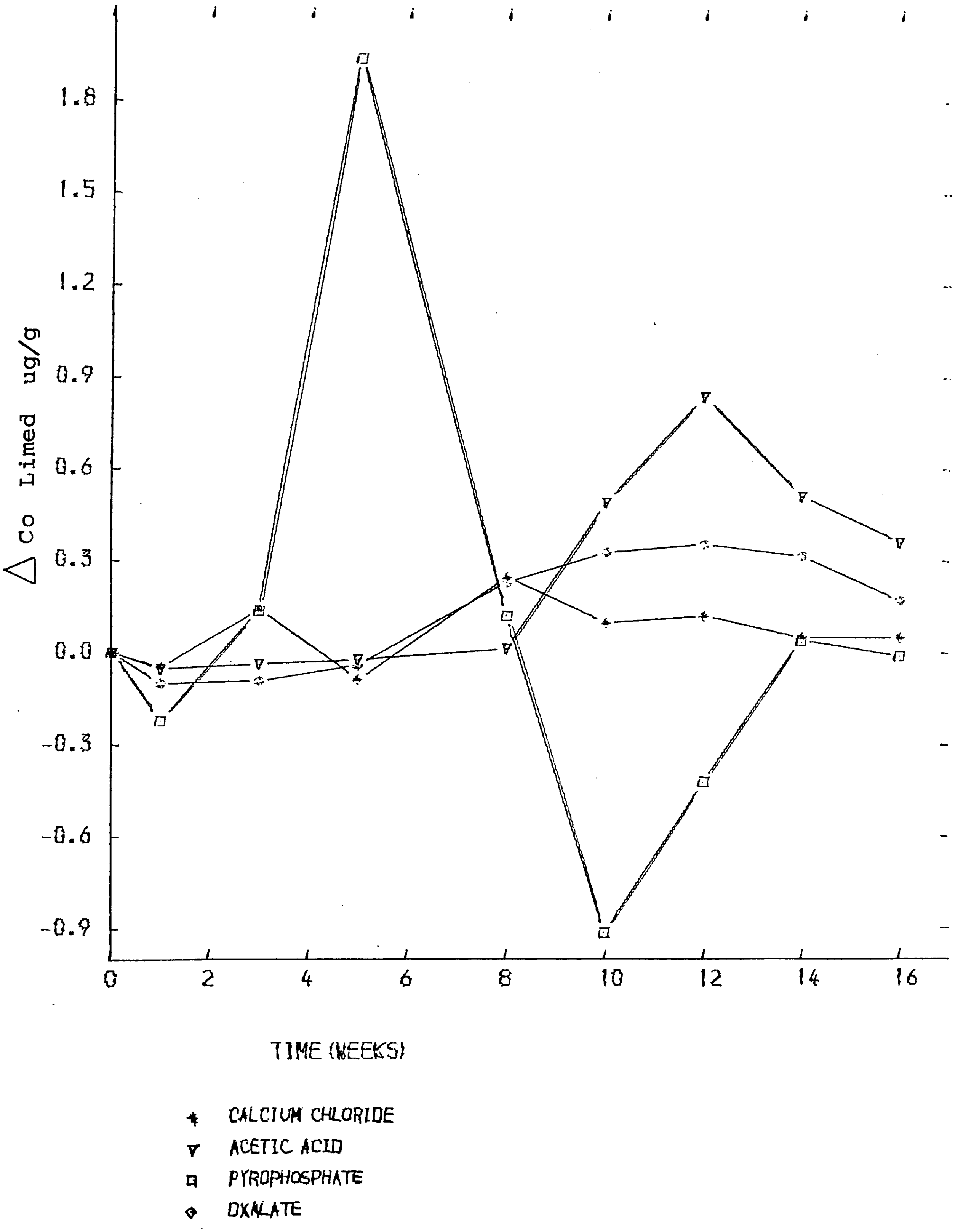
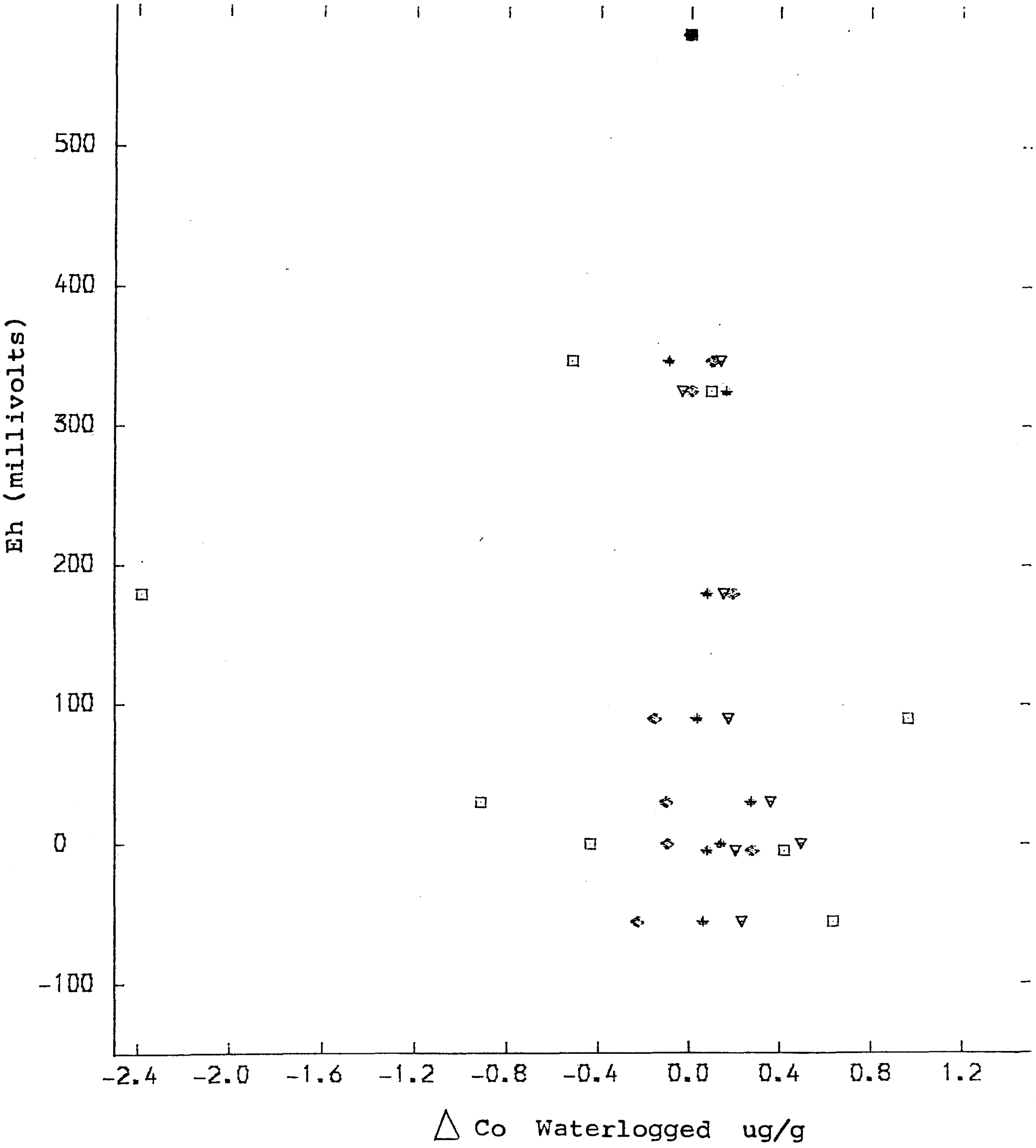


Figure 3.3.31 Changes in cobalt removed by the selective extractants with decrease in Eh. Waterlogged, no amendment. B2 horizon



+ CALCIUM CHLORIDE

▽ ACETIC ACID

□ PYROPHOSPHATE

◇ ACID OXALATE

Figure 3.3.32 Changes in cobalt removed by the selective extractants with decrease in Eh. Waterlogged + 1% dried grass. B_2 horizon

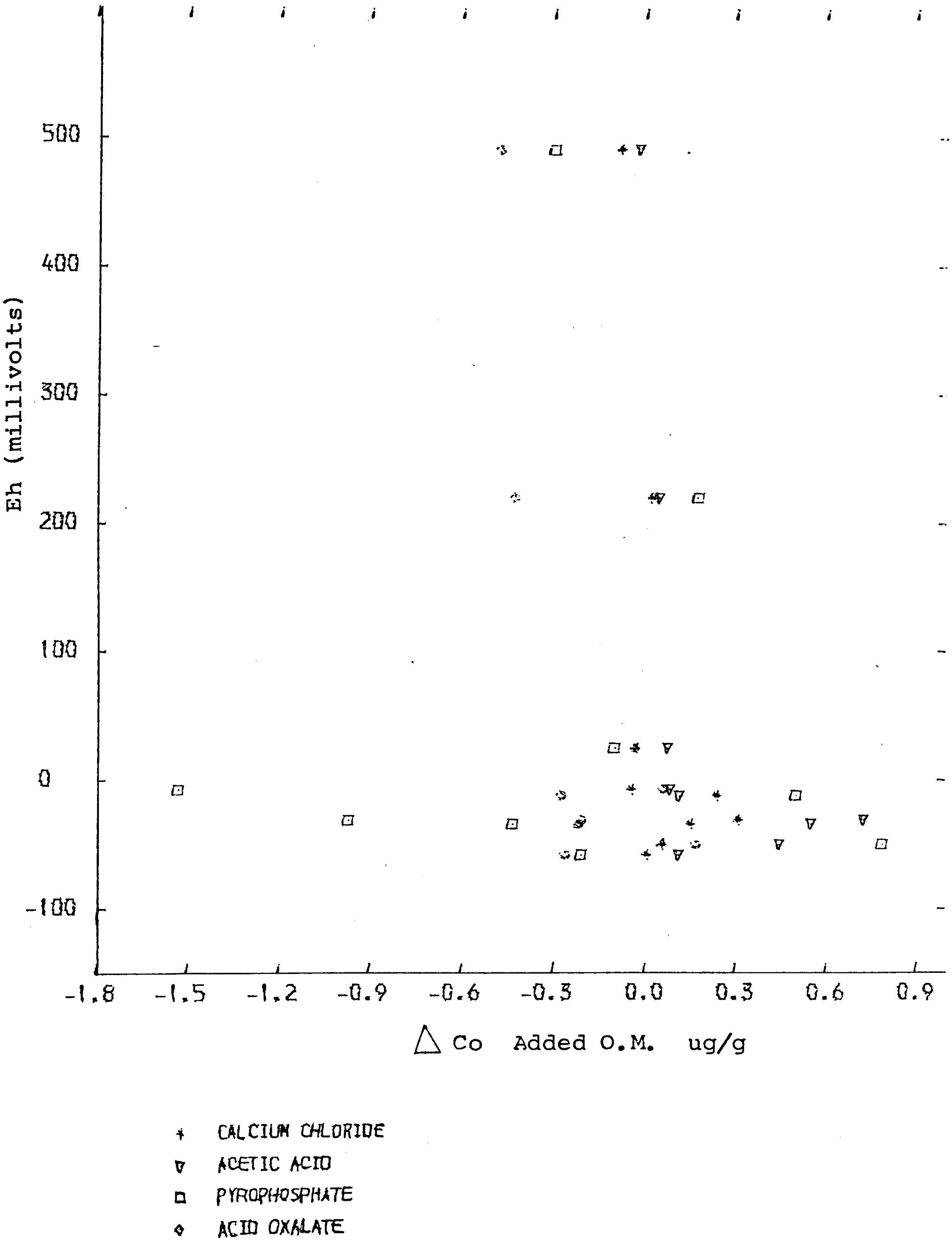
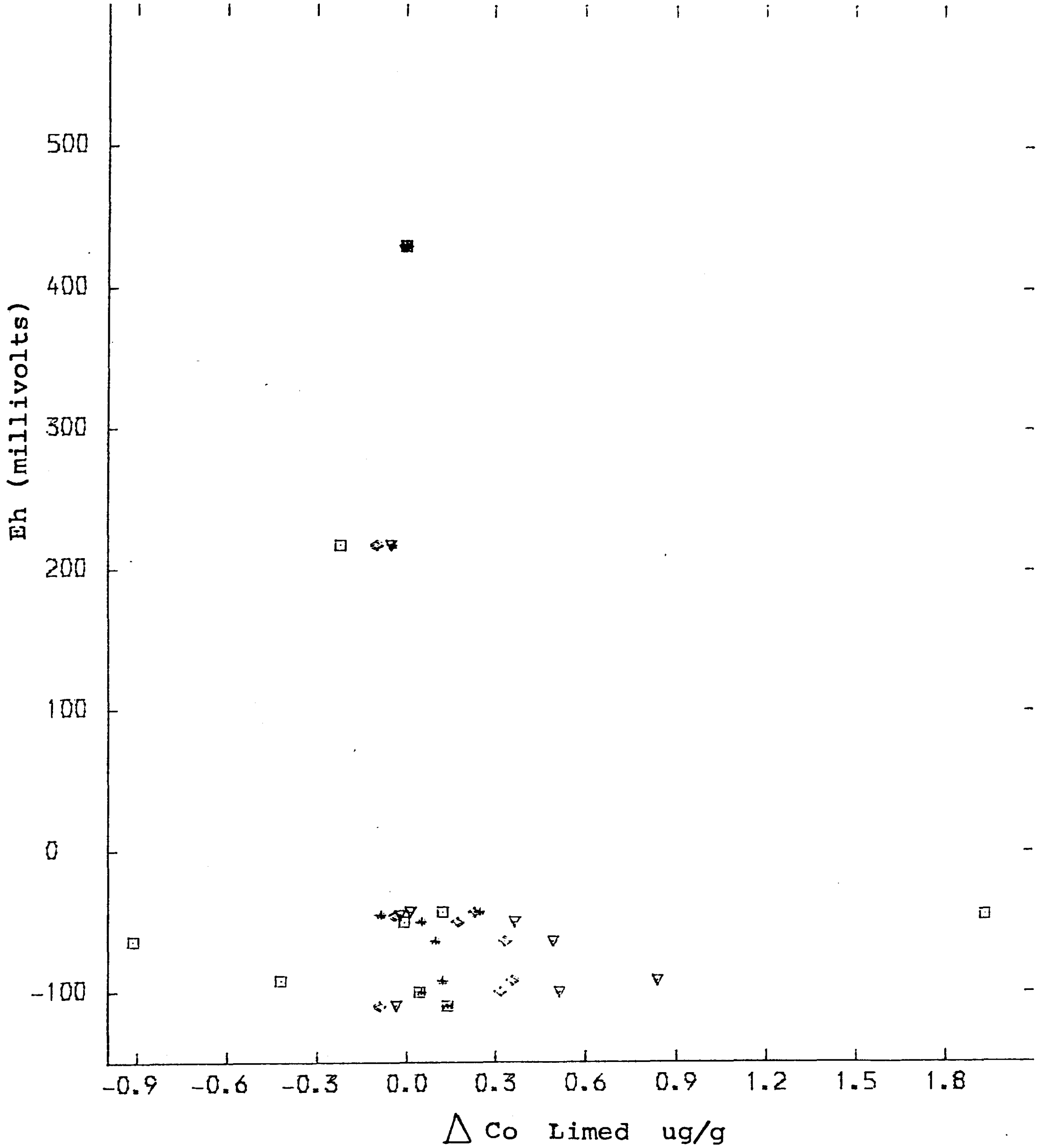


Figure 3.3.33 Changes in cobalt removed by the selective extractants with decrease in Eh. Waterlogged + 1% lime B_2 horizon



+ CALCIUM CHLORIDE

▽ ACETIC ACID

□ PYROPHOSPHATE

◇ ACID OXALATE

able cobalt found, Δpp-Co present in this section was modified by subtracting calcium chloride and acetic acid values (same modification as for other metals).

Changes in extractable cobalt in waterlogged soil with or without added organic matter were very much alike (Fig 3.3.28 and 3.3.29), and similar to those previously found in Section 3.2 (except pyrophosphate extractable fractions). Their relations with Eh are shown in Fig 3.3.31 and Fig 3.3.32. $\Delta CaCl_2$-Co and ΔHAc-Co increased and Δoxa-Co decreased on waterlogging. It seemed to suggest the increases of $\Delta CaCl_2$-Co and ΔHAc-Co were at the expense of the oxalate extractable fraction, and added organic matter would accentuate the situtation. The erratic changes of the pyrophosphate extractable fraction were not quite so easily explained. Very low concentrations of cobalt were found in pyrophosphate extracts and additional reagents were involved with further extraction procedures before analysis took place. A small amount of cobalt contamination would easily confuse the result.

Waterlogged soil with lime treatement showed different behaviour of the oxide fraction compared with other two waterlogged samples (Fig 3.3.30 and Fig 3.3.33). $\Delta CaCl_2$-Co, ΔHAc-Co and Δoxa-Co all increased. If the pyrophosphate-extractable fraction was excluded, cobalt fractions resembled iron in limed waterlogged treatment. In the previous section (Section 3.3.3.3) it was suggested that increased iron in the oxalate fraction may be due to release of iron from well crystallized oxides and adsorption of mobilized iron by calcium carbonate. Le Riche and Weir (1963) found large proportions of the total cobalt in extracted oxide fractions. Many reports suggested cobalt was associated with iron and manganese oxides (Kubota 1965, McKenzie 1975). It is possible that cobalt is associated with iron oxide in this particular soil. When well crystallized iron oxides broke down, cobalt was also released and adsorbed by calcium carbonate.

To conclude the findings in this and the previous section (Section 3.2) on the metal redistribution in B_2 soil by waterlogging, organic-bound and oxide fractions are both found to be important reserviors for indigenous metals. Metal values in calcium chloride extracts have positive relations with extractable level of metal in water extracts, while negative relations exist between water and acetic acid extracts of zinc. Break-down of well-crystallized oxides does not release the co-precipitated metals in all cases, more zinc and copper may well be adsorbed onto the increasing surface, however, the degree of adsorption depends on the competition of organic chelates.

Addition of easily decomposable organic matter or lime would quicken the redistribution of metals in waterlogged soils. But in limed samples, relations of different fractions and metals may change in some cases.

Table 3.3.2-3.3.4 show the correlation coefficients of various fractions and metals in matrix forms. In samples with or without added organic matter, manganese, iron and cobalt form a group, zinc and copper another, if based on the relations between their fractions in the former group it has been found that calcium chloride and acetic acid fractions compliment pyrophosphate and oxalate fractions; in the latter group calcium chloride and pyrophosphate fractions compliment acetic acid and oxalate. Relations of fractions in limed samples differ from the above. Positive relations between all the iron fractions show the net gain of iron in all examined fractions. Zinc and copper had lower calcium chloride extractable values in limed samples and their positive correlations between calcium chloride and acetic acid fractions are worth noting.

Table 3.3.2 Correlations between extractable metals in waterlogged soil with no amendment.

	CA-MN	AA-MN	PP-MN	OXA-MN	CA-FE	AA-FE	PP-FE	OXA-FE	CA-ZN	AA-ZN
AA-MN	0.995									
PP-MN	-0.970	-0.967								
OXA-MN	-0.960	-0.945	0.946							
CA-FE	0.992	0.981	-0.975	-0.983						
AA-FE	0.946	0.962	-0.921	-0.843	0.903					
PP-FE	-0.712	-0.723	0.721	0.824	-0.746	-0.639				
OXA-FE	-0.411	-0.383	0.522	0.551	-0.473	-0.277	0.608			
CA-ZN	-0.946	-0.935	0.932	0.933	-0.935	-0.913	0.755	0.516		
AA-ZN	0.987	0.989	-0.941	-0.935	0.977	0.928	-0.669	-0.320	-0.890	
PP-ZN	-0.887	-0.873	0.788	0.883	-0.897	-0.736	0.613	0.297	0.765	-0.924
OXA-ZN	0.961	0.956	-0.913	-0.949	0.967	0.849	-0.705	-0.442	-0.869	0.975
CA-CU	-0.511	-0.475	0.459	0.654	-0.582	-0.299	0.611	0.170	0.427	-0.520
AA-CU	0.854	0.851	-0.856	-0.889	0.857	0.798	-0.837	-0.708	-0.939	0.794
PP-CU	-0.477	-0.472	0.581	0.596	-0.507	-0.461	0.686	0.659	0.648	-0.378
OXA-CU	0.041	-0.032	0.004	-0.145	0.063	-0.060	-0.056	-0.357	-0.155	-0.049
CA-CO	0.211	0.213	-0.312	-0.382	0.286	0.124	-0.592	-0.231	-0.263	0.178
AA-CO	0.665	0.638	-0.776	-0.778	0.730	0.505	-0.646	-0.856	-0.694	0.607
PP-CO	0.417	0.462	-0.272	-0.303	0.377	0.409	-0.208	0.284	-0.236	0.534
OXA-CO	-0.196	-0.190	0.193	0.271	-0.243	-0.032	0.322	0.327	0.218	-0.209

	PP-ZN	OXA-ZN	CA-CU	AA-CU	PP-CU	OXA-CU	CA-CO	AA-CO	PP-CO
OXA-ZN	-0.960								
CA-CU	0.639	-0.541							
AA-CU	-0.710	0.834	-0.340						
PP-CU	0.205	-0.400	0.227	-0.710					
OXA-CU	-0.061	0.008	-0.194	0.155	-0.254				
CA-CO	-0.096	0.169	-0.622	0.253	-0.587	-0.178			
AA-CO	-0.540	0.689	-0.382	0.767	-0.748	0.187	0.428		
PP-CO	-0.625	0.534	-0.188	0.218	0.368	-0.544	-0.180	-0.128	
OXA-CO	0.351	-0.312	0.265	-0.345	-0.054	0.354	-0.227	-0.288	-0.420

Table 3.3.3 Correlations between extractable metals in waterlogged soils with 1% added organic matter.

	CA-MN	AA-MN	PP-MN	OX-MN	CA-FE	AA-FE	PP-FE	OX-FE	CA-ZN	AA-ZN
AA-MN	0.992									
PP-MN	-0.926	-0.924								
OX-MN	-0.909	-0.890	0.847							
CA-FE	0.956	0.928	-0.919	-0.891						
AA-FE	0.906	0.944	-0.836	-0.776	0.773					
PP-FE	-0.254	-0.285	0.223	0.209	-0.276	-0.227				
OX-FE	-0.441	-0.409	0.463	0.727	-0.471	-0.343	0.305			
CA-ZN	-0.733	-0.691	0.707	0.783	-0.880	-0.495	0.175	0.482		
AA-ZN	0.970	0.969	-0.954	-0.885	0.966	0.878	-0.292	-0.468	-0.790	
PP-ZN	-0.946	-0.914	0.833	0.894	-0.942	-0.756	0.181	0.446	0.785	-0.910
OX-ZN	0.790	0.735	-0.727	-0.818	0.858	0.561	-0.076	-0.592	-0.813	0.824
CA-CU	-0.513	-0.472	0.431	0.635	-0.490	-0.294	-0.051	0.376	0.419	-0.387
AA-CU	0.815	0.846	-0.833	-0.742	0.671	0.912	-0.309	-0.474	-0.325	0.779
PP-CU	-0.238	-0.219	0.400	0.336	-0.401	-0.148	-0.005	0.471	0.571	-0.439
OX-CU	0.391	0.323	-0.269	-0.430	0.424	0.092	-0.083	-0.351	-0.340	0.342
CA-CO	0.536	0.512	-0.553	-0.449	0.459	0.381	0.174	-0.052	-0.191	0.432
AA-CO	0.545	0.589	-0.718	-0.502	0.477	0.547	-0.062	-0.199	-0.255	0.574
PP-CO	0.081	0.115	0.213	-0.111	-0.136	0.255	0.047	-0.022	0.279	-0.048
OX-CO	0.495	0.505	-0.496	-0.572	0.609	0.385	-0.272	-0.445	-0.754	0.644

	PP-ZN	OX-ZN	CA-CU	AA-CU	PP-CU	OX-CU	CA-CO	AA-CO	PP-CO
AA-ZN									
PP-ZN									
OX-ZN	-0.890								
CA-CU	0.646	-0.442							
AA-CU	-0.621	0.438	-0.294						
PP-CU	0.252	-0.599	-0.233	-0.089					
OX-CU	-0.636	0.674	-0.645	0.049	-0.082				
CA-CO	-0.602	0.373	-0.792	0.427	0.244	0.581			
AA-CO	-0.482	0.297	-0.506	0.610	-0.112	0.213	0.733		
PP-CO	-0.133	0.013	-0.178	0.114	0.423	0.250	0.057	-0.127	
OX-CO	-0.574	0.713	-0.130	0.169	-0.743	0.348	-0.038	0.292	-0.047

Table 3.3.4 Correlations between extractable metals in waterlogged soils with 1% lime.

	CA-MN	AA-MN	PP-MN	OX-MN	CA-FE	AA-FE	PP-FE	OX-FE	CA-ZN	AA-ZN
AA-MN	0.985									
PP-MN	-0.835	-0.787								
OX-MN	-0.891	-0.893	0.565							
CA-FE	0.808	0.713	-0.746	-0.709						
AA-FE	0.898	0.953	-0.722	-0.773	0.557					
PP-FE	0.950	0.950	-0.764	-0.794	0.667	0.891				
OX-FE	0.157	0.100	-0.265	0.102	0.346	0.129	0.117			
CA-ZN	0.083	0.091	-0.172	-0.039	-0.164	0.042	0.184	-0.655		
AA-ZN	0.939	0.962	-0.794	-0.857	0.614	0.902	0.906	-0.077	0.279	
PP-ZN	-0.881	-0.853	0.742	0.856	-0.667	-0.687	-0.832	0.193	-0.415	-0.920
OX-ZN	0.954	0.905	-0.844	-0.830	0.806	0.759	0.894	0.237	0.110	0.879
CA-CU	0.607	0.606	-0.545	-0.656	0.538	0.524	0.535	-0.535	0.419	0.684
AA-CU	0.821	0.839	-0.700	-0.711	0.650	0.845	0.750	0.057	0.255	0.806
PP-CU	-0.322	-0.369	0.185	0.291	-0.241	-0.441	-0.227	-0.616	0.756	-0.262
OX-CU	-0.157	-0.242	0.045	0.177	-0.070	-0.338	-0.101	0.055	0.258	-0.290
CA-CO	0.312	0.290	-0.218	-0.191	0.237	0.280	0.481	-0.202	0.495	0.227
AA-CO	0.627	0.662	-0.756	-0.329	0.483	0.780	0.605	0.279	0.013	0.633
PP-CO	0.031	-0.041	-0.032	-0.236	0.137	-0.276	-0.099	-0.199	-0.023	0.066
OX-CO	0.741	0.742	-0.673	-0.536	0.717	0.787	0.728	0.246	-0.064	0.634

	PP-ZN	OX-ZN	CA-CU	AA-CU	PP-CU	OX-CU	CA-CO	AA-CO	PP-CO
AA-ZN									
PP-ZN									
OX-ZN	-0.901								
CA-CU	-0.717	0.476							
AA-CU	-0.657	0.705	0.605						
PP-CU	0.021	-0.285	0.233	-0.153					
OX-CU	0.075	0.005	-0.283	-0.231	0.538				
CA-CO	-0.273	0.220	0.376	0.398	0.575	0.383			
AA-CO	-0.371	0.474	0.453	0.739	-0.342	-0.458	0.211		
PP-CO	-0.327	0.226	0.102	-0.347	-0.012	0.140	-0.486	-0.510	
OX-CO	-0.457	0.563	0.575	0.800	-0.243	-0.357	0.489	0.858	-0.504

CHAPTER 4 THE INFLUENCE OF WATERLOGGING ON THE TRACE METAL UPTAKE IN PLANTS GROWN IN POTS

4.1 Introduction

Waterlogging is one of the many environmental factors occurring in soil. Its importance to plants is not just physical cut off of the oxygen supply to the soil, it also induces the soils to change chemically. When the pore spaces of soil are filled with water, the plant may be deprived of oxygen. The immediate effect of this is suppression of growth. Further than this, leaves become chlorotic, no new roots develop, shoots die back, and death ensues. In some cases, plants may survive brief periods of oxygen deficiency, however, because of the rapid increase of readily available metals in soil due to waterlogging, the effect of some toxic metals may actually be more detrimental than the effect of short-term lack of oxygen (Graven, Attoe and Smith 1965). Rice can adapt to flooded soils because it has large inter-cellular air spaces, through which oxygen can diffuse from regions of high to low oxygen supply, and generally it grows better under flooded conditions than non-flooded conditions. However, in some situations, it may suffer from excess of ferrous iron in solution (Ponnamperuma, Bradfield and Peech 1955) or shortages of other essential metals such as Zn and Cu (IRRI 1976).

Many soils are subjected to wetting and drying cycles of varying intensity and duration. Work by Nambiar (1975) showed that manganese mobility on wetting and drying soil will depend on the state of reduction to which the soil has been brought during the wet phase. Ng and Bloomfield (1962) found that a cycle of wetting and drying will increase the water-extractable and acetate-extractable metals such as Co and Cu. Mitchell (1964) remarked on the experience with Scottish profiles that any change in extractable trace elements can generally be ascribed to drainage factors. His results showed the mobilization of Co, Fe, Cu and Mn in the gleyed sub-surface layers of poorly drained profiles.

The increases of extractable metals in soils by the effect of waterlogging are also reflected by higher metal uptake by plants. Plants grown on poorly drained soils have higher metal content than on well drained soils, for example such as Mn and Co (Mitchell, Reith and Johnston 1957). According to Adams and Honeysett (1964), 2 weeks waterlogging would affect the metal status in soil and plants for longer than half a year. Even if the waterlogged soils were stored in air-dried condition for 56 days, reduction of yield and increases of metal content of plants were still significant.

Although the effect of waterlogging on trace metal availability to plant health is so important, research on this aspect is less than comprehensive. Following the previous chemical extraction experiments for Carbeth soil, a green-house experiment was set up to look at the effect on plant uptake of metals. A further experiment was set up to investigate the waterlogging effect on plant uptake while plants were growing rapidly and on the soil metal changes with plants growing on it.

4.2 The influence of waterlogging of soil on the trace metal status in subsequent French bean and maize growth

4.2.1 Procedure

Carbeth B_2 and B_{3g} horizon soils received one of three different moisture treatments, (i) 60% field capacity, (ii) periodic waterlogging, (iii) continuous waterlogging for 15 weeks as described in Section 3.2. When the 15 weeks period expired, all soils were allowed to drain freely and dry naturally for a month. Soil samples were transferred into a set of 2.5 inch diameter pots. A French bean or maize seed was planted in each pot and allowed to grow for 45 days with regular watering in a $26^{\circ}C \pm 2^{\circ}$ plant growth chamber. Triplicates were run for each treatment. After 45 days, plants were harvested at soil level and washed by distilled water. Harvests were then dried in an oven at $105^{\circ}C$ for 24 hours. Dried samples in the individual pots were stored separately and preserved for analysis.

4.2.2 Analysis

Plants in each pot were analysed individually. Reagents and methods were followed as described in Chapter 2. Digested acid solutions were analysed by flame or flameless atomic absorption depending on the metal's detection limit.

4.2.3 Results and discussion

4.2.3.1 French bean

The results for French beans are shown in Figure 4.2.1 and Table 4.2.1.

Although B_2 and B_{3g} soils had different properties, French beans grown on B_2 and B_{3g} control soils had similar manganese contents (no significant differences). In both soils, previous waterlogging increased the manganese content in bean plants significantly; by more than 100% on B_2 soil and just 40-50% on B_{3g} soil. This was in agreement with the results from soil extraction studies (Section 3.2.3, Chapter 3). There is greater mobilization of manganese due to waterlogging in B_2 soil than in B_{3g} soil, again showing the influence of natural waterlogging in the lower horizon (see Section 3.2.2). Ng and Bloomfield (1962) found that manganese released by waterlogging is not greatly affected by aeration. Drying only decreased the water extractable manganese, but the acetate extractable manganese simultaneously increased. Nambiar (1975) suggested reoxidation of manganese is mainly biological and the process is slow. Therefore the waterlogging effect is expected to persist and is reflected by manganese uptake by French bean.

The waterlogging effect was less pronounced on the plant's iron status, only periodical waterlogging of B_2 soil significantly increased the concentration of iron in French beans (Table 4.2.1). The results in Section 3.2.3 showed

Figure 4.2.1 Means of metal contents of French beans grown on soils subjected to differing waterlogging treatments (all figures: ug/g)

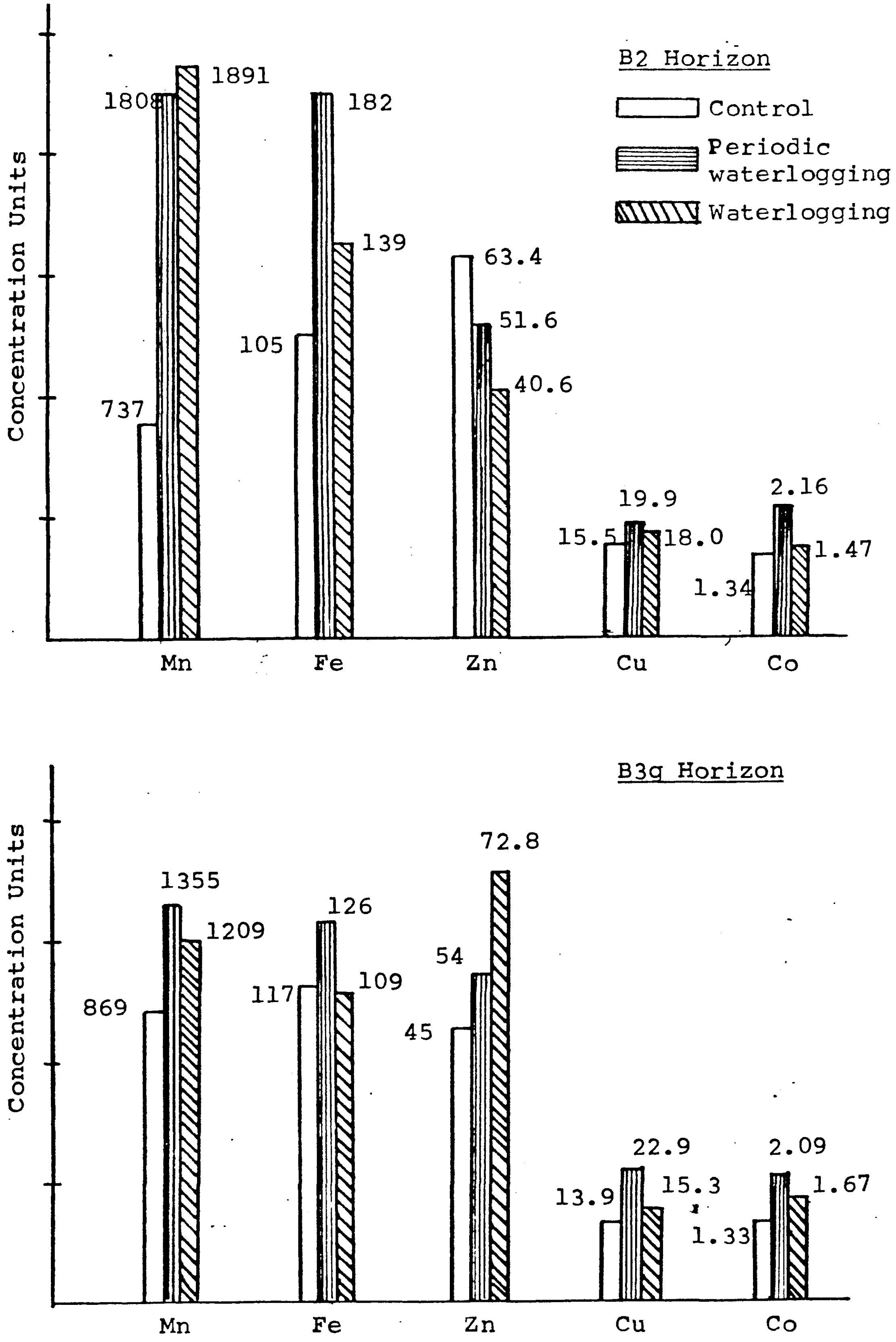


Table 4.2.1 Means and standard deviations of metal contents of French beans grown on soils subjected to differing waterlogging treatments

Soil	Waterlogging Treatment	Mn	Fe	Zn	Cu	Co
Carbeth B_2 Horizon	Control	737.9	105.7	63.41	15.48	1.335
		(86.8)	(15.8)	(3.45)	(2.32)	(0.050)
	Periodic Waterlogging	1808.0***	182.7 **	51.64	19.94 *	2.155 *
		(123.0)	(6.5)	(17.30)	(0.30)	(0.440)
	Waterlogging	1891.0 **	139.9	40.66 *	18.06	1.471
		(369.0)	(38.3)	(9.22)	(5.30)	(0.520)
B_{3g} Horizon	Control	869.4	117.5	45.05	13.98	1.334
		(110.0)	(18.5)	(4.84)	(3.63)	(0.340)
	Periodic Waterlogging	1355.0 *	126.2	54.10	22.98	2.094
		(179.0)	(23.9)	(14.45)	(5.10)	(0.380)
	Waterlogging	1209.0 *	109.3	72.88	15.32	1.670
		(126.0)	(13.2)	(19.70)	(1.65)	(0.390)

( ): standard deviations of triplicates.

*, **, ***, 95%, 99% and 99.9% significant differences with corresponding control.

large amounts of iron mobilized under waterlogged conditions. However its effect was less marked than that of manganese on subsequent bean growth. One of the reasons may be that iron is influenced by reoxidation to a greater degree, therefore, the previous waterlogging effect is diminished. Ng and Bloomfield (1962) found that mobilized iron which existed in water and neutral-acetate extractable forms during the wet phase, fell to negligible levels after soil reoxidation.

French beans had higher levels of zinc uptake on B_2 control soil than on B_{3g} control soil. The mean values for the other treatments suggest that previous waterlogging decreased uptake of zinc in French beans on B_2 soil, but increased uptake on B_{3g} soil. Decreases in zinc uptake correlated with the decrease of water and calcium chloride extractable values in B_2 waterlogged soil. Previous increases of extractable zinc in B_{3g} waterlogged soils were also reflected by increased uptake in French bean. However, only continuous waterlogging of the B_2 soil for 15 weeks changed the zinc uptake at 5% significance level.

Copper was found to decrease in the calcium chloride extractable fraction on waterlogging B_2 soil, but subsequent French bean growth on that soil showed higher copper uptake. In both B_2 and B_{3g} soils, uptake of copper was found to be highest in periodically waterlogged treatments (Table 4.2.1). This may be caused by soluble, highly stable copper chelates formed in the wetting and drying cycles.

Table 4.2.1 shows that previous waterlogging increased the cobalt content of the French bean, this was especially significant on periodically waterlogged B_2 soil. These results supported other workers' findings. Mitchell, Reith and Johnston (1957) found that plants growing on poorly drained soils contain more cobalt than those on well drained soils. Adams and Honeysett (1964) provided more details of increasing cobalt uptake by subsequent plant growth on previously waterlogged soils. The reason for increasing cobalt uptake may well be associated with dissolution of oxide materials. Cobalt has been shown to be associated particularly with manganese oxides in soil (McKenzie 1975).

4.2.3.2 Maize

Figure 4.2.2 and Table 4.2.2 show the effect of previous waterlogging on the metal content of maize plants.

Manganese content in maize was positively affected by previous waterlogging. Manganese content of plants grown on previously waterlogged soils were 30-50% higher than those on unwaterlogged soils, except for the plants on B_{3g} periodically waterlogged soil which had more than twice the manganese content compared with its corresponding control. These changes were similar to those found in beans, although the levels of metal content were different, being lower in maize.

Figure 4.2.2 Means of metal contents of Maize grown on soils subjected to differing waterlogging treatments (key to blocks as on Figure 4.2.1)

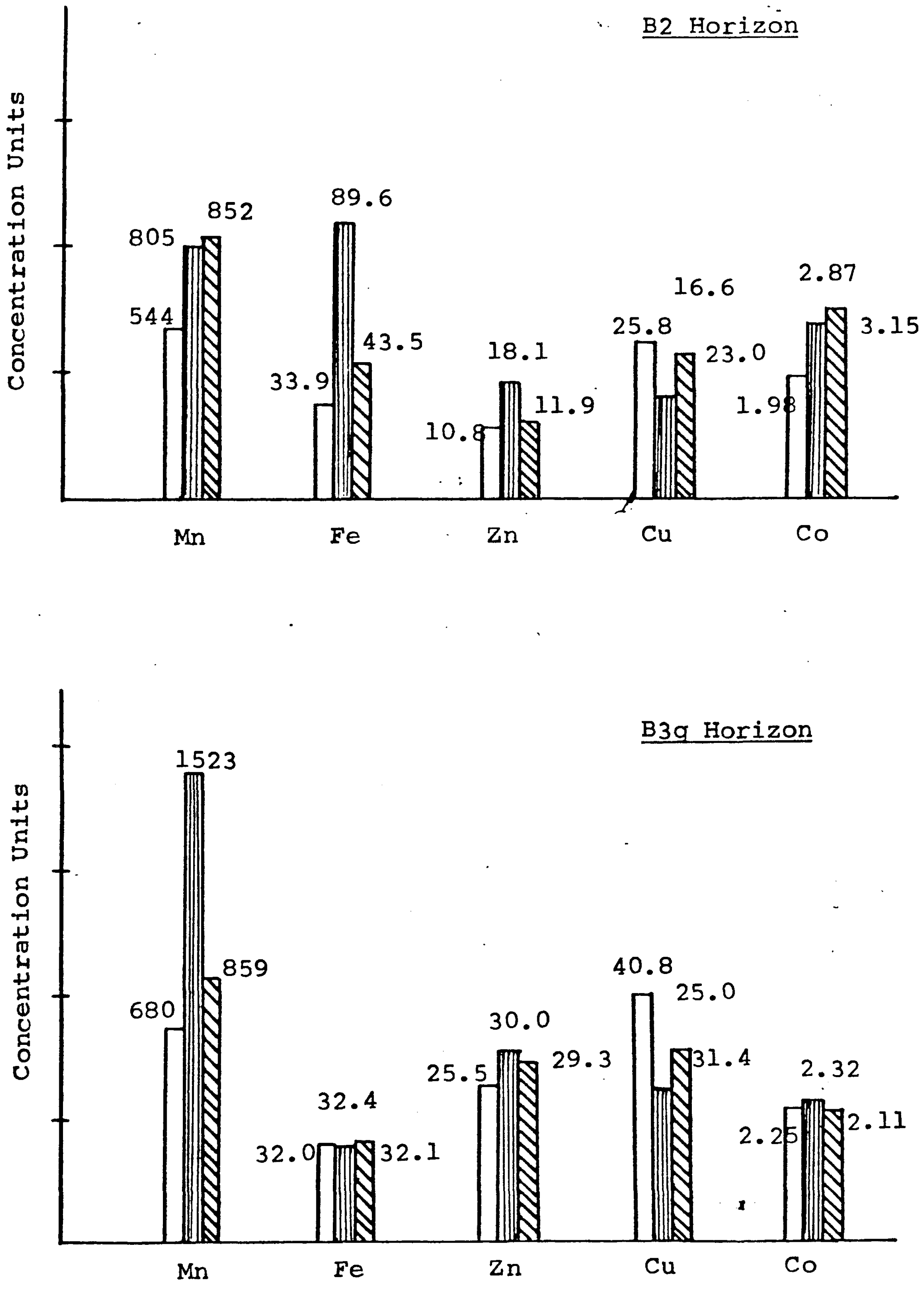


Table 4.2.2 Means and standard deviations of metal contents of maize grown on soils subjected to differing waterlogging treatment

Soil	Waterlogging Treatment	Mn	Fe	Zn	Cu	Co
Carbeth B_2 Horizon	Control	544.5 (47.2)	33.95 (1.56)	10.88 (0.99)	25.87 (21.80)	1.980 (0.390)
	Periodic Waterlogging	805.1 ** (77.8)	89.69 * (33.40)	18.13 ** (2.37)	16.66 (13.30)	2.879 * (0.380)
	Waterlogging	852.2 * (141.6)	43.57 (9.30)	11.96 (3.10)	23.07 (8.13)	3.156 * (0.390)
B_{3g} Horizon	Control	680.8 (67.5)	32.09 (7.13)	25.54 (6.90)	40.88 (18.30)	2.250 (0.660)
	Periodic Waterlogging	1523.0 * (355.0)	32.45 (15.60)	30.09 (11.40)	25.02 (11.90)	2.320 (0.040)
	Waterlogging	859.2 * (98.5)	32.19 (1.41)	29.31 (14.60)	31.46 (24.80)	2.110 (0.420)

( ): standard deviation of triplicates.

*, **, 95% and 99% significant differences with corresponding control.

Previous waterlogging affected iron uptake by maize only with B_2 soil, and no changes were found on B_{3g} soil. In B_2 soil, iron values increased from 33ppm to 89ppm and 43ppm under periodic and continuous waterlogging respectively (Table 4.2.2). Explanations for the differences of iron uptake by maize from soil which had been waterlogged would be similar to those in French bean plants.

Judging by mean values, waterlogging increased zinc uptake by maize. The most significant increase was in plants grown on B_2 periodic waterlogged soil, zinc values increased from 10.8 ppm to 18.1 ppm in dry matter. As mentioned in the previous section, reduction of French bean uptake of zinc by waterlogging treatments correlated with water and calcium chloride extractable values during the wet phase, but maize grown on B_2 soil did not follow this trend. These results might be ascribed to the different nature of the two species.

Copper exhibited a different behaviour to zinc in response to waterlogging. Soil treatment with periodic waterlogging decreased copper uptake most, followed by continuous waterlogging. The suggestion of copper chelates for increasing copper uptake by French bean could not be applied to maize. Possible explanations shall be stated in the following section.

Cobalt uptake by maize was significantly increased on B_2 soil by previous waterlogging, but not on B_{3g} soil, maize grown on B_2 soil showed similar patterns of cobalt uptake to French bean plants. Explanations for this may be as in the previous section for French beans.

4.2.3.3 Interactions of soils and plants

The different species of plants differ very considerably in the proportion of a given element they take up from a soil. Certainly, the soil condition also induces large changes in the uptake of trace metals by plants. Results from Figures 4.2.1 -4.2.2, and Tables 4.2.1-4.2.2 show soils and plants will have different degrees of influence on each other. Moreover, waterlogging treatments can have varying effects on the absorption of trace metals by plants.

French beans grown on Carbeth soil had much higher contents of Mn, Fe and Zn but lower Cu and Co than maize. Comparing absorption by plants grown on the two soils, French bean had similar contents of Mn, Fe, Cu and Co, but higher Zn on B_2 soil than on B_3 soil; maize had similar contents of Mn, Fe and Co on both soils, however, higher Zn and Cu uptake were found on B_{3g} soil. It is interesting to find that plants grown on these two soils, which have different extractable values and drainage conditions (Chapter 3), would have similar amounts of metal uptake.

In addition to the different abilities of French bean and maize to take up metals from the Carbeth B_2 and B_{3g} soils, their responses to the effects of previous waterlogging are varied. A general trend was observed. Uptake of Mn, Fe and Co by French beans and maize showed increases due to previous waterlogging of the two soils, but uptake of Zn and Cu by these two species showed different patterns.

Referring to the soil metal status in waterlogged conditions the calcium chloride extractable Mn, Fe and Co increased, but Zn and Cu decreased. This may suggest that the increases of calcium chloride extractable metal in soils will increase the metal uptake by plants. Relationships between metal uptake by the plants and changes in other extractable metal fractions will depend on selection by the plant species.

The opposite effects of waterlogging on zinc and copper uptake by maize and French bean show that the properties and behaviour of plants are extremely important in determining the fractions of soil zinc and copper which plants can obtain. That zinc uptake by maize increased, but decreased in French beans, due to waterlogging, suggests that maize has the ability to take up zinc from a soil fraction which French beans can not utilize. Increased zinc uptake by maize corresponds with increases of oxalate extractable zinc in soil which had been previously waterlogged, suggesting that maize can take up zinc from the oxide fraction. Evidence also shows that Cu and Zn apparently have a competitive effect. Bowen's (1969) report may support this point. He found zinc and copper absorption by sugarcane leaf tissue were mutually competitive, and therefore he concluded, Cu^{2+} and Zn^{2+} were absorbed through the same carrier sites.

From the statistical point of view, most of the significant changes (95% or higher) of metal uptake due to previous waterlogging were on B_2 soils. This may be caused by B_2 soil having more pronounced metal changes under waterlogged conditions. Again this reflects the differences in natural field conditions of the B_2 and B_{3g} soils. The B_{3g} having been subjected to a higher degree of waterlogging and leaching of the products of reduction.

4.3 The influence of temporary waterlogging on the metal status of soil and French bean plants

4.3.1 Procedure

230g of field moist soil (127g oven-dried basis) of the Carbeth B_2 horizon identical to that employed in the previous experiment (Chapter 3) were placed in each of 36 3.5 inch diameter pots. 2 French bean seeds were sown in each pot and allowed to grow for 11 days under free drainage conditions prior to the commencement of waterlogging.
4 pots were maintained under free drainage conditions throughout the experiment, and the other 32 pots were waterlogged for different periods of time. The plants were grown

in a growth cabinet at 26°C ± 2°. Waterlogging conditions were maintained by placing the pots in sealed containers and adding deionized water slowly. Then the exercise was set up as follows:

a) 4 pots were allowed to grow without waterlogging for 32 days.

b) 4 pots for 4 days waterlogging and 28 days well drained condition.

c) 4 pots for 8 days waterlogging and 24 days well drained condition.

d) 4 pots for 12 days waterlogging and 20 days well drained condition.

e) 4 pots for 16 days waterlogging and 16 days well drained condition.

f) 4 pots for 20 days waterlogging and 12 days well drained condition.

g) 4 pots for 24 days waterlogging and 8 days well drained condition.

h) 4 pots for 28 days waterlogging and 4 days well drained condition.

i) 4 pots for 32 days waterlogging.

To drain a pot it was simply removed from the sealed container. Soil solutions were collected and filtered through a Whatman No.42 filter paper. At day 32, all plants were harvested and washed with deionized water. These were then dried in a 105°C oven for 24 hours. Yield of tops and roots were weighed on oven-dried basis. Soil samples were analysed using chemical extraction methods as described in Section 3.2. Extractants in this case were water, acetic acid, pyrophosphate and oxalate.

4.3.2 Analysis

Plants for each treatment were bulked and sub-sampled for acid digestion. The digestion method was described in Section 2.3.2, Chapter 2. Acid digest solutions and soil extracts were analysed by flame and flameless atomic absorption. pH of the soil was measured in the pot by a glass pH electrode at different times when the pot was still flooded.

4.3.3 Results and discussion

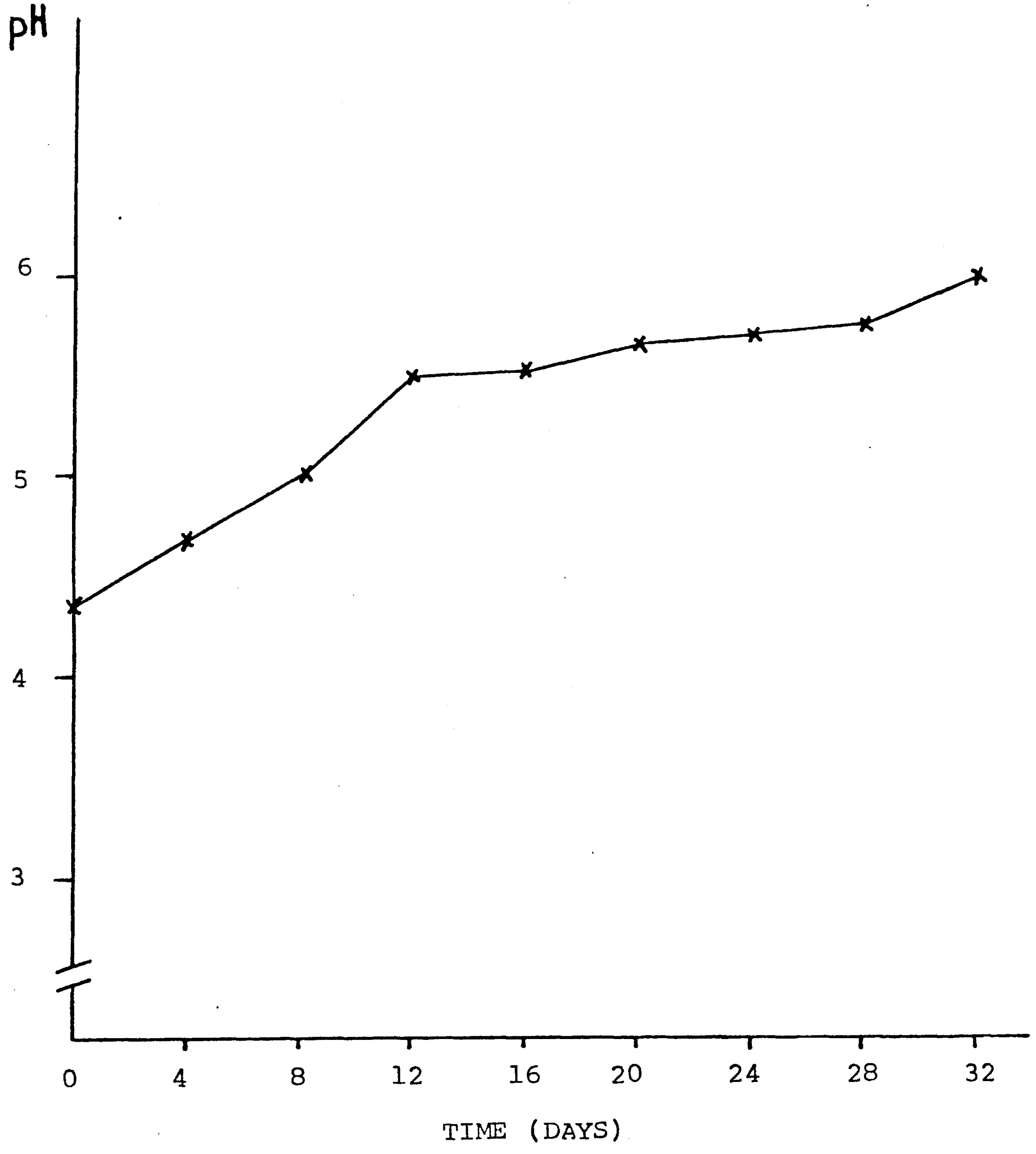
Results of soil and plant analysis will be discussed in separate sections. Extractable values of soils are presented as differences between temporary waterlogged and control (as in Section 3.2.3).

4.3.3.1 Soil analysis

4.3.3.1.1 pH of soil

Figure 4.3.1 shows the pH changes of the soils. pH values increased sharply from 4.3 to 5.5 within 12 days of waterlogging, then increased more slowly and reached pH 6 at the end of 32 days waterlogging. The pattern of changes was similar to that in soil without plants grown on it (Figure 3.2.1), but the rate of increase was much quicker in soil with plant growth than without plant growth. The higher rate of pH rise in soil with plant growth is probably caused by root respiration and stimulation of the

Figure 4.3.1 Variation in pH over 32 days temporary waterlogging.

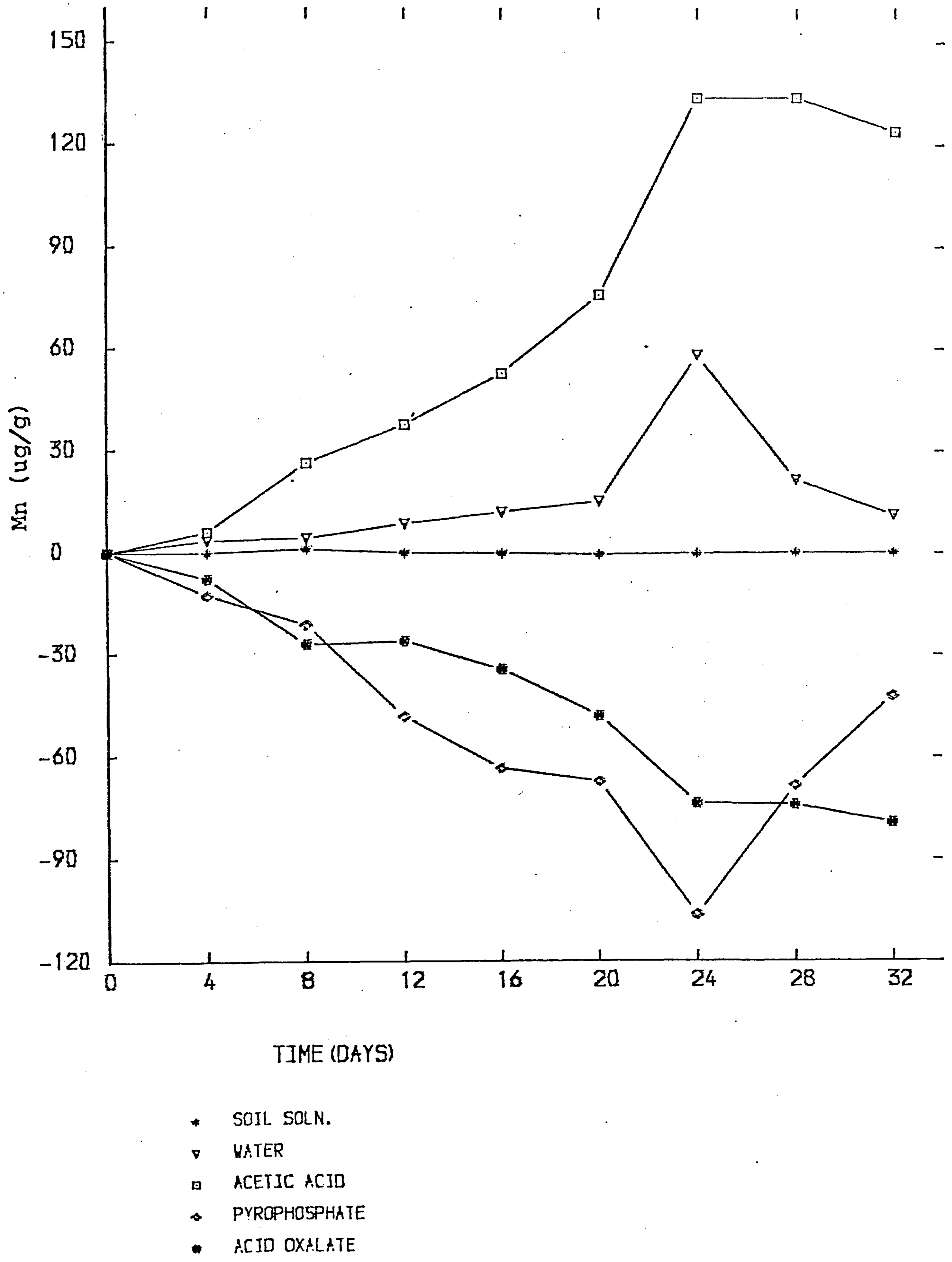


activities of micro-organisms by root exudates, which would accelerate soil reduction. Root respiration quickens the exhaustion of trapped oxygen and thus allows the higher oxide material to reach the reduced state more quickly. Roots stimulate the microbial population and increase the breakdown of insoluble oxides (Bromfield 1958a,b, Chaney, Brown and Tiffin 1972). The sharp changes of soil pH accompany the redistribution of trace metals.

4.3.3.1.2 Manganese

The changes of extractable manganese values in temporarily waterlogged soils are shown in Figure 4.3.2. Results show short term waterlogging and drainage do affect the manganese status in soil. ΔH_2O-Mn and ΔHAc-Mn increased, Δpp-Mn and Δoxa-Mn decreased, however, water leachate showed little change. The longer the waterlogging period within the 32 days incubation, the higher the effect on manganese changes. Comparing the extractable manganese values in waterlogged soil (Chapter 3, Section 3.2) and waterlogged and re-drained soil (this section), the latter had higher rates of change. Subsequent drainage did not reduce the significant effect of waterlogging on manganese. After 24 days of waterlogging in soil with bean growth, manganese in the four extracts reached either maximum or minimum values. These results show that plant growth will increase the rate of manganese changes in waterlogged soil. As mentioned in the previous section, this effect may be mainly attributed to root respiration, root exudation and higher activities

Figure 4.3.2 Variation in the levels of manganese removed by the selective extractants over 32 days temporary waterlogging, expressed as the difference in content between temporary waterlogged and aerated treatments.

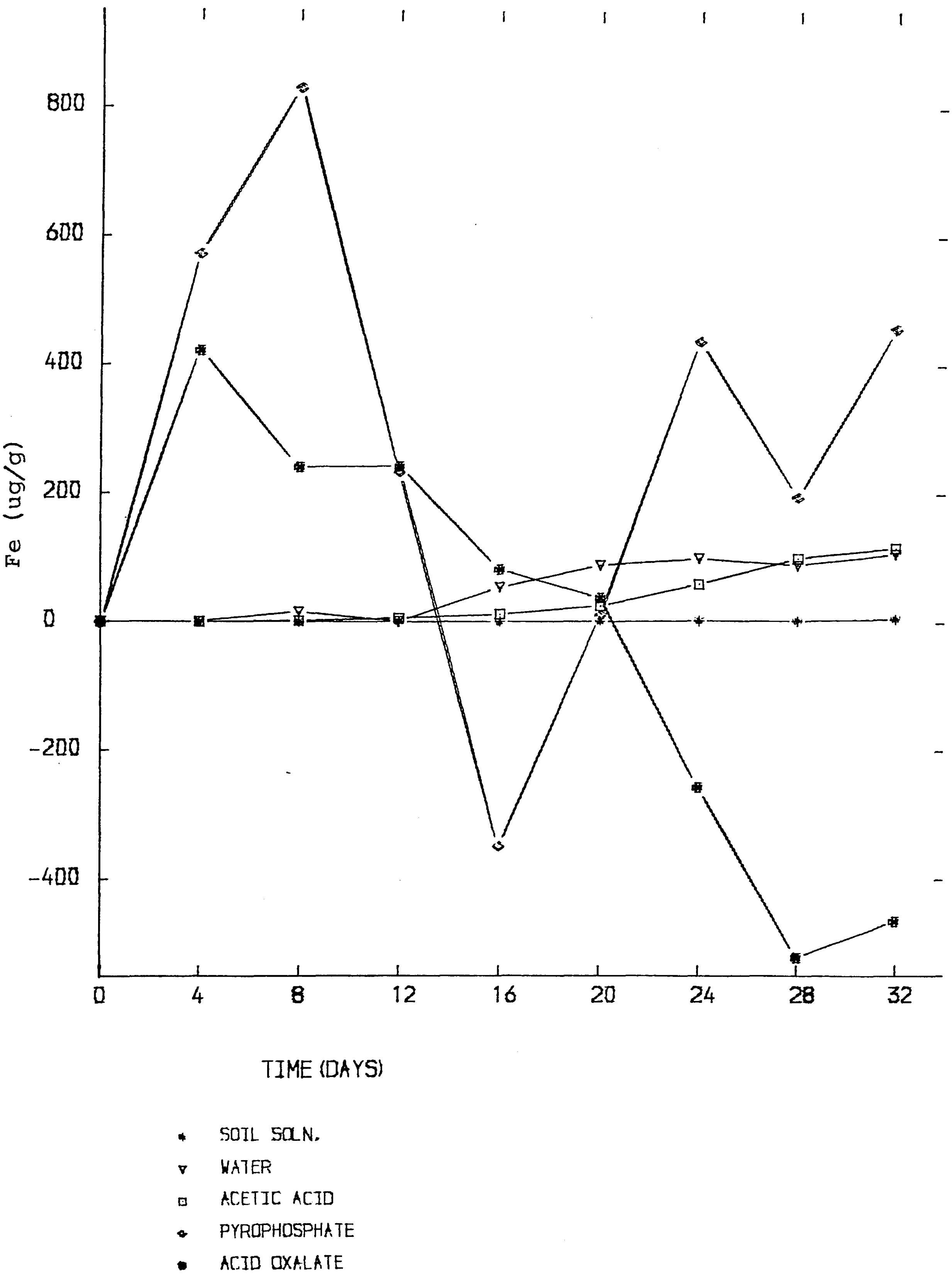


of micro-organisms. Persistance of high levels of ammonium acetate-extractable Mn in incubated soils after re-oxidizing was also reported by Ng and Bloomfield (1962) and Nambiar (1975).

4.3.3.1.3 Iron

Results in Chapter 3 showed that iron fractions in B_2 soil had similar changes to manganese brought about by waterlogging. This is also found in the temporary waterlogging of B_2 soil. Iron values in water leachate, water or acetic acid extracts increased when the soil had been previously waterlogged (Figure 4.3.3). The values increased with waterlogging time. It is noted that ΔH_2O-Fe had a quicker response than HAc-Fe to previous waterlogging and that iron values in water extracts were higher than in acetic acid extracts in some samples (see Appendix). This may be associated with formation of soluble organic iron complexes. Acetic acid extractable iron was not affected by plant growth over a short time period, which caused a similar amount of ΔHAc-Fe to be released as found in waterlogged soil without plant growth over a corresponding period (Chapter 3, Figure 3.2.4). This result may be due to inhibition by manganese which prevents large amounts of mobilized iron building up before easily reducible manganese is exhausted. Δoxa-Fe showed decreases, which may be released to water or acetic acid fractions. Δpp-Fe showed fluctuation. That may be caused by very high pp-Fe values (about 15,000 ppm) so small changes could not be detected

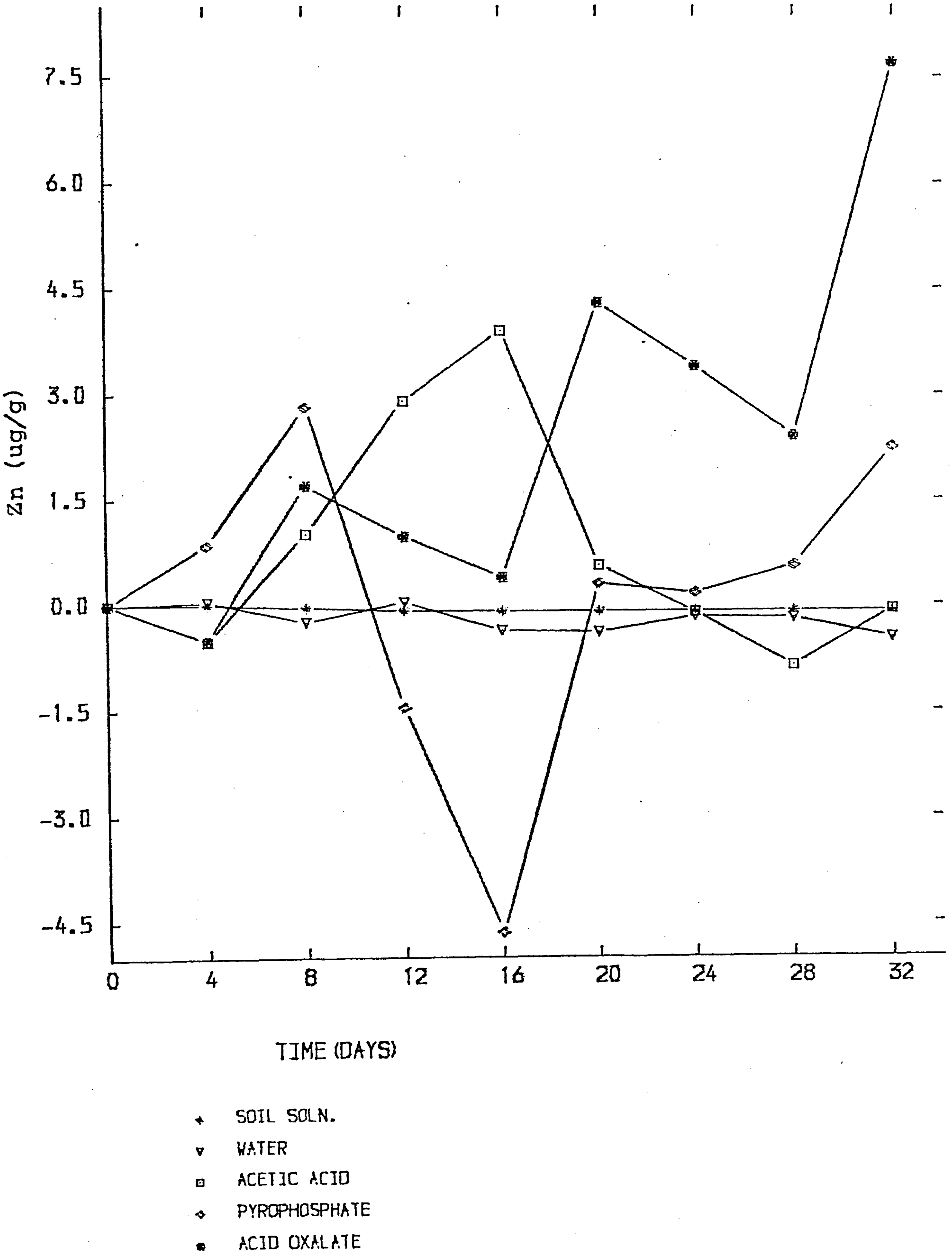
Figure 4.3.3 Variation in the levels of iron removed by the selective extractants over 32 days temporary waterlogging, expressed as the difference in content between temporary waterlogged and aerated treatments.



4.3.3.1.4 Zinc

Zinc values in water leachate and water extracts decreased with waterlogging time, ΔHAc-Zn and Δpp-Zn initially increased, then dropped back to low levels as waterlogging time increased. Δoxa-Zn increased and reached 8 ppm at the end of 32 days waterlogging (Figure 4.3.4). As mentioned in Chapter 3, water extractable zinc may decline due to an increase of adsorbing sites on oxide materials. However, the much higher oxalate extractable zinc in soil at the end of waterlogging compared with the control soil suggests that the increased value was not all due to a reduction in water extractable fractions. Other fractions of zinc did not show corresponding decreases in value as the oxalate fraction increased, indicating that some of the zinc might come from fractions which were not extractable at the beginning of the experiment. Comparing the levels of the oxalate extractable zinc fraction with oxalate extractable manganese and iron fractions, it was noticed that significant negative correlations existed, as shown on Table 4.3.1 (Zn v Mn r: -0.81, $p \geqslant 99\%$, Zn v Fe r: -0.68 $p \geqslant 95\%$). The rates of change of the oxalate extractable fractions were especially marked over the period after 12-16 days waterlogging. This may suggest that the fractions of zinc which were not extractable at the beginning of the experiment were released from the manganese and iron oxides which broke down due to waterlogging. Pyrophosphate extractable zinc was decreased by continuous waterlogging (Figures 3.2.6 and 3.3.19, Chapter 3) but not by temporary waterlogging with

Figure 4.3.4 Variation in the levels of zinc removed by the selective extractants over 32 days temporary waterlogging, expressed as the difference in content between the temporary waterlogged and aerated treatments.



plants grown and with re-drained conditions (Figure 4.3.4), suggesting that plants and/or re-drainage may influence the zinc changes in soil. The effect of temporary waterlogging and redraining on zinc in soil was more complicated than continuous waterlogging alone. Nevertheless, re-oxidation has been found to remedy zinc deficiency in some soils used for rice growth (IRRI 1976).

4.3.3.1.5 Copper

Extractable copper values in temporary waterlogged soil are shown in Figure 4.3.5. Increases of water and oxalate extractable copper correspond to decreases of pyrophosphate extractable copper and agree with results found in Chapter 3. The explanation given in Chapter 3 for copper can also be applied here. This may suggest that the copper in organic matter is released by decomposition to a readily soluble form and some released copper adsorbed onto the imperfectly crystalline oxide surfaces. Copper in water leachate and acetic acid extracts did not increase as expected. This could be caused by the short waterlogging time, which was not long enough to allow copper to get into these fractions.

4.3.3.1.6 Cobalt

Figure 4.3.6 shows water leachate, water and acetic acid extractable cobalt were increased by temporary waterlogging, but oxalate extractable cobalt was decreased. Pyrophosphate extractable cobalt values were not included

Figure 4.3.5 Variation in the levels of copper removed by the selective extractants over 32 days temporary waterlogging, expressed as the difference in content between the temporary waterlogged and aerated treatments.

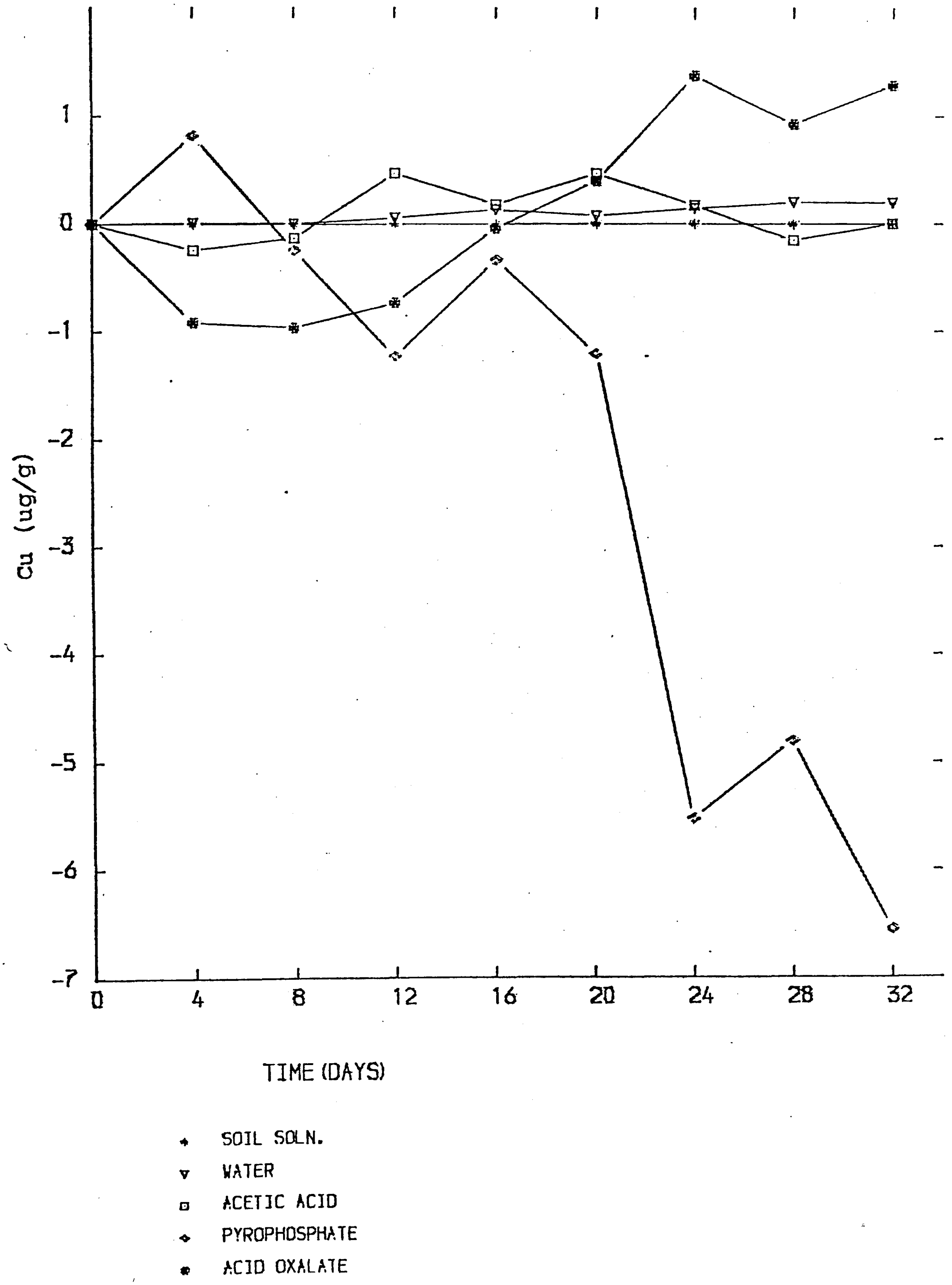
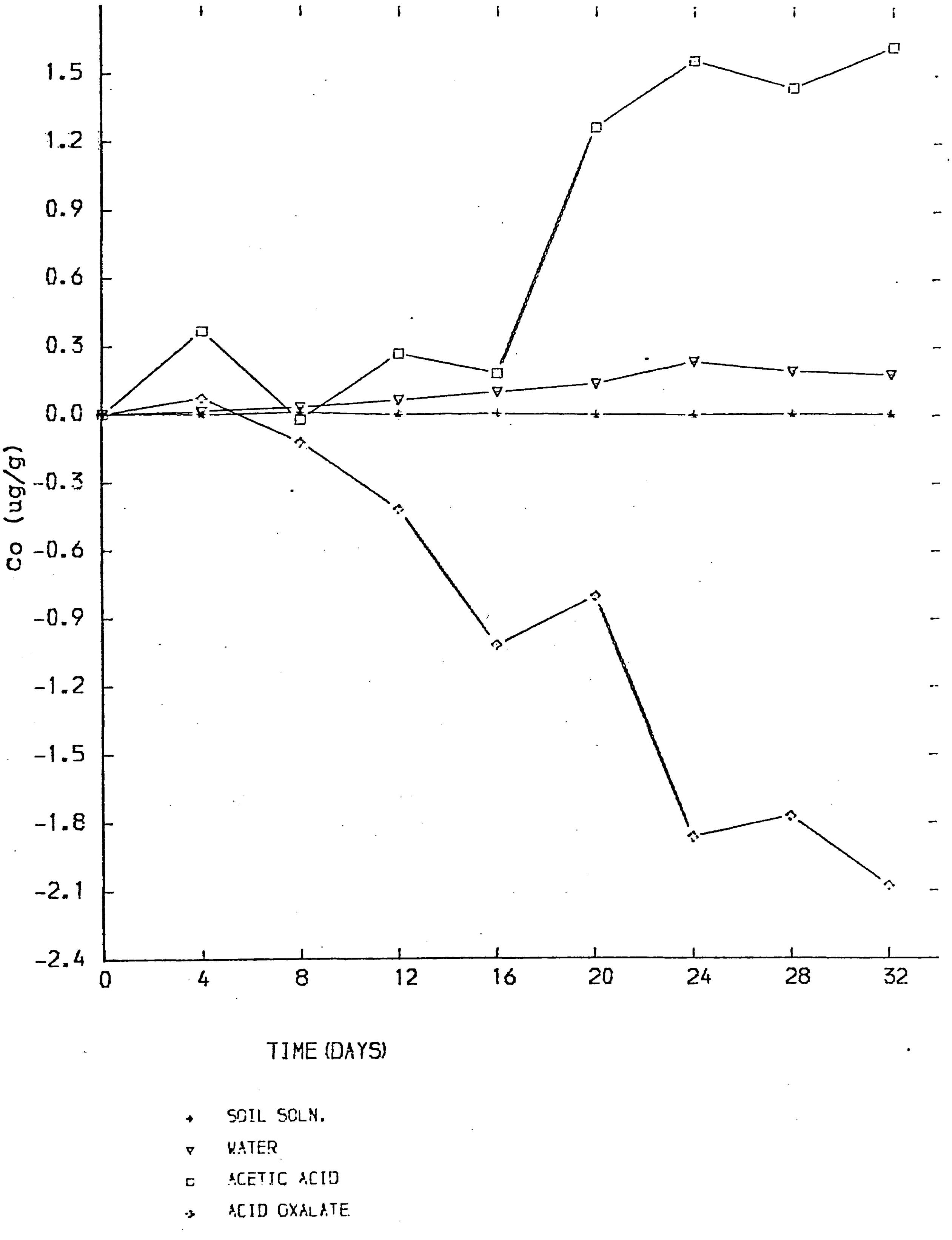


Figure 4.3.6 Variation in the levels of cobalt removed by the selective extractants over 32 days temporary waterlogging, expressed as the difference in content between the temporary waterlogged and aerated treatments.



because of low values.

This result is similar to the findings of Chapter 3, where in continuous waterlogging it was suggested that increases of $CaCl_2$-Co and HAc-Co were at the expense of the oxalate extractable fraction. Temporary waterlogging and redraining have shown the same trend. Adams and Honeysett (1964) reported waterlogging and redrying two Australian soils increased the 1 N ammonium acetate and N/10 hydrochloric acid extractable Co. Ng and Bloomfield (1962) also showed wetting and drying increased extractable Co.

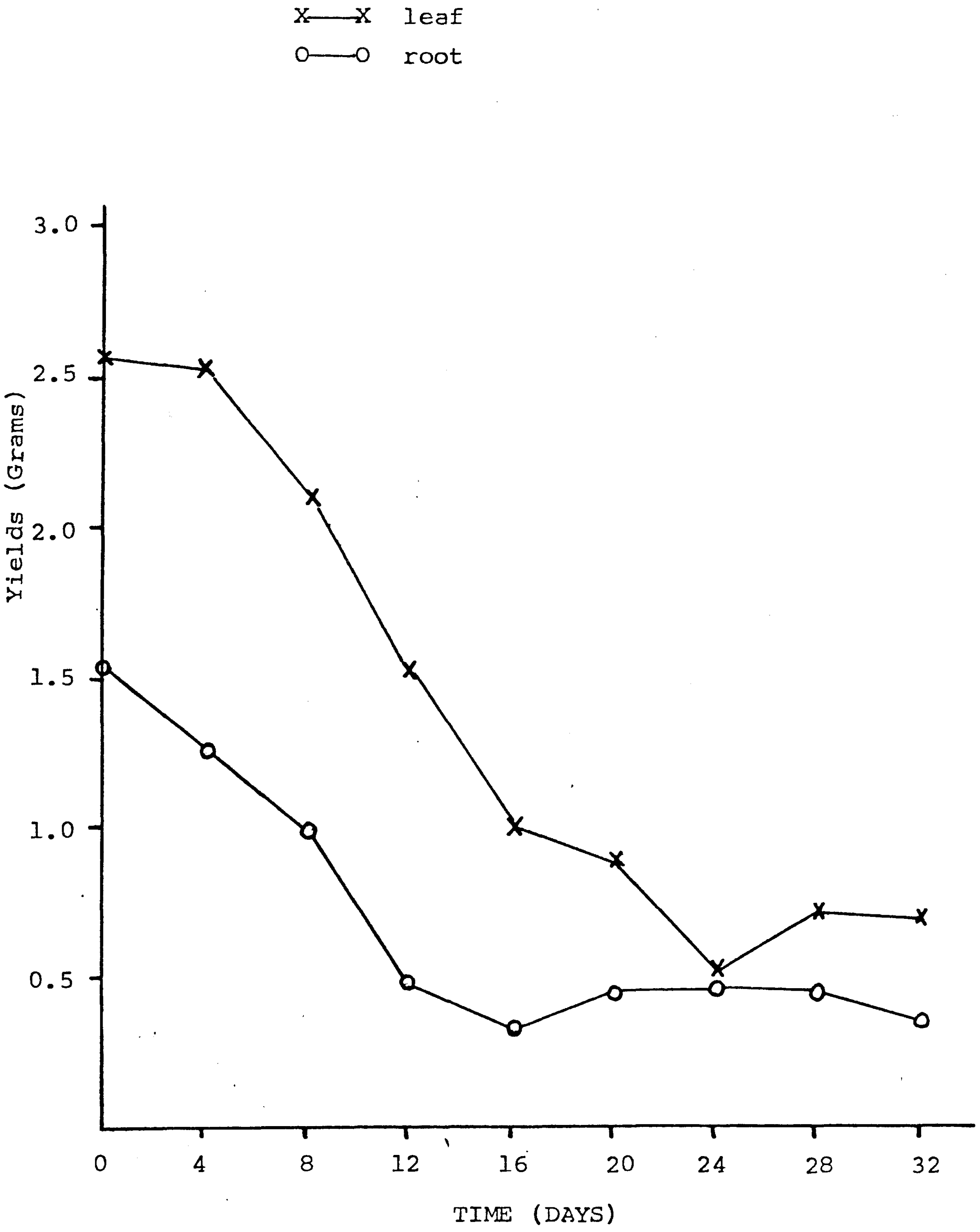
Comparing the metal changes in waterlogged soil with and without plants growing on it, it might be concluded that the transformation processes have occurred more rapidly due to respiration of plant roots. The behaviour of metal changes in soil under temporary waterlogging with plant growth resemble those under continuous waterlogging. Most changes could be explained as in Chapter 3.

4.3.3.2 Plant Analysis

4.3.3.2.1 Yield

The yield of leaves and roots were reduced by waterlogging as would be expected (Figure 4.3.7). It was noticed that waterlogging for 4 days did not reduce the yield of leaves to any great extent. Up to 12 days waterlogging, the appearance of the plants seemed healthy. New leaves could grow after drainage. However, compared with 4 days waterlogged samples, the yield of leaves on 8 days

Figure 4.3.7 Effect of temporary waterlogging on yields of bean's leaves and roots.

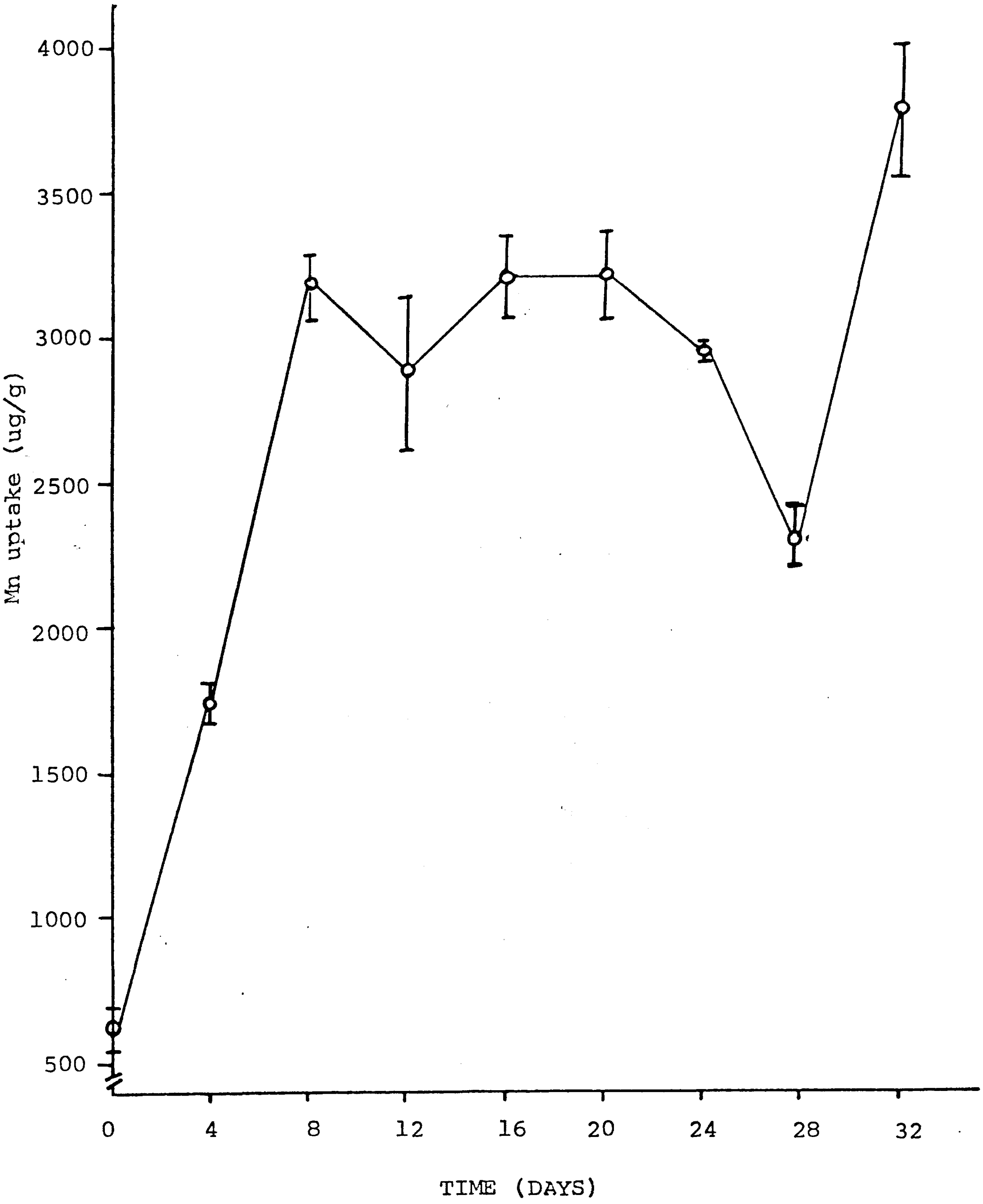


and 12 days waterlogged pots were significantly reduced. Plants subjected to more than 12 days waterlogging showed damage and might not recover after drainage. The yield of roots was immediately affected by waterlogging and continued to decline until 12 or 16 days waterlogging when a minimum value for yield of roots was found. Adventitious roots developed around the base of the stem after 4-8 days continuous anaerobic conditions. Roots near the soil surface attempted to grow upward. The reduction of plant yield could be caused mainly by lack of oxygen and higher toxic metal contents.

4.3.3.2.2 Manganese

Manganese concentration in plant tissues sharply increased from 650 ppm to more than 3,000 ppm after 8 days waterlogging (Figure 4.3.8). Longer periods of waterlogging did not increase manganese concentration further, except at 32 days. Comparing this with soil analysis data shows that bean plants may only respond to a certain high level of mobile manganese. Over this limit, bean plants could not take up the excess amount. The mechanism to inhibit beans taking up higher amounts of Mn due to higher levels of mobile Mn in soil is unknown. It may be that bean roots have selectivity which excludes manganese ions transferring to plant tissues from soil solution due to saturation of the transport mechanism (Maas, Moore and Masort 1968). The selectivity did not extent to cobalt, which will be discussed in a later section. Manganese in beans was negatively corre-

Figure 4.3.8 Effect of temporary waterlogging on manganese uptake in bean plants.

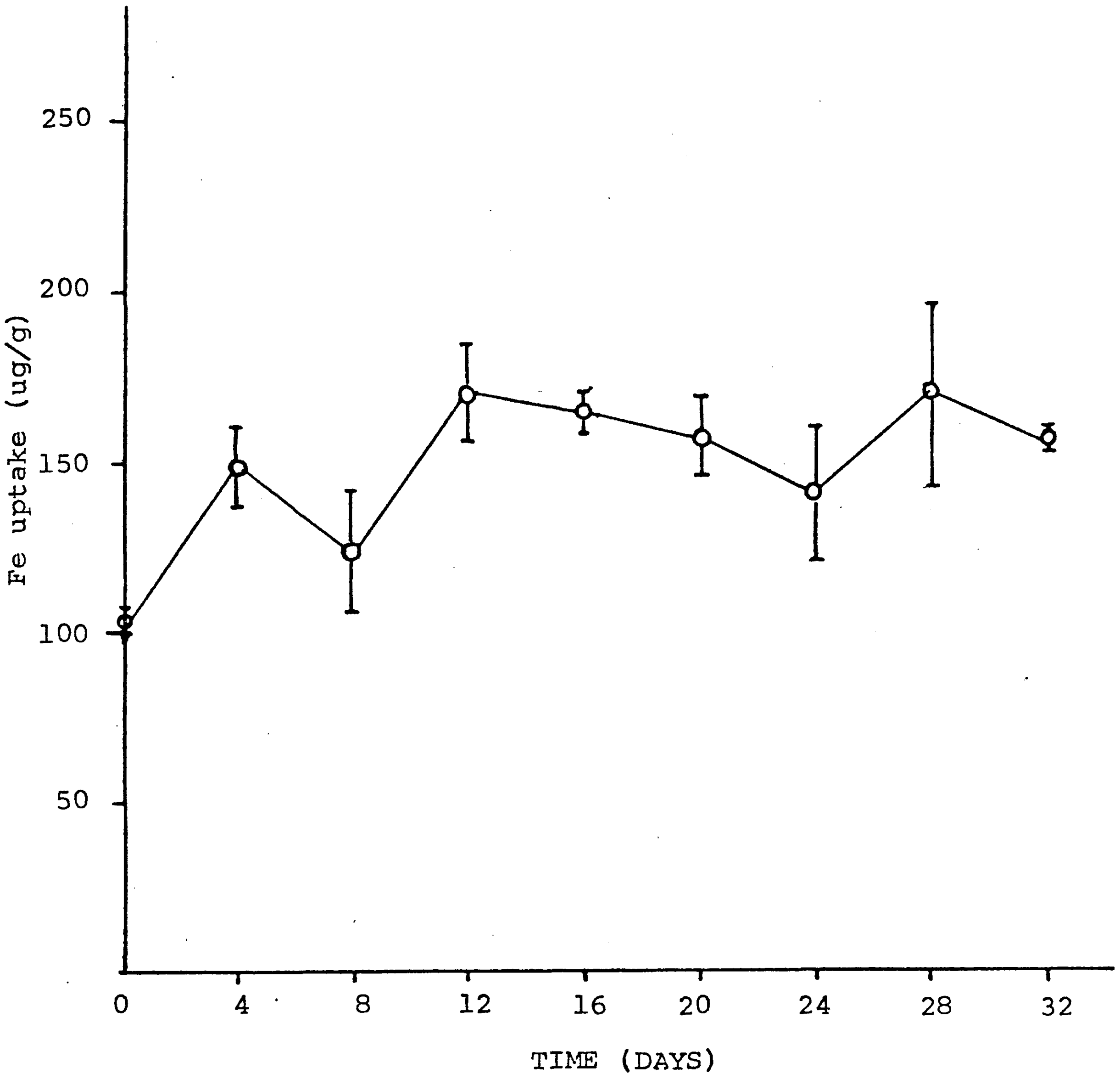


lated with pyrophosphate and oxalate extractable-Mn in waterlogged soil suggesting that it is the reduced forms of pp-Mn and oxa-Mn which beans available to plants. This relationship further supports the findings of sequential extraction of soil metal (Section 3.3.3, Chapter 3).

4.3.3.2.3 Iron

The iron content of bean tissue ranged from 100 ppm to 170 ppm in control and various waterlogged treatments (Figure 4.3.9). The changes of iron uptake by beans due to waterlogging are much less pronounced than for manganese, although they both demonstrated increases of exchangable forms. This result agrees with Graven, Attoe and Smith (1965). In their report, it was further shown that waterlogging increased the Mn : Fe ratio from 4.5 to 28 in alfalfa, as here found in beans where Mn : Fe ratio increased from 6.1 to 24.4. Chaudhrey and McLean (1963) reported that waterlogging increased the Mn content of rice but did not influence the iron content to a great extent. This could suggest that plants may have preferential absorption of manganese rather than iron, when both metals are in excess amounts. It may be also caused by large amounts of soluble manganese coming into solution before iron, then the transport mechanism is firstly saturated by manganese.

Figure 4.3.9 Effect of temporary waterlogging on iron uptake in bean plants.

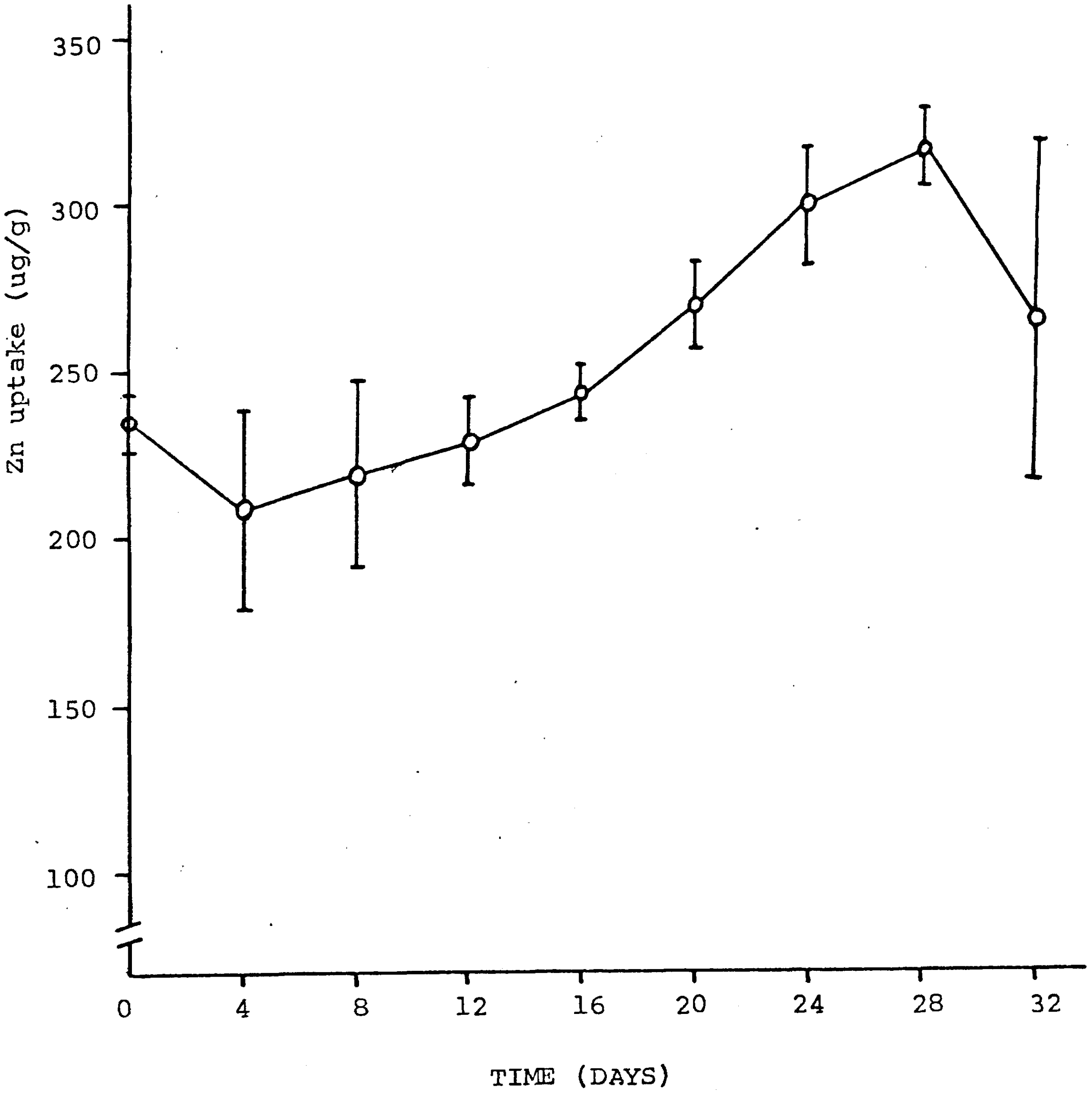


4.3.3.2.4 Zinc

Figure 4.3.10 illustrates that the zinc content in beans was slightly decreased by waterlogging for a short period (4-8 days), then gradually increased with longer periods of waterlogging. This was contrary to the result of Section 4.2.3.1, in which it was shown that bean plants grown on previously waterlogged soil had lower uptake of zinc.

This contradictory result may be explained by few points: Firstly, the increases of plant uptake of zinc coincided with increases of oxalate extractable zinc in soil and some zinc in this fraction was not removed by the sequential extraction procedure at the beginning of the experiment (Figure 4.3.4). This zinc may come from well crystalline oxides (Section 4.3.3.1.4). By bringing this extra zinc into the extractable fractions, total zinc usable by beans is increased. Secondly, the plants had for a certain period of time been grown under waterlogging condition during this period, plant roots would have more chance of contact with metal ions in soil solution and could absorb metal ions which were being redistributed. These effects could culminate in a higher metal content in plants.

Figure 4.3.10 Effect of temporary waterlogging on zinc uptake in bean plants.



4.3.3.2.5 Copper

The mean values of plant copper were initially increased by waterlogging, as waterlogging time increased they dropped and then increased again at the end of experiment. However in statistical terms, there were no significant differences (Figure 4.3.11). Adams and Honeysett (1964) reported copper was found to increase by 2% in subterranean clover after 9 days' waterlogging during the growth of the plant. Mitchell, Reith and Johnston (1957) also found higher Cu content in plants under poorly-drained conditions. On the other hand, Kubota, Lemon and Allaway (1963) found copper contents in plants did not differ consistently because of soil wetness. Results in this section could not support either of the above arguments, but the previous experiment (Section 4.2.3.1) did show beans grown on previously waterlogged soil had significant increases in copper content.

4.3.3.2.6 Cobalt

Cobalt content in beans was immediately increased under waterlogged conditions (Figure 4.3.12). It was highly correlated with water and acetic acid extractable values in sequential soil extraction, but negatively correlated with oxalate extractable values (Table 4.3.1). Increases of cobalt content in plants under waterlogged or poorly drained soils have been observed previously (Hill, Toth and Bear 1953; Alban and Kubota 1960; Kubota, Lemon and Allaway 1963; Adams and Honeysett 1964). With the results of soil analysis,

Figure 4.3.11 Effect of temporary waterlogging on copper uptake in bean plants.

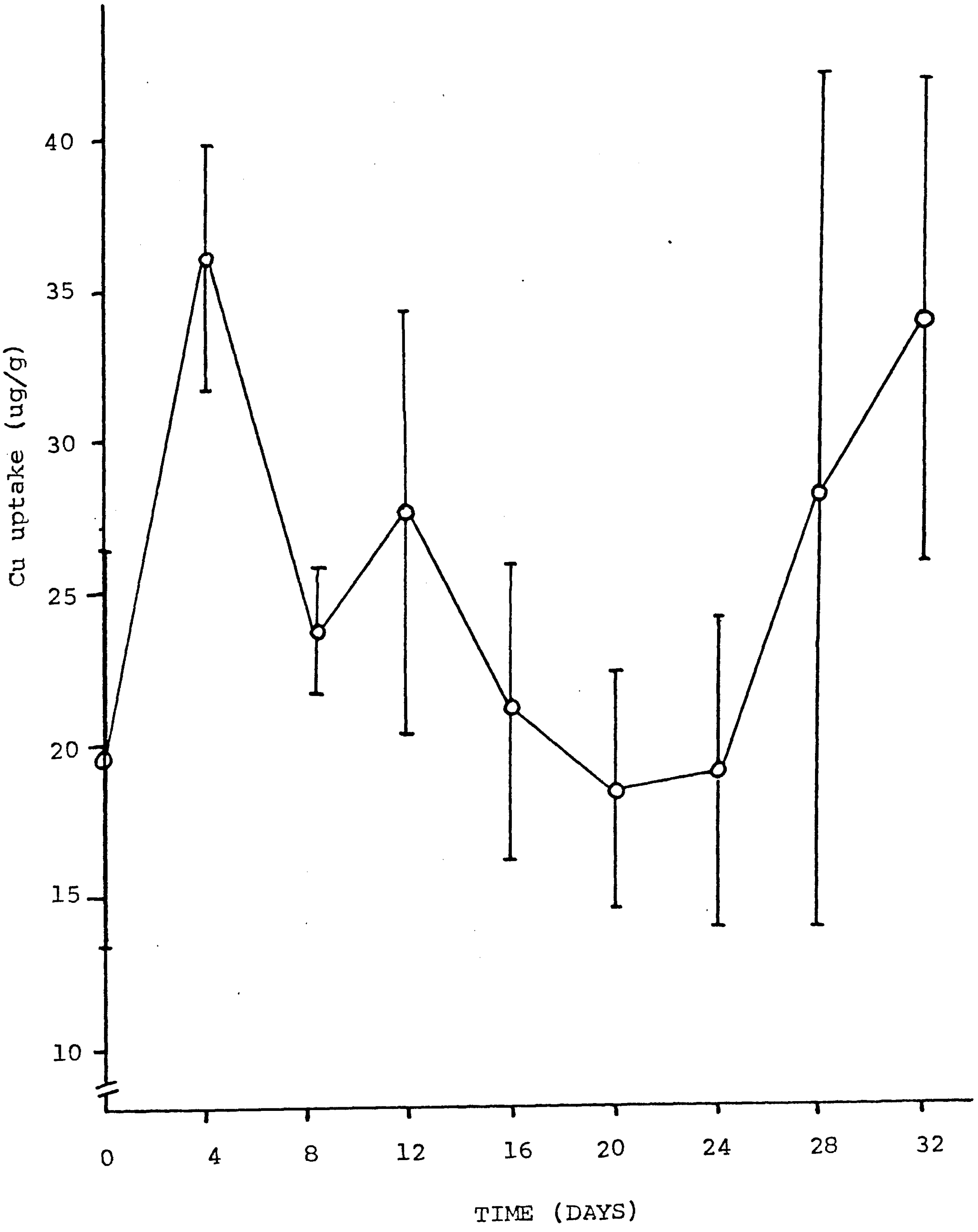
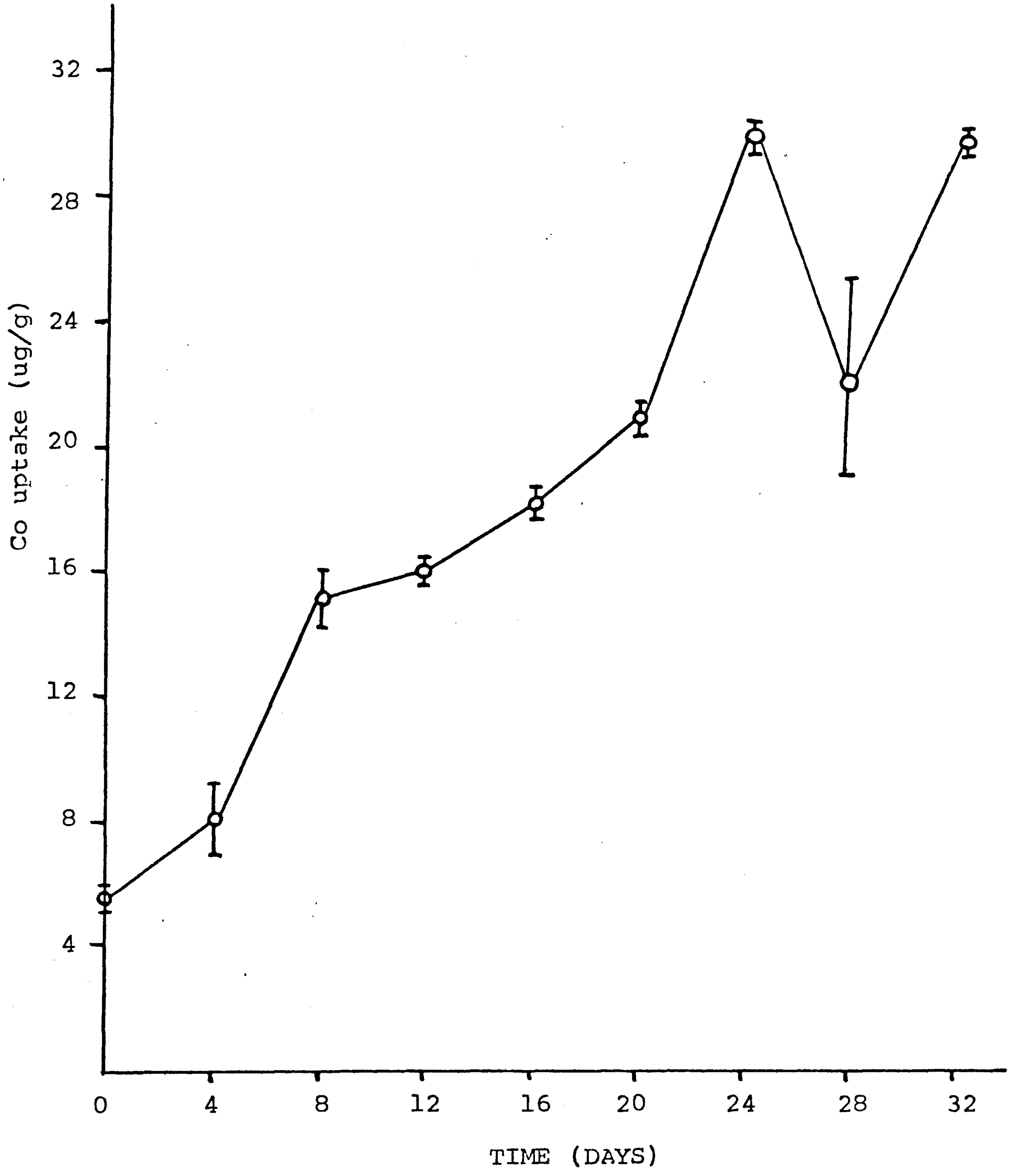


Figure 4.3.12 Effect of temporary waterlogging on cobalt uptake in bean plants.



it might have been inferred that Co associated with oxides material was mobilized or dissolved into available forms, such as water and acetic acid extractable forms, by waterlogging. Therefore, plants grown on waterlogged or poorly-drained soils would have higher cobalt contents.

4.4 Concluding section

Waterlogging has different effects on the distribution of metals in soil. Manganese, iron and cobalt all increased in water, acetic acid or calcium chloride fractions due to waterlogging. There were corresponding decreases in the pyrophosphate and oxalate fractions. This has been thought of as decomposition of organic matter and dissolution of oxide materials (Iu, Pulford and Duncan 1981a). Zinc and copper in soil have rather different responses to waterlogging, in that their pyrophosphate or calcium chloride fractions decreased at the point at which the oxalate fraction increased. The increases of oxalate extractable zinc and copper may be attributed to increases of active oxide surfaces which can adsorb more cations (Iu, Pulford and Duncan 1981b). Generally, patterns of metal changes in waterlogged soils with or without plant growth are similar. The rate of metal changes is faster in waterlogged soil with plant growth and that may be caused by respiration and excretion of plants.

Increases of water, calcium chloride and acetic acid extractable manganese, iron and cobalt in soils due to previous waterlogging are reflected by plant uptake (Table

4.2.1-4.2.2). This indicates that metals in those fractions are easily used by plant. However, reduction of zinc and copper levels in water and calcium chloride extractable fractions to other forms during waterlogging, did not necessarily reduce plant uptake. Beans and maize showed opposite responses to uptake of zinc or copper, on soil with previous waterlogging. This would suggest that beans and maize have rather different selection abilities in relation to extraction of metals from the soil fractions. It is likely that waterlogging during the growth period of some plants could increase metal uptake. Bean plants have higher levels of all five metals, manganese, iron, zinc, copper and cobalt at the harvest time, when under waterlogging for part of their growth period.

The results show that interactions between plant and soil could affect metal uptake by plants and metal status in the soil. Actions of plants, through direct excretion of substances or indirect stimulation of microbiological activities, increased the rate of metal redistribution processes (manganese and iron) and changed the patterns of zinc redistribution in waterlogged soil. Alternatively the status of metals in soil would determine metal uptake by plants, although the effects depended on plant species.

Table 4.3.1 Correlation between the concentration of metals in soil extractable fractions and French beans. Soil solution (SOL-), water (H2O-), acetic acid (AA-), pyrophosphate (PP-), oxalate (OXA-) and plant (PL-) for abbreviations.

	SOL-MN	H2O-MN	AA-MN	PP-MN	OXA-MN	PL-MN	SOL-FE	H2O-FE	AA-FE	PP-FE
H2O-MN	-0.239									
AA-MN	-0.224	0.726								
PP-MN	-0.116	0.649	0.944							
OXA-MN	0.140	-0.664	-0.988	-0.935						
PL-MN	0.147	0.293	0.540	0.378	-0.640					
SOL-FE	-0.207	0.424	0.892	0.898	-0.924	0.593				
H2O-FE	-0.318	0.639	0.931	0.856	-0.935	0.585	0.896			
AA-FE	-0.163	0.429	0.902	0.953	-0.909	0.402	0.953	0.830		
PP-FE	0.567	0.190	0.243	0.426	-0.309	0.257	0.266	0.116	0.328	
OXA-FE	0.203	-0.477	-0.869	-0.915	0.843	-0.222	-0.864	-0.810	-0.929	-0.115
PL-FE	-0.364	0.173	0.498	0.281	-0.506	0.546	0.433	0.420	0.413	-0.142
SOL-ZN	0.222	-0.559	-0.801	-0.582	0.817	-0.756	-0.695	-0.771	-0.590	0.081
H2O-ZN	-0.007	-0.153	-0.558	-0.466	0.638	-0.739	-0.711	-0.760	-0.533	-0.003
AA-ZN	0.033	-0.194	-0.294	-0.525	0.262	0.345	-0.284	-0.264	-0.450	-0.557
PP-ZN	0.760	-0.318	-0.212	-0.103	0.093	0.328	-0.057	-0.255	-0.112	0.684
OXA-ZN	-0.106	0.317	0.738	0.758	-0.805	0.661	0.904	0.808	0.787	0.381
PL-ZN	-0.383	0.790	0.843	0.745	-0.772	0.204	0.585	0.809	0.633	-0.065
SOL-CU	-0.387	0.267	0.572	0.532	-0.624	0.576	0.740	0.767	0.559	0.120
H2O-CU	-0.317	0.539	0.925	0.846	-0.915	0.518	0.895	0.868	0.898	0.017
AA-CU	-0.453	0.204	0.098	-0.146	-0.092	0.366	0.025	0.161	-0.158	-0.466
PP-CU	0.102	-0.613	-0.916	-0.981	0.914	-0.408	-0.910	-0.801	-0.949	-0.430
OXA-CU	-0.426	0.674	0.889	0.895	-0.851	0.275	0.855	0.901	0.849	0.051
PL-CU	0.076	-0.317	0.018	0.144	-0.067	0.074	0.220	-0.103	0.302	0.470
SOL-CO	0.745	0.018	0.271	0.237	-0.379	0.697	0.324	0.243	0.249	0.388
H2O-CO	-0.305	0.809	0.984	0.892	-0.963	0.554	0.837	0.937	0.821	0.169
AA-CO	-0.404	0.653	0.923	0.905	-0.909	0.431	0.870	0.914	0.870	0.294
OXA-CO	0.265	-0.663	-0.970	-0.924	0.964	-0.546	-0.933	-0.921	-0.917	-0.153
PL-CO	-0.114	0.725	0.925	0.844	-0.952	0.764	0.871	0.903	0.782	0.310

----- cont. Table 4.3.1

	OXA-FE	PL-FE	SOL-ZN	H2O-ZN	AA-ZN	PP-ZN	OXA-ZN	PL-ZN	SOL-CU	H2O-CU
PL-FE	-0.237									
SOL-ZN	0.562	-0.707								
H2O-ZN	0.497	-0.266	0.632							
AA-ZN	0.416	0.273	-0.291	-0.102						
PP-ZN	0.263	-0.261	0.143	-0.055	-0.024					
OXA-ZN	-0.680	0.255	-0.609	-0.742	-0.289	0.242				
PL-ZN	-0.761	0.312	-0.667	-0.354	-0.304	-0.476	0.442			
SOL-CU	-0.472	0.234	-0.526	-0.741	-0.214	0.046	0.894	0.409		
H2O-CU	-0.861	0.633	-0.801	-0.589	-0.103	-0.368	0.646	0.717	0.488	
AA-CU	0.094	0.338	-0.546	-0.152	0.552	-0.094	0.181	0.237	0.370	0.066
PP-CU	0.901	-0.284	0.592	0.434	0.439	0.042	-0.775	-0.662	-0.516	-0.843
OXA-CU	-0.917	0.207	-0.603	-0.529	-0.393	-0.386	0.721	0.835	0.645	0.833
PL-CU	0.005	0.359	0.170	0.144	-0.261	0.221	0.117	-0.398	-0.042	0.142
SOL-CO	-0.180	0.102	-0.419	-0.582	0.281	0.577	0.357	-0.021	0.084	0.255
H2O-CO	-0.802	0.500	-0.829	-0.545	-0.224	-0.283	0.696	0.876	0.587	0.895
AA-CO	-0.791	0.441	-0.644	-0.494	-0.502	-0.208	0.798	0.786	0.735	0.807
OXA-CO	0.890	-0.481	0.789	0.611	0.198	0.267	-0.746	-0.763	-0.578	-0.967
PL-CO	-0.709	0.437	-0.850	-0.674	-0.096	-0.004	0.821	0.690	0.677	0.827

	AA-CU	PP-CU	OXA-CU	PL-CU	SOL-CO	H2O-CO	AA-CO	OXA-CO
PP-CU	0.099							
OXA-CU	0.091	-0.872						
PL-CU	-0.492	-0.196	-0.145					
SOL-CO	-0.114	-0.261	0.012	0.005				
H2O-CO	0.206	-0.859	0.886	-0.085	0.210			
AA-CO	0.107	-0.860	0.881	0.111	0.015	0.913		
OXA-CO	-0.077	0.924	-0.911	-0.057	-0.281	-0.951	-0.872	
PL-CO	0.244	-0.850	0.797	-0.037	0.437	0.935	0.838	-0.918

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APPENDIX 1

Analytical results of metal concentration in soil extracts on B_2 and B_{3g} soils with aerated, periodically waterlogged and continuously waterlogged treatments. (Abbreviation: C. for aerobic control, P.W. for periodic waterlogging and W. for continuous waterlogging.)

Appendix 1. Manganese

Extractant	Time (weeks)	B_2 C.	B_2 P.W.	B_2 W.	B_{3g} C.	B_{3g} P.W.	B_{3g} W.
Water	0.	0.90	0.90	0.90	0.40	0.40	0.40
	1.	1.79	3.67	3.66	1.63	1.17	1.17
	2.	1.86	3.56	6.08	4.59	1.56	6.21
	3.	4.82	13.55	15.61	7.29	9.33	10.36
	7.	9.49	7.26	26.55	11.32	1.66	19.90
	9.	6.61	19.82	38.24	17.85	15.54	19.27
	11.	7.26	22.16	36.41	23.45	12.33	29.85
	13.	4.45	94.47	15.18	16.73	10.88	36.64
	15.	11.17	20.19	23.23	16.82	6.37	23.79
Acetic acid	0.	12.70	12.70	12.70	4.50	4.50	4.50
	1.	41.00	53.23	53.30	14.70	15.31	14.00
	2.	37.10	47.12	67.50	19.90	14.00	20.30
	3.	63.10	63.90	104.10	24.40	25.26	25.40
	7.	60.80	96.94	126.70	36.70	26.77	35.00
	9.	46.60	146.00	127.70	35.80	29.94	35.50
	11.	74.00	160.31	166.80	44.60	30.11	39.30
	13.	76.50	137.88	205.30	46.80	32.37	36.90
	15.	33.20	166.62	184.60	45.50	14.50	39.40
Pyrophosphate	0.	81.90	81.90	81.90	43.80	43.83	43.80
	1.	89.50	117.31	115.00	48.80	45.29	46.50
	2.	100.60	125.39	158.10	64.50	49.50	59.70
	3.	92.20	142.58	167.40	60.20	62.59	67.80
	7.	95.00	160.80	190.60	59.00	50.04	61.70
	9.	112.00	181.55	220.90	67.10	54.69	64.80
	11.	93.00	190.02	219.50	71.50	45.46	65.40
	13.	94.20	206.89	220.50	68.30	46.13	60.80
	15.	91.20	198.42	219.90	68.80	43.88	62.90
Oxalate	0.	163.30	163.31	163.30	95.58	95.58	95.58
	1.	172.10	169.47	172.55	94.16	95.70	93.93
	2.	192.85	148.36	137.85	104.44	83.92	105.70
	3.	225.18	157.96	186.77	146.29	166.41	152.93
	7.	182.46	124.95	147.68	93.89	101.89	91.84
	9.	174.59	89.50	83.15	56.77	53.77	66.64
	11.	180.01	100.92	98.61	110.90	121.66	125.45
	13.	215.54	110.78	120.92	115.38	127.74	145.80
	15.	189.85	115.08	93.15	75.16	74.76	52.87

Appendix 1. Iron

Extractant	Time (weeks)	B_2			B3g		
		C.	P.W.	W.	C.	P.W.	W.
Water	0.	0.0	0.0	0.0	0.0	0.0	0.0
	1.	0.0	29.9	29.9	0.0	0.0	0.0
	2.	0.0	39.2	62.8	0.0	0.0	0.0
	3.	0.6	50.9	35.7	0.0	0.0	0.0
	7.	3.3	120.9	38.2	0.4	3.0	0.3
	9.	0.0	66.1	0.0	0.0	0.0	0.0
	11.	0.0	40.6	44.9	0.0	0.0	0.0
	13.	0.0	0.0	54.1	0.0	0.0	0.0
	15.	0.0	70.2	32.6	0.0	0.0	0.0
Acetic acid	0.	10.7	10.7	10.7	12.1	12.1	12.1
	1.	17.4	18.8	20.4	14.2	13.7	13.7
	2.	17.2	15.7	19.1	14.0	14.8	16.3
	3.	28.8	33.4	32.7	17.7	30.4	25.8
	7.	22.7	87.8	131.0	17.1	16.0	15.0
	9.	10.5	437.6	416.9	12.1	14.0	13.0
	11.	26.6	658.0	795.2	25.6	20.0	26.8
	13.	24.2	912.2	1071.5	28.7	23.9	28.3
	15.	9.3	794.2	1222.2	14.6	8.8	16.4
Pyrophosphate	0.	10872.0	10872.0	10872.0	6394.0	6394.0	6394.0
	1.	11715.5	10408.0	10612.5	6084.0	5883.0	6125.0
	2.	11474.0	11407.0	10822.5	6085.0	5957.0	6803.0
	3.	10667.0	10493.0	10593.5	5950.0	6322.0	6223.0
	7.	10609.0	11675.0	11552.0	6193.0	6499.0	6223.0
	9.	11394.0	13092.0	12675.0	7148.0	6750.0	6766.0
	11.	10610.5	12557.0	12325.0	6782.0	6215.0	6112.0
	13.	11373.5	12777.0	12179.0	6909.0	6343.0	5989.0
	15.	11002.0	12324.0	12010.0	7322.0	6333.0	6223.0
Oxalate	0.	9721.0	9721.0	9721.5	9124.0	9124.0	9124.0
	1.	10677.0	11325.8	9830.5	7445.6	7923.7	7474.4
	2.	9698.5	9381.5	9855.0	9298.0	6912.6	9146.0
	3.	18859.0	13684.0	15460.0	17374.5	17495.0	20163.0
	7.	10255.0	9878.9	12544.4	8792.0	8977.4	8401.0
	9.	10595.0	10941.0	9507.0	6791.0	7090.8	7638.0
	11.	15136.5	13449.4	11106.5	19012.0	17088.5	15470.0
	13.	14514.0	13668.6	13201.5	13454.5	11737.3	12936.0
	15.	9421.5	10794.0	10033.5	6230.5	6051.3	6203.0

Appendix 1. Zinc

Extractant	Time (weeks)	B_2			B_{3g}		
		C.	P.W.	W.	C.	P.W.	W.
Water	0.	0.80	0.80	0.80	0.70	0.70	0.70
	1.	0.59	0.12	0.00	0.09	0.16	0.10
	2.	1.53	3.36	0.00	0.07	0.16	0.19
	3.	0.95	0.23	0.25	0.17	0.45	0.20
	7.	0.82	0.20	0.21	0.33	0.12	0.16
	9.	1.53	1.44	1.52	0.47	0.36	0.36
	11.	0.93	1.84	0.36	0.45	1.10	0.86
	13.	0.83	0.23	0.00	0.18	5.83	3.32
	15.	1.34	0.00	0.32	0.00	0.38	0.21
Acetic acid	0.	4.80	4.80	4.80	3.80	3.80	3.80
	1.	6.30	6.99	7.00	6.10	6.38	6.00
	2.	6.10	5.64	6.50	5.60	5.43	5.40
	3.	7.45	7.78	7.40	7.50	9.86	8.30
	7.	6.80	5.73	8.10	8.00	10.66	7.70
	9.	9.00	6.58	8.90	5.30	5.99	5.30
	11.	10.40	10.62	12.60	8.20	8.11	8.20
	13.	6.85	11.82	7.70	6.30	13.38	9.10
	15.	4.25	4.19	4.40	3.70	3.94	4.50
Pyrophosphate	0.	26.76	26.76	26.76	19.41	19.41	19.41
	1.	20.34	22.17	17.88	15.98	16.90	17.19
	2.	18.67	19.26	19.55	19.49	15.46	24.08
	3.	17.36	17.85	17.75	13.45	19.63	18.63
	7.	22.64	13.71	18.79	10.95	12.60	11.74
	9.	14.87	13.55	12.88	11.57	11.45	12.04
	11.	15.36	16.53	14.20	13.16	11.84	13.28
	13.	18.08	16.69	13.74	12.80	11.53	12.85
	15.	14.12	15.04	13.21	9.41	10.01	10.75
Oxalate	0.	53.02	53.02	53.02	25.28	25.28	25.28
	1.	24.89	23.52	22.24	26.96	27.92	29.63
	2.	21.03	20.92	23.67	22.90	21.71	24.61
	3.	33.74	35.05	35.82	42.63	44.85	49.50
	7.	23.39	24.26	29.12	25.61	25.24	29.98
	9.	22.16	20.27	23.43	19.36	20.47	17.70
	11.	25.23	32.50	29.49	33.97	34.41	34.33
	13.	26.21	29.75	31.56	32.97	33.75	39.83
	15.	11.81	22.55	18.45	14.67	15.32	15.76

Appendix 1. Copper

Extractant	Time (weeks)	B2 C.	B2 P.W.	B2 W.	B3g C.	B3g P.W.	B3g W.
Acetic acid	0.	1.280	1.280	1.280	0.900	0.900	0.900
	1.	0.870	0.956	0.956	0.883	0.903	0.903
	2.	0.608	0.769	0.657	0.577	0.645	0.558
	3.	0.641	0.516	0.714	0.566	0.560	0.913
	7.	0.899	1.233	1.023	0.597	0.512	0.873
	9.	1.436	1.674	1.440	0.644	0.838	0.934
	11.	2.035	4.351	2.157	1.850	2.502	2.310
	13.	0.600	0.705	0.914	0.460	0.790	0.414
	15.	0.620	1.128	0.805	0.662	0.570	1.353
Pyrophosphate	0.	8.200	8.200	8.200	3.058	3.058	3.058
	1.	7.700	5.885	5.885	2.918	3.225	3.225
	2.	14.400	13.320	16.260	11.140	13.350	3.420
	3.	4.712	5.165	4.650	2.488	3.950	2.196
	7.	7.140	4.858	9.540	3.580	7.770	3.180
	9.	18.830	18.050	16.340	20.910	15.950	13.550
	11.	14.520	11.920	13.720	18.130	23.560	10.750
	13.	17.110	23.960	22.710	25.710	17.370	13.800
	15.	4.446	5.220	4.514	4.378	1.750	1.560
Oxalate	0.	8.830	8.830	8.830	11.840	11.840	11.840
	1.	14.150	13.920	13.920	12.470	12.057	12.057
	2.	4.107	4.000	3.940	4.470	4.024	3.050
	3.	3.862	6.030	4.613	7.490	7.383	6.205
	7.	3.462	3.550	4.808	2.968	4.158	2.734
	9.	7.230	8.580	7.170	11.340	8.711	8.540
	11.	6.263	6.650	7.185	10.230	8.444	8.005
	13.	9.010	12.310	12.580	16.890	13.360	11.840
	15.	2.400	2.648	2.600	2.866	2.488	2.594

Appendix 1. Cobalt

Extractant	Time (weeks)	B_2			B_{3g}		
		C.	P.W.	W.	C.	P.W.	W.
Water	0.	0.023	0.023	0.023	0.021	0.021	0.021
	1.	0.024	0.035	0.035	0.024	0.026	0.037
	2.	0.025	0.030	0.027	0.027	0.020	0.031
	3.	0.047	0.064	0.063	0.038	0.061	0.057
	7.	0.049	0.024	0.200	0.040	0.032	0.171
	9.	0.029	0.067	0.300	0.091	0.053	0.054
	11.	0.035	0.035	0.053	0.095	0.129	0.164
	13.	0.032	0.057	0.031	0.055	0.325	0.434
	15.	0.042	0.035	0.058	0.070	0.030	0.165
Acetic acid	0.	2.200	2.200	2.200	2.800	2.800	2.800
	1.	2.350	2.800	2.440	2.800	2.800	2.800
	2.	2.100	2.000	2.400	2.700	2.700	2.900
	3.	2.300	2.600	2.500	3.000	4.500	3.700
	7.	2.600	2.700	3.500	3.000	3.400	3.000
	9.	1.600	2.800	3.000	2.600	3.100	2.500
	11.	2.400	2.800	3.400	3.400	3.000	3.600
	13.	2.500	3.600	3.300	3.100	3.200	3.100
	15.	1.800	2.700	2.600	2.200	2.100	2.000
Pyrophosphate	0	0.250	0.250	0.250	0.170	0.170	0.170
	1	0.221	0.277	0.220	0.166	0.202	0.252
	13	0.345	0.426	0.368	0.301	0.246	0.338
Oxalate	0.	2.800	2.800	2.800	4.100	4.100	4.100
	1.	3.600	2.700	2.700	4.100	3.900	4.100
	2.	4.300	3.470	3.200	6.000	5.300	5.600
	3.	5.000	4.600	4.800	6.700	7.600	6.400
	7.	4.000	3.100	3.500	4.400	4.400	4.300
	9.	5.400	4.500	4.200	9.800	11.600	10.200
	11.	8.400	8.100	7.300	11.800	12.000	14.000
	13.	7.800	8.000	8.200	12.000	12.000	12.800
	15.	4.700	4.200	4.800	7.800	9.200	8.500

APPENDIX 2

Analytical results of metal concentration in soil extracts on B_2 soils with aerated, waterlogged, aerated with 1% lime, waterlogged with 1% lime and waterlogged with 1% added organic matter treatments.

Appendix 2. Manganese

Extractant	Time (weeks)	Control	Waterlogged	Control + 1% lime	Waterlogged + 1% lime	Waterlogged + 1% O.M.
Calcium chloride	0.	1.66	1.66	1.21	1.21	2.50
	1.	1.43	1.73	0.89	1.52	2.44
	3.	1.54	3.46	0.57	9.72	15.53
	5.	1.47	9.18	0.78	30.33	32.91
	8.	1.31	31.65	0.52	39.52	46.93
	10.	0.72	21.77	0.33	21.21	26.18
	12.	1.58	50.58	0.60	44.83	56.87
	14.	1.69	54.30	0.84	43.52	58.74
	16.	1.99	57.91	1.25	46.23	61.48
Acetic acid	0.	0.57	0.57	0.91	0.91	0.59
	1.	0.20	0.19	0.59	0.59	0.19
	3.	0.53	0.58	0.47	3.92	2.08
	5.	0.41	0.58	0.73	11.04	5.01
	8.	0.49	4.63	0.78	16.18	7.09
	10.	0.34	3.53	0.58	8.49	4.57
	12.	0.43	7.92	0.96	20.11	9.56
	14.	0.52	8.80	0.91	21.53	9.77
	16.	0.56	9.67	0.82	22.72	11.33
Pyrophosphate	0.	41.35	41.35	41.56	41.56	40.89
	1.	42.03	42.53	41.60	44.49	45.04
	3.	28.73	30.51	28.69	37.01	36.30
	5.	32.09	35.84	31.36	58.80	51.85
	8.	39.03	64.41	41.03	87.09	77.78
	10.	15.75	28.45	15.51	35.83	30.07
	12.	25.56	54.58	26.57	67.03	58.47
	14.	29.46	67.67	31.06	84.49	71.49
	16.	28.23	69.48	31.71	86.74	75.25
Oxalate	0.	52.95	52.95	52.68	52.68	54.52
	1.	50.57	50.64	48.32	54.02	49.14
	3.	59.43	59.04	58.36	57.25	47.79
	5.	64.45	59.26	65.90	50.44	52.45
	8.	75.52	61.84	80.23	61.28	58.84
	10.	41.33	27.37	37.14	31.11	25.30
	12.	56.26	37.62	54.68	45.83	40.08
	14.	75.91	57.72	83.49	61.63	53.05
	16.	74.54	55.46	84.18	58.43	53.99

Appendix 2. Iron

Extractant	Time (weeks)	Control	Waterlogged	Control + 1% lime	Waterlogged + 1% lime	Waterlogged + 1% O.M.
Calcium chloride	0.	5.1	5.1	5.6	11.8	11.8
	1.	5.6	6.2	6.0	11.3	9.3
	3.	7.0	7.2	528.2	17.3	16.2
	5.	4.9	272.2	1284.1	13.0	36.6
	8.	9.3	961.7	1195.1	12.0	35.9
	10.	3.9	746.8	701.5	7.0	28.5
	12.	6.6	1499.3	1421.4	14.1	34.6
	14.	7.4	1416.9	1422.4	11.8	29.2
	16.	5.5	1505.0	1395.6	11.8	26.3
Acetic acid	0.	16.0	16.0	16.6	18.5	18.5
	1.	10.1	9.6	10.7	9.1	9.9
	3.	10.3	14.0	26.0	11.3	23.7
	5.	7.8	16.5	67.2	8.9	85.9
	8.	19.1	84.9	211.3	18.7	295.5
	10.	9.1	79.1	152.4	9.8	207.8
	12.	15.7	289.7	445.7	16.6	532.7
	14.	20.5	399.6	552.2	19.3	539.2
	16.	18.6	447.1	621.9	18.4	615.6
Pyrophosphate	0.	11826.8	11826.8	10863.0	11108.8	11108.8
	1.	11109.9	11360.2	11378.6	10737.7	10824.1
	3.	10708.2	11031.3	11197.1	10603.8	10890.4
	5.	10734.0	11133.0	11267.3	10163.2	10634.1
	8.	10456.0	11353.7	11277.4	10181.7	11384.4
	10.	5751.2	5849.4	5800.5	5235.0	5722.4
	12.	10544.5	12062.8	12231.3	10445.5	11949.8
	14.	11613.0	12937.3	13041.4	10846.7	12276.0
	16.	11706.5	13182.8	12760.8	10741.6	12173.6
Oxalate	0.	6770.9	6770.9	6798.8	7432.6	7432.6
	1.	5348.3	5835.5	5682.7	5798.3	6511.3
	3.	6024.7	5955.9	5454.7	6453.0	6278.5
	5.	6384.3	6296.4	6204.0	6260.8	6650.3
	8.	6177.6	6186.9	5904.4	6463.0	6820.4
	10.	3471.0	3042.8	2900.9	3215.5	3700.7
	12.	5893.6	5486.1	5795.9	6298.2	6916.3
	14.	7037.5	6842.4	6441.9	7512.1	7593.7
	16.	6591.3	6647.3	6291.4	7439.4	7700.4

Appendix 2. Zinc

Extractant	Time (weeks)	Control	Waterlogged	Control + 1% lime	Waterlogged + 1% lime	Waterlogged + 1% O.M.
Calcium chloride	0.	11.73	11.73	12.25	0.31	0.31
	1.	10.17	9.96	9.36	0.08	0.00
	3.	11.65	10.99	9.33	0.57	0.91
	5.	10.46	9.23	5.55	0.20	0.21
	8.	10.45	8.97	7.58	0.68	0.74
	10.	5.48	3.53	3.27	0.08	0.10
	12.	9.87	7.23	7.00	0.46	0.56
	14.	10.73	6.99	7.04	0.30	0.38
	16.	9.86	6.82	6.39	0.31	0.38
Acetic acid	0.	4.06	4.06	4.60	9.50	9.50
	1.	3.13	3.32	3.30	7.13	6.81
	3.	4.13	4.19	5.98	6.94	8.32
	5.	3.94	3.91	7.13	5.40	7.50
	8.	4.64	7.00	7.54	7.45	9.56
	10.	2.32	3.47	4.46	3.24	4.23
	12.	3.82	7.09	8.26	5.75	9.17
	14.	4.05	7.36	8.18	5.15	9.10
	16.	3.68	7.57	8.50	5.06	8.78
Pyrophosphate	0.	23.13	23.13	27.85	20.91	20.91
	1.	17.36	17.49	18.59	14.27	14.65
	3.	24.76	23.77	22.12	20.30	18.59
	5.	20.32	19.33	13.72	15.99	13.21
	8.	25.67	18.30	16.15	16.92	14.59
	10.	11.19	7.65	8.38	7.26	6.79
	12.	21.37	16.20	14.94	15.64	14.66
	14.	21.40	14.63	14.99	16.22	14.73
	16.	21.38	15.33	13.47	15.05	13.88
Oxalate	0.	9.98	9.98	11.07	11.40	11.40
	1.	6.56	6.08	7.81	9.90	11.27
	3.	8.39	9.55	20.04	13.01	15.43
	5.	10.03	9.59	20.47	13.40	19.25
	8.	9.97	17.18	22.29	11.42	17.30
	10.	5.02	9.23	10.50	6.50	9.28
	12.	8.48	16.88	19.79	12.52	18.99
	14.	9.20	17.16	19.81	13.82	19.14
	16.	10.20	18.91	22.11	14.34	20.42

Appendix 2. Copper

Extractant	Time (weeks)	Control	Waterlogged	Control + 1% lime	Waterlogged + 1% lime	Waterlogged + 1% O.M.
Calcium chloride	0.	0.937	0.937	1.054	0.061	0.061
	1.	1.006	0.937	0.911	0.222	0.172
	3.	0.744	0.931	0.678	0.019	0.020
	5.	0.334	0.220	0.253	0.018	0.019
	8.	0.642	0.351	0.280	0.017	0.019
	10.	0.506	0.300	0.182	0.017	0.019
	12.	0.368	0.230	0.169	0.016	0.019
	14.	0.393	0.304	0.254	0.107	0.128
	16.	0.371	0.200	0.236	0.016	0.019
Acetic acid	0.	3.467	3.467	3.595	2.578	2.578
	1.	3.267	3.087	3.040	1.928	2.012
	3.	2.973	3.191	2.948	1.821	2.434
	5.	2.344	2.468	2.253	1.295	1.784
	8.	3.120	3.425	3.308	2.122	2.894
	10.	2.891	3.542	3.252	1.899	3.062
	12.	2.197	2.795	2.858	1.660	2.711
	14.	2.255	3.169	3.050	1.522	2.636
	16.	2.280	2.888	2.895	1.428	2.755
Pyrophosphate	0.	22.001	22.001	20.928	21.485	21.485
	1.	25.256	24.129	24.309	23.680	21.337
	3.	25.259	23.825	20.121	21.189	24.381
	5.	21.814	19.413	17.319	21.437	20.649
	8.	25.509	25.113	24.613	20.386	21.539
	10.	24.400	20.316	22.775	23.517	24.271
	12.	22.442	19.929	19.690	21.491	21.376
	14.	21.359	19.541	19.208	21.611	20.791
	16.	20.311	17.710	17.696	20.105	19.450
Oxalate	0.	10.733	10.733	10.034	10.201	10.201
	1.	10.068	9.027	9.135	7.394	7.408
	3.	8.765	9.533	9.483	8.237	9.358
	5.	7.672	10.112	7.688	8.175	8.484
	8.	8.222	9.345	10.256	6.713	8.359
	10.	10.043	10.780	10.221	10.422	10.039
	12.	7.273	7.298	7.678	7.506	7.724
	14.	9.203	9.529	8.506	10.845	8.222
	16.	6.820	7.638	7.220	7.301	7.655

Appendix 2. Cobalt

Extractant	Time (weeks)	Control	Waterlogged	Control + 1% lime	Waterlogged + 1% lime	Waterlogged + 1% O.M.
Calcium chloride	0.	1.103	1.103	1.103	0.975	0.975
	1.	1.222	1.862	1.322	1.602	1.413
	3.	1.518	1.150	1.405	1.152	1.706
	5.	0.737	1.052	0.565	0.970	0.625
	8.	1.012	1.162	1.972	0.609	1.595
	10.	0.503	1.597	1.117	0.409	0.804
	12.	0.682	1.247	1.934	0.859	1.342
	14.	1.452	1.703	1.486	0.306	0.511
	16.	0.590	0.918	0.832	0.192	0.397
Acetic acid	0.	1.452	1.452	1.452	3.021	3.021
	1.	1.616	1.477	1.803	2.570	2.358
	3.	1.499	2.043	1.801	3.570	3.426
	5.	1.429	2.036	1.774	3.442	3.360
	8.	1.277	1.975	1.728	3.253	3.306
	10.	1.180	2.624	3.388	2.219	4.192
	12.	1.768	3.754	4.690	3.038	6.392
	14.	2.154	3.093	2.598	2.080	4.132
	16.	1.369	2.192	3.153	1.442	2.896
Pyrophosphate	0.	17.102	17.102	17.102	15.958	15.958
	1.	14.601	15.487	15.590	17.027	15.736
	3.	14.728	12.825	14.514	12.294	13.258
	5.	18.512	9.919	12.568	7.871	15.196
	8.	19.056	23.769	22.479	19.977	21.499
	10.	7.772	6.660	8.858	7.836	6.552
	12.	11.262	12.071	11.554	10.255	12.408
	14.	12.314	16.051	11.958	12.031	14.453
	16.	12.369	15.200	17.562	13.049	14.676
Oxalate	0.	4.922	4.922	4.922	4.225	4.225
	1.	4.609	4.634	2.901	4.274	3.871
	3.	7.811	8.215	7.692	8.512	8.148
	5.	7.418	8.192	7.662	8.263	8.114
	8.	10.172	9.557	9.077	9.329	10.250
	10.	8.050	7.652	7.188	7.136	8.456
	12.	8.324	7.956	7.493	7.877	9.302
	14.	8.403	7.497	7.358	7.617	8.886
	16.	7.266	8.384	7.946	8.133	8.824

APPENDIX 3

Analytical results of metal concentration in soil extracts on B_2 soils with temporary waterlogging.

Appendix 3

Metal	Time (days)	Soil solution	Water	Acetic acid	Pyrophosphate	Oxalate
Copper	0.	0.00350000	0.039200	1.22000	12.5300	3.65000
	4.	0.00350000	0.050500	0.98000	13.1200	2.74000
	8.	0.00350000	0.042800	1.09000	12.1600	2.69000
	12.	0.00350000	0.100100	1.69000	11.8300	2.92000
	16.	0.00420000	0.173000	1.40000	12.5100	3.61000
	20.	0.00900000	0.121000	1.69000	11.8800	4.05000
	24.	0.00600000	0.190000	1.39500	7.3300	5.03000
	28.	0.00400000	0.245000	1.07000	7.7800	4.58000
	32.	0.00920000	0.239000	1.22600	6.1820	4.94000
Cobalt	0.	0.0089000	0.041700	2.22000	0.0	5.61800
	4.	0.0089000	0.057200	2.59000	0.0	6.69300
	8.	0.0187000	0.076800	2.20000	0.0	5.49700
	12.	0.0121000	0.109000	2.49300	0.0	5.20300
	16.	0.0144000	0.144000	2.40500	0.0	4.60200
	20.	0.0112000	0.182000	3.49200	0.0	4.82000
	24.	0.0122000	0.281000	3.78400	0.0	3.75500
	28.	0.0132000	0.238000	3.66400	0.0	3.84800
	32.	0.0151000	0.223000	3.84600	0.0	3.53900

Appendix 3

Metal	Time (days)	Soil solution	Water	Acetic acid	Pyrophosphate	Oxalate
Manganese	0.	1.58000	1.3700	22.000	110.000	130.800
	4.	1.58000	4.9300	28.200	107.000	123.000
	8.	2.94000	5.7800	48.700	120.000	104.000
	12.	1.65000	10.1400	60.000	108.800	105.000
	16.	1.55000	13.4600	75.000	111.800	96.700
	20.	1.23000	16.6000	98.000	134.000	83.000
	24.	1.54000	59.7000	156.000	196.000	57.300
	28.	1.72000	22.8000	155.000	197.000	56.200
	32.	1.67000	12.5000	146.000	203.000	51.500
Iron	0.	0.04000	1.770	7.160	14668.0	5407.00
	4.	0.04000	2.600	7.110	15242.0	5890.00
	8.	0.57000	17.500	9.800	15517.0	5649.00
	12.	0.72000	3.270	13.090	14909.0	5550.00
	16.	1.59000	57.000	19.970	14388.0	5490.00
	20.	2.27000	92.000	33.590	14796.0	5446.00
	24.	3.06000	102.300	67.970	15268.0	5151.00
	28.	3.54000	91.270	107.810	15054.0	4885.00
	32.	5.97000	109.800	124.000	15351.0	4942.00
Zinc	0.	0.0960000	0.940000	8.5900	17.1000	12.1000
	4.	0.0960000	0.990000	8.0800	17.5000	11.6000
	8.	0.0570000	0.700000	9.6100	20.7000	13.8000
	12.	0.0200000	0.980000	11.5000	18.6000	13.0800
	16.	0.0130000	0.580000	12.5000	16.0000	12.5000
	20.	0.0130000	0.560000	9.1600	17.6000	16.4000
	24.	0.0090000	0.770000	8.4900	17.0000	15.5000
	28.	0.0130000	0.750000	7.7200	16.6000	14.5000
	32.	0.0120000	0.470000	8.5200	18.8000	19.8000